Marschner's Mineral Nutrition of Higher Plants

Third Edition

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Foreword

The publication of the first and second editions of Horst Marschner's *Mineral Nutrition of Higher Plants* established this book internationally as the leading and most widely cited textbook for graduate students and researchers in plant nutrition. The book demonstrated Horst Marschner's comprehensive understanding of the subject and ability to present this knowledge in a clear, logical form to his readers. In view of this great success it was a devastating blow to all that, soon after the appearance of the second edition, Horst Marschner died tragically from cerebral malaria contracted during a visit to Niger to see at first hand the results of field experiments. With his passing in 1996 plant nutrition lost one of its most remarkably gifted scientists of the twentieth century.

Horst Marschner is mostly remembered for his long association with the Institute of Plant Nutrition at the University of Hohenheim, where he found refuge in 1960 after fleeing from communist East Germany. Other than the period during which he was Professor at the Technical University of Berlin (1970–76), the greater part of his working life was spent at Hohenheim and it was there too that his most important contributions to research were achieved. Under his direction from 1976 to 1996, the institute flourished. Charismatic enthusiasm, devotion to work, and thoughtful leadership assured him of the support and affection of staff and students. Together with eminent colleagues and in particular Dr Volker Römheld with whom he worked closely for many years, he developed a highly

imaginative research programme covering a wide range of interests in plant nutrition from the plant cell to the field which attracted visiting research scientists from all over the world. During those years it was always a pleasure to visit my old friend Horst Marschner in Hohenheim. A visit there was to the hub of research in plant nutrition, and a discussion with Horst was always greatly invigorating, providing many ideas for my own research.

Those who had the privilege of working with Horst Marschner will remember with admiration his critical discussion and attention to detail together with his unfailing encouraging support. Undoubtedly he was a great mentor to his students, encouraging them to express their thoughts and stressing the importance of being open to new ideas. Fifteen years on from his death, the enormity of the Marschner legacy to plant nutrition is still very evident. Many of Horst Marschner's former post-graduate students are now directing their own teaching and research departments across the world, their number including the current President of the International Council on Plant Nutrition and his three immediate predecessors.

The need for a new edition of *Mineral Nutrition of Higher Plants* has been obvious for some time because of the burgeoning flow of new literature and major developments in various aspects of the subject. In discussing the way forward to produce a new edition, Horst Marschner's former colleagues and co-workers concluded that in order to maintain the quality of the second edition, individual chapters should be written by eminent selected authors. As a testament to Horst Marschner's outstanding work as teacher, communicator and research scientist in plant nutrition, a new title for the book was chosen, *Marschner's Mineral Nutrition of Higher Plants*. Changes in the development of the subject are reflected in the format of the new edition. Part I includes a new chapter on nutrition and quality and Part II divides and extends rhizosphere into two chapters, rhizosphere chemistry and rhizosphere biology. Additionally, a new chapter on nutrient cycling is included. The aim of this third edition is the same as the two previous editions: to provide a comprehensive text on plant nutrition for both graduate students and research workers.

Fortunately, Horst Marschner's daughter, Dr Petra Marschner, now at the University of Adelaide, Australia, has willingly taken on the onerous task of senior editor of the book. Without her dedication, determination and ability to persuade, cajole as well as control the contributing authors, this masterpiece would not have seen the light of day.

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Preface to First Edition

Mineral nutrients are essential for plant growth and development. Mineral nutrition of plants is thus an area of fundamental importance for both basic and applied science. Impressive progress has been made during the last decades in our understanding of the mechanisms of nutrient uptake and their functions in plant metabolism; at the same time, there have also been advances in increasing crop yields by the supply of mineral nutrients through fertilizer application. It is the main aim of this textbook to present the principles of the mineral nutrition of plants, based on our current knowledge. Although emphasis is placed on crop plants, examples are also presented from noncultivated plants including lower plants in cases where these examples are considered more suitable for demonstrating certain principles of mineral nutrition, either at a cellular level or as particular mechanisms of adaptation to adverse chemical soil conditions.

Plant nutrition as a subject is closely related to other disciplines such as soil science, plant physiology and biochemistry. In this book, mineral nutrients in soils are treated only to the extent considered necessary for an understanding of how plant roots acquire mineral nutrients from soils, or how roots modify the chemical soil properties at the soil-root interface. Fundamental processes of plant physiology and biochemistry, such as photosynthesis and respiration, are treated mainly from the viewpoint of how, and to what extent, they are affected or regulated by mineral nutrients. Crop physiology is included as an area of fundamental practical importance for agriculture and horticulture, with particular reference to source-sink relationships as affected by mineral nutrients and phytohormones.

Mineral nutrition of plants covers a wide field. It is therefore not possible to treat all aspects with the detail they deserve. In this book, certain aspects are covered in more detail, either because they have recently become particularly important to our understanding of mineral nutrition, or because many advances have been made in a particular area in the last decade. Naturally, personal research interests and evaluation are also factors which have influenced selection. Particular emphasis is placed on short- and long-distance transport of mineral elements, on source-sink relationships, and on plant-soil relationships. It is also the intention of this book to enable the reader to become better acquainted with the mechanisms of adaptation of plants to adverse chemical soil conditions. The genetical basis of mineral nutrition is therefore stressed, as well as the possibilities and limitations of "fitting crop plant to soils", especially in the tropics and subtropics.

I have written this textbook for graduate students and researchers in the various fields of agricultural, biological and environmental sciences, who already have a profound knowledge of plant physiology, biochemistry and soil science. Instead of extensive explanations of basic processes, emphasis is placed on representative examples—tables, figures, schematic presentations—illustrating the various aspects of mineral nutrition. In a textbook of such wide scope, generalizations cannot be avoided, but relevant literature is cited for further and more detailed studies. In the literature, preference has been given to more recent publications. Nevertheless, representative examples of classical contributions are also cited in the various sections. Although this book is written by one person, it is nevertheless the product of cooperation at various levels. My interest in plant nutrition and my scientific career in this field are due to the inspiration of Dr. G. Michael. The book as it is presented here would not have been accomplished without the excellent support of two colleagues. Dr. V. Roemheld and Mr. Ernest A. Kirkby. I am very much indebted to both of them. Dr. Roemheld not only prepared the drawings but also gave highly valuable advice regarding the arrangement of the tables and improvements to the text. My old friend Ernest A. Kirkby corrected the English and improved the first draft considerably by valuable suggestions and stimulating criticism. My colleagues in the institute, Dr. P. Martin, Dr. W. J. Horst and Dr. B. Sattelmacher helped me greatly, both by valuable discussions in various subject areas treated in this book and by keeping me free for some time from teaching and administrative responsibilities. Many colleagues were kind enough to supply me with their original photographs, as indicated in the legend of the corresponding figures.

The preparation of such a manuscript requires skilful technical assistance. I would especially like to thank Mrs. H. Hoderlein for typing the manuscript.

Last but not least, I have to thank my family for encouraging me to write the book and for their assistance and patience throughout this time-consuming process.

Stuttgart-Hohenheim *Horst Marschner*
August 1985

Preface to Second Edition

As mentioned in the first edition the main aim of this textbook is to present the principles of the mineral nutrition of higher plants, based on current knowledge. This ambitious aim requires that the content of the book has to be updated regularly to take into account new developments in the subject as has been done in this second edition. The structure of the textbook has not been altered and the subject matter and number of chapters remains the same. The contents of the chapters, however, have been revised and on average about half the figures, tables and references replaced. The introduction of these more recent findings was based on the principle that newer examples and references are given priority, provided the quality of the information is at least similar to that which is being replaced. In Part I more emphasis has been placed on root-shoot interactions, stress physiology, water relations, and functions of micronutrients. In view of the worldwide increasing interest in plant-soil interactions, Part II has been considerably altered and extended. This is particularly true for Chapter 14 on the effects of external and internal factors on root growth, and Chapter 15 on the root-soil interface (root exudates, rhizosphere microorganisms, mycorrhizae).

The second edition would not have been accomplished without the support of many colleagues, friends and coworkers. Of these colleagues I am particularly grateful to Dr. Ismail Cakmak, Dr. Albrecht Jungk, Dr. Volker Roemheld and Dr. Alexander Hansen. And again my old friend Ernest A. Kirkby took the most difficult task not only of correcting the English but also of improving the presentation by valuable suggestions and detailed, constructive criticism. I am also highly indebted to Dr. Eckhard George and his team for skilfully drawing the figures, to my daughter Petra and Dr. Ulrich Grauer for critically reading the text and the proofs, and to Mrs. Helga Hoderlein for the high quality of her technical assistance, especially in preparing the manuscript.

Stuttgart-Hohenheim *Horst Marschner*
December 1993

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Chapter 1

Introduction, Definition and Classification of Nutrients

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SUMMARY

This chapter provides a brief overview of the history of plant nutrition and defines the term 'essential mineral element', and groups these elements according to their biochemical behaviour and physiological functions.

1.1 GENERAL

The beneficial effect of adding mineral elements (e.g., plant ash or lime) to soils to improve plant growth has been known in agriculture for more than 2,000 years. Nevertheless, even 150 years ago it was still a matter of scientific controversy as to whether mineral elements function as nutrients for plant growth. It was mainly to the credit of Justus von Liebig (1803–1873) that the scattered information concerning the importance of certain elements for plant growth was compiled and summarized and that the mineral nutrition of plants was established as a scientific discipline. These achievements led to a rapid increase in the use of mineral fertilizers. By the end of the nineteenth century, especially in Europe, large amounts of potash, superphosphate and, later, inorganic N were used in agriculture and horticulture to improve crop growth and production.

Liebig's conclusion that the elements N, S, P, K, Ca, Mg, Si, Na and Fe are essential for plant growth was reached by observation and speculation rather than by precise experimentation. The fact that the 'mineral element theory' was based on this unsound foundation was one of the reasons for the large number of studies undertaken at the end of the nineteenth century. From these and other extensive investigations on the elemental composition of different plant species growing on various soils, it was realized as early as the beginning of the last century that neither the presence nor the concentration of an element in a plant is a criterion for essentiality. Plants have a limited capability for the selective uptake of those elements which are essential for their growth. Additionally they take up elements which are not needed for growth and which may even be toxic.

1.2 ESSENTIAL ELEMENTS FOR PLANT GROWTH

The elemental composition of plants growing in soils cannot therefore be used to establish whether an element is essential. Once this fact was appreciated, both water and sand culture experiments were carried out in which plants were deprived of particular elements and the consequent effects on growth and development studied. Such investigations made possible a more precise characterization of the essentiality of elements and helped to define their role in plant metabolism. This work also revealed two fairly distinct groups of nutrients, the macronutrients which are required and are present in relatively high concentrations in plants, and the micronutrients which are equally essential, but present in very much lower concentrations. For higher plants, the essentiality of 14 elements is now well established, although the requirement for the micronutrients Cl and Ni is as yet restricted to a limited number of plant species. Progress in this research was closely related to the development of analytical chemistry, particularly in the purification of chemicals and analysis. This relationship is reflected in the time course of the discovery of the essentiality of the micronutrients (Table 1.1).

The term *essential mineral element* (or mineral nutrient) was proposed by Arnon and Stout (1939). These authors concluded that, for an element to be considered essential, three criteria must be met:

1. A given plant must be unable to complete its lifecycle in the absence of the element.

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TABLE 1.1 Discovery of the essentiality of micronutrients for higher plants

Element (chemical symbol)	Year	Discovered by
Fe	1860	J. Sachs
Mn	1922	J.S. McHargue
B	1923	K. Warington
Zn	1926	A.L. Sommer and C.B. Lipman
Cu	1931	C.B. Lipman and G. MacKinney
Mo	1938	D.I. Arnon and P.R. Stout
Cl	1954	T.C. Broyer *et al.*
Ni	1987	P.H. Brown *et al.*

2. The function of the element must not be replaceable by another element.
3. The element must be directly involved in plant metabolism – for example, as a component of an essential plant constituent such as an enzyme – or it must be required for a distinct metabolic step such as an enzyme reaction.

According to this strict definition, an element which alleviates the toxic effects of another element (e.g., Si for Mn toxicity), or one which simply replaces another element (e.g., Na for K) may not be described as essential for plant growth.

1.3 BIOCHEMICAL BEHAVIOUR AND PHYSIOLOGICAL FUNCTIONS OF ELEMENTS IN PLANTS

In addition to their relative concentrations within the plant, elements may also be classified according to biochemical behaviour and physiological function. In a scheme proposed by Mengel and Kirkby (2001) (Table 1.2), all plant nutrients including C, H and O as well as some non-essential elements (Si and Na) are considered. Four groups can be distinguished (Table 1.2).

The first group includes the major constituents of organic plant material: C, H, O, N and S. These elements are constituents of amino acids, proteins, enzymes and nucleic acids, the building blocks of life. The assimilation of all these nutrients by plants is closely linked with oxidation-reduction reactions.

Phosphorus, B and Si constitute a second group of elements with close similarities in biochemical behaviour. All three are taken up from the soil solution as inorganic anions or acids and occur in this form in plant cells or are bound by hydroxyl groups of sugars to form phosphate, borate and silicate esters.

The third group of plant nutrients is made up of K, Na, Ca, Mg, Mn and Cl, all of which are taken up from the soil solution in the form of their ions. In plant cells, they are also present in ionic form where they have non-specific functions, e.g. in establishing electro-potentials.

TABLE 1.2 Classification of plant nutrients

Nutrient	Uptake	Biochemical functions
Group 1		
C, H, O, N, S	as CO_2, HCO_3^-, H_2O, O_2, NO_3^-, NH_4^+, N_2, SO_4^{2-}, SO_2 ions from the soil solution, gases from the atmosphere	Major constituents of organic material. Essential elements of atomic groups involved in enzymatic processes. Assimilation by oxidation-reduction reactions.
Group 2		
P, B, Si	as phosphates, boric acid or borate, silic acid from the soil solution	Esterification with alcohol groups. Phosphate esters involved in energy transfer reactions.
Group 3		
K, Na, Ca, Mg, Mn, Cl	as ions from the soil solution	Non-specific functions establishing osmotic potential. More specific functions for optimal confirmation of enzymes (enzyme activation). Bridging of reaction partners. Balancing anions. Controlling membrane permeability and electrochemical potentials.
Group 4		
Fe, Cu, Zn, Mo	as ions or chelates from the soil solution	In chelated form in prosthetic groups of enzymes. Enable electron transport by valency change.

From Mengel and Kirkby (2001) with kind permission from Springer Science Business Media.

The cations are associated with diffusible or indiffusible anions, e.g. Ca with oxalate or with the carboxylic groups of pectins in cell walls. Magnesium can also be bound very strongly by coordinate and covalent bonds (chelation) as occurs in the chlorophyll molecule. The ability of Mg, Ca and Mn to form chelates means that these elements closely resemble those of the fourth group, Fe, Cu, Zn and Mo, which are predominantly present in plants in chelated form. An important function of these latter elements is to facilitate electron transport by valency change.

Because of continuous developments and refinements in analytical techniques, especially in the purification of chemicals, the current list of essential elements might well be extended to include elements that are essential only in very low concentrations in plants (i.e., that act as micronutrients). This may possibly be the case for Na and Si, two elements abundant in the biosphere for which essentiality has already been established for some plant species (Chapter 8). Most micronutrients are predominantly constituents of enzyme molecules and are thus essential only in small amounts at the whole plant level. By contrast, the macronutrients are either constituents of organic compounds, such as proteins and nucleic acids, or act as osmotica. These differences in function are reflected in the average concentrations of mineral nutrients in plant shoots that are sufficient for adequate growth (Table 1.3). The values can vary considerably depending on plant species, plant age, and concentration of other mineral elements. This aspect is discussed in Chapters 6 to 8.

TABLE 1.3 Average concentrations of mineral elements in plant shoot dry matter sufficient for adequate growth

Element	Chemical symbol	$\mu mol\ g^{-1}$ dw	$mg\ kg^{-1}$
Molybdenum	Mo	0.001	0.1
Nickel	Ni	0.001	0.1
Copper	Cu	0.1	6
Zinc	Zn	0.3	20
Manganese	Mn	1.0	50
Iron	Fe	2.0	100
Boron	B	2.0	20
Chlorine	Cl	3.0	100
Sulphur	S	30	1,000
Phosphorus	P	60	2,000
Magnesium	Mg	80	2,000
Calcium	Ca	125	5,000
Potassium	K	250	10,000
Nitrogen	N	1,000	15,000

From Epstein (1965), Epstein and Bloom (2005), Brown *et al.* (1987b).

Chapter 2

Ion Uptake Mechanisms of Individual Cells and Roots: Short-distance Transport

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SUMMARY

The uptake of nutrients by higher plants is characterized by selectivity of transport and accumulation in specific tissues, cells or subcellular compartments. These characteristics are genetically determined and can differ both between and within plant species. This chapter reviews the environmental, physiological and developmental factors that affect the entry of nutrients into the extracellular space (apoplasm) of roots, their transport across the plasma membrane and tonoplast of root cells, and the pathways of their movement to the xylem. It describes the structure and composition of cellular membranes, the electrochemical gradients that determine the energetics of solute transport across membranes, and the mechanisms involved and the genetic identity of the proteins that facilitate the transport of nutrients across the plasma membrane and tonoplast of plant cells. The overriding influence of plant nutritional status on the expression of mechanisms by which roots acquire nutrients is emphasized.

2.1 GENERAL

As a rule, there is a great discrepancy between the concentrations of mineral nutrients in the soil and the nutrient requirements of plants. Furthermore, soil, and in some cases nutrient solutions, can contain high concentrations of mineral elements not needed for plant growth, or that are potentially harmful to plants. The mechanisms by which plants accumulate nutrients must therefore be selective. This selectivity can be demonstrated particularly well in algal cells (Table 2.1), where the external and vacuolar (cell sap) solutions are separated by only two membranes: the plasma membrane and the tonoplast.

In *Nitella*, the concentrations of K, Na, Ca, and Cl ions are higher in the cell sap than in the pond water, but the concentration ratio differs considerably between the ions. By contrast, in *Valonia* growing in seawater, only K is more concentrated in the cell sap, whereas the Na and Ca concentrations are lower in the cell sap than in the seawater.

Selective ion uptake is also a typical feature of higher plants. When plants are grown in a nutrient solution of limited volume, the external concentrations of ions change with time (Table 2.2). The concentrations of K, P and nitrate decline markedly, whereas those of Na and sulphate can even increase, indicating that water is taken up faster than either of these two ions. Uptake rates, especially for K and Ca, differ between plant species (e.g., maize and bean,

TABLE 2.1 Relationship between ion concentrations in the substrate and in the cell sap of *Nitella* and *Valonia*

	Nitella concentration (mM)			*Valonia* concentration (mM)		
	A	B	Ratio	A	B	Ratio
Ion	Pond water	Cell sap	B/A	Seawater	Cell sap	B/A
Potassium	0.05	54	1080	12	500	42
Sodium	0.22	10	45	498	90	0.18
Calcium	0.78	10	13	12	2	0.17
Chloride	0.93	91	98	580	597	1

Modified from Hoagland (1948).

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TABLE 2.2 Changes in the ion concentration of the external (nutrient) solution and in the root sap of maize and bean

	External concentration (mM)			Concentration in root sap (mM)	
		After 4 days[a]			
Ion	Initial	Maize	Bean	Maize	Bean
Potassium	2.00	0.14	0.67	160	84
Calcium	1.00	0.94	0.59	3	10
Sodium	0.32	0.51	0.58	0.6	6
Phosphate	0.25	0.06	0.09	6	12
Nitrate	2.00	0.13	0.07	38	35
Sulfate	0.67	0.61	0.81	14	6

[a] *No replacement of water lost through transpiration.*

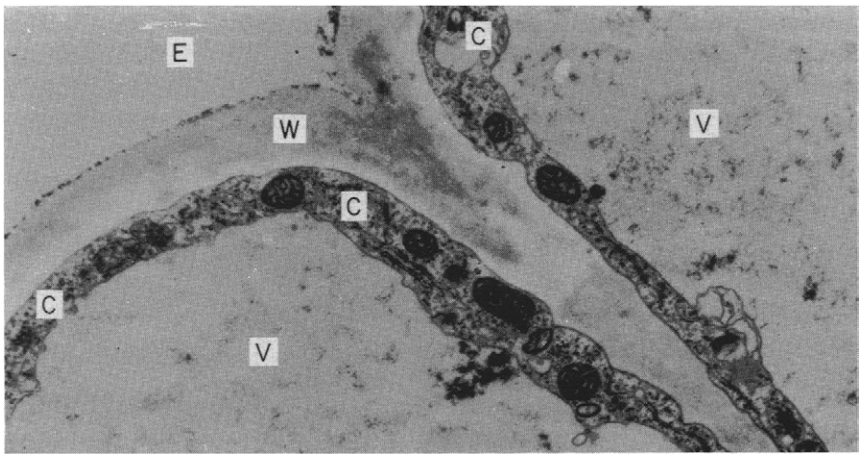

FIGURE 2.1 Cross-section of two rhizodermal cells of a maize root. V, vacuole; C, cytoplasm; W, cell wall, E, external solution. *Courtesy of C. Hecht-Buchholz.*

Table 2.2). The concentrations of ions in the root sap are generally higher than those in the nutrient solution; this is most evident in the case of K, nitrate and phosphate.

The results obtained from both lower and higher plants demonstrate that ion uptake is characterized by:

1. *Selectivity*. Certain mineral elements are taken up preferentially, while others are discriminated against or almost excluded.
2. *Accumulation.* The concentration of elements can be much higher in cell sap than in the external solution.
3. *Genotype.* There are distinct differences between plant species in their ion uptake characteristics.

These observations lead to many questions. In particular, how do individual cells and higher plants regulate the uptake of ions both to satisfy plant demand and to avoid ion toxicities? To understand the regulation of ion uptake it is necessary to follow the pathway of solutes (ions, charged and uncharged molecules) from the external solution through the cell wall and the plasma membrane into the cytoplasm and vacuoles of plant cells.

2.2 PATHWAY OF SOLUTES FROM THE EXTERNAL SOLUTION INTO ROOT CELLS

2.2.1 Influx to the Apoplasm

Movement of low-molecular-weight solutes (e.g., ions, organic acids, amino acids, sugars) from the external solution through the walls of individual root cells (the *free space*) is a non-metabolic, passive process, driven by diffusion or mass flow (Fig. 2.1). Nevertheless, cell walls can interact with solutes and, thereby, facilitate or restrict

TABLE 2.3 Diameter of cell wall pores and hydrated cations

	Diameter (nm)
Rhizodermal cell wall (maize; Fig. 2.1)	500–3,000
Cortical cell wall (maize)	100–200
Pores in cell wall	<5.0
Sucrose	1.0
Hydrated ions	
K^+	0.66
Ca^{2+}	0.82

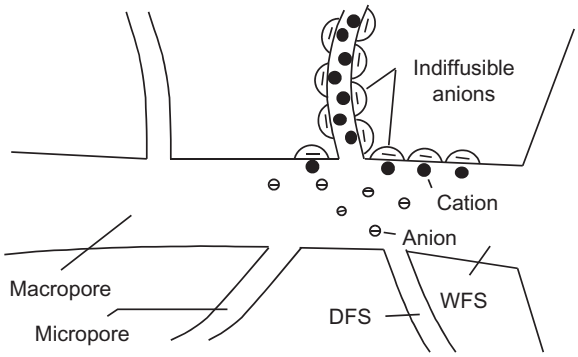

FIGURE 2.2 Schematic diagram of the pore system of the apparent free space. DFS, Donnan free space; WFS, water free space.

TABLE 2.4 Cation exchange capacity of root dry matter of different plant species

Plant species	Cation exchange capacity (mmol $(100\,g)^{-1}$ dw)
Wheat	23
Maize	29
Bean	54
Tomato	62

Based on Keller and Deuel (1957).

passage across the root and uptake across the plasma membrane of individual cells.

The primary cell wall consists of a network of cellulose, accounting for about 15–30% of its dry weight, cross-linking glycans (generally xyloglucans in Type I walls, but in the Type II walls of commelinoid monocotyledons mostly gluconoarabinoxylans) and glycoproteins, all embedded in a pectin matrix (Carpita and McCann, 2000). Type I cell walls contain more pectin than Type II cell walls. Both Ca and B are also integral components of cell walls, which can be additionally impregnated with Si. The cell wall network contains pores, the so-called interfibrillar and intermicellar spaces, which differ in size. For root hair cells of radish, a maximum diameter of 3.5 to 3.8 nm (35–38 Å) has been calculated and maximum diameters for the pores in plant cell walls are generally in the range of 5.0 nm (Table 2.3). The diameters of hydrated ions, such as K^+ and Ca^{2+}, are small in comparison. Therefore, the pores themselves would not be expected to offer any restriction to ion movement through the cell wall.

In contrast to nutrients and low-molecular-weight organic solutes, the movement of high-molecular-weight solutes (e.g., metal chelates, fulvic acids and toxins) or viruses and other pathogens through one cell walls is severely restricted by the diameter of the pores.

A variable proportion of the pectins in cell walls consist of polygalacturonic acid, originating mainly from the middle lamella. Accordingly, their carboxylic groups ($R.COO^-$) act as cation exchangers in the cell wall continuum of roots and other plant tissue, the so-called *apoplasm*. In roots, cations from the external solution can accumulate in the *free space*, whereas anions are repelled.

Hope and Stevens (1952) introduced the term *apparent free space* (AFS). This comprises the *water free space* (WFS), which is freely accessible to ions and charged and uncharged molecules, and the *Donnan free space* (DFS), where cation exchange and anion repulsion take place (Fig. 2.2). Ion distributions within the DFS are characterized by typical Donnan distributions. Trivalent cations, such as Al^{3+}, bind more strongly than divalent cations, such as Ca^{2+}, which bind more strongly than monovalent cations, such as K^+. Plant species differ considerably in their cation exchange capacity (CEC), that is, in the number of cation exchange sites (fixed anions; $R{\cdot}COO^-$), located in cell walls (Table 2.4).

As a rule, the CEC of dicotyledonous species is greater than that of monocotyledonous species (White and Broadley, 2003). As the external pH decreases, the effective CEC is reduced, particularly in monocotyledonous species (Allan and Jarrell, 1989) as protons occupy an increasing proportion of the cation binding sites. Because of apoplastic barriers within the root, such as the Casparian band of the endodermis and exodermis, only part of the AFS is directly accessible to cations from the external solution. Exchange adsorption of cations in the apoplasmic AFS is not a prerequisite for ion uptake across the plasma membrane or for the movement of ions within the apoplasm. However, fixed negative charges in the AFS can influence both the absolute and relative concentrations of cations in the apoplasm, especially when roots grow in dilute solutions (White and Broadley, 2003). Thus, root CEC can affect the rate and selectivity of ion influx into root cells and apoplasmic ion movements indirectly. It

has been speculated that this could account for the positive correlation between root CEC and the ratio of Ca^{2+} to K^+ concentration in different plant species. Alternatively, this correlation could simply reflect the predominant accumulation of Ca^{2+} in the apoplasm and of K^+ in the vacuoles of plant cells (White and Broadley, 2003; White and Karley, 2010).

The apoplasmic AFS can also serve as a transient storage pool for essential mineral elements such as iron and zinc which can be mobilized, for example, by specific root exudates such as phytosiderophores, and subsequently translocated to the shoots (Zhang *et al.*, 1991b, c; Cesco *et al.*, 2002; Liu *et al.*, 2010). For iron, the size of this storage pool possibly contributes to genotypic differences in sensitivity to iron deficiency in soybean (Longnecker and Welch, 1990). In addition, the entry of elements supplied to the root in excess of plant demand, such as Ca in calcareous soils, can be restricted by precipitation as insoluble salts.

2.2.2 Passage into the Cytoplasm

Despite some selectivity for cation binding in the cell wall, the main site of selectivity in the uptake of cations and anions, as well as solutes in general, is the *plasma membrane* of individual cells. The lipid bilayer of the plasma membrane prevents the indiscriminate movement of ions and large polar molecules from the apoplasm into the cytoplasm (influx) and from the cytoplasm into the apoplasm (efflux). Integral membrane proteins facilitate the selective transport of solutes across the plasma membrane. It can be readily demonstrated that the plasma membrane is a selective barrier to the uptake of ions. For example, when barley plants are placed in a nutrient solution containing Ca^{2+} (^{45}Ca) and K^+ (^{42}K), most of the ^{45}Ca accumulated in the roots in the first 30 min is still readily exchangeable and is almost certainly located in the apoplasmic AFS (Fig. 2.3). By contrast, only a minor fraction of the ^{42}K is readily exchangeable after this period, most of the ^{42}K having been transported across the plasma membranes of root cells. Furthermore, in most mature plant cells, the vacuole comprises more than 80–90% of the cell volume, acting as central storage compartment for ions and solutes (e.g., sugars and secondary metabolites), and it is likely that the ^{42}K is already sequestered within this cellular compartment.

Within the plant cell, membranes with contrasting lipid and protein composition separate the various cellular compartments. These membranes include the *tonoplast* (vacuolar membrane), the endoplasmic reticulum (ER), the Golgi apparatus, the nuclear membrane and the membranes surrounding vesicles, mitochondria and plastids (Staehelin and Newcomb, 2000). For these membranes, the lipid bilayer provides the barrier to solute movement, and proteins facilitate the selective transport of solutes to provide the unique transport properties required for the function of each compartment. Before the mechanisms of solute transport across membranes are discussed in greater detail (Sections 2.4 and 2.5), some fundamental aspects of the composition and structure of biological membranes will be described.

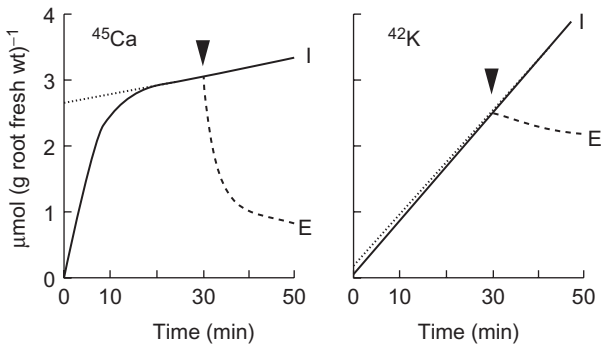

FIGURE 2.3 Time course of influx (*I*) and efflux (*E*) of ^{45}Ca and ^{42}K to isolated barley roots. After 30 min (arrow) some of the roots were transferred to solutions with no labelled Ca^{2+} and K^+. The proportion of the exchangeable fraction in the apparent free space is calculated by extrapolation to zero time.

TABLE 2.5 Reflection coefficients (δ) of some non electrolytes at the cell membranes of *Valonia utricularis*

Compound	δ[a]	Molecular radius (nm)
Raffinose	1.00	0.61
Sucrose	1.00	0.53
Glucose	0.95	0.44
Glycerol	0.81	0.27
Urea	0.76	0.20

Based on Zimmermann and Steudle (1970).
[a] *1.00 indicates that the membranes are impermeable to the solute; 0.00 indicates that the membranes are freely permeable to the solute.*

2.3 COMPOSITION OF BIOLOGICAL MEMBRANES

The capacity of plant cell membranes to regulate solute uptake has fascinated botanists since the nineteenth century. By the early years of the twentieth century some basic facts of solute permeation across biological membranes had been established, such as the inverse relationship between the diameter of uncharged molecules and the rates at which they permeate membranes (Table 2.5). High-molecular-weight organic solutes such as polyethyleneglycol are not taken up by cells and can be used at high external concentrations as osmotica to induce water

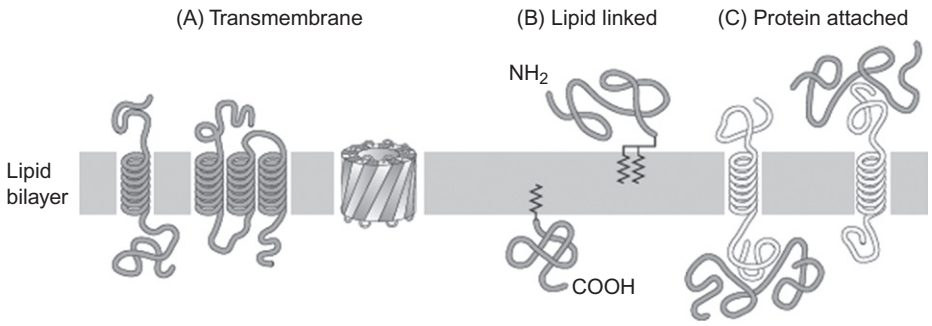

FIGURE 2.4 Protein associations with biological membranes. Integral transmembrane proteins extend through the lipid bilayer in α-helical or β-sheet structures. Peripheral proteins are attached to the membrane either by covalently attached lipid groups or through interactions with integral membrane proteins. *Based on a figure from Essential Biology of the cell by Bruce Alberts* et al. *(1998). Reproduced by permission of Garland Science/Taylor & Francis Books, Inc.*

TABLE 2.6 Lipid and fatty acid composition of the plasma membrane and tonoplast of mung bean

Lipids	Plasma membrane (μmol mg^{-1} protein)	Tonoplast (μmol mg^{-1} protein)
Phospholipids	1.29	1.93
Sterols	1.15	1.05
Glycolipids	0.20	0.80

Fatty acid composition of the phospholipids

Fatty acid	Chain length	Melting point (°C)	Plasma membrane (% of total)	Tonoplast (% of total)
Palmitic acid	C16	+62.8	35	39
Stearic acid	C18	+70.1	6	6
Oleic acid	C18:1[a]	+13.0	9	9
Linoleic acid	C18:2[a]	−5.5	21	22
Linolenic acid	C18:3[a]	−11.1	19	20
Others	–	–	10	4

Based on Yoshida and Uemura (1986).
[a] *Numeral to the right of the colon indicates the number of double bounds.*

deficiency (drought stress) in plants. However, some hydrophobic molecules penetrate membranes much faster than would be predicted on the basis of their size, which is presumably related to their ability to partition into the lipid bilayer.

Biological membranes are typically composed of a lipid bilayer and associated proteins (Fig. 2.4). However, membrane composition is sensitive to environmental conditions, and the relative abundance, and types, of both lipids and proteins in membranes surrounding cellular compartments differ (Table 2.6). The lipids in cell membranes have hydrophilic headgroups and hydrophobic tails. The most abundant membrane lipids are (i) phospholipids, in which the hydrophilic headgroup is linked to the hydrophobic tail by a phosphate group, (ii) sterols, which are based around a four-ring structure, and (iii) glycolipids, which have sugars as their hydrophilic headgroup (Fig. 2.5). Common plant phospholipids are phosphatidyl serine, phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl inositol and diphosphatidyl glycerol. Plasma membranes and mitochondria are enriched in phosphatidyl inositol and diphosphatidyl glycerol, respectively. The fatty acid moiety in phospholipids varies in both chain length and number of double bonds, but is often palmitic (length:double-bonds, 16:0), stearic (18:0), oleic (18:1), linoleic (18:2) or linolenic (18:3) acid (Table 2.6). Major plant sterols include campesterol, sitosterol and stigmasterol. The sterol content of the ER is low, but sterols can make up more than 30% of the total lipids in the plasma membrane and tonoplast (Table 2.6). Most glycolipids are found in the chloroplast (Hölzl and Dörmann, 2007), where the thylakoid membrane is predominantly composed of monogalatosyldiacyglycerol (MGDG), together with digalatosyldiacyglycerol (DGDG) and the sulpholipid, sulphoquinovosyldiacylglycerol (SQDG).

Although the lipid bilayer provides the basic structure of the membrane and forms a permeability barrier, most biological functions of membranes are performed by proteins. The membrane surrounding each cellular compartment has different types of proteins reflecting the particular function of that membrane. Membrane proteins function (i) to anchor the membrane to the cytoskeleton and/or cell wall, (ii) as receptors/transducers for compartmentalized signals, (iii) as enzymes for specific reactions, such as energy transduction processes in mitochondria and chloroplasts, and (iv) to transport specific solutes across membranes.

There are several ways by which proteins can be associated with the lipid bilayer (Staehelin and Newcomb, 2000). Many membrane proteins extend through the bilayer (Fig. 2.4). These integral transmembrane proteins have both hydrophobic and hydrophilic portions. Their

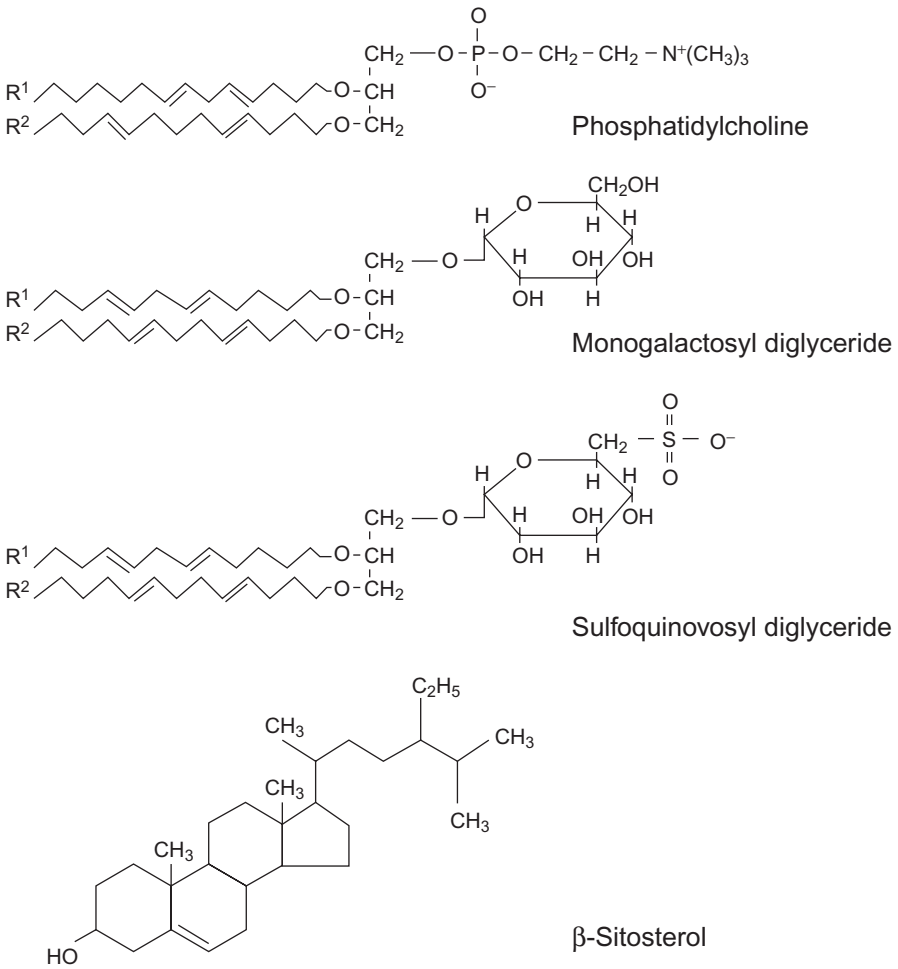

FIGURE 2.5 Chemical structures of selected membrane lipids. Phospholipids are represented by phosphatidylcholine, glycolipids by monogalactosyl diglyceride and sulphoquinovosyl diglyceride, and sterols by β-sitosterol, respectively.

hydrophobic portions lie within the bilayer, alongside the hydrophobic tails of the lipid molecules, while their hydrophilic portions extend into the aqueous environment on either side of the membrane. Other membrane proteins are located entirely outside the bilayer. These peripheral proteins are bound to the membrane through lipid groups attached covalently through prenylation (attachment of the isoprenoids farnesyldiphosphate or geranylgeranyldiphosphate), *S*-acetylation (attachment of palmitate or stearate) or *N*-myristolation (Sorek *et al.*, 2009), or are associated with other membrane proteins through ionic interactions. It is thought that lipid modification of membrane proteins also facilitates their subcellular targeting and clustering into specific domains.

Lipid composition not only differs between cellular membranes (Table 2.6), plant tissues and plant species (Staehelin and Newcomb, 2000), but is also strongly influenced by environmental factors. In leaves, for example, distinct annual variations in sterol concentrations occur (Westerman and Roddick, 1981); membrane lipid composition changes during exposure to low temperatures (e.g., Welti *et al.*, 2002; Penfield, 2008), and DGDG and SQDG can replace phospholipids in membranes of P deficient plants (Hölzl and Dörmann, 2007; White and Hammond, 2008). Similarly, the composition of root membranes is influenced by temperature, salinity and the ionic composition of the external solution (e.g., Cakmak and Marschner, 1988c; White *et al.*, 1990b; Wu *et al.*, 1998; Lindberg *et al.*, 2005; López-Pérez *et al.*, 2009). The changes in lipid composition reflect often the adaption of a plant to its environment. For example, membranes of plants growing at low temperatures have more phospholipids with charged headgroups and shorter fatty acid chains with lower degree of saturation, and greater sterol content than plants growing at higher temperatures (Staehelin and Newcomb, 2000; Wallis and Browse, 2002; Penfield, 2008). Such changes shift the freezing point (i.e., the transition temperature) of membranes to a lower temperature and may therefore be

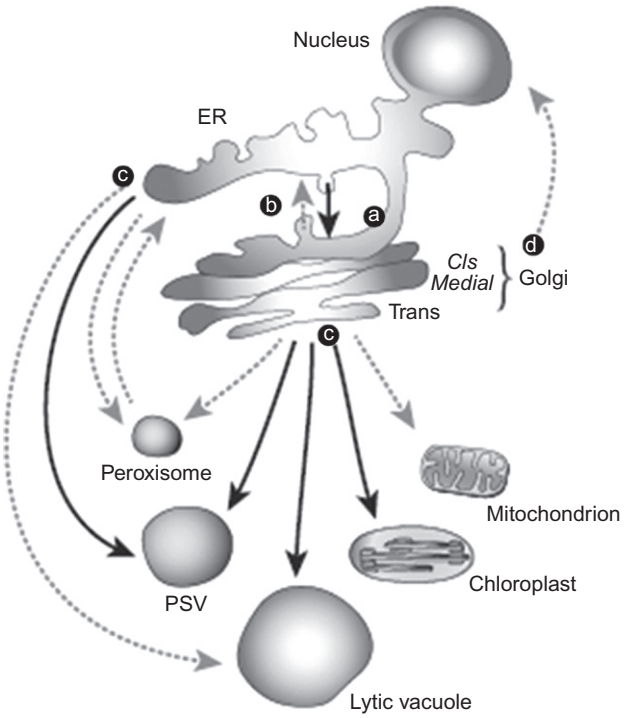

FIGURE 2.6 Pathways of membrane trafficking. The initial pathways are divided into (a) endoplasmic reticulum (ER) and Golgi integration and (b) transport of vesicles between the ER and Golgi. The subsequent pathways (c) involve transport of vesicles between the ER and peroxisomes and vacuoles, and between the Golgi and peroxisomes, vacuoles, chloroplasts, mitochondria and plasma membrane. Feedback signals from the Golgi to the nucleus (d) are thought to regulate aspects of membrane trafficking. *Figure adapted from Matheson* et al. *(2006).*

important for the maintenance of membrane functions at low temperatures.

Cellular membranes are dynamic structures that are continuously remodelled to allow the plant to respond to developmental signals, biotic challenges and environmental conditions. This remodelling occurs over minutes to months, and is supported by complex trafficking pathways that deliver lipids and proteins to and from cellular membranes (Fig. 2.6). These pathways are functionally linked through the Golgi apparatus to the endoplasmic reticulum, plasma membrane, peroxisomes, vacuoles, mitochondria and chloroplasts (Matheson *et al.*, 2006; Robinson *et al.*, 2007). The delivery of secretory vesicles to the plasma membrane can be targeted to specific locations, such as the apex of tip-growing cells, e.g. elongating root hairs or pollen tubes (Cole and Fowler, 2006; Cheung and Wu, 2008; Ishida *et al.*, 2008; Sorek *et al.*, 2009), or to plasmodesmata (Oparka, 2004; Maule, 2008; Lucas *et al.*, 2009). Thus, membranes are not homogeneous, but possess domains in which specific lipids and proteins can be clustered, stably or transiently, to improve the efficiency of biochemical and physiological processes (Opekarová *et al.*, 2010).

2.4 SOLUTE TRANSPORT ACROSS MEMBRANES

2.4.1 Thermodynamics of Solute Transport

In the experiment described in Table 2.2, the K concentration in maize root sap (which is approximately equal to the K concentration of the vacuoles) was 80 times higher than in the external solution. In contrast, the Na concentration in the root sap remained lower than that in the external solution. Such phenomena require both a source of energy and selective transport across the plasma membrane of root cells.

Transport across plant membranes is facilitated by transmembrane proteins (Fig. 2.7). These can be classified into three groups: (i) primary active transporters (pumps), in which solute transport is coupled directly to the hydrolysis of an energy substrate such as ATP or pyrophosphate (PP_i); (ii) secondary active transporters or 'coupled transporters', which harness the electrochemical gradient of (generally) H^+ to the movement of a solute in either the same (symport) or opposite (antiport) direction; and (iii) passive transporters, which catalyse the movement of solutes down their electrochemical gradient. The latter group includes a variety of carriers (uniporters) and channels. Channels can be distinguished from uniport carriers by their high catalytic rate, which can exceed 10 million ions s^{-1} which is several orders of magnitude greater than uniport carriers (White, 2003). In the next paragraphs, the driving forces for solute movement across membranes are considered in relation to facilitated diffusion, or 'passive' transport, of solutes down their electrochemical gradient by carriers and channels, and to 'active' transport of solutes against their electrochemical gradient catalysed by pumps and coupled transporters.

Under most circumstances, the driving force for the facilitated diffusion of an uncharged solute across a membrane is its concentration gradient, whereas for an ion it is its electrochemical gradient (White, 2003). The Nernst equation (Fig. 2.8) allows the direction of the net diffusive flux of an ion at a given membrane potential and temperature to be determined. When the cell membrane potential is more negative than the Nernst potential, cations can move into the cell, and anions out of the cell, by facilitated diffusion. When the membrane potential is more positive than the Nernst potential, the opposite fluxes are favoured. According to the Nernst equation, at 20°C with a membrane potential of −100 mV, K^+ or Cl^- would be in electrochemical equilibrium across the plasma membrane if their concentration in the cytosol were 52 times higher (K^+) or 52 times lower (Cl^-) than in the external solution (Fig. 2.8). At the same temperature and membrane potential, the concentrations of a divalent cation or anion would differ more than 2,700-fold between the cytosol and external medium if it was in electrochemical equilibrium.

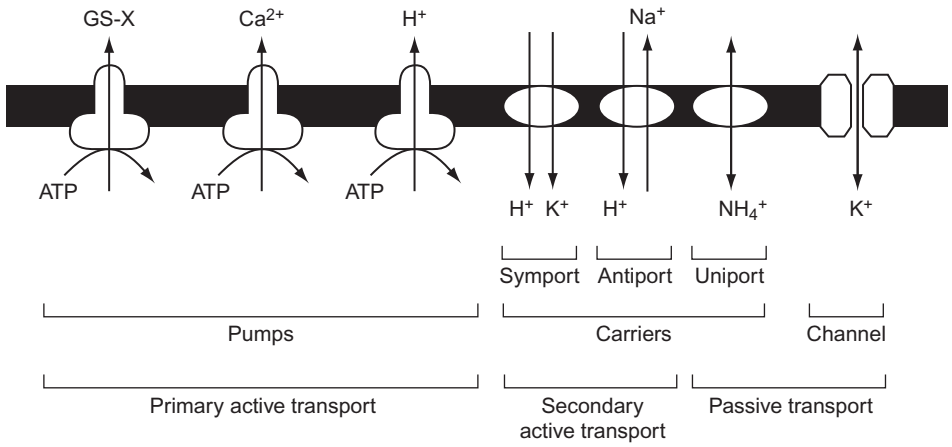

FIGURE 2.7 Nomenclature of transport proteins. Schematic representation of primary active transport mechanisms, such as ABC transporters (e.g., glutathione conjugate pump), metal transporters (e.g., Ca^{2+}-ATPase) and H^+-ATPases, secondary active transport mechanisms, such as the K^+/H^+ symporter or the Na^+/H^+ antiporter, and passive transport mechanisms, such as the NH_4^+ carrier and the K^+ channel. *Figure adapted from White (2003).*

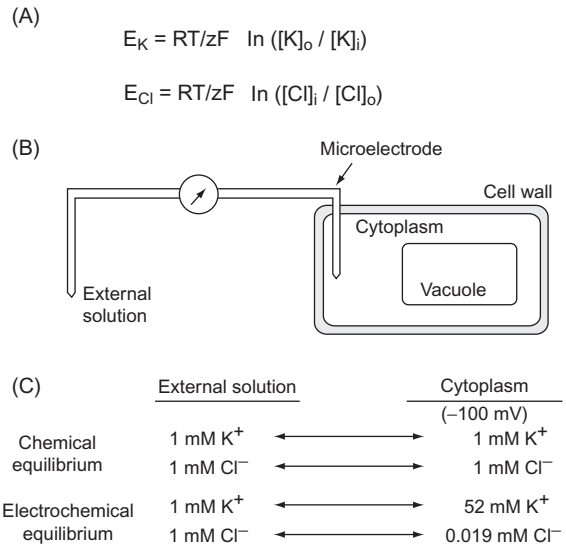

FIGURE 2.8 (A) The Nernst equation. The equilibrium potential for potassium (E_K) and chloride (E_{Cl}) are given as a function of R, the gas constant (8.314 V C K^{-1} mol^{-1}), T, the absolute temperature, z the valency of the ion, and their activity concentrations outside (subscript o) and inside (subscript i) the membrane. (B) Schematic representation of the system used for measuring the membrane potential of plant cells. (C) Example of the calculation of ion distributions at electrochemical equilibrium assuming a membrane potential of −100 mV at 20°C.

The resting membrane potential of root cells is often more negative than −100 mV (Maathuis and Sanders, 1993; Walker *et al.*, 1996; Britto and Kronzucker, 2006). It is generated primarily by the activity of plasma membrane H^+-ATPases encoded by members of the *AHA* gene family (Gaxiola *et al.*, 2007). These H^+ pumps are clustered in discrete (micro)domains of the plasma membrane and their activity is regulated by phosphorylation-dependent interactions with cytosolic 14-3-3 proteins in response to diverse environmental signals including exposure to salt and low temperatures. Under physiological conditions, many cations are in electrochemical equilibrium across the plasma membrane of root cells (White, 2003). However, there is always a large electrochemical gradient driving Ca^{2+} influx to cells, and, in saline environments, there is also a large electrochemical gradient driving Na^+ influx. On the other hand, anions cannot be concentrated in the cytoplasm by facilitated diffusion across the plasma membrane, and their influx to root cells is often facilitated by symporters coupled to the proton electrochemical gradient generated by plasma membrane H^+-ATPases.

At the molecular level, facilitated diffusion is mediated by uniporters or channels. Passive transporters facilitating the influx of 10 of the 14 mineral nutrients across the plasma membrane of root cells have been reported (Fig. 2.9; White, 2003; Gojon *et al.*, 2009; Karley and White, 2009; White and Broadley, 2001, 2003, 2009; Miwa and Fujiwara, 2010; Teakle and Tyerman, 2010). These include K-channels, such as *At*AKT1:*At*KC1 of *Arabidopsis thaliana*, voltage-dependent Ca-channels, cation channels, such as those encoded by the *cyclic nucleotide gated channel* (*CNGC*) and *glutamate receptor* (*GLR*) gene families, ammonium transporters encoded by the *ammonium transporter* (*AMT*) gene family, M, transporters, such as *At*MGT1 and *At*MGT10, members of the Zn-regulated transporter (ZRT)-, Fe-regulated transporter (IRT)-like protein (ZIP) family, which transport Fe^{2+}, Zn^{2+}, Cu^{2+} and Mn^{2+}, Cu^+ transporters encoded by *CTR*/*COPT* genes, boric acid channels, formed by nodulin-26-like intrinsic proteins (NIPs) and plasma membrane intrinsic proteins (PIPs), and, in saline environments, Cl^- channels. However, influx into root cells of nutrients present in the soil solution as anions (e.g., nitrate, phosphate, sulphate, molybdate, chloride) is not thought to be mediated

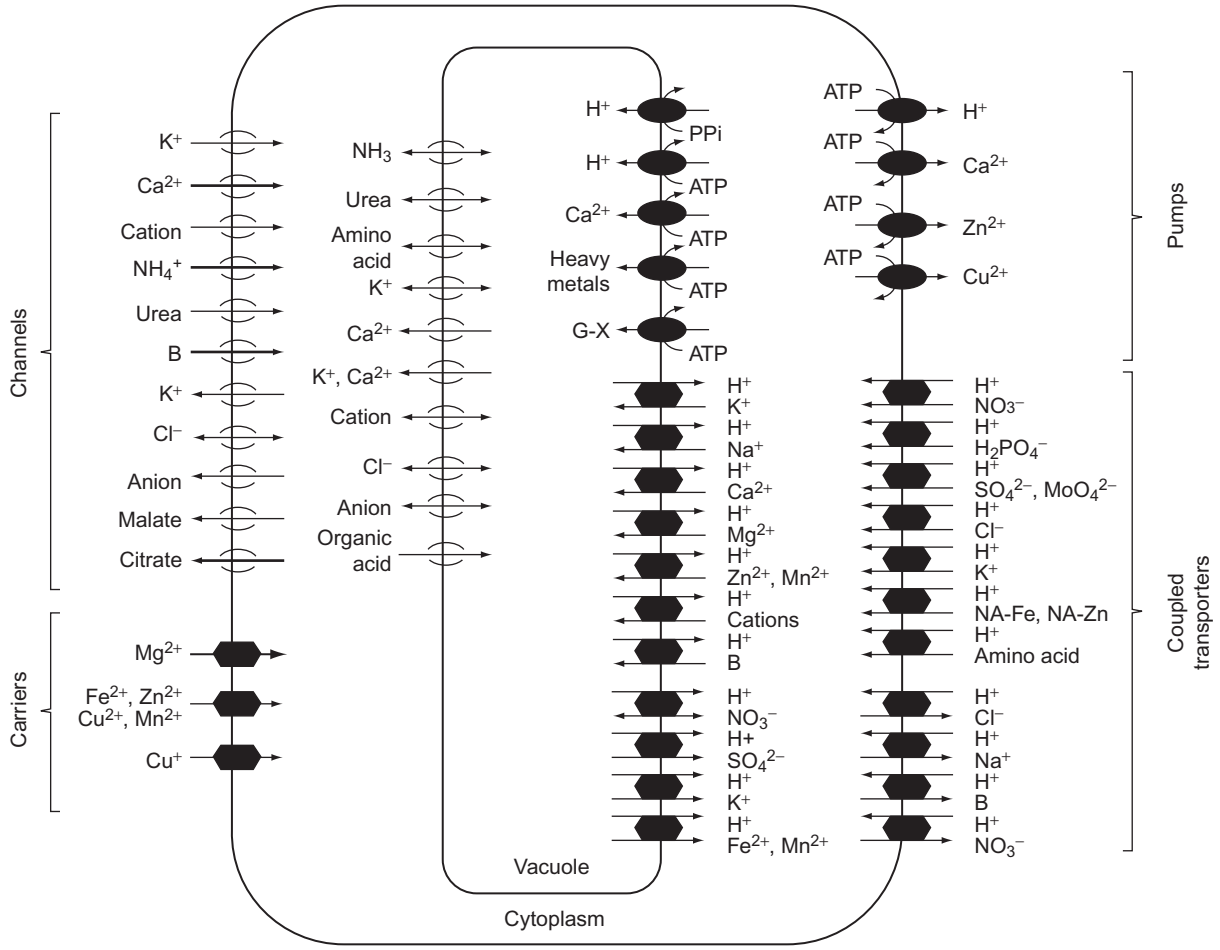

FIGURE 2.9 Transport proteins of the tonoplast and plasma membrane of plant cells. See text (Section 2.4.1) for details.

by facilitated diffusion but by active transport against their electrochemical gradient, as discussed below. In the stele, uniporters and channels facilitate the efflux of potassium, nitrate, sulphate, phosphate, chloride and organic acids from xylem parenchyma cells into xylem vessels in the direction of their electrochemical gradients (Section 3.2). Similar transport proteins are present in the plasma membranes of other plant cells, where they serve both general and specific functions. Channels in the plasma membrane of root cells facilitating the efflux of malate or citrate into the rhizosphere, such as members of the Al-activated malate transporter (ALMT) family and the multidrug and toxin extrusion (MATE) protein family, respectively, have been implicated in Al tolerance and improving P availability in acid soils (Delhaize *et al.*, 2007; Ryan *et al.*, 2011). Channels facilitating the efflux of chloride, such as the depolarization activated R-type and S-type anion channels (White and Broadley, 2001; Roberts, 2006; Teakle and Tyerman, 2010) in the plasma membrane of root cells, may be required for charge compensation of other ion fluxes. Recently, homologues of the *Arabidopsis thaliana* *At*SLAC1 protein have been proposed as candidates for the S-type anion channels of root cells (Teakle and Tyerman, 2010).

In addition to uniporters and channels, solute transport across membranes can be catalysed by primary or secondary active transporters that move solutes against their electrochemical gradient. Several ATPases are present in the plasma membrane of plant cells. These catalyse the efflux of H^+, Ca^{2+} and heavy metals from the cytoplasm. The plasma membrane H^+-ATPases catalyse H^+ efflux, which is then coupled directly, through the proton electrochemical gradient, or indirectly, via the cell membrane potential, to the movement of other solutes. The plasma membrane Ca^{2+}-ATPases remove Ca^{2+} from the cytosol to maintain the low cytosolic Ca^{2+} concentrations required for cell signalling (Section 6.6). In the stele, Ca^{2+}-ATPases and CPx-ATPases catalyse the efflux of Ca^{2+} and other divalent cations from the symplast to the xylem (Section 3.2).

A multitude of secondary active transporters are present in the plasma membranes of root cells, which couple H^+ influx to the movement of solutes against their

electrochemical gradients (Fig. 2.9). Proton-coupled transporters in the plasma membrane of root cells are responsible for the uptake of anions, such as nitrate (e.g., NRT1 and NRT2 transporters), phosphate (e.g., PHT1 transporters), sulphate (SULTR1 transporters), chloride and (probably) molybdate (White and Broadley, 2001; Buchner *et al.*, 2004; Fitzpatrick *et al.*, 2008; White and Hammond, 2008; Gojon *et al.*, 2009; Miller *et al.*, 2009; Shinmachi *et al.*, 2010). In addition, proton/potassium symporters, such as those encoded by the *KUP*/*HAK* gene family, facilitate K uptake by root cells (White and Karley, 2010), and homologues of the maize yellow stripe 1 protein (ZmYS1) allow proton-coupled symport of Fe and Zn conjugates (White and Broadley, 2009) into root cells. Proton-coupled transporters also alleviate element toxicities by removing chloride, sodium and boron from root cells (White and Broadley, 2001; Munns and Tester, 2008; Miwa and Fujiwara, 2010). In the stele, proton-coupled transporters load nitrate and B into the xylem (Miller *et al.*, 2009; Miwa and Fujiwara, 2010). Similar transport proteins are present in the plasma membranes of other plant cells, where they serve both general and specific functions. The transport of amino acids, peptides and sugars across the plasma membrane is also catalysed by proton-coupled transporters.

The tonoplast of the vacuole similarly contains a variety of primary active transporters, proton-coupled transporters, uniporters and channels (Fig. 2.9). In cells of higher plants, the electrical potential difference between the vacuole and the cytosol is about −20 to −60 mV and the pH of the vacuolar sap can be as low as pH 3 (Martinoia *et al.*, 2007). Based on estimates of solute concentrations in the cytosol and vacuole, it is thought that sequestration of K^+, Na^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Mn^{2+} and nitrate requires active transport into the vacuole, whereas the movement of other anions is likely to be passive (White and Broadley, 2001, 2003; Martinoia *et al.*, 2007; Teakle and Tyerman, 2010; White and Karley, 2010).

The tonoplast contains two distinct types of proton pumps, the H^+-ATPases and the H^+-PP_iases that generate the negative electrical potential across the tonoplast and lower the pH of the vacuole (Gaxiola *et al.*, 2007). The tonoplast H^+-ATPases of plants are complex oligomeric proteins comprising two subcomplexes: the peripheral V_1 complex, which consists of eight subunits (A, B, C, D, E, F, G and H) and is responsible for ATP hydrolysis, and the trans-membrane V_0 complex, which consists of up to five subunits (a, c, c″, d and e) and is responsible for proton translocation (Gaxiola *et al.*, 2007). These subunits are encoded by the *VHA* genes. Plants possess two distinct H^+-PP_iases, which are both single subunit enzymes. The Type I H^+-PP_iases require K^+ for their activity and are relatively insensitive to inhibition by Ca^{2+}, whereas Type II H^+-PP_iases do not require K^+ for their activity and are extremely sensitive to inhibition by Ca^{2+} (Gaxiola *et al.*, 2007; Martinoia *et al.*, 2007).

Magnesium is essential for both H^+-ATPases and H^+-PP_iases, since their substrates are Mg.ATP and Mg.PP_i (White *et al.*, 1990c; Gaxiola *et al.*, 2007). In addition, the H^+-PP_iases require Mg^{2+} for their activity (White *et al.*, 1990c; Gaxiola *et al.*, 2007). Inorganic pyrophosphate is generated in several major biosynthetic pathways, such as starch synthesis or activation of sulphate. Cytosolic PP_i concentrations generally lie in the range 50–400 μM, which is adequate to drive this proton pump (White *et al.*, 1990c). Under most circumstances, H^+-PP_iases contribute far less than H^+-ATPases to proton transport into the vacuole. Therefore it has been suggested that H^+-PP_iases act as ancillary enzymes to maintain the proton electrochemical gradient across the tonoplast when the activity of the H^+-ATPases is restricted by substrate availability, for example during anoxia (White *et al.*, 1990c), or at high temperatures, which promote protein degradation (Martinoia *et al.*, 2007).

The proton electrochemical gradient generated by the tonoplast H^+-ATPase and H^+-PP_iase supports the activities of a large number of proton-coupled transporters. These catalyse the efflux of K^+ (e.g., NHX and KEA transporters), Na^+ (NHX transporters), Ca^{2+} (CAX transporters), NO_3^- (e.g., *At*CLC-a and *At*CLC-c), sucrose (e.g., *At*SUT4), and various divalent cations, including Mg^{2+}, Zn^{2+} and Mn^{2+} (e.g., CAX, MGT and MTP transporters) from the cytosol to the vacuole, and the influx of K^+, nitrate (e.g., *At*CLC-a), SO_4^{2-} (e.g., *At*SULTR4-1; *At*SULTR4-2) and iron (e.g., *At*NRAMP3) from the vacuole to the cytosol in times of high demand for growth (Shigaki and Hirschi, 2006; Martinoia *et al.*, 2007; Gojon *et al.*, 2009; Miller *et al.*, 2009; White and Broadley, 2009; White and Karley, 2010; Zifarelli and Pusch, 2010). The sequestration of K^+, Cl^- and NO_3^- in vacuoles is important for turgor regulation and the sequestration of Na^+, Ca^{2+} and heavy metals is important to avoid cytoplasmic poisoning (Section 17.6). In addition, the sequestration of essential elements and metabolites in the vacuole provides storage for times of need (Martinoia *et al.*, 2007).

The tonoplast also contains Ca^{2+}-ATPases (e.g., *At*ACA4) that pump Ca^{2+} into the vacuole (White and Broadley, 2003) and a variety of ATP Binding Cassette (ABC) transporters that protect the cytoplasm by removing heavy metals, oxidation products conjugated to glutathione and xenobiotics from the cytosol into the vacuole (Martinoia *et al.*, 2007). These transporters are also involved in the sequestration of chlorophyll catabolites and natural pigments in the vacuole (Martinoia *et al.*, 2007).

Several ion channels have been recorded in the tonoplast. These facilitate the movement of K^+, Cl^-, NO_3^-, ammonia, amino acids, urea, Ca^{2+}, SO_4^{2-}, HPO_4^{2-}, sugars and organic acids in the direction of their electrochemical

gradients (White and Broadley, 2003; Martinoia *et al.*, 2007; Teakle and Tyerman, 2010; White and Karley, 2010). The rapid efflux of K^+ and Cl^- from the vacuole, through fast vacuolar (FV), slow vacuolar (SV) or vacuolar potassium (VK) channels and Cl^- channels, respectively, is required for stomatal closure and other osmotically driven plant movements (White and Broadley, 2001; White and Karley, 2010). The sequestration and release of NO_3^-, ammonia, amino acids and urea are central to the N economy of plants (Martinoia *et al.*, 2007). Aquaporins have been shown to facilitate the transport of ammonia (e.g., *At*TIP2;1, *At*TIP2;3) and urea (e.g., *At*TIP1;1, *At*TIP1;2; *At*TIP2;1, *At*TIP4;1) across the tonoplast (Martinoia *et al.*, 2007; Miller *et al.*, 2009). The rapid efflux of Ca^{2+} from the vacuole through SV, voltage-regulated, cADPR-regulated or IP_3-regulated channels is important for cell signalling (Section 8.6). The influx of malate through anion channels is a prerequisite for crassulacean acid metabolism (CAM), which separates CO_2 fixation from photoassimilation and allows plants to restrict water loss in arid environments by closing stomata during the day. In CAM plants at night, malate enters the vacuoles as $malate^{2-}$ through an anion channel, and is accumulated in monovalent and uncharged forms by vacuolar acidification (White and Smith, 1989; Martinoia *et al.*, 2007). Malate is subsequently released to the cytoplasm during the day for photoassimilation to occur.

From the preceding discussion, it is apparent that proton pumps are responsible for energizing solute transport across cell membranes. However, it is important to note that these pumps not only generate the proton electrochemical gradient across the tonoplast and plasma membrane, and the acidic conditions of the apoplasm (pH ~5.5) and the vacuole (pH 4.5–5.9), but also maintain cytosolic pH at its optimal value (pH 7.3–7.6; Felle, 2001).

2.4.2 Energy Demand for Solute Transport

The energy demand for ion uptake by roots can be considerable, especially during rapid vegetative growth (Table 2.7). Early calculations suggested that, in seedlings, up to 36% of the total respiratory energy cost, expressed as ATP consumption, is required for ion uptake; this proportion declines in older plants in favour of ATP demand for growth and maintenance of biomass (Table 2.7). Kurimoto *et al.* (2004) subsequently calculated that up to 76% of total respiratory energy cost was required to support low-affinity nitrate influx across the plasma membrane of cereal roots. Although several of the assumptions of these calculations have been criticized, recent studies addressing the weaknesses of the original calculations also found very high energy costs of ion uptake (Britto and Kronzucker, 2006, 2009; Teakle and Tyerman, 2010).

Recently, it has been observed that high rates of apparently 'futile' cycling of K^+, NH_4^+, NO_3^- and SO_4^{2-} across the plasma membrane of root cells occurs when these ions are present at high concentrations in the rhizosphere solution (Britto and Kronzucker, 2006). As the rhizosphere concentration increases, and the rate of unidirectional influx increases, the quotient of the unidirectional rates of ion influx and ion efflux across the plasma membrane approaches unity (see Fig. 2.13). The energy costs of this 'futile' cycling represent a substantial proportion of the total respiratory energy cost of the root. Similarly, under saline conditions, a considerable proportion (almost all) of the total energy budget of the root is expended on the removal of sodium (Britto and Kronzucker, 2006, 2009) and chloride (Teakle and Tyerman, 2010) from the symplasm.

TABLE 2.7 Respiratory energy costs for ion uptake in roots of *Carex diandra*

Proportion of total ATP demand required for	Plant age (days)		
	40	60	80
Ion uptake	36	17	10
Growth	39	43	38
Maintenance of biomass	25	40	52

[a]*Based on Van der Werf et al. (1988).*

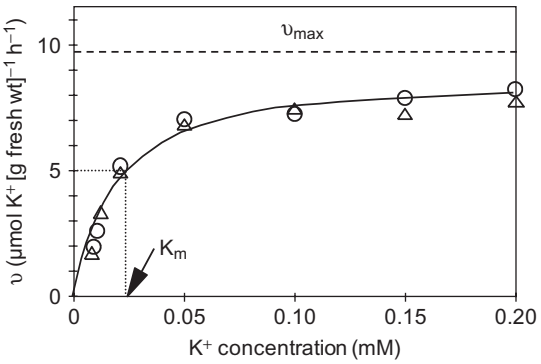

FIGURE 2.10 Rate of K^+ uptake (v) as a function of the external concentration of KCl (○) or K_2SO_4 (Δ); K_m = 0.023 mM. *Adapted from Epstein (1972).*

2.4.3 The Kinetics of Solute Transport in Plant Roots

As a general rule, the rate of solute uptake by plant cells, excised plant tissues and roots of intact plants saturates with increasing external solute concentration. Emanuel Epstein and colleagues in the early 1950s suggested that this relationship was similar to that between an enzyme and its substrate (Fig. 2.10). In their analogy, the transport protein was an enzyme that catalysed the movement of its substrate from one side of a membrane to the other. Using

the terms from enzymology, they defined the relationship between the rate of transport of a solute and its concentration by the Michaelis-Menten equation:

$$V = (V_{max} \times S)/(K_m + S)$$

where V is the rate of solute transport at a solute concentration of S, V_{max} is the maximal rate of solute transport, and K_m is the Michaelis constant, which is the solute concentration at which half the maximal transport rate is reached. The K_m value reflects the affinity of the transporter for the solute; just as in enzymatic reactions it indicates the affinity of the enzyme for its substrate.

When assayed at low concentrations in the external medium, solute uptake is often described well by this equation, as illustrated by K uptake by barley roots (Fig. 2.10). It is evident from this experiment that the relationship between K uptake and K concentration in the external medium is the same whether the source of K is KCl or K_2SO_4. However, as we shall see later, when substrate concentrations are higher, the accompanying anion can have a significant effect on the uptake rate of a cation and vice versa. As a first approximation, in the low concentration range, Michaelis-Menten kinetics can also be applied to describe uptake rates of many other solutes including the anions nitrate, phosphate, sulphate and chloride (e.g., Epstein, 1972; Deane-Drummond, 1987; Siddiqi *et al.*, 1990; Teo *et al.*, 1992; Laine *et al.*, 1993; Wang *et al.*, 1993; White and Broadley, 2001; Li *et al.*, 2007), the cations ammonium, calcium, magnesium, manganese, zinc and cadmium (e.g., Kelly and Barber, 1991; Huang *et al.*, 1992a; Kronzucker *et al.*, 1998; Rawat *et al.*, 1999; Sadana *et al.*, 2005; Broadley *et al.*, 2007; Lux *et al.*, 2011) and chelates such as Fe-phytosiderophores (von Wirén *et al.*, 1995). A low-capacity saturable uptake system can sometimes be discerned in B-deficient plants (e.g., Dannel *et al.*, 2000); but the relationship between the uptake of B and its concentration in the external solution is often reported to be linear in B-replete cells (Seresinhe and Oertli, 1991) and plants (Table 2.8). However, the relationship between solute concentration and its uptake by roots cannot always be fitted to a simple Michaelis-Menten equation. This is a consequence both of the theoretical limitations of Michaelis-Menten kinetics and the presence of multiple mechanisms for the transport of a particular solute (White, 2003).

The original concept of a single protein-mediated mechanism of ion transport (one carrier system for each ion) did not sufficiently describe the kinetics of uptake when wide concentration ranges were tested. At concentrations above 1 mM, for example, the kinetics of K uptake differ considerably from those at lower concentrations (Epstein *et al.*, 1963). The apparent selectivity of transport is lower (Na^+ competes with K^+) and the accompanying anion has a marked effect on the uptake rate. These observations led to the hypothesis of *dual systems* for K transport, with System I having a higher selectivity than System II.

In view of the usually very low concentrations, particularly of P and K, in soil solutions (Section 13.2) and results of ion uptake studies in the low concentration range (<10 µM), the term C_{min} was introduced to define the concentration at which net uptake of ions ceases before the ions are completely depleted (Fig. 2.11). The C_{min} concentration is an important factor in ion uptake from soils, because it is the lowest concentration at which roots can extract an ion from the soil solution. C_{min} concentrations differ considerably between plant species (Asher, 1978). For P, for example, a value of 0.12 µM has been found in tomato (Itoh and Barber, 1983a), 0.04 µM in soybean (Silberbush and Barber, 1984) and 0.01 µM in ryegrass (Breeze *et al.*, 1984). For K, the corresponding values were 2 µM in maize (Barber, 1979) and 1 µM in barley (Drew *et al.*, 1984). C_{min} concentrations for nitrate can vary from between more than 50 µM to less than 1 µM depending not only on the plant species but also on the

TABLE 2.8 Boron concentration in shoots and shoot dry weight of two barley genotypes with increasing B supply

	B supply (µM)			
	0	2.5	7.5	15
B concentration (mg kg^{-1} dw)				
Schooner	5.6	10.0	22.1	46.4
Sahara 3771	2.5	5.5	7.8	11.7
Shoot dry weight mg per plant				
Schooner	129	140	132	121
Sahara 3771	74	84	92	107

Based on Nable *et al.* (1990b).

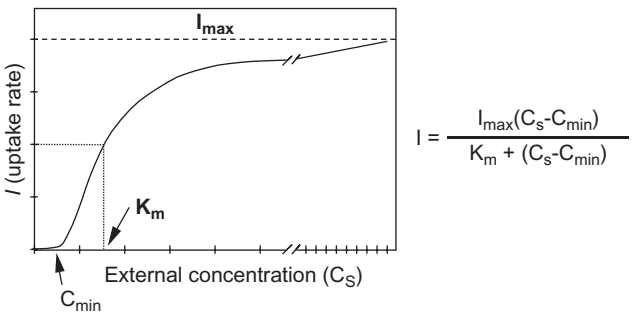

FIGURE 2.11 Schematic presentation of the relationships between uptake rates (net influx = I) of ions and their external concentrations; C_{min} = net uptake zero (influx = efflux).

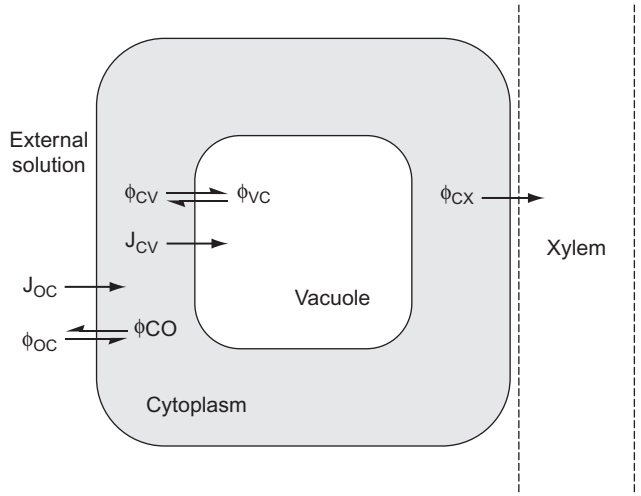

FIGURE 2.12 Nomenclature of unidirectional (ø) and net (J) solute fluxes across the plasma membrane between cytoplasm (c) and the external solution (o) or xylem (x), and across the tonoplast between the cytoplasm (c) and the vacuole (v) of a stereotypical root cell. *Figure adapted from White and Broadley (2001).*

environmental conditions (Deane-Drummond and Chaffey, 1985; Marschner *et al.*, 1991). For ammonium, C_{min} concentrations decreased from 30 to 1.5 µM as the root zone temperature increased (Marschner *et al.*, 1991).

For the kinetics of ion uptake by plants, the Michaelis-Menten equation has been modified to include the parameter C_{min}, and the term I, designating unidirectional influx, has replaced the term V (Fig. 2.11). Very often, though, only the *net uptake* of ions is determined experimentally, which is the net result of influx and efflux across the plasma membrane (Fig. 2.12). Efflux can become similar in magnitude to influx, particularly at extreme low or high external concentrations, and therefore can be an important component in determining net uptake (Fig. 2.13; Elliott *et al.*, 1984; Britto and Kronzucker, 2006). It is also noteworthy that, at a given external concentration, the efflux of a particular mineral nutrient can be many times higher from roots of plants sufficiently supplied than from roots of deficient plants (e.g., McPharlin and Bieleski, 1989; Lee *et al.*, 1990; Topa and Sisak, 1997).

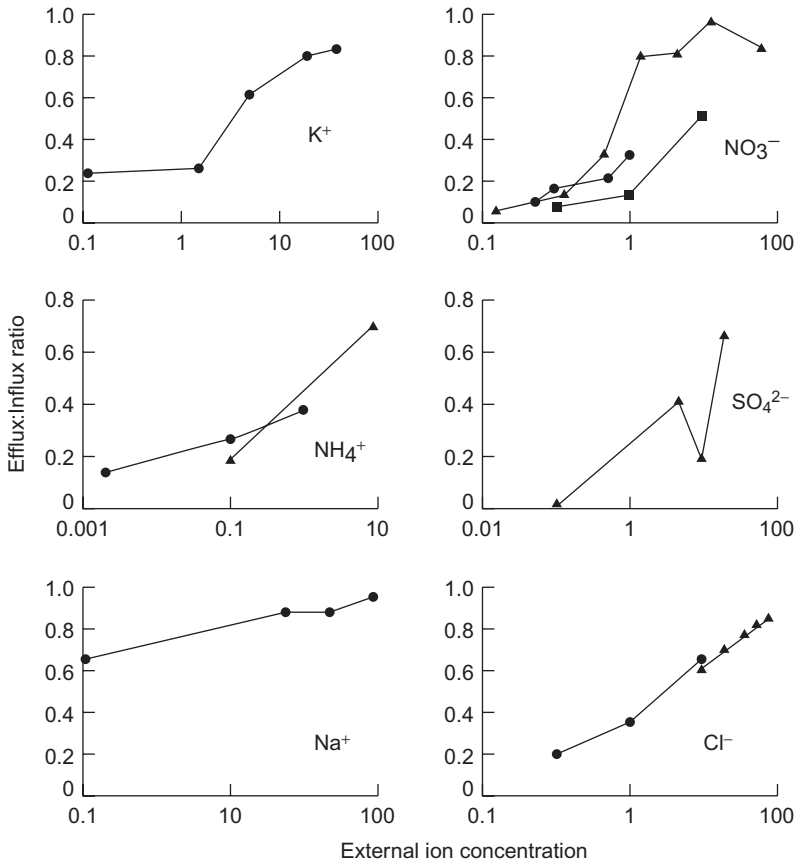

FIGURE 2.13 Ratios of efflux to influx across the plasma membrane for K^+, NO_3^-, NH_4^+, SO_4^{2-}, Na^+ and Cl^-. Data from various studies, each study plotted as a different symbol. *Adapted from Britto and Kronzucker (2006).*

The efflux of ions and other solutes is affected by several factors: (i) the integrity of the plasma membrane, (ii) the presence of transport proteins allowing efflux, (iii) the electrochemical driving force for transport, and (iv) the concentration of the solute in the cytoplasm. In pea, for example, the initial high rate of net uptake of sulphate by S-deficient roots placed in a solution containing sulphate decreases to about 30% within one hour due to a marked increase in sulphate efflux, despite a slight increase in influx (Deane-Drummond, 1987; Bell *et al.*, 1995). Similarly, for nitrate and ammonium, the efflux component can account for a high proportion – almost 40–50% of the influx – most probably due to the high concentrations of nitrate and ammonium in the cytoplasm (Britto and Kronzucker, 2003, 2006). The rapid exchange between ions in the external solution and in the cytoplasm is reflected in low half-times for exchange ($t_{\{1/2\}}$), which are between 7–14 min for ammonium (Kronzucker *et al.*, 1998), 10–50 min for potassium (White *et al.*, 1991; Szczerba *et al.*, 2006), 7–75 min for calcium (White *et al.*, 1992), 10–20 min for sulphate (Deane-Drummond, 1987; Bell *et al.*, 1995), 10–20 min for chloride (Britto *et al.*, 2004), 4–107 min for nitrate (Lee and Clarkson, 1986; Macklon *et al.*, 1990; Britto and Kronzucker, 2003) and 23–115 min for phosphate (Lee *et al.*, 1990; Macklon *et al.*, 1996). These rates of exchange with the cytoplasmic pool are usually orders of magnitude faster than the rates of exchange with the vacuole (e.g., Macklon *et al.*, 1990; White *et al.*, 1991, 1992; Bell *et al.*, 1995).

The parameters of ion uptake kinetics are also strongly affected by the nutritional status of plants. Roots of plants deficient in a particular nutrient generally exhibit a greater I_{max} and a lower C_{min} for that nutrient than plants sufficiently supplied. Occasionally, but not always, deficient plants also exhibit a lower K_m. An example is given in Table 2.9 for P. In plants with greater tissue P concentrations, I_{max} for P uptake is substantially lower, and K_m is also slightly lower. The I_{max} values were based on net uptake in this experiment, therefore the contribution of increased efflux at higher root P concentrations cannot be evaluated. However, for nitrate, ammonium, potassium, phosphate and sulphate, there is evidence that both increased efflux and reduced influx contribute to the decline in net uptake when internal concentrations are increased (Lee *et al.*, 1990; Britto and Kronzucker, 2006).

In the high concentration range (>1 mM), a linear relationship is often found between external concentrations and the rate of ion uptake by plant roots. This has been observed, for example, for the anions nitrate, phosphate, sulphate and chloride (Loneragan and Asher, 1967; Epstein, 1972; Borstlap, 1983; Clarkson and Saker, 1989; Siddiqi *et al.*, 1990; White and Broadley, 2001; Li *et al.*, 2007), the cations ammonium, potassium, sodium, calcium, magnesium, iron and zinc (Epstein, 1972; Borstlap, 1983; Wang *et al.*, 1993; Rawat *et al.*, 1999; White, 2001; Vallejo *et al.*, 2005; Broadley *et al.*, 2007), for boron (Dannel *et al.*, 2000), and for Fe-phytosiderophores (von Wirén *et al.*, 1995). Several explanations for the linear relationship (formerly defined as System II; Epstein, 1972) have been proposed. The first explanation is that it reflects influx through non-saturating transport proteins, perhaps ion channels, in the plasma membrane of root cells. The second explanation is that it is the consequence of rapid chelation or metabolism of a solute in the cytoplasm, or its removal by sequestration in the vacuole or transfer to the xylem, which maintains the electrochemical gradient, and reduces efflux, across the plasma membrane. The third explanation is that it represents a non-saturating, apoplasmic flux to the xylem. However, given the usually low ion concentrations in soil solutions, the ecological significance of the low-capacity, non-saturating mechanisms for the nutrition of plants grown in natural soils has been questioned. There are, however, at least two exceptions: plants growing in saline soils (Section 17.6), and the uptake of mineral nutrients from the apoplast following their long-distance transport in the xylem (Section 3.2) and phloem (Section 3.3).

TABLE 2.9 Short-term P uptake parameters of soybean plants with different P nutritional status

Plants grown at P concentration (μM)	P concentration (mg kg^{-1} dw)		I_{max} (mol cm^{-1} $sec^{-1} \times 10^{-14}$)	K_m (μM)
	Shoot	Root		
0.03	2.2	2.3	17.6	1.6
0.3	3.4	3.0	16.9	1.7
3.0	5.9	5.6	6.5	1.2
30.0	6.6	9.0	3.7	1.0

Based on Jungk *et al.* (1990).

2.5 FACTORS AFFECTING ION UPTAKE BY ROOTS

2.5.1 Influx to the Apoplasm

Before reaching the plasma membrane of root cells, ions must pass through the cell wall. In general, neither diffusion nor mass flow of ions or other low-molecular-weight solutes is restricted at the external surface of the roots. The cell walls and water-filled intercellular spaces of the root cortex are, to a certain extent, accessible to these solutes from the external solution.

The main barrier to solute flux through the apoplasm of young roots is the endodermis, the innermost layer of cells of the cortex (Fig. 2.14). Suberization of the radial and transverse walls of the endodermal cells (the Casparian band) creates an effective barrier against solute movement into the stele. In most angiosperm species suberization of the radial and transverse cell wall is also found in the *hypodermis*, or *exodermis* (cell layer below rhizodermis; Enstone and Peterson, 1992; Ma and Peterson, 2003). These barriers may also protect the inner cortex from colonization by microorganisms, for example preventing the colonization of sorghum roots by endophyte *Polymyxa* spp. (Galamay *et al.*, 1992).

Suberization of the exodermis generally occurs after the formation of the endodermal Casparian band, particularly in fast growing roots (Ma and Peterson, 2003). There are different views on the effectiveness of the exodermis as a barrier to solute movement through the root apoplasm (Clarkson *et al.*, 1987; Enstone and Peterson, 1992). However, since the development of the exodermis generally occurs after that of the endodermis, its function is thought to be largely structural. In plants adapted to submerged conditions, the exodermis serves another function, namely as an effective barrier against oxygen diffusion (leakage) from the root aerenchyma into the rhizosphere (e.g., Soukup *et al.*, 2007).

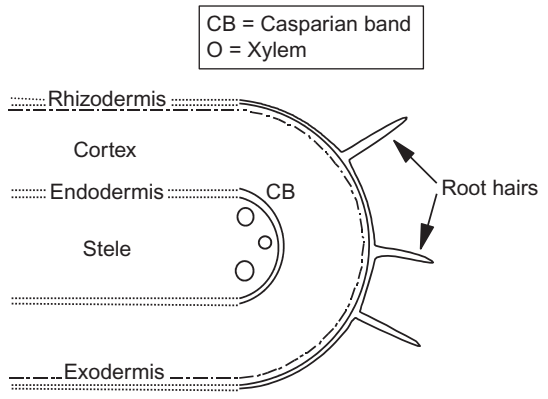

FIGURE 2.14 Schematic representation of cross section of a differentiated root zone of maize.

The volume of root tissue accessible for apoplasmic solute movement, the *free space*, represents only a small fraction of the total root volume. For example, the free space is estimated to occupy 5% of a maize root (Shone and Flood, 1985). The presence of this free space enables individual cortex cells to contribute to solute uptake from the external solution. Solute concentrations in the free space depend on various factors such as the capacity for solute uptake by epidermal cells, the presence of root hairs, the solute concentration in the rhizosphere solution and the rate of transpiration. As shown more than 50 years ago by Vakhmistrov (1967), at low external concentrations root hair formation is usually extensive and the uptake of mineral nutrients is limited mainly to the rhizodermal cell layer, i.e. the outer-most cells of the cortex. This is particularly relevant for roots growing in soil, where the importance of root hairs for the acquisition of nutrients present at low concentrations in the soil solution or with restricted soil mobility, such as P, has been clearly demonstrated (Gahoonia and Nielsen, 2004; Gahoonia *et al.*, 2006; Zhu *et al.*, 2010).

2.5.2 Effects of pH

The pH of the external solution can have profound effects on the uptake of nutrients by plant roots. These can be divided into three broad categories: (i) effects of solution pH on the chemical species present in solution, (ii) effects of apoplasmic pH on the concentrations of ions present in the apoplasm, and (iii) influence of rhizosphere pH for the proton electrochemical gradient and the driving force for proton-coupled solute transport. In addition, solution pH can affect ion transport by protonation/deprotonation of amino-acid residues of transport proteins.

The pH of the soil solution influences the availability of cations and anions for root uptake (White and Broadley, 2009). In alkaline soils, the availability of P, Zn, Fe, Mn, Cu and B is very low, whereas in acid soils, plant growth is mainly limited by toxic concentrations of Al^{3+} and Mn^{2+} in the rhizosphere. In addition, the pH of the external solution also determines the chemical species present in the rhizosphere. This is particularly relevant to the uptake of solutes that can be protonated and are transported across the plasma membrane as specific chemical species, such as boron, phosphate and ammonium. The rate of B uptake decreases strongly when the pH of the external solution is increased (Fig. 2.15). This pattern is closely related to the decrease in the ratio of boric acid, which is the substrate of the transporter catalysing boron uptake by root cells (Miwa and Fujiwara, 2010), to the borate anion. Similarly, the rate of phosphate uptake decreases as the pH of the external solution increases (Fig. 2.16). This can be explained by a decrease in the concentration of $H_2PO_4^-$, the substrate of the proton-coupled phosphate symporter in the plasma

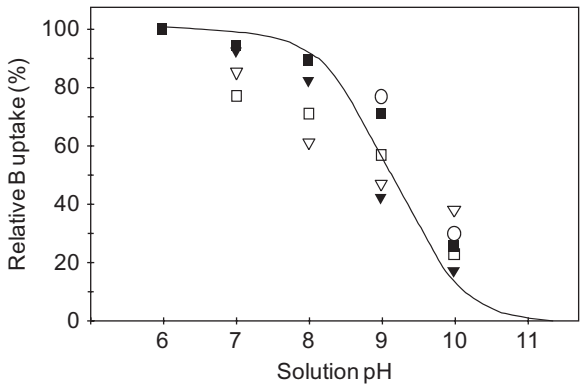

FIGURE 2.15 Relative uptake of B by barley roots as a function of the external solution pH. Uptake at pH 6 = 100 at each supply concentration. Solid line: percentage of undissociated H_3BO_3. Key for B concentrations (mg l^{-1}): 1.0 (open triangle), 2.5 (open square), 5.0 (open circle), 7.5 (filled triangle), 10.0 (filled square). *Based on Oertli and Grgurevic (1975).*

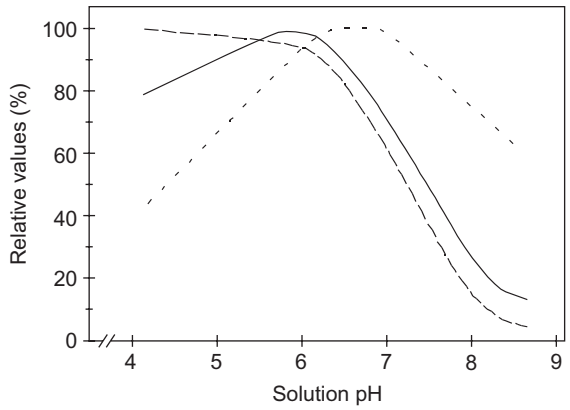

FIGURE 2.16 Relationships between solution pH and the proportion of $H_2PO_4^-$ (dashed line) in solution, and the uptake of phosphate (solid line) and sulphate (dotted line) by bean plants. Data are expressed as relative values. *Figure adapted from Hendrix (1967).*

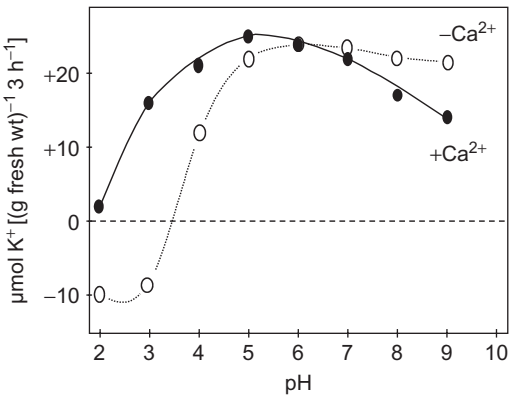

FIGURE 2.17 Net uptake of K^+ by barley roots from solutions containing 5 mM KBr as a function of the pH of an external solution with ($+Ca^{2+}$) or without calcium ($-Ca^{2+}$). *Modified from Jacobson* et al. *(1960).*

membrane, in the pH range of 5.6 to 8.5 (Fig. 2.16). In contrast, there is a smaller effect on the uptake rate of sulphate, since in this pH range only the divalent anion SO_4^{2-} occurs. The effects of the pH of the external solution on ammonium uptake by plant roots are more complex. At high external pH, ammonium uptake increases sharply, probably due to an increase in the proportion of the uncharged species NH_3 and NH_4OH.

In relation to the effects of apoplasmic pH on the uptake of solutes by root cells, it has been noted previously that both cell walls and biological membranes contain charged groups and that ions interact with these groups. Generally, the strength of these interactions increases with valency. Fixed negative charges can influence both the absolute and relative concentrations of cations in the apoplasm and, thereby, ion movements in the apoplasm and the rate and selectivity of ion influx to root cells. As the external pH is lowered, the effective CEC of the apoplasm decreases, particularly in monocotyledonous species due to binding of H^+ to the cation exchange sites. Thus, apoplasmic pH can affect ion uptake.

The pH of the rhizosphere solution can also affect ion uptake by altering both substrate (H^+) concentration and the electrochemical driving force for proton-coupled solute transport. A decrease in pH can increase the activity of proton-coupled solute transporters and enhance anion uptake. Thus, the uptake of anions is generally either not affected or stimulated by low pH. In short-term experiments with maize roots, decreasing the external pH from 8 to 4 increased nitrate influx by a factor of about 10 (McClure *et al.*, 1990b) and phosphate uptake by a factor of about 3 (Sentenac and Grignon, 1985). For phosphate, this increase at low pH was also observed when the concentration of the monovalent species ($H_2PO_4^-$) was kept constant. In contrast, the efficiency of H^+ efflux decreases as the external solution becomes more acidic and, consequently, the membrane potential of root cells decreases from about −150 mV at pH 6 to −100 mV at pH 4 (Dunlop and Bowling, 1978). Accordingly, the driving force for cation uptake is reduced. In general, the uptake of cations, such as K^+, is inhibited by low pH of the external solution, although Ca^{2+} often has an ameliorating effect (e.g., Fig. 2.17).

The contrasting effects of external pH on cation and anion uptake are well-documented phenomena, for example, in rice (Zsoldos and Haunold, 1982) and soybean (Rufty *et al.*, 1982b). In the latter case, a decrease in the pH of the external solution from 6.1 to 5.1 resulted in an increase in the ratio of anion to cation uptake from about 1.0 to 1.25. In long-term growth experiments, the contrasting effects of external pH on the uptake of cations and anions are reflected in the nutrient composition of plants

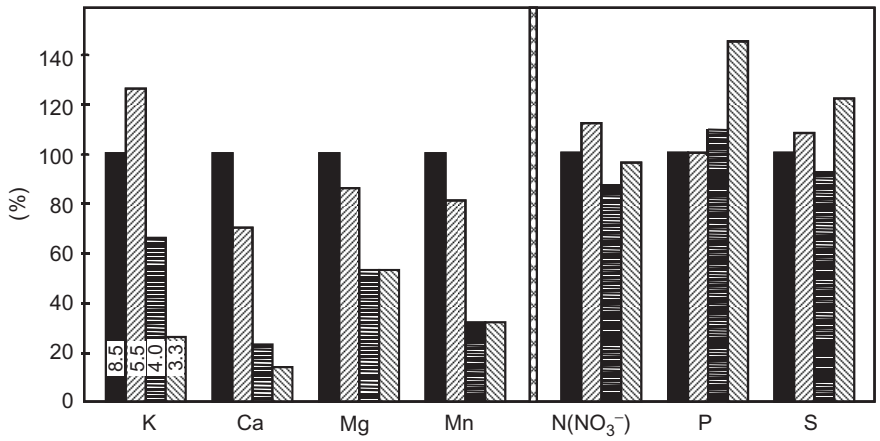

FIGURE 2.18 Nutrient concentrations (expressed on a dry weight basis and as a percentage of the concentration observed in plants grown at pH 8.5) in shoots of bean (*Phaseolus vulgaris*) grown in solutions with pH 8.5, 5.5, 4.0 and 3.3, respectively, as indicated in the columns for K. *Data recalculated from Islam* et al. *(1980).*

(Fig. 2.18), with tissue cation concentrations being more affected than tissue anion concentrations.

The effect of external pH on N uptake depends on whether N is supplied as ammonium (NH_4^+) or nitrate (NO_3^-). As is to be expected, lowering the external pH increases the uptake of NO_3^-, but decreases the uptake of NH_4^+ (Zsoldos and Haunold, 1982). Interpreting results with both forms of N at different external pH are, however, complicated by side-effects, such as changes in cation–anion balance in the plants and on root metabolism and function.

TABLE 2.10 K and P uptake by barley plants at different oxygen partial pressure around roots

Oxygen partial pressure (%)	Relative uptake[a]	
	K	P
20	100	100
5	75	56
0.5	37	30

Based on Hopkins *et al.* (1950).

2.5.3 Metabolic Activity

The accumulation of ions and other solutes against a concentration gradient requires an expenditure of energy, either directly or indirectly. The main source of energy in non-photosynthesizing cells and tissues (including roots) is respiration. Thus, all factors that affect respiration can influence ion accumulation.

Oxygen. As oxygen tension is lowered, the uptake of ions such as potassium and phosphate is decreased, particularly at very low oxygen tensions (Table 2.10). Consequently, oxygen deficiency is one of the factors that can restrict plant growth in poorly aerated substrates (e.g., waterlogged soils; Section 17.4).

Carbohydrates. The main energy substrates for respiration are carbohydrates. Therefore, in roots and other non-photosynthesizing tissues, under conditions of limited carbohydrate supply from a source (e.g., leaves), a close correlation can often be found between carbohydrate concentration and the uptake of ions. For example, carbohydrate concentration, respiration and N uptake decrease within a few hours after excising roots which cuts off the photosynthate supply from the shoot (Table 2.11). These relationships are of particular ecological importance, for example, when leaves are removed (grazing, cutting) or in dense plant stands when light supply to the basal leaves is limited, since the basal leaves are the main source of carbohydrates for the roots.

TABLE 2.11 Sugar concentration, respiration (O_2 uptake), and N uptake in barley roots after root excision

Time (h) after excision	Sugar (μmol g^{-1} dw)	Net uptake (μmol g^{-1} dw min^{-1})		
		O_2	NH_4^+	NO_3^-
0	82	4.5	1.8	1.5
3	51	3.3	1.1	1.0

Recalculated from Bloom and Caldwell (1988).

Distinct diurnal patterns in solute uptake (maxima during the day, minima during the night) have been observed for nitrate, phosphate, ammonium, potassium, iron and zinc (e.g., Clement *et al.*, 1978b; Zhang *et al.*, 1991b; Le Bot and Kirkby, 1992; Macduff *et al.*, 1997; Cesco *et al.*, 2002; Vert *et al.*, 2003; Louahlia *et al.*, 2008). Root carbohydrate concentration may act as a coarse control for ion uptake and is one of the factors responsible for the diurnal fluctuations in ion uptake. However, in maize roots, for example, diurnal fluctuations in nitrate uptake were only loosely related to root carbohydrate content (Fig. 2.19). In soybean growing under short-day conditions, the typical diurnal fluctuations of nitrate uptake could be reversed by an intervening 3 h period of low light (i.e., imitating long-day conditions, repressed flower initiation); uptake rates of nitrate were then twice as high during the night as compared with the day (Raper *et al.*, 1991).

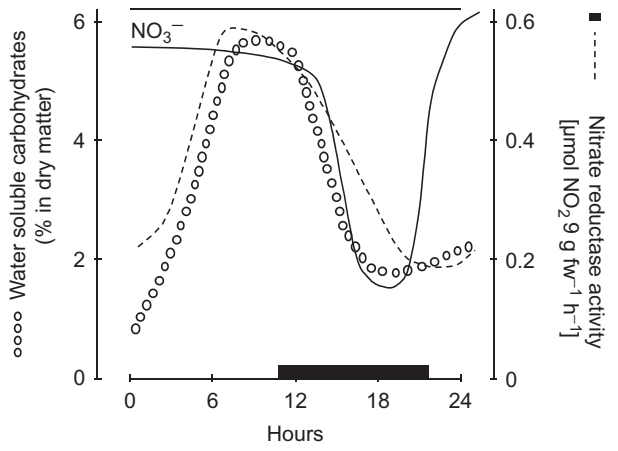

FIGURE 2.19 Diurnal fluctuations in nitrate uptake (solid line), nitrate reductase activity and concentration of water-soluble carbohydrates in maize roots. Nitrate uptake: relative values, uptake at end of the light period = 100. *Adapted from Keltjens and Nijenstein (1987).*

Recently, it has been suggested that diurnal fluctuations in the uptake of nitrate, phosphate, sulphate, ammonium, potassium and iron may be related to the delivery of sucrose to the root in the phloem through the regulation of the expression of genes encoding proteins catalysing their transport across the plasma membranes of root cells (Lejay *et al.*, 2003; Vert *et al.*, 2003; Hammond and White, 2008; Liu *et al.*, 2009; Vance, 2010).

There is evidence that the delivery of sucrose via the phloem can act as a systemic signal informing the root of the shoot N and P status (Hermans *et al.*, 2006; Hammond and White, 2008) and that the increase in uptake capacity in roots of plants lacking sufficient N or P for maximal growth (Section 2.5.6) is effected by sucrose-induced expression of nitrate and phosphate transporters.

Temperature. Physical processes such as exchange adsorption of cations in the AFS are only slightly affected by temperature (Q_{10} = 1.1–1.2, with Q_{10} referring to the change in a reaction or process imposed by a change in temperature by 10°C). However, chemical and biochemical reactions show much greater temperature dependence with $Q_{10} = 2$ and $Q_{10} > 2$, respectively. The Q_{10} value for the uptake of ions from solutions of low concentration often exceeds 2, at least within the physiological temperature range (e.g., Fig. 2.20; Clarkson *et al.*, 1988; Wang *et al.*, 1993). Ion uptake is more temperature dependent than respiration, especially at temperatures below 10°C. Furthermore, at very high temperatures root respiration further increases whereas ion uptake declines (Fig. 2.20), indicating that membrane transport and respiration are not coupled directly.

In studies of temperature effects on ion uptake, two phenomena are often studied: (i) the immediate effects of an abrupt change in root temperature, which occur within seconds and reflect the direct effects of temperature on the uptake system, and (ii) the long-term effects of growing plants at a particular root temperature. The latter effects are manifest after several days or weeks of growth at a

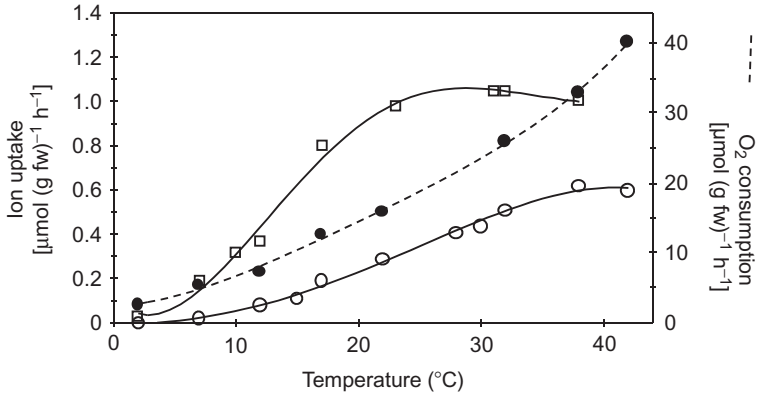

FIGURE 2.20 Rates of respiration (●) and uptake of P (○) and K (□) from solutions containing 0.25 mM K and 0.25 mM P by maize root segments at different temperatures. *Adapted from Bravo and Uribe (1981).*

particular root temperature and include adaptive responses, for example changes in root membrane properties. The latter effects are of greater ecological significance. Such studies often compare plants exposed to different root temperatures, but the same shoot temperature. This creates a temperature differential between the root and the shoot, and it is noteworthy that the shoot meristem of, for example, gramminoid plants is close to stem base. Thus, different temperatures in the rooting medium will influence cell division and cell elongation in the shoot. In the long term, such experimental systems can affect the growth rates of root and shoot quite differently and, thus, the root/shoot biomass ratio (Clarkson *et al.*, 1988, 1992). Accordingly, long-term effects of root temperature on ion uptake can include feedback regulation via plant demand, an example of which is shown for maize in Table 2.12.

In maize, low root temperatures (12°C) decrease shoot and root growth and uptake rates of nitrate and potassium, as might be expected for a cold-sensitive plant species. However, the reduction in ion uptake at low temperature was not a temperature effect on the roots *per se*, but reflected feedback regulation via lower shoot demand. This was shown by increasing the temperature of the shoot growing zone (stem base) (24/12°C). Shoot growth was strongly increased (i.e., the demand for nutrients) and so were the uptake rates of nitrate and potassium per unit root weight (Table 2.12). Similarly, in other graminaceous species, poor growth at low root temperatures is generally not caused by limited uptake of nutrients such as N, K or P (Clarkson *et al.*, 1986, 1992).

Low root temperatures can affect the uptake of nutrients differently, P uptake usually being reduced more than the uptake of other nutrients (e.g., Engels and Marschner, 1992a; Engels, 1993). The uptake rate of nitrate seems to be more strongly reduced at low root zone temperatures than ammonium in cold-sensitive plants like cucumber (Tachibana, 1987), and in cold-tolerant species, such as barley and ryegrass, the strong preference for ammonium compared with nitrate uptake is little affected by the temperature of the root zone (Macduff and Jackson, 1991; Clarkson *et al.*, 1992). Compared with Ca and Mg, uptake rates of K are often more affected by root zone temperatures. In winter wheat, the increase in K/(Ca + Mg) ratios in the shoots with increasing root zone temperature may cause tetany in grazing beef cattle on winter wheat forage (Miyasaka and Grunes, 1990).

In contrast to plants grown in solution culture, the roots of plants grown in soil must forage for many immobile nutrients (Engels and Marschner, 1990; Rengel, 2001; Lynch, 2007; White and Hammond, 2008; White and Broadley, 2009). In soil-grown plants, therefore, root temperature can affect the uptake of nutrients additionally through effects on root growth rate and root system morphology (Section 13.3).

TABLE 2.12 Shoot and root growth and uptake of nitrate and K by maize plants grown at different root zone temperatures (RZT) and the temperatures at the stem base (shoot growing zone temperature, SGT) for eight days

	Temperature treatment (SGT°C/RZT°C)		
	24/24	12/12	24/12
Shoot growth (g fw day^{-1})	1.91	0.32	1.34
Root growth (g fw day^{-1})	0.85	0.20	0.26
Nitrate uptake (pmol g^{-1} fw day^{-1})	6.40	4.20	7.60
K uptake (pmol g^{-1} fw day^{-1})	2.50	1.20	3.10

After Engels and Marschner (1992a).

2.5.4 Interactions between Ions in the Rhizosphere

In the preceding sections, for the sake of simplicity, the transport of a particular ion was treated as a singular process. In reality, however, the transporters catalysing ion uptake are rarely specific and ions can compete directly for transport. This competition is influenced by the properties of the transporter itself and by the concentrations of different ions in solution. Solutes that are not transported can also interact with transport proteins altering their activity. In addition, there may be indirect interactions between ions as a result of their transport across the plasma membrane, for example via effects on membrane potential through the movement of charge, or via effects on the proton electrochemical gradient through the coupling of solute transport to proton movements.

2.5.4.1 Competition

Transport proteins catalyse the movement of nutrients from the rhizosphere solution to the cytoplasm across the plasma membrane of root cells (Fig. 2.9). Competition between ions of the same valency for entry to a channel protein or for binding to a carrier protein is common, whether these ions are ultimately transported or merely inhibit the transport process. Such competition occurs particularly between ions with similar physicochemical properties (valency and ion diameter), for example between the alkali cations potassium (K^+), rubidium (Rb^+), cesium (Cs^+) and sodium (Na^+), or between the Group II divalent cations calcium (Ca^{2+}), strontium (Sr^{2+}) and barium (Ba^{2+}). It is important to note, however, that the inhibition of transport of a particular ion by another ion

TABLE 2.13 Interactions between the uptake of NH_4^+ and K^+ by maize roots[a]

	Concentration in roots (μmol g^{-1} fw)			
	Ammonium		Potassium	
$(NH_4)_2SO_4$ (mM)	$-K^+$	$+K^+$	$-K^+$	$+K^+$
0.00	6.9	6.7	8.2	53.7
0.15	7.3	7.1	6.7	48.4
0.50	17.1	13.5	8.9	41.1
5.00	29.4	31.5	9.3	27.1

Based on Rufty *et al.* (1982a).
[a]*Duration of the experiment: 8 h; + K indicates addition of 0.15 mM K^+; calcium concentration constant at 0.15 mM.*

TABLE 2.14 Uptake of labelled Mg^{2+} (^{28}Mg) by barley seedlings without or with supply of K^+ and Ca^{2+} (0.25 mM each)

	Mg^{2+} Uptake (μmol Mg^{2+} (10 g)$^{-1}$ fw 8 h^{-1})		
	$MgCl_2$	$MgCl_2$ + $CaSO_4$	$MgCl_2$ + $CaSO_4$ + KCl
Roots	165	115	15.0
Shoots	88	25	6.5

Based on Schimansky (1981).

TABLE 2.15 Uptake rates of Mn and Mg by roots of soybean plants at increasing Mn concentrations in the substrate

	Manganese supply (μM)		
Nutrient (μmol g^{-1} root dw)	1.8	90	275
Mn	0.5	3.1	4.8
Mg	121.8	81.1	20.2

Based on Heenan and Campbell (1981).

does not necessarily imply that the inhibitory ion is itself transported.

Radioactive rubidium (^{86}Rb) has often been used as a tracer to study K^+ transport in plants, although this can give misleading results under certain circumstances (Behl and Jeschke, 1982). In general, transport proteins catalysing K^+ transport across the plasma membrane of root cells, such as K-channels, cation-channels, and proton-coupled K^+ symporters, do not differentiate between K^+ and Rb^+ for transport (White, 1997; Maathuis and Amtmann, 1999; Vallejo *et al.*, 2005; Pyo *et al.*, 2010; White and Karley, 2010). However, the major K-channel in roots of *Arabidopsis thaliana* (*At*AKT1:*At*KC1) is relatively impermeable to Cs^+, which inhibits K^+ influx through this channel (White and Broadley, 2000). By contrast, proton-coupled K^+ symporters, such as *At*HAK5, can transport both K^+ and Cs^+ into root cells and, in plants sufficiently supplied with K, the influx of both Cs^+ and Na^+ is thought to occur largely through non-selective cation-channels (White and Broadley, 2000; Qi *et al.*, 2008; Munns and Tester, 2008).

The competition between potassium (K^+) and ammonium (NH_4^+) is difficult to explain simply by competition for a single transport process at the plasma membrane (Table 2.13). Whereas NH_4^+ is quite effective in inhibiting K^+ influx, the reverse (inhibition of NH_4^+ uptake by K^+) is rarely observed (e.g., Mengel *et al.*, 1976; Rufty *et al.*, 1982a; Shaviv *et al.*, 1987). This may be explained by two phenomena. First, it has been reported recently that, in *Arabidopsis thaliana*, NH_4^+ competes with K^+ for transport through both *At*AKT1 and *At*HAK5 (ten Hoopen *et al.*, 2010) and also reduces the expression of *AtHAK5* (Qi *et al.*, 2008), whereas K^+ does not appear to affect the expression or activity of the major NH_4^+ transporter, *At*AMT1. Second, a substantial proportion of ammonium may not be taken up as NH_4^+ through transporters such as *At*AMT1 but as NH_3 (Section 2.5.2). Thus, the uptake of ammonium by root cells will be determined not only by competition of NH_4^+ for cation transporters, but also by rhizosphere pH and cytosolic tolerance of NH_4^+ and NH_3 uptake.

Applications of K and Ca fertilizers often induce Mg deficiency in crop plants. This is partly a consequence of cations, such as K^+ and Ca^{2+}, inhibiting Mg^{2+} uptake by plant roots (Table 2.14). The presence of Mn^{2+} in the rhizosphere also inhibits Mg^{2+} uptake by roots (Table 2.15), but has little effect on the uptake of K^+ (Heenan and Campbell, 1981). This presumably reflects the contrasting specificity of the transporters responsible for the uptake of each cation.

Competition also occurs between anions for uptake by root cells. Some well-known examples are competition between sulphate and molybdate, sulphate and selenate, selenite and phosphate, and phosphate and arsenate.

Sulphate and molybdate are thought to enter root cells through the same proton-coupled symporters (Fitzpatrick *et al.*, 2008). Increasing the sulphate concentration in the rooting medium reduces molybdate uptake strongly. Hence, S fertilization may be an effective tool to reduce excessive Mo uptake thereby improving plant growth and animal nutrition in soils containing high concentrations of Mo (Pasricha *et al.*, 1977; Chatterjee *et al.*, 1992). However, the competition may become a critical factor in soils containing little Mo.

The interactions between selenate and sulphate are also quite distinct, and of considerable practical importance in view of the absolute requirement for selenium of humans and animals and the detrimental effects of excessive selenium in the diet (White and Broadley, 2005a, 2009). Sulphate and selenate are taken up into root cells through the same proton-coupled symporters (White *et al.*, 2007b). Increasing sulphate concentration in the substrate strongly decreases selenate uptake by roots and selenium accumulation by plants, suggesting direct competition between selenate and sulphate for transport (White *et al.*, 2007b). On the other hand, increasing selenate concentration in the substrate often increases sulphate uptake and accumulation by plants (White *et al.,* 2004, 2007b; Stroud *et al.,* 2010), possibly by interfering with the regulation of expression or activity of high-affinity sulphate transporters by plant S-status (White *et al.*, 2004, 2007b).

Antagonistic interactions between selenite and phosphate, and also between phosphate and arsenate, are thought to occur because these anions are transported by the same proton-coupled symporters into root cells (Li *et al*., 2008c; Zhao *et al.*, 2010). In *Holcus lanatus* L., arsenate-tolerant and non-tolerant genotypes exist, and arsenate uptake is much lower in the tolerant genotypes (Meharg and Macnair, 1992). The low arsenate uptake rate is achieved by suppression of the P deficiency-induced high affinity uptake system in the tolerant plants. Similar mechanisms of arsenic tolerance have been observed in other plant species. For example, mutants of *Arabidopsis thaliana* with defective phosphate transport are more tolerant to arsenate (Shin *et al.*, 2004). Arsenite and undissociated methylated arsenic species are taken up by roots through the silicon transport pathway via members of the nodulin 26-like intrinsic protein (NIP) family (Zhao *et al.*, 2010). Members of this family also transport a range of small neutral molecules including ammonia, urea, boric acid and silicic acid (Maurel *et al.*, 2008; Wallace *et al.*, 2006; Miwa and Fujiwara, 2010).

The inability of transport proteins to differentiate effectively between K^+ and Rb^+, Ca^{2+} and Ba^{2+}, SO_4^{2-} and SeO_4^{2-}, and phosphate and arsenate illustrates that the selectivity of transport proteins in the plasma membrane of root cells does not indicate any essential role for an element in the plant, but merely reflects the physicochemical similarities between ions. Plant roots may be unable to exclude many non-essential or toxic ions from the root symplasm. This has important practical implications, for example, for the entry of heavy metals into the food chain via their uptake and accumulation by plants (e.g., Marschner, 1983).

Another distinct type of anion competition occurs between chloride and nitrate. Chloride concentrations in plant tissues, particularly in roots, can be reduced strongly by increasing nitrate availability (Table 2.16).

TABLE 2.16 Chloride concentrations in roots and shoots of barley plants at different nitrate concentrations in the nutrient solution

Concentation in nutrient solution (mM)		Chloride content (μmol g^{-1} fw)	
Cl^-	NO_3^-	Roots	Shoot
1	0	52	93
1	0.2	26	73
1	1.0	13	54
1	5.0	9	46

Based on Glass and Siddiqi (1985).

This reduction seems to be the result of negative feedback from nitrate stored in the vacuoles of root cells (Glass and Siddiqi, 1985). Similarly, nitrate uptake is reduced when roots contain high chloride concentrations, and chloride accumulated in the vacuoles seems to be particularly effective in this respect (Cram, 1973). The active efflux of chloride and nitrate from the cytoplasm into the vacuole is catalysed, in part, by the same proton-coupled transporters (members of the CLC family) and several anion channels also facilitate the movement of both Cl^- and NO_3^- across the tonoplast (White and Broadley, 2001; Martinoia *et al.*, 2007; Teakle and Tyerman, 2010; Zifarelli and Pusch, 2010). Thus, it is possible that the two anions compete for transport across the tonoplast, which affects their accumulation in vacuoles, cytoplasmic concentrations and uptake. In addition, several anion channels and proton-coupled symporters in the plasma membranes of root cells appear to facilitate the transport of both chloride and nitrate (White and Broadley, 2001; Roberts, 2006), suggesting further interactions in the pathways of their uptake and accumulation.

Interactions between nitrate and chloride during their uptake and accumulation in vacuoles are of great importance for crop production. The competing effect of chloride can be used to decrease the nitrate content of plant species such as spinach which tend to accumulate large amounts of nitrate for use as an osmoticum. On the other hand, in saline soils, the competing effect of chloride on nitrate uptake may impair N uptake by the plants (Bernal *et al.*, 1974). Under these conditions increasing nitrate supply can be an effective means to improve the N nutritional status of the plants and simultaneously prevent chloride toxicity in sensitive plant species (Section 17.6).

An interesting case of the indirect regulation of transport by nutrients is the inhibition of nitrate uptake, and stimulation of chloride uptake, by ammonium supply (Lee and Drew, 1989; Xu *et al.*, 2000; Miller and Cramer,

2004). In almost all cases, increasing the availability of ammonium strongly suppresses nitrate uptake. By contrast, increasing nitrate supply generally has little or no effect on ammonium uptake (Breteler and Siegerist, 1984). Thus, when nitrogen is supplied as NH_4NO_3, ammonium is taken up in preference to nitrate. In Norway spruce, the rhizosphere ammonium concentration must fall below about 100 µM NH_4^+ before nitrate uptake occurs (Marschner *et al.*, 1991). In short-term experiments with barley, external ammonium inhibited net influx of nitrate within 3 min, and upon removing ammonium from the external solution net influx of nitrate resumed within 3 min (Lee and Drew, 1989). Such immediate effects suggest that they arise from the effect of ammonium on the electrochemical gradients supporting nitrate uptake across the plasma membrane.

2.5.4.2 Effects of Extracellular Calcium

An example of synergism, first discovered by Viets (1944), is the stimulation of cation and anion uptake by extracellular Ca^{2+} at low rhizosphere pH (Table 2.17; Fig. 2.17). It is thought that this phenomenon is the result of Ca^{2+} counteracting the negative effects of high H^+ concentrations on plasma membrane integrity or the activity of the plasma membrane H^+-ATPase. Calcium, as a divalent cation, stabilizes membranes through interactions with the negatively charged headgroups of phospholipids and, thereby, influences membrane function. It also contributes to the resealing of the plasma membrane following damage (Schapire *et al.*, 2009). These functions of Ca^{2+} are reflected, for example, in the higher rates of efflux of low-molecular-weight solutes across the plasma membrane of Ca-deficient cells when faced with environmental challenges, such as low temperatures or mechanical damage. Calcium can be removed fairly readily from its binding sites at the outer surface of the plasma membrane, for example by chelators (Van Steveninck, 1965), or can be exchanged by high concentrations of H^+ or metal cations including Na^+ (Lynch *et al.*, 1987), which will increase solute efflux.

Rhizosphere Ca^{2+} concentration also influences the selectivity of ion uptake, and the relative accumulation of K^+ and Na^+ in particular. For example, in the absence of Ca^{2+} there are clear differences in the K^+/Na^+ uptake ratio between the 'natrophobic' maize and the 'natrophilic' sugar beet. However, the presence of Ca^{2+} in the rhizosphere solution shifts the uptake ratio in favour of K^+ at the expense of Na^+ in both species (Table 2.18). These shifts in K^+/Na^+ uptake ratio are likely to be due to the fact that extracellular Ca^{2+} inhibits Na^+ influx through voltage-insensitive cation-channels (White, 1999; Maathuis and Amtmann, 1999; Munns and Tester, 2008), but has little effect on K^+ influx through inward-rectifying K-channels (White, 1997a; Maathuis and Amtmann, 1999). High Ca^{2+} concentrations in the soil solution are particularly beneficial for the maintenance of high K^+/Na^+ uptake ratios in saline environments as they increase plant salt tolerance.

TABLE 2.17 K^+ and Cl^- uptake in barley roots with or without Ca^{2+} supply with external pH 5.0

	Uptake rate (µmol g^{-1} dw (2 h) $^{-1}$)			
External solution (mM)	K^+ influx	K^+ net uptake	Cl^- influx	Cl^- net uptake
0.1 KCl	116 ± 3	117 ± 6	35 ± 1	34 ± 4
0.1 KCl + 1.0 $CaSO_4$	137 ± 2	140 ± 7	53 ± 3	52 ± 4

2.5.4.3 Cation–Anion Relationships

The uptake of cations and anions occurs through different transport proteins (Fig. 2.9), therefore direct interactions between cations and anions for uptake are rare. However, the uptake of one nutrient can influence the uptake of another indirectly through effects on the membrane potential, the proton electrochemical gradient or via feedback regulation through plant growth or metabolism. The stimulation of cation uptake by anions, and of anion uptake by cations, is observed frequently, and is generally

TABLE 2.18 K^+/Na^+ selectivity of roots with or without Ca^{2+} supply

	Uptake rate (µmol g^{-1} fw (4 h) $^{-1}$)					
	Maize			Sugar beet		
External solution NaCl + KCl (10 mM each)	Na^+	K^+	$Na^+ + K^+$	Na^+	K^+	$Na^+ + K^+$
−Ca	9.0	11.0	20.0	18.8	8.3	27.1
+1 mM $CaCl_2$	5.9	15.0	20.9	15.4	10.7	26.1

a consequence of the necessity to maintain charge balance. However, synergism in ion uptake can also be the result of a general increase in root metabolic activity when nutrients are supplied after a period of deprivation.

When present at low concentrations in the rhizosphere, the rate of uptake of a cation is not affected by the accompanying anion and vice versa, as shown in Table 2.19 for K^+ and Cl^-. At high external concentrations, however, an accompanying ion that is taken up relatively slowly can reduce the uptake of an oppositely charged ion that is transported at a faster rate: for example, SO_4^{2-} depresses K^+ uptake and Ca^{2+} depresses Cl^- uptake from single-salt solutions. Different uptake rates of cations and anions require both compensation of electrical charges and regulation of cellular pH. At high external concentrations these requirements become a limiting factor for the uptake of K^+ when accompanied by SO_4^{2-} and for Cl^- when accompanied by Ca^{2+} (Table 2.19).

TABLE 2.19 Rate of K^+ and Cl^- uptake by maize plants with different accompanying Ions

Concentration (mM)	Uptake rate (μmol g^{-1} fw h^{-1})			
	K^+ from		Cl^- from	
	KCl	K_2SO_4	KCl	$CaCl_2$
0.2	1.6	1.6	0.8	0.7
2.0	2.7	1.9	2.0	1.0
20.0	5.7	2.2	4.3	2.1

Recalculated from Lüttge and Laties (1966).

Different rates of cation and anion uptake by roots can cause perturbations of intracellular pH. The stabilization of cytosolic pH in the range of 7.3 to 7.6 is achieved by the so-called cellular *pH-stat*, which consists of two components: the *biophysical pH stat*, characterized by proton transport across the plasma membrane or tonoplast (Fig. 2.21), and the *biochemical pH stat*, which involves production and consumption of protons through metabolism and is achieved by the formation and removal of carboxylic groups (Britto and Kronzucker, 2005; Miller and Cramer, 2004; Peuke, 2010). The functioning of the biochemical pH stat is thought to be reflected in the net changes in organic acid concentrations in roots in response to an imbalance in cation–anion uptake ratio (Table 2.20). When K_2SO_4 is supplied, the excess cation uptake is compensated for by an equivalent synthesis of organic acid anions and when $CaCl_2$ is supplied the excess anion uptake is compensated for by an equivalent decrease in the synthesis of organic acid anions. These changes in organic acid concentrations are also reflected in the rates of CO_2 fixation in the roots (dark fixation).

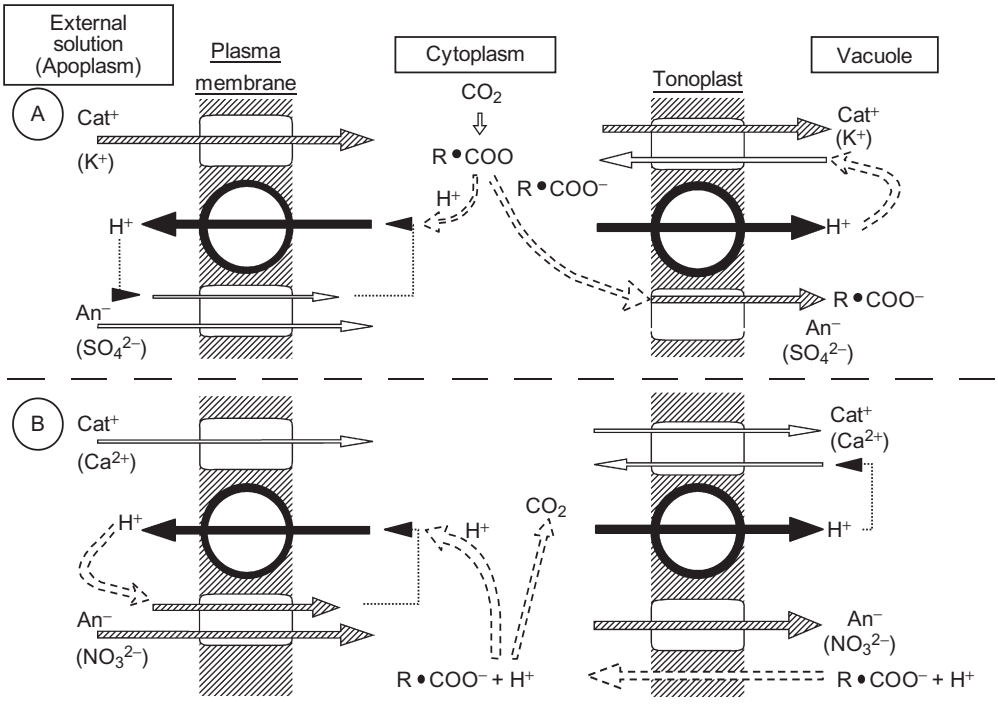

FIGURE 2.21 Model for internal pH stabilization and for charge compensation at different ratios of cation:anion uptake from the external solution. A. Excessive uptake of cations (Cat^+), for example, with K_2SO_4 supply. B. Excessive uptake of anions (An^-), for example, with $Ca(NO_3)_2$ supply.

The main reactions involved in the traditional concept of the biochemical pH stat in relation to different cation–anion uptake ratios are shown schematically in Fig. 2.21. Excessive cation uptake (A) results in an increase in cytosolic pH, which increases the synthesis of organic acids. This produces anions ($R.COO^-$) for pH stabilization and charge compensation and enables the subsequent transport of cations and anions either into the vacuole or the shoot. By contrast, excessive anion uptake (B) is correlated with a decrease in cytosolic pH, which stimulates the decarboxylation of organic acids from the storage pool (i.e., the vacuoles). This causes an increase in pH as decarboxylation consumes protons. In addition to increases or decreases in root concentrations of organic acid anions, the biochemical pH stat also affects the pH in the root apoplasm and external solution with excess cation uptake increasing proton efflux thus decreasing the external pH whereas excess anion uptake increases it. In the experiment reported in Table 2.20 when K_2SO_4 was supplied, the net H^+ efflux was 2.15 µmol g^{-1} root fresh weight h^{-1}, leading to a decrease in the pH of the external solution from 5.60 to 5.12 (Hiatt and Hendricks, 1967). The cation–anion balance in plants and the consequences for rhizosphere pH and mineral nutrition of plants has been reviewed by Haynes (1990) and, in the context of nitrogen nutrition, by Britto and Kronzucker (2005).

TABLE 2.20 Relationship between the uptake of cations and anions and the organic acid concentration of isolated barley roots

External solution (mM)	Uptake (µmol g^{-1} fw)		Change in organic acid (µmol g^{-1} fw)	$^{14}CO_2$ fixation (relative)
	Cations	Anions		
2 K_2SO_4	17	1	+15.1	145
1 KCl	28	29	−0.2	100
1 $CaCl_2$	1	15	−9.7	60

Based on Hiatt (1967a, b) and Hiatt and Hendricks (1967).

In the cytoplasm, the equilibrium between carboxylation (CO_2 fixation) and decarboxylation is thought to be regulated by the pH sensitivity of two enzymes, phosphoenolpyruvate (PEP) carboxylase and malic enzyme (Fig. 2.22). An increase in pH activates PEP carboxylase (reaction (1)), and both the rate of CO_2 fixation and the synthesis of oxaloacetate are increased. After oxaloacetate is reduced to malate by the enzyme malate dehydrogenase, the malate can be directly transported into the vacuoles (reaction (2)), where it acts as a counterion for excess cations (Fig. 2.21A). Alternatively, malate can be incorporated into the cytoplasmic pool of the organic acids of the Krebs cycle, and another organic acid from this pool (e.g., citric acid) can be transported into the vacuole. An oxalate-based biochemical pH stat may play an important role in plant species that accumulate large amounts of oxalate, such as members of the Chenopodiaceae (Davies, 1986). When anions are taken up in excess (Fig. 2.21B), the pH of the cytoplasm decreases and malic enzyme (reaction (4)) is activated, leading to the decarboxylation of malate and the production of CO_2. As a result of these reactions, the cytoplasmic pH is stabilized and the cation–anion ratio in the cells maintained. This biochemical pH stat responds rapidly to supply of K_2SO_4, PEP carboxylase activity being increased by 70% within 20 min (Chang and Roberts, 1992).

Nitrogen nutrition (NH_4^+; NO_3^-; N_2 fixation) has a strong effect on cation–anion relationships in plants

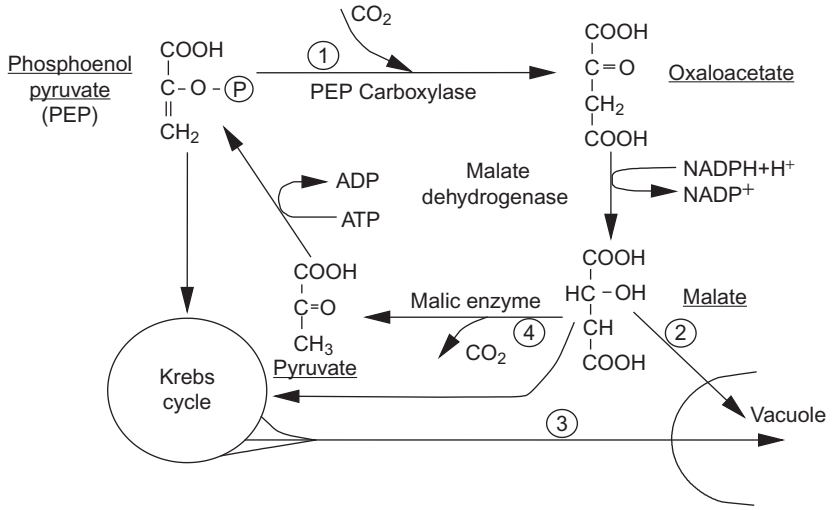

FIGURE 2.22 Model of the pathways of CO_2 fixation ('dark fixation') and decarboxylation. Reactions (1)–(4) are explained in the text.

because about 70% of the cations and anions taken up by plants are either NH_4^+ or NO_3^- (Van Beusichem *et al.*, 1988). Nitrogen nutrition affects both organic acid metabolism and the element composition of plant tissues (Table 2.21). Plants supplied NH_4^+ are generally characterized by a high cation/anion uptake ratio and plants supplied NO_3^- by a low cation/anion uptake ratio. However, the effects of NH_4^+ and NO_3^- on organic acid metabolism differ from those anticipated from Fig. 2.21, since N assimilation in roots is correlated with the production or consumption of protons. The shoots of higher plants have a limited capacity to dispose of protons, thus, NH_4^+ assimilation takes place in roots (Engels and Marschner, 1993). The assimilation of NH_4^+ produces protons, thus, despite a high total cation uptake by NH_4^+-fed plants, the pH in the cytoplasm decreases during NH_4^+ assimilation and must be stabilized both by enhanced proton excretion and the decarboxylation of organic acids (Fig. 2.21B). The protons are effluxed to the external solution in equimolar amounts to the NH_4^+ taken up (Marschner *et al.*, 1991) or to the excess cation uptake (van Beusichem *et al.*, 1988). In contrast, the assimilation of NO_3^- is correlated with an approximately equimolar consumption of H^+ (Raven, 1986). Depending on whether NO_3^- reduction and assimilation take place in the root or the shoot, carboxylates are either produced in the roots or transported in the phloem from the shoots to the roots to maintain charge balance (Peuke, 2010). Legumes dependent on biological nitrogen (N_2) fixation are characterized by a cation/anion uptake ratio >1 and have higher tissue concentrations of organic acid anions and greater proton efflux than plants supplied NH_4^+ (Allen *et al.*, 1988).

The pH of the external solution is strongly influenced by the form of plant nitrogen nutrition due to differences in cation/anion uptake ratio, nitrogen assimilation and cellular pH stabilization (Fig. 2.23). When plants with preferential NO_3^- reduction in the roots, such as sorghum, are supplied NO_3^-, the external pH usually increases considerably with time. When they are supplied NH_4NO_3, after preferential uptake of NH_4^+ and depletion of external NH_4^+, a transient decrease in the pH of the external solution during NH_4^+ uptake is followed by an increase in pH, as observed for NO_3^--fed plants. However, under conditions where NO_3^- uptake and assimilation are impaired and cation/anion uptake ratio is high, a strong decrease in the pH of the external solution has been observed. This phenomenon occurs in many plant species with, for example, P deficiency (Schjorring, 1986), Zn deficiency (Cakmak and Marschner, 1990) and Fe deficiency in dicotyledonous plants.

In plants supplied NH_4^+, net proton efflux and maintenance of the cellular pH stat becomes increasingly difficult in roots when the pH of the external solution is low. The presence of high NH_4^+ concentrations in the rhizosphere causes a reduction in both the cytoplasmic and vacuolar pH of root cells (Gerendas *et al.*, 1990). Poor growth of NH_4^+-fed plants at low external pH is probably related to the difficulty in maintaining cytosolic pH homeostasis in the face of high NH_4^+ fluxes across the plasma membrane of root cells, together with cation–anion imbalance, and the high energy costs incurred by the futile cycling of ammonium across the plasma membrane (Miller and Cramer, 2004; Britto and Kronzucker, 2006).

Maintenance of cytoplasmic pH homeostasis involves costs in terms of energy, photosynthate and water (Raven, 1985). This is particularly true in relation to N nutrition. When both NH_4^+ and NO_3^- are supplied, cytoplasmic pH homeostasis may be achieved by similar rates of H^+ production (NH_4^+ assimilation) and H^+ consumption (NO_3^- assimilation) and thus have a very low energy requirement (Raven, 1985; Allen *et al.*, 1988). This may explain, in part, why optimal growth for many plant species is usually obtained with a mixed supply of NH_4^+ and NO_3^-.

2.5.5 External Concentration

The relationship between the rate of influx (I), or uptake, of an ion and its concentration in solution (S) can usually be described by Michaelis-Menten kinetics in the low concentration range: the flux saturates, transport appears to be selective and is closely coupled to metabolism. In contrast, at high external ion concentrations, the uptake rate is often linearly related to solute concentration ($I = kS$) through a proportionality parameter (k), is not very selective, and

TABLE 2.21 Ionic balance in shoots of castor oil plants grown with different forms of N supply

	Cations				Anions					
Form of N supply	K^+	Ca^{2+}	Mg^{2+}	Total	NO_3^-	$H_2PO_4^-$	SO_4^{2-}	Cl^-	Organic acids[a]	Total
NO_3^-	99	85	28	212	44	18	11	2	137	212
NH_4^+	55	43	22	120	0	23	33	5	59	120

Van Beusichem *et al.* (1988).
[a]*Calculated from the difference of cations–anions.*

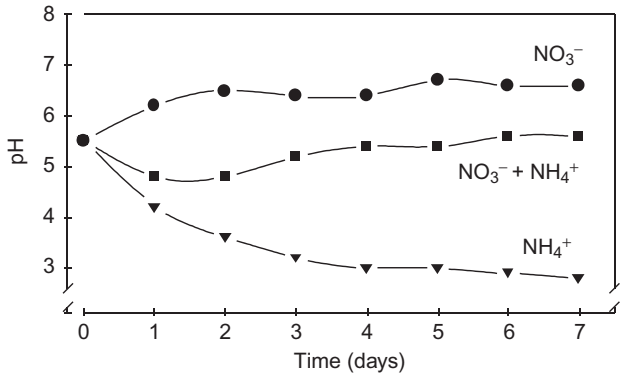

FIGURE 2.23 Time course of changes in the pH of the external solution in sorghum plants supplied with 300 mg l^{-1} total N as only NO_3^-, only NH_4^+, or both at a ratio of 8 NO_3^- to 1 NH_4^+ as their N source. *Redrawn from Clark (1982b).*

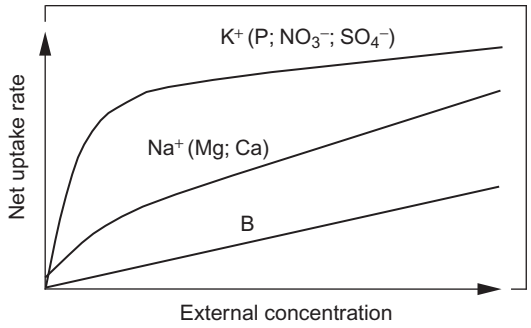

FIGURE 2.24 Schematic representation of the relationships between the rates of uptake of K, Na and B by barley roots and the concentrations of KCl, NaCl and B in the external solution. The relationships between the uptake rates of other nutrients and their concentrations in the external solution are indicated in brackets.

is not particularly sensitive to temperature or metabolic inhibitors. The typical relationship between external concentration and uptake rate of K^+ is presented in Fig. 2.24 schematically, without consideration of C_{min}. Similar uptake isotherms have been obtained for many nutrients, although their kinetic parameters (K_m, I_{max}, C_{min}, k) differ.

The concentrations in soil solutions are usually low for K^+ (<1 mM) and ammonium (<100 µM), and extremely low for phosphate (<10 µM), boron (<10 µM), nickel (<10 µM), zinc (<1 µM), copper (<1 µM), molybdate (<1 µM), manganese (<1 µM), and iron (<0.1 pM in calcareous and alkaline soils). On the other hand, the concentrations of Ca^{2+}, Mg^{2+} chloride, sulphate and nitrate are often in the millimolar range, especially in arable soils (Table 13.3; Tyler and Olsson, 2001; White and Broadley, 2001, 2009). Therefore acquisition of most nutrients will require significant energy input, and a selective, high-affinity transport system to enable the plant to satisfy plant demand and avoid ion toxicities.

TABLE 2.22 Influx of nitrate into barley roots without and with an induced high capacity nitrate uptake system

	NO_3^- Influx (µmol g^{-1} fw h^{-1})		
External conc. (mM NO_3^-)	Non-induced	Induced 1 day[a]	Induced 4 days[a]
0.02	0.10	2.75	1.54
0.30	0.39	5.27	2.86
20.0	11.54	20.87	8.02

Based on Glass *et al.* (1990).
[a]*Pretreatment with nitrate for one or four days.*

The kinetic parameters for a given nutrient are influenced greatly by plant nutritional status and nutrient availability in the rhizosphere. In general, deficiency of a nutrient leads to an increase in the capacity of the root system to take up that nutrient (Siddiqi *et al.*, 1990; Rawat *et al.*, 1999; Buchner *et al.*, 2004; Li *et al.*, 2007; White and Broadley, 2009; White and Hammond, 2010; White and Karley, 2010). For example, in plants grown in the absence of nitrate, (non-induced) roots possess only a low capacity (constitutive) influx system for nitrate (Table 2.22). This constitutive nitrate influx system does not saturate as the external nitrate concentration is increased, suggesting that the transport protein has a low affinity for nitrate. However, within 20 min of supplying nitrate, a high-affinity, high-capacity nitrate influx system is induced (Table 2.22). Four days after induction, the capacity for nitrate influx is lower, suggesting negative feedback regulation of the activity of this transport system.

As a rule, optimal growth can be achieved at concentrations in the range of the high-affinity system for nutrients such as K, P and N when these are supplied continuously to plants in the external solution (Asher and Edwards, 1983). Similarly, for the micronutrient cations Zn, Cu, Mn and Fe, the concentration of the free metal species (Zn^{2+}, Cu^{2+}, Mn^{2+}, Fe^{3+}) in the external solution required for optimal growth derived from chelate-buffered nutrient solutions, suggest that nutritional requirements can be met by extremely low concentrations of free cations in the external solution; in the order of 10^{-9} to 10^{-12} for Mn^{2+}, Zn^{2+} and Cu^{2+}, and perhaps even lower for $Fe^{2+/3+}$ provided a continuous supply is maintained (Bell *et al.*, 1991; Laurie *et al.*, 1991; Webb *et al.*, 1993; Fox *et al.*, 1996; Degryse *et al.*, 2006).

Thus, under optimal conditions in which a constant nutrient supply is maintained (e.g., in nutrient solutions), only very low concentrations of nutrients are required in the external solution for maximal plant growth. At higher supply, higher uptake rates reflect what is known as 'luxury uptake'. In soil-grown plants in general, and under field

conditions in particular, the conditions in the root environment are far from optimal and the maintenance of a constant nutrient supply to the root surface is unlikely to occur. Higher external concentrations and luxury uptake in preceding periods can be important in providing an internal reserve for periods of high demand or interrupted root supply. This also holds true for natural vegetation experiencing transient nutrient flushes under favourable weather conditions (Rorison, 1987; Millard and Grelet, 2010).

In both soil and nutrient solutions, essential elements can be supplied in concentrations so high that they become toxic to plants; e.g. Mn in acid soils, B in sodic (Na-rich) soils, and Mn and Fe in waterlogged or flooded soils (White and Brown, 2010). However, there is considerable genotypic variation in the uptake of nutrients, and these differences, both between and within plant species, have important consequences for ecology and agriculture (White and Brown, 2010). The ability to grow well in soils with high concentrations of elements allows plants to survive in these soils and also enables the development of plants for the phytoremediation of contaminated land. For example, in barley, the cultivar Sahara 3771 has a lower B uptake capacity than the cultivar Schooner, and therefore requires a higher concentration in the external solution for optimal growth (Table 2.8). This can be a disadvantage in low B soils, but is an effective mechanism in avoiding B toxicity when plants are grown in soils with high B availability.

The ability to grow well in soils with low availability of nutrients confers an ecological advantage in these environments. Genotypic variation within crop species in the nutrient supply required for optimal growth is well documented, for example for N, P and K (Hirel *et al.*, 2007; Rengel and Damon, 2008; White and Hammond, 2008; Fageria, 2009). Such genotypic variation can be used to improve fertilizer use efficiency in agriculture through the development of crops that acquire and/or utilize mineral elements more effectively.

2.5.6 Plant Nutritional Status

The rate of uptake of a nutrient at a given external concentration is often determined by plant growth rate which is thought to affect the uptake of a particular mineral nutrient through plant nutritional status (e.g., Clement *et al.*, 1978a; Clarkson *et al.*, 1988; Laine *et al.*, 1993; Walker *et al.*, 2001). Nutrient uptake responds rapidly to fluctuations in root nutrient concentrations and more slowly to long-term changes in plant demand or external nutrient supply. A rapid decrease in the net uptake of a nutrient by roots upon an abrupt increase in its external concentration can be the consequence of an increase in its cytosolic concentration and increased efflux across the plasma membrane (Britto and Kronzucker, 2006). It is also observed

TABLE 2.23 Relationship between tissue concentration and K influx to barley roots

K Concentration (μmol g^{-1} fw)	K^+ Influx (μmol g^{-1} fw h^{-1})
20.9	3.05
32.1	2.72
47.9	2.16
57.8	1.61

From Glass and Dunlop (1979).

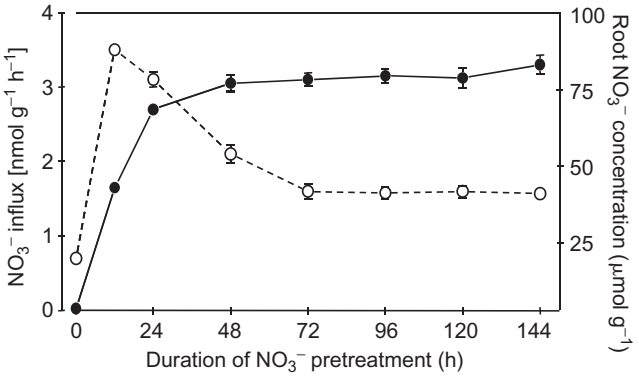

FIGURE 2.25 Nitrate concentration (●) and nitrate influx (○) to roots of barley plants with different nitrate (NO_3^-)-pretreatment. *Adapted from Siddiqi* et al. *(1989).*

that, as the tissue concentration of a particular mineral element increases, its influx declines, and vice versa. An example of this feedback regulation is shown for K influx in barley roots in Table 2.23. Similar relationships between internal concentrations and influx rate are well documented, for example, for nitrate (Fig. 2.25), P (Table 2.9), sulphate (Fig. 2.26), Fe, Zn and Cu (Broadley *et al.*, 2007; White and Broadley, 2009). Immediate effects on nutrient influx are due to post-translation modifications of regulatory components or transport proteins, whereas longer-term effects are mediated by transcriptional responses. The mechanisms that may be involved in this feedback regulation are summarized in Fig. 2.27.

The uptake of NH_4^+ and NO_3^- is closely related to the N status of plants. For example, NH_4^+ uptake capacity is negatively correlated with the concentrations of NH_4^+ and certain amino acids, such as glutamine and asparagine, in the roots (Causin and Barneix, 1993; Rawat *et al.*, 1999; von Wirén *et al.*, 2000). Accordingly, NH_4^+ uptake capacity increases rapidly within a few days after the withdrawal

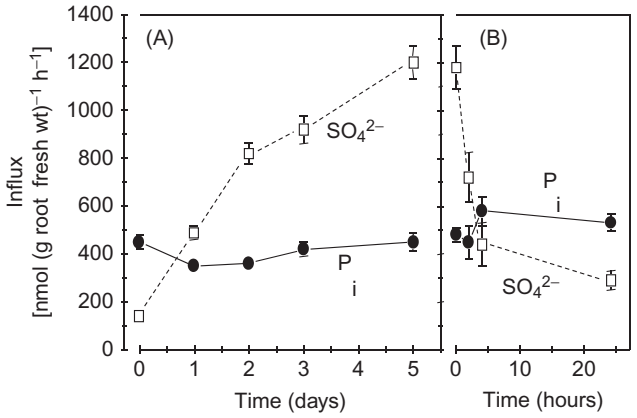

FIGURE 2.26 Time course of changes in the influx of sulphate (SO_4^{2-}) and phosphate (P_i) in roots of barley plants deprived of external sulphate supply for up to 5 days (A) and then resupplied with sulphate for up to 24 hours (B). *Adapted from Clarkson and Saker (1989)*.

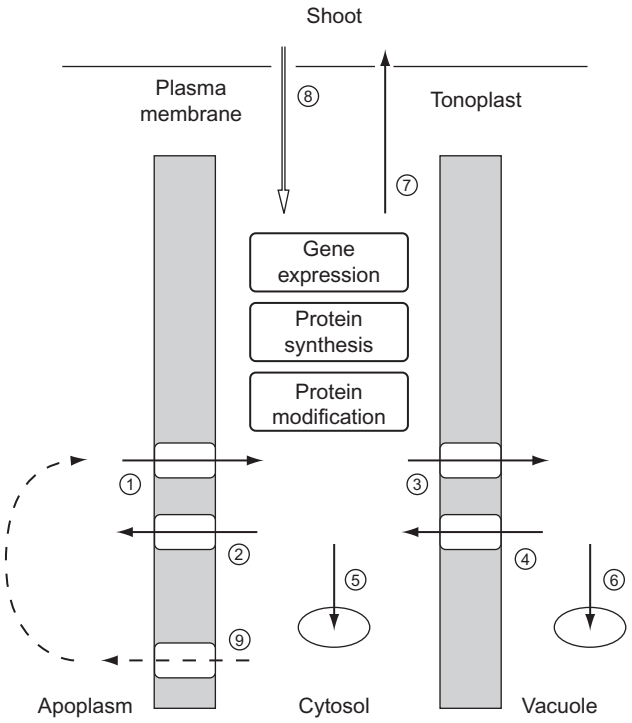

FIGURE 2.27 Model for the regulation of nutrient uptake by roots via plant nutritional status. Changes in gene expression, protein synthesis and protein modification in response to either the cytosolic concentration of the mineral nutrient or its metabolites can regulate: (1) nutrient influx via the number and activity of transporters, and the electrochemical gradient across the plasma membrane; (2) nutrient efflux via nutrient concentration in the cytosol, the number and activity of transporters, and the electrochemical gradient across the plasma membrane; (3) nutrient influx to the vacuole via nutrient concentration in the cytosol, the number and activity of transporters, and the trans-tonoplast electrochemical gradient; (4) nutrient efflux from the vacuole via nutrient concentration in the vacuole, the number and activity of transporters, and the trans-tonoplast electrochemical gradient; (5) complexation in the cytosol, sequestration in organelles and metabolism of nutrients, which affect the concentrations of the nutrient and its metabolites in the cytosol; (6) complexation or precipitation of the nutrient in the vacuole, which affects its ability to exit the vacuole; (7) transport of a nutrient or metabolite from the root to the shoot in the xylem, which affects their cytosolic concentrations; (8) transport of a nutrient or metabolite from the shoot to the root in the phloem, which affects their cytosolic concentrations; (9) modification of the rhizosphere through the secretion of protons, enzymes and organic solutes to increase the concentration of the mineral nutrient in the apoplasm, and its availability for influx.

of N supply (Lee and Rudge, 1986; Rawat *et al.*, 1999). In *Arabidopsis thaliana*, the increase in NH_4^+ uptake capacity is correlated with a decrease in glutamine and increased expression of the *AtAMT1* gene (Rawat *et al.*, 1999). A decrease in NH_4^+ efflux is also observed, but this is not the major factor responsible for increasing net NH_4^+ uptake in N-starved plants (Morgan and Jackson, 1988). The regulation of NO_3^- uptake involves the induction of a high-capacity, high-affinity uptake system and the negative feedback regulation of NO_3^- uptake by increasing internal NO_3^- concentrations (Table 2.22). In non-induced plants, NO_3^- supply rapidly increases both NO_3^- influx and NO_3^- concentrations in root tissues. Later, the increase in NO_3^- concentrations reduces NO_3^- influx. This negative feedback regulation may be caused by high NO_3^- concentrations in the vacuoles and follow a turgor-regulated event (Glass, 1983). However, negative feedback regulation by elevated concentrations of reduced N in the form of the amino acids glutamine and asparagine (Lee *et al.*, 1992; Louahlia *et al.*, 2008) or of NH_4^+ is a more likely explanation, which is consistent with the inhibitory effects of NH_4^+ supply on NO_3^- uptake. It is also noteworthy that increased root sucrose concentrations appear to increase the uptake of both ammonium and nitrate through up-regulation of genes encoding high-affinity transport proteins (Lejay *et al.*, 2003; Louahlia *et al.*, 2008; Girin *et al.*, 2010).

In a similar manner, sulphate uptake is regulated by plant S status. The dynamics of this feedback regulation on the uptake of sulphate is shown in Fig. 2.26. Without external sulphate supply, the capacity of barley roots for sulphate uptake (influx) increases rapidly within 3–5 days, but decreases strongly within a few hours of sulphate resupply and is lost within one day of sulphate resupply. The influx rate of P is unaffected by the S nutritional status. Induction of the transport system for sulphate requires transcription and protein synthesis, as does the induction of enzymes involved in the S assimilatory pathway (Hawkesford and De Kok, 2006; Hell *et al.*, 2010). Early studies suggested that sulphate stored in the vacuoles played an important role in negative feedback regulation of sulphate uptake (Cram, 1983). However, it is now thought that the accumulation of reduced S compounds, such as cysteine or reduced glutathione (GSH), are the dominant signals of tissue S status that regulate sulphate uptake

TABLE 2.24 Phosphorus concentrations in tissues of barley plants following the supply of P to plants grown without P

	P concentration (μmol P g^{-1} dw)[a]		
	8 days −P[b]	7 days −P +1 day +P[c]	7 days −P +3 days +P[d]
Shoot total	49 (20)	151 (61)	412 (176)
Youngest leaves	26 (5)	684 (141)	1647 (493)
Roots	43 (24)	86 (48)	169 (94)

Based on Clarkson and Scattergood (1982).
[a]*Numerals in parentheses are relative values; 100 represents control with continuous phosphorus supply of 150 μM P throughout the experiment.*
[b]*Eight days of growth without P.*
[c]*Seven days of growth without P and 1 day of growth upon addition of P (150 μM).*
[d]*Seven days of growth without P and 3 days of growth upon addition of P (10 μM).*

and assimilation (Hawkesford and De Kok, 2006; Hell *et al.*, 2010). These compounds can be the product of root cell metabolism or, in the case of GSH and its precursor γ-glutamylcysteine, can be translocated from the shoots to the roots as a systemic signal of shoot S status (Herschbach and Rennenberg, 2001; Hell *et al.*, 2010). The expression of genes involved in S uptake and assimilation are also controlled by the delivery of sucrose (Lejay *et al.*, 2003) and a regulatory microRNA (miRNA395; Pant *et al.*, 2008, 2009; Kragler, 2010; Liang *et al.*, 2010) from the shoot.

Similarly to the examples for nitrate and sulphate, P uptake (influx) capacity increases after P is withheld from the external solution (White and Hammond, 2008). This is correlated with increased transcription of genes encoding proton-coupled P transporters. This transcriptional response appears to be mediated through the interplay of biochemical signals indicating root and shoot P status. It is thought that low root P status initiates a complex regulatory cascade through the PHR1 transcription factor and that the increased transport of sucrose and microRNA (miR399) in the phloem acts as systemic signals of low shoot P status (Hammond and White, 2008; Buhtz *et al.*, 2010; Kragler, 2010; Vance, 2010). Although resupplying P to P-deficient plants ultimately reduces their capacity for P uptake, this response does not occur immediately (Table 2.24). Thus, resupplying P after a period of deficiency can result in greatly increased tissue P concentrations and to P toxicity (Clarkson and Scattergood, 1982; Cogliatti and Clarkson, 1983). Such rapid changes in P supply are unlikely to occur in soil grown plants. In nutrient solution culture, however, this factor should be considered, especially after the replacement of solutions.

Although the transport systems induced by nutrient deficiencies are generally specific for the nutrient the plant lacks, they can also transport other elements. For example, the uptake of selenate increases in sulphur-deficient barley plants, and the uptake rate of arsenate increases in P-deficient barley plants (Lee, 1982). This is likely to be a consequence of an increased abundance of the *Hv*SULTR1 proton-coupled sulphate symporter and of the *Hv*Pht1;1 and *Hv*Pht1;2 proton-coupled phosphate symporters, respectively (Smith *et al.*, 1997; Buchner *et al.*, 2004; Schunmann *et al.*, 2004; Christophersen *et al.*, 2009). Similarly, caesium uptake increases in K-deficient *Arabidopsis thaliana* as a consequence of the up-regulation of the *AtHAK5* gene, which encodes a high-affinity plasma membrane proton-coupled K^+ (and Cs^+) symporter (Qi *et al.*, 2008; White and Karley, 2010). Unusual and unexpected responses also occur sometimes. In tomato roots, for example, Cd uptake rate increases as the Cd concentration in the root increases (Petit *et al.*, 1978). This may reflect the induction of synthesis of compounds, such as metallothioneins or phytochelatins, which chelate and detoxify heavy metals. A similar mechanism may be involved in the differences in the rate of Cu uptake in Cu-sufficient and Cu-deficient plants: on resupplying Cu to deficient plants the uptake rate is much lower than in Cu-sufficient plants (Jarvis and Robson, 1982).

As observed in the preceding paragraphs, the relationships between the rate of uptake of a particular nutrient and plant nutritional status cannot always be explained satisfactorily by consideration of root tissue concentration alone; feedback regulation of ion uptake by shoot nutritional status is also evident (Fig. 2.27). Such feedback control is essential for the coordination of nutrient uptake by the demand of the plants for growth. Variation in the supply of sugars to the roots (Hermans *et al.*, 2006; Hammond and White, 2008; Peuke, 2010), or general differences in the rates of root export of nutrients in the xylem to the shoot, can be considered as coarse feedback mechanisms. For example, an increased supply of sucrose in the phloem leads to greater root biomass and up-regulates the expression of genes encoding transporters for nitrate, phosphate, sulphate, ammonium, potassium and iron (Lejay *et al.*, 2003; Hermans *et al.*, 2006; Hammond and White, 2008; Liu *et al.*, 2009). However, there are also fine controls specific to particular nutrients. For example, the uptake rates of P (Drew *et al.*, 1984) and K (Table 2.25) may be more closely related to the concentrations of these nutrients in the shoots than in the roots. Drew and Saker (1984) proposed models for the regulation of K and P uptake in which the fraction of these nutrients delivered to the shoot in excess of demand is translocated back to the roots to convey information concerning the nutritional status of the shoot. There is good evidence for the cycling of nutrients within the plant (Section 3.4), and that the translocation

TABLE 2.25 Relationship between the rate of K uptake by maize and the K concentrations of the root and shoot

K^+ uptake (pmol cm^{-1} sec^{-1})	K concentration (g kg^{-1}dw)	
	Root	Shoot
15.8	58.5	80.0
28.0	55.5	64.5
33.8	49.9	43.5
36.8	55.1	41.3

From Barber (1979).

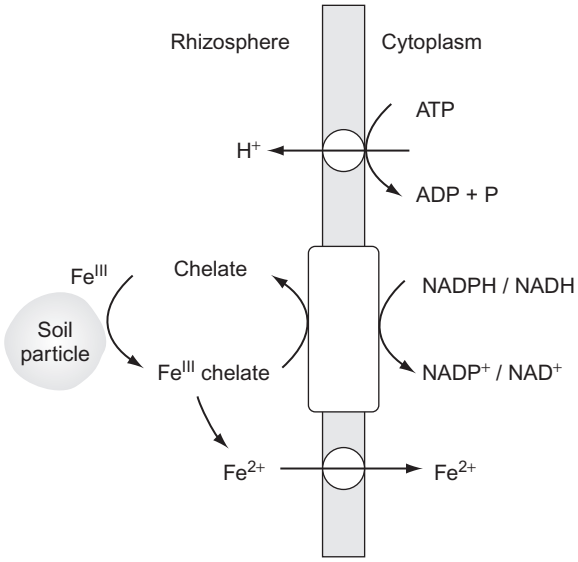

FIGURE 2.28 Model for root responses to iron deficiency in dicots and non-graminaceous monocots (Strategy I): increased acidification of the rhizosphere by H^+-ATPases, induction of ferric reductase activity, reduction of Fe(III)-chelates to Fe^{2+}, uptake of Fe^{2+} across the plasma membrane by Fe deficiency-inducible, high-affinity Fe^{2+} transporters.

of nutrients and/or their metabolites from the shoot to the roots in the phloem plays an important role in regulating ion uptake by roots. This is the case, for example, for P (Drew *et al.*, 1984; Drew and Saker, 1984) and K (White and Karley, 2010), and for N through nitrate or amino acids (Cooper and Clarkson, 1989; Liu *et al.*, 2009) and S through glutathione (Liu *et al.*, 2009; Hell *et al.*, 2010). In addition, the synthesis of specific microRNAs in shoots of nutrient-deficient plants, and their translocation to the root in the phloem, could regulate adaptive responses to limited availabilities of N, S, P, Fe and Cu (Liu *et al.*, 2009; Buhtz *et al.*, 2010; Kong and Yang, 2010; Liang *et al.*, 2010; Lundmark *et al.*, 2010; Vance, 2010; Vidal *et al.*, 2010).

In addition to regulating the uptake capacity for nutrients, signals of root and shoot nutritional status can also affect the biomass and morphology of the root system, adaptive responses to nutrient deficiency that mobilize nutrients from recalcitrant compounds, and biochemical pathways for the assimilation of nutrients such as N, S and P. For example, plant N status also influences plant shoot/root biomass ratio, root morphology and N assimilation (Hodge, 2004; Hermans *et al.*, 2006; Garnett *et al.*, 2009), plant S status influences plant shoot/root biomass ratio and modulates sulphate assimilation (Hawkesford and De Kok, 2006; Hell *et al.*, 2010), and plant P status influences plant shoot/root biomass ratio, root morphology, the release of protons, phosphatases and organic acids into the rhizosphere, and P metabolism in plants (White and Hammond, 2008). These responses serve primarily to increase the acquisition of nutrients and improve the physiological efficiency by which plants utilize nutrients for growth when they are in short supply.

Regarding their response to Fe deficiency, plants can be classified into two categories (Strategy I and Strategy II) (Marschner *et al.*, 1986a, b; Römheld, 1987a,b; Schmidt, 1999; Puig *et al.*, 2007; White and Broadley, 2009; Guerinot, 2010). In both strategies, the responses are confined to the apical zones of growing roots and are fully repressed within about one day after resupply of iron.

Strategy I is typical of dicotyledonous and non-graminaceous monocotyledonous plants. It is characterized by an increase in ferric (Fe^{3+}) reduction capacity, acidification of the rhizosphere, and the release of organic acid anions and phenolic compounds into the soil solution (Fig. 2.28). These root responses are closely related to changes in root morphology and anatomy, particularly in the formation of transfer cell-like structures in rhizodermal cells. The plasma-membrane ferric (chelate) reductases that catalyse the reduction of Fe^{3+} to Fe^{2+} in roots of Fe-deficient and Fe-sufficient plants have similar enzymatic characteristics (Holden *et al.*, 1991), and are encoded by members of the ferric reductase oxidase (*FRO*) gene family (Puig *et al.*, 2007; White and Broadley, 2009; Guerinot, 2010). Members of the ZIP transporter family, such as the *At*IRT1 transporter of *Arabidopsis thaliana*, then mediate Fe^{2+} influx to root cells (Puig *et al.*, 2007; White and Broadley, 2009; Guerinot, 2010). The expression of both *AtFRO2* and *AtIRT1* in roots of *Arabidopsis thaliana* vary diurnally, being less at night than in the day, and are increased under Fe deficiency (Vert *et al.*, 2002). An example of the root responses to Fe deficiency, and the corresponding enhanced rates of Fe uptake, of a Strategy I plant (cucumber) are shown in Table 2.26. The higher reduction rates of Fe^{3+}at the outer surface of the plasma membrane of root rhizodermal cells are correlated with enhanced rates of Fe uptake.

TABLE 2.26 Proton excretion (pH), reducing capacity of the roots and iron uptake rate[a] in cucumber (Strategy I) with or without Fe preculture

Fe nutritional status (preculture)	Chlorophyll (mg g^{-1} dw)	H^+ excretion (pH solution)	Reducing capacity (µmol Fe^{II} g^{-1} root dw (4 h) $^{-1}$)	Fe uptake (µmol (g^{-1} root dw (4 h) $^{-1}$)
+Fe[a]	12.2	6.2	3.2	0.03
−Fe	7.8	4.8	96.8	2.60

Data compiled from Römheld and Kramer (1983) and Römheld and Marschner (1990).
[a]*Supply of 1 × 10^{-6} M FeEDDHA, pH 6.2.*

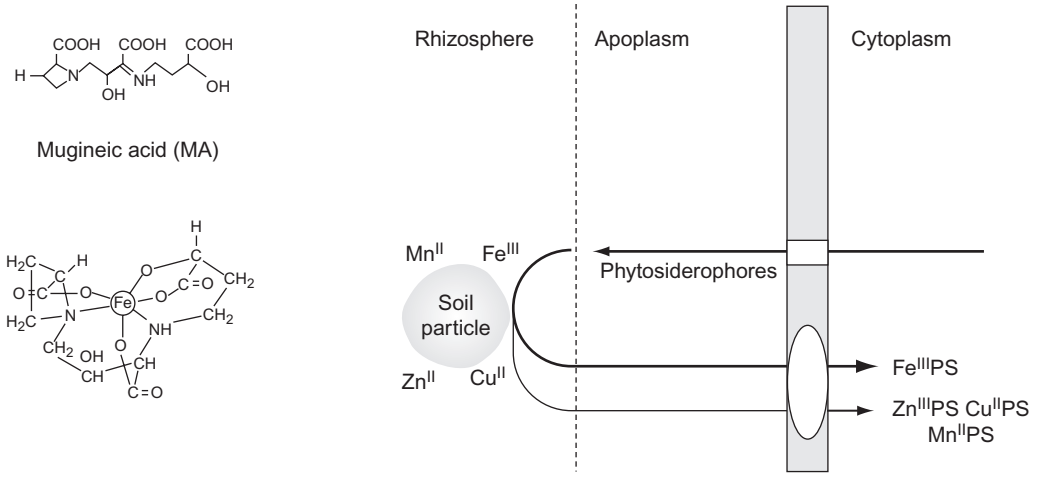

FIGURE 2.29 Model for root responses to iron deficiency in graminaceous species (Strategy II): enhanced synthesis and release of phytosiderophores into the rhizosphere, chelation of Fe^{3+}, Fe^{2+}, Cu^{2+} and Mn^{2+}, and transport of metal-phytosiderophore chelates across the plasma membrane by transport proteins. The structures of the phytosiderophore mucigenic acid and its corresponding Fe(III) chelate are also shown.

Strategy II is confined to graminaceous plant species (cereals and grasses) and characterized by an Fe deficiency-induced enhanced release of non-proteinogenic amino acids called phytosiderophores (Takagi *et al.*, 1984; von Wirén *et al.*, 1995; White and Broadley, 2009; Guerinot, 2010). The chemistry of phytosiderophores is species specific and determines the contrasting abilities of different grasses and cereals to acquire Fe (Römheld and Marschner, 1990; Bashir *et al.*, 2006; Nagasaka *et al.*, 2009). Enzymes involved in the synthesis of phytosiderophores from L-methionine include S-adenosylmethionine synthetase, nicotianamine synthase, nicotianamine amino-transferase and deoxymugineic acid synthase (Bashir *et al.*, 2006; Guerinot, 2010). The expression of genes encoding these enzymes, and also of genes involved in S uptake and methionine synthesis, is often rapidly up-regulated in response to Fe deficiency (Bashir *et al.*, 2006; White and Broadley, 2009; Guerinot, 2010). Phytosiderophores, such as mugineic acid (Fig. 2.29), form highly stable complexes with Fe^{3+}, Zn^{2+} and Cu^{2+}. The stability constant for the Fe^{3+}-mugineic acid complex in water is in the order of 10^{33} (Murakami *et al.*, 1989). The release of phytosiderophores is induced by Fe deficiency (Table 2.27) and rapidly decreases when Fe is resupplied to a Fe-deficient plant (Fig. 2.30A). Both the release of phytosiderophores and the uptake of metal-phytosiderophore complexes follow a distinct diurnal rhythm (Fig. 2.30B) being highest in the first hours after onset of light. The Fe^{3+}-phytosiderophore complex enters the root cytoplasm via proton-coupled Fe^{3+}-phytosiderophore symporters in the plasma membrane of root cells of cereals and grasses (Fig. 2.29; Römheld and Marschner, 1990; von Wirén *et al.*, 1995; Schaaf *et al.*, 2004; Ishimaru *et al.*, 2006; White and Broadley, 2009; Guerinot, 2010). Homologues of the maize yellow stripe 1 (*Zm*YS1) protein belonging to the oligopeptide transporter (OPT) family mediate Fe^{3+}-phytosiderophore uptake by Strategy II plants (von Wirén *et al.*, 1995; Schaaf *et al.*, 2004; Ishimaru *et al.*, 2006; Puig *et al.*, 2007; White and Broadley, 2009; Guerinot, 2010). The corresponding

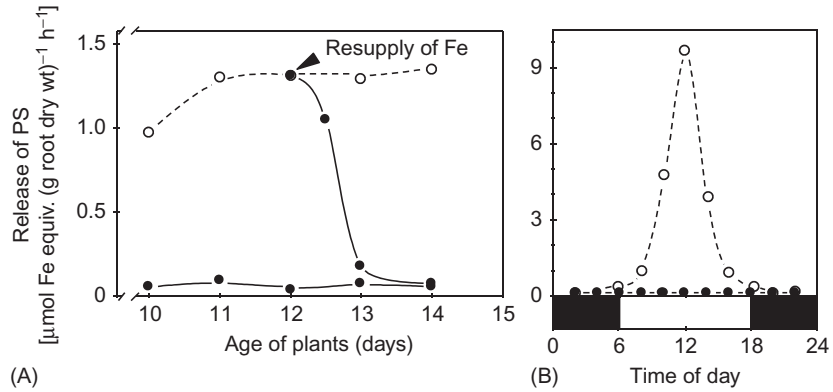

FIGURE 2.30 Release of phytosiderophores (PS) from barley roots as affected by plant Fe nutritional status (A), and diurnal rhythm of release of phytosiderophores (B). Assays were performed on iron-sufficient (●) and iron-deficient (○) plants. *Data from Römheld (1987a, b) and A. Walter, personal communication.*

TABLE 2.27 Release of phytosiderophores (PS; mugineic acid) and uptake of Fe-phytosiderophores in Fe-sufficient and Fe-deficient barley plants

Fe nutritional status (preculture)	Chlorophyll concentration (mg g^{-1} dw)	PS release (µmol g^{-1} root dw (4 h)$^{-1}$)	Fe uptake (µmol g^{-1} root dw per 4 h)$^{-1}$)
+Fe	12.8	0.4	0.4
−Fe	7.5	8.2	3.4

Römheld and Marschner (1990).

Zn^{2+} and Cu^{2+}-phytosiderophore complexes are also transported by proton/metal–phytosiderophore symporters, but often with a lower affinity and capacity than the Fe^{3+}-phytosiderophore complex (Marschner *et al.*, 1989; Ma *et al.*, 1993; von Wirén *et al*., 1996; Broadley *et al*., 2007; White and Broadley, 2009). The release of phytosiderophores can also enhance the uptake rate of these metals by increasing their mobility in the rhizosphere (Zhang *et al.*, 1991a, b, c). In addition to their ability to take up Fe^{3+}-phytosiderophores, graminaceous species can also take up Fe^{2+} (Ishimaru *et al.*, 2006; Cheng *et al.*, 2007; White and Broadley, 2009; Guerinot, 2010).

Although the phytosiderophore uptake system of Strategy II plants resembles the siderophore uptake system of microorganisms, its affinity for phytosiderophores is two to three orders of magnitude higher than that for siderophores such as ferrioxamine B (Bar-Ness *et al.*, 1991, 1992; Crowley *et al.*, 1992), or for synthetic iron chelates such as FeEDDHA (Römheld and Marschner, 1990; Bar-Ness *et al.*, 1991, 1992).

Differences exist in 'Fe efficiency' (i.e., sensitivity to Fe deficiency chlorosis) both between and within graminaceous species. These differences are, as a rule, closely related to the amount and type of the phytosiderophores released under Fe deficiency (Clark *et al.*, 1988; Römheld and Marschner, 1990; Bashir *et al*., 2006; Nagasaka *et al.*, 2009). The manipulation of phytosiderophore synthesis, their release into the rhizosphere, and the uptake of metal-phytosiderophores by roots offer strategies to improve Fe uptake by plants and thereby the generation of crops for soils with high Fe concentrations but low Fe availability.

2.5.7 Studying Nutrition at Constant Tissue Concentration

The common approach of studying plant nutrition in relation to nutrient uptake, nutrient supply, plant nutritional status and growth rate has been questioned by Ingestad and coworkers (Ingestad and Agren, 1992; Ingestad, 1997). These authors have argued that, particularly during the exponential phase of plant growth and even in a flowing solution, low external concentrations and plant tissue concentrations are difficult to maintain constant. Thus, the relationships between external supply, uptake rates and plant nutritional status are difficult to ascertain unequivocally.

In order to overcome these difficulties, and also to define the effectiveness of a nutrient in terms of biomass production per unit of nutrient at different internal concentrations, Ingestad and colleagues have used a different theoretical and experimental approach. In principle, this approach is based on relative values. To set a constant relative uptake, the relative addition rates of nutrients (i.e., the supply of nutrients) are divided by the amount of the nutrient already in the plant. Accordingly, only the amount of nutrients supplied count, and not the external concentration. Using this approach, a range of different but constant relative growth rates can be achieved at different degrees of nutrient limitations. Interestingly, although the root/shoot

TABLE 2.28 Uptake and translocation of K (^{42}K) and Ca (^{45}Ca) supplied to different zones of the seminal roots of maize[a]

Nutrient (1 mM)	Accumulation and translocation	Root zone supplied (distance from tip, cm)		
		0–3	6–9	12–15
K	Translocation to shoot	3.8	14.6	15.6
	Accumulation in zone of supply	11.5	3.8	1.9
	Translocation to root tip	–	4.3	2.0
	Total	*15.2*	*22.7*	*19.5*
Ca	Translocation to shoot	2.4	2.2	2.4
	Accumulation in zone of supply	4.1	1.6	0.4
	Translocation to root tip	–	–	–
	Total	*6.5*	*3.8*	*2.8*

Based on Marschner and Richter (1973).
[a]*Data expressed as micromoles per 12 plants in 24 h.*

dw ratio of the nutrient-limited plants is often large, visual deficiency symptoms are absent.

This highly formal concept is an interesting variation to the common approach for studying the nutrition of plants, in which the influence of external concentration and plant nutrient status on nutrient uptake, growth responses and various physiological and biochemical parameters (e.g., photosynthesis) are studied. This concept allows studying the effects of mineral nutrition under suboptimal but steady-state conditions. However, these steady-state conditions, in which the relative nutrient supply is adjusted to the relative growth rate, are not typical of those experienced by plants growing in the field. For field grown plants, fluctuations in nutrient supply to the roots are as common as fluctuations in other environmental parameters such as irradiation, temperature and water supply. To cope with these fluctuations plants possess a range of adaptive mechanisms. Fluctuations in nutrient supply are compensated for by modulating uptake capacity, changes in root morphology and physiology and root/shoot biomass ratio (Chapters 14–16), and the storage and remobilization of mineral nutrients (Chapters 3 and 6).

2.6 UPTAKE OF IONS AND WATER ALONG THE ROOT AXIS

Roots vary both anatomically and physiologically along their longitudinal axes. This should be borne in mind when models for 'the' behaviour of root tissue and root cells are based on studies with isolated roots or roots of intact plants. In the apical zone, non-vacuolated cells dominate. These cells differ in many respects from the vacuolated cells in the basal zones. The apical root zones have higher respiration rates (Thomson *et al.*, 1989b), which decrease rapidly when the carbohydrate supply to roots is interrupted, for example following excision (Brouquisse *et al.*, 1991). In general, there is a tendency for the rate of ion uptake per unit root length to decrease with distance from the root apex. However, this tendency strongly depends on the identity of the ion, plant nutritional status and plant species. When K or Ca are supplied to different regions of seminal roots of maize (Table 2.28), the uptake rate of K is slightly lower in the apical zone than the sub-apical zone, despite the high K requirement for growth. The high K concentration in root apical cells of about 200 mM (Huang and Van Steveninck, 1989a) is maintained not only by uptake from the external solution but also by delivery from more basal root zones (Table 2.28) or from the shoot via the phloem (Gould *et al.*, 2004). Similar observations have been made in other cereals (White *et al.*, 1987; Vallejo *et al.*, 2005) and also in non-mycorrhizal long roots of perennial plant species such as Norway spruce (Häussling *et al.*, 1988).

In contrast to K the uptake of Mg, and particularly of Ca, is higher in apical than in basal root zones (Marschner and Richter, 1973; Ferguson and Clarkson, 1976; Häussling *et al.*, 1988; White, 2001). This is also shown in Table 2.28. Because Ca mobility in the phloem is low, apical cells of the root must meet their Ca demand for growth by direct uptake from the external solution. Root apical zones also contribute considerably to Ca delivery to the shoot (Table 2.28; Clarkson, 1984; White, 2001). At the root tip, Ca may reach the xylem through an exclusively apoplasmic pathway or may be transported across

TABLE 2.29 Rate of P uptake by various root zones of barley plants after different pre-treatment

	P uptake rate (pmol mm^{-3} (24 h)$^{-1}$)		
	Root zone (distance from root tip, cm)		
Pretreatment for 9 days	1	2	3
With P	2,019	1,558	970
Without P	3,150	4,500	4,613

Based on Clarkson *et al.* (1987).

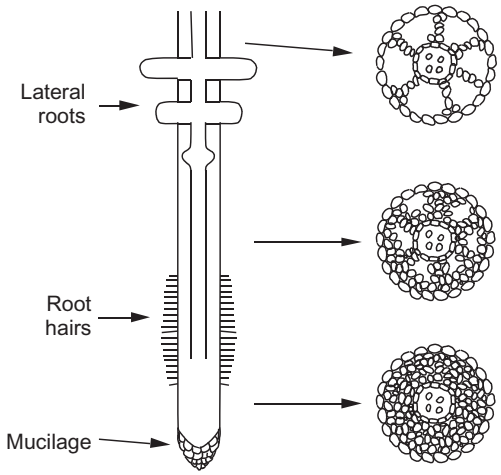

FIGURE 2.31 Schematic representation of anatomical changes along the axis of a maize nodal root. In basal zones there is degeneration of cortical cells and formation of tertiary endodermis.

the Casparian band through immature, unsuberized endodermal cells (White, 2001; Moore *et al.*, 2002). Calcium delivery to the xylem is also high in basal root zones, where lateral roots emerge from the pericycle, disrupting the integrity of the Casparian band (Clarkson, 1984; White, 2001). The apoplasmic pathway is also important for the movement of Na, Zn, Fe and Cd to the xylem (Yeo *et al.*, 1987; Taiz and Zeigler, 2006; Broadley *et al.*, 2007; Plett and Møller, 2010; Lux *et al.*, 2011). The delivery of these elements to the xylem is often greatest at the root tip.

The decline in P uptake along the root axis is much less striking than that for Ca (Ferguson and Clarkson, 1975; Clarkson *et al.*, 1978; Rubio *et al.*, 2004). In soil-grown maize this decline is mainly related to a decrease in root hair viability and, thus, in absorbing root surface area (Ernst *et al.*, 1989). The gradient in P uptake along the root axis also depends on the P nutritional status of the plant and may be reversed under deficiency in favour of the basal zones (Table 2.29). The situation is different under Fe deficiency in Strategy I plants where the apical, but not the basal, root zones increase their capacity for Fe uptake by a factor of up to 100 (Römheld and Marschner, 1981b). Apical, or immediately sub-apical, root zones generally contribute most to nitrate and ammonium uptake by intact plants irrespective of their nutritional status, although the magnitude of the decline in uptake with distance from the root apex depends greatly on root anatomy (Reidenbach and Horst, 1997; Colmer and Bloom, 1998; Sorgona *et al.*, 2010). Indeed, it should be noted that the uptake of most elements is restricted when the rhizodermis and cortex cells of basal (older) regions of the roots collapse and die.

Formation of cortical gas spaces (*aerenchyma*) particularly in more basal root zones can often be observed (Fig. 2.31). The formation of aerenchyma is a typical response to oxygen deficiency in the root zone in plant species adapted to wetland conditions (Section 16.4), but it can also be induced, for example, in maize roots under fully aerobic conditions by temporary deprivation of N or P supply (He *et al.*, 1992; Lynch, 2007). Despite these anatomical changes, the basal root zones still have a considerable capacity for ion uptake (Drew and Fourcy, 1986) and also for radial transport, indicating that the strands of cells bridging the cortex maintain sufficient ion transport capacity from the rhizodermis to the endodermis (Drew and Saker, 1986).

Water uptake can affect ion uptake both directly, through effects on the rate of radial transport of ions through the apoplasm, and indirectly, by influencing the supply of ions to the plasma membrane of root cells. Water uptake is usually low at the extreme root apex, but increases in the elongation zone and reaches a maximum in the root hair zone, where the endodermis is undergoing suberization (e.g., Sanderson, 1983; Boyer, 1985; Häussling *et al.*, 1988). Water uptake is often reduced strongly following suberization of the endodermis and, particularly, the exodermis. Water can reach the xylem through both the apoplast and via root cells (Steudle, 2000). Transport through root cells is facilitated by aquaporins (Javot and Maurel, 2002; Hachez *et al.*, 2006; Maurel *et al.*, 2008). Aquaporins are found in various membranes of root cells, including the plasma membrane and the tonoplast. Recent data, using mercury to inhibit the activity of aquaporins, suggest that rapid changes in root hydraulic conductivity in response to many stimuli, such as diurnal cycles, nutrient deficiency, salt stress, low temperatures, anoxia and drought, are the result of changes in cell membrane permeability achieved by regulation of aquaporin activity (Javot and Maurel, 2002; Maurel *et al.*, 2008). The abundance of aquaporins is often greatest in the elongation and mature root zones (Hachez *et al.*, 2006). In these root zones, strong expression of genes encoding aquaporins is observed in the endodermis and exodermis,

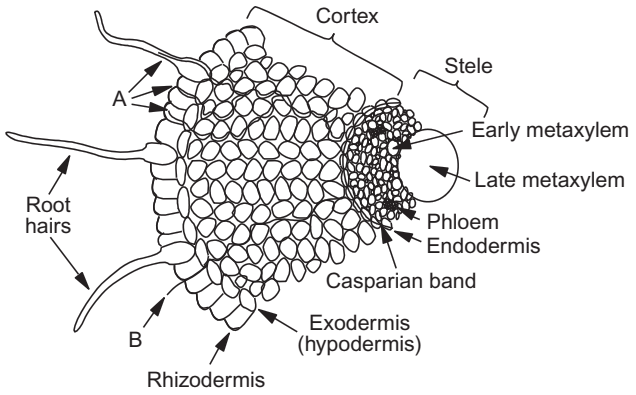

FIGURE 2.32 Segment of a transverse section of a maize root showing (A) symplasmic and (B) apoplasmic pathways of solute movement across the root.

presumably to allow water to bypass the Casparian band through a transcellular pathway (Hachez *et al.*, 2006)

2.7 RADIAL TRANSPORT OF IONS AND WATER ACROSS THE ROOT

There are two parallel pathways of movement of solutes and water across the cortex towards the stele: one passing through the apoplasm (cell walls and intercellular spaces) and another passing from cell to cell in the *symplasm* through the *plasmodesmata* (Fig. 2.32). In most of the root, the apoplasmic movement to the stele is restricted by the Casparian band in the walls of endodermal cells (White, 2001). This band is suberized and joins each endodermal cell (stage I endodermis). In the basal regions of the root, suberin lamellae cover the entire surface of endodermal cells (stage II endodermis). This prevents endodermal cells taking up solutes from the apoplasm (Moore *et al.*, 2002). Thick cellulose secondary walls are deposited over the suberin lamellae, which can be lignified (stage III endodermis). The nature and extent of these cell wall modifications are determined by both genetic and environmental factors.

In most angiosperms, another apoplasmic barrier, the exodermis, can develop in parallel with the endodermis (Ma and Peterson, 2003). The exodermis develops in the same three stages as the endodermis. Formation of an exodermis is found, for example, in *Zea mays*, *Allium cepa,* or *Helianthus annuus,* but not in *Vicia faba* or *Pisum sativum* (Enstone and Peterson, 1992). However, there are somewhat different views on the function of the exodermis as an effective barrier for transport of water and solutes in the apoplasm of the root cortex. Termination of the apoplasmic pathway at the exodermis, as suggested by Enstone and Peterson (1992), would confine the entry of solutes and water to the root symplast to the rhizodermal cells in basal root zones. Although rhizodermal cells, and in particular root hair cells, play a key role in the acquisition of mineral nutrients, especially K and P (Gahoonia and Nielsen, 2004; Gahoonia *et al.*, 2006; Jung *et al.*, 2009; Zhu *et al.*, 2010), the relative importance of the two pathways for solute transport across the root cortex is unknown. It will depend on: (i) the external concentration versus the capacity and affinity of the transport system for a particular solute at the plasma membrane of root cells; (ii) the root zone considered: depending on environmental conditions and the growth rate of the root, the exodermis can develop within a centimetre of the root apex or remain undeveloped (Ma and Peterson, 2003) and may possess 'passage cells' (Storey and Walker, 1987); and (iii) the hydraulic conductivity of the root zone considered and the transpiration rate of the shoot. For water, estimates of the contribution of the apoplasmic pathway to radial transport across roots vary between about 10 and 70% (Javot and Maurel, 2002; Hachez *et al.*, 2006; Maurel *et al.*, 2008).

The endodermis is also not a perfect barrier to the apoplasmic movement of water and solutes from the cortex to the stele (Fig. 2.32). In addition to the presence of passage cells in some plant species, this barrier may be 'leaky' at two sites along the root axis, at least. At the root apex, where the Casparian band is not yet fully developed, the apoplasmic movement of water and solutes to the stele can occur. However, the movement of some solutes, such as polyvalent cations like aluminium, through the apoplasm of the root apex can be restricted by *mucilage* formed at the external surface of the rhizodermal cells (Section 15.4). The apoplasmic pathway to the stele is also possible in basal root zones where the structural continuity of the endodermis is disrupted transiently by the emergence of lateral roots from the pericycle, as has been demonstrated, for example, for Ca (White, 2001), Al (Rasmussen, 1968) and water (Häussling *et al.*, 1988; Wang *et al.*, 1991). This 'bypass-flow' becomes particularly important for water supply to the shoot at high transpirational demand (Sanderson, 1983) and in the accumulation of Na in leaves under saline conditions (Yeo *et al.*, 1987; Plett and Møller, 2010). Both genetic and environmental factors influence the movement of water and solutes via the apoplasmic pathway through their effects on the development of the endodermis and exodermis. Accelerated deposition of suberin and lignin restricts the apoplasmic movement of cations and other solutes to the xylem (White, 2001; Enstone *et al.*, 2002; Krishnamurthy *et al.*, 2009; Lux *et al.*, 2011) and reduces hydraulic conductivity (Boyer, 1985; Cruz *et al.*, 1992).

The symplasmic pathway plays a key role in delivering most nutrients to the xylem, beginning either at the rhizodermis and the root hairs, at the exodermis, or at the endodermis. Radial transport in the symplasm requires movement through *plasmodesmata*, which connect neighbouring root cells (Fig. 2.33). Plasmodesmata have a

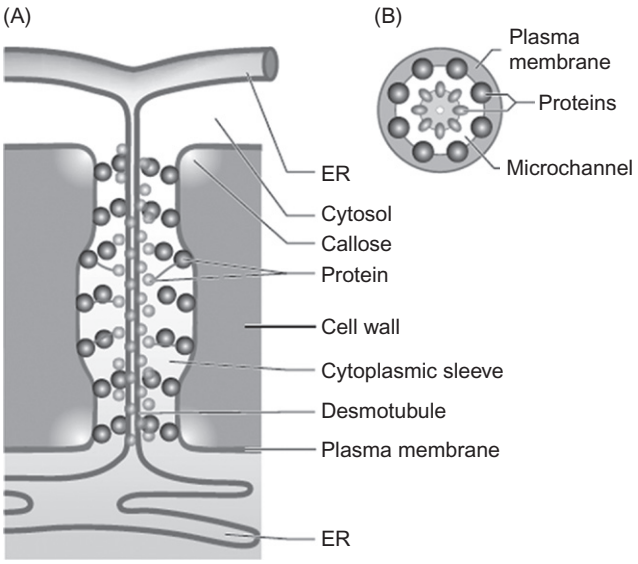

FIGURE 2.33 Schematic representation of plasmodesmata including substructural components. Solute fluxes between adjacent cells occur in the cytoplasmic sleeve, between the plasma membrane and the appressed endoplasmic reticulum (ER) forming the desmotubule. Partial control of solute fluxes by callose deposition in the cell wall. The cytoplasmic sleeve is interrupted by actin and other proteins that create microchannels through which solutes can diffuse. *Modified from Maule (2008)*.

TABLE 2.30 Intracellular K^+ activity and number of plasmodesmata in tangential walls of hair and hairless cells of the root epidermis

Plant species	Cell type	K^+ activity (mM)	Number of plasmodesmata	
			Per μm^2	Per cell junction
Trianea bogotensis	Hair	133	2.06	10,419
	Hairless	74	0.11	693
Raphanus sativus	Hair	129	0.16	273
	Hairless	124	0.07	150

From Vakhmistrov (1981).

complex structure (Lucas and Lee, 2004; Maule, 2008; Lucas *et al.*, 2009). The simplest type, which occurs in young tissues, comprises a tube of appressed endoplasmic reticulum (ER) running through the pore, the *desmotubule*. The transport of solutes and water between cells occurs in the 'cytoplasmic sleeve', i.e. the cytosol between the desmotubule and the plasma membrane (Fig. 2.33). Protein structures in the cytoplasmic sleeve create microchannels through which solutes can diffuse (Lucas and Lee, 2004; Maule, 2008; Lucas *et al.*, 2009). In more mature tissues, the structure becomes more complex through the addition of branches and the formation of central cavities (Maule, 2008; Lucas *et al.*, 2009). Plasmodesmata can be closed and opened by the production and degradation of a 'collar' of callose (β-1,3-glucan) and they generally have a size exclusion limit of about 1 kDa, which is regulated physically by the collar and also by interactions with cytosolic proteins (Lucas and Lee, 2004; Maule, 2008; Lucas *et al.*, 2009). Indeed, plasmodesmatal microchannels can dilate to allow the passage of solutes in excess of 20 kDa. The primary role of plasmodesmata appears to be cell communication, as they regulate the transport of transcription factors and microRNAs that control plant development and responses to biotic and environmental challenges (Lucas *et al.*, 2009). Addionally, the regulation of plasmodesmatal conductance represents another mechanism of cellular control of ion fluxes across the root.

High cytosolic Ca^{2+} concentrations induce closure of plasmodesmata (Tucker, 1990) and many environmental stimuli that increase cytosolic Ca^{2+} also disrupt the symplasmic movement of water and nutrients across the root. The number of plasmodesmata per cell varies considerably between plant species and cell type (Table 2.30). Rhizodermal cells that have developed into root hairs generally have more plasmodesmata than other rhizodermal cells. The relatively small number of plasmodesmata in *Rhaphanus* raises the question as to whether the root hairs are of major importance for symplasmic radial transport in this plant species. However, not only the number of plasmodesmata, but also whether they are functional must be taken into account. In the endodermis of young barley roots, on average 20,000 plasmodesmata per cell have been found (Helder and Boerma, 1969). In the tertiary (lignified) endodermis of older zones of barley roots, there are far fewer plasmodesmata, but the number appears to be sufficient to permit considerable radial transport of both water and ions through the endodermis (Clarkson *et al.*, 1971).

The mechanism of symplasmic transport of solutes seems to be chiefly by diffusion, facilitated by radial water flux and cytoplasmic streaming. During their radial transport through the symplasm, elements can be metabolized and/or sequestered in the vacuoles of root cells. When a nutrient is supplied to roots of a plant that is deficient in that nutrient ('low-salt' roots), it is accumulated in vacuoles of root cells resulting in an immediate accumulation in roots and a delay in its translocation from the roots to the shoots (Fig. 2.34). Thus, when the supply of a nutrient is suboptimal, the roots usually have higher tissue concentrations of that particular nutrient than the shoot. In long-term studies, this phenomenon is responsible, in part, for the often observed shift in the relative growth rates of roots and shoots in favour of the roots under nutrient deficiency.

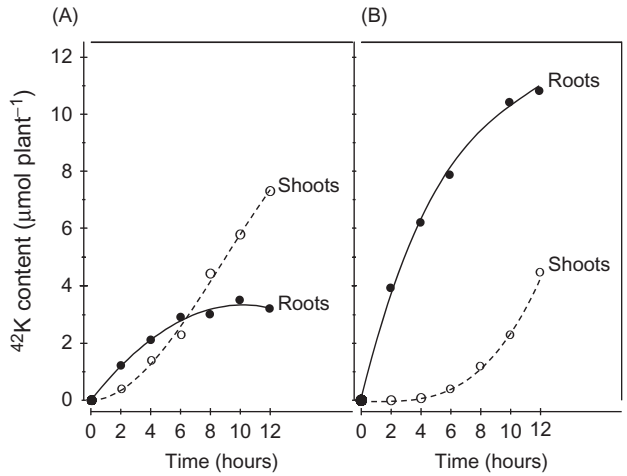

FIGURE 2.34 Accumulation and translocation rates of K^+ (^{42}K) in barley plants from a solution containing 1 mM KCl (+0.5 mM $CaSO_4$) after preculture with (A) or without (B) 1 mM KCl.

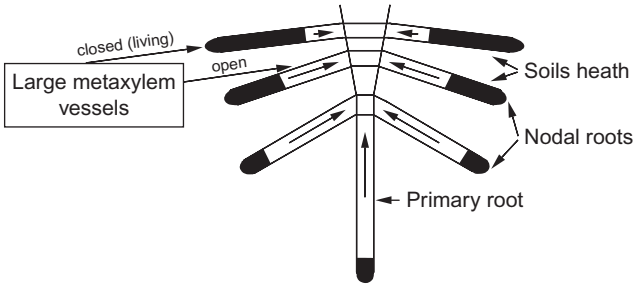

FIGURE 2.35 Model of root hydraulic conductivity and formation of a soil rhizosheath in the root system of maize. *Modified from Wenzel* et al. *(1989).*

The vacuoles of root cells also remove potentially toxic elements from the symplasmic pathway. For example, vacuolar sequestration of Na^+ in the root accounts for the restricted shoot transport of Na^+ in natrophobic plant species (Chapter 3). Preferential accumulation in roots also restricts the translocation of Ca, Mo, Cd and Al to the shoot (Conn and Gilliham, 2010). In contrast, in plants sufficiently supplied with P, symplasmic transport of P, and its translocation to the shoot, is greater than accumulation in root vacuoles (Lamaze *et al.*, 1987). The exchange rate between ions in the vacuoles of cortex cells and those in the symplasm depends on the ion species ($K^+ > Na^+$; $NO_3^- > SO_4^{2-}$), and the half-time for exchange is generally in the order of at least a few days.

The radial transport of water and solutes is strongly influenced by maturation of the xylem vessels along the root axis. For example, in graminaceous species such as maize growing in soil, two root zones can be observed: 'sheathed' zones which are covered by a layer of strongly adhering soil, the *rhizosheath* and 'bare' zones (Fig. 2.35). The development of the *rhizosheath* appears to be related to the presence of root hairs (Haling *et al.*, 2010). In the sheathed zones, the metaxylem vessels are still alive and non-conducting, whereas in the bare zones the metaxylem is mature (McCully and Canny, 1988). Accordingly, the hydraulic conductivity of bare roots is about 100 times greater than that of sheathed roots (Wenzel *et al.*, 1989). This difference in hydraulic conductivity and thus water uptake results in high water contents in the rhizosphere soil of the sheathed zones and low water contents in the rhizosphere soil of the bare zones. Living metaxylem vessels can be found up to 20–30 cm proximal to the root tip in maize (Wenzel *et al.*, 1989), and up to 17 cm proximal to the root tip in soybean (Kevekordes *et al.*, 1988). This delay in metaxylem maturation not only affects hydraulic conductivity of the roots and plant water relations (Wang *et al.*, 1991) but also the movement of solutes to the xylem and their translocation to the shoot.

2.8 RELEASE OF IONS INTO THE XYLEM

After radial transport through the symplasm to the stele, ions and organic solutes (amino acids, organic acids) are released into the xylem. This release (*xylem loading*) into fully differentiated, non-living xylem vessels occurs across the plasma membrane of xylem parenchyma cells. The membrane potential of these cells is slightly negative (Bowling, 1981) and the xylem sap has a pH between about 5.2 and 6.0 (Section 3.2). Solutes enter the xylem through ion channels or uniporters, if their electrochemical gradients allow this, or their transport is coupled to the proton electrochemical gradient generated by the plasma membrane H^+-ATPase or directly to ATP hydrolysis. The xylem parenchyma cells are also responsible for the reabsorption of solutes from the xylem sap by tissues along the pathway to the shoot.

The key role of the H^+-ATPases in the plasma membrane of parenchyma cells in xylem loading is now well established. Protons are pumped into the xylem both to generate a negative membrane potential and to acidify the xylem sap (De Boer and Volkov, 2003). The K^+ electrochemical gradient is sufficient for K to be loaded into the xylem by voltage-gated, outwardly rectified K-channels, such as the *At*SKOR K-channel of *Arabidopsis thaliana* (Gaymard *et al.*, 1998). Similarly, anion channels can facilitate the movement of nitrate, sulphate, phosphate and chloride from the symplasm to the xylem in the direction of their electrochemical gradients (White and Broadley, 2001; Köhler *et al.*, 2002; Gilliham and Tester, 2005). In addition, nitrate can be loaded into the xylem by members of the NRT1 (nitrate transporter 1) family (Lin *et al.*, 2008). Cations present at low concentrations in the root symplasm are loaded into the xylem by active transport

TABLE 2.31 Root uptake and translocation to the shoot of phosphate and sulphate in the wildtype and pho1 mutant of *Arabidopsis thaliana*

	Phosphate[a]		Sulphate	
Genotype	Root uptake (nmol $g^{-1} h^{-1}$)	Translocation to shoot (%)	Root uptake (nmol $g^{-1} h^{-1}$)	Translocation to shoot (%)
Wildtype	1,593	35	291	25
Mutant	1,559	0.9	367	12

Poirier *et al.* (1991).
[a]*Supply of 8 µM P_i.*

mechanisms. Members of the P_{2A} and P_{2B} Ca^{2+}-ATPase families load Ca into the xylem and members of the heavy metal P_{1B}-ATPase family load Zn and Cu into the xylem (White and Broadley, 2003, 2009). It is thought that Mg and Mn are also loaded into the xylem by ATPases, although the genes encoding these transporters are not yet known. Boron is loaded into the xylem by orthologues of the Arabidopsis *At*BOR1 transporter (Miwa and Fujiwara, 2010). The regulation of xylem loading separately from solute uptake offers additional possibilities to control the selectivity and rate of long-distance transport to the shoot, for example in response to shoot demand.

Separate genetic control of solute uptake and xylem loading from that of root cortex cells is in agreement with the observation that selective inhibitors of protein synthesis strongly impair xylem loading of nutrients, such as K, without affecting their accumulation in the roots (Läuchli and Pflüger, 1978; Morgan *et al.*, 1985) and that diurnal fluctuations in nutrient uptake by the roots and their delivery to the xylem do not coincide (Herdel *et al.*, 2001). Another example of the separate genetic control of solute uptake and xylem loading is shown in Table 2.31. Compared with the wildtype, the *pho1* mutant of *Arabidopsis thaliana* requires very high external P supply for normal growth. The recessive gene (*pho1*) regulates the loading of P into the xylem (Poirier *et al*., 1991). At low P supply, the mutant becomes severely P deficient because of impaired translocation of P to the shoot, although P uptake by the roots does not differ from the wildtype plant (Table 2.31). Sulfate translocation to the shoot is similar in the mutant and the wildtype plant. Evidence for tight regulation of P loading into the xylem is also shown by the inability of maize plants to meet the P demand of the shoot at low root zone temperatures (Engels and Marschner, 1992a).

The discovery of the abundance of living metaxylem vessels in more than half of the total root length in mature maize plants (Wenzel *et al.*, 1989) renewed the view of leakage as a mechanism of ion release into the xylem (McCully *et al.*, 1987). The concentrations, of K^+, for example, in the vacuoles of living metaxylem vessels are up to 400 mM. Upon maturation of the metaxylem vessels, the accumulated K^+, together with the other solutes in the vacuoles, is released into the transpiration stream. According to McCully and Canny (1988), this leakage from maturing xylem vessels could account for about 10% of the shoot demand of growing maize plants. Thus, a significant proportion of the solutes present in the xylem sap (including proteins) may derive not from active xylem loading but from maturing xylem vessels.

2.9 FACTORS AFFECTING ION RELEASE INTO THE XYLEM AND EXUDATION RATE

The permeability of plant membranes to water is higher than that to ions. Plant cells or roots therefore behave as osmometers. Ion release into the apoplasm of the stele decreases both the osmotic potential and the water potential (they become more negative) in the stele, and a corresponding net flux of water from the external solution is induced. As a result of this water flux, the hydrostatic pressure increases. As the endodermis with its Casparian band 'seals' the apoplasm of the stele, the hydrostatic pressure in the stele induces a volume flow of water and solutes in the non-living xylem vessels towards the shoot. Because of this 'root pressure' droplets are sometimes released on the tips and margins of leaves, a process known as *guttation.* This is particularly apparent in seedlings and young plants at night and in the early morning (under conditions of high relative air humidity and low transpiration). Exudation from the stumps of cut plants (e.g., freshly mown grass) is also the result of root pressure. Root pressure and the corresponding volume flow in the xylem are of particular importance for the long-distance translocation of Ca into low-transpiring organs such as fruits. Volume flow and composition of the xylem exudate can provide important information on the influence of external and internal factors on root activity and metabolism, nutrient uptake and assimilation in the roots, release into the xylem and the cycling of nutrients and organic solutes in plants.

TABLE 2.32 Relationship between external concentration, exudate concentration, and exudate volume flow in decapitated sunflower plants

External solution KNO_3 + $CaCl_2$ (m*M* each)	Exudate (mM)			Concentration factor			Exudation volume flow (mL (4 h)$^{-1}$)
	K^+	Ca^{2+}	NO_3^-	K^+	Ca^{2+}	NO_3^-	
0.1	7.3	2.8	7.4	73	28	74	4.0
1.0	10.0	3.2	10.7	10	3.2	10.1	4.5
10.0	16.6	4.2	10.3	1.7	0.4	1.0	1.6

TABLE 2.33 Exudate volume flow and K and Ca concentrations in the exudate of decapitated maize plants at different temperatures

Temperature (°C)	Exudate volume flow (mL h^{-1})	Exudation concentration (mM)		Ratio K^+/Ca^{2+}
		K^+	Ca^{2+}	
8	1.32	13.4	1.5	8.9
18	5.48	15.2	1.0	15.2
28	7.93	19.6	0.8	24.5

Marschner (1995).
[a] *Concentration of KNO_3 and $CaCl_2$ in the external solution: 1 mM each.*

For technical reasons, it is difficult to measure ion release into the xylem directly. Although secretions from xylem-feeding insects, such as the meadow spittlebug (*Philaenus spumarius*), can be obtained from intact plants (e.g., Watson *et al.*, 2001; Malone *et al.*, 2002; Teakle *et al.*, 2007), most experimental evidence on xylem loading comes from studies of xylem exudate, or xylem sap, obtained from isolated roots or decapitated plants (Section 3.2). Because of reabsorption along the xylem pathway (Section 3.2), and the contribution of solutes from maturing metaxylem vessels, the concentration of ions at the sites of collection can differ from that at the sites of loading into the non-living xylem vessels. When interpreting analyses of xylem exudate it should be kept in mind that (i) at least two separately regulated membrane transport processes are involved in symplasmic radial transport of nutrients from the external solution into the xylem (i.e., influx to the symplasm and xylem loading), (ii) an apoplasmic pathway can contribute to the delivery of water and solutes to the xylem, and (iii) xylem sap volume flow is affected by root hydraulic conductivity and rate of transpiration.

2.9.1 External and Internal Factors Affecting the Composition of Xylem Sap

As a rule, an increase in the external ion concentration leads to an increase in the concentration of ions in the xylem exudate. However, the relative concentration difference decreases as the external concentration is increased (Table 2.32). Thus, the concentration gradient ('concentration factor') between the external solution and the xylem exudate decreases, and can even fall below 1 in the case of Ca, i.e. the concentration of Ca in the xylem exudates is lower than that in the external solution. The volume flow of xylem exudation shows a somewhat different pattern, and is maximal at an external concentration of 1.0 mM in the experiment reported in Table 2.32. At 0.1 mM, this flow is limited by the ion concentration in the xylem. In contrast, at 10.0 mM, the flow is limited by water availability (i.e., the low water potential in the external solution) and the small concentration gradient between the external solution and the xylem. The increase in the concentration of nutrients in the xylem exudate with increasing external concentration from 1.0 to 10.0 mM does not compensate for the decrease in the exudation volume flow. Thus, in contrast to their accumulation in roots, which generally follows a hyperbolic relationship with the external concentration, the rate of root pressure-driven translocation of nutrients to the shoot can decline at high external solute concentrations due to limited water uptake.

An increase in the root zone temperature often has a greater effect on the exudation volume flow than on the ion concentrations in the exudate (Table 2.33). This is consistent with the expectation that a root behaves as an

TABLE 2.34 Exudation volume flow and ion concentration in the exudate of decapitated maize plants with (O_2 treatment) or without root respiration (N_2 treatment)

Treatment[a,b]	Exudation volume flow (ml h^{-1})	Exudate concentration (mM)	
		K^+	Ca^{2+}
O_2	8.83	16.6	1.8
N_2	1.90	15.2	1.7

Marschner (1995).
[a]*Concentration of KNO_3 and $CaCl_2$ in the external solution: 0.5 mM each.*
[b]*Respiration treatment consisted of bubbling oxygen or nitrogen through the external (nutrient) solution.*

TABLE 2.35 Flow rate and ion concentration in the xylem exudate of wheat seedlings[a]

Parameter	Treatment	
	KNO_3	K_2SO_4
Exudation flow rate (µl h^{-1} 50 plants^{-1})	372	180
Ion concentration (mM)		
Potassium	23.3	24.5
Calcium	9.1	9.5
Nitrate	18.1	0.0
Sulphate	0.2	0.8
Organic acids	9.6	25.8

From Triplett *et al.* (1980).
[a]*Seedlings were supplied with either KNO_3 (1 mM) or K_2SO_4 (0.5 mM) in the presence of 0.2 mM $CaSO_4$.*

osmometer. Temperature has a marked effect on the rate of solute uptake, transport through the symplasm and release into the xylem, and water moves accordingly along the water potential gradient. Since different transport proteins facilitate the movement of each nutrient across the plasma membrane of root cells, and the relative contributions of symplasmic and apoplasmic transport differ between nutrients, temperature can have differential effects on the delivery of each element to the xylem. For example, an increase in the root temperature results in an increase in K concentration but in a decrease in Ca concentration of the exudate. This shift in the K/Ca ratio may reflect temperature effects either on membrane selectivity or on the relative importance of the apoplasmic pathway of radial transport of Ca and water (Engels *et al.*, 1992). Similar shifts in the K/Ca transport ratio are also observed at different soil temperatures (Walker, 1969). This temperature effect may have important implications for the Ca nutrition of plants.

The rate of xylem loading is closely related to root respiration (Table 2.34). A lack of oxygen strongly reduces exudation volume flow but not the concentrations of K and Ca in the exudate. Oxygen deficiency seems to affect the release of ions into the xylem and root hydraulic conductivity to the same degree.

The cation–anion balance in the xylem exudate needs to be maintained. Thus, the accompanying ion can affect xylem exudation flow and sap composition even at low rhizosphere concentrations (Table 2.35). When KNO_3 is supplied, the exudation flow rate is almost twice as high as the flow rate when an equivalent concentration of K_2SO_4 is added. Since the K concentration in the exudate is the same in both treatments, the translocation rate of K supplied as K_2SO_4 is only about half the rate of K supplied as KNO_3.

In contrast to the K concentration, the concentrations of nitrate and sulphate in the exudate exhibit large differences between the treatments (Table 2.35). When plants are supplied sulphate rather than nitrate, the difference in negative charge in the exudate is approximately compensated for by elevated concentrations of organic acid anions. However, the capacity of the roots to maintain the cation–anion balance by organic acid synthesis in the K_2SO_4 treatment appears to be limited, which leads to a decrease in the rate of K and Ca release into the xylem and a corresponding decrease in exudation flow rate when compared to the KNO_3 treatment.

Because of the energy demand for ion transport processes, release of ions into the xylem and the corresponding changes in root pressure are also closely related to the carbohydrate status of the roots (Table 2.36). Variation in the length of the photoperiod one day before decapitation affected the carbohydrate status of roots and, correspondingly, the rate and duration of exudation volume flow after decapitation. Both the uptake and transport rate of K in roots with high carbohydrate content are greater than in roots with low carbohydrate content. The higher transport rate is closely related to the exudation volume flow. In roots with low carbohydrate content, reserves are rapidly depleted after decapitation and there is a corresponding decline in the rate of exudation volume flow within 8 h. This depletion of carbohydrates in the roots of decapitated plants and the consequent decline in xylem exudation flow is one of the factors restricting studies of xylem loading.

Solute fluxes into the xylem and exudation volume flow also exhibit endogenously regulated diurnal fluctuations. These are maintained in plants transferred to continuous darkness (Ferrario *et al.*, 1992a, b) but disappear in plants maintained under continuous light (Herdel *et al.*, 2001). These phenomena are related not only to the carbohydrate status of the roots but also to plant nutritional status.

TABLE 2.36 Relationship between photoperiod, carbohydrate content of roots, and uptake and translocation of K in decapitated maize plants[a]

	Photoperiod (h)	
	12/12[b]	24/0
Carbohydrate in roots (mg)	122 (48)	328 (226)[c]
Total potassium uptake (mmol)	1.3	5.0
Potassium translocation in exudation volume flow (mmol)	1.0	3.5
Exudation volume flow (mL $8\,h^{-1}$)	30.3	88.5
Relative decline in flow rate within 8 h (%)	60	12

Marschner (1995).
[a]*Data per 12 plants.*
[b]*Hours of light/hours of darkness. This pretreatment with different day lengths was for one day (i.e., the day prior to decapitation).*
[c]*Numbers in parentheses denote carbohydrate content after 8 h (decapitation).*

TABLE 2.37 Role of shoot demand on net uptake, net translocation and flux of K in the xylem exudate of maize

	K flux (μmol g^{-1} root fw h^{-1})		
Shoot demand[a]	Net uptake 0–3 days	Net translocation 0–3 days	Xylem exudates day 3
High	2.26	1.83	8.55
Low	2.28	1.17	2.46

Engels and Marschner (1992b).
[a]*Shoot demand altered by the shoot base temperature, see Table 2.12.*

2.9.2 Xylem Exudate, Root Assimilation and Cycling of Nutrients

Analyses of xylem exudates provide valuable information about assimilation of nutrients in the roots, for example the capacity of roots for nitrate reduction or N_2 fixation. In soybean and other tropical legumes, the proportion of ureides (Chapter 16) to total N in the xylem exudate reflects nodule activity and is also a suitable indicator in field-grown plants of the relative contribution of N_2 fixation to the N nutrition of legumes (Peoples *et al.*, 1989). In non-legumes, the forms of N in xylem exudate can provide information on its assimilation by roots and the relative importance of various organic and inorganic fractions in its long-distance translocation in the xylem (Van Beusichem *et al.*, 1988). Similarly, studies of the chelation of heavy metals in xylem sap have increased our understanding of their translocation within the plant, and the transport proteins involved (Section 3.2). Analyses of xylem exudate can also inform about the metabolism and translocation of hormonal signals from roots to shoots (Chapters 3 and 5). The discovery of unexpectedly high concentrations of sugars in xylem exudates of annual species provided insight to their carbon economy (Cataldo *et al.*, 1988; Canny and McCully, 1989).

There are, however, several factors that need to be considered in the interpretation of such analyses. Xylem sap collected from decapitated plants represents only the root pressure component of xylem volume flow. For evaluation of the transpirational component, xylem sap should be collected from intact plants *in situ*. This can be achieved using xylem-feeding insects (e.g., Watson *et al.*, 2001; Malone *et al.*, 2002; Teakle *et al.*, 2007), but this technique is rarely applied. Alternatively, xylem volume flow can be increased artificially by increasing the external pressure in the root zone (pressure chamber) or by collecting exudates from the cut stump under a vacuum. Using either method, xylem volume flow is increased and, hence, concentrations of nutrients are usually decreased. The estimated translocation rates to the shoot can differ between these methods and can also be quite different from the results using intact plants (Salim and Pitman, 1984; Allen *et al.*, 1988; White, 1997b). Furthermore, irrespective of the collection method, the xylem sap also contains shoot-derived nutrients recycled in the phloem and reloaded into the xylem in the roots (Section 3.4). The recirculation of water may also have to be considered in interpreting analyses of xylem sap (Tanner and Beevers, 1990). The fractions of recycled nutrients can be particularly high in the cases of K, P, N and S (Section 3.4), and may lead to misinterpretation of, for example, the capacity of roots to reduce nitrate and sulphate.

The proportion of recycled nutrients in the xylem sap depends on various factors, such as plant species and nutritional status in general and shoot demand in particular, as shown for K in Table 2.37. When shoot demand is high, K translocation in the xylem exudate increases greatly and net translocation is also higher, but net uptake is unaffected. Accordingly, root K concentration is lower in the plants with high shoot demand. The differences between net translocation and xylem transport of K reflect differences in the recirculation of K which, against expectation, was higher in plants with high shoot demand. Recycling of K to the roots in plants with high shoot demand may be explained by the role of K in xylem transport of nitrate (Section 3.4). This is an example of the important insight xylem sap analyses can provide when combined with other measurements, such as rates of net uptake and accumulation in plant tissues or the analysis of phloem sap, to the regulation of xylem loading and the recycling of nutrients within the plant.

Chapter 3

Long-distance Transport in the Xylem and Phloem

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SUMMARY

Long-distance transport of solutes in the xylem and phloem is important for shoot nutrition, the redistribution of essential elements between tissues during ontogeny, the maintenance of charge balance in leaves of nitrate-fed plants, the removal of potentially toxic elements from leaf tissues, and the systemic signalling of plant nutritional status. This chapter describes the anatomy of the xylem and phloem, the composition of xylem and phloem saps, and the movement of xylem sap from root to shoot in response to gradients of water potential generated by root pressure and transpiration and of phloem sap from source to sink tissues in response to osmotic gradients generated by differences in phloem sucrose concentration. Emphasis is placed on the pathways of solute movement within the plant and recent insight into the transport proteins catalysing the loading and unloading of elements to and from the xylem and phloem.

3.1 GENERAL

The long-distance transport of water and solutes – elements and low-molecular-weight organic compounds – takes place in the vascular system of xylem and phloem. Long-distance transport from the roots to the shoots occurs predominantly in the non-living xylem vessels. Coniferous trees lack a continuous system of xylem vessels and depend on tracheides, which are non-living conducting cells ranging in length from 2 to 6 mm (Tyree and Ewers, 1991). In annual plant species, long-distance transport in the xylem vessels may also be interrupted by tracheides, for example at the root–shoot junction (Aloni and Griffith, 1991) or in the nodes of the stem. These structures pose an internal resistance to xylem volume flow but simultaneously permit an intensive xylem–phloem solute transfer.

Xylem transport is driven by the gradient in hydrostatic pressure (root pressure) and by the gradient in water potential. Pure free water is defined as having a water potential of zero. Accordingly, values for water potential are usually negative. The gradient in water potential between roots and shoots is quite steep particularly during the day when the stomata are open. Values become less negative in the following sequence: atmosphere $>>$ leaf cells $>$ xylem sap $>$ root cells $>$ external solution. Solute flow in the xylem from the roots to the shoots is therefore unidirectional (Fig. 3.1). However, under certain conditions in the shoots a counter-flow of water in the xylem may also occur, for example, from low-transpiring fruits back to the leaves (Lang and Thorpe, 1989)

In contrast to the xylem, long-distance transport in the phloem takes place in the living sieve tube cells and is

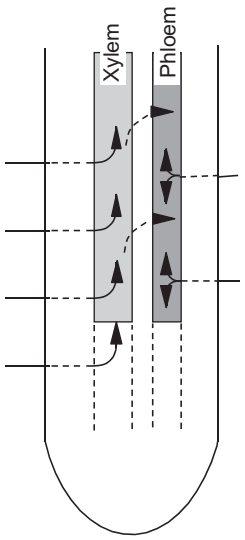

FIGURE 3.1 Direction of long-distance transport of elements in the non-living xylem vessels and in the phloem of roots.

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bidirectional. The direction of transport is determined primarily by the nutritional requirements of the various plant organs or tissues and occurs, therefore, from source to sink (Chapter 5). In addition, phloem transport is an important component in cycling of nutrients between shoots and roots and for signalling the nutritional status of the shoots to the roots. Elements can enter the phloem in either the shoot or the root. The translocation of different elements taken up by a particular zone of the root varies markedly as shown in Table 3.1 for maize seedlings. Long-distance transport from the zone of supply to the root tip must take place in the phloem. Whereas ^{45}Ca is rapidly translocated to the shoot, it is not transported to the root tip at all. In contrast, the translocation of ^{22}Na toward the shoot is severely restricted. The steep gradient in ^{22}Na content of the root sections in the direction of the shoot (basipetal) reflects retrieval by the surrounding root tissue and is a typical feature of so-called natrophobic plant species (Chapter 10). Some ^{22}Na is also translocated via the phloem to the root tip. In contrast, ^{42}K is quite mobile both in the xylem and in the phloem, and a high proportion of the K taken up in more basal root zones is translocated via the phloem toward the root tip, which acts as a sink for this nutrient.

TABLE 3.1 Accumulation and long-distance transport of ^{45}Ca, ^{22}Na, and ^{42}K in maize seedlings[a]

Plant part	Content (μmol (12 plants)$^{-1}$ 24 h^{-1})		
	^{45}Ca	^{22}Na	^{42}K
Shoot	2.20	0.01	9.07
Endosperm	0.18	0.04	2.38
Root (cm from tip)			
24–27	0.01	0.06	0.35
21–24	0.01	0.09	0.85
18–21	0.01	0.18	1.30
15–18	0.01	0.46	1.58
12–15 (zone of supply)	0.40	1.28	1.93
9–12	0	0.03	0.40
6–9	0	0.02	0.38
3–6	0	0.02	0.45
0–3	0	0.01	0.75
Total	2.82	2.20	19.44

Based on Marschner and Richter (1973).
[a]*Each seedling was supplied with 1 mM of labelled nutrient solution to the root zone 12–15 cm from the root tip. The remainder of the root system was supplied with the same solution in which the nutrients were not labelled.*

During long-distance transport, elements and organic solutes are transferred between the xylem and phloem by extensive exchange processes. Despite this interchange and internal cycling, nutrients, such as P, supplied to only one part of the root system (lateral or seminal roots) are transported preferentially to those parts of the shoots that have direct vascular connections with particular root zones (Stryker *et al.*, 1974). This distribution pattern is particularly important for the nutrition of trees that are supplied with fertilizer in a localized area of the root system.

3.2 XYLEM TRANSPORT

3.2.1 Composition of the Xylem Sap

The composition of xylem sap and concentrations of elements and organic solutes in the xylem sap depend on factors such as plant species, element supply to the roots, assimilation of nutrients in the roots and nutrient recycling. The concentrations of solutes are also strongly influenced by dilution by water (Section 2.9) and are therefore dependent on the transpiration rate and the time of the day. The composition and concentration of xylem sap also changes during plant ontogenesis (Table 3.2). In soybean during the reproductive stage, xylem sap volume flow declines and the concentrations of some nutrients in the sap decrease while those of others increase. The decline

TABLE 3.2 Xylem volume flow (pressurized exudation at 100 kPa) and nutrient concentrations in the xylem sap of soil-grown nodulated soybean during the reproductive stage

Parameter	Plant developmental stages			
	Full pod extension	Early-mid podfill	Late podfill	Early leaf yellowing
Sap volume (mL plant^{-1} 50 min^{-1})	1.43	1.13	0.94	0.43
Nutrient concentration				
K (mM)	6.1	5.0	4.0	2.4
Mg (mM)	3.8	2.6	1.9	1.2
Ca (mM)	4.8	3.9	3.9	2.2
P (mM)	2.5	1.6	0.9	0.4
S (mM)	1.8	1.6	2.1	1.5
B (mM)	1.0	1.5	1.6	3.2
Zn (μM)	23.0	29.0	32.0	42.0
Cu (μM)	2.7	3.6	2.8	6.9

Based on Noodén and Mauk (1987).

in nutrient concentrations can be prevented by removal of the pods. This eases the competition for photosynthates between pods and roots; thus, leading to higher uptake and xylem loading of nutrients (Noodén and Mauk, 1987).

In perennial species in temperate climates, the composition of the xylem sap changes with season, not only in organic solutes (e.g., remobilized in spring), but also in nitrate concentrations and pH (Glavac *et al.*, 1991). Polyvalent heavy metal cations in the xylem sap exist mainly in organic form complexed with organic acids, amino acids and peptides (White *et al.*, 1981a, b). Both the number and abundance of these complexes vary with plant age in annual species (Cataldo *et al.*, 1988).

The proportion of the various N fractions in the xylem sap depend on the form of N supply (NO_3^-; NH_4^+; N_2 fixation), the predominant site of nitrate reduction (roots or shoots) and the proportion of recycled N. Except at very high external NH_4^+ supply, the concentration of NH_4^+ in the xylem is low (Van Beusichem *et al.*, 1988), often being in the range of 1 mM, irrespective of whether N is supplied as NH_4^+ or NO_3^- (Engels and Marschner, 1993). The concentration of organic acids in the xylem sap depends primarily on the root cation–anion uptake ratio and the form of N supply (Arnozis and Findenegg, 1986). In the xylem sap of annual species, high concentrations of sugars may also occur. For example concentrations up to 5 mM may occur in maize (Canny and McCully, 1989), and sugars may account for about 15% of the total organic carbon in the sap of soybean (Cataldo *et al.*, 1988). Phytohormones are a normal constituent of xylem sap, particularly cytokinins which are mainly synthesized in the roots (Section 5.9). The concentration of abscisic acid (ABA) in the xylem sap has attracted interest as a possible non-hydraulic chemical signal to the shoot of root water status and also on the strength of the soil (Hussain *et al.*, 1999; Wilkinson and Davies, 2002). As the soil dries, stomatal conductance decreases prior to decrease in leaf turgor, and inverse relationships have been shown to occur between stomatal conductance and xylem sap ABA concentrations (Wilkinson and Davies, 2002). Under field conditions, for example in maize, stomatal conductance has been found to be closely related to the ABA concentration of the xylem sap, but not the current leaf water status nor ABA concentrations in the bulk leaf (Tardieu *et al.*, 1992). There is some evidence that high concentrations of ABA, or of 'inhibitors' other than ABA (Munns, 1992), in xylem sap reduce rates of cell extension and cell division and, thereby, reduce leaf elongation rate in response to drying or compacted soil (Tardieu *et al.*, 2010). As the soil dries, the ionic composition and pH of the xylem sap increase (Bahrun *et al.*, 2002), and this may also alter the distribution of ABA in the shoot and lead to preferential transport of ABA to the guard cells (Jiang and Hartung, 2008; Section 5.9).

The concentration of ABA in the xylem sap is also affected by N, P and K nutrition (Jeschke *et al.*, 1997b; Peuke *et al.*, 1994, 2002; Jiang and Hartung, 2008); the consequences of this for plant water relations and leaf growth are discussed in Chapter 6. The root-derived hormonal signals in the xylem sap can also affect long-distance transport of nutrients, for example via the volume flow rate in the xylem, the rate of xylem–phloem transfer, and the nutrient distribution within the shoot.

3.2.2 Xylem Loading and Unloading

Elements that traverse the root via the symplasmic pathway are loaded into the xylem by various transport proteins across the plasma membrane of xylem parenchyma cells. Evidence suggests that nitrate can be loaded into the xylem by members of the NRT1 (nitrate transporter 1) family (Lin *et al.*, 2008). In addition, anion channels can facilitate the movement of nitrate, sulphate, phosphate and chloride in the direction of their electrochemical gradients from the cytosol of xylem parenchyma cells to the xylem vessels (White and Broadley, 2001; Köhler *et al.*, 2002; Gilliham and Tester, 2005). Boron is loaded into the xylem by orthologues of the Arabidopsis AtBOR1 transporter, whose activities are regulated in response to plant B status to ensure appropriate B concentrations are maintained in the shoot (Miwa and Fujiwara, 2010).

Potassium is loaded into the xylem by voltage-gated, outwardly rectified K-channels present in the plasma membrane of root pericycle and stelar parenchyma cells, such as orthologues of the AtSKOR protein of *Arabidopsis thaliana* (Gaymard *et al.*, 1998). Cations that are present in low concentrations in the cytosol of root cells are loaded into the xylem by active transport mechanisms. Calcium is loaded into the xylem by members of the P_{2A}-Ca^{2+}-ATPase and P_{2B}-Ca^{2+}-ATPase families and members of the heavy metal P_{1B}-ATPase family load Zn^{2+} and Cu^{2+} into the xylem (White and Broadley, 2003, 2009). Similarly, it is thought that Mg^{2+} and Mn^{2+} are loaded into the xylem by ATPases, although the genes encoding these transporters are unknown. Cation carriers have also been implicated in loading Zn^{2+} and Fe^{2+} into the xylem (Song *et al.*, 2010).

A significant amount of Ca and other potentially cytotoxic elements, such as Zn, Fe and Na, can also reach the xylem through an apoplasmic route when they are present at high concentrations in the soil solution (White, 2001; Broadley *et al.*, 2007; Plett and Møller, 2010).

Nitrogen is mostly present in the xylem in its inorganic forms, although amino acids and amides have also been observed (Peuke, 2010). Similarly, phosphate and sulphate are the dominant forms of P and S in the xylem. Calcium, Mg, Mn and Zn are likely to be transported in the xylem as cations or cation complexes with organic acids (Welch, 1995; White and Broadley, 2003, 2009). Iron

is transported mainly as Fe^{3+} citrate (Welch, 1995; von Wirén *et al*., 1999). In *Arabidopsis thaliana*, a member of the multidrug and toxin efflux (MATE) transporter family, AtFRD3, is expressed in the root pericycle and appears to be involved in loading citrate into the xylem (Puig *et al*., 2007; Guerinot, 2010). Zinc can also be transported as a histidine complex, and Zn, Cu, Mn and Ni can be transported as nicotianamine (NA) complexes (Welch, 1995; von Wirén *et al*., 1999; Broadley *et al*., 2007; Curie *et al.*, 2009). During transport from the roots to the leaves in the non-living xylem vessels, important interactions take place between solutes and both the cell walls of the vessels and the surrounding xylem parenchyma cells. The major interactions are exchange adsorption of polyvalent cations in the cell walls, and retrieval (uptake) and release of elements and of organic solutes by surrounding living cells (xylem parenchyma and phloem).

3.2.2.1 Exchange Adsorption and Xylem Retrieval

The interactions between cations and the negatively charged groups in the cell walls of the xylem vessels (and tracheides) are similar to those in the AFS of the root cortex (Fig. 2.2). The long-distance transport of cations in the xylem can be compared with ion movement in a cation exchanger, i.e. it exhibits lower translocation rates of cations such as Ca^{2+} (Jacoby, 1967) and Cd^{2+} (Senden and Wolterbeek, 1990) compared to water (Thomas, 1967) or anions such as phosphate (Ferguson and Bollard, 1976). This cation-exchange adsorption is not restricted to the xylem vessels; the cell walls of the surrounding tissue also take part in these exchange reactions (Wolterbeek *et al.*, 1984).

The degree of retardation of cation translocation depends on (i) the valency of the cation ($Ca^{2+} > K^+$), (ii) its concentration and activity (McGrath and Robson, 1984), (iii) the charge density of the negative groups (dicotyledons/monocotyledons), (iv) the diameter of the xylem vessels, and (v) the pH of the xylem sap, which may vary between 5 and 7. The translocation rate of heavy metal cations in the xylem is enhanced when the ions are complexed, e.g. Cu (Smeulders and van de Geijn, 1983), Zn (McGrath and Robson, 1984) or Cd (Senden and Wolterbeek, 1990).

Solutes can also be retrieved ('scavenged' or 'unloaded', see below) from the xylem into the living cells along the pathway of the xylem sap from the roots to the leaves. For example, sulphate can be retrieved from the xylem through proteins of the SULTR2 (sulphate transporter 2) and SULTR3 families (Takahashi *et al.*, 2000; Kataoka *et al.*, 2004), and phosphate can be retrieved from the xylem through proteins of the Pht1 (phosphate transporter 1) family (Mudge *et al.*, 2002), which are all present in the plasma membrane of cells within the stele. The retrieved elements can be stored transiently or permanently in the xylem parenchyma and other stem tissue, or transferred from xylem to phloem via specialized cells.

In some plant species, the retrieval of certain elements from the xylem sap is very pronounced and can have important consequences for the nutrition of these plants. This is most evident in natrophobic plant species (Section 10.2). In these plant species (e.g., *Phaseolus vulgaris*), Na^+ is retained mainly in the roots and lower stem, whereas in natrophilic species (e.g., sugar beet), Na^+ is readily translocated into the leaves (Fig. 3.2). The restricted upward Na^+ movement in natrophobic plants, is caused by selective Na^+ accumulation in the xylem parenchyma cells (Rains, 1969; Drew and Läuchli, 1987; Blom-Zandstra *et al.*, 1998) together with re-translocation to the roots (Fig. 3.10). In castor bean, these two components led to a decrease in the Na^+ concentration of the upward moving xylem stream from 0.8 to 0.2 mM (Jeschke and Pate, 1991b).

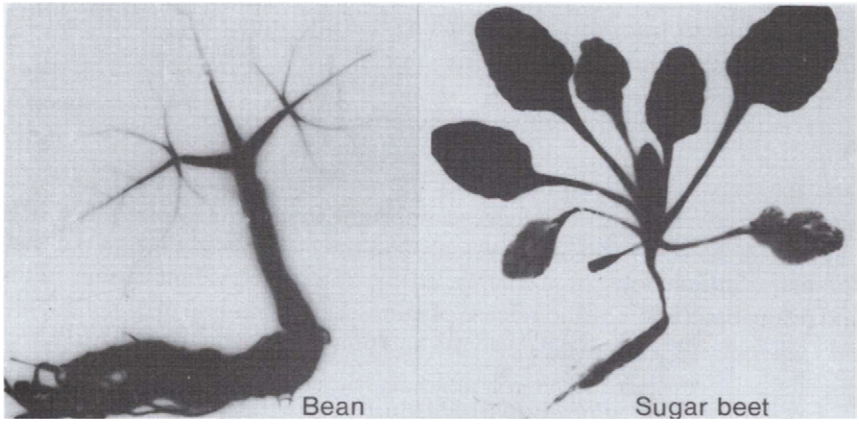

FIGURE 3.2 Distribution of Na in bean (*Phaseolus vulgaris* L.) and sugar beet (*Beta vulgaris* L.) 24 h after 5 mM $^{22}NaCl$ was supplied to the roots. Autoradiogram.

Retrieval of Na^+ from the xylem sap is therefore an effective mechanism of restricting translocation to the leaf blades. This mechanism, however, is not necessarily advantageous for the salt tolerance of plants (Drew and Läuchli, 1987; Jeschke and Pate, 1991b; see also Section 16.6) and is also a disadvantage in forage plants. For animal nutrition, the Na concentration of the forage should be at least 0.2%. As shown in Table 3.3, in *Lolium perenne* and *Trifolium repens*, Na^+ is readily translocated to the shoots, whereas in *Phleum pratense* and *Trifolium hybridum* this translocation is rather restricted. Thus, it is evident that selecting suitable plant species can be just as important as the application of Na fertilizers for increasing Na concentrations in forage.

Retrieval from the xylem sap in roots and stems can also be a determining factor in the distribution of micronutrients in plants. In certain species, such as bean and sunflower, Mo is preferentially accumulated in the xylem parenchyma of the roots and stems. In these species, a steep gradient occurs in tissue Mo concentrations from the roots to the leaves (Table 3.4). In contrast, in other species, such as tomato, Mo is readily translocated from the root to the leaves. In accordance with this finding, when the Mo supply in the nutrient medium is high, toxicity occurs much earlier in tomato than in bean or sunflower (Hecht-Buchholz, 1973).

3.2.2.2 Release or Secretion

The composition of the xylem sap along the transport pathway can also be changed by the release or secretion of solutes from the surrounding cells. For example, in non-legumes supplied with nitrate, the nitrate concentration in the xylem sap decreases as the path length increases, whereas the concentration of organic N, glutamine in particular, increases (Pate *et al*., 1964). In N_2 fixing legumes, on the other hand, the ratio of amides to amino acids is shifted in favour of the amino acids (Pate *et al.*, 1979).

Besides these specific aspects of N translocation, the release or secretion of nutrients from the xylem parenchyma (and stem tissue in general) into the xylem is of major importance for the maintenance of a continuous nutrient supply to the growing parts of the shoots. In periods of ample supply to the roots, nutrients are retrieved from the xylem sap, whereas in periods of insufficient root supply they are released into the xylem sap. Changes in the K and nitrate concentrations of the stem base reflect this functioning of the tissues along the xylem in response to changes in the nutritional status of a plant. Based on this, a rapid test for nitrate in the stem base has been developed as a means for recommending N fertilizer rates. Magnesium is also often stored in root cells and released to the xylem when shoots become Mg deficient (Hermans *et al.,* 2004).

3.2.2.3 Xylem Unloading in Leaves

Despite retrieval of elements along the pathway in the stem, most of the solutes and water are transported in the xylem vessels into the leaves. Here, water is preferentially transported in the major veins to sites of rapid evaporation such as leaf margins, or from the vein endings mainly via symplasmic movement towards the stomata (Canny, 1990; Karley *et al.*, 2000). Although the bundle sheath walls of the veins are suberized in leaves of C3 and C4 grass species, they do not provide a barrier against apoplasmic flux of water and solutes (Eastman *et al.*, 1988). Depending on the concentration and composition of solutes in the xylem sap entering the leaf, and the rate of water loss by transpiration along its stream through the leaf, the solute concentration may be enriched several fold at, for example, the leaf edges. This is particularly true when element concentrations are high in the root medium (e.g., saline substrates) and for elements such as B and Si.

TABLE 3.3 Sodium concentration of roots and shoots of pasture plants with and without Na fertilizer

Plant species	Na concentration (g kg^{-1} dw)			
	Without Na fertilizer		With Na fertilizer	
	Roots	Shoots	Roots	Shoots
Lolium perenne	0.3	2.6	0.6	11.6
Phleum pratense	1.0	0.4	2.8	3.8
Trifolium repens	2.7	2.2	7.7	19.6
Trifolium hybridum	4.5	0.3	7.7	2.2

Based on Saalbach and Aigner (1970).

TABLE 3.4 Distribution of Mo in bean and tomato plants supplied with Mo in the nutrient solution at 4 mg L^{-1}

Plant part	Mo concentration (μg g^{-1} dw)	
	Bean	Tomato
Leaves	85	325
Stems	210	123
Roots	1030	470

Based on Hecht-Buchholz (1973).

Unless some of this excessive solute accumulation at the terminal sites of the transpiration stream is removed, for example by guttation, as occurs for B (Oertli, 1962) or through epidermal glands in halophytes (Fitzgerald and Allaway, 1991), necrosis on the tips or margins of leaves occurs (Fig. 3.7). Some plants accumulate Ca in leaf trichomes (White and Broadley, 2003), or form Ca-oxalate crystals in specific cell types (Franceschi and Nakata, 2005).

Prevention of excessive solute accumulation in the leaf apoplasm by mechanisms other than uptake by the leaf cells can be achieved by the formation of salts of low solubility in the apoplasm. This strategy is utilized for the removal of soluble Ca in gymnosperms (Fink, 1991a). Calcium oxalate crystals are abundant in the needles of various gymnosperms in the cell walls of the epidermis mesophyll and phloem (Fig. 3.3). This mechanism of precipitation seems to be a safe way of coping with a continuous xylem import of Ca, which cannot be exported in the phloem. However, the xylem import of solutes into leaves and the evaporation of water does not necessarily lead to the accumulation of solutes in the leaf apoplasm. In fast growing plants with low nutrient supply, the solute concentration in the xylem sap declines sharply from the roots to the leaves and within a leaf blade from the base to the tip. For example, in barley the xylem-sap concentration of Mg decreased from 1.1 to 0.1 mM and that of K decreased from 18.0 to 8.0 mM from the leaf base to the tip (Wolf *et al.*, 1990b). Similarly, in tomato, water released by guttation from the leaf tips was virtually free of inorganic solutes (Klepper and Kaufmann, 1966).

The molecular mechanisms responsible for the uptake of solutes from the leaf apoplasm are being identified (Fig. 3.4). The cells of the bundle sheath are sites of intensive net proton excretion which acidifies the apoplasm. The electrochemical potential and the proton gradient across the plasma membrane of leaf cells act as the driving force for solute uptake. Many distinct amino acid transporters have been suggested to be present in the plasma membrane of leaf cells, which represent members of at least five different gene families (Daniel-Vedele *et al.*, 2010). Nitrate is likely to be retrieved from the xylem by transporters encoded by members of the *NRT1* and *NRT2* gene families (Li *et al.*, 2010a), and ammonium by transporters encoded by members of the *AMT1* (*Ammonium transporter* 1) gene family (Daniel-Vedele *et al.*, 2010). Phosphate and sulphate are likely to enter leaf cells through proton-coupled transporters encoded by members of the *Pht1* (*Phosphate transport 1*) and the *SULTR1* and *SULTR2* gene families, respectively (Miller *et al.*, 2009; Hell *et al.*, 2010). Boric acid channels, encoded by members of the *NIP* (*nodulin-26-like intrinsic protein*) gene family, are likely to facilitate B influx to leaf cells (Miwa and Fujiwara, 2010).

The electrochemical gradient between the xylem, shoot apoplast and the cytosol of shoot cells suggests that the influx of K and Ca to shoot cells can be mediated by cation channels in their plasma membrane (Keunecke *et al.*, 2001; Karley and White, 2009). It is likely that K influx is mediated by voltage-gated, inwardly rectified K-channels, whereas Ca influx is mediated by non-selective cation channels. The influx of other cations to shoot cells can also be facilitated by non-selective cation channels. Magnesium influx to shoot cells is thought to be catalysed by members of the MRS2 family of transport proteins (Karley and White, 2009), members of the ZIP family of transport proteins

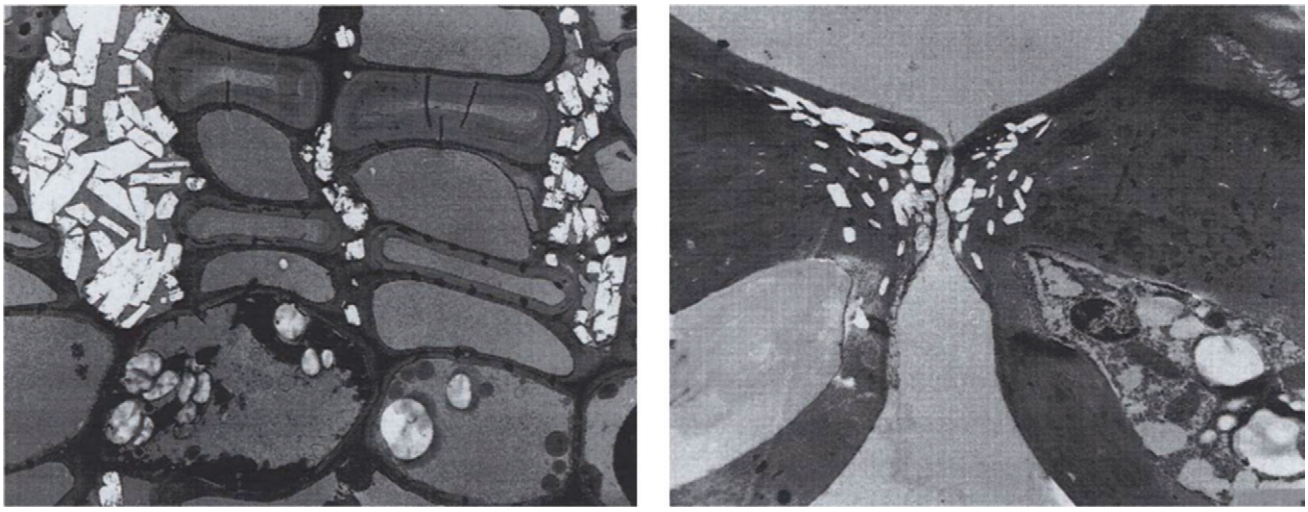

FIGURE 3.3 Calcium oxalate crystals in the apoplasm of needles. Micrograph from the phloem of a needle from *Juniperus chinensis* (*left*); micrograph of a stomatal pore in a needle from *Picea abies* (L.) Karst (*right*). *Courtesy of S. Fink, 1991a, c.*

allow Zn^{2+}, Fe^{2+}, Cu^{2+} and Mn^{2+} influx to shoot cells (White and Broadley, 2009), and members of the COPT family of transporters can mediate Cu influx to shoot cells (Cohu and Pilon, 2010). In addition, members of the YSL family may catalyse the influx of metal chelates to shoot cells (Guerinot, 2010).

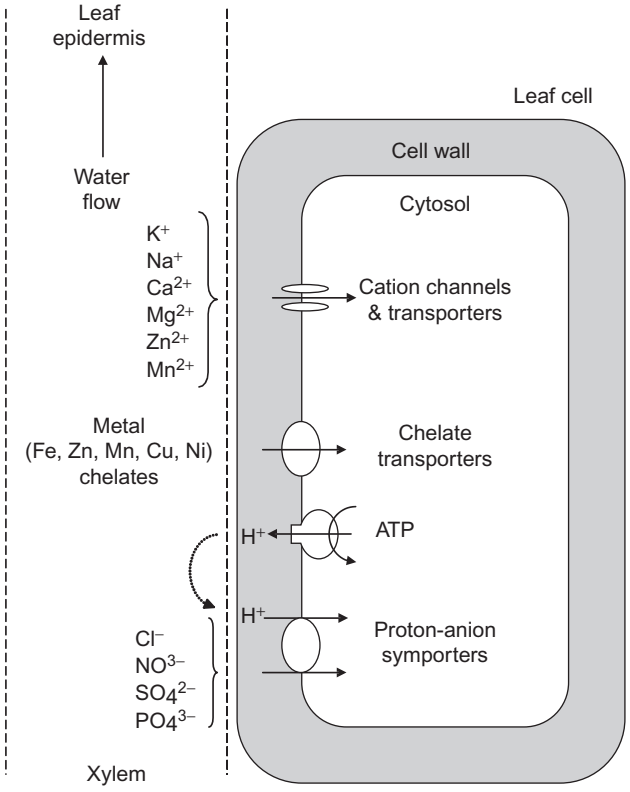

FIGURE 3.4 Model for the retrieval of major solutes from the xylem sap ('xylem unloading') in leaf cells.

3.2.3 Effect of Transpiration Rate on Uptake and Translocation

The rate of water flux across the root (short-distance transport) and in the xylem vessels (long-distance transport) is determined by both root pressure and the rate of transpiration. An increase in the transpiration rate may, or may not, enhance the uptake and translocation of elements in the xylem. Enhancement can be achieved in various ways, as shown in Fig. 3.5. Scheme A is true for elements such as B and Si except in the case of wetland rice. Scheme C may be important for soil-grown plants (Section 15.2), particularly in saline substrates (Section 17.6). Whether or not transpiration affects uptake and translocation rate of elements depends predominantly on the following factors:

Plant age. In seedlings and young plants with a low leaf surface area, increased transpiration rarely affects the accumulation of elements; water uptake and solute transport in the xylem to the shoots are determined mainly by root pressure. As age and size of the plants increase, the relative importance of transpiration, particularly for the translocation of elements, increases.

Time of day. In leaves, up to 90% of the total transpiration occurs via the stomata. During the light period, transpiration rates, and thus the potential for uptake and translocation of elements, are higher than during the dark period. Transient reductions in the translocation rates of

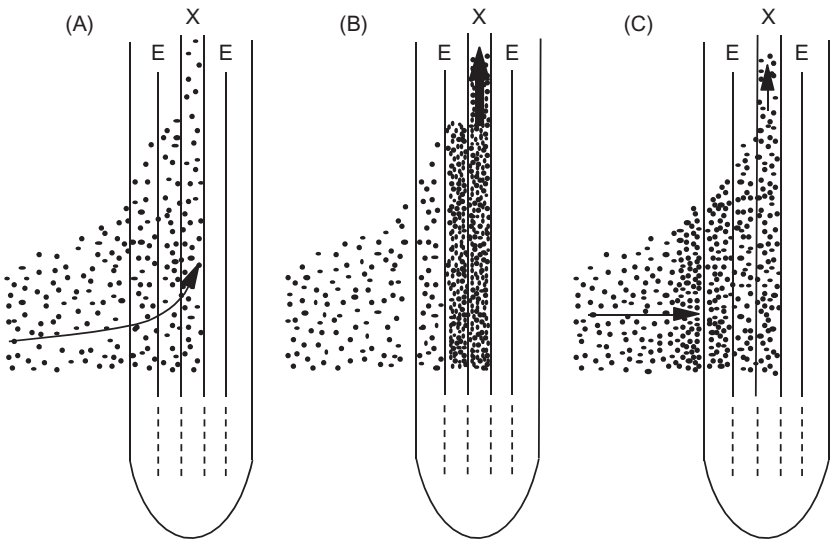

FIGURE 3.5 Models for the enhancement of uptake and translocation of elements by plant roots by increased transpiration. A. Drag of elements through the root apoplasm into the stele. B. Water flow-induced increase in the efflux of solutes from the root symplasm to the xylem vessels. C. Increased mass flow of the external solution to the rhizoplane and into the apparent free space, favouring greater uptake into the symplasm and delivery to the xylem. E, endodermis; X, xylem; arrow, water flux.

elements at the onset of the dark period reflect the change from transpiration-driven to root pressure-driven xylem volume flow (Crossett, 1968). The synchronous diurnal pattern in transpiration rate and uptake rate of K and nitrate (Le Bot and Kirkby, 1992) is probably caused by changes in carbohydrate availability in the roots or feedback control of uptake.

Nodulated legumes show a distinct diurnal pattern in shoot transport of fixed N. The strong decrease in transpiration-driven xylem volume flow during the dark period is compensated for by a strong increase in the concentration of fixed N (as ureides, see Chapter 7) in the xylem sap, thus keeping the total xylem transport rate of fixed N constant throughout the light/dark cycle (Rainbird *et al.*, 1983).

External concentration. It is well known that an increase in the concentration of elements in the nutrient medium can enhance the effect of transpiration rate on their uptake and translocation. This is most likely the result of transport as shown in schemes A and C in Fig. 3.5. Usually, translocation rates are more responsive to differences in transpiration rates than are uptake rates, as shown for K and Na in Table 3.5. Transpiration has a greater effect on translocation rate of Na than of K. On the other hand, uptake rates of K are more strongly increased by high external concentrations than are those of Na. At low external concentrations the nitrate flux in the xylem of maize plants is also unaffected by varying the transpiration rate by a factor of two; a reduction in transpiration rate to 20% is required for a major decline in nitrate flux (Shaner and Boyer, 1976).

Type of element. Under otherwise comparable conditions (e.g., plant age and external concentration), the effect of transpiration rate on the uptake and transport of elements follows a defined rank order. It is usually absent, or minor, for K, nitrate and P, but it may be significant for Na or Ca. As a rule, transpiration enhances the uptake and translocation of uncharged molecules to a greater extent than that of ions. The uptake and translocation of elements in uncharged forms is of great importance for B (boric acid; Miwa and Fujiwara, 2010) and Si (monosilicic acid; Ma and Yamaji, 2006). A close correlation between transpiration and the uptake of Si is shown for oat plants in Table 3.6.

There is a perfect agreement between Si uptake by the plants and that predicted from the product of water loss and Si concentration in the soil solution. Silicon accumulation in the shoot dry matter may therefore be a suitable parameter for calculations of the water use efficiency WUE (kg water transpired kg^{-1} dry matter produced) in cereals grown under rain-fed conditions (Walker and Lance, 1991). However, this parameter is unsuitable, for example in plants grown at different irrigation regimes (Mayland *et al.*, 1991), plants grown with nutrient solution (Jarvis, 1987), or when different genotypes within a species such as barley are compared (Nable *et al.*, 1990b). However, even in plants where close correlations between transpiration and Si accumulation are found, it should be emphasized that roots are not freely permeable to the radial transport of Si (Ma and Yamaji, 2006).

The absence of effects of reduced transpiration rates on the root to shoot transport of nutrients may indicate a high proportion of xylem to phloem transfer in the stem tissue, or a corresponding increase in xylem sap concentrations of the mineral nutrients.

TABLE 3.5 Uptake and translocation of K and Na from contrasting nutrient solutions at high or low transpiration rates in sugar beet plants. Transpiration in relative values: low transpiration = 100; high transpiration = 650

Nutrient solution concentration (mM)	K		Na	
	Transpiration			
	Low	High	Low	High
	Uptake rate (μmol plant^{-1} (4 h)$^{-1}$)			
1 K^+ + 1 Na^+	4.6	4.9	8.4	11.2
10 K^+ + 10 Na^+	10.3	11.0	12.0	19.1
	Translocation rate (μmol plant^{-1} (4 h)$^{-1}$)			
1 K^+ + 1 Na^+	2.9	3.0	2.0	3.9
10 K^+ + 10 Na^+	6.5	7.0	3.4	8.1

Based on Marschner and Schafarczyk (1967) and W. Schafarczyk (unpublished).

TABLE 3.6 Calculated and measured Si uptake in relation to transpiration (water consumption) of oat plants grown at an Si concentration in the soil solution of 54 mg L^{-1}

Harvest after (days)	Transpiration (mL plant^{-1})	Uptake (mg plant^{-1})	
		Measured	Calculated
44	67	3.4	3.6
58	175	9.4	9.4
82	910	50.0	49.1
109	2785	156.0	150.0

From Jones and Handreck (1965).

3.2.4 Effect of Transpiration Rate on Distribution within the Shoot

The distribution of an element that is transported in the xylem but not the phloem should be related solely to transpiration rates (e.g., mL (g dw day)$^{-1}$) and duration of transpiration (e.g., age of the organ). This is true, for example, for Mn (McCain and Markley, 1989) where in the same plant (maple tree), the 'sun leaves' (high transpiration rates) have higher Mn concentrations in their dry matter than 'shade leaves' (low transpiration rates) of a similar age. The distribution and concentration of Si also usually reflect the loss of water from various organs. The Si concentration increases with leaf age and is particularly high in spikelets of cereals such as barley. Even within a certain tissue, the Si distribution resembles the pathway of transpiration flow in the apoplasm. Silicon is deposited in the walls of epidermal cells (Hodson and Sangster, 1988) or in the pericarp and outer aleurone layer of grass seeds such as *Setaria italica* (Hodson and Parry, 1982).

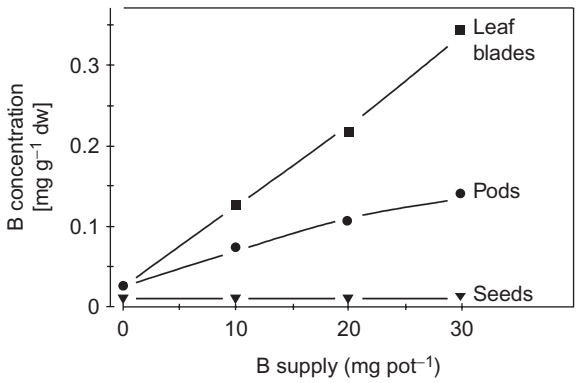

FIGURE 3.6 Distribution of B within the shoot of oilseed rape (*Brassica napus* L.) with increasing B application to the soil. *Recalculated from the data of Gerath* et al. *(1975).*

The distribution of B is also related to the loss of water from the shoot, as shown for oilseed rape in response to an increasing B supply (Fig. 3.6). The typical gradient in transpiration rates in the shoot organs (leaves > pods >> seeds) corresponds to the differences in B concentration. Even for a particular leaf, an excessive supply of B creates a steep gradient in the B concentrations: petioles < middle of the leaf blade < leaf tip (Oertli and Roth, 1969). Necrosis on the margins or leaf tips is therefore a typical symptom of B toxicity (Fig. 3.7). Similar symptoms can be observed in salt-affected plants, reflecting the transpiration-mediated distribution pattern within the shoot and its organs.

Frequently, there is a close positive correlation between Ca distribution and transpiration rates of shoot organs, as is evident from the low Ca concentrations of low transpiring fleshy fruits (<1 g kg^{-1} dw) when compared with that of the leaves (30–50 g^{-1} dw) of the same plant. Lower transpiration rates further decreases the Ca concentration of fruits (Table 3.7). The effect of transpiration on Mg distribution is less than on Ca, and the effect of transpiration on K distribution is negligible. Despite the correlations shown in Table 3.7, the interactions are much more complex between the rates of water and Ca influx into a plant organ.

The fact that transpiration rates are higher and leaf water potentials are lower in mistletoe than in the host plant presumably explains why xylem parasites such as *Loranthus* can compete effectively with the host for nutrients particularly N in the xylem fluid, (Schulze *et al.*, 1984), and also maintain a high influx of root-derived phytohormones such as cytokinins.

The influence of transpiration on the distribution of elements differs not only between elements but also between the various forms of the same element, as shown in Fig. 3.8 for N. Whereas the distribution within the shoot

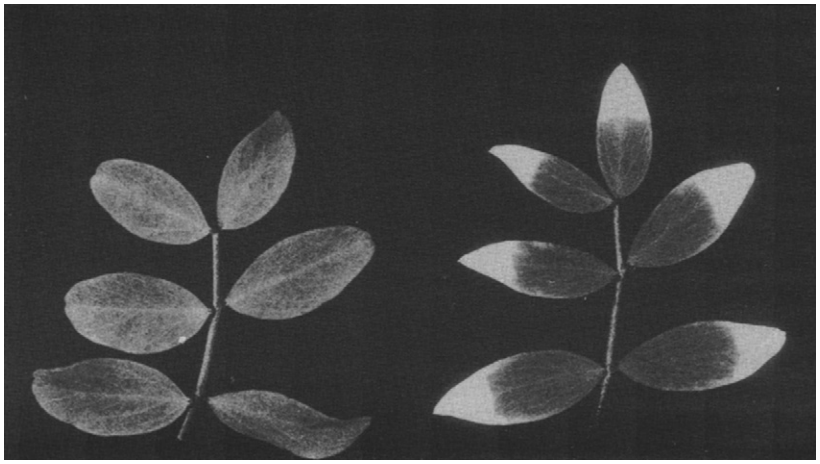

FIGURE 3.7 Boron toxicity in lentil leaves: control (*left*); B toxicity (*right*).

TABLE 3.7 Fruit K, Mg and Ca concentration in red pepper grown at high or low transpiration rates during fruit growth

Transpiration rate (relative)	Fruit weight (g dw fruit^{-1})	Concentration in fruits (mg g^{-1} dw)		
		K	Mg	Ca
100	0.62	91.0	3.0	2.75
35	0.69	88.0	2.4	1.45

From Mix and Marschner (1976b).

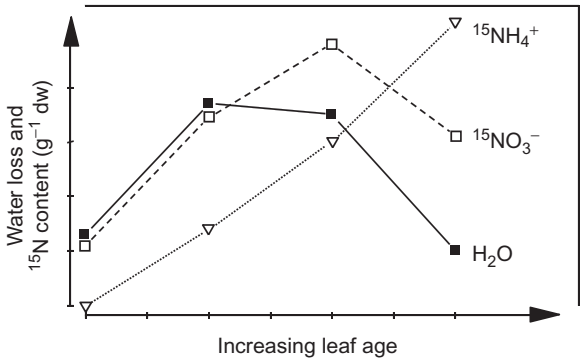

FIGURE 3.8 Transpiration rates and accumulation of labelled nitrogen (^{15}N) in leaves of bean following the application of $^{15}NO_3^-$ and $^{15}NH_4^+$ to the root. *Redrawn from Martin (1971).*

of ^{15}N from ammonium is independent of transpiration rates (water loss) of the leaves and is translocated preferentially to the shoot apex, which acts as a sink for reduced N, ^{15}N from nitrate follows the transpiration pattern quite closely. The decrease in xylem flux of water and nitrate into older leaves of plant species such as bean is due to an endogenously regulated decrease in hydraulic conductivity caused by plugging of the xylem vessels at the pulvinal junction in older leaves (Neumann, 1987). This plugging can be considered as a primary step of a programmed sequence leading to a decrease in xylem import of nutrients and phytohormones into the leaf and, thus, to leaf senescence (Neumann, 1987).

3.3 PHLOEM TRANSPORT

3.3.1 Principles of Transport and Phloem Anatomy

Long-distance transport in the phloem takes place in living cells, the sieve tubes (Fig. 3.9). The principles of the transport mechanism of the phloem were proposed as early as 1930 by Münch in a *pressure flow hypothesis (Druckstromtheorie)* based on the principle of an osmometer. This has already been discussed in Section 2.9 in relation to root pressure. Münch suggested that solutes such as sucrose are concentrated in the phloem of leaves (i.e., *phloem loading*) and water is sucked into the phloem, creating a positive internal pressure. This pressure induces a mass flow in the phloem to the sites of lower positive pressure caused by removal of solutes from the phloem. Therefore, flow rate and direction of flow are closely related to phloem unloading at the sink. This type of pressure-driven mass flow in the phloem differs from that in the xylem in three important ways: (i) organic compounds are the dominant solutes in the phloem sap, (ii) transport takes place in living cells, and (iii) the unloading of solutes at the sink plays an important role.

For nutrients, the main sites (sources) for phloem loading are located in the stem and the leaves. These supply nutrients to growth sinks (shoot apices, fruits, roots) and allow nutrient cycling within the plant. An example of sink-regulated transport of a nutrient is shown in Fig. 3.10 for P. After application to one of the two mature primary leaves, the labelled P is transported to the shoot apex and the roots whereas transport to the other primary leaf is negligible. In contrast, Na is not transported to the shoot apex but moves exclusively downwards (basipetally) to the roots where it is confined to the basal zones (Fig. 3.10). From here a considerable net efflux of Na takes place (Lessani and Marschner, 1978). This example reflects the role of phloem transport in cycling elements within the plant and specifically in prevention of Na accumulation in the shoots of natrophobic plant species. The capacity of bidirectional, ion-specific, long-distance transport is based on the physio-logy and anatomy of the phloem and its elements.

Within the phloem, the sieve tube elements are associated with companion cells and parenchyma cells (Fig. 3.9). Some of these individual sieve tube elements are stretched end to end in a long series, forming the sieve tubes which are connected by pores (inset, Fig. 3.9) called sieve plate pores. The sieve tubes are highly specialized vascular systems for the long-distance transport of solutes. The sieve tube cells contain a thin layer of cytoplasm, which forms transcellular filaments (the so-called P-protein) that pass through the sieve plate pores. The anatomical features of long-distance transport in the sieve tube across the sieve plate pores are similar to those of short-distance transport between cells in the symplasm across the plasmodesmata.

In most plant species, the sieve plate pores are lined with callose, a highly hydrated polysaccharide. There is good evidence that callose can swell rapidly and fill the pores, thus blocking long-distance transport in the sieve tubes. Callose formation is strongly enhanced by Ca^{2+} even at a concentration of a few µM (Kauss, 1987). Thus, very low concentrations of free Ca^{2+} must be maintained

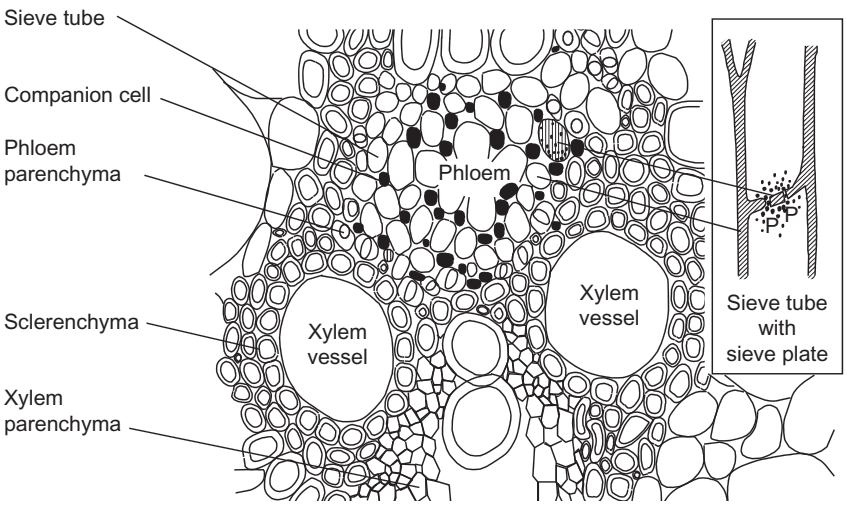

FIGURE 3.9 Cross-section of a vascular bundle from the stem of maize. Inset: Sieve tube with sieve plate pores and 'P-protein'. *Redrawn from Eschrich (1976).*

in the phloem sap for long-distance transport to occur. Plugging sieve tube pores is also induced by factors such as heat treatment or mechanical stress, as well as by mechanical injury of the sieve tubes. Incision causes a sudden reduction of the internal pressure of the sieve tubes (>10 bars), which presumably triggers the mechanism of plugging the sieve tube plates. This process can be thought of as a 'security valve' that prevents 'bleeding' when the system is injured. For experimental studies on long-distance transport this plugging mechanism is both an advantage and a disadvantage. It is an advantage in that very soon after decapitation of a plant, only xylem exudate is obtained at the stump of the root or stem; it is a disadvantage in that with a few exceptions – for example, the inflorescent stalks of certain palm tree species – it is very difficult to collect phloem exudate and thus to conduct extended studies on the element composition of the phloem sap. There are some plant species (e.g., *Ricinus* and *Lupinus* spp.) from which small amounts of phloem exudate can be collected relatively easily by careful incision. However, with the incision technique there is always a possibility of contaminating the phloem sap by cut parenchyma cells and by substances from the apoplasm. Another method to collect phloem sap is to use sucking insects such as aphids and plant hoppers. In the process of feeding, these insects insert their stylet into the phloem tissue and sieve tubes. If the stylet is severed, for example with a laser beam (Hayashi and Chino, 1990), it remains in the tissue and the high internal pressure within the sieve tubes forces the phloem sap out of the open end of the stylet. This technique, of course, is very laborious, and the amounts of exudate obtained are quite small. For these reasons our knowledge of long-distance transport based on phloem sap analysis is rather limited, particularly for nutrients.

3.3.2 Phloem Loading and the Composition of Phloem Sap

Phloem sap has a high pH (7–8) and contains high concentrations of solutes, on average 15–25% dry matter. A comprehensive analysis of phloem sap composition is shown in Table 3.8. The main component is usually sucrose, which may comprise up to 90% of the solids. The proportion of sucrose to other solutes depends on the site of phloem sap collection; it can be very high at the loading sites, for example the ear of cereals (Hayashi and Chino, 1990). In addition to sucrose, amino compounds are usually present in high concentrations in phloem sap (Table 3.8; Peuke, 2010) with the amides glutamine and asparagine representing up to 90% of this fraction. On the other hand, the concentrations of nitrate and ammonium are usually low (Van Beusichem *et al.*, 1988). Organic acids such as citrate and malate are also abundant in the phloem sap, and, in white lupin, succinate concentrations may reach the same orders of magnitude as the concentration of total amino-N (Jeschke *et al.*, 1986). A whole range of other organic compounds are also found in phloem sap, for example secondary metabolites, hormones, proteins and RNA (Turgeon and Wolf, 2009).

All plants transport sucrose in the phloem, but some plants also transport raffinose and stachyose and/or sugar alcohols (Turgeon and Wolf, 2009). These compounds enter the phloem in mature leaves. Sucrose is generally

TABLE 3.8 Comparison of concentrations of organic and inorganic solutes in the phloem (stem incision, pH 7.9–8.0) and xylem (tracheal, pH 5.6–5.9) exudates of *Nicotiana glauca*

	Phloem	Xylem	Ratio phloem/xylem
	($mg\ L^{-1}$)		
Dry matter	170–196	1.1–1.2	155–163
Sucrose	155–168	nd	
	($\mu g\ mL^{-1}$)		
Amino compounds	10,808	283	38.2
Nitrate	nd	na	
Ammonium	45.3	9.7	4.7
K	3,673.0	204.3	18.0
P	434.6	68.1	6.4
Cl	486.4	63.8	7.6
S	138.9	43.3	3.2
Ca	83.3	189.2	0.44
Mg	104.3	33.8	3.1
Na	116.3	46.2	2.5
Fe	9.4	0.60	15.7
Zn	15.9	1.47	10.8
Mn	0.87	0.23	3.8
Cu	1.20	0.11	10.9

From Hocking (1980b).
nd: not detectable; na: data not available.

loaded into the phloem from the apoplasm by sucrose-proton symporters, encoded by members of the *SUT/SUC* gene family such as *AtSUC2* from *Arabidopsis thaliana* (Sauer, 2007; Kühn and Grof, 2010), and specific transporters for sorbitol and mannitol have also been reported (Juchaux-Cachau *et al.*, 2007). Interestingly, the ability to transport B in the phloem is associated with the presence of polyols in phloem sap (Brown and Hu, 1998). A multitude of genes encoding transporters that can potentially load amino acids, ureides and short peptides into the phloem from the apoplasm have been uncovered in plant genomes, which belong to at least nine gene families (Rentsch *et al.*, 2007). Nitrate can be loaded into the phloem by transporters of the NRT1 family (Fan *et al.*, 2009).

Of the nutrients, K is usually present in the highest concentration, followed by P, Mg and S (Table 3.8). Potassium is loaded into the phloem by voltage-gated, inwardly-rectified K-channels with electrophysiological properties resembling AtAKT2/3 of *Arabidopsis thaliana* (Deeken *et al.*, 2002; Hafke *et al.*, 2007). Sulphur occurs in both the reduced form (e.g., glutathione, S-methylmethionine, methionine, cysteine) and as sulphate (Hell *et al.*, 2010). Sulphate is loaded into the phloem by orthologues of the *Arabidopsis thaliana* AtSULTR1;3 transporter, while methionine and cysteine are likely to be loaded by amino-acid transporters (Hell *et al.*, 2010). Sulphate concentrations in the phloem sap can be as high as those of phosphate (Van Beusichem *et al.*, 1988). Chloride and Na may also be present at high concentrations (Table 3.8), but this depends on their external supply and the plant species (Jeschke and Pate, 1991b; White and Broadley, 2001). In contrast, the concentration of Ca in the phloem sap is always very low, regardless of plant species.

Reliable data on micronutrient concentrations in the phloem sap are rare (Table 3.8), but B concentrations in the range of 200 to 500 µM have been reported (Huang *et al.*, 2008). Members of the ZIP family are thought to transport Zn into the phloem (Ishimaru *et al.*, 2005) and Fe, Mn, Zn and Cu are probably also loaded into the phloem by YSL proteins. In general, these elements are transported to sink tissues as metal-nicotianamine complexes or in association with small proteins (Puig *et al.*, 2007; Waters and Grusak, 2008; White and Broadley, 2009; Curie *et al.*, 2009; Guerinot, 2010). A large proportion of Fe transported in the phloem is complexed to the Iron Transport Protein (Guerinot, 2010).

With the exception of Ca, the concentrations of all solutes are usually several times greater in phloem exudate than in the xylem exudate (Table 3.8). The data in Table 3.8 on phloem sap composition are in fairly good agreement with those obtained from analyses of stems of castor bean (Van Beusichem *et al.*, 1988), white lupin (Jeschke *et al.*, 1986) and rice (Chino *et al.*, 1982). For reviews of the composition of phloem sap, the reader is referred to Ziegler (1975) and Turgeon and Wolf (2009).

3.3.3 Mobility in the Phloem

All nutrients have been found in reasonable concentrations in the phloem sap. The question arises, however, as to whether the phloem sap composition, particularly based on exudate collected by incision, reflects the mobility of elements in long-distance transport in the phloem from source to sink. Another approach to studying phloem mobility is the use of labelled elements (radioactive or stable isotopes) to follow long-distance transport after application, for example, to the tip of a leaf blade (Fig. 3.10). Because of the gradient in xylem water potential, re-translocation from the leaf tips and out of the treated leaf must take place in the phloem. On the basis of such

FIGURE 3.10 Re-translocation of labelled P (^{32}P) and Na (^{22}Na) after application to the tip of a primary leaf of bean. Autoradiogram, 24 h after application.

studies and with consideration of the data on phloem sap composition, nutrients can be classified based on their phloem mobility (Table 3.9). Sodium has been included as it is a beneficial nutrient for some plant species; its phloem mobility is of particular importance for plants growing in saline substrates.

This classification in Table 3.9 is, of course, only a first approximation as certain factors are ignored, for example genotypical differences or the nutritional status of plants. However, for the macronutrients, except Ca, phloem mobility is generally high, and for the micronutrients it is at least intermediate with the exception of Mn. For Mo, fairly high phloem mobility has been established from both, indirect (Wood *et al.*, 1986) and direct (Kannan and Ramani, 1978) measurements. Studies on B mobility in the phloem followed B translocation with time into developing fruits such as peanut (Campbell *et al.*, 1975), often with the aid of B isotopes (Chamel *et al.*, 1981; Changzhi *et al.*, 1990). Such investigations together with the fairly high concentrations of B occurring in phloem exudates clearly reveal that B is mobile in the phloem. Substantial amounts of B are translocated in the phloem to growth sinks, for example flower buds after foliar application (Hanson, 1991a). Thus, B may at least be classified as of intermediate phloem mobility.

Some long-distance transport in the phloem can be demonstrated with labelled Mn (Nable and Loneragan, 1984; El-Baz *et al.*, 1990), but its mobility is generally very low. The same holds true for Ca. Although substantial Ca concentrations can be found in the phloem sap (Table 3.8), it is appropriate to classify Ca as a nutrient with very low phloem mobility. The observed ratio of Ca/K of about 1/100 in the phloem sap in Table 3.8 (Jeschke and Pate, 1991b) is about 5 to 10 times too low to cover the Ca demand of a growth sink. A similar conclusion may be drawn from other phloem sap analyses. Thus, most of the Ca demand of growth sinks has to be covered by import via the xylem.

TABLE 3.9 Mobility of nutrients in the phloem

Mobility		
High	Intermediate	Low
K	Fe	Ca
Mg	Zn	Mn
P	Cu	
S	B	
N (amino-N)	Mo	
Cl		
(Na)		

3.3.4 Transfer between the Xylem and Phloem

In the vascular bundles, phloem and xylem are separated by only a few cells (Fig. 3.9). Exchange of solutes between the two conducting systems is very important for regulation of long-distance transport (Fig. 3.11). From the concentration differences shown in Table 3.8 it is evident that transfer from phloem to xylem can occur down a concentration gradient. In contrast, for most organic and inorganic solutes, a transfer from xylem to phloem is usually against a steep concentration gradient. Nevertheless, xylem-to-phloem transfer of nutrients is of particular importance for the mineral nutrition of plants, because xylem transport is directed mainly to the sites (organs) of highest transpiration, which are often not the sites of highest demand for

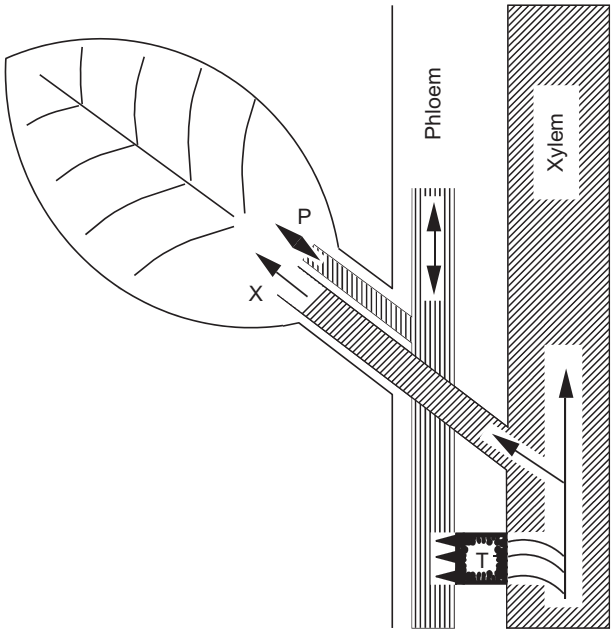

FIGURE 3.11 Schematic diagram of long-distance transport in the xylem (X) and phloem (P) in a stem with a connected leaf, and xylem-to-phloem transfer mediated by a transfer cell (T).

nutrients. The transfer of organic and inorganic solutes can take place along the entire pathway from roots to shoot, and the stem plays an important role in this respect (McNeil, 1980; Van Bel, 1984). The stem nodes are sites of intensive xylem-to-phloem transfer, which function, for example, to exchange K in cereals (Haeder and Beringer, 1984a, b) and amino acids in soybean (Da Silva and Shelp, 1990). In soybean, between 21 and 33% of the total xylem-to-phloem transfer of amino acids occurs in the stem and between 60 and 73% in the leaf blades.

The proportion of xylem-to-phloem transfer in the stem is influenced by the rate of xylem volume flow, i.e. by the transpiration rate, with high rates reducing transfer to the phloem. In tomato, doubling the volume flow rate reduced the transfer of amino acids in the stem resulting in a higher proportion being transported to the older leaves at the expense of the shoot apex (Van Bel, 1984). A diurnal rhythm in the partitioning of solutes between mature leaves and shoot apex or fruits is thus also to be expected for this reason, unless it is compensated for by a higher xylem-to-phloem transfer in the leaf blades.

Information is scarce about the opposite process, phloem-to-xylem transfer. In wheat after anthesis, retranslocation in the phloem from the flag leaf to the stem is followed by a considerable release of P, Mg and N, but not of K, into the xylem. These nutrients are subsequently transported in the xylem into the ears (Martin, 1982). In white lupin, at least in some regions of the stem, phloem-to-xylem transfer seems to be of greater importance than transfer in the opposite direction (Jeschke *et al.*, 1987).

3.4 RELATIVE IMPORTANCE OF PHLOEM AND XYLEM FOR LONG-DISTANCE TRANSPORT OF NUTRIENTS

3.4.1 General

Precise quantitative assessments of the relative importance of solute transport in the phloem and xylem into parts or organs of plants are difficult to make. For such assessments not only the concentrations of solutes are required, but also the velocity of transport and the cross-sectional area of the conducting vessels, according to the following relationship:

$$\text{Specific mass transfer } (\text{g cm}^{-2}\,\text{h}^{-1}) = \text{velocity } (\text{cm h}^{-1}) \times \text{concentration } (\text{mg cm}^{-3})$$

The velocity of transport in the xylem and phloem varies enormously. On average, velocities range between 10 and 100 cm h^{-1}, with rates in the phloem being lower than in the xylem. For example, in fruit stalks of white lupins, maximal velocities of 22 cm h^{-1} in the phloem and 147 cm h^{-1} in the xylem have been reported (Pate *et al.*, 1978).

Our present knowledge on the relative importance of xylem and phloem import and export of elements into plant parts or organs is mainly based on detailed analyses of phloem and xylem sap in different shoot parts of individual plants and the corresponding element content in the shoot parts at sequential harvests (Jeschke *et al.*, 1987; Jeschke and Pate, 1991a, b; 1992; Peuke, 2010).

3.4.2 Nutrients with High Phloem Mobility

For nutrients with high phloem mobility such as K, P or N as amino-N, the relative importance of phloem and xylem transport into an organ depends on the stage of development of the organ as shown in Table 3.10 for amino-N during the lifespan of an individual leaf.

Throughout the lifespan of the leaf of the nitrate-fed castor bean plant, N import by the xylem sap was high and only declined at the onset of senescence. Additional N import by the phloem during rapid leaf expansion was followed by a strong increase in phloem export so that export was greater than import. Nitrate represented only a small fraction of the N imported in the xylem. The rates of phloem export of N closely matched the net rates of CO_2 fixation by the lamina (Jeschke and Pate, 1992).

In principle, similar data on the time course of import and export in xylem and phloem during the lifespan of individual leaves have been obtained in barley for K (Greenway and Pitman, 1965) and for P (Greenway and

Gunn, 1966). A lack of change in the net contents of highly phloem mobile elements in fully expanded leaves is therefore a reflection either of cessation of the import or, more likely, of an equilibrium between import and export (re-translocation).

3.4.3 Nutrients of Low Phloem Mobility

Calcium is used as an example of a nutrient with low phloem mobility. Because of its low concentrations in the phloem sap the import of Ca into growth sinks such as shoot apices, young leaves or fruits takes place nearly exclusively in the xylem, whereas the import by the phloem is negligible as shown for castor bean in Table 3.11. This is in marked contrast to K of which most (terminal bud) and at least half (youngest leaves) of the total net import takes place in the phloem. For Mg, phloem import contributes to 25 and 40% of the total import.

In order to cover the relatively high Ca demand of growth sinks, particularly in dicotyledonous plant species that have a high apoplasmic cation exchange capacity (Section 6.6), a high rate of xylem volume flow into these organs is required. Fruits developing in the soil such as peanut (Hallock and Garren, 1968) and potato tubers (Krauss and Marschner, 1975) are exceptions, as they can cover part of their Ca demand by direct uptake from the soil solution. Shoot apices, young leaves, particularly those enclosed by mature leaves (e.g., cabbage), and fleshy fruits are characterized by low rates of transpiration and inherent low rates of xylem volume flow. Calcium deficiency and the so-called Ca deficiency-related disorders, such as tip-burn in lettuce, blossom end rot in tomato, and bitter pit in apple, are therefore widespread (see also Chapter 9). For reviews on this subject see Shear (1975), Marschner (1983) and Ho and White (2005).

To increase the Ca concentration in growing leaves or fruits, increasing the transpiration rates of the fruits is more effective than increasing the Ca supply in the substrate (Table 3.12). As expected, because of its high phloem mobility, the K concentration is not affected by these treatments. Furthermore, there is a negative correlation between growth rate and Ca concentration in the dry matter of growing fruits, whereas this is again not observed with K. High growth rates are based on high solute volume inflow via the phloem and thus correlated with high K, but a very low inflow of Ca. In addition, in organs with low transpiration rates, such as fleshy fruits, a high phloem solute volume flow either strongly depresses, or even reverses the direction of the xylem volume flow (Mix and Marschner, 1976c). This counter-flow of water in the xylem can be substantial, for example in grape berries (Lang and Thorpe, 1989), and may lead to the export from fruits of Ca (Mix and Marschner, 1976c) and organic solutes (Hamilton and Davies, 1988).

High transpiration rates of the whole shoot, however, often decrease rather than increase the Ca influx into low-transpiring organs such as rosettes of cauliflower (Krug *et al.*, 1972). Under these conditions, the xylem volume flow is directed to the high-transpiring outer leaves at the expense of the inner leaves or the rosettes. Inhibition of transpiration (by high relative humidity or during the dark period) usually favours the direction of the xylem volume flow towards low-transpiring organs. For example, in Chinese cabbage, an increase in relative humidity during the night increased the Ca concentrations in the inner leaves by 64% and decreased the proportion of tip-burn in heads by 90% (van Berkel, 1988). In potato plants subjected to soil drying, Ca deficiency-related tuber necrosis

TABLE 3.10 Import (+) and export (−) of N during the lifespan of a leaf of nitrate-fed castor bean plants

Days after leaf emergence	N (nmol leaf^{-1})		
	Xylem (as NO_3)	Phloem	Net change
1–12	+2.7 (0.23)	+1.4	+4.10
13–20	+2.5 (0.43)	−1.1	+1.36
21–40	+2.8 (0.63)	−3.7	−0.87
41–60	+1.4 (0.48)	−4.0	−2.63

Based on Jeschke and Pate (1992).

TABLE 3.11 Xylem and phloem import of K, Mg and Ca into the terminal bud and youngest leaves of castor bean

	Terminal bud			Youngest leaves		
	K	Mg	Ca	K	Mg	Ca
	(μmol plant^{-1} (9 d)$^{-1}$)					
Xylem	3.9	8.0	4.2	20.6	5.2	2.4
Phloem	20.4	2.0	0.03	19.3	2.0	0.03

Data recalculated from Jeschke and Pate (1991b).

TABLE 3.12 Calcium and K concentrations of red pepper fruits as affected by Ca supply, relative humidity in the fruit environment and fruit growth rate

	Concentration in fruits (μmol g^{-1} dw]	
	Ca	K
Ca supply to roots (mM)		
0.5	26.9	1,315
5.0	33.2	1,228
Relative humidity in the fruit environment (%)		
90	32.7	1,892
40	55.4	1,918
Growth rate of fruits (mg dw day^{-1})		
20	28.2	1,772
30	20.7	1,846
39	17.2	1,813

From Marschner (1983).

TABLE 3.13 Relationship between root pressure (guttation), leaf tip necrosis and Ca and Mg transport into expanding strawberry leaves. Root pressure varied according to nutrient solution concentration: concentrated (6.5 atm, high root pressure), diluted (1.6 atm, low root pressure)

Root pressure		Guttation (relative)[a]	Tip necrosis (relative)[b]	Content (μg $leaf^{-1}$)	
Day	Night			Ca	Mg
high	high	0.3	3.0	7	87
high	low	2.4	0.3	25	77
low	high	0.8	1.3	16	74
low	low	2.3	0.0	62	78

Based on Guttridge *et al.* (1981).
[a]*O = none; 3 = high.*
[b]*O = none; 5 = very severe.*

could be significantly reduced by foliar application of anti-transpirants which altered leaf-tuber water potential gradients (Win *et al.*, 1991). Diurnal shrinking (during the light period) and swelling (during the dark period) of the rosettes of cauliflower (Krug *et al.*, 1972) or of cabbage (Wiebe *et al.*, 1977) are closely correlated with corresponding changes in the xylem volume flow and Ca flux into various parts of the shoots.

At low transpiration, the rate of xylem volume flow from the roots to the shoots is determined by root pressure. The import of water and Ca via the xylem into low-transpiring organs therefore strongly depends on root pressure. Water availability in the rooting medium, particularly during the dark period, is thus crucial for the long-distance transport of Ca into low-transpiring organs with high Ca demand for growth. In agreement with this, low osmotic potential of the soil solution (e.g., soil salinity) decreases both root pressure and Ca influx into young leaves or fruits and induces Ca deficiency symptoms (Mizrahi and Pasternak, 1985; van Berkel, 1988; Ho and White, 2005).

These relationships are shown in Table 3.13 for expanding strawberry leaves. High root pressure, as indicated by the intensity of guttation, is closely correlated with an increased Ca concentration in expanding leaves and either the absence of, or only mild symptoms of, Ca deficiency (tip necrosis). Magnesium, which is highly phloem mobile, is only slightly affected by root pressure. Root pressure also strongly depends on root respiration and oxygen supply to the roots. Interruption of the aeration of the nutrient solution during the night had no effect on Ca accumulation in the roots of tomato but reduced the Ca transport into the stem by 42% and into the leaves by 82% (Tachibana, 1991). The increase in blossom end rot in tomato by poor aeration of the rooting medium is well documented (Ho and White, 2005).

Increasing Ca import to fruits and leaves is, however, not advantageous under all circumstances. In tomato, for example, environmental factors which enhanced Ca import into fruits increased the incidence of 'gold specks', which is a physiological disorder caused by an excess of Ca in the tissue (DeKreij *et al.*, 1992). In the gold speck tissue, high Ca concentrations are found together with a high density of Ca oxalate crystals. Abundant formation of Ca oxalate crystals in the apoplast of needles in gymnosperms (Fig. 3.3) is another example for excessive Ca import into an organ, and is particularly evident in trees growing on calcareous soils (Fink, 1991b).

3.4.4 Re-translocation and Cycling of Nutrients

With the exception of Ca and presumably also Mn, import of nutrients in the xylem and export (re-translocation) in the phloem is a normal feature throughout the life of an individual leaf. Several pieces of evidence indicate a rapid xylem-to-phloem transfer in leaf blades in which only a small fraction of the total leaf content ('cycling' fraction; Fig. 2.26) is involved. Considerable amounts of nutrients are re-translocated in the phloem from the shoots back to the roots and may thereby serve various functions. They may be used to convey

information about the nutritional status of the shoots and, via feedback regulation, control uptake by the roots. In natrophobic plant species, re-translocation in the phloem is an important component in maintaining low Na concentrations in the leaves (Fig. 3.10). This also holds true for some natrophilic, salt tolerant species such as reed (Matsushita and Matoh, 1992), but not for others such as barley (Munns *et al.*, 1987).

In plant species for which the shoot provides the main site of nitrate reduction (Section 6.2) re-translocation of N in reduced form in the phloem from shoot to the roots is required to meet root demand for reduced N. Frequently, however, considerable amounts of re-translocated nutrients are again loaded into the xylem of the roots to be transported back to the shoot, i.e. they cycle in the plant. For K, it has been demonstrated that, at least in certain plant species, cycling is an important process for maintenance of charge balance in shoots and roots of nitrate-fed plants (see below). In more general terms, cycling of nutrients may smooth out fluctuations in external supply to match a more consistent demand. Cycling of nutrients may also be important to compensate, at least in part, for the non-uniform distribution of nutrients in the rooting zone, for example in the case of Zn (Loneragan *et al.*, 1987; Webb and Loneragan, 1990) and Mg (Hermans *et al.*, 2004), but not in the case of Fe (Romera *et al.*, 1992). However, cycling of nutrients should not be considered solely as a specific regulatory mechanism for a particular nutrient. In many instances, cycling could well be the consequence of the mechanism and the direction of phloem transport, which is governed by sugar transport from leaves as the source to roots as a sink.

Comprehensive studies on nutrient cycling were conducted with white lupin and castor bean by Jeschke and Pate (1991b). Some of their data are summarized in Table 3.14. These give nutrient import and export from leaf laminae, re-translocation through the phloem and cycling through the roots. As had already been shown for reduced N (Table 3.10) and is also the case for K, Na and Mg, export through the phloem can comprise a major fraction of the import through the xylem. Phloem export of Ca is negligible in castor bean but unexpectedly high in white lupin. This high Ca export probably relates to the exceptionally high concentrations of organic acids (mainly succinate) in the phloem sap of white lupin (Jeschke *et al.*, 1986; Cramer *et al.*, 2005). Organic acids chelate Ca and may thus improve its phloem mobility. Between 82 and 100% of the exported elements are re-translocated in the phloem back to the roots, and a high proportion of the K and Mg cycle, i.e. they are again loaded into the xylem and transported to the shoots (Table 3.14). For Ca, no precise data can be given but cycling is of minor importance. In general, these data are consistent with other studies indicating that up to 90% of K, 80% of N, 65% of Mg, 30% of P, 30% of S, and 30% of Cl delivered to the shoot via the xylem is exported back to the root via the phloem (Armstrong and Kirkby, 1979a; Cooper and Clarkson, 1989; Jeschke *et al.*, 1997a; White, 1997b; White and Broadley, 2001; Peuke, 2010).

Nutrient cycling is of particular importance for the N nutrition of plants. In nitrate-fed barley plants, of the N translocated in the xylem to the shoots, up to 79% was re-translocated in the phloem as reduced N back to the roots; of this, about 21% was incorporated into the root tissue and the remainder cycled back in the xylem to the shoots (Simpson *et al.*, 1982). In young wheat and rye plants, over 60% of the reduced N in the xylem sap cycles within the plant (Cooper and Clarkson, 1989). In wheat throughout ontogenesis, 10–17% of N and 12–33% of S in the xylem sap derived from the fraction recycled in the phloem from shoots to roots (Larsson *et al.*, 1991). Accordingly, in nitrate-fed plants the proportion of nitrate in total N in the xylem sap can be used as an indicator for nitrate reduction in the roots only in plant species in which nitrate reduction is confined to the roots. For plant species that reduce nitrate in both roots and shoots, however, the situation is more complicated (Van Beusichem *et al.*, 1988). In castor bean, for example, about half of the nitrate reduction occurs in the roots. Most of the N reduced in the roots is translocated in the xylem to the shoots, and a considerable portion of this is re-translocated in the phloem to the roots and cycles back in the xylem to the shoots (Jeschke and Pate, 1991a). Thus, at any given

TABLE 3.14 Partitioning, translocation and cycling of K, Na, Mg and Ca in white lupin and castor bean

Parameter	Proportion of total uptake (%)							
	White lupin				Castor bean			
	K	Na	Mg	Ca	K	Na	Mg	Ca
Leaf laminae								
Import via xylem	96	45	33	29	138	11	51	39
Export via phloem	72	33	25	12	93	9	13	2
Roots								
To roots via phloem	59	33	20	9	85	9	15	1
Cycling through roots	39	–[a]	10	–	78	–	7	–

Based on Jeschke and Pate (1991b).
[a]*Could not be quantified.*

moment a substantial proportion of the reduced N in the xylem sap will have already cycled at least once through the plant. This may also hold true for reduced S (Schupp *et al.*, 1991).

The predominant site of nitrate reduction in plants, whether roots or shoots, can also have an important impact on K cycling (Fig. 3.12). Potassium plays an important role as counter-ion for nitrate transport in the xylem (Van Beusichem *et al.*, 1988; Section 2.9). After nitrate reduction in the shoots, charge balance has to be maintained by a corresponding net increase in organic acid anions. As an alternative to their storage in leaf cell vacuoles, organic acids (mainly malate) can be re-translocated in the phloem to the roots together with K as the accompanying cation. After decarboxylation of the organic acids in the roots, K may act again as counter-ion for nitrate transport in the xylem to the shoot. Strong support of this model had been provided by Touraine *et al.* (1990) in soybean, which reduces about 90% of the nitrate in shoots. In these plants, close correlations were found between nitrate reduction in the shoot, re-translocation of K and organic acids (mainly malate) in the phloem, decarboxylation in the roots and release of bicarbonate. As would be predicted from the model, illuminating the shoot results in enhanced release of bicarbonate from the roots, and stem-feeding of K malate induces an increase in net uptake of nitrate and the net consumption of protons by the roots (i.e., bicarbonate release).

3.5 REMOBILIZATION OF NUTRIENTS

3.5.1 General

Import and export of nutrients occur simultaneously during the lifespan of plant organs such as leaves (Table 3.14). As a rule, ageing (senescence) is associated with higher rates of export of nutrients than rates of import and, thus, a decrease in net content, i.e. in amount per organ (Table 3.10). In the literature the terms *redistribution* and *re-translocation* are often used to describe this process. In view of the dynamics of import and export and cycling of nutrients, these terms may lead to some confusion. In the following discussion therefore, this decrease in net content is denoted by the term *remobilization*.

Remobilization is based on a range of different physiological and biochemical processes: (i) utilization of nutrients stored in vacuoles (K, P, Mg, amino-N, etc.), (ii) breakdown of storage proteins (e.g., in vacuoles of paraveinal mesophyll cells of legumes; Klauer *et al.*, 1991), and (iii) breakdown of cell structures (e.g., chloroplasts) and enzymes thereby transforming structurally bound nutrients (e.g., Mg in chlorophyll, micronutrients in enzymes) into a mobile form.

Remobilization of nutrients is important during ontogenesis of a plant at the following stages: seed germination; periods of insufficient supply to the roots during vegetative growth; reproductive growth; and, in perennials, the period before leaf drop.

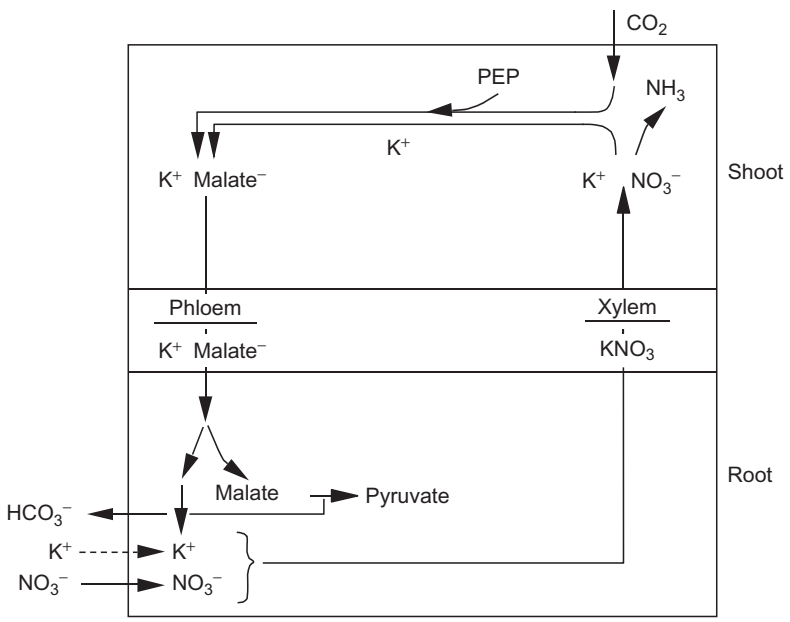

FIGURE 3.12 Model for the circulation of K between root and shoot in relation to nitrate and malate transport based on Ben-Zioni *et al.* (1971) and Kirkby and Knight (1977). PEP = phosphenol pyruvate.

3.5.2 Seed Germination

During the germination of seeds (or storage organs such as tubers), nutrients are remobilized within the tissue and translocated in the phloem and/or xylem to the developing roots and shoots. Consequently, seedlings are able to grow for at least several days without an external supply of nutrients. In seeds, many nutrients (e.g., K, Mg, Ca, Mn, Zn, Fe) are usually present as phytate salts and remobilization of these nutrients, and also of P, is correlated with phytase activity (Lott *et al.*, 1995). In legume seeds, a higher proportion of the nutrients (including Ca) stored in the cotyledons is remobilized (Hocking, 1980a) than, for example, in cucumber (Ockenden and Lott, 1988).

3.5.3 Vegetative Stage

During vegetative growth, nutrient supply to the roots is often either permanently insufficient (as in the case of low soil availability) or temporarily interrupted (when, for example, there is a lack or excess of soil water content). Remobilization of nutrients from mature leaves to areas of new growth is thus of key importance for the completion of the life cycle of plants under such conditions. This behaviour is typical for fast-growing crop species, whereas many wild species simply cease to grow under adverse environmental conditions and, therefore, redistribution of nutrients plays a less important role (Chapin, 1983).

The extent to which remobilization takes place, however, also differs between nutrients and this is reasonably well reflected in the distribution of visible deficiency symptoms in plants. Deficiency symptoms which predominantly occur in young leaves and apical meristems reflect insufficient remobilization. In the latter case, either phloem mobility is insufficient or only a relatively small fraction of the nutrients can be transformed into a mobile form in the fully expanded older leaves.

The extent of remobilization is also important for diagnosis of the nutritional status of plants (Chapter 11). Leaves and other organs that respond to an insufficient supply of a particular nutrient to the roots by rapidly increasing the remobilization of that nutrient are more suitable for foliar (plant) analysis than less responsive leaves or other organs. However, discrepancies do exist in this respect. For example, Scott and Robson (1991) showed that, despite the normally high mobility of Mg in plants, interruption of Mg supply to the roots of young wheat plants resulted in a faster decrease in Mg concentrations in the fully expanded young leaves than in the older leaves. Such a sudden interruption of a nutrient supply to the roots under otherwise optimal growth conditions, however, does not reflect the conditions in the field where nutrient availability would change more slowly. Thus a somewhat different pattern of nutrient remobilization would occur under field conditions. Accordingly, the so-called critical deficiency concentrations of nutrients in shoots of young plants (Chapter 12) obtained by the procedure of sudden interruption of root supply are higher (Burns, 1992) than those from field-grown plants.

3.5.4 Reproductive Stage

Remobilization of nutrients is particularly important during reproductive growth when seeds, fruits and storage organs are formed. At this growth stage, root activity and nutrient uptake generally decrease, mainly as a result of decreasing carbohydrate supply to the roots ('sink competition', Chapter 5). The nutrient concentrations of vegetative parts therefore often decline sharply during the reproductive stage (Fig. 3.13).

The extent of this remobilization depends on various factors, including (i) the specific requirement of seeds, fruits and tubers for a given nutrient, (ii) the mineral nutrient status of the vegetative parts, (iii) the ratio between vegetative mass (source size) and number and size of seeds, fruits or tubers (sink size), and (iv) the nutrient uptake rate by the roots during the reproductive stage. Cereal grains, for example, are characterized by high concentrations of N and P, and low concentrations of K, Mg and Ca, whereas fleshy fruits (e.g., tomatoes) or storage organs (e.g., potato tubers) are high in K but relatively low in N and P (White and Broadley, 2005, 2009).

A typical example of the differences in the extent of remobilization of these nutrients from vegetative shoots is shown in Table 3.15 for pea plants grown under field conditions. The percentage of remobilization of N and P is very high, whereas there is a lack of remobilization of

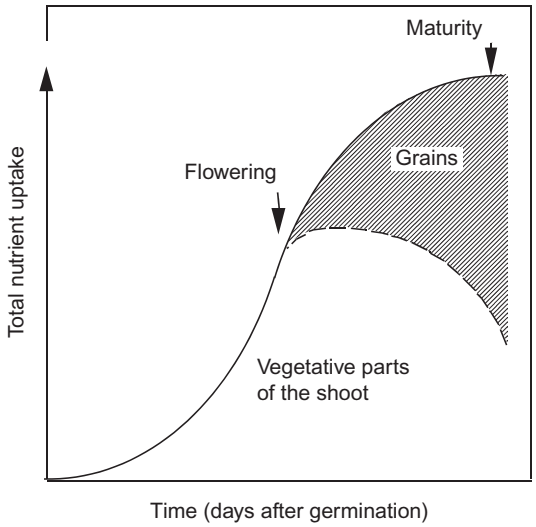

FIGURE 3.13 Schematic representation of the distribution of nutrients in a cereal plant throughout ontogeny.

Mg and Ca; instead, a net increase in these nutrients takes place in the vegetative organs, as has also been shown for soil-grown soybean plants (Wood *et al.*, 1986). Relatively high concentrations of nutrients in the soil solution leading to continuous uptake by the roots and the import of nutrients into leaves after anthesis are the reasons for the lack of remobilization of Mg and Ca. An inherent low capacity for remobilization of Ca is also a contributing factor.

In cereals such as wheat, up to about 90% of the total P in grains can be attributed to remobilization from vegetative parts. Lower proportions are only found when the roots are continuously well supplied with P in sand culture (Batten *et al.*, 1986). For N a comparison of remobilization in different wheat cultivars under field conditions gave an average value for remobilization of 83%, but values ranged from 51 to 91% depending on the total N uptake of the cultivars (Van Sanford and MacKown, 1987).

The remobilization of highly phloem-mobile nutrients can lead to such a rapid decline in their concentration in the vegetative shoots that senescence is induced and plants behave as 'self-destructing' systems. From experiments with soybean, remobilization of nutrients as a senescence-inducing factor has been questioned (Wood *et al.*, 1986; Mauk and Noodén, 1992). However, there are various examples (see also Chapter 5) showing this phenomenon, including the remobilization of P and senescence of the flag leaf in wheat (Batten and Wardlaw, 1987a, b), remobilization of P and disruption of carbon metabolism in source leaves of P-deficient soybean (Lauer *et al.*, 1989b), or for N remobilization and senescence in field-grown bean (Fig. 3.14). Despite the high potential for N_2 fixation of the field bean genotype G5059, the enhanced remobilization (and export) of N from the leaves to the pods and developing seeds soon after flowering strongly limited the rate of photosynthesis of the leaves and, thus, also seed yield of beans grown in the tropics (Fig. 3.14; Lynch and White, 1992).

Another example of strong remobilization during reproductive growth is shown in Table 3.16 for K in two tomato cultivars. The cultivar VF-13L was developed for mechanical harvesting and is characterized by a heavy fruit load combined with an early and uniform maturation. Severe K deficiency symptoms during fruit ripening occur in this cultivar even in plants growing in soils with high K availability. In this genotype, a particularly strong sink competition for carbohydrates between fruits and roots causes a rapid decline in root uptake of K during the period of high K demand for fruit growth. This is also an instructive example of a specific yield limitation induced by a nutrient (Chapter 5) and demonstrates some of the physiological limitations of plant breeding for higher yield.

Remobilization is highly nutrient selective. This selectivity and the corresponding discrimination against elements which are either not essential or required only in very low concentrations is quite impressive, as shown in Table 3.17 for barley grown in saline substrates. In the vegetative shoots, the concentration of K is lower than that of Na and Cl. During remobilization, however, K is highly preferred and the ratio of three elements is reversed in the ears.

TABLE 3.15 Remobilization of nutrients in a pea crop between flowering and ripening

	N	P	K	Mg	Ca
	Content in stems and leaves (kg ha^{-1})				
Harvest					
June 8 (flowering)	64	7	53	5	31
June 22	87	10	66	8	60
July 1	60	7	61	8	69
July 12 (ripening)	32	3	46	9	76
	Increase or decrease after June 22 (%)				
	−63	−73	−30	+10	+21
	In seeds (% of total shoot content)				
	76	82	29	26	4

Based on Garz (1966).

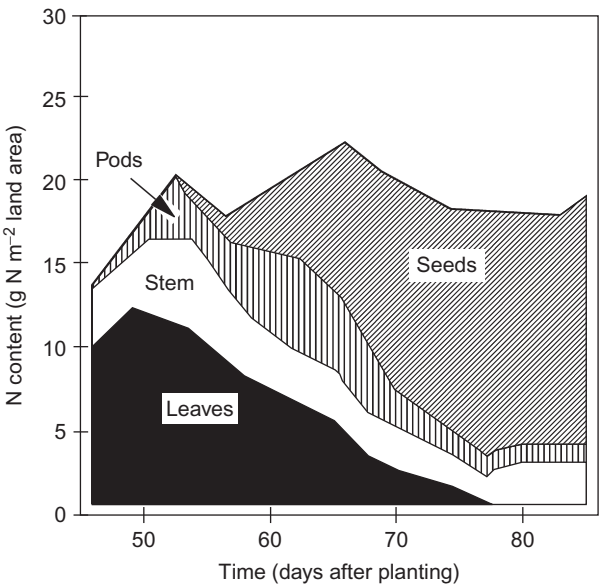

FIGURE 3.14 Nitrogen partitioning in field-grown bean (*Phaseolus vulgaris* L.,) during reproductive growth *(Lynch and White, 1992)*.

An additional step in the selection of nutrients takes place before their entry into the grains.

During the reproductive stage, the degree of remobilization of micronutrients and of Ca is often surprisingly high compared with that during vegetative growth. In white lupin (*Lupinus albus*), for example, up to 50% of micronutrients and 18% of Ca that originally accumulated in the leaves were re-translocated to the fruits (Hocking and Pate, 1978). Substantial remobilization of at least some of the micronutrients also occurs in soil-grown plants as shown in Table 3.18 for soybean. Remobilization of Mo is particularly high, a result which has also been confirmed by Mauk and Noodén (1992).

The extent of remobilization of micronutrients depends strongly on their concentrations in the fully expanded leaves (Loneragan *et al.*, 1976). During grain development in wheat, leaves with a high Cu concentration lost more than 70% of their Cu, whereas leaves of Cu-deficient plants lost less than 20% (Hill *et al.*, 1978). This relationship between leaf nutrient status and degree of remobilization contrasts with that for the highly mobile nutrients, such as Na and K, where a higher proportion is remobilized in deficient plants. The inverse relationship between leaf concentration and the degree of remobilization of micronutrients is caused by the greater proportion of firmly-bound micronutrients (structural constituents, for example in enzymes, cell membranes and cell walls) in deficient leaves. The same relationship has been observed in fruit tree leaves after foliar application of boron (^{10}B). Whereas the foliar-applied B was almost completely exported within the following weeks, the content of soil-derived leaf B (already present before foliar application of the isotope) remained unchanged (Hanson, 1991a).

The extent of remobilization of the micronutrients Cu, Fe and Zn, but not Mn, is also closely related to leaf senescence (Nable and Loneragan, 1984; Himelblau and Amasino, 2001; Waters *et al.*, 2009). This is reflected in the close positive correlation between the remobilization of N and of Cu (Fig. 3.15). The onset of senescence can be accelerated by shading and this is associated with a more rapid remobilization of both N and Cu; in Cu-deficient plants most of the Cu can then be remobilized. Nitrogen deficiency, like shading, also enhances Cu remobilization (Hill *et al.*, 1978). The same is true for Zn (Hill *et al.*, 1979b). These relationships may in part be responsible for the results of field experiments showing a particularly high Cu demand in plants supplied with high levels of N fertilizers which lead to a delay in leaf senescence (Section 6.4).

Remobilization of nutrients requires several steps: (i) mobilization within individual leaf cells, (ii) short-distance transport in the symplasm to the phloem, (iii) phloem loading, and (iv) phloem transport. Discrepancies between high or intermediate phloem mobility (Table 3.9), on the one hand, and low rates of remobilization, on the

TABLE 3.16 Potassium concentration of petioles of two tomato cultivars at different growth stages

Cultivar	Full bloom (third cluster)	Mature green (first cluster)	Pink fruit (first cluster)	Ripe (50% of fruits)
	K concentration (g kg^{-1} dw)			
VFN-8	53.0	68.3	34.8	9.7
VF-13L	52.4	58.6	18.0	4.0

Based on Lingle and Lorenz (1969).

TABLE 3.17 Potassium, Na and Cl concentrations in barley grown in a saline substrate containing 6 mM K^+ and 125 mM Na^+ (as NaCl)

Plant part	Concentration (μmol g^{-1} dw)		
	K	Na	Cl
Vegetative shoot	0.22	2.27	1.52
Ears (rachis, glume, awn)	0.56	0.42	0.43
Grain	0.13	0.04	0.04

Based on Greenway (1962).

TABLE 3.18 Changes in fresh weight and micronutrient concentration of leaf blades of soybean during podfill

	Early-mid podfill (day 64)	Late podfill (day 88)
	Fresh weight (g (3 leaflets)$^{-1}$)	
	1.96	2.57
	Concentration (μg g^{-1} fw)	
Fe	48.9	30.2
Zn	45.1	21.6
Mn	36.3	56.2
Cu	1.01	0.87
B	17.4	24.2
Mo	0.45	0.09

Based on Wood *et al.* (1986).

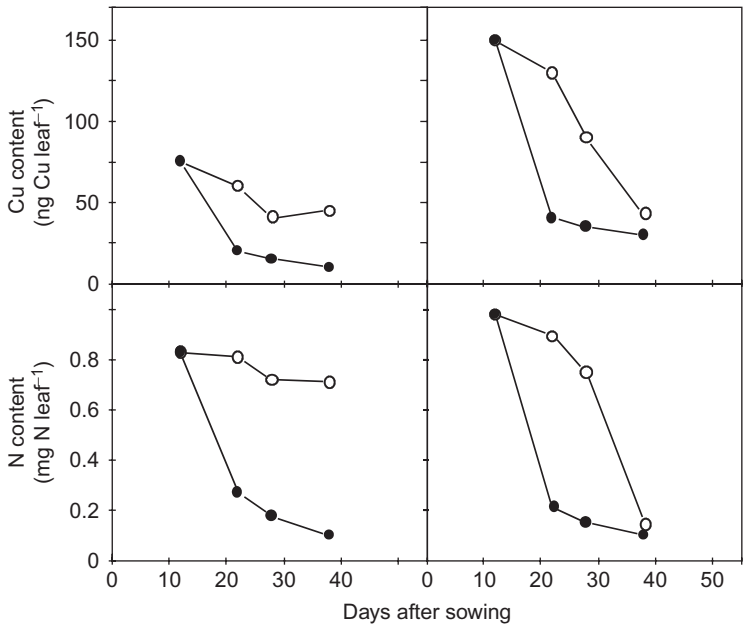

FIGURE 3.15 Copper and N content of the oldest leaf at low (*left*) or high (*right*) Cu supply in ○, unshaded or ●, shaded wheat. *Adapted from Hill* et al. *(1979a).*

other, particularly during the vegetative growth stage, are most likely caused by insufficient mobilization within the leaf cells. A large proportion of micronutrients are incorporated into cell structures and high-molecular-weight organic compounds (e.g., enzymes). This is most likely the reason why, despite the high to moderate phloem mobility of Fe, Zn, Cu, Mo and also B, deficiency symptoms of these micronutrients during the vegetative growth first appear in young leaves and the shoot apex. However, during reproductive growth, seed- and fruit-induced leaf senescence overcomes the most limiting step (mobilization within individual leaf cells) of remobilization for most micronutrients.

The extent of remobilization of nutrients is attracting increasing attention in connection with the selection and breeding of genotypes with high 'nutrient efficiency' (White and Brown, 2010). Better growth on soils with low nutrient availability can be conferred not only by improved acquisition of nutrients, but also by increasing the efficiency of their use at the physiological level. Genotypic variation has been observed in tissue utilization of many elements including those commonly used as fertilizers, such as N, P and K (Hirel *et al.*, 2007; Rengel and Damon, 2008; White and Hammond, 2008; Sylvester-Bradley and Kindred, 2009). In addition, the necessity to increase the concentrations of elements essential for human and animal nutrition in edible produce is driving the selection of genotypes that remobilize a greater proportion of micronutrients from leaves to seeds, fruit and storage organs (Cakmak, 2008; White and Broadley, 2009).

3.5.5 Period before Leaf Drop (Perennials)

Remobilization of nutrients (except Ca and Mn) from the leaves to woody parts is a typical feature of perennial species before leaf drop in temperate climates, and is closely related to the discoloration of leaves in the autumn. As a rule, and similar to annual species, the extent of retranslocation is high for N, K, P and Zn, whereas the leaf concentrations of Ca, Mg, B, Fe and Mn increase until leaf drop (Sanchez-Alonso and Lachica, 1987a). During this period, typical visible deficiency symptoms are often observed, indicating deficiency of a particular nutrient during the growing period. In plants growing on saline substrates, preferential remobilization of certain nutrients (Table 3.17) often gives rise to toxicity symptoms in leaf margins, indicating a further shift toward extreme ion imbalance before leaf drop.

Chapter 4

Uptake and Release of Elements by Leaves and Other Aerial Plant Parts

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SUMMARY

Although leaves and other aerial plant parts are protected by the cuticle and by stomata against the uncontrolled exchange of matter with the environment, elements may penetrate the external plant surface either through the cuticle (solutes) or through stomata (gases and solutes). Gases, such as ammonia (NH_3) and sulfur dioxide (SO_2), may be taken up or released through open stomata. Dissolved nutrients may cross the leaf surface in both directions, resulting both in foliar uptake of solutes originating from atmospheric deposition or foliar fertilization and in leaching of nutrients out of leaves. This chapter gives an overview on the importance of uptake and release of gases through stomata and summarizes the current knowledge about the barrier properties of both the cuticle and stomata against the penetration of solutes. Practical aspects of foliar fertilization and the ecological consequences of nutrient uptake and release are outlined.

4.1 GENERAL

To minimize uncontrolled exchange of matter with the environment, leaf surfaces of terrestrial plants are covered by a cuticle. The cuticle is a non-living, hydrophobic skin with a low permeability for water, gases and solutes. To enable CO_2 uptake, leaves are furthermore equipped with stomata as adjustable apertures in the leaf surface, which optimize the trade-off between CO_2 uptake and water loss of plants. The evolutionary development of the cuticle and stomata as barriers against the uncontrolled exchange of matter was the prerequisite for the colonization of the land surface by higher plants, but these barriers do not fully impede the exchange of both gaseous and dissolved nutrients. Gases, such as ammonia (NH_3) and sulphur dioxide (SO_2), may be taken up or released through open stomata. Dissolved nutrients may penetrate the leaf surface in both directions, resulting both in foliar uptake of solutes originating from atmospheric deposition or foliar fertilization and in leaching of nutrients from leaves.

4.2 UPTAKE AND RELEASE OF GASES AND OTHER VOLATILE COMPOUNDS THROUGH STOMATA

In terrestrial plants, the stomata (Fig. 4.1) are the sites of exchange of gases (mainly CO_2, O_2) with the atmosphere. Their number per mm^2 of leaf surface varies between about 20 in succulents (CAM species), 100–200 in most annual species, and more than 800 in certain tree species (e.g., *Acer montanum*). The stomata are usually more abundant (most annual species) or confined (many tree species, e.g. *Fagus sylvatica*) to the lower (abaxial) leaf surface. Nutrients in the form of gases, such as SO_2, NH_3 and NO_2, also enter the leaves predominantly through the stomata and are rapidly metabolized in the leaves. In recent years, foliar uptake of these gases has attracted much interest as they are major components of air pollution and their uptake can be substantial. Moreover, depending on concentration and the plant species, they can either reduce or enhance plant growth. For many gases, plant surfaces can act both as a source or a sink. The compensation point, i.e. the external gas concentration at which the net flux is zero, depends mainly on the type of gas, plant species, plant nutritional status and climatic conditions.

4.2.1 Volatile Nitrogen Compounds

On a global scale, agriculture is the major source for atmospheric ammonia (NH_3) emissions with an estimated share of 80% in the USA and Europe (Clarisse *et al.*, 2010). Motor vehicles are, due to the implementation of

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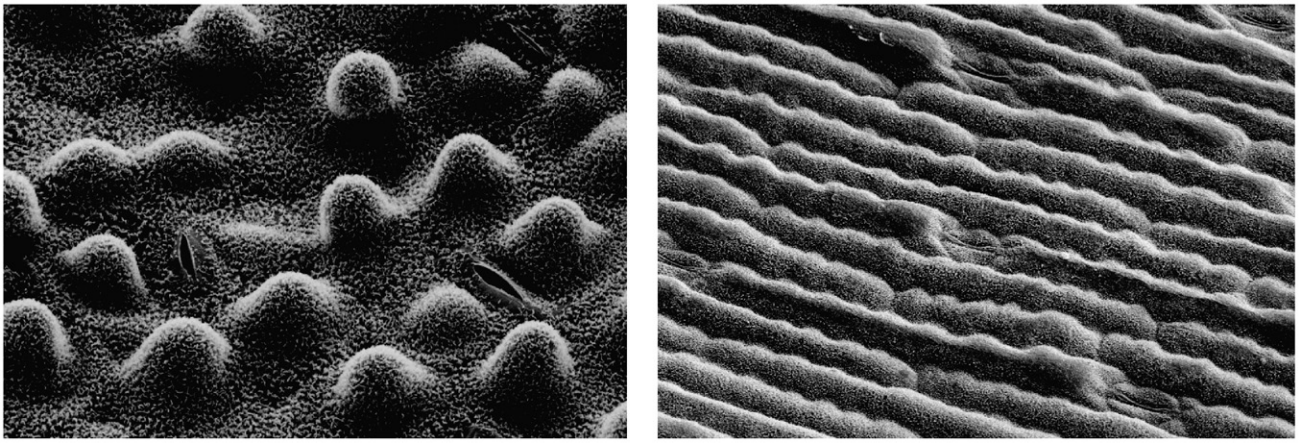

FIGURE 4.1 Scanning electron micrograph of lower (abaxial) leaf surfaces of *Apocynum cannabinum* (left) and *Zea mays* (right). *Courtesy of W. Barthlott*.

catalytic converters, also significant NH_3 sources with an estimated contribution of 5% in the USA in 2000 (EPA, 2003). In areas remote from significant sources, NH_3 concentrations can be quite low, $<35\,\mu g\,m^{-3}$, whereas in agricultural areas they may be about three orders of magnitude higher (Krupa, 2003). Concentrations of NH_3 can reach $20–30\,\mu g\,m^{-3}$ immediately after the application of mineral N fertilizers (Herrmann *et al.*, 2001; Hensen *et al.*, 2009). Organic N fertilizers may cause even higher NH_3 emissions; after sewage sludge application, NH_3 concentrations of up to $100–2400\,\mu g\,m^{-3}$ were measured (Beauchamp *et al.*, 1978). In the past years, research has focused on the development of application methods for organic materials, such as manure and slurry, to minimize the emissions of volatile N compounds (Webb *et al.*, 2010).

Plants may rapidly absorb and utilize NH_3 at concentrations above the compensation point (Table 4.1), which depends on a range of factors, such as plant species, root N supply and the N form (Wichink Kruit *et al.*, 2010). In oilseed rape, the compensation point ranged from 0.8 to $12.2\,\mu g\,m^{-3}$, depending on the level of N root supply (Massad *et al.*, 2009), whereas it was $0.5–2.5\,\mu g\,m^{-3}$ in a grass/clover pasture (Herrmann *et al.*, 2001). In grassland, a high intra-specific diversity of compensation points was observed, resulting in the coexistence of sink species and source species (Mattsson *et al.*, 2009). Under high root N supply, the exposure to NH_3 reduced the biomass production in *Brassica oleracea* and induced a down-regulation of root nitrate uptake, whereas in N-deficient plants shoot growth was increased (Castro *et al.*, 2006). Under controlled experimental conditions exposure to gaseous NH_3 can even fully replace root N uptake (Stulen *et al.*, 1998).

As a result of variations in release by the soil and uptake by the canopy, net concentrations of NH_3 in the field can be subject to distinct diurnal fluctuations. At night a steep concentration gradient of NH_3 can occur within the canopy from the base (soil surface) to the atmosphere above the canopy (the free atmosphere above the vegetation). During the day, however, the NH_3 concentration within the canopy may drop to a very low level as a result of NH_3 uptake through the stomata (Lemon and van Houtte, 1980). Daily uptake rates of NH_3 by leaves in a pasture have been calculated to be between 100 and $450\,g\,N\,ha^{-1}$ (Cowling and Lockyer, 1981), but in certain periods as much as 10–20% of the N in pasture plants can originate from gaseous NH_3 (Whitehead and Lockyer, 1987). Up to 70% of soil NH_3 emissions resulting from N fertilization were directly absorbed and utilized by corn leaves (Bash *et al.*, 2010).

Nitrogen fertilization usually results in a net emission of NH_3 from the canopy. In barley, annual nitrogen losses due to NH_3 emission were calculated to reach $0.5–1.5\,kg\,N\,ha^{-1}$ (Schjoerring *et al.*, 1993). In wheat,

TABLE 4.1 Shoot dry weight (dw), N concentration and uptake of NH_3–N from the atmosphere in Italian ryegrass grown at low soil nitrate concentrations and exposed to different NH_3 concentrations for 33 Days

NH_3 concentration ($\mu g\,m^{-3}$)	Shoot dw ($g\,pot^{-1}$)	Shoot N concentration ($g\,kg^{-1}$ dw)	Total plant N derived from NH_3 ($mg\,pot^{-1}$)
14	6.4	8.9	8
123	7.8	11.4	42
297	9.0	14.7	121
498	10.2	19.2	230
709	10.7	28.0	341

Recalculated from Whitehead and Lockyer (1987).

cumulative N losses of 2.8– 4.4 kg ha^{-1} were reported (Parton *et al.*, 1988). The highest emission rates are usually observed during senescence and grain filling (Mattsson and Schjoerring, 1996). In wheat, losses of NH_3 by the leaves during senescence were reported to reach about 7 kg N ha^{-1}, an equivalent of 21% of the fertilizer N applied to the soil (Harper *et al.*, 1987).

In urban areas, the main source of N oxides, NO_x, i.e. the sum of N oxide (NO) and N dioxide (NO_2), is fossil fuel combustion, whereas in rural areas the use of N fertilizers and the resulting microbial NO production are responsible for substantial NO_x emissions (Williams *et al.*, 1992). Nitrogen oxides can be both emitted from and deposited on plant surfaces (Teklemariam and Sparks, 2006). Nitrogen oxide acts as a gaseous signal in plants (Neill *et al.*, 2003). The compensation points for NO_x were reported to range from <0.2 to 34 μg m^{-3} and to be highly variable (Raivonen *et al.*, 2009). The uptake of atmospheric NO_2 through stomata is linearly related to the external concentration and its metabolism is rapid (Thoene *et al.*, 1991). Long-term exposure of plants to NO_2 can contribute considerably to their N nutrition (Gupta and Narayanan, 1992). Nitrogen dioxide uptake by maize shoots accounted for more than 25% of the soil-emitted NO_x (Hereid and Monson, 2001).

Peroxyacetyl nitrate (PAN) is a toxic organic nitrate formed by photochemical reactions in the atmosphere (Teklemariam and Sparks, 2004). Its uptake is controlled by stomata aperture; it was estimated that 3% of global N oxide emissions could be removed by foliar uptake of PAN (Sparks *et al.*, 2003).

4.2.2 Volatile Sulphur Compounds

Sulphur dioxide (SO_2) is readily taken up through stomata. In sensitive plants, SO_2 can be phytotoxic at relatively low atmospheric concentrations (0.1 mg m^{-3}), but susceptibility to SO_2 shows a high inter-specific variability (van der Kooij *et al.*, 1997). In SO_2 fumigated Norway spruce seedlings (Kaiser *et al.*, 1993), *Arabidopis thaliana* (van der Kooij *et al.*, 1997), and Chinese cabbage (Yang *et al.*, 2006b) S accumulation in the leaves or needles increased linearly with increasing atmospheric SO_2 concentrations. Short-term exposure to high concentrations (50 mg SO_2 m^{-3}) causes a long-term reduction in net photosynthesis (Keller, 1981). With long-term exposure of tobacco plants to moderate concentrations (1.5 mg m^{-3}), SO_2 had a similar effect on growth as sulphate supplied to the roots (Faller, 1972). In short-term experiments with Chinese cabbage exposure to 0.3 mg m^{-3} SO_2 was estimated to be sufficient to cover the S requirements for growth (Yang *et al.*, 2006b).

In oats and oilseed rape grown under field conditions in an S-deficient soil, nearly half of the total S taken up over the vegetation period was derived from atmospheric volatile S compounds (Siman and Jansson, 1976), most probably via SO_2 foliar absorption. However, plants grown in a non-polluted atmosphere and supplied only in the form of sulphate in the soil also release substantial amounts of volatile S compounds through the stomata (Table 4.2). The main component found in this experiment was SO_2 and its emission increased with the sulphate concentration in the soil. For oats and oilseed rape, emissions of volatile S compounds occur within 35 days after the onset of growth and vary between 0.2 and 2–3 kg S ha^{-1} depending on whether the plants were grown in a soil with low or high sulphate concentration (Siman and Jansson, 1976).

TABLE 4.2 Relation between soil sulphate concentration and S concentration of, and volatile S emissions by, needles of Norway spruce

	Sulphate (mg SO_4 − S kg^{-1} soil)		
Parameter	97	129	181
Total S (mg g^{-1} needle dw)	1.0	0.9	1.2
H_2S emission (nmol mol^{-1} H_2O)	0.9	1.1	1.0
SO_2 emission (nmol m^{-2} (2 h)$^{-1}$)	4.1	8.8	10.4

Based on Rennenberg *et al.* (1990).

In alfalfa, the emissions of volatile S compounds follow a distinct diurnal rhythm with maximal rates occurring around midday (Grundon and Asher, 1986). Both the amounts and the spectrum of the emitted volatile S compounds vary between plant species, and in the case of rape may represent up to 1% of the total S in the plant per day. It was estimated that in cotton between a few hundred grams and a few kilograms S per ha are emitted during the growing season (Grundon and Asher, 1988).

Uptake and release of hydrogen sulphide (H_2S) by leaves, which follows a distinct diurnal pattern, is closely related to the stomatal aperture (Winner *et al.*, 1981; Rennenberg *et al.*, 1990). Hydrogen sulphide is toxic to sensitive plant species such as spinach even at concentrations below 0.7 mg m^{-3} (DeKok *et al.*, 1989). Below toxic concentrations and under low S supply to the roots, foliar H_2S uptake may improve the S status of plants; under certain conditions it may even fully replace root uptake (DeKok *et al.*, 1997). Foliar uptake of H_2S can reduce root uptake of sulphate suggesting the existence of coordinated shoot to root signals (Westerman *et al.*, 2001). It has also been proposed that in plants H_2S may act as a signalling

molecule (Zhang *et al.*, 2010). Plants may also release H_2S and other S-containing gases into the atmosphere (Rennenberg *et al.*, 1990), with average annual release of 2–3 kg H_2S ha^{-1} (Schröder, 1993). It has been suggested that the emission of volatile S may be a mechanism to dispose of excess S taken up by the plants. Release of H_2S can also be part of a defence mechanism against pathogen attack, the so-called 'sulphur-induced resistance' (Bloem *et al.*, 2005) or 'sulphur-enhanced defence' (Rausch and Wachter, 2005).

Plants are also net sinks for other volatile S compounds, such as carbonyl sulphide (COS) and carbon disulphide (CS_2) (Xu *et al.*, 2002). The uptake of COS is closely correlated with CO_2 uptake because both involve the conversion by carbonic anhydrase in the plants (Protoschill-Krebs *et al.*, 1996). The measurement of COS fluxes between atmosphere and vegetation can therefore be used for the large-scale estimation of CO_2 uptake and photosynthesis (Campbell *et al.*, 2008; Stimler *et al.*, 2010).

4.3 UPTAKE OF SOLUTES

4.3.1 General

Foliar-applied nutrients may penetrate the leaf surface via both the cuticle and stomata, and the relative importance of the pathways is still under debate. There is evidence that both pathways can be of equal importance (Eichert and Goldbach, 2008), but this also depends on the properties of the compound under consideration (e.g., water solubility and size) and of the specific leaf surface (e.g., wettability, composition of the cuticle, stomata density).

The penetration of leaf surfaces by solutes is a passive process driven by the concentration difference between the surface and the leaf interior. Therefore, the frequently used term foliar 'uptake' is strictly speaking inappropriate because this implies an active role of the plant. Nevertheless, because of its widespread use this denotation will be also used in this chapter. Furthermore, uptake into the leaf has to be separated from the subsequent uptake of the substances into the leaf cells. Both processes may be affected by similar controlling factors, such as light or temperature, but since there is no strict feedback loop between solute uptake rates into the leaf cells and uptake rates through the leaf surface, this chapter will exclusively focus on the initial process of leaf penetration.

In aquatic plants the leaves, not the roots, are the main sites of nutrient uptake. In terrestrial plants, on the other hand, the uptake of solutes by the surface of leaves and other aerial parts is severely restricted by the outer wall of the epidermal cells and the overlaying cuticle. The principal structure of the outer epidermal wall is shown schematically in Fig. 4.2, and in Fig. 4.3 in an example of a cross-section through the leaf epidermis.

FIGURE 4.2 Schematic diagram of the different layers of a typical outer epidermal wall. The epidermal cells are covered by the cuticle. The cuticle consists of the cutin matrix (light grey) with embedded waxes (crosses). The lower *cuticular layer* impregnates the epidermal cell wall (dark grey) and thus contains significant amounts of cellulose and pectin. The outermost *cuticle proper* is free of cell wall material.

4.3.2 Structure of the Cuticle

The surface of plants is covered by the cuticle, a bio-polymer synthesized by epidermal cells (Pollard *et al.*, 2008). Structure and composition of the cuticle vary greatly among plant species, varieties, organs and developmental stages (Heredia-Guerrero *et al.*, 2008) and are in addition affected by environmental conditions during development.

The cuticle consists mainly of cutin, a polyester matrix of polymerized long-chain fatty acids in which waxes are embedded (intracuticular waxes) (Pollard *et al.*, 2008). Waxes are a mixture of hydrophobic compounds mainly composed of aliphatic lipids. The cuticle is covered by epicuticular waxes which are often well and typically structured (Barthlott *et al.*, 1998). Variable amounts of polysaccharide fibrils and pectin lamellae may extend from the epidermal cell wall, binding the cuticle to the underlying tissue (Jeffree, 2006). As a consequence, two cuticular layers can be distinguished: the inner layer (cuticular layer) which contains polysaccharides from the epidermal cell wall, and the outermost layer (cuticle proper) which is free of cell wall extensions (Fig. 4.2). Due to this layered structure, the chemical and physical properties of the cuticle differ between outer and inner surfaces, a distinct gradient occurring from the hydrophobic (lipophilic) outer surface to a more hydrophilic inner surface of the cutinized layer.

The cuticle has diverse functions. A major function is to protect the leaf from excessive water loss by transpiration. The control of water economy in terrestrial higher plants by the stomata is dependent on the remaining surface of the plant having very low hydraulic conductivity. The other main function of the cuticle is to protect the leaf against excessive leaching of inorganic and organic solutes by rain. Nutrients and other solutes entering the leaves via the xylem are in the apoplasm of the leaf tissue, therefore a 'waterproof' barrier is required to act as an apoplastic boundary, thereby playing a similar role to that of the Casparian band in the endodermis of the roots (Chapter 2).

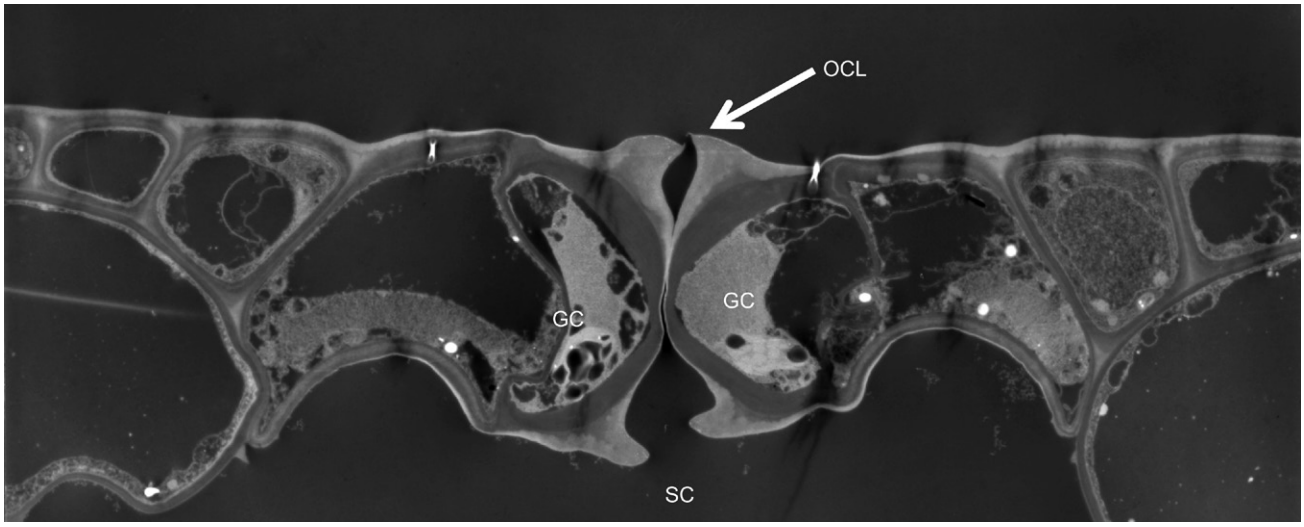

FIGURE 4.3 Cross-section of the epidermis of a *Heliconia choconiana* leaf. GC: guard cell, OCL: outer cuticular ledge, SC: substomatal cavity. The cuticle can be identified as a light-coloured layer covering the epidermal cells including the GC. *Courtesy of W. Barthlott.*

The relative importance of these two main functions of the cuticle depends on climatic conditions (arid zones versus humid tropics). In addition, the cuticle is involved in temperature control, optical properties of leaves and plays a role in defence against pests and diseases (Chapter 11).

4.3.3 Nutrient Uptake through Cuticles

The hydrophobic nature of the cuticle makes it an effective barrier against the penetration by hydrophilic, polar solutes, whereas lipophilic molecules may penetrate cuticles at much higher rates (Schönherr, 2006). The penetration of cuticles by lipophilic molecules is described by the solution-diffusion model (Riederer and Friedmann, 2006). This model predicts penetration rates of a molecule from its solubility and mobility in the cuticle according to Eq. (4.1) (Schreiber, 2006):

$$P = D * K/\Delta x \tag{4.1}$$

where P is the permeance ($m\,s^{-1}$), D ($m^2\,s^{-1}$) is the diffusion coefficient in the cuticle and Δx (m) is the path length of diffusion.

On a molecular level, solubilization and diffusion of a molecule in the cuticle can be viewed as moving into and between voids in the three-dimensional cutin network (Schönherr, 2006). The solubility parameter takes into account the chemical affinity between the permeating molecule and the cutin matrix, whereas the diffusion coefficient is determined by the size of the molecule as compared to the size of the voids in the cutin matrix. The cuticle is highly size selective (Buchholz *et al.*, 1998) because the size of voids, which is in the same order of magnitude as the solutes on the leaf surface, hinders diffusion and sets the size limits for penetrating molecules. There is evidence that the size distribution of voids follows a log–normal distribution (Baur, 1997).

With a few exceptions, such as boric acid or urea, foliar fertilizers are applied as ions which have a very low solubility in the cuticle (Schönherr, 2000, 2006). According to the solution-diffusion model, this should result in very low cuticule penetration rates. However, both laboratory studies using isolated cuticles and greenhouse or field studies showed that ion uptake can be substantial. To solve this apparent contradiction, a second penetration pathway for hydrophilic solutes, named 'polar pores', was postulated. These 'pores' are thought to be created by clusters of water molecules sorbed by the cuticle (Tyree *et al.*, 1990), both from the inner, epidermal, side and from atmospheric water vapor absorbed from the other side. Under dry atmospheric conditions, only small amounts of water will be absorbed by the outer cuticle, and hence less functional 'pores' traversing the cuticle will exist (Fig. 4.4). This hypothesis is supported by the observation that penetration rates of ions across isolated cuticles strongly increased with increasing relative humidity (Schönherr, 2000, 2001, 2002; Schönherr and Luber, 2001). Cuticles may also have different size selectivities for lipophilic and hydrophilic molecules, which was taken as further evidence for the existence of two spatially separate cuticular penetration pathways for these classes of compounds (Schönherr and Schreiber, 2004; Schreiber, 2005). In other studies, however, such differences were less evident (Popp *et al.*, 2005). To date, it is still debated if polar 'pores' as an independent pathway for hydrophilic solutes actually exist. Alternatively, it was suggested that the dependence of ion penetration on cuticle hydration may simply be caused

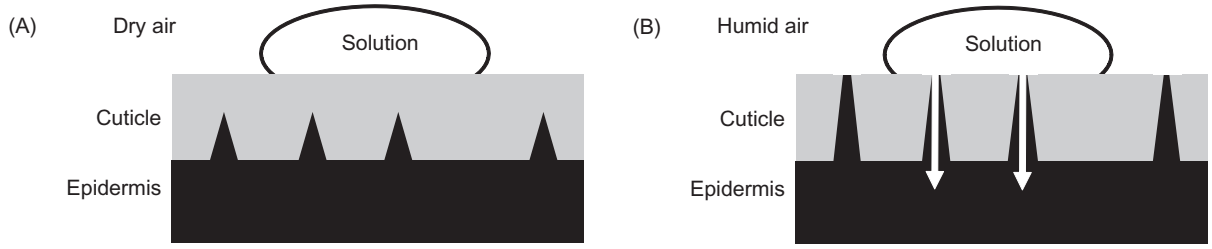

FIGURE 4.4 Schematic diagram of the effect of air relative humidity on cuticular penetration of polar solutes according to the 'pore model'. In dry air (A), water supplied by epidermal cells is mainly sorbed in the lower cuticular layer, whereas water sorption in the outermost layer, the cuticle proper, is low. In humid air (B), water sorption in the cuticle proper increases, water clusters eventually traverse the cuticle, and 'pores' emerge which enable the penetration of polar solutes through the cuticle.

by the resulting increase in overall hydrophilicity of the cuticle which will, in turn, increase the solubility of polar solutes (Fernández and Eichert, 2009). In this case, the solution-diffusion model may still be valid and can be used to predict the permeability of cuticles for both hydrophilic and lipophilic solutes.

Irrespective of the nature and location of the hydrophilic cuticule penetration pathway, the question arises as to the size relations of the uptake rate in relation to the size of the permeating molecule. The diameter of hydrated ions such as metal cations, NO_3^- or NH_4^+, is well below 1 nm, whereas organic compounds such as sugars or chelates may be larger. Early estimations of 'pore' sizes in de-waxed isolated citrus cuticles yielded diameters of about 1 nm (Schönherr, 1976), and a similar value was reported for isolated ivy cuticles (Popp *et al.*, 2005). Such pore sizes would exclude the penetration of larger molecules. It has to be considered, however, that these values represent the average pore size which implies that some pores will be larger. In intact poplar or coffee leaves, average diameters of 4–5 nm were found (Eichert and Goldbach, 2008).

4.3.4 Penetration of Stomata

Stomata are adjustable apertures which enable the controlled entry of CO_2 into the leaf mesophyll required to sustain photosynthesis while water loss via transpiration is minimized. Stomata were initially assumed to be involved in foliar penetration of solutes via mass-flow into the leaf mesophyll. However, stomata are protected against capillary infiltration of aqueous solutions due to their specific architecture (Schönherr and Bukovac, 1972). Stomata penetration by mass-flow can only be induced by certain surface active compounds, such as organosilicone surfactants (Field and Bishop, 1988; Zabkiewicz *et al.*, 1993) which lower the surface tension of the foliar-applied solution below a critical threshold value. Thus mass-flow of foliar-applied solutions is negligible in most cases; however, many studies indicate a promoting effect of stomata on foliar solute penetration. Foliar uptake rates into hypostomatous leaves were higher through the lower (abaxial) leaf sides which have stomata than though the upper (adaxial) side lacking them (Kannan, 1969; Knoche and Bukovac, 1992; Eichert and Goldbach, 2008). Other studies reported positive correlations between uptake rates and stomata density (Schönherr and Bukovac, 1978; Eichert and Burkhardt, 2001) or stomata aperture (Sands and Bachelard, 1973; Eichert *et al.*, 1998; Schlegel and Schönherr, 2002; Fernández *et al.*, 2005; Schlegel *et al.*, 2006).

The promoting effect of stomata on penetration rates may be due to the higher permeability of the peristomatal cuticle covering the guard cells (Fig. 4.5). Specific morphological structures of the guard cell cuticle, the cuticular ledges (see Fig. 4.3), could be preferred entry points for foliar-applied solutes, with their permeability increasing with increasing stomatal aperture (Schönherr and Bukovac, 1978), but evidence for this is still lacking (Fernández and Eichert, 2009).

There is increasing evidence that penetration of solutes may occur directly through the stomatal pores without passage of the cuticle (Fig. 4.5). Hydrophilic particles (43 nm diameter) suspended in water can enter leaves through stomata by diffusion along the walls of the pore (Eichert *et al.*, 2008). However, only a small portion, usually less than 10% of stomata covered by a foliar-applied droplet of solution, are penetrated (Eichert and Burkhardt, 2001; Eichert and Goldbach, 2008). It is therefore likely that the penetrability of stomata is not a native, *a priori* property of stomata but that it is acquired *a posteriori*, possibly by modifications of the, initially rather hydrophobic, pore wall cuticle, due to the effect of, for example, deposited hygroscopic particles, microbes growing in the stomata chamber or salts ascending the pore, rendering it more wettable (Eichert *et al.*, 1998; Eichert and Burkhardt, 2001).

4.3.5 Role of External Factors

4.3.5.1 Environmental Effects on the Barrier Properties during Ontogenesis

The environmental conditions during plant growth have a direct influence on the leaf surface in terms of cuticle

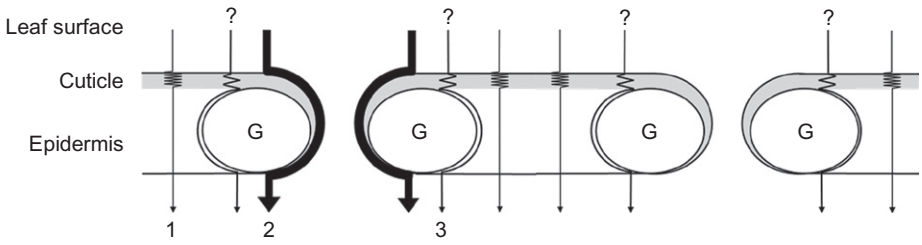

FIGURE 4.5 Schematic diagram of solute penetration across the leaf surface. 1, penetration of the cuticle, 2, stomata penetration, 3, preferential penetration of the peristomatal cuticle as suggested by some authors (see text). Thickness indicates the relative permeabilities of the pathways. Note that not all stomata are penetrable. Not to scale. G: guard cell.

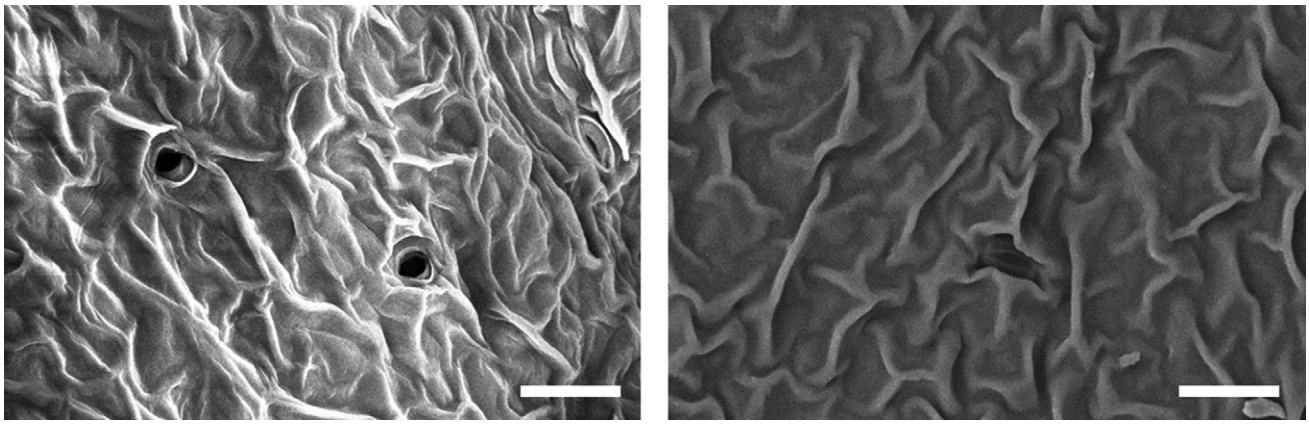

FIGURE 4.6 Lower (abaxial) leaf surfaces of *Glycine max* grown under sufficient (*left*) or deficient (*right*) B supply. Bars = 20 µm.

thickness or amount and composition of epicuticular waxes (Bird and Gray, 2003; Koch *et al.*, 2006), which will, in turn, affect wettability and permeability. Shading during plant development may induce a decrease in the amount of wax per leaf area (Whitecross and Armstrong, 1972; Baker, 1974), whereas high temperatures can modify the morphology and composition of epicuticular waxes (Riederer and Schneider, 1990; Welker and Haas, 1999). Relative humidity affects the amount of wax per leaf area and wax crystal morphology (Baker, 1974; Koch *et al.*, 2006).

In general, young, partially expanded leaves are more penetrable than fully expanded leaves (Sargent and Blackman, 1962). However, the barrier function of the surfaces of older leaves can be modified by passive, for example accumulation of injuries and pathogens, and active processes, i.e. regulated responses to the environment (Jordan and Brodribb, 2007; Munné-Bosch, 2007). Leaf surface injuries caused by, for example, abrasion by particles or microorganisms can have a strong effect on the permeability of leaves.

Nutrient deficiency may also affect structure and anatomy of the leaf surface. Iron chlorosis decreased the size of stomatal pores and reduced stomata conductance, the cuticle weight per unit surface, and the concentrations of soluble cuticular lipids (Fernández *et al.*, 2008a). Manganese deficiency increased water permeability and altered light reflectance of barley leaves, indicating effects at the leaf epidermal level (Hebbern *et al*., 2009). Boron deficiency can alter stomata morphology and functionality (see Fig. 4.6; Rosolem and Leite, 2007). In olive trees, K deficiency decreased the rate of uptake of foliar-applied K fertilizer (Restrepo-Díaz *et al*., 2008)

4.3.5.2 Humidity Effects on Concentration

Foliar-applied solutions are usually rather dilute, not in equilibrium with the water potential of the atmosphere and will therefore evaporate. This will result in droplet drying, accompanied by an increase in solute concentrations. Depending on external air relative humidity (RH) and the type of solute, solutions may dry out completely or remain liquid. For each solute, there is a certain threshold RH above which the solution remains liquid and below which it dries out. This threshold is called deliquescence relative humidity (DRH) or deliquescence point (Burkhardt and Eiden, 1994). At and just above the DRH, solute concentrations are maximal. With increasing RH above the DRH, concentrations will decrease. Since the concentration gradient between the solution on the leaf surface and the leaf interior is the

driving force of foliar uptake, RH will have a strong impact on penetration rates. Moreover, at RH below DRH uptake will cease due to immobilization on the leaf surface. The weather conditions and the diurnal rhythm of RH and temperature will result in fluctuating concentrations, making the prediction of uptake rates difficult under field conditions.

4.3.5.3 Humidity Effects on the Permeability of the Leaf Surface

Increasing RH not only decreases the solute concentration on the leaf surface due to dilution by water absorbed from the atmosphere, but also increases water sorption of the cuticle and thus the permeability for polar solutes. An increase of RH from 50% to saturation may increase the permeability of isolated cuticles for ions by two orders of magnitude (Fernández and Eichert, 2009), indicating that the effect of cuticle hydration overrides the dilution effect.

On the other hand, air humidity, more precisely water vapour deficit, also affects stomata aperture, which is correlated with foliar penetration rates. Control and adjustment of stomata aperture, however, is a complex process governed by a range of additional interacting factors, such as soil water availability, water status of the plant and irradiation. Therefore, no strict relationship between RH and aperture exists and the stomata penetration pathway will thus probably be less RH dependent than the cuticular pathway.

4.3.5.4 Adjuvants

Adjuvants are chemicals added to foliar-applied solutions to optimize the penetration process. The usually low uptake rates of ions through cuticles can be substantially increased by the use of such substances. In laboratory studies, surface active adjuvants increased the penetration of Ca^{2+} by a factor of six (Schönherr, 2000). Some specific adjuvants (plasticizers) may increase the fluidity of the cuticle, thereby enhancing the penetration rates of solutes (Schönherr, 1993; Schönherr and Baur, 1994). Surface-active adjuvants will also increase stomata penetration by improving the contact area between the foliar-applied solution and the leaf surface. Selection of an appropriate adjuvant is often crucial for improving penetration rates, and this will depend on a range of factors such as properties of the target surface, the compound to be applied and its concentration.

4.4 FOLIAR APPLICATION OF NUTRIENTS

4.4.1 General

Foliar sprays are widely used in agricultural production as an alternative or complementary strategy to soil fertilization. Theoretically, this method of application is more targeted and environmentally friendly than soil treatments, since nutrients are directly delivered to sink organs and there is a lower risk of environmental contamination due to, for example, nutrient leaching. Plant responses to elements supplied via foliar sprays are normally more rapid than to soil treatment. However, complete reliance on foliar sprays to meet the nutrient demand cannot be achieved in commercial plant production, since crop yields and quality may be negatively affected, particularly in the case of macronutrients (Johnson *et al.*, 2001).

Appropriate timing and management of foliar sprays according to plant phenology is key to improving the performance of foliar treatments as a strategy to increase yields and quality of horticultural crops (Southwick *et al.*, 1996; Lovatt, 1999).

The efficacy of a foliar fertilizer is determined by many environmental, physico–chemical and physiological factors associated with the plant and the properties of the spray formulation, which are currently not fully understood (Fernández and Eichert, 2009). An array of problems limiting the effectiveness of foliar fertilization may occur in response to foliar sprays, such as:

1. Limited leaf wetting and spreading of the applied nutrient solution when treating hydrophobic leaves and when applying unformulated sprays (i.e., in absence of adjuvants).
2. Spray run-off due to low solution retention rates.
3. Washing off by rain following the application of nutrient sprays.
4. Low penetration rates of the applied nutrient solution due to, for example, the nature of the leaf surface treated or to the effect of environmental conditions on solution physico-chemistry and plant performance (e.g., stomata opening or closure).
5. Rapid drying of spray solutions, particularly at low relative humidity and high temperature.
6. Limited rates of distribution of certain nutrients such as Ca or Fe, from the sites of foliar uptake to other plant parts due to low phloem mobility.
7. Limited amounts of foliar-applied macronutrients to meet the plant demand that can be supplied by one foliar spray without inducing phytotoxicity; on average, 1% concentration (at $4001ha^{-1}$), an exception being urea, which can supplied up to a 10% concentration, normally prior to leaf senescence.
8. Leaf damage (necrosis and 'burning'; see below).
9. Foliar fertilization may modify phyllosphere microbial populations and change the rate of spore germination and colony growth of pathogens such as *Erysiphe graminis* (powdery mildew), as observed after the application of urea sprays (Gooding and Davies, 1992).
10. Potential nutrient imbalances when single nutrient sprays are supplied to the plants.

TABLE 4.3 Urease activity, leaf tip necrosis and urea and ammonia concentrations in soybean leaves after foliar application of 15 mg urea leaf^{-1} with or without the urease inhibitor phenylphosphorodiamidate (Ppd)

PPD (μg per leaf)	Urease activity (μmol N h^{-1} g^{-1} fw)	Leaf tip necrosis (%)	Concentration (g kg^{-1})	
			Urea	Ammonia
0	16.1	1.3	1.0	0.31
75	5.8	5.7	5.5	0.17

Based on Krogmeier *et al.* (1989).

The occurrence of leaf injuries caused by foliar fertilization is a serious risk when applying foliar sprays. Different degrees of damage ranging from the appearance of necrotic spots to complete defoliation can occur with excessive nutrient concentrations, hygroscopic active ingredients (e.g., mineral salts with low points of deliquescence) or in the presence of surfactants, which significantly increase the rate of penetration of the applied nutrients and are often phyto-toxic when applied at high concentrations (Kannan and Chamel, 1986; Fernández and Eichert, 2009; Kraemer *et al.*, 2009; Burkhardt, 2010). Since plant cuticles are poly-electrolytes (Schönherr and Huber, 1976) the pH of the spray solution may affect the rate of penetration and the degree of phyto-toxicity of the foliar treatment.

Leaf tip necrosis following foliar application of urea is not caused by ammonia formed through hydrolysis of urea by plant urease. As shown in Table 4.3, inhibiting plant urease and thus decreasing ammonia concentrations in the leaves increased the incidence of leaf tip necrosis rather than decreasing it. Thus, accumulation of urea in the leaf tissue is the causal factor for the leaf tip necrosis, a result which is of particular interest in view of the function of nickel as a metal component of urease (Krogmeier *et al.*, 1991).

4.4.2 Practical Importance of Foliar Application of Nutrients

Although plant leaves are organs specialized in capturing light and CO_2, their capacity to absorb water and nutrients has long being recognized and exploited in agriculture (Fernández and Eichert, 2009). Despite the constraints described above, foliar fertilization is of increasing importance in agricultural production worldwide.

Either used as a supplement for soil applications or under conditions of limited soil nutrient availability, the application of foliar sprays can help to preserve crop yields and quality, with low environmental impact (Zhang and Brown, 1999; Fageria *et al.*, 2009; Fernández-Escobar *et al.*, 2009). Traditionally, foliar fertilization was used to correct nutrient deficiencies (Weinbaum, 1988; Kannan, 2010); however, there is an increasing trend to using nutrient sprays in the absence of deficiency symptoms, particularly for elements with limited phloem mobility such as Ca, B, Zn, Fe or Mn (Fernández and Eichert, 2009).

Foliar fertilization has great practical utility under certain conditions which will be briefly described below.

4.4.2.1 Low Nutrient Availability in Soils

Certain soil properties can limit element solubility and uptake of nutrients by plant roots. For example, approximately 50% of the world's potentially arable land is acidic with widespread occurrence of P deficiency (Zheng, 2010; see Chapter 8). Another example is the limited solubility of several micronutrients (e.g., Fe, Mn, Cu and Zn) in high pH, calcareous soils (see Chapter 9).

The application of micronutrient sprays to control or avoid the occurrence of micronutrient deficiencies under conditions of limited plant availability in the soil is one of the most important practical uses of foliar fertilization worldwide (Fageria *et al.*, 2009; Kannan, 2010). However, the effectiveness of foliar micronutrient treatments may vary significantly among plant species and also in relation to the active ingredients (e.g., salts, complexes or chelates; Zhang and Brown, 1999; Wójcik, 2004; Fernández and Ebert, 2005).

In high pH, calcareous soils found in many arid and semi-arid areas of the world, the solubility of Fe is very low and iron deficiency-chlorosis (lime-induced chlorosis) is a common physiological disorder affecting plants (Nikolic and Römheld, 2003). Foliar Fe sprays are effective in re-greening chlorotic leaves, particularly when adjuvants are added to the formulation (Neumann and Prinz, 1974; Levy and Horesh, 1984; Fernández and Ebert, 2005). Iron sprays have been shown to restore some leaf physiological parameters such as the rate of photosynthesis or transpiration (Eichert *et al.*, 2010). However, there may also be problems due to the limited distribution within different plant parts and the metabolic functionality of the applied Fe (Fernández and Ebert, 2005; Fernández *et al.*, 2008b).

Manganese foliar sprays are also widely used and can be effective in overcoming Mn deficiency (e.g., Dordas, 2009; see Table 4.4 as example). However, after foliar application, Mn remobilization to other plant parts is low due to its poor phloem mobility (e.g., Gettier *et al.*, 1985; Papadakis *et al.*, 2007). Zinc foliar sprays are commonly applied to horticultural crops worldwide, often before leaf fall in the case of fruit trees (Castagnoli *et al.*, 1990; Zhang and Brown, 1999).

4.4.2.2 Dry Topsoil

In semi-arid regions, a lack of available water in the topsoil and a corresponding reduction in nutrient availability

TABLE 4.4 Rates of Mn fertilizers (as $MnSO_4$) required for optimal yield of soybean grown in Mn-deficient soil

Mode of Mn fertilizer application	Requirement for optimal yield (kg Mn ha^{-1})
Broadcast	14
Banded	3
Foliar sprays (2 ×)	0.1

Based on Mascagni and Cox (1985).

TABLE 4.5 Growth parameters and grain yield in wheat with Cu supplied as Cu-sulphate via soil (2.5 and 10 kg Ha^{-1}) or foliar (2 kg Ha^{-1}) application once (at stem extension) or twice (at stem extension and at booting)

Treatment	Ears (m^{-2})	Grains per ear	Grain yield (g m^{-2})
No application	37	0.14	0.03
Soil (kg ha^{-1})			
2.5	29	2.3	1.0
10	59	2.9	2.3
Foliar (2 kg ha^{-1})			
At stem extension	64	17.1	14.0
At stem extension and at booting stage	127	52.7	79.7

Based on Grundon (1980).

during the growing season are common phenomena. Even though water may still be available in the subsoil, nutrition becomes the growth-limiting factor. For example, soils inducing chronic micronutrient deficiencies are often high pH, calcareous soils occurring in seasonally dry climates (Ascher-Ellis *et al.*, 2001). Under such conditions, a normal practice to preserve crop yield and quality is to complement soil micronutrient applications with foliar sprays (Ascher-Ellis *et al.*, 2001).

Under dry conditions, soil treatments may be less effective than foliar nutrient sprays, as shown in an example where Cu was applied to wheat under field conditions in a semi-arid region of Australia (Table 4.5). However, a lower uptake of foliar-applied K was observed in water-stressed compared to irrigated olive trees (Restrepo-Díaz *et al.*, 2008).

4.4.2.3 Decrease in Root Activity during the Reproductive Stage

As a result of sink competition for carbohydrates, root activity and thus nutrient uptake by the roots decline with the onset of the reproductive stage. Foliar sprays containing nutrients can compensate for this decline (Trobisch and Schilling, 1970; Weinbaum, 1988). In legumes that rely on symbiotic N_2 fixation, sink competition for carbohydrates between developing seeds and root nodules may cause a marked decrease in the rate of N_2 fixation (Chapter 7). Often, although not always (Neumann, 1982), foliar application of N supplied as, for example, urea after flowering and at pod filling can be quite effective in increasing the yield of nodulated legumes, as shown in an example for soybean in Table 4.6. The application of urea both alone and particularly in combination with a sucrose-containing surfactant (SFE) strongly increased yield and N content of the plants (Table 4.6). By labelling urea with ^{15}N, it could be shown that most of this increase in N was due to enhanced N_2 fixation, as a result of delayed leaf senescence, thus prolonging the supply of carbohydrates to the roots and nodules (Ikeda *et al.*, 1991). Similarly, in wheat grown in a P-deficient soil, foliar application of P after anthesis can considerably delay senescence of the flag leaf and, thus, increase the leaf area duration (Batten and Wardlaw, 1987b). Boron plays a crucial role in plant reproduction, thus it is important to ensure adequate B status to preserve crop yield and quality (Brown *et al.*, 2002; see Chapter 6). The mobility of B in plants is species dependent (Brown *et al.*, 2002). In *Ricinus communis*, B mobility was increased when the transpiration rate (i.e., the xylem flow) was low (Eichert and Goldbach, 2010). In sunflower, yields were improved by application of B sprays before flowering (Asad *et al.*, 2003). In fruit trees species with high B phloem mobility, foliar application of B in autumn is a very effective procedure for increasing the B concentrations in reproductive and vegetative tissues, and improving fruit set in the following season (Nyomora *et al.*, 1997; Christensen *et al.*, 2006).

4.4.2.4 Increasing the Protein Content of Cereal Grains

In cereals such as wheat, the protein content of the grains and thus their quality for certain purposes (e.g., baking, animal feeds) can be increased by the foliar application of N at the later stages of growth (e.g., Kara and Uysal, 2009). Nitrogen supplied at these stages is rapidly re-translocated from the leaves and directly transported to the developing grains (Section 8.2.5). Although the recovery rates of N from foliar sprays with urea are usually quite high, for example in wheat about 70% (Powlson *et al.*, 1989), losses by volatilization of NH_3 also occur from the leaves and can be higher than with soil application. There are contradictory reports on the levels

TABLE 4.6 Dry matter production and N content of nodulated soybean without or with foliar application of urea (1%) alone or in combination with 0.1% surfactant (sucrose mono- and diester of long-chain fatty acids) at flowering and podfill

Treatment	Dry matter (g plant^{-1})		N content (mg plant^{-1})		
	Seeds	Total	Seeds	Total	From urea
Control	4.6	21.4	234	342	–
1% urea	10.2	38.1	518	680	99
1% urea + 0.1% surfactant	20.7	54.9	1,204	1,476	169

Based on Ikeda *et al.* (1991).

of N losses by ammonia volatilization from leaves following foliar sprays with urea. Values range from 4% of the applied N in wheat (Smith *et al.*, 1991) to $>$ 30% in Kentucky bluegrass (Wesely *et al.*, 1987); high leaf surface moisture followed by rapid drying seems to be the major responsible factor for high losses.

4.4.2.5 Avoiding the Occurrence of Physiological Disorders in Horticulture

The occurrence of Ca-related imbalances is a problem of major economic significance in horticultural crops, with more than 30 different Ca-related disorders in fruits and vegetables, such as bitter-pit in apple or blossom end rot in tomato. Such physiological disorders, which develop during fruit growth, have generally been related to localized Ca deficiencies in plant organs (Saure, 2005; Ferguson and Watkins, 1989). The low transpiration rate of fruits in combination with the mobility of Ca in the phloem (see Chapter 3) poses serious problems enhancing the distribution of this element to the fruit via Ca application to the root system (Bangerth, 1979). Therefore, treatment of aerial plant parts with Ca sprays is recommended and applied in many fruit production areas of the world (Lurie and Crisosto, 2005), either as routine applications to prevent the occurrence of localized Ca deficiencies or to improve quality of commodities (Fallahi *et al.*, 1997; Schmitz-Eiberger *et al.*, 2002; Fernández *et al.*, 2009; Liebisch *et al.*, 2009). However, inconsistent results regarding improvements in tissue Ca concentrations, fruit quality and storability have been often reported (e.g., Val *et al.*, 2008; Bonomelli and Ruiz, 2010; Sotiropoulos *et al.*, 2010).

4.4.2.6 Bio-fortification

The use of foliar nutrient sprays as an approach to enhance the nutritional value of crops for human consumption is a field of rising interest in agriculture, particularly for staple foods (Cakmak, 2002; Grusak, 2002; Cakmak *et al.*, 2010; Shi *et al.*, 2010; White and Brown, 2010). Foliar sprays applied alone or in combination with soil treatments can be used to increase the concentration of micronutrients in foods and alleviate micronutrient deficiencies in human populations around the world (Rengel *et al.*, 1999; Cakmak, 2008). For example, treatment with Zn-containing foliar sprays increased grain Zn concentrations under field conditions in Turkey (Cakmak *et al.*, 2010). Application of Fe and B sprays to rice increased the nutritional value and Fe content of rice grains (Jin *et al.*, 2008; Zhang *et al.*, 2009)

4.4.3 Foliar Uptake and Irrigation Methods

Water shortage and water quality for irrigation purposes is of increasing importance, especially in arid and semi-arid areas of the world. The competition for water resources leads to the use of low quality water for crop irrigation in some areas (Singh *et al.*, 2009a), while conservation strategies such as the use of recycled waste water are being implemented in some countries (Jordan *et al.*, 2001; Devitt *et al.*, 2005; Valdez-Aguilar *et al.*, 2009).

Water supply to plants via sprinkler irrigation is increasingly being used in agriculture, because it provides a higher irrigation efficiency, automation benefits and cuts down labour costs (Isla and Aragués, 2010). However, foliar uptake of elements may occur as a negative side-effect of sprinkler irrigation with low-quality water, which often contains high amounts of soluble salts (Devitt *et al.*, 2005; Singh *et al.*, 2009a). As shown as an example in Fig. 4.7, specific ion toxicity (particularly Na^+ and $Cl^{-)}$ and decreased yields have been reported for several crops (Bernstein and Francois, 1975; Grattan *et al.*, 1994; Isla and Aragués, 2009, 2010).

Sprinkler irrigation with saline water has been shown to strongly increase the concentration of Na and Cl in crops such as pepper, maize, wheat, barley, alfalfa or ornamentals (e.g., Grattan *et al.*, 1994; Jordan *et al.*, 2001; Devitt *et al.*, 2005; Singh *et al.*, 2009a; Isla and Aragués, 2009, 2010). The concentrations of these two ions in the leaves may become toxic quite rapidly, decreasing yield and quality as

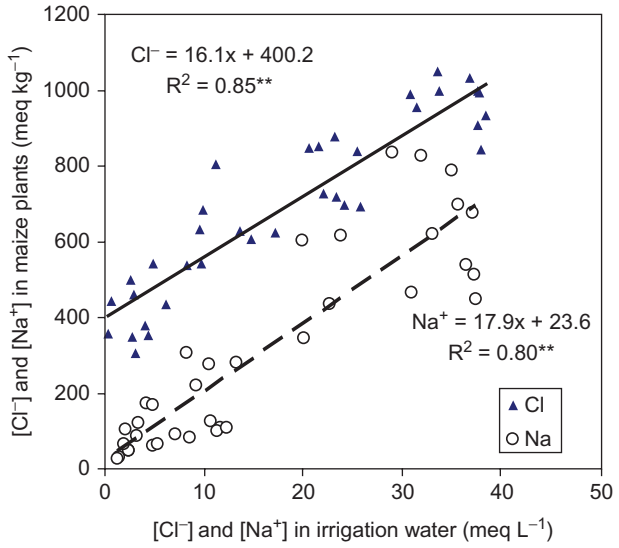

FIGURE 4.7 Relationship between tissue Na and Cl concentrations in maize at harvest and Na and Cl concentrations in the sprinkler irrigation water. *Courtesy of Isla and Aragués 2010.*

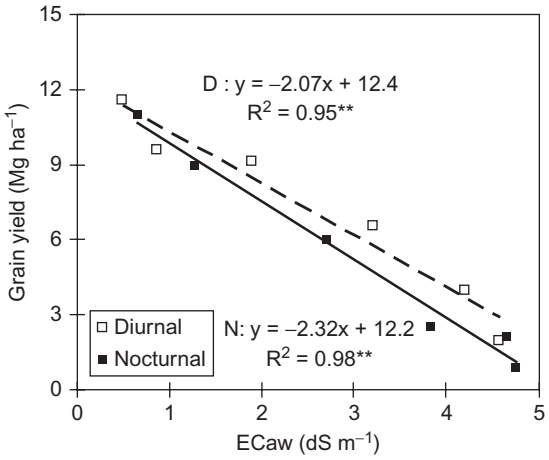

FIGURE 4.8 Relationship between maize grain yield and electrical conductivity of applied water (ECaw) for the diurnal and nocturnal sprinkler irrigations. *Based on Isla and Aragués (2010).*

shown in Fig. 4.8 (Francois and Clark, 1979a; Maas, 1985; Isla and Aragués, 2010). Moreover, increasing salt concentrations in the irrigation water may decrease plant K concentrations as there is a negative relationship between tissue Na and K concentration (e.g., Isla and Aragués, 2009, 2010).

Salinity problems may be minimized by irrigating at night rather than during the day because of lower evaporation of saline water from the wetted leaves and decreased tissue ion accumulation (Ehlig and Bernstein, 1959). However, the effects of salinity are not always alleviated by irrigation at night (see Fig. 4.8; Isla and Aragués, 2009, 2010).

In general, the sensitivity to foliar injury by irrigation with saline water depends on the nature of the leaf surface (e.g., surface properties and chemical composition). For example, deciduous fruit trees (e.g., almond, apricot) are more sensitive to leaf injury by saline sprinkler water than cotton and sunflower (Maas, 1985).

4.5 LEACHING OF ELEMENTS FROM LEAVES

Compounds can be lost from inside plants via: (i) active excretion to the external surface, such as excretion of lipophilic or hydrophilic compounds by glandular trichomes (Werker, 2000); (ii) excretion of inorganic solutes at tips and margins of leaves by guttation (root pressure induced); and (iii) leaching from damaged plant parts, such as leaf tips or margins or from intact plant tissues.

In this section, the term 'leaching' refers to the removal of inorganic and organic substances from aerial plant parts by the action of aqueous solutions such as rain, dew, mist, irrigation and fog. Substances leached from plants may include an array of inorganic elements and organic compounds such as carbohydrates, amino acids, vitamins, hormones or phenols (Tukey, 1970, 1971). However, the quantity and quality of leached materials may vary between plant species and varieties as well as elements, for example K and Mg are more easily leached than Fe and Zn (Tukey, 1970). Leaching is thought to be a passive process resulting in the temporary reduction in concentrations of foliar compounds (Potter, 1991). However, the processes by which compounds are leached from plant surfaces remain unclear and may be related to the mechanisms of foliar uptake.

Leaching rate increases with duration and intensity of rainfall as well as with leaf age. Younger leaves are generally more hydrophobic and the accumulation of substances in the apoplast of mature leaves may result in a steeper concentration gradient across the cuticle that may favour the process of leaching (Tukey, 1970; Wetselaar and Farquhar, 1980; Turner and van Broekhuizen, 1992; Borer *et al*., 2005).

Leaching of elements such as K, Ca, Mg and Mn (Tukey, 1970; Jones *et al*., 1998b) which may explain the generally lower nutrient concentrations in leaves of plants grown under field conditions as compared with plants grown in the same soil, but indoors.

Stress conditions such as prolonged darkness, water shortage and high temperatures or air pollutants such as ozone, dry deposition or high acidity of the rain water or fog can also increase the leaching rate of nutrients from leaves (Tukey and Morgan, 1963; Tukey, 1970; Potter, 1991; González-Arias *et al*., 2000; Schaberg *et al*., 2000; Borer *et al*., 2005). High acidity of rain water increases leaching of solutes, due to more rapid leaf senescence and structural damage at the cuticular or tissue level. Cations are

TABLE 4.7 Element input in bulk precipitation (wet deposition) and dry deposition, throughfall and leaching in a 40 year-old Scots pine forest

		Element Input (kg ha^{-1} $year^{-1}$)				
Parameter	(mm $year^{-1}$)	Ca	Mg	K	Mn	Na
Wet deposition	550	12.7	1.4	1.9	0.24	4.1
Dry deposition	–	10.2	1.1	1.5	0.19	3.7
Throughfall	397	27.8	4.3	15.1	2.50	7.4
Leaching from canopy	–	4.9	1.8	11.7	2.07	–

Based on Marschner *et al.* (1991).

particularly affected because they are replaced from their binding sites by protons in the rain water. For example, acid mist has been found to deplete the labile and physiologically available pool of membrane-associated Ca in red spruce (Schaberg *et al*., 2000; Borer *et al*., 2005). On average, a decrease in the pH of rain water or fog from about 5.5 to 3.5–3.0 may increase the leaching of K, Ca, Mg, Mn and Zn by a factor between 2 and 10 (Mengel *et al*., 1987; Leisen and Marschner, 1990; Turner and Tingey, 1990).

Although leaching of cations usually does not represent more than 1% of the total content of the leaves (Pfirrmann *et al*., 1990), it may reach up to 10% of the annual net incorporation of cations into aerial biomass (Schuepp and Hendershot, 1989). The leaching of specific cations from aerial plant parts may be compensated by higher uptake rates of these cations by the roots (Mengel *et al*., 1987; Kaupenjohann *et al*., 1988). As a consequence of higher cation uptake rate, the rhizosphere pH may decrease (Kaupenjohann *et al*., 1988; Leonardi and Flückiger, 1989) (see also Chapter 14) and, thus, part of the acid load of the canopy may indirectly be carried into the rhizosphere.

4.6 ECOLOGICAL IMPORTANCE OF UPTAKE AND LEACHING OF SOLUTES FROM LEAVES

Uptake and leaching of solutes by/from leaves and other aerial plant parts are continuously taking place in different ecosystems throughout the world. Leaching and uptake of solutes can contribute to the cycling of nutrients in the plant due to either foliar or root absorption of the leachates (Tukey, 1970). For example, in perennial ecosystems such as forest trees, these processes can be a dominant component in nutrition, influencing internal nutrient cycling, as well as the element input and output of forest ecosystems and their long-term stability (Chabot and Hicks, 1982). Nitrogen deposited as gaseous or dissolved compounds can be readily taken up by the forest canopy (Hinko-Najera Umana and Wanek, 2010). In central Europe and northern America, a considerable amount of the dry and wet deposition of N compounds (between 3 and 10 kg N ha^{-1} per year; Brumme *et al*., 1992) can be taken up by the foliage (Garten and Hanson, 1990; Wilson, 1992; Krupa, 2003). Between 40 and 90% of N deposited in forests is retained by their canopies (Lovett and Lindberg, 1993; Clark *et al.*, 1998; Gaige *et al.*, 2007) which could satisfy a substantial proportion of the annual N demand of temperate and boreal forests (Schulze, 2000; Hinko-Najera Umana and Wanek, 2010).

Rain forest plants may be more resistant to leaching via mechanisms such as increased surface hydrophobicity as compared to agricultural plants adapted to lower rainfall areas, such as banana or sugar cane (Tukey, 1970). However, due to the frequent and prolonged precipitation in tropical rain forests, the amounts of nutrients leached from the canopy can be very high. For example, the following annual leaching losses (kg ha^{-1}) have been reported: K 100–200; N 12–60; Mn 18–45; Ca 25–29; P 4–10 (Nye and Greenland, 1960; Bernhard-Reversat, 1975). This magnitude of nutrient leaching is similar to the annual rate of nutrients supplied to the soil surface from the throughfall and is thus an important component of nutrient recycling, particularly in ecosystems with low amounts of available nutrients in the soil, for example in highly weathered tropical soils. Reabsorption of leached nutrients also offers the possibility for plants to supply sites of nutrient demand (e.g., new growth) with nutrients that are re-translocated within the plant to only a very limited extent (e.g., Ca, Mn) (Tukey, 1970).

In temperate climates where rainfall is lower, losses by leaching from aerial plant parts are lower, but still considerable (Table 4.7). Compared with their concentrations in the leaves, the amounts of leached Ca and particularly Mn are often very high. However, quantification of losses by leaching is difficult because ‘dry deposition’ (particulate and gaseous) may constitute a substantial portion of the nutrients in the throughfall (Table 4.7).

Besides inorganic elements, substantial amounts of organic solutes can also be leached from a forest canopy, reaching amounts between 25 and 60 kg organic C ha^{-1} per year in temperate climates (Bartels, 1990), and several hundreds of kilograms in tropical forests. As a side effect, leaching of inorganic elements and organic compounds such as phenolics, organic acids and amino acids (Tyagi and Chauhan, 1982) can affect other plant species within the canopy as well as soil microorganisms, soil fertility and soil forming processes (Tukey, 1970).

The ecological significance of foliar water uptake is still not fully understood, but recent studies on trees, understorey ferns and shrubs of the redwood forest in California showed the ability of leaves to rehydrate upon fog exposure (Burns Limm *et al*., 2009). While crown-wetting events due to, for example, fog or dew may only provide limited amounts of water to the plant or the entire ecosystem, they may be an important water source to aerial plant parts during periods of water shortage (Grammatikopoulos and Manetas, 1994; Burgess and Dawson, 2004; Oliveira *et al*., 2005).

Chapter 5

Mineral Nutrition, Yield and Source–Sink Relationships

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SUMMARY

This chapter describes the role of nutrients in regulating plant processes underlying yield formation. The yield of crop plants is controlled by biomass production and its partitioning to harvested plant organs. Biomass production is dependent on photosynthetic activity of leaves, i.e. source activity, and leaf area, i.e. source size. Nutrients are directly required for leaf growth and as integral constituents of the photosynthetic apparatus. Nutrient supply indirectly controls photosynthesis and leaf senescence via photooxidation, hydraulic and hormonal signals as well as by sugar signalling. Nutrients also affect respiration as constituents of the respiratory electron chain and by their influence on the efficiency of respiratory ATP synthesis. The chapter further describes how photosynthate partitioning to harvested plant organs is controlled by the ability of these organs to utilize assimilates for growth and storage, i.e. their sink strength and how this is influenced by nutrient supply. Nutrients play an important role in regulating sink formation, for example by their effects on flowering, pollination, and tuber initiation, as well as in controlling storage processes in the sink organs. Nutrient supply also modifies endogenous concentrations of phytohormones, which, in turn, regulate sink–source relationships. In higher plants source and sink organs are physically separated from one another. Therefore, long-distance transport of photosynthates and nutrients in the phloem from source to sink is essential for growth and plant yield. The principles of phloem loading of assimilates at source sites, phloem transport and phloem unloading at the sink sites are also described.

5.1 GENERAL

More than 90% of plant dry matter consists of organic compounds such as cellulose, starch, lipids and proteins. The total dry matter production of plants, the *biological yield*, is therefore directly related to photosynthesis, the primary process of synthesis of organic compounds in green plants. In crop plants, *economic yield* is usually defined as the dry matter production of those plant organs for which particular crops are cultivated and harvested (e.g., grains and tubers) (Barnett and Pearce, 1983). Thus, in many crop plants it is not only total dry matter production that is of importance but also partitioning of the dry matter. The so-called *harvest index* represents the proportion of the total plant dry matter production present in the harvested parts of the crop. The partitioning of biomass among plant organs and its controlling mechanisms are therefore of crucial importance in crop production. For the quality of food plants, not only the organic compounds synthesized in the primary and secondary metabolism but also the content of nutrients and the distribution of nutrients among plant organs are important (Welch and Graham, 1999; Karley and White, 2009; Chapter 3).

In this chapter some principles of photosynthesis are discussed, as are the related processes of photophosphorylation and photorespiration, and examples of the involvement of nutrients are given. This discussion includes aspects of photoinhibition and photooxidation and mechanisms protecting the photosynthetic apparatus against this damage.

In higher plants the main sites of photosynthesis – the source (mature green leaves) – and the sites of consumption and storage – the sink (roots, shoot apices, seeds and fruits) – are separated. The long-distance transport of photosynthates in the phloem from source to sink is therefore essential for growth and plant yield. It is, thus, necessary to have a basic understanding of the processes of phloem

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loading of photosynthates at the source sites, phloem transport and phloem unloading at the sink sites and the regulation of these processes, particularly the role of phytohormones. Finally, the source–sink relationship and the question of whether yield can be limited by source or sink are discussed.

5.2 RELATIONSHIPS BETWEEN NUTRIENT SUPPLY AND YIELD

Various factors are required for plant growth such as light, CO_2, water and nutrients. Increasing the supply of any of these factors from the deficiency range increases the growth rate and yield although the response diminishes as the supply of the growth factor is increased. This relationship was formulated mathematically for nutrients by Mitscherlich as the *law of diminishing yield increment* (Mitscherlich, 1954; von Boguslawski, 1958). According to this law, the yield response curves for a particular nutrient are asymptotic; when the supply of one nutrient (or growth factor) is increased, other nutrients (or growth factors) or the genetic potential of crop plants become limiting factors. Typical yield response curves for nutrients are shown in Fig. 5.1. The slopes of the three curves differ. Micronutrients have the steepest and N the smallest slope, if the nutrient supply is expressed in the same mass units. The slopes reflect the different demands of plants for particular nutrients.

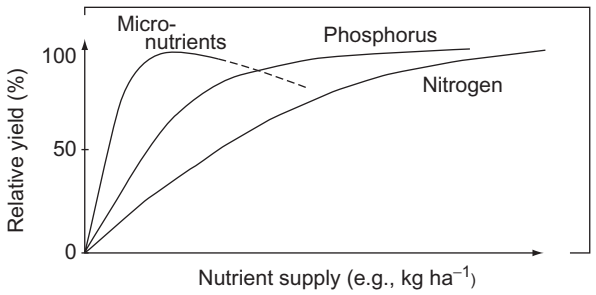


FIGURE 5.1 Yield response curves for N, P and micronutrients.

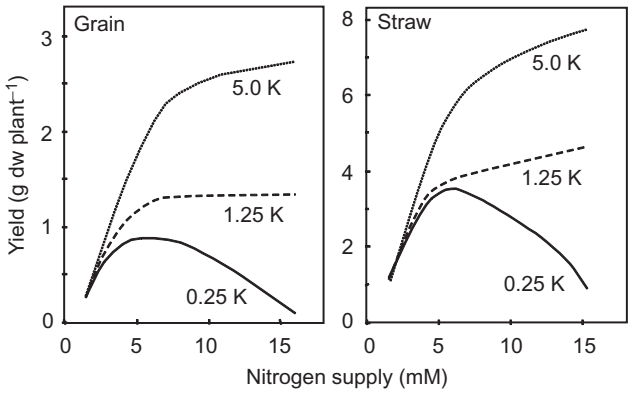


FIGURE 5.2 Grain and straw yield of barley grown in nutrient solution at three K concentrations and increasing N supply. *Based on MacLeod (1969).*

It is now established that some of the assumptions made by Mitscherlich were incorrect. The slope of the yield response curve for a particular nutrient cannot be described by a constant factor, nor is the curve asymptotic. Also when there is an abundant supply of nutrients, a point of inversion is obtained, as shown for micronutrients in Fig. 5.1. This inversion can be caused by a number of factors including toxicity of a nutrient *per se* or induced deficiency of another nutrient. With high N supply to cereals, grain yield may be reduced due to lodging. High N supply may also reduce yield due to its effect on phytohormone concentrations and thus on development processes (Section 5.9). Furthermore, yield response differs from the typical curves (Fig. 5.1) when nutrients are supplied in very low amounts to nutrient fixing soils. In this case seed set is either prevented or severely inhibited.

An example of the effect of interaction between nutrients on yield is given in Fig. 5.2. At the lowest K supply, the response to increasing N supply is small and at high N supply the yield is strongly depressed. Under field conditions, however, yield depressions caused by excessive nutrient supply are usually less marked.

Yield response curves differ between grain and straw, particularly at lower K supply (Fig. 5.2). In contrast to straw yield, grain yield levels off when N supply is high, reflecting sink limitation (e.g., small grain number per ear), sink competition (e.g., enhanced formation of tillers), or source limitation (e.g., mutual shading of leaves).

Yield response curves are strongly modulated by interactions between nutrients and other growth factors. Under field conditions, the interactions between water availability and N supply are particularly important. In maize, for example, with increasing N supply at different soil water content, grain yield response curves obtained by Shimshi (1969) were similar to those for K in Fig 5.2. At the lowest soil water content, optimum yield was obtained at low N and increasing N supply depressed yield. Depressions in yield at high N application rates at low soil water content may be caused by several factors such as (i) delay in stomatal response to water deficiency (Section 5.9), (ii) higher water consumption of vegetative biomass, and, correspondingly, higher risk of drought stress at critical periods of grain formation, and (iii) increase in shoot–root dry weight ratio with increasing N supply (Section 8.2.), an effect which may be stronger in C3 than in C4 plant species (Hocking and Meyer, 1991).

The yield response curves also differ depending on the yield component of harvested products. In most crops, both quantity (e.g., dry matter yield in tons per hectare) and quality (e.g., content of sugars or protein) are important yield components. As shown schematically in Fig. 5.3,

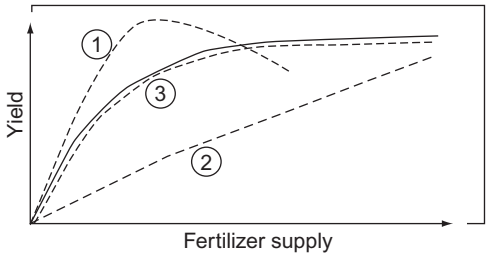


FIGURE 5.3 Schematic representation of yield response curves of harvested products. —— quantitative yield (e.g., dry matter per hectare); - - - qualitative yield (e.g., content of sugar, protein and elements). For explanation see text.

maximum quality can be obtained either before (curve (1)) or after (curve (2)) the maximum dry matter yield has been reached, or both yield components can have a synchronous pattern (curve (3)). Examples for the different curves are (1) accumulation of nitrate in spinach and sucrose in sugar beet in response to increasing N fertilizer rates; (2) increases in protein content of cereal grains or forage plants with increasing N supply or the change in content of certain elements (e.g., Fe, Mg, Na) in food and forage plants with increasing nutrient supply; and (3) positive relationship between number of either reproductive sinks (e.g., grains) or vegetative storage sinks (e.g., tubers) with nutrient supply.

5.3 PHOTOSYNTHETIC ACTIVITY AND RELATED PROCESSES

In order to meet the growing demands of the rapidly growing world population for food, and renewable primary products including biofuels, bioenergy and biomaterials, yields of agricultural and horticultural crops have to be significantly increased. In the past, increasing yields of many crops were mainly achieved by raising the harvest index, i.e. by increasing the ratio of biomass in harvestable plant organs (for review see Fischer, 2007). Modern wheat varieties have harvest indices between 0.45 and 0.5, whereas the best rice and maize varieties have harvest indices exceeding 0.5. For wheat, a maximum harvest index of 0.6 has been predicted (Austin *et al*., 1980). Thus, there is some scope for further increase of harvest index. However, further yield increases also necessitate increased biomass production, i.e. net photosynthesis. In principle, photosynthesis can be enhanced by increasing the photosynthetic activity of the leaves (source activity) and/or the photosynthetic area (source size). In this section, the processes contributing to source activity are described, and examples presented which demonstrate the role of nutrients in the regulation of source activity.

5.3.1 Photosynthetic Energy Flow and Photophosphorylation

The conversion of light energy to chemical energy is brought about by a flow of electrons through pigment systems. In the chloroplasts these pigment systems are embedded in thylakoid membranes. Often, the thylakoid membranes are stacked into piles which appear as grains or 'grana' under the light microscope. The principles involved in the process of electron flow are illustrated in Fig. 5.4. Light energy is absorbed by two pigment systems: photosystem II (PS II) and photosystem I (PS I). In each of these photosystems, between 400 and 500 individual chlorophyll molecules and accessory pigments (e.g., carotinoids) act as 'antenna' to trap light energy (photons), which is then transferred to a chlorophyll molecule with maximum absorbance at 680 nm in PS II, and 700 nm in PS I. In both photosystems, the absorption of light energy induces the emission and up-hill transport of two electrons against the electrical gradients. The electrons required for this process are derived from the photolysis of water, mediated by PS II. In higher plants, PS II and PS I act in series (Z scheme; for reviews see Chitnis, 2001; Diner and Rappaport, 2002; Renger and Renger, 2008). At the end of the up-hill transport chain, the electrons are taken up by an acceptor chlorophyll a molecule (A_0) and transferred through a chain of redox centres that includes phylloquinones (A_l) and three Fe-S-clusters (F_x, F_a and F_b) to ferredoxin. The reduced ferredoxin is a strong reductant and is able to reduce $NADP^+$ (nicotinamide adenine dinucleotide phosphate), as well as other compounds (see below).

Several nutrients are directly involved in this photosynthetic electron transport chain (Raven *et al*., 1999; Fig. 5.4). The chlorophyll molecules in PS II and PS I with their central Mg atom absorb photons, thereby initiating the electron flow. The photolysis (splitting) of water is mediated by an Mn and Ca-containing enzyme complex attached to PS II. Additionally, Cl is an inorganic cofactor of the water-splitting system. In this water-splitting system Mn clusters store energy prior to the oxidation of two molecules of water. Manganese may also act as the binding site for the water molecules which are oxidized (Rutherford, 1989). Cytochromes (Cyt b–f) which contain a central Fe atom as well as an Fe-S complex mediate the electron flow between PS II and PS I (Marder and Barber, 1989). One of the electron acceptors in this chain is plastocyanin, a Cu-containing protein. In PS I, the electrons are transferred via three Fe-S clusters (F_x, F_a and F_b) to ferredoxin. Ferredoxin is a 9 kDa Fe-S protein which is soluble in the stroma, and acts as transmitter of electrons to $NADP^+$. This is reduced to NADPH by the ferredoxin-$NADP^+$-oxidoreductase which is anchored to the thylakoid surface.

Reduced ferredoxin in the chloroplasts can also function as an electron donor for other acceptors. The

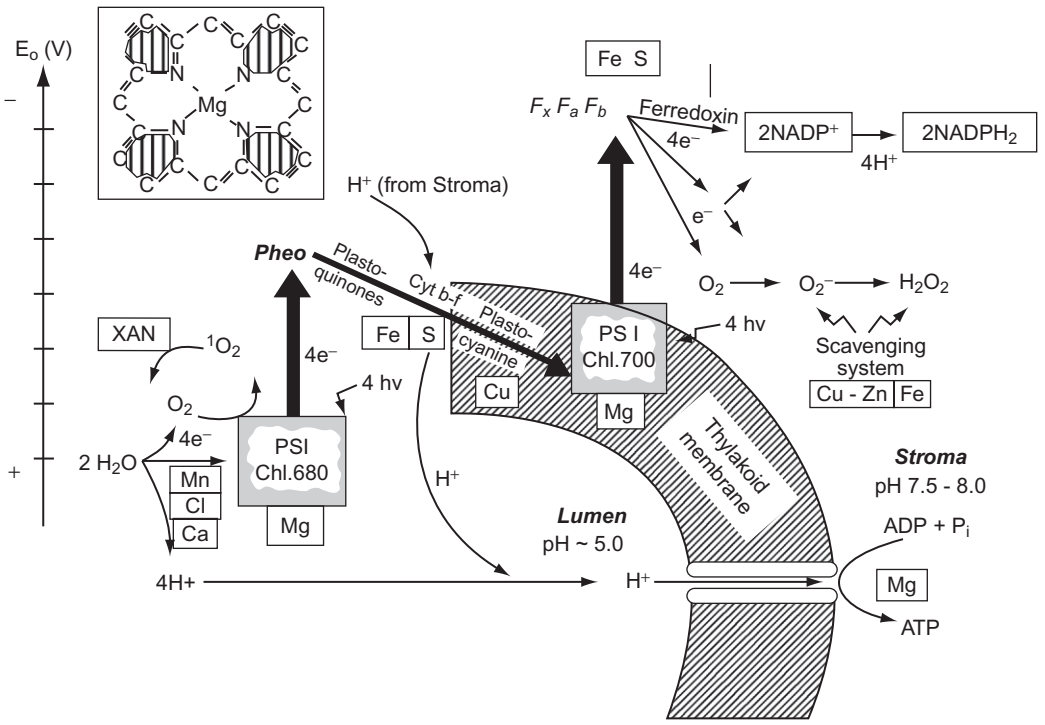
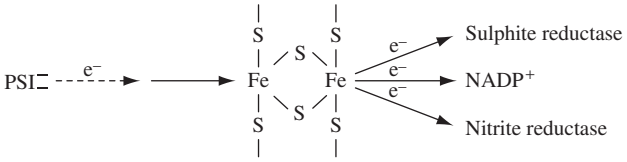


FIGURE 5.4 Photosynthetic electron transport chain with photosystems II and I (PS II; PS I) and photophosphorylation. Pheo: pheophytins; Cyt: cytochrome; F_x F_a F_b: Fe-S-clusters which transfer electrons from primary acceptors (A0, A1) to ferredoxin; XAN: xantophyll cycle. Inset: section of the porphyrin structure of chlorophyll with the central Mg atom.

ferredoxin-mediated reduction of nitrite and sulphite is of particular importance for the nutrition of plants:



Both nitrite and sulphite compete within the chloroplasts with $NADP^+$ for reduction. In leaves, the rates of reduction of nitrite and sulphite are higher during the light period. This coupling of nitrite and sulphite reduction with light is also an example of a more general regulatory mechanism, since photosynthesis supplies the structures (carbon skeletons) required for the incorporation of reduced nitrogen (-NH_2) and sulphur (-SH) into organic compounds such as amino acids.

Water splitting and the passage of electrons through the electron transport chain in the thylakoid membrane are coupled with the pumping of protons into the thylakoid lumen (Fig. 5.4), leading to acidification to about pH 5. The light-induced accumulation of H^+ (positive charge) in the lumen is counterbalanced by Mg efflux. On the other hand, protons are consumed at the terminal site of the electron transport chain (formation of NADPH), raising the stroma pH to 7.5–8.0. The corresponding electrochemical potential gradient across the thylakoid membrane is used for *photophosphorylation*, a proton-driven ATP synthesis by an Mg-ATPase. An additional component in the formation of the proton gradient is the *cyclic photophosphorylation*, a pumping system for protons between PS II and PS I (Fig. 5.4). The downhill transport of three protons across the thylakoid membrane is thought to result in the production of one ATP molecule. In the stroma, ATP is required at various steps of CO_2 assimilation, carbohydrate synthesis as well as other ferredoxin-mediated processes (see above).

5.3.2 Photoinhibition and Photooxidation

Light absorbed by PS II and PS I is not necessarily balanced by a corresponding electron flow and formation of reduced ferredoxin, and the consumption of electrons (e.g., in CO_2 assimilation). Imbalances occur at high light intensity in general and particularly when high light intensity is combined with other environmental stress factors such as drought, low temperatures or nutrient deficiencies. Excess excitation energy depresses photosynthesis and quantum yield, which although usually reversible (*dynamic photoinhibition*), may also lead in the long term to irreversible damage of the photosynthetic apparatus which results in decreased maximum photosynthesis (*chronic photoinhibition*) as well as chlorosis and necrosis of the leaves

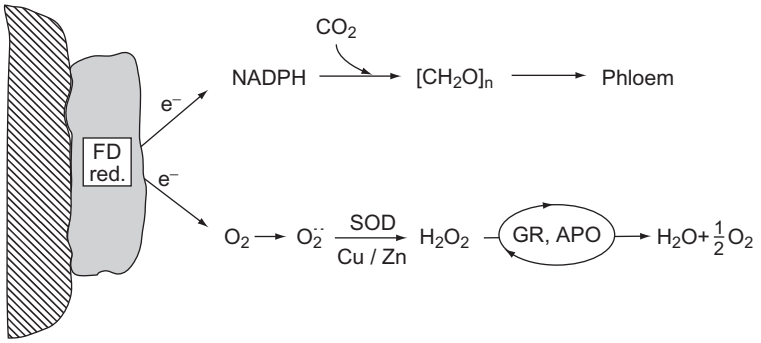


FIGURE 5.5 Alternative utilization of photoreductants for CO_2 assimilation or activation of molecular oxygen and detoxification (scavenger) systems. SOD: superoxide dismutase; GR: glutathione reductase; APO: ascorbate peroxidase.

(*photooxidation*). These latter symptoms are caused by the formation of reactive oxygen species (ROS) (Asada, 1999; Apel and Hirt, 2004). It has been suggested that nitrite-dependent nitric oxide (NO) production in chloroplasts and subsequent formation of active N species is also involved in photoinhibition (Yamasaki, 2000).

Plants possess a range of protective adaptations and systems to reduce damage by ROS. These include, for example, light reflecting wax cover of leaf epidermis, change in leaf angle, leaf rolling and chloroplast movement to reduce light absorption. If excess light energy is absorbed, plants can (i) dissipate the energy in the form of heat (Ort, 2001), (ii) activate detoxification mechanisms against damage by ROS (Niyogi, 1999), and (iii) repair photo-damaged PSII by fast and efficient turnover of the D1 protein (Nishiyama *et al*., 2006).

The primary target for photoinhibition is PS II. This photosystem produces molecular oxygen to which the excessive excitation energy can be transferred to form the highly toxic singlet oxygen (Fig. 5.4). As a self-protecting mechanism, carotenoids (xanthophylls in particular) play an important role in both scavenging singlet oxygen and discharging excess photon flux energy as heat (*thermal dissipation*, *non-photochemical quenching*) (Ort, 2001; Johnson *et al*., 2007). In a process induced by low lumen pH, PS II is transformed from a high efficiency state, which uses most of the absorbed light energy for photochemical processes, to a photoprotected state, which dissipates excess light energy via the xanthophyll cycle in the form of heat. The transition from the photoprotected state back to the high efficiency state is a relatively slow process, particularly in thermophilic plant species at low temperatures (Zhu *et al*., 2004). Thus, in leaf canopies in the field, where there are short-term fluctuations in light intensity, ongoing thermal energy dissipation after transfer from high to low light may cause substantial losses in carbon gain by crop species (Long *et al*., 2006).

The capacity of plants for rapid increases in xanthophyll cycle-dependent energy dissipation is enhanced by environmental stresses that depress the photosynthetic rate of the plant, such as low temperatures and low N supply (Demmig-Adams and Adams, 1996). However, despite this acclimation, the lower CO_2 assimilation capacity of N-deficient plants leads to increased susceptibility of PS II to photoinhibition, as shown, for example, in Norway spruce (Grassi *et al*., 2001) and rice (Kumagai *et al*., 2010). High ultraviolet-B (UV-B) radiation may also cause inhibition of photosynthesis and photooxidation of pigments (Jordan, 2002). However, the effects of two stresses are not necessarily additive. For example, in a field study with maize, N-deficient plants were less sensitive to increased UV-B radiation than plants that were well supplied with N (Correia *et al*., 2005).

Another main site of formation of ROS is the stroma of chloroplasts, where reduced ferredoxin can use molecular oxygen (O_2) as an electron acceptor leading to reduction of O_2 to the superoxide anion ($O_2^{\cdot -}$; Figs 5.4 and 5.5). This reductive O_2 activation in chloroplasts is unavoidable and enhanced under conditions which increase in the NADPH/$NADP^+$ ratio, for example, low CO_2 supply or impaired CO_2 fixation, caused by a range of environmental stress factors such as low temperatures in chilling sensitive plant species (Hodgson and Raison, 1991), salinity, drought and nutrient deficiency. Reductive O_2 activation is also enhanced by low or inhibited export rates of photosynthates from source leaves under nutrient deficiency (Marschner and Cakmak, 1989; Cakmak and Kirkby, 2008). In C3 species, photorespiration (i.e., the release of CO_2 in the light) may be an important protective mechanism consuming ATP and reducing equivalents which prevents over-reduction of the photosynthetic electron transport chain and photoinhibition (Wingler *et al*., 2000).

Other systems, however, play a key role in preventing elevated levels of ROS, photoinhibition and photooxidation by detoxifying $O_2^{\cdot -}$ and related compounds such as H_2O_2. In chloroplasts, where catalase is absent, $O_2^{\cdot -}$ is detoxified by Cu–Zn superoxide dismutase (SOD) producing H_2O_2 which is reduced to H_2O by the ascorbate

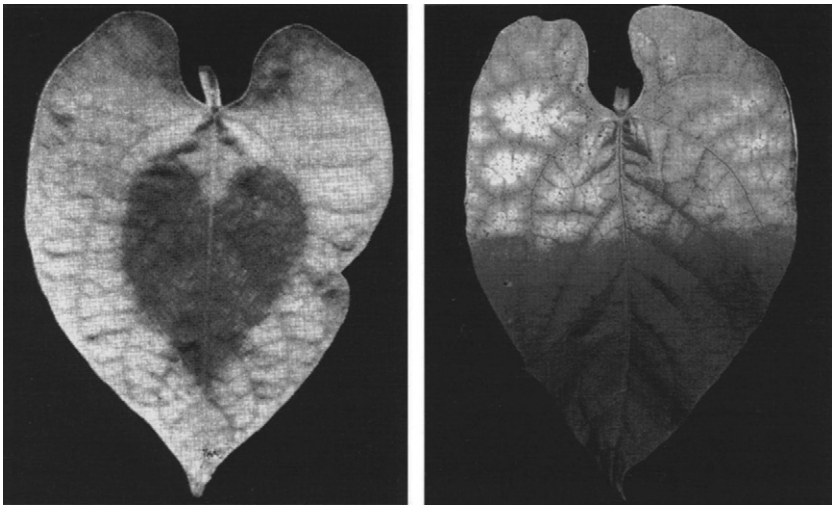


FIGURE 5.6 Chlorosis and necrosis in partially shaded primary leaves of Zn-deficient (*left*) and Mg-deficient (*right*) *Phaseolus vulgaris* plants exposed to high light intensity (480 $\mu E\,m^{-2}\,s^{-1}$). *From Marschner and Cakmak* et al. *(1989) with permission from Elsevier.*

peroxidase–glutathione reductase cycle (Asada, 1999; Fig. 5.5). In leaves about 70–80% of the ascorbate-dependent H_2O_2 scavenging enzymes are located in the chloroplasts (Strother, 1988).

Elevated activity of the detoxifying enzymes (Fig. 5.5) and increased concentrations of their metabolites (glutathione, ascorbate) are indicators of oxidative stress, particularly under high light intensity, for example in pine needles during winter (Anderson *et al.*, 1990), spruce needles at noon (Schupp and Rennenberg, 1988) and in bean leaves under Mg deficiency (Cakmak and Marschner, 1992). There is substantial evidence that ROS are also involved in senescence of cells and organs such as leaves, and that quite often the appearance of chlorosis and necrosis of leaves as visual symptoms of nutrient deficiency can be explained by elevated concentrations of ROS. An example of this in bean leaves is shown in Fig. 5.6. Under Zn deficiency the concentration of ROS is high (Cakmak and Marschner, 1988a,b; Cakmak, 2000) because of both depressed SOD activity and lower export rates of carbohydrates as a result of low sink activity (Marschner and Cakmak, 1989). Under Mg deficiency and high light intensity, oxidative stress is caused by impaired phloem loading of carbohydrates (Cakmak and Marschner, 1992; Cakmak and Kirkby, 2008). In both cases the production of photooxidants and thus photooxidation of leaf pigments could almost be completely prevented by partial shading of the leaf blades (Fig. 5.6). In agreement with this observation, inhibited phloem loading of sucrose in genetically manipulated tobacco and tomato plants is associated with severe chlorosis and necrosis of the leaf blades (von Schaewen *et al.*, 1990). However, chlorosis and necrosis

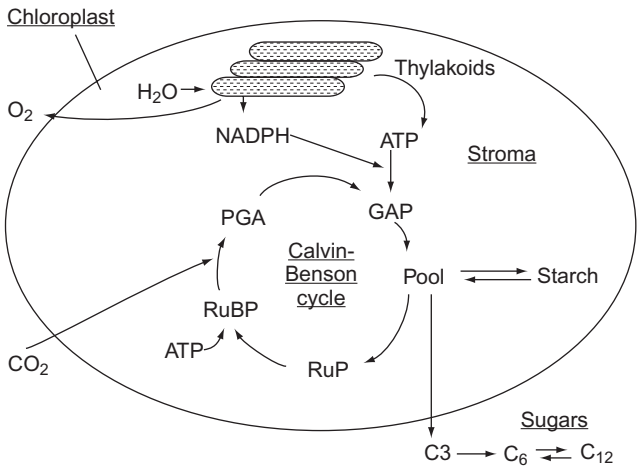


FIGURE 5.7 Simplified scheme of CO_2 fixation and carbohydrate synthesis in the Calvin-Benson cycle in C3 plants. *Modified from Larcher (1980).*

in leaves following sugar accumulation may also be caused by regulation of photosynthetic and senescence genes by sugars (Rolland *et al.*, 2006) and redox signals (Pfannschmidt *et al.*, 2009).

5.3.3 Carbon Dioxide Assimilation and Photorespiration

The reduction equivalents (NADPH) and ATP produced in the light reactions of photosynthesis are used for CO_2 assimilation in the Calvin (Calvin-Benson) cycle (so-called *dark reactions* or *light-independent reactions*). The principles of CO_2 fixation by the so-called C3 pathway in chloroplasts are shown in Fig. 5.7. The enzymes

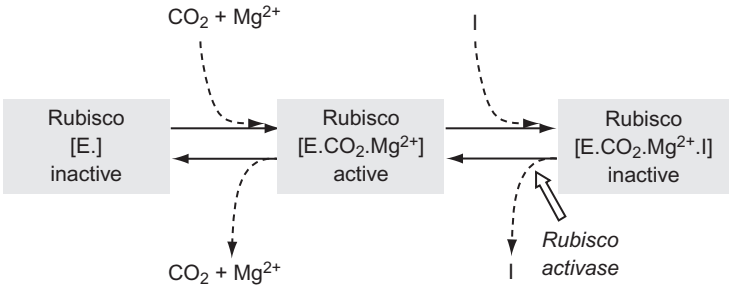
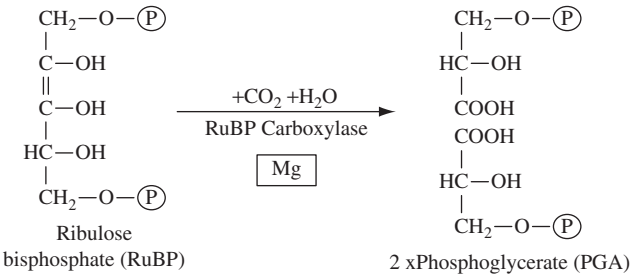


FIGURE 5.8 Principles of regulation of Rubisco activity. The unmodified enzyme [E.] is inactive; reversible reaction with CO_2 and Mg^{2+} leads to the formation of an active state of the enzyme [E.CO_2.Mg^{2+}]; the active state is transformed to an inactive state through binding of inhibitors (I); removal of the inhibitors is mediated by the enzyme Rubisco activase. *Modified from Parry et al. (2008).*

catalysing the individual steps of the Calvin cycle are located in the stroma of the chloroplasts, whereas NADPH and ATP are supplied by the thylakoids. The first step in the Calvin cycle (carbon fixation phase) is catalysed by the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), which binds CO_2 to the C_5 compound ribulose-1,5-bisphosphate (RuBP):



Ribulose bisphosphate (RuBP)

2 xPhosphoglycerate (PGA)

After carboxylation of RuBP, two molecules of the C_3 compound phosphoglycerate (PGA) are formed. This route of CO_2 assimilation is thus referred to as the C_3 pathway and those plant species for which this is the main form of CO_2 acquisition are known as C_3 species. In the reductive phase of the Calvin cycle, in which NADPH and ATP are used, PGA is reduced to glyceraldehyde 3-phosphate (GAP) and further transformed to triosephosphates. Of the six triosephosphate molecules generated by the carboxylation of three molecules of RuBP, five are used to regenerate three RuBP molecules which then can again act as acceptors for CO_2; one is available either for synthesis of transitory starch in the chloroplast or exported through the chloroplast envelope into the cytosol for further synthesis of mono- and disaccharides. The rate of release of C_3 compounds from the chloroplasts is controlled by the concentration of inorganic phosphate (P_i) in the cytoplasm; P_i therefore has a strong regulatory effect on the ratio of starch accumulation to sugar release from the chloroplast (Section 6.4).

The Calvin cycle comprises 11 different enzymes. Molecular approaches to increase efficiency of photosynthetic CO_2 fixation are focusing on those processes and enzymes that limit photosynthesis. The use of antisense plants with reduced Calvin cycle enzyme concentrations allows investigation of the contribution of individual enzymes to the control of C flux through the Calvin cycle ('metabolic control analysis'; for a review see Raines, 2003). Enzymes exerting considerable control of C flux include Rubisco, sedoheptulose-1,7-bisphosphate, plastid aldolase and transketolase (Raines, 2003). The influence of specific enzymes is strongly dependent on environmental conditions. For example, the impact of decreased Rubisco concentrations on photosynthesis was greater either at low N supply, or when photosynthesis was measured under saturating light conditions (Stitt and Schulze, 1994).

Potential Rubisco activity is determined not only by the concentraiton of the Rubisco protein, but also by activation and inhibition of the protein (Fig. 5.8; Parry *et al*., 2008). The enzyme is activated by carbamylation (i.e., reversible reaction of a molecule of CO_2 with a lysine residue of Rubisco to form a carbamate) and subsequent stabilization of the resulting carbamate by Mg, and retransformed to its inactivated form by release of CO_2 and Mg. Additionally, organic inhibitors can bind to the Rubisco protein and block the active site of the enzyme. Such inhibitors include 2-carboxy-D-arabinitol 1-phosphate that is formed in the chloroplast during periods of low irradiance or darkness, and pentadiulose 1,5-bisphosphate which is produced under conditions favouring photorespiration, such as high temperature and drought. Rubisco activity is also regulated by Rubisco activase. This enzyme is required for the removal of inhibitors from the catalytic site of Rubisco (Portis *et al*., 2008). The activity of Rubisco activase is increased by illumination, and is very sensitive to heat stress (Parry *et al*., 2008).

In C3 species, light enhances not only the incorporation of CO_2 but also its evolution, which is stimulated by the presence of O_2. Incorporation and release of CO_2 are dependent on Rubisco. This light-driven evolution of CO_2 (so-called *photorespiration*) occurs simultaneously with the incorporation of CO_2 and is a

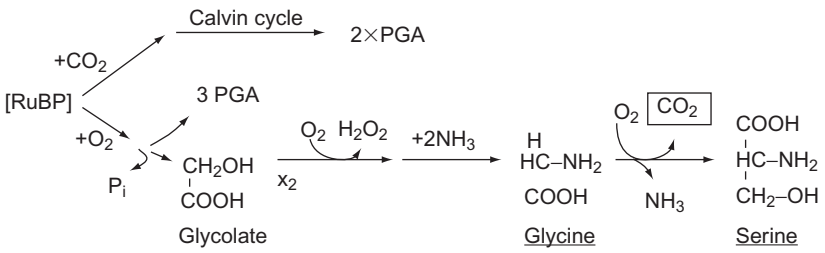


FIGURE 5.9 Photorespiration, glycolate pathway and synthesis of the amino acids glycine and serine.

consequence of the oxygenation of RuBP catalysed by Rubisco (Reumann and Weber, 2006). In the oxygenase reaction (Fig. 5.9), the C_5 compound RuBP is split into 3-PGA (C3) and glycolate (C_2), the first compound of the 'glycolate pathway'. Glycolate is released from the chloroplasts into the cytosol and transferred to peroxisomes in which glycolate is transformed to glyoxylate which acts as an acceptor for ammonia, forming the amino acid glycine. After translocation into the mitochondria, two molecules of glycine are converted into the amino acid serine with simultaneous release of CO_2 (photorespiration) and ammonia (Fig. 5.9). Both ammonia toxicity and losses by volatilization are avoided by the reassimilation of ammonia via the formation of glutamine from glutamate in the chloroplast (Chapter 8). This 'photorespiratory N cycle' has been reviewed by Wallsgrove *et al.* (1983).

Serine is translocated into the peroxisomes, where it is transformed to glycerate. Finally, glycerate is transferred into the chloroplasts and transformed to 3-PGA which can enter the Calvin cycle. In total, for every two molecules of glycolate (C_2) formed during photorespiration, one molecule of 3-PGA (C3) is regenerated. Furthermore, ATP and NADPH are consumed in the phosphorylation of glycerate and the reassimilation of ammonia in the chloroplast (Wingler *et al*., 2000). The photorespiratory N cycle represents the largest component of ammonia incorporation in leaves of most C3 plants in the light (Yu and Wo, 1991). In *Arabidopsis thaliana*, the introduction of a glycolate catabolic pathway from *Escherichia coli* significantly increased biomass formation (Kebeish *et al*., 2007). This pathway is confined to the chloroplasts, and thus avoids the consumption of ATP and reducing equivalents for ammonia assimilation, and releases CO_2 in the stroma in the vicinity of Rubisco.

A key factor regulating the rate of photorespiration is the CO_2/O_2 ratio at the active site of Rubisco. In C3 species, the CO_2 concentration in the stroma of chloroplasts ranges from 100 to 60% of the ambient CO_2 concentration (Sharkey, 1988). Any increase in ambient CO_2 concentration therefore decreases the rate of photorespiration in C3 plants. Photorespiration of C3 plants is strongly increased by high temperatures, because the solubility of CO_2 relative to that of O_2 decreases. Thus, the relative rate of oxygenation by Rubisco increases compared to that of carboxylation (Brooks and Farquhar, 1985). Photorespiration is also increased by mild to moderate drought stress, which leads to closure of stomata and decrease in internal CO_2 concentrations in leaves.

Photorespiration also takes place in C4 plants (see below); however, at much lower rates. In the C4 plant maize, for example, under ambient conditions (21% O_2, 0.035% CO_2), photorespiratory CO_2 loss was about 6% of net photosynthesis as compared with 27% in the C3 plant wheat (De Veau and Burris, 1989). The lower photorespiration in C4 plants is due to the higher CO_2 concentrations in the bundle sheath cells, i.e. at the sites of CO_2 assimilation by Rubisco in C4 plants (see below). The higher rates of photorespiration in C3 plants explain why under otherwise optimal environmental conditions (high light and high temperatures), rates of net photosynthesis and biomass production are considerably lower than in C4 plants.

Photorespiration appears to be a wasteful process in which CO_2 and ammonia are produced and ATP and reducing equivalents are consumed. However, photorespiration is an important pathway of amino acid synthesis in leaf cells (Fig. 5.9). De Veau and Burris (1989) found that the rate of serine synthesis per unit leaf area was about twice as large in wheat (C3) than in maize (C4). Furthermore, photorespiration may also play a protective role against abiotic stresses such as drought and salinity (Wingler *et al*., 2000) and biotic stresses such as fungal pathogens (Taler *et al*., 2004). This protective effect may be due to prevention of over-reduction of the photosynthetic electron transport chain and photoinhibition. Additionally, glycine may be used for the synthesis of glutathione which is also involved in stress alleviation. Furthermore, it has been shown in *Arabidopsis* and wheat that inhibition of photorespiration, for example at elevated atmospheric CO_2 concentrations, is associated with strong reduction of nitrate assimilation in shoots suggesting inhibition of nitrate assimilation if photorespiration is depressed (Rachmilevitch *et al*., 2004).

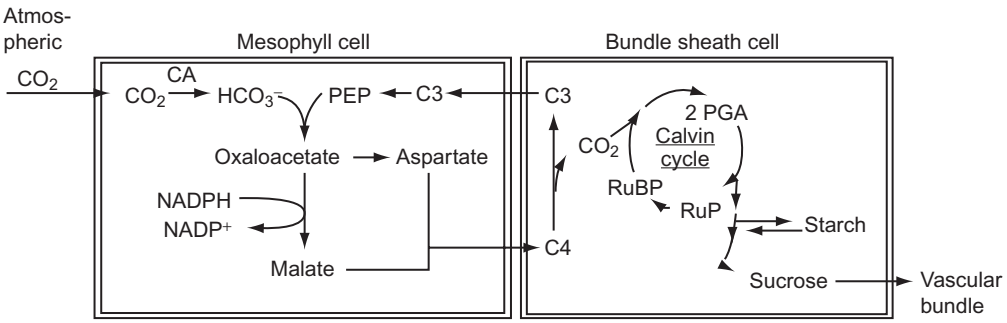
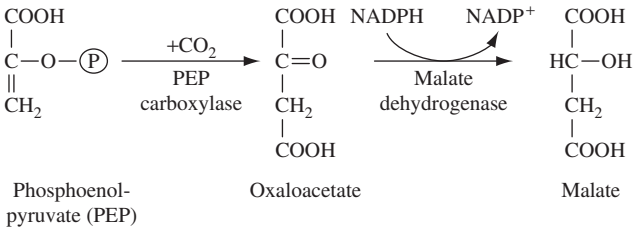


FIGURE 5.10 Simplified scheme of CO_2 fixation and compartmentation in C4 plants. CA: carbonic anhydrase.

5.3.4 C4 Pathway of Photosynthesis and Crassulacean Acid Metabolism

The incorporation of CO_2 into organic compounds is not restricted to the C3 pathway described above. As described earlier (Section 2.5), an imbalance of cation–anion uptake by roots in favour of cations has to be compensated for by the incorporation of CO_2 via the PEP carboxylase (PEPCase) and formation of organic acids. In principle the same pathway of CO_2 incorporation occurs in the chloroplasts of C4 plants.



Phosphoenol pyruvate (PEP) acts as CO_2 acceptor to form oxaloacetate (OAA) which is reduced to malate. This fixation of CO_2 in the chloroplasts of mesophyll cells is dependent on the Zn containing cytosolic enzyme carbonic anhydrase (CA) which converts CO_2 to hydrogen carbonate (Badger and Price, 1994) prior to assimilation in the chloroplasts by PEPCase. The products of this CO_2 incorporation are C4 compounds, either malate or the amino acid aspartate. These C4 compounds are transported from the mesophyll cells to the bundle sheath cells where they are decarboxylated and where Rubisco is located which fixes the released CO_2 (Fig. 5.10). The release of CO_2 leads to a rise in CO_2 concentration that almost saturates Rubisco at a temperature range between 20 and 30°C (von Caemmerer and Furbank, 1999). CO_2 fixed by Rubisco is channelled into the Calvin cycle. The remaining C3 acids in the bundle sheath cells are translocated back to the mesophyll cells where PEP is regenerated via PEPCase to act again as a CO_2 acceptor. The C4 pathway was first identified in sugar cane by Kortschak *et al.* (1965) and Hatch and Slack (1966). In plant species with this C4 pathway, the final

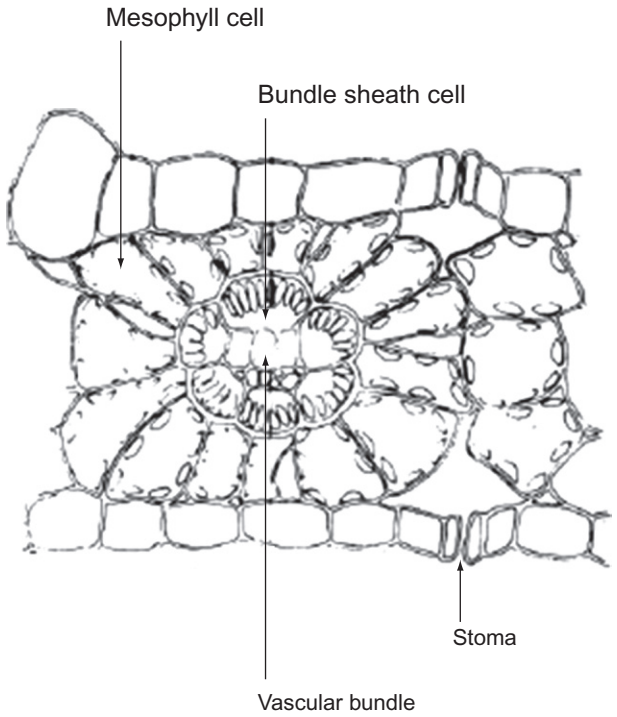


FIGURE 5.11 Diagrammatic representation of a transverse section of a leaf with C4 Kranz anatomy. *Courtesy of Dr A.J. Keys.*

fixation and reduction of CO_2 in the bundle sheath chloroplasts is identical with that in C3 plants, but in C4 plants the two forms of CO_2 fixation are spatially separated, usually the mesophyll and bundle sheath cells as described above (Fig. 5.10). However, in three C4 species of the Chenopdiaceae, the C4 pathway occurs in single photosynthesizing cells (Voznesenskaya *et al.*, 2001).

In most C4 species, the two cell types are arranged in the so-called Kranz-type leaf anatomy. The minor veins of the vascular bundles are surrounded by bundle sheath cells, forming a Kranz, or wreath and the bundle sheath cells in turn are surrounded by a concentric layer of large mesophyll cells (Fig. 5.11; Hibberd *et al.*, 2008). Additionally, in C4 species, the chloroplasts are dimorphic, those in the

bundle sheath cells being larger and having grana that are not as well developed as those of the mesophyll cells. On the other hand, the starch synthesizing enzymes are confined to the bundle sheath chloroplasts where almost all of the leaf starch is accumulated (Spilatro and Preiss, 1987). Both cell types have anatomical features which favour the C4 pathway including a high frequency of plasmodesmata linking the mesophyll cell and bundle sheath cell cytosol, and suberin deposition in the cell walls of bundle sheath cells to restrict CO_2 leakage (Hatch, 1987). The differentiation of chloroplasts between mesophyll and bundle sheath cells to accommodate the C4 pathway not only influences processes associated with CO_2 assimilation and C and N metabolism, but also is important for fatty acid synthesis and isoprenoid and sulphur metabolism (Friso *et al*., 2010).

C4 plants are generally categorized into three subtypes based on enzymes used to release CO_2 in the bundle sheath cells. These three enzymes are: NADP-malic enzyme, NAD-malic enzyme and PEP carboxykinase (Buchanan *et al*., 2000). The NAD-malic enzyme has an absolute requirement for Mn for activation; to produce maximum biomass the NAD-malic C4 plants, pearl millet and amaranthus, require an approximately 25-fold higher Mn supply compared to some other C3 and C4 species at similar photosynthetic rates (Kering *et al*., 2009).

Many C4 plants species are of tropical and subtropical origin, have high photosynthetic rates and produce large amounts of dry matter. These include the highly productive C4 crops (e.g., sugar cane, sorghum, maize, *Miscanthus* and switchgrass and various Chenopodiaceae) which are very efficient in use of resources including light, CO_2, water and N. In C4 plants, radiation use efficiency is about 50% higher than in C3 plants (Long *et al*., 2006). Large differences, however, occur among C4 crop species in use of these resources, for example *Miscanthus* is more efficient than switchgrass (Heaton *et al*., 2008; Dohleman *et al*., 2009).

The higher efficiency of water use by C4 plants compared to C3 plants can be explained by the lower internal CO_2 partial pressure in leaves and the correspondingly steeper CO_2 gradient from the ambient atmosphere through the open stomata into the leaf tissue. Lower internal CO_2 concentrations are achieved by efficient conversion of CO_2 to HCO_3^- in the cytosol by CA and high affinity of PEPCase to HCO_3^- in the mesophyll cytosol (Badger and Price, 1994). Thus, in C4 plants there is a relatively greater inward diffusion of CO_2 through the stomata per unit of water vapour lost, which can be utilized for photosynthesis and dry matter production. In addition, when stomata partially close in response to water deficit, the decrease in CO_2 influx is less in C4 than in C3 plants, because the internal recycling of CO_2 maintains a lower CO_2 concentration in the leaf tissue of C4 plants. Correspondingly, the relative efficiency of water use is around 200–300 g water transpired g^{-1} dry matter produced in C4 species compared to usually more than 500 in C3 species.

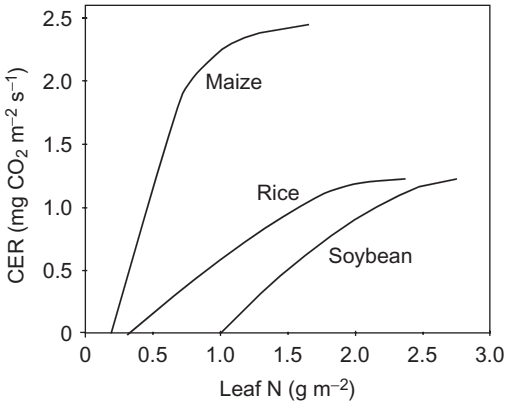


FIGURE 5.12 Leaf CO_2 exchange rate (CER) at light saturation for maize, rice and soybean as a function of leaf N concentration. *Based on Sinclair and Horie (1989).*

In general, C4 plants have greater photosynthetic N use efficiency (PNUE, CO_2 assimilation rate per unit leaf organic N concentration; Poorter and Evans (1998)) than C3 plants (Monson, 1989). An example of this is shown in Fig. 5.12: maize had not only higher rates of CO_2 fixation than the two other C3 crop species, but these higher rates were achieved at a lower leaf N concentration. In C3 plants, Rubisco has a slow catalytic rate, a low affinity for atmospheric CO_2 and uses O_2 as an alternative resulting in photorespiration (see above) (Spreitzer and Salvucci, 2002). As a result, C3 plants require high concentrations of Rubisco to maintain high rates of photosynthesis; the Rubisco protein accounts for as much as 20–30% of the leaf N content (Makino *et al*., 2003). The higher PNUE in C4 plants is possible because Rubisco in the bundle sheath cells operates at CO_2 saturation because of the high concentrations of CO_2 in the vicinity of Rubisco, hence photorespiration is repressed allowing the enzyme to function near saturation. Thus, in C4 species a high assimilation rate can be maintained with only one third to one quarter of the Rubisco required in C3 species. In comparison with this saving, the N cost for the C4 cycle enzymes is low (Makino *et al*., 2003; Friso *et al*., 2010). Furthermore, in many C4 species the catalytic efficiency of Rubisco (k_{cat} in mol CO_2 mol^{-1} Rubisco active sites s^{-1}) is higher than in C3 species (Sage, 2002). Photosynthetic N use efficiency varies among C4 species of the different subtypes, being greater in the NADP-malic enzyme subtype than in the NAD-malic enzyme subtype (Ghannoum *et al*., 2005). Also PNUE can vary, for example between cultivars differing in N use efficiency as observed in maize (Paponov and Engels, 2003).

Phosphorus use efficiency is less well understood in C4 than in C3 plants. The enzymes of the additional steps and membrane transport processes that characterize the

C4 pathway are regulated by P_i. These include P_i activation of the enzymes PEPCase and pyruvate:orthophosphate dikinase, and PEP/P_i transport in the mesophyll chloroplast envelope (Iglesias *et al*., 1993). Measurements of CO_2 assimilation rates in leaves of C3 and C4 tropical grasses showed higher P use efficiency in the C4 species (higher CO_2 assimilation rates at low leaf P_i concentrations). However, the C4 grasses were more sensitive to P deficiency (Ghannoum *et al*., 2008).

It should be emphasized that the higher nutrient use efficiency of C4 species (e.g., maize and sugar cane) in general does not imply a lower fertilizer demand. Indeed, because of their potential to produce very high dry matter yields, C4 crops often have higher nutrient requirements than C3 crops. Accordingly, C4 grassland species may respond more strongly to N and P fertilization than C3 species (Rubio *et al*., 2010).

The rise in atmospheric CO_2 concentration stimulates C3 photosynthesis more than C4 photosynthesis, because in C4 plants, Rubisco is already saturated with CO_2. On the other hand, the associated warmer climate favours C4 photosynthesis because in C3 plants, high photorespiration is expected to constrain net CO_2 fixation at higher temperatures (Sage and Kubien, 2003). In view of the high photosynthetic energy conversion efficiency and the high N use and water use efficiency (see above), the C4 pathway is attracting attention in agriculture (Long *et al*., 2006) and genetic engineering of the C4 photosynthetic machinery into C3 plants is regarded as an important strategy to increase yields of C3 crops like rice (Zhu *et al*., 2010). Introduction of the *Escherichia coli* glycolate catabolic pathway into *Arabidopsis thaliana* chloroplasts to reduce the loss of fixed carbon by photorespiration has been shown to increase biomass production (Kebeish *et al*., 2007). However, engineering the whole C4 photosynthetic pathway into crop plants such as rice or wheat remains a challenging task for the future (Zhu *et al*., 2010).

Fixation of CO_2 via the PEP carboxylase pathway (*crassulacean acid metabolism* (CAM)) is also a characteristic feature of plant species in certain families, such as Crassulaceae and Bromeliaceae, which are particularly well adapted to dry and saline habitats. These plants are mostly succulent; that is, they have a low surface area per unit of fresh weight. The CAM pathway of CO_2 fixation was identified by Thomas and his colleagues at Newcastle on Tyne in the UK over 60 years ago (Ranson and Thomas, 1960), although at that time its relevance to photosynthesis was not appreciated (Walker, 1992). CAM plants differ from C4 species in a number of features: (i) the stomata of CAM species are open at night, (ii) CO_2 enters the leaves and is fixed by PEP carboxylase in the cytosol with subsequent reduction to malic acid, which is stored in the vacuoles during the night, and (iii) during the day malic acid is released from the vacuoles and decarboxylated. This release of CO_2 promotes stomatal closure and increases CO_2 concentrations around Rubisco, thus minimizing photorespiration. CO_2 is fixed and reduced in the chloroplasts following the C3 pathway. Accordingly, large day–night changes in vacuolar pH occur in the leaves of CAM plants (Lüttge, 1988), and both proton pumping systems (ATPase and PP_iase) are probably involved in the transport of malate into the vacuole (Marquardt and Lüttge, 1987).

In contrast to the spatial separation of the two steps of CO_2 fixation in C4 species, the separation of the three steps of CO_2 fixation in CAM species is temporal (*diurnal acid rhythm*). The combination of CAM and succulence is of particular advantage for adaptation to dry habitats or high salinity or both. Aboveground productivity of CAM crop plants such as pineapple (*Ananas comosus* L.) and *Opuntia ficus-indica* can be similar to that of C4 and C3 species, but with only 20% of the water required for growth (Borland *et al*., 2009).

Depending on plant species, plant developmental stage and environmental conditions, CAM may operate in different modes: (i) obligate CAM; (ii) facultative or inducible CAM (C3-CAM) in which CAM metabolism is induced by factors such as drought, salinity, high photon flux, N and P deficiency; (iii) CAM-cycling, with daytime CO_2 fixation and acid accumulation but closed stomata during the night; (iv) idling, with little acid accumulation and stomatal closure during the day and night in severely stressed plants (Cushman, 2001; Herrera, 2009). In facultative CAM and CAM-cycling species, dark CO_2 fixation does not only contribute to water saving but also plays an important role in photo-protection and reproduction (Herrera, 2009).

5.3.5 Effect of Leaf Maturation on Source Function

During its life cycle, each leaf undergoes a shift in which its function changes from a sink to a source for both nutrients and photosynthates. For nutrients, this shift is correlated with a change in the prevailing long-distance transport in the phloem and xylem (Section 3.4). The long-distance transport of sugars such as sucrose, however, is restricted to the phloem, and thus, the sink–source transition of leaves is associated with a corresponding shift from phloem unloading (import) to phloem loading (export). As shown in Fig. 5.13, in sugar beet this transition from net import to net export occurs when the leaf has reached about 40–50% of its final area and net photosynthetic capacity. Similarly, this transition occurs in other dicotyledonous species when the leaves are 30–60% expanded.

Leaf maturation is not uniform within the leaf blade. The sink–source transition progresses basipetally along

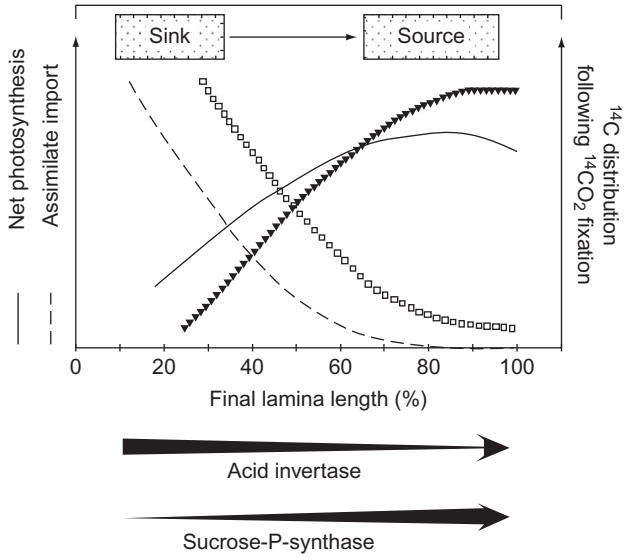


FIGURE 5.13 Relationship between assimilate import, net photosynthesis, rate of sugar synthesis (▼: sucrose; □: glucose) and enzyme activity during maturation of sugar beet leaves. *Based on Giaquinta (1978).*

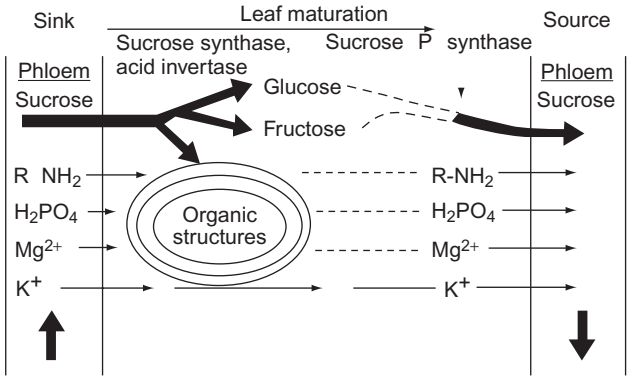


FIGURE 5.14 Schematic representation of the sink–source transition during leaf maturation: the shift from import to export of assimilates and nutrients.

the leaf. Therefore, there is a period when bidirectional phloem transport occurs in a single leaf, with some vascular bundles importing and others exporting photosynthates (Turgeon, 1989). As leaves expand, their photosynthetic rate increases (Schurr *et al*., 2000; Li *et al*., 2008b). For tobacco leaves, partial shading of a sink leaf delays sink–source transition (Wright *et al.*, 2003). However, the photosynthetic capacity *per se* of a developing leaf is not a regulatory factor in sink–source transition (Turgeon, 2006).

The sink–source transition of leaves is associated with biochemical, physiological and anatomical changes. In maturing sugar beet leaves a shift occurs in the incorporation of carbon into sugars, as can be demonstrated by supplying $^{14}CO_2$ to leaves of different age (Fig. 5.13). The shift in favour of sucrose synthesis is closely correlated with changes in the activity of enzyme associated with carbohydrate metabolism in the leaves: a decrease in activities of acid invertase and sucrose synthase (sucrose hydrolysis) and an increase in sucrose-P-synthase activity (sucrose synthesis) (Schurr *et al.*, 2000; Li *et al*., 2008b). The correlation with acid invertase is probably a reflection of high rates of cell wall synthesis and the provision of hexoses for synthesis rather than of regulatory functions of this enzyme in phloem unloading of sucrose (Haupt *et al*., 2001). In sink leaves, the activity of the cytosolic enzyme sucrose synthase is also high and rapidly declines during sink–source transition (Turgeon, 1989). The correlation between a decrease in sucrose synthase and an increase in sucrose-P-synthase (sucrose synthesizing enzyme) is correlated with the transition from sink to source (Fig. 5.13) in plants where sucrose is the dominant sugar in the phloem sap, because the functioning of a leaf as a source relies on the induction and activity of this sucrose-synthesizing enzyme. Results similar to those obtained with sugar beet leaves have been found for soybean (Silvius *et al.*, 1978), *Ricinus* (Schurr *et al*., 2000) and rice leaves (Li *et al*., 2008b) during maturation. The sink–source transition of leaves is also associated with changes in the frequency and architecture of plasmodesmata in the mesophyll and epidermal cells which reduce symplasmic continuity (Turgeon, 2006).

The mechanism by which the import and export of nutrients are regulated during leaf maturation is not very clear. From a consideration of both, the mechanism of phloem transport (solute volume flow) and the average composition of phloem sap in the stem of plants during vegetative growth (Section 3.3) it is to be expected that there should be a positive correlation between the import rate of sugars such as sucrose into a sink leaf and the import rate of nutrients such as K and P, and also amino acids (Fig. 5.14) if phloem unloading of these solutes is regulated by the requirement for growth processes. However, preferential phloem transport from source to sink can also be observed when a non-proteinogenic amino acid (α-aminoisobutyric acid) is supplied to a source leaf (Schilling and Trobisch, 1971) or to the stem (Van Bel, 1984). This amino acid accumulates in the sink in the soluble N fraction, indicating that it is not the sink demand for a particular amino acid that regulates transport from source to utilizing sinks such as developing leaves, but rather the direction of solute volume flow in the phloem and the unloading of other solutes, sugars in particular.

With the onset of leaf maturation and the capacity for synthesis of sucrose and other export sugars (e.g., mannose), the leaf becomes a new source as loading of sugars into the phloem begins and an increase in the volume flow rate in the phloem from the leaf is induced. Thus, the export of other solutes in the phloem such as nutrients and

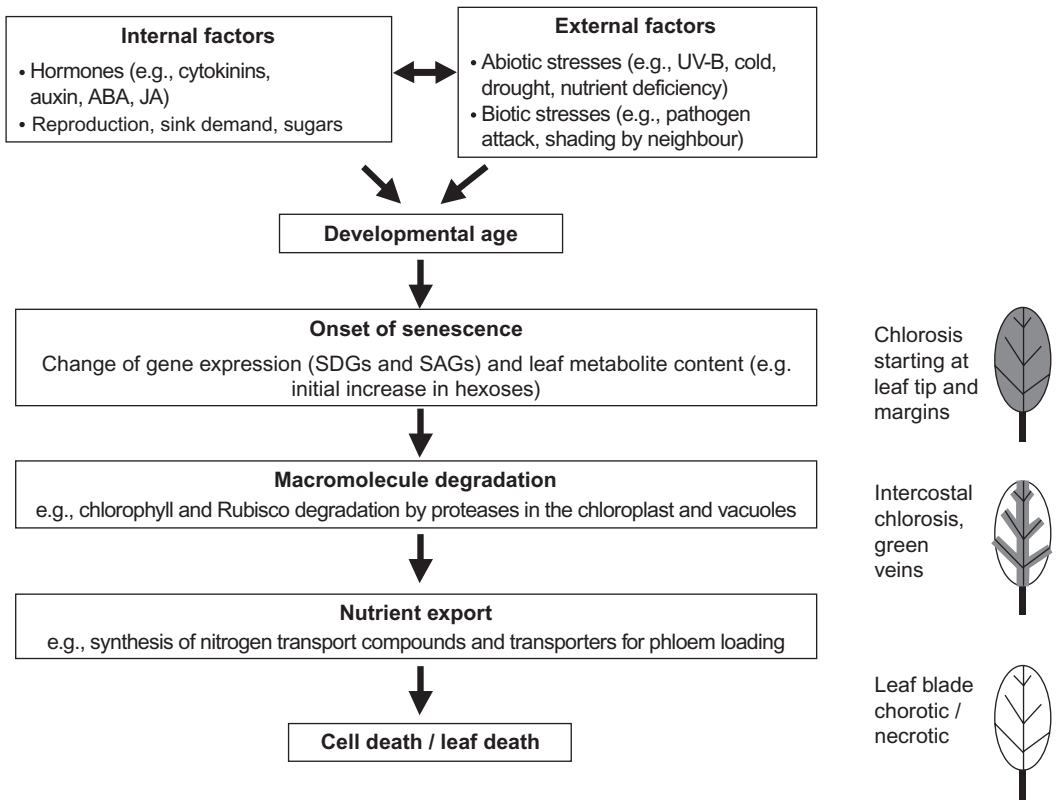


FIGURE 5.15 Overview of the factors involved in the onset of leaf senescence and processes related to senescence-induced nutrient export; for further explanation see text.

amino compounds can also increase. As discussed previously (Section 3.4) for highly phloem mobile nutrients such as K and P, import via the xylem and export via the phloem can be in equilibrium in mature leaves. The degree to which mature leaves also act as a source of nutrients depends, however, not only on the rate of photosynthate export but also on the nutrient concentration of the source leaf and the demand of the sink.

5.3.6 Leaf Senescence

Leaf senescence is an important developmental phase which, on the one hand, restricts the lifespan and photosynthetic activity of the leaf and, on the other, allows recycling C and nutrients within the plant (Himelblau and Amasino, 2001; Feller *et al*., 2008). Leaf senescence involves coordinated action at cellular, tissue, organ and organism levels under the control of a highly regulated genetic programme (Lim *et al*., 2007). The expression of many genes is down-regulated (senescence-down-regulated genes, SDGs), for example genes encoding photosynthesis-related proteins. However, for many other genes expression is up-regulated (senescence-associated genes, SAGs). These include genes for degrading enzymes like proteinases and lipases and genes encoding nutrient transporters needed for remobilization in the phloem (Fig. 5.15). The onset of leaf senescence is governed by developmental age, but also influenced by various internal and external factors (Fig. 5.15). Internal factors regulating leaf senescence include sugars (Wingler and Roitsch, 2008) and plant hormones such as cytokinins (CYT) which delay senescence, and abscisic acid (ABA), jasmonic acid (JA) and ethylene which promote senescence (Lim *et al*., 2007; see below). Both abiotic and biotic external factors may also induce leaf senescence. Abiotic factors include drought, nutrient limitation, extreme temperatures, oxidative stress by UV-B irradiation and ozone whereas biotic factors are pathogen infection and shading by neighbouring plants. In many cases environmental cues induce a change in hormone synthesis and translocation to the leaves (see below).

Leaf senescence is strongly influenced by plant nutrition. For example, leaf senescence is induced by K deficiency (Armengaud *et al*., 2004). In *Arabidopsis*, K deficiency is associated with up-regulation of genes encoding enzymes involved in JA biosynthesis (Armengaud *et al*., 2004) and increased leaf concentrations of JA, and can be prevented or delayed by application of salicylate and aspirin, which are inhibitors of JA synthesis (Cao *et al*., 2006). Limited N availability and high tissue C/N

TABLE 5.1 Leaf composition in wheat with and without N and 6-benzylaminopurine (BAP); +N: continuous N supply; −N: interruption of N supply for 48 h

	+N	−N	+N/+BAP	−N/−BAP
Protein (mg g^{-1} fw)	18.0	16.6	20.9	19.1
Chlorophyll (mg g^{-1} fw)	1.50	1.31	1.74	1.63
Starch (µmol glucose eq. g^{-1} fw)	31.3	23.1	40.8	33.5
Isopentenyl adenosine (pmol g^{-1} fw)	15.8	1.5	322	200

Based on Criado *et al.* (2009).

are important regulators of leaf senescence (Parrott *et al.*, 2010). As shown in an example for wheat in Table 5.1, after 48 h of N starvation (−N), chlorophyll, protein and starch concentrations in an older leaf decreased compared to well supplied (+N) plants. This decrease was associated with a strong reduction in leaf concentration of isopentenyl adenosine which is an active form of cytokinin. Supply of the cytokinin 6-benzylaminopurine via the roots significantly increased the leaf concentrations of isopentenyl adenosine, and in turn also chlorophyll, protein and starch concentrations. This finding indicates that N deficiency-induced acceleration of leaf senescence could be due to reduced supply of root-derived CYT to the leaves.

Leaf senescence in the form of chlorosis of source leaves can readily be induced by high light combined with Zn, Mg and K deficiency (Marschner and Cakmak, 1989) where senescence is not induced by decrease in CYT import, but rather by inhibited export of photosynthates and accumulation of large amounts of non-structural carbohydrates in the source leaves. In this type of premature leaf senescence, toxic oxygen species and photooxidation of chloroplast pigments are involved. Increased concentrations of free radicals, which are the result of both elevated formation of radicals and reduced activity of antioxidative enzymes, play an important role during developmentally and stress-induced senescence (Zimmermann and Zentgraf, 2005). Further examples of the effect of nutrient supply on leaf senescence are given in Section 5.4.3.

At the whole leaf level, natural senescence usually begins in the tips or margins toward the base of a leaf (Fig. 5.15). Cell death, which is the final result of senescence, starts in the mesophyll cells and then proceeds to other cell types. Cells close to the veins are often the last areas that senesce, presumably because they are needed for nutrient export (Quirino *et al.*, 2000). At the cellular level, earliest structural changes occur in chloroplasts, whereas the nucleus and mitochondria which are essential for gene expression and energy production, respectively, remain intact until the last stages of senescence, when visible disintegration of the plasma and vacuolar membranes appears (Lim *et al.*, 2007).

The senescence-induced breakdown of chloroplasts is associated with degradation of chlorophyll and Rubisco (Fig. 5.15) and mobilization of large amounts of N and other nutrients in the chloroplasts (Himelblau and Amasino, 2001). The initial steps of degradation are likely to take place within the chloroplast itself (Martínez *et al.*, 2008) in a process in which ROS and senescence-induced chloroplast proteases are involved. Presumably, chloroplast components are then transferred to the central vacuole that remains intact for a longer period during senescence where they are further degraded by vacuolar (acid) proteases (Gregersen *et al.*, 2008).

The proteolysis of chloroplast proteins and related transamination reactions including deaminating activity of glutamate dehydrogenase lead to the release of ammonia which in turn is reassimilated by a cytosolic form of glutamine synthetase (GS1) to form glutamine for export to sink organs via the phloem (Fig. 5.15). Genes encoding a specific form of GS1, aminotransferases and asparagine synthetase are up-regulated during senescence suggesting a role of these enzymes in N recycling during leaf senescence (Masclaux-Daubresse *et al.*, 2010). Furthermore, amino acid and peptide transporters are also induced during senescence and the export of amino acids and other phloem mobile nutrients from leaf blades is increased (Masclaux-Daubresse *et al.*, 2010; see Section 3.5).

Other nutrients are also remobilized during leaf senescence in *Arabidopsis* (Himelblau and Amasino, 2001) and other plant species. In senescing tomato, remobilization of RNA-bound P is associated with the induction of specific ribonucleases (Lers *et al.*, 2006). In petunia, expression of a phosphate transporter gene (*PhPT1*) is up-regulated and may function in P translocation during ethylene-induced senescence (Chapin and Jones, 2009). Also in *Arabidopsis*, specific members of the Pht1 family of P transporters (Pht1;5) that are localized in the phloem of leaves are induced during senescence, suggesting a role for these transporters for redistribution of P from old leaves (Mudge *et al.*, 2002). Expression of genes encoding yellow stripe-like (YSL) transporters also increases during senescence. Since these transporters play an important role in the mobilization of Cu and Zn in the form of metal–nicotianamine complexes, they may be involved in the remobilization of these nutrients from senescing leaves (Curie *et al.*, 2009; see Section 3.3).

In agriculture and horticulture, leaf senescence may restrict yield in crop plants by limiting growth duration and may also cause postharvest spoilage such as leaf

yellowing and nutrient loss in vegetable crops. In different plant species, including many crops, 'stay green' mutants have been identified which are delayed in senescence (Thomas and Howarth, 2000). Possible advantages of 'stay green' genotypes include increased biomass production and yield (Spano *et al*., 2003), increased N acquisition (Martin *et al*., 2005) and water uptake from deep soil layers (Christopher *et al*., 2008), as well as increased tolerance to extreme drought (Rivero *et al*., 2007), and extended shelf-life of vegetables (Barry, 2009). In grain crops such as maize or wheat, however, the 'stay green' phenotype may be associated with lower N use efficiency and N harvest index, if the delay in leaf senescence is not associated with a faster rate of senescence and N remobilization before maturity (Thomas and Howarth, 2000).

Quality of plant products may also be improved by accelerated senescence. In durum wheat, a gene which encodes a member of the NAC transcription factor family, belonging to the NAM subgroup (*NAM-B1*) is involved in the regulation of leaf senescence (Uauy *et al.*, 2006). The gene confers accelerated flag leaf senescence and this is associated with higher grain contents of protein and micronutrients such as Fe and Zn (Diestelfeld *et al.*, 2007).

5.3.7 Feedback Regulation of Photosynthesis by Sink Demand for Carbohydrates

Many studies have shown that photosynthesis in source leaves responds to the demand for carbohydrates in sink organs (Paul and Foyer, 2001). The rate of photosynthesis of a specific leaf is increased when the photosynthetic capacity of other leaves is reduced, for example by abscission (Römer, 1971), herbivory (Nabity *et al*., 2009) or shading (McCormick *et al.*, 2008). Also increased carbohydrate drain to rhizobia and mycorrhiza can increase photosynthesis (Kaschuk *et al*., 2009). An example is given in Table 5.2 for young mustard plants in the vegetative growing phase. The removal of four source leaves led to an approximate doubling of both the rate of photosynthesis and the export of photosynthates from the remaining source leaf.

On the other hand, reduction of the demand for carbohydrates, for example by removal of sink organs (Iglesias *et al*., 2002), can reduce photosynthesis (so-called *feedback inhibition of photosynthesis*). Reduction of photosynthesis induced by low sink demand is often associated with increased leaf carbohydrate concentrations. Various mechanisms have been suggested to explain feedback inhibition of photosynthesis by high concentrations of sugars and starch in the leaves (for review see Stitt, 1991). These include (i) chloroplast damage, (ii) negative effects on CO_2 diffusion by excessive starch accumulation, (iii) limitation of photosynthesis by P deficiency within the chloroplasts which is induced by accumulation of sugar phosphates in the cytosol, and (iv) sugar-induced repression of photosynthetic genes. High leaf carbohydrate concentrations, particularly hexose, inhibit transcription of genes coding for enzymes involved in photosynthesis (Rolland *et al*., 2006). Sugar-induced repression of photosynthetic genes is also involved in the reduction of photosynthesis associated with leaf senescence (Rolland *et al*., 2006).

TABLE 5.2 Photosynthesis and assimilate export of a remaining source leaf (#2) after removal of source leaves (#3-6) of white mustard

Treatment	Photosynthetic rate of leaf no. 2 (%)	^{14}C export from leaf no. 2 (%)
Control	100	36
Source leaves (#3–6) removed	187	62

Based on Römer (1971).

Sugar-mediated regulation of photosynthetic genes has been found to be dependent on nutrition. In tobacco seedlings, chlorophyll content and Rubisco activity were strongly decreased by feeding sugar to N-deficient plants, whereas in plants which were either P deficient or well supplied with N and P, sugar feeding had no effect (Nielsen *et al*., 1998). The difference between N- and P-deficient plants in sensitivity to sugar-mediated reduction in chlorophyll content and Rubisco activity corresponds to visual appearance and photosynthetic activity of the plants. In contrast to N deficiency, P deficiency is not associated with rapid reduction of chlorophyll content and radiation use efficiency (Plénet *et al*., 2000; Fletcher *et al*., 2008). The rapid loss of chlorophyll and leaf photosynthetic activity in N-deficient plants may also be related to direct effects of N on gene expression. Nitrate deficiency directly represses genes involved in photosynthesis, including chlorophyll synthesis, and induces genes involved in protein degradation and senescence (Peng *et al*., 2007).

The rate of net photosynthesis often increases after fruit or seed set due to increased sink demand. However, high demand of sink organs for carbohydrates is not necessarily associated with high net photosynthesis and biomass production at the whole plant level because increased biomass allocation to storage sink organs can be at the expense of biomass allocation for new leaf construction. In perennials with indeterminate vegetative growth during the reproductive phase, sink competition by the developing fruits can be quite dramatic, for example in citrus trees (Table 5.3). With increasing fruit load, and thus fruit dry weight, the amount of water lost per kg leaf dry weight

TABLE 5.3 Dry matter production and distribution and water consumption of *Citrus madurensis* at different number of fruits per plant (fruit load)

	Number of fruits per plant		
	0	50	100
Dry weight (g plant^{-1})			
Fruits	–	134	175
Vegetative shoots and flowers	457	305	118
Roots	68	49	17
Total dry wt	525	488	310
Water transpired			
L plant^{-1}	91	90	59
L kg^{-1} leaf dw	370	520	1,030

Based on Lenz and Döring (1975).

TABLE 5.4 Nodule weight and N content of soybean plants after 60 days of growth as affected by defoliation or removal of flowers and developing pods

	Dry weight of root nodules	Nitrogen
Treatment	(mg plant^{-1})	(mg plant^{-1})
Control	298	475
Defoliation	176	266
Removal of flowers and pods	430	548

Based on Bethlenfalvay *et al.* (1978).

was strongly increased. As water loss through transpiration is closely associated with CO_2 uptake, this indicates higher rates of photosynthesis per unit leaf weight. However, increasing fruit load reduced not only the growth of vegetative shoots and roots, but also total plant growth and the total amount of water lost per plant, i.e. net photosynthesis per plant. Heavy fruit load decreased root dry weight more than the total amount of water loss per plant; plants with a heavy fruit load are therefore more sensitive to inadequate supplies of water and nutrients (Lenz, 1970) because their shoots place a higher demand on their small root system than do the shoots of plants without fruits or with only a small number of fruits.

The effect of sink demand on photosynthesis is even more complicated in legumes which fix atmospheric N (N_2). In these plants, the root nodules represent an additional sink for carbohydrates supplied from the leaves. As shown in Table 5.4, the removal of source leaves leads to a decrease in both nodule growth and N_2 fixation, whereas the removal of flowers and pods (competing sinks) results in an increase in both nodule weight and N_2 fixation to values that are higher than those of untreated control plants. This shows that in legumes high sink demand of generative organs can decrease the N supply to leaves from the rhizobial symbionts and thus can decrease leaf photosynthesis and accelerate leaf senescence (see also Chapter 16).

5.3.8 Nutrition and Photosynthesis

The rate of net photosynthesis may be influenced by nutrition through various modes of action (Fig. 5.16). The direct involvement of some nutrients in the light and dark reaction of photosynthesis has been discussed in Sections 5.3.1 and 5.3.3. An example for direct involvement of nutrients in the light reaction is the light-induced efflux of Mg and K from the lumen to the stroma of chloroplasts for maintenance of charge balance in light-induced influx and generation of protons in the lumen (Fig. 5.4). In the dark reaction, light-induced influx of K into the guard cells leads to opening of stomata, and thus uptake of CO_2 into the leaf, which is required for CO_2 assimilation. An example of direct involvement of nutrients in the dark reaction is the control of triosephosphate transport across the chloroplast envelope into the cytosol by the concentration of inorganic P (Heldt *et al*., 1977; see Section 6.4).

Nutrients are also required for biosynthesis of the photosynthetic apparatus, either as cofactors of enzymes involved in biosynthetic pathways (e.g., Fe for chlorophyll synthesis, see also Chapters 6 and 7), or as integral components of the photosynthetic apparatus (Fig. 5.4). Deficiency of nutrients that are involved in synthesis of protein or chloroplast pigments or electron transfer results in the formation of chloroplasts with lower photosynthetic efficiency (Spencer and Possingham, 1960), and also in a change in the fine structure of chloroplasts (Hecht-Buchholz, 1972; Chen *et al*., 2008b). In leaves of spinach about 24% of the total N is in the thylakoid membranes; N nutrition therefore also affects the amount of thylakoids per unit leaf area (Terashima and Evans, 1988). In Mn-deficient leaves, the photosynthetic efficiency per unit chlorophyll is strongly decreased and can be restored within two days after foliar application of Mn, indicating a direct effect on photosystem II (Fig. 5.4) rather than an indirect effect via source–sink relationships (Kriedemann *et al.*, 1985).

In the range between suboptimal and optimal nutrient supply, positive correlations are often observed between nutrient concentration of leaves and the rate of net photosynthesis (Paponov *et al*., 2005a; Flechter *et al*., 2008, Fig. 5.12). In field-grown wheat, rates of net photosynthesis at low light intensity were similar in N-deficient plants

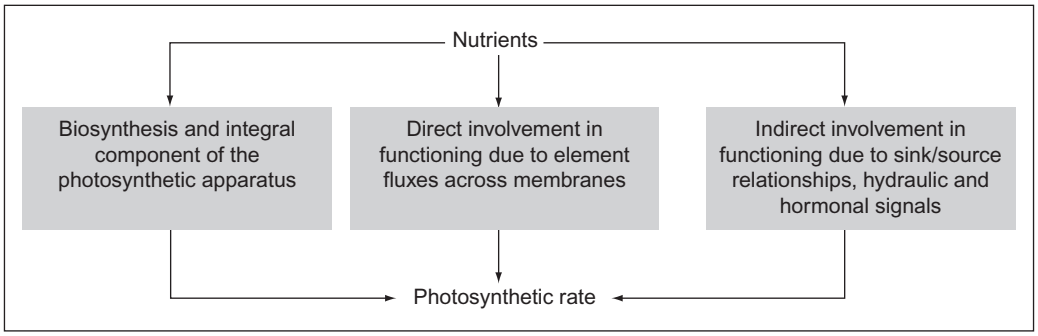


FIGURE 5.16 Modes of action of nutrients in the regulation of photosynthesis.

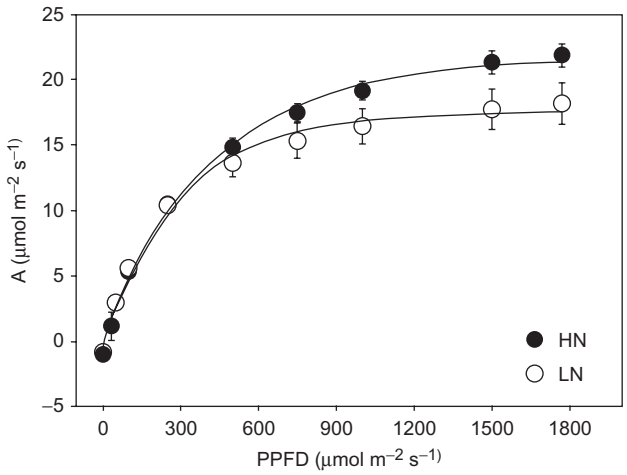


FIGURE 5.17 Light response curves of N-deficient (LN) and N-sufficient (HN) field-grown wheat plants; A: rate of net photosynthesis; PPFD: photosynthetically active photon flux density. *Based on Cabrera-Bosquet* et al. *(2009).*

and plants which were well supplied with N (Fig. 5.17). In contrast, at high light intensity net rates of photosynthesis were lower in N deficient plants. In N-deficient plants, with increasing light intensity an increasing proportion of the absorbed light energy is not used in photochemical reactions but dissipated as heat (Demming and Winter, 1988; de Groot *et al*., 2003). Similar changes in the light response curves are also found under P deficiency (Lauer *et al.*, 1989a), K deficiency (Weng *et al*., 2007) and deficiency of a range of other nutrients.

At the whole plant level, the rate of net photosynthesis may also be indirectly influenced by nutrient supply via the effects of nutrition on growth and source–sink relationships (Fig. 5.16). Despite poor radiation use efficiency at high light intensities, carbohydrates may accumulate in leaves (Rao *et al.*, 1990) and also in roots (Khamis *et al.*, 1990a) of P deficient plants. Thus, low photosynthetic efficiency of source leaves from nutrient-deficient plants is often the result of feedback regulation induced by a lower demand for photosynthates at the sink sites (Pieters *et al*., 2001). An example for this is shown for Zn deficiency in Table 5.5. With increasing light intensity, plant dry weight increases in the Zn-sufficient plants but not in the Zn-deficient plants. Although the chlorophyll concentration decreases with increasing light intensities, particularly in the Zn-deficient plants, the carbohydrate concentration increases, indicating that the lack of growth response to increasing light intensities reflects a sink and not source limitation.

Accumulation of photosynthates under high light intensity in source leaves of deficient plants not only decreases utilization of light energy but also poses a stress. This high light stress is indicated, for example, by an increase in the antioxidative defence mechanisms in the deficient leaves (Cakmak and Marschner, 1992; Fig. 5.5), photooxidation of chloroplast pigments (Table 5.5) and enhanced leaf senescence. These side effects of nutrient deficiency decrease not only current photosynthesis and *leaf area index* (LAI) but also *leaf area duration* (LAD), i.e. the length of time in which the source leaves supply photosynthates to sink sites, an aspect that is discussed in Section 5.4.3.

In N-deficient plants, sugars also accumulate due to low sugar utilization for N assimilation and growth. This N deficiency-induced sugar accumulation, in turn, may lead to suppression of photosynthetic rate (Fig. 5.18). In tobacco leaves, photosynthetic rate decreased after withdrawal of N, the decrease being associated with a strong decrease in the amount and activity of Rubisco. This decrease was prevented when leaves were shaded (Fig. 5.18). The shading had a negligible effect on amino acids, but strongly decreased hexose concentration in the leaves (Paul and Driscoll, 1997).

Another example of an indirect mode of action of nutrients on photosynthesis is the decrease of photosynthetic rates in plants via hydraulic or hormonal signals which reduce stomatal conductance, and thus leaf gas exchange (Fig. 5.16; see Cramer *et al*., 2009). Transpiration can be regulated by root hydraulic conductance, which in turn is affected by nutrients through control of aquaporins, for example by nitrate, P and S (Maurel *et al*., 2008). Nutrient

TABLE 5.5 Shoot growth and concentrations of chlorophyll and carbohydrates in primary leaves of common bean (*Phaseolus vulgaris*) at different light intensities and with or without Zn addition

Light intensity	Shoot dw		Chlorophyll		Carbohydrates			
					Sucrose		Total[a]	
	(g plant^{-1})		(mg g^{-1} dw)		(mg glucose equiv g^{-1} dw)			
(μE m^{2}s^{-1})	+Zn	−Zn	+Zn	−Zn	+Zn	−Zn	+Zn	−Zn
80	1.24	1.13	19.2	17.3	10	11	40	42
230	2.38	1.13	16.6	7.8	11	54	42	124
490	3.80	1.16	11.2	4.5	17	82	77	138

Based on Marschner and Cakmak (1989).
[a]*Sucrose, reducing sugars and starch.*

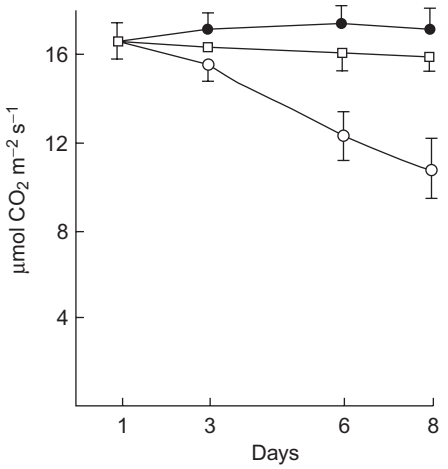


FIGURE 5.18 Rates of photosynthesis (μmoles CO_2 m^{-2} s^{-1}) in shaded and unshaded tobacco leaves grown with sufficient after withdrawal of N for 8 days. ○: unshaded plants with N deficiency; ●: shaded plants with N deficiency; □, unshaded N-sufficient control plants. *Based on Paul and Driscoll (1997).*

deficiency can regulate stomatal conductance also via alteration of supply of guard cells with root- or leaf-sourced hormones such as ABA and CYT (Wilkinson *et al*., 2007).

5.4 PHOTOSYNTHETIC AREA

The ability of plants to produce assimilates is not only related to photosynthetic activity (*source activity*, Section 5.3) but also to size of the photosynthetic area (*source size*) including leaves, stems, husks and other green organs of the plant. For example, spike organs of grain crops such as barley may substantially contribute to plant photosynthesis during grain filling, particularly under drought and in dense crop stands, where mutual shading and senescence limit leaf photosynthesis (Tambussi *et al.*, 2007). In this section only leaves are considered. Since all nutrients are constituents of leaves, they are required in the formation of leaf biomass. Control of leaf growth has been associated with many processes including cell cycle regulation, tissue extensibility, as well as hydraulic, sugar and hormonal signalling (for reviews see Granier and Tardieu, 2009; Walter *et al*., 2009) and there is evidence that specific nutrients are involved in these regulating biochemical and biophysical processes. In this section some examples are presented explaining these functions of nutrients in the regulation of leaf growth at levels of individual leaves, whole plants and canopies. Further examples are given in Chapters 6 and 7.

5.4.1 Individual Leaf Area

The area of individual leaves of a plant is dependent on leaf position and environmental conditions during leaf development. Environmental stresses, for example low temperatures, drought, salinity and nutrient deficiency (Granier and Tardieu, 2009), reduce final leaf area, with this depression being dependent on genotype. This is demonstrated in Fig. 5.19 in an example of two soil-grown maize genotypes differing in N efficiency (i.e., grain yield obtained under low N supply in the field). Regardless of genotype, individual leaf area increased from the basal leaf positions to leaf 8 and decreased again from leaf 10 towards the apical leaf positions. The area of individual leaves was dependent on N fertilization, the effect only becoming significant in leaves 10 to 15, presumably because in non-fertilized plants, N supply from seeds and mineralization of soil organic matter was sufficient to meet growth demands in the initial phase of plant development. N deficiency-induced reduction of leaf area was less pronounced in the N-efficient genotype, which under conditions of low N supply, produced higher grain yields than the inefficient genotype. The lower leaf N concentration

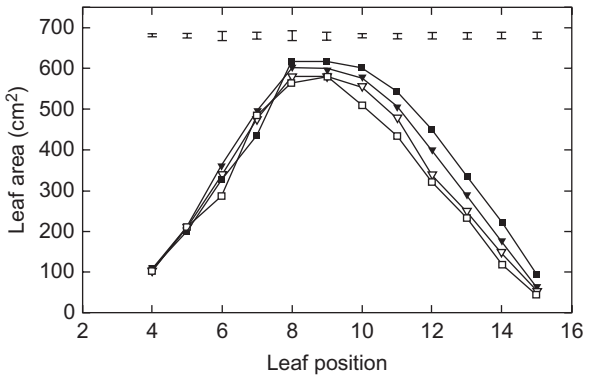


FIGURE 5.19 Individual area of leaves in different positions (leaf position 15 = apical leaf) in an N-efficient maize genotype (triangles) and a non-efficient genotype (squares) grown at high (filled symbols) or low N supply (open symbols). *From Paponov and Engels (2003) with permission from Wiley VCH.*

TABLE 5.6 Leaf elongation rate, cell production rate and final cell length in leaves of *Lolium perenne* at high or low N supply

	High N	Low N
Leaf elongation rate (mm h^{-1})	1.70	0.97
Cell production rate (cells h^{-1})	1.67	1.21
Final cell length (mm)	1.03	0.82

Based on Kavanová *et al.* (2008).

of the efficient as compared to the inefficient genotype (Paponov and Engels, 2003), indicates lower sensitivity of leaf growth to suboptimal internal leaf N status. Similarly to N deficiency, deficiencies of other nutrients such as K (Jordan-Meille and Pellerin, 2004) and P (Fletcher *et al*., 2008) reduce leaf elongation rates and final leaf area of field-grown plants.

Leaf growth is controlled by cell division and cell expansion. From a biophysical viewpoint, cell expansion is dependent on cell turgor as driving force together with cell wall properties which regulate wall expansion (for reviews see Fricke, 2002; Cosgrove, 2005). These biophysical cell properties, in turn, are regulated by an internal circadian clock and external cues such as temperature, light and nutrients via complex signalling cascades involving phytohormones (Section 5.9) and sugar signals (Walter *et al*., 2009; Poiré *et al*., 2010). In the long term, wall expansion must be matched by synthesis and integration of new wall materials, and thus is dependent on assimilate supply. In N-deficient plants, leaf elongation rates may decline before there is any reduction in net photosynthesis (Chapin *et al.*, 1988). Besides hormonal effects (see below), lower water availability in expanding leaf blades may also be involved (Radin and Boyer, 1982; see below).

Cell division and expansion in the leaf growth zone are particularly sensitive to plant supply of P (Assuero *et al.*, 2004) and N (MacAdam *et al.*, 1989; Roggatz *et al*., 1999). For example, in perennial ryegrass, leaf elongation rate was reduced by 43% in plants with low as compared to high N supply (Table 5.6). This reduction is the result of changes of similar magnitude in both cell production rate (−28%) and final cell length (−20%). In this study, the number of meristematic cells was not affected by low N supply, implying that the lower rate of cell production ensued from a decrease in the rate of cell division (Kavanová *et al*., 2008). The final cell length at low N supply was reduced despite the longer duration of the elongation phase. Similar effects on cell production and elongation have been found under P deficiency (Kavanová *et al*., 2006).

There is evidence that K is also needed for cell division and cell expansion. In *Arabidopsis* (Elumalai *et al*., 2002) and barley (Boscari *et al*., 2009), genes encoding K transporters and channels have been identified, which are expressed in growing leaf tissue. In tobacco protoplasts, the K deficiency-induced decrease in cell division was partially reversed by addition of alkalizing reagents, which suggests a role of K in increasing cytosolic pH (Sano *et al*., 2007).

Post-mitotic cell expansion is mainly due to increase in vacuolar volume. Nutrients such as K are required for cell expansion as solutes which decrease the vacuolar osmotic potential, and thus drive water influx and increase cell turgor (Section 6.7). Furthermore, supply of P and nitrate can directly modify the number and activity of aquaporins which mediate water influx (Gorska *et al*., 2008; Maurel *et al*., 2008).

The effect of N deficiency on leaf expansion differs between monocotyledons and dicotyledons (Table 5.7). In monocotyledons, cell expansion is inhibited to the same extent during day and night whereas in dicotyledons, inhibition is more severe during the day. This difference in response is related to morphological differences among species and corresponding differences in competition for the water available for transpiration and for cell expansion. In dicotyledons, cell expansion occurs in leaf blades which are exposed to the atmosphere and which therefore experience a high rate of transpiration during the day. In cereals, however, cell expansion occurs at the base of the leaf blade, a zone which is protected from the atmosphere by the sheath of the preceding leaf, so that little transpiration occurs during elongation. In contrast to leaf expansion, net photosynthesis per unit leaf area is depressed to a similar extent in both groups of plants by N deficiency. Similar results to those in Table 5.7 showing the marked effect of N deficiency in inhibiting leaf growth in dicotyledons have also been obtained for P deficiency in cotton plants (Radin and Eidenbock, 1984) where leaf growth rate

TABLE 5.7 Inhibition of leaf growth by N deficiency in different plant species

	Average growth inhibition (%)	
Plant species	Day	Night
Cereals (wheat, barley, maize, sorghum)	16	18
Dicotyledons (sunflower, cotton, soybean, radish)	53	8

Based on Radin (1983).

TABLE 5.8 Phyllochron and number of tillers per plant in wheat at different rates of P fertilization

	P fertilization (kg P ha^{-1})			
	7	15	60	300
Phyllochron (degree days $leaf^{-1}$)	124	108	110	94
Tiller number $plant^{-1}$	1.1	1.6	2.8	3.2

Based on Rodríguez *et al.* (1998).

is only during the day. Interestingly, monocotyledons and dicotyledons also differ in diel time course changes of leaf growth and its dependence on environmental conditions (Poiré *et al.*, 2010). In monocotyledons, diurnal variation in leaf growth is dependent on environmental conditions with no circadian oscillation when plants are grown in continuous light. By contrast, in dicotyledons leaf growth is regulated by an endogenous circadian clock and shows a clear circadian oscillation even under constant day–night conditions (Poiré *et al.*, 2010).

Turgor above a threshold value (*yield threshold*) induces cell expansion which depends on cell wall extensibility (Fricke, 2002). Regulators of wall extensibility include cell wall loosening expansins, xyloglucan endotransglucosylase/hydrolase and hydroxyl radicals which may be produced non-enzymatically by Cu ions bound to the cell wall or by wall peroxidases (Cosgrove, 2005). The activity of expansins is regulated by phytohormones including auxin, ethylene, gibberellin and cytokinin (Downes *et al*., 2001; Sánchez-Rodríguez *et al*., 2010). Phytohormone supply to leaves, in turn, is affected by nutrition and other external cues such as drought and salinity (see below). For example, the stimulation of leaf growth by nitrate and the inhibition by ammonium nutrition is related to xylem transport and leaf concentrations of active forms of cytokinins, which are increased by nitrate and decreased by ammonium in tobacco (Walch-Liu *et al*., 2000) and tomato (Rahayu *et al*., 2005).

5.4.2 Leaf Area per Plant

Leaf area development at the whole plant level is also dependent on the rate of leaf development, and on tillering and formation of axillary branches. The rate of leaf development is strongly regulated by temperature and can be described on the basis of thermal time, i.e. the product of time and temperature exceeding a minimal threshold below which development is completely arrested (Granier *et al*., 2002). Nutrient deficiency may delay plant development. For example, in barley the number of days to reach the booting stage is about twice as high for Mn-efficient than for Mn-sufficient plants (Longnecker *et al.*, 1991b). The thermal time elapsing between the visual appearance of two successive leaf tips (phyllochron in degree days $leaf^{-1}$) is also influenced by nutrition. As shown in an example for wheat in Table 5.8, phyllochron was reduced from 124 degree days $leaf^{-1}$ in P-deficient plants to 94 degree days $leaf^{-1}$ in highly fertilized plants. Furthermore, the total number of tillers per plant was also significantly increased by high P supply in this P-deficient soil. Nitrogen deficiency has also been shown to increase the phyllochron (Adamowicz and Le Bot, 2008). It is well known in agricultural and horticultural plant production that tillering and axillary branching of field grown plants can be stimulated by N fertilization.

5.4.3 Canopy Leaf Area (LAI, LAD)

At the canopy level, photosynthetic area is often expressed in terms of the *leaf area index* (LAI), which is defined as leaf area of plants per unit area of soil. For example, an LAI of 5 means that there are 5 m^2 leaf area per m^2 soil area. LAI values below 3 are often associated with incomplete interception of incoming solar radiation, whereas LAI values above 6 indicate strong shading, and thus negative net photosynthesis of lower leaves. Light distribution within the canopy is also influenced by leaf architecture (Horton, 2000; Long *et al.*, 2006). Compared to horizontal leaves, erect leaves reduce excessive light interception of the top leaf layer in a crop stand, and thus photoinhibition. In contrast, light incidence is increased onto lower leaves within the canopy, in which photosynthesis is limited by low light. Model simulations have shown that in a canopy with an LAI of 3, the efficiency with which the intercepted light is converted to biomass through photosynthesis is about 40% higher in erect leaves compared to horizontal leaves (Long *et al.*, 2006). Source size is not only determined by leaf area but also *leaf area duration* (LAD, which is the sum of LAI integrated over a period of time),

that is the length of time in which the source leaves supply photosynthates to sink sites. In crop species, LAD is of crucial importance for the length of the sink filling period and often is closely positively correlated with yield (see, e.g., Cabrera-Bosquet *et al*., 2009).

Nutrition influences leaf growth and leaf senescence (see above), and thus also LAI and LAD. Nutrient deficiency under high light intensity is often associated with accumulation of photosynthates in source leaves. Photosynthate accumulation not only decreases utilization of light energy but also poses a stress. This high light stress is indicated by an increase in the antioxidative defence mechanisms in the deficient leaves (Cakmak and Marschner, 1992; Fig. 5.5), photooxidation of chloroplast pigments (Table 5.5) and enhanced leaf senescence. These side effects of nutrient deficiency decrease not only current photosynthesis and LAI but also LAD.

In the initial phase of development, leaves are sink organs which utilize assimilates exported from source organs. In crop species with vegetative storage organs like root and tuber crops, there is competition for assimilates between leaf area construction and storage processes. This has to be considered, for example in N fertilization of potato. On the one hand, a high N supply is important for rapid leaf expansion and for obtaining an LAI between 4 and 6, a value considered as necessary for high tuber yields (Kleinkopf *et al.*, 1981). On the other hand, high N supply delays tuberization and/or the onset of the linear phase of tuber growth. The principles of these interactions are demonstrated in Fig. 5.20. At low N supply, the advantage of earlier tuberization is offset by a low LAI and earlier leaf senescence, i.e. a short LAD and a correspondingly lower tuber yield. When the nitrogen supply is high, both LAI and LAD, and thus final tuber yield, are much higher. However, higher tuber yield induced by a large N supply can be achieved only when the vegetation period is sufficiently long, i.e. in the absence of early frost (Clutterbuck and Simpson, 1978) or in the absence of severe drought stress.

The early decline in LAI at low N supply (Fig. 5.20) indicates that the final tuber yield is limited by the source. One of the reasons for this source limitation is that in potato plants at maturity, between 60 and 80% of the total N is located in the tubers (Kleinkopf *et al.*, 1981). Thus, when the N supply is low, exhaustion of N in the source leaves presumably plays a key role in leaf senescence and in the termination of tuber growth. However, these simple relationships between N supply, LAI, LAD and tuber yield (Fig. 5.20) are not only modified by the length of the growing period but also by the mineralization rate of soil N and by soil temperature during tuber growth. At high N supply and high LAI, mutual shading of the basal leaves may not only drastically decrease their net photosynthesis but also the LAD by rapid leaf senescence (Firman and

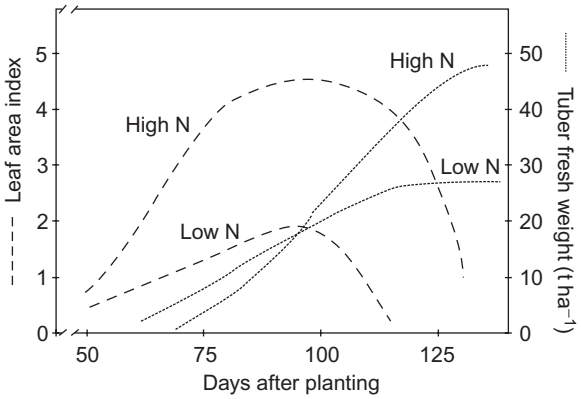


FIGURE 5.20 Time course of leaf area index and fresh weight of potato tubers at high or low N supply. *Based on Ivins and Bremner (1964) and Kleinkopf* et al. *(1981).*

Allen, 1988), a process which is further enhanced at high temperatures (Manrique and Bartholomew, 1991). Thus, a lower, but more continuous supply of N which allows earlier tuberization and continuous root growth and CYT production, and which is more effective on LAD than on LAI, may often lead to higher tuber yields than a rapid establishment of a high LAI by high N supply during early growth.

LAI and LAD are dependent on leaf area per plant and plant density, which in agricultural crop stands is also influenced by sowing density. The increase in wheat and maize grain yield of modern varieties has been largely attributed to increases in LAI and LAD (Austin, 1989; Lee and Tollenaar, 2007). Interestingly, in modern maize hybrids leaf area per plant is similar to that of old hybrids. Improvements in LAI have thus mainly resulted from greater crowding tolerance (tolerance to intraspecific competition among neighbouring plants) allowing higher plant densities (Boomsma *et al*., 2009).

5.5 RESPIRATION AND OXIDATIVE PHOSPHORYLATION

In non-green tissue (e.g., roots, seeds and tubers) or in green tissue during the dark period, respiratory carbohydrate decomposition is the main source of energy for energy-consuming processes such as synthesis and transport. Respiration consumes 30–70% of the carbon assimilated during photosynthesis (Amthor, 2000). Respiration can be partitioned into two functional components: growth and maintenance respiration. Growth respiration is defined as respiratory energy required for biosynthesis of new plant constituents. Maintenance respiration is the respiratory energy required for all processes that maintain cellular structure, for example turnover of cellular components and maintenance of intracellular ion gradients. For roots,

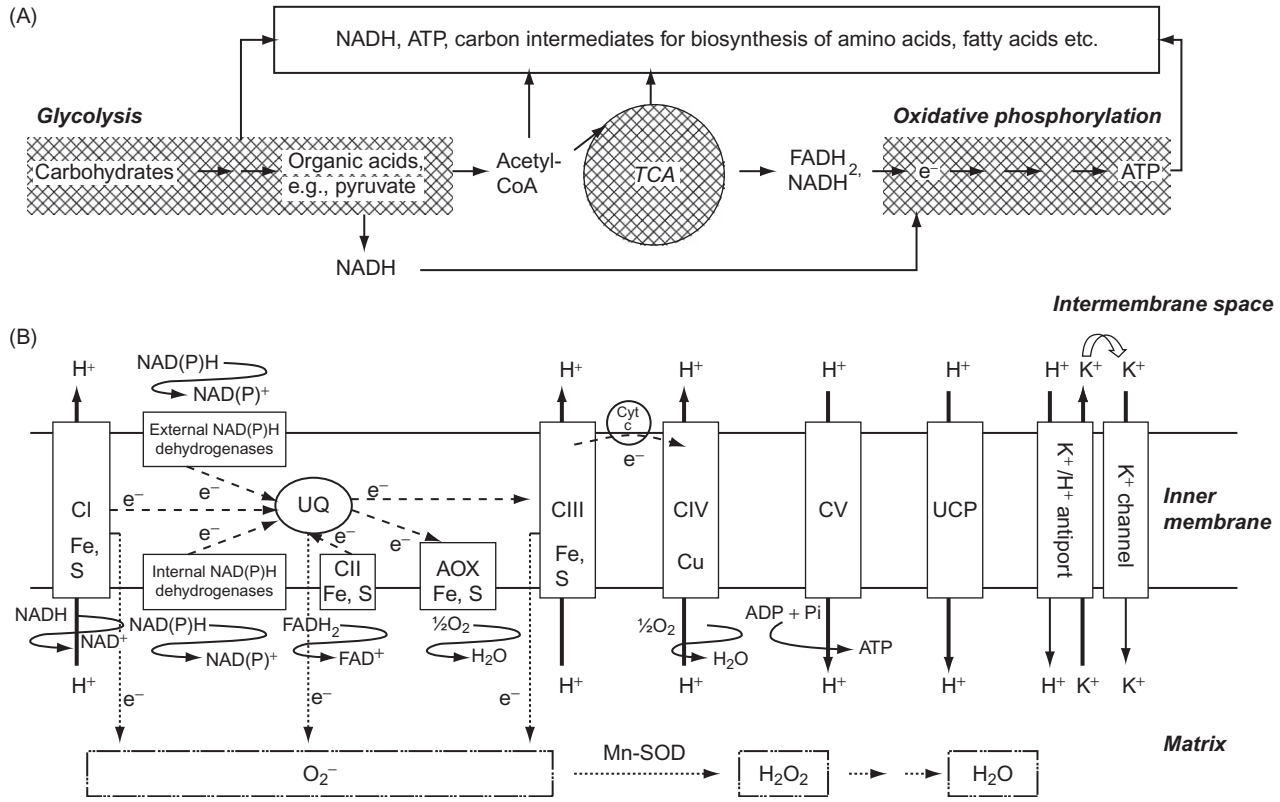


FIGURE 5.21 Scheme of respiration. (A) Main steps of respiration: glycolysis in the cytosol and plastids, tricarboxylic acid cycle (TCA) and oxidative phosphorylation in mitochondria; (B) organization of the electron transport processes on the inner membrane of mitochondria; CI complex I (NADH dehydrogenase), CII complex II (succinate dehydrogenase), CIII complex III (cytrochrome bc_1 complex), CIV complex IV (cytochrome c oxidase), CV complex V (ATP synthase); UQ: ubiquinone; cyt c: cytochrome c; UCP: uncoupling protein; Mn-SOD: Mn superoxidedismutase. *Based on Plaxton and Podesta (2006), Navrot* et al. *(2007) and Atkin and Macherel (2009).*

a third functional component is the energy needed for nutrient uptake (Section 2.4). There is some evidence, for example from field-grown maize, that there is a potential for increasing crop yields through reduction of maintenance respiration (Earl and Tollenaar, 1998).

Respiration can be divided into three major steps (Fig. 5.21A). Glycolysis is the decomposition of sugars to organic acids, for example pyruvate, which yields a small amount of ATP and reduced nicotianamide dinucleotide (NADH). In the tricarboxylic acid cycle (TCA, also called the Krebs cycle or citric acid cycle), pyruvate is completely oxidized to CO_2 and a considerable amount of reducing power (NADH and reduced flavin adenine dinucleotide $FADH_2$) is produced. In oxidative phosphorylation, electrons from the donors NADH and $FADH_2$ are transferred along an electron transport chain in the inner mitochondrial membrane to oxygen (Fig. 5.21B). The individual electron transport proteins are organized into four multi-protein complexes (CI to CIV). Electrons are transferred from CI (NADH dehydrogenase) and CII (succinate dehydrogenase) via ubiquinone (UQ) to CIII (cytochrome bc_1). Cytochrome c transfers the electrons to CIV (cytochrome oxidase), the terminal oxidase which transfers the electrons to molecular oxygen.

Several nutrients are directly involved in this mitochondrial electron transport chain (Fig. 5.21B). In CI, CII and CIII, electrons are transferred via Fe-S proteins. CIV contains two Cu centres. Electron transport via CI, CIII and CIV is coupled to proton pumping across the inner mitochondrial membrane, and the resultant electrochemical gradient is used by an ATP synthase (also called complex V) for ATP production (Fig. 5.21B). Thus, the principles involved in ATP synthesis in the mitochondria are the same as those of ATP synthesis in the chloroplasts: charge separation by a membrane with a corresponding proton (pH) gradient across the membrane constituting the electromotive force for ATP synthesis.

The NADH synthesized in the decarboxylation reactions represents a universal reducing agent in non-green tissue and is therefore also required for various synthetic processes involving reduction, such as amino acid and fatty acid synthesis. Furthermore, the various intermediates of carbohydrate decomposition are essential structures (carbon skeletons), for example, for the synthesis of amino

acids and fatty acids. The rate of respiration is therefore regulated not only by environmental factors such as temperature or by energy requirements (e.g., ATP for ion uptake in the roots), but also by the demand for reducing equivalents and intermediates.

Depending on the metabolic process, the demand for ATP (activating agent) relative to that for NADH (reducing agent) and carbon intermediates can vary markedly. For example, for transport processes across membranes, mainly ATP is needed, whereas the biosynthesis of lipids or amino acids requires large amounts of NADH and carbon intermediates. This variable demand for respiratory products is met by metabolic 'bypasses' in the three steps of respiration: glycolysis, TCA and oxidative phosphorylation (Plaxton and Podestá, 2006; Sweetlove *et al*., 2010). These bypasses yield different amounts of respiratory products. For example, the proton gradient across the inner mitochondrial membrane generated by electron transport via CI, CIII and CIV can be dissipated by an uncoupling protein (UCP) which allows proton diffusion into the matrix without ATP production (Plaxton and Podestá, 2006; Fig. 5.21B). There is also evidence for a role of K in the dissipation of the transmembrane proton gradient (Fig. 5.21B). In this case, protons are exported to the matrix in exchange for K import via a K^+/H^+ antiporter. Subsequently, K is released into the matrix via a K channel (Pastore *et al*., 1999). These energy dissipating pathways may help to avoid over-reduction of the electron transport chain which is a major mechanism for ROS production (Fig. 5.21B), for example under salt and drought stress (Pastore *et al*., 2007).

Another example of plasticity in plant respiration is the engagement of mitochondrial electron transport pathways that allow electron transfer to oxygen which circumvent proton-pumping sites (the complexes CI, CIII and CIV). Electrons from NADH and NADPH can be transferred via external and internal NAD(P)H dehydrogenases to ubiquinone (Fig. 5.21B). From ubiquinone, electrons can be further transferred via the alternative oxidase (AOX) to molecular oxygen. This alternative pathway of electron transport is not coupled to H^+ transport across the mitochondrial membrane, hence it is not associated with ATP synthesis. The lower efficiency of the alternative pathways in energy conversion in the form of ATP results in higher energy dissipation in the form of heat (*thermogenesis*). Apart from heat production, there are two major hypotheses regarding the function of AOX in overall metabolism (Molen *et al*., 2006). One hypothesis is that AOX reduces mitochondrial ROS formation under stress conditions. AOX provides a pathway of electron flow, and thus may prevent over-reduction of electron transport components, particularly UQ. Over-reducing UQ would exacerbate the formation of toxic ROS thereby overburdening the ability of plants to detoxify superoxide ions (O_2^-) via the Mn-containing superoxide dismutase and the ascorbate-glutathione pathway to H_2O (Navrot *et al*., 2007; Fig. 5.21B). The other hypothesis is that engagement of alternative pathway respiration provides the plant with metabolic flexibility to upstream carbon metabolism and ATP production (Molen *et al*., 2006). AOX may allow continued operation of glycolysis and TCA (Fig. 5.21B) when the cytochrome chain is inhibited by specific stress factors or otherwise restricted by a high cellular ATP/ADP ratio.

The proportion of the alternative pathway can vary between less than 10% and up to more than 80% of the total respiration in the roots and leaves (Poorter *et al*., 1991; Florez-Sarasa *et al*., 2007). Factors contributing to this variation include time of day (Siedow and Berthold, 1986), P nutritional status (Theodorou and Plaxton, 1993; González-Meler *et al*., 2001), plant species, developmental stage and plant organ. The proportion of the alternative pathway is also dependent on the level (Scheible *et al*., 2004) and form of N supply (Barneix *et al*., 1984; Escobar *et al*., 2006). In N-deficient *Arabidopsis*, the transcription of AOX genes was reduced by resupplying nitrate, and increased by resupplying ammonium (Escobar *et al*., 2006). Similar results were observed at adequate N supply when nitrate and ammonium sources were switched. These effects of form of N supply on AOX capacity are in accordance with a role of the alternative pathway for redox balancing (Escobar *et al*., 2006). Nitrate nutrition has a high demand for NADH for nitrate reduction. Thus, there is no excess of NADH for electron transport via the alternative pathway. Ammonium nutrition, on the other hand, has a lower demand for NADH so that NADH is oxidized via the alternative pathway. It was found that decreased transcription of *AOX* in nitrate-fed plants was mediated by the nitrate ion itself, whereas ammonium regulation of *AOX* transcription was dependent upon assimilation and affected by ammonium-induced changes in apoplasmic pH (Escobar *et al*., 2006).

5.6 PHLOEM TRANSPORT OF ASSIMILATES AND ITS REGULATION

Long distance transport of assimilates, sugars, amino acids and nutrients from mature source leaves to sink organs occurs in the phloem. The phloem system can be subdivided into three sectors as shown in Fig. 5.22. In the collection phloem, photosynthates are loaded into the minor veins of source leaves. The transport phloem in the main veins, leaf sheaths, petioles, stems and roots transports photosynthates to the sink organs, where they are released into the sink cells in the release phloem (van Bel, 2003). The processes associated with the transport of photosynthates and amino acids in the different sectors of the phloem are briefly described.

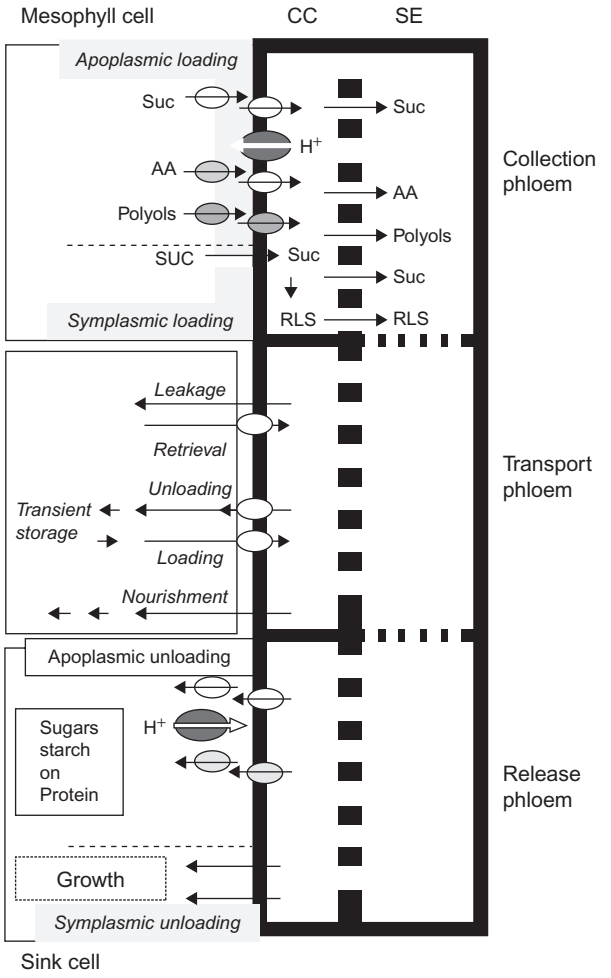


FIGURE 5.22 Long-distance transport of sugars and amino acids via the phloem; for further explanation see text. *Based on Lalonde* et al. *(2003).*

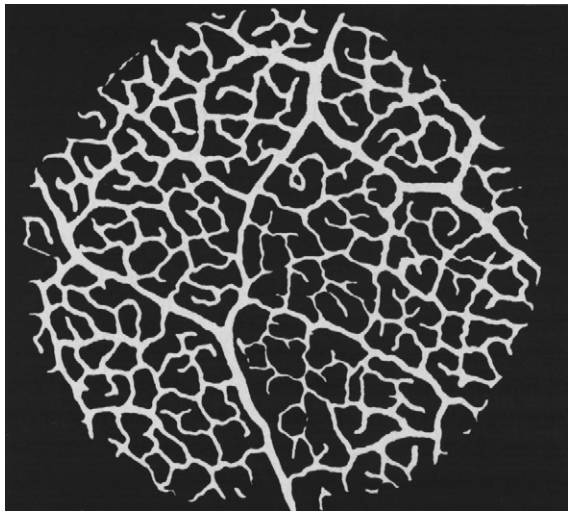


FIGURE 5.23 Autoradiograph of phloem loading of [^{14}C]sucrose into source leaf tissue of bean. Sucrose concentration, 1 mM; accumulation period, 30 min. White areas = minor veins with ^{14}C. *From Giaquinta and Geiger (1977) with permission from the American Society of Plant Biologists.*

5.6.1 Phloem Loading of Assimilates

The first step in supplying young leaves and other sinks with assimilates from the source leaves is short-distance transport of the assimilates from individual leaf cells to the phloem parenchyma cells of the vascular bundles which is followed by loading into the sieve elements (SEs) or companion cells (CCs). Sieve elements and CCs are symplasmically connected by special plasmodesmata (*plasmodesmata-pore units*) which are highly permeable (van Bel, 2003; Turgeon and Wolf, 2009). The conductivity of plasmodesmata is dependent on the diameter of their pores. This diameter may be expressed as size exclusion limit, i.e. maximum size or mass of a molecule that can pass through the plasmodesmata passively. The branched plasmodesmata connecting SEs and CCs are characterized by a high size exclusion limit that allows transfer of large molecules (20–40 kDa; van Bel, 2003). The SEs lack many structures normally found in living cells such as nuclei, ribosomes and vacuoles. They have large pores in their cell walls and are highly specialized for transport of water and solutes by mass flow. The essential metabolic functions lacking in SEs such as protein synthesis are taken over by the CCs. Thus, SEs and CCs form a functional unit which is referred to as the sieve element companion cell complex (SE–CC complex).

As a rule, sugars represent 80–90% of the assimilates exported in the SEs from the source leaves (see Chapter 3, Table 3.8). In most plant species, sucrose is the dominant sugar in the phloem sap, but in some plant species raffinose, stachyose (e.g., cucurbits) or sugar alcohols like mannitol (e.g., celery, parsley, carrot and olive) or sorbitol (e.g., apple and cherry) are also transported (Turgeon and Wolf, 2009). The preferential sites for phloem loading of sugars are the minor veins of a source leaf as shown in Fig. 5.23 where ^{14}C-labelled sucrose was infiltrated into the leaf.

Depending on the plant species, time of the day, and also the site of collection, the sucrose concentrations in the phloem sap are in the range of 200 to 1,000 mM. In plant species which additionally transport the sugar alcohols mannitol and sorbitol, concentrations of between 300 and 700 mM have been measured (Nadwodnik and Lohaus, 2008). In order to achieve these high concentrations, a loading step from the mesophyll or phloem parenchyma cells into the SE–CC complex of the minor veins is required in most plant species (Fig. 5.22, collection phloem upper part). Estimates of whole-leaf apoplasmic concentrations of sucrose are in the range of 1–5 mM, and of apoplasmic sucrose concentrations in the vicinity

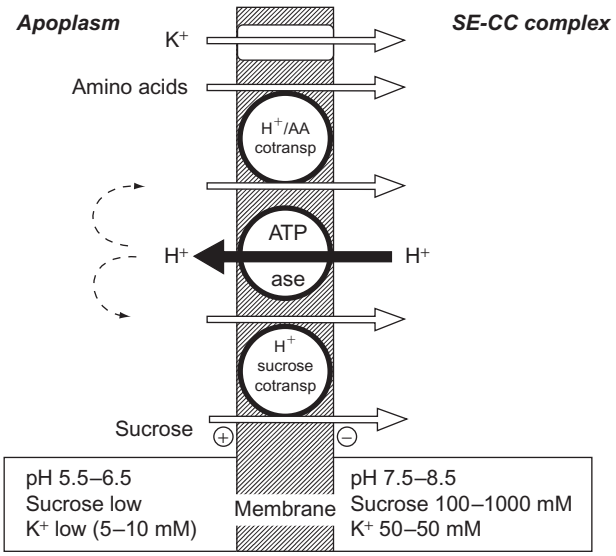


FIGURE 5.24 Model for phloem loading of sucrose mediated by proton–sucrose cotransport, proton–amino acid (AA) cotransport, and uniport of K. *Based on Baker* et al. *(1980) and Giaquinta (1977).*

TABLE 5.9 Concentrations of sucrose and amino acids in different cell compartments of barley leaves

	After 8 h light		After 5 h dark	
	Cytosol	Phloem	Cytosol	Phloem
Sucrose (mM)	150	1,030	43	930
Amino acids (mM)	156	186	58	244
Ratio Aa/Suc	1.04	0.18	1.35	0.26

Based on Winter *et al.* (1992).

of minor veins, 27–133 mM (Lalonde *et al.*, 2003). In plant species which additionally transport sugar alcohols, sugar alcohol concentrations in the leaf apoplast have also been found to be substantially lower than in the phloem (Nadwodknik and Lohaus, 2008). Loading of sucrose and sugar alcohols from the apoplasm into the phloem must therefore be energized by the proton motive force generated by a proton-pumping ATPase in the plasma membrane of the SE–CC complex (Lalonde *et al*., 2003). This ATPase creates a steep transmembrane potential gradient as well as a pH gradient between the lumen ('symplasm') of the SE–CC complex and the apoplasm (Fig. 5.24). This gradient acts as a driving force for the transport of sucrose from the apoplasm into the SE–CC complex in the form of H^+/sucrose cotransport (symport) mediated by phloem-specific sucrose transporters (Lalonde *et al*., 2003). This transport model follows the same principle as has been described in Fig. 2.9 for the proton–anion cotransport at the plasma membrane of root cells. In plants species which transport sugar alcohols, H^+/sugar alcohol symporters localized in the CCs of the phloem in source leaves have been identified (Fig. 5.22, collection phloem upper part), suggesting that they play a role in phloem loading of sorbitol and mannitol (Ramsperger-Gleixner *et al*., 2004).

In some plant species, abundant plasmodesmata occur along the possible solute pathway from the mesophyll to the SE–CC complex (Rennie and Turgeon, 2009) suggesting symplasmic phloem loading (Fig. 5.22, collection phloem lower part). In leaves of willow and other woody species, the gradient of sucrose concentrations between mesophyll cells and phloem allows passive diffusion-driven transport of sucrose through plasmodesmata into the phloem (Rennie and Turgeon, 2009). In other species (e.g., cucurbits) which load sugars via the symplasm, raffinose-like sugars are transported in the phloem. In these species, sucrose is transported from the mesophyll cells into specialized companion cells in the minor veins known as intermediary cells. In these cells sucrose is converted to raffinose and stachyose (RLS, raffinose-like sugars; Fig. 5.22, collection phloem lower part), i.e. to molecules that are larger than sucrose. Diffusion back to the mesophyll cells is prevented through the small size exclusion limit of the plasmodesmata that connect the intermediary and the mesophyll cells (*polymer trapping*), whereas diffusion into the SEs is made possible by the larger size exclusion limit of the plasmodesmata that connect the intermediary cells with the SEs (Rennie and Turgeon, 2009).

Phloem loading of amino acids is rather selective. In castor bean, for example, glutamine is loaded preferentially compared to glutamate or arginine (Schobert and Komor, 1989). Amino acid loading is also depressed by simultaneous loading of sucrose, and vice versa. In maize leaves, asparagine is preferentially loaded into the phloem where its concentration is about eight times higher than in the cytosol of the leaf cells (Weiner *et al.*, 1991). However, as a rule, the up-hill transport of sucrose from mesophyll cells into the phloem is steeper than for most of the amino acids. An example of this is shown in Table 5.9. In barley leaves, sucrose was preferentially loaded into the phloem compared to the amino acids, as reflected by the ratio of amino acids/sucrose in the cytosol of about one in contrast to that of about 0.2 in the phloem sap (Table 5.9). In barley, the overall phloem transport of amino acids seems to depend on sucrose loading and mass flow in the phloem (Winter *et al.*, 1992). Phloem loading of amino acids in barley is thus presumably mediated by a uniport similar to that for K (Fig. 5.24).

Compared to sugar concentrations, amino acid concentrations in the phloem sap are usually lower, in the range of 50 to 200 mM. However, in individual SEs of wheat, amino acid concentrations above 1,000 mM have been

TABLE 5.10 Loading and transport of [^{14}C] alanine in the phloem of castor bean petioles with supply of K or Na at different pH

Treatment		[^{14}C] alanine in the phloem exudate
Ion	pH	(^{14}C counts × 10^3 mL^{-1})
K^+	5	27.8
	8	8.4
Na^+	5	13.8
	8	4.1

Based on Baker *et al.* (1980).

measured (Gattolin *et al.*, 2008) and oilseed rape phloem sap can contain up to 650 mM amino acids, more than four times higher than in the cytosol of the mesophyll cells (Tilsner *et al.*, 2005). Thus, in many plant species loading of amino acids into the SE–CC complex may be as important as sucrose loading. Baker *et al.* (1980) showed that the loading and transport of amino acids in the phloem is also strongly depressed by a high external pH (Table 5.10) suggesting a proton-amino acid co-transport similar to that for sucrose. Indeed, there is increasing evidence of amino acid transporters located to the phloem tissue of leaves, which mediate the uptake of amino acids into the SE–CC complex by H^+/amino acid cotransport (Fig. 5.24; Lalonde *et al.*, 2003; Rentsch *et al.*, 2007). In potato, the importance of the leaf H^+/amino acid-symporter StAAP1 for phloem loading was demonstrated by antisense inhibition (Koch *et al.*, 2003). Transgenic plants with antisense *StAAP1* showed up to 50% reduction in free amino acid concentrations in tubers. In *Arabidopsis*, inactivation of the gene *AAP6*, which encodes an H^+/amino acid cotransporter, significantly reduced the concentrations of amino acids in the phloem sap (Hunt *et al.*, 2010). In addition to amino acid transporters, transporters for allantoin and peptides are expressed in the phloem, suggesting a role of the transporters for phloem loading of these N-containing organic compounds (Rentsch *et al.*, 2007).

Loading and transport rate of amino acids also depends on the cation present in the external solution, K being more stimulatory than Na. It is also well established that phloem loading of sucrose is enhanced by K (Peel and Rogers, 1982), unless excessive external K concentrations lead to depolarization of the membrane potential and thus impairment of the H^+/sucrose cotransport. It is not clear, however, whether stimulation by K is a direct effect on the loading mechanism (e.g., maintenance of the transmembrane pH gradient) or an indirect one via an increase in osmotic potential of phloem sap and, thus, the rate of mass flow in the sieve tubes. In *Arabidopsis*, a K channel of the AKT2/3 family is localized in phloem cells (Lacombe *et al.*, 2000). In an AKT2/3 loss-of-function mutant, sucrose concentration in the phloem sap was only half that of the wildtype (Deeken *et al.*, 2002) suggesting a regulatory role of this K channel for phloem loading of sucrose.

Whereas the transporters mediating influx of sucrose and amino acids from the apoplast into the SE–CC complex are well characterized, little is known about the transport systems for efflux from the mesophyll or phloem parenchyma cells to the apoplasm. It has been reported that the concentrations of sucrose and sugar alcohols (Nadwodnik and Lohaus, 2008), and of amino acids (Tilsner *et al.*, 2005) are substantially lower in the leaf apoplasm than in the cytosol of mesophyll cells. The concentration gradient would thus allow passive export to the apoplasm by efflux transporters. In *Arabidopsis*, an amino acid transporter (BAT1) has been identified that mediates not only proton coupled uptake into the cells but also passive efflux of amino acids into the apoplasm (Dündar and Bush, 2009).

5.6.2 Mechanism of Phloem Transport of Assimilates

The principles regulating transport in the sieve tubes, the anatomy of the phloem, and transport direction (from source to sink) have been discussed in Chapter 3 in relation to long-distance transport of nutrients. In brief, according to the *pressure flow hypothesis* (Münch, 1930) solutes are loaded into the sieve tubes of leaves and water is sucked into the sieve tubes creating a positive internal pressure. As sucrose and other sugars are the dominant osmotically active solutes in the sieve tubes of leaves, volume flow rates are determined primarily by phloem loading of photosynthates (including amino acids) at the source and unloading at the sink. Water availability in the source leaves is also an important factor for volume flow rates in the sieve tubes (Smith and Milburn, 1980), and phloem loading is associated with lateral water transport in the leaves towards the phloem (Minchin and Thorpe, 1982).

Of the nutrients, K is usually present at the highest concentrations in the phloem sap (Section 3.3). Thus, K contributes substantially to the volume flow rates in sieve tubes as shown in Table 5.11 for castor bean. In plants well supplied with K, the concentration of K is the phloem sap, and particularly the volume flow rate (exudation rate), are higher than in plants with low K supply. The sucrose concentration in the phloem sap remains more or less unaffected, and a high K supply increases the transport rate of sucrose in the phloem by a factor of ~2. There could be several reasons for this enhancement of the volume flow rate by K, including higher rates of sucrose synthesis, enhanced of phloem loading (Deeken *et al.*, 2002;

TABLE 5.11 Composition of phloem sap and rate of phloem sap exudation of castor bean plants at different K supply

	K supply in the growth medium	
	0.4 mM	1.0 mM
Phloem sap concentration (mM)		
Potassium	47	66
Sucrose	228	238
Osmotic potential (bars)	−12.5	−14.5
Exudation rate (ml 3 h^{-1})	1.4	2.5

Based on Mengel and Haeder (1977).

TABLE 5.12 Concentration (mM) of various solutes in phloem sap of rice plants

	Site of collection	
Solutes	Leaf sheath (7–8 leaf stage)	Uppermost internode (One week after anthesis)
Sucrose	206	574
Amino acids	103	125
K	147	40
ATP	1.63	1.76

Hayashi and Chino (1990).

Section 5.6.1), or direct osmotic effects of K within the sieve tubes.

Along the pathway between source and sink, concentrations and composition of the phloem sap may change considerably for various reasons including leakage, unloading to and reloading from transient storage compartments along the axial pathway (Fig. 5.22, transport phloem) and xylem–phloem transfer (Atkins, 2000). Photosynthates may leak from the sieve tubes, hence retrieval becomes important to drive the pressure flow and to supply the sink (Minchin and Thorpe, 1987). Along the pathway, retrieval of sucrose is mediated by the same mechanism (sucrose–proton cotransport) as phloem loading in the source tissue (Aoki *et al.*, 2004). Leakage (or unloading) along the pathway may serve several functions such as (i) supply sucrose as an energy source for surrounding tissues, (ii) transient storage of starch or fructans in leaf sheaths and stem tissues of cereals and forage grasses (Schnyder, 1993; Berthier *et al.*, 2009), and (iii) adjustment of the solute composition in the sieve tubes according to the demand of the sink. In soybean, for example, sucrose concentration was found to decrease from 336 mM in the leaves to 155 mM in the roots as a growth (utilization) sink, with a corresponding increase in the osmotic potential from –6.0 to –1.8 bars, i.e. no compensation of osmotically active solutes in the phloem sap (Fisher, 1978). By contrast, in rice plants the solute concentration in the phloem sap increases from the source leaves towards the ear as a storage sink (Table 5.12). This increase is due to sucrose as the K concentration decreases. Despite a similar total concentration of amino acids (Table 5.12), the composition differed: towards the sink, the proportion of glutamine and arginine increased at the expense of glutamate and asparagine (Hayashi and Chino, 1990).

The shift in proportion of sucrose/K in the phloem sap (Table 5.12) reflects the demand at the sink sites. Developing grains of cereals as starch storing organs with low water content have a high demand for sucrose but a low K demand, particularly at the later stages of grain filling. In agreement with this suggestion, the contribution of K to the total osmotic potential of the phloem sap in the peduncles of wheat ears decreases from 8% to 2% within five weeks after anthesis (Fisher, 1987). The increase in sucrose/K ratio (Table 5.12) is most likely the result of mobilization of carbohydrates (starch, fructans) in the stem tissue and subsequent sucrose loading into the phloem (Fig. 5.22, transport phloem). Thus, K is replaced by sucrose and presumably transferred into the vacuoles of the stem tissue, demonstrating that the turgor of individual sieve tubes may be regulated along the pathway from source to sink and that K plays an important role in this regulation (Lang, 1983). Along the pathway, K may therefore not only fulfil the functions in phloem loading of sucrose but also represent a means of fine regulation within the coarse regulation of pressure-driven solute flow from source to sink (Martin, 1989).

5.6.3 Phloem Unloading

The release of solutes from the phloem into the surrounding tissue at the sink sites is strongly regulated by the sink strength, i.e. the capacity of a tissue or organ to accumulate or metabolize photosynthates (Zhou *et al.*, 2009; Fig. 5.22, release phloem). In this section, only the transport of assimilates from the SE–CC complex to the adjacent sink cells is considered, including the transport processes in the post-sieve element pathway but excluding uptake into sink cells which is described in Section 5.8.

The sinks for solutes (sugars, amino acids, nutrients) delivered in the sieve elements can be differentiated into (i) utilization sinks such as root tips, shoot apices and stem

elongation zones in which photosynthates are utilized for growth, and (ii) storage sinks in which photosynthates are mainly accumulated. Storage sinks include storage roots (e.g., sugar beet), stems (e.g., sugar cane) and other vegetative shoot organs (e.g., tubers), as well as generative organs like fleshy fruits and seeds. In utilization sinks there is evidence for symplasmic phloem unloading from electronmicroscopic studies which show many plasmodesmata linking SEs with adjacent meristem cells (Patrick, 1997; Fig. 5.22, release phloem lower part). For utilization sinks such as vegetative apices, young leaves and root tips, it has been demonstrated that a 27 kDa protein (the jellyfish green fluorescent protein (GFP)) can be unloaded symplasmically from the phloem into sink tissues (Imlau *et al.*, 1999), indicating a high size exclusion limit of plasmodesmata on the post-phloem pathway of these sinks.

In generative storage organs such as seeds of cereals and grain legumes, the filial tissues (embryo, endosperm) are symplasmically isolated from the phloem in the maternal seed tissues (seed coat). Therefore, the transport of photoassimilates and nutrients from the phloem to the filial tissues is always associated with unloading from maternal tissues into the apoplasm and subsequent loading into the symplasm of filial tissues (Patrick, 1997; Fig. 5.22 release phloem upper part). Apoplasmic unloading of solutes delivered in the phloem generally does not occur at the SE–CC complex, but at sites more distant from the phloem at the interface between maternal and filial tissues (Patrick and Offler, 2001).

In other storage sink organs, the mode of phloem unloading can change during development. A switch from apoplasmic phloem unloading in early stages of organ development to symplasmic phloem unloading in later stages has been found in vegetative storage sinks such as potato tubers (Viola *et al*., 2001) and sugar beet storage roots (Godt and Roitsch, 2006). This switch may be caused by modification of the conductivity of plasmodesmata connecting the phloem with the surrounding sink cells (Ruan *et al*., 2001) and is also associated with changes in the expression of sugar transporters (Lalonde *et al*., 2003) and metabolism. For example, in sugar beet, the transition from apoplasmic to symplasmic unloading was associated with reduction of the activity of the cell wall invertase which cleaves sucrose into fructose and glucose in the apoplasm, and thus maintains low apoplasmic sucrose concentrations (Godt and Roitsch, 2006).

Compared to import of sucrose and amino acids from the apoplasm into cells of the phloem tissue which is mediated by proton-coupled sucrose symporters and amino acid symporters, little is known about the transporters involved in efflux from cells along the post-sieve element pathway into the apoplast, and the energy demand for solute efflux (Lalonde *et al*., 2003). Various mechanisms for this efflux have been suggested, including carriers supporting facilitated diffusion of sucrose and sucrose/H^+ antiporters (Lalonde *et al*., 2003). In maize, a sucrose transporter (ZmSUT1) has been localized in the phloem that is capable of mediating both sucrose uptake into the phloem as well as sucrose release from the phloem into the surrounding tissues (Carpaneto *et al.*, 2005). In *Arabidopsis*, an amino acid transporter (bidirectional amino acid transporter 1, BAT1) has been found, which displays both export and import activity for various amino acids, depending on the electrochemical potential gradient across the membranes (Dündar and Bush, 2009). The mRNA of the *BAT1* gene was expressed in various organs and tissues including the vascular tissue. Thus BAT1 is possibly an amino acid transporter which mediates the efflux of amino acids in apoplasmic phloem loading in leaves (efflux from mesophyll cells to the apoplasm) and phloem unloading (efflux from phloem parenchyma cells) in sink organs.

Whether phloem unloading *per se* is an active or passive process (leakage) is controversial, with arguments in favour (van Bel and Patrick, 1985) and against (Farrar and Minchin, 1991) an active process. Symplasmic unloading from the SE–CC complex through plasmodesmata can take place by diffusion and/or mass flow and is a passive process that is, however, linked to metabolism and compartmentation in sink cells (Lalonde *et al*., 2003). Sucrose unloading from cells along the post-sieve element pathway to the apoplasm in some sink organs may passively follow a transmembrane concentration gradient established by extracellular invertases that cleave sucrose to hexoses. In the seed coats of French and broad beans, however, energy-coupled sucrose release to the apoplasm may account for 50% of the total sucrose flux (Lalonde *et al*., 2003). The phytohormone abscisic acid (ABA) seems to be involved in the unloading of sucrose (Schussler *et al*., 1984); even low concentrations of ABA increase the rate of sucrose efflux from phloem tissue (Ross *et al.*, 1987). The induction of a localized increase in the membrane permeability of the phloem cells of the host seems to be the mechanism by which stem parasites such as *Cuscuta europea* acts as a sink, acquiring the assimilates and nutrients they require for growth (Wolswinkel *et al.*, 1984). A particular mechanism of phloem unloading exists in *Mimosa pudica*, where seismonastic responses in leaf movement are based on an action potential arising from the touched leaf, travelling through the phloem (1–10 cm s^{-1}) and leading to unloading of the phloem in the exterior region of the motor cell cortex (Fromm, 1991).

Negative feedback regulations on phloem unloading are exerted by high sucrose concentrations in a utilization sink such as growing roots (Farrar and Minchin, 1991). In seeds of grain legumes, a turgor-sensitive component is involved in phloem unloading. Enhanced sucrose uptake by filial tissues decreases the osmolarity of the seed apoplasmic solution, and consequently raises the turgor of seed coat cells (maternal tissue). If seed coat

turgor exceeds a set point (about 2 kPa), the activity of sucrose transporters responsible for release into the apoplasm is enhanced (short-term turgor regulation), and in the long term rates of phloem import are increased (Zhang *et al*., 2007). The increase of seed coat turgor also leads to increased efflux of K and accompanying anions (Walker *et al*., 2000) which is presumably mediated by K/H^+ antiporters and non-selective channels that allow passage of K and Cl (Zhang *et al*., 2002).

5.7 SINK FORMATION

In crop species in which storage organs such as fruits, seeds and tubers represent yield, the effects of nutrient supply on yield response curves often reflect sink limitations imposed either by a deficiency or an excess supply of nutrients during critical periods of plant development, including flower induction, pollination and tuber initiation. These effects can be both direct (e.g., deficiency of a nutrient needed for a particular metabolic step) and indirect (e.g., nutrient deficiency-induced alteration of concentrations of photosynthates or phytohormones).

5.7.1 Flower Initiation

Floret development in wheat and barley is strongly influenced by the availability of photosynthates, and N- and P-containing assimilates during the critical growth period immediately before heading (Abbate *et al*., 1995; Prystupa *et al*., 2004). In field experiments with durum wheat (Ferrante *et al*., 2010) and barley (Arisnabarreta and Miralles, 2010), N fertilizer application increased the number of fertile florets mainly by reducing the degeneration of initiated florets during the late part of stem elongation. Floret initiation was not promoted. In apple trees, flower formation is affected to a greater extent by the time and/or form of N application than by the rate of N supply. Compared to continuous nitrate supply, a short-term application of ammonium to the roots more than doubled both the percentage of buds developing inflorescences and the arginine concentration in the stem (Table 5.13). Arginine is a precursor of polyamines which also accumulate particularly in leaves of plants supplied with high rates of ammonium (Gerendás and Sattelmacher, 1990).

The involvement of polyamines in ammonium-induced enhancement of inflorescence development in apple trees is indicated by the similar effects obtained by infiltrating polyamines or ammonium into the petioles (Table 5.13). The flower-inducing effects of ammonium supply confirm earlier results of Grasmanis and Edwards (1974). Since the apple trees in this study were well supplied with N throughout the growing season, it is unlikely that these effects on flower initiation (i.e., on developmental processes) are related to a direct nutritional role of N. It is more likely that some N compounds such as polyamines function as secondary messenger in flower initiation. The involvement of polyamines in the biochemical control of the events leading to gametophyte formation, fertilization and fruit development has been demonstrated, for example, in apricot (Alburquerque *et al*., 2006), kiwi (Falasca *et al*., 2010) and maize (Liang and Lur, 2002).

TABLE 5.13 Flower initiation in apple trees supplied with N and polyamines

Treatment	Percentage flowering	Stem arginine concentration (mg g^{-1} dw)
Control, nitrate continuously	15	1.1
NH_4 for 24 h[a]	37	2.6
NH_4 for 1 week	40	2.3
Putrescine[b]	51	–
Spermine[b]	47	–
NH_4 for 24 h[a]	50	–

Based on Rohozinski *et al.* (1986).
[a] *8 mM NH_4^+ in the nutrient solution.*
[b] *8 mM petiole infiltration.*

Most probably, changes in phytohormone concentration in general and of CYT in particular are involved in the enhancing effect of ammonium supply on flowering (Buban *et al.*, 1978). In apple root stocks, ammonium supply compared to nitrate, not only increased flower bud formation but also CYT concentration in the xylem exudate and the number of flower-bearing lateral branches, whereas the total shoot length was reduced (Table 5.14). Promotion of flower morphogenesis by CYT is well documented for various plant species (Herzog, 1981; Bonhomme *et al*., 2000).

Flower formation in apple trees (Bould and Parfitt, 1973), tomato (Menary and Van Staden, 1976) and wheat (Rahman and Wilson, 1977) is also positively correlated with P supply. The positive correlations between the number of flowers and CYT concentration in tomato (Menary and Van Staden, 1976), on the one hand, and between the P supply and the CYT concentration, on the other (Horgan and Wareing, 1980), provide additional evidence that CYT also contributes to the stimulating effect of P on flower formation. In principle, similar conclusions have been drawn from the effects of K on flower formation in *Solanum sisymbrifolium* (Wakhloo, 1975a, b). Low K concentrations in the leaves were correlated with a high proportion of sterile female flowers. This sterility did not occur in plants of either high or low K status when the plants had been sprayed with CYT.

TABLE 5.14 Shoot growth, flower induction and cytokinin (CYT) concentration in xylem exudate of apple root stock at different forms of N supply

Form of N supply	Shoot length (cm)	No. lateral shoots (spurs)	Flowering bud (% of emerged)	CYT (nmol $100\,g^{-1}$ shoot fw)
NO_3	326	6.4	7.4	0.002
NH_4NO_3	268	6.0	8.2	0.373
NH_4	209	8.9	20.7	0.830

Based on Gao *et al.* (1992).

TABLE 5.15 Number of tillers, straw and grain yield of wheat grown in Cu-deficient soil at different Cu supply (4 plants pot^{-1})

	Cu supply (mg pot^{-1})			
	0	0.1	0.4	2.0
Number of tillers pot^{-1}	22	15	13	10
Straw yield (g pot^{-1})	7.7	9.0	10.3	10.9
Grain yield (g pot^{-1})	0.0	0.5	3.5	11.8

Based on Nambiar (1976c).

These results confirm the supposition that the effects of mineral nutrient supply on flower formation are due to changes in phytohormone concentration. The same is also true for the beneficial effects of N fertilizer application before anthesis in increasing grain number per ear in wheat (Herzog, 1981) or seed number per plant in sunflower (Steer *et al.*, 1984). However, seed number per plant can also be increased by high concentrations of sucrose prior to flower initiation (Waters *et al.*, 1984), high light intensity (Stockman *et al.*, 1983; Reynolds *et al.*, 2005) or stem injection of sucrose under drought stress conditions (Boyle *et al.*, 1991; Boyer and Westgate, 2004). Therefore, the nutritional status may also affect flower initiation and seed set by increasing the supply of photosynthates during critical periods of the reproductive phase (Corbesier *et al.*, 1998; Arisnabarreta and Miralles, 2010).

5.7.2 Pollination and Seed Development

The number of seeds and/or fruits per plant can also be directly affected by nutrient supply. This is clearly the case with various micronutrients. In cereals in particular, Cu deficiency affects the reproductive phase (Table 5.15). The critical period in Cu-deficient plants is the early booting stage at the onset of pollen formation (microsporogenesis). When Cu deficiency is severe, no grains are produced even though the straw yield is quite high as a consequence of enhanced tiller formation (due to the loss of apical dominance of the main stem). With increasing Cu supply, grain yield increases more strongly than straw yield. These results are a good example of both sink limitation on yield and deviation from the typical response curve (Fig. 5.1) between grain yield and nutrient supply.

The primary causes of failure of grain set in Cu-deficient plants are inhibition of anther formation, the production of a much smaller number of pollen grains per anther, and particularly the loss of pollen viability (Graham, 1975), in part because of lack of supply of carbohydrates to the developing pollen grains (Jewell *et al.*, 1988). In transgenic *Arabidopsis* plants with low expression of the Cu transporter COPT1, the percentage of pollen abnormalities was enhanced and the formation of abnormal pollen could be considerably reduced by Cu addition (Sancenón *et al.*, 2004). This finding further underscores the role of Cu in pollen development.

In principle, similar results to those found for Cu deficiency (Table 5.15) are obtained with Zn and Mn deficiency. In maize, Zn deficiency prior to microsporogenesis (~35 days after germination) decreased pollen viability and cob dry weight by about 75% (Sharma *et al.*, 1990). Zinc-finger proteins are essential for the proper progression of male and possibly also female meiosis (Kapoor and Taktsuji, 2006) and participate in processes that influence shedding of floral organs (Cai and Lashbrook, 2008).

Under Mn deficiency vegetative growth of maize is less depressed than grain yield (Table 5.16). In the deficient plants anther development is delayed and fewer and smaller pollen grains are produced with very low germination rates. In contrast, ovule fertility is not significantly affected by Mn deficiency (Sharma *et al.*, 1991), a result which is in agreement with the effect of Cu deficiency in wheat (Graham, 1975).

There is evidence that Fe is also involved in inflorescence formation and pollen production (Takahashi *et al.*, 2003). In transgenic tobacco with low internal nicotianamine (NA) concentration due to constitutive expression of the nicotianamine consuming enzyme nicotianamine aminotransferase,

TABLE 5.16 Growth, fertilization and grain yield in maize at high or low Mn supply

	Dry weight			Pollen	
Mn supply ($\mu g\ L^{-1}$)	Shoot ($g\ plant^{-1}$)	Grain ($g\ plant^{-1}$)	Single grain (mg)	Number (no. $anther^{-1}$)	Germination (%)
550	82.5	69.3	302	2,770	85.6
5.5	57.8	11.8	358	1,060	9.4

Sharma *et al.* (1991).

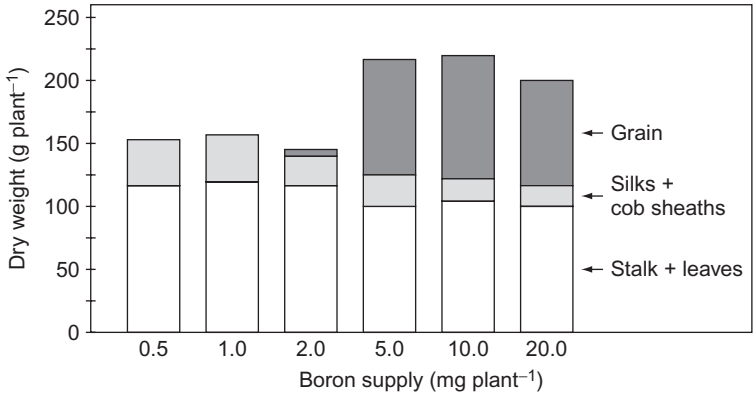


FIGURE 5.25 Production and distribution of dry matter in maize plants grown at different B supply. *Based on Vaughan (1977).*

flowers were abnormally shaped and sterile. Application of a solution containing NA with Fe(III) citrate reversed the morphological abnormalities in flowers, and adequate amounts of pollen were produced (Takahasi *et al*., 2003).

Both production and viability of pollen are also affected by Mo (Kaiser *et al*., 2005). In maize, a decrease in the Mo concentration in pollen was correlated with a decrease in the number of pollen grains per anther as well as a decrease in the size and viability of the pollen grains (Section 7.6). It is not known to which extent Mo deficiency also depresses fertilization and grain set. However, it is well documented that pre-harvest sprouting in maize and wheat (Cairns and Kirtzinger, 1992) causing severe yield losses in certain areas is very high in seeds with low Mo concentration and can be decreased by Mo supply to the soil or as foliar spray. In grapevines, Mo deficiency may be a primary cause of the bunch development disorder 'Millerandage' or 'hen and chicken'. Millerandage is characterized by grapevine bunches which develop unevenly: in the same bunch, fully matured berries are present alongside a large number of fertilized underdeveloped berries as well as unfertilized swollen green ovaries (Kaiser *et al*., 2005). It has been shown that this disorder can be prevented by foliar sprays of Mo before flowering (Williams *et al*., 2004).

Boron is another nutrient that affects fertility. Boron is essential for pollen tube growth (Section 7.7); B deficiency results in a decrease in the number of grains per head in rice (Garg *et al.*, 1979) or the total lack of fertilization in barley and rice (Ambak and Tadano, 1991). Failure of seed formation in B-deficient maize is caused by the non-receptiveness of the silks to the pollen (Vaughan, 1977). With increasing B supply, vegetative growth, including structural growth of the silks, is either not affected or is even depressed whereas grain formation is increased (Fig. 5.25). There is a minimum B requirement for fertilization and grain set, which is in the range of 3 mg B per maize plant. In wheat, the B requirement of anthers and carpels is higher than that of leaves (Rerkasem and Jamjod, 2004). Figure 5.25 provides another example of a strict sink limitation induced by nutrient deficiency and a yield response curve quite different from the typical curve. In wheat (Nachiangmai *et al*., 2004) and barley (Jamjod and Rerkasem, 1999), genetic variation occurs in the degree of pollen sterility or the ability to set grain under B deficiency. In wheat, B concentrations in the ear were higher in an efficient than in an inefficient genotype, and this was associated with higher ability of the efficient genotype in long-distance transport of B from the rooting medium to the ear via the xylem (Nachiangmai *et al*., 2004). Low B supply not only inhibits flowering and seed development, but may also result

in low B concentration in seeds, even in plants without visual symptoms of B deficiency. Low B seeds have a low germination rate and produce a high percentage of abnormal seedlings (Bell *et al.*, 1989).

In lowland rice, grain yield may be considerably decreased by spikelet sterility induced by low temperatures (below 20°C) during anthesis. This temperature sensitivity can be decreased by high supply of K (Haque, 1988). Increasing K concentrations in the panicles from 0.6 to 2.4% in the dry matter decreased spikelet sterility after three days from 75 to 11%. The reasons for this protective effect of K are not known, but high N concentrations in the low K plants may be involved (Haque, 1988).

In certain plant species, such as grain legumes, drop of flowers and developing pods is a major yield-limiting factor (Patrick and Stoddard, 2010). Nitrogen or P deficiency during the flowering period enhances flower and pod drop and thus depresses seed yield (Streeter, 1978; Lauer and Blevins, 1989). Supplying ample amounts of N and P during this critical phase is therefore quite effective in reducing flower and pod drop and in increasing final seed yield in soybean (Brevedan *et al.*, 1978; Lauer and Blevins, 1989). It is well documented that N deficiency also decreases grain number in cereals such as wheat (Abbate *et al*., 1995; Demotes-Mainard *et al*., 1999) and maize (Uhart and Andrade, 1995; Paponov *et al*., 2005b). The reduction of grain number in N-deficient plants is associated with decreased supply of the generative organs with assimilates and N during the critical period determining grain set around flowering, which in turn may result from low photosynthetic activity/area and/or reduced assimilate partitioning to the generative organs (Paponov and Engels, 2005; D'Andrea *et al.*, 2008).

Competition for N rather than for carbohydrates supplied from the source leaves can be the main limiting factor for seed yield in mustard and oilseed rape (Trobisch and Schilling, 1969; Schilling and Trobisch, 1970). In mustard plants, developing seeds and leaves compete for N so that seed set, seed growth and final seed yield are determined primarily by the size of the N pool in the vegetative parts. In crucifers, flower differentiation at the auxiliary stems occurs after the onset of flowering of the main stem and is strongly dependent on the availability of N during this period. Additional N application at the onset of flowering therefore leads to an increase in seed number and yield (Fig. 5.26). This example demonstrates that source limitation can be imposed by N rather than carbohydrates.

Phytohormones, especially CYT and ABA, are also involved in the regulation of grain set. Sufficient N supply increases CYT and decreases ABA and hence decreases flower and pod drop, as would be expected from the specific role of ABA in the formation of abscission layers. Accordingly, maize kernel abortion can be reduced by either foliar application of CYT or supplying the roots with ammonium (Smiciklas and Below, 1992), the latter increasing the CYT contents in the plants (Table 5.14).

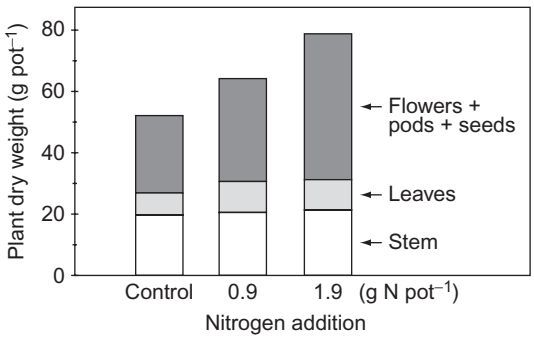


FIGURE 5.26 Total dry weight and dry weight distribution in shoots of white mustard plants with addition of 0.9 and 1.9 g N at the onset of flowering. *Based on Trobisch and Schilling (1970).*

TABLE 5.17 Growth rate of potato tubers at different rates of nitrate supply to the roots of potato plants

Nitrate concentration (meq L^{-1})	Nitrate uptake (meq day^{-1} plant^{-1})	Tuber growth rate (cm^3 day^{-1} plant^{-1})
1.5	1.18	3.24
3.5	2.10	4.06
7.0	6.04	0.44
Nitrogen supply withheld for 6 days	–	3.89

Krauss and Marschner (1971).

5.7.3 Formation of Vegetative Sink Organs

In root and tuber crops such as sugar beet or potato, the induction and growth rate of the storage organs are strongly influenced by environmental factors. In root and tuber crops there is often a strong sink competition between vegetative shoot growth and storage tissue growth for fairly long periods after the onset of storage growth. This competition is particularly evident in so-called indeterminate genotypes of crop species, for example potato (Kleinkopf *et al.*, 1981). In general, environmental factors (e.g., high N supply) which increase vegetative shoot growth, delay the initiation of the storage process and decrease growth rate and photosynthate accumulation in storage organs, for example in sugar beet (Forster, 1970) and potato (Ivins and Bremner, 1964; Gunasena and Harris, 1971).

A high and continuous N supply to the roots of potatoes delays or even prevents tuberization (Krauss and Marschner, 1971). After tuberization, the tuber growth rate is also reduced by high N supply, whereas the growth rate of the vegetative shoot is enhanced. The effect of N supply on tuber growth rate is illustrated in Table 5.17. Resumption of the tuber growth rate after interruption of the N supply indicates that sink competition between the vegetative shoot and tubers can readily be manipulated by N supply.

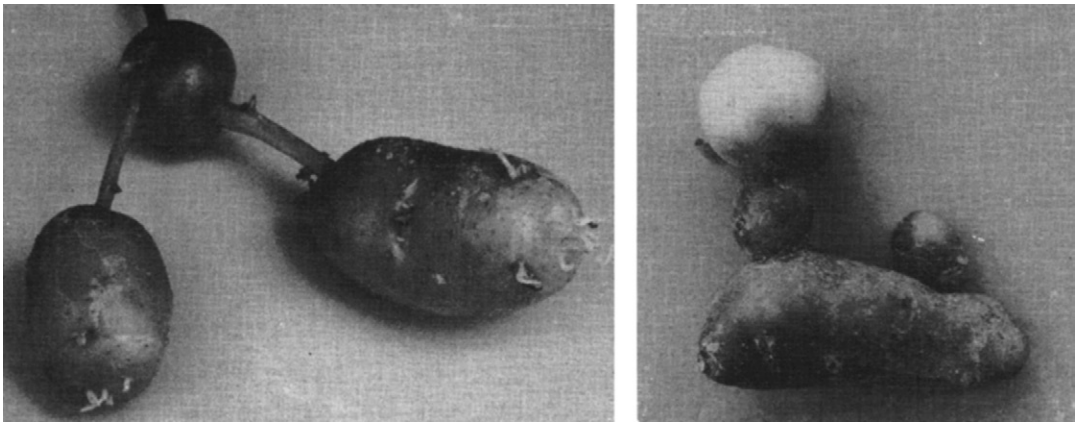


FIGURE 5.27 Secondary growth and malformation of potato tubers induced by alternating high and low N supply to the roots. *Courtesy of A. Krauss.*

In potato, cessation of tuber growth caused by a sudden increase in N supply to the roots induces 'regrowth' of the tubers, i.e. the formation of stolons on the tuber apex (Krauss and Marschner, 1976, 1982). Interruption and resupply of N, therefore, can result in the production of chain-like tubers or so-called secondary growth (Fig. 5.27). After a temporary cessation of growth, resumption of the normal growth rate is usually restricted to a certain area of the tubers (meristems or 'eyes'), leading to typical malformations and knobbly tubers, which are often observed under field conditions after periods of transient drought. Similar effects on cessation of tuber growth and regrowth occur when growing tubers are exposed to high temperatures, which rapidly inhibit starch synthesis and lead to the accumulation of sugars in the tubers (Krauss and Marschner, 1984; Van den Berg *et al*., 1991), followed by a decrease in ABA concentrations in the tubers and regrowth.

The effects of N supply on tuber growth rate and regrowth are due to N-induced changes in the phytohormone balance in the vegetative shoots and in the tubers. Interruption of N supply results in a decrease in CYT export from roots to shoots as well as in the sink strength and growth rate of the vegetative shoot. A corresponding increase in the ABA/GA ratio of the shoots seems to trigger tuberization (for a review on tuberization see Rodríguez-Falcón *et al*., 2006). In agreement with this, tuberization can also be induced by the application of either ABA or the GA antagonist CCC (Krauss and Marschner, 1976) or by the removal of the shoot apices, the main sites of GA synthesis (Hammes and Beyers, 1973). On the other hand, a sudden increase in N supply is correlated with a decrease in ABA/GA ratio not only in the vegetative shoots but also in the tubers, where the GA concentration increases by a factor of 2, while the ABA level decreases to less than 5% of that in normal growing tubers (Krauss, 1978b).

5.8 SINK ACTIVITY

Partitioning of phloem-delivered compounds (e.g., sugars, amino acids, nutrients) among competing sink organs is governed by sink activity, i.e. the relative ability of specific sink organs to unload nutrients from the phloem and to use them for growth and storage. Phloem unloading from the SE–CC complex or the post-sieve element pathway was discussed above under phloem unloading. In this section, uptake of assimilates and nutrients into sink cells of storage organs is discussed. Examples are given which demonstrate the regulation of sink activity by processes associated with storage such as membrane transport of assimilates and biosynthesis of storage compounds.

The development of generative sink organs, for example seeds of cereals and grain legumes, can be divided into a pre-storage phase that is dominated by cell division and cell extension, and a storage phase in which storage compounds are accumulated (Weber *et al*., 2005; Patrick and Stoddard, 2010). The pre-storage phase is often characterized by high activity of invertase in the cell wall and vacuoles of sink organs (Weber *et al*., 2005). Sucrose unloaded from the phloem is cleaved into hexoses, and sugar uptake into sink cells is presumably mediated by H^+/hexose symporters. The high intracellular glucose/sucrose ratio appears to be a key component of the regulatory complex that induces and sustains mitotic activity in this phase, and therefore determines potential seed size (Weber *et al*., 2005; Ruan *et al*., 2010).

In the storage phase, cell wall invertase activity is low. In legumes and temperate cereals, sucrose released from the phloem is imported into sink cells via sucrose/H^+ symporters. In plant species such as broad bean, common bean, barley and wheat, lower intracellular glucose concentrations and high ethylene concentrations induce the formation of transfer cells in the filial tissues. These transfer cells are characterized by wall ingrowths to increase the membrane

surface area, and a high density of H^+-ATPases, sucrose transporters and amino acid transporters in their plasma membrane (Patrick and Offler, 2001). High intracellular sucrose concentrations may induce the expression of key enzymes involved in starch biosynthesis such as sucrose synthase and ADP-glucose-pyrophosphorylase (Weber *et al*., 2005). For starch synthesis, sucrose is cleaved within the storage cells by sucrose synthase to UDP-glucose and fructose. After further metabolic conversion of sugars, glucose-6 phosphate is imported through the plastid membranes into the amyloplasts and ADP-glucose is synthesized by the plastidic ADP-glucose-pyrophosphorylase. From ADP-glucose, the glucose can be transferred to starch by various forms of starch synthase and the starch branching enzyme. In cereals, ADP-glucose is synthesized by cytosolic ADP-glucose-pyrophosphorylase and then imported into the amyloplasts for starch synthesis (Smith, 2008).

In sugar cane and sugar beet, sucrose is the main storage compound. The sucrose concentration in the vacuoles of storage cells can exceed 500 mM, which is about 10 times higher than in the cytosol of storage cells (Saftner *et al*., 1983). Tonoplast-located H^+-ATPases and pyrophosphatases maintain a low pH inside the vacuole and provide a source of energy for active transport across the membrane against a concentration gradient (Maeshima, 2001). Transporters using the H^+ gradient as a driving force may act as H^+/antiporters and a number of cation transporters of this type have been identified (Maeshima, 2001). Vacuolar sucrose transporters have been identified in mesophyll cells of barley and *Arabidopsis* (Endler *et al*., 2006; Neuhaus, 2007). Sucrose antiporter activity has been reported in membranes from sugar beet (Briskin *et al.*, 1985) and red beet roots (Getz and Klein, 1995). In storage cells of sugar beet roots, the accumulation of sucrose is stimulated by K (Fig. 5.28). Sodium has an even greater stimulatory effect on sucrose accumulation (Saftner and Wyse, 1980; Willenbrink *et al.*, 1984). The sites of stimulation may be located at the tonoplast and activate the membrane-bound proton pumps or maintain high cytosolic pH required to compensate for protons of the proton–sucrose antiporter.

An example of the direct role of nutrients in sucrose transport into vacuoles is shown in Table 5.18. Sucrose accumulation depends on Mg and is stimulated by K. This strongly supports the view that a membrane-bound, Mg-dependent proton pump is also involved in the sucrose transport into the vacuoles of storage cells. Activation of Mg-ATPases by K is a well-known phenomenon in ion transport at the plasma membrane of root cells (Section 2.4). However, at the tonoplast, K stimulates only the Mg-PP_iase, and not the Mg-ATPase, indicating the involvement of a proton pump energized by PP_i.

The yield of crop plants is influenced by the length of the storage phase, which is reduced, for example, by drought stress, high temperatures (for a review see Barnabás *et al*., 2008), and nutrient deficiency which reduce weight (size) of the sink organs, but not the number of sink organs. There is substantial evidence that hormones are involved in premature ripening. An example of this is shown in Table 5.19 for K-deficient wheat. In these plants, and particularly 4–6 weeks after anthesis, the concentrations of ABA in the grains are higher than those in the grains of plants well supplied with K. Correspondingly, the grain-filling period in K-deficient plants is shorter and the weight of a single grain at maturity is lower than that in K-sufficient plants. High ABA concentrations in grains coincide with a strong decline in their sink activity. Therefore, the high ABA concentrations in the flag leaves of K-deficient wheat plants (Haeder and Beringer, 1981) and a correspondingly higher ABA import to the developing grains may be responsible for the premature ripening and not the source limitation of a nutrient *per se*.

In cereals and grain legumes, proteins are important storage compounds in sink organs (Shewry, 2007). Thus, high import of amino acids into sink organs is needed. Amino-N unloaded from the phloem is imported into storage cells by facilitated diffusion and/or amino acid/H^+ symport (Zhang *et al*., 2007). In addition, peptide

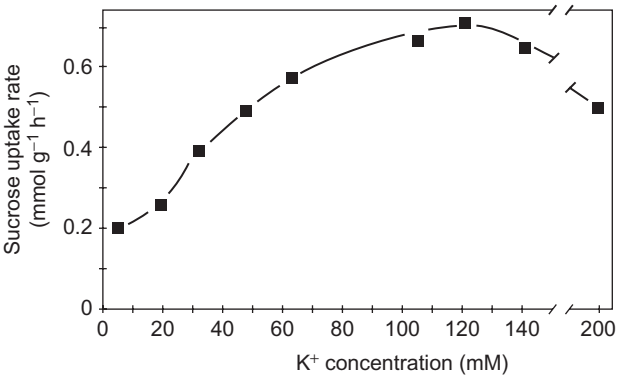


FIGURE 5.28 Sucrose uptake rates by slices of sugar beet storage roots with different K concentration. Sucrose concentration: 40 mM. *Based on Saftner and Wyse (1980).*

TABLE 5.18 Sucrose transport into vacuoles isolated from red beet tissue with and without Mg and K

Mg^{2+}	K^+	Uptake rate of sucrose (nmol unit^{-1} β-cyanin h^{-1})
–	–	4.9
+	–	42.3
+	+	55.3

Based on Doll *et al.* (1979).

TABLE 5.19 Absiscic acid (ABA) content and weight of grains of wheat at different days after anthesis and high or low (deficient) K supply

	ABA content (ng per grain) days after anthesis				Days from anthesis to full ripening	Weight of a single grain (mg)
K supply	28	35				
Low	7.7	13.4	16.5	2.2	46	16.0
High	3.7	4.4	nd[a]	9.4	75	34.4

Based on Haeder and Beringer (1981).
[a]*nd: not determined.*

transporters may play a role in providing peptides for protein deposition during seed development (Miranda *et al.*, 2003). In the storage phase of seed development, the onset of storage protein accumulation is linked to a sucrose signal from the onset of sucrose/H^+ symporter activity (Rosche *et al*., 2002).

Results from many N fertilization experiments show that in cereals grain, total protein content is strongly regulated by N supply to the developing grains (Barneix, 2007). On the other hand, in the *opaque 2* mutant in maize, down-regulation of a specific class of storage proteins is associated with a compensatory increase in N storage in other seed proteins. Thus, the total amount of reduced N stored in mutants and non-mutants does not differ (Tabe *et al*., 2002). Moreover, the expression of transgenes encoding specific storage proteins does not increase the total amount of amino acids that are stored in seeds (Tabe *et al*., 2002). In other crop species, however, there is evidence for sink limitation of protein contents in storage organs. In potato, tuber-specific expression of a seed protein from *Amaranth* (AmA1, Amaranth Albumin 1) increased tuber protein concentration by up to 60%, indicating that in wildtype potato, protein concentration was limited by sink activity, i.e. the capacity of tuber tissue for protein synthesis (Chakraborty *et al*., 2010).

In addition to organic compounds such as carbohydrates, proteins, oils and nutrients, for example, Fe and Zn are also accumulated in storage organs. Little is known about transporters involved in nutrient uptake into sink tissue, and the factors and processes regulating sink strength for nutrients. In developing seeds of bean (*Phaseolus vulgaris*), uptake of K and other univalent cations into cells of developing cotyledons is mediated by a non-selective K channel (Zhang *et al.*, 2004). For uptake of Fe into developing seeds of *Arabidopsis*, AtOPT3 may play an important role (Stacey *et al*., 2008). AtOPT3 is a member of the oligopeptide transporter family and reduced expression in *Arabidopsis* mutants results in decreased accumulation of Fe in seeds despite Fe accumulation in other tissues of the mutants (Stacey *et al*., 2008). For loading of seeds with Fe and other metals including Mn and Cu, transporters of the Yellow Stripe Like transporter family YSL which mediate membrane transport of metals complexed with nicotianamine also play an important role, for example OsYSL 2 in rice (Ishimaru *et al*., 2010) and AtYSL1 in *Arabidopsis* (Curie *et al*., 2009). The transport of Fe and other metals in plants is facilitated by chelating compounds such as citrate, nicotianamine and deoxymugineic acid, which form soluble complexes with metals (Morrissey and Guerinot, 2009), and the availability of chelators can regulate seed metal concentrations. For example, over-expression of the barley nicotianamine gene *HvNAS1* in rice increased endogenous nicotianamine and phytosiderophore concentrations in shoots, roots and seeds, and increased Fe and Zn concentrations in seeds three- and two-fold, respectively (Masuda *et al*., 2009).

In *Arabidopsis* seeds, Mn, Zn and Fe are stored in the vacuoles in complexes with phytate or other chelators, for example nicotianamine (Otegui *et al*., 2002; Roschzttardtz *et al*., 2009; Morrissey and Guerinot, 2009). Iron import into the vacuole may be mediated by VIT1 (vacuolar ion transporter 1) that transports Fe^{2+} (Kim *et al*., 2006) or YSL4 and YSL6 (of the Yellow Stripe Like transporter family) that transport Fe (and Mn)–nicotianamine complexes (Morrissey and Guerinot, 2009). Iron export from the vacuoles during seed germination is mediated by NRAMP3 and NRAMP4 which belong to the Natural Resistance-Associated Macrophage Protein family (Lanquar *et al*., 2005).

In addition to the activity of transporters mediating nutrient loading of seeds and the availability of chelating compounds enhancing the mobility of metals in plants, the storage capacity of sink tissues for metals also plays an important role in regulating seed metal concentrations. For Fe, ferritin is the principal iron storage protein in all living aerobic organisms, it can store up to 4,500 Fe(III) atoms in its cavity in a soluble and bioavailable form (Harrison and Arosio, 1996). In seeds of cereals and legumes, ferritins are also proposed as a major storage form for Fe (Briat *et al*., 2010). Transgenic rice plants expressing soybean ferritin under control of a seed-specific promoter, accumulated up to three times more seed Fe than wildtype

plants (Goto *et al*., 1999). Transgenic rice plants expressing simultaneously *AtNAS1*, an *Arabidopsis* gene encoding a nicotianamine-synthesizing enzyme, and *Pvferritin*, a *Phaseolus* gene encoding ferritin, accumulated six times more Fe in the endosperm than wildtype plants (Wirth *et al*., 2009).

5.9 ROLE OF PHYTOHORMONES IN THE REGULATION OF THE SINK–SOURCE RELATIONSHIPS

Phytohormones play an important role in the regulation of the growth and development of higher plants; for example, by affecting sink–source relationships. The synthesis and action of phytohormones are modulated by environmental factors, such as nutrient supply. At least some of the effects of nutrient deficiencies on plant growth and yield are caused by their influence on phytohormone concentrations in the plant. Some examples of these effects are given in the following sections.

Phytohormones are chemical messengers, or 'signal' molecules for which sites of synthesis and sites of action are usually physically separated. Transport either from cell to cell or from organ to organ is therefore necessary. With the exception of ethylene and the brassinosteroids, phytohormones can be translocated in the phloem and the xylem (Wilkinson and Davies 2002; Hirose *et al*., 2008; Robert and Friml, 2009). The prevailing direction of transport depends on their site of synthesis and the developmental stage of the plant. Each phytohormone has a broad spectrum of actions; the same phytohormone can affect various processes depending on its concentration and conditions at the sites of action.

5.9.1 Structure, Sites of Biosynthesis and Main Effects of Phytohormones

The importance of the five 'classical' classes of phytohormones in higher plants is well established. These are auxins (IAA), cytokinins (CYT), gibberellins (GA), abscisic acid (ABA) and ethylene (ET). More recently, several other molecules have also been recognized as phytohormones. These include jasmonic acid (JA) and its derivates, salicylic acid, brassinosteroids and polyamines. Additionally, strigalactones may affect shoot and root architecture, and their classification as phytohormones is being considered (Dun *et al*., 2009). The basic molecular structures of the various phytohormone classes are shown in Fig. 5.29, and some of their major characteristics are summarized in Table 5.20.

Auxins are indole derivates of the amino acid tryptophan, the most prominent being indole-3-acetic acid (IAA or 'auxin'). They are synthesized in meristems or young expanding tissues (Crozier *et al*., 2000). They can be transported in the phloem, and are redistributed locally from cell to cell with the direction determined by the polar locations in the plasma membrane of the AUX/LAX auxin influx carriers: the PIN auxin efflux carriers and auxin efflux transporters of the multi-drug-resistant/P-glycoprotein (MDR/PGP) subfamily of ATP-binding cassette (ABC) proteins (Robert and Friml, 2009). Several pathways for irreversible IAA catabolism have been elucidated, but reversible inactivation by *O*-glycosylation allows IAA-ester conjugates to be stored (Crozier *et al*., 2000). Auxin promotes cell expansion and cell division, and is implicated in apical dominance, shoot elongation, adventitious root development, xylogenesis and plant tropism (Table 5.20).

Cytokinins are synthesized from purine derivatives (Crozier *et al*., 2000; Hirose *et al*., 2008; Argueso *et al*., 2009). They are readily mobile within plants. Although the major sites of their biosynthesis are in roots, and root to shoot xylem transport dominates, cytokinins are also mobile in the phloem and are transported from source leaves into inflorescences and developing seeds (Hirose *et al*., 2008). Cytokinins are degraded by cytokinin oxidases, which are induced in response to increasing tissue CYT concentrations, and reversibly inactivated by glucosylation (Crozier *et al*., 2000; Argueso *et al*., 2009). Cytokinins promote cell division and differentiation, stimulate transcription and protein synthesis, and delay protein degradation. They suppress auxin-induced apical dominance and delay leaf senescence (Table 5.20).

The terpenoid pathway produces the gibbane carbon skeleton, which gives rise to over 100 giberellin structures (GAs) in plants (Crozier *et al*., 2000; Yamaguchi, 2008). Different plant species produce different GAs, and physiological responses are often specific to a subset of GAs, which is likely to be the result of structural specificity in the GA-receptors of the target cells. GA concentrations are greater in developing seeds than in vegetative tissues. Giberellins are implicated in stimulating shoot elongation, delaying leaf and fruit senescence, breaking dormancy of buds and seeds, promoting seed germination and inducing flowering (Table 5.20). In addition to free GAs, plants contain biologically inactive GA conjugates such as GA-O-β-glucosides and β-glucosyl esters (Crozier *et al*., 2000; Yamaguchi, 2008).

The synthesis of the 'stress hormone' abscisic acid (ABA) occurs rapidly in response to environmental factors, especially a lack of water or N (Wilkinson and Davies, 2002). The precursors for ABA biosynthesis are the carotinoids violaxanthin and neoxanthin (Crozier *et al*., 2000). Roots and shoots are important sites of ABA biosynthesis and ABA is highly mobile in both the xylem and phloem and can circulate within the plant (Jiang and Hartung, 2008). In white lupin, when water supply was adequate, 28% of the ABA in xylem sap originated from biosynthesis in the roots, whereas under drought conditions this proportion increased to about 55% (Wolf *et al.*, 1990a). In

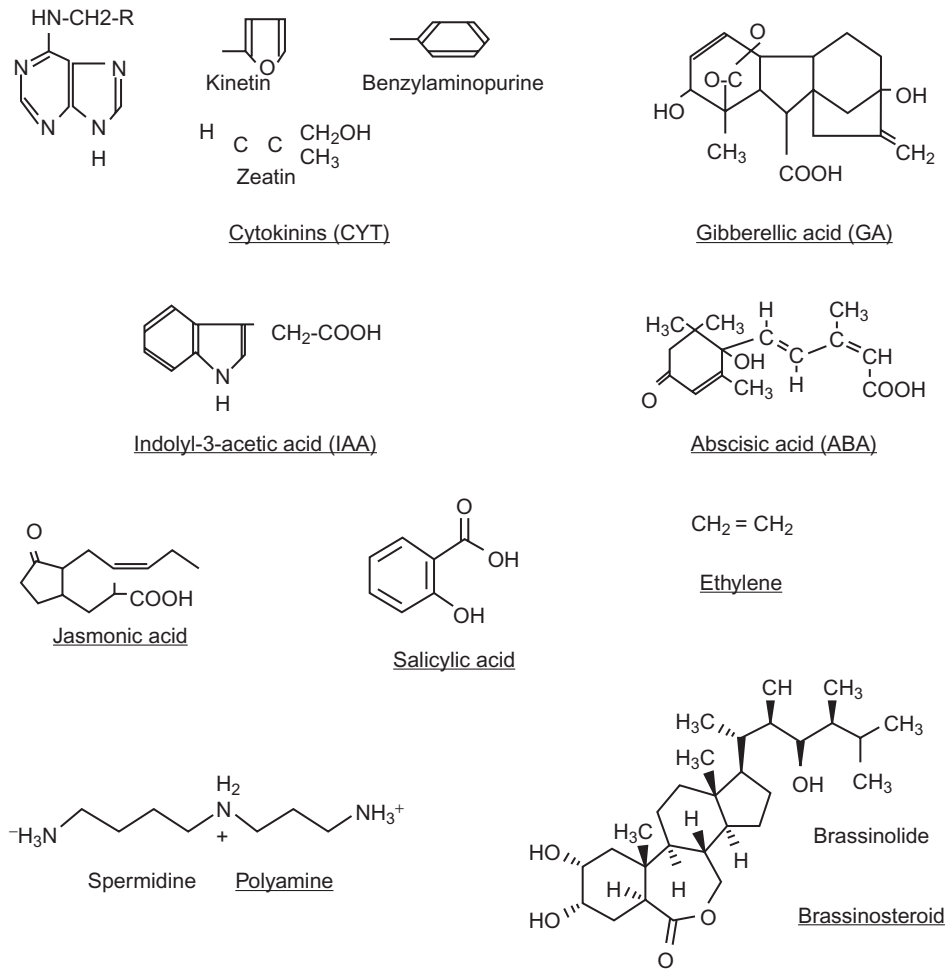


FIGURE 5.29 Molecular structure of phytohormones.

TABLE 5.20 Pathways and main sites of biosynthesis and some major effects of phytohormones

Auxins (IAA)
Biosynthetic precursors: Indole derivates of the amino acid tryptophan, the most prominent being IAA ('auxin').
Main sites of biosynthesis: Meristems or young expanding tissues; in dicots mainly the apical meristems and young leaves; prevailing direction of transport basipetally: polar from cell to cell, and some long distance in the vicinity of the phloem.
Effects: Promote cell division and expansion, apical dominance, adventitious root development, tropisms.
Antagonists/inhibitors: ABA, coumarins, TIBA, 2,4-D, NAA and other synthetic auxins.
Cytokinins (CYT)
Biosynthetic precursors: Purine derivates (adenine).
Main sites of biosynthesis: Primarily root meristems, but also shoot meristems and embryo in seeds. Prevailing long-distance transport via xylem from roots to shoot.
Effects: Promote cell division and expansion, stimulate RNA and protein synthesis, suppress auxin-induced apical dominance, delay senescence.
Gibberellins (GA)
Biosynthesis: Hemiterpenes to the gibbane carbon skeleton; more than 100 gibberellins with this basic structure have been found.

(*Continued*)

TABLE 5.20 (Continued)

Main sites of biosynthesis: Seeds and developing tissues.
Effects: Promote cell expansion, induce enzymic activities (e.g., hydrolases), stimulate shoot elongation, delay leaf and fruit senescence, break dormancy of buds and seeds, induce flowering.
Inhibitors of biosynthesis: Chlorocholine chloride (CCC), ancymidol, triazoles.
Abscisic acid (ABA)
Biosynthetic precursors: The carotenoids violaxanthin and neoxanthin.
Main sites of biosynthesis: Fully differentiated tissues of shoots and roots.
Effects: Inhibits cell extension, induces stomatal closure, favours abscission of leaves and fruits and enhances or induces dormancy of seeds and buds.
Antagonists/inhibitors: IAA, CYT, GA, fusicoccin.
Ethylene (ET)
Biosynthetic precursor: Methionine.
Main sites of biosynthesis: Various plant parts and organs.
Effects: Promotes seed germination, formation of root hairs, formation of root aerenchyma, epinastic curvature of leaves, flowering, ripening and senescence, defence responses to pests and pathogens.
Antagonists/inhibitors: Co, Ag, polyamines.
Jasmonic acid (JA)
Biosynthetic precursor: Linolenic acid.
Main sites of biosynthesis: Roots, shoot, fruits.
Effects: Inhibits seed germination, root and shoot growth, promotes leaf senescence, fruit ripening and tuber formation, induces tendril coiling, induces defence responses to pests and pathogens.
Antagonist: CYT.
Salicylic acid
Biosynthetic precursor: Phenylalanine.
Main sites of biosynthesis: Present in all tissues.
Effects: Inhibits leaf senescence and induces flowering, induces thermogenesis in the spadix of voodoo lily, induces defence responses to pests and pathogen.
Brassinosteroids (BR)
Biosynthesis: From isopentyl diphosphate with campesterol as an important intermediate.
Main sites of biosynthesis: Pollen, seeds, vegetative tissues.
Effects: Affect cell division and cell elongation, promote stem elongation and apical dominance, prevent leaf abscission, enhance stress resistance.
Antagonist: Brassinazole.
Polyamines (PA)
Biosynthetic precursors: Arginine and ornithine.
Main sites of biosynthesis: Present in all tissues.
Effects: Stimulate cell division, the synthesis of DNA, RNA and proteins, root initiation, embryogenesis, flower development, fruit ripening and tuber formation, delay leaf senescence.

response to water deficit, ABA rapidly induces stomatal closure, which occurs through the opening of ion channels in the tonoplast and plasma membrane of guard cells (Wilkinson and Davies, 2002; Amtmann and Blatt, 2009). Abscisic acid can be converted to various biologically inactive metabolites including phaseic acid, dihydrophaseic acid and glucose conjugates (Crozier *et al*., 2000; Jiang and Hartung, 2008). In addition to its role in preventing water loss from leaves, ABA promotes desiccation tolerance of seeds and induces dormancy of seeds and buds.

Ethylene (ET) is produced in response to abiotic (e.g., flooding, chilling, dehydration) and biotic stresses. It is synthesized from methionine by the sequential action of S-adenosyl-L-methionine synthase, 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase (Crozier *et al*., 2000; Dugardeyn and Van Der Straeten 2008; Lin *et al*., 2009). Unlike other phytohormones, ET is a gas and its sites of synthesis and action are located in the same tissue. Responses to ET often show concentration optima. Ethylene (i) enhances or represses root, stem and leaf growth (Pierik *et al.*, 2006; Dugardeyn and Van Der Straeten, 2008), (ii) induces aerenchyma formation in roots in response to flooding (Section 16.4.3), (iii) induces senescence of leaves and flowers (Lin *et al*., 2009), (iv) is required for the ripening of climacteric fruits, such as bananas, apples, avocado and tomatoes (Lin *et al.*, 2009), (iv) is important for plant tropism (Dugardeyn and Van Der Straeten, 2008), (v) accelerates germination, and (vi) may play a role in defence responses to pests and pathogens (Bari and Jones, 2009). Enhanced biosynthesis of ET in shoots in response to O_2 deficiency in the rooting medium is thought to be mediated by an increase in xylem transport of ACC (1-aminocyclopropan-1 carboxylic acid), the precursor of ET, to the shoot (Jackson, 1990a). The action of ET as a local signal is demonstrated by its stimulation of root hair development in response to patches of high P availability (Zhang *et al*., 2003; White and Hammond, 2008).

Jasmonates (JA) are synthesized from linolenic acid (Crozier *et al*., 2000; Browse, 2009). The concentration of jasmonoyl-isoleucine, the active form of JA, increases in response to various abiotic stresses including drought, UV radiation and ozone (Parthier, 1991; Browse, 2009). Jasmonate concentrations also increase when plants are challenged by specific pests and pathogens. Jasmonates are highly phloem mobile, and are thought to act as systemic signals inducing defence responses (Bari and Jones 2009; Browse, 2009). Jasmonate inhibits seed germination and root and shoot growth, and promotes fruit ripening and tuber induction, and accelerates fruit- and seed-induced leaf senescence (Creelman and Mullet, 1997).

Salicylic acid is synthesized from phenylalanine (Crozier *et al*., 2000) and is present in all plant tissues. Increasing salicylic acid concentration slows leaf senescence and induces flowering, probably by reducing the rate of ET synthesis. The synthesis of salicylic acid is also associated with local hypersensitive response and the induction of systemic resistance to the spread of some fungal, bacterial and viral diseases (Crozier *et al*., 2000; Bari and Jones, 2009).

Brassinosteroids (BR) are synthesized from isopentyl diphosphate through campesterol as an important intermediate (Crozier *et al*., 2000). They have the same basic structure as sterols in plant membranes, such as campesterol, sitosterol and stigmasterol (Section 2.3). Since their first isolation from the pollen of oilseed rape (*Brassica napus*), over 60 brassinosteroids have been identified in various plant species (Clouse and Sasse, 1998). Brassinosteroids have strong effects on plant growth and development. They are lipophilic compounds that increase cell elongation and division, acting synergistically to IAA and GA. They promote apical dominance, stem elongation and the bending of grass leaves even at very low concentrations. For example, at concentrations as low as 10^{-10}M, brassinosteroids stimulate elongation growth. Impressive beneficial effects on horticultural crop species have been achieved by application of brassinolids (Rao *et al*., 2002). Several pathways for BR catabolism have been elucidated that can affect BR concentrations in tissues (Crozier *et al*., 2000). Brassinosteroids are not transported long-distance, although they can be transported locally between cells (Symons *et al*., 2008).

Polyamines (PA) can be considered as another class of phytohormones. The major polyamines are the diamine putrescine (NH_2–CH_2–CH_2–CH_2–NH_2), the triamine spermidine and the tetramine spermine. In legumes, cadaverine (1,5-diaminopentane) can also be found at high concentrations. They are synthesized from arginine and ornithine (Alcázar *et al*., 2010), are ubiquitous in plant cells and mobile in both xylem and phloem. Depending upon environmental conditions, their concentrations can be in the micromolar and millimolar range. In cereals, PA biosynthesis is increased rapidly under a range of environmental stresses, including drought, heat and salinity (Crozier *et al*., 2000). Polyamines also accumulate under K deficiency (Section 8.7; White and Karley, 2010), or when NH_4^+ is the main N source (Gerendás and Sattelmacher, 1990). In contrast, PA concentrations are very low under N deficiency, also when in combination with K deficiency (Altman *et al.*, 1989). Polyamines stimulate cell division, the synthesis of DNA, RNA and proteins, root initiation, embryogenesis, flower development, fruit ripening and tuber formation. They also delay senescence, acting synergistically to CYT. They can act as compatible osmotica to protect cells from dehydration when plants are exposed to stresses such as drought, salinity and chilling (Alcázar *et al.*, 2010) and act as antioxidants to protect cells from oxidative damage, for example when plants are exposed to

TABLE 5.21 Patterns of auxin (IAA), abscisic acid (ABA) and zeatin and zeatin riboside (CYT) concentration during the growth of trifoliate leaves of bean (***Phaseolus vulgaris***)

Area of the trifoliate leaf (cm^2)	Phytohormone concentration (ng g^{-1} dw)		
	IAA	ABA	CYT
1.3	419	568	23
6.8	336	245	19
23.4	297	146	14
57.6	217	57	11
110.0	153	106	10
191.0[a]	166	156	10

From Cakmak *et al.* (1989).
[a]*Fully expanded leaf.*

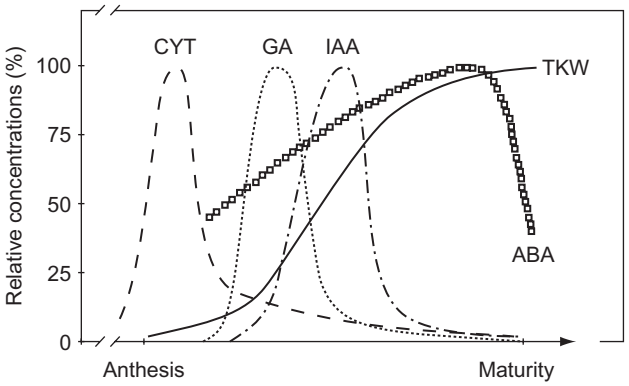


FIGURE 5.30 Generalized patterns of relative phytohormone concentrations (CYT, GA, IAA, ABA) in cereal grains during grain development. TKW: thousand kernel weight. *Data compiled from Rademacher (1978); Radley (1978); Michael and Beringer (1980); Mounla* et al. *(1980) and Jameson* et al. *(1982).*

ozone or heavy metal stresses (Sharma and Dietz, 2006). They accumulate in the 'green islands' of senescing leaves (Walters and Wylie, 1986). Polyamines are effective inhibitors of ethylene biosynthesis; during fruit ripening a decline in PA content is correlated with a strong increase in ethylene production (Winer and Apfelbaum, 1986).

Irrespective of the various effects of phytohormones on plant growth and development (Table 5.20) and the effects of environmental factors on their biosynthesis, a typical pattern occurs in the concentrations of the individual phytohormones in a given organ during its growth and development. Such a pattern is shown in Table 5.21 for trifoliate leaves of bean plants. The concentrations of IAA, ABA and CYT are high in very young leaves and decrease rapidly during early leaf development. 'Dilution effects' by cell wall material are certainly involved in this decrease in concentration in the dry matter. Thereafter, IAA and CYT concentrations remain constant, whereas the concentration of ABA increases.

The phytohormones in developing leaves can originate from biosynthesis within the leaves themselves or be imported from other plant tissues. In view of the main sites of biosynthesis in plants (Table 5.20), IAA most likely originates from the leaf itself, and the gradient in IAA concentrations correlates with the shift from sink to source of a leaf. On the other hand, ABA is mainly synthesized in mature (source) leaves and exported with the photosynthates in the phloem to young (sink) leaves. The changes in ABA concentration in a leaf (Table 5.21) may reflect the shift in its physiology from sink to source during its development. Leaf CYT concentrations change less than those of IAA and ABA (Table 5.21). The high CYT concentrations in very young leaves may be attributed to a combination of both local biosynthesis and phloem import, and the subsequent decrease could be due to export to the xylem.

Changes in the concentrations of phytohormones during the development of reproductive sinks, such as seeds and fruits, also follow a characteristic sequence (Fig. 5.30), which is different from that observed in developing leaves (Table 5.21). In cereal grains, maximum CYT concentrations are reached a few days after anthesis, which coincides with the maximum rate of cell division (Jameson *et al.*, 1982). Maxima of GA and IAA concentrations are reached when rates of dry matter accumulation are highest, i.e. when both sink activity and rate of phloem unloading are greatest. In contrast, ABA concentrations increase later and reach a maximum during the period of rapid decline in the rate of dry matter accumulation. The peak in ABA concentration is correlated with rapid water loss and the corresponding desiccation of the grains. Similar patterns in endogenous phytohormone concentrations also occur in fruits such as tomatoes (Desai and Chism, 1978) and grapes (Alleweldt *et al.*, 1975).

There is a well-established positive correlation between final grain weight and the number of endosperm cells (Singh and Jenner, 1982) as well as the length of the grain-filling period (days between anthesis and maturity). In agreement with this, single grain weight can be increased by application of CYT to the roots shortly before anthesis (Herzog and Geisler, 1977) and decreased by elevated ABA concentrations, induced, for example, by high leaf temperatures during the grain-filling period (Goldbach and Michael, 1976). In maize, elevated ABA concentrations during early kernel development decrease the rate of cell division in the endosperm and, therefore, the storage capacity of the kernels (Myers *et al.*, 1990).

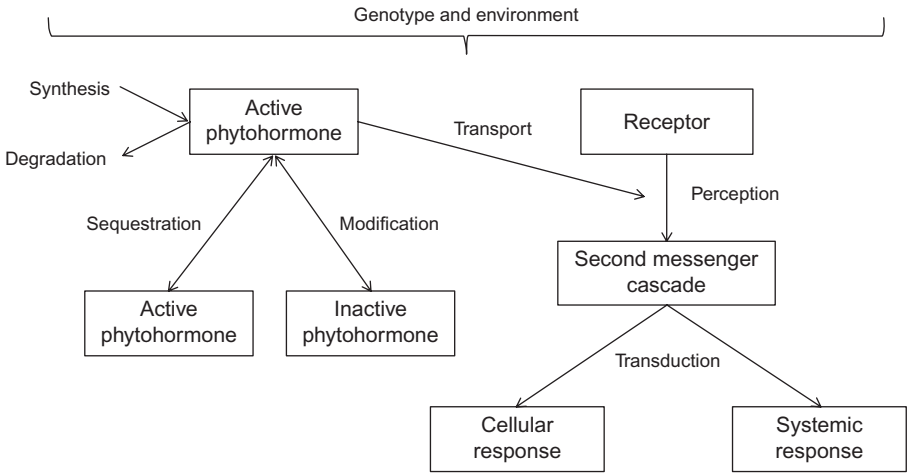


FIGURE 5.31 Relationships controlling the concentration of active phytohormone, its perception by the cell, and its eliciting of a physiological or developmental response.

The dependency of developing seeds and fruits on the import of phytohormones from the xylem (e.g., CYT) and phloem (ABA, GA) is unclear. However, at least for cereals such as wheat, it has been demonstrated that there is no such dependency. In cultures of isolated ears, even when isolated prior to anthesis, normal kernel development can be achieved in the absence of phytohormones with only exogenous supply of sugars and N (Lee *et al.*, 1989).

Based on knowledge of the effects of phytohormones on plant growth and development, and their typical concentrations during organ development, 'bioregulators' that mimic or alter the concentrations or activity of endogenous phytohormones have been developed to improve crop production. For example, synthetic plant hormones, such as kinetin, and growth retardants, such as CCC (chlorocholine chloride) and TIBA (2,3,5-tri-iodobenzoic acid), can regulate vegetative and reproductive growth, as well as senescence and abscission. Bioregulators are used on a large scale (Nitsche *et al.*, 1985), the most successful being the 'anti-gibberellins' which interfere with the biosynthesis of GAs (Grossmann, 1990) and brassinosteroids (Rao *et al*., 2002).

5.9.2 Phytohormones, Signal Perception and Signal Transduction

There are often poor correlations between the concentrations of endogenous phytohormones, as determined by chemical methods or bioassays, and their actions in plants. These poor correlations are attributed to the ability of target cells and organs to receive, perceive and transduce the phytohormone signal into a physiological response (Fig. 5.31).

Following their biosynthesis, phytohormones must be transported to their site of action. Usually, only a fraction of the total phytohormone synthesized remains in its biologically active form. The remainder is either degraded or modified, transiently or permanently, to biologically inactive compounds. Phytohormones or their modified products can also be sequestered in the vacuoles of plant cells. Sequestration in cellular organelles is particularly important for ABA, GA and IAA (Hartung and Slovik, 1991). In target tissues, cells must be competent to respond to the phytohormone, i.e. they must possess a receptor for the phytohormone. The synthesis, degradation, modification, sequestration and transport of phytohormones are affected by plant genotype and environment, as are the abundance and activity of the phytohormone receptors. The molecular identity of receptors for IAA, ABA, CYT, GA, ET, JA and BR have been revealed (Chow and McCourt, 2006; Argueso *et al*., 2009; Browse, 2009; Lin *et al*., 2009; Wolters and Jürgens, 2009; Kline *et al*., 2010). Similarly, the interaction between a phytohormone and its receptor will initiate intracellular signal transduction cascades only in cells competent to respond to the phytohormone. The expression of these biochemical cascades is also determined by genetic and environmental factors. Enzymes, metabolites, ions and electrical events involved in these signal transduction cascades are called second messengers. These signal transduction cascades alter solute transport across cellular membranes, cell metabolism and gene expression and thereby physiological and developmental responses (Fig. 5.32). During cell and tissue differentiation, and organ maturation, both the perception (sensitivity) and the response to a given phytohormone can change.

The biochemistry of signal transduction cascades initiated by various phytohormones has been elucidated. Several of these signal transduction cascades involve electrical events at the plasma membrane and changes in

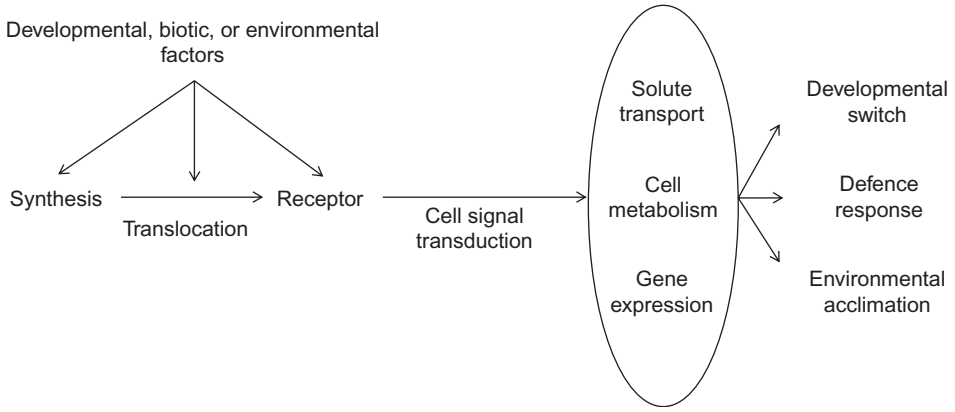


FIGURE 5.32 Response of phytohormone synthesis, translocation or perception to developmental, environmental or biotic factors. For explanations see text.

cytosolic Ca^{2+} concentrations (Section 6.6). For example, changes in cytosolic Ca^{2+} concentrations appear to be necessary for cellular responses to changes in the concentrations of (i) IAA to affect root development and tropisms, (ii) ABA to affect stomatal closure and responses to drought and chilling, (iii) GA during seed germination, and (iv) ET in combination with reactive-oxygen species to affect responses to pests and pathogens, oxidative stress and heavy metals (White and Broadley, 2003). Calcium can enter the cytosol through cation channels present in the apoplasm, vacuole or intracellular organelles, and the cellular origin of Ca^{2+} entering to the cytosol can differ between phytohormones (White and Broadley, 2003).

Other typical components of phytohormone signal transduction are multi-step phospho-relay cascades. For example, the CYT signal transduction cascades consist of sensor histidine kinase receptors, histidine phosphotransfer proteins and cystolic response regulators (Argueso *et al*., 2009), the ET signal transduction cascades are initiated by sensor histidine kinase receptors (Chow and McCourt, 2006; Lin *et al*., 2009), and the BR signal transduction cascades are initiated by sensor serine/threonine kinase receptors (Chow and McCourt, 2006; Wolters and Jürgens, 2009). The ABA signal transduction cascades are initiated by protein phosphatases that modulate the activities of diverse protein kinases (Kline *et al*., 2010). Such signalling cascades are integrated with mitogen-activated protein kinase (MAPK) cascades, as well as other protein kinase and phosphatase enzymes and Ca/calmodulin systems (Browse, 2009; Alcázar *et al*., 2010). Receptors for IAA, GA, ET and JA are F-box subunits of E3 ubiquitin ligase complexes, which result in the activation of transcriptional regulators (Chow and McCourt, 2006; Browse, 2009; Wolters and Jürgens, 2009). Recent studies have identified a large number of transcriptional regulators that modulate gene expression in target cells in response to changes in IAA, ABA, GA, CYT, ET, JA or BR concentrations (Argueso *et al*., 2009; Wolters and Jürgens, 2009; Kline *et al*., 2010).

Phytohormones and intracellular second messengers are integral parts of an extensive signal transduction chain that induces appropriate plant responses to environmental challenges. For example, low soil water content in the rhizosphere increases the synthesis and export of ABA from the roots to the shoot (Wilkinson and Davies, 2002). Some of this ABA binds to the ABA-receptors in guard cells and, through an intracellular signal transduction cascade involving changes in cytosolic Ca^{2+}, cytosolic pH and a variety of kinase cascades, opens K^+ and Cl^- channels in their plasma membrane and tonoplast (Amtmann and Blatt, 2009). This results in a loss of osmotica from the guard cells, causing them to shrink and close the stomatal pore, thereby preventing further water loss.

In addition to producing cellular or local effects, signalling cascades can also initiate systemic second messengers that induce physiological or developmental responses in other tissues. Examples of this are phloem-mobile signals that influence root biochemistry and morphology to increase root uptake and translocation of elements to the shoot (Section 2.5.6). Systemic second messengers can include recycling essential elements, sucrose, or specific microRNAs (Hammond and White 2008; Buhtz *et al.*, 2010; Liu *et al.*, 2009). The interactions between phytohormones and other systemic signals integrate the physiology of the whole plant. Since phytohormones interact and form parts of an integrated, whole-plant, signal transduction system, care is required in interpreting their endogenous levels in terms of expected effects on plant growth and development. However, the endogenous concentrations do provide valuable information as to whether, for example, an environmental stress was sufficiently severe to elicit a distinct hormonal signal.

TABLE 5.22 Export of CYT from roots of potato with continuous or interrupted N supply

	N supply	
	Continuous	Interrupted
Plant age at zero time[a] (days)	CYT exported (ng plant^{-1} 24 h^{-1})	
0	196	196
3	420	26
6	561	17
9[b]	–	132

Based on Sattelmacher and Marschner (1978a).
[a] *30 days after sprouting.*
[b] *Restoration of N supply after 6 days without N.*

TABLE 5.23 CYT concentration of roots and leaves of sunflower plants grown in nutrient solution at sufficient or insufficient supply of N, P or K

	CYT (kinetin equivalents μg kg^{-1} fw)	
Treatment (15 days)	Roots	Leaves
Control	2.38	3.36
1/10 N[a]	0.94	1.06
1/10 P	1.06	1.28
1/10 K	1.06	2.02

From Salama and Wareing (1979).
[a] *Indicates proportion of nutrients in relation to fully concentrated control solution.*

5.9.3 Effects of Nutrition on the Endogenous Concentrations of Phytohormones

The synthesis, degradation and action of phytohormones are affected by environmental factors such as temperature, day length and water and nutrient supply. Some of these factors are of particular ecological importance and can be influenced relatively easily by agronomic and horticultural practices. Thus, growth and development of plants, and ultimately economic yield, can be improved via manipulation of endogenous phytohormone concentrations. The focus of the following discussion is on the effects of nutrition on endogenous phytohormone concentrations.

The main sites of CYT synthesis are root meristems. There is a close relationship between the number of root meristems, root system development and CYT production in roots (Forsyth and Van Staden, 1981). Local antagonistic interactions between CYT and IAA determine root meristem size and the growth rate of the root system (Perilli *et al*., 2010). Of the nutrients, N exerts the most obvious influence on root growth as well as the production and export of CYT to the shoots (Argueso *et al*., 2009). Because CYT is exported mainly in the xylem, collecting xylem exudate is a simple method of obtaining information on this N effect. As shown in Table 5.22 for potato plants, when the N supply is continuous, CYT export increases with plant age, whereas when N supply is interrupted, there is a rapid decrease in CYT export from the roots. After restoring the N supply, CYT export is rapidly enhanced. When tomato plants pre-cultured in NH_4^+ were supplied NO_3^- , the resultant increase in leaf expansion was associated with an increase in the CYT concentration in the xylem sap (Rahayu *et al*., 2005). The synthesis and export of CYT from roots are also affected by P, S and K supply (Hirose *et al*., 2008), although their effect is not as pronounced as with N (Table 5.23). Similar results have been obtained in a variety of plants, including both annuals and perennials (Sakakibara *et al*., 2006; Wilkinson *et al*., 2007; Argueso *et al*., 2009). The expression of genes responsible for CYT biosynthesis is down-regulated in roots of plants with inadequate nitrate, sulphate and phosphate supply whereas the expression of genes encoding transporters for these anions is increased (Hirose *et al*., 2008).

The role of CYT in the reduction of plant growth at low supply of nutrients is shown in Table 5.24. When plantain (*Plantago major*) was grown for a long period at low nutrient supply (2%), its growth rate and tissue CYT concentration were lower than in control plants grown at high nutrient supply (100%). Within two days after transfer from high to low nutrient supply (100→2%), CYT concentrations in shoots and roots and shoot growth rate strongly declined, whereas root growth rate slightly increased. The decline in shoot growth rate could be prevented by adding 10^{-8}M benzyladenine (CYT) to the nutrient solution. During these short-term responses in growth rates, shoot concentrations of nutrients did not change significantly (Kuiper *et al*., 1988), suggesting that the changes in shoot growth rates in response to altered nutrient supply were mediated indirectly by tissue CYT concentrations (Kuiper *et al*., 1989). It is likely that the plants in these experiments were responding to an interrupted N supply. In *Urtica dioica*, photosynthates were preferentially allocated to roots at low N supply whereas at high N supply, or following direct application of BA to the roots, photosynthates were preferentially allocated to the shoot apex (Fetene *et al*., 1993).

Enhanced synthesis and higher concentrations of ABA in roots and shoots are also typical of N-deficient plants (Wilkinson and Davies, 2002; Jiang and Hartung, 2008). An example of the effects of N supply on shoot ABA

TABLE 5.24 Relative growth rate of ***Plantago major*** and CYT concentration at high or low nutrient supply with and without addition of benzyladenine (BA, 10^{-8} M)

		Relative growth rate (mg dw g^{-1} day^{-1})		CYT concentration (pmol g^{-1} fw)	
Nutrient supply[a]	BA	Shoot		Roots	Shoot
100%	–	208	159	78	105
2%	–	49	76	21	39
100% → 2%	–	73	183	34	50
100% → 2%	+	220	163	81	110

Compiled data from Kuiper (1988) and Kuiper *et al.* (1988).
[a]*Full concentrated nutrient solution (100%) or diluted to 2%; treatments 100→2% for two days.*

TABLE 5.25 ABA concentrations in shoots of sunflower plants grown in nutrient solution with or without N

Plant part	With N	Without N (7 days)
	ABA (μg g^{-1} fw)	
Leaves		
Old	8.1	29.8
Fully expanded	6.8	21.0
Young	13.5	24.0
Stem	2.5	4.9

Based on Goldbach *et al.* (1975).

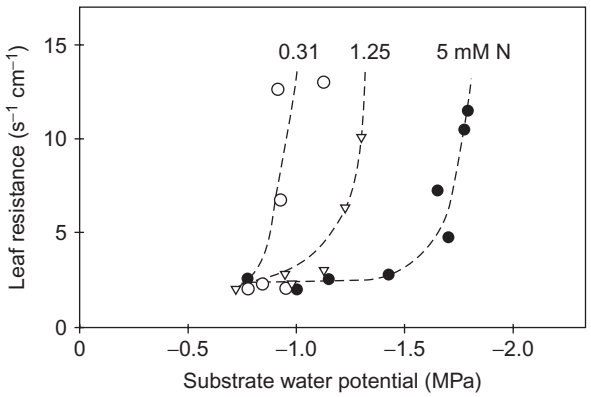


FIGURE 5.33 Relationship between N supply (mM nitrate N), leaf resistance to water vapour diffusion and substrate water potential in cotton plants. *Based on Radin and Ackerson (1981).*

concentrations is shown in Table 5.25. When the N supply to sunflower plants was interrupted, ABA concentrations in all parts of the shoot increased strongly within 7 days (Goldbach *et al.*, 1975). In potato plants, this response can be observed within 3 days, and this effect is even more apparent in roots and xylem exudate than in shoots (Krauss, 1978a). Similar changes have been observed in other plant species, although it is not universally observed (Wilkinson and Davies, 2002).

In many crop species, a reduction in leaf elongation rate is an immediate response to restricted N supply (Chapin *et al.*, 1988; Kavanová *et al.*, 2008). Net photosynthesis, however, is not affected immediately, and sugars accumulate (Chapin *et al.*, 1988; Hermans *et al.*, 2005). This short-term response in leaf elongation rate has been associated with a decrease in CYT translocation from the root to the shoot (Römheld *et al.*, 2008). The expansion of leaf cells is mediated by expansins, whose abundance and activity are influenced by the concentrations of phytohormones including CYT, IAA, GA and ET (Downes *et al.*, 2001; Sánchez-Rodríguez *et al.*, 2010). The failure to restore shoot growth in potato plants by foliar application of N when the N supply to the roots is interrupted suggests that systemic signals from the roots cause the reduction in shoot growth (Sattelmacher and Marschner, 1979; Krauss and Marschner, 1982). In tall fescue (*Festuca arundinacea*), low N supply reduces the number of epidermal cells as well as their elongation rate, and the duration of epidermal cell elongation is about 20 h shorter than in plants with high N supply (MacAdam *et al.*, 1989).

The effects of N supply on ABA production are important for the water balance of plants. Under water deficiency (e.g., in dry soils or soils with a high salt concentration), elevated ABA levels in the leaves favour stomatal closure and prevent excessive water loss (Fig. 5.33). When plants are N-deficient, or are supplied with suboptimal amounts of N, they respond to a shortage of available water in the substrate (i.e., a decrease in substrate water potential) by a more rapid stomatal closure (indicated

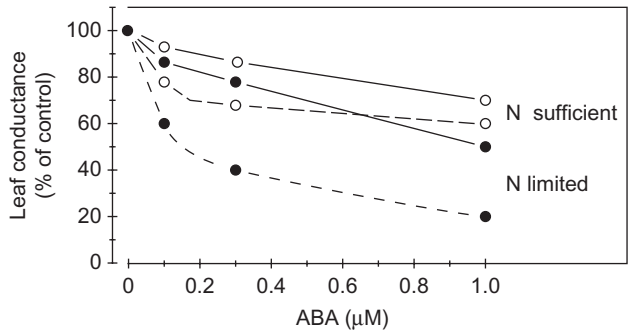


FIGURE 5.34 Stomatal conductance of expanded (○) and old (●) excised cotton leaves from N-sufficient (—) and N-deficient (---) plants at different ABA concentrations. *Adapted from Radin and Hendrix (1988).*

by an increase in leaf resistance to water vapour diffusion) than do plants well supplied with N (Fig. 5.33). The faster stomatal response in leaves of N-deficient plants is due not only to higher ABA concentrations in the xylem sap, but is also a consequence of greater responsiveness of the stomata to elevated ABA concentrations (Fig. 5.34). The responsiveness of stomata to ABA concentrations is greater in older leaves than in younger leaves, and for a given leaf age, greater in leaves from N-deficient than from N-sufficient plants.

The higher stomatal responsiveness to ABA in N-limited plants is also related to lower CYT concentrations (Wilkinson *et al*., 2007). It is well documented that CYT and ABA have opposite effects on stomatal aperture (Radin *et al.*, 1982). In agreement with this, the higher stomatal responsiveness of the older, N-limited leaves to ABA (Fig. 5.34) could be at least partially reversed by a simultaneous supply of CYT (Radin and Hendrix, 1988). The higher drought resistance of low-N plants (Radin and Parker, 1979) is therefore the result not only of morphological changes in root growth or leaf anatomy (e.g., smaller leaf blades), but also of physiological changes such as an increase in the ABA/CYT ratio.

Somewhat similar relationships to those described for N have also been reported for P (Radin, 1984). In P-deficient cotton plants, more ABA is accumulated in leaves in response to drought stress than in P-sufficient plants: in the deficient plants, the stomata close at leaf water potentials of approximately −1.2 MPa, compared with 1.6 MPa in the sufficient plants. As for N-deficient plants (Fig. 5.34) the sensitivity of the stomata to ABA is increased and can be reversed by CYT under P deficiency.

The well-documented increase in root/shoot biomass ratio which occurs when plants lack N may be explained, at least in part, by an increase in ABA and decrease in CYT concentrations. This response to N deficiency, and also to drought stress, is, in most instances, advantageous to plants growing in soils with limited availability of N and water.

Tissue concentrations of GA are also influenced by plant nutrition. For example, interrupting the N supply to the roots of potato plants causes a rapid decrease in their shoot GA concentration and an increase in shoot ABA concentration (Krauss and Marschner, 1982). After restoring the N supply, shoot concentrations of GA and ABA respond rapidly in the opposite direction, with GA concentration increasing and ABA concentration falling. Similar changes in GA and ABA concentrations induced by N supply can also be observed in the tubers of potato plants (Krauss, 1978b), where the changes are correlated with differences in tuber growth and development.

The effects of N on GA concentrations are presumably indirect. The main sites of GA synthesis during vegetative growth are the shoot apex and the expanding leaves. Thus, environmental factors which favour shoot growth (e.g., high N supply, sufficient water supply), also indirectly favour GA synthesis, which is reflected in changes in plant morphology. For example, at high N supply to cereals, stem elongation is enhanced and the plants become prone to lodging. In order to counteract these effects, growth retardants, such as CCC, which depress GA synthesis, are often applied to cereals receiving high quantities of N fertilizer.

The phytohormone balance of the plant is influenced not only by the quantity but also the form of N fertilizer supplied to the roots (Römheld *et al*., 2008). Thus, plant growth and development and, ultimately, plant yield can be affected indirectly by plant N nutrition through specific effects on phytohormone synthesis, transport or perception. For example, deep placement of urea fertilizer close to cereal roots reduces CYT concentration in the xylem sap, which correlates well with decreased tillering (Bauer *et al*., 2009).

An increase in the exudation of strigolactones from roots is observed when plants are N or P deficient, and it is possible that these compounds are involved in changes to root and shoot architecture observed under N and P deficiency (Dun *et al*., 2009).

5.9.4 Phytohormones and Sink Action

During the growth and development of a plant organ the concentrations of different phytohormones vary substantially (Table 5.21; Fig. 5.30) and are usually correlated with the sink strength and, in leaves, the transition from sink to source. Phytohormone concentrations are also important for sink competition, for example between the reproductive and vegetative sinks of a plant (Römheld *et al*., 2008). In this section examples are given of the involvement of phytohormones in determining the distribution of photosynthates and nutrients within the plant.

Expanding leaves act as strong sinks for photosynthates, and the application of ABA not only reduces leaf expansion, similarly to the effect of drought stress, but also

TABLE 5.26 Rate of photosynthesis and increase in leaf area expansion and dry weight in soybean during an 8 hour light period exposed to ABA (1 nmol leaflet^{-1}), drought stress or low light intensity

	Treatments			
Parameter	Control	ABA	Drought	Low light
Photosynthesis (μmol CO_2 $m^{-2}s^{-1}$)	14.8	14.0	14.6	2.4
Area increase (cm^2)	4.5	3.2	2.7	4.2
Dry weight increase (mg leaflet^{-1})	33	26	27	7

Based on Bunce (1990).

TABLE 5.27 Concentrations of leaf-applied ^{32}P and ^{14}C in peduncles of bean with seeds or fruits removed and treated with hormones at cut end after fruit removal

	^{32}P	^{14}C from $^{14}CO_2$ Applied mature leaf
Treatment		(cpm)
Control (intact fruit)	373	
Seeds removed	189	
Fruit removed	34	
Fruit removed and cut end treated with		
Lanoline	6	320
Kinetin	20	
IAA	235	5,520
IAA + kinetin	471	

Based on Seth and Wareing (1967).

increases the rate of export of photosynthates (Table 5.26). ABA reduced dry mass gain although the rate of photosynthesis was not affected. This ABA-induced shift from sink to source may be due to a decrease in IAA concentration leading to decrease in cell elongation and the sink strength of the leaf. In flowers of melon, an increase in ABA concentration reduces the concentration of IAA and the proportion of free IAA (Dunlap and Robacker, 1990) which is considered as a main hormonal component responsible for the sink strength of an organ. As early as 1950, Nitsch demonstrated the role of IAA in the sink action of developing strawberry fruits. Removal of the seeds from the developing fruits resulted in the immediate cessation of fruit growth. Application of IAA to the seedless fruits restored the growth rate of the fruits, indicating that solute volume flow via the phloem into developing strawberry fruits depended on IAA produced in the seeds.

Phytohormones in general, and IAA in particular, are also involved in the action of tissues as sinks for nutrients, as shown in Table 5.27 for P in bean plants. Removal of the seeds and, especially, the fruit reduced the accumulation of ^{32}P in the peduncles. The movement of ^{32}P to the peduncles could be partly restored by the application of IAA to the cut end of the stump, and was strongly stimulated by the application of IAA in combination with kinetin. These data suggest that the action of the fruit as a sink for ^{32}P is governed by its phytohormone balance. The accumulation of ^{14}C in the peduncle following the exposure of a mature leaf to $^{14}CO_2$ is also stimulated by the application of IAA.

Auxins also play an important role in dominance phenomena. Dominance phenomena are widespread in the plant kingdom and are particularly common in reproductive sinks between fruits (e.g., individual tomato fruits on the same truss), seeds (e.g., grains in medial, proximal and acropetal position within an ear) and in utilization

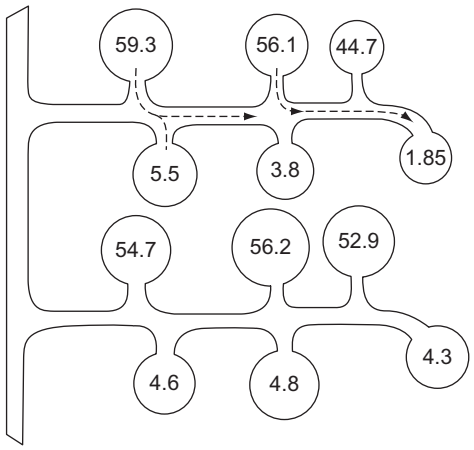


FIGURE 5.35 Tomato trusses with natural sequence of fruit development (upper truss) and 'synchronized' fruit development by pollination at the same day (lower truss). Numbers in fruits represent final fruit weight in g (upper fruits) and polar IAA export (ng fruit^{-1} day^{-1}) of 10-day-old fruits (lower fruits). *Based on Bangerth (1989).*

(vegetative) sinks (e.g., terminal versus lateral buds). Competition for photosynthates may be an explanation for this phenomenon. However, dominance frequently occurs very early in the ontogeny of reproductive and utilization sinks, when competition for the limited amount of photosynthates available is less likely and may even be discounted. A dominance signal may account for this effect. An example of this is shown in Fig. 5.35. Fruits developing earliest dominate over those developing later, and dominance is achieved by higher polar, basipetal transport

of IAA as a 'signal' of higher sink activity. The higher IAA export from dominating sinks seems to have additional repressing effects ('auto-inhibition') on those fruits which are dominated (Bangerth, 1989). The same phenomenon is observed in the dominance of apical versus lateral buds on a stem (Li and Bangerth, 1992; Shimizu-Sato *et al*., 2009). Auxin from the apical bud inhibits CYT biosynthesis and suppresses axillary bud outgrowth. However, more than one of the dominance mechanisms can be involved. In potato plants, for example, dominance phenomena in the early stages of tuber development may have a hormonal basis whereas competition for photosynthates operates at later stages (Engels and Marschner, 1986).

Although it is relatively easy to modify the concentrations of the endogenous phytohormones by environmental factors such as N supply, these changes are complex and the plant system is not an easy one to manipulate. Direct application of phytohormones for increasing sink activity appears more straightforward, but has been successful only in a few cases. Some reasons for the difficulties in manipulating reproductive sinks have been discussed by Michael and Beringer (1980; see also Fig. 5.31). One of the few successful examples is increased seed yield of faba bean by foliar application of GA at the six-leaf stage (Belucci *et al.*, 1982). The yield increase obtained was mainly the result of an increased number of pods and seeds per plant. In faba beans a high proportion of flowers are aborted and the application of GA decreases this abortion.

A similar mode of action may be responsible for the increase in grain yield and harvest index in maize after foliar application of CYT (Smiciklas and Below, 1992) which reduced kernel abortion. Interestingly, this effect of CYT was dependent on the form of N supplied. In plants which were predominantly supplied with nitrate, kernel abortion was higher than in plants supplied with a mixture of ammonium and nitrate. This negative effect of sole nitrate nutrition on kernel abortion was reversed by foliar application of CYT, whereas in plants supplied with a mixture of ammonium and nitrate, CYT application had no effect on kernel number.

In plants with vegetative storage sinks, such as tuber and root crops, manipulations of sink activity by phytohormone application appears somewhat easier and successful results have been reported on storage roots of winter radish (Starck *et al.*, 1980) and potato tubers (Ahmed and Sagar, 1981). In these experiments, however, phytohormones were not applied to the sink organs directly, therefore their effects were indirect. An example is shown in Table 5.28. The application of kinetin (CYT) and GA as foliar sprays strongly increased the shoot growth of carrot plants, but this increase was largely at the expense of the growth of the storage root. This is a typical example of sink competition between shoot and root and the effects of phytohormones on the sink strength of tissues and organs.

TABLE 5.28 Shoot and root growth of carrot plants with foliar application of phytohormones once per week for 7 weeks

	Dry weight (g plant^{-1})			Ratio
Spray	Shoot	Root	Total	Shoot/root
H_2O	3.2	10.9	14.1	0.29
Kinetin	7.3	8.8	16.1	0.83
GA	9.9	5.7	15.6	1.74
CCC	2.8	10.8	13.6	0.26

From Linser *et al.* (1974).

CCC inhibits shoot growth without affecting the storage root growth; i.e., it supports the sink strength of the storage root and produces plants similar to the untreated control, but with a larger harvest index (storage root/shoot ratio). This example again demonstrates that the use of 'bioregulators' which influence phytohormone biosynthesis or the action of a phytohormone in plants are, generally, more effective in modifying activity and strength of sinks than direct application of phytohormones.

5.10 SOURCE AND SINK LIMITATIONS ON YIELD

The growth rate of sink tissues and organs such as roots, shoot apices and storage organs can be limited either by supply of photosynthates from source organs (*source limitation*) or capacity of the sink to utilize these photosynthates (*sink limitation*). In storage organs, sink limitation can result from low rates of phloem unloading and conversion of photosynthates to storage compounds (e.g., starch), as well as from a low number of storage cells per sink organ or sink organs (e.g., grains) per plant or land area. Sink limitation, in turn, can lead to inhibition of photosynthesis. Sink–source limitations are strongly affected by interactions between genotype and environment. In the following examples, both types of limitation are considered with particular emphasis on different phases of plant development and environmental factors.

The potential sink capacity is determined by the number of sink organs (e.g., grain or tuber number), the number of storage cells per organ (e.g., number of endosperm cells per grain) and the number of storage organelles per cell (e.g., number of amyloplasts per endosperm cell). In crop species with vegetative storage organs such as potato, the fixation of potential sink capacity is not restricted to a specific phase of plant development. Depending on environmental conditions, additional

TABLE 5.29 Seed set and grain yield of maize at normal or low leaf water potential during anthesis and with stem injections of culture medium (murashige with 150 g sucrose L^{-1}) only, or culture medium with CYT + 2,4-D

		Seed		
Treatment	Leaf potential (−MPa)	Number (number ear^{-1})	Weight per seed (mg $seed^{-1}$)	Weight per plant (g $plant^{-1}$)
Control	−0.63	431	176	75
Low	−1.81	0	0	0
Low + CYT + 2,4-D	−1.71	19	225	4
Low + culture medium only	−1.62	302	203	60

Based on Boyle *et al.* (1991).

tubers and/or storage cells in existing tubers can be formed until shortly before maturity. Thus, sink capacity and tuber growth rates can be continuously adjusted to current photosynthate supply suggesting that tuber growth is mainly source limited (Engels and Marschner, 1987), unless tuber initiation or growth are directly inhibited, for example, by extreme soil temperatures. In sugar cane, on the other hand, there is evidence of sink limitation during sugar accumulation (McCormick *et al*., 2009). Transgenic sugar cane producing the sucrose isomer isomaltulose exhibited a substantial increase in both overall sugar concentration in the stalk and leaf photosynthetic rates, suggesting that in wildtype sugar cane, photosynthetic capacity is not completely used because of limited sugar storage capacity of the culms (Wu and Birch, 2007).

In crops with generative storage organs, the potential sink capacity is established in a relatively short period around anthesis (the so-called *critical period*). Processes related to sexual reproduction such as meiosis, pollination and zygote formation are particularly sensitive to stressful conditions such as heat, drought and nutrient deficiency (Barnabás *et al*., 2008; Hedhly *et al*., 2009). Thus, supraoptimal temperatures, drought stress or nutrient deficiency during anthesis reduce seed and fruit set which leads to sink limitation of yield and decrease of harvest index (Porter and Semonov, 2005). Stress-induced decrease in grain set may be due to elevated concentrations of ABA (Zeng and King, 1986; Setter and Parra, 2010) or ethylene (Hays *et al*., 2007) in the reproductive organs. In wheat, for example, drought stress during meiosis of pollen mother cells decreased the proportion of fertile spikelets from 68% (well watered) to 44%, and simultaneously increased ABA concentrations in the ears from 35 to 111 µg g^{-1} fw (Morgan, 1980). Application of ABA to the ears of well-watered plants also decreased spikelet fertility from 68 to 37%. During grain filling, in contrast, grain ABA concentrations were positively correlated to grain-filling rate in wheat (Yang *et al*., 2006a).

Conclusions about the role of hormones as the 'signals' of drought stress and also for depressing fertilization and grain set have to be drawn with care. In maize, drought is associated with a decrease in photosynthate influx into kernels and depletion of carbohydrate reserves (for review see Ruan *et al*., 2010). Short-term interruption of carbohydrate supply to the flowers during anthesis can strongly affect seed set by increasing kernel abortion (Table 5.29). Stem injection of a liquid medium from tissue culture with high sucrose concentrations (150 g l^{-1}) over a 5- or 7-day water deficit period prevented failure of reproduction, regardless of whether the liquid medium contained the phytohormones (IAA, CYT). It has been suggested that the sugar reserve status of the florets provides a signal to genes regulating senescence and kernel abortion (Ruan *et al*., 2010). Drought at anthesis is associated with up-regulation of genes for senescence enzymes in maize ovaries leading to kernel abortion. This up-regulation during water deficit is prevented by sucrose feeding (Ruan *et al*., 2010).

In the absence of abiotic stresses which inhibit sexual propagation, the establishment of potential sink capacity is often closely related to assimilate supply to generative organs during the critical period (Andrade *et al*., 1999; Fischer, 2007). Assimilate supply, in turn, is dependent on photosynthesis (source strength) and partitioning of assimilates to generative organs (strength of alternative sinks relative to that of generative organs). In wheat, considerable genotypic variation exists for dry matter partitioning to sinks at anthesis including leaves, stems and roots (for a review see Foulkes *et al*., 2011). Lower partitioning to roots and leaves involves the risk of reduced acquisition of soil resources and photosynthesis, but there is scope for reducing allocation of assimilates to stems in favour of increased allocation to the spikes (Foulkes *et al*., 2011).

The actual yield is dependent not only on the potential sink capacity which is fixed during the critical period, but also on assimilate supply after anthesis. Whether yield is limited by source or sink is dependent on environmental conditions during the critical period and the post-anthesis period. Transient stress during the critical period may reduce the potential sink capacity to such an extent that yield is mainly limited by the ability of sink organs to store assimilates (i.e., sink limitation of yield). In contrast, abiotic and biotic stresses during the post-anthesis period which reduce photosynthesis and accelerate leaf senescence can reduce yield by source limitation. Under these conditions reserve pools accumulated in vegetative organs prior to anthesis become an important source of assimilates for grain filling. In graminaceous species of cool and temperate climate (e.g., *Agrostis* species, or wheat), the main transient storage carbohydrates in stems are fructans which are accumulated prior to and also during the first weeks after anthesis, and utilized thereafter for grain filling (Schnyder, 1993). In wheat, the contribution of reserve pools to individual grain weight and yield varies greatly depending on genotype and environmental conditions (Dreccer *et al*., 2009). With drought stress after flowering, remobilization of water-soluble carbohydrates (mainly fructans) may contribute up to 50% to yield (Blum, 1998). In maize, large amounts of N are transiently stored in the stem, and nearly half the N in the grains may derive from this source (Ta, 1991).

In the absence of stresses, grain filling and yield are often sink limited (Borrás *et al*., 2004; Fischer, 2007). This is shown in Table 5.30 in an example of field-grown wheat cultivars. In this experiment light penetration into the canopy was artificially increased for 12 days prior to anthesis which increased the kernel number per spike, and thus sink strength. The increase in sink strength, in turn, enhanced light-saturated rates of flag leaf photosynthesis during grain filling and grain yield. The extent to which yield of grain crops is limited by sink and source is dependent on species and genotype. During seed filling, wheat yield was mainly sink limited whereas in soybean, yield was limited by sink and by source (Borrás *et al*., 2004). In wheat varieties released from 1940 to 2005, breeding has decreased the degree of sink limitation during post-anthesis from nearly complete sink limitation in the oldest varieties to limitation of sink and source in the most modern varieties (Acreche and Slafer, 2009). These findings point to the importance of considering plant traits related to assimilate supply from source organs (source strength) as well as to assimilate storage in sink organs (i.e., sink strength) in conventional and molecular plant breeding to increase yields of grain crops. Sink strength may become even more important for yield with rising atmospheric CO_2 concentration.

TABLE 5.30 Kernel number per spike, light saturated net assimilation rate of the flag leaf of wheat during grain filling and grain yield at normal or transiently increased light penetration into the canopy for 12 days during booting stage

Treatment	Kernel no spike^{-1}	Net assimilation rate (μmol CO_2 m^{-2} s^{-1})	Grain yield (g m^{-2})
Control	40.3	25.9	790
Increased light[a]	43.3	28.6	950

Based on Reynolds *et al*. (2009).
[a]*Light penetration into the canopy was increased by bending back and holding neighbouring plants at an angle of approximately 45° from the vertical from 8 am until 5 pm prior to anthesis.*

Chapter 6

Functions of Macronutrients

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Summary

In this chapter, the role of the macronutrients nitrogen (N), sulphur (S), phosphorus (P), magnesium (Mg), calcium (Ca) and potassium (K) in plant metabolism and growth are described as well as symptoms of deficiency and toxicity. After carbon, N is the element required in largest quantity by plants; it plays a central role in plant metabolism as a constituent of proteins, nucleic acids, chlorophyll, co-enzymes, phytohormones and secondary metabolites. Upon uptake as ammonium or nitrate, N is assimilated into amino acids either in the roots or shoots. Within the plant, N is translocated as nitrate or amino acids. Sulphur is taken up as sulphate and assimilated into S-containing amino acids such as cysteine which are used to synthesize S-containing enzymes and co-enzymes as well as secondary compounds such as phytochelatins (detoxification of metals) or aliins and glucosinolates (feeding deterrents). Phosphorus is a structural element in nucleic acids and plays a key role in energy transfer as a component of adenosine phosphates. It is also essential for transfer of carbohydrates in leaf cells. Magnesium is a component of chlorophyll and is required for photosynthesis and protein synthesis. Calcium is important for cell wall and membrane stabilization, osmoregulation and as second messenger allowing plants to regulate developmental processes in response to environmental stimuli. The main role of K is osmoregulation which is important for cell extension and stomata movement. Potassium further affects loading of sucrose and the rate of mass flow-driven solute movement within the plant.

6.1 NITROGEN

After carbon, nitrogen (N) is the element required in largest amounts by plants: about 1–5% of total plant dry matter consists of N, which is an integral constituent of proteins, nucleic acids, chlorophyll, co-enzymes, phytohormones and secondary metabolites. The availability of N to roots is therefore a decisive factor for plant growth. Atmospheric N_2 is only available to plants that are capable of forming symbiosis with N_2-fixing soil bacteria (Chapter 16). Most plants therefore depend on other N compounds for their growth. The major sources of N taken up by the roots of higher plants are nitrate (NO_3^-) and ammonium (NH_4^+). In order to increase crop production, approximately 100 million tons of N fertilizers were applied globally in 2008 (FAO, 2008). A large proportion, approx. 60%, of the total N fertilizer used is for cereal crops (FAO, 2006a). Generally, only 40–50% of the applied N fertilizer is utilized by the crop (Sylvester-Bradley and Kindred, 2009). The N which is lost from the plant–soil system can result in environmental problems, including water and air pollution.

This chapter describes N acquisition by roots, N assimilation, functions of N compounds and the effect of N on plant growth and composition. Classical physiological observations of N responses are linked with the underlying molecular mechanisms that have recently been unravelled in order to provide integrated insight into the principles of N nutrition of higher plants.

Nitrate and ammonium are the major sources of inorganic N taken up by the roots of higher plants. Nitrate is generally present in higher concentrations (1–5 mM) than ammonium (20–200 μM) in the soil solution of agricultural soils (Owen and Jones, 2001). Nitrate is also more mobile in the soil than ammonium and therefore more available to plants (Miller and Cramer, 2004).

In unfertilized agricultural soils, ammonium can be present in higher concentrations than nitrate, and amino acids provide an additional source of N. Amino acid concentrations in the soil solution range between 0.1 and 100 μM and they dominate the pool of N bound to soil particles (Jones *et al.*, 2002; Jämtgård *et al.*, 2010).

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Ammonium and amino acids are also the dominating plant-available N forms in acid forest soils (Rennenberg *et al*., 2009). Due to limited nitrification in anaerobic soils, rice paddy soils also contain more ammonium than nitrate (Kronzucker *et al.*, 2000).

The availability of N sources in the soil varies substantially in time and space, depending on soil properties such as texture, pH, moisture and microbial activity (Robinson, 1994). As a consequence, plants have evolved mechanisms to modulate their N acquisition efficiency in response to availability and form of external N as well as to plant N demand during their life cycle (von Wirén *et al.*, 2000; Forde, 2002). This includes having several N transport systems which mediate uptake at different external concentrations as well as being able to change root system architecture to allow better exploration of a particular soil volume or further exploration of a larger volume.

The uptake of nitrate and ammonium into plant roots is mediated by transport proteins located in the plasma membrane of the epidermal and cortical root cells. Several physiological transport systems mediate uptake of nitrate or ammonium with different affinities. The high-affinity transport systems (HATS) operate at low concentrations ($<$0.5 mM) of external nitrate or ammonium. At higher concentrations, $>$0.5 mM, uptake is primarily via the low-affinity transport systems (LATS), allowing large influxes of substrate at high substrate availability. Both uptake systems have inducible and constitutive components.

The molecular constituents of the physiological transport systems are unravelled as more nitrate and ammonium transport proteins are identified and characterized. Predominantly, the genes encoding nitrate or ammonium transporters belong to the NRT, CLC and AMT families.

6.1.1 Nitrate Transport in Plants

6.1.1.1 Nitrate Uptake by Roots

In higher plants, there are two types of transporters involved in root nitrate uptake. These transporters belong to the NRT1 or NRT2 families. It is generally assumed that the NRT1 and NRT2 transporters mediate the low- and high-affinity transport of nitrate into roots, respectively, with the exception of AtNRT1.1 which can exhibit both high and low affinity (Tsay *et al*., 2007). Irrespective of the type of nitrate transporter, the inward transport of nitrate across the plasma membrane occurs against a steep electrochemical potential gradient because the negatively charged nitrate ion has to overcome both the negative plasma–membrane potential as well as an uphill concentration gradient. Nitrate influx therefore requires metabolic energy. Nitrate transporters of the NRT1 and NRT2 families transport nitrate across the plasma membrane in symport with protons (Forde, 2000), which in turn requires the expenditure of ATP by the H^+-ATPase for proton extrusion in order to maintain the proton gradient over the plasma membrane (Fig. 6.1).

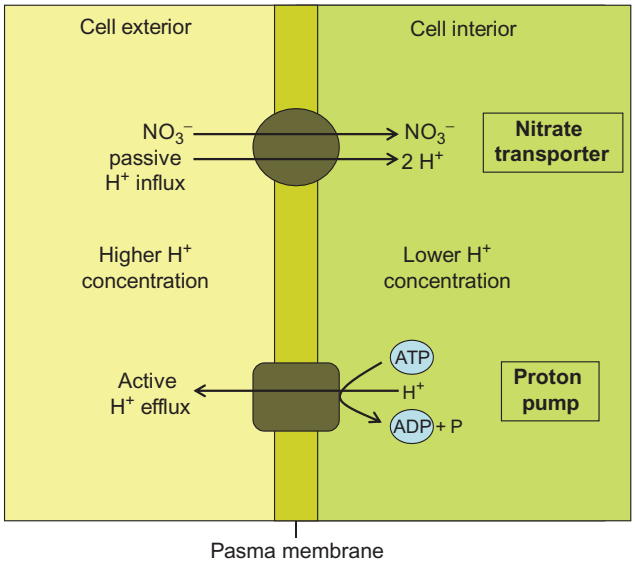


FIGURE 6.1 Schematic diagram of transport of nitrate across the plasma membrane of plant cells.

In *Arabidopsis*, the NRT1 family has 53 members, of which nine have been functionally characterized to transport nitrate (Tsay *et al*., 2007). The *NRT1* family includes the first nitrate transporter gene to be cloned from plants, *AtNRT1.1* (*CHL1*). The NRT2 family has seven members in *Arabidopsis* (Orsel *et al.*, 2002a; Okamoto *et al.*, 2003). The DNA sequencing of the complete genomes of several grass species has made the identification of their *NRT1* and *NRT2* orthologues possible. A comparison between *Arabidopsis*, rice, sorghum, maize and *Brachypodium* has revealed that the grasses have multiple copies of some of the *Arabidopsis NRTs*, but are missing others (Plett *et al*., 2010).

The NRT1 and NRT2 proteins both have the same topology of 12 transmembrane domains divided into two sets of six helices connected by a cytosolic loop (Forde, 2000). Although the protein structure is similar, there is no DNA sequence homology between the *NRT1* and *NRT2* families of genes (Orsel *et al*., 2002b).

AtNRT1.1 is expressed in the epidermal cells in young roots and root tips of *Arabidopsis* (Huang *et al*., 1996), which is in accordance with its role in nitrate uptake from the soil. Further away from the root tip, *AtNRT1.1* is expressed in cortex and endodermis (Huang *et al*., 1996) suggesting a role for AtNRT1.1 in radial transport of nitrate through the root. AtNRT1.1 functions in both the high- and low-affinity range and the two modes of activity are switched by phosphorylation and dephosphorylation (Liu and Tsay, 2003; Ho *et al*., 2009). Phosphorylated AtNRT1.1 functions as a high-affinity nitrate transporter.

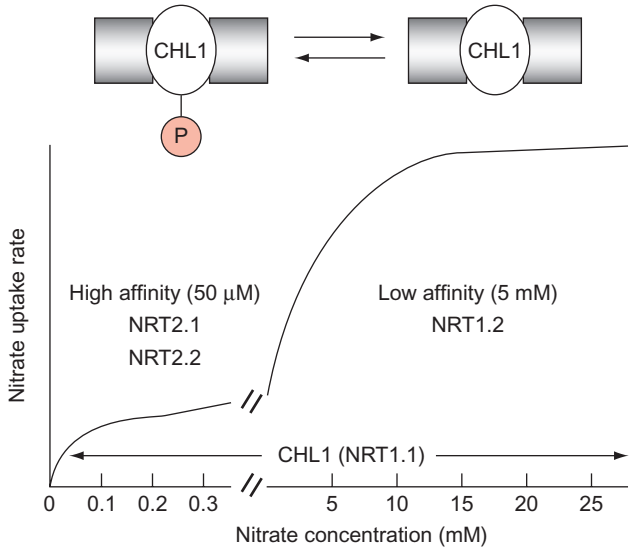


FIGURE 6.2 Nitrate uptake systems in *Arabidopsis* roots in response to increasing nitrate concentrations. *From Tsay* et al. *(2007) with permission from Elsevier.*

The phosphorylation levels of AtNRT1.1 are regulated in response to changes in the external nitrate concentrations allowing the AtNRT1.1 protein itself to function as a nitrate sensor (Liu and Tsay, 2003; Ho *et al*., 2009). At low N concentrations, the AtNRT1.1 protein is phosphorylated, which enables the plant the ability to take up nitrate even when present at low concentrations. High N availability will lead to de-phosphorylation of the AtNRT1.1 protein, which will then adopt a low affinity to nitrate (Fig. 6.2). Thus, AtNRT1.1 senses the external nitrate concentration, to control its own mode of transport and further control the expression of another nitrate transporter involved in nitrate uptake, AtNRT2.1, through an unknown signal (Ho *et al*., 2009). Furthermore, nitrate signalling mediated by AtNRT1.1 leads to up-regulation of genes involved in assimilation of N and regulation of the root system architecture.

Another *NRT1* family member, *AtNRT1.2*, is also expressed in the root epidermal cells and the root tip and is also involved in nitrate uptake from the soil into roots (Tsay *et al*., 1993; Huang *et al*., 1999). AtNRT1.2 is solely a low-affinity transporter and is responsible for the constitutive low-affinity nitrate uptake capacity of roots being expressed even if nitrate is not present (Huang *et al*., 1996, 1999).

Two members of the *NRT2* family are also important for nitrate uptake into roots. *AtNRT2.1* and *AtNRT2.2* are involved in high-affinity nitrate uptake (Li *et al*., 2007). The main component of nitrate uptake at low nitrate concentrations (<0.5 mM) is mediated by AtNRT2.1. Expression of *AtNRT2.1* is induced rapidly upon resupply of nitrate to N-starved roots (Zhuo *et al*., 1999; Remans *et al*., 2006a; Li *et al*., 2007). This initial induction is followed by feedback repression (Lejay *et al*., 1999; Girin *et al*., 2007); the amino acid glutamine represents the main signal for the shoot to communicate its N status to the roots for repression of *AtNRT2.1* (Nazoa *et al*., 2003; Gansel *et al*., 2001). This allows regulation of the rate of N uptake according to N demand during plant growth. Nitrate uptake and *AtNRT2.1* expression in plants show a diurnal pattern, with the maximum towards the end of the light period (Lejay *et al*., 1999; Glass *et al*., 2002). In contrast to the NRT1 nitrate transporters, NRT2 transporters from higher plants require an additional protein component for activity. This component, named NRT3, is a protein with a single transmembrane domain which directly interacts with NRT2. Separately, neither NRT2 nor NRT3 can mediate nitrate transport (Orsel *et al*., 2006).

In many plant species, nitrate uptake varies during the life cycle of the plant. Commonly, nitrate uptake is reduced substantially at flowering (Malagoli *et al*., 2004).

In conclusion, molecular genetics in *Arabidopsis* have shown that at least four nitrate transporters are involved in nitrate uptake into roots, namely AtNRT1.1, AtNRT1.2, AtNRT2.1 and AtNRT2.2. However, there is no simple one-to-one relationship between these genes and the physiological uptake systems. Rather, multiple genes are involved in each uptake system and sometimes the same gene can be involved in more than one uptake system (Tsay *et al*., 2007). An overview of the functions of the NRT1 and NRT2 nitrate transporters involved in uptake from the soil or nitrate transport within the *Arabidopsis* plant is presented in Fig. 6.3.

6.1.1.2 Nitrate Efflux by Roots

While the influx of N compounds into epidermal and cortical root cells is essential for plant growth, efflux of nitrate, ammonium and amino acids back into the soil solution can also occur. This seemingly energetically wasteful process may particularly occur with excess N, as nitrate induces nitrate efflux in barley roots (Aslam *et al*., 1996; Kronzucker *et al*., 1999). The physiological importance of nitrate efflux remains unclear, but the process may play a role in the sensing of nitrate availability by providing a dynamic and flexible regulation of cytosolic nitrate homeostasis (Miller and Smith, 2008) and nitrate net uptake (influx minus efflux).

At low external nitrate, the chemical gradient and the electrical gradient favour passive efflux of nitrate from the cytosol across the plasma membrane (Miller and Smith, 1996). Although this gradient is present for many hours after N deprivation, the efflux nevertheless decreases and ceases after a few hours, suggesting that in the absence of external nitrate, nitrate efflux is down-regulated (van der Leij *et al*., 1998).

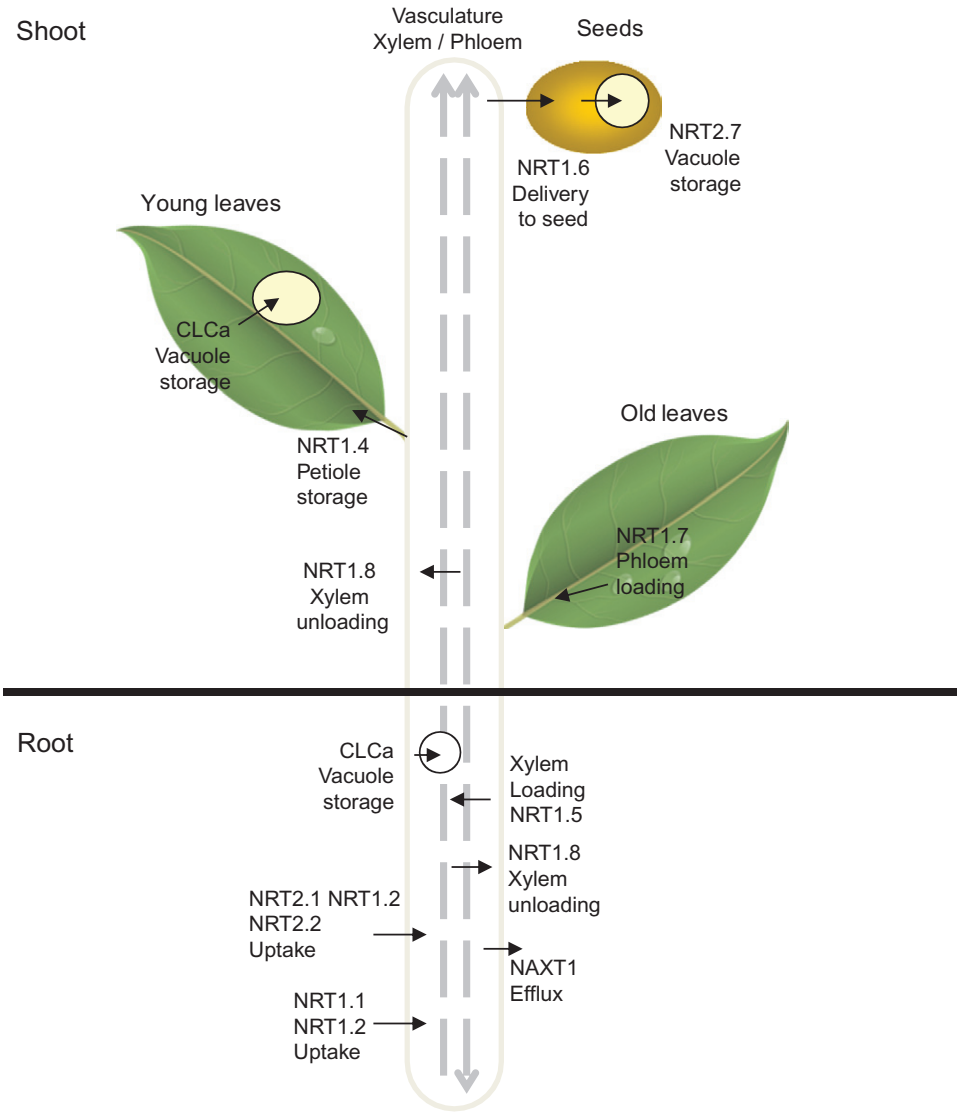


FIGURE 6.3 Schematic representation of nitrate transport steps in *Arabidopsis* plants. *From Dechorgnat* et al. *(2011) with permission from Oxford University Press.*

A molecular component mediating nitrate efflux has been characterized in *Arabidopsis* and belongs to the NRT1 family of transporters; it has been named NAXT1 for nitrate excretion transporter (Segonzac *et al*., 2007). NAXT1 is expressed in the root cortex and is located on the plasma membrane, in accordance with a function in nitrate efflux from roots (Fig. 6.3). A NAXT member is also strongly expressed in the root stele of *Arabidopsis*, raising the possibility that NAXT transporters are also involved in nitrate efflux during xylem loading (Segonzac *et al*., 2007).

6.1.1.3 Radial Transport of Nitrate across the Root and Xylem Loading

Once nitrate has been taken up into the root symplast (the continuum of cell cytoplasm connected via plasmodesmata), nitrate can move radially across the different cell types of the root and pass the endodermal Casparian strip. For nitrate to be transported to the shoot, nitrate is loaded from the symplast of the stele cells into the apoplast of the xylem for long-distance transport via the transpiration stream. In *Arabidopsis*, the nitrate transporter gene *AtNRT1.5* is expressed in the pericycle cells adjacent to the protoxylem where AtNRT1.5 mediates low-affinity proton-coupled efflux of nitrate from the root cells into the xylem (Lin *et al*., 2008). Expression of *AtNRT1.5* is induced by nitrate, in accordance with greater N transport to the shoot in high N conditions. Knockout of *AtNRT1.5* slowed but did not prevent nitrate transport to the shoot, indicating that AtNRT1.5 is only one of several mechanisms which control long-distance transport of nitrate to the shoot. The diurnal regulation of *AtNRT1.5* ensures that more nitrate is loaded

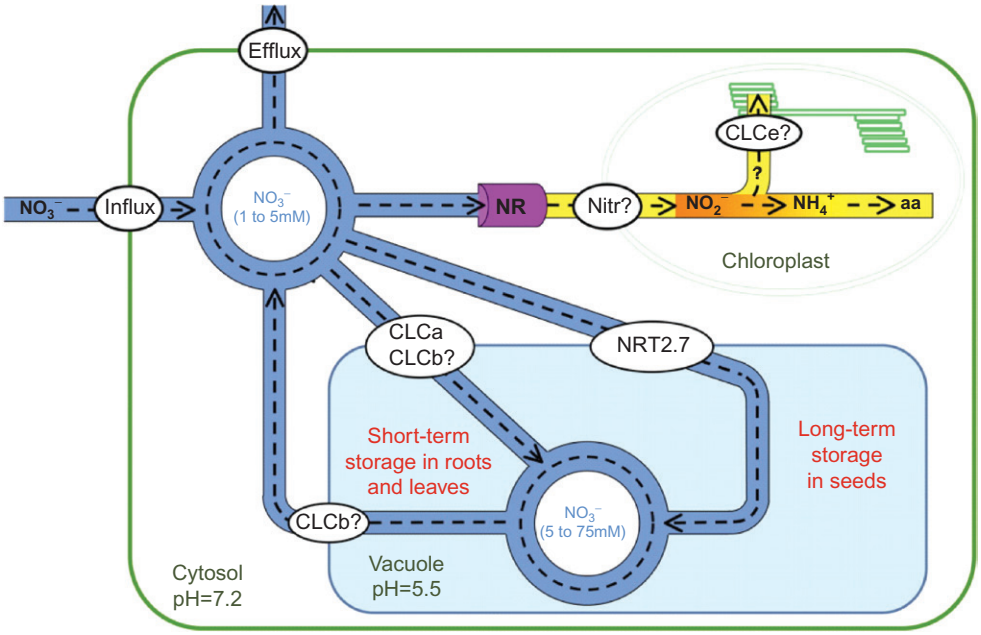


FIGURE 6.4 Schematic representation of the nitrate transport processes at the cellular level. *From Dechorgnat* et al. *(2011) with permission from Oxford University Press.*

in the xylem and transported to the shoot during the day when nitrate reductase is most active.

Another *NRT1* family member, *AtNRT1.8*, is also expressed in xylem parenchyma cells of the root, but AtNRT1.8 is involved in the retrieval of nitrate from the xylem sap (Li *et al*., 2010). AtNRT1.8 mediates low-affinity influx of nitrate into the xylem parenchyma enabling removal of part of the nitrate loaded in the long-distance transport route before it reaches the shoot (Li *et al*., 2010a). Thus, AtNRT1.5 and AtNRT1.8 are involved in nitrate transport in opposite directions which may allow fine regulation of nitrate transport to the shoot as well as distribution of nitrate within the root system (Fig. 6.3).

6.1.1.4 Nitrate Transport within the Cell

In contrast to ammonium, which is predominantly incorporated into organic compounds in the root, nitrate is more readily distributed throughout the plant (Dechorgnat *et al*., 2011). Nitrate is accumulated in the vacuoles relative to the cytosol (Miller and Smith, 1992) (Fig. 6.4) and can be stored in the vacuoles of root, shoots and storage organs, from where it can be retrieved (Rossato *et al*., 2001). Nitrate stored in the vacuoles may be a reservoir of N to be used when the external N supply is low (van der Leij *et al*., 1998; Richard-Molard *et al*., 2008). However, this N reserve is, in most cases, very small compared to organic N in the plant, and the store of nitrate in the vacuoles is depleted within 12–48 h of nitrate starvation (Richard-Molard *et al*., 2008). Nitrate is stored in vacuoles transiently, for example during the night when nitrate is not metabolized by nitrate reductase. This suggests that storage of nitrate in vacuoles serves as a nitrate buffer for transport processes rather than as N storage.

In *Arabidopsis*, nitrate transport across the tonoplast is mediated by some of the members of the CLC family of voltage-dependent chloride channels. The seven CLC family members in *Arabidopsis* have been named AtCLC-a to AtCLC-g (De Angeli *et al*., 2009).

AtCLC-a, b and c have been shown to be involved in nitrate transport across the tonoplast in *Arabidopsis* (Harada *et al*., 2004; De Angeli *et al*., 2006; Lv *et al*., 2009; von der Fecht-Bartenbach *et al*., 2010). Nitrate transport across the tonoplast is less understood in other higher plants.

6.1.1.5 Nitrate Transport within the Shoot

Several members of the NRT1 family of nitrate transporters have been found to exert a specific role in controlling nitrate distribution within the shoot (Fig. 6.3). As in roots, AtNRT1.8 is likely to mediate unloading of nitrate from the xylem for uptake into leaf cells in the shoot (Li *et al*., 2010a). AtNRT1.7 is thought to transport nitrate across the plasma membrane into the phloem of older leaves (Fan *et al*., 2009). This would allow nitrate remobilization from older (source) leaves to N-demanding (sink) tissues. However, nitrate concentrations in the phloem are usually very low (μM), thus, the physiological importance of this process is probably limited. In *Arabidopsis* several *NRT2* genes are expressed in the shoot and might be involved in the distribution of nitrate between different organs and cell

types in the shoot. Nitrate primary uptake can also occur in the leaves, which is important for epiphytes and for foliar fertilization of crop plants.

6.1.2 Ammonium Transport into and within Plants

Ammonium (NH_4^+) is in equilibrium with ammonia (NH_3), which is a weak base with a pK_a of 9.25. In most soils, the pH is considerably lower than this pK_a, therefore NH_3 concentrations are usually very low. Thus, NH_4^+ is the main form taken up by roots, and protein-mediated influx of NH_3 into roots plays a minor role (Loqué and von Wirén, 2004).

6.1.2.1 *Ammonium Uptake by Roots*

The high-affinity transport system (HATS) is a saturable ammonium uptake system which operates at ammonium concentrations less than 0.5 mM (Glass and Siddiqi, 1995; Kronzucker *et al.*, 1996). The ammonium low-affinity transport system (LATS) dominates at ammonium concentrations above 0.5 mM. All plants express a non-saturable, low-affinity influx system (Wang *et al.*, 1993; Kronzucker *et al.*, 1996; Rawat *et al.*, 1999), which is at least partially protein-mediated. The ammonium LATS is responsible for ammonium uptake at high concentrations of ammonium, which may result in toxicity.

Throughout the plant, ammonium transport is to a very large extent carried out by members of the ammonium transporter family (AMT/MEP/Rh) (von Wirén and Merrick, 2004): the AMT1 subfamily which transports ammonium via NH_4^+ uniport or NH_3/H^+ symport (Ludewig, 2006) or the AMT2/MEP subfamily which includes the NH_3 channel AmtB from *E. coli* and the Mep1-3 transporters from yeast. The AMT transporters in plants are predicted to have an extracytosolic N-terminus, 11 transmembrane domains and a cytosolic C-terminal end (Loqué and von Wirén, 2004).

Ammonium transporters of the AMT1 family represent the major entry pathway for root uptake of ammonium (Loqué and von Wirén, 2004).

In *Arabidopsis*, the AtAMT1;1, AtAMT1;2 and AtAMT1;3 transporters equally contribute to the root ammonium HATS activity (Yuan *et al.*, 2007), whereas AtAMT1;5 plays a minor role in ammonium uptake. An overview of the HATS AMT1s is presented in Fig. 6.5. *AtAMT1;1* and *AtAMT1;3* are expressed in the root cortical and epidermal cells (Loqué *et al.*, 2006) in agreement with a direct role in ammonium uptake from the soil. *AtAMT1;2* is expressed in cortical and endodermal root cells, suggesting that AtAmt1.2 is also involved in uptake of ammonium from the apoplast for radial transport of ammonium (Yuan *et al.*, 2007). The affinity for ammonium is higher for AtAMT1;1 (K_m ≈50 µM) and AtAMT1;3 (K_m ≈60 µM) than for AtAMT1;2 (K_m ≈150–230 µM).

The expression levels of the *AMT1* genes is up-regulated in N-starved plants (Gazzarrini *et al.*, 1999; Rawat *et al.*, 1999; Lejay *et al.*, 2003; Yuan *et al.*, 2007) but

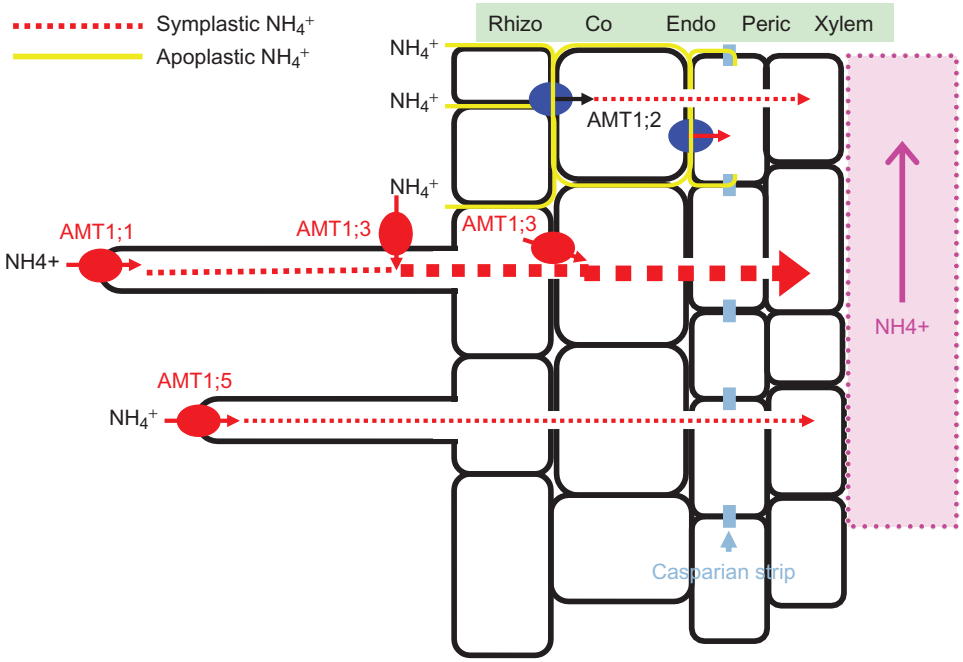


FIGURE 6.5 Schematic representation of the functions of the AMT1 transporters in the ammonium HATS in *Arabidopsis* roots. Rhizo: rhizodermis; co: cortex; endo: endodermis; peric: pericycle; xyl: xylem. *From Yuan* et al. *(2007) with permission from the American Society of Plant Biologists.*

reduced upon resupply of ammonium. *AtAMT1;1* is particularly up-regulated in the portion of the root system which is experiencing N-starvation (Gansel *et al.*, 2001). Following resupply of ammonium to N-starved plants, the expression of the HATS *AMT1* genes is reduced (Rawat *et al.*, 1999). Rather than a systemic signal, it appears to be the local concentration of ammonium or glutamine which inhibits ammonium influx.

NH_4^+ resembles K^+ in terms of ionic radius and size of hydration shell (Howitt and Udvardi, 2000), thus ammonium ions may be able to permeate K^+ channels (White, 1996; ten Hoopen *et al*., 2010). The low K^+ concentrations often observed in ammonium-fed plants may lead to up-regulation of K^+ channels in order to improve the plant K^+ uptake, potentially resulting in further ammonium influx through them (ten Hoopen *et al.*, 2010).

Similarly to the diurnal pattern of nitrate uptake, ammonium uptake (and the expression of *AtAMT1;3*) also increases during the day with a maximum at the end of the light period after which the uptake decreases (Gazzarrini *et al*., 1999; Glass *et al.*, 2002). This diurnal pattern shows that N uptake is regulated by C supply (Liu *et al.*, 2009). Indeed, external supply of photoassimilates leads to ammonium influx in the dark via up-regulation of transcription of the *AtAMT1* genes as well as of the nitrate transporter *AtNRT2.1* (Lejay *et al.*, 2003).

In ammonium-sensitive barley plants, high ammonium influx is counteracted by an active efflux of ammonium back to the soil (Britto *et al.*, 2001; Kronzucker *et al*., 2001). This results in an apparently futile cycling of ammonium ions across the plasma membrane (Britto *et al*., 2001). However, the physiological significance of ammonium efflux is as yet unclear.

6.1.2.2 Ammonium in the Shoot

Ammonium taken up by the roots is assimilated or stored in vacuoles in the root or is transported to the aerial parts. Generally it has been assumed that ammonium is not used for long-distance transport of N within the plant; however, the ammonium concentration of xylem can be in the millimolar range (Finnemann and Schjoerring, 1999; Rawat *et al.*, 1999; Yuan *et al.*, 2007), suggesting that ammonium is transported from roots to shoots. The transporters involved in xylem loading in the root and unloading in the shoot are unknown at present.

Ammonium is generated by photorespiration in chloroplasts of illuminated leaf cells, lignin biosynthesis, amino acid catabolism and protein breakdown in senescing tissue and is also supplied from the nodules following nitrogen fixation in legumes. Therefore transporters of ammonium are important throughout the plant in order to move ammonium from sources to sinks. The transporters involved in distribution of ammonium within the shoot and within plant cells are largely unknown. An exception to this is AMT1;4, which is specifically expressed in pollen and mediates uptake of ammonium into the pollen grains (Yuan *et al*., 2009).

Generally, concentrations of ammonium in the cytosol range from 1 to 30 mM (Miller *et al*., 2001). Excessive accumulation of ammonium in the cytosol may lead to necrosis of plant tissue. The ammonium concentration in the cytosol is a function of (i) influx into cells and efflux of ammonium to the apoplast, (ii) compartmentation of ammonium into vacuoles, and (iii) ammonium assimilation in the cytoplasm or plastids (Nielsen and Schjoerring, 1998).

In vacuoles, the ammonium concentration of non-stressed plants ranges from 2 to 45 mM (Miller *et al*., 2001). Cytosolic NH_3 is passively transported across the tonoplast where the acidic environment traps NH_3 as NH_4^+. NH_3 and water have similar sizes and polarity, allowing NH_3 to permeate water channels in some cases. Accordingly, members of the tonoplast intrinsic proteins have been shown to play a role in NH_3 import into the vacuole (Jahn *et al*., 2004; Loqué *et al.*, 2005).

6.1.3 Organic Nitrogen Uptake

In addition to inorganic N acquisition, uptake of organic N also contributes to plant nutrition (Näsholm *et al.*, 2009). Organic N is the main form of N in soils: in the organic matter and in the form of peptides and proteins, amino acids and urea (Miller and Cramer, 2004).

6.1.3.1 Amino Acid Uptake

Peptides and proteins are broken down to amino acids in the soil by proteases released by soil microorganisms (Miller and Cramer, 2004). The concentration of free amino acids in agricultural soils is in the range of 1 to 100 µM (Jones *et al.*, 2002), constituting the largest fraction of low-molecular-weight dissolved organic N (Jones *et al*., 2005). Amino acid uptake by plants is in strong competition with microbes and the extent to which plants access organic N from the soil is still under investigation. However, several amino acid transporters have been described in plants (Lipson and Näsholm, 2001). In *Arabidopsis* roots, the three amino acid transporters AAP1, AAP5 and LHT1 have been shown to have a role in amino acid uptake. They each have different specificity and affinity for amino acids (Hirner *et al*., 2006; Lee *et al*., 2007; Svennerstam *et al*., 2008).

6.1.3.2 Urea Uptake and Metabolism

In agriculture, urea is used as N fertilizer and is also a naturally occurring and readily available N source in soils. Urea is hydrolysed to ammonium in the soil by the enzyme urease produced by soil microorganisms, but

plants can also take up urea directly (Kojima *et al*., 2007; Witte, 2011). Most plants have a single urease gene, with the multiple urease genes in soybean an exception (Witte, 2011). Urease is activated by incorporation of Ni (Witte, 2011).

Urea transporters involved in uptake from the soil likely include the AtDUR3 transporter in *Arabidopsis*, which appears to be high-affinity urea transport in symport with protons. Expression of *AtDUR3* in *Arabidopsis* roots was up-regulated by N deficiency (Liu *et al*., 2003).

Passive urea uptake is mediated by some members of the major intrinsic proteins (MIP) family of aquaporins. Of those, some are likely to mediate urea transport across the plasma membrane, while others mediate urea transport across the tonoplast or the mitochondrial membrane (Witte, 2011).

Arginine is a major N storage form and is catabolized during N remobilization from source tissue or during senescence. Arginine catabolism takes place in the mitochondria and produces urea, which is transported to the cytosol. There, urea is hydrolysed to ammonium which is then re-assimilated. A role for AtDUR3 in internal urea transport is indicated by expression of *AtDUR3* near the root xylem and in the shoot (Kojima *et al.*, 2007).

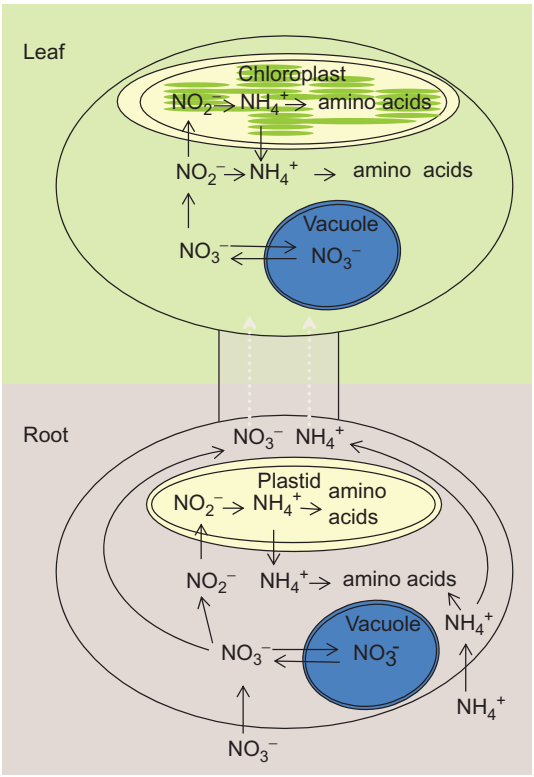


FIGURE 6.6 Overview of N uptake and N assimilation in plants.

6.1.4 Nitrogen Assimilation

Nitrate (NO_3^-) is readily mobile in the xylem and can also be stored in the vacuoles of roots, shoots and storage organs. In order for the N in nitrate to be incorporated into organic structures, nitrate has to be reduced to ammonium (NH_4^+). Most of the ammonium, whether originating from nitrate reduction or from direct uptake from the soil solution, is normally incorporated into organic compounds in the roots, although some NH_4^+ may also be translocated to the shoot even in plants receiving nitrate as the sole N form (Schjoerring *et al*., 2002). The importance of reduction and assimilation of nitrate for the life of plants is similar to that of the reduction and assimilation of CO_2 in photosynthesis. Nitrogen assimilation is intricately regulated. This is necessary in order to integrate environmental signals with carbon metabolism so that N assimilation is coupled with the availability of N in the soil and the demand for synthesis of various N-containing compounds as well as with the availability of C skeletons, energy and reductants for the assimilatory pathway (Nunes-Nesi *et al*., 2010). An overview of N assimilation is given in Fig. 6.6.

6.1.4.1 Nitrate Reduction

The reduction of nitrate to ammonium is mediated by two enzymes: *nitrate reductase*, which catalyses the two-electron reduction of nitrate to nitrite (NO_2^-), and *nitrite reductase*, which transforms nitrite to ammonium in a six-electron transfer process (Fig. 6.7). The net reaction is:

$$NO_3^- + 2H^+ + [4NAD(P)H + 4H^+] \rightarrow NH_4^+ + [4NAD(P)^+] + H_2O \quad (6.1)$$

Nitrate reductase (NR) is a cytosolic enzyme consisting of two identical subunits, each with three co-factors covalently bound to specific domains of the enzyme (Fig. 6.7). The three co-factors which participate in the transfer of electrons from NADH/NADPH to nitrate are flavine adenine dinucleotide (FAD), a heme (bound to a domain which resembles a family of cytochromes) and molybdopterin (a molybdenum containing co-factor). Most plant species have two nitrate reductase (*NIA*) genes (Crawford and Arst, 1993) which are expressed in shoots and roots.

The nitrite generated by nitrate reductase is transported to the chloroplast for reduction to ammonium by nitrite reductase. Nitrite reductase is encoded by a single gene in higher plants (Rastogi *et al*., 1997; Kant *et al*., 2011). It is localized in the chloroplasts in leaves and in the proplastids of roots and other non-green tissues. In green leaves, the electron donor is reduced ferredoxin, generated by photosystem I during photosynthetic electron transport in the light. Electrons from the reduced ferredoxin are passed to nitrite via a ferredoxin-binding domain, an

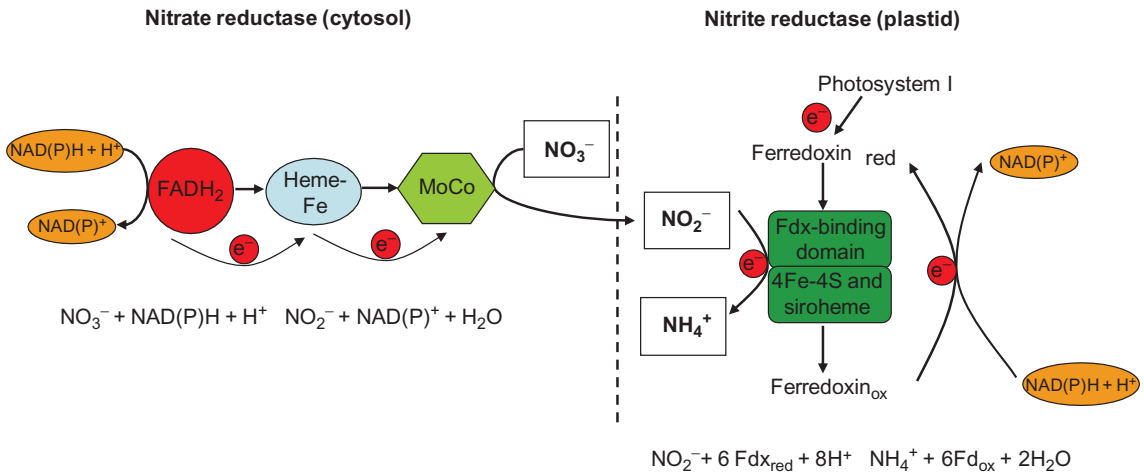


FIGURE 6.7 Schematic representation of the sequence of nitrate assimilation.

iron–sulphur cluster, and a siroheme co-factor bound to the nitrite reductase enzyme (Fig. 6.7). In the root plastids, reduced ferredoxin is generated via NADPH in the pentose phosphate pathway coupled with ferredoxin-$NADP^+$ reductase (Bowsher *et al*., 2007).

To prevent accumulation of nitrite which is toxic to plant cells, nitrate reductase activity is regulated by several mechanisms (Lillo, 2008). The regulation is exerted at different levels, including enzyme synthesis, degradation and reversible inactivation as well as regulation of effectors and the concentration of substrate. The enzyme has a half-life of only a few hours and is absent in plants not receiving nitrate. The expression of the nitrate reductase genes is strongly and rapidly induced by nitrate, leading to active protein within a few hours following addition of nitrate (Patterson *et al.*, 2010). Additionally, the concentration of nitrate reductase protein is increased by light, sucrose and cytokinin, whereas glutamine, a primary product of N assimilation, represses nitrate reductase (Krapp *et al.*, 1998). This regulation links the capacity for nitrate assimilation with the availability of sugars to provide C skeletons. Elevated atmospheric carbon dioxide can reduce the assimilation of nitrate because the reductants produced by photosynthesis are necessary for both carbon and nitrate assimilation (Bloom *et al*., 2010).

Nitrate reductase is further regulated by several post-translational mechanisms. A protein kinase phosphorylates nitrate reductase and thereby enables binding of a protein which inactivates the enzyme. The inactivation of nitrate reductase by protein binding is inhibited by triose and hexose phosphates. This ensures that nitrate reductase is maintained in an active state when there is ample supply of C skeletons for amino acid synthesis. Also, enzyme activity can be restored by dephosphorylation by a phosphatase which prevents protein binding and inhibition. During short-term light–dark transitions, post-translational

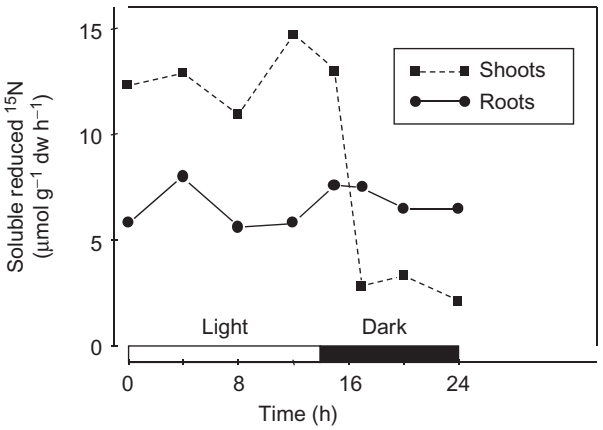


FIGURE 6.8 Concentration of soluble reduced N in roots and shoots of maize during a 24 h period of $^{15}NO_3$ supply to the roots. *Based on Pearson* et al*., 1981.*

inhibition of nitrate reductase occurs within a few minutes, preventing accumulation of nitrite (Lea *et al*., 2006).

The close correlation between light intensity and nitrate reduction in green leaves (Fig. 6.8) may reflect fluctuations in carbohydrate concentrations and in the corresponding supply of reducing equivalents and C skeletons (Anjana *et al.*, 2007). The diurnal fluctuations in nitrate reductase activity may lead to a decrease in the foliar nitrate concentrations during the light period (Table 6.1; Neely *et al.*, 2010). Plants grown permanently under low-light conditions (e.g., in glasshouses during winter) may contain nitrate concentrations which are several fold higher than those of plants grown under high-light conditions (e.g., in an open field during the summer). This is particularly evident in certain vegetables belonging to the *Brassicaea* or *Chenopodiacea* (Santamaria, 2006); for example, spinach has a high preference for nitrate accumulation in the shoots

TABLE 6.1 Time course of nitrate content in spinach leaves during the light period from 9:00 to 18:00

		Nitrate concentration (mg kg^{-1} fw)	
	Time of day	Leaf blade	Petioles
	8:30	228	830
light	9:30	167	725
light	13:30	101	546
light	17:30	91	504
	18:30	106	578

Based on Steingröver *et al.* (1982).

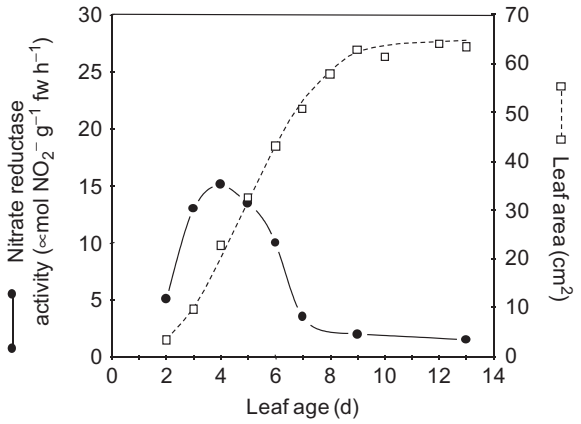


FIGURE 6.9 Nitrate reductase activity and leaf area development during the ontogeny of the first trifoliate leaf of soybean. *Modified from Santoro and Magalhaes, 1983.*

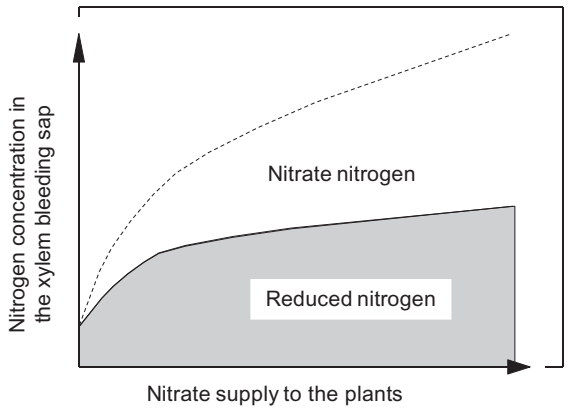


FIGURE 6.10 Schematic representation of the concentration of N in the xylem breeding sap in the form of nitrate and reduced N. *Data recalculated from Wallace and Pate, 1965.*

and uses nitrate accumulation in vacuoles for osmoregulation. Under low-light conditions, nitrate concentrations in spinach leaves can reach 100 mM nitrate, corresponding to 6,000 mg kg^{-1} fresh weight (Burns *et al.*, 2011).

Vacuolar nitrate is rapidly remobilized to sustain cytosolic nitrate concentrations when plants are deprived of external nitrate or when nitrate reductase activity is high at high light intensities (Cookson *et al.*, 2006). Accordingly, the rate of release of nitrate from the vacuoles in leaf cells does not appear to be a rate limiting step for the utilization of stored nitrate. However, nitrate reductase activity peaks when the rate of leaf expansion is maximal and becomes very low in fully expanded leaves (Reed *et al.*, 1980; Fig. 6.9); this may limit utilization of stored nitrate in senescing leaves. In roots, nitrate reductase activity is high in expanding cells of the apical zones and declines rapidly towards the basal root zones (Di Laurenzio *et al.*, 1996).

In most plant species, both roots and shoots are capable of nitrate reduction, and roots may reduce between 5 and 95% of the nitrate taken up. The proportion of reduction carried out in roots and shoots depends on various factors, including level of nitrate supply, plant species and plant age (Andrews, 1986a). In general, when the external nitrate supply is low, a high proportion of nitrate is reduced in the roots. With increasing supply of nitrate, the capacity for nitrate reduction in the roots becomes a limiting factor and an increasing proportion of the total N is translocated to the shoots in the form of nitrate (Fig. 6.10).

There is a general pattern between plant species in partitioning of nitrate reduction and assimilation between roots and shoots. In temperate perennial species as well as in temperate annual legumes, most of the nitrate is reduced in the roots when the external concentration is relatively low. In contrast, tropical and subtropical annual and perennial species tend to reduce a large proportion of the nitrate in the shoots, even at low external supply with no change in the proportion between root and shoot reduction when the external concentration is increased. There are exceptions to this generalization; for example, in Australian open forest plants (Stewart *et al.*, 1990) or woody plants growing in cerrado and forest communities in Brazil (Stewart *et al.*, 1992), at least in some under- and overstorey species, the capacity for nitrate reduction in the leaves is low compared to the roots. With high nitrate availability, shoots appear to be the predominant site of nitrate reduction in both fast- and slow-growing grass species (Scheurwater *et al.*, 2002).

The uptake rate of the accompanying cation also affects the proportion of nitrate reduced in roots. With K as accompanying cation, translocation of both K and nitrate to the shoots is rapid; correspondingly, nitrate reduction in the roots is relatively low (Ruiz and Romero, 2002). In contrast, when Ca or Na is the accompanying cation, nitrate reduction in the roots is considerably higher (Cramer *et al.*, 1995).

The preferential site of nitrate reduction, roots or shoots, may have an important impact on carbon economy of plants, and probably also has ecological consequences for the adaptation of plants to low-light and high-light conditions. The energy requirement for reduction and assimilation of nitrate is high and are costly processes when carried out in roots (Schilling *et al*., 2006; Gavrichkova and Kuzyakov, 2009). When expressed in ATP equivalents, the energy requirement for the reduction of one mole of nitrate is 15 moles ATP with an additional 5 moles ATP for ammonium assimilation (Salsac *et al*., 1987). In barley, where a high proportion of nitrate reduction occurs in the roots, up to 23% of the energy from root respiration is required for absorption (5%), reduction (15%) and assimilation of the reduced nitrogen (3%), compared to only 14% for assimilation when ammonium is supplied (Bloom *et al.*, 1992). In contrast, for nitrate reduction in leaves reducing equivalents can be directly provided by photosystem I and ATP from photophosphorylation. Under low-light conditions or in fruiting plants (Hucklesby and Blanke, 1992) this may lead to competition between CO_2 and nitrate reduction. On the other hand, under high-light conditions and excessive light absorption (photoinhibition, photooxidation), nitrate reduction in leaves may not only use energy reserves but also alleviate high-light stress. Competition with CO_2 reduction under elevated CO_2 may lead to acclimation and reduced CO_2 response when nitrate is the N source, while acclimation is less pronounced under ammonium nutrition (Bloom *et al*., 2010).

In C4 plants, mesophyll and bundle sheath cells differ in their functions not only in CO_2 assimilation but also in nitrate assimilation. Nitrate reductase and nitrite reductase are localized in the mesophyll cells and are absent in the bundle sheath cells. This division of labour in C4 plants, whereby mesophyll cells utilize light energy for nitrate reduction and assimilation and bundle sheath cells for CO_2 reduction, is most probably the cause for higher photosynthetic N use efficiency (NUE) in C4 compared with C3 plants (Sage *et al*., 1987). Because of the particular CO_2 concentration mechanism in the bundle sheath cells (see also Chapter 5), less RuBP carboxylase (Rubisco) is required in C4 than in C3 plants. In C3 plants, N in Rubisco accounts for 20–30% of the total leaf nitrogen compared with less than 10% in C4 plants, plus 2–5% N for PEP carboxylase in C4 plants (Sage *et al*., 1987).

In plant species in which most or all nitrate assimilation occurs in the shoots, organic acid anions are synthesized in the cytoplasm and stored in the vacuole in order to maintain both cation–anion balance and intracellular pH. The latter is required because nitrate reduction consumes two protons per nitrate reduced. This may lead to osmotic problems if nitrate reduction proceeds after the termination of leaf cell expansion (Raven and Smith, 1976; Britto and Kronzucker, 2005). However, several mechanisms exist for the removal of excess osmotic solutes from the shoot tissue: (i) precipitation of excess solutes in an osmotically inactive form, for example synthesis of oxalic acid for charge compensation in nitrate reduction and precipitation as calcium oxalate are common in plants, including sugar beet; (ii) retranslocation of reduced N (amino acids and amides) together with phloem-mobile cations, such as K and Mg, to areas of new growth; (iii) re-translocation of organic acid anions, predominately malate, together with K into the roots and release of CO_2 after decarboxylation.

6.1.4.2 Ammonium Assimilation

Ammonium is a central intermediate in plant N metabolism. Besides uptake from the soil by roots, ammonium is constantly generated in high rates in plant tissues by processes such as nitrate reduction, photorespiration, lignin biosynthesis, senescence-induced N remobilization and N_2 fixation in legumes (Joy, 1988). Irrespective of the source of ammonium or the organ in which it is assimilated (roots, root nodules and leaves) the key enzymes involved are glutamine synthetase (GS) and glutamate synthase (GOGAT; glutamine-oxoglutarate aminotransferase). Both enzymes are present in roots, in chloroplasts and in N_2-fixing microorganisms. Assimilation of most, if not all, ammonium derived from ammonium uptake, N_2 fixation, nitrate reduction and photorespiration is mediated by the glutamine synthetase–glutamate synthase pathway. In this pathway the amino acid glutamate acts as the acceptor for ammonium, forming the amide glutamine (Fig. 6.11). The net reaction is:

$$\text{Glutamate} + \text{2-Oxoglutarate} + NH_4^+ \rightarrow \text{2-Glutamate} + H^+ \quad (6.2)$$

Glutamine synthetase exists in multiple enzyme forms located in the cytosol and in plastids (Hirel and Lea, 2001; Bernard *et al*., 2008). Cytosolic GS has multiple metabolic functions such as assimilation of ammonium into glutamine for transport and distribution throughout the plant. During leaf senescence cytosolic GS fulfils a key function in the assimilation and recycling of ammonium generated from various catabolic processes (Masclaux-Daubresse *et al*., 2010). This role is particularly important after anthesis and during grain development and filling in cereals, when N is remobilized to the reproductive sinks (Martin *et al*., 2006). Several of the isoenzymes of the cytosolic GS1 gene family are abundantly expressed in roots and can be classified into high-affinity or low-affinity subtypes differing in V_{max} values (Ishiyama *et al.*, 2004). Some are more abundant under N deficiency while others dominate under high external ammonium supply. This dynamic regulation may contribute to the homeostatic control of glutamine synthesis in roots.

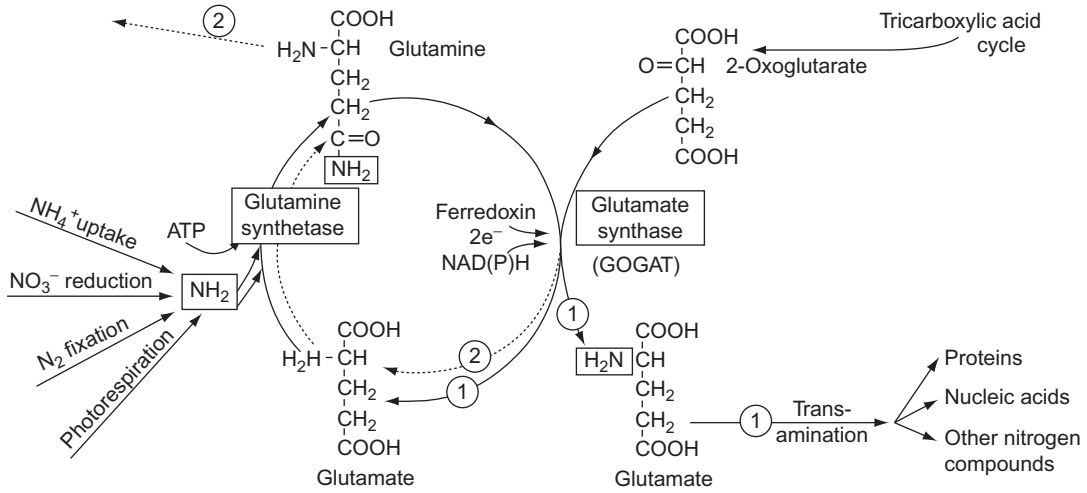


FIGURE 6.11 Model of ammonium assimilation via the glutamine synthetase–glutamate synthase cycle. Pathway at low (1) and at high (2) ammonium supply.

In chloroplasts, light-stimulated nitrate reduction and enhanced ammonium assimilation are coordinated through import of 2-oxoglutarate from the stroma and export of glutamate from the stroma of chloroplasts into the cytoplasm, thus preventing high ammonium concentrations. Chloroplast GS is activated by high pH and high concentrations of Mg and ATP, and all three factors are increased in the chloroplast stroma upon illumination. In cytosol and chloroplasts, GS is also subject to post-translational regulation by phosphorylation and subsequent interactions with proteins (Finnemann and Schjoerring, 2000; Lima *et al.*, 2006).

The other enzyme involved in ammonium assimilation, glutamate synthase (GOGAT), catalyses the transfer of the amide group (-NH_2) from glutamine to 2-oxoglutarate, which is a product of the tricarboxylic acid cycle. The conversion of glutamine to glutamate takes place in plastids which have two isoforms of GOGAT. One form accepts electrons from reduced ferredoxin (from photosystem I), the other from NADPH from respiration. The ferredoxin-linked GOGAT isoform dominates in leaves, particularly in the chloroplasts of phloem companion cells in leaf veins (Masclaux-Daubresse *et al.*, 2007), whereas the NADPH isoform is prevalent in roots (Tabuchi *et al.*, 2007). Both forms contain an Fe-S cluster which transfers electrons during the reductive synthesis of two glutamate molecules from one 2-oxoglutarate and one glutamine molecule. One of the two produced molecules of glutamate is required for the maintenance of the ammonium assimilation cycle and the other can be transported from the sites of assimilation and utilized elsewhere for biosynthesis of proteins. When the ammonium supply is high, both glutamate molecules can act as ammonium acceptors, and one molecule of glutamine leaves the cycle (Fig. 6.11). Two Fd-GOGAT genes (GLU1 and GLU2) have been characterized in higher plants, with *GLU1* playing a major role in the assimilation of ammonium derived from photorespiration, whereas the *GLU2* gene may play a major role in the primary nitrogen assimilation in roots (Suzuki and Knaff, 2005).

The enzyme glutamate dehydrogenase (GDH) was for many years assumed to be involved in ammonium assimilation. However, it is now evident that GDH mainly is involved in the liberation of ammonium during senescence via catalysing the oxidative deamination of glutamate, thereby providing carbon skeletons for respiration and oxidative phosphorylation. Thus, the GDH enzyme, in conjunction with NADH-GOGAT, contributes to the control of leaf glutamate homeostasis, an amino acid which plays a central role in signalling at the interface of the C and N assimilatory pathways (Labboun *et al.*, 2009).

6.1.4.3 Low molecular Weight Organic N Compounds

The inorganic N assimilated into glutamate and glutamine can readily be used for the synthesis of other amides as well as amino acids, ureides, amines, peptides, proteins, nucleic acids and other N-containing compounds (Fig. 6.12). In higher plants, low-molecular-weight organic compounds not only act as intermediates between the assimilation of inorganic N and the synthesis of high-molecular-weight compounds, they are also important for transfer of N from source organs to sink tissues and to build up reserves during periods of high N availability.

In contrast to lower plants, animals and humans, higher plants are not capable of excreting substantial amounts of organically bound N, for example as urea. Although plants can store large amounts of nitrate, they can not re-oxidize organically bound nitrogen to nitrate, which could be a safe storage form in periods of enhanced protein degradation in, for example, senescing leaves. In plants, amino acids and

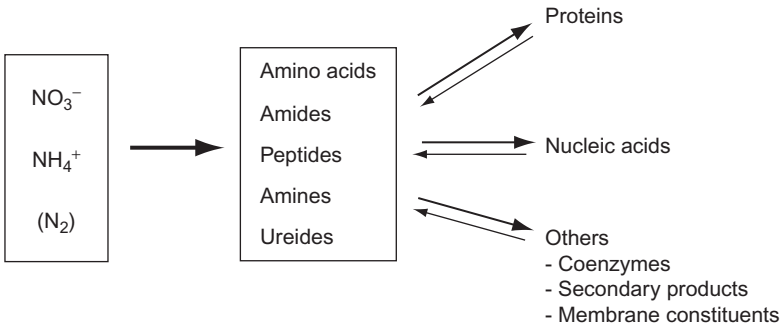


FIGURE 6.12 Major classes of nitrogen compounds in plants.

amides act as buffer and transient storage, in addition to their function in long-distance transport of reduced N.

Glutamate, glutamine, aspartate and asparagine occupy a central position in amino acid metabolism and in C–N interactions in plants. Indeed, glutamine and glutamate are the major entry points of ammonia into organic compounds, and the amino groups in glutamate and aspartate and the amide group in glutamine are the N source for most plant N compounds, including other amino acids (Morot-Gaudry *et al*., 2001). Aspartate is a metabolically reactive amino acid that serves as donor in numerous aminotransferase reactions, while asparagine is relatively inert and serves primarily as an N storage compound.

Ammonium assimilation in roots has a large requirement for carbon skeletons for amino acid synthesis. These carbon skeletons are provided by the tricarboxylic acid cycle (TCA), and the removed intermediates have to be replenished by increased activity of PEP carboxylase. Compared to nitrate supply, net carbon fixation in roots with ammonium is several-fold higher (Viktor and Cramer, 2005). In order to minimize the carbon costs for root-to-shoot transport, the bulk of the N assimilated in the roots is transported in the form of N-rich compounds with N/C ratios >0.4. One, rarely two or more, of the following compounds dominate in the xylem exudate of the roots: the amides glutamine (2N/5C) and asparagine (2N/4C); the amino acid arginine (4N/6C); and the ureides allantoin and allantoic acid (4N/4C). In phloem transport to developing fruits, which are non-photosynthetic sinks, amino acids with an N/C ratio of greater than 0.4 are the predominant transport forms of nitrogen.

The low-molecular-weight organic N compounds used predominantly for long-distance transport or for storage in individual cells differ among plant families. Glutamine and asparagine are the dominating transport amides in *Graminae*. Asparagine is the dominant transport form in legume species such as clover, lucerne, pea and lupin, which have indeterminate nodules. In other legume species such as soybean, cowpea and common bean, characterized by determinate nodules, the majority of the fixed N transported in the xylem of nodulated roots is incorporated into the ureides allantoin and allantoic acid (Pélissier *et al*., 2010; see also Chapter 16). The concentrations of these transport amino acids vary and are modulated by factors such as light and N availability. Glutamine is mainly synthesized in the light, while asparagine is preferentially synthesized in the dark.

An important class of low-molecular-weight organic N compounds are amines and polyamines, their biosynthesis being mediated by decarboxylation of amino acids, for example serine which forms the basis for synthesis of ethanolamine which is contained in the lipid fraction of biomembranes. Arginine is the main precursor for polyamines which are important secondary messengers (Kusano *et al*., 2008). Putrescine is usually the dominating polyamine in plants and may constitute up to 1.2% of the plant dry matter. The polyamine concentration is particularly high in meristematic tissues of plants supplied with high concentrations of ammonium (Gerendas and Sattelmacher, 1990) and under K deficiency (Watson and Malmberg, 1996).

Another low-molecular-weight organic N compound is betaine (glycine betaine) which is involved in osmoregulation. Under salt or drought stress, the synthesis of betaine and its accumulation particularly in the cytoplasm are strongly enhanced. Betain is important for the adaptation of plants to drought or salinity, because it acts as compatible solute to counteract the osmotic perturbation caused by high vacuolar concentrations of inorganic ions such as Cl and Na which would inhibit cytoplasmic metabolism (Guo *et al*., 2009b; Missihoun *et al*., 2011).

Although plants may contain up to 200 different amino acids, only about 20 of them are required for protein synthesis. Not much is known about the role of the large number of non-proteinogenic amino acids in plants. However, at least some of them are important for plant nutrition. Nicotianamine is an effective chelator of Fe^{2+} and plays a role in iron homeostasis and in phloem transport of Fe, Zn and Mn (Suzuki *et al.*, 2008b; Ishimaru *et al.*, 2010; see also Chapter 7). In addition, nicotianamine is precursor of a group of other non-proteinogenic amino acids, the so-called phytosiderophores which are

of particular importance for acquisition of Fe in graminaceous plant species (Suzuki *et al.*, 2006; see also Section 7.1 and Chapters 2 and 14).

6.1.5 N Supply, Plant Growth and Composition

6.1.5.1 Synergy between Ammonium and Nitrate Nutrition

Whether ammonium or nitrate as sole source of N supply is better for growth and yield formation of plants depends on many factors. Generally, plants adapted to soils which are acid (calcifuge species) or have a low redox potential (e.g., wetlands) have a preference for ammonium (Lee, 1999). In contrast, plants adapted to calcareous, high pH soils (calcicole species) utilize nitrate preferentially. However, highest growth rates and plant yields are obtained by combined supply of both ammonium and nitrate.

Ammonium is preferentially taken up by many plants when supplied in equimolar concentrations with nitrate, particularly when the N supply is low (Gazzarrini *et al*., 1999). The preference for ammonium relative to nitrate increases strongly with decreasing temperatures, and below 5°C uptake of ammonium can still proceed, while that of nitrate ceases (Macduff and Jackson, 1991). This may reflect the greater costs of metabolic energy associated with absorption and assimilation of nitrate compared to ammonium. On the other hand, ammonium is predominantly assimilated in the roots, imposing a direct demand for carbon skeletons which is reflected in higher activities of PEP carboxylase. Compared with ammonium, nitrate has the advantage of allowing more flexible distribution of assimilation between roots and shoots and can be stored in higher amounts than ammonium in the vacuoles.

As ammonium or nitrate comprises about 80% of the total cations and anions taken up by plants, the form of N has a strong impact on the uptake of other cations and anions, on cellular pH regulation and on rhizosphere pH (see also Chapter 14). The assimilation of ammonium in roots produces about one proton per molecule of ammonium (Raven and Smith, 1976). The generated protons are to a large extent excreted into the external medium in order to maintain cellular pH and electro-neutrality, the latter compensating for the excess uptake of cation equivalents over anion equivalents, which are generally associated with ammonium nutrition. Under mixed N nutrition, the proton generated by ammonium assimilation can be used for nitrate reduction; therefore it is easier for plants to regulate intracellular pH when both forms of nitrogen are supplied.

Rhizosphere chemistry can be affected by the form of N taken up: ammonium supply may reduce rhizosphere pH through a net excretion of protons, whereas nitrate supply may increase rhizosphere pH through a net uptake of protons from the rhizosphere (Hinsinger *et al.*, 2003). The implications of this for the availability of other nutrients such as P and micronutrients are discussed in Chapter 14.

The form in which N is taken up is important for the biosynthesis and function of phytohormones, especially cytokinins (Inoue *et al.*, 2001). For example, the enzymes required for the synthesis of cytokinins are specifically induced by nitrate supply and not by other nutrients (Miyawaki *et al*., 2004). In wheat plants supplied with ammonium, the presence of nitrate at even very low concentrations (100 μM) can stimulate increases in the concentration of the active cytokinin forms zeatine, trans-zeatine riboside and isopentenyl adenosine (Garnica *et al*., 2010). The higher cytokinin concentrations in nitrate-fed plants may be accompanied by higher shoot concentrations of auxin (IAA) (Garnica *et al.*, 2010). These results suggest that the beneficial effect of nitrate on the growth of plants predominately fed with ammonium is mediated by a coordinated effect on the levels of cytokinins and IAA in the shoot. Conversely, in nitrate-fed plants, reproductive growth may be delayed due to excessive concentrations of cytokinins. Under such circumstances, provision of ammonium may induce flowering, probably via increased biosynthesis of polyamines acting as secondary messengers (Rohozinski *et al*., 1986).

6.1.5.2 Ammonium Toxicity

Plant species differ in tolerance to ammonium (Britto and Kronzucker, 2002). Among crop plants, barley is ammonium-sensitive, whereas rice is ammonium-tolerant. The symptoms of ammonium toxicity include leaf chlorosis, stunted growth and eventually necrotic leaves and plant death.

Various hypotheses have been put forward to explain the physiological processes underlying ammonium toxicity. When whole tissue of ammonium-fed plants is analyzed, several chemical changes are observed. Generally, compared to nitrate-fed plants, there is an accumulation of ammonium ions, inorganic anions such as chloride, sulphate and phosphate as well as of amino acids. In contrast, there is a reduction in the concentration of the essential cations such as K^+, Ca^{2+} and Mg^{2+} as well as organic acids such as malate (Britto and Kronzucker, 2002). These and other observations have led to the hypotheses that ammonium toxicity may be the result of (i) decreased uptake of essential cations (Siddiqi *et al*., 2002; Roosta and Schjoerring, 2007), (ii) ammonium-induced disorders in pH regulation (Walch-Liu *et al*., 2000), or (iii) excessive consumption of sugars for ammonium assimilation causing carbohydrate limitation (Finnemann and Schjoerring, 1999).

Ammonium influx into the roots of the ammonium-sensitive species barley appears to be much higher than into the ammonium-tolerant species rice (Britto *et al*.,

2001), suggesting that rice can control the influx of ammonium into the roots. Barley on the other hand, releases ammonium back into the soil. This has led to the hypothesis that in ammonium-sensitive species, the apparently futile transmembrane cycling of ammonium and the operation of an energy-intensive ammonium efflux mechanism may be the cause of ammonium toxicity (Britto *et al*., 2001).

Additionally, the acidification of the rhizosphere induced by ammonium uptake may in itself pose a stress to plants, particularly in acid soils where it can increase Al toxicity.

Each of these factors may contribute to plant ammonium toxicity depending on the plant species and particular growth conditions.

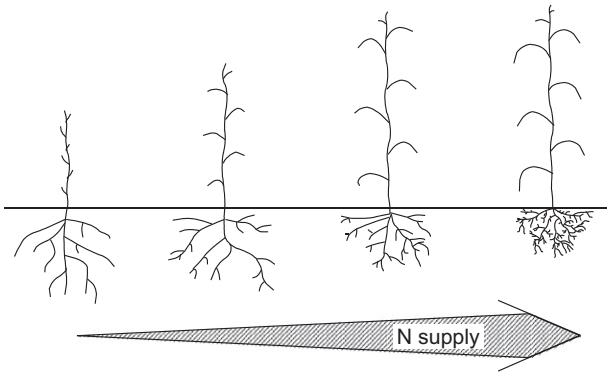


FIGURE 6.13 Schematic representation of shoot and root growth in cereal plants with increasing N supply.

6.1.5.3 N Deficiency

In order to achieve efficient growth, development and reproduction, plants require adequate, but not excessive, amounts of N. Therefore, low soil N availability or a decline in root uptake capacity will negatively affect plant productivity and ecological competiveness. Nitrogen-deficient plants are typically stunted, with narrow leaves. Chlorosis caused by N deficiency typically begins in the older leaves as N is remobilized to younger leaves. At the field scale, N-deficient crops appear pale green or even yellow. The canopy height is lower and, in grasses, tillering as well as the number of seeds per inflorescense are reduced compared to plants growing with adequate N.

With temporary N starvation in the root medium, plants display a two-phase response. In the first phase, the leaf elongation rate is reduced without affecting photosynthesis (Anandacoomaraswamy *et al.*, 2002). Root growth is maintained or even stimulated by transport of assimilated carbon to the roots, which results in a lower shoot/root biomass ratio (Fig. 6.13) (Richard-Molard *et al*., 2008). Concomitantly, N compounds, particularly nitrate, are mobilized in order to maintain N metabolism and the capacity to take up nitrate from the soil is increased. In the second phase, upon continued N starvation, the breakdown of leaf nucleic acids and proteins is triggered. This is usually associated with leaf senescence (Hortensteiner and Feller, 2002). The breakdown of Rubisco leads to a decrease in the maximum photosynthetic capacity of the plant, ultimately inhibiting whole plant growth.

Plants have evolved multifaceted strategies to respond to variations in N availability in the soil, i.e. metabolic, physiological and developmental adaptations, which, in part, depend on changes in gene expression. The expression of many genes is changed within minutes in response to nitrate concentrations (Wang *et al*., 2000). In *Arabidopsis*, N deprivation or limitation leads to a coordinated repression of genes involved in photosynthesis, chlorophyll synthesis, plastid protein synthesis, while genes involved in secondary metabolism and protein degradation are induced (Scheible *et al*., 2004).

6.1.5.4 Changes in Root System Architecture in Response to N Supply

One of the most striking examples of plasticity to changing N supply is the modulation of the root system architecture. Generally, a uniformly high nutrient supply suppresses root branching. However, when overall N availability is limited, plants may respond to a spatially restricted availability of N by enhancing lateral root development into N-rich patches. An example of such proliferation of lateral roots within a localized nitrate-rich zone is shown in Fig. 6.14 for barley plants.

Nitrate and ammonium are locally sensed and trigger a signalling pathway which stimulates elongation of lateral roots in a confined soil volume (Zhang *et al*., 2009). Whereas nitrate stimulates lateral root elongation, ammonium triggers the initiation of lateral root growth (Lima *et al*., 2010).

6.1.5.5 Storage Proteins

The amino acids formed by nitrate assimilation can be stored in dedicated storage proteins, which have neither metabolic nor structural roles (Heldt and Piechulla, 2011) and often have a relatively high proportion of N-rich amino acids, particularly arginine and the amides. Storage proteins accumulate transiently, and upon protein degradation, the amino acids can be used directly for *de novo* protein synthesis or may be metabolized.

Vegetative storage proteins (VSP) have been identified in a large number of plant species (Staswick, 1994; Ourry *et al.*, 2001) and can constitute up to 50% of the total soluble proteins in various vegetative storage organs, for example in the taproot of lucerne where four VSPs have been identified (Ourry *et al.*, 2001).

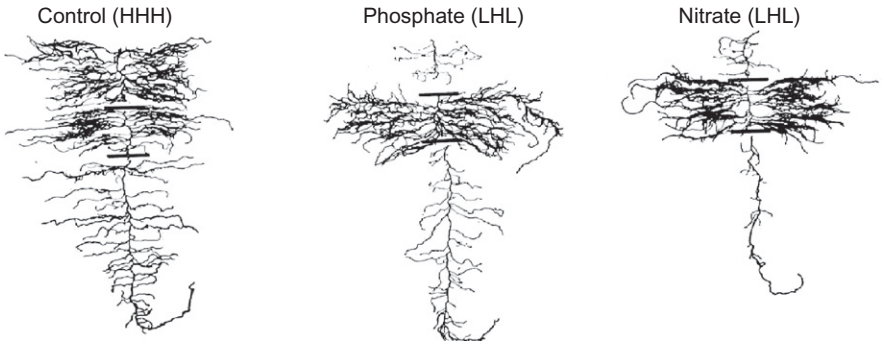


FIGURE 6.14 Root growth of barley plants with complete nutrient solution to all parts of the root system (*left*) or complete nutrient solution in the middle zone only with the top and bottom parts of the root system supplied with nutrient solution deficient in either phosphate (*middle*) or nitrate (*right*). *Adapted from Drew (1975).*

Vegetative storage proteins differ from seed storage proteins in that they accumulate transiently and are degraded within the life cycle of the plant. The accumulation of VSP can be indirectly affected by changes in source–sink relationships in relation to N within the plant (Staswick, 1994; Ourry *et al*., 2001), or directly by exogenous stimuli such as methyl-jasmonate (Noquet *et al*., 2001; Meuriot *et al*., 2004) or modifications of soil N availability (Meuriot *et al*., 2003). For example, in lucerne, the rate of regeneration of new photosynthetic tissues was linearly related to taproot VSP concentration on the day of cutting (Avice *et al*., 1996). Vegetative storage proteins may also be important for autumn hardening and overwintering of lucerne (Dhont *et al*., 2006).

The concentration of protein in seeds varies from 10 to 15% of the dry weight in cereals to 40 to 50% in some legumes (e.g., soybean). In cereal seeds, protein concentrations on a dry weight basis are in the range of 5.8–7.7% in rice, 8–15% in barley, 7–22% in wheat and 9–11% in maize (Barneix, 2007; Holding and Larkins, 2008; Shewry, 2007). About 50–85% of these proteins are storage proteins (Shewry, 2007). Seed storage proteins are synthesized during seed development and serve as the principal source of amino acids for germination and seedling growth. They are initially synthesized on the rough endoplasmic reticulum, transported into the lumen and finally deposited in the protein bodies (Kumamaru *et al*., 2007).

Seed storage proteins share a number of common properties: i.e. (i) high rate of synthesis in specific tissues, (ii) presence in mature seeds in discrete deposits called protein bodies, and (iii) being mixtures of components that exhibit polymorphism both within single genotypes and among genotypes of the same species.

Because of their abundance and economic importance in agricultural crops, seed storage proteins have been studied for more than 250 years. Based on their solubility properties, they were originally classified into: albumins (soluble in pure water), globulins (soluble in dilute salt solutions), glutelins (soluble in diluted solutions of alkali and acids) and prolamins (soluble in aqueous ethanol) (Osborne, 1924). The structures of glutelins and prolamins are closely related, therefore glutelins are now regarded as members of the prolamins (Heldt and Piechulla, 2011).

The predominant storage proteins of cereals are the prolamins, except for oats and rice, in which the major storage proteins are globulins. In legumes, globulins are the major legume storage proteins. Globulins are particularly rich in the amino acids glutamine and asparagines. They are found in nearly all plants and are the most widely distributed group of storage proteins (Holding and Larkins, 2008). Prolamins are only present in grasses (Shewry and Halford, 2002; Holding and Larkins, 2008). In the major cereals, prolamins usually account for about 50% of the total grain nitrogen. Exceptions to this general rule are oats and rice in which prolamins represent only about 5 to 10% of the total seed protein. Albumins are a heterogeneous group of proteins for which the only unifying criterion is that they have a sedimentation coefficient of about 2 Svedberg (Holding and Larkins, 2008). They are widely distributed in seeds of dicot species.

Beside the classical storage proteins, seeds contain additional proteins that are associated with defence mechanisms developed by plants against pests and pathogens. These special proteins include proteinase inhibitors, lectins and lectin-like proteins, ribosome-inactivating proteins, lipid transfer proteins, glucanases and chitinases. Seed proteins also include hydrolases such as amylases; proteinases and lipases mobilize several types of associated reserve compounds, the products of which are used during germination for the synthesis of new tissues (Shewry *et al.*, 1995).

In many plant storage proteins, the concentration of nutritionally essential amino acids is low. Since these amino acids cannot be synthesized by the human metabolism, humans have to absorb essential amino acids from their food. In cereals, for example, the storage proteins are low in threonine, tryptophan and particularly in lysine, whereas

in legumes there is a shortage of methionine. The aleurone and embryo tissues of grains contain higher concentrations of essential amino acids, but these are often not available for human nutrition as they are removed by milling.

The increase in grain protein with high N fertilization is due to greater synthesis and accumulation of storage proteins. Several studies in wheat have shown that increases in grain N are associated with increased proportions of the monomeric gliadins and a decreased proportion of large glutenin polymers, resulting in increased dough extensibility (Kindred *et al*., 2008; Godfrey *et al.*, 2010).

6.1.6 Nitrogen Use Efficiency (NUE)

Nitrogen use efficiency can have several meanings in the context of crop production (Good *et al.*, 2004; Fageria and Baligar, 2008). In general, NUE is the ratio between the total biomass of output (e.g., grain yield) and the N input (e.g., N supplied in fertilizers and/or residual N present in the soil). NUE is divided into two components: N uptake efficiency (NupE; the ability of the plant to remove N from the soil) and the utilization efficiency (NutE; the ability to use N to produce biomass or grain yield).

In crops, and particularly in cereals, large amounts of N fertilizer are required to attain maximum yield and NUE is estimated to be less than 50% (Zhu, 2000; Raun and Johnson, 1999). The resulting N losses from agricultural land give rise to soil and water pollution. In addition, incomplete capture and poor conversion of fertilizer-derived N causes global warming through emissions of nitrous oxide. As a consequence, plant breeding aiming at development of new crop genotypes with better N use efficiency have a high priority (Hirel *et al*., 2007). Plant breeding for better NUE is focused on the different physiological processes which affect N uptake from the soil, N translocation, N assimilation and N redistribution (Foulkes *et al*., 2009; Masclaux-Daubresse *et al*., 2010). Identification of genotypes which grow and yield well under low N conditions are particularly needed for a successful outcome (Barraclough *et al*., 2010).

With regard to N uptake efficiency, capture of nitrate in low concentrations in the topsoil requires a high rooting density (Dunbabin *et al*., 2003). The primary root traits affecting nutrient uptake are root axis number, rooting depth and rooting density. Prolific root systems are more effective at capturing nutrients than sparse systems, but inter-root competition sets a natural threshold for optimal root density. Further root traits which could increase N capture include enhanced root longevity for N uptake after flowering (Garnett *et al*., 2009).

Expression of both nitrate and ammonium transporter genes is regulated by supply and demand for N (Tsay *et al*., 2007). Higher threshold levels of down-regulation of the transporter genes may allow greater influx which in turn may drive increased N assimilation. Alternatively, decreasing the activity of efflux systems could also improve the efficiency of uptake.

For N utilization efficiency, the cytosolic isoforms of the enzyme glutamine synthetase (GS1) appear to play an important role in nitrogen management, growth rate, grain yield and grain filling (for a review see Bernard and Habash, 2009). GS isoforms can be critical for N assimilation and remobilization, and specific manipulation of some isoforms in a developmentally controlled manner may offer prospects for gains in NutE. Increased conversion of N into grain yield may be achieved by improving the efficiency of CO_2 fixation (Long *et al*., 2006).

To attain maximum yields, modern crop cultivars require large amounts of fertilizers, in particular N. This reflects that the genotypes currently cultivated in developed countries have mostly been selected under non-limiting fertilization conditions (Presterl *et al.*, 2003). Although plant breeders have consistently targeted improved grain yield under high inputs of fertilizer and crop protection chemicals, N efficiency *per se* has only recently been a target. Differences in N efficiency between varieties show that there is a potential to exploit genotypic differences in N responsiveness of maize, wheat and rice (Cirilo *et al*., 2009; Barraclough *et al.*, 2010). Although it is well known that there is genetic variability in maximum N uptake in rice and wheat, the physiological and genetic basis underlying this variability is poorly understood. High maximum N uptake could allow storage of greater quantities of nitrogen during periods of abundant nitrogen supply, thus reducing N losses in the soil.

6.2 SULPHUR

6.2.1 General

Although atmospheric SO_2 is taken up and utilized by the aerial parts of higher plants (Chapter 4), the most important source of S is sulphate taken up by the roots. In the physiological pH range, the divalent sulphate anion (SO_4^{2-}) is taken up by root cells and then transported in the xylem and phloem (Chapter 3), with transmembrane transport steps catalysed by a family of sulphate transporters (Hawkesford, 2003). In several respects, sulphur assimilation has many common features with nitrate assimilation. For example, reduction is necessary for the incorporation of sulphur into amino acids, proteins and coenzymes, and in green leaves ferredoxin is the reductant for sulphate. Unlike nitrate nitrogen, however, sulphate can also be utilized without reduction and incorporated into essential organic structures such as sulpholipids in membranes or polysaccharides such as agar. Also in contrast to N, reduced S can be reoxidized in plants. In this oxidation reaction the reduced S of cysteine is converted

to sulphate (Sekiya *et al.*, 1982a), the 'safest' storage form of S in plants. Sulphite oxidase, localized to peroxisomes, is a potential component of such a pathway (Hänsch *et al.*, 2007).

6.2.2 Sulphate Uptake, Assimilation and Reduction

For comprehensive reviews the reader is referred to Schmidt (1992), Leustek *et al.* (2000), Saito (2004), Kopriva (2006) and Hawkesford and DeKok (2006). Sulphate uptake into root cells is a high affinity H^+ cotransport with the expression of the respective genes being strongly induced by S deficiency. Lower affinity transporters from the same gene family are involved in cell to cell distribution of sulphate across plasma membranes, and in storage and remobilization from vacuoles across the tonoplast. The transporters for delivery of sulphate into the chloroplasts, the site of activation and reduction, remain unknown. In higher plants and in green algae, the first step of S assimilation is the activation of the sulphate ion by ATP (Fig. 6.15). In this reaction the enzyme ATP sulphurylase catalyses the replacement of two phosphate groups of the ATP by the sulphuryl group, which leads to the formation of adenosine phosphosulphate (APS) and pyrophosphate (Fig. 6.15). This enzyme is regulated by various external (e.g., light) and internal (e.g., reduced sulphur compounds) factors. The activated sulphate, adenosine phosphosulphate (APS), can serve as substrate for the synthesis of sulphate esters or sulphate reduction. For the synthesis of sulphate esters such as sulpholipids, the enzyme APS kinase catalyses the formation of phosphoadenine phosphosulphate (PAPS) in an ATP-dependent reaction (Fig. 6.15). From PAPS, the activated sulphate can be transferred to a hydroxyl group forming a sulphate ester.

For sulphate reduction, the activated sulphate of APS is reduced to sulphite (SO_3^{2-}) by APS reductase (sometimes called APS sulphotransferase) requiring two electrons supplied from glutathione (Fig. 6.16). Subsequently, six electrons from ferredoxin are required to produce sulphide (S^{2-}), catalysed by sulphite reductase, the sole reaction of the pathway which only occurs in the chloroplast (Fig. 6.16). The newly formed sulphide is transferred to *O*-acetylserine, by the enzyme *O*-acetylserine(thiol)lyase (OASTL). The substrate *O*-acetylserine is synthesized from serine and acetyl CoA catalysed by serine actyl transferase (SAT). However, this enzyme is only active when it occurs in a complex with OASTL (in contrast, OASTL is inactive in the complexed state). Excess *O*-acetylserine (occurring when sulphide is limiting) disrupts the complex, resulting in inactive SAT, and limiting futher *O*-acetylserine production and consumption of acetyl CoA (Hell and Wirtz, 2008). In addition, *O*-acetylserine is thought to be part of a signalling pathway which stimulates

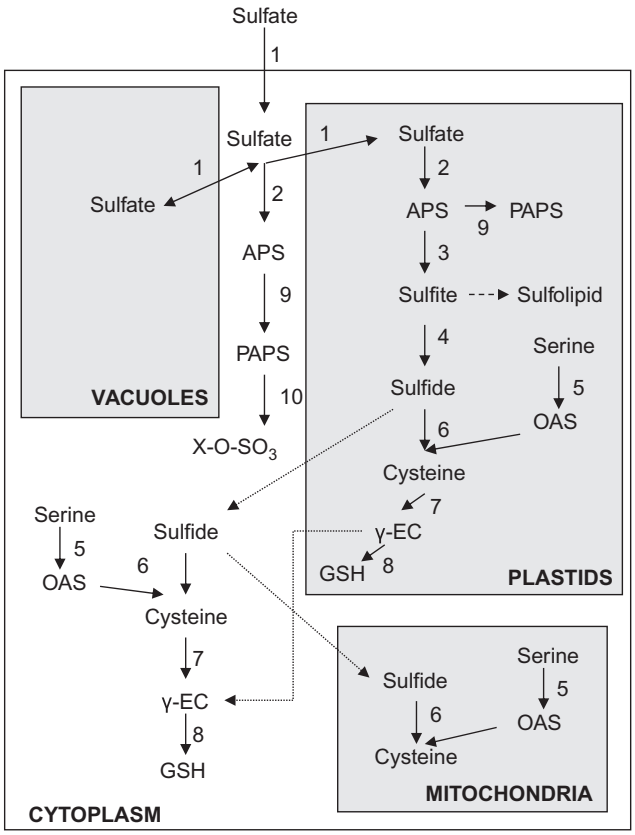


FIGURE 6.15 Plant S assimilation and subcellular localization of its major steps. Numbers represent enzymes as follows: 1, sulphate transporter; 2, ATP sulphurylase; 3, APS reductase; 4, sulphite reductase; 5, serine acetyltransferase; 6, *O*-acetylserine(thiol)lyase; 7, γ-glutamylcysteine synthetase; 8, glutathione synthetase; 9, APS kinase; 10, sulphotransferase. Solid lines represent multiple reaction steps; dotted lines indicate unconfirmed transport steps. *Modified from Kopriva, 2006.*

expression of genes for the transporters and APS reductase to enhance sulphate acquisition and S flux to sulphide. Such positive regulation of expression may balance an apparent repression of gene expression of the sulphate transporters and APS reductase caused by reduced S compounds (Hawkesford and DeKok, 2006). Cysteine, the first stable product of the assimilatory SO_4^{2-} reduction, acts as a precursor for the synthesis of all other organic compounds containing reduced S including glutathione and methionine (Nikiforova *et al.*, 2004), as well as for other biosynthetic pathways, such as the formation of ethylene (Miyazaki and Yang, 1987).

Sulphate uptake and assimilatory reduction are regulated at various levels (Stulen and DeKok, 1993; Vauclare *et al.*, 2002; Hawkesford and DeKok, 2006) by: (i) regulation of expression of the sulphate transporters, (ii) modulation of the activity of ATP sulphurylase, (iii) the availability of sulphate as a substrate for ATP sulphurylase, (iv) change in the level of APS reductase expression

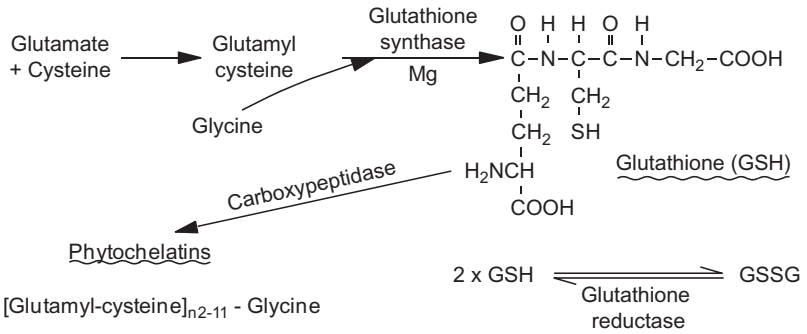


FIGURE 6.16 Biosynthesis of glutathione and phytochelatins.

and activity, and (v) the state of complexation of SAT and *O*-acetylserine(thiol)lyase which may act as both a sensor (of plant S nutritional status) and a regulator (of cysteine biosynthesis).

At high cellular concentrations of either cysteine (Sekiya *et al.*, 1982a) or SO_2 (Sekiya *et al.*, 1982b), the evolution of hydrogen sulphide (H_2S) from green cells is strongly enhanced by light. The light-dependent SO_2 reduction coupled with H_2S release from green leaves (Chapter 4) is considered an important mechanism for the detoxification of SO_2 in leaves and needles (Sekiya *et al.*, 1982b). This type of sulphate reduction may be considered a modification of the dissimilatory sulphate reduction pathway in prokaryotic anaerobes such as *Desulfovibrio* which use sulphate as an oxidant in the formation of ATP and sulphide during respiration (Schiff, 1983).

In higher plants the isoforms of enzymes of the assimilatory sulphate reduction pathway occur in various subcellular compartments (see Fig. 6.15, and Kopriva, 2006) in both leaves and in roots. In many, but not all (Kopriva and Koprivova, 2005), C4 plants, the bundle sheath chloroplasts are the main sites of sulphate assimilation (Schmutz and Brunold, 1984), whereas the mesophyll chloroplasts are the sites of nitrate assimilation (see Section 6.1). Mesophyll chloroplasts, however, do contain at least sulphite reductase and cysteine synthase (Schmidt, 1986). Glutathione biosynthesis occurs in both cell types.

In general, sulphate reduction is several times higher in green leaves than in roots, and in leaves the reaction is strongly stimulated by light (Willenbrink, 1964; Fankhauser and Brunold, 1978). This light enhancement is to be expected because of the requirement for glutathione and ferredoxin as reductants for APS and sulphite, respectively. In addition, expression of several of the genes for enzymes of the reductive assimilation pathway (e.g., genes encoding for ATPS, APR, SiR and OASTL; Hell *et al.*, 1997) appear to be under light and/or diurnal regulation. The stimulation of sulphate reduction by light may also be related to higher levels of serine (acetylserine; Fig. 6.16) synthesized during photorespiration. Reduced sulphur compounds, mainly glutathione, are exported from the leaves via the phloem (Rennenberg, 1989) to sites of demand for protein synthesis (e.g., in the shoot apex, fruits, but also roots) and may also be involved in regulation of sulphate uptake by roots (Rennenberg, 1989; see also Chapter 3). During leaf development, the pattern of sulphate reduction is similar to that of nitrate reduction; that is, it is maximal during leaf expansion, but declines rapidly after leaf maturation (Schmutz and Brunold, 1982). Compared with nitrate reduction, the reduction of sulphate seems to be under a strict negative feedback control as high concentrations of reduced sulphur compounds are rare. Secondary plant products are an exception.

6.2.3 Metabolic Functions of S

Sulphur is a constituent of the amino acids cysteine and methionine, and hence of proteins. Both amino acids are precursors of other S-containing compounds such as coenzymes and secondary plant products. Sulphur is a structural constituent of these compounds (e.g., R_1-C-S-C-R_2) or acts as a functional group (e.g., R-SH) directly involved in metabolic reactions. About 2% of the organically reduced S in plants is present in the water-soluble thiol (-SH) fraction, and under normal conditions the tripeptide glutathione accounts for more than 90% of this fraction (DeKok and Stulen, 1993). Glutathione has many functions in plants and its roles in metabolism have been extensively reviewed, for example by Bergmann and Rennenberg (1993) and Rouhier *et al.* (2008). The synthesis of glutathione occurs in two steps (Fig. 6.16). In the first step, glutacysteine is produced from glutamate and cysteine. In the second step, glycine is coupled to glutamylcysteine, mediated by glutathione synthase, an enzyme which requires Mg for activity (Hell and Bergmann, 1988). In some legume species in the second step, alanine rather than glycine is used by glutathione synthase, forming homo-glutathione which functions similarly to glutathione (Rennenberg and Lamoureux, 1990).

In plants the glutathione concentration is usually higher in leaves than in roots, and in leaves more than 50% of it is localized in the chloroplasts where it may reach millimolar concentrations (Rennenberg and Lamoureux, 1990). Also in root apical zones, for example of maize, the glutathione concentration is in the range of 0.7 mmol kg^{-1} fw, about four times higher than that of cysteine (Nieto-Sotelo and Ho, 1986). Glutathione is readily water soluble and a powerful antioxidant in plants, probably of much greater importance than the cysteine–cystine redox system. Particularly in the chloroplasts, the antioxidants glutathione and ascorbate play a key role in detoxification of oxygen radicals and hydrogen peroxide, for example in the ascorbate peroxidase–glutathione reductase cycle (see also Chapter 5). In the cells, glutathione is maintained in its reduced form by the enzyme glutathione reductase (Fig. 6.16). The antioxidative role of glutathione is reflected, for example, in the increase in glutathione reductase activity at high light intensities in Mg-deficient plants (Cakmak and Marschner, 1992), or in response to other oxidative stresses such as ozone or sulphur dioxide (Smith *et al.*, 1990b). Conjugation of reduced glutathione to a number of xenobiotics such as atrazine (used for weed control) is also the mechanism of detoxification and, thus, of resistance of some plant species to certain xenobiotics (Schröder *et al.*, 1990; Labrou *et al.*, 2005).

Glutathione may function as a transient storage pool of reduced S (Schütz *et al.*, 1991) and thereby maintain a certain cellular cysteine concentration (Schmidt and Jäger, 1992). Glutathione is also the precursor of phytochelatins (Fig. 6.16), which are important in detoxifying certain heavy metals in higher plants (Grill *et al.*, 1987; Rauser, 1990; Cobbett and Goldsbrough, 2002). Plant cells respond to exposure to high concentrations of heavy metals such as Cu, Cd and Zn, by increasing the synthesis of phytochelatins, and additionally, the synthesis of cysteine-rich polypeptides (metallothioneins) (Rauser, 1990; Cobbett and Goldsbrough, 2002).

Phytochelatins consist of repetitive glutamyl-cysteine units (between 2 and more than 10) with a terminal glycine, and are synthesized by degradation of glutathione mediated by a carboxypeptidase (Fig. 6.16). Phytochelatins bind heavy metal cations via thiol coordination and thereby detoxify them (Grill *et al.*, 1987). The synthesis of phytochelatins in roots is most strongly stimulated by Cd, less so by Zn and Cu and negligibly by Ni (Tuckendorf and Rauser, 1990). An example for Cd is shown in Table 6.2. Synthesis of phytochelatins is strongly increased by exposure of the roots to 3 µM Cd, and this increase is accompanied by a rapid decline in the glutathione concentration. This inverse relationship is evident soon after 1–2 h exposure to Cd. Phytochelatin synthesis is induced by exposure as low as 0.05 µM Cd, and synthesis by far exceeds the amount required for detoxification of the heavy metal (Tuckendorf and Rauser, 1990).

Differences between ecotypes of *Silene vulgaris* in Cd tolerance are presumably related to differences in phytochelatin synthesis (Verkleij *et al.*, 1990). However, a general key role of phytochelatins in heavy metal tolerance of plants, for example Zn tolerance, has been questioned (Rauser, 1990).

Thioredoxins are another important family of thiols in higher plants, besides glutathione and its related compounds. Thioredoxins are low-molecular-weight proteins of about 12 kDa with two well-conserved cysteine residues which form a redox-active, intermolecular disulphide bridge. Plant cells contain two different systems capable of reducing thioredoxins: the ferredoxin/thioredoxin system in chloroplasts, and the NADP/thioredoxin system in the cytoplasm (Schürmann, 1993). In chloroplasts, thioredoxins function primarily as regulatory proteins in carbon metabolism. In the reduced form, thioredoxins activate, for example, fructose-1,6-bisphosphatase and several enzymes of the Calvin cycle and thus act as regulatory link between provision of reducing equivalents (PS II) and assimilation of CO_2.

Reduced S is a structural constituent of several coenzymes and prosthetic groups such as ferredoxin (Section 7.1), biotin (vitamin H) and thiamine pyrophosphate (vitamin B_1). In many enzymes and coenzymes such as urease, sulphotransferases (Fig. 6.15) and coenzyme A, the

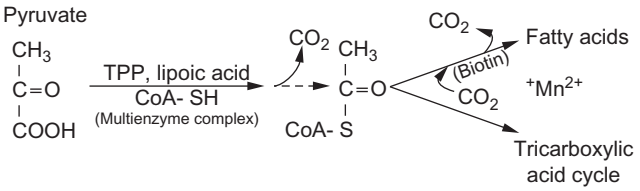
TABLE 6.2 Concentrations of free cysteine, total glutathione, and phytochelatins and Cd in the apical 10 cm of maize roots exposed to 0 or 3 µM Cd for 24 h

	Thiol (nmol g^{-1} fw)			Cd in roots (nmol g^{-1} fw)
Cd (µM)	Cysteine	Glutathione	Phytochelatins	
0	43	421	3	nd
3	44	156	230	13

Based on Tuckendorf and Rauser (1990).
nd: not determined.

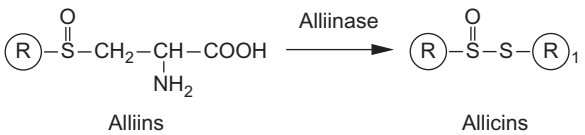
-SH groups act as functional groups in the enzyme reaction. In the glycolytic pathway, for example, decarboxylation of pyruvate and the formation of acetyl coenzyme A are catalysed by a multienzyme complex involving three S-containing coenzymes: thiamine pyrophosphate (TPP), the sulphhydryl–disulphide redox system of lipoic acid, and the sulphhydryl group of coenzyme A:

The acetyl group (-CO-CH_3) of coenzyme A is then transferred to the tricarboxylic acid cycle or to the fatty acid synthesis pathway. The coupling of C_2 units in the synthesis of long-chain fatty acids requires transient carboxylation, which is mediated by the S-containing coenzyme biotin and activated by Mn.



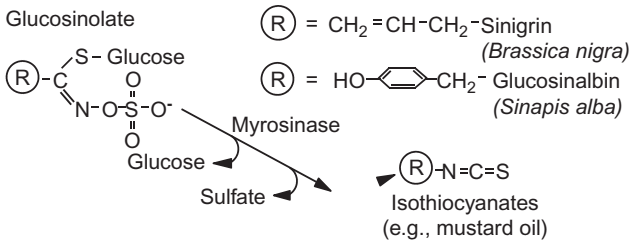
Also as a structural component, cysteine has particular effects on structure and function of proteins. The reversible formation of disulphide bonds between two adjacent cysteine residues (cysteinyl moiety) in the polypeptide chain is of fundamental importance for the tertiary structure and thus the function of enzyme proteins. This bond may form a permanent (covalent) cross-link between polypeptide chains or a reversible dipeptide bridge, comparable with the redox functions of glutathione (Fig. 6.15). During dehydration, the number of disulphide bonds in proteins increases at the expense of the -SH groups, and this shift is associated with protein aggregation and denaturation (Tomati and Galli, 1979). The protection of -SH groups in proteins from the formation of disulphide bridges is important for providing cellular resistance to dehydration (caused by drought and heat) and frost damage (Levitt, 1980).

The most important S-containing compounds of secondary metabolism are *alliins* and *glucosinolates*. They are of particular relevance for horticulture and agriculture (Schnug, 1993; Jones *et al*., 2004c). Alliin is the common name for S-alk(en)ylcysteine sulphoxides which are the characteristic compounds of the genus *Allium*:



More than 80% of the total S in *Allium* species may be bound to such compounds, in onion (*Allium cepa*) for example as S-propylcysteine sulphoxide (R = -CH_2-CH_2-CH_3). Enzymatic cleavage of alliins is mediated by alliinase. Loss in cellular compartmentation by mechanical damage of the tissue greatly enhances enzyme activity through increased availability of the substrate and leads to the formation of allicins as precursor of a large number of volatile substances such as mono- and disulphides with a characteristic odour.

Glucosinolates are characteristic compounds of the secondary metabolism of at least 15 dicotyledonous taxa, including the Brassicaceae (for a recent review see Halkier and Gershenzon, 2006). Glucosinolates contain S both as a sulphhydryl and a sulpho group, the side chain R varies between plant species:



Glucosinolates are stored in vacuoles and their hydrolysis is catalysed by the cytosolic enzyme myrosinase which is present in only a very small number of cells in a given organ such as a leaf or seed (Höglund *et al*., 1991; Wink, 1993; McCully *et al*., 2008). Hydrolysis leads to the liberation of glucose, sulphate and volatile compounds such as isothiocyanates in *Brassica napus*. As for alliinase, myrosinase activity in cells is greatly enhanced by mechanical damage of cells.

The role of many secondary S compounds is not fully understood. They definitely act as defence substances (phytocids, feeding deterrents) although the importance of this defence mechanism may have been overestimated in the past (Ernst, 1993). This is certainly true for glucosinolates which have important functions as S storage in plants. During periods of low S supply to the roots but high plant demand (e.g., rapid vegetative growth, seed formation) glucosinolates are degraded by myrosinase and both S molecules are reutilized through the S assimilation pathway (Schnug, 1993). Roles for S-containing compounds in defence against both abiotic and biotic stresses have been recently revisited, with sufficient or even excess S fertilization, having a positive impact on resistance to stress (Rausch and Wachter, 2005).

Sulphur in its non-reduced form, i.e. as sulphate ester, is a component of sulpholipids and is thus a structural constituent of all biological membranes. In sulpholipids the sulpho group is coupled by an ester bond to a C_6 sugar, for example glucose.

Sulpholipids are particularly abundant in the thylakoid membranes of chloroplasts, about 5% of the chloroplast lipids are sulpholipids (Schmidt, 1986). Sulpholipids may also be involved in the regulation of ion transport across biomembranes. Sulpholipid levels in roots have been shown to be positively correlated with salt tolerance (Erdei *et al*., 1980; Stuiver *et al.*, 1981).

6.2.4 S Supply, Plant Growth and Plant Composition

Sulphur requirement for optimal growth varies between 0.1 and 0.5% of the dry weight of plants. For the families of crop plants, the requirement increases in the order Gramineae < Leguminosae < Cruciferae and this is also reflected in corresponding differences in the S concentration ($g\,kg^{-1}$) of their seeds: 1.8–1.9, 2.5–3.0 and 11–17, respectively (Deloch, 1960). The protein S concentration also varies considerably both between the protein fractions of individual cells (Table 6.3) and among plant species. On average, proteins from legumes contain less S than proteins from cereals, the N/S ratios being 40:1 and 30:1, respectively (Dijkshoorn and van Wijk, 1967).

As with N deficiency, under S deficiency shoot growth is more reduced than root growth, leading, for example in tomato, to a decrease in shoot/root ratio from 4.4 in S-sufficient to 2.0 in S-deficient plants (Edelbauer, 1980). Interruption of S supply decreases root hydraulic conductivity, stomatal aperture and net photosynthesis (Karmoker *et al.*, 1991). The reduced leaf area in S deficient plants is the result of both smaller size and particularly the number of leaf cells (Burke *et al.*, 1986). The number of chloroplasts per mesophyll cell may or may not be affected, for example in wheat (Burke *et al*., 1986), or strongly decreased, for example in spinach (Dietz, 1989).

A drastic decrease in chlorophyll and protein concentration of leaves is a typical feature of sulphur deficiency (Burke *et al.*, 1986; Dietz, 1989; Gilbert *et al.*, 1997). This is to be expected, as in leaves a high proportion of the protein is located in the chloroplasts where the chlorophyll molecules comprise prosthetic groups of the chromoproteid complex. Accordingly, under S deficiency, shortage of the S-containing amino acids cysteine and methionine not only inhibits protein synthesis but also decreases the chlorophyll concentration in leaves (Table 6.3). In contrast, starch may accumulate as a consequence either of impaired carbohydrate metabolism at the sites of production (the source) or of low demand at the sink sites (growth inhibition).

In S-deficient plants, inhibition of protein synthesis is correlated with an accumulation of soluble organic N and nitrate (Table 6.4). Sulphur deficiency increases the concentration of amides as well as their proportions in the soluble N fraction (Freney *et al.*, 1978; Karmoker *et al.*, 1991). The sulphate concentration is extremely low in deficient plants and increases markedly when the sulphate supply is sufficient for optimal growth. The sulphate concentration of plants is therefore a more sensitive indicator of S nutritional status than the total S concentration, the best indicators being the proportion of sulphate-S in the total S (Freney *et al.*, 1978), or the ratio of sulphate to malate (which also accumulates under S deficiency) (Blake-Kalff *et al*., 2000). Sulphur deficiency also leads to accumulation of the sulphate analogues, selenate and molybdenate, in plant tissues due to both decreased competition by sulphate for uptake and enhanced sulphate transporter expression (Shinmachi *et al*., 2010).

Chlorosis is characteristic for S and of N deficiency. Unlike N, however, S is more uniformly distributed between old and new leaves and its concentration is similarly affected in old and young leaves by the level of sulphate supply (Freney *et al*., 1978). Furthermore, the distribution of S in S-deficient plants is also affected by the N supply. Sulphur deficiency symptoms may occur either in young (in combination with sufficient N) or in old (in combination with low N) leaves (Robson and Pitman, 1983), indicating that the extent of remobilization and retranslocation from older leaves depends on the rate of N deficiency-induced leaf senescence, a relationship which is also found for the micronutrients Cu and Zn (see also Chapter 3). In legumes, during the early stages of S deficiency, nitrogenase activity in the root nodules is more strongly reduced than

TABLE 6.3 Leaf composition in tomato with or without S supply

	Concentration in leaves (mg kg^{-1} dw)			Protein S concentration (μg mg^{-1} protein)	
S supply	Chlorophyll	Protein	Starch	Cytoplasm	Chloroplast
+S	58	480	28	14	7
0S	9	35	270	4	5

Based on Willenbrink (1967).

photosynthesis (DeBoer and Duke, 1982). Symptoms of S deficiency in N_2-fixing legumes are therefore indistinguishable from N-deficiency symptoms (Anderson and Spencer, 1950). However, in root nodules of S-deficient legumes, the bacteroids may still be well supplied with S (O'Hara *et al.*, 1987). The high sensitivity of nitrogenase activity to S deficiency therefore reflects either impaired host plant metabolism or a direct effect on nitrogenase activity (see also Chapter 16).

In S-deficient plants, not only the protein concentration decreases but also the S concentration in storage proteins (Table 6.3), indicating that proteins with lower proportion of methionine and cysteine but higher proportions of other amino acids such as arginine and aspartate are synthesized (Table 6.5). The decrease in S-rich proteins under S deficiency has been shown in wheat (Zhao *et al*., 1999a, b; Table 6.12) and also in other cereals and legumes (Randall and Wrigley, 1986). Under S deficiency in wheat, the proportion of a low-molecular-weight S-rich polypeptide decreases (Castle and Randall, 1987), and in maize, the proportion of the major storage protein zein, which has a low S concentration, increases by about 30%, whereas the proportion of the S-rich glutelin decreases by 36 to 71% (Baudet *et al.*, 1986). The lower S concentration of proteins influences the nutritional quality considerably: methionine is an essential amino acid in human nutrition and often a limiting factor in diets in which seeds are a major source of protein (Arora and Luchra, 1970). Furthermore, a decrease in the cysteine concentration of cereal grains reduces the baking quality of flour, since disulphide bridging during dough preparation is responsible for the polymerization of the glutelin fraction (Ewart, 1978). There are prospects to enhance nutritional quality of seeds, for example methionine concentration, by pathway engineering (Tabe and Higgins, 1998).

In Cruciferae, the concentration of glucosinolates and their volatile metabolites is closely related to sulphate supply. Their concentrations in plants can be increased beyond the level at which sulphate supply affects growth (Table 6.6). From the qualitative viewpoint this increase can be favourable (e.g., because it enhances the taste of vegetables, making them spicier) or unfavourable (e.g., because it decreases acceptability as animal feed).

In highly industrialized areas the S requirement of plants is often met fully or to a substantial degree by atmospheric SO_2 pollution. In Northern Europe, however, industrial SO_2 emissions were drastically decreased at the end of the 20th century. Thus, S deficiency is becoming more widespread in Northern Europe in agricultural areas, affecting both yield and quality (Schnug, 1993; Zhao *et al*., 1999a). The application of S fertilizers is effective in remediating this problem. Worldwide, S deficiency in crop production is quite common in rural areas, particularly in

TABLE 6.5 Amino acid composition of endosperm protein from S-sufficient (2.5 g S kg^{-1} dw) and S-deficient (1.0 g S kg^{-1} dw) wheat

	Amino acid concentration (nmol g^{-1} protein N)	
Amino acid	S-sufficient	S-deficient
Methionine	0.9	0.3
Cysteine	1.3	0.4
Arginine	1.7	2.1
Aspartate	2.1	5.8

Based on Wrigley *et al.* (1980).

TABLE 6.4 Fresh weight and S and N concentration of cotton leaves at different S supply in nutrient solution

		Concentration (g kg^{-1} dw)				
		S		N		
S supply (mg SO_4^{2-} L^{-1})	Leaf dw (g dw $plant^{-1}$)	Sulphate	Organic	Nitrate	Soluble organic	Protein
0.1	1.1	0.03	1.1	13.9	22.3	9.6
1.0	2.4	0.03	1.2	13.7	22.1	12.8
10.0	3.4	0.09	1.7	0.6	11.9	25.6
50.0	4.7	1.0	2.6	0.0	5.1	32.5
200.0	4.7	3.6	2.5	01.0	4.5	3.2

Based on Ergle and Eaton (1951).

TABLE 6.6 Yield and mustard oil concentration of the shoots of *Brassica juncea* at different S supply

S supply (mg S pot^{-1})	Shoot fresh weight (g fw pot^{-1})	Mustard oil concentration (mg kg^{-1} fw)
1.5	80	28
15.0	208	81
45.0	285	307
405.0	261	531
1,215.0	275	521

Based on Marquard *et al.* (1968).

high rainfall areas, for example in the humid tropics and temperate climates (Murphy and Boggan, 1990) and in highly leached soils. Under these conditions, the application of N fertilizers is ineffective unless S is applied simultaneously (Wang *et al.*, 1976).

6.3 PHOSPHORUS

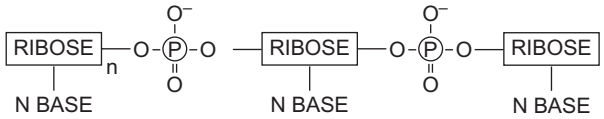
6.3.1 General

Most of the phosphate that is used in fertilizers is derived from rock phosphate, which is a non-renewable resource. Global phosphate resources are predicted to be depleted within the next 50–100 years in an era when more P fertilizers are needed to produce more food and fibre to sustain a growing global population (Cordell *et al*., 2009; Gilbert, 2009).

Unlike nitrate and sulphate, phosphate is not reduced in plants, but remains in its highest oxidized form. Therefore, even though the more reduced oxide of phosphorus (phosphite) is sometimes advertised as a fertilizer, it is harmful when given to plants that are already short of phosphate, because it is an analogue of phosphate and inhibits its uptake (Carswell *et al.*, 1996; Ratjen and Gerendás, 2009). After uptake – at physiological pH mainly as $H_2PO_4^-$ – phosphate either remains as inorganic phosphate (P_i) or it is esterified through a hydroxyl group to a carbon chain (C-O-P) as a simple phosphate ester (e.g., sugar phosphate) or attached to another phosphate by the energy-rich pyrophosphate bond (P)~(P) (e.g., in ATP). The exchange between P_i and the (P) in ester and the pyrophosphate bond is very fast. For example, P_i taken up by roots is incorporated within minutes into organic (P), but released again as P_i into the xylem (see also Chapter 2). Another type of phosphate bond is the relatively stable diester (C-(P)-C). In this association phosphate forms a bridging group connecting units to more complex or macromolecular structures.

6.3.2 P as a Structural Element

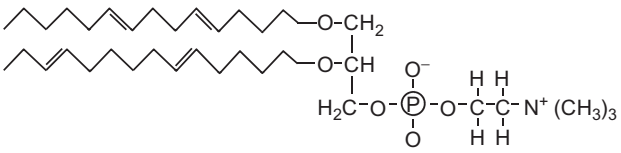
The function of phosphorus as a component of macromolecular structures is most prominent in nucleic acids, which, as components of DNA, are the carriers of genetic information and, as units of RNA, are the structures responsible for the translation of the genetic information. In both DNA and RNA, phosphate forms a bridge between ribonucleoside units to form macromolecules:



(Section of DNA or RNA molecule)

Phosphate is responsible for the strongly acidic nature of nucleic acids and thus for the high cation concentrations in DNA and RNA. The proportion of P in ribonucleic acids to total organically bound P differs among tissues and cells; it is high in expanding leaves, where a large amount of ribosomal RNA is required for rapid protein synthesis, lower in mature leaves, and very low in senescing leaves (Suzuki *et al*., 2001).

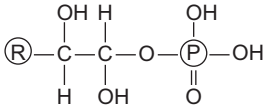
The bridging form of P diester is also abundant in phospholipids of biomembranes. There it forms a bridge between a diglyceride and another molecule (amino acid, amine, or alcohol). In biomembranes, amine choline is often the dominant partner, forming phosphatidylcholine (lecithin):



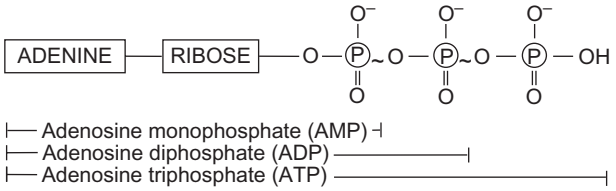
The functions of phospholipids (and also of sulpholipids) are related to their molecular structure. There is a lipophilic region (consisting of two long-chain fatty acid moieties) and a hydrophilic region in one molecule; at a lipid–water interface, the molecules are oriented so that the boundary layer is stabilized. The electrical charge of the hydrophilic region plays an important role in the interactions between biomembrane surfaces and ions in the surrounding medium. Charged ions are either attracted or repelled by the charge of the hydrophilic regions, whereas ions do not interact with the hydrophobic regions. Under P deficiency, plants may replace phospholipids by galactolipids (Andersson *et al*., 2003; Gaude *et al*., 2008) or sulpholipids (Maathuis, 2009; Byrne *et al.*, 2011).

6.3.3 Role in Energy Transfer

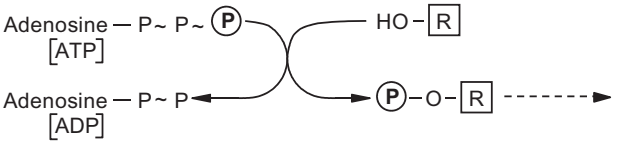
Although present in cells in relatively low concentrations, phosphate esters (C-(P)) and energy-rich phosphates ((P)~(P)) represent the metabolic energy of cells. Up to 50 esters formed from phosphate and sugars and alcohols have been identified, about 10 of which, including glucose 6-phosphate and phosphoglyceraldehyde, are most abundant. The common structure of phosphate esters is:



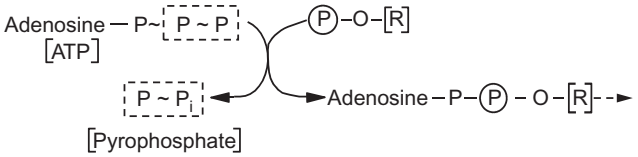
Most phosphate esters are intermediates in metabolic pathways of biosynthesis and degradation. Their function and formation are directly related to the energy metabolism of the cells and to energy-rich phosphates. The energy required, for example, for biosynthesis of starch or ion uptake is supplied by an energy-rich intermediate or coenzyme, predominantly ATP:



Energy liberated during glycolysis, aerobic respiration, or photosynthesis (see also Chapter 5) is utilized for the synthesis of the energy-rich pyrophosphate bond, and upon hydrolysis of this bond ~30 kJ per mole ATP are released. This energy can be transferred with the phosphoryl group in a phosphorylation reaction to another compound which results in the activation (priming reaction) of this compound:



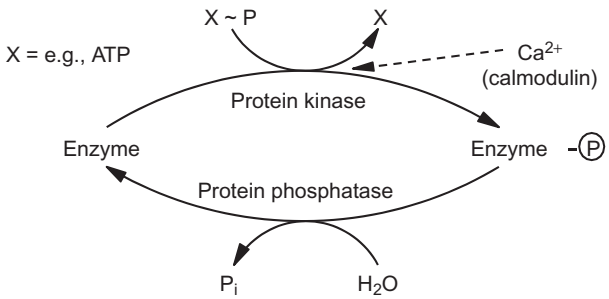
ATP is the principal energy-rich phosphate required for starch synthesis. The energy-rich pyrophosphate bonds of ATP can also be transmitted to other coenzymes, which differ from ATP only in the nitrogen base, for example uridine triphosphate (UTP) and guanosine triphosphate (GTP), which are required for the synthesis of sucrose and cellulose, respectively. The activity of ATPases, mediating the hydrolysis and, thus, energy transfer, is affected by many factors, including nutrients such as Mg (Section 6.5), Ca (Section 6.6) and K (Section 6.7; Chapter 2). In some phosphorylation reactions the energy-rich inorganic pyrophosphate (PP_i) is liberated and the adenosine (or uridine) moiety remains attached to the substrate:



Liberation of PP_i takes place in all of the major biosynthetic pathways, for example acylation of CoA in fatty acid synthesis, formation of APS in sulphate activation (Fig. 6.15), of starch in chloroplasts, and of sucrose in the cytosol (Fig. 6.17). Various enzymes can make use of PP_i, for example the UDP-glucosephosphorylase (Fig. 6.20) and the proton-pumping inorganic pyrophosphatase at the tonoplast (see also Chapter 2). The cellular concentrations of PP_i are in the range of 100–200 nmol per gram fresh weight which similar to the range of ATP (Duff *et al.*, 1989). In leaves, PP_i concentrations are similar in the cytosol and stroma of chloroplasts and kept stable during the light–dark cycle (Eberl *et al.*, 1992).

In rapidly metabolizing cells, energy-rich phosphates are characterized by very high rates of turnover. From pulse-labelling experiments with ^{32}P, the turnover rates of various P compounds can be calculated, as shown in Table 6.7. Obviously, a very small amount of ATP satisfies the energy requirement of plant cells. For example, 1 g of rapidly metabolizing maize root tips synthesizes about 5 g ATP per day (Pradet and Raymond, 1983). The amounts of phospholipids and RNA are considerably higher, but these are also more stable, with a relatively low rate of synthesis (Table 6.7).

Phosphorylation of enzyme proteins by ATP, GTP, or ADP is another mechanism by which energy-rich phosphates can modulate enzyme activities:



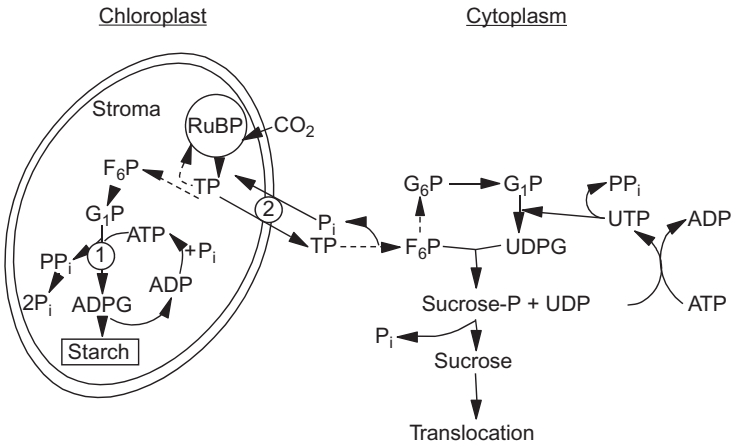


FIGURE 6.17 Involvement and regulatory role of P in starch synthesis and carbohydrate transport in a leaf cell. (1) ADP-glucose pyrophosphatase: regulates the rate of starch synthesis, inhibited by P_i and stimulated by PGA. (2) Phosphate translocator: regulates the release of photosynthates from chloroplasts, enhanced by P_i. TP: triosephosphate; GAP: glyceraldehydes-3-P; DHAP: dihydroxyacetone P; F_6P: fructose-6-P; G_6P: glucose-6-P. *Based on Walker (1980).*

TABLE 6.7 Turnover times and rates of synthesis of organic P fractions in *Spirodela*

P Fraction	Concentration (nmol g^{-1} fw)	Turnover (min)	Synthesis rate (nmol P g^{-1} fw min^{-1})
ATP	170	0.5	340
Glucose-6-P	670	7	95
Phospholipids	2,700	130	20
RNA	4,900	2,800	2
DNA	560	2,800	0.2

Based on Bieleski and Ferguson (1983).

This regulatory phosphorylation is mediated by protein kinases and can result in activation, inactivation and/or changes in the allosteric properties of the target protein (Budde and Chollet, 1988). Dephosphorylation is generally a hydrolytic reaction catalysed by phosphatases. Protein phosphorylation is considered a key factor in signal transduction, for example in phytochrome-mediated responses of plants (Shen *et al*., 2009). An example of this is the light-stimulated enhancement of nitrate assimilation in leaves (Fig. 6.8). PEP carboxylase is one of the key enzymes regulated by phosphorylation, in both C3 and C4 plants. In C4 plants and in CAM plants (see also Chapter 5) phosphorylation increases the activity of PEP carboxylase and simultaneously the enzyme becomes less sensitive to negative feedback control by high malate concentrations (Budde and Chollet, 1988).

6.3.4 Compartmentation and Regulatory Role of Inorganic P

In many enzyme reactions, P_i is either a substrate or an end-product (e.g., ATP→ADP + P_i). Furthermore, P_i controls some key enzyme reactions. Compartmentation of P_i is therefore essential for the regulation of metabolic pathways in the cytosol and chloroplasts. In fruit tissue of tomato, for example, P_i released from the vacuoles into the cytosol can stimulate phosphofructokinase activity (Woodrow and Rowan, 1979) which is a key enzyme in the regulation of substrate flux in glycolysis. Thus the release of P_i from vacuoles can initiate the respiratory burst during fruit ripening.

In vacuolated cells of higher plants the vacuole acts as storage pool, or 'non-metabolic pool', of P, and at adequate P supply ~85–95% of the total P of the cell is located in the vacuoles as P_i (Lauer *et al*., 1989b). In contrast, in leaves of P-deficient plants most P_i is found in the cytosol and chloroplasts, i.e. in the 'metabolic pool' (Lauer *et al.*, 1989a). In leaves, the total P concentration may vary by a factor of 20 without strongly affecting photosynthesis, as the P_i concentration in the cytosol is regulated in a narrow range by an effective phosphate homeostasis in which the P_i in the vacuole acts as buffer (Mimura *et al.*, 1990). The same is true for roots where the cytosolic P_i concentration is maintained at 6.0 mM (maize) and 4.2 mM (pea), also under P deficiency, unless the vacuolar pool is depleted (Lee *et al.*, 1990). Under severe P deficiency, cytosolic P_i concentrations in leaves may decrease from about 5 mM to less than 0.2 mM, and the concentrations of energy-rich phosphates drop to 20–30% of the original level.

In leaves, photosynthesis and carbon partitioning in the light–dark cycle are strongly affected by the P_i concentrations in the stroma of chloroplasts and the compartmentation between chloroplasts and cytosol (Fig. 6.20). In the light, for maximum photosynthesis, a P_i concentration in chloroplasts of 2.0–2.5 mM is required, and photosynthesis is almost completely inhibited when the P_i concentration falls below 1.4–1.0 mM (Robinson and Giersch, 1987; Heber *et al.*, 1989). Due to the high demand of P_i for phosphorylated intermediates of photosynthesis (Fig. 6.17), the P_i concentrations in leaves of P-deficient plants (i.e., without vacuolar buffer) may drop to 50% after onset of light (Sicher and Kremer, 1988).

The role of P_i in carbon partitioning between chloroplasts and cytosol has been demonstrated with isolated chloroplasts (Heldt *et al.*, 1977). An increase in external P_i concentration up to about 1 mM stimulates net photosynthesis, but decreases incorporation of the fixed carbon into starch. At a P_i concentration of 5 mM in the stroma, starch synthesis is severely inhibited. The inhibition of starch synthesis by high concentrations of P_i is caused by two separate mechanisms in the chloroplasts. The key enzyme of starch synthesis in chloroplasts, ADP-glucose pyrophosphorylase (pathway (1), Fig. 6.17), is allosterically inhibited by P_i and stimulated by triosephosphates. The ratio of P_i to triosephosphates therefore strongly influences the rate of starch synthesis in chloroplasts (Portis, 1982); at high ratios the enzyme is inactive. The other mechanism regulated by P_i is the release from the chloroplasts of triosephosphates (glyceraldehyde-4-phosphate and dihydroxyacetone phosphate), the main products of CO_2 fixation. This release is mediated by a phosphate transporter, located in the inner membrane of the chloroplast envelope (pathway (2), Fig. 6.17) and facilitating the exchange $P_i \leftrightarrow$ triosephosphate (Heldt *et al.*, 1991). In C4 plants and CAM plants this translocator also transports phosphoenolpyruvate (PEP). Via the phosphate translocator, the net uptake of P_i into the chloroplasts regulates the release of photosynthates from the chloroplast. High P_i concentrations in the cytosol, therefore, deplete the stroma of triosephosphates, which serve both as substrates for and activators of starch synthesis. Thus, inhibition of starch synthesis by high P_i concentrations is also the result of substrate depletion.

In guard cells of pea, the phosphate transporter in the chloroplast envelope (pathway (2), Fig. 6.17) enables uptake of glucose-6-phosphate, similarly as in amyloplasts in storage cells. This mechanism enables guard cells to synthesize starch although they lack fructose-1,6-bisphosphate synthase, the enzyme required for C3→C6 biosynthesis (Overlach *et al.*, 1993).

CO_2 fixation in the Calvin cycle is a process in which five-sixths of the carboxylation products are required in the chloroplast stroma to regenerate the CO_2 acceptor ribulose bisphosphate (RuBP). Excessive export of triosephosphates induced by high P_i concentrations in the cytosol leads to the depletion of these metabolites, which are required for the regeneration of RuBP (Fig. 6.20). In isolated chloroplasts, high external P_i concentrations, therefore, inhibit CO_2 fixation (Flügge *et al.*, 1980). However, in intact plants low P_i concentrations in the cytosol and chloroplasts are more common, for example, under severe P deficiency (Lauer *et al.*, 1989a). Due to the inhibition of triose export from the chloroplast, accumulation of large amounts of starch in the chloroplasts is a typical feature of P deficiency (Table 6.10, Fig. 6.17). The shift towards utilizing triosephosphates for starch synthesis may even reduce Calvin cycle activity and CO_2 fixation by limiting regeneration of RuBP (Fredeen *et al.*, 1990). This starch is not completely mobilized at night (Qiu and Israel, 1992) or during reproductive growth (Giaquinta and Quebedeaux, 1980).

Accumulation of starch and sugars in leaves of P-deficient plants can also result from lower export due to lack of ATP for sucrose-proton cotransport in phloem loading (see also Chapter 5), and lower demand at the sink sites (Rao *et al.*, 1990). However, under P deficiency shoot growth is more suppressed than photosynthesis (Plénet *et al.*, 2000b), especially when plants are grown under low-light conditions (De Groot *et al.*, 2003). The finely tuned homeostasis of P_i in the cytosol and chloroplasts is one reason for this and a higher activity of various enzymes of carbohydrate metabolism and, thus, turnover of P_i may be another (Rao *et al.*, 1990).

In principle, similar regulation of starch synthesis takes place in amyloplasts of storage cells. ADP-glucose pyrophosphorylase is also the key enzyme in the regulation of starch synthesis in potato tubers (Mohabir and John, 1988) and in grains, for example maize (Plaxton and Preiss, 1987). When isolated from these storage tissues, the enzyme is severely inhibited by P_i. In contrast, starch accumulation in the endosperm of wheat grains is not affected by high P_i concentrations (Rijven and Gifford, 1983) which suggests that these cells have a particularly large capacity for effective P_i sequestration.

In storage cells, the transport of phosphorylated trioses from the cytosol into the amyloplasts also proceeds by strict countertransport with P_i; however, the P transporter also accepts glucose-6-phosphate and releases P_i in a C6-P_i shuttle (Heldt *et al.*, 1991).

6.3.5 P Fractions and the Role of Phytate

When the P supply is increased from deficiency to the sufficiency range, the concentrations of major P fractions in vegetative plant organs also increase (Chapin and Bieleski, 1982), as shown in a typical example for leaves in Table 6.8.

With further increase in supply, only P_i as the major storage form of P in highly vacuolated tissue increases (Shane *et al.*, 2004c). However, plants may also store P in two other major forms, namely phytate (Lott *et al.*, 2000) and inorganic polyphosphates (Seufferheld and Curzi, 2010).

The storage of phosphate in cells as inorganic polyphosphates is widespread among bacteria, fungi and green algae (Kornberg, 1995). It has also been found in higher plants. Polyphosphates synthesized by plants are linear polymers of P_i ($>$ 500 molecules) with pyrophosphate linkages energetically equivalent to ATP. Polyphosphates may therefore function as energy storage compounds and as compounds controlling the P_i concentration in the metabolic pool of the cells. They also function as cation exchangers, for example for K in *Chlorella* (Peverly *et al.*, 1978) and for Ca in mycorrhizal fungi (Strullu *et al.*, 1982). Polyphosphate formation in the hyphae of mycorrhizal fungi plays a key role in P nutrition of mycorrhizal plants (Kuga *et al.*, 2008). Hyphae take up P_i from the soil solution and synthesize polyphosphates; these act as a transient storage pool of P in the hyphae, and are subsequently transported as polyphosphates toward the host roots (Ezawa *et al.*, 2004) (see also Chapter 15).

Phytate is the typical storage form of P in grains and other seeds (Lott *et al.*, 2009). Phytates are the salts of phytic acid, *myo*-inositol, hexa*kis*phosphate. Phytic acid is synthesized from the cyclic alcohol *myo*inositol by esterification of the hydroxyl groups with phosphoryl groups (Josefsen *et al.*, 2007):

The sparingly soluble Ca-Mg salt of phytic acid is termed phytate. Phytic acid also has a high affinity for Zn and Fe (Wang *et al.*, 2008a). In legume seeds and cereal grains the main phytates are the K-Mg salts (Ockenden *et al.*, 2004). The proportions of K, Mg and also of Ca associated with phytic acid vary considerably among plant species and even between different tissues of a seed (Lott *et al.*, 2009). Phytate P makes up ~50% of the total P in legume seeds, 60–70% in cereal grains, and about 86% in wheat bran. In cereals and legumes, phytates are deposited in electron-dense globoid crystals inside membrane-bound intracellular protein bodies, in cereal grains mainly in the aleuron layer, and in legumes in cotyledons and embryo axes. In grains and other seeds, phytates are the main storage sites of K and Mg, in some instances also of Ca and Zn (Lott *et al.*, 1985).

Phytate in the form of the K-Mg-Ca salt is also the major form of P in pollen grains (Scott and Loewus, 1986), where it is deposited in the form of discrete particles and degraded by phytase during pollen germination (Baldi *et al.*, 1987). Phytates are also found in roots and tubers in crops such as carrot, artichoke and potato, representing 15–23% of total P (Campbell *et al.*, 1991). The high affinity of phytic acid for Zn, Fe and other heavy metals may be important for heavy-metal binding and, thereby, detoxification in roots. In cortical root cells of zinc-tolerant ecotypes of *Deschampsia caespitosa* (Van Steveninck *et al.*, 1987a) and a range of crop species (Van Steveninck *et al.*, 1994), up to 60% of the charges of phytic acid are

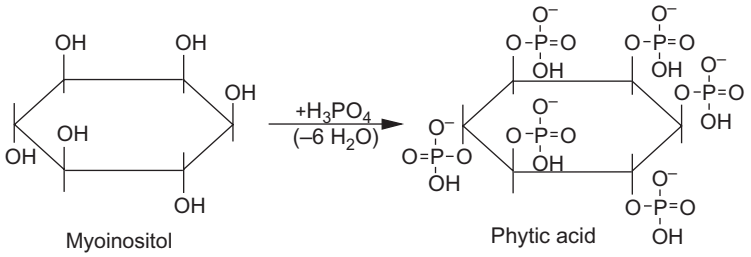


TABLE 6.8 Phosphorus fractions in tobacco leaves at different P supply

P supply (mg L^{-1})	Leaf dry weight (g $plant^{-1}$)	P fraction (g P kg^{-1} leaf dw)			
		Lipid	Nucleic acid	Ester	Inorganic
2	0.82	0.32	0.74	0.36	0.33
6	1.08	0.83	1.34	0.91	0.83
8	1.10	0.89	1.33	1.04	1.23
20	1.06	0.91	1.42	1.09	3.38

Based on Kakie (1969).

occupied by Zn. Cadmium is not bound by the phytate, even when this is added together with Zn (Van Steveninck *et al*., 1994). Phytic acid can also be a major component of soil organic P, but this is a different isomer from that stored in roots, tubers, grains, seeds and pollen; the origin of phytate in soil is unclear (Richardson *et al.*, 2007).

During the early stages of seed and grain development in legumes and cereals, the concentration of phytate is low (Fig. 6.18), but increases sharply during the period of rapid starch synthesis (Raboy and Dickinson, 1987). In contrast, the concentration of P_i during the early stages of seed and grain development is generally low and further declines during rapid phytate formation. When the P supply to the roots is increased after anthesis, phytate is the only P fraction that increases in grains (Michael *et al.*, 1980).

Phytates are presumably involved in the regulation of starch synthesis during grain filling or tuber growth as the synthesis of phytate results in a decrease in P_i concentration in the grains (Fig. 6.18; Michael *et al.*, 1980). In addition, with the onset of desiccation in grains and seeds in the final stage of the filling period, phytic acid acts as a major cation trap that eliminates excessive cellular concentrations of K and Mg.

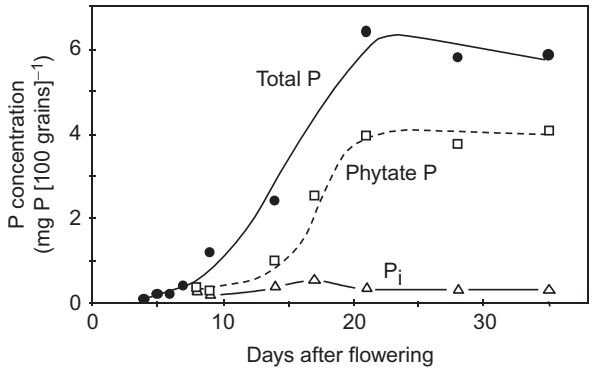


FIGURE 6.18 Total P, phytate P and P_i concentration in rice grains during grain development. *Based on Ogawa* et al.*, 1979.*

Some P is associated with the starch fraction and is incorporated into the starch grains. In cereals this is only a small proportion, but in potato tubers up to 40% of the total P may be incorporated in starch. Starch-bound P may reflect another type of compartmentation of P_i allowing control of its concentration at the sites of starch synthesis. It could also act as a source of P for sugar export from the amyloplasts during sprouting of tubers.

The function of phytate is to provide the germinating seedling with a source of P for synthesis of membrane lipids and nucleic acids. In agreement with this, digestion of the globoid crystals containing phytate is one of the earliest changes in cotyledons during germination (Lott and Vollmer, 1973). Degradation of phytate, catalysed by phytases, leads to a rapid decline in phytate-bound P (Table 6.9). In germinating rice seeds (Table 6.9), most of the P released from phytate within the first 24 h is incorporated into phospholipids, indicating membrane synthesis, which is essential for compartmentation and thus for the regulation of metabolic processes within cells. An increase in P_i and phosphate-ester concentrations reflects the onset of enhanced respiration, phosphorylation and related processes. The degradation of phytate continues with time, and finally the concentrations of DNA and RNA P increase, indicating enhanced cell division and net protein synthesis. The rate of phytate degradation is also controlled by P_i; high concentrations of P_i suppress the synthesis of phytase (Sartirana and Bianchetti, 1967). During degradation of phytate, various inositol phosphates with a lower P concentration occur as intermediates, and some of them constitute a significant proportion of the phospholipid fraction of membranes. In addition, inositol-1,4,5(tri)phosphate serves as a secondary messenger regulating Ca channels in membranes of plant cells (Isayenkov *et al*., 2010).

In animals, phytates interfere with intestinal absorption of mineral elements, especially Zn, Fe and Ca, thereby causing nutritional deficiencies in both monogastric animals (Welch *et al.*, 1974) and humans (Kumar *et al.*, 2010), especially children (Hambidge and

TABLE 6.9 Phosphorus fractions in rice seeds during germination

	P fraction (mg P g^{-1} dw)				
Duration of germination (h)	Phytate	Lipid	Inorganic	Ester	RNA + DNA
0	2.67	0.43	0.24	0.08	0.06
24	1.48	1.19	0.64	0.10	0.05
48	10.6	1.54	0.89	0.11	0.08
72	0.80	1.71	0.86	0.12	0.12

From Mukherji *et al.* (1971).

Walravens, 1976). For a given supply, the amount of Zn absorbed by the intestine is determined by the Zn/phytate ratio in the diet (Lantzsch *et al.*, 1980). In humans on cereal diets, Zn deficiency results from both the low Zn concentration of the grains and the consumption of phytate-rich unleavened wholemeal bread (Reinhold *et al.*, 1973). This problem can be alleviated by Zn supplementation in the diet or by an increase in the zinc/phytate ratio in seeds and grains through the application of zinc fertilizers (Peck *et al.*, 1980). Breeding for low-phytic acid content is another alternative (Raboy, 2001; Shi *et al.*, 2007). In rice, low-phytic acid concentration is associated with reduced grain yield and seed viability (Zhao *et al.*, 2008). There is obviously a tradeoff between human health benefits and crop performance which must be taken into account when using the breeding strategy to reduce phytic acid in the grain (Lopez *et al.*, 2002).

6.3.6 P supply, Plant Growth and Plant Composition

The P requirement for optimal growth is in the range of 3 to 5 mg g^{-1} dw during the vegetative stage of growth, but some plants that have evolved on severely P-impoverished soils contain an order of magnitude less P in their leaves (Lambers *et al.*, 2010). The probability of P toxicity increases at concentrations higher than 10 mg g^{-1} dw. P toxicity in plants is rare, because plants down-regulate their Pi transporters involved in net P uptake from the root environment when supplied with more P than required for optimum growth (Dong *et al.*, 1999). However, many species from severely nutrient-impoverished soils in Australia and South Africa cannot down-regulate their net P uptake and show P toxicity symptoms when fertilized with P (Shane *et al.*, 2004a). Some tropical food legumes are rather sensitive to P; toxicity may occur already at P concentrations in the shoot dry matter of 3–4 mg g^{-1} in pigeon pea and 6–7 mg g^{-1} in black gram (Bell *et al.*, 1990). At the other end of the spectrum, *Ptilotus polystachyus*, a fast-growing non-mycorrhizal Australian native herb, accumulates P to approximately 40 mg g^{-1} shoot dw, without signs of P toxicity (Ryan *et al.*, 2009a).

P-starvation responses in plants are mediated via sugar signalling (Karthikeyan *et al.*, 2007). Signalling of the shoot P status also involves specific microRNA molecules (Doerner, 2008). In P-deficient plants, reduction in leaf expansion (Fredeen *et al.*, 1989) and also number of leaves (Lynch *et al.*, 1991) are the most obvious effects (Table 6.10). The average length of the cell division zone is decreased in P-deficient maize leaves, and both cell production and cell division rates are reduced (Assuero *et al.*, 2004). Leaf expansion is strongly related to the expansion of epidermal cells, and this process may be impaired in P-deficient plants because of a decrease in root hydraulic conductivity, due to a decreased expression of genes encoding aquaporins (Clarkson *et al.*, 2000). In contrast to the severe inhibition of leaf expansion under P deficiency, the concentrations of protein (Rao and Terry, 1989) and chlorophyll per unit leaf area are less affected (Table 6.10). The chlorophyll concentration tends to increase even under P deficiency (Rao and Terry, 1989), and P-deficient leaves have a darker green colour, because leaf expansion is more strongly inhibited than chlorophyll formation (Hecht-Buchholz, 1967).

TABLE 6.10 Growth parameters and concentrations of P and carbohydrates in soybean at high or low P supply

Parameter		High P	Low P
Leaf area (dm^2)		12.1	1.8
No. primary trifoliates		7	4
Shoot/root ratio		4.2	1.0
Chlorophyll (mg dm^{-1})		3.0	2.8
P concentration (mg g^{-1} dw)			
Leaf	Inorganic P	4.4	0.3
	Organic P	2.4	0.6
Total P	Stems and petioles	5.8	1.1
	Roots	10.5	1.3
Total root P/total shoot P		0.5	1.6
Carbohydrates in leaves (g m^{-2} leaf)	Starch	0.4	12.8
	Sucrose	0.7	0.2
Carbohydrates in roots (mg g^{-1} fw)	Starch	23	160
	Sucrose	16	177

Based on Fredeen *et al.* (1989).

Compared with shoot growth, root growth is less inhibited under P deficiency, leading to a typical decrease in shoot/root ratio (Table 6.10). This decrease in shoot/root ratio is due to the increase in partitioning of carbohydrates towards the roots, indicated by a strong increase particularly in sucrose concentration of the roots of P-deficient plants (Table 6.10). Under P starvation, the elongation rate of individual root cells and of the roots may be enhanced (Anuradha and Narayana, 1991). In *Stylosanthes hamata*, under P deficiency shoot growth declines rapidly, but roots continue to grow, not only because of reduced transport of P to the shoot, but also due to additional net translocation of P from the shoot to the roots (Smith *et al.*, 1990a). In certain plant species, P-deficiency-induced formation of 'cluster' or 'dauciform' root clusters is another P-starvation response (Lambers *et al.*, 2006) (see also Chapter 13). Root clusters are common on the world's most

P-impoverished soils (Lambers *et al*., 2010); they may also play an important role when a large fraction of the soil P is poorly available, because of a very high or very low pH and/or high concentrations of Fe and Al (Lambers *et al*., 2011). Due to the release of carboxylates in an 'exudative burst' (Watt and Evans, 1999; Shane *et al*., 2004b), root clusters efficiently 'mine' P (Lambers *et al.*, 2008) (see also Chapter 14).

Despite a wide range of adaptive responses in plants to P deficiency (Lambers *et al.*, 2006), triggered by intricate P-starvation signalling pathways (Rolland *et al*., 2006), shoot growth rate is inhibited under P limitation as is the formation of reproductive organs. Flower initiation is delayed (Rossiter, 1978), the number of flowers is decreased (Bould and Parfitt, 1973) and seed formation is restricted (Barry and Miller, 1989). Premature senescence of leaves is another factor limiting seed yield in P-deficient plants.

Challenges for the future, when P reserves are being depleted (Gilbert, 2009) include the development of crops and pastures and agriculture management systems that require less P while maintaining productivity. There may well be lessons to be learned from native species that evolved in severely P-impoverished landscapes (Lambers *et al.*, 2011), but this remains to be explored.

6.4 MAGNESIUM

6.4.1 General

The ionic radius of Mg^{2+} is substantially smaller (0.065 nm) and its hydrated radius substantially larger (0.476 nm) than that of K^+ and Ca^{2+}. Thus, the volume of the hydrated Mg^{2+} ion is about 400 times larger than the dehydrated ion. Since ions are transported through biological membranes as dehydrated cations, Mg^{2+} transport proteins must possess specific features (Maguire and Cowan, 2002). Only recently Mg^{2+} transporters in higher plants have been identified. In *Arabidopsis* an *AtMRS2*/*AtMGT* gene family encoding Mg^{2+} transport proteins that are homologous to the bacterial CorA Mg^{2+} transporter has been described (Schock *et al*., 2000; Li *et al*., 2001). Complementing in knockout mutants and over-expressing *AtMRS2-7* in *Arabidopsis* enhanced growth at limiting Mg supply (Gebert *et al*., 2009). These proteins are channels facilitating the transport of Mg^{2+} through membranes along the gradient in electro-chemical potential. The uptake of Mg^{2+} can be strongly depressed by other cations, such as K^+, NH_4^+ (Kurvits and Kirkby, 1980), Ca^{2+} and Mn^{2+} (Heenan and Campbell, 1981), as well as by H^+, that is, by low pH. Magnesium deficiency induced by competing cations is thus a fairly widespread phenomenon.

The functions of Mg in plants are mainly related to its capacity to interact with strongly nucleophilic ligands. The Mg ion tends to adopt octrahedral coordination with a marked preference for oxygen-donor ligands or water and binds electrostatically particularly to negatively charged P groups (Sreedhara and Cowan, 2002). The interactions of Mg^{2+} with proteins can be grouped into two general reaction classes: (i) Mg^{2+} may bind directly to a protein/enzyme and determines its structure and/or serves a catalytic role, such as central atom of the chlorophyll molecule or as bridging element for the aggregation of ribosomes; (ii) Mg^{2+} may bind the substrate of an enzyme thus increasing the efficiency of the catalytic reaction, such as in Mg–ATP phosphorylation and the Mg-isocitrate isocytrate-lyase reaction (Cowan, 2002). The specific role of Mg^{2+} in enzyme catalysis mainly depends on its ability to position a water molecule for participation in the catalytic reaction (outer sphere complexation; Maguire and Cowan, 2002). Magnesium forms ternary complexes with enzymes in which bridging cations are required for establishing a precise geometry between enzyme and substrate (Clarkson and Hanson, 1980), for example in RuBP carboxylase (Pierce, 1986). Magnesium generally binds weakly to proteins and enzymes in the cytosol, thus their activity depends on the strict control of the cytosolic free Mg^{2+} concentration in the range of 0.5 mM. Beyond its role in enzyme regulation, a substantial proportion of the total Mg^{2+} in the cell is involved in the regulation of cellular pH and the cation–anion balance.

6.4.2 Binding Form, Compartmentation and Homeostasis

A major function of Mg in green leaves is as the central atom of the chlorophyll molecule (see also Chapter 5). The proportion of total Mg bound to chlorophyll depends on Mg supply (Michael, 1941). In leaves of subterranean clover, this proportion ranges from 6% in plants with high Mg supply to 35% in Mg-deficient plants (Scott and Robson, 1990a). Under low-light conditions, the proportion of total Mg bound in chlorophyll may even be $> 50\%$, for example in Mg-deficient poplar (Dorenstouter *et al*., 1985). Depending on the Mg nutritional status, between 6 and 25% of total Mg is bound to chlorophyll. As a rule, another 5–10% of total Mg in leaves and needles is firmly bound to pectin in cell walls or precipitated as sparingly soluble salts in the vacuole (e.g., as Mg-phosphate), and the remaining 60–90% are extractable with water (Table 6.11).

In cells of mature leaf tissue, ~15% of the whole cell volume is occupied by the chloroplast, the cytoplasm and the cell wall (~5% each), the remaining 85% by the vacuole (Cowan *et al.*, 1982; Leigh and Wyn Jones, 1986). Similarly to inorganic P (P_i), the concentration of Mg^{2+} in the 'metabolic pool' (i.e., in the cytoplasm and chloroplasts) has also to be strictly regulated. The concentration of Mg^{2+} in the metabolic pool of leaf cells is assumed to

TABLE 6.11 Concentration and binding form of Mg in one-year-old needles of Norway spruce growing on two soil types

Soil type	Total Mg concentration (mg g^{-1} dw)	Proportion of total Mg		
		Water-soluble	Pectate, phosphate	Chlorophyll
Rendzina	1.47	91.2	2.6	6.2
Podsol	0.31	64.8	10.0	25.2

Based on Fink (1992a).

be in the range of 2–10 mM (Leigh and Wyn Jones, 1986). However, the free Mg^{2+} (non-complexed) is expected to be lower (about 0.4 mM) (Yazaki *et al.*, 1988). As for P_i, the vacuole is also the main storage pool required for maintenance of Mg^{2+} homeostasis in the 'metabolic pool'. Physiological and molecular evidence indicate that Mg^{2+} influx into the vacuole is mediated by an Mg^{2+}/H^+ exchanger such as *AtMHX* (Shaul, 2002). In needles of Mg-sufficient Norway spruce, Mg^{2+} concentrations in the vacuole were 13–17 mM in mesophyll cells and 16–120 mM in endodermis cells. These high concentrations function as a buffer in maintaining Mg^{2+} homeostasis in other cells throughout the season (Stelzer *et al.*, 1990). In addition, vacuolar Mg^{2+} is also important for cation–anion balance and turgor regulation of cells.

Within the 'metabolic pool', the Mg^{2+} distribution between the cytosol and the chloroplast has to be well regulated. In isolated chloroplasts, photosynthesis is strongly inhibited even by 5 mM Mg^{2+} in the external solution (i.e., cytosol). This inhibition is caused by decrease in K^+ influx and corresponding acidification of the stroma upon illumination (Wu *et al.*, 1991; Section 6.6). Inhibition of photosynthesis by high Mg^{2+} concentrations in the 'metabolic pool' may occur in intact plants under drought stress.

6.4.3 Chlorophyll and Protein Synthesis

Chlorophyll and heme synthesis share a common pathway up to the level of protoporphyrin IX. The first step of chlorophyll biosynthesis, insertion of Mg^{2+} into the porphyrin structure is catalysed by Mg chelatase (Walker and Weinstein, 1991). Activation of this enzyme also requires ATP and, thus, additional Mg (Kobayashi, *et al*., 2008). Release of Mg during chlorophyll breakdown requires two steps, a chlorophyllase hydrolysing chlorophyll to chlorophyllide and phytol (Tsuchiya *et al.*, 1999) and Mg-dechelatase yielding Mg^{2+} and pheophytin (Ougham *et al*., 2008; Schelbert *et al*., 2009).

Magnesium also has an essential function as a bridging element for the aggregation of ribosome subunits (Cammarano *et al.*, 1972), a process that is necessary for protein synthesis. Under Mg deficiency, or in the presence of high concentrations of K^+ (Sperrazza and Spremulli, 1983), the subunits dissociate and protein synthesis ceases. Magnesium plays a critical role in stabilizing specific confirmations of nucleic acids required for their synthesis and functions, and for the activities of nucleic acid polymerases and nucleases (Sreedhara and Cowan, 2002).

Net synthesis of RNA ceased immediately in response to Mg deficiency, and synthesis resumes rapidly after the addition of Mg (Galling, 1963; Fig. 6.19). In contrast, protein synthesis remained unaffected for more than 5 h, but it rapidly declined thereafter. The requirement for Mg in protein synthesis was also directly demonstrated in chloroplasts (Bamji and Jagendorf, 1966; Table 6.12). As Mg^{2+} readily permeates the chloroplast envelope (possibly via Mg^{2+} channels such as MRS2-11; Drummond *et al*., 2006), a concentration of at least 0.25 to 0.40 mM Mg^{2+} is required in the cytosol to prevent net efflux of Mg^{2+} from the chloroplast and, thus, to maintain protein synthesis (Deshaies *et al.*, 1984).

In leaf cells at least 25% of the total protein is localized in chloroplasts. This explains why a deficiency of Mg particularly affects the size, structure and function of chloroplasts, including electron transfer in photosystem II (McSwaine *et al.*, 1976). In Mg-deficient plants, Mg transport from mature to young leaves is enhanced and, thus, visual deficiency symptoms typically appear on mature leaves, indicated by enhanced rates of protein degradation, including structural proteins of the thylakoids. The breakdown of the thylakoids also explains why in Mg-deficient plants, the other plastid pigments are often similarly affected as chlorophyll (Baszynski *et al*., 1980; Table 6.13). Regardless of this decline in chloroplast pigments, starch accumulates in Mg-deficient chloroplasts which may explain the increase in dry matter of Mg-deficient leaves (Scott and Robson, 1990a and Table 6.13). Impaired export of photosynthates is another factor leading to enhanced degradation of chlorophyll in Mg-deficient source leaves.

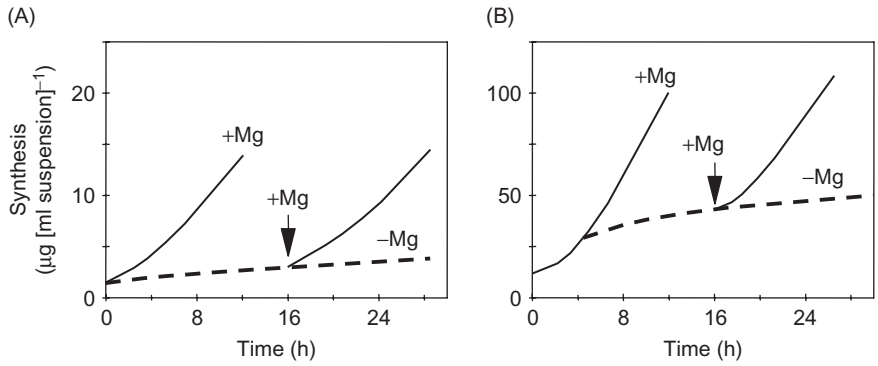


FIGURE 6.19 (A) RNA and (B) protein synthesis in *Chlorella pyrenoidosa* suspension culture at Mg deficiency and Mg resupply. *Based on Galling (1963).*

TABLE 6.12 Incorporation of ^{14}C (leucine) into the protein fraction of isolated wheat chloroplasts at different Mg supply

Mg concentration (mM)	^{14}C incorporation (cpm mg^{-1} chlorophyll)	Relative value
0	412	11.5
0.5	688	19.5
5.0	3,550	100.0

Based on Bamji and Jagendorf (1966).

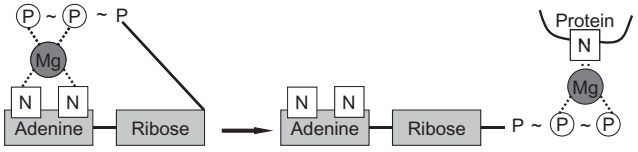
TABLE 6.13 Magnesium deficiency-induced changes in plastid pigments and leaf dry matter in oilseed rape

Treatment	Chlorophyll (a and b) concentration	Carotenoid concentration	Leaf dry matter
	(mg g^{-1} fw)		(%)
Control	2.33	0.21	13.6
Mg-deficient	1.33	0.11	17.7

Based on Baszynski *et al.* (1980).

6.4.4 Enzyme Activation, Phosphorylation and Photosynthesis

There is a long list of enzymes and enzyme reactions which require or are strongly promoted by Mg, for example glutathione synthase (Section 6.2) or PEP carboxylase. For this latter enzyme in the presence of Mg, the substrate phosphoenolpyruvate (PEP) is bound in greater quantities and more tightly (Wedding and Black, 1988). Most of the Mg-dependent reactions can be grouped into general types such as the transfer of phosphate (e.g., phosphatases and ATPases) or of carboxyl groups (e.g., carboxylase). In these reactions, Mg^{2+} is preferentially bound to N bases and phosphoryl groups and this is also, for example, the case in ATP:



The substrate for ATPases, as well as inorganic PP_iases (Rea and Sanders, 1987), is Mg–ATP rather than free ATP. The Mg–ATP complex is stable above pH 6, and this complex can be utilized by the active sites of ATPases for the transfer of the energy-rich phosphoryl group (Balke and Hodges, 1975). An example of the Mg^{2+} requirement of membrane-bound ATPases is shown by Leonard and Hotchkiss (1976; Fig. 6.20). Maximal activity requires the presence of both Mg^{2+} and K^+. In meristematic cells of Mg-sufficient roots about 90% of the cytoplasmic ATP is complexed with Mg and the concentration of free Mg^{2+} is only about 0.4 mM as compared with total Mg concentrations of 3.9 mM in the tissue (Yazaki *et al.*, 1988).

The synthesis of ATP (phosphorylation: ADP + $P_i \rightarrow$ATP) has an absolute requirement for Mg^{2+} as a bridging component between ADP and the enzyme. As shown by Lin and Noble (1971; Table 6.14), ATP synthesis in isolated chloroplasts (photophosphorylation, see also Chapter 5) is increased considerably by external supply of Mg^{2+}. The addition of Ca^{2+} severely inhibits photophosphorylation. Hence, a low Ca^{2+} concentration has to be maintained within the chloroplasts at the sites of photophosphorylation (Section 6.5).

Another key reaction of Mg is the modulation of RuBP carboxylase in the stroma of chloroplasts (Pierce,

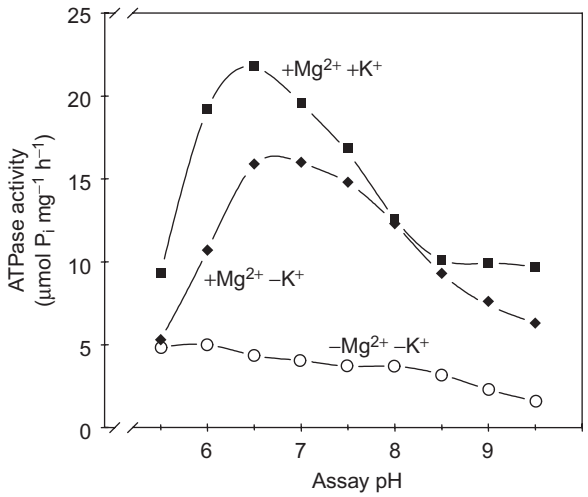


FIGURE 6.20 ATPase activity of the plasma-membrane protein of maize roots at different pH and addition of Mg (3 mM) and K (50 mM). *Based on Leonard and Hotchkiss (1976).*

TABLE 6.14 Photophosphorylation of isolated pea chloroplasts with or without Mg or Ca in the incubation medium containing ADP and P_i

Cation in the incubation medium	Photophosphorylation rate (µmol ATP formed mg^{-1} chlorophyll h^{-1})
None	12.3
5 mM Mg	34.3
5 mM Ca	4.3

Based on Lin and Nobel (1971).

1986). The activity of this enzyme is highly dependent on both Mg^{2+} and pH (Fig. 6.21A). Binding of Mg to the enzyme increases its affinity (K_m) for the substrate CO_2 and the turnover rate V_{max} (Sugiyama *et al.*, 1968). Magnesium also shifts the pH optimum of the reaction towards the physiological range (below 8). In chloroplasts, the light-triggered activation of RuBP carboxylase results in increases in pH and Mg^{2+} concentrations in the stroma. As shown in Fig. 6.21B, upon illumination, protons are pumped from the stroma into the inter-thylakoid space, creating the proton gradient required for ATP synthesis (Kramer *et al.*, 2003). The light-induced transport of protons from the stroma is counterbalanced by transport of Mg^{2+} (and H^+) from the inter-thylakoid space into the stroma which becomes more alkaline (Oja *et al.*, 1986). In wheat leaf chloroplasts, stroma pH may increase from about 7.6 in the dark to about 8.0 in the light (Heineke and Heldt, 1988). This light-triggered reaction increases the Mg^{2+} concentration of the stroma. Using an Mg-sensitive fluorescent indicator, free Mg^{2+} concentrations of 0.5 mM and 2.0 mM have been measured in the stroma of dark and illuminated spinach chloroplasts, respectively (Ishijima *et al.*, 2003); generally confirming earlier measurents of ~2 mM in the dark to ~4 mM in the light (Portis and Heldt, 1976; Portis, 1981). Changes of this magnitude in both pH and Mg^{2+} concentration are sufficient to increase the activity of RuBP carboxylase and also of other stromal enzymes which depend on high Mg^{2+} concentrations and which have a pH optimum above 6.

One of the key enzymes with high Mg requirement and high pH optimum is fructose-1,6-bisphosphatase which, for example, regulates assimilate partitioning between starch synthesis and export of triose phosphates in chloroplasts (Gerhardt *et al.*, 1987). Another key enzyme with high Mg requirement is glutamine synthetase (O'Neal and Joy, 1974). A light-induced increase in nitrite reduction

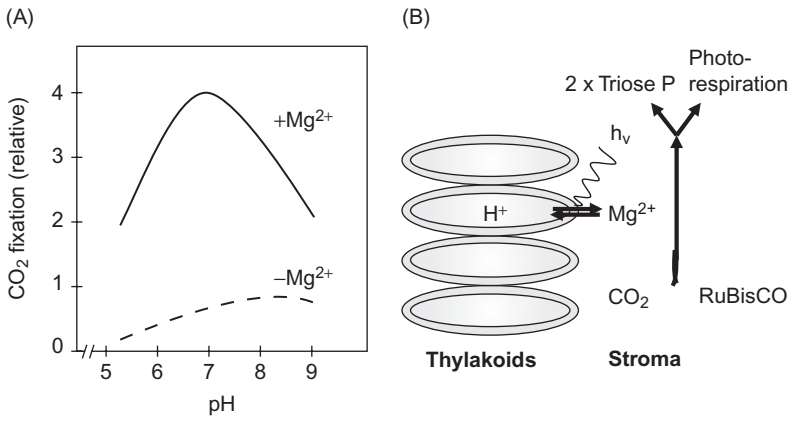


FIGURE 6.21 (A) Activation of ribulose-1,5-bisphosphate (RuBP) carboxylase from spinach leaves by Mg (modified from Sugiyama *et al.*, 1969). (B) Model for light-induced Mg transport from the intra-thylakoid space into the stroma of chloroplasts with subsequent activation of the RuBP carboxylase/oxygenase.

and thus NH_3 production requires a simultaneous increase in the activity of enzymes such as glutamine synthetase regulating ammonia assimilation within the chloroplasts. Thus the model of regulation for CO_2 fixation and reduction (Fig. 6.21B) also applies, in principle, for nitrite reduction and ammonia assimilation.

6.4.5 Carbohydrate Partitioning

The accumulation of non-structural carbohydrates (starch, sugars) is a typical feature in source leaves of Mg-deficient plants (Fischer and Bussler, 1988; Cakmak *et al*., 1994a; Table 6.15) and can be detected well in advance of the appearance of Mg deficiency symptoms and inhibition of photosynthesis (Hermans *et al*., 2004; Hermans and Verbruggen, 2005). Thus, inhibition of photosynthesis appears to be a response to increasing sugar concentrations serving as important signals in the regulation of plant metabolism and development (Wingler and Roitsch, 2008). Accumulation of starch, which is a typical feature of Mg-deficient leaves (Hermans *et al*., 2005; Hermans and Verbruggen, 2005), is also found in P-deficient leaves, but the latter is associated with high chlorophyll concentrations in the leaves (Table 6.15).

Accumulation of carbohydrates in source leaves of Mg-deficient plants is the result of inhibited export from the leaves via the phloem (Cakmak *et al*., 1994a; Hermans *et al*., 2005), leading to lower carbohydrate export to and thus lower concentrations in sink organs such as pods and roots in common bean (Fischer and Bussler, 1988; Cakmak *et al*., 1994a, b; Fig. 6.22) or growing sink leaves in sugar beet (Hermans *et al*., 2005). Impairment of carbohydrate supply to the roots by Mg deficiency leads to strongly reduced root growth in young common bean plants (Table 6.15; Fig. 6.22). This effect of Mg deficiency on root growth is similar to the effect of K deficiency but just the opposite of what is observed under P deficiency (Fig. 6.22). In sugar beet and *Arabidopsis*, inhibition of root growth was not a primary response of the plants to Mg deficiency. In these plant species, shoot growth was more sensitive of Mg deficiency than root growth (Hermans *et al*., 2004; Hermans and Verbruggen, 2005). This may indicate a plant species-specific response to Mg deficiency or just reflect differences in age of the

TABLE 6.15 Shoot and root dry weight and carbohydrate content (glucose equivalents) in primary leaves and roots of Mg- and P-deficient common bean

	Dry weight (g plant^{-1})				Carbohydrates (mg g^{-1} dry wt)			
					Leaves		Roots	
Treatment	Shoots	Roots	S/R	Chlorophyll (mg g^{-1} dry wt)	Starch	Sugars	Starch	Sugars
Control	2.5	0.5	5.0	11	10	27	4	51
−Mg	1.5	0.15	10.0	4	77	166	4	11
−P	0.9	0.48	1.9	12	43	34	8	35

Compiled from Cakmak *et al.* (1994a).

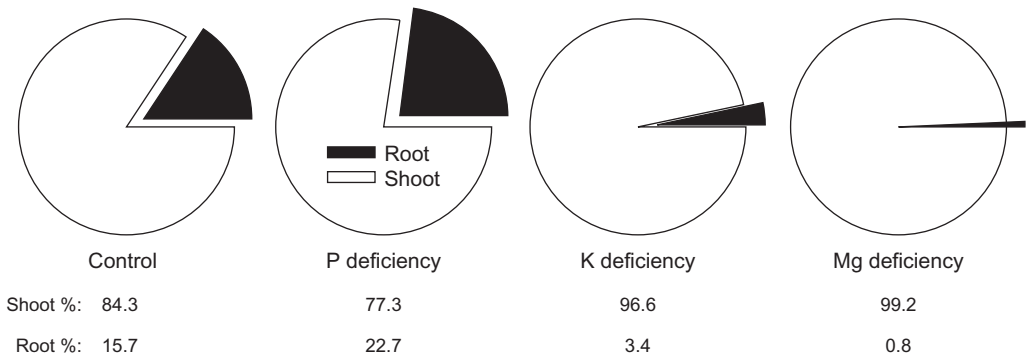


FIGURE 6.22 Relative distribution of carbohydrates (sum of reducing sugars, sucrose and starch) between shoot and roots of 12-day-old common bean plants grown for 12 days in nutrient solution with sufficient (control) or deficient supply of P, K, or Mg. *From Cakmak* et al. *(1994a) with permission from Oxford University Press.*

TABLE 6.16 Chlorophyll and antioxidants concentration and activity of oxygen radical and H_2O_2-scavenging enzymes in primary leaves of common bean grown at low or high Mg supply. SOD, superoxide dismutase; AsPox, ascorbate peroxidase; GR, glutathione reductase

Mg supply	Chlorophyll	Ascorbate	Soluble thiols (SH)	Enzyme activities (relative values)		
(μM)	(mg g^{-1} dry wt)	(μmol g^{-1} fresh wt)	(nmol g^{-1} fresh wt)	SOD	AsPox	GR
1,000	11.3	0.9	0.6	100	100	100
20	5.3	6.2	2.3	229	752	310

Based on Cakmak and Marschner (1992).

experimental plants, because Mg deficiency primarily affects assimilate transport from young, fully expanded leaves while older leaves maintain their assimilate export to roots (Hermans *et al*., 2005).

Inhibition of phloem loading of sucrose in Mg-deficient source leaves is most likely the reason for the shift in carbohydrate partitioning. The key role of a proton-pumping ATPase for phloem loading of sucrose (proton-sucrose cotransport) is discussed in Chapter 5. For optimal activity, this enzyme requires an Mg^{2+} concentration of about 2 mM (Williams and Hall, 1987); in deficient leaves, the concentration of Mg^{2+} is most likely much lower in the 'metabolic pool' in general and at the plasma membrane of sieve tube cells in particular. In agreement with this assumption, the up-regulation in sugar beet of the *Bv*SUT1 gene encoding a phloem companion-cell sucrose/H^+ symporter supports the assumption of a defective sucrose loading into the phloem under Mg deficiency (Hermans *et al*., 2005). Also in common bean, phloem loading of sucrose can be restored within one day after resupply of Mg to Mg-deficient plants (Cakmak *et al*., 1994b).

Accumulation of photosynthates in leaves exerts a feedback regulation of RuBP carboxylase/oxygenase in favour of the oxygenase reaction and, thus, enhanced O_2 activation (Cakmak and Kirkby, 2008). Accordingly, in Mg-deficient leaves the formation of superoxide radicals ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2) and, in response to this, the content of antioxidants such as ascorbate, and the activity of superoxide radical and H_2O_2 scavenging enzymes, is enhanced (Cakmak and Marschner, 1992; Cakmak, 1994; Table 6.16). Magnesium-deficient leaves and needles are, therefore, highly photosensitive, and symptoms of chlorosis and necrosis strongly increase with light intensity (Marschner and Cakmak, 1989; Cakmak and Kirkby, 2008). A transcriptomic study of Mg starvation in *Arabidopsis* confirms the involvement of oxidative stress and the impairment of the photosynthetic apparatus, but also revealed a dysfunction of the circadian clock and the triggering of ethylene signalling in the response to Mg deficiency (Hermans *et al*., 2010a, b). The physiological significance of these findings for the understanding of Mg functions in plants remains to be elucidated.

6.4.6 Mg Supply, Plant Growth and Composition

The Mg requirement for optimal plant growth is 1.5–3.5 g kg^{-1} in vegetative parts. Chlorosis of fully expanded leaves is the most obvious visible symptom of Mg deficiency. In accordance with the function of Mg in protein synthesis, Mg deficiency results in a lower proportion of protein N while the proportion of non-protein N is increased. The rate of photosynthesis per unit leaf area or unit chlorophyll is lower in leaves of Mg-deficient plants and carbohydrates accumulate (negative feedback regulation). Slight and transient Mg deficiency symptoms during the vegetative growth stage, however, do not necessarily result in low yield unless irreversible changes, such as a reduction in grain number per ear in cereals, occur (Forster, 1980). At permanently insufficient root supply, remobilization of Mg from mature leaves reduces their longevity. For example, in perennials such as Norway spruce concentrations of Mg and chlorophyll as well as rate of photosynthesis of the older needles decrease in spring when the new needles develop (Lange *et al.*, 1987).

There is increasing evidence that Mg deficiency is widespread in forest ecosystems in Central Europe (Liu and Hüttl, 1991), exacerbated by other stress factors, in particular air pollution (Schulze, 1989) and soil acidification (Marschner, 1992). Impairment of root growth which is also typical for declining Mg-deficient spruce stands (Roberts *et al.*, 1989) has a considerable impact on acquisition not only of Mg but also of other nutrients and of water and, thus, on drought resistance and adaptation to nutrient-poor sites.

When Mg is deficient and the export of carbohydrates from source to sink sites is impaired, the starch concentration in storage tissues such as potato tubers (Werner, 1959)

and the single-grain weight of cereals decrease (Beringer and Forster, 1981). In cereal grains, however, Mg may play an additional role in the regulation of starch synthesis through its effect on the concentration of P_i and phytate. As discussed above, high P_i concentrations inhibit starch synthesis. In Mg-deficient wheat grains, twice as much P remains as P_i, and there is a correspondingly smaller proportion of phytate-P, compared with the grains adequately supplied with Mg (Beringer and Forster, 1981).

Increasing the Mg supply beyond the growth-limiting level results in additional Mg being stored mainly in the vacuoles, as buffer for Mg^{2+} homeostasis in the 'metabolic pool' and for charge compensation and osmoregulation in the vacuole. However, high Mg concentrations in the leaves (e.g., 15 g kg^{-1}) may be dertrimental under drought stress. As the leaf water potential declines, the Mg^{2+} concentration in the 'metabolic pool' increases from 3–5 mM up to 8–13 mM in sunflower. Such high concentrations, for example in the stroma of chloroplasts, inhibit photophosphorylation and photosynthesis (Rao *et al.*, 1987). In pea under drought stress, Mg^{2+} concentrations in the chloroplasts may increase up to 24 mM (Kaiser, 1987).

Generally, high Mg concentrations improve the nutritional quality of plants (Chapter 9). For example, hypomagnesaemia (grass tetany) is a serious disorder of ruminants caused by low Mg concentrations in feed and reduced efficiency of Mg resorption (Grunes *et al.*, 1970). An increase in Mg concentrations of forage grasses by Mg fertilization is relatively easy to achieve. Breeding for high leaf Mg concentrations, for example in Italian ryegrass, could be an alternative (Moseley and Baker, 1991). Insufficient Mg intake with the human diet leading to an Mg-deficiency syndrome has attracted considerable attention (Tong and Rude, 2005).

6.5 CALCIUM

6.5.1 General

Calcium is a relatively large divalent cation with a hydrated ionic radius of 0.412 nm and a hydration energy of 1577 J mol^{-1}. In the apoplasm, part of the Ca is firmly bound in structures, while another part is exchangeable at the cell walls and at the exterior surface of the plasma membrane. A high amount of Ca is often sequestered in vacuoles, whereas its concentration in the cytosol is low. The mobility of Ca in the symplasm and in the phloem is also low. Most of the functions of Ca as a structural or regulatory component of macromolecules are related to its capacity for coordination, by which it provides stable but reversible molecular linkages. Calcium can be supplied at high concentrations and can reach more than 10% of the dry weight, for example in mature leaves, without symptoms of toxicity or serious inhibition of plant growth. The functions of Ca in plants have been reviewed by White and Broadley (2003). In recent years, Ca has attracted much interest in plant physiology and molecular biology because of its role as second messenger linking environmental and developmental stimuli to their physiological responses. This role is related to perturbations in cytosolic free Ca^{2+} concentration.

TABLE 6.17 Relationship between Ca supply and proportion of total Ca in various binding forms in young sugar beet plants

	Ca supply (mM)	
	0.33	5.0
Binding form of Ca		
Water soluble	27	19
Pectate	51	31
Phosphate	17	19
Oxalate	4	25
Residue	1	6

Based on Mostafa and Ulrich (1976).

6.5.2 Binding Form and Compartmentation

In contrast to other macronutrients, a high proportion of the total Ca in plant tissues is often located in cell walls (apoplasm). This unique distribution is mainly the result of the large number of binding sites for Ca in the cell walls (Table 6.17). In the middle lamella it is bound to R-COO$^-$ groups of polygalacturonic acids (pectins) in a readily exchangeable form. In dicotyledons such as sugar beet, which have a large cation-exchange capacity, and particularly when the Ca supply is low, up to 50% of the total Ca can be bound as pectates (Table 6.17; Armstrong and Kirkby, 1979b). Compared to other plant species, the Ca requirement of commelinoid monocotyledons is low which is due to their low concentration of cell wall pectate (White and Broadley, 2003).

When Ca supply is increased, excess Ca is generally accumulated in the vacuole. Three distinct physio-types for Ca nutrition exist: 'calciotrophes', 'oxalate plants' and 'potassium plants', which show contrasting responses to Ca supply (Fig. 6.23; Kinzel, 1982; White, 2005). Calciotrophes, such as *Sedum album*, contain high concentrations of water-soluble Ca complexes in their vacuoles and their accumulation of Ca is stimulated greatly by increasing Ca supply. The oxalate plants are divided into species whose vacuoles contain either soluble oxalate, such as *Oxalis acetosa*, or Ca-oxalate crystals, such as

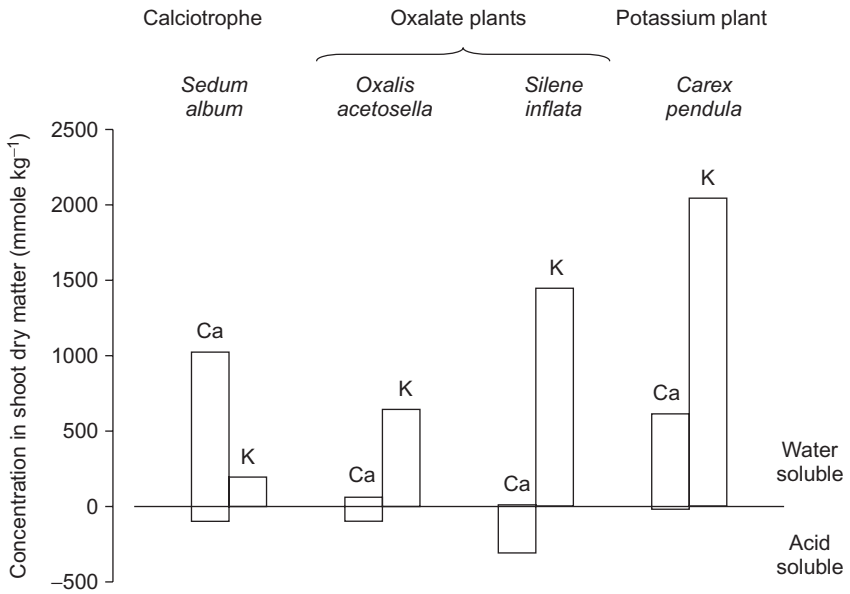


FIGURE 6.23 Physiotypes for calcium nutrition. *Adapted from White (2005), based on data from Horak and Kinzel (1971) and Longin and Neirinckx (1977).*

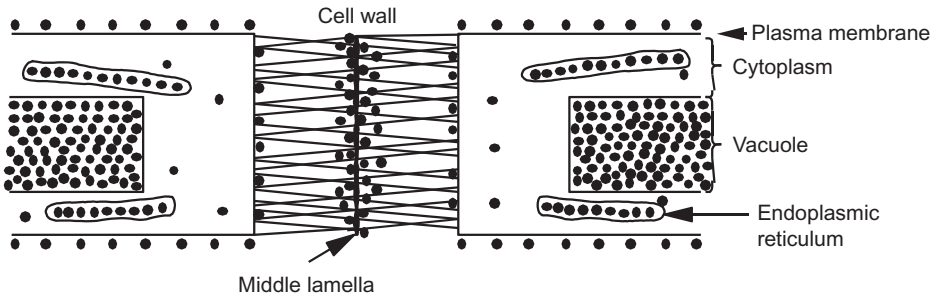


FIGURE 6.24 Schematic diagram of two adjacent cells with typical distribution of Ca, •.

Silene inflata. Increasing Ca supply increases Ca accumulation in plants that precipitate Ca-oxalate, but not in plants containing soluble oxalate. Potassium plants, such as *Carex pendula*, contain little mineralized or water-soluble Ca and maintain a high tissue K:Ca ratio. Calcium can also be precipitated in the apoplasm as Ca-oxalate or Ca-carbonate (Kinzel, 1989; Fink, 1991a–c). The shape and distribution of Ca oxalate crystals differs between plant species and has proven useful as a taxanomic character (Prychid and Rudall, 1999; Franceschi and Nakata, 2005).

A typical distribution of Ca in cells of fully expanded tissue with high cation exchange capacity of the cell walls is shown in Fig. 6.24. There are distinct areas and compartments with high or very low Ca concentrations. High Ca concentrations are found in the middle lamella of the cell wall, at the exterior surface of the plasma membrane, in the endoplasmic reticulum (ER), and in the vacuole. Most of the water soluble Ca in a plant tissue is located in the vacuoles, accompanied with organic anions (e.g., malate) or inorganic anions (e.g., nitrate, chloride). Calcium in the ER is associated with Ca^{2+}-binding proteins. In contrast to the cell wall, ER and vacuole, the concentration of Ca in the cytosol is low (0.1–1.0 mM) and free Ca^{2+} is buffered at 0.1–0.2 µM by Ca^{2+}-binding proteins and active Ca^{2+} efflux to the apoplasm, vacuole and ER (White and Broadley, 2003; McAinsh and Pittman, 2009). Such low Ca^{2+} concentrations are essential for various reasons, such as (i) prevention of P_i precipitation, (ii) competition with Mg^{2+} for binding sites, and (iii) as a prerequisite for the function of Ca^{2+} as a second messenger. The major transporters catalysing Ca^{2+} efflux from the cytosol to the apoplast and ER are Ca^{2+}-ATPases (Fig. 6.25). At the tonoplast, both Ca^{2+}-ATPases and Ca^{2+}/H^+ antiporters catalyse Ca^{2+} efflux from the cytosol to the vacuole. The latter is energized by the proton electrochemical gradient generated by tonoplast H^+-ATPase and H^+-PP_iase activities. Chloroplasts can also contain large

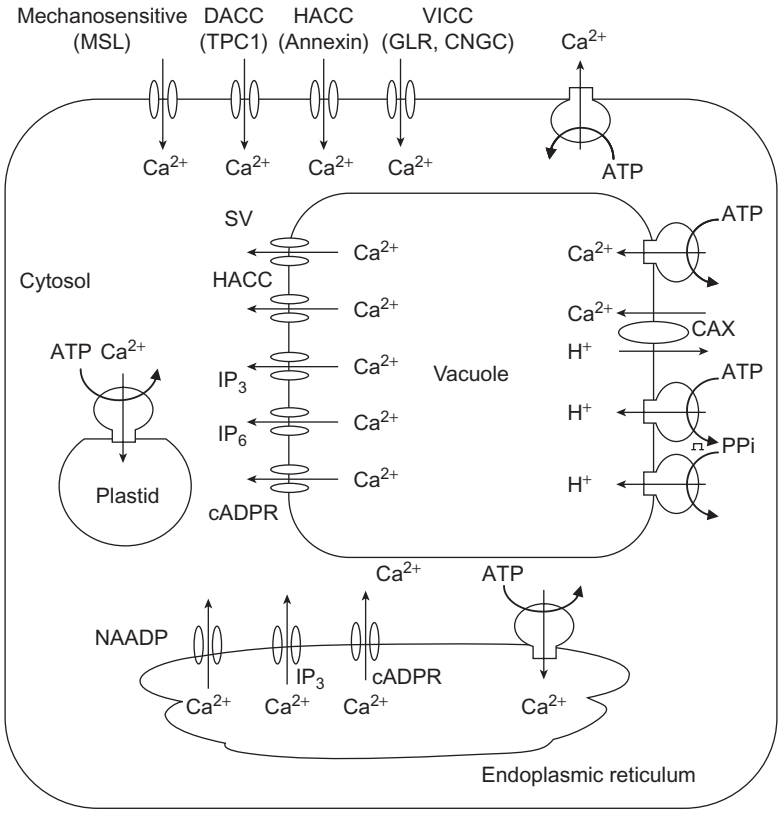


FIGURE 6.25 Calcium transport processes in plant cells. *Adapted from White and Broadley (2003).*

TABLE 6.18 Activity of cytosolic fructose-1,6-bisphosphatase from spinach leaves at different Ca and Mg concentrations

Ca^{2+} concentration (μM)	0	0.1	1.0	10	100
Mg^{2+} concentration (mM)	Enzyme activity (nmol mg^{-1} protein min^{-1})				
1.0	300	250	80	20	–
4.0	760	760	710	620	250

Recalculated from Brauer *et al.* (1990).

amounts of Ca (6.5–15 mM total Ca, mostly bound to thylakoid membranes), but in the stroma the concentrations of free Ca^{2+} is only in the range 2.4–6.3 μM (Kreimer *et al.*, 1988). A plastid Ca^{2+}-ATPase catalyses Ca^{2+} uptake by plastids (Fig. 6.25).

The importance of low cytosolic free Ca^{2+} concentrations for the functioning of certain key enzymes is illustrated in Table 6.18. The cytosolic enzyme fructose-1,6-bisphosphatase regulates sucrose synthesis from triosephosphates delivered by the chloroplasts. As little as 1 μM Ca^{2+} severely inhibits the activity of this enzyme, even in the presence of 1,000 times higher concentrations of Mg (1 mM).

6.5.3 Cell Wall Stabilization

Calcium bound as Ca-pectate in the middle lamella is essential for strengthening cell walls and plant tissues. This function of Ca is clearly reflected in the positive correlation between cation exchange capacity of cell walls and Ca concentration in plant tissues required for optimal growth. The degradation of pectates is mediated by polygalacturonase, which is strongly inhibited by high Ca concentrations (Table 6.19; Wehr *et al.*, 2004). Hence, in Ca-deficient tissue polygalacturonase activity is increased (Konno *et al.*, 1984), and a typical symptom of Ca deficiency is the disintegration of cell walls and the collapse of

TABLE 6.19 Hydrolysis of sodium pectate by polygalacturonase at different Ca concentrations

Ca^{2+} concentration (mg L^{-1})	Galacturonic acid released (μmol h^{-1})
0	0.875
40	0.625
200	0.150
400	0.050

Based on Corden (1965).

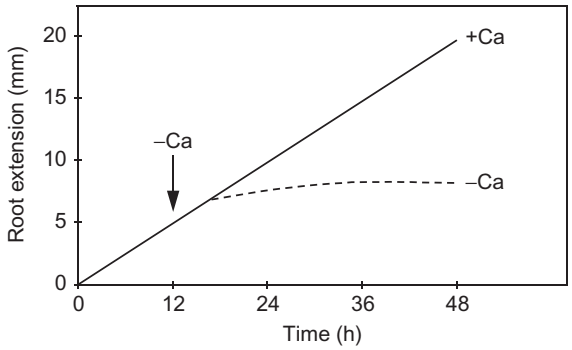


FIGURE 6.26 Extension of primary roots of bean without or with 2 mM Ca in the nutrient solution. *Based on Marschner and Richter (1974).*

the affected tissues, such as petioles, upper parts of stems and fruits (Shear, 1975; Ho and White, 2005).

In leaves of plants receiving high amounts of Ca during growth, or when grown under conditions of high light intensity, a large proportion of the pectic material is in the form of Ca-pectate. This makes the tissue highly resistant to degradation by polygalacturonase. The proportion of Ca-pectate in the cell walls is also of importance for the susceptibility of the tissue to fungal and bacterial infections (Chapter 10) and for the ripening of fruits (Ferguson, 1984). In tomato fruit, the Ca concentration of the cell walls increases to the fully grown immature stage, but this is followed by a decline in Ca concentration and a change in its bound form just before ripening (Rigney and Wills, 1981). Increasing the Ca concentration in fruits, for example, by spraying several times with Ca salts during fruit development or by post-harvest dipping in $CaCl_2$ solution, leads to an increase in the firmness of the fruit and delays fruit ripening (Ferguson, 1984; Oms-Oliu *et al.*, 2010).

6.5.4 Cell Extension and Secretory Processes

In the absence of an exogenous Ca supply, root extension ceases within a few hours (Fig. 6.26). This is due to impaired cell elongation, rather than lack of cell division, and is more obvious in a Ca-free nutrient solution than in distilled water, an observation consistent with the role of Ca in counterbalancing the harmful effects of high concentrations of other cations. Cell elongation in roots and shoots requires acidification of the apoplasm and replacement of Ca from the cross-links of the pectic chain, although this is only part of the process (Carpita and McCann, 2000). An increase in cytosolic free Ca^{2+} concentration stimulates the synthesis of cell wall precursors and their secretion into the apoplasm. The latter process is inhibited by removing apoplasmic Ca. The elongation of root hairs and pollen tubes also relies on the availability of apoplasmic Ca. Calcium influx from the apoplasm is restricted to the apex of these cells and increases local cytosolic Ca^{2+} concentration, which acts as focus for the exocytosis of cell wall material and establishes a polarity for cell elongation (White and Broadley, 2003; Cole and Fowler, 2006; Krichevsky *et al.*, 2007). In root caps, the secretion of mucilage also depends on the presence of apoplasmic Ca.

Callose formation is another example of a calcium-induced secretory process. Under normal conditions, cells synthesize cellulose (1.4 β-glucan units). However, in response to injury or the presence of toxic cations such as aluminium, a switch to callose (1.3 β-glucan units) production can occur (Kauss, 1987; Kartusch, 2003; Rengel and Zhang, 2003). This switch is triggered by an increase in cytosolic free Ca^{2+} concentration (Kauss, 1987).

Stimulation of α-amylase activity in germinating cereal seeds and the aleuron is one of the few examples of enzyme stimulation by high (millimolar) Ca concentrations. Calcium is a constituent of α-amylase, which is synthesized on the rough ER. Transport of Ca^{2+} through the ER membranes is enhanced by GA and inhibited by ABA, leading to the typical stimulation (GA) and inhibition (ABA) of α-amylase activity in aleurone cells (Lovegrove and Hooley, 2000).

6.5.5 Membrane Stabilization

Calcium plays a fundamental role of Ca in membrane stability and cell integrity. This is evident in the increased leakage of low-molecular-weight solutes from cells of Ca-deficient tissue and, in severely deficient plants, by a general disintegration of membrane structures and loss of cell compartmentation.

Calcium stabilizes cell membranes by bridging phosphate and carboxylate groups of phospholipids and proteins. Calcium can be exchanged for other cations at these binding sites; the exchange of plasma membrane-bound Ca for Na, heavy metals, or Al can contribute to salinity, heavy metal and aluminium toxicity (Cramer, 2002; Horst *et al.*, 2010). To prevent indiscriminate solute leakage and

TABLE 6.20 Carbohydrate loss from cotton roots at different temperatures, O_2 supply and Ca concentrations

Aeration	Treatment temperature (°C)	Solution	Carbohydrate loss (μg seedling^{-1} min^{-1})
O_2	31	Distilled water	18
O_2	5	Distilled water	57
O_2	5	10^{-5} M Ca^{2+}	7
N_2	31	Distilled water	89
N_2	31	10^{-5} M Ca^{2+}	7

Based on Christiansen *et al.* (1970).

influx of toxic solutes, Ca must always be present in the external solution. The membrane-stabilizing effect of Ca is most prominent under stress conditions such as freezing, low temperature and anaerobiosis. The loss of low-molecular-weight solutes, such as sugars and K ions, in response to chilling or anaerobiosis is reduced by increasing the Ca concentration in the external solution (Table 6.20). In addition to its role in stabilizing membranes, cytosolic Ca^{2+} acts as a secondary messenger to initiate membrane repair (Schapire *et al.*, 2009) and adaptive responses to freezing, low temperature and anaerobiosis (White and Broadley, 2003; Ruelland *et al.*, 2009).

6.5.6 Cation–anion Balance and Osmoregulation

In vacuolated cells of leaves in particular, a large proportion of Ca is localized in the vacuoles, where it may contribute to the cation–anion balance by acting as a counter-ion for inorganic and organic anions (White and Broadley, 2003). In plant species that preferentially synthesize oxalate in response to nitrate reduction, the formation of Ca oxalate in vacuoles is important for the maintenance of a low cytosolic free Ca^{2+} concentration (Kinzel, 1989). The same holds true for plant species with preferential formation of Ca oxalate in the apoplasm. The formation of sparingly soluble Ca oxalate is also important for salt accumulation in vacuoles of nitrate-fed plants without increasing the osmotic pressure in the vacuoles (Osmond, 1967). Additionally, Ca plays a key role in osmoregulation through its involvement as a second messenger in the cell. Stomatal movements and nyctinastic and seismonastic movements are turgor-regulated processes induced by turgor changes in individual cells (guard cells) or tissues (e.g., motor cells of pulvini). These turgor changes are driven by fluxes mainly of K (Section 6.6), Cl and malate (Section 7.8) as osmotically active solutes. It is now well established that a transient change of cytosolic free Ca^{2+} concentration is required for transduction of the signals (e.g., light, touch) to the physiological response (Moran, 2007; Amtmann and Blatt, 2009; Karley and White, 2009; Kim *et al.*, 2010).

6.5.7 Ca as Second Messenger

The ability of Ca to function as second messenger is based on the very low cytosolic free Ca^{2+} concentrations in plant cells and the chemistry of Ca^{2+} which allows it to alter the conformation of proteins to which it binds (White and Broadley, 2003). Environmental and developmental signals can activate Ca^{2+} channels in cell membranes that catalyse rapid Ca^{2+} influx to the cytosol and increase cytosolic free Ca^{2+} concentrations (Fig. 6.27). Environmental signals include light intensity and day length, extreme temperatures, drought, osmotic stress, salinity, aluminium stress, oxidative stress, mechanical stimulation, anoxia, nodulation and attack by pathogens (White and Broadley, 2003; Lecourieux *et al.*, 2006; McAinsh and Pittman, 2009). Changes in cytosolic Ca^{2+} concentrations also regulate developmental processes including cell division, cell elongation, cell polarity after fertilization and in the elongation of pollen tubes and root hairs, germination, circadian rhythms, trophic responses, senescence and apoptosis.

Calcium influx to the cytosol is mediated by Ca^{2+} channels located on the cell membrane (Fig. 6.25). The specificity of a response to an environmental or developmental signal is encoded by an explicit spatial and temporal perturbation in cytosolic Ca^{2+} concentration (White and Broadley, 2003; Lecourieux *et al.*, 2006; McAinsh and Pittman, 2009). Calcium channels in the plasma membrane have been classified on the basis of their voltage dependence (Fig. 6.25). They include (i) depolarization-activated Ca channels (DACCs), which may be encoded by homologues of the *AtTPC1* gene, (ii) hyperpolarization-activated Ca channels (HACCs), thought to be formed by plant

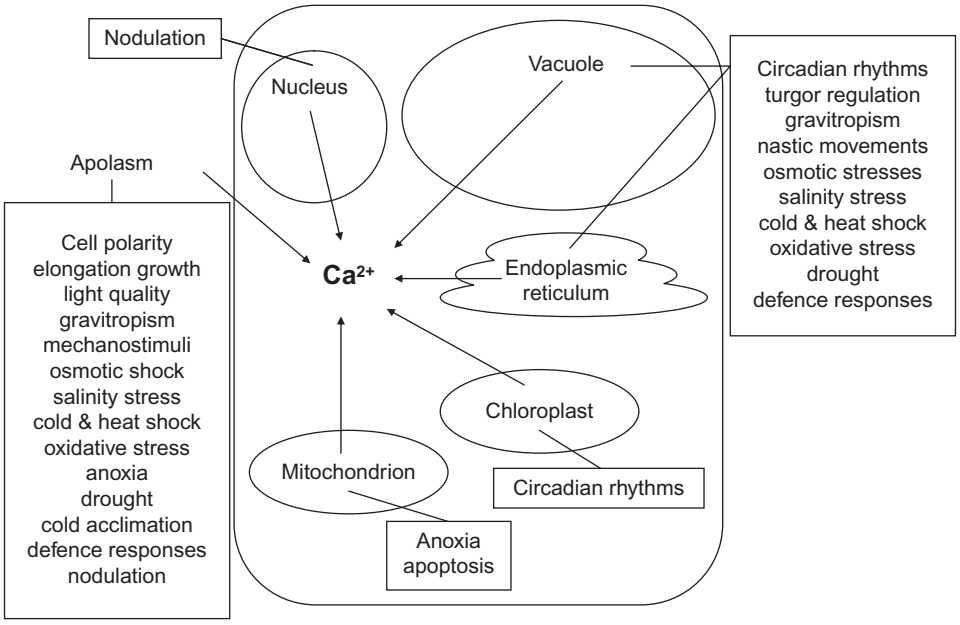


FIGURE 6.27 The origins of Ca influx to the cytosol implicated in plant cell development and responses to environmental signals.

annexins, and (iii) voltage-insensitive Ca channels (VICCs), which are thought to be encoded by members of the *cyclic nucleotide gated channel* (*CNGC*) and *glutamate receptor* (*GLR*) gene families (Karley and White, 2009; McAinsh and Pittman, 2009; Laohavisit and Davies, 2009). The plasma membrane may also contain mechano-sensitive Ca channels encoded by members of the *MSL* gene family (Haswell, 2007). Membrane depolarization occurs in response to many challenges and DACCs are thought to initiate general responses to stresses, including adaption to low temperatures (White and Broadley, 2003; White, 2009). The HACCs are involved in cell elongation, trophism and response to pathogens and oxidative stress (White and Broadley, 2003; Lecourieux *et al.*, 2006; Miedema *et al.*, 2008). The VICCs are thought to be responsible for regulating the basal cytosolic Ca^{2+} concentration of a resting cell (White and Broadley, 2003). Calcium can be released from the vacuole through various cation channels (White and Broadley, 2003; Pottosin and Schönknecht, 2007; Karley and White, 2009). These include (i) hyperpolarization-activated channels, which could be formed by annexins, (ii) depolarization-activated channels, such as the ubiquitous slow-vacuolar (SV) channel, and (iii) channels gated by ligands such as inositol-1,4,5-triphosphate (IP3) and cyclic ADP-ribose (cADPR). The IP3-gated Ca^{2+} channels in the tonoplast may be involved in turgor regulation during stomatal movements, cell elongation, tropism and response to salt and hyperosmotic stress (White and Broadley, 2003; Moran, 2007; Amtmann and Blatt, 2009; Kim *et al.*, 2010). These channels may also have a role in coordinating cellular responses to pathogens. The cADPR-gated channels in the tonoplast may be involved in stomata movement, circadian rhythms, cold adaption, desiccation tolerance and response to pathogens. A variety of voltage-dependent and ligand-gated channels are also found in the endoplasmic reticulum, plastid and nuclear membranes, but their roles in signal transduction are not yet clear.

In the cytosol, the primary target of Ca signals are Ca^{2+}-binding proteins (White and Broadley, 2003). These include calmodulins (CaMs), CaM-like proteins, calcineurin-B-like (CBL) proteins, Ca^{2+}-dependent protein kinases (CDPKs) and other Ca^{2+}-binding proteins, such as annexins. The binding of Ca^{2+} to these proteins alters their structure or enzymatic properties which can change solute transport, metabolism, cell morphology and gene expression. Calmodulins and CaM-like proteins are involved in the Ca^{2+}-dependent initiation of diverse developmental processes, adaption to numerous adverse environmental conditions and response to a variety of pathogens. Important targets for calmodulins are CaM-binding transcription activators (CAMTAs), which control gene expression (Bouché *et al.*, 2002; Doherty *et al.*, 2009). Similarly, the CBL proteins, together with their target proteins like the CIPK protein kinases, play a role in a wide variety of signalling cascades including those initiated by cold, drought, salinity, wounding or nutrient starvation. The CDPKs, which implement cytosolic Ca^{2+} signals through the phosphorylation of diverse target proteins, are involved in a multitude of cellular responses to diverse stimuli. Plant annexins play a role in Ca^{2+}-dependent membrane repair, secretory processes, cell elongation and responses to drought and salinity (Laohavisit and Davies,

TABLE 6.21 Relative growth rates of ryegrass and tomato and shoot Ca concentration at different Ca concentrations in the nutrient solution

	Ca concentration (μM)				
Plant species	0.8	2.5	10	100	1,000
	Relative growth rate				
Ryegrass	42	100	94	94	93
Tomato	3	19	52	100	80
	Ca concentration (mg g^{-1} dw)				
Ryegrass	0.6	0.7	1.5	1.7	10.8
Tomato	2.1	1.3	3.0	12.9	24.9

Based on Loneragan *et al.* (1968) and Loneragan and Snowball (1969).

2009). The spatial and temporal perturbations of cytosolic Ca^{2+} and the types of Ca^{2+}-binding proteins are specific for individual cells and particular stimuli; this is thought to ensure not only an appropriate response to a given challenge but also phenotypic plasticity of the response.

Calcium-binding proteins in the ER include calreticulin, calsequestrin, calnexin and BiP. These proteins are involved in cellular Ca^{2+} homeostasis, protein folding and post-translational modifications.

6.5.8 Ca Supply, Plant Growth, and Plant Composition

The Ca concentration of plants varies between 1 and $>50\,g\,kg^{-1}$ depending on the growing conditions, plant species and plant organ. The Ca requirement for optimum growth is much lower in monocotyledons than in dicotyledons (Table 6.21; Loneragan *et al.*, 1968; Loneragan and Snowball, 1969). In well-balanced, flowing nutrient solutions with controlled pH, maximal growth rates were obtained at Ca concentrations of 2.5 (ryegrass) and 100 μM (tomato), i.e. differing by a factor of 40. This difference is mainly a reflection of the Ca demand at the tissue level, which is lower in ryegrass ($0.7\,mg\,kg^{-1}$) than in tomato ($12.9\,mg\,kg^{-1}$). Differences in Ca requirements between genotypes are closely related to Ca^{2+}-binding sites in the cell walls, i.e. the cation-exchange capacity (White and Broadley, 2003).

The differences between monocotyledons and dicotyledons in Ca demand shown for ryegrass and tomato (Table 6.21) have been confirmed for a large number of plant species (Islam *et al.*, 1987). However, the dicotyledon *Lupinus angustifolius* had a Ca requirement (in terms of supply and tissue concentration) which was comparable to monocotyledons, and the growth of this species was severely depressed at higher Ca concentration in the tissue. Consequently, *L. angustifolius* prefers acidic soils and grows poorly in calcareous soils. Such typical calcifuge behaviour may be related to insufficient capacity for compartmentation and/or physiological inactivation of Ca.

TABLE 6.22 Growth rate of seminal roots of soybean at different Ca concentrations and solution pH

Ca^{2+} concentration ($mg\,L^{-1}$)	Root growth rate ($mm\,h^{-1}$)	
	pH 5.6	pH 4.5
0.05	2.66	0.04
0.5	2.87	1.36
2.5	2.70	2.38

Based on Lund (1970).

Another factor determining the Ca requirement for optimum growth is the concentration of other cations in the external solution. Because Ca is readily replaced by other cations from its binding sites at the exterior surface of the plasma membrane, Ca requirement increases with increasing external concentrations of heavy metals (Wallace *et al.*, 1966), Al, Na (LaHaye and Epstein, 1971; see also Chapter 17), or protons (Table 6.22). At low compared to high pH, the Ca^{2+} concentration in the external solution has to be several times higher in order to counteract the adverse effect of high H^+ concentrations on root elongation (Table 6.22). A similar relationship exists between external pH and the Ca requirement for nodulation of legumes (see also Chapter 16). In order to protect roots against the adverse effects of high concentrations of various other cations in the soil solution, the Ca^{2+}

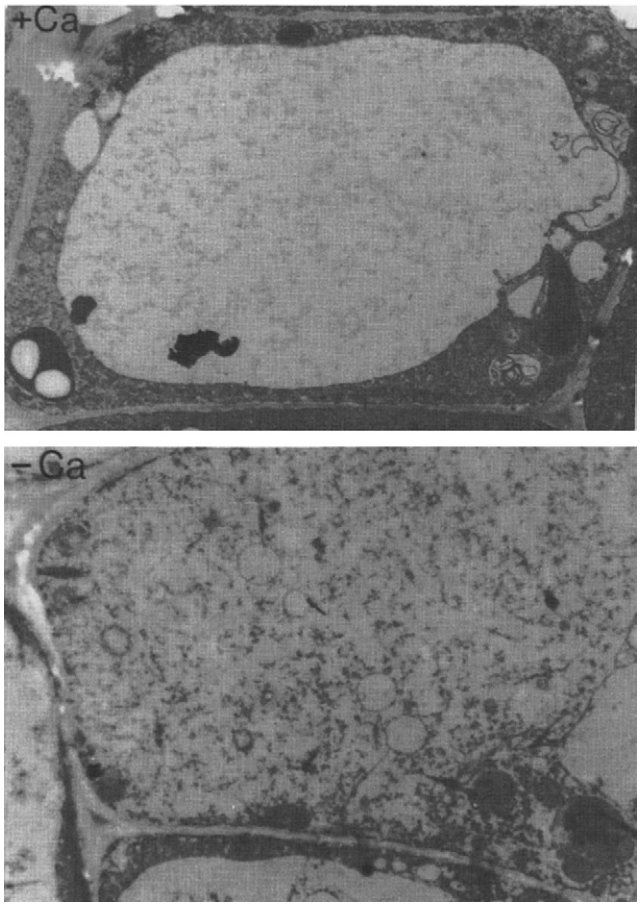


FIGURE 6.28 Fine structure of potato sprouts: Ca-sufficient (top), Ca-deficient (bottom). *Courtesy of Ch. Hecht-Buchholz.*

concentrations required for optimal growth have to be substantially higher in soil solutions than in balanced flowing nutrient solutions (Asher and Edwards, 1983).

An increase in the concentration of Ca^{2+} in the external solution often leads to an increase in the Ca concentration in the leaves, but not necessarily in low-transpiring organs such as fleshy fruits or tubers, which are supplied predominantly via the phloem. The mobility of Ca in the phloem is extremely low (see also Chapter 3) which can protect these organs against excessive Ca accumulation. However, high growth rates of low-transpiring organs increase the risk that tissue Ca concentration falls below the critical level required for cell wall stabilization and membrane integrity, and perhaps also its functioning as second messenger. In rapidly growing tissues, Ca deficiency-related disorders are widespread, such as tipburn in lettuce, blackheart in celery, blossom end rot in tomato or watermelon, and bitter pit in apple (Shear, 1975; White and Broadley, 2003; Ho and White, 2005; Fig. 6.28).

Low Ca concentrations in fleshy fruits and tubers also increase the losses caused by enhanced senescence of the tissue and by fungal infections. Even a relatively small increase in the Ca concentration of fruits can be effective in reducing or preventing economic losses caused by storage disorders (Table 6.23).

TABLE 6.23 Calcium concentrations and percentage of wastage during storage (3 months at 3.5°C) of 'Cox' apples receiving Ca sprays during the growing season or left unsprayed[a]

	Unsprayed	Sprayed
Calcium concentration (mg kg^{-1} fresh wt)	33.5	39.0
Storage disorders (wastage (%)		
Lenticel blotch pit	10.4	0.0
Senescence breakdown	10.9	0.0
Internal bitter pit	30.0	3.4
Gloesporium rots	9.1	1.7

From Sharpless and Johnson (1977).
[a]*Sprays containing 1% calcium nitrate were applied four times during the growing season.*

6.6 POTASSIUM

6.6.1 General

Potassium is a univalent cation with a hydrated ionic radius of 0.331 nm and a hydration energy of 314 J mol^{-1}. Its uptake is highly selective and closely coupled to metabolic activity (Chapter 2). It is characterized by high mobility in plants at all levels – within individual cells, within tissues, as well as in long-distance transport via the xylem and phloem. Uptake and transport of K^+ throughout the plant is facilitated by integral membrane proteins (transporters and cation channels) which enable its movement across the plasma membrane (Chapters 2 and 3). Potassium is the most abundant cation in the cytosol and K^+ and its accompanying anions contribute substantially to the osmotic potential of cells and tissues of glycophytic plant species. For various reasons, K^+ has an outstanding role in plant–water relations (Hsiao and Läuchli, 1986). Potassium is not metabolized and it forms only weak complexes in which it is readily exchangeable (Wyn Jones *et al.*, 1979). Therefore, K^+ does not strongly compete for binding sites of divalent cations (e.g., Mg^{2+}). On the other hand, due to its high concentrations in the cytosol and chloroplasts, it balances the charge of soluble (e.g., organic acid anions and inorganic anions) and insoluble anions and thus facilitates stabilizing the pH between 7 and 8 in these compartments, which is the optimum for most enzyme reactions.

6.6.2 Compartmentation and Cellular Concentrations

Generally, K concentrations are maintained at 100–200 mM in the cytosol (Leigh and Wyn Jones, 1984), and chloroplasts (Schröppel-Meier and Kaiser, 1988). In these compartments, it has important metabolic functions and cannot be replaced by other inorganic cations such as Na^+ (Section 8.2). In contrast, the vacuolar K^+ concentrations may vary between 10 and 200 mM (Hsiao and Läuchli, 1986) or even reach up to 500 mM in guard cells of stomata (Outlaw, 1983). The functions of K^+ in cell extension and other turgor-driven processes are related to the K^+ concentration in the vacuoles where it can be replaced to a varying degree by other cations (Na^+, Mg^{2+}, Ca^{2+}) or organic solutes (e.g., sugars). In contrast to Ca^{2+}, K^+ concentrations in the apoplast are usually low (Mühling and Sattelmacher, 1997), with the exception of specialized cells or tissues (stomata, pulvini), where apoplastic K^+ concentrations may transiently increase up to 100 mM.

For rapid uptake and transport of K^+ throughout the plant and between different cell compartments and cells within a tissue, membrane proteins are required to facilitate movement of K^+ through membranes. These transport proteins include high-affinity transporters and ion channels encoded by a number of genes, resulting in a large range of functional, regulatory and tissue-specific properties (Véry and Sentenac, 2003; Lebaudy *et al.*, 2007). Among the K^+ channels, voltage-regulated ('gated') channels play a major role in the control of K^+ influx and K^+ efflux (Amtmann and Blatt, 2009) (see also Chapter 2). Permeation rates through these channels are at least three orders of magnitude faster than those catalysed by pumps and carriers (Tester, 1990). The gating characteristics of such channels in response to environmental signals play a major role in the plant response to biotic and abiotic stresses.

Although K^+ channels are, in principle, similar to Ca^{2+} channels (Sanders *et al.*, 2002), their function is different. Potassium ions act directly as solutes, changing the osmotic potential in the compartments and thereby turgor, and, as carrier of charges, also the membrane potential.

6.6.3 Enzyme Activation

A large number of enzymes are either completely dependent on or are stimulated by K^+ (Suelter, 1970). Potassium and other univalent cations activate enzymes by inducing conformational changes in the protein. All macromolecules are highly hydrated and stabilized by firmly bound water molecules forming an electrical double layer. Maximum suppression of this electrical double layer and optimization of the protein hydration occur at univalent salt concentrations of about 100 to 150 mM (Wyn Jones and Pollard, 1983). This concentration range agrees well with the K^+ concentrations in the cytosol and in the stroma of plants well supplied with K^+ (Leigh and Wyn Jones, 1984). In general, K^+-induced conformational changes of enzymes increase the rate of catalytic reactions, V_{max}, and in some cases also the affinity for the substrate, K_m (Evans and Wildes, 1971).

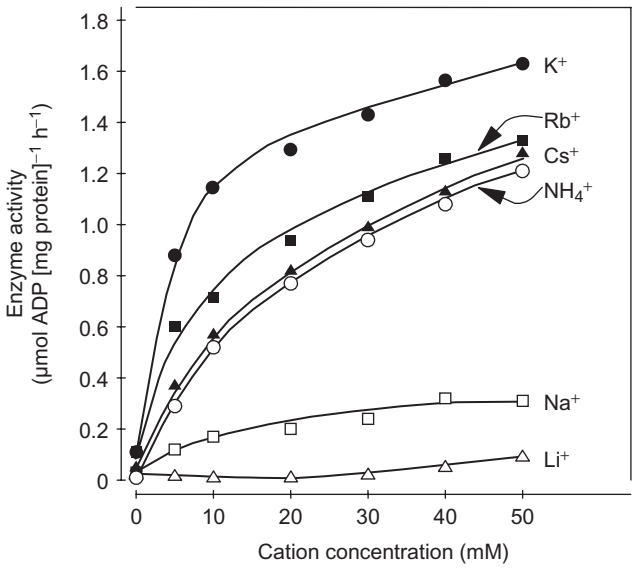


FIGURE 6.29 Activity of ADP-glucose starch synthase from maize with supply of different univalent cations (as chlorides). *Based on Nitsos and Evans (1969).*

When bulk leaf K concentrations decrease under K deficiency, cytosolic K^+ concentrations are maintained rather constant, whereas vacuolar K^+ concentrations strongly decrease (Walker *et al.*, 1996). However, with prolonged K deficiency, cytosolic K^+ concentrations also decline. This has severe consequences for the activity of cytosolic enzymes, not only because of the lack of enzyme activation but also because of the inability to maintain the optimum cytosolic pH. Among the enzymes most sensitive to K deprivation are pyruvate kinase and phosphofructokinase (Läuchli and Pflüger, 1978). Based on a multi-level analysis of the response of the primary metabolism of *Arabidopsis* to low K supply, Armengaud *et al.* (2009) concluded that the primary cause of metabolic disorders in low-K plants is the direct inhibition of pyruvate kinase by low cytoplasmic K^+ in root cells.

The activity of starch synthase is also highly dependent on univalent cations, and of these K^+ is the most effective (Nitsos and Evans, 1969; Fig. 6.29). The enzyme catalyses the transfer of glucose to starch molecules:

$$\text{ADP-Glucose} + \text{starch} \leftrightarrows \text{ADP} + \text{glucosyl} - \text{starch}$$

Potassium similarly activates starch synthase isolated from a variety of plant species and organs (e.g., leaves,

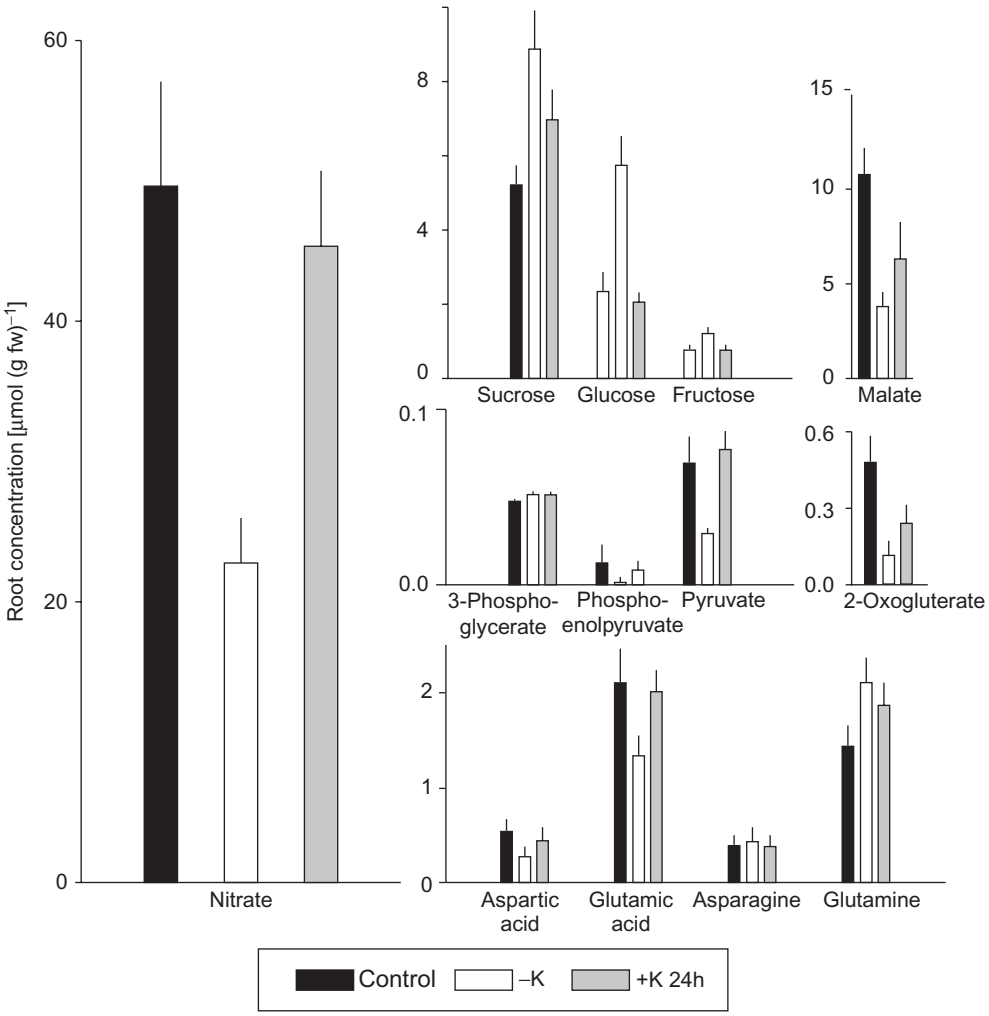


FIGURE 6.30 Concentrations of selected metabolite in roots of *Arabidopsis* induced by low K supply for 14 days and resupply of K for 24 h. *From Armengaud* et al. *(2009) with permission from the American Society of Plant Biologists.*

seeds and tubers), with maximum activation at 50 to 100 mM K^+ (Nitsos and Evans, 1969). Higher concentrations, however, may be inhibitory (Preusser *et al.*, 1981).

Another key function of K^+ is the activation of membrane-bound proton-pumping ATPases (Gibrat *et al.*, 1990; see also Chapter 2). This activation not only facilitates the transport of K^+ from the external solution across the plasma membrane into the root cells, but also makes K the most important element in cell extension and osmoregulation. Potassium also specifically activates vacuolar (tonoplast) pyrophosphatase isoforms involved in the transport of H^+ into the vacuoles (Darley *et al.*, 1998). Potassium deficiency increases the activity of certain hydrolases or oxidases such as polyphenol oxidase.

These changes in enzyme activities in K-deficient plant tissues lead to typical changes in the metabolite pattern: an increase in soluble carbohydrates, particularly reducing sugars, and soluble organic N compounds, particularly N-rich and positively charged amino acids, whereas the concentrations of nitrate, organic acids, negatively charged amino acids and pyruvate are decreased (Armengaud *et al.*, 2009; Fig. 6.30).

It is not clear to which degree these changes in enzyme activities are caused by direct or indirect effects of K^+ on enzyme activity. An indirect effect may be the role of K^+ in maintaining the cytosolic pH and the anion–cation charge balance. An instructive example of indirect effects is the accumulation of the diamine putrescine in K-deficient plants by a factor of 80–100 (Houman *et al.*, 1991; Tachimoto *et al.*, 1992). The enzymes which catalyse the synthesis of putrescine from arginine via agmatine are inhibited by high K^+ concentrations (Reggiani *et al.*, 1993) and stimulated by low cellular pH. Putrescine, a divalent cation, can replace K^+ in maintenance of high cytoplasmic pH; in K-deficient plants, putrescine concentrations may account for up to 30%

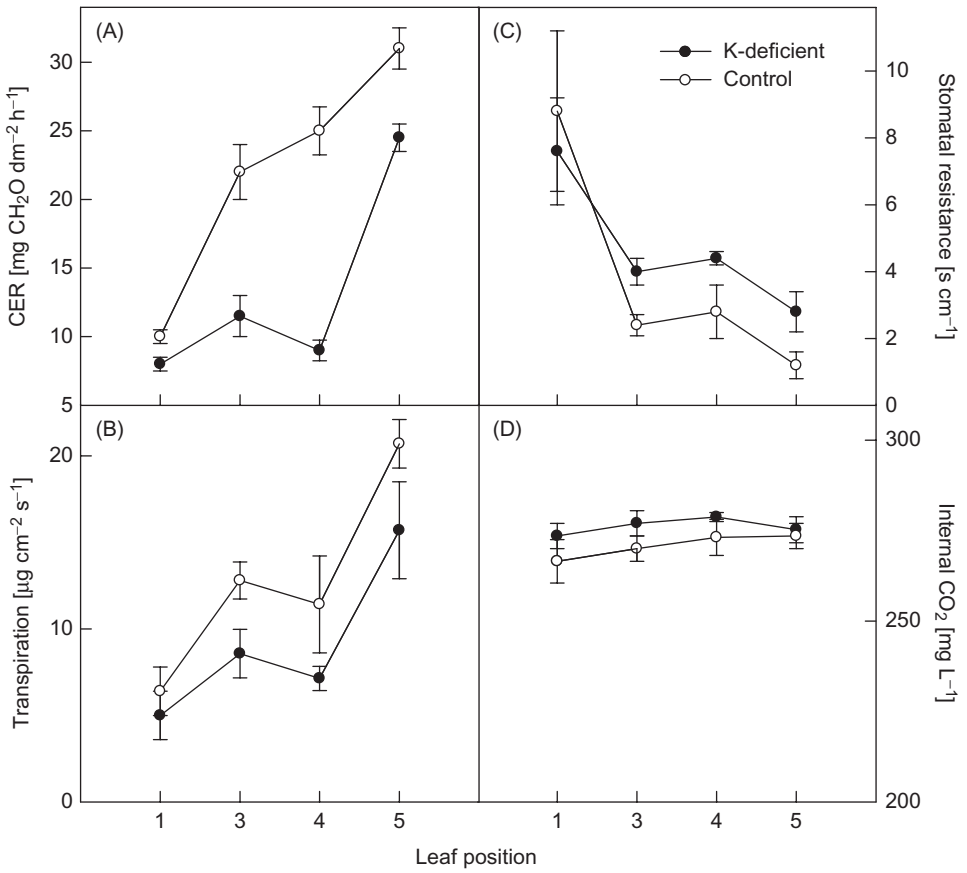


FIGURE 6.31 (A) Carbon exchange rate (CER), (B) transpiration, (C) stomatal resistance, and (D) internal CO_2 in soybean plants under K deficiency. *Adapted from Huber (1984).*

of the deficit in K^+ equivalents (Murty *et al.*, 1971). In agreement with this compensatory function of putrescine, external supply of putrescine to K-deficient plants enhanced growth and prevented visual symptoms of K deficiency (Tachimoto *et al.*, 1992).

Potassium deficiency alters assimilate partitioning and thus changes metabolite concentrations in vegetative plant organs. Accumulation of sugars in mature leaves is the consequence of inhibited export from the leaves and a lower demand by sink organs such as growing leaves (Gerardeaux *et al*., 2010) and fleshy fruits such as tomato (Kanai *et al*., 2007).

6.6.4 Protein Synthesis

Potassium is required in higher concentrations for protein synthesis than for enzyme activation, which is maximal already at about 50 mM K^+ (e.g., Fig. 6.29). In cell-free systems, the rate of protein synthesis by ribosomes isolated from wheat germ is optimal at 130 mM K^+ and ~2 mM Mg^{2+} (Wyn Jones *et al.*, 1979). It has been suggested that K^+ is involved in several steps of the translation process, including the binding of tRNA to ribosomes (Wyn Jones *et al.*, 1979). In green leaves, the chloroplasts account for about half of both leaf RNA and leaf protein. In C3 species, the majority of the chloroplast protein is RuBP carboxylase. Accordingly, the synthesis of this enzyme is particularly impaired under K deficiency and responds rapidly to resupply of K (Peoples and Koch, 1979; Table 6.24). Maximum activation was obtained at 10 mM K^+ in the external solution. This concentration must have been sufficient to obtain a more than 10-fold higher K^+ concentration in the chloroplasts which is required for high rates of protein synthesis.

The role of K in protein synthesis is not only reflected in the accumulation of soluble N compounds (e.g., amino acids, amides and nitrate) in K-deficient plants (Mengel and Helal, 1968) but can also be demonstrated directly through incorporation of ^{15}N-labelled inorganic N into the protein fraction (Koch and Mengel, 1974). Pflüger and Wiedemann (1977) suggested that K^+ not only activates nitrate reductase, but is also required for the synthesis

TABLE 6.24 Incorporation of ^{14}C-leucine into RuBP carboxylase in the leaves of K-deficient alfalfa plants pre-incubated at different K concentrations in the light for 20 hours

Preincubation medium (mM KNO_3)	^{14}C-leucine incorporation (dpm mg^{-1} RUBP carboxylase 24 h^{-1})
0.00	99
0.01	167
0.10	220
1.00	274
10.00	526
Control (K-sufficient plants)	656

From Peoples and Koch (1979).

TABLE 6.25 Relationship between K concentration in leaves, carbon dioxide exchange, RuBP carboxylase activity, photo and dark respiration in lucerne

	Leaf K concentration (mg g^{-1} dw)		
	12.8	19.8	38.4
Stomata resistance (s cm^{-1})	9.3	6.8	5.9
Photosynthesis (mg CO_2 dm^{-2} h^{-1})	11.9	21.7	34.0
RUBP carboxylase activity (µmol CO_2 mg^{-1} protein h^{-1})	1.8	4.5	6.1
Photorespiration (dpm dm^{-2})	4.0	5.9	9.0
Dark respiration (mg CO_2 dm^{-2} h^{-1})	7.6	5.3	3.1

From Peoples and Koch (1979).

of this enzyme; a conclusion which is supported by the results of Armengaud *et al*. (2009).

6.6.5 Photosynthesis

Photosynthesis is strongly reduced in K-deficient leaves. Potassium affects photosynthesis at various levels. The K nutritional status affects photosynthesis via its function in stomatal regulation with K deficiency increasing stomatal resistance to CO_2. However, the higher leaf internal CO_2 concentration (Fig. 6.31) clearly shows that the leaf mesophyll resistance is more important than stomatal resistance in limiting photosynthesis in K-deficient leaves. Potassium is the dominant counter-ion to the light-induced H^+ flux across the thylakoid membranes (Tester and Blatt, 1989) and for the establishment of the transmembrane pH gradient necessary for the synthesis of ATP (photophosphorylation), in analogy to ATP synthesis in mitochondria.

The role of K in CO_2 fixation has been most clearly demonstrated with isolated chloroplasts (Pflüger and Cassier, 1977). An increase in the external K^+ concentration to 100 mM, which is equivalent to the K^+ concentration in the cytosol of intact cells, stimulated CO_2 fixation more than three-fold. Upon illumination, additional influx of K^+ from the cytosol is required for the maintenance of a high pH in the stroma necessary for optimal RuBP carboxylase activity. This additional influx is mediated by an H^+/K^+ counterflow through the chloroplast envelope (Wu *et al*., 1991). For maximum H^+-ATPase activity, an external K^+ concentration of about 100 mM is necessary (Wu and Berkowitz, 1992).

With decreasing leaf K concentration, not only the rate of photosynthesis and RuBP carboxylase activity, but also photorespiration is decreased (Table 6.25). This may be due to a depletion of CO_2 at the catalytic sites of the enzyme. On the other hand, dark respiration increases. Higher respiration rates are a typical feature of K deficiency (Bottrill *et al.*, 1970) and may reflect the higher substrate (sugars) availability for respiration.

6.6.6 Osmoregulation

In Chapter 3 it was shown that a high osmotic potential in the stele of roots is a prerequisite for turgor pressure-driven solute transport in the xylem and for the water balance of plants. The role of K^+ in maintaining xylem-sap flow is evident from the reduced night-time stem expansion and enhanced day-time stem shrinkage in K-deficient tomato plants (Kanai *et al.*, 2007). In principle, at the level of individual cells or in certain tissues, the same mechanisms are responsible for cell extension and various types of movement. Potassium, as the most prominent inorganic solute, plays a key role in these processes (Hsiao and Läuchli, 1986).

6.6.6.1 Cell Extension

Cell extension involves the formation of a large central vacuole occupying 80–90% of the cell volume. There are three major requirements for cell extension: (i) cell extensibility (rearrangement or loosening of the existing cell wall), (ii) synthesis and deposition of newly formed wall components, and (iii) solute accumulation to create

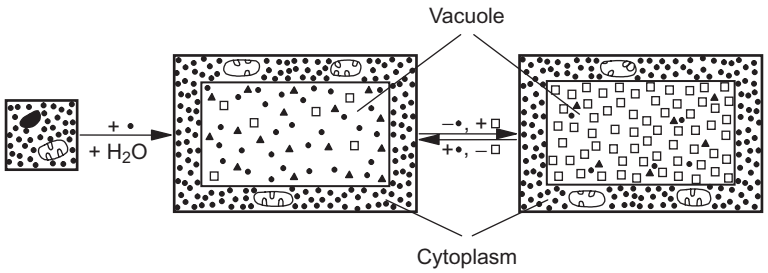


FIGURE 6.32 Model of the role of K and other solutes in cell extension and osmoregulation. ●: K^+; □: reducing sugars, sucrose, Na^+; ▲: organic acid anions.

TABLE 6.26 Plant height and concentrations of sugars and K in the shoots of sunflower plants at different K and gibberellic acid (GA) supply

Treatment			Concentration (μmol g^{-1} fw)		
KCl (mM)	GA (mg L^{-1})	Plant height (cm)	Reducing sugars	Sucrose	Potassium
0.5	0	7.0	19.1	5.0	10.2
0.5	100	18.5	38.5	5.4	13.2
5.0	0	11.5	4.6	4.1	86.5
5.0	100	26.0	8.4	2.5	77.8

Based on De la Guardia and Benlloch (1980).

the necessary internal osmotic potential for turgor pressure (Fig. 6.32). In most cases, cell extension is due to K^+ accumulation in the cells, which is required for both stabilizing the pH in the apoplast and the cytoplasm and increasing the osmotic potential in the vacuoles. A decrease in apoplastic pH is necessary to activate enzymes involved in cell wall loosening (Hager, 2003). Potassium is required to electrochemically counterbalance the ATPase-driven H^+ release into the cell wall (Stiles and van Volkenburgh, 2004). In *Avena* coleoptiles, IAA-stimulated H^+ efflux was electrochemically balanced by a stoichiometric K^+ influx; in the absence of external K^+, IAA-induced elongation declined and ceased after a few hours (Haschke and Lüttge, 1975).

Potassium associated with either inorganic anions or organic acid anions is the main solute required in the vacuoles for turgor-driven cell extension. Thus, cell extension not only in leaves but also in roots (Dolan and Davies, 2004) is positively correlated with their K concentration. Potassium deficiency significantly reduced turgor, cell size and leaf area in expanding leaves of bean plants (Mengel and Arneke, 1982). Reduced leaf extension rate was a most sensitive indicator of K deficiency in maize grown in the field (Jordan-Meille and Pellerin, 2004) and under controlled conditions in hydroponics (Jordan-Meille and Pellerin, 2008). This inverse relationship between K concentration in plants and cell size also holds true for storage tissues such as carrot (Pfeiffenschneider and Beringer, 1989) and tomato (Kanai *et al.*, 2007).

As shown by De la Guardia and Benlloch (1980; Table 6.26), the stimulation of stem elongation by gibberellic acid (GA) is also dependent on K supply. Potassium and GA act synergistically, the highest elongation rate being obtained when both GA and K are applied. Furthermore, the results indicate that K^+ and reducing sugars act in a complementary manner to produce the turgor potential required for cell extension. At low K supply, however, GA-stimulated growth was correlated with a marked increase in K^+ concentration in the elongation zone to a level similar to that of the reducing sugars (De la Guardia and Benlloch, 1980). As K^+ was supplied together with Cl^- (as KCl), a substantial proportion of the effects on plant growth and sugar concentrations may be due to the combined effects of K^+ and Cl^- on osmotic potential.

The extent to which sugars and other low-molecular-weight organic solutes contribute to the osmotic potential and turgor-driven cell expansion depends on the K nutritional status of plants, as well as on plant species and specific organs. For example, in the elongation zone of leaf blades of tall fescue, about half of the imported sugars are used for accumulation of osmotically active fructanes in the vacuoles (Schnyder *et al.*, 1988).

After completion of cell extension, K^+ can be fairly readily replaced for maintenance of the cell turgor in the

vacuoles by other solutes such as Na^+ or reducing sugars (Fig. 6.32). At later stages of leaf extension, sugars even overcompensated leaf-tissue K^+ deficiency in cotton (Gerardeaux *et al.*, 2010). Generally, there is a negative relationship between tissue concentrations of K^+ and sugars, reducing sugars in particular (Pitman *et al.*, 1971) which can also be observed during the growth of storage tissues. As shown by Steingröver (1983), the osmotic potential of the press sap from the storage root of carrot remains constant throughout growth. Before sugar storage begins, K^+ and organic acids are the dominant osmotic substances. During sugar storage, however, an increase in the concentration of reducing sugars is compensated for by a corresponding decrease in the concentration of K^+ and organic acid anions. In storage roots of sugar beet, the same holds true for the concentrations of sucrose and K^+ (Beringer *et al.*, 1986).

6.6.6.2 Stomata Movement

In most plant species K^+, associated with an anion, plays a major role in turgor changes in the guard cells during stomata movement. Increasing K^+ concentration in the guard cells increases their osmotic pressure and results in the uptake of water from the adjacent cells which results in an increase in turgor in the guard cells and thus stomata opening as shown for faba bean by Humble and Raschke (1971) (Table 6.27). The accumulation of K^+ in guard cells of open stomata can also be shown by X-ray microprobe analysis (Fig. 6.33). Closure of the stomata in the dark is correlated with K^+ efflux and a corresponding decrease in the osmotic pressure of the guard cells.

The metabolic and transport systems involved in stomata opening are shown schematically in Fig. 6.34.

TABLE 6.27 Relationship between stomatal aperture and characteristics of guard cells of faba bean

		Open stomata	Closed stomata
Stomatal aperture (μm)		12	2
Content per stoma (10^{-14} mol)	K	424	20
	Cl	22	0
Gaurd cell volume (10^{-12} L per stoma)		4.8	2.6
Gaurd cell osmotic pressure (MPa)		3.5	1.9

From Humble and Raschke (1971).

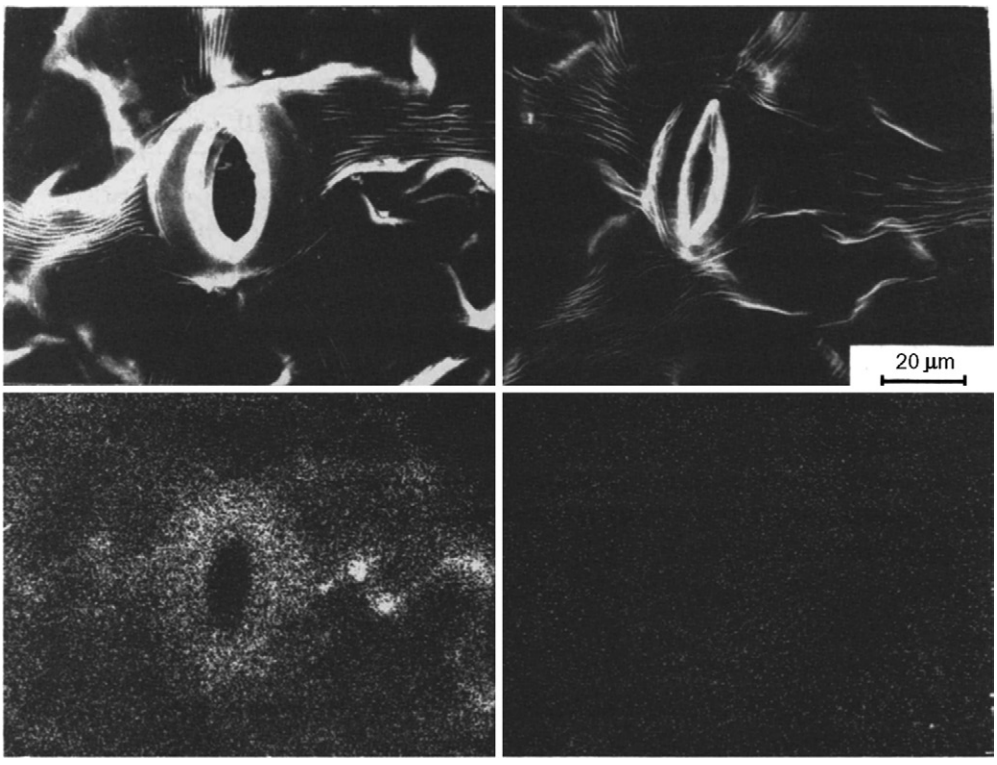


FIGURE 6.33 Electron-probe analyser image (*top*) and corresponding X-ray microprobe images of K distribution (*bottom*) in open and closed stomata of faba bean. *Courtesy of B. Wurster.*

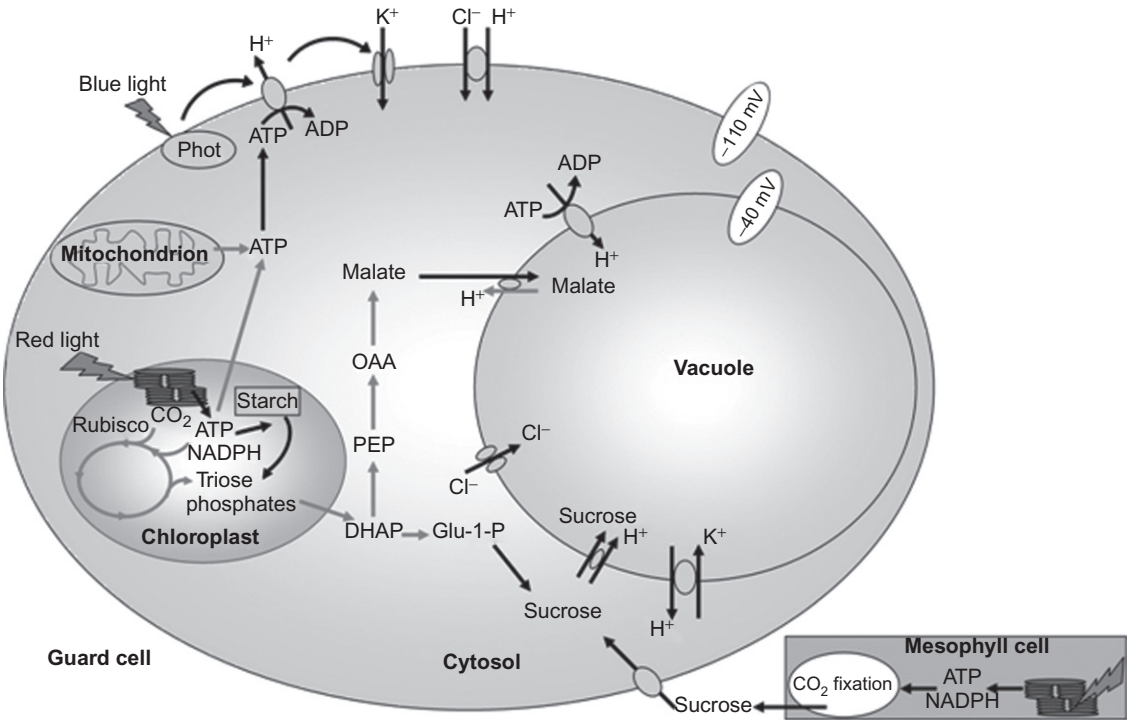


FIGURE 6.34 Schematic diagram of possible osmoregulatory pathways in guard cells for stomata opening. The diagram is not to scale. For explanations see text. Inspired by and redrawn from Roelfsema and Hedrich (2005) and Lawson (2009). DHAP = dihydroxyacetonphosphat; PEP = phosphoenolpyruvate; OAA = oxalacetate.

Light-induced accumulation of K^+ in the guard cell cytoplasm through inward-rectifying K^+ channels is driven by electrical potential differences established by a plasma membrane-bound proton pumping ATPase. Accordingly, stomata opening is preceded by a decrease in the pH of the guard cell apoplast (Edwards *et al.*, 1988). Against the electrical potential, K^+ is then pumped into the vacuole via an H^+-driven antiporter. The accumulation of K^+ in the vacuoles has to be balanced by a counter-anion, mainly $malate^{2-}$ or Cl^-, depending on the plant species and concentrations of Cl^- in the vicinity of the guard cells. In faba bean, K^+ can be counterbalanced exclusively by malate (Raschke and Humble, 1973). In epidermal cells of wheat leaves, on the other hand, Cl^- appears to be the counter-ion, because Cl^- concentrations are often substantially higher than in the mesophyll cells (Hodson and Sangster, 1988). The transport of Cl^- into the guard cell cytoplasm is mediated by a Cl^-/H^+ symporter at the plasma membrane and along the electrical potential across the tonoplast via anion channels into the vacuole (Fig. 6.34).

At low Cl^- availability, or in plant species which do not use Cl^- as accompanying anion for K^+ in guard cells, the H^+-driven K^+ influx activates PEP carboxylase in the cytoplasm. The synthesized $malate^{2-}$ in the guard cell cytosol is transported into the vacuole through anion channels and/or a malate carrier and serves as accompanying anion for K^+ in the vacuole. The C3 compound phosphoenolpyruvate (PEP) required for malate synthesis is supplied primarily via starch degradation in the guard cell chloroplasts (Outlaw and Manchester, 1979). In plant species such as onion, which lack starch in the guard cell chloroplasts, on the other hand, Cl^- is the main counter-ion for K^+influx, at least for stomatal regulation (Schnabl, 1980).

Sugars have been discussed as alternative osmotic solutes for stomatal opening (Tallman and Zeiger, 1988). Sugars may be produced directly through photosynthesis or starch degradation in the guard-cell chloroplasts or derive from uptake from the apoplast of sugars released into the guard-cell apoplast by mesophyll cells. They are taken up and loaded into the vacuole by sugar transporters (Fig. 6.34). However, the rate of sugar uptake and production in guard cells is insufficient to meet the high requirement for rapid stomatal opening (Reckmann *et al.*, 1990). Nevertheless, sugars may be important for the sustained opening of the stomata (Talbott and Zeiger, 1998), and particularly under K deficiency sugars may contribute substantially to osmoregulation in guard cells (Poffenroth *et al.*, 1992).

Closure of the stomata is induced by darkness, dehydration and ABA, and is associated with rapid efflux of K^+ and accompanying anions from the guard cells. Whereas stomata opening is based on active transport,

closure is due to the release of solutes along their concentration gradients via channels (Roelfsema and Hedrich, 2005). Stomatal closure is associated with a strong increase in K^+ and Cl^- concentrations in the apoplast of guard cells; for example, in *Commelina communis*, from 3 mM K^+ and 4.8 mM Cl^- in open stomata to 100 mM K^+ and 33 mM Cl^- in closed stomata (Bowling, 1987). In roots and shoots of angiosperm parasites such as *Striga* and *Loranthus*, stomata remain open permanently and do not respond to darkness, ABA or drought stress. This anomalous behaviour is caused by exceptionally high K^+ concentrations in the leaves of these parasites (which lack a phloem) and the lack of the capability of release of K^+ from the guard cells, required for stomata closure (Smith and Stewart, 1990).

Dark-induced stomata closure is initiated by a strong depolarization of the vacuolar and plasma membrane which activates the outward-rectifying K^+ and anion channels. The membrane depolarization is triggered by the cessation of the 'blue light' activation of H^+-ATPases and the 'red light'-dependent CO_2 assimilation giving rise to elevated intracellular CO_2 concentrations (Roelfsema and Hedrich, 2005).

The induction of stomatal closure by ABA derives from the roots via the xylem as 'non-hydraulic' signal (Davies and Meinzer, 1990; see also Chapter 5). However, endogenous ABA from guard cells may also serve this function; ABA concentrations in the guard cells are in the range of 2.5 mM compared with about 0.9 mM in other epidermal cells in faba bean (Brinckmann *et al.*, 1990). ABA-induced stomatal closure is triggered by plasma membrane depolarization via activation of anion channels (Roelfsema *et al.*, 2004), reduced H^+-ATPase activity (Brault *et al.*, 2004) and an increase in cytoplasmic Ca^{2+} concentration through stimulation of Ca^{2+} channels (Roelfsema and Hedrich, 2010).

It is unclear how sugars are released from the vacuole and cytoplasm of guard cells. Sugars are released rather slowly upon stimuli of stomata closure, particularly under K deficiency. This slow response of sugar-loaded guard cells is presumably the reason for the 'sluggish movement' and incomplete opening and closure of stomata in K-deficient plants (Hsiao and Läuchli, 1986). The incomplete closure of the stomata is responsible for the typical wilting of K-deficient plants exposed to drought stress.

6.6.6.3 Photonastic and Seismonastic Movements

In leaves of many plants, particularly in Leguminosae, leaves reorientate their laminae photonastically in response to light signals either to non-directional light signals (*circadian rhythm*, for example leaf blades folded in the dark and unfolded in the light), or directional light signals (e.g., reorientation towards the light source). These photonastic responses either increase light interception or allow avoidance of damage by excess light (Koller, 1990). The movements of leaves, and also of leaflets, are brought about by reversible turgor changes in specialized tissues, the motor organs (or pulvini). Turgor changes cause shrinking and swelling of cells in opposing regions (extensor and flexor) of the motor organ. The major solutes involved in osmoregulation are K^+, Cl^- and malate^{2-} inducing water flow through the membrane matrix and particularly aquaporins (Moshelion *et al.*, 2002) and thus volume change and leaflet movement (Satter *et al.*, 1988). The principles of the mechanisms responsible for stomata movement also apply to leaf and leaflet movement, only the scales are different, individual cells versus specialized tissues. Also, in contrast to guard cells, the extensor and the flexor regions in the motor organ respond to these signals in opposite ways (Moran, 2007).

In leaflet movement the driving force for K^+ influx in the flexor is also a plasma membrane-bound H^+-ATPase (Satter *et al.*, 1988) and, thus, leaflet movement can be prevented by anaerobiosis or vanadate (Antkowiak *et al.*, 1992). In the primary leaf pulvinus of *Phaseolus vulgaris* during circadian leaf movement, the concentration of H^+ and K^+ in the apoplast of the extensor change in opposite manner: at swelling (upward movement of the leaf lamina), the pH decreases from 6.7 to 5.9 and the K^+ concentration from 50 to 10 mM, and vice versa when the extensor cells shrink (Starrach and Mayer, 1989). The extensor cell walls have a particularly high cation exchange capacity and, thus, are an important reservoir of K^+ and H^+ (Starrach *et al.*, 1985). Similar to stomata movement, in the leaf movements, environmental signals (light, mechanical stimulation) activate Ca^{2+} channels in the plasma membrane and/or mobilize Ca^{2+} from internal stores and, thereby, increase cytosolic free Ca^{2+} concentrations in the flexor (Roblin *et al.*, 1989; Moyen *et al.*, 1995).

Although similar mechanisms are responsible for the movement of leaves and other plant parts in response to light and mechanical stimulus, there are differences in the speed of the response to *seismonastic* signals, for example in insectivorous plants or in *Mimosa*. In *Mimosa pudica*, the leaflets fold within a few seconds and reopen after about 30 min (Campbell and Thomson, 1977). This turgor-regulated response is correlated with redistribution of K^+ within the motor organ (Allen, 1969) and sudden release of sucrose from the phloem (Fromm and Eschrich, 1988). In seismonastic reactions, a rapid long-distance transport of the 'signal' from the touched leaflet to other leaflets also takes place. This 'signal' is an action potential, travelling in the phloem to the motor organs at a speed of 1–10 cm sec^{-1} inducing phloem unloading of sucrose in the motor organ (Fromm, 1991).

TABLE 6.28 Potato tuber yield, K concentration in leaves and percentage of leaves damaged by frost at different K supply. Average values of 14 locations

K supply (kg ha^{-1})	Tuber yield (tons ha^{-1})	K concentration in leaves (mg g^{-1} dw)	Percent of foliage damaged by frost (%)
0	2.39	24.4	30
42	2.72	27.6	16
84	2.87	30.0	7

Based on Grewal and Singh (1980).

6.6.7 Phloem Transport

Potassium has important functions in both the loading of sucrose and the rate of the mass flow-driven solute transport in the sieve tubes of the phloem (see also Chapter 3). This function of K^+ is related to two factors: (i) the necessity of maintaining a high pH in the sieve tubes for sucrose loading, and (ii) the contribution of K^+ to the osmotic potential in the sieve tubes and, thus, the transport rates of photosynthates from source to sink. The role of K^+ in phloem loading and assimilate partitioning is evident by comparing the relative distribution of non-structural carbohydrates between shoots and roots in K-sufficient compared to K-deficient plants (see Fig. 6.32). Similar to Mg deficiency but unlike P deficiency, assimilate transport to the roots is strongly reduced in K-deficient plants (Cakmak *et al*., 1994a). In K-sufficient plants within 90 min about half of the ^{14}C-labelled photosynthates are exported from the source leaf to other organs, with about 20% transported to the stalk as main storage organ in sugar cane. In contrast, in the K-deficient plants the export rates were much lower, even after 4 hours.

A lower assimilate transport to sinks is also evident in the reduced root growth in K-deficient plants (Cakmak, 1994; Cakmak *et al*., 1994b). Compared to K-deficient plants, root nodules in legumes with adequate K supply have a greater supply of sugars, which increases their rates of N_2 fixation and export of fixed N (Mengel *et al.*, 1974; Collins and Duke, 1981).

6.6.8 Energy Transfer

In addition to the role in assimilate transport in the phloem, K^+ circulating in the phloem may serve as a decentralized energy store that can be used to overcome local energy limitations induced by, for example, shading. This role of K is suggested by a study of the regulation of the K^+ channel AKT2 using an *Arabidopsis* knockout mutant grown under K and light-sufficient and limiting conditions (Gajdanowicz *et al*., 2010). AKT2 mediates K^+ uptake and release from the phloem which accompanies phloem loading and unloading of assimilates. Simulation of H^+, sucrose and K^+ transport in the phloem and phloem companion cells supports the conclusion that post-translational modification of AKT2 switches on a 'K power source' that assists the H^+-ATPase in generating the energy necessary to sustain transmembrane transport processes under energy-limiting conditions.

6.6.9 Cation–Anion Balance

In charge compensation, K^+ is the dominant cation for counterbalancing immobile anions in the cytoplasm, chloroplasts and quite often also for mobile anions in vacuoles, the xylem and the phloem. The accumulation of organic acid anions in plant tissues is often the consequence of K^+ transport without an accompanying anion into the cytoplasm (e.g., root or guard cells). The role of K^+ in the cation–anion balance is also reflected in nitrate metabolism, in which K^+ often is the dominant counter-ion for NO_3^- in long-distance transport in the xylem as well as for storage in vacuoles. As a consequence of NO_3^- reduction in leaves, the remaining K^+ requires the stoichiometric synthesis of organic acids for charge balance; part of this newly formed K-malate may be transported to the roots for subsequent utilization of K^+ as a counter-ion for NO_3^- within the root cells and for xylem transport (see also Chapter 3). In nodulated legumes, this recirculation of K^+ may serve a similar function in the xylem transport of amino acids (Jeschke *et al.*, 1985).

6.6.10 Stress Resistance

The frequently observed positive effects of K fertilization on crop yields under adverse conditions have been interpreted as evidence that K increases the resistance of plants against biotic (Prabhu *et al*., 2007) and abiotic stress (Cakmak, 2005). However, the evidence of enhanced stress resistance beyond the K nutritional status needed for optimum growth is not unequivocal in most cases. The frequently described higher K fertilizer requirement under low rainfall conditions does not reflect a higher K requirement of the plants, but is rather a reflection of the lower K uptake due to low K mobility in the soil at low water content (Kuchenbuch *et al*., 1986) which can be compensated for by higher K fertilizer rates.

However, it is well established that K-deficient plants are more susceptible to abiotic and biotic stresses. Examples are the enhanced plant injury of K-deficient

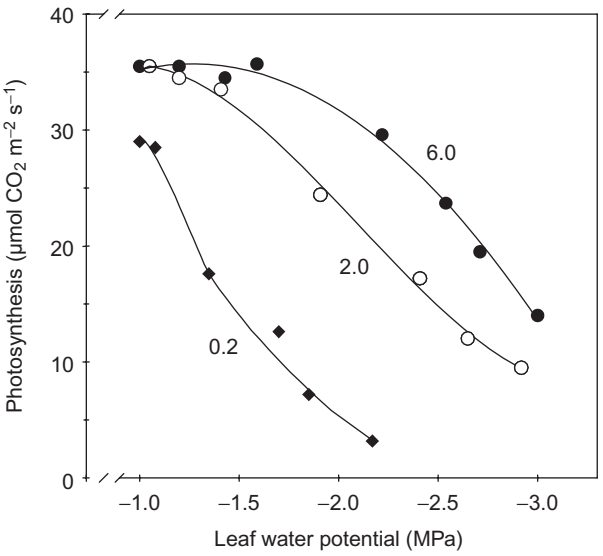


FIGURE 6.35 Photosynthesis of leaves at declining leaf water potentials in wheat at different K supply (mM). *Based on Sen Gupta et al. (1989).*

plants under high-light intensity (Marschner and Cakmak, 1989), drought (Sen Gupta *et al*., 1989; Fig. 6.35), low temperature (Grewal and Singh, 1980; Table 6.28), iron toxicity (Li *et al*., 2001) and pest and disease pressure (Amtmann *et al*., 2008; see also Chapter 10). Thus, when exposed to such stresses an optimum K nutritional status is critical for stress resistance of plants. One of the reasons for decreased stress resistance under K deficiency is an enhanced production of reactive oxygen species (ROS) which result in stress-induced oxidative stress (Cakmak, 2005). Potassium deficiency causes reduction in photosynthetic CO_2 fixation and assimilate transport, leading to enhanced transfer of photosynthetically produced electrons to O_2 and thus production of ROS. However, the lower drought sensitivity of K-sufficient plants appears to be due to additional factors (Lindhauer, 1985): (i) oxidative stress avoidance, (ii) stomata regulation, which is the major mechanism controlling the water regime of higher plants, and (iii) high osmotic pressure in the vacuoles, maintaining a high tissue water content even under drought conditions. Furthermore, drought resistance may require higher leaf K concentrations than required for optimal growth. The H^+/K^+ counterflow necessary for pH stabilization in the chloroplast stroma (Section 6.6.5) is impaired under drought stress. During dehydration, isolated chloroplasts lose large amounts of their K^+, and photosynthesis decreases; this decrease can be overcome by high extrachloroplastic concentrations of K^+ (Pier and Berkowitz, 1987). Similarly in intact plants, the decrease in photosynthesis under drought stress is less severe at high K^+ supply (Sen Gupta *et al*., 1989; Fig. 6.35). Supply of 2 mM K^+ resulted in maximal photosynthesis in well-watered plants but not under drought stress. The decrease in photosynthesis under drought stress was less severe in plants supplied with 6 mM K^+ (Fig. 6.35). This ameliorating effect of K was associated with higher leaf K concentrations (Pier and Berkowitz, 1987). The higher K requirement in leaves of plants exposed to drought or salinity stress (Chow *et al.*, 1990) is primarily caused by the necessity to maintain high stromal K^+ concentrations under these conditions (Sen Gupta *et al.*, 1989).

The greater frost damage to K-deficient plants (Larsen, 1976) is related to water deficiency at the cellular level. An example of this effect is shown by Grewal and Singh (1980; Table 6.28). Frost damage was negatively related to the K concentration of the leaves, at least in the range in which the increase in K is still correlated with an increase in tuber yield. Inadequate K supply is, therefore, one factor leading to an increase in the risk of frost damage.

Iron toxicity in K-deficient paddy rice plants is mainly related to the accumulation of low-molecular-weight organic metabolites and their enhanced release from the roots leading to a higher density of heterotrophic microorganisms in the rhizosphere. This impairs the oxidation capacity of the roots and increases the supply and uptake of Fe^{2+} causing Fe toxicity in the leaves (Trolldenier, 1977; Benckiser *et al*., 1984).

The greater susceptibility of K-deficient plants to pathogens and insects is thought to be due to changes in enzyme activities and metabolite concentrations leading to facilitated entry and development in the plant tissue. These results are, so far, difficult to reconcile with the molecular characterization of the response of *Arabidopsis* to K deficiency (Amtmann *et al*., 2008). They show that K deficiency induces not only the expression of high-affinity K^+ transporters and enhances root-hair elongation mediated by ethylene and ROS, but also activates signalling cascades similar to drought, wounding and biotic stresses involving phytohormones and jasmonic and salicylic acids (Ashley *et al*., 2006; Amtmann *et al*., 2008); the latter two being important for pathogen resistance. Thus a higher stress resistance of K-deficient plants could have been expected.

6.6.11 K Supply, Plant Growth and Plant Composition

After N, K is the nutrient required in the largest amount by plants. The K requirement for optimal plant growth is 20–50 g kg^{-1} in vegetative parts, fleshy fruits and tubers. In natrophilic species, however, the requirement for K^+ can be lower because in these plant species K^+ can be replaced by Na^+. When K is deficient, growth is retarded, and net transport of K^+ from mature leaves and stems is enhanced. Under severe deficiency these organs become chlorotic

and necrotic, depending on the light intensity to which the leaves are exposed (Marschner and Cakmak, 1989). Also lignification of vascular bundles is impaired (Pissarek, 1973), a factor which may contribute to the higher susceptibility of K-deficient plants to lodging.

The changes in composition also affect the nutritional and technological (processing) quality of harvested products. This is most obvious in fleshy fruits and tubers with their high K requirement. In tomato fruits, for example, the incidence of so-called ripening disorders ('greenback') increases with inadequate K supply (Lune and Goor, 1977), and in potato tubers a whole range of quality criteria are affected by the K concentration of the tuber tissue (see also Chapter 9).

By increasing the K supply to plant roots it is relatively easy to increase the K concentration of various organs except grains and seeds, which maintain a relatively constant K concentration of $3\,g\,kg^{-1}$. When the K supply is abundant, 'luxury consumption' of K often occurs in vegetative tissues and fleshy reproductive organs, which deserves attention for its possible interference with the uptake and physiological availability of Mg and Ca and thus the incidence of K-induced deficiencies.

Chapter 7

Function of Nutrients: Micronutrients

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SUMMARY

In this chapter, the functions of iron, manganese, copper, zinc, nickel, molybdenum, boron and chlorine in plants are discussed. Iron (Fe) plays a crucial role in redox systems in cells and in various enzymes. The strategies of plants to acquire Fe in dicotyledonous and graminaceous plants are described. Manganese (Mn) and copper (Cu) are important for redox systems, as activators of various enzymes including those involved in the detoxification of superoxide radicals, and for the synthesis of lignin. Zinc (Zn) plays a role in the detoxification of superoxide radicals, membrane integrity as well as the synthesis of proteins and the phytohormone IAA. Nickel (Ni) is involved in N metabolism as metal component of the enzyme urease. Molybdenum (Mo) is important for N metabolism as metal component of the nitrogenase (N_2 fixation) and nitrate reductase enzymes. Boron (B) is crucial for cell wall and membrane integrity whereas chlorine plays a role in osmoregulation and stomata movement. For each micronutrient, the effects of deficiency and toxicity are described.

7.1 IRON

7.1.1 General

Iron is the second most abundant metal in the earth's crust after aluminium. Solubility of Fe is, however, extremely low, especially in aerated alkaline soils. In aerated systems in the physiological pH range, the concentrations of ionic Fe^{3+} and Fe^{2+} are below 10^{-15}M due to formation of Fe hydroxides, oxyhydroxides and oxides (Lemanceau *et al*., 2007). Chelates of Fe(III) and occasionally of Fe(II) are therefore the dominant forms of soluble Fe in soil and nutrient solutions. As a rule, Fe(II) is taken up preferentially compared with Fe(III), but this also depends on the plant species (Strategies I and II, Chapters 2 and 14). In long-distance transport in the xylem, there is a predominance of Fe(III) complexes (Chapter 3).

As a transition element, Fe is characterized by the relative ease by which it may change its oxidation state:

$$Fe^{3+} \leftrightarrow Fe^{2+}$$

and by its ability to form octahedral complexes with various ligands. Depending on the ligand, the redox potential of Fe(II/III) varies widely. This variability explains the importance of Fe biological redox systems. Due to the high affinity of Fe for various ligands (e.g., organic acids or inorganic phosphate) ionic Fe^{3+} or Fe^{2+} do not play a role in short- or long-distance transport in plants. In aerobic systems many low-molecular-weight iron chelates, and free iron in particular (either Fe^{3+} or Fe^{2+}), produce reactive oxygen species (ROS) such as superoxide radical and hydroxyl radical (Halliwell and Gutteridge, 1986; Halliwell, 2009) and related compounds, for example:

$$O_2 + Fe^{2+} \rightarrow O_2{\cdot}^- + Fe^{3+}$$

or in the Fenton reaction:

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH\cdot$$

or in the Haber-Weiss reaction:

$$O_2{\cdot}^- + H_2O_2 \xrightarrow{Fe} O_2 + OH\cdot + OH^-$$

These radicals are highly toxic and responsible for peroxidation of polyunsaturated fatty acids of membrane lipids and proteins. To prevent oxidative cell damage, Fe has to be either tightly bound or incorporated into structures (e.g., heme and non-heme proteins) which allow controlled reversible oxidation–reduction reactions.

$$Fe(II) \underset{+e^-}{\overset{-e^-}{\rightleftharpoons}} Fe(III)$$

including those in antioxidant protection.

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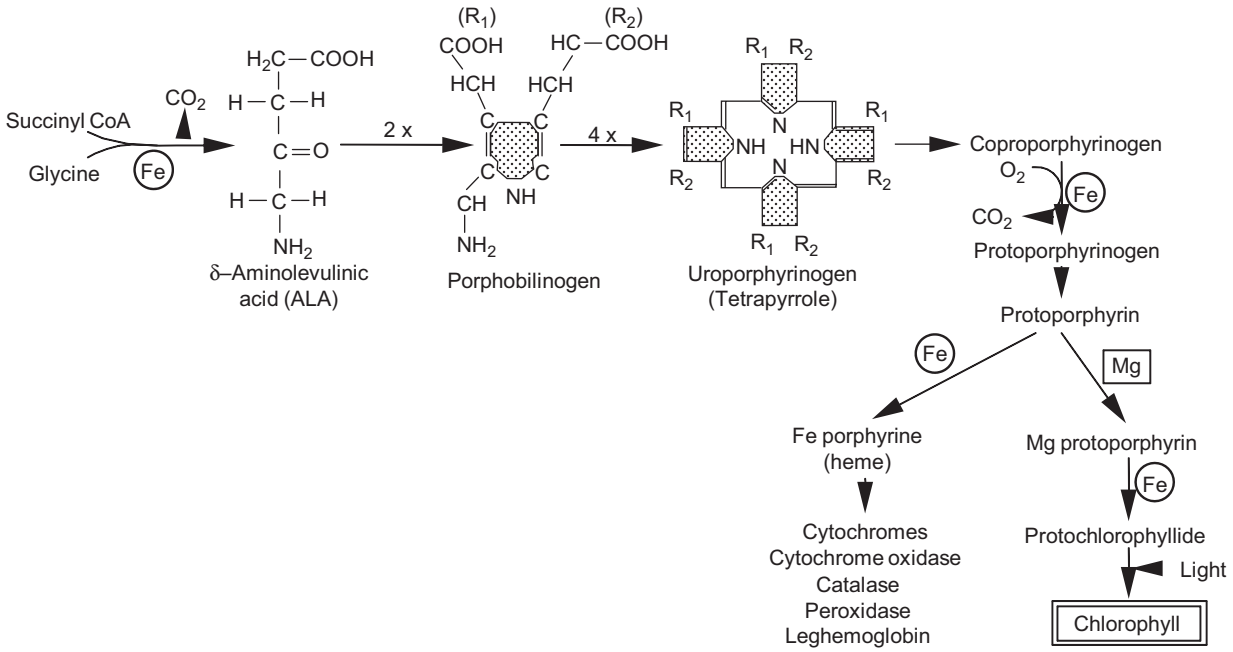


FIGURE 7.1 Role of Fe in the biosynthesis of heme coenzymes and chlorophyll.

7.1.2 Iron-containing Constituents of Redox Systems

7.1.2.1 Heme Proteins

The most well-known heme proteins are the cytochromes, which contain a heme Fe–porphyrin complex (Fig. 7.1) as a prosthetic group. Cytochromes are constituents of the redox systems in chloroplasts, in mitochondria and also a component in the redox chain in nitrate reductase. The particular role of Fe in leghemoglobin and nitrogenase is discussed in Chapter 16. Small amounts of leghemoglobin may also be present in the roots of plants which are not capable of forming root nodules (Appleby *et al.*, 1988). This leghemoglobin may act as signal molecule indicating O_2 deficiency; initiating a metabolic shift towards fermentation.

Other heme enzymes are catalase and peroxidases which are susceptible to low supply of Fe. Under conditions of Fe deficiency, the activity of both enzymes rapidly decreases in plant tissues, particularly catalase in genotypes susceptible to Fe deficiency, for example tomato (Table 7.1). Despite similar leaf Fe concentrations under low Fe supply, catalase activity was lower in the susceptible tomato (inefficient) genotype than in the tolerant (efficient) genotype. The activity of this enzyme is, therefore, an indicator of the Fe nutritional status of plants (Chapter 11). Similarly, the activity of ascorbate peroxidase decreases at low Fe supply in the inefficient genotype (Table 7.1).

Catalase facilitates detoxification of H_2O_2 to water and O_2 according to the reaction:

$$H_2O_2 \rightarrow H_2O + 1/2\,O_2$$

The enzyme plays an important role in association with superoxide dismutase, as well as in photorespiration and the glycolate pathway.

Various isoenzymes of peroxidases are present in plants. They catalyse the following reactions:

$$XH_2 + H_2O_2 \rightarrow X + 2H_2O$$

and

$$XH + XH + H_2O_2 \rightarrow X\text{---}X + 2H_2O$$

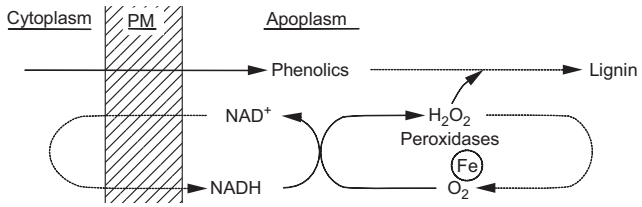
An example of the first type of reaction is the detoxification of H_2O_2 in chloroplasts catalysed by ascorbate peroxidase. In the second type of reaction, cell wall-bound peroxidases catalyse the polymerization of phenols to lignin. The alterations in cell wall formation of rhizodermal cells under Fe deficiency (see Fig. 7.5) may be related to impaired peroxidase activity. Peroxidases are abundant in cell walls of the epidermis (Hendricks and Van Loon, 1990) and rhizodermis (Codignola *et al.*, 1989) and are required for biosynthesis of lignin and suberin. Both synthetic pathways require phenolic compounds and H_2O_2 as substrates. The formation of H_2O_2 is catalysed by the oxidation of NADH at the plasma membrane/cell wall

TABLE 7.1 Fe concentrations and activities of H_2O_2-scavenging enzymes in leaves of tomato genotypes target (Fe-inefficient) and pakmor (Fe-efficient) grown 50 days in nutrient solution with low and sufficient Fe supply

	Target (Fe-inefficient)		Pakmore (Fe-efficient)	
	Sufficient Fe	Low Fe	Sufficient Fe	Low Fe
Leaf Fe concentration (μg g^{-1}dw)	226	21	200	21
Enzyme activity (μmol g^{-1} fw min^{-1})				
Catalase	198	35	244	63
Guaiacol proxidase	412	136	304	214
Ascorbate peroxidase	613	133	584	192

Based on Dasgan *et al.* (2003).

interface (Mäder and Füssl, 1982). The principles of these reactions are as follows:



In Fe-deficient roots, peroxidase activity is strongly depressed (Sijmons *et al.*, 1985; Ranieri *et al.*, 2001). Consequently, H_2O_2 production is increased (Ranieri *et al.*, 2001) and phenolics accumulate (Römheld and Marschner, 1981a). Phenolics are also released at higher rates from the roots of Fe-deficient compared with Fe-sufficient plants (Hether *et al.*, 1984; Marschner *et al.*, 1986a; Jin *et al.*, 2007). Certain phenolics, such as caffeic acid, are very effective in chelation and reduction of inorganic Fe(III), and a component of Strategy I in Fe acquisition (Chapter 2). In response to Fe deficiency, red clover releases high amounts of phenolics which contribute to utilization and remobilization of root apolastic Fe (Jin *et al*., 2007).

7.1.2.2 Fe-S Proteins

In the non-heme Fe-S proteins, Fe is coordinated to the thiol group of cysteine or to inorganic S as clusters, or to both. The most well-known Fe-S protein is ferredoxin, which acts as an electron transmitter in a number of metabolic processes according to the principle:

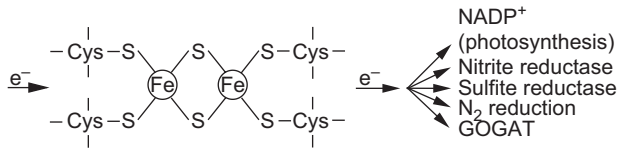


TABLE 7.2 Concentration of chlorophyll and ferredoxin and nitrate reductase activity in citrus leaves with different Fe concentration

Fe concentration (μg g^{-1} dw)	Chlorophyll (mg g^{-1} dw)	Ferredoxin (mg g^{-1} dw)	Nitrate reductase (nmol NO_2 g^{-1} fw h^{-1})
96	1.80	0.82	937
62	1.15	0.44	408
47	0.55	0.35	310
47→81[a]	–	0.63	943

Based on Alcaraz *et al.* (1986).
[a] *40 h after infiltration of intact Fe-deficient leaves with 0.2% $FeSO_4$.*

Details of the function of ferredoxin in these processes are discussed in the relevant sections. In Fe-deficient leaves, the concentrations of ferredoxin and chlorophyll are decreased to a similar extent (Table 7.2) with the low ferredoxin concentration correlated with lower nitrate reductase activity (NRA). Both ferredoxin concentration and NRA can be restored by resupplying Fe. Due to the involvement of Fe at various steps in nitrate reduction (Section 6.1), positive correlations between Fe supply, ferredoxin concentration and nitrate reduction are to be expected.

Another example of Fe-S proteins are the isoenzymes of superoxide dismutase (SOD) which contain Fe as a metal component of the prosthetic group (FeSOD). Superoxide dismutases detoxify superoxide anion free radicals ($O_2\cdot^-$) by formation of H_2O_2 and may contain Cu, Zn, Mn or Fe as metal components (Fridovich, 1983; Sevilla *et al.*, 1984). In chloroplasts, FeSOD is the main

TABLE 7.3 Relationship between Fe supply, chlorophyll concentration in leaves and organic acid concentration in roots of oats

Treatment	Chlorophyll concentration (relative)	Organic acid concentration (μg (10 g)$^{-1}$ fw)			
		Malic	Citric	Other	Total
+Fe	100	39	11	23	73
−Fe	12	93	67	78	238

Based on Landsberg (1981).

isoenzyme of SOD (Kwiatowsky *et al.*, 1985), but it may also occur in mitochondria and peroxisomes in the cytoplasm (Droillard and Paulin, 1990). In Fe-deficient plants, FeSOD activity is low (Iturbe-Ormaetxe, 1995), whereas the activity of CuZnSOD is increased, resulting in high production of H_2O_2 (Tewari *et al.*, 2005). Although Fe-deficient plants have reduced levels of antioxidative enzymes such as catalase and ascorbate peroxidase and increased concentrations of H_2O_2, there does not appear to be enhanced oxidative cell damage (e.g., lipid peroxidation) (Raineri *et al*., 2001), which may be due to the very low concentrations of active Fe required for ROS generation through the Haber-Weiss and/or Fenton reactions.

Iron deficiency stress is associated with enhanced production of organic acids, particularly citrate. Reduced aconitase activity may explain the enhanced production of organic acids in Fe-deficient plant tissues. Aconitase is an Fe-S protein (Broquisse *et al.*, 1986) which catalyses the isomeration of citrate to isocitrate in the tricarboxylic acid cycle. Iron as metal component of the prosthetic group is required for stability and activity of the enzyme (Hsu and Miller, 1968), and the Fe cluster of the enzyme is responsible for the spatial orientation of the substrates (citrate and isocitrate); valency changes are not involved in the reaction (Beinert and Kennedy, 1987). In Fe-deficient plants, aconitase activity is lower (De Vos *et al.*, 1986), and reactions in the tricarboxylic acid cycle are disturbed leading to organic acids, particularly citric and malic acid (Table 7.3). In roots of Fe-deficient Strategy I and Strategy II plants, citrate concentrations were 3.7- to 8.8-fold and 3.8- and 11.1-fold higher, respectively (Abadía *et al.*, 2002). Similar increases in concentration of organic acids were also found in xylem exudates and leaf apoplasmic fluids of Fe-deficient plants (Nikolic and Römheld, 1999; López-Millán *et al*., 2000). Such high citrate concentrations in the xylem may indicate Fe transport as stable, water soluble Fe-citrate complexes. In roots of Fe-deficient tomato plants, the increase in organic acid concentration is correlated with enhanced CO_2 dark fixation and net excretion of H^+, i.e. acidification of the rhizosphere (Miller *et al.*, 1990). The relationship between lower aconitase activity and organic acid accumulation in roots of Fe-deficient plants are still a matter of controversy (De Vos *et al.*, 1986; Pich *et al.*, 1991). Iron deficiency-induced CO_2 fixation and high PEPC activity in root cells may be major reasons for accumulation of organic acids in Fe-deficient plants (Abadia *et al.*, 2002). López-Millán *et al.* (2009) showed that Fe deficiency resulted in a 10-fold increase in PEPC activity in tomato root tip extracts which were associated with increased citrate concentration by about 20-fold in roots and 17-fold xylem sap. Increased PEPC activity may also be linked to Fe deficiency-induced root adaptive responses like proton release and Fe reduction capacity (Rambolla *et al.*, 2002; M'sehli *et al*., 2009).

Recently, existence of a tri-Fe (III), tri-citrate complex (Fe3Cits) in xylem exudates of tomato plants was shown (Rellan-Alvarez *et al*., 2010), confirming previous speculations that Fe is transported in xylem in form of Fe–citrate complex.

Riboflavin also accumulates in most dicotyledenous plant species under Fe deficiency, and its release from roots may be enhanced by a factor of 200 in Fe-deficient plants (Welkie and Miller, 1989; Andaluz *et al*., 2009). Increased root concentrations of riboflavin are associated with the activity of 6,7-dimethyl-8-ribityllumazine synthase which contributes to the final step of riboflavin biosynthesis (Andaluz *et al*., 2009). Accumulation of riboflavin is presumably the result of alterations in purine metabolism due to impairment of xanthine oxidase (Schlee *et al.*, 1968), another enzyme with Fe-S clusters as a prosthetic group.

7.1.3 Other Fe-requiring Enzymes

There are a number of less well-characterized enzymes in which Fe acts either as a metal component in redox reactions or as a bridging element between enzyme and substrate. In Fe-deficient plants, the activities of some of these enzymes are low which may result in gross changes in metabolic processes.

Methionine is the principal precursor for biosynthesis of ethylene. Along the biosynthetic pathway in the

conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene, a two-step one-electron oxidation takes place, catalysed by Fe(II) (see Fig. 7.6). Accordingly, ethylene formation is very low in Fe-deficient cells and is restored immediately upon resupply of Fe, without the involvement of protein synthesis (Bouzayen *et al.*, 1991).

Lipoxygenases are enzymes containing one atom Fe per molecule (Hildebrand, 1989), which catalyse the peroxidation of linolic and linolenic acid, i.e. of long chain polyunsaturated fatty acids which are important components of cell membranes. Hence, high lipoxygenase activity is typical for fast growing tissues and organs, and may be critical for membrane stability.

Low chlorophyll concentration (chlorosis) of young leaves is the most obvious visible symptom of Fe deficiency. Various factors are responsible for this decrease, the most direct one being the role of Fe in the biosynthesis of chlorophyll (Fig. 7.1). The common precursor of chlorophyll and heme synthesis is aminolevulinic acid (ALA), and the rate of ALA formation is controlled by Fe (Pushnik and Miller, 1989). Iron is also required for the formation of proto-chlorophyllide from Mg-protoporphyrin (Fig. 7.1). Feeding ALA to Fe-deficient leaf tissue leads to an increase in the Mg-protoporphyrin concentration whereas the protochlorophyllide and chlorophyll concentrations remain low compared to those in leaf tissue adequately supplied with Fe (Spiller *et al.*, 1982; Pushnik *et al.*, 1984).

7.1.4 Chloroplast Development and Photosynthesis

As a rule, Fe deficiency has less effect on leaf growth, cell number per unit area, or number of chloroplasts per cell than on the size of the chloroplasts and protein content per chloroplast (Table 7.4). Iron is required for protein synthesis, and the number of ribosomes – the sites of protein synthesis – decrease in Fe-deficient leaf cells (Lin and Stocking, 1978). In Fe-deficient maize leaves, for example, the total protein content decreases by 25% but that of the chloroplasts by 82% (Perur *et al.*, 1961), most probably because of a particular high Fe requirement of chloroplastic mRNA and rRNA (Spiller *et al.*, 1987). In sugar beet leaves, Fe is important for RNA synthesis and a decrease in Fe concentration is associated with a strong decrease in protein synthesis (Nishio et al., 1985). Decreases in leaf protein content under Fe deficiency are particularly pronounced for the Rubisco protein that represents nearly 50% of the chloroplast soluble proteins (Ellis, 1979; see also Table 7.6)

In the thylakoid membranes, about 20 Fe atoms are directly involved in the electron transport chain. Photosystem (PS) I is a strong sink for Fe due to its higher Fe content (12 atoms of Fe per complex) compared to PS II (3 atoms of Fe per complex) and the Cyt *bf* complex (5 atoms of Fe per complex) (Raven et al., 1999). The high

TABLE 7.4 Properties of leaves of sugar beet with sufficient and mild or severe Fe deficiency

Parameter	Control	Mild deficiency	Severe deficiency
Chlorophyll concentration ($mg\,cm^{-2}$)	>40	20–40	<20
Soluble protein (mg cm^{-2} leaf area)	0.57	0.56	0.53
Mean leaf cell volume ($10^{-8}\,cm^3$)	2.64	2.78	2.75
Chloroplasts (no. $cell^{-1}$)	72	77	83
Chloroplast volume (μm^3)	42	37	21
Protein N (pg $chloroplast^{-1}$)	1.88	1.34	1.24

From Terry (1980).

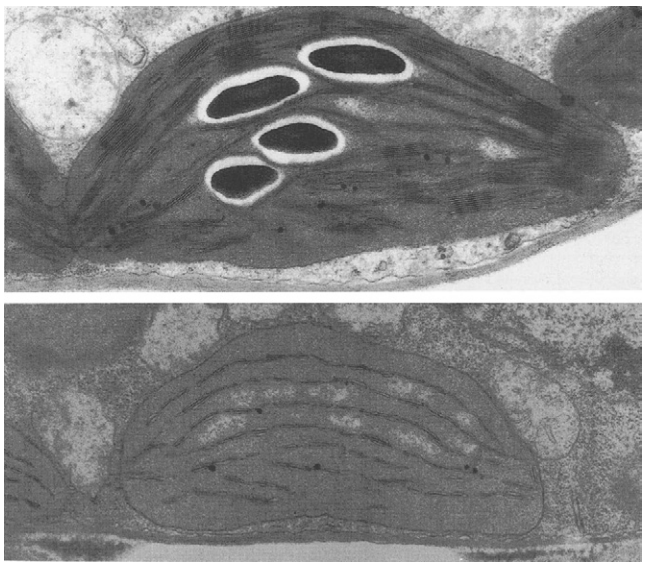


FIGURE 7.2 Fine structure of chloroplasts from Fe-sufficient (*top*) and Fe-deficient (*bottom*) soybean (*Glycine max.* L.) plants (×24,000). *Courtesy of Ch. Hecht-Buchholz.*

Fe requirement for the structural and functional integrity of the thylakoid membranes, and the additional Fe requirement for ferredoxin and the biosynthesis of chlorophyll explain the particular sensitivity of chloroplasts in general, and the thylakoids in particular, to Fe deficiency (Fig. 7.2). In Fe-deficient leaves, however, not all photosynthetic pigments and components of the electron transport chain are decreased to the same extent (Table 7.5). The activity of PS I is more depressed than of PS II under Fe deficiency,

TABLE 7.5 Concentrations of Fe, chlorophyll and components of photosystem I (PS I) and photosynthetic electron transport capacity of PS II and PS I with sufficient or deficient Fe supply

	Fe	Chlorophyll	PS I components			Fe-transport capacity ($\mu eq\,cm^{-2}\,leaf\,h^{-1}$)	
	($\mu g\,cm^{-2}$ leaf)		P700	Cytochromes ($pmol\,cm^{-2}$)	Protein ($\mu g\,cm^{-2}$)	PS II	PS II
+Fe	1.44	89	545	599	108	56	840
−Fe	0.25	26	220	201	38	30	390
−Fe +Fe[a]	1.16	24	474	474	79	36	764

Recalculated from Pushnik and Miller (1989).
[a] *10 days after foliar application of Fe.*

TABLE 7.6 Concentration of chlorophyll and carotenoids and maximum velocity of rubisco carboxylation ($V_{c\,max}$) in leaves of hydroponically grown sugar beet and field-grown pear and peach plants as affected by Fe deficiency

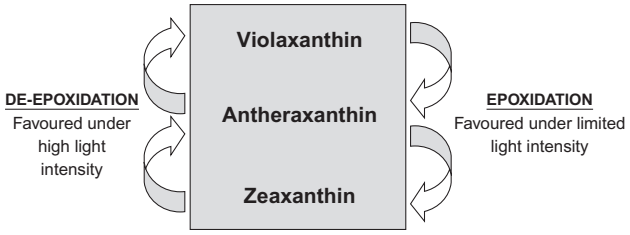
		Total carotenoids	Chlorophyll		$V_{c\,max}$
Species	Fe supply	$\mu mol\,m^{-2}$	a + b	a/b	$\mu mol\,m^{-2}\,s^{-1}$
Sugar beet	+Fe	126	389	3.7	21.7
	Severe −Fe	34	79	4.6	11.9
	Extreme −Fe	19	32	5.7	6.7
Pear	+Fe	95	248	3.5	131.4
	Severe −Fe	60	83	5.7	79.7
	Extreme −Fe	26	24	5.9	17.2
Peach	+Fe	103	199	3.7	56.2
	Severe −Fe	50	70	4.8	14.6
	Extreme −Fe	29	37	4.9	7.8

Based on Larbi *et al.* (2006).

probably due to a higher amount of Fe per PS I than PS II (Table 7.5). The Cyt *bf* complex involved in electron and proton transfer in photosynthetic organisms is also decreased under Fe deficiency, but at a lesser degree than PS I (Nishio *et al.*, 1985). Resupplying Fe to chlorotic leaves increases the function of PS I as an electron transmitter more strongly than that of PS II. As Fe deficiency becomes more severe, the activity of PS II also decreases and is more difficult to restore (Table 7.4; Morales *et al.*, 1991).

Generally, carotenoids are less affected than chlorophylls, and chlorophyll a is more sensitive to Fe deficiency than chlorophyll b, leading to higher chlorophyll b/chlorophyll a ratios in Fe-deficient leaf tissues as shown in Table 7.6. The amount and composition of xanthophyll cycle pigments are also markedly affected from Fe nutritional status of plants. With Fe deficiency, there is a significant increase in the de-epoxidized xanthophyll pigments zeaxanthine and violaxanthin, while the epoxidated form violaxanthin declines greatly, especially under high light intensity (Timperio *et al.*, 2007). In a short-term experiment, it has been shown that about 70% of the total xanthophylls is converted to de-epoxidized forms (A + Z) in Fe-deficient leaves when exposed to a high irradiation for 3 h, while the extent of the de-epoxidation was only 40%

in the Fe-adequate plants (Jiang *et al*., 2001). Resupply of Fe to Fe-deficient plants rapidly increased the concentration of the epoxidated form violaxanthin at the expense of zeaxanthin (Larbi *et al*., 2004). Xanthophyll cycle pigments have photoprotective effects in chloroplasts by coping with excess light energy through conversion of zeaxanthin to violaxanthin:



Under Fe deficiency, leaves generally have low photosynthetic activity due to several reasons discussed below; but they absorb more light energy per chlorophyll molecule than required for photosynthesis, especially under high radiation (Abadía *et al*., 1999). This results in a high risk for photoinhibitory and photooxidative damages in Fe-deficient leaves. Nevertheless, in contrast to Zn-deficient or Mg-deficient plants, there appears to be little photooxidative damage in Fe-deficient plants. Absence of serious photoxidative damage in Fe-deficient leaves is, most probably, related to the rapid increases in levels of de-epoxidized xanthophyll pigments and the low concentrations of catalytic Fe required in ROS generation.

Iron-deficient leaves are characterized by low concentrations of starch and sugars (Arulanathan *et al.*, 1990). This is to be expected due to the low concentrations of chlorophyll and ferredoxin, impairment of photosynthetic electron transport and the decreased regeneration of reduced ferredoxin. Reduction in photosynthesis is a characteristic physiological response of plants to Fe deficiency. As presented in Fig. 7.3, Fe-deficient plants respond to Fe resupply by a rapid increase in photosynthesis. Decrease in photosynthesis under Fe deficiency is attributed to reduced photosynthetic electron transport and thus impaired carboxylation due to low availability of ATP and NADPH for the Calvin cycle (Table 7.6). The low concentration of Rubisco protein is a further important reason for the low photosynthesis in Fe-deficient plants (Larbi *et al*., 2004, 2006; Timperio *et al.*, 2007).

7.1.5 Localization and Binding State of Fe

When plants are grown under controlled conditions, about 80% of the Fe is localized in the chloroplasts in rapidly growing leaves, regardless of Fe nutritional status (Fig. 7.4). With Fe deficiency, a shift in the distribution of Fe occurs only within the chloroplasts, whereby the lamellar Fe concentration increases at the expense of the stroma Fe.

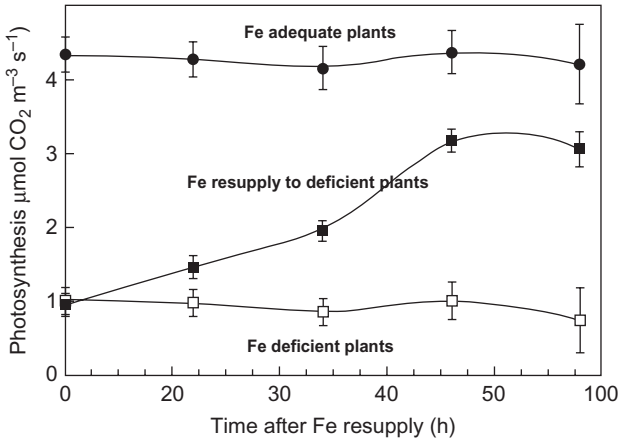


FIGURE 7.3 Photosynthesis in Fe-deficient (open squares), Fe-sufficient (solid circles) and Fe-deficient plants resupplied with Fe (solid squares) in hydroponically grown sugar beet plants. *Based on Larbi* et al.*, 2004.*

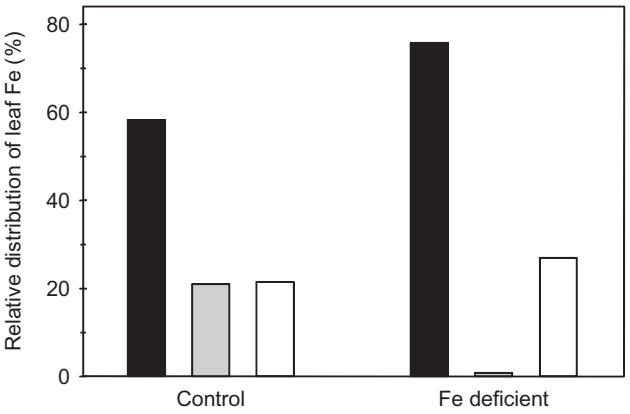


FIGURE 7.4 Intercelluar distribution of Fe in leaf blades of Fe-suficient and Fe-deficient sugar beet plants. Solid bars: lamellar Fe; grey bars: stromal Fe; white bars: extra-chloroplastic Fe. *Redrawn from Terry and Low, 1982.*

Iron can be stored in the stroma of plastids as phytoferritin (plant ferritin). It consists of a hollow protein shell which can store up to 5,000 atoms of iron as Fe(III) (Fe content 12–23% dw). Phytoferritin often has a well-defined crystalline form with the proposed formula $(FeO.OH)_8$. $(FeO.OPO_3H_2)$ (Seckbach, 1982). Its concentration is high in dark-grown leaves (up to 50% of the total Fe), but it rapidly disappears during regreening (Mark *et al.*, 1981) and is very low in green leaves. In young leaf tissues, ferritin-bound Fe represents an important Fe source for biosynthesis of Fe-containing proteins in photosynthesis (Briat *et al*., 2010). Ferritin is a vital compound in maintenance of Fe homeostasis and protection against oxidative damage. By sequestration of large amounts of Fe, ferritin exerts a critical protective role against peroxidative cell damage catalysed by Fe-induced

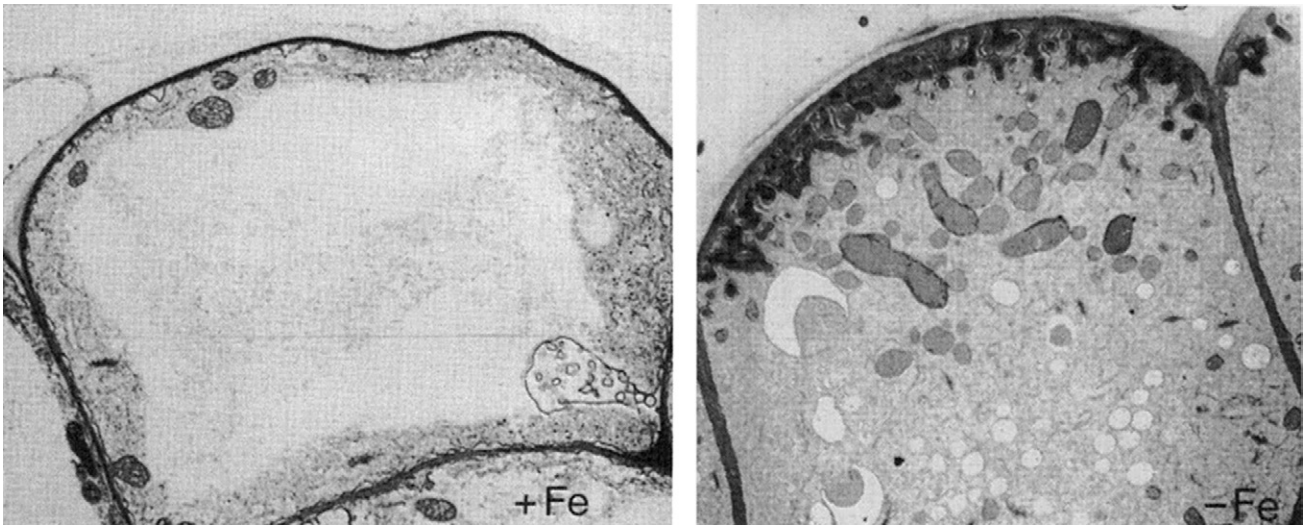


FIGURE 7.5 Sections of rhizodermal cells of sunflower. (*Left*) Fe-sufficient. (*Right*) Fe-deficient. *Courtesy of D. Kramer.*

formation of ROS (Ravet *et al.*, 2009; Briat *et al.*, 2010). In plants without ferritin, ROS accumulate resulting in impairment of plant growth and development (Ravet *et al*., 2009). Ferritin is not only present in chloroplasts: it can also be found in the xylem and phloem (Smith et al., 1984). Additionally, ferritin is abundant in seeds. In pea plants, ferritin-bound Fe represented 92% of the total Fe in seed embryos, indicating that ferritin is probably a major form of Fe storage in seeds (Marentes and Grusak, 1998). However, there is a large genetic variation in seed concentration of ferritin-bound Fe among plant species. In legume species, ferritin-Fe concentration ranges from 15% of the total Fe in kidney beans up to 69% in lentils (Hoppler *et al.*, 2009). During seed germination, ferritin is rapidly degraded, probably catalysed by the released Fe^{2+} and generation of hydroxyl radicals which destroy the protein shell (Bienfait, 1989; Lobreaux and Briat, 1991). Phytoferritin may also act as storage for Fe in nodules of legumes, for heme synthesis during nodule development and heme degradation during senescence (Ko *et al.*, 1987).

Bioavailability of Fe in seeds or grains is an important issue for nutritional quality and human nutrition (see Chapter 9). Iron from ferritin in soybean and wheat seeds is bioavailable and absorbed well and suggests that ferritin Fe is a valuable dietary source and could be a target compound for biofortification of food crops with Fe (Loennerdahl, 2009; Zhao *et al.*, 2010; Wirth *et al.*, 2009). Phytate is abundant in seeds and can bind Fe. Phytate has a high binding affinity to Fe and forms insoluble complexes with Fe (Minihane and Rimbach, 2002) Therefore phytate-rich diets (e.g., cereal-based foods) may be a key factor in high prevalence of Fe deficiency in humans (see Chapter 9) (Hurrell and Egli, 2010).

If plants are grown under controlled conditions (e.g., in nutrient solutions), there is a close positive correlation between total leaf concentration of Fe and that of chlorophyll when the supply of Fe (as chelates) is suboptimal (Römheld and Marschner, 1981a; Terry and Abadia, 1986). This correlation, however, is often poor or absent in plants grown in calcareous soils (Mengel, 1994b; Römheld, 2000) where the Fe concentration in chlorotic leaves may be similar to or even higher than that in green leaves. This phenomenon has been termed 'chlorosis paradox' (Römheld, 2000). Previously, inactivation of Fe in chlorotic leaves of plants grown in calcareous soils has been discussed as a plausible explanation for the same or even higher Fe concentrations in chlorotic than green leaves (Mengel, 1994b). However, inactivation of Fe in leaf tissue could not be detected in later studies (Nikolic and Römheld, 1999, 2002). Instead, the high Fe concentrations in chlorotic young leaf tissues may be the result of restricted leaf expansion growth and consequently diminished dilution of Fe concentrations by growth (Morales *et al.*, 1998; Römheld, 2000).

7.1.6 Root Responses to Fe Deficiency

In leaves, the major symptom of Fe deficiency is inhibition of chloroplast development. For roots, however, Fe deficiency induces morphological and physiological changes which depend upon plant species (Strategies I and II, Chapter 2). In dicotyledonous and monocotyledonous plant species, with the exception of the grasses (graminaceous species), Fe deficiency is associated with inhibition of root elongation, increase in the diameter of apical root zones, and abundant root hair formation (Römheld and Marschner, 1981a; Chaney *et al.*, 1992b; Schmidt, 2003). These morphological changes are often associated with

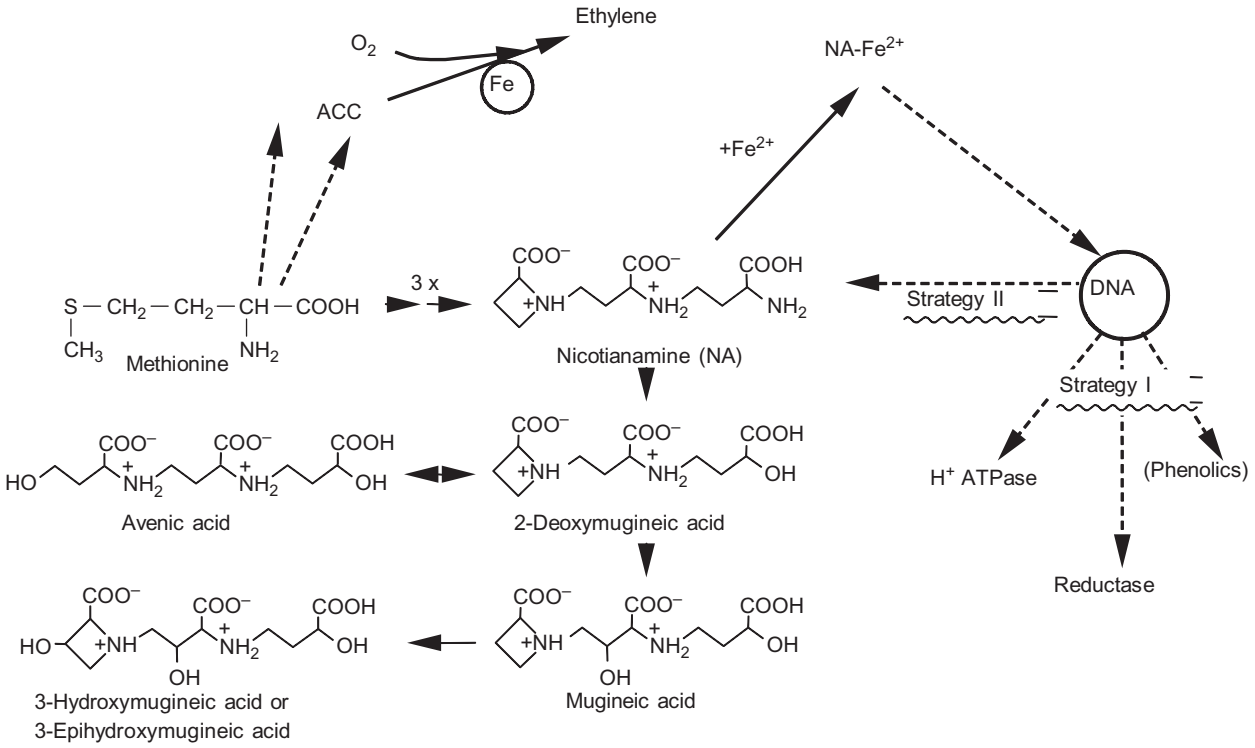


FIGURE 7.6 Model of phytosiderophore biosynthesis and other Fe-related factors in roots. *Based on Shojima* et al.*, 1989 and Scholz* et al.*, 1992.*

the formation of cells with a distinct wall labyrinth typical of transfer cells (Fig. 7.5). These transfer cells may be induced either in the rhizodermis (Fig. 7.6) or in the hypodermis (Landsberg, 1989). The Fe deficiency-induced formation of rhizodermal transfer cells (Kramer *et al.*, 1980) is part of a mechanism for enhancing iron uptake. The transfer cells are most likely the sites of Fe deficiency-induced root responses of Strategy I, namely enhanced net excretion of protons and reducing capacity as well as of release of phenolic compounds. After the resupply of Fe, not only do the physiological root responses disappear, but also the transfer cells degenerate within 1 to 2 days. In perennial and annual dicotyledenous species such as *Ficus benjamina* (Rosenfield *et al.*, 1991) and *Lupinus cosentinii* (White and Robson, 1989), the formation of cluster roots is enhanced in response to P deficiency but also to Fe deficiency. Cluster roots have a high capacity to reduce Fe(III) and excrete protons (Marschner *et al.*, 1986a, b; Rosenfield *et al.*, 1991).

In recent years, impressive progress has been made in genetic and physiological characterization of root responses to Fe deficiency in Strategy I plants (see also Chapter 2). The importance of ferric reductase activity in development of Fe deficiency tolerance was demonstrated by over-expression of a yeast ferric reductase gene in rice plants. The transgenic rice plants with elevated ferric reductase activity showed higher tolerance to Fe deficiency and had greater grain yield on an iron-deficient calcareous soil when compared to non-transgenic rice plants (Ishimura *et al.*, 2007).

In graminaceous species (Strategy II), the Fe deficiency-induced morphological and physiological changes described above for Strategy I plants are absent. Instead, roots release phytosiderophores (PS) as chelators for Fe(III). The pathway of PS biosynthesis is understood reasonably well (Fig. 7.6). L-methionine is the dominant precursor (Mori and Nishizawa, 1987), and three molecules of methionine form one molecule of nicotianamine which, after deamination and hydroxylation, is converted to 2-deoxymugineic acid and further to other PS (Fig. 7.6), which vary with plant species (Römheld and Marschner, 1990). Over the past decade, the understanding of genetic regulation and physiological characterization of root release of PS and their role in Fe nutrition of graminaceous species has improved. Various transporters have been identified and described for root uptake, shoot transport and seed deposition of Fe, for example the Yellow Stripe-Like (YSL) family of proteins which contribute to Fe transport in plants (Curie *et al.*, 2009).

Nicotianamine (NA) is not only a precursor of PS biosynthesis but is also a strong chelator of Fe(II), but not of Fe(III) (Scholz *et al.*, 1988). Nicotianamine is also essential for the proper functioning of Fe(II)-dependent processes (Pich *et al.*, 1991). It plays an important role in Fe

homeostasis within cells and cellular compartments (Fig. 7.6) as well as in phloem transport and seed deposition of Fe (Haydon and Cobbett, 2007). In a recent study, overexpression of the NA-synthase gene in rice grains resulted in an about three-fold increase in grain Fe concentration (Lee *et al*., 2009).

7.1.7 Fe Deficiency and Toxicity

The critical deficiency concentration of Fe in leaves is in the range of 50–150 mg Fe kg^{-1} dw. This refers to total Fe and is, therefore, only of limited value for characterization of the Fe nutritional status of field-grown plants. In general, C4 species require a higher Fe supply than C3 species, but their critical deficiency concentrations are similar, namely 72 mg Fe kg^{-1} in C3 species and 66 mg Fe kg^{-1} in C4 species (Smith *et al.*, 1984). In fast growing meristematic and expanding tissues, for example shoot apices, the critical deficiency concentrations are higher, in the range of 200 mg Fe^{v} kg^{-1} dw of total Fe (Häussling *et al.*, 1985). In legumes, the Fe demand for nodule development is particularly high (see Chapter 16).

Iron deficiency is a worldwide problem in crop production on calcareous soils. It is the major factor responsible for so-called lime-induced chlorosis. Iron deficiency also represents an important nutrient deficiency problem in oceans, limiting CO_2 fixation and N_2 fixation capacity of the phytoplankton (Greene *et al*., 1992; Berman-Frank *et al*., 2001).

On the other hand, Fe toxicity ('bronzing') is a serious problem in crop production on waterlogged soils; it is the second-most severe yield-limiting factor in wetland rice. The critical toxicity concentrations are above 500 mg Fe kg^{-1} leaf dw, but depend on other factors such as concentration of other nutrients (Yamauchi, 1989). Iron toxicity may also occur under dryland conditions: drought-induced damage in photosynthetic tissue is caused by Fe-catalysed formation ROS in the chloroplasts (Price and Hendry, 1991). Iron toxicity damage is generally associated with formation of ROS, and therefore induction of antioxidative enzymes such as ascorbate peroxidase and Fe-binding proteins such as ferritin represent an important cellular defence mechanism against iron toxicity damage (Fourcroy *et al.*, 2004; Briat *et al*., 2010).

7.2 MANGANESE

7.2.1 General

Manganese can exist in the oxidation states I, II, III, IV, VI and VII. In biological systems, however, it mainly occurs in oxidation states II, III and IV, with MnII and MnIV being fairly stable and MnIII unstable (Hughes and Williams, 1988). In plants, MnII is by far the dominant form, but it can readily be oxidized to MnIII and MnIV. Manganese therefore plays an important role in redox processes.

The ionic radius of Mn^{2+} (0.075 nm) lies between Mg^{2+} (0.065 nm) and Ca^{2+} (0.099 nm), and it can therefore substitute, or compete with, either of these ions in various reactions. The binding strength of all three ions for ligands based on oxygen donors is quite similar (Hughes and Williams, 1988) or may be higher for Mn^{2+}, for example, by a factor of about four in case of ATP (Burnell, 1988). This has important consequences for the compartmentation of Mn^{2+} in cells and interactions between Mn and Mg.

7.2.2 Mn-containing Enzymes

Although a relatively large number of enzymes are activated by Mn^{2+}, there are only a small number of Mn-containing enzymes, namely the Mn-protein in PS II, the Mn-containing superoxide dismutase (MnSOD) and oxalate oxidase. Oxalate oxidase is a secreted multimeric glycosylated Mn-containing enzyme (Kanauchi *et al*., 2009). It is a homohexamer and belongs to a large family of germin-like proteins termed cupins because of their conserved β-barrel fold. The active site is in the centre of the β-barrel and contains an Mn ion (Dunwell *et al.*, 2001). Initial reports on an Mn-containing purple acid phosphatase (Uehara *et al.*, 1974) were followed by a subsequent work suggesting that this enzyme contains two Fe atoms per molecule, thus requiring Fe rather than Mn for its activity (Hefler and Averill, 1987). However, more recent opinion is that both Mn-containing and Fe-containing purple acid phosphatase may exist (Wieghardt, 2003).

Superoxide dismutases (SOD) are present in all aerobic organisms and play an essential role in the survival of these organisms in the presence of oxygen (Elstner, 1982; Fridovich, 1983). They protect tissues from the deleterious effect of the oxygen radical O_2^- formed in various enzyme reactions in which a single electron is transmitted to O_2:

$$O_2 + e^- \longrightarrow O_2^{\cdot-}\text{(Superoxide)}$$

$$O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \xrightarrow[\text{dismutase (SOD)}]{\text{Superoxide–}} H_2O_2\,\text{(Hydrogen peroxide)} + O_2$$

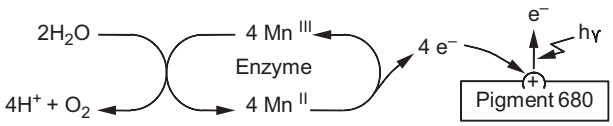
$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

The conversion of O_2^- is catalysed by SOD, and the subsequent dismutation of H_2O_2 into H_2O and O_2 is facilitated by either peroxidases, catalase (Elstner, 1982) or, in chloroplasts, by an ascorbate-specific peroxidase or ascorbate free radical reductase (Kroeniger *et al.*, 1992). In illuminated green cells, the chloroplasts are the organelles with the highest rate of oxygen turnover, including the

formation of O_2^- and H_2O_2. Hence, in green leaves more than 90% of the SOD is located in the chloroplasts and only 4–5% in the mitochondria (Jackson *et al.*, 1978).

The SOD isoenzymes differ in their metal component, which may be Fe (FeSOD), Mn (MnSOD) or Cu + Zn (CuZnSOD). The FeSOD is mainly confined to chloroplasts. CuZnSOD is found in chloroplasts, but also occurs in the cytoplasm in peroxisomes and mitochondria (Palma *et al.*, 1986). MnSOD is not widely distributed in higher plants (Sandmann and Böger, 1983) and mainly located in mitochondria and peroxisomes. There are controversial reports concerning the occurrence of MnSOD in chloroplasts. It is absent in pea (Palma *et al.*, 1986), but present in tobacco (Bowler *et al.*, 1991). MnSOD is present in chloroplast thylakoids of most eukaryotic algae (e.g., Grace, 1990). Numerous transgenic plants have been produced over the last two decades with MnSOD targeted to the choloroplasts; such plants showed increased tolerance to a range of abiotic stresses (e.g., salinity, Tanaka *et al.*, 1999; drought, Wang *et al.*, 2005a) and Mn deficiency (Yu *et al.*, 1999a). Free-living and symbiotic rhizobia (bacteroids) possess only MnSOD, whereas in the cytosol of nodules both MnSOD and CuZnSOD are present (Becana and Salim, 1989; Matamoros *et al.*, 2003).

The most well-known and best documented example of an Mn-containing enzyme is the 33 kDa polypeptide of the water-splitting system in PS II. In this system, four Mn atoms arranged as a cluster (which also contains one Ca atom, hence Mn4Ca) which stores positive charges prior to the four-electron oxidation of two molecules of water:



The functioning of the Mn atoms in both transient electron storing and electron transmitting is coupled with fluctuations in the oxidation state of Mn between MnII and MnIV (Rutherford, 1989). The Mn4Ca catalytic cluster cycles through five oxidation states coupling the one-electron photochemistry of the reaction centre with the four-electron redox chemistry of water oxidation (Yano, 2010). The precise structure of the cluster as well as the structural changes linked to the catalytic cycle are yet to be deciphered. In photosynthesizing cells, PS II is the most sensitive function impaired by Mn deficiency.

7.2.3 Mn-dependent or Activated Enzymes

Manganese acts as cofactor, activating about 35 different enzymes (Burnell, 1988). Most of these enzymes catalyse oxidation-reduction, decarboxylation and hydrolytic reactions. Manganese has a primary role in the tricarboxylic acid cycle (TCA) in oxidative and non-oxidative decarboxylation reactions, for example in the NADPH-specific decarboxylating malate dehydrogenase, malic enzyme and isocitrate dehydrogenase:

Malic enzyme catalysing the reaction:

$$\text{Malate} + \text{NADP}^+ \xrightarrow[Mn^{2+},\ Mg^{2+}]{} \text{Pyruvate} + \text{NADPH} + H^+ + CO_2$$

Isocitrate dehydrogenase catalysing the reaction:

$$\text{Isocitrate} + \text{NADP+} \xrightarrow[Mn^{2+},\ Mg^{2+}]{} \text{Oxalosuccinate} + \text{NADPH}$$

Most studies on Mn activation of enzymes have been carried out *in vitro*, and in many cases Mn^{2+} can be replaced by Mg^{2+}, or vice versa. Given that the concentration of Mg^{2+} in the cells is on average about 50 to 100 times higher than that of Mn^{2+}, activation of enzymes by Mn^{2+} *in vivo* is presumably only important for those enzymes where Mn^{2+} is a more effective cofactor than Mg^{2+}. An example of the higher effectivity of Mn^{2+} is the chloroplast RNA polymerase whose activation requires about 10 times lower concentrations of Mn^{2+} than Mg^{2+} (Ness and Woolhouse, 1980).

An absolute requirement for Mn occurs in the bundle sheath chloroplasts of those C4 plants in which oxaloacetate acts as the carbon shuttle and where decarboxylation is catalysed by PEP carboxykinase. This enzyme has an absolute requirement for Mn that cannot be replaced by Mg (Fig. 7.7). Maximum activity occurs at an Mn/ATP ratio of one, suggesting that the substrate for the enzyme is the Mn–ATP complex (Burnell, 1986), rather than Mg–ATP as in most other reactions (see Section 6.4).

Manganese activates several enzymes of the shikimic acid pathway, and subsequent pathways, leading to the biosynthesis of aromatic amino acids (such as tyrosine), various secondary products (such as lignin, flavonoids)

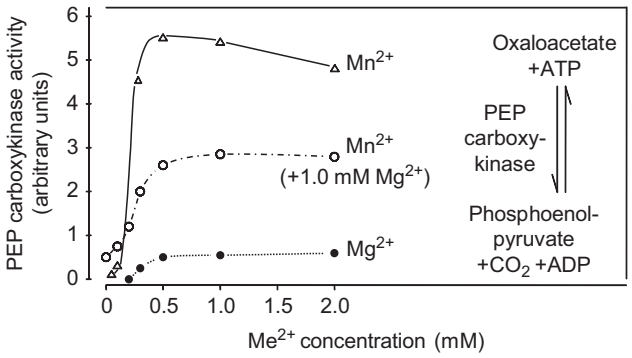


FIGURE 7.7 Activity of PEP carboxykinase from *Urochloa panicoides* with addition of Mn or Mg or Mn + Mg. ATP concentration was kept constant at 0.25 mM. *Based on Burnell (1986).*

and IAA (Burnell, 1988; Hughes and Williams, 1988). For example, Mn stabilizes the active conformation of phenylalanine ammonia lyase (PAL) (Wall *et al.*, 2008), stimulates peroxidases and works as a diffusible redox shuttle in combination with peroxidases in lignin biosynthesis (Önnerud *et al.*, 2002). In leaves suffering from Mn deficiency or Mn toxicity, the IAA oxidase activity is high (Morgan *et al.*, 1976). Manganese-dependent enzymes (e.g., phytoene synthetase) have also been found in the biosynthetic pathway of isoprenoids as precursors of carotenoids, sterols and GA (Wilkinson and Ohki, 1988).

In nodulated legumes such as soybean, which mainly transport N in the form of allantoin and allantoate to the shoot (see Chapter 16), the degradation of these ureides in the leaves (Winkler *et al.*, 1985) and in the seed coat (Winkler *et al.*, 1987) is catalysed by the enzyme allantoate amidohydrolase that has an absolute requirement for Mn (Werner *et al.*, 2008). Arginase is another Mn-dependent enzyme in N metabolism (Dabir *et al.*, 2005).

A role of Mn in nitrate reductase activity was presumed because of an increase in nitrate conentration in Mn-deficient leaves. However, this accumulation of nitrate is the consequence of a shortage of (i) reducing equivalents in the chloroplasts and (ii) carbohydrates in the cytoplasm, as well as of negative feedback regulation resulting from lower demand for reduced N in the new growth of deficient plants. There is no evidence of a direct role of Mn in nitrate reductase activity (Leidi and Gomes, 1985).

Manganese (Mn^{2+}) can readily displace Mg^{2+} from ATP because Mn^{2+} binds ATP four times more strongly than Mg^{2+}. At high concentrations of Mn^{2+}, ATP in the cytoplasm is readily saturated by Mn^{2+} (Pfeffer *et al.*, 1986). Hence, for the normal functioning of Mg–ATP as the main energy-transmitting system, the concentrations of Mn^{2+} in the cytosol and the stroma of chloroplasts have to be maintained at a low level. In agreement with this, most Mn^{2+} is sequestered in the vacuoles (Pfeffer *et al.*, 1986; Clarkson, 1988) or in other cell compartments such as Golgi vesicles (Hughes and Williams, 1988). Depression of net photosynthesis in leaves high in Mn may be due to decreased chlorophyll concentration (e.g., in bean genotypes; Gonzales and Lynch, 1997) or inhibition of the RuBP carboxylase (Houtz *et al.*, 1988), with such inhibition unlikely to be due to the replacement of Mg^{2+} by Mn^{2+} (Chatterjee *et al.*, 1994).

7.2.4 Photosynthesis and Oxygen Evolution

The role of Mn in photosynthesis was discovered in green algae (Pirson, 1937). In *Chlorella*, the Mn requirement for optimal growth is about 1,000 times lower under heterotrophic (darkness and external supply of carbohydrates) compared with autotrophic conditions, i.e. carbohydrate supply via photosynthesis (Eyster *et al.*, 1958). Also in higher plants, photosynthesis in general and photosynthetic O_2 evolution in PS II in particular are the processes that are most strongly depressed by Mn deficiency (Fig. 7.8) (Shenker *et al.*, 2004; Husted *et al.*, 2010). Photosynthetic O_2 evolution was reduced by more than 50% with a decrease in Mn concentration in young leaves of subterranean clover, whereas there was only a small effect on chlorophyll concentration (Fig. 7.8) or leaf dry weight (Nable *et al.*, 1984). Resupplying Mn to deficient leaves restored photosynthetic O_2 evolution within one day to the levels measured in leaves adequately supplied with Mn. Similar results have been obtained in wheat (Kriedemann *et al.*, 1985) and maize (Gong *et al.*, 2010). Manganese deficiency-induced alterations in O_2 evolution were correlated with changes in the ultrastructure of thylakoid membranes, namely the loss of PS II functional units in the stacked areas of thylakoid membranes (cf. Husted *et al.*, 2010). Resupplying Mn restored the number of the PS II protein-pigment units in the thylakoid membranes (Simpson and Robinson, 1984; Gong *et al.*, 2010).

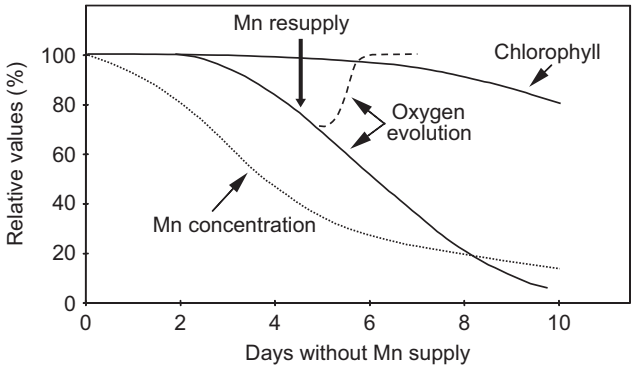


FIGURE 7.8 Concentration of Mn and chlorophyll and photosynthetic O_2 evolution in young leaves of *Trifolium subterraneum* with withdrawal of Mn supply and resupply of Mn. *Recalculated from Nable* et al.*, 1984.*

With increasing severity of Mn deficiency, the chlorophyll concentration decreases and the ultrastructure of the thylakoids is drastically changed (Kroengier *et al.*, 1993). These ultrastructural alterations are either very difficult to restore, or irreversible, and are presumably caused by inhibition of biosynthesis of lipids and carotenoids. They are not brought about by enhanced photooxidation (lipid peroxidation) of the thylakoids and chlorophyll.

7.2.5 Proteins, Carbohydrates and Lipids

Although Mn activates RNA polymerase (Ness and Woolhouse, 1980), protein synthesis is not specifically impaired in Mn-deficient tissues. The protein concentration of deficient plants is either similar to (Table 7.7) or somewhat higher than that of plants adequately supplied with Mn (Lerer and Bar-Akiva, 1976). The accumulation

TABLE 7.7 Growth and composition of bean plants with or without Mn supply

	Leaves		Stems		Roots	
	+Mn	−Mn	+Mn	−Mn	+Mn	−Mn
Dry weight (g plant^{-1})	0.64	0.46	0.55	0.38	0.21	0.14
Protein-N (mg g^{-1} dw)	52.7	51.2	13.0	14.4	27.0	25.6
Soluble-N (mg g^{-1} dw)	6.8	11.9	10.0	16.2	17.2	21.7
Soluble carbohydrates (mg g^{-1} dw)	17.5	4.0	35.6	14.5	7.6	0.9

From Vielemeyer *et al.* (1969).

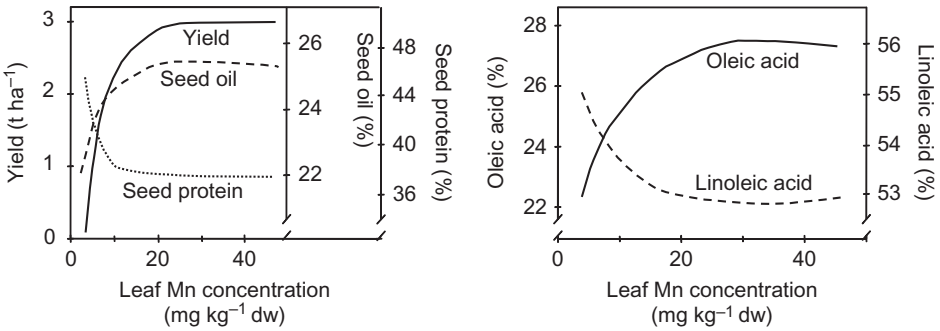


FIGURE 7.9 Relationships between leaf Mn concentration, seed yield and seed composition of soybean. *Adapted from Wilson* et al. *(1982).*

of soluble N in Mn-deficient tissues is due to a shortage of reducing equivalents and carbohydrates for nitrate reduction, as well as a lower demand for reduced N. Manganese deficiency has the most severe effect on the concentration of non-structural carbohydrates, as shown in Table 7.7 for the soluble (sugar) fraction. This decrease in carbohydrate concentration is particularly evident in roots and is most likely a key factor responsible for the depression in root growth of Mn-deficient plants (Table 7.7; Marcar and Graham, 1987).

The role of Mn in lipid metabolism is more complex. In Mn-deficient leaves, the concentration of thylakoid-membrane constituents such as glycolipids and polyunsaturated fatty acids may be decreased by up to 50% (Constantopoulus, 1970). This depression in lipid concentration in chloroplasts can be attributed to the role of Mn in biosynthesis of fatty acids, carotenoids and related compounds.

Manganese supply affects the lipid concentration and composition in the seeds (Fig. 7.9). In the deficiency range, the Mn concentration in leaves and both the seed yield and oil concentration were positively correlated. The fatty acid composition of the oil was also markedly altered, with the concentration of linoleic acid (Fig. 7.9) and certain other fatty acids increasing (Wilson *et al.*, 1982). This was counteracted by a decrease in oleic acid concentration. The lower oil concentration in the seeds of deficient plants probably resulted mainly from lower rates of photosynthesis and thus a decreased supply of carbon skeletons for fatty acid synthesis. In addition, a direct involvement of Mn in the biosynthesis of fatty acids may be a contributing factor.

The lower lignin concentration in Mn-deficient plants (Table 7.8) is a reflection of the requirement for Mn in various steps of lignin biosynthesis. Given that Mn is a cofactor for (i) phenylalanine ammonia-lyase which mediates production of cinnamic acid and various other phenolic compounds, and (ii) peroxidase involved in polymerization of cinnamyl alcohols into lignin, deficiency of Mn may reduce phenolics and lignin concentrations (Brown *et al.*, 1984; Rengel *et al.*, 1993) which are considered important defence against fungal infection (Rengel, 2003). A decrease in lignin concentration is particularly evident in roots, and is an important factor responsible for the lower resistance of Mn-deficient plants to root-infecting pathogens.

7.2.6 Cell Division and Extension

Inhibition of root growth in Mn-deficient plants is caused by shortage of carbohydrates as well as by a direct Mn requirement for growth (Campbell and Nable, 1988; Sadana

TABLE 7.8 Relationship between Mn and lignin concentration in shoots and roots of young wheat plants

	Mn concentration ($mg\,kg^{-1}$ dw)			
	4.2	7.8	12.1	18.9
Lignin concentration (% of dw)				
Shoots	4.0	5.8	6.0	6.1
Roots	3.2	12.8	15.0	15.2

Recalculated from Brown *et al.* (1984).

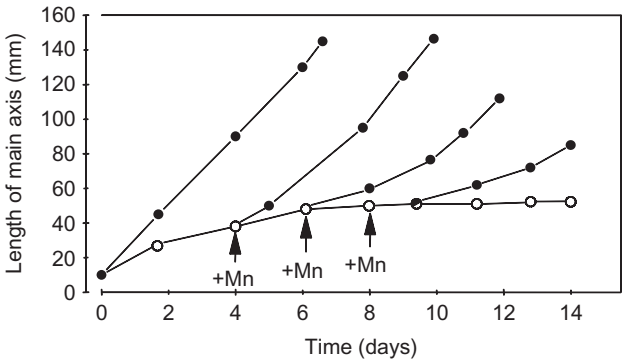


FIGURE 7.10 Growth of main axis of excised tomato roots after transfer from Mn-deficient to complete medium. ○ 0 Mn; ● + Mn. *Based on Abbott, 1967.*

et al., 2002). The rate of elongation appears to respond more rapidly to Mn deficiency than the rate of cell division. As shown in Fig. 7.10 with isolated tomato roots in sterile culture and an ample supply of carbohydrates (but without Mn), there was a decline in extension of the main axis in less than 2 days. Resupplying Mn rapidly restored the growth rate to normal levels if the deficiency was not too severe. In Mn-deficient plants, the formation of lateral roots ceased completely (Abbott, 1967). Compared to Mn-sufficient plants, there was a greater abundance of small non-vacuolated cells in Mn-deficient roots, indicating that Mn deficiency impairs cell elongation more strongly than cell division, an observation also supported by tissue culture experiments (Neumann and Steward, 1968).

7.2.7 Mn Deficiency and Toxicity

Manganese deficiency is abundant in plants growing in soils derived from parent material inherently low in Mn, and in highly leached tropical soils. It is also common in soils of high pH containing free carbonates, particularly when combined with high organic matter content (Farley and Draycott, 1973).

In Mn-deficient plants, dry matter production (Ohki *et al.*, 1979), net photosynthesis and chlorophyll content decline rapidly (Shenker *et al.*, 2004), whereas rates of respiration and transpiration remain unaffected (Ohki *et al.*, 1979). Manganese-deficient plants are more susceptible to damage by freezing temperatures (Buntje, 1979), a range of soil-borne root-rotting fungal diseases (e.g., take-all, Huber and McCay-Buis, 1993; Rengel *et al.*, 1993) and require twice as long to reach booting stage than Mn-sufficient plants (Longnecker *et al.*, 1991b). A decrease in grain number and grain yield in Mn-deficient plants is presumably a combination of low pollen fertility (Sharma *et al.*, 1991) and shortage of carbohydrate supply for grain filling (Longnecker *et al.*, 1991b).

In dicotyledonous plants, intercostal chlorosis of the younger leaves is the most distinct symptom of Mn deficiency, whereas in cereals, greenish grey spots on the older leaves ('grey speck') are the major symptoms. In legumes, Mn-deficiency symptoms on the cotyledons are known as 'marsh spot' in peas or 'split seed' disorder in lupins; the latter disorder includes discoloration, splitting and deformity of seeds (Campbell and Nable, 1988).

Manganese deficiency can be corrected by soil (Brennan *et al.*, 2001) or foliar application of $MnSO_4$ (Reuter *et al.*, 1988), but the latter method has limitations. High Mn concentrations in seeds, either supplied naturally from the parent plants or artificially by soaking the seeds in $MnSO_4$, can considerably improve plant growth and seed yield on soils with low Mn availability as has been shown for barley (Longnecker *et al.*, 1991a). In wheat, high seed Mn was more effective than Mn fertilization in achieving good yield in soils with low Mn availability (Moussavi-Nik *et al.*, 1997; Khabaz-Saberi *et al.*, 2000). Also, wheat produced from seed with high Mn concentration had increased tolerance to take-all disease (McCay-Buis *et al.*, 1995).

Plant species and genotypes within a species differ considerably in susceptibility to Mn deficiency when grown on soils with low Mn availability (Rengel, 2001). Oat, wheat, soybean and peaches are susceptible, whereas maize and rye are not (Reuter *et al.*, 1988). Differential Mn efficiency was reported among genotypes of bread wheat (e.g., Sadana *et al.*, 2002), durum wheat (e.g., Khabaz-Saberi *et al.*, 2000), barley (e.g., Hebbern *et al.*, 2005), and other crops. Despite differences in efficiency among plant species, the critical deficiency concentrations of Mn in plants are similar, varying between 10 and $20\,mg\,Mn\,kg^{-1}$ dw in fully expanded leaves, regardless of plant species or cultivar or prevailing environmental conditions. Only *Lupinus angustifolius* has a critical deficiency concentration which is twice as high as that of other plant species (Hannam and Ohki, 1988; Brennan *et al.*, 2001).

In contrast to the narrow range of critical deficiency concentration of Mn, the critical toxicity concentration

TABLE 7.9 Critical toxicity concentrations of Mn (at which dry matter production is reduced by 10%) in the shoots of various plant species

Species	Critical toxicity concentration (mg Mn kg^{-1} dw)
Maize	200
Pigeon pea	300
Soybean	600
Cotton	750
Sweet potato	1,380
Sunflower	5,300

Based on Edwards and Asher (1982).

varies widely among plant species and environmental conditions. An example of the differences among crop species is given in Table 7.9. Even within a species, the critical toxicity concentration can vary substantially among cultivars (Edwards and Asher, 1982; Horst, 1988; Wang *et al.*, 2002; Khabaz-Saberi *et al.*, 2010).

Of the environmental factors affecting critical toxicity concentrations, temperature and the presence of silicon are of particular importance. At high temperatures, the Mn concentration in leaves is often higher than that at low temperatures (Heenan and Carter, 1977; Rufty *et al.*, 1979) or when supplied with silicon (Iwasaki *et al.*, 2002; Doncheva *et al.*, 2009; Führs *et al.*, 2009), indicating greater tissue tolerance to Mn. In a maize genotype tolerant to Mn toxicity, silicon substantially increased the thickness of the epidermal leaf layers where excess Mn was stored (Doncheva *et al.*, 2009). Maintaining sufficient concentrations of ascorbic acid in leaf apoplast can contribute to tolerance to Mn toxicity in cowpea and common bean cultivars, but is not a determining factor (Gonzales *et al.*, 1998; Fecht-Christoffers and Horst, 2005). Nevertheless, increased activity of antioxidative enzymes and increased concentrations of antioxidants contribute to alleviation of Mn toxicity stress in many plant species (e.g., Gonzáles *et al.*, 1998; Fecht-Christoffers *et al.*, 2006; Rosas *et al.*, 2007; Führs *et al.*, 2009; Mora *et al.*, 2009; Gangwar *et al.*, 2010). There are conflicting views on the effect of high light intensity on Mn toxicity, with reports of increasing the severity of toxicity symptoms (Horiguchi, 1988; Nable *et al.*, 1988; Gonzáles *et al.*, 1998) or lessening them (Wissemeier and Horst, 1987). The diversity of Mn toxicity symptoms may be a major reason for these contradictory results.

In many plant species, symptoms of Mn toxicity are brown speckles on mature leaves (e.g., Wissemeier and Horst, 1987). Although these brown speckles contain oxidized Mn, the brown colour derives not from Mn, but from oxidized polyphenols (Wissemeier and Horst, 1987; Führs *et al.*, 2009). The formation of brown speckles is preceded by enhanced callose formation in the same area (Horst *et al.*, 1999), indicating toxic effects of Mn on the plasma membrane and enhanced Ca^{2+} influx (Wissemeier and Horst, 1987) as a signal for callose formation. The intensity of formation of brown speckles can be used as a simple and rapid method for screening different cultivars for Mn tolerance (e.g., Wissemeier and Horst, 1991; Doncheva *et al.*, 2009).

In leaves of Mn-tolerant plant species such as sunflower or stinging nettle growing at high Mn concentrations, brown spots are also often found around the base of trichomes (Blamey *et al.*, 1986; Hughes and Williams, 1988) which contain Mn oxides and may therefore be considered as mechanism to reduce soluble Mn concentrations.

Interveinal chlorosis and necrosis are further symptoms of Mn toxicity (Nable *et al.*, 1988; Horiguchi, 1988; Gonzáles and Lynch, 1997; Gonzáles *et al.*, 1998; Fecht-Christoffers *et al.*, 2007). Particularly in dicots such as bean (Horst and Marschner, 1978b), soybean (Heenan and Campbell, 1980), cotton (Foy *et al.*, 1981) and blueberry (Bañados *et al.*, 2009), these symptoms are combined with deformations of young leaves ('crinkle leaf'), which is a typical symptom of Ca deficiency. Hence, Mn toxicity is accompanied by induced deficiencies of other nutrients such as Ca, Mg, Fe (Horst, 1988) and Zn (de Varennes *et al.*, 2001). Induced deficiency of Fe and Mg is caused by inhibited uptake across the plasma membrane (see also Chapter 2) and competition (or imbalance) at the cellular level. Accordingly, Mn toxicity can often be counteracted by a high supply of Mg (Löhnis, 1960; Davis, 1996).

In contrast to Fe and Mg, induction of Ca deficiency symptoms ('crinkle leaf') by high tissue concentrations of Mn is most likely an indirect effect on the Ca transport to expanding leaves. Acropetal Ca transport is mediated by a basipetal counter-transport of IAA (Chapter 3), and high IAA oxidase activity, or polyphenoloxidase activity in general, is frequently measured in tissues with high Mn concentration (Horst, 1988; Fecht-Christoffers *et al.*, 2007). Calcium deficiency symptoms induced by Mn toxicity are therefore most likely caused by enhanced degradation of IAA, a process which is aggravated, for example, by high light intensity (Horst, 1988). Loss of apical dominance and enhanced formation of auxiliary shoots ('witches' broom') is another symptom of Mn toxicity (Kang and Fox, 1980, Bañados *et al.*, 2009), further supporting the hypothesis of a relationship between impaired basipetal IAA transport and Mn toxicity (Gangwar *et al.*, 2010).

7.3 COPPER

7.3.1 General

Copper is a redox-active transition element with roles in photosynthesis, respiration, C and N metabolism, and protection against oxidative stress. Like Fe, it forms highly stable complexes and participates in electron transfer reactions. Divalent Cu is reduced readily to monovalent Cu which is unstable.

Most of the functions of Cu as a plant nutrient are based on enzymatically bound Cu which catalyses redox reactions. In redox reactions of the terminal oxidases, Cu enzymes react directly with molecular oxygen. Terminal oxidation in living cells is therefore catalysed by Cu and not by Fe.

Copper has a high affinity for peptide and sulphhydryl groups, and thus to cysteine-rich proteins, as well as also for carboxylic and phenolic groups. Therefore, more than 98% of the Cu in plants is present in complexed forms and the concentrations of free Cu^{2+} and Cu^{+} is extremely low in the cytoplasm.

There has been rapid progress in understanding Cu transport into cells and organelles in recent years (reviewed by Burkhead *et al*., 2009; Yruela *et al*., 2009). Within the Cu transport (COPT) protein family, of which there are six members in Arabidopsis, COPT1 is thought to mediate uptake of Cu into cells, whereas other members may mediate intracellular transport. Zn/Fe permeases (ZIPs) may also be involved in divalent Cu^{2+} transport at the plasma membrane, alongside P_{1B}-type ATPase (HMA) transporters on organelle and plasma membranes which are selective for both monovalent and divalent Cu forms. Yellow Stripe Like (YSL) transporters are likely to mediate Cu^{2+}-nicotianamine transport at the plasma membrane. In addition to transport, several Cu chaperones have a central role in cellular Cu homeostasis.

7.3.2 Cu Proteins

There are more than 100 different Cu-containing proteins in plants (Yruela, 2009). About 50% of Cu found in plants is present in chloroplasts, bound to plastocyanin, where it participates in photosynthetic reactions (Hänsch and Mendel, 2009). Other major forms include Cu-binding chaperones and numerous enzymes, particularly single and multi Cu-containing oxidase enzymes (Burkhead *et al.*, 2009). Copper is also part of the ethylene receptor and is involved in Mo-cofactor biosynthesis. In legumes, Cu deficiency reduces nodulation and N_2 fixation. Under Cu deficiency, the activity of these Cu enzymes decreases rapidly, and in most, but not all, cases, these decreases are correlated with metabolic changes and inhibition of plant growth.

TABLE 7.10 Relationship between Cu concentration and various chloroplast constituents and activities of Cu-containing enzymes in pea leaves

	Cu concentration ($\mu g\,g^{-1}$ dw)		
	6.9	3.8	2.2
Chlorophyll ($\mu mol\ g^{-1}$ dw)	4.9	3.9	4.4
Plastocyanin (nmol μmol^{-1}chlorophyll)	2.4	1.1	0.3
Photosynthetic e-transport PS I (relative)	100	54	19
Enzyme activity (EU mg^{-1} protein)			
Diamine oxidase	0.86	0.43	0.24
Ascorbate oxidase	730	470	220
CuZnSOD	22.9	13.5	3.6

Based on Ayala and Sandmann (1989).

7.3.2.1 Plastocyanin

Plastocyanin is a component of the electron transport chain of PS I. This protein has a molecular weight of ~10 kDa and contains one Cu atom per molecule. There are 3 to 4 molecules of plastocyanin per 1,000 molecules of chlorophyll (Sandmann and Böger, 1983).

Under Cu deficiency, a close relationship exists between the Cu concentration of leaves and the plastocyanin concentration and, thus, the activity of PS I, whereas the chlorophyll concentration is only slightly affected (Table 7.10). The activity of PS II is usually less depressed by Cu deficiency (Table 7.11). Lower activity of PS II in Cu-deficient plants is related to other functions of Cu in chloroplasts. For example, Cu is required for the synthesis of quinones; the decrease in plastoquinone in Cu-deficient chloroplasts (Table 7.11) may reflect this function. In Cu-deficient chloroplasts, electron transport is further inhibited by the lack of two polypeptides in the chloroplast membrane, which are probably necessary to maintain appropriate membrane fluidity to ensure the mobility of plastoquinone molecules to transport electrons between the two photosystems (Droppa *et al.*, 1984).

7.3.2.2 Superoxide Dismutase

The various types of SOD isoenzymes and their role in the detoxification of superoxide radicals ($O_2^{\cdot -}$) have been discussed in Section 7.2. The copper–zinc SOD (CuZnSOD) has a molecular weight of 32.5 kDa, and at the active site one Cu and one Zn atom share a common histidine ligand. The Cu atom in CuZnSOD is directly involved in

TABLE 7.11 Chloroplast pigments and photosynthetic electron transport in photosystems II and I in spinach leaves with or without Cu supply

	Chloroplast pigment concentration ($\mu g\,g^{-1}$ fw)			Plastocyanin ($nmol\,mg^{-1}$ chlorophyll)	Photosystem activity (relative)	
	Chlorophyll	Carotenoids	Plastoquinone		PS II	PS I
+Cu	1,310	248	106	5.2	100	100
−Cu	980	156	57	2.1	66	22

Based on Baszynski *et al.* (1978).

the detoxification of $O_2^{\cdot -}$ generated in photosynthesis (Elstner, 1982). There are at least three major isoforms of CuZnSOD in plants (Yruela, 2009). These occur in the cytosol (CSD1), in chloroplast stroma together with FeSOD (CSD2, Section 7.1), and in peroxisomes (CSD3).

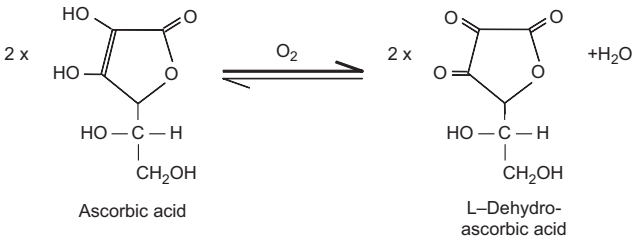
Under Cu deficiency, CuZnSOD activity strongly declines in leaves (Table 7.10). This decline occurs in the chloroplastic and the cytoplasmic compartments. Copper deficiency appears to lead to increases in the activity of several other SOD isoforms, including FeSOD and MnSOD (Ayala and Sandmann, 1988; Burkhead *et al.*, 2009). There are a number of mechanisms by which the Cu status regulates CuZnSOD, including direct transcriptional control of SOD isoforms by Cu, and through post-transcriptional activity of microRNAs on CuZnSOD RNA metabolism, for example miR398 (Sunkar *et al.*, 2006; Yruela, 2009).

7.3.2.3 Cytochrome c Oxidase

Cytochrome c oxidase (CcO) is a large integral membrane protein which is encoded in the mitochondrial genome. It is a terminal oxidase of the mitochondrial electron transport chain, and it is expressed in the mitochondrial inner membrane. Assembly of the active oxidase complex is dependent on the insertion of three Cu ions, along with two heme Fe, and individual Zn, Mg and Na ions (Carr and Winge, 2003). The activity of CcO can be blocked by cyanide; the remaining respiratory O_2 consumption of cells is then mediated by the cyanide-insensitive quinol oxidase known as the 'alternative oxidase' pathway (in the 'alternative pathway', see Chapter 5). This enzyme contains Cu, but no heme Fe, and it is therefore unlikely that the alternative respiration can function to compensate low CcO activity in Cu-deficient cells. Since respiration is not greatly affected by Cu deficiency, CcO appears to be present in large excess in the mitochondria (Ayala and Sandmann, 1988).

7.3.2.4 Ascorbate Oxidase

Ascorbate oxidase is a multi-Cu oxidase which catalyses the oxidation of ascorbic acid to L-dehydro ascorbic acid according to the equation:



Ascorbic acid — L–Dehydro-ascorbic acid

The enzyme contains at least four Cu atoms per molecule and catalyses a four-electron reduction of O_2 to water. The enzyme is thought to occur primarily in the cell wall apoplasm and act as a terminal respiratory oxidase; however, it may also act in combination with polyphenol oxidases. Ascorbate oxidase activity decreases in Cu-deficient plants (Table 7.10) and is a sensitive indicator of the Cu nutritional status of a plant (Fig. 7.11). This correlation has been used to develop a rapid and simple colorimetric field test to diagnose Cu deficiency (Delhaize *et al.*, 1982). Resupplying Cu to deficient plants can restore the activity of ascorbate oxidase in very young, but not in mature leaves (Table 7.12), suggesting that the enzyme can only be synthesized in leaf blades during their very early development. This is in contrast to plastocyanin, the activity of which can also be restored in mature leaves upon resupply of Cu (Droppa *et al.*, 1984).

7.3.2.5 Diamine Oxidases

Polyamine oxidases are flavoproteins which catalyse the aerobic degradation of polyamines, for example spermidine to form putrescine, H_2O_2 and NH_3. Polyamine oxidases preferentially degrade tri- and tetraamines which are the main forms present in graminaceous species (Federico *et al.*, 1990). However, the degradation of

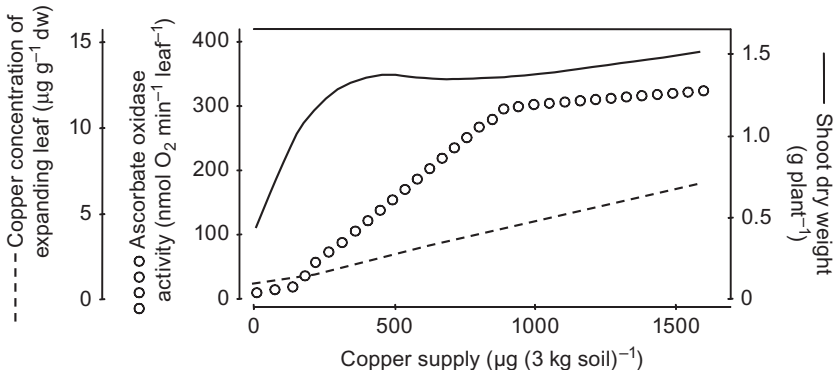


FIGURE 7.11 Relationships between Cu supply, shoot dw, ascorbate activity and Cu concentration of subterranean clover. *Modified from Loneragan* et al.*, 1982a.*

TABLE 7.12 Cu concentration, ascorbate oxidase activity (AOA) and protein concentration in very young and mature leaves of subterranean clover with and without Cu supply or Cu resupply to Cu-deficient plants (−Cu + Cu)

		Cu supply		
Leaf age	Parameter	−Cu	−Cu + Cu	+Cu
Very young	Cu concentration (µg Cu g^{-1} dw)	<0.5	17.9	13.4
	AOA (nmol O_2 $leaf^{-1}$ min^{-1})	10	245	240
	Protein (mg g^{-1} dw)	17.6	38.4	40.7
Mature	Cu concentration (µg Cu g^{-1} dw)	1.0	7.9	10.0
	AOA (nmol O_2 $leaf^{-1}$ min^{-1})	5.0	5.0	34.0
	Protein (mg g^{-1} dw)	36.6	43.9	40.0

Based on Delhaize *et al.* (1985).

putrescine (diamine) and, to some extent, spermidine (triamine) is mediated by diamine oxidase, a Cu-containing enzyme. Diamine oxidase is widespread in different plant species, particularly in legumes. H_2O_2 may be involved in production of structural defence-related compounds, as a signal molecule, or as an antimicrobial compound in host resistance (Walters, 2003). The activity of diamine oxidase decreases in Cu-deficient plants (Table 7.10) and can be restored by resupplying Cu (Delhaize *et al.*, 1985). Similarly to ascorbate oxidase (Table 7.12), this restoration of activity is confined to very young leaves.

Diamine oxidase is mainly located in the apoplasm, including the epidermis and the xylem of mature tissues, where the H_2O_2 produced can sustain the activity of peroxidases involved in lignification and suberinization (Angelini *et al.*, 1990; Walters, 2003).

7.3.2.6 Polyphenol Oxidases

Polyphenol oxidases (also known as catechol oxidases, diphenol oxidases and tyrosinases) contain two Cu ions. They catalyse the oxygenation reactions of plant phenols in

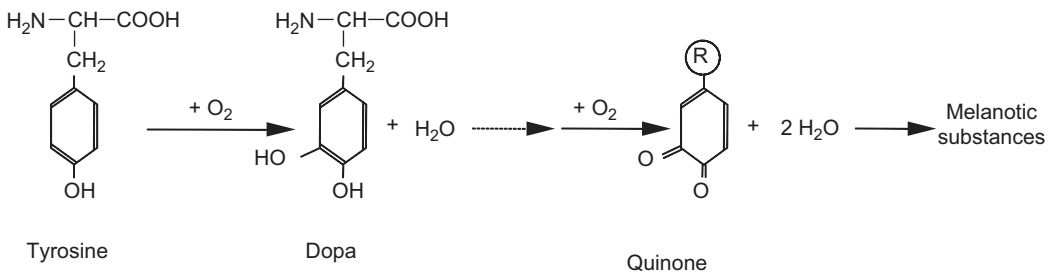


TABLE 7.13 Cu concentration, flowering and enzyme activities in Cu-sufficient or Cu-deficient *Chrysanthemum morifolium*

	Cu-sufficient	Cu-deficient
Cu concentration (μg Cu g^{-1} dw)	7.9	2.4
No. flowering shoots $plant^{-1}$	14.2	8.3
No. open flowers $plant^{-1}$	13.1	0.5
Enzyme activity (relative)		
Polyphenol oxidase	100	26
IAA oxidase	100	52
Peroxidase	100	41

Based on Davies *et al.* (1978).

which molecular oxygen is inserted into an aromatic ring, followed by oxidation of dihydroxyphenols to orthoquinones, which are powerful oxidants (Parveen *et al.*, 2010) (see bottom of page 208).

Both reactions require molecular oxygen. They are coupled to each other if monophenols are the substrates. They are named according to their most important substrates as monophenol oxidases, polyphenol oxidases, phenolases, DOPA oxidases, tyrosinases, etc. Their specificity is rather low.

Phenol oxidases are abundant in cell walls but are also located in the thylakoid membranes of chloroplasts. Polyphenol oxidases are involved in the biosynthesis of lignin and alkaloids and in the formation of brown melanotic substances, which may be formed when tissues are wounded (e.g., in apples and potatoes). The melanotic substances are also active as phytoalexins, which inhibit spore germination and fungal growth. Under Cu deficiency, polyphenol oxidase activity is strongly inhibited (Table 7.13) which leads to an accumulation of phenolics and a decrease in the formation of melanotic substances. A decline in polyphenol oxidase activity with Cu deficiency may be at least indirectly responsible for the delay in flowering and maturation often observed in the Cu-deficient plants (Reuter *et al.*, 1981) and shown for the flowering of *Chrysanthemum* in Table 7.13. Copper deficiency led to a decrease in the number of flowering shoots, but particularly prevented the opening of flowers. As would be expected, polyphenol oxidase activity was lower in Cu-deficient plants, but the activity of IAA oxidase and peroxidase was also lower. On the other hand, in tissue cultures regeneration of plants is often severely impaired by high activity of polyphenol oxidase. Accordingly, the percentage of shoot-regenerating explants is negatively correlated with the Cu concentration of the stock plants, and best regeneration is achieved with explants from severely Cu-deficient stock plants (Schum *et al.*, 1988).

7.3.3 Carbohydrate, Lipid and N Metabolism

Due to the role of Cu in PS I, it is not surprising that Cu-deficient plants have low rates of photosynthesis and reduced carbohydrate synthesis, at least during the vegetative stage. In Cu-deficient wheat plants, the concentration of soluble carbohydrates during the vegetative stage is lower than in Cu-sufficient plants (Brown and Clark, 1977). However, when grains have developed as a dominant sink after anthesis, Cu-deficient plants produce few grains, remain green (i.e., they remain actively photosynthesizing) and have high concentrations of soluble carbohydrates in leaves and roots (Fig. 7.12). The reduction in net CO_2 fixation in severely Cu-deficient plants to about 50% expressed both in terms of unit chlorophyll (Botrill *et al.*, 1970) or leaf area (Casimiro *et al.*, 1990), cannot be attributed solely to lower activities of PS I. Lower activity of PS II must also be a contributing factor. In Cu-sufficient plants, 11 Cu atoms per 1,000 chlorophyll molecules are located in the PS II complex (Ayala *et al.*, 1992). Under severe Cu deficiency, polypeptides of PS II are altered (Droppa *et al.*, 1984; Yruela, 2009) and the lipid composition changes in favour of the less unsaturated fatty acids, for example 18:3→18:2 (Ayala *et al.*, 1992). These changes in fatty acid composition in the thylakoids and in the PS II complex are probably related to functions of Cu in the desaturation of long-chain fatty acids (e.g., 18:2→18:3).

The low carbohydrate concentrations in Cu-deficient plants can explain the impaired pollen formation and fertilization, and are the main reason for reduced nodulation and N_2 fixation in Cu-deficient legumes (Cartwright and Hallsworth, 1970). Symptoms of N deficiency in Cu-deficient plants can be overcome by the application of mineral N. However, it has been shown that N application promotes Cu deficiency, and when N supply is high, application of Cu fertilizers may be required for maximum yield (Thiel and Finck, 1973; Robson and Reuter, 1981). In addition to non-specific growth enhancement by N, N affects Cu availability and mobility within the plant, including (i) a higher proportion of Cu complexed to amino acids and proteins in mature tissue and, (ii) a decrease in the rate of re-translocation of Cu from old leaves to areas of new growth. Re-translocation of Cu is closely related to leaf senescence (Chapter 3) and because high N supply delays senescence, it also retards Cu re-translocation (Hill *et al.*, 1978). In agreement with this, the critical deficiency concentration of Cu in the shoot required for maximum growth increases with increasing N supply (Thiel and Finck, 1973).

7.3.4 Lignification

Impaired lignification of cell walls is a typical anatomical change induced by Cu deficiency in higher plants. This results in the characteristic distortion of young leaves,

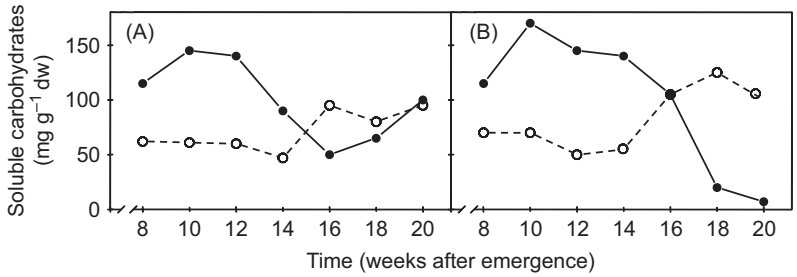


FIGURE 7.12 Concentrations of soluble carbohydrates in flag leaves (A) and roots (B) of wheat plants grown two Cu levels as a function of plant age. Key: ● + Cu; ○ − Cu. *Modified from Graham, 1980a.*

TABLE 7.14 Cell wall composition of the youngest fully emerged leaves of wheat with or without Cu supply

	+Cu	−Cu
Cu concentration (μg Cu g^{-1} dw)	7.1	1.0
Cell wall concentration (% of dw)	46.2	42.9
Cell wall composition (% of cell walls)		
α-cellulose	46.8	55.3
Hemicellulose	46.7	41.4
Lignin	6.5	3.3
Total phenolics (% of dw)	0.73	0.82
Ferulic acid (% of dw)	0.50	0.69

From Robson *et al.* (1981).

bending and twisting of stems and twigs (stem deformation and 'pendula' forms in trees; Oldenkamp and Smilde, 1966; Hopmans, 1990) and an increase in the lodging susceptibility of cereals, particularly in combination with a high N supply (Vetter and Teichmann, 1968).

As shown in Table 7.14, Cu has a strong effect on the formation and chemical composition of cell walls. In Cu-deficient leaves, the ratio of cell wall material to the total dry matter and the lignin concentration decrease, whereas the proportion of α-cellulose and hemicellulose increases compared to leaves adequately supplied with Cu. This effect on lignification is even more pronounced in the scelenchyma cells of stem tissue (Fig. 7.13). In severely Cu-deficient plants, the xylem vessels are also insufficiently lignified. A decrease in lignification occurs even with mild Cu deficiency and is thus a suitable indicator of the Cu nutritional status of a plant (Rahimi and Bussler, 1974; Pissarek, 1974).

Lignification responds rapidly to Cu supply; transient periods of Cu deficiency during growth can be readily identified by variations in the degree of lignification in stem sections (Bussler, 1981b).

The inhibiton of lignification in Cu-deficient tissue (Table 7.14) is related to a direct role of at least two Cu enzymes in lignin biosynthesis: polyphenol oxidase catalyses the oxidation of phenolics as precursors of lignin, and diamine oxidase provides the H_2O_2 required for oxidation by peroxidases. Accordingly, the activity of both enzymes is lower in Cu-deficient tissues and phenolics accumulate.

7.3.5 Pollen Formation and Fertilization

Copper deficiency affects grain, seed and fruit formation more strongly than vegetative growth. A typical example is shown in Table 7.15. Supplying 0.5 μg Cu produced maximum dry weight of roots and shoots, but flower formation was impaired, and no fruits were formed. For fruit formation a much higher Cu supply was required, >1.0 μg Cu, and with 10 μg Cu, toxicity occurred.

The main reason for the decrease in the formation of generative organs is the non-viability of pollen from Cu-deficient plants (Graham, 1975). The critical stage of Cu deficiency-induced pollen sterility is microsporogenesis. Reduced seed set in Cu-deficient plants may be the result of the inhibition of pollen release, since lignification of the anther cell walls is required to rupture the stamen and release the pollen. In Cu-deficient plants, lignification of the anther cell wall is reduced or absent (Dell, 1981); the anther cell wall expands instead of supplying the developing pollen with nutrients. Following grain set in wheat (Hill *et al.*, 1979c) and seed set in subterranean clover (Reuter *et al.*, 1981), further grain and seed growth, are not influenced by the Cu status of the plants, even though the Cu concentration of wheat grains in plants adequately supplied with Cu is five to six times higher than in deficient plants.

7.3.6 Cu Deficiency and Toxicity

7.3.6.1 Cu Deficiency

Copper deficiency is often observed in plants growing on soils either low in total Cu (e.g., ferrallitic and ferruginous coarse textured soils, or calcareous soils derived from chalk) and on soils high in organic matter where Cu

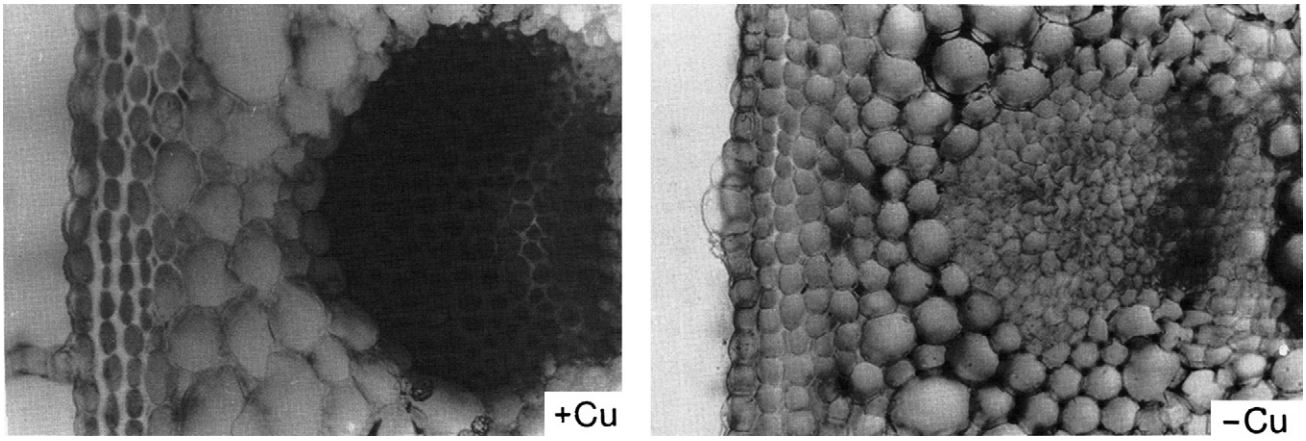


FIGURE 7.13 Stem sections of sunflower plants grown with sufficient Cu supply (50 µg $Cu1^{-1}$) (*left*) and without Cu supply (*right*) *Courtesy of A. Rahimi.*

TABLE 7.15 Relationship between Cu supply and growth and dry matter distribution in red pepper

	Dry weight (g dw $plant^{-1}$)			
Cu supply (µg pot^{-1})	Roots	Leaves and stems	Buds and flowers	Fruit
0.0	0.8	1.7	0.16	none
0.5	1.6	3.3	0.28	none
1.0	1.5	3.2	0.38	0.87
5.0	1.4	3.0	0.36	1.81
10	1.2	2.0	0.28	1.99

From Rahimi (1970).

is complexed with organic substances (Alloway and Tills, 1984). As mentioned above, high N availability can also lead to Cu deficiency.

The critical deficiency concentration of Cu in vegetative plant parts is generally in the range of 1–5 µg g^{-1} dw, depending on plant species, plant organ, developmental stage and N supply (Thiel and Finck, 1973; Robson and Reuter, 1981) with the critical deficiency concentration in the youngest emerged leaf being less affected by environmental factors than that of older leaves. Plant species differ considerably in sensitivity to Cu deficiency: wheat, oats and spinach are more sensitive than, for example, pea, rye and oilseed rape (Alloway and Tills, 1984). Stunted growth, distortion of young leaves, chlorosis/necrosis starting at the apical meristem extending down the leaf margins, and bleaching of young leaves ('white tip' or 'reclamation disease' of cereals grown in organic soils), and/or 'summer dieback' in trees are typical visible symptoms of Cu deficiency (Rahimi and Bussler, 1973). Enhanced formation of tillers in cereals and of auxiliary shoots in dicotyledons are secondary symptoms caused by necrosis of the apical meristem. Wilting in young leaves, also characteristic of Cu-deficient plants, is either the result of impaired water transport due to insufficient lignification of the xylem vessels (Rahimi and Bussler, 1973; Pissarek, 1974) or of structural weaknesses in the cell wall system rather than the result of a low water content *per se* (Graham, 1976). According to Yreula (2009) the molecular responses to Cu deficiency are increased expression of metal reductases and transporters, and prioritizing Cu to essential enzymatic pathways including compensatory increases in FeSOD and MnSOD in place of CuZnSOD.

The availability of Cu can be low in many soils and this can be corrected by soil or foliar applications (Gupta, 1979b). Soil applications of inorganic copper as $CuSO_4$ or oxide forms, or slow-release metal compounds, sewage sludges or manures are often appropriate for long-term effects. Foliar applications of Cu in the form of inorganic salts, oxides, or chelates can be used to rapidly correct Cu deficiency in soil grown plants. The use of Cu-containing fertilizers can be used to increase the Cu concentration of the edible portions of crops where there are dietary deficiencies of Cu in humans and livestock (White and Broadley, 2009). However, Cu fertilization must be managed appropriately since high Cu concentrations can be toxic to plants and animals. Selecting genotypes which are highly efficient in Cu uptake, translocation from the roots to the shoots and re-translocation within the shoot is a promising longer-term approach to the prevention of Cu deficiency.

7.3.6.2 Cu Toxicity

Toxic levels of Cu can occur under natural conditions or due to anthropogenic inputs. Anthropogenic inputs include those from the long-term use of Cu-containing fungicides

TABLE 7.16 Relationship between Cu supply, dry weight and Cu concentrations of different plant parts in tomato growing in nutrient solution

	Dry weight ($g\,dw\,plant^{-1}$)		Cu concentration ($mg\,kg^{-1}\,dw$)		
Cu supply ($\mu g\,L^{-1}$)	Roots	Shoots	Roots	Stems and petioles	Leaves
0	0.3	2.6	4.0	2.8	3.0
2.5	2.5	9.4	3.8	2.1	3.2
5.0	3.2	11.2	6.4	2.4	4.1
20.0	3.4	12.0	64.0	4.3	14.6
250.0	1.6	9.7	360.0	6.2	20.3

From Rahimi and Bussler (1974).

(e.g., in vineyards), industrial and urban activities (air pollution, urban waste and sewage sludge), and the application of pig and poultry slurries. For most crop species, the critical toxicity level of Cu in the leaves is above 20 to $30\,\mu g\,g^{-1}\,dw$ (Von Hodenberg and Finck, 1975; Robson and Reuter, 1981). There are, however, marked differences in Cu tolerance between plant species. Among certain Cu-tolerant species ('metallophytes'), particularly among the flora of the Cu-rich soils in the Democratic Republic of Congo, there have been field or herbarium reports that the Cu concentration in leaves can be as high as $1{,}000\,\mu g\,g^{-1}\,dw$. However, while these species may have an elevated requirement for Cu and are certainly highly tolerant of Cu, Cu 'hyperaccumulation' has not been demonstrated under controlled conditions, suggesting that some of these records may be due to leaf contamination with dust (Macnair, 2003; Chipeng *et al.*, 2010).

A high Cu supply usually inhibits root growth before shoot growth (Lexmond and Vorm, 1981). This does not mean that roots are inherently more sensitive to high Cu concentrations; rather, they are the sites of preferential Cu accumulation when the external Cu supply is high, as shown in Table 7.16 for tomato plants. With high supply, the Cu concentration of the roots increases proportionally to the concentration of Cu in the external medium, whereas transport to the shoot is still highly restricted. Critical toxicity concentrations of Cu in the shoots may therefore not necessarily reflect the Cu tolerance of plants. This is an important consideration when genotypes are compared. Even at high supply, up to 60% of the total Cu in roots can be bound to the cell wall fraction and the cell wall–plasma membrane interface (Iwasaki *et al.*, 1990). In addition to immobilization of Cu in the root, or reductions in uptake *per se* through binding of extracellular Cu by root exudates, cellular mechanisms of Cu tolerance are likely to include: (i) enhanced binding to cell walls, (ii) restricted influx through the plasma membrane, (iii) stimulation of efflux from the cytoplasm, including via HMA proteins, (iv) compartmentation of Cu by export to the vacuole, (v) chelation at the cell wall–plasma membrane interface, and (vi) intracellular chelation of Cu by organic acids, glutathione-derived phytochelatins and cysteine-rich metallothioneines in the cytoplasm (Fig. 7.14; see also Burkhead *et al.*, 2009; Yruela, 2009). In perennials, root colonization with ectomycorrhiza may play an important role in heavy metal tolerance of the host plant (see Chapter 15).

7.4 ZINC

7.4.1 General

Zinc (Zn) is the second most abundant transition metal in living organisms after Fe. Average total Zn concentration in cultivated soils is around $65\,mg\,kg^{-1}$ (Alloway, 2009). Zinc is taken up predominantly as a divalent cation (Zn^{2+}); at high pH, it is presumably also taken up as a monovalent cation ($ZnOH^{+}$). In long-distance transport in the xylem, Zn is either bound to organic acids or occurs as the free divalent cation (Chapter 3). In the phloem sap, the Zn concentrations are fairly high, with Zn possibly complexed by low-molecular-weight organic solutes (Kochian, 1991). In plants as well as in other biological systems, Zn exists only as ZnII, and does not take part in redox reactions. The metabolic functions of Zn are based on its strong tendency to form tetrahedral complexes with N-, O- and particularly S-ligands through which it plays a functional (catalytic) and a structural role in enzyme reactions (Vallee and Auld, 1990). In the last decade, impressive progress has been made on identification and characterization of catalytic

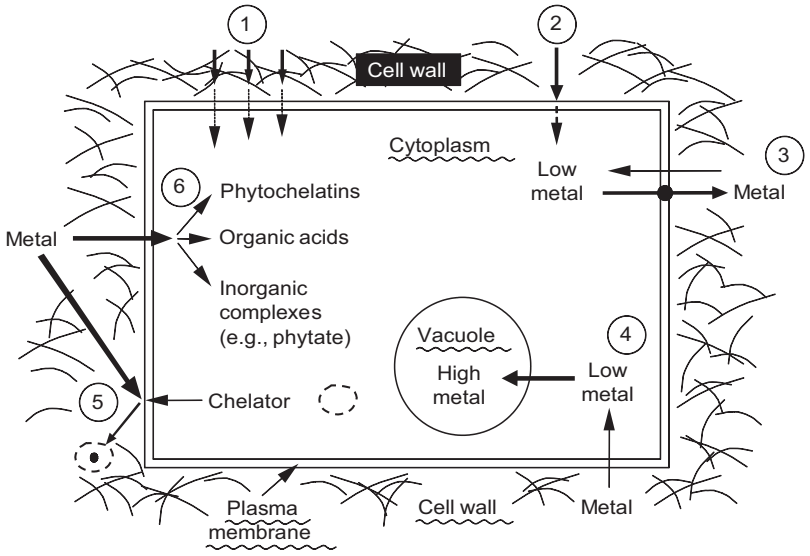
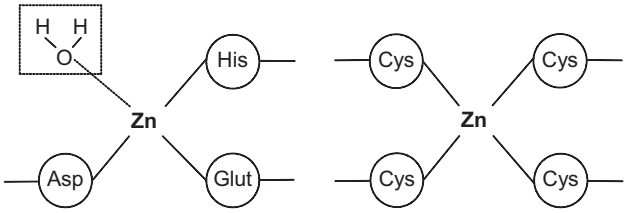


FIGURE 7.14 Possible mechanisms of heavy metal tolerance of plants. (1) Binding to cell wall; (2) restricted influx through plasma membrane; (3) active flux; (4) compartmentation in vacuole; (5) chelation at the cell wall–plasma membrane interface; (6) chelation in the cytoplasm. *Modified from Tomsett and Thurman, 1988.*

and structural Zn sites in proteins. Recent studies show the existence of a large number of proteins containing or binding Zn. It is estimated that up to 10% of the proteins in the human genome is proteins which require Zn for their structural or functional activities, indicating that at least 2,800 proteins are Zn dependent (Andreini *et al.*, 2009; Maret and Li, 2009). The role of Zn in protein molecules involved in DNA replication and in regulation of gene expression has attracted growing interest (Coleman, 1992; Broadley *et al*., 2007). Changes in metabolism induced by Zn deficiency are quite complex. Nevertheless, some of the changes are typical and can be explained by the functions of Zn in specific enzyme reactions or steps in particular metabolic pathways. By affecting expression and regulation of genes and defence mechanisms, Zn contributes to plant tolerance to environmental stress factors (Cakmak, 2000).

7.4.2 Zn-containing Enzymes

In biological systems, Zn is the only metal that is present in enzymes of all six enzyme classes including oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases (Sousa *et al.*, 2009). In these enzymes, four types of Zn-binding sites have been identified: (i) catalytic, (ii) structural, (iii) co-catalytic, and (iv) protein interface which determine the biological activity of the enzymes. In enzymes with catalytic Zn sites (e.g., carbonic anhydrase), Zn ions are coordinated to three protein ligands and one water molecule. Histidine is the most common ligand to these catalytic sites:



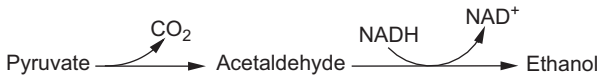
Structural Zn sites contribute to maintenance of the structure of enzymes (e.g., alcohol dehydrogenase, and proteins involved in DNA replication and gene expression). In these proteins, Zn ions are mostly coordinated to four cysteine residues. Cocatalytic Zn sites are present in enzymes containing two or more Zn atoms with aspartic acid and histidine being the most common ligands in these cocatalytic sites. At the protein interface, Zn bridges proteins or subunits and affects the protein–protein interactions (Auld and Bergman, 2009; Auld, 2009). In these Zn-binding sites, the most frequent amino acid ligand is histidine, accounting for 28% of all the Zn-binding ligands. As shown in Fig. 7.15, cysteine is the second important Zn-binding amino acid ligand and aspartic acid and glutamic acid are further important Zn ligands. Water molecules are also important Zn ligands within the protein structure.

7.4.2.1 Alcohol Dehydrogenase

Most Zn enzymes have only one Zn atom per molecule, the alcohol dehydrogenase being an exception. This

enzyme contains two Zn atoms per molecule, one with catalytic and the other with structural functions (Coleman, 1992; Auld and Bergman, 2008). The catalytic Zn sites are bound to two cysteins, one histidine and one water molecule, while the structural Zn-binding sites are generally complexed by four cysteins.

The enzyme catalyses the reduction of acetaldehyde to ethanol:



In higher plants under anaerobic conditions, ethanol formation takes place mainly in meristematic tissues, such as root apices. In Zn-deficient plants, alcohol dehydrogenase activity decreases, but the consequences for plant metabolism when grown in aerobic soils are not known. The situation is different in plants grown in waterlogged or submerged soils. In lowland rice, flooding stimulates the activity of root alcohol dehydrogenase twice as much in Zn-sufficient than in Zn-deficient plants. The lower activity of this key enzyme in anaerobic metabolism may impair root functions of submerged rice (Moore and Patrick, 1988).

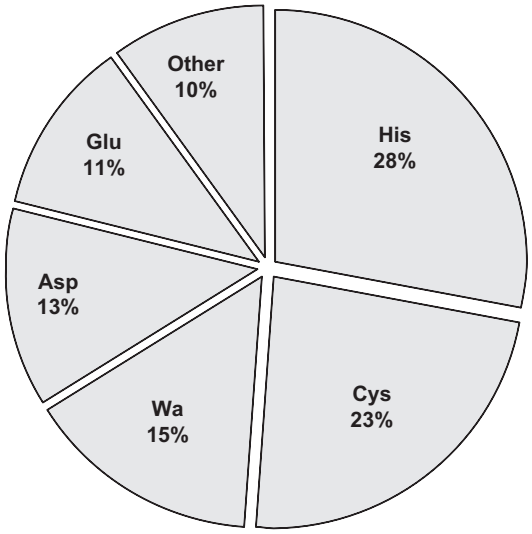


FIGURE 7.15 Overview of the percentage of Zn-binding ligands in the Zn proteome as present in the Protein Data Bank. Asp: aspartic acid; His: histidine; Cys: cysteine; Glu: glutamic acid; Wa: water *(Sousa* et al. *(2009) with permission from the Royal Society of Chemistry.*

7.4.2.2 Carbonic Anhydrase

The carbonic anhydrase contains a single Zn atom which catalyses the hydration of CO_2:

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$$

Carbonic anhydrase (CA) from dicotyledons consists of six subunits, has a molecular weight of 180 kDa, and six Zn atoms per molecule (Sandmann and Böger, 1983). The enzyme is localized in the chloroplasts and in the cytoplasm (Fig. 7.16).

The role of CA, and particularly that in chloroplasts, differs between C3 and C4 plants and, in C4 plants, between mesophyll and bundle sheath chloroplasts (see also Chapter 5). In C3 plants, CA is required to facilitate the diffusion of CO_2 to the sites of carboxylation by Rubisco. In C4 plants, CA meditates conversion of CO_2 to HCO_3^- to be used by phosphoenolpyruvate carboxylase (Fig. 7.16 and Badger and Price, 1994).

In C3 plants, there is no direct relationship between CA activity and photosynthetic CO_2 assimilation of plants with different Zn nutritional status (Fig. 7.16). In rice plants, Zn deficiency resulted in a decrease in the expression of mRNAs for CA, indicating that the decrease in CA activity by Zn deficiency is due to a reduced amount of the enzyme (Sasaki *et al.*, 1998). With extreme Zn deficiency, CA activity is completely inhibited, but even when CA activity is low, maximum net photosynthesis can occur (Fig. 7.17).

In C4 plants, however, the situation is different (Burnell and Hatch, 1988; Hatch and Burnell, 1990). A high CA activity is required in the mesophyll chloroplasts

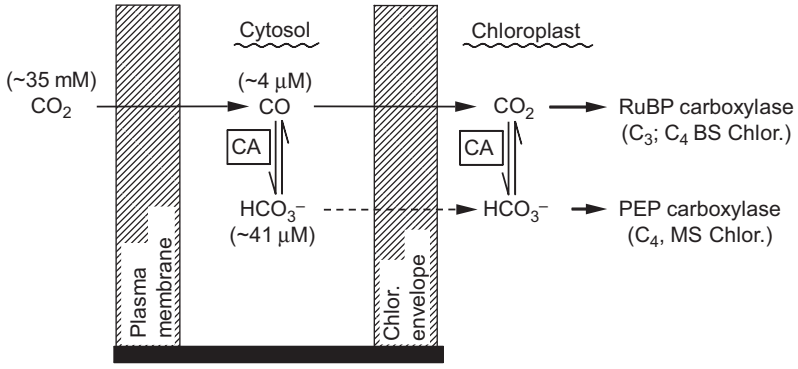


FIGURE 7.16 Functioning of carbonic anhydrase (CA) in leaf cells of C3 and C4 plants. BS = bundle sheath chloroplasts; MS = meshophyll chloroplasts. *Based on Edwards and Walker, 1983 and Hatch and Burnell, 1990.*

to shift the equilibrium in favour of HCO_3^- , the substrate for PEP carboxylase (Fig. 7.16), which forms C_4 compounds (e.g., malate) for the shuttle into the bundle sheath chloroplasts (see Chapter 5). Here CO_2 is released and serves as a substrate for RuBP carboxylase. In agreement with this, despite similar total activities in leaves of C3 and C4 plants, only 1% of the total CA activity in C4 plants is located in the bundle sheath chloroplasts (Burnell and Hatch, 1988), whereas 20–60% is associated with the plasma membrane (Utsunomiya and Muto, 1993).

At least in C4 plants, the *in vivo* activity of CA appears to be just sufficient to prevent the rate of conversion of CO_2 to HCO_3^- from limiting photosynthesis (Hatch and Burnell, 1990). Accordingly, Zn deficiency may have a more dramatic effect on the rate of photosynthesis in C4 compared with C3 plants (Burnell *et al.*, 1990).

7.4.2.3 CuZn Superoxide Dismutase

The CuZn superoxide dismutase (CuZnSOD) is the most abundant SOD in plant cells. Most likely, the Cu atom represents the catalytic metal component and Zn the structural. In the enzyme, Zn is bound to two histidines and one aspartate and contributes to structural stability of the enzyme (Abreu and Cabelli, 2010). The localization and role of CuZnSOD have been discussed in Section 7.3. As reviewed by Cakmak (2000), in a number of plant species Zn deficiency reduces CuZnSOD activity and a resupply of Zn rapidly restores enzyme activity, indicating that the Zn atom is an essential structural component for the normal functioning of CuZnSOD. CuZnSOD activity is strongly reduced to low supply of Zn and is therefore a better indicator of Zn deficiency tolerance than the total Zn concentration of leaf tissue (Cakmak *et al.*, 1997b; Yu *et al.*, 1999b; Hacisalihoglu *et al.*, 2003).

The decrease in SOD activity under Zn deficiency is particularly critical because of the simultaneous increase in the rate of $O_2^{\cdot -}$ generation (Table 7.17). The higher concentration of the toxic $O_2^{\cdot -}$ radicals and related oxidants leads to peroxidation of membrane lipids and an increase in membrane permeability (Cakmak and Marschner, 1988c). Accordingly, over-expression of CuZnSOD in transgenic plants increases their tolerance to various abiotic stress factors (Cakmak, 2000; Kim *et al.*, 2010).

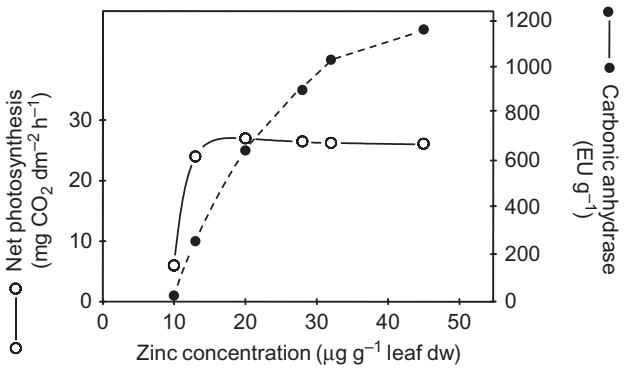


FIGURE 7.17 Relationship between the Zn concentration of leaf blades and net photosynthesis and carbonic anhydrase activity in cotton. EU: enzyme units. *Modified from Ohki, 1976.*

7.4.2.4 Other Zn-containing Enzymes

Zinc is the metal component in a number of other enzymes (Coleman, 1992, 1998), for example,

1. alkaline phosphatase
2. phospholipase: both these enzymes contain three Zn atoms each, of which at least one of has catalytic functions
3. carboxypeptidase, which hydrolyses peptide cleavages, and contains a single Zn atom with catalytical functions
4. RNA polymerase which contains two Zn atoms per molecule, and is inactive if Zn is removed (Prask and Plocke, 1971; Falchuk *et al.*, 1977). With the exception of green algae, RNA polymerase activity in relation to the Zn nutritional status has been studied extensively in bacteria, animals and humans. However, there is little information on this relationship in higher plants.

TABLE 7.17 Dry weight of roots and shoots and generation of superoxide radicals ($O_2\cdot^-$) and activity of superoxide dismutase (SOD) in roots of cotton with or without Zn supply

	Dry weight (g (4 plants)$^{-1}$)		Activity	
Zn supply	Shoots	Roots	$O_2\cdot^-$ generation (nmol min^{-1} mg^{-1} protein)	SOD (EU mg^{-1} protein)
+Zn	3.1	0.8	1.3	75
−Zn	1.8	0.5	3.7	35

Cakmak and Marschner (1988a).

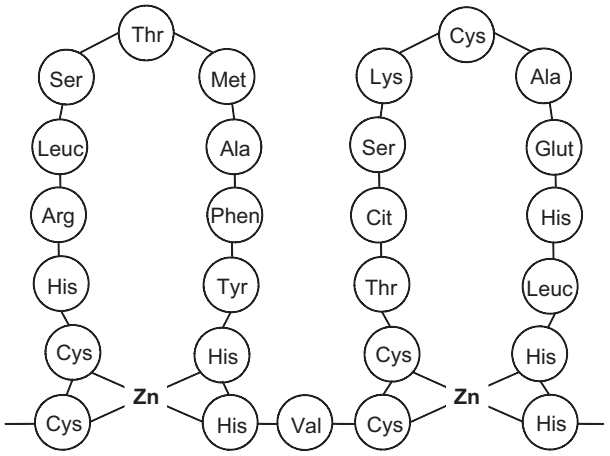


FIGURE 7.18 Schematic presentation of role of Zn in tertiary structure of the peptide chain in replication proteins ('zinc finger'). *Based on Coleman, 1992 and Vallee and Falchuk, 1993.*

TABLE 7.18 Shoot dry weight and composition of young leaves and shoot apex of bean plants with Zn supply (1 µmol Zn), without Zn supply or without Zn supply and then resupply of 3 µmol Zn for 3 days (−Zn +Zn)

	Zn Supply		
	+Zn	−Zn	−Zn +Zn
Shoot dw (g^{-1} dw $plant^{-1}$)	8.2	3.7	4.5
Zn concentration (µg g^{-1} dw)	52	13	141
Free amino acids (µmol g^{-1} dw)	82	533	118
Protein (mg g^{-1} fw)	28	14	30
Tryptophan (µmol g^{-1} dw)	0.4	1.3	0.3
IAA tryptophan (nmol g^{-1} fw)	239	118	198

From Cakmak *et al.* (1989).

7.4.3 Zn-activated Enzymes

In higher plants, Zn is either required for, or at least modulates, the activity of a large number of various types of enzymes, including dehydrogenases, aldolases, isomerases, transphosphorylases and RNA and DNA polymerases. Some examples are given below.

Inorganic pyrophosphatases (PP_iase) are important components of the proton-pumping activity in the tonoplast (Chapter 2). Besides the well-known Mg^{2+} dependent enzyme (Mg.PP_iase), a PP_iase isoenzyme in leaves is Zn^{2+} dependent (Zn.PP_iase). In rice leaves, the activity ratios of Mg./Zn.PP_iase vary between 3 and 6, and the two pyrophosphatases are most likely different isoenzymes (Lin and Kao, 1990).

The role of Zn in DNA and RNA metabolism, in cell division, and protein synthesis has been documented for many years, but only recently has a new class of Zn-dependent proteins (Zn-metallo proteins) have been identified which are involved in DNA replication, transcription and, thus, regulation of gene expression (Coleman, 1992; Vallee and Falchuk, 1993; Andreini *et al*., 2009). For transcription, Zn is required in these proteins for binding to specific genes by forming tetrahedral complexes with amino acid residues of the polypeptide chain (Fig. 7.18). In eukaryotic cells, 44% of the Zn-dependent proteins are used in regulating DNA transcription (Andreini *et al.*, 2009). The Cys(2)His(2) Zn finger is one of the most common DNA-binding motifs in eukaryotic cells with diverse functions in biological systems (Papworth *et al*., 2006). In these DNA-binding proteins, Zn is directly involved in the translation step of gene expression and activation or repression of DNA elements.

7.4.4 Protein Synthesis

In Zn-deficient plants, the rate of protein synthesis and the protein concentration are strongly reduced, whereas amino acids accumulate (Table 7.18). Upon resupply of Zn to deficient plants, protein synthesis resumes quite rapidly. Besides the functions of Zn described above, at least two other functions of Zn in protein metabolism are responsible for these changes. Zinc is a structural component of ribosomes and essential for their structural integrity. The Zn concentration of ribosomal RNA in Zn-sufficient cells of *Euglena* is in the range of 650 to 1280 µg g^{-1} RNA, whereas in Zn deficient cells it is 300 to 380 µg g^{-1} RNA (Prask and Plocke, 1971). In the absence of Zn, ribosomes disintegrate, but can be reconstituted after resumption of Zn supply.

In shoot meristems of rice, disintegration of the 80S ribosomes (soluble fraction in the cytoplasm) takes place when the Zn concentration is below 100 µg g^{-1} dw. Considerably lower Zn concentrations are required to decrease protein concentration (Fig. 7.19). In tobacco tissue culture cells, the corresponding concentrations were 70 µg Zn for a decrease in 80S ribosomes and 50 µg Zn for a decrease in protein concentration (Obata and Umebayashi, 1988).

A particularly high Zn requirement for protein synthesis has been also shown in pollen tubes, where the Zn concentration at the growing tip was about 150 µg g^{-1} dw compared with about 50 µg g^{-1} in more basal regions (Ender *et al.*, 1983). In the newly emerged root tips of wheat plants, Zn concentrations are about 220 µg g^{-1} (Ozturk *et al.*, 2006). In the shoot meristems, and presumably also in other meristematic tissues, a Zn concentration of at least 100 µg g^{-1} dw is required for maintenance of protein synthesis. As shown in Table 7.19, this is about

TABLE 7.19 Element concentration in meristematic tissue of the youngest leaf and of mature leaves blades of Zn-sufficient rice plants

	Element concentration in dry matter				
	Zn	Mn	Mg	Ca	K
	($\mu g\,g^{-1}$ dw)		($mg\,g^{-1}$ dw)		
Meristem	204	188	4.2	2.3	30.1
Mature leaves	18	540	8.9	6.0	12.8

Based on Kitagishi and Obata (1986).

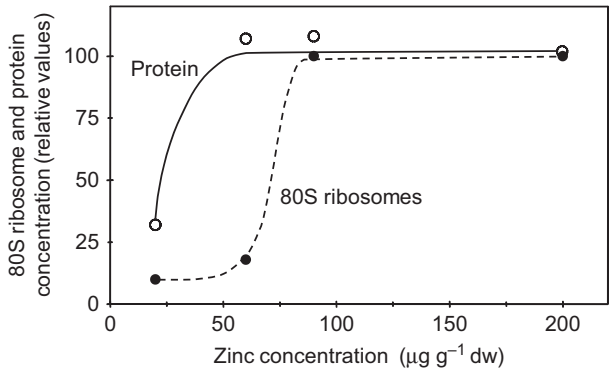


FIGURE 7.19 Relationship between concentration of Zn, 80S ribosomes and protein in the soluble fraction of rice shoot meristematic tissue. *Based on Kitagishi* et al. *(1987).*

TABLE 7.20 Fresh weight, RNase activity and protein-N in concentration perennial soybean (*Glycine wightii*) at different rates of Zn supply

Zn supply ($\mu g\,L^{-1}$)	Fresh weight ($g\,fw\,plant^{-1}$)	RNAse activity (% hydrolysis)	Protein-N ($mg\,g^{-1}\,fw$)
5	4.0	74	18.2
10	5.1	58	22.5
50	6.6	48	27.8
100	10.0	40	36.5

Based on Johnson and Simons (1979).

TABLE 7.21 Activity of enzymes in leaves of maize plants grown without Zn supply for 5, 10 and 15 days

	Decrease in activity after days without Zn supply (% of sufficient plants)		
Enzyme	5	10	15
Fructose-1,6-bisphosphatase	36	50	65
Carbonic anhydrase	84	76	84
PEP carboxylase	<1	5	34
RuBP carboxylase	9	41	38
Malic enzyme	<1	22	37

Based on Shrotri *et al.* (1983).

5–10 times more than the adequate Zn concentration in mature leaf blades. For other nutrients this gradient is usually less steep. To meet the high Zn demand in the shoot meristem, most of the root-supplied Zn is preferentially translocated to the shoot meristem, via xylem–phloem transfer in the stem (Kitagishi and Obata, 1986).

Low protein and high amino acid concentration in Zn-deficient plants are not only the result of reduced transcription and translation but also of enhanced rates of RNA degradation due to high RNAse activity under Zn deficiency (Sharma *et al.*, 1982). There is a negative relationship between Zn supply and RNAse activity, and also between RNAse activity and protein concentration (Table 7.20).

7.4.5 Carbohydrate Metabolism

Many Zn-dependent enzymes are involved in carbohydrate metabolism in general and of leaves in particular. In Zn-deficient leaves, the rapid decrease in carbonic anhydrase activity is the most sensitive and obvious change in activity of enzymes of the carbohydrate metabolism (Table 7.21). The activity of fructose 1,6 bisphosphatase also declines fairly rapidly, whereas the activity of other enzymes is affected to a much lesser extent, particularly with mild Zn deficiency.

Despite a decrease in enzyme activities and in the rate of photosynthesis (as indicated by the activity of the Hill reaction), sugars and starch often accumulate in Zn-deficient plants (Table 7.22). As early as 24 h after the Zn supply is restored, the sugar concentration and the Hill reaction activity are again comparable to those of the adequately supplied control plants continuously

TABLE 7.22 Zn and carbohydrate concentrations of cabbage leaves at high or low Zn supply and 24 h after resumption of Zn supply (2 μM Zn) to Zn-deficient plants

	Zn supply (μM)		
	0.001	0.001	0.001 + 2.0
Zn concentration (mg kg^{-1} dw)	21	14	30
Sugars (mg kg^{-1} dw)	4	9	5
Starch (mg kg^{-1} dw)	8	26	19
Hill reaction activity (%)	100	48	66

Based on Sharma *et al.* (1982).

receiving 1.0 μM Zn. The accumulation of carbohydrates in Zn-deficient leaves increases with light intensity (Marschner and Cakmak, 1989) and is an expression of impaired new growth, particularly of the shoot apices, i.e. of lower sink activity probably due to reduced concentrations of phytohormones which stimulate cell elongation.

Most experimental evidence obtained with green plants supports the view that Zn deficiency-induced changes in carbohydrate metabolism are not primarily responsible for either growth retardation or the visible symptoms of Zn deficiency.

7.4.6 Tryptophan and Indoleacetic Acid Synthesis

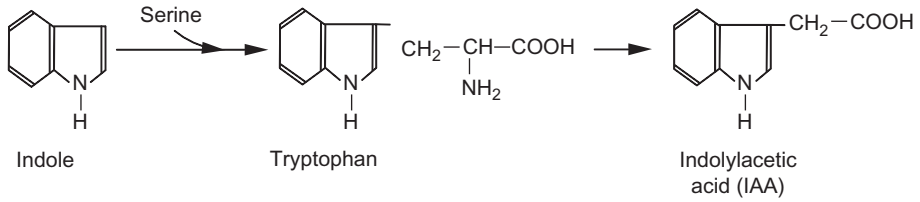
The most distinct Zn deficiency symptoms – stunted growth and 'little leaf' – are presumably related to disturbance in the metabolism of auxins, indoleacetic acid (IAA) in particular. The mode of action of Zn in auxin metabolism is still unclear. In Zn-deficient tomato plants, retarded stem elongation is correlated with a decrease in IAA concentration; upon resupply of Zn, stem elongation and IAA concentrations increase. The response to the Zn treatment was more rapid for IAA concentrations than for elongation growth (Tsui, 1948). Low concentrations of IAA in Zn-deficient plants may be the result of inhibited synthesis or enhanced degradation of IAA (Cakmak *et al.*, 1989). Tryptophan is most likely the precursor for the biosynthesis of IAA:

In leaves of Zn-deficient plants, tryptophan concentrations increase similarly to other amino acids (Cakmak *et al.*, 1989; Domingo *et al.*, 1992), most likely as a result of impaired protein synthesis as shown in Table 7.18. Although the lower IAA concentration in Zn-deficient leaves may indicate a role for Zn in the biosynthesis of IAA from tryptophan, as postulated by Salami and Kenefick (1970), lower IAA concentrations are more likely the result of enhanced oxidative degradation of IAA (Fig. 7.20). Adequate Zn nutrition also increases the concentrations of endogenous gibberellins (Sekimoto *et al.*, 1997). The low concentrations of IAA and gibberellins may be the cause for the stunted growth and 'little leaf' formation under Zn deficiency.

7.4.7 Membrane Integrity and Lipid Peroxidation

Zinc is required for maintenance of integrity of biomembranes. It binds to phospholipid and sulphhydryl groups of membrane constituents or forms tetrahedral complexes with cysteine residues of polypeptide chains and thereby protects membrane lipids and proteins against oxidative damage. In its function as a metal component in CuZnSOD, Zn may also control the generation of toxic oxygen radicals by interfering with the oxidation of NADPH, as well as by scavenging $O_2^{\cdot -}$ (Cakmak and Marschner, 1988a, b). Accordingly, there is a typical increase in plasma membrane permeability, for example in roots under Zn deficiency (Welch *et al.*, 1982) indicated by leakage of low-molecular-weight solutes, a decrease in phospholipid concentration and in the degree of unsaturation of fatty acids in membrane lipids (Table 7.23). As early as 12 hours after resupplying Zn, some restoration of membrane integrity can be observed. Plasma membrane vesicles, isolated from Zn-deficient roots, also have a higher passive permeability than vesicles from Zn-sufficient roots (Pinton *et al.*, 1993).

Increased membrane permeability in Zn-deficient plants is due to higher rates of $O_2^{\cdot -}$ generation (Table 7.17) as a result of increased activity of an NADPH-dependent $O_2^{\cdot -}$ generating oxidase (Table 7.24). Higher activity of this oxidase is either a reflection of a direct role of Zn in regulation of enzyme activity, or an indirect result of the alterations in structure and composition of the membranes (Table 7.23).



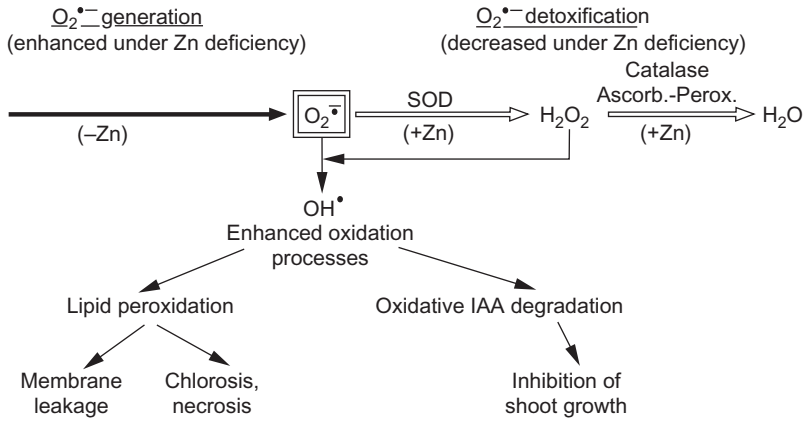


FIGURE 7.20 Involvement of Zn in the generation and detoxification of superoxide radicals, and effects of oxygen-free radicals on membrane function and IAA metabolism. *Compiled from Cakmak and Marschner, 1988a, b and Cakmak* et al.*, 1989.*

TABLE 7.23 Leakage of low-molecular-weight solutes (root exudates) and lipid composition of roots of cotton plants with or without Zn supply or with resupply of Zn to deficient plants for 12 h (−Zn +Zn)

	Zn supply		
	+Zn	−Zn	−Zn +Zn
Root Zn concentration (μg g^{-1} dw)	258	16	121
Root exudates (g^{-1} dw 6 h^{-1})			
Amino acids (μg)	48	165	94
Sugars (μg)	375	751	652
Phenolics (μg)	117	161	130
K (mg)	1.7	3.7	2.3
Lipid composition			
Phospholipids (mg g^{-1} fw)	2.2	1.5	nd
Fatty acid ratio (saturated/unsaturated)	0.8	0.9	nd

Based on Cakmak and Marschner (1988c).
nd: not determined.

TABLE 7.24 Zinc concentration in roots and shoots, chlorophyll concentration and superoxide generation and NADPH oxidation in root extracts of bean plants with or without Zn supply or with resupply of Zn to deficient plants for 2 days (−Zn +Zn)

	Zn supply		
	+Zn	−Zn	−Zn +Zn
Zn concentration (μg g^{-1} dw)			
Roots	44	11	69
Shoots	37	10	71
Chlorophyll (mg g^{-1} dw)	7.4	3.6	4.1
$O_2 \cdot^-$ generation (nmol mg^{-1} protein min^{-1})	2.2	6.6	4.3
NADPH oxidation (nmol mg^{-1} protein min^{-1})	18.3	61.0	40.0

Cakmak and Marschner (1988a).

Many of the most obvious symptoms of Zn deficiency such as leaf chlorosis and necrosis, inhibited shoot elongation and increased membrane permeability are expressions of oxidative stress brought about by higher generation of reactive oxygen species (ROS) and an impaired detoxification system in Zn-deficient plants. In Zn-deficient plants, the activity of H_2O_2 scavenging enzymes, such as catalase and ascorbate peroxidase, is also reduced, probably due to inhibited protein synthesis (Yu *et al*., 1999b; Cakmak, 2000), leading to accumulation of H_2O_2 and stimulation of lipid peroxidation in Zn-deficient tissues (Chen *et al*., 2008b). These events are summarized schematically in Fig. 7.20.

7.4.8 P-Zn Interactions

High application rates of P fertilizers to soils low in available Zn can induce Zn deficiency (*P-induced Zn deficiency*; Robson and Pitman, 1983), by altering either soil or plant factors. In soil, high P concentrations can decrease solubility of Zn (Marschner and Schropp, 1977; Loneragan *et al.*, 1979). However, this is not always the case (Pasricha *et al.*, 1987). High P supply is often associated with a reduction in root growth and a lesser degree of colonization of roots with arbuscular mycorrhiza (AM) (Ryan *et al*., 2008; see also Chapters 13 and 15). Both these factors are important for the acquisition of Zn. In wheat, P fertilization reduced grain Zn concentration by 33 to 39% and root colonization with AM by 33 to 75% (Ryan

TABLE 7.25 Growth and Zn and P concentration of the shoots of ochra (*Abelmoschus esculentum L.*) grown at different Zn supply and P supply in nutrient solution

	Dry weight ($g\,plant^{-1}$)		Zn concentration ($\mu g\,g^{-1}\,dw$)		P concentration ($mg\,g^{-1}\,dw$)	
P supply (mM P)	0.25	2.0	0.25	2.0	0.25	2.0
Zn supply (μM)						
0	8.3	9.5	15	15	11	24
0.25	9.6	9.9	27	27	10	20
1.0	9.8	11.6	54	57	9	12

Based on Loneragan *et al.* (1982b).

et al., 2008). The decrease in grain Zn concentration by P fertilization was also related to dilution of Zn due to increased grain yield with P fertilization. Similarly, the decrease in Zn concentration in shoots and an induction of Zn deficiency symptoms by high P supply is the result of enhanced shoot growth and, thus, 'dilution' of Zn in the plants (Loneragan *et al.*, 1979; Neilsen and Hogue, 1986). There are, however, additional physiological interactions between P and Zn within the plants involved. With increasing P concentration in the shoot, Zn deficiency symptoms become more severe, although the Zn concentration is not decreased (Table 7.25; Cakmak and Marschner, 1987). However, the physiological availability of Zn is decreased as indicated, for example, in lower proportions of water extractable Zn and lower SOD activity in leaves (Cakmak and Marschner, 1987). P concentration in the shoot may therefore decrease solubility and mobility of Zn both within the cells and in long-distance transport to the shoot apex.

In solution culture at high P but low Zn supply, the P-induced Zn deficiency is often associated with very high P concentrations and symptoms of P toxicity in mature leaves (Loneragan *et al.*, 1979; Cakmak and Marschner, 1986; Parker, 1997), which may be mistaken for evidence of accentuation of Zn deficiency because of the large P/Zn ratio. As shown in Table 7.26, Zn uptake is not affected by increasing P concentrations in the external solution. In the absence of Zn, or with low external concentrations, however, the P concentration in the shoot is very high, leading to toxicity symptoms. In general, a P concentration greater than $20\,mg\,kg^{-1}$ in leaves can be considered as toxic.

The main reason for the high P concentration in the leaves is that Zn deficiency enhances the P uptake rate by the roots and its translocation to the shoots (Tables 7.26 and 7.27). Zinc deficiency also increases the permeability of the plasma membrane of root cells to P, as well as to Cl (Welch *et al.*, 1982) and B, and may even lead to B toxicity (Graham *et al.*, 1987b; Singh *et al.*, 1990b).

TABLE 7.26 Dry weight and P concentration in roots and shoots of cotton plants without micronutrient deficiency, or deficient in Zn, Fe, Mn or Cu

	Dry weight ($mg\,plant^{-1}$)		P concentration ($mg\,g^{-1}\,dw$)	
	Roots	Shoots	Roots	Shoots
Control	180	1,210	1.03	1.10
−Zn	130	700	1.15	2.65
−Fe	160	980	1.00	0.90
−Mn	150	930	0.96	1.20
−Cu	160	1,000	1.38	1.40

Cakmak and Marschner (1986).

Thus, enhanced P uptake in Zn-deficient plants can in part be due to higher passive permeability of the plasma membranes of root cells or impaired control of xylem loading.

The high P concentration in the shoots of Zn-deficient plants is also the result of a specific impairment of re-translocation of P in the phloem (Table 7.27) and, thus, of an important 'signal' in shoot control on P uptake by the roots (Chapter 3). The mechanism by which Zn deficiency impairs re-translocation of P from the shoots is unclear, as in Zn-deficient plants neither the re-translocation of ^{86}Rb nor ^{36}Cl are impaired (Table 7.27). Zinc deficiency has been also found to enhance the abundance of high affinity P transporter proteins in barley roots irrespective of P nutritional status of plants (Huang *et al*., 2000). Normally, expression of the P transporter proteins is down-regulated by high P nutritional status of plants. However, under Zn deficiency, down-regulation of the expression of the P transporter genes is impaired, leading to high root uptake and shoot accumulation of P (Huang *et al*., 2000).

TABLE 7.27 Distribution of ^{32}P, ^{86}Rb and ^{36}Cl between shoots and roots (% of total in plant) of Zn-sufficient and Zn-deficient cotton plants, 19 h after stem application

	^{32}P		^{86}Rb		^{36}Cl	
Zn supply	Shoots	Roots	Shoots	Roots	Shoots	Roots
+Zn	66	34	62	38	29	71
−Zn	92	8	66	34	32	68

Marschner and Cakmak (1986).

TABLE 7.28 Element concentration in germ and protein bodies in the germ of maize kernels

	Zn	Fe	Mn	Cu	Ca	K	Mg	P	
								Total	Phytate
	($\mu g\,g^{-1}$ dw)				($mg\,g^{-1}$ dw)				
Germ	163	186	30	12	449	27	10	30	23
Protein bodies	565	490	170	11	1,645	68	44	89	88

Marschner, Ehret and Haug (unpublished).

7.4.9 Zn binding Forms and Bioavailability

Much is known about the localization and binding forms of Zn in seeds and grains; however less is known for vegetative organs. In grains and seeds, most of the Zn and other nutrients are localized in so-called 'protein bodies' in the form of discrete particles, the *globoid crystals* (Lott and Buttrose, 1978; Welch, 1986). These globoids mainly consist of phytate, i.e. salts of phytic acid (Table 7.28). In wheat seeds, similarly high Zn concentrations ($600\,\mu g\,g^{-1}$ dw) were found in the scutellum (Mazzolini *et al.*, 1985). Zinc, Fe and proteins are generally co-localized within seed tissues (Cakmak *et al.*, 2010b) and there is a very high positive correlation between the concentrations of Zn, Fe and protein in seeds of a number of germplasms (Peterson *et al.*, 1986; Morgounov *et al.*, 2007; Zhao *et al.*, 2009; Cakmak *et al.*, 2010b). These results suggest that protein is a sink for Zn and Fe. A recent speciation analysis in the barley embryo fraction showed that Fe is bound to phytic acid whereas Zn is mainly associated with proteins or peptides (Persson *et al.*, 2009).

Phytic acid is a strong negatively charged compound and has high affinity to bind divalent cations such as Zn, forming insoluble or unavailable Zn-phytate complexes in seeds (Lönnerdal, 2002; Schlemmer *et al.*, 2009). The strong binding of Zn to phytic acid is of concern to nutritionists as it reduces the bioavailability of Zn for monogastric animals and man. A negative correlation occurs, for example, in soybean products between phytic acid (phytate) concentration and zinc bioavailability for rats (Zhou *et al.*, 1992; Lönnerdal, 2000). It is possible to reduce the phytate concentration of seeds and grains by selection and breeding, or by P deficiency; however, a lower phytate concentration of seeds is associated with various negative effects such as reduced seedling emergence and poor agronomic performance (Oltmans *et al.*, 2005). The formation of phytate is not confined to reproductive organs, therefore, decreased physiological availability of Zn in vegetative plant organs resulting from the formation of phytate may also be important, particularly in the context of P-induced Zn deficiency.

TABLE 7.29 Concentrations of Zn in the cytoplasm and vacuoles of roots of a Zn-tolerant and Zn-sensitive clone of *Deschampsia caespitosa* at low or high Zn supply

Zn supply (mM Zn)	Bound Zn in the cytoplasm (mM)		Soluble Zn in the vacuole (mM)	
	Sensitive	Tolerant	Sensitive	Tolerant
0.10	7.1	10.6	3.7	5.3
0.75	33.4	6.2	2.1	33.4

Based on Brookes *et al.* (1981).

7.4.10 Zn Deficiency and Toxicity

7.4.10.1 Zn Deficiency

Zinc deficiency is widespread among plants grown in highly weathered acid soils and in calcareous soils. In the latter case, Zn deficiency is often associated with Fe deficiency ('lime chlorosis'). The low availability of Zn in calcareous soils of high pH is mainly due to the adsorption of Zn to clay or $CaCO_3$, rather than from the formation of sparingly soluble $Zn(OH)_2$ or $ZnCO_3$ (Trehan and Sekhon, 1977). In addition, Zn uptake and translocation to the shoot are inhibited by high concentrations of bicarbonate, HCO_3^- (Forno *et al.*, 1975; Dogar and van Hai, 1980). This effect is very similar to the effect of HCO_3^- on Fe. In contrast to Fe deficiency, however, Zn deficiency in plants grown in calcareous soils can be corrected quite readily by application of inorganic Zn salts such as $ZnSO_4$ to the soil (Nayyar and Takkar, 1980; Cakmak *et al*., 1996a).

The most characteristic visible symptoms of Zn deficiency in dicotyledonous plants are stunted growth due to shortening of internodes ('rosetting') and a drastic decrease in leaf size ('little leaf'), as shown in Fig. 7.21. Under severe Zn deficiency, the shoot apices die ('dieback') as, for example, in forest plantations in South Australia (Boardman and McGuire, 1990). Quite often these symptoms are combined with chlorosis, which is either highly contrasting or diffusive ('mottle leaf'). These symptoms are usually more severe at high light intensity than in partial shade (Boardman and McGuire, 1990). Similarly, plants are more susceptible to low Zn supply when exposed to heat and drought stress (Bagci *et al*., 2007; Peck and McDonald, 2010). In cereals such as wheat, typical symptoms are reduction in shoot elongation and development of whitish-brown necrotic patches on middle-aged leaves, whereas young leaves remain yellowish green in colour, but show no necrotic lesions (Cakmak *et al*., 1996a). Symptoms of chlorosis and necrosis in older leaves of Zn-deficient plants are often secondary effects caused by P or B toxicity, or by photooxidation resulting from impaired export of photosynthates.

Under Zn deficiency, shoot growth is usually more inhibited than root growth (Zhang *et al.*, 1991a), and root growth may even be enhanced at the expense of the shoot growth (Cumbus, 1985; Cakmak *et al*., 1996b). Zinc deficiency increases root exudation of low-molecular-weight solutes. In dicotyledonous plants, amino acids, sugars, phenolics and potassium dominate (Table 7.23), whereas in graminaceous species the main solutes are phytosiderophores (Zhang *et al.*, 1991a) which are released in a distinct diurnal pattern (Zhang *et al.*, 1991b; Cakmak *et al.*, 1994c), as is typical for Fe deficiency (see also Chapter 2). Enhanced release of phytosiderophores under both Zn and Fe deficiency are separately regulated and not related to a Zn deficiency-induced disturbance of Fe metabolism in the plants (Suzuki *et al*., 2006).

FIGURE 7.21 Symptoms of Zn deficiency in apple with typical inhibition of internode elongation ('rosetting') and reduction in leaf size ('little leaf').

In leaves, the critical deficiency concentrations are below 15–20 μg Zn g^{-1} dw (but see Section 7.4.4). Grain and seed yield are depressed to a greater extent by Zn deficiency than the total dry matter production, probably due at least in part to impaired pollen fertility in deficient plants. Plant species differ in their sensitivity to Zn deficiency, with maize, rice and apples being more sensitive than, for example, rye, oats, or pea. Among the cereal species, rye has the highest tolerance to Zn deficiency, followed by triticale, barley, bread wheat, oats and durum wheat (Cakmak *et al*., 1997a).

7.4.10.2 Zn Toxicity

Zinc toxicity is observed very rarely in crop plants and occurs mainly in soils contaminated by mining and smelting activities and treated with sewage sludge (Broadley *et al.*, 2007). At very high Zn supply, Zn toxicity can readily be induced in non-tolerant plants with inhibition of root elongation being a very sensitive parameter (Godbold *et al.*, 1983; Ruano *et al.*, 1988). Quite often, Zn toxicity leads to chlorosis in young leaves. This may be an induced deficiency of, for example, Mg or Fe, because of the similar ion radius of Zn^{2+} and Fe^{2+} (Woolhouse, 1983; Sagardoy *et al*., 2009) and Zn^{2+} and Mg^{2+} (Boardman and McGuire, 1990; Sagardoy *et al*., 2009). Induced Mn deficiency may also be of importance, as high Zn supply strongly decreases the Mn concentration of plants (Ruano *et al.*, 1987).

In bean plants, Zn toxicity inhibits photosynthesis at various steps and through different mechanisms. Depressed RuBP carboxylase activity is presumably caused by

competition with Mg (Van Assche and Clijsters, 1986a). High Zn supply to sugar beet plants strongly reduces photosynthesis by depleting CO_2 at the Rubisco as a consequence of severe reductions in stomatal conductance (70%) and mesophyll conductance (44%) to CO_2 (Sagardoy *et al*., 2009). Excess Zn can also inhibit PS II activity by replacing Mn in the thylakoid membranes (Van Assche and Clijsters, 1986b). Whereas in the thylakoid membranes of control plants about 6 atoms of both Mn and Zn are bound per 400 chlorophyll molecules, under Zn toxicity this proportion shifts to 2 Mn and 30 Zn atoms.

The critical toxicity concentrations in leaves of crop plants range from 100 µg Zn g^{-1} dw (Ruano *et al.*, 1988) to more than 300 µg Zn g^{-1}, the latter values being more typical. Increasing soil pH by liming is the most effective strategy for decreasing Zn concentration and zinc toxicity in plants (White *et al.*, 1979). In comparison with the genotypical differences between wild plants, differences in zinc tolerance between crop plants are small, but nevertheless marked, even within the same species.

7.4.10.3 Zn Tolerance

As with Cu tolerance, the mechanisms responsible for Zn tolerance have long been of major interest in ecophysiology (Baker and Walker, 1989a, b; Verkleij and Schat, 1989). Zinc tolerance is also of interest in agriculture and crop physiology, as Zn is the heavy metal found to occur in the greatest concentrations in the majority of wastes arising in modern, industrialized communities (Boardman and McGuire, 1990; Hall, 2002).

The principal mechanisms of heavy metal tolerance are illustrated in Fig. 7.13 and reviewed comprehensively by Hall (2002). In contrast to Cu, exclusion from uptake, or binding to the cell walls, does not seem to be important for Zn tolerance (Qureshi *et al*., 1985; Vazquez *et al.*, 1992). However, a particular mechanism of exclusion may exist in forest tree species such as *Pinus sylvestris,* where certain ectomycorrhizal fungi retain most of the zinc in their mycelium and, thus, strongly increase the Zn tolerance of the host plant (Colpaert and Van Assche, 1992; Jentschke and Godbold, 2000; see also Chapter 15).

In the case of Zn, tolerance is achieved mainly through sequestering Zn in the vacuoles as shown in Table 7.29. In the sensitive clone receiving a high supply of Zn, Zn is preferentially accumulated in the cytoplasm. In the tolerant clone, on the other hand, the Zn concentration in the cytoplasm remains low; instead, Zn is sequestered in the vacuoles. Vacuolar membranes possess a Zn transporter metal tolerance protein (MTP) that mediates Zn transport from the cytosol into the vacuole leading to detoxification of excessive Zn (Kawachi *et al*., 2009; Gustin *et al*., 2009). There are positive correlations in tolerant genotypes between accumulation of organic acids such as malate and citrate, and accumulation of Zn, indicating that complexation of Zn with organic acids in the vacuoles may be an important mechanism of Zn tolerance (Godbold *et al.*, 1983, 1984).

Previous reports indicated that phytochelatins are not involved in Zn tolerance (Grill *et al.*, 1988; Robinson, 1990; Davies *et al.*, 1991a). However, recently there is evidence showing important roles of phytochelatins in plant tolerance to Zn toxicity. Arabidopsis mutants deficient in phytochelatins were highly susceptible to Zn toxicity (Tennstedt *et al.*, 2009; Clemens and Peršoh, 2009). In non-vacuolated, meristematic tissues such as root apices, other tolerance mechanisms have to exist such as sequestering of Zn by binding to phytate as it occurs in a Zn-tolerant ecotype of *Deschampsia caespitosa* (Van Steveninck *et al.*, 1987a, b).

7.5 NICKEL

7.5.1 General

Nickel is chemically related to Fe and Co. Its preferred oxidation state in biological systems is Ni^{2+} (Ni II), but it can also exist in the redox states Ni I and Ni III (Cammack *et al.*, 1988). Nickel forms stable complexes, for example with histidine, cysteine and citrate (Thauer *et al*., 1980), and in Ni-enzymes it is coordinated to various ligands (Li and Zamble, 2009).

The first clear evidence for the function of Ni in urease in higher plants was provided by Dixon *et al.* (1975). Later, a requirement of Ni in legumes (Eskew *et al*., 1984) and subsequently in a number of non-legumes grown with varying N sources was demonstrated (Brown *et al*., 1987a,b). Nickel deficiency in crops was discovered in pecan (*Carya illinoiensis* (Wangh.) K. Koch) trees growing in sandy, poorly draining soils with low cation exchange capacity of south-eastern USA (Wood *et al.*, 2004).

7.5.2 Ni-containing Enzymes

Nickel is involved in the function of at least nine proteins (Li and Zamble, 2009) including methyl-coenzyme M reductase, superoxide dismutase, Ni-dependent glyoxylase, aci-reductone dioxygenase, NiFe-hydrogenase, carbon monoxide dehydrogenase, acetyl-CoA decarbonylase synthase and methyleneurease, of which urease and the Ni-urease accessory protein (Eu3) (Freyermuth *et al*., 2000) have roles in plants. Symptoms of Ni deficiency suggest additional essential roles are likely (Bai *et al.*, 2006; Brown *et al*., 1990).

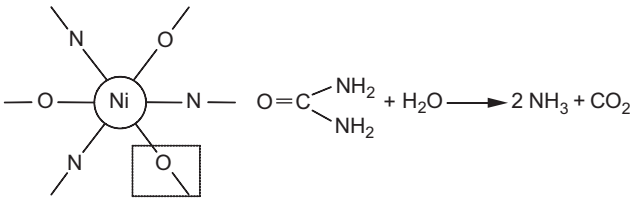
Urease isolated from jack bean (*Canavalia ensiformis* L.) has a molecular weight of 590 kDa, and consists of six subunits, each subunit containing two Ni atoms (Dixon *et al.*, 1980). In the subunits, Ni is coordinated to N- and O-ligands, and one of the Ni–O bonds can

TABLE 7.30 Leaf tip necrosis, urea concentration and urease activity in soybean plants with and without Ni supply and with three rates of foliar application of urea

Ni supply ($\mu g\ L^{-1}$)	Foliar application (mg urea $leaf^{-1}$)	Leaf tip necrosis (%)	Urea concentration ($\mu g\ g^{-1}$ dw)	Urease activity ($\mu mol\ NH_3\ h^{-1}\ g^{-1}$ dw)
0	0	<0.1	64	2.2
	3	5.2	1,038	2.7
	6	13.6	6,099	2.4
100	0	0	0	11.8
	3	2.0	299	11.3
	6	3.5	1,583	9.6

Based on Krogmeier *et al.* (1991).

possibly be displaced by water molecules during hydrolytic reactions:



Nickel is not required for the synthesis of the urease protein (Winkler *et al.*, 1983) but, as the metal component, is essential for the structure and catalytic function of the enzyme (Klucas *et al.*, 1983). The Ni-urease accessory protein (Eu3) is required for urease function (Freyermuth *et al.*, 2000).

In hydrogenases from sulphate-reducing, photosynthetic and hydrogen-oxidizing bacteria (thus also the hydrogen uptake hydrogenases of rhizobia) (Chapter 16), Ni is associated with Fe-S clusters (Li and Zamble, 2009). *Rhizobium* and *Bradyrhizobium* produce hydrogen-uptake hydrogenase when free-living and as bacteroids in the root nodules (Maier *et al.*, 1990). In free-living rhizobia, without Ni supply, the hydrogenase activity is very low, but can be restored within 3 hours by resupplying Ni (Maier *et al.*, 1990).

7.5.3 Role of Ni in N Metabolism

When supplied with urea as sole N source and in the absence of Ni, growth of *Lemna* (Gordon *et al.*, 1978) and of tobacco, zucchini, tomato, rice and canola is reduced (Gerendas *et al.*, 1999; Nicoulaud and Bloom, 1998). Soil or foliar addition of Ni increased growth, decreased urea concentrations and increased urease activity. In low Ni plants supplied with urea, not only is the utilization of this form of N impaired, but also urea toxicity occurs. Foliar application of urea is often associated with urea toxicity, and the severity of toxicity symptoms are related to the Ni nutritional status of the plants as shown in Table 7.30 for soybean. In plants without Ni supply through the roots, urease activity in leaves was low and foliar application of urea led to accumulation of urea and severe necrosis of the leaf tips. In plants supplied with Ni, on the other hand, urease activity was higher and urea accumulation and necrosis lower.

In nodulated legumes such as soybean, ureides are the dominant form of N transported to the shoots (Chapter 16) where they are degraded to NH_3 and CO_2 without involving urea metabolism. Accordingly, nodulated soybean and other ureide-type legumes have a low Ni requirement compared to soybean supplied with mineral N (Winkler *et al.*, 1988). Regardless of the form of N nutrition (urea, ammonium, nitrate, N_2 fixation) in soybean and cowpea, without Ni supply, large amounts of urea accumulate in the leaves and symptoms of leaf tip necrosis are severe (Eskew *et al.*, 1984). As shown in Table 7.31, there is an accumulation of urea (up to 3% of the dry weight) towards the tip of the leaf blade in Ni-deficient plants. In soybean, ureide concentrations are low and unaffected by Ni supply, which is also true for free purines and uric acid (Walker *et al.*, 1985). In contrast, Ni deficiency in pecan (a ureide transporting species) resulted in marked accumulation of xanthine, allantoic acid, ureidoglycolate and citrulline, but total ureides, urea concentration and urease activity were reduced (Bai *et al.*, 2006).

Seedlings of wheat, barley and oat from plants grown under low Ni conditions accumulate urea and show severe leaf tip necrosis when grown without Ni supply (Brown *et al.*, 1987b). Root and shoot growth was significantly lower in the Ni-deficient plants, which were less green, developed interveinal chlorosis and necrosis, and the terminal 2 cm of the leaves failed to unfold.

TABLE 7.31 Concentrations of urea, ureides and Ni in different parts of mature leaves of cowpea supplied with NH_4NO_3 with or without Ni supply

	Urea ($\mu mol\,g^{-1}\,dw$)		Ureides ($\mu mol\,g^{-1}\,dw$)		Ni ($\mu g\,g^{-1}\,dw$)	
	+Ni	−Ni	+Ni	−Ni	+Ni	−Ni
Petiole	0.11	0	nd	nd	nd	nd
Blade base	0.56	18.1	3.6	4.5	3.7	0.1
Blade tip	2.16	238.4	nd	nd	nd	nd

Based on Walker *et al.* (1985).
nd: not determined.

In barley seeds from plants grown at low Ni supply, there is a close relationship between the Ni concentration, viability, germination rate and seedling vigour (Brown *et al.*, 1987a). This relationship is shown for germination rate in Fig. 7.22.

Viability of the Ni-deficient seeds could not be restored by soaking the seeds in a solution containing Ni, demonstrating that Ni is essential for normal seed development in the maternal plants and, thus, for completing the life cycle of the barley plant (Brown *et al.*, 1987a).

Changes in concentration of organic acids and other solutes may result from secondary events of disturbances in N metabolism in Ni-deficient plants (Bai *et al.*, 2006; Brown *et al.*, 1990). It is not clear if these various effects of Ni deficiency are directly related to the function of Ni in the urease. However, these studies demonstrate that in N metabolism, urea is a normal metabolite whose concentration has to be maintained at a low level in order to prevent toxicity. Various pathways of urea biosynthesis in plants are known (Fig. 7.23). The ornithine cycle for urea biosynthesis is likely to be of general importance, as well as the higher rate of urea formation during protein degradation, for example in mature leaves, at onset of reproductive growth (Eskew *et al.*, 1984) and in germination of legume seeds (Horak, 1985b).

7.5.4 Ni Concentration in Plants

The Ni concentration in plants grown on uncontaminated soil ranges from 0.05 to $5.0\,\mu g\,g^{-1}\,dw$ (Welch, 1981; Brooks, 1980). The adequate range for Ni is between $0.01\,\mu g\,g^{-1}\,dw$ and $>10\,\mu g\,g^{-1}\,dw$ which is a wide range as compared to other elements (Gerendas *et al.*, 1999; Brown *et al.*, 1987a). This range mainly reflects the differences between plant species in uptake and root-to-shoot transport of Ni (Rebafka *et al.*, 1990). The critical Ni concentration required for seed germination in barley, shoot growth in oats, barley and wheat, and shoot growth of urea-fed tomato, rice and zucchini was $100\,ng\,g^{-1}$ dry weight (Brown *et al.*, 1987a, b; Gerendas and Sattelmacher, 1997).

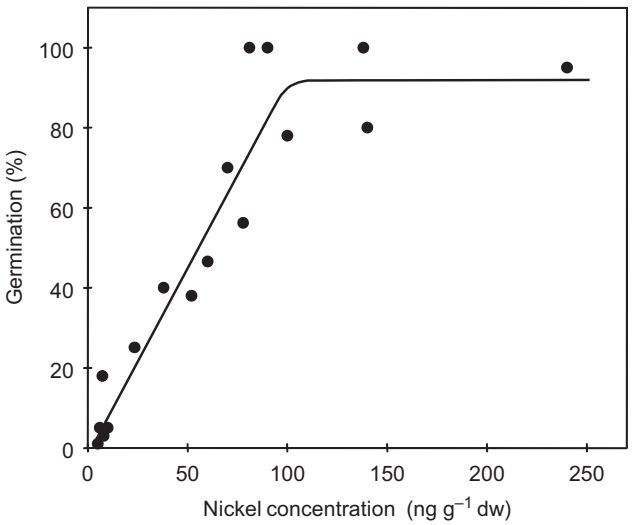


FIGURE 7.22 Relationship between Ni concentration in seeds and germination percentage in barley. *Redrawn from Brown* et al., *1987a.*

7.5.5 Nickel Deficiency and Toxicity

The existence of Ni deficiency in crops in the field was discovered in pecan trees growing in sandy, poorly draining soils with low cation exchange capacity of south-eastern USA (Wood *et al.*, 2004). A number of containerized crops have shown responses to foliar and soil applications, particularly, though not exclusively, when provided with urea as N source (Ruter, 2004; Bai *et al.*, 2006; Gheibi *et al.*, 2009) as well as in species that utilize ureides as a main transport form of N. Examples of ureide transporting crop genera are *Annona*, *Carya*, *Diospyros*, *Juglans* and *Vitis* (Brown, 2008). The clearest agronomic responses to Ni have been observed when N is supplied as urea or by N_2 fixation. Plants without Ni supply have low urease activity in the leaves, and foliar application of urea leads to an accumulation of urea and severe necrosis of the leaf tips (Eskew *et al.*, 1984). Nicoulaud and Bloom (1998) observed that in tomato seedlings growing with foliar urea as the only N source, addition of Ni increased growth.

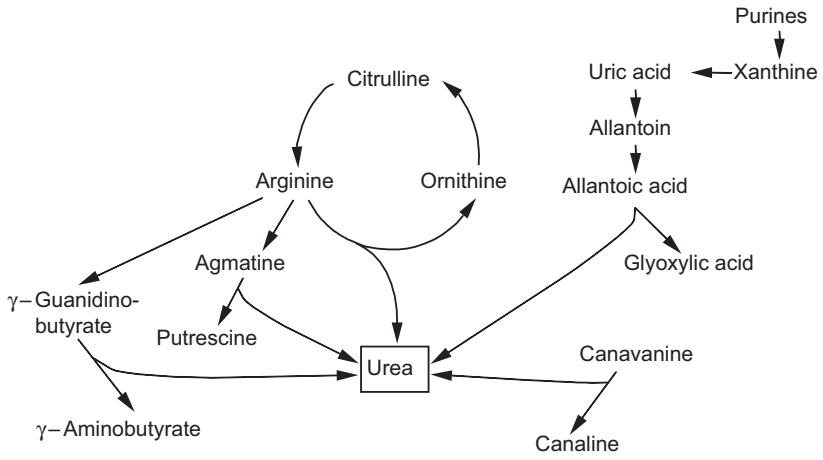


FIGURE 7.23 Pathways of urea biosynthesis in plants. *Modified from Walker* et al. *(1985).*

In legumes and other dicots, Ni deficiency results in decreased activity of urease and subsequently in urea toxicity, exhibited as leaflet tip necrosis (Eskew *et al.*, 1984). In graminaceous species deficiency symptoms include chlorosis similar to that induced by Fe deficiency (Brown *et al.*, 1987a,b), including interveinal chlorosis and patchy necrosis in the youngest leaves. Nickel deficiency also results in a marked enhancement in plant senescence and a reduction in tissue Fe concentrations. In both monocotyledonous and dicotyledonous plants, the accumulation of urea in leaf tips can be used to detect Ni deficiency (Eskew *et al.*, 1984). In early stages of Ni toxicity, there are no clear symptoms, although shoot and root growth may be reduced. In pecan, Ni deficiency results in deformed leaves, a symptom referred to as 'mouse-ear' (Wood *et al.*, 2004).

In general, in crop plants there is more concern about nickel toxicity, which may occur after application of sewage sludge which is often high in Ni (Marschner, 1983; Brown *et al.*, 1989). Critical toxicity levels in crop species are in the range of $>10\,\mu g\,g^{-1}$ dw in sensitive to $>50\,\mu g\,g^{-1}$ dw in moderately tolerant species (Asher, 1991). In wheat, the critical toxicity levels increased from 63 to $112\,\mu g\,g^{-1}$ dw with increasing supply of urea (Singh *et al.*, 1990a). In sensitive species, root growth is severely inhibited even below 5 µM Ni when the Ca^{2+} concentration is low (Gabbrielli *et al.*, 1990).

7.5.6 Ni Tolerance

Serpentine (ultramafic) soils are usually very high in Fe, Mg, Ni, Cr and Co, but low in Ca. The flora on these soils include many species exhibiting hyperaccumulation of nickel (e.g., of the genus *Alyssum*), in which the Ni concentration in the leaves may reach 10–30 $mg\,g^{-1}$ dw (Baker and Walker, 1989a, b; Homer *et al.*, 1991). Tolerance in these hyperaccumulators is mainly achieved by complexation of Ni with organic acids, malic and citric acid in particular; the stability of the citric acid complexes being about 150 times higher than those formed with malic acid (Homer *et al.*, 1991). In soils underneath the canopy of hyperaccumulating trees there is a higher proportion of Ni resistant bacteria than beyond the canopy, indicating high rate of Ni cycling in the micro-ecosystem of these trees (Schlegel *et al.*, 1991).

7.6 MOLYBDENUM

7.6.1 General

Molybdenum is a transition element; it is present in small amounts in the lithosphere (average $2.4\,mg\,kg^{-1}$) and in soils (ranging from 0.2 to $36\,mg\,kg^{-1}$) (Barber, 1984). In aqueous solution with a pH >4.3, Mo occurs mainly as the molybdate oxyanion, MoO_4^{2-}, in its highest oxidized form (Mo(VI)). At lower pH (<4.3), protonated species ($HMoO_4^-$, $MoO_3(H_2O)_3$) become the prevailing forms. At high concentrations ($>10^{-4}$ M) and low pH, molybdate can polymerize; but this is unlikely to occur in soil solution because soluble Mo is usually $<10^{-6}$ M (Smith *et al.*, 1997). Due to its electron configuration, Mo(VI) shares many chemical similarities with vanadium (V) and, particularly, tungsten (W). In fact, many anaerobic archaea and some bacteria require tungsten, but not Mo (Schwarz *et al.*, 2009). Several properties of the molybdate anion MoO_4^{2-} also resemble those of the divalent inorganic anion sulphate (SO_4^{2-}), which has important implications for Mo availability in soils and uptake by plants. In long-distance transport in plants, Mo is readily mobile in xylem and phloem (Kannan and Ramani, 1978; Kaiser *et al.*, 2005). The form in which Mo is translocated is unknown, but its chemical properties indicate that it is most likely transported as MoO_4^{2-} rather than in complexed form.

The requirement of plants for Mo is lower than that for any of the other nutrients. The functions of Mo as a plant nutrient are related to the valency changes it undergoes as a metal component of enzymes. Within these enzymes, Mo shuttles between three oxidation states (+4, +5 and +6), thereby catalysing two-electron transfer reactions (Schwarz *et al*., 2009). In higher plants, only few enzymes have been found to contain Mo as a cofactor, including nitrate reductase, xanthine dehydrogenase, aldehyde oxidase and sulphite reductase. In addition, Mo is a cofactor of nitrogenase in N_2-fixing bacteria. The functions of Mo are therefore closely related to N metabolism, and the Mo requirement strongly depends on the mode of N supply.

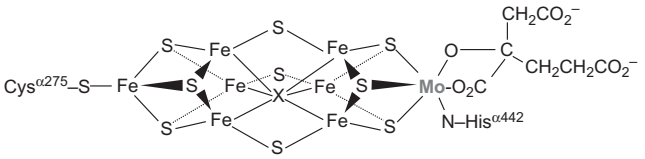
7.6.2 Mo Uptake

A molybdate-specific transporter has been identified in *Arabidopsis thaliana* (Tomatsu *et al.*, 2007; Baxter *et al*., 2008). This transporter, MOT1, has a high affinity for MoO_4^{2-} with a K_m of 20 nM in an uptake assay with yeast expressing the *MOT1* gene (Tomatsu *et al.*, 2007). MOT1 belongs to the sulphate transporter superfamily, but does not appear to mediate sulphate transport. It is expressed in both roots and leaves and the protein appears to be localized in the mitochondria (Baxter *et al*., 2008). Mutants lacking MOT1 had markedly decreased Mo concentrations in roots and shoots (Tomatsu *et al*., 2007). Natural variation in Mo accumulation among different accessions of *Arabidopsis thaliana* is, to a large extent, related to the expression level of *MOT1* (Baxter *et al*., 2008).

In addition to MOT1, it is likely that some non-specific transporters also contribute to Mo uptake by plants, particularly sulphate transporters. For example, the high-affinity sulphate transporter from the tropical legume *Stylosanthes hamata*, SHST1, is able to mediate MoO_4^{2-} uptake into yeast cells expressing the *SHST1* gene from the external medium containing nM concentrations of molybdate (Fitzpatrick *et al*., 2008). There are also numerous reports in the literature showing that molybdate uptake is suppressed by sulphate (reviewed by Macleod *et al*., 1997). The effect of sulphate can be two-fold: a direct competition for the transporters and regulation of the expression of sulphate transporter genes by plant S status. In field-grown wheat, S deficiency resulted in greatly increased transcript abundance of sulphate transporters such as *Sultr1;1* and *Sultr4;1*, but not *Sultr5;2* which is the wheat homologue of the *Arabidopsis MOT1*. Molybdenum concentrations in leaves and ears of S-deficient wheat were about double of those in S-sufficient plants (Shinmachi *et al*., 2010).

7.6.3 Nitrogenase

Nitrogenase is the key enzyme complex unique to all N_2-fixing microorganisms. It consists of two Fe proteins, one of which is the FeMo protein containing two unique metal centres, the P-cluster (8Fe-7S) and the FeMo cofactor (Mo-7Fe-9S-X-homocitrate cluster, where X may be C, O or N) (Schwarz *et al*., 2009):



Details of the structural arrangement and catalytical functions of Mo in nitrogenase are discussed in Chapter 16. In some free-living diazotrophic bacteria (e.g., *Azotobacter chroococcum*) in addition to the Mo-nitrogenase, another nitrogenase occurs in which Mo is replaced by vanadium (Dilworth *et al.*, 1988).

Legumes and non-legumes dependent on N_2 fixation have a high Mo requirement, particularly in root nodules. When the external supply is low, the Mo concentration of the nodules is usually higher than that of leaves, whereas when the external supply is high, the concentration in the leaves increases more strongly than in the nodules (Brodrick and Giller, 1991a). When Mo is limiting, preferential accumulation in root nodules may lead to a considerably lower Mo concentration in the shoot and seeds of nodulated legumes (Ishizuka, 1982). However, the relative allocation of Mo to the various plant organs varies considerably not only between plant species, but also between genotypes within a species, for example in *Phaseolus vulgaris* (Brodrick and Giller, 1991b).

As would be expected, the growth of plants relying on N_2 fixation is particularly stimulated by the application of Mo to deficient soils (Becking, 1961) and nodule dry weight can increase 18-fold which indirectly reflects the increase in the capacity for N_2 fixation by improved Mo supply.

In soils low in Mo availability, the effect of application of Mo to legumes depends on the form of N supply. As shown in Table 7.32, Mo applied to nodulating and non-nodulating soybean plants increased N concentration and seed yield only in the nodulated plants without or with insufficient supply of N fertilizer. This demonstrates the greater requirement for molybdenum in N_2 fixation than in nitrate reduction. It also indicates that on soils with low Mo availability, it is possible to replace the application of N fertilizer to legumes by application of Mo fertilizer combined with rhizobiom inoculation.

Low availability of Mo in tropical forest soil may limit N_2 fixation by free-living heterotrophic bacteria, thus impacting on N cycling. Barron *et al.* (2009) showed that Mo addition to weathered tropical forest soils from Panama significantly increased N_2 fixation.

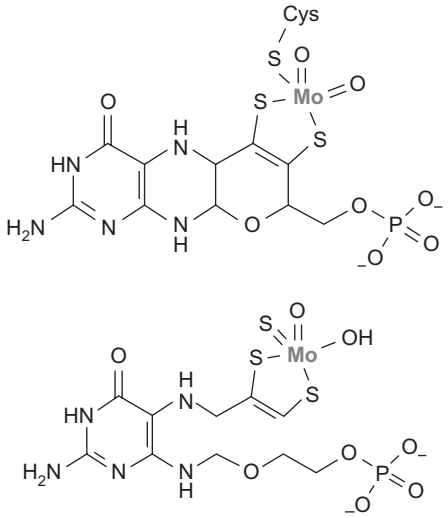
TABLE 7.32 Leaf N concentration and seed yield of non-nodulating and nodulating soybean at different rates of Mo and N supply

	Mo supply ($g\,Mo\,ha^{-1}$)	N concentration ($mg\,g^{-1}\,dw$)		Seed yield ($t\,ha^{-1}$)	
		0	34	0	34
N supply ($kg\,N\,ha^{-1}$)					
Non-nodulating					
	0	31	36	1.7	1.6
	67	46	47	2.7	2.7
	134	53	53	3.0	2.9
	201	56	56	3.2	3.2
Nodulating					
	0	43	57	2.5	3.1
	67	51	55	2.8	3.1
	134	54	56	3.1	3.2
	201	56	56	3.1	3.1

Based on Parker and Harris (1977).

7.6.4 Nitrate Reductase

Nitrate reductase is a homodimeric enzyme with three electron-transferring prosthetic groups per subunit: flavin (FAD), heme and Mo cofactor (Moco). Moco consists of Mo covalently bound to two S atoms in the tricyclic molecule pterin. Molybdenum in Moco is bound to a third S-ligand either of the cysteine residue (below, top molecule) or of a terminal S (below, bottom molecule). Moco of the first type is used in nitrate reductase and sulphite oxidase. The second type is found in xanthine dehydrogenase and aldehyde oxidase.



During nitrate reduction, electrons are transferred directly from Mo to nitrate. Details of this reduction process are described in Section 6.1.

Nitrate reductase activity (NRA) is low in leaves of Mo-deficient plants, but can be readily induced within a few hours by infiltrating the leaf segments with Mo. As shown in Fig. 7.24, in nitrate-fed plants there is a positive relation between Mo supply, NRA of the leaves and yield of spinach. Incubation of the leaf segments for 2 h with Mo increased NRA only in those from deficient plants. 'Inducible NRA' can therefore be used as a test for the Mo nutritional status of plants (Shaked and Bar-Akiva, 1967).

The Mo requirement for plant growth is strongly dependent on whether N is supplied as nitrate or ammonium (Table 7.33). In nitrate-fed plants not supplied with Mo, growth is poor, the concentrations of chlorophyll and ascorbic acid are low (mainly located in the chloroplasts), but that of nitrate is high. Leaves show typical symptoms of Mo deficiency ('whiptail', see Fig. 7.24). When ammonium is supplied, the response to Mo is less marked, but still present in terms of the effect on plant dry weight and ascorbic acid concentration. Without Mo supply, ammonium-fed plants also develop whiptail symptoms.

It is unclear if there is any requirement for Mo when plants are supplied with reduced N such as ammonium or urea. The results shown in Table 7.33 are inconclusive in this respect because under the non-sterile culture conditions, nitrification of ammonium occurred in the substrate and, thus, nitrate was taken up. In cauliflower plants growing under

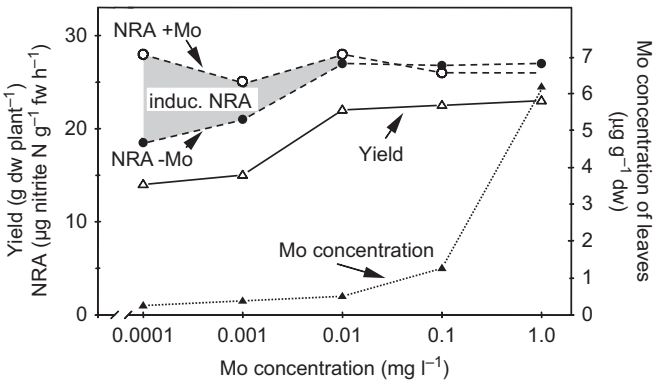


FIGURE 7.24 Nitrate reductase activity (NRA) in spinach leaves from plants grown with different Mo supply. Leaf segments were incubated with (NRA + Mo) or without (NRA – Mo) Mo for 2 h. Stippled area represents 'inducible NRA'. *Redrawn from Witt and Jungk, 1977.*

TABLE 7.33 Growth and chlorophyll, nitrate and ascorbic acid concentration of tomato grown with ammonium or nitrate N and with and without Mo supply

	N form			
	Nitrate		Ammonium	
	−Mo	+Mo	−Mo	+Mo
Dry weight (g plant^{-1})	9.6	25.0	15.9	19.4
Chlorophyll (mg (100 g)$^{-1}$ fw)	8.9	15.8	21.6	17.4
Nitrate (mg g^{-1})	73	9	10	9
Ascorbic acid (mg (100 g)$^{-1}$ fw)	99	195	126	184

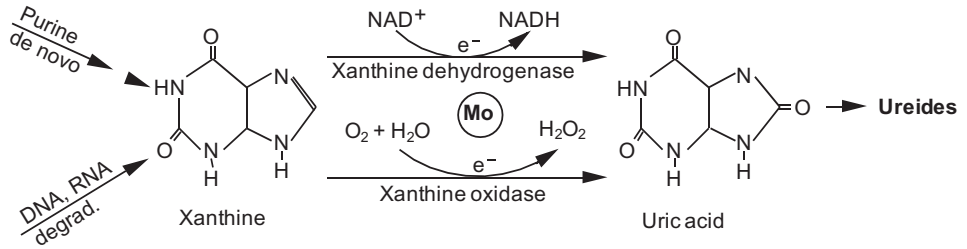
Based on Hewitt and McCready (1956).
The pH of the substrate (quartz sand) was buffered with $CaCO_3$.

sterile conditions (Hewitt and Gundry, 1970), those supplied with ammonium but without Mo did not develop deficiency symptoms and seemed to have no Mo requirement, a result which confirms corresponding results in green algae (Ichioka and Arnon, 1955). It has been suggested (Hewitt and Gundry, 1970) that even low nitrate concentrations induce the synthesis of nitrate reductase and, in absence of the Mo cofactor, may have other catalytical properties leading to metabolic disturbances similar to those induced by high concentrations of superoxide radicals, such as peroxidation of membrane lipids (Fido *et al.*, 1977). When tungsten (W) was applied to Mo-deficient plants, it was incorporated into the nitrate reductase apo-enzyme, but did not restore NRA. It is well known that certain metalloenzymes, even within the same plant species, are not absolutely metal specific. Similar metals can be incorporated and may either restore the original catalytic reaction (Sandmann and Böger, 1983), or lead to a modified type of catalytic reaction.

In tobacco, replacement of Mo by W in the apo-enzyme of nitrate reductase strongly reduces NRA within a few hours, but leads to a progressive increase not only of the apo-enzyme but also the corresponding mRNA to concentrations that are several-fold higher than in plants supplied with Mo. This response suggests that W inactivates nitrate reductase, but simultaneously leads to over-expression of the nitrate reductase genes (Deng *et al.*, 1989). These genes are suppressed in Mo-supplied plants, probably by higher concentrations of reduced N.

7.6.5 Other Mo-containing Enzymes

Three other Mo-containing enzymes have been identified in plants, including xanthine dehydrogenase, aldehyde oxidase and sulphite oxidase; they play important roles in the response and resistance to various stresses (Schwarz and Mendel, 2006). Xanthine dehydrogenase is a homodimeric metalloflavoprotein, each subunit of which contains one Moco together with one molecule of FAD and two [2Fe-2S] centres. Xanthine dehydrogenase catalyses the oxidation of hypoxanthine to xanthine and of xanthine to uric acid:



The enzyme is involved in the catabolism of purines and, thus, in the biosynthetic pathway of ureides which are oxidation products of purines. In legumes such as soybean and cowpea, in which ureides are the most prevalent N compounds formed in root nodules (Chapter 16), xanthine dehydrogenase plays a key role in N metabolism. In the cytosol of the nodules, purines (e.g., xanthine) are oxidized to uric acid, the precursor of ureides. In nodulated legumes of the ureide type under Mo deficiency, growth inhibition and low N_2 fixation rates can result from low nitrogenase activity and/or impaired purine catabolism in the nodules. Besides purine degradation, xanthine dehydrogenase may also play a role in plant–pathogen interactions, cell death associated with hypersensitive response and natural senescence (Schwarz and Mendel, 2006).

Aldehyde oxidase is very similar to xanthine dehydrogenase with respect to amino acid sequence, and contains FAD, [2Fe-2S] cluster and Moco. Aldehyde oxidase catalyses the conversion of abscisic aldehyde to abscisic acid (ABA), which is the last step in ABA biosynthesis. ABA is a phytohormone involved in developmental processes and responses to biotic and abotic stress (see also Chapter 5). An *Arabidopsis thaliana* mutant defective in Moco sulphurase, which adds the terminal sulphur to Moco, had lower concentrations of ABA and was less tolerant to freezing, salinity and drought (Xiong *et al*., 2001). Molybdenum applications to deficient wheat plants increase ABA concentration and cold tolerance (Sun *et al.*, 2009). Aldehyde oxidase may also be involved in the biosynthesis of the phytohormone indole-3-acetic acid (IAA) by catalysing the conversion of indole-3-acetaldehyde to IAA (Schwarz and Mendel, 2006).

Compared with other Mo-containing enzymes in plants, sulphite oxidase is smaller and simpler, possessing only Moco as its redox centre (Eilers *et al.*, 2001). Sulphite oxidase catalyses the oxidation of sulphite (SO_3^{2-}) to sulphate (SO_4^{2-}) inside peroxisomes, using O_2 as the terminal electron acceptor and producing hydrogen peroxide. Sulphite is a toxic metabolite, which is produced when plants are exposed to sulphur dioxide (SO_2) gas or during the decomposition of sulphur-containing amino acids. Therefore, sulphite oxidase plays an important role in protecting plants against the damage caused by sulphur dioxide (Lang *et al*., 2007).

7.6.6 Gross Metabolic Changes

In legumes dependent on N_2 fixation as N source, N deficiency and the corresponding metabolic changes are the most prevalent effects of Mo deficiency. This also often holds true for nitrate-fed plants provided the Mo deficiency is not severe. With severe Mo deficiency, the visual symptoms (e.g., whiptail; Chatterjee *et al.*, 1985; shortening of internodes and chlorosis of young leaves; Agarwala *et al.*, 1978), as well as a range of metabolic changes are different from those of N deficiency. These differences may relate to the role of Mo in xanthine dehydrogenase and aldehyde oxidase. For example, in Mo-deficient plants, organic acids (Höfner and Grieb, 1979) and amino acids (Gruhn, 1961) accumulate, and the activity of ribonuclease is high, whereas that of alanine transferase is low (Agarwala *et al.*, 1978) as are the leaf concentrations of RNA and DNA (Chatterjee *et al.*, 1985). Molybdenum-deficient plants are more sensitive to low temperature stress and waterlogging (Vunkova-Radeva *et al.*, 1988; Sun *et al.*, 2009) due to the effect on ABA biosynthesis. Molybdenum deficiency also has strong effects on pollen formation in maize (Table 7.34). In deficient plants, not only was tasseling delayed, but a large proportion of the flowers failed to open and the capacity of the anther for pollen production was reduced. Furthermore, the pollen grains were smaller, free of starch, had lower invertase activity, and showed poor germination.

TABLE 7.34 Pollen production and viability of maize plants at different rates of Mo supply

	Mo supply ($mg\,kg^{-1}$)		
	20	0.1	0.01
Mo concentration in pollen grains ($\mu g\,g^{-1}\,dw$)	92	61	17
Pollen-producing capacity (no. of pollen grains $anther^{-1}$)	2,437	1,937	1,300
Pollen diameter (μm)	94	85	68
Pollen viability (% germination)	86	51	27

From Agarwala *et al.* (1979).

As shown in Fig. 7.25, the risk of premature sprouting of maize grains in standing crops increases when the Mo concentration is below $0.03\,\mu g\,g^{-1}$ in the grains, or below $0.02\,\mu g\,g^{-1}$ in the grains and $0.10\,\mu g\,g^{-1}$ in the leaves (Farwell *et al.*, 1991). Premature sprouting is also a serious problem in some wheat-growing areas and can be aleviated by foliar sprays of Mo (Cairns and Kritzinger, 1992). In maize, the extent of premature sprouting is also related to the time of N application (Tanner, 1978). Little sprouting occurred when top dressing with ammonium-nitrate took place within 60 days after germination. On the other hand, sprouting of grains low in Mo was enhanced by very late N application. Molybdenum deficiency may result in a lack of seed dormancy, thus increased premature sprouting, due to reduced ABA biosynthesis because ABA stimulates dormancy and reduces germination (Modi and Cairns, 1994).

In grapevine, Mo deficiency is associated with a symptom called Millerandage, which is characterized by

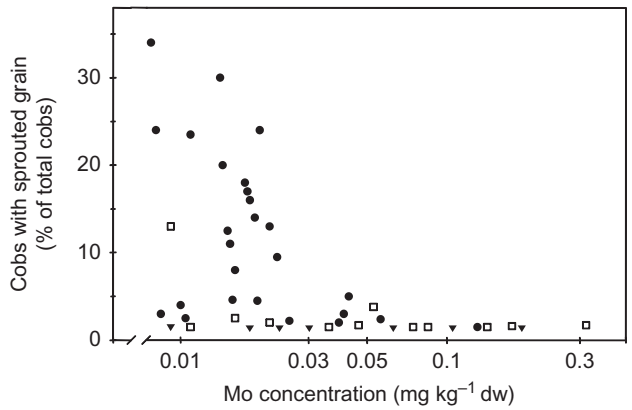


FIGURE 7.25 Relationship between Mo concentration of maize grains, time of nitrogen top dressing and percentage of sprouted cobs of maize. Top dressing with nitrogen at (▼) 30 days; (□) 40–55 days; (●) 70–85 days. *Based on Tanner, 1978.*

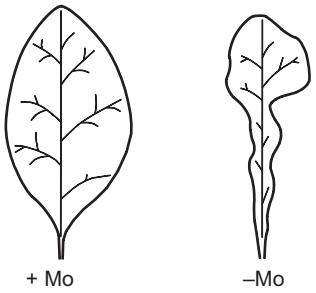


FIGURE 7.26 Schematic representation of changes in leaf morphology in Mo-deficient cauliflower ('whiptail' symptom).

unevenly developed grape bunches of berries with varying size and degree of maturity (Kaiser *et al.*, 2005). The exact reason for this symptom is unclear, but may be related to the effect of Mo on phytohormones.

7.6.7 Mo Deficiency and Toxicity

Depending on plant species and N source, the critical deficiency levels of Mo vary between 0.1 and 1.0 μg g^{-1} leaf dw (Gupta and Lipsett, 1981; Bergmann, 1992). In seeds the Mo concentration is highly variable (see below) but, in general, much higher in legumes than in non-legumes. Molybdenum is unique among the essential elements in that normal seeds of some plants may store more Mo than required by the next generation plant (Meagher *et al*., 1952).

In Mo-deficient plants, symptoms of N deficiency and stunted growth and chlorosis in young leaves are common. In dicotyledonous species, a strong reduction in size and irregularities in leaf blade formation (whiptail) are the most typical visual symptom (Fig. 7.26), caused by local necrosis in the tissue and insufficient differentiation of vascular bundles in the early stages of leaf development (Bussler, 1970).

Local chlorosis and necrosis along the main veins of mature leaves (e.g., 'Yellow spot' in citrus) and whiptail in young leaves may reflect the same type of local metabolic disturbances, occurring however, at different stages of leaf development (Bussler, 1970). When there is severe deficiency, marginal chlorosis and necrosis on mature leaves with a high nitrate concentration also occur.

Molybdenum deficiency is widespread in legumes and certain other plant species (e.g., cauliflower and maize) grown in acid mineral soils with large concentrations of reactive Fe oxidihydrate and thus a high capacity for adsorbing MoO_4^{2-}. Furthermore, adsorption of molybdate

TABLE 7.35 Relationship between soil pH, Mo supply and dry weight and shoot Mo concentration in soybean

	Mo supply (mg pot^{-1})	Soil pH		
		5.0	6.0	7.0
Dry weight (g pot^{-1})	0	15	19	23
	5	20	20	20
Shoot Mo concentration (μg g^{-1} dw)	0	0.1	0.8	0.9
	5	2.0	6.3	18.5

Based on Mortvedt (1981).

increases with decreasing soil pH. As shown in Table 7.35, regardless of whether Mo is supplied or not, the Mo concentration of the shoots of soybean increases by a factor of 10 when the soil pH is increased from 5.0 to 7.0 by liming. The effect of the liming treatment alone on the plant dry weight is similar to the application of Mo to the unlimed soil. Thus, quite often liming and Mo application can be seen as alternatives for stimulating legume growth on acid mineral soils. Responses of legume growth to liming therefore also strongly depend on the Mo availability in the soils (Adams *et al.*, 1990). A combination of both liming and Mo supply often leads to luxury uptake and very high Mo concentrations in the vegetative parts of the shoots and seeds.

A high Mo concentration in seeds ensures proper seedling growth and high final grain yields in plants growing in soils low in available Mo (Table 7.36). Hence, the effect of Mo application to a deficient soil on plant growth is negatively related to the seed Mo concentration (Tanner, 1982) and the amount of Mo applied to the seed crop (Weir and Hudson, 1966).

Compared with the uptake rates of other micronutrients, the rate of Mo uptake by soybean plants during the first 4 weeks after germination is very low; thus the Mo

TABLE 7.36 Relationship between the Mo concentration of soybean seeds and the subsequent seed yield of plants growing in an Mo-deficient soil

Mo concentration of seeds ($mg\,kg^{-1}$ dw)	Seed yield of the subsequent crop ($kg\,ha^{-1}$)
0.05	1,505
19.0	2,332
48.4	2,755

Based on Gurley and Giddens (1969).

TABLE 7.37 Dry matter production and N concentration of the subtropical pasture legume ***Desmodium intartum*** grown in a soil with pH 4.7 without Mo supply or Mo supplied to soil or seeds

Mo application ($g\,ha^{-1}$)	Dry weight ($kg\,ha^{-1}$)	N concentration ($mg\,g^{-1}$)
0	70	19
100 (soil application)	1,220	32
100 (seed pelleting)	1,380	34

From Kerridge *et al.* (1973).

requirement for growth has to be met mainly by re-translocation from the seed (Ishizuka, 1982). Large-seeded cultivars combined with high Mo availability during the seed-filling period are therefore very effective in the production of seeds suitable for soils low in available Mo (Franco and Munns, 1981).

Seed pelleting with Mo is another procedure for preventing deficiency during early growth and establishing a vigorous root system for subsequent uptake from soils low in available Mo (Tanner, 1982). As shown in Table 7.37, seed pelleting with the relatively insoluble MoO_3 at a rate of $100\,g\,Mo\,ha^{-1}$ is even somewhat more effective than soil application. Seed pelleting with 100 g Mo in legumes such as groundnut increased dry matter production and the amount of N in the plants more than an application of $60\,kg\,ha^{-1}$ of mineral fertilizer N (Hafner *et al*., 1992).

As Mo is highly phloem-mobile, foliar application is an appropriate and easy procedure for correcting acute Mo deficiency (Gupta and Lipsett, 1981; Kaiser *et al*., 2005). In legumes, Mo applied as a foliar spray in the early growth stages is preferentially translocated into the nodules (Brodrick and Giller, 1991a) and very effective in increasing final yield, for example, in soybean (Adams *et al.*, 1990) or groundnut (Table 7.38). Compared with soil application, foliar application to groundnut not only increases yield but also N uptake and the Mo concentration in the shoots, seeds and nodules. Foliar sprays of Mo applied before flowering are effective in correcting Mo deficiency in grapevine (Williams *et al*., 2004).

A lower effectivity of soil compared with foliar applied Mo may reflect fixation of Mo in the soil; however, it is often also the result of impaired uptake by the roots. Sulphate and molybdate are strongly competing anions during uptake by the roots. Therefore, sulphate-containing soil amendments such as gypsum (Stout *et al.*, 1951; Pasricha *et al.*, 1977), as well as single superphosphate (SSP, which contains sulphate), reduce Mo uptake (Table 7.39). Unlike SSP, TSPP does not contain sulphate and therefore leads to higher Mo uptake and thus higher yield and N uptake compared with SSP. Moreover, TSP increased seed Mo concentrations thereby increasing seed quality in terms of suitability for use in Mo-deficient soils.

The reduction in Mo uptake by sulphate may also be of significance for natural ecosystems. In red cedar trees, there is a negative relationship between Mo and S concentration of tree rings, the increase in S concentration being closely related to the historical trend in coal production and, thus, SO_2 emission in the area in which the trees were growing (Guyette *et al.*, 1989).

A unique feature of Mo nutrition is the wide variation between the critical deficiency and toxicity concentrations which may differ by a factor of up to 10^4 (e.g., 0.1–$1{,}000\,\mu g\,Mo\,g^{-1}$ dw) as compared with a factor of 10 or less for B or Mn. Plants are generally quite tolerant to Mo toxicity. Under Mo toxicity, malformation of the leaves and a golden yellow discoloration of the shoot tissue occur, most likely due to the formation of molybdocatechol complexes in the vacuoles (Hecht-Buchholz, 1973). In oilseed rape and tomato, the most striking symptoms of Mo toxicity is a dark blue coloration of stems (McGrath *et al*., 2010), which is due to the formation of molybdenum–anthocyanin complexes (Hale *et al.*, 2001). Genotypic differences in tolerance to Mo toxicity are closely related to differences in the translocation of Mo from roots to shoots.

High, but non-toxic, concentrations of Mo in plants are advantageous for seed production, but such concentrations in forage plants may be dangerous for animals, and for ruminants in particular, which are very sensitive to excessive concentrations of Mo. Molybdenum concentrations above 5 to $10\,mg\,kg^{-1}$ dw of forage can induce toxicity known as molybdenosis (or 'teart'). This occurs, for example, in western parts of the United States, Australia and New Zealand, often in soils with poor drainage and high in organic matter content (Gupta and Lipsett, 1981), or on pastures established on retorted oil shale disposal piles (Stark and Redente, 1990). Molybdenosis is caused by an imbalance of Mo and Cu in the ruminant diet, i.e. an

TABLE 7.38 Dry matter production, N uptake and Mo concentration in groundnut grown on a low Mo, acid sandy soil without Mo supply or Mo supplied to soil or as foliar spray

Mo application ($g\,ha^{-1}$)	Dry matter ($kg\,ha^{-1}$)	N uptake ($kg\,ha^{-1}$)	Mo concentration ($\mu g\,g^{-1}$ dw)		
			Shoots	Nodules	Seeds
0	2,685	70	0.02	0.4	0.02
200 (soil)	3,413	90	0.02	1.5	0.20
200 (foliar)	3,737	101	0.05	3.7	0.53

Based on Rebafka (1993).

TABLE 7.39 Dry matter production, N uptake and Mo concentration in groundnut grown on a low Mo, acid sandy soil without or with P supply ($13\,kg\,ha^{-1}$) as single superphosphate (SSP) or triple superphosphate (TSP)

P fertilizer	Dry matter ($kg\,ha^{-1}$)	N uptake ($kg\,ha^{-1}$)	Mo concentration ($\mu g\,g^{-1}$ dw)		
			Shoots[a]	Nodules	Seeds
−P	2,000	52	0.22	4.0	1.0
+SSP	2,550	62	0.09	1.5	0.1
+TSP	3,150	81	0.31	8.2	3.1

Based on Rebafka *et al.* (1993).
[a]at flowering.

induced Cu deficiency (Stark and Redente, 1990; Miller *et al.*, 1991). The depressing effect of sulphate on molybdate uptake (Table 7.39) can be used to reduce the Mo concentrations in plants to non-toxic levels (Pasricha *et al.*, 1977; Chatterjee *et al.*, 1992) either for the plants themselves or for the ruminants.

Molybdenum nutrition of plants growing in mixed pastures of legumes, herbs and grasses therefore requires special consideration. On the one hand, the relatively large requirement of legumes for N_2 fixation and for Mo in the seeds must be met, but at the same time toxic concentrations in the forage of grazing animals must be avoided.

7.7 BORON

7.7.1 General

Boron is a member of the metalloid group of elements which also includes silicon (Si) and germanium (Ge). These elements are intermediate in properties between metals and non-metals, and also share many features common in plants. The boron atom is small and has only three valencies. Boric acid is a very weak acid, with a pKa of 9.24, and at the pH found in the cytoplasm (pH 7.5), more than 98% of B is in the form of free $B(OH)_3$ and less than 2% as $B(OH)_4^-$. At pH values found in the apoplast (pH 5.5), >99.95% of boron is in the form of $B(OH)_3$ and less than 0.05% in the form of $B(OH)_4^-$.

$$B(OH)_3 + 2H_2O \leftrightarrow B(OH)_4^- + H_3O^+$$

Boric acid and borate can readily react with many types of biological molecules, and under normal biological conditions available B-binding molecules will typically exceed the concentration of free B. Boric acid forms spontaneous esters with mono, di and polyhydroxy compounds.

Only the monomeric species $B(OH)_3$ and $B(OH)_4^-$ are usually present in aqueous solutions at low B concentrations (<25 mM); thus polymeric B species are unlikely to occur in plants, except under B toxicity.

Boron uptake is closely related to the external B concentration over a wide concentration range. Boron availability is strongly affected by soil water content, and becomes limiting in dry conditions where mass flow to roots is reduced (Shorrocks, 1997). Its distribution in plant species that utilize sucrose as primary transported carbohydrates is primarily governed by the transpiration stream, whereas in plant species that transport C as polyols, B is freely phloem-mobile and distribution patterns resemble those of a phloem-mobile element (Brown and Shelp, 1997).

Boron is a micronutrient for vascular plants, diatoms, yeast, bacteria and some species of green algae, whereas it is apparently not required by fungi (Loomis and Durst, 1992). Boron is required by cyanobacteria when depending on N_2 fixation. The role of B in plant nutrition is still the least understood of all the nutrients and what is known of B requirement arises mainly from studies in which B was withheld or resupplied after deficiency. This lack of information is surprising, because on a molar basis, the requirement for B, at least for dicotyledonous plants, is higher than that for any other micronutrient. Withholding B very rapidly induces a range of distinct metabolic changes and visible deficiency symptoms in certain plant species (e.g., sunflower). Boron is neither an enzyme constituent nor is there convincing evidence that it directly affects enzyme activities. There are many postulated roles of B (Parr and Loughman, 1983): (i) sugar transport, (ii) cell wall synthesis, (iii) lignification, (iv) cell wall structure, (v) carbohydrate metabolism, (vi) RNA metabolism, (vii) respiration, (viii) indole acetic acid (IAA) metabolism, (ix) phenol metabolism, and (x) membranes. This long list might indicate (a) that B is involved in a number of metabolic pathways, or (b) deficiency results in a 'cascade effect' due to disruption of a critical and central cellular process. There is increasing evidence for the latter; the primary role of B in the cell wall biosynthesis and structure results in a cascade of metabolic disruptions that can explain most, but not all, observed effects of B deficiency. Additional functions of B await discovery. Several reviews on the chemistry and biology of B are available (Loomis and Durst, 1992; Cakmak and Römheld, 1997; Dell and Huang, 1997; Brown *et al*., 2002).

7.7.2 B Complexes with Organic Structures

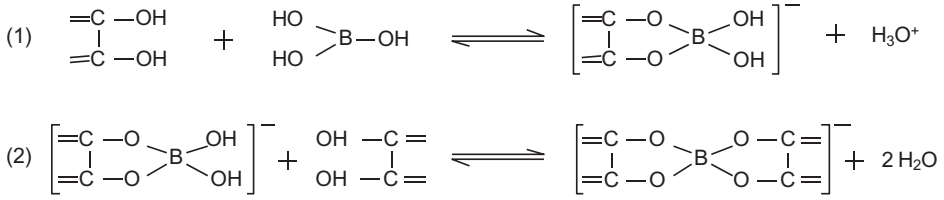
Boric acid has an outstanding capacity to form complexes with diols and polyols, particularly with *cis*-diols, either as monoester (Eq. (1)) or diester (Eq. (2)).

Polyhydroxyl compounds with an adjacent *cis*-diol configuration are required for the formation of such complexes; the compounds include a number of sugars and their derivatives (e.g., sugar alcohols and uronic acids), in particular mannitol, mannan and polymannuronic acid. These compounds serve, for example, as constituents of the hemicellulose fraction of cell walls. In contrast, glucose, fructose and galactose and their derivatives (e.g., sucrose) do not have this *cis*-diol configuration and thus do not form stable borate complexes. The most stable borate diesters are formed with *cis*-diols on a furanoid ring, namely the pentoses ribiose and apiose, the latter being a universal component of the cell walls of vascular plants (Loomis and Durst, 1992). The high B requirement of gum-producing plants is most likely related to the function of B in forming cross-links with the various polyhydroxy polymers such as galactomannan (Loomis and Durst, 1992). Boron has the capacity to form readily exchangeable complexes with ribose, the principal sugar component of RNA, but also with NAD^+ (Ralston and Hunt, 2000) and a wide range of organic molecules. As borate contains two pairs of hydroxyl moieties, it allows borate to form diester complexes with two molecules on each side of the borate ion, thus serving a cross-linking or bridging function. Diester complexes are believed to be energetically more favourable than monoester complexes as evidenced by the fact that all known B complexes in nature are diester cross-linked.

In higher plants, a substantial proportion of the total B is complexed as *cis*-diol esters in the cell walls associated with cell wall pectins and is often correlated with the whole plant B requirement (Hu and Brown, 1994; Hu *et al*., 1996). The higher B requirement in dicotyledonous plants compared with graminaceous species is related to higher proportions of compounds with the *cis*-diol configuration in the cell walls of the former, namely pectic substances and polygalacturonans (Loomis and Durst, 1992). The concentration of strongly complexed B in the root cell walls is 3–5 $\mu g\,g^{-1}$ dw in graminaceous species such as wheat, and up to 30 $\mu g\,g^{-1}$ dw in dicotyledonous species such as sunflower (Tanaka, 1967). These differences roughly reflect the differences between the species in B requirement for optimal growth (Hu *et al.*, 1996).

7.7.3 Function of B

Numerous difficulties have hindered progress in understanding B function in plants, including (i) the difficulty in measuring the low cellular B concentrations present, (ii) the labile nature of B and its complexes, (iii) lack of radioisotopes and (iv) the ability of B to rapidly and reversibly bind to diverse molecules. Upon removal of B from growing media growth is almost immediately inhibited followed by manifestation of numerous secondary effects.



Primary effects on plant processes including changes in cell wall dynamics (Findeklee and Goldbach, 1996; Goldbach *et al.*, 2001; Yu *et al.*, 2003), the cytoskeleton and plasma membrane associated processes, occur within 10–15 min, whereas secondary effects including oxidative stress responses occur within 30 min (Lukaszewski and Blevins, 1996; Kobayashi *et al.*, 2004; Koshiba *et al.*, 2009). Accumulation of phenolics under B deficiency is also typical for many plant species, although this does not take place quickly (Cakmak and Römheld, 1997). However, phenol accumulation in B-deficient tissue may have adverse impacts on plants, particularly during the reproductive growth stage with long-term exposure to high light intensity. Boron deficiency reduces utilization of absorbed light energy in photosynthesis, inducing oxidation of phenolics and impairing the antioxidative defence mechanisms of plants, thereby enhancing the susceptibility of plants to high light intensity and generation of ROS. Most probably, these changes do not occur within minutes in B-deficient tissues, but may be an important problem for plants grown under field conditions (Cakmak and Römheld, 1997).

Boron deficiency causes a wide range of anatomical, physiological and biochemical symptoms. These include inhibition of apical growth, necrosis of terminal buds, reduction in leaf expansion, breaking of tissues due to brittleness and fragility, abortion of flower initials and shedding of fruits (Goldbach, 1997; Brown *et al.*, 2002). Most anatomical deficiency symptoms have been associated with cell wall abnormalities (Loomis and Durst, 1992; Brown *et al.*, 2002) and the numerous physiological and biochemical effects observed under B deficiency have been interpreted as secondary effects of cell wall damage (Blevins and Lukaszewski, 1998; Brown *et al.*, 2002). In plants, elevated (mM) concentrations of B are toxic. Boron toxicity reduces shoot growth, primarily in expanding tissues, followed by chlorosis, beginning at the older leaf tips and margins, before finally causing necrosis (Nable *et al.*, 1997; Reid *et al.*, 2004; Reid and Fitzpatrick, 2009). The mechanism of B toxicity is unknown.

7.7.3.1 Cell Wall Structure

A role of B in cell wall structure has long been recognized. In B-deficient plants, the cell walls are strongly altered which is evident at macroscopic (e.g., 'cracked stem'; 'stem corkiness'; 'hollow stem disorder') and microscopic levels (Loomis and Durst, 1992; Shorrocks, 1997). Most anatomical deficiency symptoms are associated with cell wall abnormalities (Loomis and Durst, 1992; Brown *et al.*, 2002) and the numerous biochemical and physiological effects often observed under B deficiency have been interpreted to be secondary effects of cell wall damage (Goldbach, 1997; Blevins and Lukaszewski, 1998; Brown *et al.*, 2002; Bolanos *et al.*, 2004).

The most prominent symptoms of B deficiency are associated with primary cell walls and include abnormally formed walls that are often thick, brittle, have altered mechanical properties and do not expand normally (Brown *et al.*, 2002). Loomis and Durst (1992) first hypothesized that apiose, a rare sugar specific to the pectic fraction of cell walls, may form esters with borate under physiological conditions and hence influence cell wall structure. A high proportion of total plant B is associated with cell wall pectins (Hu and Brown, 1994). Isolation of a B-polysaccharide complex (Matoh *et al.*, 1993) later identified as RGII (Kobayashi *et al.*, 1996; O'Neill *et al.*, 1996), demonstrated that B in the cell wall predominantly cross-links the apiosyl residue in the A side chain of each of two neighbouring monomeric RGII molecules to form a dimeric B-dRGII pectin complex (Kobayashi *et al.*, 1996; Ishii and Matsunaga, 1996; O'Neill *et al.*, 1996; Pellerin *et al.*, 1996; Ishii *et al.*, 1999). This role for B in cell walls and its importance to plant growth and development was confirmed with the *Arabidopsis mur1* mutant. In *mur1*, shoot RGII, which has a substituted sugar residue, forms B-dRGII less rapidly and once formed is less stable than RGII from wildtype plants (O'Neill *et al.*, 2001). mur1 plants are dwarfed with brittle stems, but show normal growth with added B. A role of B cross-linked RGII (Fig. 7.27) in intercellular attachment of tissues was shown in another RGII biosynthesis mutant (Iwai *et al.*, 2002).

Boron does not appear to be directly involved in the synthesis of the cell wall; however, B may influence the incorporation of proteins, pectins and/or precursors into the existing and extending cell wall (Brown *et al.*, 2002). Fleischer *et al.* (1999) demonstrated that B deficiency rapidly increased cell wall pore size, which resulted in cell death once cells entered the elongation phase of growth. The inability of B-deficient cells to form a pectic network with appropriate pore size may influence physiologically important processes, including the incorporation of polymers into the wall and the transport of wall-modifying enzymes or proteins to their substrates and the transport of polymers from the protoplast into the cell wall (Fleischer *et al.*, 1999; Brown *et al.*, 2002). Boron may be necessary for cell-to-wall adhesion and the organization of the architectural integrity of the cell (Bassil *et al.*, 2004).

The changes in cell wall formation and composition result in serious physiological disturbances in plants grown under low B supply. For example, B deficiency enhances the number of Al-binding sites in cell walls, possibly due to increasing amount of unmethylated pectin in the root tips, resulting in higher Al concentrations and greater Al damage in roots (Stass *et al.*, 2007; Yu *et al.*, 2009). Impairments in development and organization of primary cell walls associated with B deficiency have adverse impacts on form, wood quality and cold tolerance of trees (Lehto *et al.*, 2010).

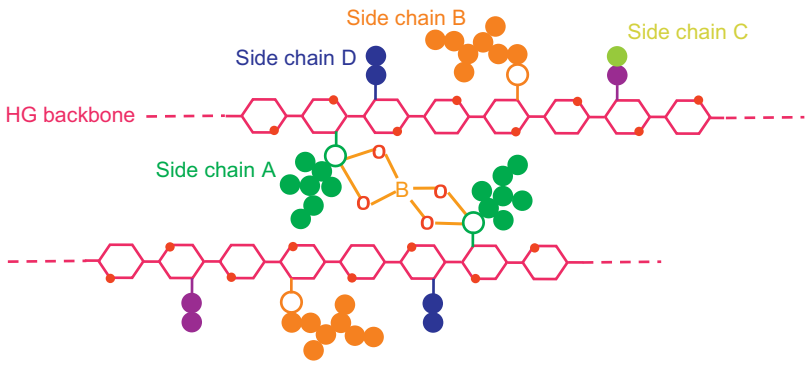


FIGURE 7.27 Structure of borate cross-linked rhamnogalacturonan II dimer (○○) residues on side chain A. *(Courtesy of Malcolm O'Neil).*

Boron is also required for legume–*Rhizobium* symbiotic interactions (see also Chapter 16). Boron plays a role in the maintenance of nodule cell wall and membrane structure (Bolanos *et al*., 1994; Bonilla *et al*., 1997), for rhizobial infection and nodule cell invasion processes (Bolanos *et al.*, 1996; Redondo-Nieto *et al.*, 2001) as well as for symbiosome development and bacteroid maturation. More recent studies have shown the participation of B in nodule organogenesis and in plant–bacteria interactions, suggesting that B has a wide range of functions beyond its role in cell wall structure (Redondo-Nieto *et al*., 2001; Reguera *et al*., 2009, 2010).

7.7.3.2 Metabolism

Boron deficiency has a rapid and profound effect on meristematic activity and causes many secondary disruptions to cellular metabolism which have been wrongly interpreted as evidence for a specific function of B in metabolic processes (Brown *et al.*, 2002). It had been proposed that B plays a key role in higher plants by facilitating short- and long-distance transport of sugars via the formation of borate–sugar complexes. However, this is unlikley because sucrose, the prevalent sugar transported in the phloem of most species, forms only weak complexes with B, and B is not involved in phloem loading of sucrose (see also Chapter 3). Boron deficiency is associated with a range of morphological alterations and changes in differentiation of tissues, similar to those induced by either suboptimal or supraoptimal concentrations of IAA. The relationships between B nutrition, auxin concentration, differentiation and lignification, however, are not clear. The interactions between B and IAA and tissue differentiation may be secondary events caused by primary effects of B on cell wall growth leading to growth inhibition which, in turn, affects phenol metabolism. Certain phenolics not only are effective inhibitors of root elongation growth but also simultaneously enhance radial cell division; that is, they induce anatomical changes that are similar to those caused by IAA. Whether the effects of B deficiency on IAA are direct or merely a consequence of disruptions in cell wall formation and the subsequent effects on apical dominance remains to be resolved.

TABLE 7.40 Phenol concentration, polyphenol oxidase activity and K efflux from leaf segments of sunflower plants grown at different light intensities and sufficient (10^{-5} M) or deficient (10^{-7} M) B supply

		Light intensity ($\mu E\,m^{-2}\,s^{-1}$)		
	B supply	100	250	580
Phenol concentration (μg caffeic acid equiv. (6 segments)$^{-1}$)	10^{-5} M	30	45	75
	10^{-7} M	35	90	265
Polyphenol oxidase activity (relative)	10^{-5} M	1.0	0.8	0.6
	10^{-7} M	1.4	2.1	4.2
K efflux (μg K (6 segments)$^{-1}$ 2 h^{-1})	10^{-5} M	10	12	25
	10^{-7} M	23	63	238

From Cakmak *et al.* (1995) and Cakmak and Römheld (1997).

Accumulation of phenols is a typical feature of B-deficient plants. It has been suggested that the formation of borate complexes with certain phenols may be involved in the regulation of the concentration of free phenols and the rate of synthesis of phenol alcohols as precursors of lignin biosynthesis (Pilbeam and Kirkby, 1983). Accordingly, under B deficiency phenols accumulate and polyphenol oxidase activity is increased (Table 7.40). While phenols clearly accumulate in B-deficient plants, simple stoichiometric analysis of cellular B concentrations and the concentrations of potential B-complexing molecules suggests that B concentrations are not sufficient to influence phenol metabolism through complexation. It has also been shown that the role of B in maintaining plasma membrane

integrity is not due to a role for B in complexing phenols or inhibiting PPO activity (Brown *et al*., 2002). A high proportion of phenols as well as the corresponding enzyme systems are located in the cell walls of the epidermis, thus disruptions of cell wall synthesis under B deficiency may result in secondary disruptions to phenol metabolism. The secondary disruptions of phenol metabolism may in turn result in tissue necrosis as the toxic products of phenol oxidation accumulate. These processes are probably relevant for the long-term effects of B deficiency, especially for plants grown under field conditions. Accumulation of phenolic compounds and related alterations in lignin concentration may also affect plant defence systems against herbivory and pathogens (Lehto *et al*., 2010).

Boron deficiency affects a number of metabolic processes for which the underlying mechanism has not been adequately resolved. A close relationship between B status and the ascorbate/glutathione cycle has been observed by several researchers (Cakmak amd Römheld, 1997; Blevins and Lukaszewski, 1998; Koshiba *et al.*, 2009). Ascorbate and glutathione concentrations are strongly reduced under B deficiency (Cakmak and Römheld, 1997), probably due to inhibiting ascorbate reductase and glutathione reductase. External application of ascorbate temporarily overcame the root growth reduction caused by B deficiency (Blevins and Lukaszewski, 1998). Boron has been implicated in N metabolism and both B deficiency and B toxicity can decrease nitrate reductase activity (Bonilla *et al.*, 1988; Cervilla *et al.*, 2009; Bellaoui *et al*., 2010). The effect of B on nitrate reductase may be mediated through a disruption of membrane transport processes (Cervilla *et al.*, 2009). Boron deficiency also influences photosynthesis, resulting in lower quantum yield and a less efficient PS II, probably as a result of lipid oxidation of the thylakoidal membranes. These effects of B on metabolism may occur as a result of B deficiency effects on membrane structure and function (Brown *et al.*, 2002).

7.7.3.3 Membrane Function

There is considerable evidence in support of a role of B in membrane integrity and functioning. The formation and maintenance of membrane potentials induced by infrared light or by gravity require the presence of B (Tanada, 1978). Boron also influences the turgor-regulated nycinastic movements of leaflets of *Albizzia* (Tanada, 1982) and enhances ^{86}Rb influx and stomata opening in *Commelina communis* (Roth-Bejerano and Itai, 1981).

Uptake rates of P are lower in the root tips of B-deficient compared to sufficient bean and maize plants (Table 7.41). Boron pretreatment of the root tips for only 1 h enhances P uptake in both B-sufficient and -deficient roots and restores the uptake rate of the originally B-deficient roots. A similar effect of B was found for uptake of Cl and Rb (Pollard *et al.*, 1977). Furthermore, membrane-bound ATPase activity, which was low in B-deficient maize roots, was restored to the same level as that in B-sufficient roots within 1 h after resupply of B.

TABLE 7.41 Phosphorus uptake of root tip zones (0–2 cm from the apex) of faba bean and maize grown with or without B supply after pre-treatment of the root tips without or with B for 1 h

	P uptake (nmol g^{-1} h^{-1})			
	Faba bean		Maize	
Growth of plants	+B	−B	+B	−B
Pre-treatment of root tips				
−B	112	52	116	66
10^{-5} mM $B(OH)_3$	152	108	190	171

From Pollard *et al.* (1977).

These effects of B on uptake of ions are mediated by direct or indirect effects of B on membrane structure and hence function of various membrane transport processes including plasma membrane-bound H^+-pumping ATPase (Goldbach and Wimmer, 2007) (see also Chapter 2). In suspension-cultured tobacco cells the effect of B on the H^+ATPase requires the presence of IAA, and B is required for the enhanced H^+ excretion induced by IAA (Goldbach *et al*., 1990). The particular role of B for plasma membrane integrity and H^+ pumping activity was also demonstrated *in vitro* with membrane vesicles from B-sufficient and B-deficient roots of several species (Goldbach and Wimmer, 2007).

Although B may have a direct effect on the plasma membrane-bound H^+ATPase, it is more likely that these effects are indirect, mediated, for example by B complexing *cis*-diol groups of plasma membrane constituents such as glycoproteins or glycolipids at the cell wall–plasma membrane interface and thereby acting as a stabilizing and structural factor required for the integrity and functioning of the plasma membrane (Brown *et al.*, 2002; Bassil *et al.*, 2004). This is supported by the high B concentration of isolated plasma membranes and the presence of B in plasma membrane constituents (Tanada, 1983; Wimmer *et al*., 2009). Further support of the role of B in plasma membrane integrity and function is shown in Fig. 7.28 on K efflux from expanding sunflower leaves of B-sufficient and B-deficient plants (Cakmak *et al*., 1995). The leaves were isolated and immersed either in distilled water or increasing concentrations of B. Compared with the B-sufficient leaves, K efflux was higher in the B-deficient leaves. Potassium efflux from B-deficient leaves could be decreased by

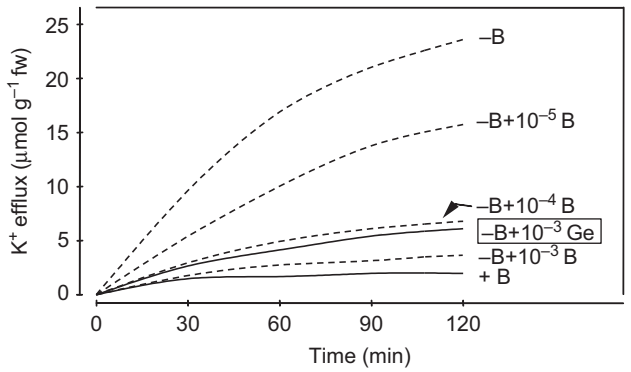


FIGURE 7.28 Potassium efflux from intact B-sufficient (+B) and B-deficient (−B) expanding sunflower leaves and effect of external supply (10^{-5}–10^{-3} M) of B or germanium (Ge) at zero time (−B +B; −B +Ge treatment) *(Cakmak and Kurz, unpublished)*.

external B supply during the efflux period with the decrease dependent on the external B concentration and evident after less than 30 min. Similarly to K efflux, the efflux of sugars, amino acids and phenols was also higher in the B-deficient leaves and could be decreased by external B supply (Cakmak *et al*., 1995). A similar decrease in K efflux could be achieved with external supply of Ge (Fig. 7.28), indicating a substitution of B by Ge not only in cell wall stability and functions, but also in plasma membrane integrity (Cakmak *et al*., 1995).

Boron is also essential for other organisms, including yeast, bacteria and animal embryos that lack a cell wall, which suggests functions of B beyond those in the cell wall. Additional effects of B deficiency include (i) swelling of liposomes, (ii) increased fluidity of microsomes, and (iii) disruption of membrane transport processes. In plant cells, B is necessary for cell to wall adhesion and the architectural integrity of the cell wall (Fleischer *et al*., 1998; Bassil *et al*., 2004) and B may stabilize membrane raft formation through glycolipid binding and hence maintain membrane function (Brown *et al*., 2002).

7.7.3.4 Reproductive Growth and Development

In many agronomic and horticultural crops, B deficiency results in a decrease in reproductive success as a result of poor flower production, pollen production and pollen viability, as well as infertility and premature flower and fruit drop. These reproductive effects can often be observed in the absence of vegetative symptoms or growth reduction suggesting that the B requirement for reproductive tissues is greater than for vegetative tissues or that delivery of B to reproductive structures is limited. Evidence suggests that both higher demand and restricted delivery contribute to the higher sensitivity of reproduction to B deficiency.

The role of B in cell wall structure and plasma membrane integrity is clearly expressed in pollen tube growth and development. The gene responsible for borate cross-linking of pectin rhamnogalacturonan-II is highly active in pollen tubes and is required for plant reproductive tissue development (Loomis and Durst, 1992) and fertilization (Iwai *et al*., 2006). Boron is essential for *in vitro* pollen cultures of most plant species (Robbertse *et al*., 1990). In the absence of adequate B supply, pollen germination is reduced, pollen tubes may burst and the rate of pollen tube extension is reduced (Nyomora *et al*., 2000; Perica *et al*., 2001). After germination, pollen tubes extend by tip growth through the activity of secretory vesicles which are transported to tube tips by cytoplasmic streaming where they fuse with existing pollen tube plasma membrane and their contents (polysaccharides and pectins) are discharged to the outside, where they contribute to cell wall formation. Boron may play a critical role in the control of secretory activities in pollen tubes (Jackson, 1989). In growing pollen tubes, abnormal swelling or bursting of the tip region within 2–3 min of removal of external B (Schmucker, 1934; Jackson, 1989; Nyomora *et al*., 2000). As shown in Fig. 7.29, B deficiency generally has a greater effect on pollen tube growth than on pollen viability or germination (Nyomora *et al*., 2000).

In flowers, the B required for pollen tube growth has to be provided by the stigma or the silk. In maize a minimum B concentration of 3 µg g^{-1} dw in the silk is required for pollen germination and fertilization (Vaughan, 1977). The critical deficiency concentration in the stigma may, however, vary considerably between cultivars and species (Nyomora *et al*., 2000). In grapevine (*Vitis vinifera*) which is known for its high B requirement, with sufficient B supply, the B concentration of the stigma is 50–60 µg g^{-1} dw and fertilization is impaired at concentrations of 8–20 µg g^{-1} dw (Gärtel, 1974).

It is clear that reproductive tissues have a high requirement for B due to their rapid growth rates and pectin-rich cell walls; however, this does not adequately explain why reproductive B deficiency often occurs in absence of vegetative deficiency. Dell and Huang (1997) suggested that the apparent higher requirement for B by reproductive tissues occurs because reproductive structures are not well supplied by vascular bundles and low transpiration rates reduce B supply. This is supported by observations that foliar application of B to developing reproductive tissues can increase reproductive success even in the presence of soil B sufficient for vegetative growth (Nyomora *et al*., 1999; Dordas, 2006). Factors that influence transpiration such as temperature, humidity and water supply interact to affect the occurrence of reproductive B deficiency (Dell and Huang, 1997). Foliar applications of B in soybean improves nitrate reductase and nitrogenase activities of plants and improves concentrations of seed proteins and oleic acid (Bellaloui *et al*., 2010).

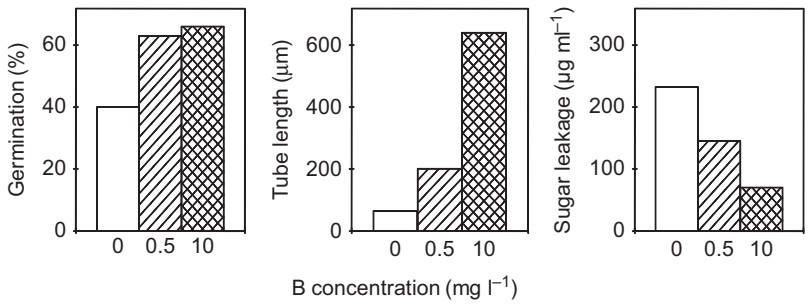


FIGURE 7.29 Growth of pollen germination tube and leakage of sugar to the medium in lily (*Lilium longiflorum* L.) at different B concentrations. *Based on Dickinson (1978).*

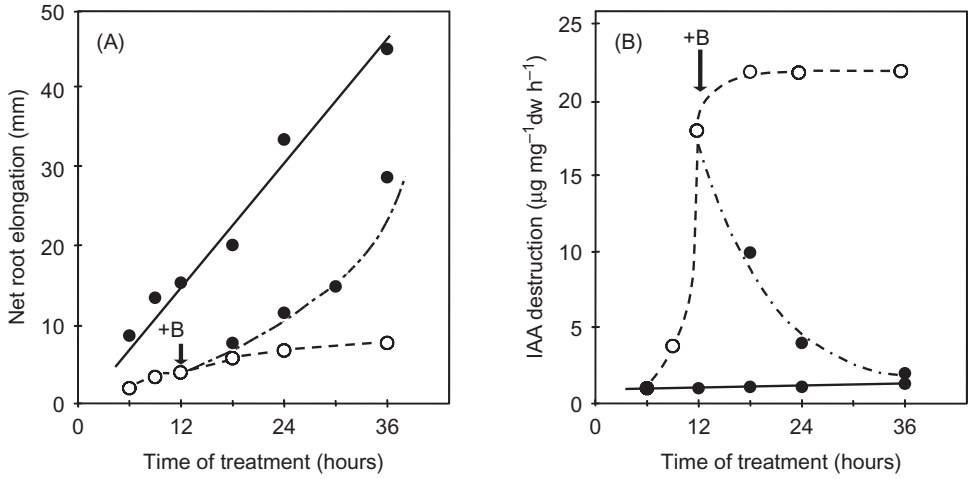


FIGURE 7.30 Root elongation (A) and IAA oxidase activity (B) in apical 5 mm root sections of squash with or without B supply or resumption of B supply after 12 h (arrow) of B deficiency. Key: ●—●, + B; ○- -○, − B. *Based on Bohnsack and Albert (1977).*

The particular role of B in pollen tube growth as well as limitations in B transport to reproductive structures are the major factors responsible for the usually higher demand of B supply for seed and grain production compared to that for vegetative growth. This has been shown to be the case, for example, for maize (Vaughan, 1977) or white clover (Johnson and Wear, 1967), alfalfa (Dordas, 2006), almond (Nyomora *et al.*, 1999) and olive (Perica *et al.*, 2001). In mango, irregular and periodic fruit set caused by suboptimal temperatures during pollination can be, at least in part, compensated by increasing the B concentrations in the pistil and pollen grains (De Wet *et al.*, 1989). Boron also affects fertilization by increasing the pollen-producing capacity of the anthers and pollen grain viability (Dell and Huang, 1997). Indirect effects may also be important, such as increase in amount and composition of sugars of the nectar, whereby the flowers of species that rely on pollinating insects become more attractive to insects (Eriksson, 1979).

7.7.3.5 Root Elongation and Shoot Growth

One of the most rapid responses to B deficiency is inhibition or cessation of root elongation and shoot meristematic growth. In roots, B deficiency results in the roots with a stubby and bushy appearance. In shoots, complete inhibition of meristematic growth occurs in many species, whereas meristem death occurs in others. As shown in Fig. 7.30A, inhibition of root elongation occurs as soon as 3 h after the B supply is interrupted, becoming more severe after 6 h, and finally ceasing after 24 h. Twelve hours after the B supply is restored to roots deprived of B for the same period of time, elongation growth again increases. Between 6 and 12 h after the B supply is stopped, there is an increase in the activity of IAA oxidase in the roots (Fig. 7.30B), which decreases rapidly when B is resupplied. The similarities in the responses of root elongation and IAA oxidase activity to B deficiency and resupply are striking. There is, however, a distinct difference in the time

of response to deficiency: root elongation is inhibited 3 h before IAA oxidase activity increases. Thus, the increase in IAA oxidase activity is a secondary response to B deficiency.

An inhibition of shoot growth is a typical early symptom of B deficiency. In some species, growth inhibition is followed by tissue death and B resupply results in a bushy shoot development as lateral shoots emerge. Other responses to B deficiency include (i) growth inhibition and mild chlorosis, or (ii) growth inhibition with no secondary symptoms. In both cases, B resupply can result in renewed growth of the existing meristem. The chlorosis and tissue death seen in some species is probably due to the inability to synthesize new cell walls in the absence of adequate B and the disruption of cell membrane integrity.

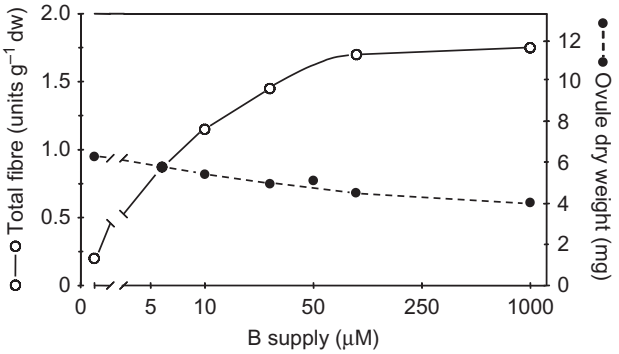


FIGURE 7.31 Dry weight and fibre development of unfertilized cotton ovules cultured in the presence of IAA, gibberellic acid and cytokinin with increasing B supply. Total fibre units represent the ratio of fibre length to g dw. *Based on Birnbaum* et al. *(1974).*

Similar responses of elongation growth to B can be demonstrated in cotton ovules cultured *in vitro* (Birnbaum *et al*., 1974). Epidermal cells of cotton ovules that form lint fibres begin to elongate on the day of anthesis. The degree of extension is closely related to the external B concentration, as shown in Fig. 7.31. Boron is necessary for fibre elongation and the prevention of callusing of the epidermal cells, as indicated indirectly by the decrease in ovule dry weight. Additional observations suggest that B is required primarily for cell elongation rather than for cell division (Birnbaum *et al*., 1974). In root tips, B deficiency results in a reduction in elongation growth associated with changes in cell division from a normal longitudinal to a radial direction (Robertson and Loughman, 1974). Enhanced cell division in a radial direction with a proliferation of cambial cells and impaired xylem differentiation are also features typical of the subapical shoot tissue of B-deficient plants (Fig. 7.32).

7.7.3.6 Integrated Assessment of the Function of B in Plants

From the above discussion, it can be concluded that in higher plants, B exerts its primary influence in the cell wall and at the plasma membrane–cell wall interface, as summarized in a model in Fig. 7.33. Changes in the cell wall and at this interface are considered as primary effects of B deficiency leading to a cascade of secondary effects in metabolism, growth and plant composition. It should be remembered that changes in plasma membrane potential act as a signal for many changes in the cytoplasm, and also for a shift in excretion of cell wall material.

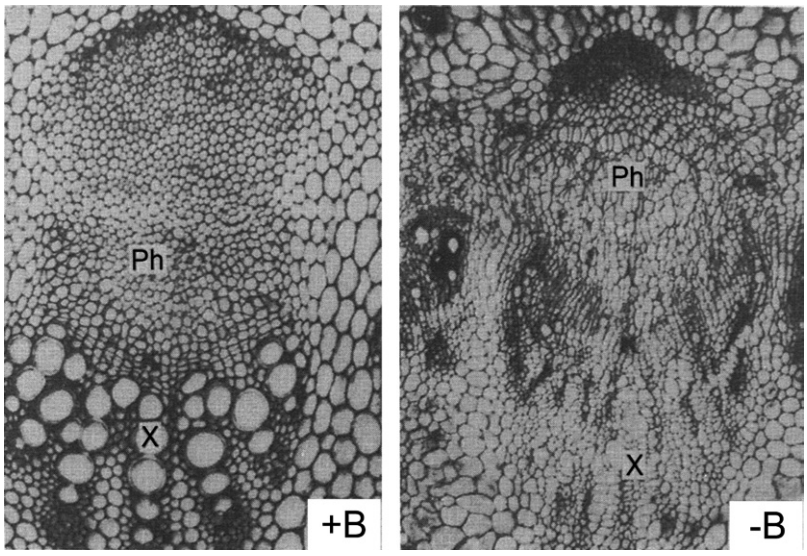


FIGURE 7.32 Cross-section of a vascular bundle of an upper internode of a B-sufficient (*left*) and a B-deficient (*right*) sunflower plant. X, xylem; Ph, phoem. *From Pissarek (1980) with permission from Wiley VCH.*

7.7.4 B Deficiency and Toxicity

7.7.4.1 B Deficiency

Boron deficiency is a widespread nutritional disorder. Under high rainfall conditions, B is readily leached from soils as $B(OH)_3$. Boron availability to plants decreases with increasing soil pH, particularly in calcareous soils and soils with a high clay content, presumably as a result of the formation of $B(OH)_4^-$ and subsequent anion adsorption. Boron deficiency occurs in diverse cropping systems throughout the world and across a wide range of climates and is not restricted to specific soil types or crops. Boron deficiency is more prevalent on leached sandy, alkaline and heavily limed soils, however, B is easily leached from most soils and deficiencies often occur in areas of high rainfall (South-East Asia, Japan and Brazil) or in irrigated systems utilizing water with low B concentration content ($<0.3\,\mu g\,ml^{-1}$). Boron availability is significantly affected by soil water content, and becomes limiting in dry soils due to reduced mass flow (Shorrocks, 1997). The main soil factors affecting B availability include pH, soil texture, organic matter and clay mineralogy, which influence the extent of adsorption of B to soil surfaces (Goldberg, 1997).

Plant species differ in their capacity to take up B (Table 7.42), which generally reflects typical species differences in the requirement of B for growth. For example, the critical deficiency range increases from about 5–10 $mg\,kg^{-1}$ dw in graminaceous species (e.g., wheat) to 20–70 $mg\,kg^{-1}$ dw in most dicotyledonous species (e.g., clover) to 80–100 $mg\,kg^{-1}$ dw in gum-bearing plants such as poppy (Bergmann, 1992). For evaluation of critical deficiency concentrations of B, the elongation rate of the youngest leaf is a more suitable parameter than, for example, shoot dry weight (Kirk and Loneragan, 1988).

TABLE 7.42 B concentration of the leaf tissue of plant species from the same location

Plant species	B concentration ($mg\,kg^{-1}$ dw)
Wheat	6
Maize	9
Timothy	15
Tobacco	29
Red clover	32
Alfalfa (lucern)	37
Brussel sprouts	50
Carrots	75
Sugar beet	102

Based on Gupta (1979a).

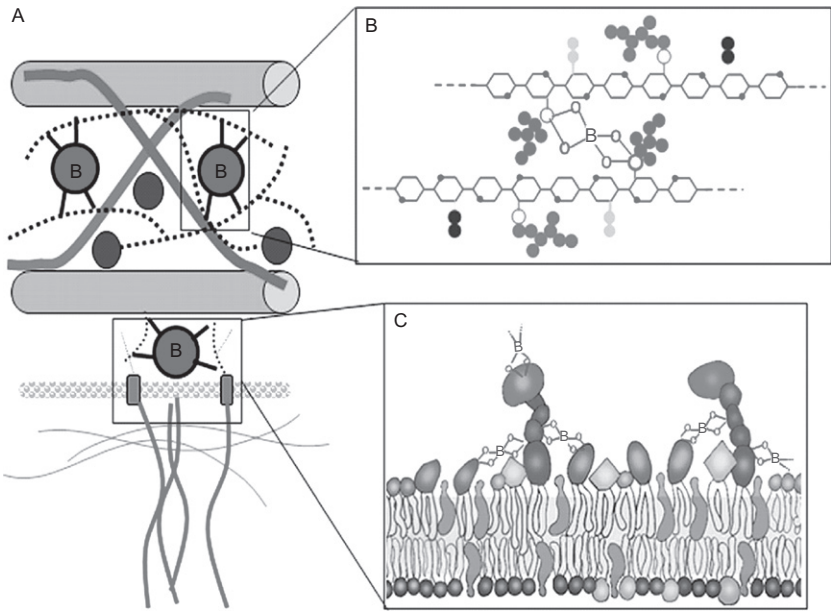


FIGURE 7.33

A. Network of cellulose fibrils (), hemicelluloses (), pectins () and cell wall proteins (). Plasma membrane () with attachment sites () of actin () and tubulin ().

B. Galacturonic and backbone (-○-○-) with various side chains linked by B (modified with permission from Malcolm O'Neill).

C. Membrane bilayer showing glycosphingolipids (), sphingomyelins, (), glycosylphosphatidyl-inositol anchored proteins () and other membrane components. *Modified with permission from Brown* et al. *(2002).*

The differences in B demand particularly between graminaceous and dicotyledonous species is most likely related to the differences in their cell wall composition. In graminaceous species, the primary cell walls contain very little pectic material and have also a lower Ca requirement (Section 6.5). These two plant groups also differ in their capacity for Si uptake which is usually negatively related to the B and Ca requirement (Loomis and Durst, 1992). All three elements are mainly located in the cell walls. Symptoms of B deficiency in the shoots are noticeable at the terminal buds or youngest leaves, which become deformed, and depending upon species, may become discolored and die. Internodes are shorter, giving the plants a bushy or rosette appearance. In some species, interveinal chlorosis may occur while misshaped leaf blades are common. The differences among species in expression of B deficiency is not well understood, but may reflect differences in species response to the inhibition of cell wall formation and the changes in cellular metabolism. Many plant species accumulate large amounts of phenols under B deficiency which can result in increased concentrations of oxidized phenols and other reactive species which can cause cell death. Other plant species respond to B deficiency with a cessation of shoot growth and deformed leaf blades, but do not exhibit chlorosis or necrosis, presumably because toxic metabolites did not accumulate.

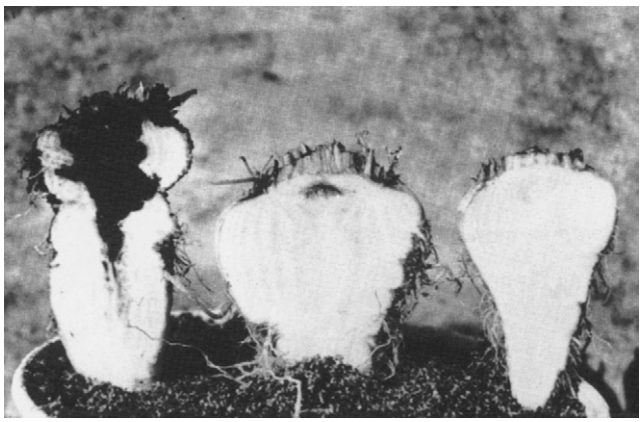


FIGURE 7.34 B deficiency in sugar beet: severe B deficiency (heart and crown rot) (*left*); mild B deficiency (heart rot) (*middle*); B-sufficient (*right*). *Courtesy of W. Bussler.*

An increase in the diameter of petioles and stems is particularly common and may lead to symptoms such as 'stem crack' in celery or 'hollow stem disorder' in broccoli (Shelp, 1988). Drop of buds, flowers and developing fruits is also a typical symptom of B deficiency. In the heads of vegetable crops (e.g., lettuce), water-soaked areas, tipburn, and brown- or blackheart occur. In storage roots of celery or sugar beet, necrosis of the growing areas leads to heart rot (Fig. 7.34). With severe deficiency, the young leaves also turn brown and die, often followed by rotting and microbial infections of the damaged tissue. In B-deficient fleshy fruits, the growth rate is lower, and the quality may also be severely affected by malformation (e.g., 'internal cork' in apple) or, in citrus, by a decrease in the pulp/peel ratio.

Boron deficiency-induced reduction or even failure of seed and fruit set are well known. However, even when seed yield is not depressed in plants grown in a low B soil, the seeds produced may have a lower quality in terms of viability as shown in Table 7.43 for black gram. Despite the same seed dry weight, the seeds with the lower B concentration had a lower viability and produced a high percentage of abnormal seedlings. A B concentration of $6\,mg\,kg^{-1}$ seed dw is considered as critical for growth of normal seedlings in black gram.

For the application of B either to the soil or as a foliar spray, different sodium borates, including borax or sodium tetraborate, can be used. Boric acid or sodium borate are effective as foliar sprays, for example, to increase flower and fruit set in fruit trees (Hanson, 1991a,b; Nyomora *et al.*, 1997) or in soybean and alfalfa (Dordas, 2006). The amount of B applied varies from 0.3 to $3.0\,kg\,ha^{-1}$, depending on the requirement and sensitivity of the crop to B toxicity. The high solubility of many B fertilizers and the possibility of inducing toxicity require special care in the application of B fertilizers.

TABLE 7.43 Yield, seed B concentration, seed viability and germination of black gram (*Vigna mungo* L.) grown with or without B supply

	Seed yield ($g\,dw\,plant^{-1}$)	B concentration ($mg\,kg^{-1}$ seed)	Percentage of seedlings		
			Normal	Weak/ abnormal	Non-viable
−B	5.0	3.4	57	40	3
+B	5.1	7.4	92	6	2

Based on Bell *et al.* (1989).

7.7.4.2 B Toxicity and Tolerance

Boron toxicity is most common in arid and semi-arid regions in plants growing on soils formed from parent material of marine origin, or related to the use of irrigation water high in B (Nable *et al.*, 1997). Boron toxicity may also occur when large amounts of municipal compost are applied. Plant species, and to some extent also cultivars within a species, differ in their B tolerance. For example, the critical toxicity concentrations ($mg\,kg^{-1}\,dw$) in leaves are in the range of 100 in maize, 400 in cucumber and 1,000 in squash, and between 100 and 270 in wheat genotypes (Paull *et al.*, 1992a), or about 100 in snap bean and over 330 in cowpea (Francois and Clark, 1979b). Typical symptoms of B toxicity in mature leaves are marginal or tip chlorosis or both, and necrosis. They reflect the distribution of B in shoots which is related to the transpiration stream. Visual symptoms of B toxicity on leaves may occur at lower B concentrations than required for depression of grain yield, for example in wheat (Kluge, 1990). The B concentration in wheat grains can be increased more than 20-fold without negative effects on seed germination and seedlings growth (Paull *et al.*, 1992a).

Critical toxicity concentrations of B in leaves have to be interpreted with reservation for various reasons. There is a steep gradient in B concentration within a leaf blade (see also Chapter 3). In barley, this gradient from the base to the tip of the leaf blade is from about 80 to $2{,}500\,\mu g\,B\,g^{-1}\,dw$, but the average for the leaf is $208\,\mu g\,g^{-1}$ (Nable *et al.*, 1990b). Furthermore, the critical toxicity concentrations are often lower in field-grown plants compared with plants grown in a greenhouse. This difference is partially related to leaching of B from leaves by rain (Nable *et al.*, 1990b).

The physiology of B tolerance and B toxicity is not well understood. There is a positive correlation between critical deficiency and toxicity concentrations for a wide range of plant species. In many cases, B concentrations of leaves or whole shoot are, however, not well related to differential tolerance to B toxicity of plants (Nable *et al.*, 1990a; Torun *et al.*, 2003; Choi *et al.*, 2006). Thus, the severity of leaf symptoms of B toxicity and decreases in shoot growth may be better parameters than leaf B concentrations in ranking genotypes for their tolerance to B toxicity (Torun *et al.*, 2003; Choi *et al.*, 2006). Differential expression of B toxicity tolerance among the genotypes despite similarly high leaf B concentration seems to be related to better redistribution of B by efflux transporters from sensitive symplastic compartments into the leaf apoplasm (Reid and Fitzpatrick, 2009).

Species with high B demand may have also a higher capacity to sequester B in the cell walls (Fig. 7.33). When B supply is excessive, inactivation as soluble complexes seems to be less important, with the exception of certain halophytes which use compatible solutes (Section 17.6). such as sorbitol for this purpose (Rozema *et al.*, 1992). If these detoxification mechanisms become limiting, the B concentration in the cytosol may increase causing metabolic disturbances by complexing with, for example, NAD^+, or ribose of RNA (Loomis and Durst, 1992), or inhibiting ureide metabolism in the leaves of nodulated soybean (Lukaszewski *et al.*, 1992).

Within species such as barley, wheat, annual medics (*Medicago* spp.) and field peas (*Pisum sativum* L.), large genotypic differences exist in the capacity to tolerate high B concentrations in soil or nutrient solution (Paull *et al.*, 1992b; Nable *et al.*, 1997). These differences are based on restrictions in B uptake by the roots and the rate of exudation of B from roots to soil (Reid, 2007). In wheat cultivars varying in sensitivity to high B in soils, a close correlation between root and shoot B concentrations, and root B concentration and shoot yield was observed, suggesting that the main control over B toxicity is exerted at the root level by regulation of root B concentrations. In wheat and barley tolerance is due to lower root B concentrations, thereby restricting transfer of B to the shoot, not to high B tolerance of the tissue (Nable *et al.*, 1997). Root B concentration is reduced in tolerant cultivars by B efflux via an efflux transporter in the BOR family (Sutton *et al.*, 2007). This is a different mechanism than in tomato, where root-to-shoot transport of B rather than root B concentration was the most distinct difference between genotypes (Bellaloui and Brown, 1998). In barley, genotypical differences in restriction of uptake by roots and transport of B into the leaves are closely correlated with similar restrictions in uptake and transport of Si (Nable *et al.*, 1990a).

In barley, the differences in capacity to reduce B uptake are already well defined genetically (Paull *et al.*, 1988a; Nable *et al.*, 1997), and are likely based on both restricted passive movement of B through the plasma membrane of root cells (Huang and Graham, 1990), as well as the function of a B efflux transporter, BOR4 (Miwa *et al.*, 2007; Sutton *et al.*, 2007), and not on differences in root anatomy or transpiration rates (Nable *et al.*, 1997). This restriction in uptake holds true over the whole range of applied B concentrations (Nable *et al.*, 1990a).

7.8 CHLORINE

7.8.1 General

Chlorine is ubiquitous in nature, and occurs in aqueous solution as the monovalent ion chloride (Cl^-). Its salts are readily soluble, the mobility of Cl in the soil is high, and its concentration in the soil solution varies over a wide range. Chloride is readily taken up by plants and its

mobility in short- and long-distance transport is high. In plants, Cl occurs mainly as a free anion or is loosely bound to exchange sites. However, higher plants also contain more than 130 chlorinated organic compounds (Engvild, 1986). With the exception of a role in PS II, the importance of these compounds in terms of functional requirement of Cl for higher plants is not known. Chloride acts as a counter anion to stabilize the membrane potential and is involved in turgor and pH regulation and, at concentrations present in most environments, Cl is the most abundant inorganic anion in plant cells. Average Cl concentrations in plants are in the range of 2–20 $mg\,g^{-1}$ dw, which is typical of the concentration of a macronutrient. In most plant species, the minimum Cl requirement for plant growth, however, is in the range of 0.2–0.4 $mg\,g^{-1}$ dw, i.e. about 10 to 100 times lower. Thus, Cl plays a quantitatively important role in ion balance when Cl is abundant, but other anions (nitrate, malate) can fulfil this role when Cl supply is reduced. Chlorine is usually supplied to plants as chloride from various sources (soil reserves, irrigation water, rain, fertilizers, air pollution), therefore on a worldwide basis deficiencies are rare and there is greater concern about Cl toxicity (see also Section 17.6). Indeed, to induce Cl deficiency, in most plant species particular precautions are required to reduce the 'contamination' by Cl from seeds, chemicals, water and air. Using these precautions, Broyer *et al.* (1954) were able to demonstrate the requirement of Cl as a micronutrient for higher plants. For more recent summaries of plant species where Cl deficiency has been demonstrated the reader is referred to Flowers (1988) and Heckman (2007).

7.8.2 Uptake, Transport and Homeostasis

Comparatively little is known about Cl transport in plants though a number of genes involved in Cl transport have been identified. Chloride transporters in plants include members of the CLC protein family (chloride channels) which includes both Cl^- channels and $(Cl^-/NO_3^-)/H^+$ antiporters (Lv *et al.*, 2009; Zifarelli and Pusch, 2010) and CCC (cation chloride transporters) which likely function as Na^+:K^+:Cl^- cotransporters (Colmenero-Flores *et al.*, 2007).

Seven chloride channel (CLC) members have been identified in the *Arabidopsis* genome (Isayenkov *et al.*, 2010). *AtCLCe* is localized in thylakoid membranes, *AtCLCf* in Golgi vesicles, *AtCLCc* is expressed in guard cells, *AtCLCb* in roots, and all *AtCLC* members are expressed in vascular tissues in both roots and shoots and may be involved in long-distance ion transport within the plant (De Angeli *et al.*, 2007; Lv *et al.*, 2009; Isayenkov *et al.*, 2010; Zifarelli and Pusch, 2010). Further characterization of Cl transporters in plants under non-salt stress conditions is required to better define the functional role of these transporters in Cl uptake and homeostasis.

Chloride is relatively mobile within the phloem and the recirculation of Cl (defined as the ratio of phloem–xylem nutrient fluxes) is about 20% in a number of plants (White and Broadley, 2001) with the phloem Cl concentration positively correlating with the Cl solution in which plants are grown.

7.8.3 Photosynthetic O_2 Evolution

In 1946, Warburg and Lüttgens showed that Cl is required in the water oxidation complex (WOC) of PS II; since then, the involvement of Cl in the splitting of water at the oxidizing site of PS II, i.e. for O_2 evolution (see also Chapter 5), has been confirmed in a large number of studies. The Cl in PS II is located close to the entrance of putative proton transfer pathways and its participation in proton release from the Mn_4O_xCa cluster is very likely (Guskov *et al.*, 2010). One or two Cl ions are required for the water oxidation cycle to proceed, and a depletion of Cl has been shown to inhibit the S2→S3 and S3→S0 transitions. Proposed roles of Cl in the water oxidation complex of PS II include: (i) ligation to Mn or Ca atoms, (ii) regulation of the redox potential of the Mn_4O_xCa cluster, (iii) maintaining a hydrogen bond network, and (iv) activation of the substrate water.

Establishing the requirement for Cl in photosynthetic O_2 evolution in experiments with whole plants and intact chloroplasts are inconclusive as Cl concentrations are relatively high even in chloroplasts from Cl-deficient plants and since effects on plant growth generally occur well before effects on photosynthesis (Fig. 7.35; Terry, 1977). The determination that only one or two Cl atoms are required for each WOC within PS II suggests that the real Cl requirement for PS II function is lower than 1 mM; a conclusion that is supported by the observation that near maximal O_2 evolution was maintained at less than 30 µM Cl in purified PS II of *Thermosynechococcus vulcanus* (Kawakami *et al.*, 2009). The true requirement for Cl for function of the WOC of PS II *in planta* remains unknown; however, it appears unlikely that disruption of PS II function is a primary consequence of Cl deficiency (Fig. 7.35).

7.8.4 Proton-pumping V-type ATPase

Membrane-bound proton-pumping ATPases and PP_iases are stimulated by various cations and anions (Table 7.44). The importance of these pumps for pH regulation of the cytosol, acidification of endomembrane or intracellular compartments, ion uptake and plant growth in roots is discussed in Chapter 2. The proton-pumping ATPase at the plasma membrane is stimulated by monovalent cations, K^+ in particular, whereas the proton-pumping V-type ATPase on endomembranes is specifically stimulated by

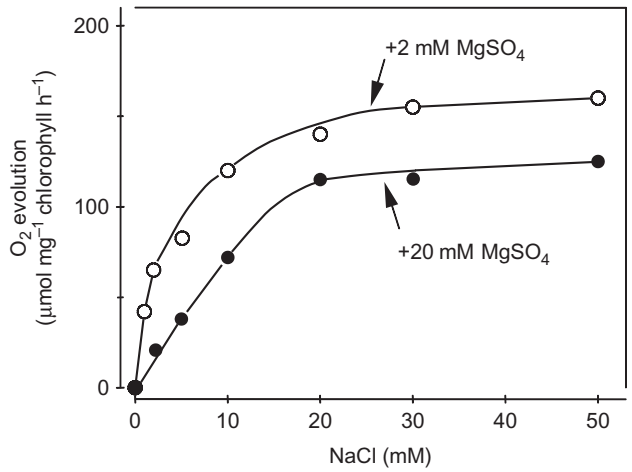


FIGURE 7.35 O_2 evolution of Cl-depleted PS II particles of spinach chloroplasts at different concentrations of NaCl and 2 or 20 mM $MgSO_4$. *Based on Itoh and Uwano, 1986.*

TABLE 7.44 Proton-pumping ATPase activity of tonoplast vesicles with different salt forms

Salt (10 mM monovalent ion)	ATPase stimulation (% of control)
No monovalent ion	10
KCl (control)	100
NaCl	102
NaBr	87
KNO_3	21
K_2SO_4	3

Based on Mettler *et al.* (1982).

Cl and other anions (Sze, 1985). Fluorescent-labelled AtClC-d is colocalized with V-type ATPase in the trans-Golgi network and mutations in either the AtCLC-d or V-ATPase result in similar phenotypes. In addition, Cl^- dissipates the electrical potential (positive inside) generated by the electrogenic H^+-V-ATPase in vesicles, which is accompanied by an increase in the difference in pH between inside and outside (acid inside). These results suggest a functional linkage between AtCLC-d and V-ATPase. A strong interdependence between CLC activity and acidification of intracellular compartments has also been established in mammals.

A functional linkage between AtCLC-d and V-ATPase may also explain the similarities between the Cl-stimulated V-ATPase and the mechanisms regulating elongation of coleoptiles (Hager and Helmle, 1981). Plants with a loss of function mutation of AtCLC-d had impaired root growth and cell elongation, an effect that was also reported following H^+-ATPase knock-down by RNAi (Padmanaban *et al.*, 2004). Severe inhibition of root elongation in Cl-deficient plants may be related to the function of Cl in stimulating V-ATPase mediated compartmental acidification and plant growth.

7.8.5 Stomatal Regulation

Chlorine can play an essential role in stomatal regulation of some species. Opening and closure of stomata is mediated by fluxes of K and accompanying anions such as malate and Cl^- (Roelfsema and Hedrich, 2005) and it has long been hypothesized that tonoplast Cl^-/H^+ antiporters mediate stomatal opening (Pierce and Higinbotham, 1970). Recently, the Cl^- transporters AtCLC-c and SLAC1 have been localized in the guard cell vacuole and endomembrane compartments, but their function in Cl transport and stomata opening has not been resolved.

In plant species such as *Allium cepa* which do not synthesize malate in their guard cells, Cl is essential for stomatal functioning, and stomatal opening is inhibited in the absence of Cl (Schnabl, 1980). Members of the *Palmaceae* such as coconut (*Cocus nucifera* L.) and oil palm (*Elaeis guineensis* Jacq.), which may possess chloroplasts containing starch in their guard cells (Braconnier and d'Auzac, 1990), also require Cl for stomatal functioning.

In coconut, there is a close correlation between K and Cl fluxes during stomata opening from the subsidiary cells into the guard cells and vice versa during stomata closure; in Cl-deficient plants, stomatal opening is delayed by about 3 h (Braconnier and d'Auzac, 1990). Impairment of stomatal regulation in palm trees is considered as a major factor responsible for growth depression and wilting symptoms in Cl-deficient plants (Von Uexküll, 1985; Braconnier and d'Auzac, 1990).

7.8.6 Cl Supply and Plant Growth

In most plants the principal effects of Cl deficiency are wilting and a reduction in leaf surface area and thereby plant dry weight (Fig. 7.36). This decrease in leaf area is the result of a reduction in cell division rates (Terry, 1977) and cell extension, and not of net photosynthesis per unit chlorophyll, indicating a lower Cl requirement for photosynthetic O_2 evolution than for other Cl-dependent processes. In sugar beet, the critical deficiency concentration in leaf blades is between 20 (Fig. 7.36) and 50 µmol Cl g^{-1} dw (Ulrich and Ohki, 1956) or 0.7 and 1.7 mg Cl g^{-1} dw, respectively.

The plant species plays a major role in determining the critical deficiency concentration of Cl in the shoot dry weight, and growth depression when Cl supply is interrupted as shown in Fig. 7.37 for various plant species

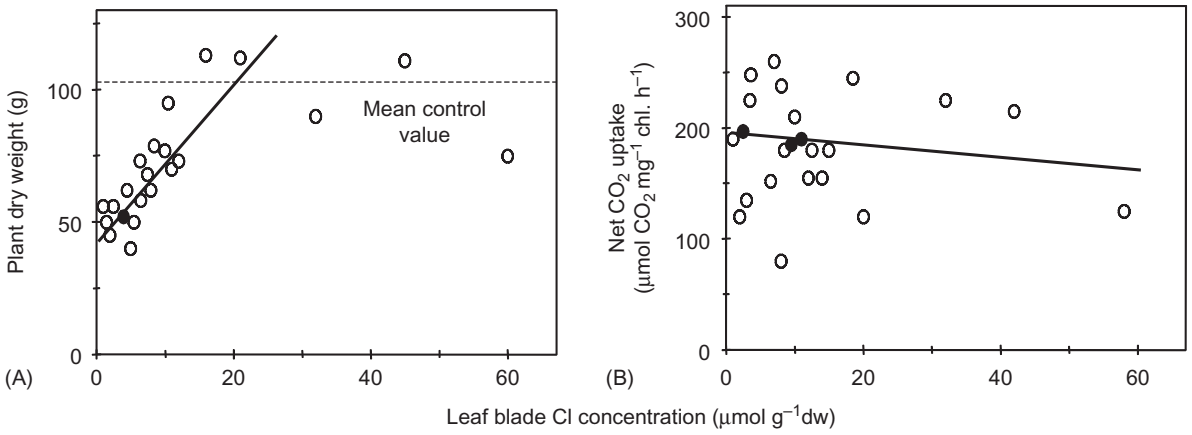


FIGURE 7.36 Growth (A) and photosynthesis (B) of sugar beet at different Cl concentrations in the leaf blade. *Based on Terry (1977).*

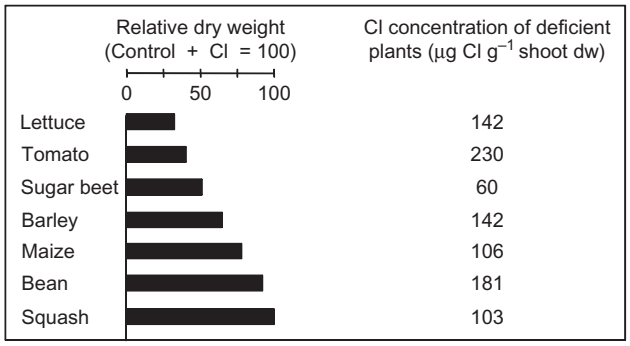


FIGURE 7.37 Relative shoot dry weight and Cl concentrations of Cl-deficient plants. *Redrawn from Johnson* et al. *(1957).*

grown in nutrient solutions under controlled environmental conditions. By withholding Cl supply, growth was not affected in squash, but strongly reduced in lettuce. Resupply of Cl to the deficient plants restored growth within a few days.

Growth reduction and Cl deficiency symptoms could be restored to 90% of the levels in plants adequately supplied with Cl by supplying bromide (Br) (Broyer, 1966) and Br can replace Cl in photosynthesis of purified PS II protein (Kawakami *et al*., 2009). Chloride and bromide have similar physico-chemical properties; for example, their hydrated ionic radii are nearly the same: 0.332 nm (Cl^-) and 0.330 nm (Br^-). Substitution of Cl by Br is of no practical significance, however, because of the difference in their natural abundance. In the earth crust, the sea and the air, as well as in plants, Cl is ~1,000 times more abundant than Br (McClendon, 1976).

Compared with most other plant species (with the exception of palm trees), kiwifruit (*Actinidia deliciosa*) has a very high Cl requirement (Table 7.45). In Cl-deficient plants, dry weight and leaf size are strongly reduced and interveinal chlorosis occurs in mature leaf blades. The critical deficiency concentration in leaves is about 2 mg Cl g^{-1} dw and, thus, Cl deficiency can readily be induced in this species. The reasons for the high Cl requirement of kiwifruit are unclear. In the experiment shown in Table 7.45, the Cl effects on growth were not related to changes in cation–anion balance in the plants, as increasing Cl concentrations in the leaves were counterbalanced by equimolar decreases in nitrate concentration (Smith *et al.*, 1987).

Not much is known of a specific role of Cl as a micronutrient, for example, in cell division and extension, or in N metabolism. The concentrations of certain amino acids and amides are high in Cl-deficient cabbage and cauliflower plants (Freney *et al.*, 1959) as a result of either inhibition of synthesis or degradation of proteins. A role of Cl in N metabolism is indicated by its stimulating effect on asparagine synthetase, which uses glutamine as a substrate:

$$\text{Glutamine} \xrightarrow[\text{Asparagine synthetase}]{(NH_3)} \text{Asparagine} + \text{Glutamic acid}$$

Chloride or B enhances this transfer by a factor of 7, whereas sulphate has an inhibitory effect. Furthermore, Cl increases the affinity of the enzyme for the substrate by a factor of 50 (Rognes, 1980). In plant species in which asparagine is the major compound in the long-distance transport of soluble N (Section 6.1), chloride may therefore also play a role in N metabolism.

Some of the Cl-containing organic compounds in plants may act as antibiotics and fungicides (Engvild, 1986). Chlorine may stimulate extension growth in some legume species such as peas and faba bean which contain substantial amounts of chlorinated IAA in their seeds. Chlorinated IAA enhances hypocotyl elongation 10-fold more than IAA itself, probably because of its higher

TABLE 7.45 Cl concentration in the youngest leaf and growth of kiwifruit (*Actinida deliciosa*) at different rates of Cl supply

Cl supply (μM)	Concentration in youngest leaf ($mg\,g^{-1}$ dw)	Total dry weight ($g\,plant^{-1}$)	Main leaf area (m^2 leaf)
0	0.7	8	0.17
350	1.5	32	0.41
700	2.1	37	0.50
1,400	4.0	34	0.43

Based on Smith *et al.* (1987).

resistance against degradation by peroxidases (Hofinger and Böttger, 1979).

7.8.7 Cl Supply and Osmoregulation

The critical deficiency concentration is $2\,g\,kg^{-1}$ dw which is equivalent to $6\,\mu mol\,Cl\,g^{-1}$ dw, or a concentration of about 6 mM Cl^- in fresh tissue. This concentration is too low to be of general importance in osmoregulation of the bulk plant tissue, unless Cl is preferentially accumulated in certain tissues (e.g., extension zones) or cell compartments (e.g., guard cells). As a rule, however, Cl concentrations in plants exceed this critical deficiency level by two orders of magnitude and become important in osmotic adjustment and plant water relations (Flowers, 1988), including a role in xylem volume flow and root pressure (see also Chapter 2). In this concentration range, Cl represents the dominant inorganic anion in the vacuole. In the phloem sap, Cl concentrations may be in the order of 120 mM and seem to play a role in phloem loading and unloading of sugars; for example, in barley leaves (Fromm and Eschrich, 1989), and in osmoregulation in the pulvini of *Mimosa pudica* during seismonastic leaf movement. In the latter process, Cl is unloaded together with K and sugars (Fromm and Eschrich, 1989; Moran, 2007).

Chloride, together with K, has a particular function in osmoregulation in the stigma of grasses (Heslop-Harrison and Reger, 1986). At anthesis, the stigma of grasses such as *Pennisetum americum* L. often extend within minutes by cell elongation and this is mainly mediated by rapid transfer of K and Cl from the surrounding tissue into the stigma primordium.

Thus, Cl has important functions in osmoregulation at different levels. At the normal plant Cl concentrations, it is a main osmoticum in the vacuoles of the bulk tissue (50–150 mM Cl), together with K. At low concentrations which are in the range of a micronutrient (~1 mM Cl^- or below), these osmoregulatory functions of Cl are presumably confined to specialized tissues or cells, such as the extension zones of roots and shoots, pulvini and stigma, and guard cells, where the Cl concentrations may be substantially higher than the average of the bulk tissue. The stimulation by Cl of the proton-pumping ATPase at the tonoplast is in accordance with the particular role of Cl in osmoregulation.

7.8.8 Chlorine Deficiency and Toxicity

Wilting of leaves, especially at leaf margins, is a typical symptom of Cl deficiency, even in water culture, when plants are exposed to full sunlight (Broyer *et al.*, 1954). With severe deficiency curling of the youngest leaves followed by shrivelling and necrosis may occur (Whitehead, 1985). In palm trees, which have a particularly high Cl requirement (about $6\,mg\,Cl\,g^{-1}$ leaf dw; Ollagnier and Wahyuni, 1986), besides wilting and premature senescence of leaves, frond fracture and stem cracking are typical symptoms of Cl deficiency (Table 7.46).

In leaves and roots, cell division and particularly cell extension are impaired in deficient plants, and in roots this is associated with subapical swelling (Smith *et al.*, 1987; Bergmann, 1992) and enhanced formation of short laterals, giving the roots a stubby appearance (Johnson *et al.*, 1957).

In plant species such as red clover with relatively low Cl requirements ($<1\,mg\,Cl\,g^{-1}$ leaf dw) the demand can be covered by a concentration of 100 μM Cl in the nutrient solution. At 10 μM Cl supply, shoot dw decreases by 50% (Chisholm and Blair, 1981), indicating that the selectivity of Cl uptake is not very high compared to, for example, P where the higher requirement in the leaf dw (Section 6.2) can be covered by supply of even less than 10 μM.

The question arises if Cl deficiency may occur under field conditions. Assuming a critical deficiency concentration of $1\,mg\,Cl\,g^{-1}$ shoot dw, the crop requirement would be in the range of $4–8\,kg\,Cl\,ha^{-1}$, which is about

TABLE 7.46 Cl and K concentrations in leaves and growth disorders in coconut (*Cocos nucifera* L.) trees at different rates of KCl fertilization

Fertilization (kg KCl tree^{-1})	Leaf concentration (mg g^{-1} dw)		Growth disorders (%)	
	K	Cl	Frond fracture	Stem cracking
0	1.61	0.07	11.6	27.0
2.25	1.64	0.41	1.7	8.1
4.50	1.66	0.51	1.2	4.5

Based on Uexküll (1985).

the input from rain in areas distant from oceans, and about 10 times lower than the input from rain at sites near oceans. However, in highly leached soils with a low Cl input from rain and other sources, Cl deficiency may occur even in plant species with low Cl requirement (Ozanne, 1958). The probability of Cl deficiency and thus response to Cl fertilizers is higher in plant species with a high Cl requirement such as kiwifruit (Smith *et al.*, 1987; Buwalda and Smith, 1991) and palm trees in particular (Ollagnier and Wahyuni, 1986; Braconnier and d'Auzac, 1990; Table 7.45).

There are also reports of field experiments with wheat and other cereals (which have a relatively low Cl requirement) in which increases in grain yield by chloride fertilization (e.g., KCl instead of K_2SO_4) occurred. The best documented example of agricultural Cl deficiency is in the wheat growing regions of the Great Plains of the USA (Fixen, 1993). These regions are characterized by very low Cl deposition in rain (<0.5 kg ha^{-1}; Xu *et al.*, 2000), leached soils with low Cl concentrations and high production/high demand species (wheat, barley) (Fixen, 1993). The yield increases with KCl may be a combination of various effects, including alleviation of Cl deficiency (Fixen *et al.*, 1986b), suppression of root rot diseases (Timm *et al.*, 1986), or a combination of suppression of diseases and improving plant water relations (Fixen *et al.*, 1986a). In kiwifruit, enhanced K uptake rates and improving the K nutritional status may be additional side-effects of Cl fertilizer application (Buwalda and Smith, 1991).

Chlorine toxicity occurs worldwide and a general stress factor limiting plant growth particularly in arid and semi-arid regions (Teakle and Tyerman, 2010). On average, Cl concentrations in the external solution of more than 20 mM can lead to Cl toxicity in sensitive plant species, whereas in tolerant species the external concentration can be four to five times higher without reducing growth. Differences in Cl toxicity concentrations are mainly related to differences in the sensitivity of leaf tissue to high Cl concentrations. More than 3.5 mg Cl g^{-1} leaf dw (10 mM Cl in the leaf water) are toxic to sensitive species such as most fruit trees, as well as to bean and cotton. In contrast, 20–30 mg Cl g^{-1} leaf dw ($\sim$60–90 mM Cl in the leaf water) are not harmful to tolerant species such as barley, spinach, lettuce and sugar beet. Genotypical differences in Cl tolerance are closely related to salt tolerance mechanisms, which are discussed in Section 17.6.

Chapter 8

Beneficial Elements

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SUMMARY

In this chapter, the roles of sodium (Na), silicon (Si), cobalt (Co), selenium (Se) and aluminium (Al) are described. These elements are termed beneficial because they stimulate growth, but are not essential, or are essential only for certain plant species, or under specific conditions. Sodium is essential for halophytes, but can also stimulate growth of other plants, particularly some C4 plants. In C4 plants, Na aids the movement of substrates between the mesophyll and the bundle sheath. Sodium can also to some extent replace K in its role as osmoticum. Silicon has a number of similarities to B and plays an important role in cell wall stability, by both bridging between polyuronides and stimulating lignin synthesis. It can improve plant and leaf erectness, water use and protect plants from pests and diseases. Cobalt is essential for N_2 fixing plants because it is part of the coenzyme cobalamin (vitamin B_{12}) which is important in nodule metabolism. Therefore Co deficiency results in poor nodulation and low N_2 fixation rates. The chemistry of Se is similar to that of S and can replace, to some extent, S in proteins, particularly in Se hyperaccumulating plants. Selenium is essential for animals, therefore Se fertilization may be beneficial for human and animal health in areas with Se-deficient soils. Aluminium is beneficial to some plants such as tea, but mechanisms of this beneficial effect are unknown. It may aleviate proton; toxicity and increase the activity of anti-oxidant enzymes.

8.1 DEFINITION

Elements that stimulate growth, but are not essential (for a definition of essentiality see Chapter 1), or are essential only for certain plant species, or under specific conditions, are termed *beneficial elements*. This definition applies in particular to sodium (Na), silicon (Si) and cobalt (Co). The distinction between beneficial and essential is especially difficult in the case of some trace elements. Developments in analytical chemistry and in methods to minimize contamination during growth experiments may well lead to a lengthening of the list of micronutrient elements and a corresponding shortening in the list of beneficial elements. Nickel is the most recent example of such development.

8.2 SODIUM

8.2.1 General

The sodium (Na) concentration of the earth's crust is ~2.8% (w/w) compared with 2.6% (w/w) for K. In temperate regions, the Na concentration in the soil solution is on average 0.1–1 mM, thus similar to, or higher than, the K concentration. In semi-arid and arid regions, particularly under irrigation, concentrations of 50 to 100 mM Na^+ (mostly as NaCl) in the soil solution are typical and may have a detrimental effect on the growth of most crop plants (Section 17.6). The hydrated sodium ion (Na^+) has a radius of 0.358 nm, whereas that of the potassium ion (K^+) is 0.331 nm. Most higher plants have developed high selectivity in the uptake of K compared to Na, and this is particularly obvious in transport to the shoot (Chapter 3). Plant species are characterized as *natrophilic* or *natrophobic,* depending on their growth response to Na and their differential capacity to take up Na by roots and transport it to shoots (e.g., Phillips *et al.*, 2000). The differences in capacity of Na uptake and long-distance transport are large among plant species as well as genotypes within a species. Genotypic differences in uptake by roots are related to factors such as (i) differential activity/capacity of Na efflux pumps (e.g., Flowers and Hadjibagheri, 2001; Aktas *et al.*, 2006; Guo *et al.*, 2009a) (Chapter 2), (ii) passive Na permeability of the root plasma membranes

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(Schubert and Läuchli, 1990) and (iii) xylem loading of Na (i.e., root-to-shoot transport) (Davenport *et al*., 2005), but presumably not to differences in response of the root plasma membrane-bound ATPase to Na (Mills and Hodges, 1988).

For the role of Na in nutrition of plants, three aspects are important: (i) its essentiality for certain plant species, (ii) the extent to which it can replace K functions in plants, and (iii) its growth enhancement effect.

8.2.2 Essentiality: Na as Nutrient

In 1965, it was established by Brownell that Na is an essential element, i.e. a nutrient, for the halophyte *Atriplex vesicaria*. When Na contamination in the basal nutrient solution was kept to a minimum (below 0.1 µM Na^+), plants became chlorotic and necrotic and no further growth occurred, despite a high K concentration in the plants (Table 8.1). The growth response to Na at low concentration (0.02 mM) was quite strong, although the Na tissue concentration (~1 g kg^{-1} dw) was in a range more typical for a micronutrient. At higher supply, however, the Na tissue concentration was more typical of a macronutrient, with growth responses presumably related to replacing the functions of K, such as in osmoregulation.

In further studies on various halophytes and non-halophytes (glycophytes), responses to Na similar to those shown in Table 8.1 were found in species characterized by the C4 photosynthetic pathway (Brownell and Crossland, 1972) and the CAM pathway (Brownell and Crossland, 1974). Without Na supply, all C4 species grew poorly and showed visual deficiency symptoms such as chlorosis and necrosis, or even failure to form flowers. Supply of 100 µM Na^+ enhanced growth and alleviated the visual symptoms. According to these studies and their later confirmation (Johnston *et al.*, 1988), Na may be classified as a nutrient for at least some of the C4 species in the families Amaranthaceae, Chenopodiaceae and Cyperaceae (Brownell, 1979). The amounts of Na required by these plant species are similar to those for a micronutrient rather than a macronutrient. However, the conclusion by Brownell and Crossland (1972) and Brownell (1979), that Na is essential for all higher plant species with the C4 pathway, is not correct. In these studies, C4 species maize or sugar cane have not been included; species that are typically natrophobic and have similar growth rates in the absence and presence of Na (Hewitt, 1983). According to the present knowledge, Na is essential for many, but not all C4 species, and it is not essential for C3 species. However, the literature on Na as essential and/or beneficial nutrient is relatively scarce (cf. Pilon-Smits *et al.*, 2009).

TABLE 8.1 Growth and Na and K concentrations in leaves of *Atriplex vesicaria* L. at different Na concentrations in a nutrient solution with 6 mM K

Na concentration (mM Na)	Dry weight (mg (4 plants)$^{-1}$)	Concentration in leaves (mmol kg^{-1} dw)	
		Na	K
0	86	10	2,834
0.02	398	48	4,450
0.04	581	78	2,504
0.20	771	296	2,225
1.20	1,101	1,129	1,688

From Brownell (1965).

Growth of many halophytes, whether C3 or C4 species, is enhanced by high Na concentrations in the substrate (generally, 10–100 mM Na, but up to 510 mM Na in extreme cases; Redondo-Gómez *et al.*, 2010). Growth responses of halophytes to Na reflect a high salt requirement for osmotic adjustment (Flowers and Läuchli, 1983), a process in which Na can be more suitable than K (Eshel, 1985).

8.2.3 Role in C4 Species

The principle of the C4 photosynthetic pathway is the shuttle of metabolites between mesophyll and bundle sheath cells (see also Chapter 5) and an increase in CO_2 concentration in the bundle sheath cells to optimize the Calvin cycle. This advantage of C4 plants over C3 plants becomes particularly evident at low ambient CO_2 concentrations, provided the C4 plants are supplied with Na (Fig. 8.1). In the shoots of *Amaranthus tricolor*, Na concentrations as low as 0.2 g kg^{-1} dw were needed for the high efficiency in CO_2 utilization at low ambient concentrations. However, in Na-deficient *Amaranthus tricolor,* plant growth was poor and chlorosis was severe at low ambient CO_2 concentration. Increasing ambient CO_2 concentrations enhanced growth of *Amaranthus tricolor* similarly to the C3 species tomato, and Na effects on *Amaranthus tricolor* growth or CO_2 utilization were absent.

The different growth response curves in *Amaranthus tricolor* in presence and absence of Na (Fig. 8.1) suggest that in Na-deficient C4 plants, the mechanism to concentrate CO_2 in the leaves is impaired or not operating. For the mechanism to be operative, the flow of metabolites between mesophyll and bundle sheath cells is mediated through plasmodesmata and driven by the concentration gradient of the metabolites in the cytosol:

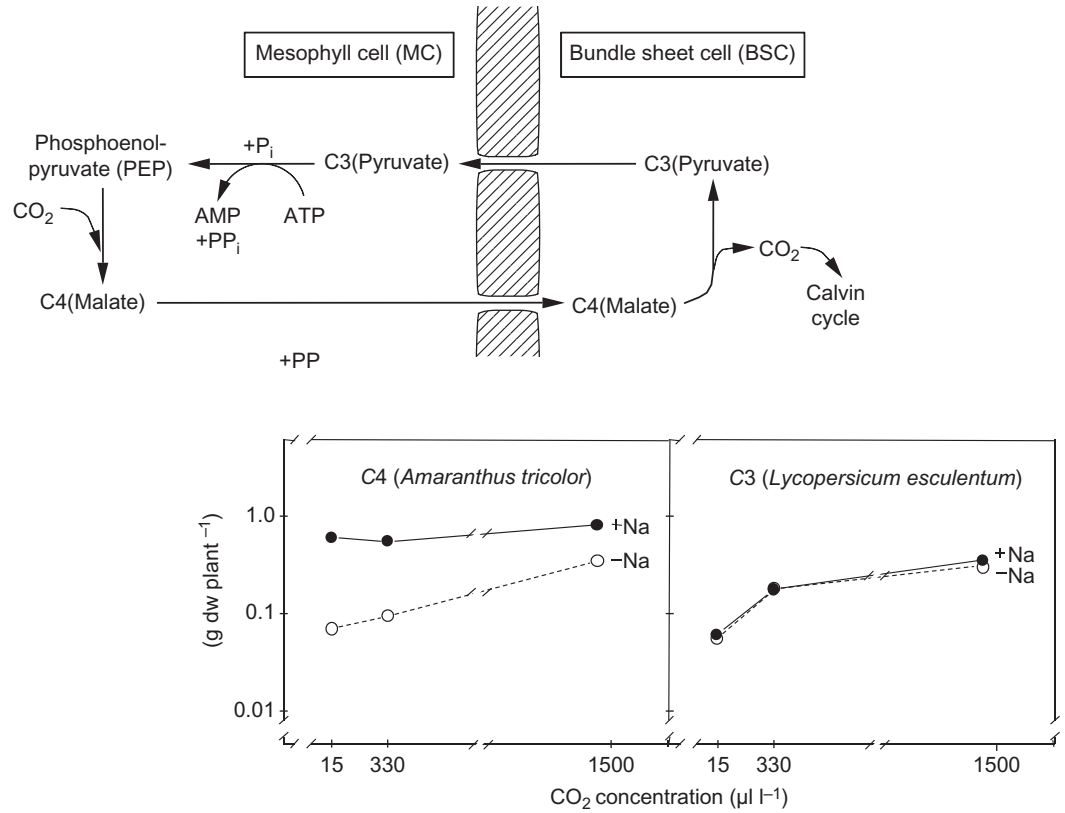


FIGURE 8.1 Growth of a C4 (*Amaranthus tricolor*) and a C3 plant (*Lycopersicum esculentum*) with increasing ambient CO_2 concentrations with and without Na. *Based on Johnston* et al. *(1984).*

Sodium deficiency particularly impairs the conversion of pyruvate to PEP, which takes place in the mesophyll chloroplasts and has a high energy requirement. Under Na deficiency in the C4 species *Amaranthus tricolor*, the C_3 metabolites alanine and pyruvate accumulated, whereas the C_4 metabolites PEP, malate and aspartate decreased (Table 8.2), suggesting that the functioning of the mesophyll chloroplasts is impaired in C4 plants under Na deficiency. In contrast, in tomato (C3 species), the concentration of these metabolites was not influenced by Na. In Na-deficient *Amaranthus tricolor* and *Kochia childsii*, the activity of the PS II in the mesophyll chloroplasts was reduced and the ultrastructure of the chloroplasts altered, whereas these parameters were not affected in the bundle sheath chloroplasts (Johnston *et al.*, 1989; Grof *et al.*, 1989). Resupplying Na restored PS II activity and changed metabolite concentrations in less than 3 days.

TABLE 8.2 Concentration of various metabolites in shoots of *Amaranthus tricolor* (C4) and *Lycopersicon esculentum* (C3) with (0.1 mM Na) or without Na supply

	A. tricolor		*L. esculentum*	
Concentration ($\mu mol\, g^{-1}$ fw)	−Na	+Na	−Na	+Na
Alanine	13.1	6.0	2.5	2.6
Pyruvate	1.7	0.9	0.1	0.1
PEPyruvate	0.9	2.3	0.2	0.2
Malate	2.7	4.8	11.3	11.3
Aspartate	1.6	3.7	1.9	1.9

Based on Johnston *et al.* (1988).

The mechanism by which Na affects metabolism and fine structure in the mesophyll chloroplasts of responsive C4 species is unclear. Protection from photo-destruction may be involved (Grof *et al.*, 1989). In C4 species, the CO_2 scavenging system and also nitrate assimilation take place in the mesophyll cells. Thus, in C4 species such as *Amaranthus tricolor*, nitrate reductase activity is very low in leaves of Na-deficient plants and can be restored in less than 2 days after resupplying Na (Ohta *et al.*, 1987). Sodium enhances nitrate uptake by the roots and nitrate assimilation in the leaves (Ohta *et al.*, 1989). Nitrate

uptake is achieved by an Na/nitrate symporter (e.g., in the marine higher plant *Zostera marina*; García-Sánchez *et al.*, 2000; Rubio *et al.*, 2005). Stimulation of nitrate reductase activity and growth enhancement by Na were absent when ammonium was provided or when nitrate combined with tungsten, an inhibitor of the nitrate reductase. Thus, in Na-deficient C4 species, particularly of the aspartate type, N deficiency may be an additional factor involved in impairment of the functioning of the C4 pathway.

A new insight into the role of Na in mesophyll chloroplasts of different types of C4 species was provided by Ohnishi and Kanai (1987) and Ohnishi *et al.* (1990) from experiments using isolated chloroplasts (Fig. 8.2). In chloroplasts of *Panicum miliaceum*, Na-enhanced pyruvate uptake had a stoichiometry of about 1:1, suggesting Na/pyruvate cotransport through the envelope into the chloroplast, driven by a light-stimulated Na efflux pump (Fig. 8.2). In contrast, such an Na effect on pyruvate uptake was absent in mesophyll chloroplasts of *Zea mays*. In C4 species of the NADP-ME type (Table 8.3), such as *Zea mays* and *Sorghum bicolor*, H^+/pyruvate rather than Na/pyruvate cotransport may operate in the envelope of mesophyll chloroplasts (Ohnishi *et al.*, 1990). This result further stresses the necessity of (i) differentiating between the various C4 metabolic types in studying the role of Na, and (ii) including species for which Na is not essential for metabolic functions in the C4 photosynthetic pathway.

8.2.4 Substitution of K by Na

The beneficial effects of Na on the growth of non-halophytes (glycophytes) are well known in agriculture and horticulture (for reviews, see, for example, Marschner, 1971; Pilon-Smits *et al.*, 2009). In general, plant species can be classified into four groups according to the differences in their growth response to Na (Fig. 8.3).

In group A, a high proportion of K is replaced by Na without a growth decline, and additional growth stimulation occurs that cannot be achieved by increasing the K concentration in plant tissues. In group B, specific growth

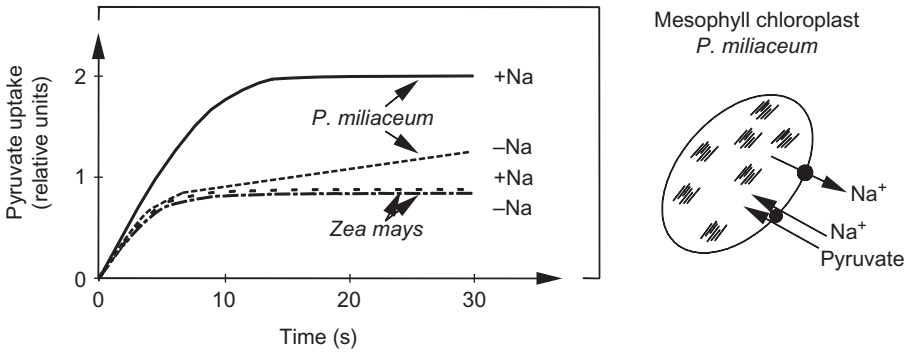


FIGURE 8.2 Pyruvate uptake into mesophyll chloroplasts of *Panicum miliaceum* (NAD^+-malic enzyme type) and *Zea mays* ($NADP^+$-malic enzyme type) and proposed Na^+/pyruvate co-transport in *P. miliaceum* with and without 1 mM NaCl. *Based on Ohnishi* et al. *(1990).*

TABLE 8.3 Variations in the biochemistry of C4 photosynthesis found in some C4 plants

Major BSC decarboxylases	Energetics of decarboxylation in BSC per CO_2	Major substrates moving from[b] MC→BSC	Major substrates moving from[b] BSC→MC	Representative species
$NADP^+$[a] ME	Production 1 NADPH	Malate	Pyruvate	*Zea mays*
				Digitaria sanguinalis
NAD^+ ME	Production 1 NADH	Aspartate	Alanine/pyruvate	*Atriplex spongiosa*
				Portulaca oleracea
PEP carboxykinase	Consumption 1 ATP	Aspartate	PEP	*Panicum maximum*
				Sporobolus poiretti

From Ray and Black (1979).
[a]*ME = malic enzyme.*
[b]*MC = mesophyll chloroplasts; BSC = bundle sheath chloroplasts.*

responses to Na are observed, but they are less distinct than in species of group A. Also, a smaller proportion of K can be replaced without decreasing growth. In group C, substitution of K can only take place to a very limited extent, and Na has no specific effect on growth. In group D, K cannot be replaced by Na. This classification is not absolute, because it does not take into account, for example, differences between cultivars within a species in the substitution of K by Na. These genotypic differences can be substantial, as has been shown in tomato (Figdore *et al.*, 1987, 1989) or cotton (Liaqat *et al.*, 2009).

The differences in the growth responses of natrophilic and natrophobic species to Na are related to differences in uptake, particularly in the translocation of Na to the shoots (Chapter 3). In pasture plants, the differential strategies for regulating Na transport to the shoots have important consequences for animal nutrition and in crop plants in general for salt tolerance (Greenway and Munns, 1980). In sugar beet (a natrophilic species), Na is readily translocated to shoots (Fig. 8.4) (see also Wakeel *et al.*, 2010), where it replaces most of the K. This substitution increased plant dry weight above that of K-deficient plants (0.05 mM K) as well as above those of plants receiving a large K supply (5.0 mM K). In contrast, the growth of K-deficient bean plants (0.5 mM K) was further depressed by Na. A lack of growth response in bean (group D species) is likely due to an *exclusion mechanism* in roots blocking Na transport to the shoots (Chapter 3). The potential for replacement of K by Na is therefore very limited (e.g., Valdez-Aguilar and Reed, 2010) or absent in group D species.

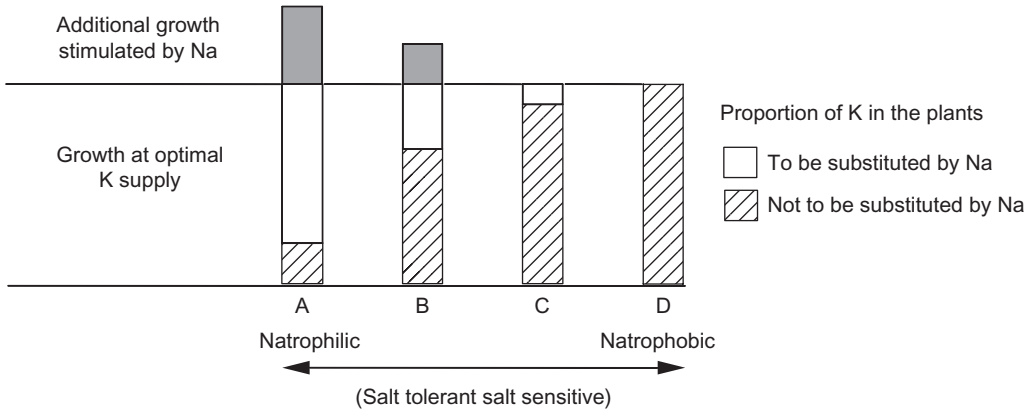


FIGURE 8.3 Tentative schematic diagram for the classification of crop plants according to the extent to which Na can be replaced by K in plants, and additional growth stimulation by Na. Group A: mainly members of Chenopodiaceae (e.g., sugar beet, table beet, turnip, Swiss chard) and many C4 grasses (e.g., Rhodes grass). Group B: cabbage, radish, cotton, pea, flax, wheat and spinach. Group C: barley, millet, rice, oat, tomato, potato and ryegrass. Group D: maize, rye, soybean, *Phaseolus* bean and timothy.

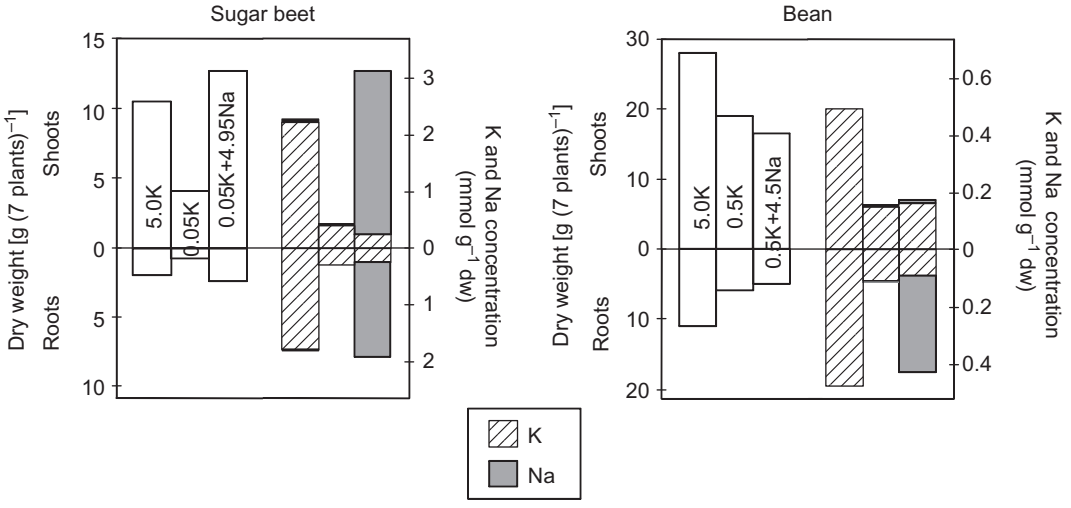


FIGURE 8.4 Dry weight and K and Na concentration of sugar beet and bean grown in nutrient solutions with different concentrations of K and N. Concentrations in mM indicated in the columns. *Based on Hawker* et al. *(1974).*

TABLE 8.4 Potassium and Na concentrations in sugar beet leaves at different K and Na concentrations in the nutrient solution

	mmol g^{-1} dw							
	Whole shoot		Old leaves		Middle leaves		Young leaves	
K and Na supply (mM)	K	Na	K	Na	K	Na	K	Na
5.0 K	3.0	<0.03	3.43	<0.03	2.36	<0.03	1.78	<0.03
0.25 K + 4.75 Na	0.24	2.72	0.18	3.05	0.34	2.01	0.52	1.75
0.10 K + 4.90 Na	0.10	3.29	0.05	4.20	0.14	2.97	0.48	1.82

From Marschner *et al.* (1981b).

Among forage grasses, ryegrass and cocksfoot are considered to be natrophilic, and timothy and kikuyu natrophobic (e.g., Smith *et al.*, 1980; Phillips *et al.*, 1999, 2000; Grieve *et al.*, 2004). Hence, Na fertilization has positive effects on growth and nutritional quality of ryegrass, but not timothy (Huhtanen *et al.*, 2000).

The majority of agriculturally important crops are natrophobic (i.e., *excluders*) (groups C and D; Fig. 8.1) and have a low salt tolerance. In contrast, natrophilic species, especially those in group A, have a moderate to high salt tolerance and are *includers*. Under saline conditions, they accumulate Na in the shoots, where it is utilized in the vacuoles of leaf cells for osmotic adjustment (e.g., Flowers and Läuchli, 1983; Gonzales *et al.*, 2002; see also Section 17.6). An interesting exception is *Populus euphratica* which achieves osmotic adjustment by accumulating Na in the apoplast rather than the vacuoles (Ottow *et al.*, 2005). Parasitic plants (e.g., *Cuscuta attenuata*) are also *includers*, because they require high internal Na concentrations as osmoticum to aid in water and nutrient extraction from the host plants (Kelly and Horning, 1999).

Even in natrophilic species, substitution of K by Na in the shoots is limited. The extent of substitution differs among individual organs and cell compartments, being large in the vacuoles, but limited in the cytoplasm (Leigh *et al.*, 1986). In tomato, for example, replacement of K by Na takes place mainly in the petioles of expanded leaves (Besford, 1978a). In sugar beet, the substitution can be high in mature leaves, but lower in expanding leaves (Lindhauer *et al.*, 1990), leading to an opposite gradient in the K/Na ratios of leaves of different age (Table 8.4). Hence, average values for substitution in the whole shoot are misleading and underestimate the essentiality of K for growth and metabolism.

In old leaves, nearly all K can be replaced by Na for specific functions in meristematic and expanding tissues. In contrast, in young expanding leaves there is a threshold level of substitution of ~0.5 mmol K g^{-1} dw (Table 8.4), which corresponds to a concentration of ~50 mM K kg^{-1} fw, and 100–150 mM K required in the cytoplasm (Leigh *et al.*, 1986).

In natrophobic species such as maize and bean, there is an absolute requirement for K in most of its metabolic functions (Section 6.6). Replacement of K by Na may occur to some extent in the root vacuoles, whereas such substitution in the cytoplasm causes substantial changes in the fine structure of the cytoplasm and its organelles (Hecht-Buchholz *et al.*, 1971).

8.2.5 Growth Stimulation by Na

In addition to K substitution, growth stimulation by Na is of practical and scientific interest. It raises the possibility of applying inexpensive, low-grade potash fertilizers with a high proportion of Na, and it increases the potential of selecting and breeding for crop plants adapted to saline soils.

Responses to Na differ not only among plant species, but also among genotypes of a species, as shown in Table 8.5. Compared with the effect of K supply only, substitution of half the K in the substrate by Na led to an increase in the plant dry weight and the amount of sucrose in the storage root in all three sugar beet genotypes. When 95% of the K in the substrate (and ~90% in the plants) was replaced by Na, plant dry weight was not affected further; the amount of sucrose per storage root was enhanced in one genotype (Fia) and severely reduced in the other two genotypes (Monohill and Ada). The decrease in sucrose amount per storage root in Monohill was due to a lower sucrose concentration (Table 8.5) and in Ada can be explained by an increase in shoot growth at the expense of storage root growth (Marschner *et al.*, 1981b), an effect which is typical in sugar beet at high Na and low K supply (Lindhauer *et al.*, 1990). Salt tolerance differed among the three genotypes, in agreement with the general pattern of classification (Fig. 8.3). At 150 mM NaCl in the external medium, growth of genotype Fia was not affected, whereas growth was severely depressed in the other two genotypes (Marschner *et al.*, 1981a).

TABLE 8.5 Genotypic differences in sucrose concentration and amount in storage roots of different sugar beet cultivars at different K and Na concentrations in the nutrient solution

	Treatment (mM)			Sucrose in storage root	
Genotype	K	Na	Dry weight (mg plant^{-1})	Concentration (g kg^{-1} fw)	Content (g root^{-1})
Monohill	5.0	0	115	92	54
	2.5	2.5	133	119	50
	0.25	4.75	126	76	34
Ada	5.0	0	86	49	19
	2.5	2.5	131	71	43
	0.25	4.75	132	77	21
Fia	5.0	0	44	100	14
	2.5	2.5	65	104	20
	0.25	4.75	84	112	28

From Marschner *et al.* (1981b).

Growth stimulation by Na is caused mainly by its effect on cell expansion and on plant water balance. Sodium can replace K in its contribution to the solute potential in the vacuoles and consequently in the generation of turgor and cell expansion (Section 6.6) and may even surpass K in this respect because it accumulates preferentially in the vacuoles (Jeschke, 1977; Nunes *et al.*, 1984). The superiority of Na can be demonstrated by the expansion of sugar beet leaf segments *in vitro* (Marschner and Possingham, 1975), and in intact sugar beet plants, where leaf area, thickness and succulence are greater when a high proportion of K is replaced by Na (Milford *et al.*, 1977). An example of this effect is shown in Table 8.6. With a large proportion of K replaced by Na, the leaves are more succulent, thicker and store more water per unit leaf area. Succulence is a morphological adaptation that is usually observed in salt-tolerant species growing in saline substrates (Jennings, 1976; Ottow *et al.*, 2005) and is considered an important buffer mechanism against deleterious changes in leaf water potential at moderate drought stress. Better osmotic adjustment by Na compared with K is also a major factor in growth stimulation of halophytes by high Na supply (Flowers and Läuchli, 1983).

High Na supply increases the leaf area and also the number of stomata per unit leaf area (Table 8.7) whereas it decreases the chlorophyll concentration (also, for example, in maize, Turan *et al.*, 2009). The latter may explain the lower rate of net photosynthesis per unit leaf area. Therefore, the higher growth rates of sugar beet plants at high Na but low K supply are not due to increased photosynthetic efficiency, but rather to a larger leaf area (Lawlor and Milford, 1973).

TABLE 8.6 Leaf properties and K and Na concentrations in sugar beet at different K and Na concentrations in the nutrient solution

	Treatment (mM)	
	5.0 K	0.25 K + 4.75 Na
Leaf area (cm^2 leaf^{-1})	233	302
Leaf thickness (μm)	274	319
Succulence (g H_2O dm^{-1})	3.1	3.7
Leaf dry weight (g plant^{-1})	7.6	9.7
Concentrations in leaves (mmol g^{-1} dw)		
K	2.67	0.43
Na	0.03	2.45

Based on Hampe and Marschner (1982).

When the availability of water in the substrate is high, Na increases the water consumption per unit fresh weight increment in sugar beet (Table 8.7), thus decreasing the water use efficiency as has also been observed in many other species (e.g., cauliflower; Sharma and Singh, 1990; tomato; Al-Karaki, 2000). However, Na improves the water balance of plants when the water supply is limited via stomatal regulation (Fig. 8.5). With a sudden decrease in the availability of water in the substrate (*drought stress*), the stomata of plants supplied with Na closed more rapidly than those of plants supplied with K only; after stress removal, opening of stomata of the K-supplied plants was

delayed compared to the plants supplied with Na. Thus, in plants supplied with Na, the relative leaf water content remained higher, even at low substrate water availability (drought periods, saline soils). Replacement of K by Na in its role in stomatal opening has been shown in epidermal strips of *Commelina* species (Raghavendra *et al.*, 1976; Perera *et al.*, 1997), but K remains crucial in regulating stomatal opening in some halophytes (e.g., *Aster tripolium*; Perera *et al*., 1997).

The replacement at the cellular level of high proportion of K by Na may also affect the activity of enzymes that particularly respond to K (Section 6.6). For example, K is four times more effective than Na in activating starch synthase that catalyses the conversion of ADP-glucose into starch (Hawker *et al.*, 1974). Thus, in leaves in which a high proportion of K is replaced by Na, the starch concentration is lower, whereas the concentration of soluble carbohydrates, particularly sucrose (Hawker *et al.*, 1974) or maltose (Kempa *et al.*, 2008) is higher. This shift in carbohydrate metabolism may favour cell expansion in the leaf tissue. Furthermore, Na is more effective than K in stimulating sucrose accumulation in the storage tissue of sugar beet. The effect of Na on sucrose storage appears to be related to stimulation of ATPase activity at the tonoplast of beet storage cells (Willenbrink, 1983). The existence of ATPases that require the presence of both K and Na for maximal activity is well documented in roots of natrophilic species (Kylin and Hansson, 1971).

TABLE 8.7 Properties of sugar beet leaves and water consumption at different K and Na concentrations and at different osmotic potential (± mannitol) of the nutrient solution

		Treatment (mM)	
		5.0 K	**0.25 K + 4.75 Na**
Stomata number on lower leaf surface (no. cm^{-2})		11,807	15,127
Chlorophyll concentration ($mg\,g^{-1}\,dw$)		12.1	9.2
Net photosynthesis ($mg\,CO_2\,cm^{-2}\,h^{-1}$)		15.2	14.4
Water consumption ($g\,H_2O\,g^{-1}$ fw increment)			
Osmotic potential of the nutrient solution MPa):	−0.02	17.7	26.5
	−0.40	28.2	24.6

Based on Hampe and Marschner (1982).

8.2.6 Application of Na Fertilizers

Given the genotypical differences in growth response to Na and the abundance of Na in the biosphere, one can expect the application of Na to have beneficial effects (i) in natrophilic plant species, (ii) when the concentrations of available K and/or Na are low, and (iii) in areas with irregular rainfall and/or transient drought during the growing season. In addition, Na fertilization and substitution of K may be important in soils that are highly K fixing (Wakeel *et al.*, 2010).

The application of Na fertilizers to sugar beet results in an increase in the leaf area index early in the growing season and thus an increase in light interception, improving water use efficiency of leaves under conditions of moderate drought stress during the growing season (Durrant *et al.*, 1978). The potential replacement of K by Na can be taken into account when applying fertilizers to natrophilic species. When Na concentrations in leaves are high, the leaf K concentrations required for optimal growth decrease from 35 to $8\,g\,kg^{-1}\,dw$ in Italian ryegrass (Hylton *et al.*, 1967)

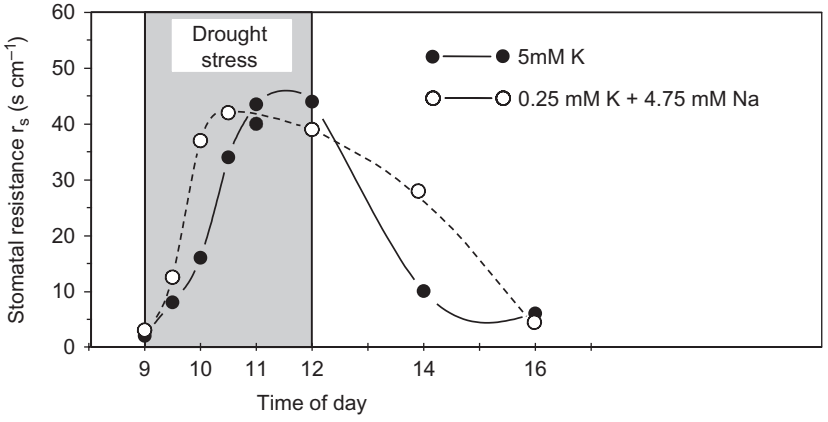


FIGURE 8.5 Stomata resistance to water vapour exchange in leaves of sugar beet with transient drought stress (decrease in solution water potential to −0.75 MPa by the addition of mannitol). *Based on Hampe and Marschner (1982).*

and from 27 to 5 g kg^{-1} in Rhodes grass (Smith, 1974), or 43 to 10 g kg^{-1} in lettuce (Costigan and Mead, 1987).

The Na concentration of forage and pasture plants is an important factor in animal nutrition. The Na requirement for lactating dairy cows is ~2.0 g kg^{-1} dw of the forage (Smith *et al.*, 1978; Zehler, 1981), which is higher than the average Na concentration of natrophobic pasture species (Smith *et al.*, 1980; Phillips *et al.*, 2000). In contrast, the K concentration in these natrophobic species is usually at least adequate, but often in excess of animal needs, which is in the range of 20 to 25 g kg^{-1} dw. The use of Na fertilizer to increase the Na concentration of forage and pasture plants is thus important in large areas of the world. Furthermore, a high Na concentration increases the acceptability of forage to animals and enhances daily food intake (Zehler, 1981). However, Na fertilizers are effective only when applied to grassland or mixed pastures with a reasonably high proportion of natrophilic species (Phillips *et al.*, 2000).

8.3 SILICON

8.3.1 General

Silicon (Si) is the second most abundant element in the earth's crust. In soil solution at pH below 9.0, the prevailing form is monosilicic acid, $Si(OH)_4$, an uncharged form, with a solubility in water (at 25°C) of ~2 mM (equivalent to 56 mg Si L^{-1}) (Fig. 8.6). On average, the concentration in the soil solution is 14 to 20 mg Si L^{-1} (with a range between 3.5 and 40 mg) with a tendency to lower concentrations at high pH (>7) and when large amounts of sesquioxides are present in soils and anion adsorption is dominant (Jones and Handreck, 1965). Such conditions are widespread in highly weathered tropical soils. Concentrations of Si in aqueous solutions higher than 56 mg Si L^{-1} indicate either supersaturation of $Si(OH)_4$ or partial polymerization of monosilicic acid.

Silicic acid, $Si(OH)_4$, has a number of similarities with boric acid $B(OH)_3$; both are very weak acids in aqueous solutions, interact with pectins and polyphenols in the cell walls, and are mainly located in the cell walls. In contrast to B, the essentiality of Si for higher plants has been demonstrated so far only in a few plant species, but it is beneficial for many species and, under certain circumstances, for most higher plants.

$$nSiO_2 + nH_2O \underset{>2\ mM}{\overset{<2\ mM}{\rightleftharpoons}} nSi(OH)_4 \underset{<pH\ 9}{\overset{>pH\ 9}{\rightleftharpoons}} n(OH)_3\,SiO^{-1} + nH^+$$

FIGURE 8.6 Forms of Si at different concentrations and pH values.

8.3.2 Uptake, Concentration and Distribution

All plants grown in soil will contain some Si in their tissues. However, the Si concentration in the shoots varies considerably among plant species, ranging from 1 to 100 mg Si g^{-1} dw (Epstein, 1999; Ma and Takahashi, 2002; Table 8.8). Grown under the same conditions, rice contains 39.1 mg Si g^{-1} in the shoots, but chickpea contains only 3.0 mg Si g^{-1} (Table 8.8). In general, plants belonging to Bryophyta, Lycopsida, and Equisetopsida in Pteridophyta show high Si accumulation, whereas those belonging to Filicopsida in Pteridophyta, Gymnospermae, and Angiospermae show low Si accumulation. In higher plants, some families accumulate high to moderate amounts of Si with Si concentrations varying between >40 mg kg^{-1} in Gramineae, Cyperacea and Balsaminaceae and 20–40 mg kg^{-1} in Cucurbitales, Urticales, and Commelinaceae, whereas most other plants species show low Si accumulation (Ma and Takahashi, 2002; Hodson *et al.*, 2005). The differences in Si accumulation between species can be attributed to differential ability of roots to take up Si (Ma and Takahashi, 2002).

Plant roots take up Si in the form of silicic acid ($Si(OH)_4$). There are three different modes for Si uptake; active, passive and rejective uptake, depending on plant species. Recently, transporters involved in active Si uptake have been identified in Si-accumulating species including rice, barley and maize.

Lsi1 from rice is the first Si transporter identified in higher plants (Ma *et al.*, 2006). Lsi1 belongs to a Nod26-like major intrinsic protein (NIP) subfamily of aquaporin-like proteins and shows influx activity for silicic acid in *Xenopus* oocytes. The predicted amino acid sequence has six transmembrane domains and two Asn-Pro-Ala (NPA) motifs, which is well conserved in typical aquaporins. *Lsi1* is constitutively expressed in the roots, but its expression is decreased to 25% when Si is added. Within the root, the

TABLE 8.8 Si concentration in different plant species grown under the same conditions

Plant species	Si concentration (mg g^{-1} dw)
Rice	39.1
Wheat	15.4
Pumpkin	13.4
Zucchini	19.8
Chickpea	3.0
Cucumber	22.9
Maize	21.0

expression of *Lsi1* and Si uptake are lower in the root tip region between 0 and 10 mm (comprising the apical meristem and the elongation zone) than in the basal regions of the root (>10 mm) (Yamaji and Ma, 2007). Therefore, Si uptake occurs in the mature regions of the roots rather than in the root tips. In the roots including seminal, lateral and crown roots, the Lsi1 protein is localized to the plasma membrane of both exodermis and endodermis, where the Casparian strips prevent apoplastic transport into the root stele.

On the other hand, Lsi2 is an efflux transporter of Si in rice (Ma *et al*., 2006). Lsi2 belongs to a putative anion transporter without any similarity with the Si influx transporter Lsi1. The expression pattern and tissue and cellular localization of Lsi2 is the same as that of Lsi1. Transport of Si by Lsi2 is driven by the proton gradient (Ma *et al*., 2006). Both Lsi1 and Lsi2 are important for Si uptake; knockout of either of them results in a significant decrease in Si uptake.

Similar transporters of Lsi1 and Lsi2 have also been identified in barley and maize (Mitani *et al*., 2009a, 2011; Chiba *et al*., 2009). However, their localization and expression patterns are different from those in rice. HvLsi1 from barley and ZmLsi1 from maize are localized in epidermal, hypodermal and cortical cells. Furthermore, the expression levels of both *HvLsi1* and *ZmLsi1* are unaffected by Si. These differences result in differential pathways of Si from the external solution to the xylem between barley and maize and rice. In barley and maize, Si can be taken up from external solution (soil solution) by HvLsi1/ZmLsi1 by epidermal, hypodermal and cortical cells and then transported in the symplasm to the endodermis where it is released to the stele by HvLsi2/ZmLsi2. In contrast, in rice, Si is only taken up by the exodermal cells by OsLsi1, then Si is released into the apoplasm and transported into the stele by both OsLsi1 and OsLsi2 at the endodermal cells.

Following uptake by the roots through Lsi1 and Lsi2, Si is translocated to the shoot in the xylem. More than 90% of Si taken up by the roots is translocated to the shoots. In rice, the Si concentration in the xylem sap can be as high as 20 mM. In the xylem sap is present as monosilicic acid (Casey *et al*., 2003; Mitani *et al*., 2005). Such very high concentrations are probably only present transiently because silicic acid polymerizes into silica gel ($SiO_2 \cdot H_2O$) when the concentration of silicic acid exceeds 2 mM *in vitro* (Mitani *et al*., 2005).

Relatively large amounts of Si are deposited in the cell walls of xylem vessels (Balasta *et al.*, 1989) where it may prevent compression of the vessels when the transpiration rates are high (Raven, 1983). A transporter, Lsi6, is responsible for this process (Yamaji and Ma, 2009). Lsi6 is a homologue of Lsi1 and also shows transport activity for silicic acid. However, in contrast to Lsi1 and Lsi2, Lsi6 is also expressed in the leaf sheaths and leaf blades. Knockout of Lsi6 does not affect the uptake of Si by the roots, but affects Si deposition pattern in the leaf blades and sheaths and causes increased excretion of Si in the guttation fluid (Yamaji *et al*., 2009).

At the reproduction stage, Lsi6 is also highly expressed in node I below the panicles where it is mainly localized at the xylem transfer cells located at the outer boundary region of the enlarged large vascular bundles in node I. Therefore, Lsi6 appears to be a transporter involved in transfer of Si from the large vascular bundles coming from the roots to the diffuse vascular bundles connected to the panicles. Knockout of Lsi6 results in decreased Si accumulation in the panicles, but increased Si accumulation in the flag leaf.

Due to the transport of Si from roots to shoots via the xylem, the distribution of Si within the shoot and shoot organs is determined by the transpiration rate of the organ (Jones and Handreck, 1969) and for a given organ such as a leaf, depends on leaf age. Most of the Si remains in the apoplasm and is deposited after water evaporation as amorphous silica ($SiO_2\ nH_2O$) at the termini of the transpiration stream, mainly the outer walls of the epidermal cells on both surfaces of the leaves as well as in the inflorescence bracts of graminaceous species (Hodson and Sangster, 1989b; Ma and Takahashi, 2002) and trichomes (Lanning and Eleuterius, 1989). The epidermal cell walls are impregnated with a layer of Si and become effective barriers against water loss by cuticular transpiration and fungal infections (Chapter 10). In grasses, a considerable proportion of Si in the epidermis of both leaf surfaces is also located intracellularly, in so-called *silica cells* (Sangster, 1970), 'bulliform' cells (Takeoka *et al.*, 1984) or phytoliths.

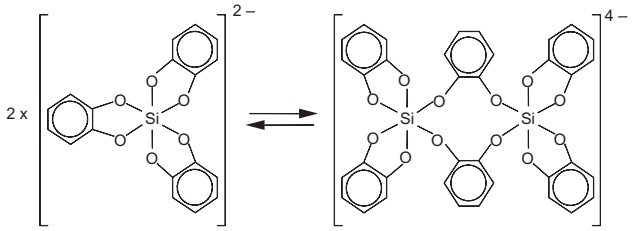
The deposition of Si in hairs on leaves, culms, inflorescence bracts and brush hairs of cereal grains such as wheat pose a potential threat to human health (Hodson and Sangster, 1989b). The inflorescence bracts of grasses of the genus *Phalaris* and foxtail millet (*Setaria italica*) contain sharp, elongated siliceous fibres which fall into the critical size range of fibres that have been classified as carcinogenic (Sangster *et al.*, 1983). The occurrence of oesophageal cancer is correlated with the consumption of either foxtail millet in north China (Parry and Hodson, 1982), or of wheat contaminated with *Phalaris* in the Middle East (Sangster *et al.*, 1983).

8.3.3 Role in Metabolism

The essentiality of Si in unicellular organisms such as diatoms is well documented, and many details of its metabolic functions in these organisms are known (Werner and Roth, 1983). In higher plants, the essentiality of Si is reasonably well established for *silicophile* species

such as *Equisetum arvense* (Chen and Lewin, 1969) and certain wetland grass species (Takahashi and Miyake, 1977). In Si-deficient lowland rice, vegetative growth and grain production are reduced and deficiency symptoms, such as necrosis on mature leaves and wilting of plants, may occur (Lewin and Reimann, 1969), suggesting, but not proving, that Si is essential for the growth of rice. However, failure to complete the life cycle has not yet been demonstrated. Because of its abundance in the biosphere, the essentiality of Si as a micronutrient for higher plants is very difficult to prove. Even highly purified water contains 2×10^{-5} mM Si (Werner and Roth, 1983), and the leaves of Si accumulator plants that were subjected to a so-called no-Si treatment usually contain between 1 and 4 mg SiO_2 g^{-1} leaf dw.

There have been only a few in-depth studies on metabolic changes in higher plants when Si is omitted from the external solution or with addition of a specific inhibitor of Si metabolism, germanic acid (Werner, 1967). In the absence of Si, the incorporation of inorganic phosphate into ATP, ADP and sugar phosphates is reduced in sugar cane (Wong You Cheong and Chan, 1973); in wheat root cell walls, the proportion of lignin declines and that of phenolic compounds increases (Jones *et al.*, 1978). This latter aspect deserves particular attention for various reasons. Some of the cell wall-bound Si is presumably present as an ester-like derivative of silicic acid (R1-O-Si-O-R2), acting as a bridge in the structural organization of polyuronides (Jones, 1978). Furthermore, Si seems to influence the concentration and metabolism of polyphenols in xylem cell walls (Parry and Kelso, 1975). As shown by Weiss and Herzog (1978) silicic acid, like boric acid, has a high affinity for *o*-diphenols such as caffeic acid and corresponding esters, forming mono-, di- and polymeric Si complexes of high stability and low solubility:



Silicon may therefore affect the stability of higher plants, as an inert deposition in lignified cell walls and also by modulating lignin biosynthesis. As stressed by Raven (1983), Si as a structural material requires less energy than lignin. About 2 g of glucose are necessary for the synthesis of 1 g of lignin; the ratio of the energy requirement for lignin to that of Si is 20:1.

Silicon not only contributes to cell wall rigidity and strengthening but may also increase cell wall elasticity during extension growth. In the primary cell walls, Si interacts with cell wall constituents such as pectins and polyphenols, and these cross-links increase cell wall elasticity during extension growth (Emadian and Newton, 1989). This is similar to the role of Si in cotton fibre growth. During the early phase of elongation growth, the Si concentration of cotton fibre is fairly high (5 mg Si g^{-1} dw) and decreases with secondary wall thickening, i.e. cellulose deposition (Boylston, 1988). The highest Si concentration has been found in cotton varieties with long fine fibres (Boylston *et al.*, 1990). This effect of Si in the primary cell walls is opposite to what is usually observed, for example, in leaves when large amounts of Si are incorporated into secondary cell walls, but it has similarities with the function of B in cell walls (Section 7.7). The relative importance of B and Si in primary cell walls may depend upon plant species (Loomis and Durst, 1992). Graminaceous and dicotyledenous species differ strongly in their cell wall composition and in their B requirement which is negatively related to their capacity of Si uptake and growth responses to Si supply.

8.3.4 Beneficial Effects

Silicon has a number of other, well-documented and readily visible and/or measurable beneficial effects. Under field conditions, particularly in dense stands of cereals, Si can stimulate growth and yield directly and indirectly (Ma and Takahashi, 2002). These include decreasing mutual shading by improving leaf erectness and alleviating abiotic and biotic stresses.

Leaf erectness is an important factor affecting light interception in dense plant stands. For a given cultivar, leaf erectness decreases with increasing N supply (Section 6.1). Silicon increases leaf erectness and thus to a large extent counteracts the negative effects of high N supply on light interception. Similarly, Si counteracts the negative effects of an increasing N supply on haulm stability and lodging susceptibility (Idris *et al.*, 1975).

Silicon enhances the resistance of plants to diseases caused by both fungi and bacteria (see also Chapter 10). In rice, Si reduces the severity of both leaf and panicle blast. In soil deficient in Si, application of silicate fertilizer is as effective as fungicide application in controlling rice blast (Datnoff *et al*., 1997). Si also decreases the incidence of powdery mildew in cucumber, barley and wheat; sheath blight in rice, ring spot in sugar cane, rust in cowpea, leaf spot in bermuda grass and grey leaf spot in St Augustine grass and perennial ryegrass (Fauteux *et al*., 2005).

Silicon also suppresses pests such as stem borer and various hoppers, leaf spiders and mites (Savant *et al*.,

1997). In a field study, there was a positive relationship between the Si concentration of rice and resistance to the brown plant hopper (Sujatha *et al*., 1987).

Two mechanisms for Si-enhanced resistance to diseases and pests have been proposed. One is that Si acts as a physical barrier. Silicon is deposited beneath the cuticle to form a cuticle-Si double layer (Fauteux *et al*., 2005; Ma and Yamaji, 2006). This layer can mechanically impede penetration by fungi and pest, thereby inhibiting infection. However, according to Heine *et al.* (2007), inhibition of infection and spread of *Phytium aphanidermatum* in roots of tomato plants by Si is related to symplasmic Si, not apoplasmic Si. Another mechanism is that soluble Si acts as a modulator of host resistance to pathogens. Several studies in monocotyledonous (rice and wheat) and dicotyledonous (cucumber) have shown that plants supplied with Si produce phenolics, lignin, H_2O_2 and phytoalexins in response to fungal infection (Belanger *et al*., 2003; Remus-Borel *et al*., 2005; Rodrigues *et al*., 2004; Sun *et al.*, 2010b). Further studies are required for a better understanding and characterization of the physiological effects of Si in biological systems.

Silicon alleviates various abiotic stresses including physical stress (lodging, drought, radiation, high and low temperature, freezing, UV irradiation) and chemical stress (salt, metal toxicity, nutrient imbalance) (Ma, 2004, 2005; Ma and Yamaji, 2006).

The beneficial effect of Si on alleviation of UV stress in rice may be related to biosynthesis of phenolic compounds (Goto *et al*., 2003). Silicon can alleviate water stress by decreasing transpiration in rice (Ma *et al*., 2001a). Transpiration from the leaves occurs mainly through the stomata and partly through the cuticle. As Si is deposited beneath the cuticle of the leaves, transpiration through the cuticle may decrease.

Silicon application in rice is effective in alleviating the damage caused by climatic stress such as strong wind, low temperature and insufficient sunshine during the summer season (Ma *et al*., 2001a). Strong winds can cause lodging and sterility in rice, resulting in a considerable reduction in rice yield. Deposition of Si in rice enhances the strength of the stem by increasing the thickness of the culm wall and the size of the vascular bundles (Shimoyama, 1958), thereby preventing lodging. Strong winds also cause excess water loss from the spikelets, resulting in sterility. Silicon deposited on the hull is effective in preventing excess water loss.

The beneficial effects of Si under P deficiency stress have been observed in many plants including rice and barley (Ma and Takahashi, 1989). This effect may be attributed to the enhanced availability of internal P through a decrease of excess Fe and Mn uptake. Silicon can also alleviate the damage by very high concentrations of P by reducing P uptake and/or the Si-induced decrease in transpiration. The positive effect of Si on rice yield under high N fertilizer application is due to decreased lodging, mutual shading and susceptibility to diseases.

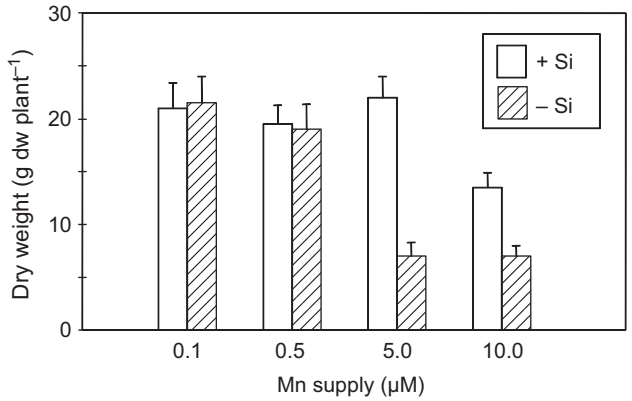


FIGURE 8.7 Dry weight of beans with (1.6 mg L^{-1}) or without Si at different Mn supply. *Modified from Horst and Marschner (1978a).*

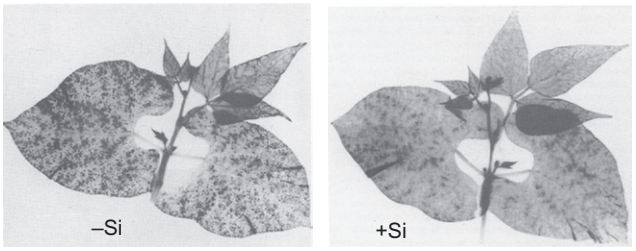


FIGURE 8.8 Autoradiograph showing the effect of Si (0.75 mg SiO_2 L^{-1}) on ^{54}Mn distribution in bean leaves supplied with 0.1 mM ^{54}Mn for 6 days. Mn concentration of the primary leaves: –Si: 22 μg g^{-1} dw and + Si: 17 μg g^{-1} dw. *Horst and Marschner, 1978a With kind permission from Springer Science+Business Media.*

Silicon may also alleviate Mn toxicity in hydroponically cultured rice (Okuda and Takahashi, 1962), barley (Williams and Vlamis, 1957; Horiguchi and Morita, 1987), bean (Fig. 8.7) and pumpkin (Iwasaki and Matsumura, 1999). Three different mechanisms seem to be involved, depending on the plant species. In rice, Si reduced Mn uptake by promoting the Mn oxidizing power of the roots (Okuda and Takahashi, 1962). In bean (Horst and Marschner, 1978a) and barley (Williams and Vlamis, 1957), Si did not reduce the Mn uptake, but led to a homogeneous distribution of Mn in the leaf blade (Fig. 8.8). The mechanism for this homogeneous distribution is unclear, but may be related to the Si-induced larger binding capacity of the cell wall, resulting in decreased apoplasmic Mn concentration in cowpea (Horst *et al*., 1999). Alleviation of Mn toxicity damage in plant cells by Si supply may also be related to stimulation of antioxidative defence systems against oxidative cell damage by ROS (Inal *et al.*, 2009)

Silicon was also effective in alleviating toxicity of other metals including Fe, Al, Cd and Zn which can be attributed to the interaction between Si and metals in the

apoplasm or symplasm. A beneficial effect of Si under salt stress has been observed in rice (Matoh *et al*., 1986; Yeo *et al*., 1999; Gong *et al*., 2006), wheat (Ahmad *et al*., 1992) and tomato (Romero-Aranda *et al*., 2006). This beneficial effect of Si may be due to the Si-induced decrease of transpiration (Matoh *et al*., 1986) and to the partial blockage of the transpirational bypass flow, the pathway by which a large proportion of the uptake of Na in rice occurs (Yeo *et al*., 1999). However, the inhibitory effects of Si on Na accumulation and salt damage in rice plants have been found to be related to Si deposition in roots, not directly to transpirational flow. Transport of K in the xylem is not affected by Si application, whereas the Na concentration in the xylem sap is reduced from 6.2 to 2.8 mM in rice plants which may be explained by inhibited apolasmic transport of Na across the root (Gong *et al.*, 2006).

Silicon is particularly important for growth and high production of rice, which can accumulate Si to over 10% of the dry weight in the shoots. When Si concentrations are insufficient, the yield is reduced due to decreased fertility (Okuda and Takahashi, 1965; Tamai and Ma, 2009). For this reason, Si fertilizers are applied in paddy fields in some countries. Sugar cane is also a Si accumulator which strongly responds to Si application. Under field conditions, at least 1% Si is required for optimal cane yield and the yield is reduced by 50% at 0.25% Si (Andersen, 1991). Such drastic yield reductions are associated with typical visible deficiency symptoms ('leaf freckling') on leaf blades directly exposed to full sunlight (Elawad *et al.*, 1982a, b).

Silicon is an essential element for animals (Nielsen, 1984), where it is a constituent of certain mucopolysaccharides in connective tissues (Jones, 1978). On the other hand, in grazing animals the uptake of a large amount of phytoliths may lead to excessive abrasion of the rumen wall, and dissolved Si may form secondary deposits in the kidney, thereby causing serious economic loss (Jones and Handreck, 1969).

8.4 COBALT

Cobalt (Co) is an essential element for prokaryotes (including blue-green algae) and animals, but an essential role in plants has not been demonstrated. However, Co promotes growth for some plant species, by enhancing growth of root symbionts, and it is therefore considered a key beneficial element (Pilon-Smits *et al.*, 2009). The role of Co as an essential element for animals was discovered in 1935 in field investigations of ruminant livestock production in Australia. The requirement of Co for N_2 fixation in legumes and in root nodules of non-legumes (e.g., alder) was established 25 years later (Ahmed and Evans, 1960). When *Medicago sativa* was grown under controlled environmental conditions with a minimum of Co contamination, plants dependent on N_2 fixation grew poorly and growth was strongly enhanced by Co supply; in contrast, nitrate-fed plants grew equally well without and with supply of Co (Delwiche *et al.*, 1961). Subsequently, Kliewer and Evans (1963a) isolated the cobalamin coenzyme B_{12} from root nodules of legumes and non-legumes, and demonstrated the interdependence of Co supply, the B_{12} coenzyme concentration of *Rhizobium*, the formation of leghemoglobin, and N_2 fixation (Kliewer and Evans, 1963b). Since then, it been established that *Rhizobium* and other N_2-fixing microorganisms have an absolute Co requirement whether or not they are growing within nodules and regardless of whether they are dependent on an N supply from N_2 fixation or from mineral N.

8.4.1 Role of Co in Plants

The main biological role of Co is in the coenzyme cobalamin (vitamin B_{12} and its derivatives). In cobalamin, Co is chelated to four N atoms at the centre of a porphyrin-like structure, corrin, and has a similar role to that of Fe in hemoglobin. In addition to cobalamin, several non-corrin Co-containing enzymes have also been identified in prokaryotes, but these are not necessarily Co specific (Zhang and Gladyshev, 2009; Randaccio *et al.*, 2010). Cobalamin has a complex biochemistry, and there are a number of cobalamin-dependent enzymes. There are three primary classes of cobalamin enzymes: (i) methylcobalamin-dependent methyltransferase (methionine synthase), (ii) adenosylcobalamin-dependent isomerase (methylmalonyl-CoA mutase), and (iii) B_{12}-dependent reductive dehalogenase (Zhang and Gladyshev, 2009). Cobalamin-dependent enzymes and Co-induced changes in their activities, nodulation and N_2 fixation, have been identified in *Rhizobium* (and *Bradyrhizobium*) species. For example, under Co deficiency, methionine synthesis is depressed (Table 8.9) which presumably leads to lower protein synthesis and contributes to the smaller size of the bacteroids (bacteria in the nodules capable of N_2 fixation). Furthermore, methylmalonyl-coA mutase is involved in the synthesis of heme (iron porphyrins) in the bacteroids and thus in the synthesis of leghemoglobin. Therefore Co deficiency impairs the synthesis of leghemoglobin (see also Chapter 16).

8.4.2 Co Deficiency and Toxicity

Cobalt deficiency affects nodule development and function at different levels and degrees as shown in Table 8.10 (Dilworth *et al.*, 1979). When lupins grown in a Co-deficient soil are supplied with Co, the weight and Co concentration of the nodules increases, as well as the number of bacteroids and amount of cobalamin and leghemoglobin per unit nodule fw.

TABLE 8.9 Characteristics of co-sufficient and co-deficient crown nodules of *Lupinus angustifolius* L.

Co treatment	Volume of bacteroids (μm^3)	DNA concentration ($pg\,cell^{-1}$)	Methionine (% of total amino-N)
+ Co	3.2	12	1.3
− Co	2.6	8	1.0

Based on Dilworth and Bisseling (1984).

TABLE 8.10 Nodule growth and composition in *Lupinus angustifolius* inoculated with *Rhizobium lupini* grown in a Co-deficient soil with (0.19 mg Co pot^{-1}) or without Co addition

	Co treatment	
	− Co	+ Co
Crown nodule fresh weight ($g\,plant^{-1}$)	0.1	0.6
Co content ($ng\,g^{-1}$ nodule dw)	45	105
No. bacteroids ($\times 10^9\,g^{-1}$ nodule fw)	15	27
Cobalamin ($ng\,g^{-1}$ nodule fw)	5.9	28.3
Leghemoglobin ($mg\,g^{-1}$ nodule fw)	0.7	1.9

Based on Dilworth *et al.* (1979).

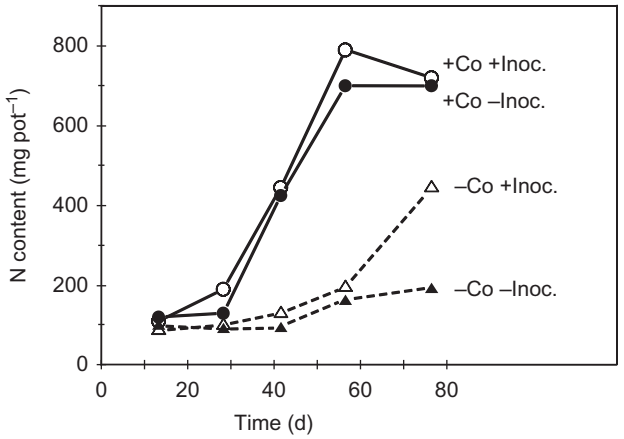


FIGURE 8.9 Time course of N accumulation in *Lupinus angustifolius* L. grown in a Co-deficient soil with or without addition of Co and with or without inoculation with Rhizobium. *Based on Dilworth* et al. *(1979).*

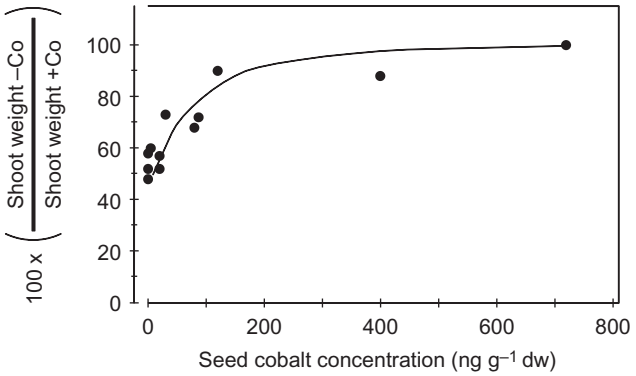


FIGURE 8.10 Relationship between cobalt concentration of seeds and response of shoot growth *of Lupinus angusfolius* L. to different extent Co. *Based on Robson and Snowball (1987).*

In legumes grown in Co-deficient soils, the nodule activity is lower in plants without Co addition. This lower activity results in reduced nitrogenase activity or N content of the plants (Fig. 8.9). Furthermore, *Rhizobium* infection is often lower than in plants supplied with Co, and the onset of N_2 fixation, as indicated by N accumulation in the plants, is delayed for several weeks. In legumes dependent on N_2 fixation, Co deficiency is therefore associated with symptoms of N deficiency (Dilworth *et al.*, 1979; Robson and Snowball, 1987). Under Co deficiency, there is a preferential accumulation of Co in the nodules. In deficient plants, the Co concentration in the nodules varies between 20 and 170 $\mu g\,g^{-1}$ nodule fw, depending on the plant species (Robson *et al.*, 1979). The Co concentration of seeds of the same species varies between plants grown in different locations, for example between 6 and 730 $ng\,g^{-1}$ in *Lupinus angustifolius* (Robson and Mead, 1980). When grown in Co-deficient soils and dependent on N_2 fixation, there is a close relationship between seed Co concentration, plant growth, N concentration and severity of the visual N deficiency symptoms (Robson and Snowball, 1987). As shown in Fig. 8.10, the shoot growth response to increasing seed Co concentrations is very strong up to about 200 $ng\,g^{-1}$ seed dw.

In large-seeded lupins, a Co concentration of ~100 ng Co g^{-1} seed dw is sufficient to prevent Co deficiency in plants grown in Co-deficient soils (Gladstones *et al.*, 1977). Treating seeds with Co is an effective procedure for supporting N_2 fixation and growth of legumes on Co-deficient soils (Reddy and Raj, 1975). Field responses to Co fertilization of nodulated legumes are rare but have been demonstrated, for example, on poor siliceous sandy soils (Ozanne *et al.*, 1963; Powrie, 1964). Foliar sprays can be effective, but less so than combining seed treatments and foliar sprays (Table 8.11). In peanut and pigeon pea, the combined seed and foliar application of Co had the strongest effect on the leghemoglobin concentration, which was 3–4-fold greater (Shiv Raj, 1987). The effectiveness of

TABLE 8.11 Peanut yield, total N concentration and number of nodules with different forms of Co application

Co treatment	Pod yield ($kg\,ha^{-1}$)	Total N at maturity ($g\,kg^{-1}$)	Nodulation (no. nodules $plant^{-1}$)
Control (−Co)	1,232	2.4	91
Seed treatment	1,687	2.6	150
Foliar spray (2×)	1,752	3.1	123
Seed treatment + foliar spray (2×)	1,844	3.4	166

Based on Reddy and Raj (1975).

foliar sprays indicates a reasonable re-translocation of Co from leaves, as has also been shown after the application of labelled Co to clover and alfalfa leaves (Handreck and Riceman, 1969). In the phloem, Co seems to be translocated largely as a negatively charged complex (Wiersma and van Goor, 1979).

Non-ruminants, including humans, have a requirement for vitamin B_{12}, but not Co. On the other hand, Co is essential for ruminants because they depend on the rumen microflora to synthesize sufficient vitamin B_{12}. Cobalt deficiency is widespread in grazing ruminants on soils low in Co (Miller *et al.*, 1991). In Co-deficient soils, Co application may therefore not only enhance the N_2 fixation of legumes, but also improve the nutritional quality of forage plants. The critical Co concentration for ruminants is about $0.07\,mg\,kg^{-1}$ dw of forage, which is higher than the critical concentration for N_2 fixation in legumes. A survey of angiosperm species has shown that leaf Co concentrations are typically $<0.20\,mg\,kg^{-1}$ with a few species having up to $0.50\,mg\,kg^{-1}$ (Watanabe *et al.*, 2007; Pilon-Smits *et al.*, 2009). However, in a small number of plant species that are highly adapted to metalliferous soils, leaf Co concentration can reach several thousand milligrams per kilogram dw (Brookes and Malaisse, 1989). These species undoubtedly have very high Co uptake rates; however, there is debate as to the role of uptake and translocation versus surface contamination in contributing to some of the highest values reported in the literature for both Co and Cu hyperaccumulators (e.g., Faucon *et al.*, 2007).

There are contradictory reports on typical critical toxicity concentrations of Co, with values varying from $0.4\,mg\,kg^{-1}$ dw in clover (Ozanne *et al.*, 1963) up to a few milligrams per kilogram dw in bean and cabbage (Bollard, 1983). In crop and pasture species, there are also genotypic differences in tolerance to high concentrations of Co in the shoots, which opens up the possibility for breeding for improved performance on Co-enriched soils.

8.5 SELENIUM

8.5.1 General

The chemistry of selenium (Se) has features in common with sulphur. Selenium, like sulphur, can exist in the −2 (selenide Se^{2-}), 0 (elemental selenium), +4 (selenite SeO_3^{2-}) and +6 (selenate SeO_4^{2-}) oxidation states. Selenium is present in soil in small amounts (typically ranging from 0.01 to $2\,mg\,kg^{-1}$); high concentrations ($>5\,mg\,kg^{-1}$) are found in seleniferous soils (Mayland *et al.*, 1989). Soil pH and Eh affect the chemical species of Se present in soil. Thermodynamic calculations show that the predominant form of selenium is selenate in alkaline and well-oxidized soils (pe + pH >15), selenite in well-drained mineral soils with pH from acidic to neutral (7.5 < pe + pH <15), and selenide under reduced soil conditions (pe + pH <7.5) (Elrashidi *et al.*, 1987).

Selenium is an essential micronutrient for animals, but the essentiality has not been established for higher plants (Terry *et al.*, 2000; Sors *et al.*, 2005b). Deficiency of Se in humans is common; it has been estimated that between 0.5 and 1 billion people worldwide may have insufficient intake of Se (Combs, 2001). Because plant-based foods are an important source of Se to humans and domestic animals, it is important to understand how plants take up and metabolize Se.

8.5.2 Uptake

Selenate is a chemical analogue of sulphate; they compete for the same transporters during root uptake and, thus, selenate uptake can be strongly decreased by high sulphate supply (Mikkelson and Wan, 1990; Zayed and Terry, 1992). The affinity constants (K_m) for sulphate and selenate uptake into barley roots were found to be similar, 19 and 15 μM, respectively (Leggett and Epstein, 1956). Selenate also competitively inhibits sulphate uptake from nutrient solutions; but this inhibition is unlikely to be significant in soil-grown plants because the concentration of selenate in soil solution is lower than that of sulphate. Recent studies have identified a number of selenate-resistant mutants of *Arbidopsis thaliana*; the phenotype is caused by a mutation in the high-affinity sulphate transporter Sultr1;2 resulting in decreased uptake of both sulphate and selenate (Shibagaki *et al.*, 2002; El Kassis *et al.*, 2007). Sultr1;2 is localized in the root tip, root cortex and lateral roots, and its expression is enhanced by S deficiency. This transporter represents the major entry route for sulphate and selenate into the roots of *Arabidopsis thaliana* (Shibagaki *et al.*, 2002; El Kassis *et al.*, 2007; Barberon *et al.*, 2008). Sulphate supply influences selenate uptake not only through a direct competition for membrane transporters, but also through a

regulation of the expression of sulphate transporter genes. Sulphur-deficient plants up-regulate the expression of sulphate transporter genes, leading to a strong increase in the capacity for selenate uptake (Li *et al*., 2008; Shinmachi *et al*., 2010).

Plant species differ strongly in Se uptake and accumulation in the shoots and also in their capacity to tolerate high Se concentrations in the rooting medium and/or in the shoot tissue. An example of the differences between plant species in Se accumulation is shown in Table 8.12. Based on these differences plants can be classified into *Se-accumulators* and *non-accumulators*, and those between both types as *Se-indicators*. Some species of the genera *Astragalus*, *Xylorrhiza* and *Stanleyea* are typical Se-accumulators, and capable of growing on high Se soils (seleniferous soils) without detrimental effect on growth and reaching shoot Se concentrations as high as 20–30 mg Se g^{-1} dw (Rosenfeld and Beath, 1964). However, within the genus *Astragalus* there are large differences among species and ecotypes in their capacity to accumulate Se, the Se concentration in accumulators being 100–200-fold higher than in non-accumulators (Shrift, 1969; Davis, 1986). Members of the Brassicaceae such as black mustard (*Brassica nigra* L.) and broccoli (*Brassica oleracea botrytis* L.) also accumulate relatively large amounts of Se and may contain, and tolerate, several hundred μg Se g^{-1} shoot dw (Zayed and Terry, 1992). On the other hand, most agricultural and horticultural plant species are non-accumulators (Shrift, 1981) and Se toxicity can occur even at concentrations below 100 μg Se g^{-1} (Mikkelsen *et al.*, 1990). White *et al.* (2007a) compared selenate and sulphate uptake by 39 plant species grown in hydroponic culture under the same conditions. They found that, among the 37 species of Se non-accumulators, there was a very close positive relationship between leaf S and leaf Se concentration (Fig. 8.11A), indicating that selenate and sulphate accumulation are strongly linked. In general, Brassicaceae species are able to accumulate more Se because they have a greater ability to accumulate S. Two Se accumulators (*Astragalus racemosus* and *Stanleya pinnata*) included in this study deviate from this relationship by having higher Se concentrations in the leaves. The ability to accumulate selenate relative to sulphate can be measured by the selenate/sulphate discrimination index, which is the molar ratio of [leaf Se/leaf S]/[solution selenate/solution sulphate]. Most of the plant species tested by White *et al.* (2007a) have a discrimination index of around 1, indicating no clear discrimination between the two anions (Fig. 8.11B). Figure 8.11B also includes data from the study of Bell *et al*. (1989) testing two plant species with different ratios of selenate/sulphate in the nutrient solution. Some plant species (e.g., *Astragalus glycyphyllos*, *Beta vulgaris* and *Medicago sativa*) have an index of below 1, suggesting that the transporter(s) may have a higher affinity for sulphate than for selenate. In contrast, Se-accumulators (*Astragalus racemosus*, *Stanleya pinnata* and *Astragalus bisulcatus*) have a discrimination index of between 2 and 10 which is strong evidence that some transporters in these species have a higher selectivity for selenate.

TABLE 8.12 Se concentrations in shoots of accumulator and non-accumulator species growing on a soil with 2–4 mg Se kg^{-1}

	Se concentration (mg kg^{-1} dw)
Astragalus pectinalus	4,000
Stanleya pinnata	330
Gutierrezia fremontii	70
Zea mays	10
Helianthus annuus	2

Based on Shrift (1981).

Selenite may also be present in soil (Stroud *et al*., 2010), although its availability to plants is lower than that of selenate because of a stronger adsorption by iron oxides/hydroxides in soil (Barrow and Whelan, 1989). The mechanisms of selenite uptake by plants are not well understood. Earlier studies suggested that selenite may enter root cells passively by diffusion (Terry *et al*., 2000). However, selenite uptake is, at least partly, active (Arvy, 1993; Li *et al*., 2008c). Selenite uptake is inhibited by the presence of phosphate in the medium, but is enhanced by P deficiency in plants (Hopper and Parker, 1989; Li *et al*., 2008c), suggesting a possible involvement of the phosphate transporters in selenite uptake. At low pH (<4.0), a significant proportion of selenite is undissociated as H_2SeO_3, and this neutral molecule can permeate through the rice NIP2;1 aquaporin channel (Zhao *et al.*, 2010), which is a silicic acid transporter (see Section 8.3).

A marked difference between selenate and selenite is that the former is rapidly translocated from roots to shoots, whereas the latter is readily assimilated into organic forms in plant roots with a limited root-to-shoot translocation (Asher *et al*., 1977; De Souza *et al*., 1998; Li *et al*., 2008c). This difference, together with a stronger adsorption of selenite in soil, explains why applications of selenate are more effective than selenite in increasing the Se concentration in crops.

Seleno-amino acids such as selenomethionine are readily taken up by wheat seedlings (Abrams *et al.*, 1990). However, the significance of this uptake in soil-grown plants is unclear.

8.5.3 Assimilation and Metabolism

Selenium is assimilated in plants via the S assimilation pathway (Fig. 8.12) (Terry *et al.*, 2000; Sors *et al*., 2005b).

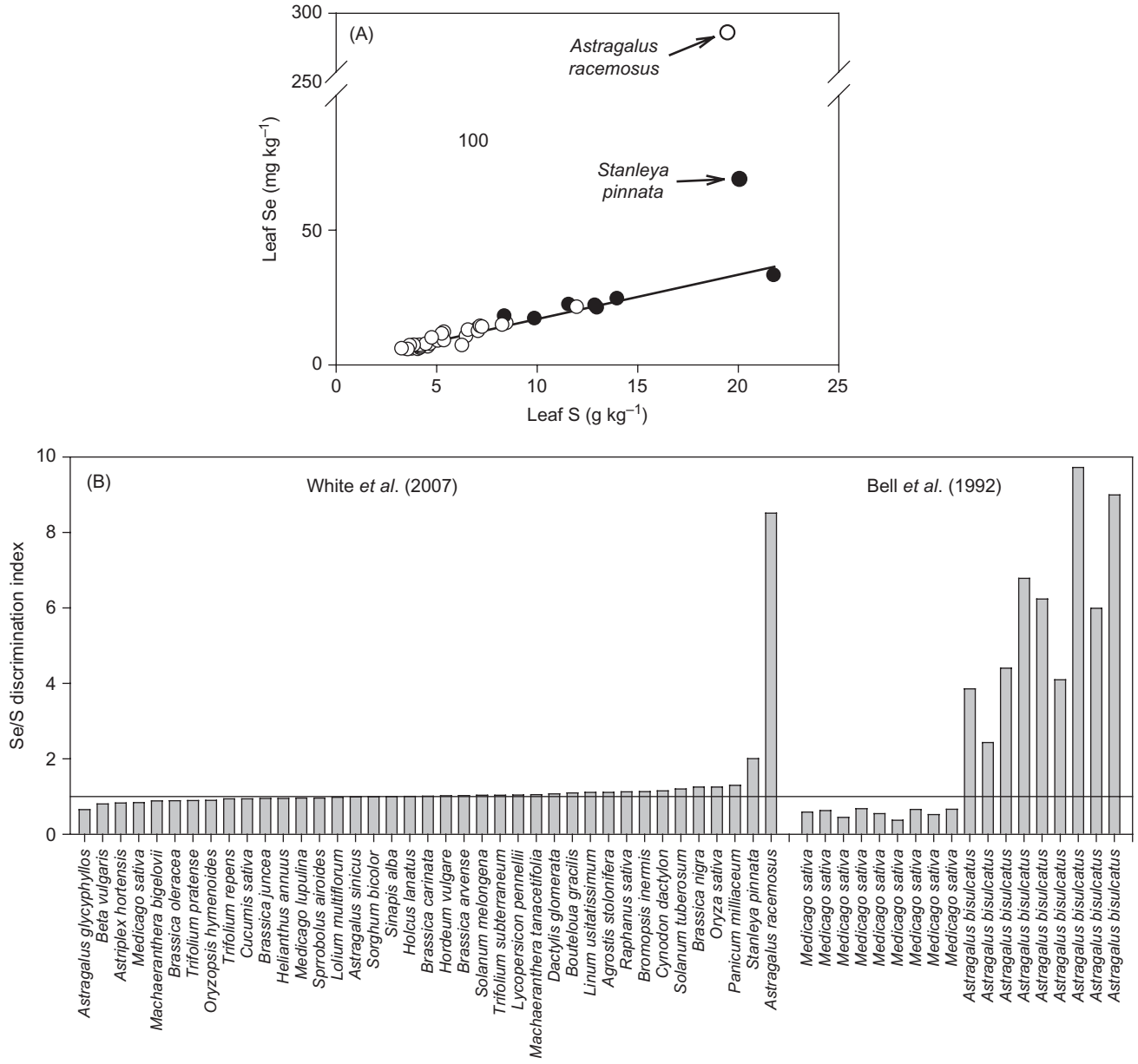


FIGURE 8.11 (A) Relationship between leaf Se and S concentrations in 39 plant species grown hydroponically with 0.91 mM sulphate and 0.63 μM selenate (redrawn from White *et al*. (2007)). Closed symbols represent Brassicaceae species. (B) Selenate/sulphate discrimination index calculated from the data of White *et al*. (2007a) and Bell *et al.* (1992).

In this pathway, selenate is activated by ATP sulphurylase to adenosine 5′-phosphoselenate (APSe), which is then reduced to selenite by APS reductase. Activation of selenate seems to be the rate-limiting step for selenate reduction, and can be overcome in transgenic plants overexpressing ATP sulphurylase (Pilon-Smits *et al.*, 1999). This rate-limiting step also explains why selenite is much more readily assimilated in plants than selenate (De Souza *et al*., 1998; Li *et al*., 2008c). Selenite is further reduced to selenide possibly via non-enzymatic reactions using reduced glutathione (GSH) as a reductant (Sors *et al.*, 2005b). Selenide is assimilated into the amino acid selenocysteine catalysed by the cysteine synthase complex, and is further assimilated into selenomethionine via the methionine biosynthetic pathway. Both selenocysteine and selenomethionine are readily incorporated into proteins in non-accumulator plants through the non-specific substitution of cysteine and methionine, respectively, and it is primarily this substitution that causes toxicity to plants, because the proteins become non-functioning or are less

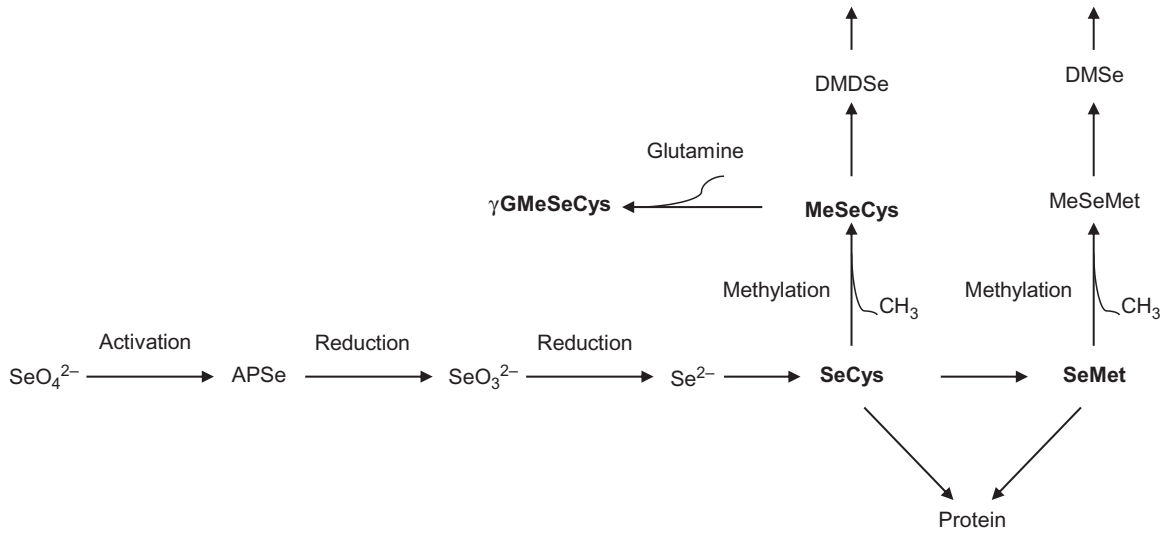


FIGURE 8.12 Schematic diagram of Se assimilation and metabolism in plants. Compounds in bold are common organic selenium species in plants. Abbreviations: APSe, adenosine 5′-phosphoselenate; SeCys, selenocysteine; SeMet, selenomethionine; MeSeCys, Se-methylselenocysteine; γ-GMeSeCys, γ-glutamyl-Se-methylselenocysteine; MeSeMet, methylselenomethionine; DMDSe, dimethyldiselenide; DMSe, dimethylselenide. *Based on Terry* et al. *(2000) and Sors* et al. *(2005).*

functional as enzymes than the corresponding S-containing proteins (Eustice *et al.*, 1981; Brown and Shrift, 1982). Incorporation of selenoamino acids is presumably particularly critical in enzymes with a sulphhydryl group (–SH) as catalytic site.

Both selenocysteine and selenomethionine can be methylated, and then are no longer able to substitute cysteine and methionine in protein synthesis. Selenium accumulators differ from non-accumulators in possessing a strong ability to convert selenocysteine into various non-protein selenoamino acids, such as Se-methylselenocysteine and γ-glutamyl-Se-methylselenocysteine. Methylation of selenocysteine is an important mechanism of Se detoxification in accumulator plants. The methylation step is catalysed by selenocysteine methyltransferase (Neuhierl and Bock, 1996), and the enzyme activity was found to correlate closely with the Se accumulation ability in eight *Astragalus* species (Sors *et al*., 2005b). In the Se accumulators *Astragalus bisulcatus* and *Stanleya pinnata*, young leaves contain high concentrations of Se, with Se-methylselenocysteine accounting for more than 70% of the total Se (Pickering *et al.*, 2003; Freeman *et al.*, 2006). Se-methylselenocysteine and its γ-glutamyl-derivatives are also found in some edible plants, including garlic, onions, broccoli and others of the *Allium* and *Brassica* families, particularly when grown in Se-enriched environments (Whanger, 2002; Rayman *et al.*, 2008). In contrast, cereal grains contain mainly selenomethionine (Whanger, 2002; Rayman *et al.*, 2008).

Similarities between S and Se metabolism in plants also exist in the production of volatile compounds released by aerial parts of plants (see also Chapter 4). The main volatile selenide compound is dimethylselenide (DMSe), of which selenomethionine is the precursor (Fig. 8.12). Plants can also volatilize dimethyldiselenide (DMDSe) which is produced via methylation and subsequent oxidation of selenocysteine. The rates of Se volatilization vary considerably between crop species. With a supply of 20 μM selenate, rice, broccoli and cabbage volatilized 200–350 $\mu g\,Se\,m^{-2}$ leaf area day^{-1} compared to less than 15 $\mu g\,Se\,m^{-2}$ leaf area day^{-1} in sugar beet, lettuce and onion (Terry *et al.*, 1992). In broccoli, which accumulates up to several hundred $\mu g\,Se\,g^{-1}$ dw, the release rate of volatile Se compounds is about seven times higher at low S supply compared to high S supply, due to inhibition of selenate uptake and competition within the plant at the sites of S assimilation by the latter (Zayed and Terry, 1992). In Indian mustard (*Brassica juncea*), more Se was volatilized when plants were supplied with selenite than with selenate (De Souza *et al.*, 1998). Rhizosphere bacteria also appear to play an important role in Se volatilization (Terry *et al.*, 2000). The ability of plants and their associated rhizosphere microorganisms to volatilize Se, or to accumulate Se in the plant biomass, may be exploited as a phytoremediation strategy to clean up Se-contaminated soils (Terry *et al.*, 2000).

8.5.4 Beneficial Effects on Plant Growth

Selenium is essential for humans and animals because of the requirement of Se in a number of enzymes, such as glutathione peroxidase, in which selenocysteine serves as

the catalytic site. Although there are glutathione peroxidase-like enzymes in higher plants, they appear to contain cysteine, not selenocysteine, at the active site of the enzyme (Terry *et al*., 2000). Despite the lack of definitive evidence for Se essentiality in higher plants, there are reports that small doses of Se improve plant growth or reproduction. Hartikainen and coworkers reported that small amounts of Se added to soil increased growth of ryegrass, delayed senescence of lettuce, and enhanced resistance of lettuce and ryegrass to UV irradiation (Hartikainen, 2005). These effects appear to be associated with enhanced activity of glutathione peroxidase and reduced lipid peroxidation. Lyons *et al.* (2009) showed that an addition of 20–50 nM Na selenite to the nutrient solution increased seed production of *Brassica rapa* by 43%, while having no effect on the total plant biomass. Selenite-treated plants had higher total respiratory activity in leaves and flowers, as well as increased concentration of the cytochrome oxidase protein in flowers. This experiment was conducted with the +Se treatment and the control being housed in two separate controlled-environment chambers in order to preclude transfer of volatile Se from the treatment to the control.

High Se concentrations in accumulator plants may offer a protection against herbivory (e.g., Galeas *et al*., 2008), thus conferring an adaptive advantage to the plants. This observation supports the elemental defence hypothesis put forward to explain the evolution of the metal or metalloid hyperaccumulation trait (Boyd, 2007). The large differences in Se concentrations in plants first attracted attention in the 1930s when it was realized that Se toxicity is responsible for certain disorders in animals grazing on native vegetation of seleniferous soils (Brown and Shrift, 1982; Miller *et al.*, 1991).

8.5.5 Biofortification

There is now a greater awareness of the importance of Se to human health than in the past, as is the realization that a considerable percentage of the population in many countries has inadequate intakes of Se (Combs, 2001; Rayman, 2008). Selenium enters the food chain primarily through plant uptake from the soil. Selenium concentration in food crops is highly variable as a result of the variation in the underlying geology and soil conditions; human intake of Se also varies considerably between countries and regions of countries, reflecting the variation in the Se concentrations in foods (Combs, 2001; Rayman, 2008). The minimum Se concentration for animals and humans is about 50–100 $\mu g\,Se\,kg^{-1}$ dw in fodder/food (Gissel-Nielsen *et al.*, 1984). A strategy to increase human intake of Se is to biofortify crops, either through the use of Se fertilizers (agronomic biofortification) or by genetic improvement in crop Se accumulation.

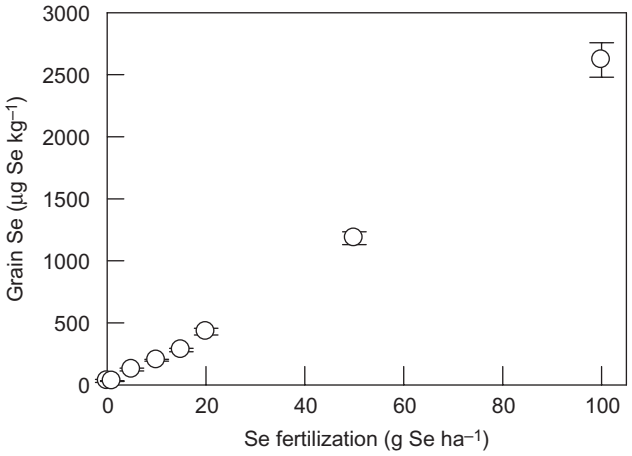


FIGURE 8.13 Relationship between Se fertilization and grain Se concentration of winter wheat. *Redrawn from Broadley* et al. *(2010).*

Agronomic biofortification has been practised in Finland since the mid-1980s with mandatory additions of small amounts of Se as Na selenate to all multi-nutrient fertilizers (6–16 $mg\,Se\,kg^{-1}$ fertilizer). This practice has raised Se concentrations in cereals, vegetables and animal products and more than doubled the Se intake by the Finnish population (Hartikainen, 2005). Compared to direct Se supplementation, agronomic biofortification is considered to be advantageous in that inorganic Se is assimilated by plants into organic forms, which are more bioavailable to humans. In addition, plants act as an effective buffer that can prevent accidental excessive Se intake by humans that may occur with direct supplementation (Hartikainen, 2005). Unlike other micronutrients such as Fe and Zn, it is relatively easy to increase Se concentrations in food crops by fertilization, because selenate is highly bioavailable to plants and is readily transported from roots to shoots, where it is assimilated into different organic forms. This is demonstrated in field studies with winter wheat, showing that Se concentration in the grain increased linearly from 30 $\mu g\,kg^{-1}$ in the control to 2,600 $\mu g\,kg^{-1}$ in the treatment receiving an additional 100 $g\,Se\,ha^{-1}$ in the form of Na selenate (Broadley *et al.*, 2010) (Fig. 8.13). Moreover, Se-enriched wheat flour contains predominantly selenomethionine (~80%), with selenocysteine, Se-methylselenocysteine and inorganic Se also being present in small proportions (Hart *et al.*, 2011). Total recovery of applied Se by the wheat crop was 20–35%; the remainder was likely leached out of the rooting depth leaving little residual effect to the subsequent crop (Broadley *et al*., 2010; Stroud *et al.*, 2010). In general, applications of selenate are more effective in increasing crop Se content than selenite (Mikkelsen *et al*., 1990; Hawkesford and Zhao, 2007).

Biofortification of Se in crops through genetic improvement requires genetic variation in the uptake and/or assimilation of Se. Within the genotypes of bread wheat tested, genetic variation in grain Se concentration is small compared to environmental variation (Lyons *et al*., 2005; Zhao *et al.*, 2009). Inter-species variation is much wider, with Se accumulators representing an extreme phenotype (Fig. 8.11). A number of studies have reported several-fold increase in Se uptake by transgenic plants over-expressing various genes involved in S/Se assimilation, but these studies generally aimed to enhance Se uptake for the purpose of phytoremediation of Se-contaminated soils (Pilon-Smits, 2009). Whether transgenic plants can accumulate more Se from soils with limited Se availability remains unclear.

8.6 ALUMINIUM

Aluminium (Al) is an abundant element representing about 8% of the earth's crust. Aluminium concentrations in mineral soil solutions are usually below 1 mg l^{-1} (~37 µM) at pH values higher than 5.5, but rise strongly at lower pH. The main interest in Al has been concerned with the ability of some plant species (accumulators) to tolerate high Al concentrations in their tissue, and the toxic effects on plant growth by high Al concentrations in soil or nutrient solutions (Section 17.3).

Although Al shows toxicity to most plants, its beneficial effect on plant growth has been observed in some plant species such as tea, Melastoma, and *Quercus serrata* Thunb under certain growth conditions. The Al concentrations at which growth stimulations have been observed vary between 71.4 µM and 185 µM in nutrient solution in sugar beet, maize and some tropical legumes. In the tea plant, which is one of the most Al-tolerant crop species, growth stimulation has been observed at Al concentrations as high as 1,000 µM (Matsumoto *et al*., 1976) or even at 6,400 µM (Konishi *et al.*, 1985). Root elongation was enhanced 2.5-fold in tea grown in a nutrient solution containing 0.5 mM Al at pH 4.3 (Ghanati *et al*., 2005). The root biomass of *Quercus serrata* increased with increasing Al concentrations up to 2.5 mM in a solution with pH 3.5 (Tomioka *et al*., 2005). In Melastoma, Al increased the root activity and stimulated the elongation of root cells (Watanabe *et al*., 2005).

The exact mechanisms for the beneficial effects are unknown, but several possible mechanisms have been suggested. One is that Al probably ameliorates proton toxicity in roots, because the beneficial effect of Al is usually observed at low pH (Kinraide, 1993), which is just the opposite of the alleviation of aluminium toxicity by high H^+ concentrations (Section 17.3). However, a recent study with *Quercus serrata* showed that Al-induced growth enhancement is not due to the amelioration of H^+ toxicity by Al (Tomioka *et al*., 2005). Another mechanism by which Al may have a beneficial effect is that Al alleviates toxicity caused by other elements, particularly the P and Cu (Asher, 1991). In peanut, root and shoot growth were enhanced at Al concentrations in the nutrient solution between 49 and 20.4 µM, which was due to reduced Zn uptake and shoot Zn concentrations which were in the toxic range in plants without Al supply (Asher, 1991).

Recently, other possible mechanisms for Al-induced beneficial effects have been reported. Aluminium increased the activities of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) in the roots of both intact tea plants and cultured cells (Ghanati *et al*., 2005). Al-induced increase in the activity of these antioxidant enzymes may cause increased membrane integrity, delayed lignification and ageing, resulting in a stimulation of growth. In Melastoma, the primary reason for the Al-induced growth enhancement is proposed to be the alleviation of Fe toxicity by Al (Watanabe *et al*., 2006). Growth was enhanced by Al more strongly under excess of Fe, and the Fe concentration was decreased by Al in both the roots and shoots. Excess Fe, as may occur in acid soils, induces the production of reactive oxygen species, leading to disruption of various cell functions. Therefore, the Al-induced alleviation of Fe toxicity may be important in these soils (Watanabe *et al*., 2006).

In conclusion, low concentrations of Al may have beneficial effects on growth under certain conditions, and this beneficial effect is probably a more general phenomenon in plant species with high Al tolerance and high capacity of Al uptake (accumulators). However, in non-accumulators, negative effects of Al on plant growth in soils of low pH are the rule (Section 17.3).

8.7 OTHER ELEMENTS

The requirement for such elements as iodine (I) and vanadium (V) is fairly well established for certain lower plant species, including marine algae (I) and freshwater algae (V) and fungi (V). The reports on the stimulation of growth of higher plants by other elements are rare and vague. Examples of this are the effect of V on the growth of tomato (Basiouny, 1984), or the effect of titanium (Ti) on the growth (Pais, 1983), enzyme activities and photosynthesis (Dumon and Ernst, 1988) of various crop species. For further information on V and I see Bollard (1983), and on Ti see Dumon and Ernst (1988).

More recently interest has increased in the rare earth elements lanthanium (La) and cerium (Ce) for enhancement of plant growth. Mixtures of both elements are used on a large scale in China as foliar sprays or seed treatment of agricultural and horticultural crop species. The amounts supplied are in the range typical for micronutrients. There are reports of substantial increases in plant growth and

yield under field conditions which, however, require more careful documentation and reproduction under controlled conditions. For further information see Asher (1991).

There are a vast number of reports on the presence of heavy metals, such as cadmium, chromium, lead and mercury, in higher plants. Most of these reports are concerned mainly with environmental pollution, the presence of heavy metals in the food chain, and genotypical differences in the critical toxicity concentrations of heavy metals in plants (Ernst and Joose-van Damme, 1983). Convincing evidence of beneficial effects of these heavy metals on growth of higher plants is lacking.

Chapter 9

Nutrition and Quality

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SUMMARY

Plant quality (i.e., appearance, nutritional, sensory and technical quality, shelf life) is a highly complex trait due to the large number of individual properties which determine quality (i.e., physical characteristics and chemical composition), and the various factors which control them (i.e., genetic and exogenous factors). This chapter discusses the relationship between nutrient supply, yield and quality showing that optimal quality may be attained at fertilizer rates suboptimal for yield. Agronomic measures, but also plant breeding, can be used to improve acquisition, biosynthesis, translocation and storage of quality-improving compounds, but also to reduce the uptake of toxic compounds and the biosynthesis of so-called antinutrients.

9.1 INTRODUCTION

Although recognized for many years (e.g., Pfützner *et al.*, 1952; Amberger, 1974; Jungk, 1975; Waterlow and Payne, 1975; Welch and Gabelman, 1984; Olson and Frey, 1987), the influence of plant nutrition on plant quality only received attention in the 1990s, triggered by reports indicating that there is a lack of adequate, balanced nutrition in many countries, resulting in poor health, low productivity and an increase of chronic diseases, particularly in low-income families (Welch and Graham, 1999; Stein, 2010). In addition to the nutritional value of food and feed crops, quality aspects are also important for processing ('technical quality') and marketing ('market value') of plant products. The large number of individual properties which determine quality as well as the various genetic and exogenous factors which control them make plant quality a highly complex trait. These aspects will be outlined in this chapter, followed by the presentation of selected examples demonstrating the effects of nutrition on plant quality.

9.1.1 Properties Determining Plant Quality

Overall quality can be defined as the sum of individual properties which enable a plant or plant product to meet the requirements of a user. Overall quality depends on physical and chemical plant properties. Physical properties determine nearly exclusively appearance and thus the marketable yield of vegetables and fruits for direct consumption. Nutritional and sensory quality is mainly determined by the chemical composition of a plant, including both quality-improving and quality-impairing compounds. Quality may be improved by high concentrations of essential nutrients, carbohydrates, essential amino acids, lipids, organic acids, flavours, vitamins and bioactive compounds (secondary compounds or accessory health factors). Plants or plant products may however also contain quality-reducing compounds, for example heavy metals, oxalate and so-called anti-nutrients. A number of compounds (e.g., phytic acid, dietary fibre) show features of an anti-nutrient (e.g., due to adverse effects on element bioavailability), as well those of an accessory health factor (e.g., due to anti-carcinogenic activity). In contrast to marketable yield and nutritional quality, processing quality depends on both physical (e.g., grain size in brewing barley) and chemical properties (e.g., concentration of sucrose and harmful N in sugar beet, gluten in baking wheat) of the harvested plant part.

9.1.2 Factors Controlling Plant Quality

Plant quality is predominantly controlled by genetic and physiological factors. Quality varies with species (e.g., low protein concentration in cassava roots compared with cereal grains), cultivars (e.g., protein nutritional quality of conventional and high-lysine barley), plant organs (e.g., nitrate concentration in vegetative and reproductive

Marschner's Mineral Nutrition of Higher Plants. DOI: 10.1016/B978-0-12-384905-2.00009-1

plant organs) and tissues (e.g., nutrients and essential amino acids in the bran and endosperm of cereal grains). Existence of sufficient genetic variation indicates that nutritional quality of crops may be improved by both conventional breeding and transgenic approaches (Frossard *et al*., 2000; Zhu *et al*., 2007; White and Broadley, 2009). However, within existing genetic boundaries, exogenous factors, either natural (e.g., climate and soil fertility, pest pressure) or anthropogenic (e.g., soil cultivation, fertilization, harvest method, processing) may considerably modify the quality of plant products (Wang *et al*., 2008b; Martínez-Ballesta *et al.,* 2010).

Nutrient supply may influence quality traits but also yield which is another important agronomic factor, and yield and quality are often not increased synchronously by nutrient supply. As shown schematically in Fig. 9.1, maximum quality can be obtained either before (curve 2) or after (curve 3) the maximum dry or fresh matter yield has been reached. A synchronous pattern of yield and quality curves (curve 1) is rather the exception than the rule. Examples for curve 2 are N supply and quality characteristics such as low nitrate concentrations in vegetables and nutritional value of proteins. On the other hand, maximum quality may require a higher nutrient supply than necessary for maximum yield (curve 3), for example to achieve high gluten concentrations in baking wheat. Figure 9.1 indicates that reverse relationships may exist between element supply and different quality properties, for example between protein concentration and nutritional value of proteins.

9.2 NUTRITION AND APPEARANCE

Appearance is of particular importance in fresh fruit and vegetable production, since it is a primary criterion for certain marketing standards and in making purchasing decisions (Kays, 1999). Among appearance parameters size (i.e., length, width, weight or volume of a product) is influenced by plant nutrition. Nutrient deficiency often reduces product size of fruits and vegetables, which may lead to application of high rates of N application to achieve the required quality standards regarding both size and uniform size distribution in vegetables (Sørensen *et al.*, 1995). Product size is also influenced by the N form. In tomatoes, Pill and Lambeth (1980) found that ammonium sulphate favoured flower formation and the number of fruits per plant, but reduced the mean single fruit weight compared with potassium nitrate. In cereals, grain size influences, for example, flour yield of baking wheat or malting suitability of brewing barley. Grain size is influenced by those nutrients affecting source strength but also those that influence source to sink transport of assimilates and sink strength (Maidl *et al.*, 1998; see also Chapter 5). Application of N to increase the crude protein concentration has little effect on single grain weight (Barraclough and Haynes, 1996; Varga and Svečnjak, 2006).

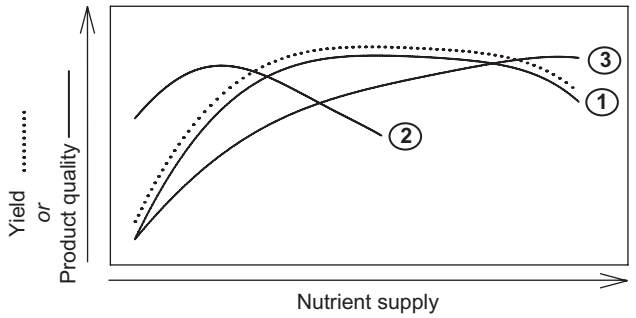

FIGURE 9.1 Nutrient supply and yield (dotted curve) and product quality (curves 1, 2 and 3). For explanation and examples see text. *Based on Marschner (1995).*

Shape and form are predominantly genetically fixed and thus an important characteristic of cultivars, for example in potatoes or apples. Shape may additionally be affected by environmental factors and cultural practices such as soil texture, water and nutrient supply. Irregularities in shape or deformations impair marketable and technical quality of fresh fruits, vegetables, potatoes or sugar beet substantially. For example, in potato production, a sudden increase in N supply to the roots may cause cessation of tuber growth and the induction of stolon formation on the tuber apex (Krauss and Marschner, 1976, 1982). Repeated interruption and resupply of N can result in the formation of chain-like tubers. After a temporary cessation of growth, resumption of the normal growth rate is usually restricted to a certain area of the tubers (meristems or 'eyes'), leading to typical malformations and knobby tubers, which are often observed under field conditions after transient drought periods (see Fig. 5.27).

Texture describes the structural, physical and chemical properties of a product. Among these, firmness of vegetables and fruits is one of the most important parameters. In apple production, N application such as regular urea sprays may increase fruit weight, but decrease fruit firmness (Ferree and Cahoon, 1987). This may be explained by the fact that small fruits have a similar number of cells as larger fruits but have a greater percentage of their volume in cell wall material (Sams, 1999). Increased K supply can increase firmness (Beringer *et al*., 1983; Tong *et al*., 1999) by an increased fruit-tissue pressure potential, resulting from a greater accumulation of K and other osmolytes such as sugars (Lester *et al.*, 2006; see also Section 6.6). Calcium is the nutrient most frequently associated with fruit firmness (Sams, 1999). Both pre-harvest Ca sprays (Raese and Drake, 1993) and post-harvest treatments such as Ca infiltration (Stow, 1993; Picchioni *et al.*, 1998) may increase fruit firmness and delay fruit softening during storage. These effects may be explained by

improved cross-linkage of pectins in the middle lamella and preservation of membrane integrity through Ca (see also Section 6.5).

The colour of ornamentals, fruits and vegetables plays an important role in consumer appeal. To achieve green coloration (i.e., high chlorophyll concentration) of vegetables, growers often apply very high rates of N. Red apple skin colour is achieved by high anthocyanin concentrations together with low chlorophyll concentrations. Anthocyanin synthesis can be affected by nutrient supply. For example, foliar urea spray increases chlorophyll and carotenoid concentrations in apples, but decreases anthocyanin concentrations (Table 9.1; Reay *et al.*, 1998).

Biological (e.g., pathogens and pests), environmental (e.g., water and nutrient supply, radiation, imissions, hail) and physiological (e.g., nutrient deficiency or toxicity) factors may cause a number of quality-impairing defects. For example, induced or physiological Ca deficiency is a widespread problem in vegetable, fruit and ornamental production. Since Ca transport in the phloem is negligible (see Chapter 2), low transpiring organs such as shoot apices, young and inner leaves and fleshy fruits are particularly affected (see also Section 6.6). Typical Ca deficiency-related disorders are, for example, watercore and bitter pit in apples, veinal tipburn and normal tipburn in lettuce (Fig. 9.2A), internal tipburn in cabbage, bract necroses in poinsettia (Fig. 9.2B) (Shear, 1975; Wissemeier, 1996). Calcium deficiency is induced, for example, by high concentrations of competing cations such as ammonium, K and Mg or salts in the substrate (Pill and Lambeth, 1980; Mizrahi and Pasternak, 1985; Francois *et al.*, 1991; Strømme *et al.*, 1994). Leaf or fruit application rather than soil application of Ca has been found to be effective in increasing the Ca concentration of concerned organs (Fernández *et al.*, 2009).

Boron deficiency can also lead to severe quality impairing defects such as, for example, heart and crown rot, watercore of the tissue, loosened and brown heads (cauliflower; Fig. 9.2C), and flower deformation and discoloration in ornamental plants. Soil and leaf application can be used to avoid B deficiency. On the other hand, B toxicity impairs quality by leaf marginal or tip chloroses and necroses.

Potassium deficiency in potato can lead to discoloration before or after cooking due to oxidation of phenols and phenol-like compounds and reactions of reducing sugars and amino acids under heat to amino sugars (Maillard reaction). Sufficient K supply alleviates these symptoms due to increased citrate concentrations which may inhibit phenol oxidase or decreased concentration of reducing sugars (Welte and Müller, 1966; Marschner and Krauss, 1980).

TABLE 9.1 Chlorophyll, carotenoid and anthocyanin concentration in the fruit skin of 'Gala' apples with or without periodic urea foliar applications after flowering (eight sprays at weekly intervals)

Foliar urea application	Chlorophyll ($nm\,cm^{-2}$)	Carotenoids ($nm\,cm^{-2}$)	Anthocyanins ($nm\,cm^{-2}$)
0	1.76	1.06	27.8
+	2.16	1.45	24.2

From Reay *et al.* (1998).

9.3 NUTRITION AND CHEMICAL COMPOSITION

9.3.1 Nutrients

Nutrients are essential constituents in food and feed. Furthermore, they influence the technical quality (e.g., concentration of molasses; influenced by K and Na concentration in sugar beet) and the sensory quality (e.g., as a compound wine) of plant products. And, finally, biosynthesis of all quality-determining organic compounds strongly depends on an adequate plant element status.

(A) (B) (C)

FIGURE 9.2 Ca deficiency in lettuce: tipburn (A), Ca deficiency in poinsettia: bract necrosis (B), (C) B deficiency in cauliflower: hollow stem disorder and brown, loosened heads (C). *Courtesy of A. Wissemeier.*

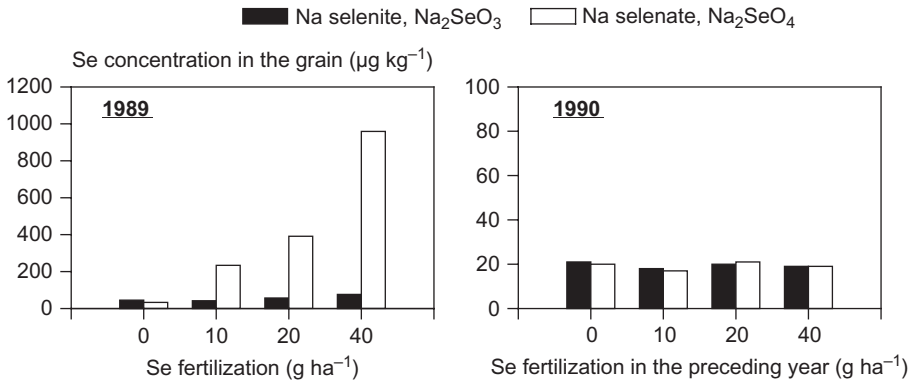

FIGURE 9.3 Selenium concentrations in grains in the year of Se fertilization (1989) and the following year in barley supplied with selenite or selenate. *From Gupta* et al. *(1993). With permission from Taylor and Francis.*

All elements essential for plant growth and additionally several other elements (Na, I, F, Se, Cr) are considered essential for humans and animals (Van Campen, 1991; Stein, 2010). In addition to their essentiality for growth and metabolism and their effects in preventing the classical mineral deficiency diseases (e.g., anaemia caused by Fe deficiency, goiter and cretinism caused by iodine deficiency), some elements are known to be significant contributors to the risk reduction of chronic diseases such as cancer, cardiovascular diseases and degenerative diseases associated with ageing. On the other hand, all essential elements may cause nutrient imbalances and toxicity in humans when intake persistently exceeds requirement. Health risks may also be caused by excess intake of other trace elements such as cadmium (Cd), arsenic (As), mercury (Hg) or lead (Pb).

Mineral malnutrition of humans is widespread, but there are large variations in prevalence of specific deficiencies across geographical and socioeconomic divisions. Nutrients of particular concern are Ca, Mg, Fe, Zn, Cu, I and Se (White and Broadley, 2009; Stein, 2010). So-called hidden hunger may affect up to two billion people in the case of Fe and the same number in case of Zn deficiency (Pinstrup-Andersen, 2005). Dietary element deficiency may be remedied by different strategies, for example dietary diversification, supplementation or fortification of the food with nutrients, biofortification and appropriate preparation of the food (White and Broadley, 2005a). It is undisputed that food diversity is very effective for an adequate and balanced nutrition. Supplementation and fortification of the food may also be successful, but may have a number of disadvantages such as high costs, side-effects regarding colour and flavour of the food, low bioavailability, etc. (Frossard *et al.*, 2000). Biofortification is considered as a more sustainable and cost-effective strategy to combat element malnutrition. It includes the increase of the concentration and/or the bioavailability of nutrients in plants through agronomic measures ('agronomic biofortification') or plant breeding approaches ('genetic biofortification') (Zhu *et al.*, 2007; Cakmak, 2008).

9.3.1.1 Agronomic Biofortification

Liming of acid soils to improve plant growth and increase the Ca concentration in the plant (Norhayati, 1995), however, as mentioned above may not be effective in increasing Ca concentrations in low transpiring organs such as fruit. In contrast, application of Mg fertilizers can increase Mg concentration in leaves and also in seeds and grains (Beringer and Forster, 1981; Draycott and Allison, 1998).

Substantial research has been conducted to determine the most effective and economical methods of preventing and correcting Fe deficiency through agronomic approaches (Mortvedt, 1991; Shuman, 1998; Rengel *et al*., 1999; Cakmak, 2008; Morikawa and Saigusa, 2008; Rodríguez-Lucena *et al*., 2010). However, the Fe concentration in cereal grains cannot easily be increased, either by soil or by leaf application of Fe. In barley, foliar application of $FeSO_4$ or an Fe chelate was an effective measure to increase the Fe concentration in the vegetative shoot tissue but not in the grain (Gupta, 1991). Soil application had no effect on Fe concentration in the shoot or the grain. On the other hand, in a calcareous soil, Moraghan (2004) showed that Fe concentrations in seeds of common bean and soybean were increased by soil application of Fe-EDDHA with application at flowering being more effective than pre-plant application. Inconsistent success in increasing the Fe concentration in reproductive organs by Fe fertilizer application, even at increased leaf Fe concentrations, has been explained by, for example, precipitation of Fe in the apoplasm (Garnett and Graham, 2005), limited availability of chelators needed for phloem transport

TABLE 9.2 Seed yield and seed Fe concentration of common bean and soybean with different time of Fe application as Fe EDDHA

Time of Fe application		Seed dw (g dw plant^{-1})		Seed Fe concentration (μg dw g^{-1})	
Pre-plant	Flowering	Bean	Soybean	Bean	Soybean
0	0	7.3	1.5	56	34
+	0	7.5	4.7	58	69
0	+	7.3	3.6	72	97
+	+	7.4	4.3	70	80

Data recalculated from Moraghan (2004).

TABLE 9.3 Zinc concentration in bran, embryo and endosperm of durum wheat with or without Zn soil application and at different timing of Zn foliar application (0.5% $ZnSO_4$)

Soil Zn application (kg $ZnSO_4$ ha^{-1})	Growth stage of foliar Zn sprays	Grain Zn concentration (mg kg^{-1})		
		Bran	Embryo	Endosperm
0	0	20	38	8
0	Stem + Booting	28	47	10
0	Booting + Milk	35	62	15
0	Milk + Dough	41	63	15
50	0	33	52	11
50	Stem + Booting	34	58	13
50	Booting + Milk	44	68	17
50	Milk + Dough	45	69	16

From Cakmak *et al.* (2010a).

(Grusak, 1994), and restricted unloading, transport and storage capacity in the grains (Borg *et al*., 2009).

Similarly to Fe, Zn deficiency is most prevalent on calcareous or alkaline soils in arid and semi-arid regions (Alloway, 2004). However, in contrast to Fe, soil application of Zn can increase yield and Zn concentration in both vegetative and reproductive plant parts and the increase of grain Zn is often greater than that of grain Fe (Zhang *et al*., 2010). This is presumably due to a better reproductive partitioning (i.e., translocation from vegetative to reproductive organs within the plant) of Zn compared to Fe (Kutman *et al*., 2011). The timing of foliar Zn application is an important factor in maximizing Zn accumulation in wheat grain. Cakmak *et al.* (2010a) showed that foliar Zn sprays late in the growing season (e.g., milk and dough stage) of field-grown wheat caused a greater increase in grain Zn concentration than applications at earlier growth stages (Table 9.3). Increased concentration of endosperm Zn is particularly important, because this is the most commonly eaten part of wheat in many countries where Zn deficiency in human populations is widespread.

Selenium**,** though non-essential, is considered as a beneficial element for higher plants (Lyons *et al*., 2009; see also Section 8.5) and is both an essential trace element and a potential toxicant for animals and humans. The amount of Se ingested by animals and humans with plant products is largely dependent on the availability of Se in soils. Extensive areas where crops are generally low in Se include, for example, parts of the north-east and north-west USA, Canada, Australia, New Zealand, UK, Turkey, Greece, Denmark, Finland and China (Reilly, 1998).

In soil, selenite (SeO_3) is more strongly adsorbed by mineral surfaces and Fe(Al) oxides than selenate (SeO_4), especially at lower pH. Thus, selenite is less available to plants than selenate at equal rates of soil application (Hopper and Parker, 1999; Sharma *et al.*, 2010). On the other hand, selenate is easily leached, has a relatively short period of efficacy and may result in toxic Se concentrations in plants at excess supply. Differences in the efficacy of Se forms have been demonstrated in field experiments of Gupta *et al.* (1993), showing that low rates of Na selenate were sufficient to increase concentrations in barley grains in the year of application (Fig. 9.3) whereas Na selenite was ineffective. One year after application neither selenate nor selenite had a residual effect on grain Se concentration of the following crop.

In Finland, all agricultural compound fertilizers have to be supplemented with Na selenate to 16 (grain production) and 6 (fodder production) mg Se kg^{-1} fertilizer. This measure increased the Se concentration in the whole food chain, including meat and dairy products, resulting in a markedly greater human Se intake and blood Se concentration (Eurola *et al.*, 2003; Hartikainen, 2005). In addition to oxidation state and soil solution concentration, competing ions may affect plant uptake of Se. Moderate inhibition of selenite uptake by very high phosphate concentrations and strong inhibition of selenate uptake by sulphate has been documented, even at typical sulphate soil solution concentrations (Hopper and Parker, 1999; Hawkesford and Zhao, 2007).

Iodine deficiency disorders are recognized as a major international health problem because of the large number of people at risk due to their I-deficient environments (Hetzel

and Dunn, 1989) as a result of leaching of I by high rainfall or flooding. Iodine prophylaxis can be achieved by supplementing the diet, commonly by use of iodized table salt (Rendig, 1984). In terms of agronomic measures, addition of K iodate to irrigation water was an effective method to increase the I concentration in wheat (Cao *et al*., 1994).

9.3.1.2 Genetic Biofortification

There is considerable within-species genetic variation in element concentration in edible plant parts of staple food crops, particularly when wild plants and old varieties are included (Cakmak, 2008; Palmgren *et al*., 2008). Comprehensive data surveys are provided in a series of reviews, for example in Frossard *et al.* (2000), Welch and Graham (2004) and White and Broadley (2009). Developing nutrient-enriched plant foods through traditional breeding methods, use of mutants or wild relatives and via molecular biological techniques has been proposed as a powerful tool to cope with malnutrition in humans. A prerequisite for enhancing nutrient density through breeding is that yields of modified crops are maintained or even increased. In the past, the introduction of new, high-yielding varieties was often associated with a decrease in the micronutrient concentration (McGrath *et al.*, 2007). However, more recently breeding programmes, such as HarvestPlus, have been developed that show both higher element concentrations in the edible plant part and higher yields can be achieved (Cakmak, 2008).

Understanding the molecular mechanisms governing the accumulation of nutrients is important for developing functional markers for conventional breeding and a prerequisite for adopting molecular biological techniques in plant breeding (White and Broadley, 2005). Breeding or transgenic approaches may target to improve/increase (i) the mobilization of nutrients in the rhizosphere, (ii) the efficiency of the root uptake system, (iii) the nutrient translocation to harvest organs, and (iv) the sink strength and sink capacity of harvest organs for nutrients including the distribution of nutrients in the grain (Palmgren *et al*., 2008).

For example, current strategies to increase the Fe concentration in the endosperm of cereals are often focused on the expression of legume ferritin genes under the control of endosperm-specific promoters. Vasconcelos *et al.* (2003) showed that over-expression of soybean ferritin genes in rice considerably increased the Fe (and Zn) concentration in the whole grain including the endosperm. To avoid low leaf Fe concentrations in the plants, such modifications have to be accompanied by increased Fe uptake, for example increased release of phytosiderophores in grasses (White and Broadley, 2009; see also Section 7.1)

Proposed strategies to increase grain Zn concentrations are to increase grain protein concentration (which is associated with grain Zn concentration), for example through introduction of the Grain Protein Content B1 (GPCB1) locus (Distelfeld *et al*., 2007) and manipulation transporters involved in Zn translocation, for example members of the Zinc/Iron-regulated transporter Protein (ZIP) family (Palmgren *et al.*, 2008). Stimulating leaf senescence by genetic modifications has also been shown to increase remobilization and seed translocation of Zn and Fe (Uauy *et al*., 2006).

9.3.2 Concentration of Potentially Toxic Elements

Both essential and non-essential elements may be toxic to humans at high concentrations in the diet, with heavy metals being of primary concern in this respect. Factors leading to elevated concentrations of heavy metals in food include (i) high geogenic soil concentrations (Rebafka *et al.*, 1990), (ii) contamination of soils through anthropogenic activities (e.g., overfertilization, impurities in fertilizers, use of municipal sewage wastes, irrigation, use of metal-based pesticides, sedimentation of mining material, atmospheric deposition), (iii) high soil/plant transfer, (iv) high transfer into the harvest organ, and (v) direct contamination of plants by soil, air and dust.

Of the potentially toxic elements, cadmium (Cd) is of primary concern, mainly because of its relatively high soil/plant transfer and its relatively high human but low plant toxicity (Chaney, 1980). Cadmium concentrations in food vary, with leafy vegetables and root crops generally having higher concentrations than fruits or seeds, except seeds of oil crops such as linseed (McLaughlin *et al.*, 1999). Cadmium may also be present in rock phosphates and therefore also in P fertilizers processed from them. Often, long-term use of Cd-containing P fertilizers can lead to increased Cd concentrations in vegetative and also reproductive plant parts (Andersson and Siman, 1991; He and Singh, 1994; Grant *et al.*, 1996, 2010).

Agronomic practices, which may influence Cd availability and uptake, have been comprehensively reviewed by McLaughlin *et al.* (1999) and Sarwar *et al*. (2010) (Table 9.4). Liming may decrease Cd uptake, due to increased pH and the competition between Ca and Cd for uptake. On the other hand, liming may also increase Cd uptake by reducing Zn concentrations (less competition for Cd uptake) and competition between Ca and Cd for binding sites in the soils. Increasing N, P and K fertilizer rates can increase Cd uptake in crops (Grant *et al*., 1996; Perilli *et al.*, 2010). Increased Cd uptake through N fertilizers may be attributed to soil acidification, increased ionic strength, Cd desorption from binding sites in the soil and improved root growth. Potassium fertilization increases ionic strength and, when applied in the chloride form, may lead to the formation of complexes between Cd and chloride in the soil solution, thereby increasing Cd availability.

TABLE 9.4 Effects of agronomic measures on Cd mobility in soil and Cd uptake of plants

Measure	Inhibition of uptake	Increase of uptake
Liming	Reduced Cd availability through high pH	Reduced Zn concentration in the soil solution
	Increased competition between Ca and Cd for uptake	Increased competition between Ca and Cd for binding sites in the soil
P fertilization	Immobilization of Cd	Increased Cd input to the soil
	Dilution effect	Decreased Zn uptake
N fertilization	Dilution effect	Increased ionic strength
		Cd desorption from binding sites in the soil
		Soil acidification
		Improved root growth
K fertilization		Increased ionic strength
		Formation of Cd/Cl complexes in soil
		Increased Cd mobility within the plant
Zn fertilization	Increased competition between Zn and Cd for uptake	Desorption of Cd from binding sites in the soil
Org. fertilization	Impaired uptake of chelated Cd	Increased mobility of chelated Cd

Based on McLaughlin *et al.* (1999), Grant and Bailey (1997) and Sarwar *et al.* (2010).

Cadmium chloride complexes may also increase the Cd transport within the plants (Ozkutlu *et al.*, 2007). There are also, however, some studies showing no differences in Cd uptake when different potassium salts were compared (Römheld and Kirkby, 2010).

9.3.3 Bioavailability of Elements

Health risks caused by inadequate or excess supply of elements not only depend on the concentration, but also on element bioavailability and, ultimately, its absorption from the diet. The absorption of elements, especially those of particular concern in human diets, is often low and may vary, for example, between 5 and 70% (Ca), 3 and 25% (Fe) and 30 and 40% (Zn). Bioavailability is influenced by the chemical form of the element in the food (e.g., heme and non-heme Fe) and the presence of inhibitors or promoters of element absorption in the human digestive system. A number of naturally occurring substances including phytate, oxalate, polyphenolics, some plant proteins and fibre components can inhibit cation absorption (Baynes and Bothwell, 1990; Fairweather-Tait and Hurell, 1996). Furthermore, high concentrations of one element may inhibit the absorption of another, for example absorption of Fe may be reduced by high Ca or P concentrations in the diet. An example of a compound affecting bioavailability is phytate.

Seed and grain concentration of phytate can vary considerably depending on genotype and soil P supply (Frossard *et al.*, 2000). Phosphorus fertilizer application increases both yield and phytate-P concentration in grains and seeds, whereas the concentration of other elements may decrease, resulting, for example, in an increase of the phytate/Zn ratio (Buerkert *et al.*, 1998; Ryan *et al.*, 2008). Phytic acid has potential negative effects on element bioavailability because *in vitro* it forms largely insoluble complexes with metal cations (e.g., Mg, Ca, Fe, Zn, Cd). When added to semi-synthetic diets, it markedly reduces absorption of cations such as Zn (Rimbach *et al.*, 1995). However, *in vivo* studies using naturally occurring phytate in seeds and grains are inconclusive. This may be due to both the presence of compounds in natural diets that counteract the inhibitory effect of phytic acid (e.g., ascorbic acid, protein, tannins, polyphenolics) and the presence of other inhibitors (e.g., fibres) or metal ions with synergistic binding effects. Moreover, food processing and preparation (e.g., fermentation, heat treatment) may reduce the phytic acid concentration in grain products from various cereals. This was demonstrated in a study of Lopez *et al.* (2003), showing that fermentation reduced phytic acid concentration in yeast and particularly sour dough bread compared to the respective whole wheat flour.

Phytic acid concentration can also be lowered by use of existing genotypical differences either in phytic acid concentration or in endogenous phytase activity (Barrier-Guillot *et al.*, 1996). Furthermore, breeding may modify these traits. A number of low-phytate mutants, inbreds and hybrids have been developed by non-transgenic techniques in maize and also in rice, wheat, barley and soybean (Raboy, 2007). Consumption of tortillas prepared from low-phytate hybrids resulted in a significantly higher fractional Zn absorption compared with tortillas prepared from the wildtype isohybrids, indicating considerable potential benefits when such varieties are used for human nutrition (Hambidge *et al.*, 2004).

Use of transgenic techniques may allow a more target-oriented improvement of nutrient bioavailability in the future. Approaches to reduce phytate concentrations in the food so far have been to suppress phytate biosynthesis (Kuwano *et al.*, 2006, 2009) or phytate transfer from the cytosol to the vacuole (Shi *et al.*, 2007). Over-expression

of phytase may be another approach to improve bioavailability of cations in reproductive plant organs (e.g., Lucca *et al*., 2001; Brinch-Pedersen *et al.*, 2006; Chen *et al*., 2008a). When assessing the benefits of these approaches from a view of overall quality, it should be kept in mind that the ingestion of phytic acid may contribute to the prevention of many health-threatening diseases including cancer. These aspects should also be considered when manipulating other antinutrients such as polyphenols.

9.3.4 N Compounds

Nitrogenous compounds, namely proteins, are essential constituents in food and feed, needed to supply amino acids for synthesis of body protein and other essential N compounds. Furthermore, certain amino acids may be important by improving Fe and Zn absorption and may contribute to detoxification of harmful compounds, for example cyanogenic glycosides, which produce toxic HCN (Friedmann, 1996). Some N compounds may reduce nutritional quality (e.g., nitrate, toxic amino acids, acrylamide, lectins, alkaloids, cyanogens). Furthermore, N compounds influence the appearance (chlorophyll concentration), sensory quality (aromatic and flavouring substances) and processing quality (e.g., gluten in baking wheat) of plants or plant products.

9.3.4.1 Protein Concentration

Nitrogen nutrition is largely considered as the main factor affecting crude protein concentration in plants. In general, the concentration of crude protein continues to increase with N applied in amounts beyond those needed to obtain the maximum growth and yield of cereals, legumes, root/tuber crops and vegetables. Additional N above the amount needed for maximum yield is commonly applied by farmers to increase grain protein concentration of bread-making wheat in order to improve the technological quality of the flour. A field study of Dampney and Salmon (1990) illustrates the effects of different rates of extra N applied at the 2nd node stage on relative grain yield, relative grain crude protein concentration and apparent recovery of applied N in the grain (Fig. 9.4). In contrast to grain yield, grain crude protein concentration was increased by extra N up to the highest rate tested which was well above the level of N fertilizer required for yield only. However, recovery of the additional N was generally low and decreased progressively with increasing N rates. Late application of additional N near anthesis, rather than applied earlier in the growing season, can increase grain protein concentration in wheat, barley, maize and rice (Below *et al.*, 1984; Souza *et al.* 1999; Varga and Svecnjak, 2006) suggesting that late applied N may be preferentially allocated to the grains.

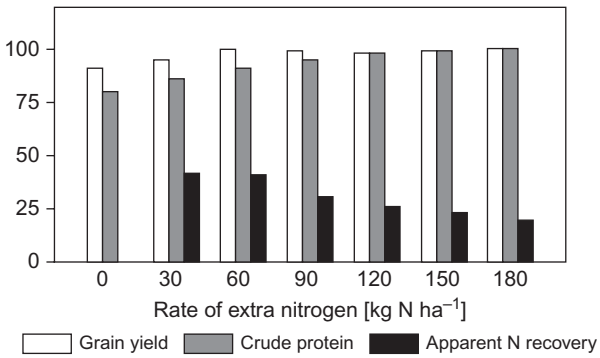

FIGURE 9.4 Relative grain yield, relative grain crude protein and apparent recovery of extra N in grain of bread wheat at different rates of ammonium nitrate at the 2nd node stage to the soil ('extra N'). Means of six field sites in England. *Recalculated from Dampney and Salmon (1990).*

On the other hand, correction of P and K deficiency decreased the crude protein concentration in a number of grain crops considerably due to dilution through improved plant growth (Buerkert *et al*., 1998).

9.3.4.2 Amino Acid Composition

The nutritional value of a protein is related mainly to its essential amino acid composition and digestibility (Damodaran, 1996). Since excess intake of a certain amino acid can lead to amino acid antagonism, both concentration and proportion of amino acids are important characteristics in human diets. Proteins of major food plants are often deficient in at least one of the essential amino acids. For example, seeds of cereals such as wheat, barley, maize and sorghum are very low in threonine, tryptophan and especially lysine, whereas seeds of many legume species are deficient in the S-containing amino acids methionine and cysteine (Shewry and Halford, 2002).

High rates of N application which increase yields and grain protein concentration in cereals may reduce the nutritional value of the grain protein. This is due to a decline of the proportion of essential amino acids in the grain protein, particularly lysine and threonine (Table 9.5), even though the concentration of these amino acids may increase (Bulman *et al*., 1994). In vegetables, N-induced increases in protein concentration are often associated with a reduction in the proportion of essential amino acids and a corresponding increase of the proportion of non-essential amino acids (Brunsgaard *et al.*, 1997).

The concentrations of S-containing amino acids cysteine and methionine are reduced by S deficiency in cereal grains, legume seeds or vegetative parts of vegetables (Byers and Bolton, 1979).

Protein quality of cereal grains. Use of mutants (e.g., high-Lys opaque2 mutant) resulted, for example, in

TABLE 9.5 Amino acid composition of barley grain protein at different rates of N supply

	Nitrogen fertilization ($kg\,ha^{-1}$)		
Amino Acid	0	100	150
Essential amino acids			
Lysine	39	37	36
Threonine	33	33	30
Valine	57	55	53
Methionine	27	25	24
Isoleucine	40	39	39
Leucine	75	75	73
Phenylalanine	55	58	58
Tryptophan	11	11	11
Arginine	56	53	51
Histidine	25	22	26
Non-essential amino acids			
Glutamate	261	266	275
Proline	101	116	125
Aspartate	39	41	34
Glycine	38	34	33
Serine	41	37	38
Alanine	38	35	33
Cystine	27	25	24
Tyrosine	39	37	37

From Bulman *et al.* (1994).

'Quality Protein Maize' or 'High-Lysine Barley' which have increased concentrations of essential amino acids, namely lysine and tryptophan (Joergensen *et al*., 1997; Krivanek *et al.*, 2007).

Before a protein can serve as a nutritional source of amino acids, it must be digested via proteolysis. Protein digestibility depends on several factors, including protein conformation, binding of proteins (e.g., to fibrous polysaccharides, tannins, phytic acid) and inhibition of proteolysis by antinutritional factors (e.g., proteinase inhibitors such as trypsin and chymotrypsin inhibitors, lectins).

Improved true digestibility with increasing N supply may be explained by both reduced concentrations of dietary fibre and/or an increased deposition of the extra protein in a readily available form rather than being tightly bound to the fibre fraction (Brunsgard *et al.*, 1997). However, due to often observed adverse effects on amino acid composition, the percentage of the N absorbed (biological value), which is actually retained by the body, may decrease.

The biological value and net protein utilization from legume seeds is often poor compared with cereal grains. This has been explained by high concentrations of proteinase inhibitors. Proteinase inhibitors reduce digestion of proteins and retention of N (Armour *et al.*, 1998).

9.3.4.3 Potentially Toxic N Compounds

Among the potentially toxic nitrogenous compounds, nitrate in plants has received particular attention during the past decades because of its precursor role in forming nitrite. Nitrite may cause methemoglobinemia in infants (cyanosis, blue baby disease) or may under certain conditions react with secondary amino groups to form carcinogenic nitrosamines (Santamaria, 2006). However, these negative health effects of nitrate have been questioned in recent years (Addiscott and Benjamin, 2004). Adequate prediction of N fertilizer demand is a prerequisite to produce vegetables with low nitrate levels.

Potentially toxic organic nitrogenous compounds include toxic amino acids, lectins, glucosinolates, alkaloids and cyanogens (D'Mello *et al.*, 1991). Occurrence of potentially toxic compounds in harmful concentrations is often confined to certain plant families, for example legumes (toxic amino acids) or crucifers (glucosinolates). Fertilization may influence the synthesis of these compounds. For example, N supply affects the concentration of glucosinolates, alkaloids and allergenic amines.

In the past years, contamination of carbohydrate-rich foods such as potato or cereal products with acrylamide has attracted considerable attention. Acrylamide is formed during food processing at high temperatures as a result of the Maillard reaction from asparagine and reducing sugars (Mottram *et al*., 2002). The potential for acrylamide formation increases with increasing N supply (Claus *et al*., 2006; Weber *et al*., 2008), whereas sufficient S supply can reduce the formation of acrylamide by reducing asparagine concentrations (Granvogl *et al*., 2007).

9.3.4.4 N Compounds and Technical Quality

Examples of the influence of nitrogenous compounds on the technical quality of plants are concentration and composition of storage proteins and quality of baking wheat and wine (Spayd *et al*., 1994), protein concentration and malting quality of barley (Varvel and Severson, 1987) and concentration of 'harmful N' in sugar beets (Burba, 1996).

In sugar beet, 'harmful N' is N which is not precipitated during beet processing and which accumulates in the raw juice. It reduces the crystallization of the juice and leads to higher molasses losses, thus reduced white sugar yield.

In wheat, the amount and composition of endosperm storage proteins has a marked influence on the rheological properties of the dough. Gluten is the major component of the wheat flour protein. When flour is mixed with water and kneaded, a three-dimensional gluten network is formed which is stretched and extended to a visco-elastic coat, trapping CO_2. This allows the dough to be expanded by fermentation and baked into leavened bread or processed into a range of other foods such as pasta. Chemical composition, structure and effects on rheological properties of these groups are highly complex and have been comprehensively discussed in various reviews (Zhao *et al*., 1999a; Shewry and Halford, 2002; Wieser, 2007; Naeen, 2008).

Among the various plant nutrients, N (Wieser and Seilmeier, 1998) and S (Wieser *et al.*, 2004) have the strongest influence on the proportion of gluten proteins, dough properties and bread volume. For example, S application can decrease the resistance of the dough to extension and increase dough extensibility and also bread volume (Zhao *et al*., 1999a; Table 9.6).

9.3.5 Carbohydrates

Carbohydrates, such as mono-, oligo- and polysaccharides, may influence the quality of plant products because they (i) are an important energy source for humans and animals and may have additional effects on health (e.g., through cholesterol-lowering effects of β-glucans), (ii) have direct (e.g., sugar/acid ratio in fruits) and indirect (e.g., concentration of fermentable carbohydrates in grapes) effects on taste, (iii) are substrate for the synthesis of many other organic compounds (e.g., amino acids, organic acids, vitamins), and (iv) may serve as an energy source as substitutes for fossil fuels (e.g., sucrose in sugar cane or sugar beet for ethanol production) or a renewable resource for the industry (e.g., starch, cellulose).

Carbohydrate concentration in storage organs is influenced by (i) source strength (leaf area, photosynthetic activity per unit leaf area, mobilization of carbohydrate reserves), (ii) source to sink transport (phloem loading, transport and unloading) and (iii) sink strength (sink number, for example seeds per ear, sink capacity, for example endosperm cells/seed and sink activity, for example rate of syntheses of storage carbohydrates. These processes are substantially influenced by nutrition of plants and are discussed in Chapter 5. In brief, P, K and Mg are crucial for carbohydrate synthesis and short-/long-distance transport of assimilates to storage organs (Cakmak *et al*., 1994b; Pettigrew, 2008). In the range of suboptimal to optimal N supply, additional N increases protein concentration, leaf growth and CO_2 assimilation per unit surface area and does not substantially depress other biosynthetic pathways related to carbohydrates. However, if the N supply is further increased, the increase in leaf area index has no effect on the rate of net photosynthesis due to mutual shading. Since carbon skeleton demand for N assimilation still increases, a growing competition for assimilates often leads to a reduction in the concentration of non-structural (sugars, starch, polyfructosans) and structural carbohydrates (cellulose). Similarly, other organic constituents such as storage lipids or ascorbic acid decrease at high N supply.

As mentioned above, high concentrations of N can negatively affect white sugar yields in sugar beet. Thus, the optimum N supply for beet yield was 230 kg N ha^{-1}, whereas the optimum N supply for white sugar yield, i.e. the economic optimum for the farmer, was only 160 kg N ha^{-1} (Engels, 1993).

In potato production, increasing N rates may decrease the starch concentration, but to have no effect on the concentration of reducing sugars (Table 9.7; Leszczýnski and Lisínska, 1988). High N supply also decreases the concentration of major storage carbohydrates, starch and

TABLE 9.6 Grain protein and grain S concentration, dough resistance and extensibility and volume of bread prepared from wheat fertilized with two N rates and three S rates

	N supply (kg ha^{-1})					
	230			280		
S supply (kg ha^{-1})	0	20	100	0	20	100
Grain S concentration (mg g^{-1} dw)	1.4	1.6	1.7	1.4	1.6	1.7
Grain protein concentration (%)	11.6	11.9	11.8	11.8	12.1	12.2
Dough resistance (Bu)	363	342	334	392	340	315
Dough extensibility (cm)	16	18	18	16	19	19
Loaf volume (mL)	1,625	1,622	1,717	1,634	1,707	1,701

Data recalculated from Zhao *et al.* (1999a).

polyfructosans in grasses (Hehl and Mengel, 1972) and sugar concentrations in fruit and vegetables (Bénard *et al.*, 2009).

High N supply may also increase the concentration of some specific carbohydrates. For example, in oat and barley, high N supply may increase the concentration of β-glucans, which have the potential to reduce blood cholesterol concentrations (Brunner and Freed, 1994; Güler, 2003).

Potassium is involved in many physiological processes which influence carbohydrate metabolism and concentrations, for example water relations, photosynthesis, assimilate transport, and enzyme activation (Pettigrew, 2008; Römheld and Kirkby, 2010; see also Section 6.6). In K-deficient plants, low- molecular-weight mono-saccharides may accumulate (Marschner and Krauss, 1980). In many fruits, high K supply increased the sugar concentration and thus influenced the sensory quality of the fruit (see comprehensive reviews of Lester *et al*., 2010).

9.3.6 Lipids

Lipids can be subdivided into simple, non-hydrolysable lipids (e.g., free fatty acids, isoprenoid lipids, tocopherols) or composite, hydrolysable lipids, consisting of triacylglycerols (i.e., 'vegetable oils': glycerol, fatty acids), phospholipids, glycolipids and waxes (long-chain alcohols, fatty acids). Vegetable oils influence the quality of plants significantly, since they are an important source of energy in food and feed, supply essential fatty acids, which cannot be synthesized by humans (linoleic and linolenic acid), and influence the cholesterol concentration in the blood. Furthermore, they are carriers of fat-soluble vitamins (vitamins A, D, E, K), are an important renewable resource for the chemical industry, and may be used as biodiesel.

Oil and crude protein concentrations are negatively correlated, thus increasing N supply decreases the concentration of storage lipids and oils in reproductive organs (Zhao *et al.*, 1993; Table 9.8). This is due to the fact that synthesis of amino acids and fatty acids compete for the same substrate, i.e. acetyl-CoA. Since N nutrition stimulates amino acid biosynthesis, the reduced availability of acetyl-CoA may limit fatty acid biosynthesis. Table 9.8 also shows, however, that the increased seed yield with high N supply may overcompensate decreasing seed oil concentrations, resulting in similar optimum N rates for seed and oil yield.

Sulphur fertilization has been shown to increase storage oil concentration in many oil crops such as *Brassica* (Malhi *et al*., 2007).

Fatty acid composition, which is of great interest for the industry, is predominately determined by the plant species (or genotype) and to some extent by environmental factors such as temperature (Canvin, 1965), but is only indirectly affected by nutrient supply (Wiesler, 1998).

Plant breeding is a very promising approach in improving fatty acid composition according to the demand of the industry (Velasco and Fernández-Martinez, 2002; Wittkop *et al*., 2009).

TABLE 9.7 Concentrations of starch and reducing sugars in field-grown potato tubers at different rates of N supply

	N supply ($kg\,ha^{-1}$)		
	40	120	200
Starch (%)	14.9	14.5	13.9
Reducing sugars (%)	0.9	0.9	0.9

From Leszczýnski and Lisínska (1988).

9.3.7 Vitamins

Vitamins (i.e., fat-soluble vitamins A, D, E and K; water-soluble vitamins C, B1, B2, B12, folic acid, biotin, pantothenic acid) are organic compounds which cannot be synthesized by humans at all or not in sufficient amounts and thus must be taken up. They have no structural functions in tissues or organs, but are essential to maintain physiological functions. On a global scale, vitamin A deficiency has the highest prevalence, causing growth retardation and health problems (blindness, night blindness,

TABLE 9.8 Seed yield, crude protein and oil concentration and oil yield in oilseed rape at different rates of N supply

N supply ($kg\,ha^{-1}$)	Seed yield ($t\,ha^{-1}$)	Protein concentration (%)	Oil concentration (%)	Oil yield ($t\,ha^{-1}$)
0	3.9	18.1	45.4	1.8
100	5.2	18.3	45.1	2.3
200	5.7	21.7	44.2	2.5
300	5.7	22.4	42.1	2.4

From Zhao *et al.* (1993).

TABLE 9.9 Concentrations of ascorbic acid, carotene, thiamin and riboflavin in the edible parts of fruits and vegetables as affected by N, P and K fertilization. Percentage of the total number of experiments showing an increase, a decrease or no effect

	Increase (%)	Decrease (%)	No effect (%)	Number of experiments
Ascorbic acid				
N	27	60	13	146
P	43	36	20	44
K	78	12	10	78
Carotene				
N	91	9	0	33
P	17	33	50	6
K	40	50	10	10
Thiamin (B1)				
N	100	0	0	22
Riboflavin (B2)				
N	69	23	8	13

From Mozafar (1994).

xerophthalmia) of 500 million people, particularly in Africa and Asia (Pinstrup-Andersen, 2005; Faber and van Jaarsveld, 2007). Vitamin concentration may be influenced by cultivar, climatic factors, nutrition, harvest time, processing, storage and food preparation (Davey and Keulemans, 2004; Kopsell and Kopsell, 2006).

Mozafar (1994) analysed all available studies on the effects of fertilization on the concentration of vitamins in a comprehensive review, showing inconsistent results and indicating that the effect of nutrient supply on plant vitamin concentration depends on the vitamin considered (Table 9.9). For example, the majority of experiments (but not all) show that high rates of N supply decrease the ascorbic acid concentration in many fruits and vegetables. In contrast to ascorbic acid, the concentrations of carotene, thiamin and riboflavin often increase with increasing N supply. Negative effects of N supply on ascorbic acid concentration have been explained by dilution effects through enhanced growth and mutual shading of plants (Lee and Kader, 2000). On the other hand, carotenoids are constituents of chloroplasts, which increase with increasing N supply (Cunningham and Gantt, 1998).

Vitamin concentrations may also be increased by breeding. The most prominent example is *Golden Rice*, which is a genetically modified rice capable of synthesizing and accumulating β-carotene in the endosperm (Ye *et al*., 2000; Schaub *et al.*, 2005). It is beyond the scope of this chapter to deal with genetic biofortification of vitamins in more detail, the reader is referred to recent reviews, for example Kopsell and Kopsell (2006) and Zhu *et al.* (2007).

In addition to vitamins, there is growing interest in understanding the influence of agronomic measures on the concentration of bioactive compounds in plants such as carotenoids, phytic acid, glucosinolates or phenolics. These are regarded as an accessory health factor, for example due to anticarcinogenic activity. Recently published reviews revealed that there is still considerable inconsistency regarding the effects of nutrition on the concentration of bioactive compounds (Poiroux-Gonord *et al.*, 2010; Treutter, 2010).

Chapter 10

Relationship between Nutrition, Plant Diseases and Pests

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SUMMARY

There are various ways in which plant nutrient supply or concentration affects plant diseases and pests. The supply of nutrients changes the resistance of plants to pathogens and pests by altering growth and tissue composition (e.g., concentration of soluble compounds or defence compounds). Depending on the pathogen/pest and nutrient, nutrient supply for optimal plant growth may increase or decrease disease incidence, with differences in response between facultative and obligate parasites. This chapter provides examples of the effect of nutrient supply on foliar and soil-borne fungal and bacterial diseases as well as on pests. Via their stabilizing effect on cell walls and membranes, Ca and B inhibit pathogen invasion. Silicon and Mn play important roles in the defence reaction to infection or attack, whereas N and K exert their effect mainly via modulating the concentration of soluble compounds in plant tissues. Lastly, effects of management (e.g., timing of fertilizer application and liming) on disease incidence are outlined.

10.1 GENERAL

The effects of nutrients on plant growth and yield are usually explained in terms of the functions of these elements in plant metabolism. However, nutrition may also have secondary, often unpredicted effects on the growth and yield of crop plants by inducing changes in growth pattern, plant morphology and anatomy or chemical composition, which may either increase or decrease the resistance or tolerance of plants to pathogens and pests. Resistance is mainly determined by the ability of the host to limit penetration, development and/or reproduction of the invading pathogen, or limit the feeding of pests. Tolerance is the ability of the host plant to maintain its growth despite the infection or pest attack. Depending on the nutrient, the nutritional status of the plant, plant species and type of pathogen or pest, nutrition may affect resistance or tolerance of the plant, or virulence of the pathogen. Plant diseases can reduce nutrient availability, uptake, distribution, or utilization by the plant; and symptoms of disease may reflect the altered nutritional status of the plant. In this section, examples are given of the effects of nutrition on both resistance and tolerance.

Considerable progress has been made in breeding and selection for increased resistance or tolerance to diseases and pests. Resistance can be increased by three mechanisms: (i) changes in anatomy (e.g., a higher degree of lignification and/or silification), (ii) physiological and biochemical changes leading to higher production of inhibitory or repelling substances, and (iii) restriction of nutrient transfer to the pathogen which it requires for growth or development (Zeyen *et al.*, 2002; Agrios, 2005; Nürnberger *et al.*, 2004; Walters and Bingham, 2007). Apparent resistance can be achieved when the most susceptible growth stages of the host plant occur at a different time than the period of highest activity of pathogens and pests (known as 'escape from attack' or 'outgrowing' the pathogen; Huber, 1980).

On the other hand, species or strains of the parasitic organisms are continuously evolving, enabling them either to evade or to suppress defence mechanisms of their specific host plants (Anderson *et al.*, 2010). Therefore, virulence, i.e. the ability of a parasite to successfully feed on host tissues and induce symptoms of disease, depends on the compatibility of both host and parasite factors (Jones and Takemoto, 2004).

Although resistance and tolerance are genetically controlled, they are significantly influenced by environmental factors. Nutrition of plants can be considered as an environmental factor that can be manipulated relatively easily

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TABLE 10.1 Incidence of leaf blotch (*Rhynchosporium scalis*) in spring barley cultivars at different rates of N fertilizer supply

Nitrogen supply ($kg\,ha^{-1}$)	Flag leaf area infected by leaf blotch (%)		
	Proctor	Cambrinus	Deba Abed
0	0.4	15.4	3.6
66	1.3	21.3	20.5
132	4.5	30.5	57.3

Based on Jenkyn (1976).

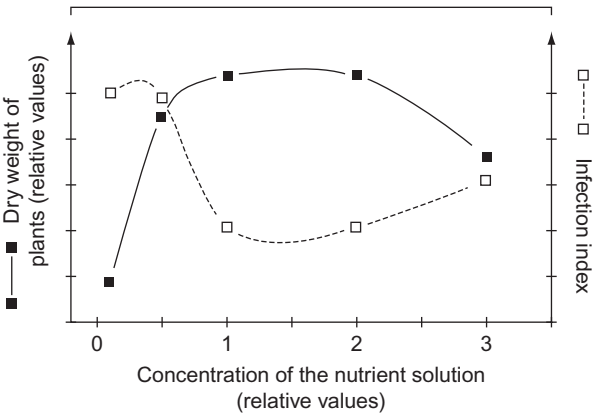

FIGURE 10.1 Growth of non-infected *Pelargonium* plants and degree of infection in plants inoculated with bacterial stem (*Xanthomonas pelargonii*) at different nutrient supply. Relative values; water only = 0; basic nutrient solution = 1; two-fold concentration of nutrient solution = 2; three-fold concentration of nutrient solution = 3. *Modified from Kivilaan and Scheffer (1958).*

by application of nutrients for mitigation of biotic stresses. Although frequently unrecognized, this factor has always been an important component of disease control (Huber and Wilhelm, 1988). For example, application of fertilizers in different amounts and forms not only affects the growth and composition of the plants directly, but also has effects on microbial activity in the soil and rhizosphere, which indirectly induces secondary and cascading effects on plant resistance and tolerance to root and shoot pathogens and pests. On the other hand, symptoms of nutrient deficiency in plants may be induced by soil-borne root diseases or pests impairing root growth and activity and thus acquisition of nutrients. This aspect should be considered for diagnosis of symptoms induced by nutritional disorders or by diseases or pests (see Chapter 11).

The impact of nutrition on plant resistance is relatively small in highly susceptible or highly resistant cultivars, but can be substantial in moderately susceptible or partially resistant cultivars. This is illustrated in Table 10.1 with the effects of N fertilizer on leaf blotch (*Ramularia* sp.) in three barley cultivars. With increasing N supply, the incidence of leaf blotch increases in all three cultivars. However, the absolute levels of infection, as expressed as a percentage of flag leaf affected, are different. In Proctor, a highly resistant cultivar, the increase is small and unlikely to affect plant growth. In the other two cultivars, however, the high disease incidence at high N supply is likely to have detrimental effects on photosynthesis and grain yield would be expected.

10.2 RELATIONSHIP BETWEEN SUSCEPTIBILITY AND NUTRITIONAL STATUS OF PLANTS

The close correlation between N supply and leaf blotch shown in Table 10.1, cannot be generalized to all fungal and parasitic diseases. Usually, a 'balanced' nutrient supply that ensures optimal plant growth is also optimal for plant resistance. Such an ideal situation, however, is not the rule, as shown in Fig. 10.1 for *Pelargonium* plants. Increasing nutrient supply stimulates plant growth, but depresses bacterial infection. From this finding, one can conclude that plants with an optimal nutritional status have the highest resistance to disease and that susceptibility increases as nutritional status deviates from this optimum.

Figure 10.2 shows different relationships between nutritional status of plants (increasing nutrient supply), growth and disease/pest incidence. Type A (Fig. 10.2), where high nutrient supply stimulates growth but decreases disease/pest incidence, can be considered as ideal because optimal growth is combined with high resistance, for example as for a facultative parasite such as *Alternaria* ssp. (leaf spot disease). Type B (Fig. 10.2), where high nutrient supply stimulates disease incidence, is typical for an obligate parasite, such as leaf blotch in spring barley (Table 10.1). However, research by Hoffland *et al.* (2000) comparing three different pathogens of tomato (*Pseudomonas syrigae*, *Fusarium oxysporium* and *Oidium lycopersicum*) at different tissue N concentrations suggests that the proposed distinction between typical obligate and facultative parasites regarding the effect of increasing N supply on disease incidence (Table 10.2) may not always hold true. Generally, plants suffering from nutrient deficiency have lower tolerance to diseases and pests and tolerance can be increased by supplying the deficient nutrient. Such a relationship is based on the fact that more vigorously growing plants usually have a higher capacity to compensate, for example, for losses of photosynthates or leaf and root surface area that may result from infection or feeding (Type A, Fig. 19.2).

Nutrients play a complex role in the interactions of higher plants with parasites and pests. Nevertheless, there

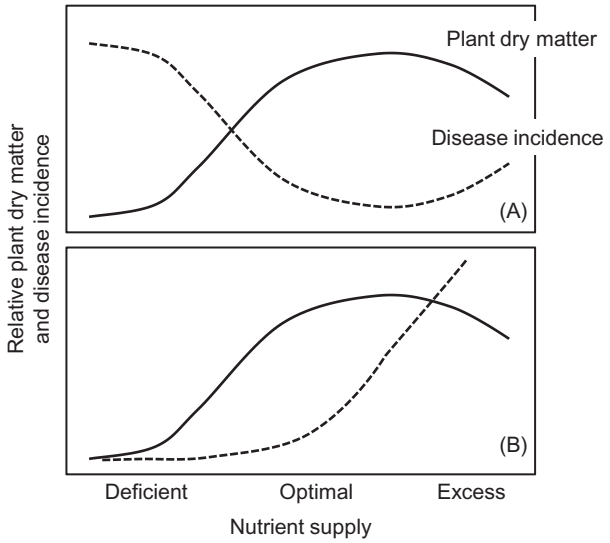

FIGURE 10.2 Schematic presentation of relationships between increasing nutrient supply, plant growth and disease/pest incidence. See text for further details.

TABLE 10.2 Incidence of diseases caused by parasites at high or low N and K levels, with + indicating low disease incidence and ++++ high disease incidence

	N		K	
Pathogen and disease	Low	High	Low	High
Obligate parasites				
Puccinia spp. (rust diseases)	+	+++	++++	+
Erysiphe graminis (powdery mildew)	+	+++	++++	+
Facultative parasites				
Alternaria spp. (leaf spot diseases)	+++	+	++++	+
Fusarium oxysporum (wilt and rot disease)	+++	+	++++	+
Xanthomonas spp. (bacterial spots and wilt)	+++	+	++++	+

Based on Kiraly (1976) and Perrenoud (1977).

are some principal areas of host–parasite interactions where the roles of nutrients and beneficial elements are not only well established, but also predictable and can readily be demonstrated. In this chapter, examples of these interactions are provided to demonstrate both the potential and the limitations of disease and pest control by nutrition and fertilizer application. Comprehensive reviews on these interactions can be found in general (Bergmann, 1992; Datnoff *et al.*, 2007a and references cited therein; Huber and Haneklaus, 2007), for micronutrients (Graham and Webb, 1991) and for particular nutrients such as N (Huber and Watson, 1974), K (Perrenoud, 1977) and Mn (Huber and Wilhelm, 1988).

10.3 FUNGAL DISEASES

10.3.1 Principles of Infection

The germination of spores on leaf and root surfaces is stimulated by plant exudates. The release of exudates by roots and leaves contributes to the success or failure of infection by many air- and soil-borne fungal pathogens. The release rate and composition of exudates depends on their cellular concentration and the diffusion gradient (Fig. 10.3). Potassium deficiency results in high concentrations of sugars and amino acids in leaves whereas excessive N supply leads to high amino acid concentrations (see also Sections 6.1 and 6.6). The concentration of photosynthates in the apoplast at the leaf surface, or root surface, depends on the permeability of the plasma membrane. On average, the concentrations of amino acids and sugars in the apoplasm of leaf and stem tissue are in the range of 1–8 mM (Hancock and Huisman, 1981), but may increase with Ca, B and Zn deficiency (which causes increased membrane permeability) and K deficiency (which impairs polymer synthesis).

The concentration of soluble assimilates in the apoplasm of the host is an important factor for the growth of parasites during penetration and post-infection because only a few groups of plant parasites are truly intracellular with direct access to assimilates in the symplasm (Hancock and Huisman, 1981). Some parasites, such as powdery mildew of barley, have access only to epidermal cells. In these cases, the physical and chemical properties of the epidermal cells are of greater importance for susceptibility and resistance than those of the bulk leaf tissue (Hwang *et al.*, 1983). In epidermal cells of barley, more than 90% of the soluble carbohydrates are β-cyanoglucosides, which appear to be important in resistance against powdery mildew (Pourmohseni and Ibenthal, 1991). Epidermal cells of leaves (Kojima and Conn, 1982), stems and roots (Barz, 1977) are also characterized by higher concentrations of phenolic compounds and flavonoids (i.e., substances with fungistatic properties). The role of nutrients in phenol metabolism is well documented, and examples of phenol accumulation have been discussed in relation to B and Cu deficiency (see Sections 7.3 and 7.7).

Many parasitic fungi and bacteria invade the apoplasm by releasing pectolytic enzymes, which dissolve the middle lamella (Fig. 10.3). The activity of some of these enzymes is strongly inhibited by Ca^{2+}, which explains the positive correlation between the Ca concentration of tissues and their resistance to these fungal and bacterial diseases. The activity

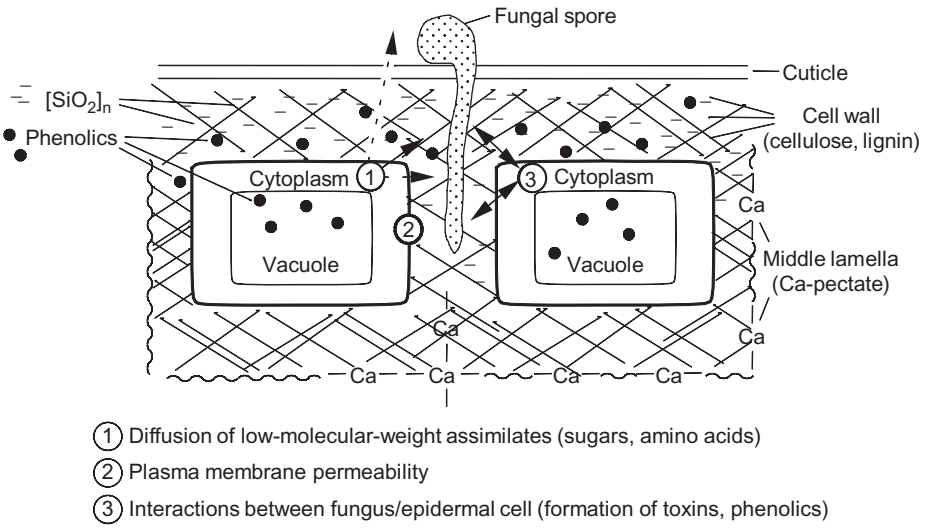

FIGURE 10.3 Schematic representation of penetration of fungal hypha into the epidermal cell layer (apoplasm), and factors which affect the penetration and growth rate of the hypha.

of other pectolytic enzymes, however, can be stimulated by Ca (e.g., exo-polygalacturonate trans-eliminase), or they may even be Ca dependent (Bateman and Millar, 1966).

During fungal infection, a range of interactions occurs between hyphae and host cells (Fig. 10.3). Inducible resistance mechanisms are associated mainly with the epidermis and the effectiveness of these mechanisms depends on the type of pathogen and the resistance of the host as well as on the nutritional status of the plant. Pectolytic enzymes of the parasite not only dissolve the middle lamella, but these enzymes or the products of pectin breakdown also increase passive permeability of the plasma membrane and enhance K^+ efflux and H^+ influx which may trigger hypersensitive reactions such as localized necrosis (Atkinson *et al.*, 1986). In other pathogenic diseases, such as leaf spot (*Helminthosporium cynodontis* Marig.), fungal toxins enhance K^+ efflux and thereby deplete cells and infected tissues of K. Thus, the severity of disease symptoms (leaf spotting) is negatively correlated with K concentration in the leaves (Richardson and Croughan, 1989).

Phenolic compounds play a key role in the early stages of infection (Fig. 10.3), either as phytoalexins or as precursors of lignin and suberin biosynthesis. For example, glucans of the cell wall of *Phytophthora megasperma* elicit the synthesis of isoflavones, which function as phytoalexins and contribute to the rapid accumulation of phenolic polymers at the infection sites (Graham and Graham, 1991). Within a few hours after infection, a signal is transmitted to non-infected leaves, which elicits an increase in their phenol synthesis (Rasmussen *et al.*, 1991). Several nutrients, B and Cu in particular, affect biosynthesis and binding form of phenols (Sections 7.3 and 7.7), and therefore defence responses (Cakmak and Römheld, 1997; Pfeffer *et al.*, 1998). The concentration of phenolics and their fungistatic effects is often high in N-deficient plants whereas it may be reduced at high N supply (Kiraly, 1964). In grapevine leaves, there is a negative relationship between N supply and the concentration of the phytoalexin stilben and resistance to downy mildew (Bavaresco and Eibach, 1987).

The production of oxygen radicals (e.g., O^- and $HO^{\cdot}$) and hydrogen peroxide (H_2O_2) may also increase in response to pathogen infection as a component of the plant defence response (Sutherland, 1991). They may contribute to hypersensitive reactions (oxidation of membrane lipids, leading to cell death), initiation of cell wall lignification and inhibition of pathogens. The role of Cu, Zn, Fe and Mn in the generation and detoxification of oxygen radicals and hydrogen peroxide (see Chapter 7) may explain their role in plant resistance to pathogens.

As tissues (particularly leaves) mature, lignification or the accumulation and deposition of Si in epidermal cells may form an effective physical barrier to hyphal penetration (Fig. 10.3). Lignification and Si deposition provide the main structural resistance of plants to diseases (and pests), especially in the leaves of grasses (Sherwood and Vance, 1980), or the endodermis of roots and are affected by nutrition in various ways.

10.3.2 Role of Si

Grasses in general, and paddy rice in particular, are Si accumulator plants. In rice, the Si concentration of leaves is negatively correlated with the number of eyespots caused by such as rice blast, indicating greater resistance to the disease (Fig. 10.4).

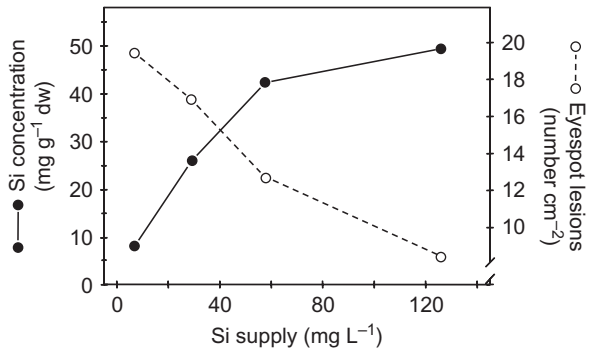

FIGURE 10.4 Silicon concentration and susceptibility to blast fungus (*Pyricularia oryzae* Cav.) of fully expanded rice leaves. *Modified from Volk* et al. *(1958).*

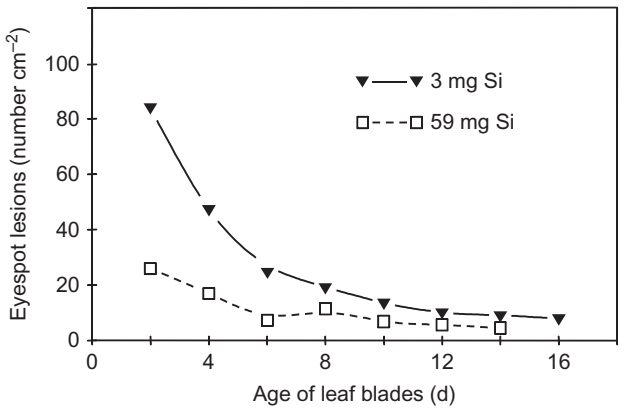

FIGURE 10.5 Eyespot lesions in rice leaves of different ages and with low or high Si supply. *Modified from Volk* et al. *(1958).*

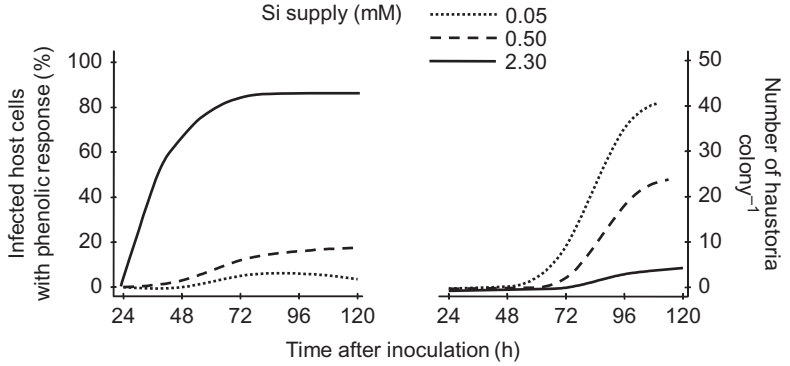

FIGURE 10.6 Percentage of host cells with phenolic response and number of haustoria of *Sphaerotheca fuiliginea* in leaf segments of cucumber (*Cucumis sativus*) at different Si supply. *Based on Menzies* et al. *(1991).*

The limitations of Si in controlling fungal diseases are also evident from Fig. 10.4. Silicon is translocated in the xylem preferentially to mature leaves, whereas rice blast infection occurs mainly in young leaves. As shown in Fig. 10.5, the number of lesions decreases with maturation (full expansion at about day 8) and ageing of the leaves, irrespective of Si supply. On the other hand, Si supply strongly reduces the number of lesions on young leaves, indicating its importance for resistance to the disease, particularly at high N supply (Osuna-Canizales *et al.*, 1991). The inhibitory effect of Si on fungal diseases is not confined to grasses, but can be found in many other plant species, for example powdery mildews in cucumber or grapevine (Miyake and Takahashi, 1983; Adatia and Besford, 1986; Samuels *et al.*, 1991; Bowen *et al.*, 1992; Datnoff *et al.*, 2007b).

Formation of a physical barrier in epidermal cells against the penetration of hyphae (Fig. 10.3) or feeding of insects such as aphids can be very localized and rapid. Silicon is rapidly deposited around the infection peg (Heath and Stumpf, 1986), for example Si accumulates at the sites of hyphal penetration of powdery mildew in wheat (Leusch and Buchenauer, 1988a) and barley within 20 h, and this accumulation is 3–4 times higher around unsuccessful infection sites than around successful ones (Carver *et al.*, 1987). The preferential accumulation of Si at the point of pathogen penetration requires a continuous supply of Si from the roots (Samuels *et al.*, 1991) or foliar sprays of Si (Bowen *et al.*, 1992), indicating that Si once deposited and polymerized in leaf tissue cannot be remobilized.

Despite the positive relationship between Si accumulation at the sites of penetration and inhibition of hyphal invasion and formation of haustoria in plant cells, the protective effect is not due to Si alone. Rather, the presence of soluble Si appears to facilitate the rapid deposition of phenolics or phytoalexins at the sites of infection (Fig. 10.6), which is a general defence mechanism to pathogen attack (Menzies *et al.*, 1991). The mechanism by which Si induces accumulation of phenolics at the infection sites is unclear. Leusch and Buchenauer (1988b) showed that not only Si, but also Mn accumulates at the sites of hyphal

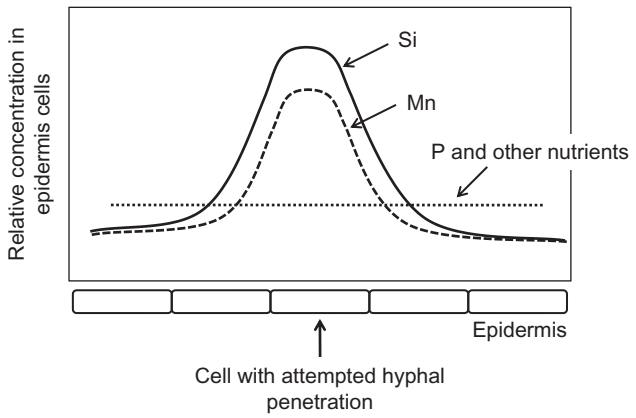

FIGURE 10.7 Schematic presentation of the relative concentrations of Si and Mn at the infection site of an unsuccessful pathogen penetration (e.g., powdery mildew) on the leaf epidermis.

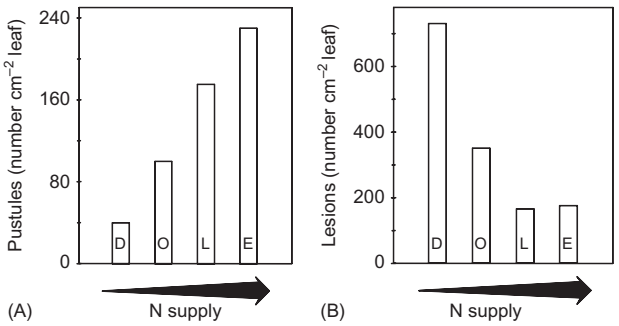

FIGURE 10.8 Number of pustules of stem rust (*Puccinia graminis* spp. *tritici*) in wheat (A) and number of necrotic lesions caused by bacterial spot (*Xanthomonas vesicatoria*) in tomato (B) grown in nutrient solutions with increasing N concentration. D: deficient, O: optimal; L: luxurious, E: excessive. *Based on Kiraly (1976).*

penetration of powdery mildew and that high Si concentrations in leaf tissues are necessary for the mobility and short-term allocation of Mn (Fig. 10.7). Hence, the stimulation of phenolic deposition by Si may be indirect via its effect on Mn; Mn plays an important role in biosynthesis of phenolics and pytoalexins (see also Section 7.2). Silicon may also form weak complexes with phenolics (see also Section 8.3) and thereby enhance their synthesis and mobility in the apoplasm. Furthermore, Si appears to have a general signaling function in modulating the resistance of plants against stresses at the level of gene transcription (Walters and Bingham, 2007; Brunings *et al.*, 2009a).

10.3.3 Role of N and K

There are many studies on the effects of N and K on parasitic diseases because their role in modulating disease resistance is quite readily demonstrated and can be of particular importance for fertilizer application.

However, the results for N may be inconsistent, and in some cases controversial, for various reasons: (i) it is not clearly stated whether the supply of these nutrients is low, optimal or excessive (see Figs 10.1 and 10.2), (ii) the effect depends on the form of N supplied (e.g., ammonium or nitrate which are metabolized differently), or (iii) the differences in infection patterns between obligate and facultative parasites are not considered.

The principal differences in the response of obligate and facultative parasites to N are shown in Fig. 10.8 and Fig. 10.2B. The susceptibility of wheat plants to stem rust, caused by an obligate parasite, increases with increasing N supply. In contrast, the susceptibility of tomato plants to bacterial leaf spot caused by a facultative parasite, decreases with increasing N supply. These differences in response are based on the nutritional requirements of the two types of parasite. Obligate parasites rely on assimilates supplied by living cells. On the other hand, facultative parasites are semi-saprophytes which prefer senescing tissues or which release toxins that damage or kill the host plant cells. Thus, factors which support the metabolic activities of host cells and delay senescence of the host plant increase resistance or tolerance to facultative parasites. This general effect of N on the disease susceptibility of plants may, however, be modified by additional factors such as the plant species and plant growth conditions. Hoffland *et al*. (1999), for example, reported that resistance to the grey mould fungus *Botrytis cinerea* of tomato plants (a facultative parasite), which were grown under controlled climate conditions, increased with increasing shoot N concentrations. On the other hand, the common field observation that high N supply increases bunch rot of wine grapes induced by the same pathogen has been attributed to N-induced increases in canopy density, which results in a microclimate that is more conducive to the development of the disease (Mundy and Beresford, 2007).

The increasing susceptibility of host plants to obligate fungal parasites with increasing N supply (Table 10.1) can be explained by the nutritional requirements of the parasite and changes in the anatomy and physiology of the host plant in response to N. As discussed in Section 6.1, N enhances growth rate so that during the vegetative growth stage, the proportion of young to mature tissue increases with the young tissue being more susceptible to parasitic attack. In addition, an increase in amino acid concentration in the apoplasm and at the leaf surface induced by high N supply may stimulate germination and growth of conidia (Robinson and Hodges, 1981). Moreover, high N supply may reduce the activity of some key enzymes of phenol metabolism (Matsuyama and Dimond, 1973), the concentration of phenolics (Kiraly, 1964) and deposition of lignin. For example, the lignin concentration of rice leaves from plants supplied with high N was 5 mg kg^{-1} dw as compared

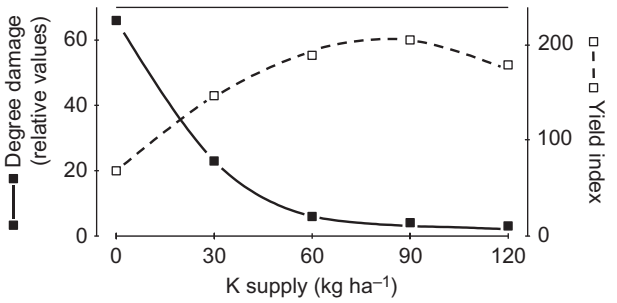

FIGURE 10.9 Grain yield of wetland rice and incidence of stem rot (*Helminthosporium sigmoideum*) at different K supply (with kg ha^{-1} 120 N and 60 P). *Based on Ismunadji (1976).*

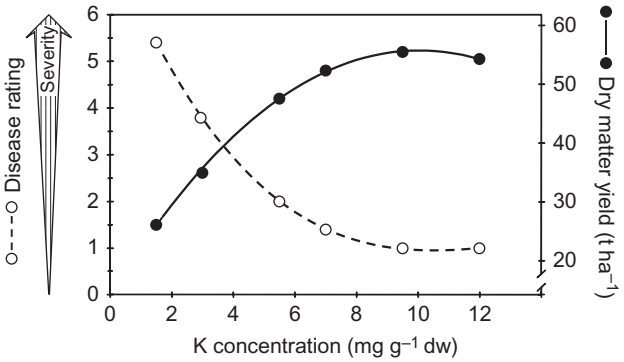

FIGURE 10.10 Severity of leaf spot disease (*Helminthosporium cynodontis*) and dry matter in coastal Bermuda grass (*Cynodon dactylon* L. Pers.) at different leaf K concentration. *Based on Matocha and Smith (1980).*

with 11 mg kg^{-1} dw in low N plants (Matsuyama, 1975). High N supply may also decrease the Si concentrations in plants (Grosse-Brauckmann, 1957; Volk *et al*., 1958). This, however, is an unspecific response and due to the growth stimulation by N thereby diluting the concentration of other nutrients if their uptake remains constant.

The various anatomical and biochemical changes, together with the increase in the concentration of low-molecular-weight organic N compounds which are substrates for the parasites, are the main factors responsible for the close correlation between N supply and susceptibility to obligate parasites. This effect of high N supply is further enhanced by high membrane permeability induced by B, Ca and Zn deficiency.

Recently it was found that the gaseous NO may play a role in regulation of various processes of plant development and also defence of pathogens; particularly in systemic resistance where NO acts as a signal molecule by binding reversibly with cystein in various regulative proteins (Misra *et al*., 2010).

Potassium deficiency increases the susceptibility of host plants to obligate and facultative parasites. As shown in Fig. 10.9, increasing K supply decreased stem rot incidence in rice and enhanced shoot growth, indicating that high K supply increases resistance/tolerance of plants. Results similar to those shown in Fig. 10.10 in rice have been obtained with oil palms infected with *Fusarium* (Ollagnier and Renard, 1976) and wheat infected with stripe rust (Kovanci and Colakoglu, 1976). However, beyond optimal K supply for growth, there is no further increase in resistance with increasing K addition or K concentration in the plants. Hence, K addition is only effective in disease control if it alleviates K deficiency.

The high susceptibility of K-deficient plants to disease is related to the metabolic functions of K (see also Section 6.6). Potassium deficiency reduces the synthesis of high-molecular-weight compounds (proteins, starch and cellulose) and thus leads to accumulation of low-molecular-weight organic compounds which can serve as easily available nutrient sources for the parasites. However, K supply

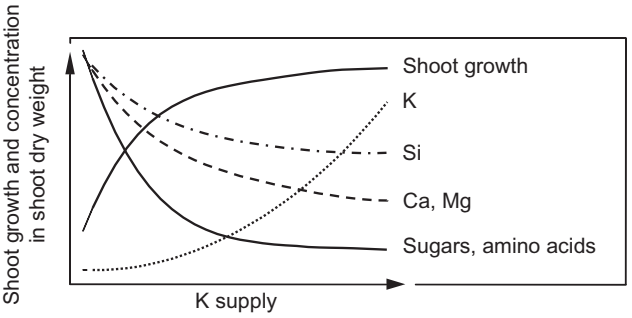

FIGURE 10.11 Schematic diagram of relationship between growth response, changes in plant composition and K supply.

decreases the concentration of low-molecular-weight organic compounds only until growth is maximal. A further increase in K supply and plant K concentration has no effect on synthesis of high-molecular-weight compounds, and as mentioned above, has no further effect on resistance or tolerance (Fig. 10.11). However, disease susceptibility and metabolic profile of K-deficient plants are variable, hence it is difficult to prove a general causal relationship (Amtmann *et al*., 2008). Furthermore, high K supply may reduce the concentrations of other nutrients by enhancing plant growth (dilution effect) or competition at uptake sites with other cations such as Mg and Ca (see also Chapter 2).

The relationship between K and resistance is more complex in seeds and fruits that are supplied with K primarily by re-translocation from vegetative organs. In some soybean cultivars, the rapid increase in incidence of blight in the upper pods formed late in the season was correlated with a decrease in K concentration of these pods compared to those formed earlier during phases with higher root activity, soil moisture and K availability in the soil. With very high soil application of K (410–1,640 kg ha^{-1}), the percentage of infected seeds can be reduced from 75% to 13%, whereas seed yield is only marginally increased (Crittenden and Svec, 1974).

TABLE 10.3 Relationship between cation concentration and infection rate with *Botrytis cinera* Pars. in lettuce

Cation concentration (mg g^{-1} dw)			Infection with *Botrytis*[a]
K	Ca	Mg	
14.4	10.6	3.2	4
23.8	5.4	4.1	7
34.2	2.2	4.7	13
48.9	1.8	4.2	15

Based on Krauss (1971).
[a]*Infection index: 0–5 slight; 6–10 moderate; 11–15 severe.*

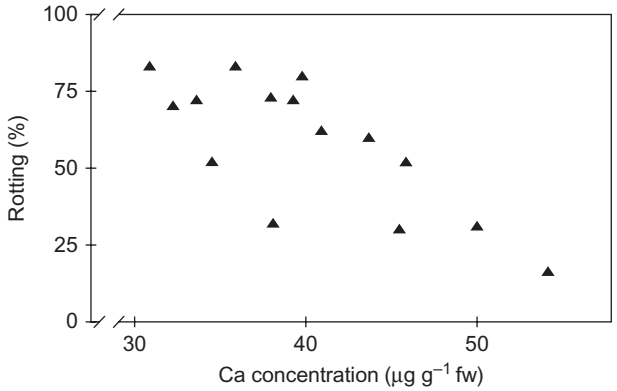

FIGURE 10.12 Percentage of rotted apples (cv Cox orange) due to *Gloesporium perennans* infection after 3 months' storage at 3°C at different Ca concentration of apples. *Modified from Sharpless and Johnson (1977).*

10.3.4 Role of Ca

The Ca concentration of plant tissues affects the incidence of parasitic diseases by three mechanisms. Firstly, Ca plays a key role in recognition of pathogenic invaders at the plasma membrane. Within seconds of pathogen invasion, there is a change in membrane potential and an increase in cytoplasmic Ca concentration which acts as a second messenger. In addition, Ca-transporting proteins may be involved in early defence signals (Yang *et al*., 1997). Secondly, Ca is essential for the stability of biomembranes; thus Ca deficiency increases the efflux of low-molecular-weight compounds (e.g., sugars) from the cytoplasm into the apoplasm (see also Section 6.5). Thirdly, Ca-polygalacturonates are required in the middle lamella for cell wall stability. Many parasitic fungi and bacteria invade plant tissues by producing extracellular pectolytic enzymes such as polygalacturonase, which dissolve the middle lamella. The activity of some polygalacturonase is inhibited by Ca (Bateman and Lumsden, 1965); thus the susceptibility of plants to infection with parasites that rely on these enzymes during their infection process decreases with increasing Ca concentration of the tissue, as shown in Table 10.3. In this experiment, the total concentration in the nutrient solution of three cations, K, Ca, and Mg, was kept constant and only the K^+/Ca^{2+} ratio was altered. Thus, a decrease in the Ca concentration in the plants was correlated with an increase in K concentration. Additional experiments showed that an increase in K concentration does not lead to an increase in infection as long as the Ca concentration is high.

On many acid tropical soils, soybean may develop 'twin stem' abnormality which is induced with low Ca availability. Calcium deficiency results in necrosis of the apical meristem and loss of apical dominance, and the plants are at the same time heavily infected with *Scelerotium* ssp. (Muchovej and Muchovej, 1982). Increasing the Ca supply suppresses fungal infection as well as twin stem formation.

Various parasitic fungi preferentially invade the xylem and dissolve the cell walls of conducting vessels. This leads to plugging of the vessels and subsequent wilting symptoms (e.g., *Fusarium* wilt). In tomato plants infected with *Fusarium oxysporum*, the plants with the highest level of infection have Ca concentration in the xylem sap below about 25 mM (Corden, 1965). Correspondingly, this and other *Fusarium* wilt diseases can be effectively controlled by Ca application as lime or Ca carbonate (Datnoff *et al*., 2007a)

Low Ca concentrations in plant tissues increase their susceptible to parasitic diseases during storage. This is of particular concern for fleshy fruits with their typically low Ca concentrations (Fig. 10.12). Calcium treatment of fruits before storage is therefore an effective procedure for preventing losses from fruit rotting. Since B has similar effects on stabilization of cell walls and membranes as Ca (see also Section 7.7), treatments of fruits before storage should with a combination of B may be more effective than with Ca alone (Xuan *et al*., 2005; Liebisch *et al*., 2009).

10.3.5 Role of Phosphate and Phosphite

Information on the effect of soil P application (in the form of phosphate, PO_4^{3-}) on host resistance is scanty. In general, optimal P supply enhances disease resistance in plants (Prabhu *et al*., 2007). Indeed, Graham and Menge (1982) attributed the suppression of take-all disease in wheat by arbuscular mycorrhiza to an improved plant P uptake and thus plant growth.

In contrast to these less pronounced effects of soil P applications, foliar phosphate applications may be very effective against some airborne pathogens by conferring local or systemic resistance. The resistance is based on release of elictor-active compounds from plant cell walls (Gottstein and Ku, 1989; Walters and Murray, 1992) or initiation of localized cell death known as hypersensitive response (Orober *et al*., 2002); however, the mechanism by

which P induces this response is not clear. Nevertheless, phosphate applied to roots of hydroponically grown cucumber plants enhanced their resistance against powdery mildew (*Sphaerothera fuliginea*), indicating a nutritional-based defence mechanism (Reuveni *et al*., 2000).

Phosphorus in the form of phosphite (PO_3^{3-}), which contains one less oxygen than phosphate, promotes plant growth even in the absence of plant pathogens (Lovatt, 1990; Rickard, 2000). However, recent studies have clearly shown that plants cannot use phosphite as a P source without microbial transformation to phosphate (Thao and Yamakow, 2000; Ratjen and Gerendas, 2009). There is, however, growing evidence that foliar application of phosphite, as phosphoric acid (H_3PO_3) or its salts, can inhibit pathogens such as *Phytophthora* and other members of the *Peronsporales* (Lobato *et al*., 2008; Brunings *et al*., 2009b). In south-western Australia, phosphite is considered an effective agent only against the dieback of natural forests due to *Phytophthora cinnamomi* (Sherarer and Fairman, 2007). It appears that the inhibiting effect of phosphite is due to direct toxic effects on the pathogens and/or inhibition of the metabolism of the pathogen. Of interest is that application of phosphite, for example for suppression of *Phytophthora cinnamomi* in Australian forests on soils with low P availability, results in increased P deficiency in plants by disruption of their typical starvation-induced responses for active phosphate acquisition (Ticconi *et al*., 2001; Lambers *et al.*, 2006; Ratjen and Gerendas, 2009).

10.3.6 Role of other Nutrients

Other macronutrients such as S (Haneklaus *et al.*, 2007) and Mg (Jones and Huber, 2007) can affect plant disease incidence. The mechanisms causing S-induced resistance have not been fully elucidated, but the free cystein pool may be related to resistance and non-protein cystein is a precursor of all of the relevant sulphur-containing metabolites putatively involved in systemic reduced resistance. Furthermore, salicylic acid and H_2O_2 initiate and maintain systemic resistance and salicylic acid accumulation is linked to S metabolism (Haneklaus *et al.*, 2007). Little information is available on direct effects of Mg on pathogenesis (Jones and Huber, 2007).

It is well established that various micronutrients affect parasitic diseases, as reviewed by Bergmann (1992), Huber and Graham (1999), Kirkby and Römheld (2004) and Datnoff *et al.* (2007a). Of the various plant defence mechanisms, those involving phenolics and lignin are the best understood, and the micronutrients B, Mn and Cu play key roles in phenol metabolism and lignin biosynthesis (see also Chapter 7). Micronutrients can also affect resistance indirectly. In deficient plants, defence mechanisms may be impaired, but the plants may also be more suitable feeding substrate. In *Hevea brasiliensis*, Zn deficiency increases leakage of sugars to the leaf surface and increases the severity of infection by an *Oidium* species (Bolle-Jones and Hilton, 1956). Infection of B-deficient wheat plants with powdery mildew is several times greater than in B sufficient plants, and the fungus spreads more rapidly over the leaves of deficient plants (Schütte, 1967; Stangoulis and Graham, 2007), which may be due to increased leakage through the plasma membrane under B deficiency (Cakmak *et al.*, 1995).

TABLE 10.4 Stem melanosis (caused by *Pseudomonas cichorii*) in wheat grown on a soil with low Cu availability without and with different forms of Cu application

Treatment	Cu rate ($kg\,Cu\,ha^{-1}$)	Disease (%)	Grain yield ($kg\,ha^{-1}$)
Nil	–	92	294
$CuSO_4$, banded	10	76	511
$CuSO_4$, incorporated	10	34	2,016
$CuSO_4$, foliar spray	10	6	2,116
Cu-chelate, foliar spray	2	7	2,505

Based on Malhi *et al.* (1989).

Copper has been extensively used as a fungicide, but the amounts required are at least 10–100 times higher than those that are nutritionally required by the plants or used as foliar sprays to correct Cu deficiency. However, increased Cu supply to Cu-deficient plants either to the soil or as foliar application can reduce leaf infections, for example by powdery mildews and ergot (*Claviceps* sp.) in wheat (Evans *et al.*, 2007), or to control stem pathogens (Table 10.4). For suppression of stem and leaf pathogens, foliar Cu application is often more effective than soil application, because of the low availability of Cu applied to soil.

10.4 BACTERIAL AND VIRAL DISEASES

10.4.1 Bacterial Diseases

Bacterial diseases caused by various facultative parasites can be divided into three main types: leaf spot diseases, soft rots and vascular diseases (Grossmann, 1976). In leaf spot diseases (e.g., bacterial leaf blight, *Xanthomonas oryzae*), pathogens usually enter the host plant through the stomata. Having entered the plant, the bacteria spread and multiply in the intercellular spaces. The effect of the nutritional status of the host plant on spread and multiplication

TABLE 10.5 Relationship between the Ca concentration of bean, the activity of pectolytic enzymes in the plant tissue, and the severity of soft rot disease without (−) or with (+) inoculation with *Erwinia carotovora*

	Pectolytic activity (%)				
	Poly galacturonase		Pectate transeliminase		Severity of symptoms (after 6 days)
Inoculation	−	+	−	+	
	Ca concentration ($mg\,g^{-1}$ dw)				
6.8	0	62	0	7	High
16	0	48	0	5	High
34	0	21	0	0	None

From Platero and Tejerina (1976).

of the bacterial pathogen is similar to that on facultative fungal parasites: for example, multiplication and severity of leaf blight is enhanced by K and Ca deficiency, and often (Kiraly, 1976) but not always, by N deficiency (Huber and Thompson, 2007).

Soft rot bacteria release polygalacturonases and related pectolytic enzymes to spread within the host tissue. As mentioned above, Ca is important for membrane stability and inhibits the activity of some polygalacturonases. Hence, in bean the activity of pectolytic enzymes is reduced at high tissue Ca concentrations (Table 10.5).

Bacterial vascular wilt diseases spread within plants through the xylem and lead to 'slime' formation that plugs the vessels ('bacterial wilt'). In tomato, alleviation of Ca deficiency suppresses bacterial canker (Table 10.6). Calcium reduces disease severity in both susceptible and resistant cultivars, indicating that resistance of a cultivar may be dependent on an adequate Ca supply.

Calcium may affect plant resistance to bacterial diseases by stabilization of the middle lamella and through its involvement in hypersensitive responses to bacterial infections. In tobacco, hypersensitive reactions induced by *Pseudomonas syringae* require a strong influx of Ca from the apoplasm into the cytoplasm through Ca channels in the plasma membrane. This leads to enhanced K^+/H^+ exchange, cytoplasm acidification and death of the host cells at the infection site (Atkinson *et al*., 1990) comparable to hypersensitive responses to attacking fungal pathogens (see above).

Infections by endophytic bacteria such as *Xylella* spp. cause little damage, except in plants that are deficient in micronutrients such as Mn and Zn (Yamada *et al.*, 2007). Deficiency of Mn and Zn may be induced by high pH or extensive use of the herbicide glyphosate (Kirkby and Römheld, 2004; Tesfamariam *et al*., 2009; Bott *et al.*, 2011).

TABLE 10.6 Relationship between Ca supply, Ca concentration in shoots and bacterial canker disease (*Clavibacter michiganense* subsp. *Michiganense* (Smith)) in a susceptible (Moneymaker) and a resistant tomato cultivar (Plovdiv 8/12)

	Ca concentration ($g\,kg^{-1}$ dw)		Disease development (% wilted leaves)	
Ca Supply ($mg\,L^{-1}$)	Moneymaker	Plovdiv 8/12	Moneymaker	Plovdiv 8/12
0	1.2	1.4	84	56
100	3.7	4.2	27	12
200	4.3	5.5	37	6
300	4.4	5.8	27	8

Based on Berry *et al.* (1988).

10.4.2 Viral Diseases

Viruses can only multiply in living cells, and their nutritional requirements are restricted to amino acids and nucleotides. Compared with fungal and bacterial diseases and pests, little is known about the effects of plant nutrition on viral diseases. In general, nutritional factors that favour rapid growth and high tissue water content favour viral multiplication. This holds true particularly for N and P (Prabhu *et al*., 2007; Huber and Thompson, 2007), but also for K (Perrenoud, 1977). The relationship between nutrition and viral diseases is often not clear for various reasons. Alleviation of nutrient deficiency may eliminate symptoms of viral disease because the plants 'outgrow' the disease, or the symptoms are hidden. For example, symptoms of sugar beet yellow or potato leaf roll viral infections may disappear with sufficient N supply, even though the plants are severely infected.

In many cases, the effect of the nutritional status of the host plant on viral diseases is indirect via their fungal and insect vectors. It is assumed that about 60% of plant viruses are spread by aphids (Dreyer and Campbell, 1978), and the severity of aphid infestation of plants is strongly affected by nutritional status. In water cress, infection with the fungal pathogen *Spongospora subterranea*, which causes crook root disease, can be depressed by a supraoptimal Zn supply, i.e. levels that exceed requirements for host plant growth (Tomlinson and Hunt, 1987). By controlling *S. subterranea*, the high Zn supply also suppressed watercress chlorotic leaf spot virus that is spread by this fungus.

10.5 SOIL-BORNE FUNGAL AND BACTERIAL DISEASES

The population density of microorganisms on the root surface and in the rhizosphere is several times higher than that in bulk soil (see also Chapter 15). The range of root-associated microorganisms, which externally colonize or invade and infect root tissues, includes various pathogens, while others (e.g., rhizobia or mycorrhiza) can be beneficial for plants. Competition among and repression of microorganisms, as well as chemical barriers (e.g., high concentrations of polyphenols in the rhizodermis; Barz, 1977) and physical barriers (e.g., Si deposition at the endodermis), ensure that microbial invasion of roots, and shoots via the roots, is restricted. Nutrition affects soil-borne fungal and bacterial diseases in various ways. For example, in Norway spruce Mn-deficiency reduces the fungistatic activity against *Fomes annosus* (Fr.) Cook in the inner bark of roots, leading to heart rot disease (Wenzel and Kreutzer, 1971). Supply with high Mn and low N concentrations leads to an increase in the fungistatic activity of the inner bark (Alcubilla *et al*., 1971). The incidence of common scab infection of potato tubers by *Streptomyces scabies* is suppressed either by lowering the soil pH or by application of Mn (Thompson and Huber, 2007 and references cited therein). The suppressive effect on Mn is due to (i) increased resistance of the tuber tissue to the pathogen and (ii) inhibition of the vegetative growth of *S. scabies* before the onset of infection (Huber and Wilhelm, 1988; Thompson and Huber, 2007).

In peanut, pre-harvest pod rot caused by infection with *Pythium myriotylum* and *Rhizoctonia solani* is particularly severe in pods with low Ca concentrations and can be suppressed by soil application of Ca (e.g., as gypsum) (Hallock and Garren, 1968). Calcium deficiency also increases bacterial soft rot disease of potato caused by various species of *Erwinia*. Soft rot can be suppressed by increasing the Ca concentration in the peel (Kelman *et al*., 1989).

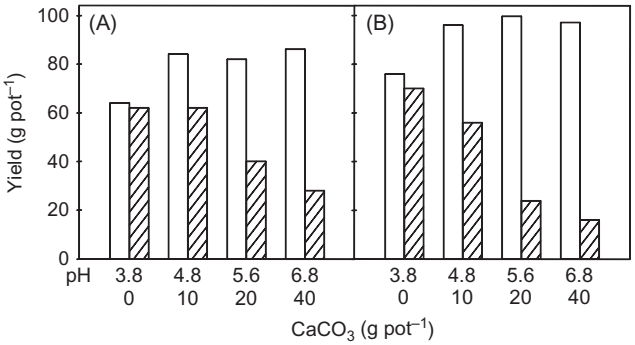

FIGURE 10.13 Straw (A) and grain yield (B) of spring wheat (*Triticum sativum*) with different lime application rates and without or with inoculation with *Gaeumanomyces graminis* var. *tritici* (take-all). Open bars: non-inoculated, striped bars: inoculated. *Modified from Trolldenier (1981).*

The root rot disease of wheat and barley (take-all) caused by *Gaeumannomyces graminis* (take-all) is capable of seriously limiting grain production in many regions of the world, but disease severity can be effectively controlled by nutrition of the host plant (for reviews see Huber and Wilhelm, 1988; Graham and Webb, 1991; Bergmann, 1992; Huber and McCay-Buis, 1993; Thompson and Huber, 2007). The fungus has a growth optimum at pH 7 and is very sensitive to low pH (Römheld, 1990), liming of acid soils therefore increases the risk of root infections and yield losses by take-all. Figure 10.13 shows that, in a soil of pH 3.8, inoculation with *G. graminis* was without significant effect on growth or yield. Liming increased soil pH, enhanced yield in non-infected plants, but reduced yield of infected plants. Manganese availability in the rhizosphere and Mn concentration of root tissues play a key role in root infection and severity of take-all, as well as other soil-borne fungal diseases (Huber and Wilhelm, 1988; Graham and Webb, 1991; Thompson and Huber, 2007). All factors which decrease the availability of Mn increase the severity of take-all (e.g., increase in soil pH by liming, nitrate versus ammonium fertilizer; Table 10.7; see also Section 7.2). Manganese deficiency also increases the severity of rice blast, *Phymatotrichum* root rot of cotton and potato scab (Thompson and Huber, 2007). In Mn-deficient plants, the capacity of the roots to restrict penetration of fungal hyphae into the root tissue by enhanced lignification at the infection site is impaired because Mn is required for the biosynthesis of phenolics, phytoalexins and lignin (Graham and Webb, 1991; see also Section 7.2). Furthermore, *G. graminis* oxidizes Mn, thereby reducing Mn availability to the plant. Differences between isolates in their oxidation power are related to their capacity to decrease Mn availability and cause disease (Wilhelm *et al*., 1990; Thompson and Huber, 2007) with isolates that could not oxidize Mn being avirulent and not able to infect wheat roots.

TABLE 10.7 Cultural conditions affecting Mn availability, nitrification and severity of take-all caused by *Gaeumannomyces graminis*

		Take-all	Nitrification	Mn availability
Soil	Acid	↓	↓	↑
	Alkaline	↑	↑	↓
	Cool, wet	↑		↓
Fertilization	Ammonium N	↓		↑
	Nitrate N	↑		↓
	Cl	↓	↓	↑
	Mn	↓		↑
Inhibition of nitrification		↓	↓	↑
Liming ($CaCO_3$)		↑	↑	↓
Pre-crop	Lupin	↓	↓	↑
	Paddy rice	↓	↓	↑
	Oat	↓		↑
	Soybean or lucerne	↑	↑	↓
Seedbed	Firm	↓	↓	↑
	Loose	↑	↑	↓
Dense seeding		↑		↓
Tolerant cultivars		↓		↑
Animal manure		↑	↑	↓

Based on Thompson and Huber (2007).

TABLE 10.8 Growth, yield and root infection with take-all (*Gaeumannomyces graminis*) of winter wheat grown in a soil with low Cu availability without and with application of Cu and gypsum

Treatment	Dry weight ($g\,pot^{-1}$)	Ears (no. pot^{-1})	Grain ($g\,pot^{-1}$)	Infected plants %
Nil	8.5	2.8	4.3	100
$CuSO_4$, soil	12.7	3.7	6.5	83
$CuSO_4$, foliar	12.8	3.3	6.1	100
$CaSO_4$, soil	9.8	2.7	5.4	83
$CuSO_4$ + $CaSO_4$, soil	17.0	4.7	9.0	0

Based on Gardner and Flynn (1988).

Suppression of take-all by soil application of Mn fertilizers is possible under field conditions (Brennan, 1992a), but has its limitations on calcareous soils, because of rapid oxidation and immobilization of Mn. Foliar Mn sprays are not effective in suppression of root pathogens because of the poor phloem mobility of Mn (see also Chapter 3 and Section 7.2). In contrast, the use of ammonium instead of nitrate N fertilizer is an effective procedure in control of take-all (see below).

Another approach to control take-all is biocontrol by *Pseudomonas fluorescens* spp. or other Mn-reducing organisms such as *Trichoderma* spp., which suppress growth of *G. graminis* var. *tritici in vitro*. However, suppression *in vivo* is related not only to the Mn-reducing capacity of the bacterial strains, but also to Mn-oxidizing potential of the pathogen and the availability of Mn in the soil (Marschner *et al*., 1991; Huber and McCay-Buis, 1993). The reduction of the growth of soil-borne pathogens by some *P. fluorescens* strains may also be related to the production of toxic substances such as cyanide (Sarniguet *et al*., 1992a, b). At high soil pH, the suppression of *G. graminis* by applying ammonium fertilizer is probably not only due to rhizosphere acidification, but also to quantitative and qualitative changes in *P. fluorescens* spp. populations, favouring those which increase Mn availability and are antagonistic to *G. graminis* (Sarniguet *et al*., 1992a, b; McCay, 1998).

The capacity of *P. fluorescens* strains to produce siderophores may also be involved in suppression of soil-borne pathogens by reducing Fe availability to the pathogens (Kloepper *et al*., 1980; Höfte *et al*., 1991). However, siderophores are less important in disease suppression than the release of toxins or anti-fungal compounds (Schippers *et al*., 1990). Root infection with arbuscular mycorrhiza is another factor which may suppress soil-borne pathogens such as *Fusarium oxysporum* in tomato (Dehne and Schönbeck, 1979a, b) or wilt diseases in casuarina (Gunjal and Paril, 1992; see also Chapter 15).

The severity of take-all in wheat is increased not only by Mn deficiency, but also by deficiency of N, P or Cu (Brennan, 1989, 1992b; Table 10.8). The decrease in severity with application of N and P fertilizer to deficient plants is most likely due to a greater tolerance by more vigorous growth rather than an increase in physiological resistance. In contrast, Cu deficiency results in impaired biosynthesis of lignin and supplying Cu fertilizer overcomes this impairment and thereby increases resistance, with soil and foliar applications having different effects (Table 10.8). Foliar application increased yield, but did not depress root infection with take-all indicating that, despite its phloem

mobility, Cu concentrations at the infection sites were not high enough for suppression of the pathogen. The greatest effect was achieved by a combination of Cu and Ca (gypsum) applied to the soil, probably by enhanced desorption and mobility of Cu in the soil. In wheat, Zn deficiency increases the severity of *Rhizoctonia* root rot (Thongbai *et al*., 1993).

10.6 PESTS

Pests are animals such as insects, mites and nematodes which are harmful to plants. In contrast to fungal and bacterial pathogens, they have digestive and excretory systems and their dietary requirements are often less specific. Furthermore, visual factors such as colour of leaves are important for recognition or orientation. For example, many aphid species tend to settle on yellow-reflecting surfaces common with nutrient deficiency (Beck, 1965). The main types of resistance of host plants to pests are: (i) physical (e.g., colour, surface properties, hairs), (ii) mechanical (e.g., fibres, Si), and (iii) chemical/biochemical (e.g., concentration of stimulants, toxins, repellents). Plant nutrition can affect all three factors to varying degrees.

Generally, young or rapidly growing plants are more likely to be attacked by pests than old and slow-growing plants. Therefore, there is often a positive correlation between N application and pest attack, as has been shown for the white-backed plant hopper *Sogatella furcifera* (Horwath) in rice (Salim and Saxena, 1991). In contrast, K deficiency increases pest attack. Although the increased concentration of sugars in K-deficient plants can act as a feeding stimulant (Beck, 1965), most sucking insects such as the rice brown plant hopper (Sogawa, 1982) depend more strongly on amino acids (Dreyer and Campbell, 1987). This is illustrated in Table 10.9 for squash bugs, where N-deficient plants had the lowest density of squash bugs and the number of squash bugs per plant was related to the concentration of total soluble N in leaves. In contrast, the protein concentration of the leaves did not affect pest density (Benepal and Hall, 1967).

The severity of attack by sucking parasites increases with the concentration of amino acids in plants and high N supply or impaired protein synthesis due to deficiencies of K or Zn enhance amino acid concentrations. An example of nutrient imbalance in plants induced by fertilizer application is shown in Table 10.10 for oak trees attacked by cup-shield lice. Magnesium applied alone, or when applied in combination with N and P, increased the nutrient imbalance by inducing K deficiency which resulted in an increase in soluble N concentration (i.e., more favourable conditions for the lice). Application of K, on the other hand, decreased lice numbers. Similar results have been found in citrus infestation by purple and black scale (Chaboussou, 1976).

TABLE 10.9 Relationship between nutrient supply, number of squash bugs (*Anasa tristis*) per plant and soluble N concentration in squash

Nutrient supply	Squash bugs (no. plant^{-1})	Soluble nitrogen (μg g^{-1} fw)
Complete	1.7	32
−N	0.7	5
−P	2.1	94
−K	2.5	99
−S	3.4	144

From Benepal and Hall (1967).

TABLE 10.10 Infestation of oak trees (*Quercus pendula*) by cup shield lice (*Eulecanium refulum* Ckll.) in a soil with low K availability and addition of fertilizers

Fertilizer	Lice density (no. of lice (10 cm stem section)$^{-1}$)
K + Mg	0.7
N + P + K + Mg	0.8
Mg	4.3
N + P + Mg	8.8

Based on Brüning (1967).

The close positive correlation between N supply, amino acid concentration and attack by pests is sometimes generalized for other plants and ecosystems (Scriber and Slansky, 1981; Chapin *et al.*, 1987). However, the interactions between plants and pests are more complex and not confined to the amino acid concentration and the C/N ratio of the plant tissue. This is particularly evident in trees where a pest attack often depends more strongly on the presence of repellents or toxic compounds than on N concentration. For example, in *Salix dasylados* grown at different nutrient concentrations and light intensities, damage of the leaves by the herbivore *Galerucella lineola* was negatively correlated with the phenol concentration (high light >> low light) and the N concentration of the leaves (Larsson *et al.*, 1986). In Scots pine (*Pinus sylvestris*), high N fertilization increased the concentration of N and di-terpenoids in the needles. Di-terpenoids act as deterrents of herbivorous insects and thereby counteract the effect of N on amino acid concentrations in the leaves. Similarly, N fertilization did not affect sawfly and caterpillar feeding on needles or leaves of forest trees (Merker, 1961; Bjorkman *et al*., 1991).

TABLE 10.11 Relationship between B supply, cyanidin concentration and intensity of red spider mite (*Tetranychus pieroei*) attack on oil palm (*Elaeis guieensis*)

B supply ($mg L^{-1}$)	Mites (no. m^{-2})	Feeding holes (no. cm^{-2})	Cyanidin concentration ($\mu g g^{-1}$)
0	1.8	67	2–5
0.5	1.7	60	10–18
5.0	1.2	30	
50	1.0	20	20–32
500	0.9	17	
1,000	0.9	12	

Based on Rajaratnam and Hock (1975).

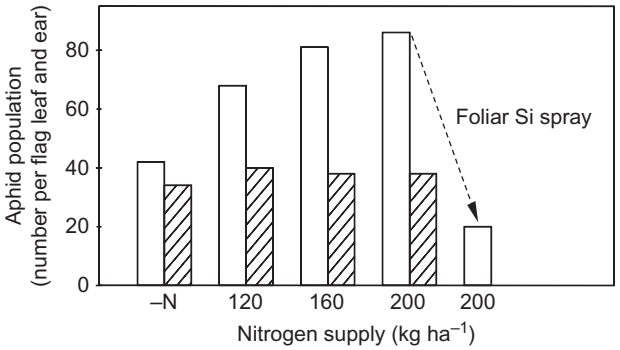

FIGURE 10.14 Population density of two aphid species in winter wheat with different N supply and foliar Si application. Striped bars, *Metopolophium dirhodum*; open bars, *Sitobion avenae*; –N: N-deficient control plant. *Based on Hanisch (1980).*

Differences in leaf concentrations of allelochemicals (products of secondary metabolism involved in interactions with living organisms) are also responsible for the negative relationships between B concentration and attack of oil palm leaves by red spider mites (Table 10.11). In plants without or with very low B supply, the attack was very high, but depressed as the B supply and leaf concentration of the flavonoid cyanidin increased. The positive effect of B supply on cyanidin concentrations can be explained by the fact that B is required for the biosynthesis of cyanidin and related polyphenols.

Silicon in the epidermal cell walls acts as a mechanical barrier to the penetration by the stylet and mandibles of sucking and biting insects. The mandibles of larvae of the rice stem borer are damaged when the Si concentration of rice plants is high (Datnoff *et al*., 2007a). The physical properties of leaf surfaces are also of importance in regulating the severity of attack by sucking insects. Labial exploration of the surface takes place before insertion of the stylet into the tissue (Sogawa, 1982). Changes in the surface properties of leaves were presumably the main reason for a decrease in the attack of wheat plants by aphids when several foliar sprays of sodium silicate were applied (Fig. 10.14). Increasing N supply enhanced the number of *Sitobion avenae* aphids, whereas foliar sprays with Si reduced the number of aphids below that in N-deficient (–N) plants. The results of this experiment also illustrate the difficulties of making generalizations about the relationship between increasing N supply and attack by sucking insects. In contrast to *S. avenae*, which is a typical ear feeder, the density of other aphids species such as *Metoplopium dirhodum*, did not increase with an increasing N supply. Differences in feeding habits and preferences for different plant organs (*M. dirhodum* prefers leaf blades) are possible reasons for the differential response to N supply. In wetland rice, several species of leaf hoppers pose a more serious threat as vectors of viruses than as juice-sucking pests (Beck, 1965). Thus, another important reason for controlling sucking insects is to reduce dissemination of viruses.

The strong depression of aphid populations on leaves after foliar application of Si (Fig. 10.14) is not only due to changes in surface properties of the leaves, but also to an increase in soluble Si within the leaf tissue. Soluble silicic acid, rather than the deposited Si in leaves, is an effective sucking inhibitor of the rice brown plant hopper. Silicon concentrations as low as $10 mg L^{-1}$ appear to be effectively inhibiting sucking insects (Sogawa, 1982). As mentioned above, soluble Si appears to facilitate the rapid deposition of phenolics or phytoalexins at the sites of invasion of plant tissue.

Growth of apple trees can be strongly depressed by nematodes such as cereal cyst nematodes (*Heterodera avenae*) or root lesion nematodes (*Pratylenchus penetrans*). Root exudates might act as signals for recognition or as repellents, but it is not clear whether nutrition plays an important role in either context. There are, however, many examples showing that nematodes depress root growth and activity, thereby influencing nutrient uptake and the nutritional status of the plants. For example, nematodes are mainly responsible for K deficiency in the apple replant disease (Merwin and Stiles, 1989). Nematode attacks have less or no effect on cotton shoot growth at high K availability, but depress shoot growth severely when the K supply in the soil is low even though the total number of nematodes was higher on plants with high K supply (Oteifa and Elgindi, 1986). This is a typical example of an increase in tolerance to pests and diseases resulting from high nutrient supply. This can also be demonstrated for micronutrients (Fig. 10.15). In barley plants grown in a soil with low Mn availability, addition of Mn had no effect on the number of infections (immature females), but

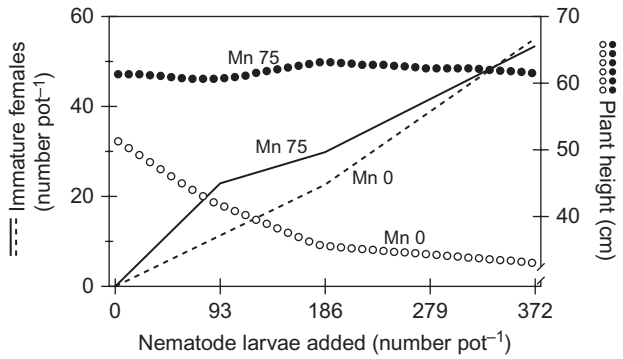

FIGURE 10.15 Number of infections (immature females) and height of barley plants growing in a soil low in manganese supplied without (Mn 0) or with 75 mg Mn $(450\,g)^{-1}$ soil (Mn 75) and different nematode (*Heteroa avenae*) densities. *Based on Wilhelm* et al. *(1985).*

growth was severely depressed only in plants which were not supplied with Mn. In this case, Mn application possibly compensated for the impaired capacity for Mn acquisition caused by nematode infection.

10.7 DIRECT AND INDIRECT EFFECTS OF FERTILIZER APPLICATION ON THE PERFORMANCE OF PLANTS AND THEIR PARASITES

Under field conditions, fertilizers affect the performance of plants and their parasites directly via their effects on plant nutrition and indirectly by changing the biotic and abiotic environment which affects pathogen and pest survival and function. Dense stands and alterations in light interception and humidity within a crop change the microenvironment, thus favouring several foliar pathogens, but increased plant vigour or hastened maturity may reduce other diseases and pests. In addition, the timing of fertilizer application is an important factor especially for N. For example, as shown in Table 10.12, severity of take-all infection of spring wheat is high without N fertilization and is increased by application of ammonium in the autumn, leading to yield depressions because of increased disease severity. In contrast, the same amount of ammonium N supplied in spring suppresses take-all, and high grain yields are obtained. The low yield with split application of N in autumn and spring demonstrates that the effects of N fertilizer application on grain yield was governed more by the effects on take-all than on the N nutritional status of wheat *per se*. Ammonium N applied in the autumn is rapidly nitrified and nitrate intensifies take-all in non-suppressive soils. The use of timed ammonium fertilizer application is therefore a practical approach to suppress take-all, and variations in suppression between years and locations (Christensen *et al*., 1987; MacNish, 1988) are probably related to rate of nitrification prior to N uptake by the crop. An opposite relationship to time of N application is observed with eye spot on winter wheat, where a spring application of N increases this disease (Huber, 1980).

TABLE 10.12 Take-all (*Gaeumanomyces graminis*) root infection and grain yield of winter wheat at different times and rates of ammonium N fertilizer application

Time of application	Rate ($kg\,N\,ha^{-1}$)	Take-all infection (%)	Grain yield ($kg\,ha^{-1}$)
0	0	1.9	2,610
Autumn	83	2.8	1,740
Spring	83	0.1	5,290
Autumn + spring	83 + 28	1.9	2,350

Based on Huber (1989b).

TABLE 10.13 Leaf Si concentration and disease incidence of powdery mildew (*Erysiphe graminis*) in spring wheat grown in soil amended with either lime ($CaCO_3$) or blast furnace lime (BFL) and different forms of N fertilizer

Nitrogen Form	Si concentration (% SiO_2)		Disease incidence (% leaf area affected)	
	$CaCO_3$	BFL	$CaCO_3$	BFL
$Ca(NO_3)_2$	1.2	2.3	27.5	11.5
$(NH_4)_2\,SO_4$	2.1	7.3	18.0	2.0

Recalculated from Leusch and Buchenauer (1988b).

The form of N fertilizer applied may also have other implications on pathogens. Solubility of Si in soils is dependent on various factors and increases, for example, as the soil pH declines. Thus, the Si concentration in plants is not only dependent on Si fertilization but also, at least to some extent, on the form of N fertilizer applied. Compared with Ca nitrate, ammonium sulphate increases the Si concentration in spring wheat and, therefore, depresses the incidence of powdery mildew (Table 10.13). Ammonium application may result in a decrease in soil pH via nitrification or proton release by the roots upon ammonium uptake.

There are also many reports that chloride fertilizer application in amounts similar to those of macronutrients may suppress various diseases: soil-borne diseases such as take-all in wheat (Christensen *et al*., 1987) or root rot (*Cochliobolus sativus*) in barley (Timm *et al*, 1986), and leaf diseases such as leaf rust (*Puccinia recondite*) in wheat (Fixen *et al*., 1986a, b; Elmer, 2007). The mechanism for the disease-suppressive effect of chloride fertilizers is not clear.

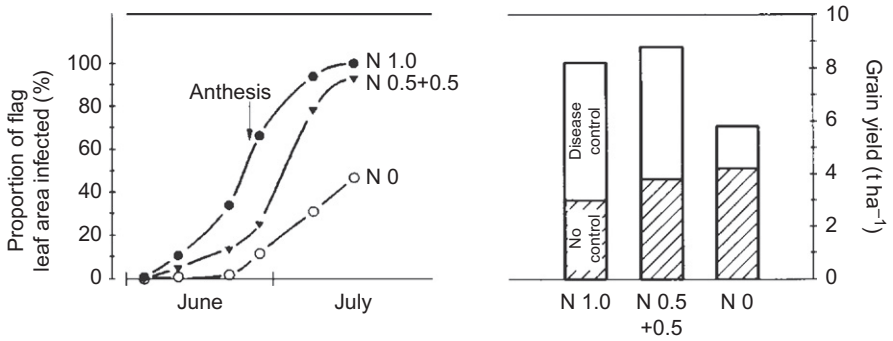

FIGURE 10.16 Yellow rust infections (*Puccinia striiformis* Westend) and grain yield of winter wheat with and without chemical disease control at different rates and timing of N supply (N1.0: 160 kg N ha^{-1} as early dressing, N0.5 + 0.5: 80 kg N early and 80 kg N at anthesis); N0: no N addition. *Based on Darwinkel (1980a).*

TABLE 10.14 Factors affecting N, Mn availability and severity of some diseases (potato scab, rice blast, take-all of cereals, phymatotrichum root rot, maize stalk rot)

		Nitrification	Mn availability	Disease severity
Soil pH	Low	↓	↑	↓
	High	↑	↓	↑
N fertilizer	Ammonium	↓	↑	↓
	Nitrate		↓	↑
Nitrification inhibitors		↓	↑	↓
Metal sulphides		↓	↑	↓
Liming		↑	↓	↑
Manure	Rye green	↓	↑	↓
	Animal	↑	↓	↑
Soil fumigation		↓	↑	↓
Glyphosate herbicide		↑	↓	↑
Seed bed	Loose	↑	↓	↑
	Firm	↓	↑	↓
Irrigation		↓	↑	↓
Low soil water content		↑	↓	↑

After Thompson and Huber (2007).

Chloride may act directly in the plant by improving the water balance and, thereby, tolerance to disease; or indirectly in the soil by inhibition of nitrification or enhanced mobilization of Mn (Graham and Webb, 1991; Elmer, 2007).

Cultural controls used to decrease disease severity may exert their effect by modifying the availability or form of nutrients, particularly N and Mn (Table 10.14). Conditions that inhibit nitrification or increase the availability of Mn can reduce the severity of potato scab, rice blast, take-all, maize stalk rot (*Gibberella*) and *Phymatotricum* root rots (Thompson and Huber, 2007). In contrast, those conditions that stimulate nitrification and decrease Mn availability for plant uptake may increase these diseases.

The various effects of nutritional status and of fertilizer application on disease and pests are of direct relevance to disease and pest control by fungicides, pesticides and other chemicals. Fertilizer application may substitute, or at least reduce, the demand for chemical disease control in some cases, but may increase the demand in others. These interactions are illustrated in Fig. 10.16 for winter wheat naturally infected by yellow rust. In temperate climates, high N application rates to winter wheat early in the growing season favour abundant tillering and dense, tall crop stands, which provide conditions favourable for infection. Rust infection was greatest with a large early single dressing (N 1.0). Split application of N decreased infection in the early growth stages, but fungal growth increased rapidly after the second application (at anthesis). Nevertheless, the disease was significantly postponed by the split N application. In plants not receiving N (N0), infection remained low. Without chemical disease control, stripe rust infection lowered grain yield in all treatments (Fig. 10.16). The extent to which the yield was decreased by the rust, however, differed between treatments; being greater in the treatments receiving N compared to plants without N addition. Thus, without chemical disease control, the highest grain yield was obtained in plants that received no N while with disease control; the highest yield was obtained in plants receiving the split N application. Similar results have been reported for wheat infected with powdery mildew (Darwinkel, 1980b).

Chapter 11

Diagnosis of Deficiency and Toxicity of Nutrients

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SUMMARY

In this chapter, the general relationship between nutrient concentration and plant growth/yield are described followed by a description of visual symptoms of nutritional disorders. Fertilizer recommendations are often based on plant analysis. Therefore this chapter also discusses nutrient concentrations considered to be in the deficient, adequate and toxic range. Of particular interest are the critical deficiency concentrations, i.e. concentrations required to obtain a certain percentage of maximal growth/yield. Examples are given showing that critical deficiency concentrations are dependent on plant age, plant part, concentrations of other nutrients as well as on environmental factors. Other methods of plant analysis such as determination of a fraction of a nutrient in the plant or biochemical methods, for example enzyme activity, are also described. Lastly, the usefulness of plant versus soil analysis is discussed.

11.1 GENERAL

Worldwide, inadequate or lack of recommendation of fertilizer use often results in pronounced yield losses, low product quality and unnecessary environmental impact such as elevated nitrate leaching or gaseous N emissions (Römheld and Kirkby, 2010). Appropriate recommendation becomes increasingly important due to (i) growing demand in the global world market for good quality, (ii) increasing fertilizer costs, (iii) increasing awareness of environmental problems caused by agriculture, and (iv) increasing weather extremes such as drought, flooding and heavy storm events. Short-term variable weather conditions often require a higher nutritional status of crop plants for specific nutrients such as K, Mg or Zn to mitigate at least partly the stress. This, in turn, requires decisions by farmers on selective fertilizer applications (Karim *et al*., 2011).

For a better recommendation of fertilizer use by farmers, correct diagnosis of deficiency is important and should be part of an integrative approach to crop production (Bergmann, 1992; Zorn *et al*., 2006).

11.2 NUTRIENT SUPPLY AND GROWTH RESPONSE

The well-known growth response curve which shows the relationship between growth (dry matter production) and nutrient supply has three clearly defined regions (Fig. 11.1): (1) growth increases with increasing nutrient supply (deficiency range); (2) growth is at its maximum and remains more or less unaffected by changes in nutrient supply (adequate range); (3) growth decreases with increasing nutrient supply (toxicity range).

In crop production, optimal nutrient supply is usually achieved by application of fertilizer. Appropriate and cost-effective fertilizer application requires information on the nutrient availability in the soil, on the one hand, and the

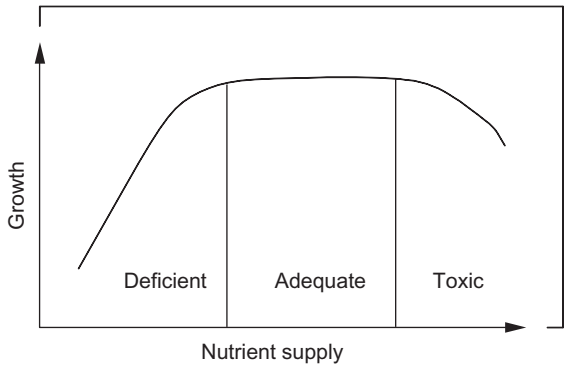


FIGURE 11.1 Relationship between nutrient supply and growth.

Marschner's Mineral Nutrition of Higher Plants. DOI: 10.1016/B978-0-12-384905-2.00011-X

nutritional status of the plants, on the other. The possibilities and limitations of using visual diagnosis and plant analysis together with soil analysis for recommendations on type and amount of fertilizer will be discussed in the following sections.

11.3 DIAGNOSIS OF NUTRITIONAL DISORDERS BY VISIBLE SYMPTOMS

In general, nutritional disorders that reduce growth and yield only slightly are not characterized by specific visible symptoms. Symptoms become clearly visible when a deficiency is acute and the growth rate and yield are reduced. However, there are exceptions. For example, transient visible symptoms of Mg deficiency in cereals, which may be observed under field conditions during stem extension, are without detrimental effect to the final grain yield (Pissarek, 1979). Furthermore, in natural vegetation, many annual and perennial plant species, particularly those adapted to nutrient-poor sites, adjust their growth rate to the most limiting nutrient and, thus, do not develop visual deficiency symptoms (Chapin, 1983, 1988).

Diagnosis based on visible symptoms requires a systematic approach as summarized in Table 11.1. Symptoms appear preferentially on either older or younger leaves, depending on whether the nutrient in question is readily retranslocated (see also Chapter 3). The distribution pattern of symptoms may also be modified by how the deficiency is induced: (i) permanent insufficient supply or sudden interruption of a high supply, or (ii) an interruption of an adequate nutrient supply by a transient drought event (Fig. 11.2).

Chlorosis or necrosis and their pattern are further important criteria for diagnosis. Generally, visible symptoms of nutrient deficiency are more specific than those of nutrient toxicity, unless the toxicity of a nutrient induces a deficiency of another. Visible deficiency symptoms of individual nutrients are described briefly in Chapters 6 and 7. For details (including colour images) of symptoms of nutrient disorders the reader is referred to Bergmann (1992) and Zorn *et al.* (2006) as well as the website www.tll.de/visuplant.

Diagnosis may be particularly difficult in field-grown plants when more than one nutrient is deficient or when

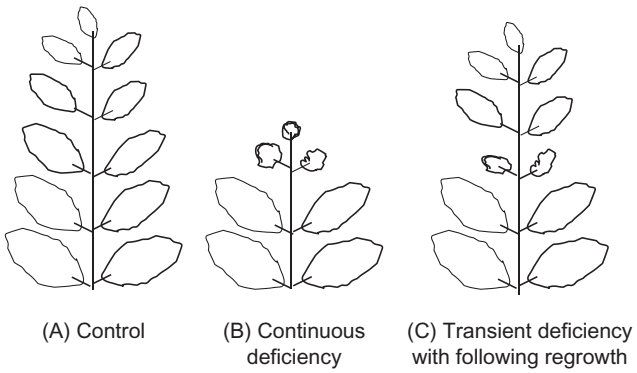


FIGURE 11.2 Schematic presentation of B deficiency symptoms with adequate (A) or continuous (B) or transient deficient supply (C).

TABLE 11.1 Principles of visual diagnosis of nutritional disorders

Plant part	Main symptom		Disorder
			Deficiency
Old and mature leaf blades	Chlorosis	Uniform	N (S)
		Interveinal or blotched	Mg (Mn)
	Necrosis	Tip and marginal scorch	K
		Interveinal	Mg (Mn)
Young leaf blades and apex	Chlorosis	Uniform	Fe (S)
		Interveinal or blotched	Zn (Mn)
	Necrosis (chlorosis)		Ca, B, Cu
	Deformations		Mo (Zn, B)
			Toxicity
Old and mature leaf blades	Necrosis	Spots	Mn (B)
		Tip and marginal scorch	B, salt (spray injury)
	Chlorosis, necrosis		Non-specific toxicity

a deficiency of one nutrient is accompanied by toxicity of another. Such simultaneously occurring deficiencies and toxicities can be found, for example, in waterlogged acid soils, where both Mn toxicity and Mg deficiency may occur. Diagnosis may be further complicated by the presence of diseases, pests and other symptoms caused, for example, by mechanical injuries or spray damage (Bergmann, 1992). In order to differentiate the symptoms of nutritional disorders from these other symptoms, it is important to bear in mind that nutritional disorders always have a typical symmetric pattern: leaves of the same or similar position (physiological age) on a plant show nearly identical patterns of symptoms, and there is a marked gradation in the severity of the symptoms from old to young leaves (Figs. 11.2 and 11.3A). In contrast, symptoms induced by the presence of diseases and pests are non-symmetric or randomly positioned in individual plants as well as within a field, particularly at an advanced phase of infections (Fig. 11.3).

To aid visual diagnosis, it is helpful to acquire additional information, including soil pH, results of soil testing for nutrients, soil water status (dry/waterlogged), weather conditions (low temperature or frost) and the application of fertilizers, fungicides, or pesticides. In some cases, visual diagnosis provides enough information for a recommendation on type and amount of fertilizer to be used. This is true, for example, for foliar sprays containing micronutrients (Fe, Zn or Mn) or Mg. With some exceptions (e.g., Fe deficiency chlorosis), however, visual diagnosis is an inadequate basis for making fertilizer recommendations. Nevertheless, it offers the possibility of focusing further attention on chemical and biochemical analysis of leaves and other plant parts (plant analysis) of selected nutrients. This is particularly important for annual crops where the results are required very quickly.

11.4 PLANT ANALYSIS

11.4.1 General

The use of chemical analysis of plant material for diagnostic purposes is based on the assumption that the growth rate of plants is affected by nutrient concentration in the shoot dry or fresh matter, or the nutrient concentration in the tissue press sap. Element composition of plant tissues can be expressed as *concentration* (e.g., $mg\,g^{-1}\,dw$ or fw) or as *content* (e.g., $mg\,leaf^{-1}$). Depending on the nutrient, plant species and age, the most suitable plant part or organ for analysis differs, as well as whether or not the total concentration or only a certain fraction of the nutrient (e.g., water extractable) should be determined. In general, the nutritional status of a plant is better reflected in the element concentration of leaves than in that of other plant organs. Thus, leaves are usually used for plant analysis. For some species and for certain nutrients, nutrient concentrations may differ considerably between leaf blades and petioles, and sometimes the petioles are a more suitable indicator of nutritional status (Bouma, 1983). In fruit trees, analysis of flowers is thought to be a more sensitive indicator of Fe nutritional status and other disorders than analysis of leaves (Khelil *et al.*, 2010). Analysis of Ca and B in the fruits or even distinct parts of a fruit provides the best indication of quality and storage properties (Liebisch *et al.*, 2009). Under certain climatic conditions, drought stress during seed filling in particular, the Zn concentration in legume seeds may be a

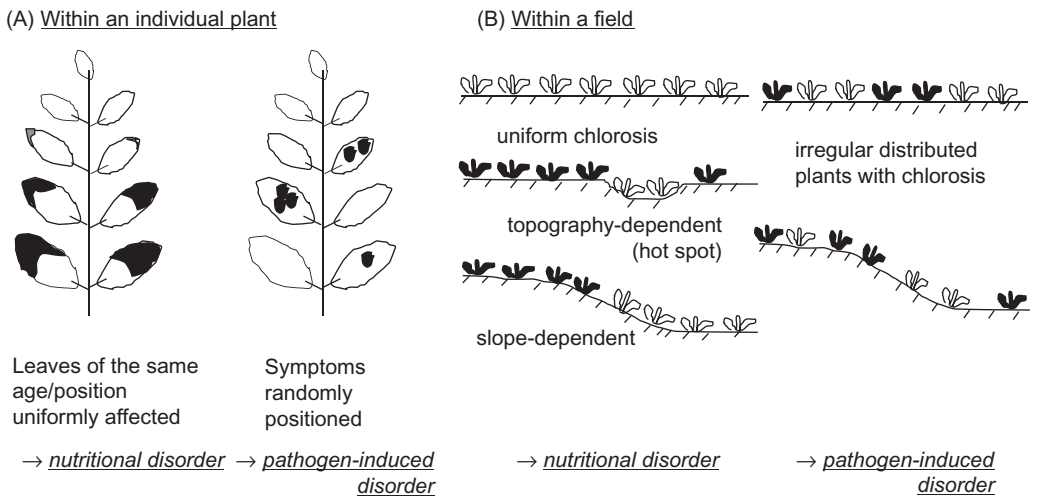


FIGURE 11.3 Schematic presentation of distribution of symptoms (chlorosis, necrosis) with nutritional and pathogen-induced disorders within individual plants (A) and the field (B).

more sensitive indicator of Zn supply than foliar analysis (Rashid and Fox, 1992).

Samples from field-grown plants are often contaminated by dust or sprays and require washing. However, washing may result in loss of elements with the loss differing among nutrients. For B, washing of leaves with water for a few minutes can result in high losses due to passive diffusion of B across plasma membranes (Brown *et al*., 2002). On the other hand, washing with diluted acids or chelating reagents may not completely remove surface contaminations of Fe, Zn and Cu (Masalha, 1998). The greatest challenge in the use of plant analysis for diagnostic purposes is, however, the often short-term fluctuations in nutrient concentrations (e.g., 'dilution effects' by fast growth or transient drought effects). Thus, the nutrient concentration reflecting deficiency, sufficiency or toxicity range varies with environmental factors as well as with plant genotype and developmental stage of plants and leaves. For example, the percentage of dry matter usually increases with age of plants or organs (Walworth and Sumner, 1988) or at elevated CO_2 concentrations because of starch accumulation (Kuehny *et al.*, 1991) which results in a decline in the critical deficiency concentrations (i.e., the concentration required for 90% of dw or yield, CDC; Bouma, 1983; Ohki, 1984) of nutrients in plants. For example, the K concentration on dry matter basis declines with plant age whereas the K concentration in the plant cell sap remains relatively constant during plant growth (Leigh and Johnston, 1983; Milford *et al*., 2008; Römheld and Kirkby, 2010). Strict standardization of sampling procedure and availability of corresponding and suitable reference data are therefore crucial for foliar analysis. The use of nutrient ratios instead of concentration is another approach to meet this difficulty. For reviews on plant analysis for diagnostic purposes the reader is referred to Reuter and Robinson (1986), Westerman (1990), Bergmann (1992), Mills and Benton-Jones (1996) and Breuer *et al.* (2003).

11.4.2 Relationship between Growth Rate and Nutrient Concentration

A representative example on the relationship between plant growth and nutrient concentration in shoots is shown in Fig. 11.4 for Mn. The CDC for Mn of the youngest emerged leaf blade of barley plants is in the range of 10–15 $\mu g\, g^{-1}$ dw. It should be kept in mind that the CDC varies with plant part, for example it may differ between the youngest emerged leaf blade and the sites of new growth, the shoot meristem, where the CDC may be substantially higher.

Usually, 90% of the maximum dry matter yield is taken as a reference point for definition of the CDC of a nutrient (Bouma, 1983; Ohki, 1984). In low input systems, however, the reference point may be 80% of the maximum dry matter yield. Hence, the CDCs are considerably lower (Smyth and Cravo, 1990).

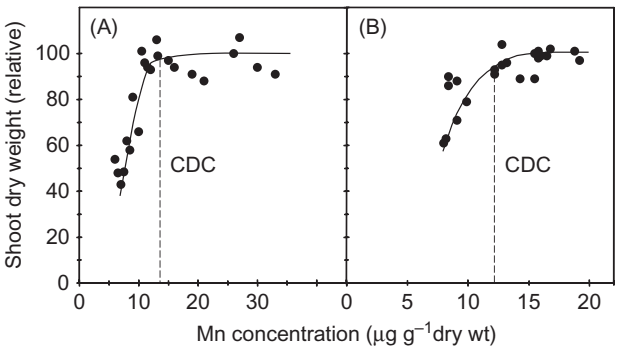
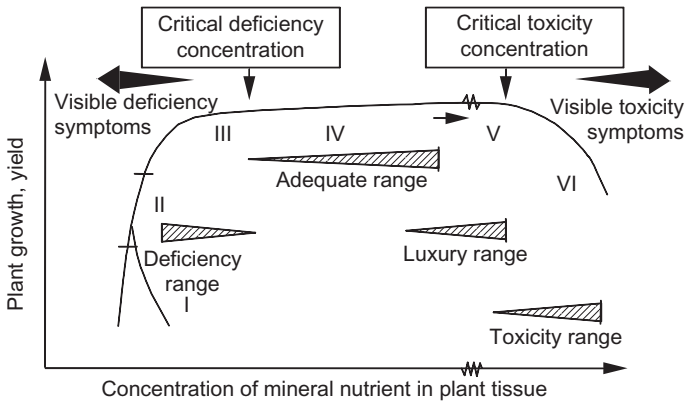


FIGURE 11.4 Relationship between Mn concentrations in youngest emerged leaf blades and shoot dry weight in barley grown in a growth chamber (A) and under field conditions (B). Dashed line indicates the critical deficiency concentration (CDC). *Based on Hannam* et al. *(1987).*

The CDC for plants provided permanently with a low supply may be quite different to those in which a high supply was suddenly interrupted (Scott and Robson, 1990b). Such a sudden interruption may occur, for example, at the onset of transient drought. This leads not only to a changed pattern of symptoms (Fig. 11.2), but also to very high CDCs for various nutrients (Burns, 1992), because fast-growing plants become suddenly completely dependent on remobilization and re-translocation of nutrients.

Therefore, the use of a CDC range instead of a single value is more appropriate. If a single value is used, it should be borne in mind that it encompasses a range of concentrations and that the probability of deficiency or sufficiency increases with the extent of deviation from this single value. The general pattern of relationships between plant growth and nutrient concentrations in plant tissue is shown in Fig. 11.5 in a schematic presentation. There is an ascending part of the curve where growth either increases without change in nutrient concentration (I and II) or where increases in growth and nutrient concentration are closely related (III). This is followed by a more or less level part where growth is not nutrient limited (IV and V) and, finally, by a part where the excessive nutrient concentration causes toxicity and a corresponding decline in growth (VI).

Occasionally, with an extreme deficiency of, for example, Cu (Reuter *et al.*, 1981) or Zn (Howeler *et al.*, 1982b), a C-shaped response curve is obtained (Fig. 11.5, region I, dashed line) in which a nutrient-induced increase in growth rate is accompanied by a decrease in its concentration, which is often referred to as the 'Piper-Steenbjerg' effect (Bates, 1971). A possible explanation for this type of response is a lack of remobilization from old leaves and stem (Reuter *et al.*, 1981).



	I	II	II	III	IV	V	VI
	Deficient		Low		Adequate	High	Toxic
P (g kg^{-1})	<1.6		1.6–2.5		2.6–5.0	5.1–8.0	>8.0
N (g kg^{-1})	<12.6		12.6–17.0		17.1–25.0	25.1–27.5	>27.5
Mn (mg kg^{-1})	<15		15–20		21–100	101–250	>250

FIGURE 11.5 Relationship between nutrient concentrations and growth or yield (*top*) and examples of the nutrient concentration in the dry matter of soybean leaves at different nutrient supply ranges (bottom). *Based on Jones (1967).*

Concentration and dilution effects of nutrients in plants are common phenomena which should be considered when interpreting nutrient concentrations in terms of ion antagonism and/or synergism during uptake; particularly when the nutrient concentrations are in the deficiency or toxicity range (Jarrell and Beverly, 1981). If, for example, the concentrations of two nutrients are in the deficiency range and only one of them is supplied, growth enhancement causes a 'dilution' of the other nutrient (a decrease in its concentration) and severe deficiency is induced without competition in uptake or within the plant.

Central to the use of plant analysis for diagnostic purposes are the critical deficiency and toxicity concentrations (Fig. 11.5). Growth is maximal between the critical deficiency and toxicity concentrations, but for practical reasons the nutrient concentration resulting in 90–95% of maximal growth used rather than maximal growth is used. The nutrient concentrations can be grouped into ranges, as shown in the lower portion of Fig. 11.5 for soybean. If nutrient concentrations are in the adequate range there is a high probability that these nutrients are not growth-limiting factors. Concentrations in the luxury range further decrease the risk that these nutrients will become deficient under conditions unfavourable for root uptake (e.g., dry topsoil) or when the demand is very high (e.g., re-translocation to fruits). However, there is a greater risk of growth reduction by direct toxicity of these nutrients or by inducing a deficiency of other nutrients, i.e. nutrient imbalance. In defining critical toxicity concentrations the non-uniform distribution of a nutrient within a plant organ has to be considered, for example of B in leaf blades.

11.4.3 Developmental Stage of Plant and Age of Leaves

In general, for recommendation of fertilizer use, leaves or needles should be collected during the period of the most intensive growth with the highest nutrient demand (Bergmann, 1992, 1993). However, after nutrient supply, the physiological age of a plant or plant part is the most important factor affecting nutrient concentration in the plant. With the exception of Ca and B, there is usually a decrease in nutrient concentration as plants and organs age. This decline is caused mainly by a relative increase in the proportion of structural material (cell walls and lignin) and of storage compounds (e.g., starch), as shown, for example, in fast growing seedlings for Fe (Venkat-Raju and Marschner, 1981) and other micronutrients (Drossopoulos *et al.*, 1994). However, such a dilution in nutrient concentration may not occur if fast growth is inhibited by environmental factors. Such inhibition of leaf expansion growth can result in very high concentrations of, for example, Fe in youngest leaves of grapevine (Häussling *et al.*, 1985) (Fig. 11.6).

Due to the increasing concentration of structural material with plant age, the adequate or critical deficiency range is lower in old than in young plants. For example, in grain sorghum the CDC of P in the leaf dry matter decreased from about 4 to 2 g kg^{-1} during the growing

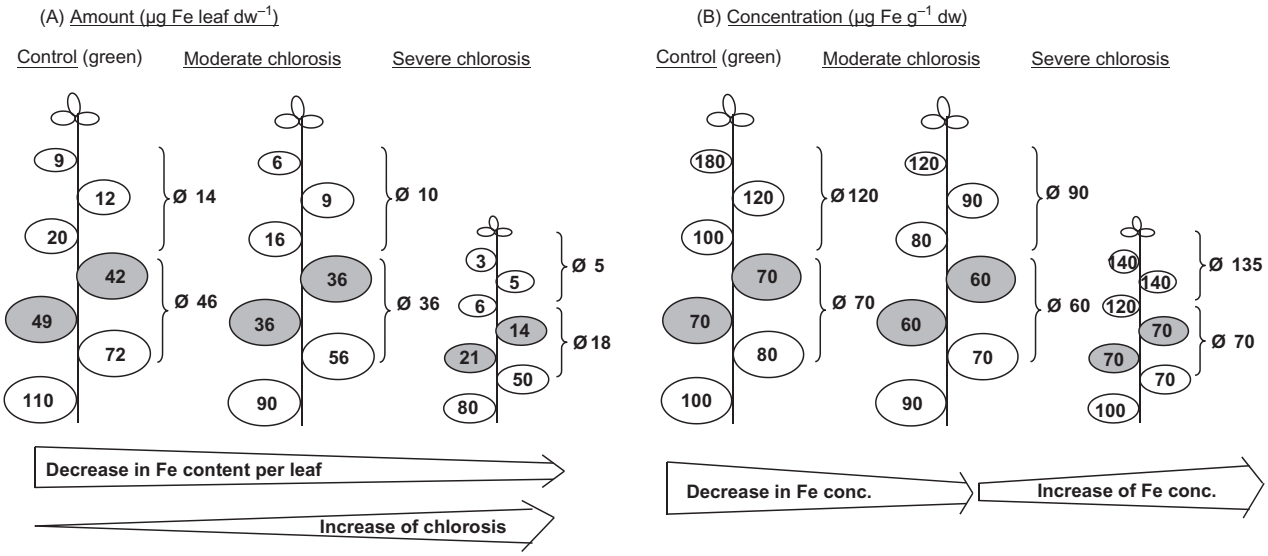


FIGURE 11.6 Schematic presentation of the amount (µg leaf^{-1}) and concentration (µg g^{-1} dw) of Fe in leaves of grapevine with different extent of chlorosis in relation with leaf expansion growth. *Based on Römheld (2000).*

TABLE 11.2 Critical deficiency concentration for Cu for maximum yield in subterranean clover whole plants or youngest open leaf blade at different plant ages

	Critical deficiency concentration (mg kg^{-1} dw)				
	Age of plants (d after sowing)				
	26	40	55	98	Early flowering
Whole plant tops	3.9	3.0	2.5	1.6	1.0
Youngest open leaf blade	3.2	3	3	3	3

Based on Reuter *et al.* (1981).

season (Myers *et al.*, 1987). In field-grown barley, the shoot K concentration decreased from 50–60 g kg^{-1} in young plants to about 10 g kg^{-1} at maturation, although the plants were well supplied with K (Leigh *et al.*, 1982). In this case, the decline in concentration was exclusively a 'dilution effect' as the K concentration in the tissue water (i.e., the vacuolar solution) remained fairly constant at ~100 mM throughout the season.

Complications arising from changes in the CDC with age can be minimized by tissue sampling at specific physiological ages. For example, as shown in Table 11.2, the CDC of Cu in the whole clover tops decreases with age, but remains fairly constant throughout the season at ~3 µg g^{-1} in the youngest leaf blades.

The use of the youngest leaves, however, is suitable only for those nutrients which either are not re-translocated or are re-translocated to only a very limited extent from the mature leaves to areas of new growth, i.e. when deficiency occurs first in young leaves and at the shoot apex (Table 11.1). The situation is different for K, N and Mg since the concentrations of these nutrients are maintained relatively constant in the youngest expanded leaves by translocation from the mature leaves. Thus, the mature leaves are a better indicator of the nutritional status of a plant, as shown for K in Fig. 11.7. The K concentration in the youngest leaf is not a suitable indicator because the K concentrations indicating deficiency and toxicity vary only between 30 and 35 g kg^{-1}, respectively, compared with 15 and 55 g kg^{-1} in mature leaves. This illustrates the necessity of using mature leaves to assess the nutritional status for nutrients which are readily re-translocated in plants.

If young and old leaves of the same plant are analyzed separately, additional information can be obtained on the nutritional status of those nutrients which are readily re-translocated. For example, a higher K concentration in the mature leaves than in young leaves indicates luxury consumption or even toxicity. The reverse gradient, a higher concentration in the young leaves, is an indicator of the

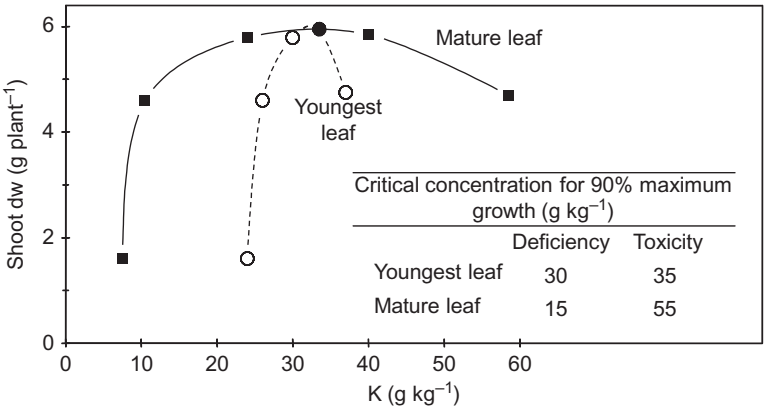


FIGURE 11.7 Relationship between shoot dry weight and K concentrations of mature and youngest leaves of tomato plants grown in nutrient solution with different K concentration and critical concentrations for 90% of maximum growth.

TABLE 11.3 Critical deficiency and adequate concentrations of NO_3-N in press sap of leaf sheath from basal stem at different developmental stages of maize and estimated amounts of NO_3-N stored in the above-ground biomass

	Concentration range (mg NO_3-N L^{-1})		Estimated stored (kg NO_3-N ha^{-1})	
Developmental stage	Critical deficiency	Adequate	Critical deficiency	Adequate
4–5 leaf stage	800	1,400	3.6	6.3
Onset of shooting	375	700	6.2	11.6
Shooting	250	550	7.0	15.3
Heading	250	550	10.6	23.3

Based on Geyer and Marschner (1990) and Geyer, unpublished.

transition stage between the adequate and deficient ranges; if this gradient is steep, a latent or even acute deficiency may exist. Such comparisons between young and old leaves are particularly useful when relevant reference data on critical concentrations are lacking (e.g., for a species or cultivar). If toxicity is suspected, the old leaves are the most suitable organs for plant analysis because toxic elements are often accumulated in the older leaves.

When choosing a given plant organ such as the most recently developed, fully expanded leaf for analysis it should be taken into account that the CDC value will decline throughout plant development, even when expressed as a concentration in the plant sap. For K, for example, in soybean the CDC decreases between podset and podfilling from 65 to 29 mM (Bell *et al.*, 1987). This decline during plant development is particularly evident for nitrate which acts as a storage form of N in the leaves and as an indicator of the N nutritional status of the plants. In petioles of potato leaves the CDC of nitrate-N decreases from 27 g kg^{-1} dw at the onset of tuberization to 10–16 g kg^{-1} in the later stages (Williams and Maier, 1990) and in the midribs of cauliflower from 11 g kg^{-1} at the 4–6 leaf stage to 1.5 g kg^{-1} at preharvest (Gardner and Roth, 1990). A similar decline occurs for sulphate as the main storage form of S in plants (Huang *et al.*, 1992c). For changes in nutrient concentrations during development in various crops, the reader is referred to Bergmann (1993).

This decline in CDC for a given organ with age can occur for various reasons. For example, as plants become older there is a decrease in demand for nutrients for new growth. However, the main reason is the increase in total shoot biomass and, thus, storage capacity of nutrients in the shoots, as illustrated in an example for maize in Table 11.3. Between the 4–5 leaf stage and heading, the CDC of nitrate-N in the press sap as well as the concentrations considered as adequate decline during plant development. The increasing amount of stored N in the larger plants can act as an internal buffer and maintain similar growth rates for several days when supply from the soil declines. Using a model which takes into account changes in growth rates and biomass as a parameter for internal demand, a single critical leaf sap concentration of 380 mg nitrate-N L^{-1} was calculated for Brussels sprouts at all growth stages and over various growing seasons (Scaife, 1988).

Compared with the changes in the nutrient concentration in annual species, the fluctuations throughout the growing season of the nutrient concentration of leaves and needles of trees are relatively small because of the nutrient buffering capacity of twigs and trunk. In evergreen trees, the simultaneous analysis of leaves or needles differing in age provides more reliable data which is little affected by short-term fluctuations (Table 11.4). With increasing age of the needles, the concentration of all macronutrients decreased, except that of Ca. This decrease may in part indicate remobilization, but is presumably mainly an expression of a dilution effect resulting from increased lignification of the old needles. Only with Ca is dilution overcompensated for by a high influx into the old needles via the transpiration stream. With the exception of Mg, the data in Table 11.4 indicate that the trees are well supplied with macronutrients. In Norway spruce, the Si concentration in the needles also increases with needle age (Wyttenbach *et al.*, 1991) as it is, together with Ca, transported via the transpiration stream.

TABLE 11.4 Nutrient concentration of Norway spruce (***Picea abies*** Karst) needles of different age

Nutrient	Age of needles (years)			
	1	2	3	4
	($g\,kg^{-1}$)			
N	17.9	17.6	14.6	12.2
P	2.0	1.7	1.4	1.3
K	6.3	5.6	4.7	4.4
Mg	0.4	0.4	0.3	0.3
Ca	2.8	4.0	5.0	5.9

Based on Bosch (1983).

11.4.4 Plant Species

Adequate and CDC concentrations differ between plant species even when comparing the same organs at the same physiological age. These variations are mainly based on differences in the plant metabolism and plant composition, for example differences in the role of Ca and B in cell walls. When grown under the same conditions the CDC of B in fully expanded youngest leaf is (in $\mu g\,g^{-1}$ dw) 3 in wheat, 5 in rice, but as high as 25 in soybean and 34 in sunflower (Rerkasem *et al.,* 1988). Native plant species from nutrient-rich habitats may have higher CDC of K in the shoots (~100 mM) than species from nutrient-poor habitats (~50 mM; Hommels *et al.*, 1989a). Representative data for adequate nutrient ranges of selected species are given in Table 11.5. More extensive and detailed data, including deficiency and toxicity concentrations, can be found in Chapman (1966), Bergmann and Neubert (1967), Bergmann (1992) and Mills and Benton-Jones (1996).

As shown in Table 11.5, the concentrations of macronutrients in the adequate range are of similar order of magnitude in the plant species; an exception is Ca, the concentration of which is lower in monocotyledons. In all species, the adequate range is relatively narrow for N, because luxury concentrations of N have negative effects on growth and plant composition (see also Section 6.1). In apple leaves, for example, an N concentration of more than $24\,g\,kg^{-1}$ often affects fruit colour and storage adversely (Bould, 1966). On the other hand, the adequate range for Mg is usually broader, due mainly to competing effects of K; at high K concentrations, high Mg concentrations are also required to ensure an adequate Mg nutritional status.

The concentrations of micronutrients in the adequate range vary by a factor of 2 or more (Table 11.5). Manganese shows the greatest variation, indicating that leaf tissue is capable of buffering fluctuations in the root uptake of Mn. This is probably an evolutionary adaption because in plants growing in soil, fluctuations in uptake Mn may be stronger than those of other nutrients, depending on variations in soil redox potential and thus on the concentrations of Mn^{2+} (Section 7.2).

The data given in Table 11.5 are average values and should be regarded as a guide as to whether a nutrient is in the deficient, adequate, or toxic range. Analyzing only one or a few nutrients may be insufficient because possible nutrient interactions cannot be adequately considered.

The CDC of Na and Cl are in general closely related to genotypic differences in salt tolerance. The interpretation of these concentrations is complicated because in saline substrates a reduction in growth is often caused primarily by effects on the water balance of plants and not necessarily by direct toxicity of Na or Cl in the leaf tissue (Section 17.6).

11.4.5 Nutrient Interactions and Ratios

There are a whole range of non-specific as well as specific interactions between nutrients in plants (Robson and Pitman, 1983) which affect critical concentrations. An example of a non-specific interaction is shown in Table 11.6 for N and P. The CDC of N increases as the P concentration increases and vice versa. Similarly in maize, at low P concentration an increase in N concentration of the ear leaf from 21 to $29\,g\,kg^{-1}$ had little effect on yield, but at high P concentration yield continued to increase as ear leaf N concentrations rose well above $30\,g\,kg^{-1}$ (Sumner and Farina, 1986).

TABLE 11.5 Nutrient concentrations in the adequate range of some annual and perennial species

	Concentrations									
	($g\,kg^{-1}$ dw)					($mg\,kg^{-1}$ dw)				
	N	P	K	Ca	Mg	B	Mo	Mn	Zn	Cu
Spring wheat (whole shoot, booting stage)	30–45	3.0–5	29–38	4–10	1.5–3	5–10	0.1–0.3	30–100	20–70	5–10
Ryegrass (whole shoot)	30–42	3.5–5	25–35	6–12	2–5	6–12	0.15–0.5	40–100	20–50	6–12
Sugar beet (mature leaf)	40–60	3.5–6	35–60	7–20	3–7	40–100	0.25–1.0	35–100	20–80	7–15
Cotton (mature leaf)	36–47	3–5	17–35	6–15	3.5–8	20–80	0.6–2.0	35–100	25–80	8–20
Tomato (mature leaf)	40–55	4–6.5	30–60	3–4	3.5–8	40–80	0.3–1.0	40–100	30–80	6–12
Alfalfa (upper shoot)	35–50	3–6	25–38	1–2.5	3–8	35–80	0.5–2.0	30–100	25–70	6–15
Apple (mature leaf)	22–28	1.8–3	11–15	13–22	2–3.5	30–50	0.1–0.3	35–100	20–50	5–12
Orange (*Citrus* spp.) (mature leaf)	24–35	1.5–3	12–20	30–70	2.5–7	30–70	0.2–0.5	25–125	25–60	6–15
Norway spruce (1–2-year-old needles)	14–17	1.3–2.5	5–12	3.5–8	1–2.5	15–50	0.04–0.2	50–500	15–60	4–10
Oak, Beech (mature leaves)	19–30	1.5–3	10–15	3–5	1.5–3	15–40	0.05–0.2	35–100	15–50	6–12

Based on Bergmann (1992).

TABLE 11.6 Critical deficiency concentration (CDC) of N and P in *Araucaria cunninghamii* at different foliage P and N concentrations

Foliage P concentration	CDC of N	Foliage N concentration	CDC of P
	($g\,kg^{-1}$)		
0.6	10.7	6.0	0.7
0.9	11.8	10.5	0.8
1.2	12.4	13.5	1.0
1.6	13.1	16.5	1.1
2.1	13.5	18.0	1.2

Based on Richards and Bevege (1969).

Unspecific interactions between two nutrients are important when the concentrations of both are at or near the critical deficiency concentrations. Increasing the supply of only one nutrient stimulates growth, which in turn can induce a deficiency of the other by dilution. Optimal ratios between nutrients in plants are therefore often as important as absolute concentrations. For example, a ratio of N to S of ~17 is considered to be adequate for the S nutrition of wheat (Rasmussen *et al.*, 1977) and soybean (Bansal *et al.*, 1983). However, optimal ratios considered alone are insufficient, because they can also be obtained when both nutrients are in the deficiency or the toxicity range (Jarrell and Beverly, 1981).

Specific interactions which affect CDC were discussed in Chapters 6 and 7; therefore, only two examples are given here: (i) competition between K and Mg at the cellular level, which may lead to K-induced Mg deficiency; and (ii) replacement of K by Na in natrophilic species, which has to be considered in the evaluation of K concentration.

Specific interactions are also important in evaluating CDCs. The CDC of Mn, for example, differs among species and cultivars of a species (Section 7.2), but also within the same cultivar, depending on Si supply. In bean leaves, the CDC of Mn can increase from $100\,mg\,kg^{-1}$ in the absence of Si to ~$1{,}000\,mg\,kg^{-1}$ in the presence of Si (Horst and Marschner, 1978a) and by a factor of 3–4 in different cowpea genotypes (Horst, 1982).

Due to the problems arising from different CDCs during plant development, and the importance of nutrient ratios in plant analysis for diagnostic purposes, Beaufils' Diagnosis and Recommendation Integrated System (DRIS) was developed. This system is based on a large amount of data on plant nutrient concentrations of (mainly macronutrients) which was used to calculate optimal nutrient ratios (so-called *nutrient indices*) – for example, ratios of N/P, N/K, etc. (Sumner, 1977). The nutrient indices calculated through DRIS are less sensitive to changes during leaf maturation and ontogenesis, but depend to some extent on location. For example, for maize ear leaf tissue N/P indices are on average 10.1, but 8.9 for South Africa and 11.1 for the south-east of the USA (Walworth and Sumner, 1988). This system requires a large number of data on concentrations of different nutrients in the plants from different locations and years. The calculated ratios are thus mean values obtained from several thousand field experiments. For certain crops and under certain conditions (high yielding sites, large-scale farming), the higher analytical input may pay off by permitting a refinement in the interpretation of the data in terms of fertilizer recommendations, as has been demonstrated for sugar cane (Elwali and Gascho, 1984), maize and fruit trees (Walworth and Sumner, 1988). However, recommendations based on DRIS are not always accurate (Reuter and Robinson, 1986), and it is certainly not the method of choice in cropping systems with a wide diversity of annual species, or low input and small-scale farming systems.

11.4.6 Environmental Factors

Fluctuations in environmental factors such as temperature and soil water content can affect the nutrient concentration of leaves considerably. These factors influence both the availability and uptake of nutrients by the roots and the shoot growth rate. Their effects are more distinct in shallow-rooted annual species than in deep-rooted perennial species, which have a higher nutrient buffer capacity within the shoot. This aspect must be considered in interpreting of critical deficiency and toxicity concentrations in leaf analysis. If fluctuations in soil water content are high, then for a given plant species, the CDC of nutrients such as K and P are higher in order to ensure a higher capacity for re-translocation during periods of limited root supply. The effects of irradiation and temperature on the nutrient concentration of leaves are described in detail by Bates (1971).

For example, under high-light intensity, the CDC in leaves of B and Zn are higher than under low-light intensity (Sections 7.4 and 7.7). In tomato, the CDC of P in mature leaves increases from 1.8 to $3.8\,mg\,kg^{-1}$ dw when the external salt concentration is increased from 10 to 100 mM (Awad *et al.*, 1990). The physiological mechanism for this higher internal requirement of P is not clear, involvement in osmotic adjustment in the mature leaves, or restricted re-translocation to expanding leaves may be involved. In addition, it should be taken into account that strong rainfall or irrigation can result in high losses of nutrients such as K and Mg from leaves and inducing K and Mg deficiency.

TABLE 11.7 Relationship between dry matter production and N concentration of C3 (***Lolium perenne*** and ***Phalaris tuberose***) and C4 grasses (***Digitaria macroglossa*** and ***Paspalum dilatatum***)

N supply ($kg\,ha^{-1}$)	Dry matter ($g\,pot^{-1}$)		N concentration ($g\,kg^{-1}$)	
	C3	C4	C3	C4
0	11	22	18.2	9.1
67	20	35	26.3	11.8
134	27	35	27.7	16.1
269	35	48	27.8	20.0

Based on Colman and Lazemby (1970).

11.4.7 Nutrient Efficiency

Genotypical differences in the CDC of a nutrient can also be due to differences in the utilization of a nutrient. In a physiological sense, this may be expressed in terms of unit dry matter produced per unit nutrient in the dry matter (e.g., $g\,P\,kg^{-1}\,dw$). As an example, the difference in N efficiency between C3 and C4 grasses is shown in Table 11.7. Per unit leaf N, C4 grasses produce more dry matter than C3 species; this is also observed in other comparisons of C3 and C4 grasses (Brown, 1985). The higher N efficiency of C4 species may be related to the lower investment of N in enzyme proteins used in chloroplasts for CO_2 fixation. In C4 species, only 5–10% of the soluble leaf protein is found in RuBP carboxylase, compared with 30–60% in C3 species (see also Chapter 5). The lower CDC of N in C4 plants is of advantage for biomass production on N-poor sites, but not necessarily of advantage in view of the nutritional quality of forage (Brown, 1985).

Differences in the utilization of nutrients are also found between cultivars, strains and lines of a species. These differences are a component of the nutrient efficiency in general as discussed in detail in Section 17.2. In agronomic terms, nutrient efficiency is usually expressed by the yield differences of genotypes growing in a soil with insufficient amounts of nutrients. In many cases, high nutrient efficiency is related primarily to root growth and activity, but may also be due to the transport from the roots to the shoots (Läuchli, 1976b). There are fewer examples indicating a higher nutrient efficiency in terms of utilization within the shoots – for example, utilization of P in bean (Whiteaker *et al.*, 1976; Youngdahl, 1990) and maize genotypes (Elliott and Läuchli, 1985), K in bean and tomato (Shea *et al.*, 1967; Gerloff and Gabelman, 1983) and Ca in tomato (English and Barker, 1987; Behling *et al.*, 1989).

In principle, higher nutrient efficiency, as reflected by lower CDC, in one genotype compared to another genotype of the same species can be based on various mechanisms:

1. Higher rates of re-translocation during either vegetative or reproductive growth, for example Zn in maize (Massey and Loeffel, 1967), N in pearl millet (Alagarswamy *et al.*, 1988) or P in bean (Youngdahl, 1990).
2. Higher nitrate reductase activity in the leaves and thus more efficient utilization of N for protein storage, for example in wheat grains (Dalling *et al.*, 1975) and potato tubers (Kapoor and Li, 1982).
3. Higher proportion of replacement of K by Na and thus lower CDC of K, for example in tomato (Gerloff and Gabelman, 1983).
4. Lower proportion of nutrients which are not – or only poorly – available for metabolic processes, either due to compartmentation or chemical binding, for example P in maize genotypes (Elliott and Läuchli, 1985). This aspect is particularly relevant for Ca; in efficient genotypes of tomato a higher proportion of Ca is translocated to the shoot apex where more Ca also remains in the water-soluble fraction (Behling *et al.*, 1989). The lower CDC of $2.5\,g\,Ca\,kg^{-1}$ in the shoot of an efficient tomato genotype compared to $4.0\,g\,Ca\,kg^{-1}$ in an inefficient genotype was associated with higher K concentrations in the shoot of the inefficient genotype (English and Barker, 1987), stressing the importance of physiologically based nutrient ratios for the CDC.
5. Differences in the ratio of vegetative shoot growth (source) to the growth of reproductive or storage organs (sink). This aspect is probably in part responsible for the general pattern in modern cultivars of many crop species with a high harvest index (harvestable product per unit shoot mass) in which the CDC of nutrients in the leaves are usually higher than those of traditional cultivars with greater vegetative growth.

11.4.8 Total Analysis versus Fractionated Extraction

Usually the total concentration of a nutrient is determined in plant analysis (e.g., after ashing). However, determination of only a fraction of the total amount – for example, that is soluble in water or in dilute acids or chelators – sometimes provides a better indication of the nutritional status. In terms of plant analysis as basis of fertilizer recommendations this is particularly true for nitrate which is an important storage form of N in many plant species (see also Section 6.1). In those species, the nitrate concentration is usually a better indicator of the N nutritional status than the total N concentration, and a better basis for recommendations of top dressing of N fertilizers.

TABLE 11.8 Total and soluble Ca (Ca minus oxalic acid) and oxalic acid concentration of two cultivars of Burley tobacco differing in susceptibility of Ca deficiency

		Concentration in buds (meq g^{-1} dw)			Concentration in upper leaves (meq g^{-1} dw)		
Cultivar	Plants with Ca-deficiency symptoms (% of total)	Ca	Oxalic acid	Soluble Ca	Ca	Oxalic acid	Soluble Ca
Ky 10	0	0.25	0.08	0.17	0.28	0.11	0.17
B 21	50	0.23	0.16	0.07	0.30	0.15	0.15

Based on Brumagen and Hiatt (1966).

This method is a satisfactory predictor of responsiveness of a crop to N fertilizer, for example, in winter cereals (Wollring and Wehrmann, 1990), irrigated wheat (Knowles *et al.*, 1991), potato (Westcott *et al.*, 1991), cabbage (Gardner and Roth, 1989) and other vegetable crops (Scaife, 1988). There are only a few cases where this was not the case (Fox *et al.*, 1989). This method is not suitable for plant species which preferentially reduce nitrate in the roots (e.g., members of the Rosaceae), or when ammonium is supplied and taken up prior to nitrification in soils. The latter may occur in soils high in organic N with high mineralization rates during the stages of high N demand of the crop.

In Norway spruce, determination of the arginine concentration in needles may be a better indicator than the total N concentration for assessing the N nutritional status and particularly of nutrient imbalances in stands with different levels of atmospheric N input (Ericsson *et al.*, 1993).

For assessing the S nutritional status of plants the concentration of sulphate as main storage form of S is also a better indicator than the total S concentration. In various legume species the CDC of SO_4-S in fully expanded leaves decreases during ontogenesis, for example in alfalfa from 3.9 to 1.0 g kg^{-1} (Huang *et al.*, 1992c). In some cases, the ratio of SO_4-S to total S may even be a better indicator, for example in wheat (Freney *et al.*, 1978) or rice (Islam and Ponnamperuma, 1982).

There are conflicting reports on the suitability of using only the fraction of inorganic (or readily extractable) P, instead of total P, as diagnostic criteria of the P nutritional status of plants. This approach seems to be suitable in grapevine (Skinner *et al.*, 1987), but not for subterranean clover (Lewis, 1992).

Determination of only a fraction of a nutrient may allow better characterization of the reserves stored in plants (e.g., nitrate, sulphate) and also of the physiological availability of a nutrient in the plant tissue. For example, extraction of leaves with diluted acids or chelators of Fe^{2+} for determination of the so-called 'active iron' may improve the correlations between Fe and chlorophyll concentrations in leaves in field-grown plants, but not necessarily so in plants grown under controlled environmental conditions in nutrient solutions (Lucena *et al.*, 1990). Determination of water-extractable Zn in leaves may better reflect the Zn nutritional status of plants than total Zn (Rahimi and Schropp, 1984), particularly in plants suffering from P-induced Zn deficiency (Cakmak and Marschner, 1987).

Another example of the advantage of determination of a fraction of a nutrient for characterization of physiological availability is shown in Table 11.8. Differences in the susceptibility of tobacco cultivars to Ca deficiency were not related to the total Ca concentration but to the soluble fraction in the buds. These differences were caused by variations in the rate of oxalic acid synthesis and thus in the precipitation of sparingly soluble Ca oxalate. Accordingly, the critical deficiency level of total Ca was higher in B 21 than in Ky 10.

11.5 HISTOCHEMICAL AND BIOCHEMICAL METHODS

Nutritional disorders are generally related to typical changes in the fine structure of cells and their organelles (Vesk *et al.*, 1966; Hecht-Buchholz, 1972; Niegengerd and Hecht-Buchholz, 1983) and of tissue. Light microscopic studies on changes in anatomy and morphology of leaf and stem tissue can be helpful in the diagnosis of deficiencies of Co, B and Mo (Pissarek, 1980; Bussler, 1981a). A combination of histological and histochemical methods is useful in the diagnosis of Cu and P deficiencies (Besford and Syred, 1979).

Enzymatic methods involving marker enzymes offer another approach to assessing the nutritional status of plants. These methods are based on the fact that the activity of certain enzymes is lower or higher (depending on the nutrient) in deficient than in normal tissue. Examples are given

TABLE 11.9 Growth, P concentration and phosphatase activity of young wheat plants

			Phosphatase activity (μmol NPP[a] g^{-1} fw min^{-1})		
P supply	Shoot dw (mg $plant^{-1}$)	P concentration (g kg^{-1})	Total	Fraction A	Fraction B
High	223	8.0	5.6	4.4	0.5
Low	135	3.0	11.1	6.7	2.9

From Barrett-Lennard and Greenway (1982).
[a]*NPP, p-nitrophenylphosphate.*

in Chapter 7 for Cu and ascorbate oxidase; Zn and aldolase or carbonic anhydrase; and Mo and nitrate reductase. Either the actual enzyme activity is determined in the tissue after extraction or the leaves are incubated with the nutrient in question to determine the inducible enzyme activities of, for example, peroxidase activity by Fe (Bar-Akiva *et al.*, 1970) and nitrate reductase by Mo. For assessing the Mn nutritional status, the activity of MnSOD (Section 7.3) in leaves may be used as biochemical marker (Leidi *et al.*, 1987) or, as a non-destructive method, specific chlorophyll fluorescence (Kriedemann and Anderson, 1988).

Biochemical methods can also be used for assessing nutritional status of plants in relation to macronutrients. The accumulation of putrescine in K-deficient plants (Section 6.6) is a biochemical indicator of the K requirement in lucerne (Smith *et al.*, 1982). Inducible nitrate reductase activity can be used as an indicator of N nutritional status (Witt and Jungk, 1974; Dias and Oliveira, 1987). Pyruvate kinase activity in leaf extracts is related to the K and Mg concentration of the leaf tissue (Besford, 1978b). In P-deficient tissue, phosphatase activity is higher, especially that of a certain fraction (Fraction B; isoenzyme) of the enzyme (Table 11.9). The increase in phosphatase activity in deficient tissue indicates enhanced turnover rates or to remobilization of P (Smyth and Chevalier, 1984). In eucalyptus, acid phosphatase activity was a more sensitive parameter for diagnosis of growth limitations by P than total P in leaves and stems (O'Connell and Grove, 1985), whereas in maize acid phosphatase activity increased significantly only under severe P deficiency and therefore may be suitable as a means of confirming visual diagnosis, but is not sensitive enough to indicate latent P deficiency (Elliott and Läuchli, 1986).

Enzymatic, biochemical and biophysical methods can be very valuable if the total concentration or the soluble fraction of a nutrient is poorly correlated with its physiological availability. Whether these enzymatic, biochemical and biophysical methods can realistically be used as alternatives to chemical analysis as a basis for making fertilizer recommendations depends on their selectivity, accuracy, and particularly whether they are sufficiently simple to provide a rapid test. These requirements may be met in the case of Fe and peroxidase (Bar-Akiva *et al.*, 1978; Bar-Akiva, 1984) and Cu and ascorbate oxidase (Delhaize *et al.*, 1982). Nevertheless, calibration of the methods remains a problem when a suitable standard (non-deficient plants) is not available and there are no visible deficiency symptoms. These methods in foliar analysis for diagnostic purposes are suitable for solving particular problems of nutritional disorders and to supplement total and fractionated foliar analysis, rather than to replace them.

11.6 PLANT ANALYSIS VERSUS SOIL ANALYSIS

There is a long history of controversy as to whether soil or plant analysis provides a more suitable basis for making fertilizer recommendations. Both methods rely in a similar manner on calibration, i.e. the determination of the relationship between concentrations in soils or plants and the corresponding growth and yield response curves, usually obtained in pot or field experiments using different concentrations of fertilizers. Both methods have advantages and limitations, and they also give qualitatively different results (Schlichting, 1976). Chemical soil analysis indicates the potential availability of nutrients that roots may take up under conditions favourable for root growth and root activity. Plant analysis in the strict sense reflects only the actual nutritional status of plants. Therefore, a combination of both methods provides a better basis for recommending fertilizer applications than one alone. The relative importance of each method for making recommendations differs, however, depending on plant species, soil properties and the nutrient in question.

In fruit or forest trees, soil analysis alone is not a satisfactory guide for fertilizer recommendations, mainly because of the difficulty of determining with sufficient accuracy the root zones from which deep-rooting plants take up most of their nutrients. On the other hand, in these perennial plants seasonal fluctuations in the nutrient concentration of leaves and needles are relatively

small compared with those in annual species because of the buffering capacity of the trunks. The nutrient concentration of mature leaves and needles is therefore also an accurate reflection of the long-term nutritional status of a plant. Furthermore, calibrations of CDC and its adequate range can be made rather precisely and refined for a special location, plant species and even cultivar. Therefore, in perennial species foliar and needle analysis is in most cases the method of choice. However, chemical soil analysis, performed at least once for a given site, is necessary for characterizing the overall level of potentially available nutrients.

In pastures, plant analysis is used more frequently than soil analysis, not only because of the peculiarities of the rooting pattern in mixed pastures (deep- and shallow-rooting species) but also because of the importance of the nutrient composition of pasture and forage plants for animal nutrition.

In annual crops, the short-term fluctuations of nutrient concentrations place a severe limitation on plant analysis as a basis for making fertilizer recommendations. Soil analysis is required for predicting the range of variation in plant nutrient concentration throughout the growing season. In annual crops, a large proportion of the nutrients are taken up from the topsoil, which makes soil analysis easier and increases its importance as a tool for making fertilizer recommendations. Nutrient imbalances in plants, especially latent micronutrient deficiencies, are a worldwide problem particularly in intensive agriculture (Franck and Finck, 1980; Welch *et al.*, 1991), with consequences for plant yield and also for plant tolerance to diseases and pests (Chapter 10), as well as for animal and human nutrition in general (Kubota *et al.*, 1987; Chapter 9).

Chapter 12

Nutrient Availability in Soils

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SUMMARY

Only a proportion of the total nutrient amount in soil can be taken up and utilized by plants. This proportion varies with nutrient, and is affected by a range of soil, plant and environmental factors. In this chapter, methods for estimating nutrient availability and their limitations are discussed. In the soil, nutrients move to the soil surface by mass flow and diffusion, the relative importance of these two processes varying with nutrient. Nutrient movement by diffusion is slow, therefore depletion zones develop around roots. The factors affecting the extent of this depletion zone, namely root hair length and release of nutrient mobilizing exudates are described. The roles of root density, soil structure and soil water content on nutrient availability are discussed. The chapter concludes with a critical assessment of the usefulness of soil tests and novel modelling approaches to improve the understanding of nutrient availability in soils.

12.1 GENERAL

Only a proportion of the total nutrient amount in soil can be taken up and utilized by plants. The magnitude of this available fraction depends on a range of soil, plant and environmental factors. Estimating the plant-available nutrient fraction in soil is routinely done by chemical extractions that have been calibrated against field responses of crops to fertilizers. However, such extractions cannot simulate the temporal and spatial dynamics of the soil nutrient supply or the crop (genotype) nutrient demand. Such dynamics are particularly important in the rhizosphere, where root exudation and microbial activity can substantially alter nutrient availability (see also Chapter 14). Nutrient availability is also dependent on soil water content, which influences nutrient movement in soil. In this chapter, various factors influencing the ratio between the total amount of soil nutrients and the plant-available fraction are discussed.

12.2 CHEMICAL SOIL ANALYSIS

The most direct way of determining nutrient availability in soils is to measure the growth responses of plants by means of field plot fertilizer trials. However, this is time and labour intensive, and the results are not easily extrapolated from one location to another. In contrast, chemical soil analysis – soil testing – is a comparatively rapid and inexpensive procedure for obtaining information on nutrient availability in soils as a basis for recommending fertilizer application. Soil testing has been practised in agriculture and horticulture for many years with relative success. The effectiveness of the procedure is closely related to (i) the extent to which the data can be calibrated with field fertilizer trials, and (ii) the interpretation of the analysis. Quite often, far more is expected from soil testing than the methods allow. The limitations of soil testing are discussed in detail in this chapter, with special reference to P and K.

Soil testing uses a range of conventional extraction methods involving different forms of dilute acids, salts, or complexing agents, as well as water. Depending on the method used, quite different amounts of plant nutrients may be extracted from a given soil, as shown for P in Table 12.1. As a guide, $10\,mg\,P\,kg^{-1}$ soil is equivalent to ~$30\,kg\,P\,ha^{-1}$ in the top 20 cm of the profile (soil bulk density 1.5 = 3 million kg soil ha^{-1}). Weak extractants such as water or sodium bicarbonate (Table 12.1) reflect mainly the intensity of supply (concentration in soil solution), whereas strong extractants primarily indicate the capacity of the soil to supply nutrients to the soil solution (buffer capacity). Particularly for poorly mobile nutrients such as P, conventional soil tests may overestimate plant availability. Novel methods that mimic the root as a sink and are therefore better indicators of nutrient availability include anion exchange resins (Kuono *et al*., 1995) and diffusive gradients in thin-films (DGT) (Mason *et al*., 2010).

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TABLE 12.1 Mean concentration of readily soluble P in 40 soils extracted with various solutions

Extraction solution	pH	Readily soluble P ($mg\,kg^{-1}$ soil)
Neutral NH_4F	7.0	148
Acidic NH_4F	<2.0	74
Truog, $H_2SO_4 + (NH_4)_2SO_4$	3.0	36
Acetic acid	2.6	25
Bicarbonate, $NaHCO_3$	8.5	24
Calcium lactate	3.8	12

Based on Williams and Knight (1963).

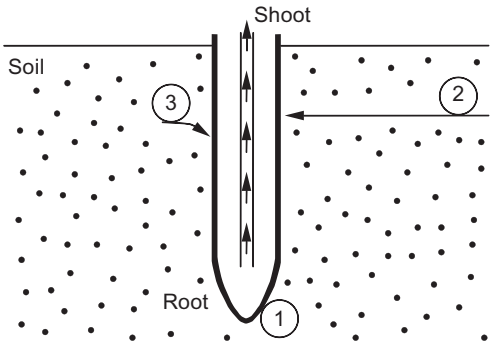


FIGURE 12.1 Schematic presentation of the movement of elements to the root surface of soil-grown plants. (1) Root interception: soil volume displaced by roots. (2) Mass flow: transport of soil solution along the water potential gradient (driven by transpiration). (3) Diffusion: element transport along a concentration gradient. • = available nutrients (as determined, e.g. by soil testing).

All methods used to characterize the availability of a given nutrient for the plants, and thus to predict fertilizer response, must be evaluated by growth experiments.

Quite often, a number of methods are equally suitable for soil testing for a particular nutrient (Vetter *et al.*, 1978; Bolland and Gilkes, 1992). For P, for example, water extraction (Van Noordwijk *et al.*, 1990) can be as satisfactory an extractant for determining availability as dilute acids, despite the difference in amounts of P extracted by these methods (Schachtschabel and Beyme, 1980). Typically, as is the case for P, soil testing methods provide a good indication of nutrient status of the soil, and the likelihood of fertilizer response, when the soil is either acutely deficient or abundantly supplied (Bolland and Gilkes, 1992). However, in the middle part of the response curve relating nutrient supply to plant growth (Fig. 11.1), soil chemical analysis alone is unsatisfactory for predicting the effects of fertilizer application.

Soil analysis mainly provides an indication of the capacity of a soil to supply nutrients to the plant, but does not adequately (and in some cases does not at all) characterize the mobility of the nutrients in the soil. Additionally, it fails to provide information about soil structure, or microbial activity, and plant factors, such as root growth and root-induced changes in the rhizosphere, which are critical for nutrient uptake under field conditions. In the following text these factors are discussed, beginning with nutrient availability in relation to mobility in soils and root growth. For comprehensive treatments of this topic the reader is referred to Jungk (1991), Barber (1995) and Tinker and Nye (2000).

12.3 MOVEMENT OF NUTRIENTS TO THE ROOT SURFACE

12.3.1 Principles of Calculations

The importance of the mobility of nutrients in soils in relation to availability to plants was emphasized by Barber (1962) and these ideas, which were refined and further developed, were summarized in a concept of 'bioavailability of nutrients' (Barber, 1995). Although this concept is focused on aerated soils, its principles may also be applied to submerged soils and plant species such as lowland rice. The three components in the concept are: root interception, mass flow and diffusion (Fig. 12.1). As roots grow through the soil, they move into spaces containing available nutrients, for example adsorbed to clay surfaces. Root surfaces may thus intercept nutrients during growth (Barber, 1995). Calculations of the importance of root interception for plant nutrient uptake are based on (i) the amounts of available nutrients in the soil volume occupied by roots; (ii) root volume as a percentage of the total soil volume – on average 1% for the topsoil; and (iii) the proportion of the total soil volume occupied by pores, on average 50%, but strongly dependent on soil bulk density (Section 12.5). In general, only a small proportion of the total nutrient requirement can be met by root interception (Table 12.2).

The second component is the mass flow of water and dissolved nutrients to the root surface, which is driven by transpiration. Estimates of the nutrient amount supplied to plants by mass flow are based on the nutrient concentration in the soil solution and the amount of water transpired either per unit weight of shoot tissue (transpiration coefficient, for example 300–600 L H_2O kg^{-1} shoot dw) or per hectare. The contribution of diffusion, the third component influencing nutrient supply to the root surface, can be calculated on the basis of the effective diffusion coefficients. Such data are more difficult to obtain than those on mass flow. Estimates of the contribution of diffusion can also be based on differences between total uptake by plants and the sum of the amounts supplied by root

TABLE 12.2 Nutrient demand of a maize crop and estimates of nutrient supply from the soil by root interception, mass flow and diffusion

		Estimates of amounts ($kg\,ha^{-1}$) supplied by		
Nutrient	Demand ($kg\,ha^{-1}$)	Interception	Mass flow	Diffusion
K	195	4	35	156
N	190	2	150	38
P	40	1	2	37
Mg	45	15	100	0

From Barber (1995).

interception + mass flow. An example of such a calculation is given in Table 12.2, showing the variable importance of the three components for different nutrients. It is apparent that, in this soil, N and Mg are supplied mainly by mass flow, whereas the supply of K and P depends mainly on diffusion. Furthermore, for Mg, supply by mass flow is greater than uptake, therefore this nutrient would be expected to accumulate at the root surface, as indeed is often found. Similarly, Ca is also accumulated at the root surface (see also Chapter 14).

Concepts of solute movement in the soil–root system usually consider only mass flow and diffusion, and include root interception in the diffusion component (Barber, 1995; Tinker and Nye, 2000). However, conditions at the soil–root interface are sometimes considerably different in a number of aspects from those at a distance from the roots (Chapters 14, 15). These conditions are insufficiently described by mechanistic models that treat roots primarily as a sink for nutrients supplied by mass flow or diffusion or both. An additional complication is that the soil structure at the root–soil interface plays an important role in determining soil water content and thus nutrient movement.

12.3.2 Concentration of Nutrients in the Soil Solution

To meet the nutrient demand of soil-grown plants, nutrients must reach the root surface, and this is mainly mediated by movement or transport in the soil solution. The concentration of nutrients in the soil solution is therefore critical for nutrient supply to roots. However, root growth or extension of root hairs or hyphae of mycorrhizal fungi are also important, because these processes reduce the distance across which nutrients have to be transported in the soil. The nutrient concentration in the soil solution varies widely, depending on such factors as soil water content, soil depth, pH, cation-exchange capacity, redox potential, quantity of soil organic matter and microbial activity, season, and fertilizer application (Asher, 1978). The concentrations of nutrients in the soil solution, particularly N, are usually very low in many natural ecosystems, for example the tundra (Chapin, 1988), compared to arable soils. An example of annual average concentrations of nutrients in the soil solution of an arable soil is shown in Table 12.3. Generally, in aerobic soils of neutral pH, the concentrations of Ca, Mg, sulphate (SO_4^{2-}) and nitrate are fairly high, whereas those of ammonium and particularly phosphate are very low. The concentration of K is mainly a function of clay content and clay mineral composition.

The concentration of nutrients in the soil solution is an indicator of the mobility of nutrients toward the root surface and in the vertical direction (i.e., in humid climates it indicates the potential for leaching). Compared with the concentration of other nutrients, that of P is extremely low (Table 12.3), thus leaching or transport by mass flow to root surfaces is generally of minor importance in mineral soils. In contrast to other anions such as nitrate and sulphate, phosphate strongly interacts with surface-active sesquioxides and hydroxides of clay minerals. In mineral soils, the P concentration in the soil solution and P mobility are enhanced by complexation of sesquioxides with organic ligands because this process reduces the number of potential binding sites. Organic ligands may further increase P availability by anion exchange, i.e. replacing P from the binding sites (Gerke 1993, 1994). Particularly effective in P mobilization are organic acid anions which are released by roots and microorganisms or produced during organic matter decomposition (Xu *et al*., 2006; see also Chapter 14).

In arable soils supporting high-yielding crops, the concentrations of nutrients, particularly N, but also P, are usually high and fluctuate over time particularly when fertilizer is applied (Table 12.4). In such soils, nutrient transport to the root surface does not limit uptake by the crop; even at soil solution concentrations of 10 µM P and 87 µM N, supply by diffusion is adequate for oilseed rape (Barraclough, 1989).

The concentration of the micronutrients Mn, Fe, Zn and Cu in the soil solution mainly depends on soil pH, redox potential and soil organic matter content, and in a temperate climate, may fluctuate throughout the season, with a maximum in early summer (Sinclair *et al.*, 1990). A decrease in pH or redox potential can increase the concentration of Mn, Fe, Zn and Cu (Sims and Patrick, 1978; Herms and Brümmer, 1980; Sanders, 1983; Miao *et al*., 2006).

Chelation by low-molecular-weight organic substances is another factor which strongly affects the concentration of micronutrient cations in the soil solution and their transport to the root surfaces by mass flow and diffusion. In the soil solution of calcareous soils, between 40 and 75% of

TABLE 12.3 Annual average concentrations of nutrients in the soil solution (0−20 cm) of an arable soil (Luvisol, pH 7.7)

Concentration (μM)								
K	Ca	Mg	$NH_4 - N$	$NO_3 - N$	$SO_4 - S$	$PO_4 - P$	Zn	Mn
510	1650	490	48	3100	590	1.5	0.48	0.002

Recalculated from Peters (1990).

TABLE 12.4 Time course of nutrient concentrations in the soil solution of the topsoil (0–20 cm) of a high-yielding winter oilseed rape (*Brassica napus*) crop

	Concentration (μM)		
Nutrient	22 February	28 March[a]	15 May
$NO_3 - N$	620	11,300	1,843
$NH_4 - N$	29	1,100	< 1
$PO_4 - P$	14	14	10
K	91	202	133
Ca	1,106	5,258	1,558
Mg	34	84	52

Barraclough (1989).
[a]*Split application of 265 kg N ha^{-1} as Ca $(NO_3)_2$ on 25 February and 25 March.*

Zn and 98–99% of Cu are in organic complexes (Hodgson *et al.*, 1966; Sanders, 1983). As a rule, dependent on soil organic matter content, the proportion of complexed cations increases in the order Mn < Zn < Cu, for example 55%, 75% and 80% at low organic matter content, and 50%, 85% and >98%, respectively, at high organic matter content (McGrath *et al.*, 1988). The importance of complexed micronutrients in the soil solution for nutrient uptake is also indicated by the fact that soil extractions with synthetic chelators provide suitable soil tests for the estimation of available micronutrients (Sims and Johnson, 1991).

The metal–organic complexes in the soil solution differ in both electrical charge (Sims and Patrick, 1978) and size which affects their interactions with charged soil surfaces of clays and organic matter (Hernandez-Apaolaza and Lucena, 2001). In nutrient solution experiments, the rate of uptake of metal cations from metal–organic complexes is lower than that of free cations (Chapter 2) and decreases with the size of the organic ligand, as has been demonstrated for Cu (Jarvis, 1987). In soil, however, chelation of micronutrient cations such as Cu and Ni increases plant uptake (Alvarez and Rico, 2003). This is due to an increase in concentration of these nutrients in soil solution and thus also in mobility and transport to the root surface (Table 12.5).

In view of the importance of concentration and binding forms of nutrients in the soil solution for transport of nutrients to the roots and for leaching from the rooting zone, various new techniques have been developed and older methods modified in order to obtain representative samples of soil solution. For many of these methods, soils are dried and ground prior to rewetting and collection of soil solution by displacement or centrifugation. Drying and grinding may strongly affect nutrient availability, either increasing nutrient availability by release of nutrients from inside of aggregates or decreasing availability by adsorption and precipitation. Hence, to characterize soil solutions of relevance for field-grown plants, collection by suction cups or from undisturbed soil cores by centrifugation or percolation is preferable.

Not only the concentration of nutrients in the soil solution (the so-called *intensity*), but also the soil buffer capacity (the so-called *capacity*) is important for nutrient supply to plants. Intensity and capacity are linked; fluctuations in the concentrations of a given nutrient in the soil solution throughout the season (for example, by plant uptake, leaching or fertilization) are minimized by release from, or sorption to, the soil (buffer capacity) (e.g., P in Table 12.4).

12.3.3 Role of Mass Flow

Mass flow is the convective transport of nutrients dissolved in the soil solution from the surrounding soil to the root surface. Calculations of the contribution of mass flow to the nutrient supply of field-grown plants therefore rely on detailed data on the concentration of nutrients in the soil solution throughout the season and on the water consumption of plants. Expressed as average values for the whole growing season, the contribution of mass flow to total supply differs not only between nutrients but also between plant species (Table 12.6). Mass flow is more than sufficient to supply Ca in both plant species shown and for supply of Mg in spring wheat, but not in sugar beet. In contrast, due to its low concentration in the soil solution, mass flow is negligible for K supply, and K was mainly supplied via diffusion. Therefore, the soil around the roots was depleted of K, whereas there

TABLE 12.5 Trace element concentration in bean leaves grown in nutrient solution or soil without or with the metal chelator diethylethylenetriamine pentaacetate (DPTA)

	Concentration in leaves (mg kg^{-1} dw)				
	Zn	Cu	Fe	Mn	Ni
Nutrient solution					
Control	34	37	125	132	33
+10^{-4} M DTPA	19	4	149	118	0
Soil					
Control	23	8	124	108	2
+10^{-3} M DTPA	27	19	230	136	13

Based on Wallace (1980a, b).

TABLE 12.6 Plant uptake and estimates of supply to the roots by massflow of K, Mg and Ca in spring wheat and sugar beet grown in a silty loam soil

	Amounts (kg ha^{-1})					
	Spring wheat			Sugar beet		
	K	Mg	Ca	K	Mg	Ca
Plant uptake	215	13	35	326	44	104
Mass flow	5	17	272	3	10	236
% of total uptake	2	131	777	1	23	227

From Strebel and Duynisveld (1989).

was a substantial accumulation of Ca and Mg at the root surface (Barber, 1995; Chapter 14).

From a four-year field study with different cereal crops and sugar beet (Table 12.6), the average contribution of mass flow was between 15 and 33% of the total N supply (Strebel and Duynisveld, 1989). No data are given in Table 12.6 for P, but a rough calculation can be made. The amount of water transpired by a crop varies from 2 to 4 ML ha^{-1} (Strebel *et al.*, 1983; Barber, 1995). Assuming a P concentration in the soil solution of 5 µM (0.15 mg L^{-1}) and total water consumption by the crop via transpiration of 3 ML, the amount of P supplied by mass flow can be calculated as about 0.45 kg. This value is equivalent to about 2 to 3% of the total P demand of a crop and agrees well with the 1 to 4% found in field experiments with winter wheat and sugar beet (Claassen, 1990).

The contribution of mass flow depends on the plant species (Table 12.6) and may, for example, be higher in onion than in maize as onion roots have a higher water uptake rate per unit length (Baligar and Barber, 1978). The relative contribution of mass flow also varies with the plant age (Brewster and Tinker, 1970), and the time of the day, as both influence transpiration and thus water uptake rate.

When the soil water content is high (e.g., at field capacity), mass flow is unrestricted and the water content (potential) at the root surface is similar to that of the bulk soil. As the soil water content decreases, the rate of water uptake by the roots can exceed the supply by mass flow and the soil at the soil–root interface may become dry. This is observed around the roots, particularly when the transpiration rate is high (Nye and Tinker, 1977; Doussan *et al.*, 2003, 2006), and often occurs in the topsoil during the growing season. The dry soil surrounding the roots will limit or even eliminate transport of nutrients via mass flow. Under field conditions, the rainfall pattern (or irrigation cycle) therefore strongly affects the contribution of mass flow to the total nutrient supply.

Mass flow and diffusion to the root surface usually occur simultaneously, therefore it is not possible to strictly separate these processes. The term ‘apparent mass flow’

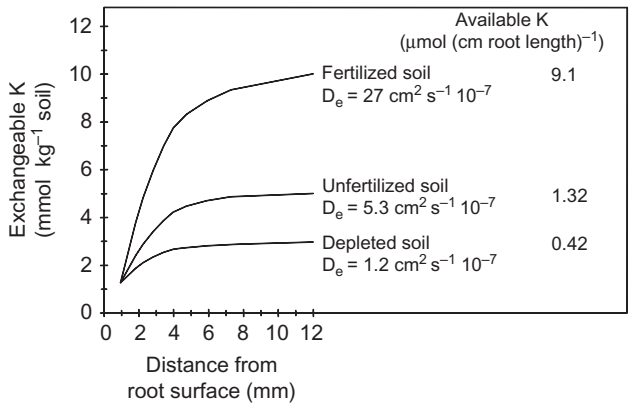


FIGURE 12.2 Concentration gradient around roots of 7-day-old oilseed rape (*Brassica napus*) seedlings grown in a soil with different concentrations of exchangeable K. *Modified from Kuchenbuch and Jungk (1984).*

TABLE 12.7 Estimates of diffusion coefficients ($m^2 s^{-1}$) of ions in water (D_l) and in soil (D_e) and of movement per day at average values of D_e

	Diffusion Coefficient			
Ion	Water (D_l)	Soil (D_e)	Average D_e in soil	Movement in soil (mm day^{-1})
NO_3^-	1.9×10^{-9}	10^{-10}–10^{-11}	5×10^{-11}	3.0
K^+	2.0×10^{-9}	10^{-11}–10^{-12}	5×10^{-12}	0.9
$H_2PO_4^-$	0.9×10^{-9}	10^{-12}–10^{-15}	1×10^{-13}	0.13

From Jungk (1991).

has therefore been recommended to define the amount of solutes transported to the root by mass flow (Nye and Tinker, 1977). A principal limitation of these calculations by mechanistic models is the assumption that uptake rates of nutrients and water are uniform along the axis of individual roots, which is not the case (Chapter 2).

12.3.4 Role of Diffusion

Diffusion is the main mechanism for the movement of P and K and other nutrients with low concentrations in the soil solution to the root surface. The driving force for diffusion is a concentration gradient. In soil-grown plants, a concentration gradient between the adjacent soil and the root surface is formed when the uptake rate of ions exceeds the supply by mass flow (Roose and Kirk, 2009). Depletion profiles develop over time and their shape depends mainly on the balance between uptake by roots, replenishment from soil, and mobility of ions by diffusion (Fig. 12.2). Furthermore, the shape is influenced by root hair length and mycorrhizal colonization.

The mobility of ions is defined in terms of the diffusion coefficient. Diffusion coefficients in homogeneous media such as water (D_1) are fairly similar for different ions and orders of magnitude higher than in non-uniform porous media such as aerated soils (Table 12.7). This is true particularly for P. In aerated soils, ions diffuse only in pore spaces that are filled with water or in the water film surrounding soil particles. Additionally, ions in the soil solution interact with the solid phase of the soil. For describing the diffusion of ions in soils the term 'effective diffusion coefficient' D_e was introduced by Nye and Tinker (1977), which is much smaller than the diffusion coefficient in water, because of the many physical and chemical interactions that ions encounter within the soil's solid phase (Tinker and Nye, 2000):

$$D_e = D_l \theta \frac{1}{f} \frac{dCl}{dCs}$$

where

D_e = the effective diffusion coefficient in the soil ($m^2 s^{-1}$)
D_l = the diffusion coefficient in water ($m^2 s^{-1}$)
θ = the volumetric water content of the soil ($m^3 m^{-3}$)
f = the impedance factor which takes into account the tortuous pathway of ions and other solutes through water-filled soil pores, increasing the path length. It becomes larger as the soil water content decreases.

$$\frac{dCl}{dCs} = \text{the reciprocal of the soil buffer power for the ion concerned}$$

where

C_l = the concentration of the ion in the soil solution
C_s = the sum of ions in the soil solution and those which can be released from the solid phase (e.g., exchangeable K). Soils with high adsorption capacity (e.g., clay soils for K^+) have a high buffer capacity, and thus a low $\frac{dCl}{dCs}$.

12.3.4.1 Soil Factors

As a rule, the concentration of K and P is substantially lower at the root surface than in the bulk soil, creating a depletion zone around roots (Gahoonia and Nielsen, 1991; Wang *et al.*, 2005c). For P, depletion zones can be found not only for inorganic P, but also for organic P (Gahoonia and Nielsen, 1992). Depletion of organic P is due to the release of phosphatases by roots and rhizosphere microorganisms which mineralize organic into inorganic P (Tarafdar and Jungk, 1987; Chapter 14) and the subsequent uptake of inorganic P.

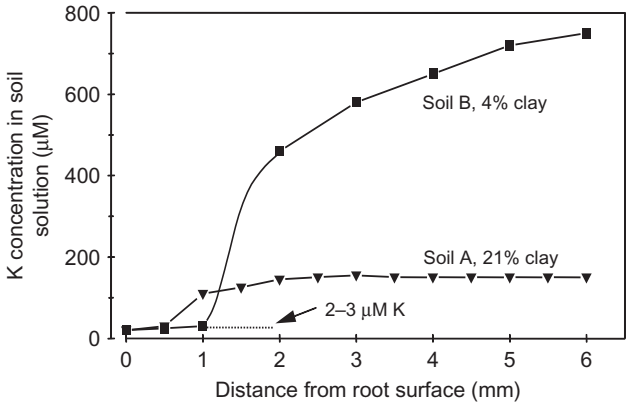


FIGURE 12.3 Concentration gradient of K in the soil solution around maize roots growing in soils with different clay contents. *Modified from Claassen and Jungk (1982).*

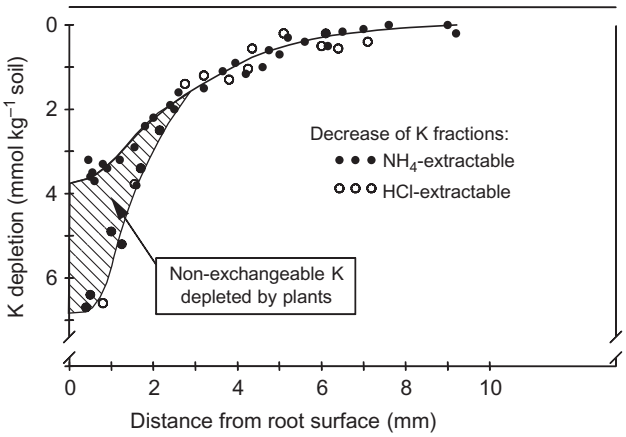


FIGURE 12.4 Concentration of different K fractions in the rhizosphere of 7-day-old oilseed rape (*Brassica napus*) seedlings. *From Jungk and Claassen (1986). Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.*

As shown in Fig. 12.2, D_e increases with increasing K concentration. The extent of the K-depletion zone surrounding the roots also increased from ~4 mm in depleted soil (by previous intensive cropping) to 5.3 mm in unfertilized and 6.3 mm in fertilized soil. Hence, raising the concentration of exchangeable K by fertilizer application increased the amount of K supplied via diffusion by a factor of more than 20, i.e. much more than would be expected from the increase in the amount of exchangeable K per unit soil weight only. Application of NaCl or $MgCl_2$ also increased the extent of the depletion zone and thus transport of K to the root surface (Kuchenbuch and Jungk, 1984). This is probably due to Na^+ and Mg^{2+} occupying potential K sorption sites or exchanging K^+ from sorption sites.

For K, shape and width of the depletion zone in different soils strongly depends on their clay content (cation-exchange capacity), which is an important parameter of the buffer capacity for K (Fig. 12.3). In soil A, with 21% clay and a correspondingly higher cation-exchange capacity, the equilibrium concentration of K in the soil solution was lower than in soil B, with only 4% clay. In both soils, the K concentration in the soil solution at the root surface was about 2 to 3 μM K^+. However, the depletion zone was wider in soil B than soil A, reflecting the lower capacity of soil B to replenish K in the soil solution.

Particularly in soils low in exchangeable K, plant demand may by far exceed K supply, and a large proportion of the K taken up derives from the non-exchangeable fraction. In the experiment shown in Fig. 12.4, the proportion of K from the non-exchangeable fraction increased from 20% in the fertilized soil to 71% in the unfertilized and 83% in the depleted soil (Kuchenbuch and Jungk, 1984), i.e. in the latter two cases the oilseed rape seedlings received most of their K from a fraction which is either not, or only to a minor extent, characterized as plant available by soil testing methods. Similarly high proportions of K from the non-exchangeable fraction have been found in ryegrass, and part of this K originated from the interlayer of clay minerals (Kong and Steffens, 1989; Hinsinger *et al.*, 1992). The release of K can be explained by the decrease in solution K concentration due to K uptake by roots which results in selective dissolution of clay minerals (Hinsinger *et al.*, 2006).

Soil water content is another important factor affecting D_e. Increasing the volumetric soil water content increases the cross-sectional area available for diffusion of ions which results in an increase in the reciprocal of the impedance factor and the soil buffer capacity (Table 12.8). As a consequence, D_e increases more than two-fold. For P, this effect of soil water content on D_e is even more pronounced. Increasing the volumetric soil water content in a Luvisol from 0.12 to 0.33 g cm^{-3} increased D_e from 0.10 to 4.5 × 10^{-13} m^2 s^{-1}, whereas changes in the bulk density of the soil had only a relatively small effect (Bhadoria *et al.*, 1991).

This pronounced effect of water content on D_e is also of importance in comparisons between soils of different texture (e.g., sandy vs. clay soils), because soils of different texture can have quite different water contents at the same soil water potential (pF or −MPa). At the same pF, the water content increases with clay content as shown by Cox and Barber (1992) by using four different soils where at −33 kPa, the water content varied between 0.13 and 0.40 g cm^{-3}. In order to achieve the same P uptake by maize plants, in the soil with the highest water content (0.4 g cm^{-3}), a concentration in the soil solution of only 10 μM P was necessary as compared with 200 μM in the soil with the lowest water content (0.13 g cm^{-3}) (Cox and Barber, 1992).

It is well known from field and greenhouse experiments that at low soil water contents – or in dry years – the uptake of K and P is more impaired than that of Ca and

TABLE 12.8 Reciprocal of the impedance factor $\frac{1}{f}$, buffer capacity b and effective diffusion coefficient D_e of K at different volumetric water content

Water content θ ($cm^3\,cm^{-3}$)	Reciprocal of the impedance factor $\frac{1}{f}$	Buffer capacity b	Effective diffusion coefficient D_e ($cm^2\,s^{-1}$)
0.19	0.20	2.68	2.6×10^{-7}
0.26	0.30	3.09	4.9×10^{-7}
0.34	0.45	4.42	6.4×10^{-7}

Based on Kuchenbuch *et al.* (1986).

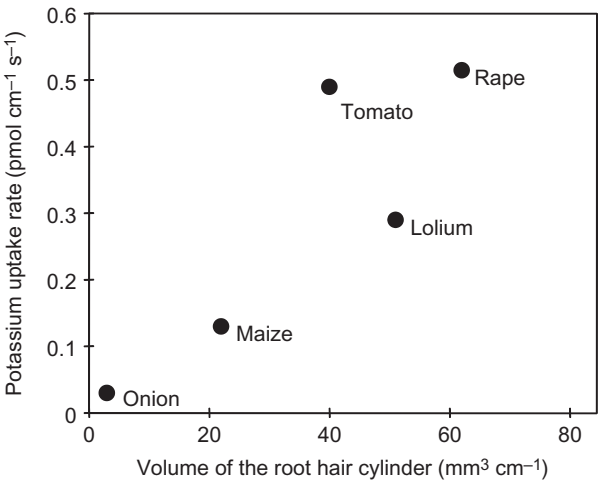


FIGURE 12.5 Rate of K uptake per unit root length in relation to the volume of the root hair cylinder. *Modified from Jungk* et al. *(1982).*

Mg which may even be increased (Talha *et al.*, 1979). At low soil water contents (Zur *et al.*, 1982) or high transpiration rates (Garrigues *et al*., 2006), the water content at the root surface is substantially lower than that of the bulk soil. Contact between root surface and soil via the soil solution can thus be lost. Consequently, the extent of the depletion zone in dry soil is strongly reduced compared to high water contents (Gahoonia *et al*., 1994).

At low soil water content, the mechanical impedance of the soil increases and root growth is inhibited, which further limits nutrient supply to the root surface by diffusion. However, root hair growth is strongly enhanced at low soil water content (Mackay and Barber, 1985, 1987; Watt *et al*., 1994) and thus may in part compensate for a decrease in surface area from impeded root growth. Moreover, root hairs and exopolysaccharides produced by roots and microorganisms in the rhizosphere (mucilage) can improve soil–root contact and modify water content and water movement and thus impact on mass flow and diffusion. For example, it has been shown that in drying soils, the water content is higher in the rhizosphere compared to the bulk soil (Young, 1995; Carminati *et al*., 2010). Additionally, exopolysaccharides can increase aggregate stability (Morel *et al*., 1991; Czarnes *et al*., 2000), thereby maintaining pore continuity as the soil dries. Liebersbach *et al*. (2004) suggested that oat and sugar beet are able to maintain higher than expected P diffusion rates in dry soil by increasing the release of polysaccharides.

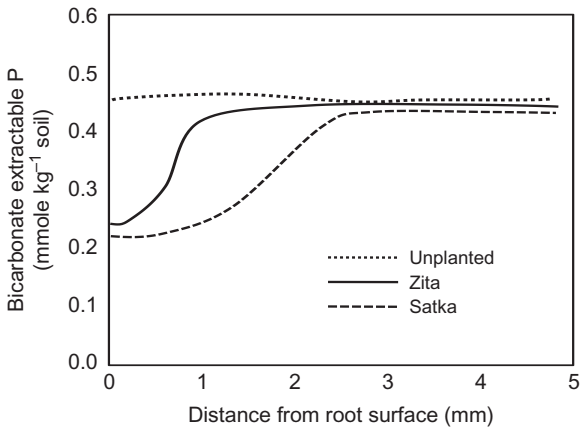
Roots may also increase the water content in the rhizosphere by redistributing water from wetter soil regions deeper in the soil profile to drier regions in the topsoil by hydraulic lift. Water taken up by deep roots is released from roots in the dry topsoil when transpiration ceases (e.g., at night) and soil water potential in the dry top soil is more negative than plant water potential (Horton and Hart, 1998).

12.3.4.2 Plant Factors

As shown in Figure 12.5, the volume of the root hair cyclinder as an indicator of root hair length is positively correlated with K uptake (Jungk *et al*, 1982). Per unit length of root, only 2 to 3 mm^3 soil was available to onion which had virtually no root hairs, compared with ~60 mm^3 for oilseed rape which had the longest root hairs.

In non-mycorrhizal plants, the extension of the P depletion zones is also positively related to root hair length (Itoh and Barber, 1983a; Gahoonia and Nielsen, 1997, 2001). For example, in maize and oilseed rape, the distance of maximum P depletion in the rhizosphere was closely related to the average root hair length, which was 0.7 mm in maize and 1.3 mm in oilseed rape (Hendriks *et al*, 1981). In agreement with this, even within a given plant species, the extent of P deletion in the rhizosphere and plant P uptake was greater in barley genotype Satka (long root hairs) than in Zita (short root hairs) (Fig. 12.6; Gahoonia and Nielsen, 1997). Moreover, P uptake in the field was greater. Similar differences among genotypes in root hair length and P uptake were also found in clover (Caradus, 1982).

The importance of root hairs for P uptake from soils is also reflected in simulation models for predicting P uptake by different plant species (Föhse *et al.*, 1988, 1991; Ma *et al*., 2001c; Leitner *et al*., 2010). The inclusion of root hairs leads to a better agreement with experimentally measured values. In soils low in extractable P, the contribution of root hairs can account for 50–90% of the total uptake (Föhse *et al.*, 1991; Nigussie *et al*, 2003). Moreover, on the basis of influx per unit area, root hairs are more effective in absorbing P than the root cylinder because of a smaller diameter and a specific geometric arrangement of the root hairs which maintain higher P diffusion rates (Jungk and Claassen, 1986; Claassen, 1990).



	Zita	Satka
Root hair length (mm)	0.5	1.1
Root hair density (no. mm^{-1})	21	32

FIGURE 12.6 Depletion of bicarbonate soluble P in the rhizosphere of two barley cultivars differing in root hair length. *From Gahoonia and Nielsen (1997). With kind permission from Springer Science & Business Media.*

The close relationship between the root hair length and the width of the depletion zone of P and K, however, is not always found. For example, in a given soil, the width of P depletion zone was confined mainly to the root hair cylinder in oilseed rape which possesses long root hairs (>0.5 mm), but considerably exceeded the root hair cylinder in species with short root hairs (~0.2 mm), for example cotton (Misra *et al.*, 1988). The extension of the depletion zone beyond the root hair cylinder in non-mycorrhizal plants can be explained by root-induced changes in the rhizosphere (e.g., release of root exudates, pH changes; Chapter 14) or higher efficiency in uptake parameters (K_m, I_{max}; Chapter 2). In mycorrhizal plants, the P depletion zone exceeds by far the root hair cylinder (Jungk and Claassen, 1986, Schnepf *et al*., 2008); for example, in red clover it can be up to 11 cm (Li *et al.*, 1991a).

Both the degree of depletion within the root hair cylinder and the width of the depletion zone can be affected by the minimum concentration of nutrients to which the roots can deplete the soil (C_{min}). The C_{min} value differs between plant species and even genotypes within a given species. In general, compared with solution cultures, C_{min} in soil-grown plants is usually higher due to the soil buffer capacity which counteracts a decrease in nutrient concentration in the rhizosphere soil solution caused by plant uptake. Average C_{min} values for soil-grown plants are 2–3 µM for K (Claassen and Jungk, 1982) and 1 µM for P (Hendriks *et al.*, 1981). For the rate of P replenishment in the soil solution of the root hair cylinder, the following calculation can be made: concentration in the soil solution: 5 µM = 0.15 mg P L^{-1}; amount of soil solution in the topsoil (0–30 cm): ~500,000 L = 75 g P per hectare; requirement during the rapid growth phase (e.g., in cereals between tillering and heading): ~300–500 g P ha^{-1} d^{-1}. Given that only ~25% of the topsoil is explored by roots during one growing season (Jungk, 1984), the rate of replenishment within the root hair cylinder has to be at least 10–20 times per day in order to meet plant requirement. For K, too, the rate of replenishment in the root hair cylinder has to be high. Within 2.5 days, more than half of the K taken up by maize was derived from the so-called non-exchangeable fraction of the soil in the root hair cylinder (Claassen and Jungk, 1982). In oilseed rape after 7 days, the contribution by the non-exchangeable fraction in an unfertilized and fertilized soil was 71% and 20%, respectively, of the total uptake (Kuchenbuch and Jungk, 1984). From these data it can be concluded that field-grown plants do not uniformly deplete even the densely rooted topsoil; near the root surface a high proportion of the non-exchangeable K contributes to the total uptake, whereas in the bulk soil, even the readily exchangeable K is not utilized. An example giving estimates of the proportion of soil delivering P and K to maize roots is shown in Table 12.9. Because of the lower D_e values for P compared to K, the proportion of soil supplying P is lower.

TABLE 12.9 Estimates of proportions of soil contributing to the P and K nutrition of field-grown maize

Root length density (cm cm^{-3})	Proportion of soil contributing (%)	
	P	K
>2	20	50
<2	5	12

Fusseder and Krauss (1986).

Table 12.9 also demonstrates the importance of the other main morphological component of nutrient

acquisition of non-mycorrhizal roots from soils, the root length. It is expressed either as total root length per plant or total root length per unit soil volume (root density).

12.4 ROLE OF ROOT DENSITY

Although a high root density and long root hairs are important factors in the uptake of nutrients supplied by diffusion, the relationship between root density and uptake rate may be linear (e.g., Kristensen and Thorup-Kristensen, 2004a,b), but as shown in Fig. 12.7, this is not always the case. At high root density, the uptake rate levels off. This is caused by overlap of the depletion zones of individual roots and reflects inter-root competition for nutrients (Fig. 12.8). For a given inter-root distance, the degree of competition mainly depends on the diffusion coefficient D_e; for maize, it is usually higher for nitrate than K and is of minor importance for P, at least under field conditions (Fusseder *et al.*, 1988). However, modelling by Ge *et al*. (2000) suggested that inter-root competition was important for P uptake in common bean. Decreased root density at depth (hence, lower root competition) accompanied by greater root proliferation in the topsoil was effective in increasing P uptake in soils with low P availability. Moreover, in poorly structured soils, roots may be aggregated or clustered; in those zones, inter-root competition for nutrients can become important even for P (Fusseder and Kraus, 1986). This also generally holds true for zones of high root density induced by localized fertilizer placement. It also should be borne in mind that root density is genetically controlled (with a number of relevant QTLs identified in *Arabidopsis*; Loudet *et al.*, 2005); hence genotypes of the same species may react differentially to fertilizer placement.

Inter-root competition is important in relating root density, for example in different soil layers or horizons, to their contribution to nutrient supply. The same curvilinear relationship as shown in Fig. 12.7 should exist between the rate of P uptake and root hair density because of competition between individual root hairs (Itoh and Barber, 1983b).

In some plant species (e.g., Proteacea, white lupin), P deficiency induces the formation of cluster roots which are characterized by dense clusters of short laterals, which in turn are covered by root hairs (Lambers *et al*., 2006; Watt and Evans, 1999). Competition among lateral roots and also among root hairs is thought to be intense, effectively depleting available P in the soil volume within the cluster roots (Neumann *et al*., 2000). Cluster roots release P-mobilizing exudates which further mobilize P (Chapter 14). However, cluster roots are active for only a few days (Watt and Evans, 1999), indicating that continuous P uptake is only possible by formation of new cluster roots in as yet undepleted soil. Similarly, modelling by Steingrobe *et al.*, (2001) suggested that root turnover, i.e. death of old roots and formation of new ones in undepleted soil, was important for P and K uptake.

In field-grown plants root density gradients occur between topsoil and subsoil (Table 12.10). The high root density in the topsoil (e.g., Liedgens *et al.*, 2000) is mainly caused by the usually more favourable physical, chemical and biological conditions in the topsoil compared with the subsoil (e.g., Lofkvist *et al.*, 2005; Adcock *et al.*, 2007). As an average of annual agricultural and horticultural crops, the logarithm of root density declines linearly with increasing depth (Greenwood *et al.*, 1982). However, at least in cereal species and maize this gradient becomes less steep during the growing season, and root density in the subsoil increases (Barber and Mackay, 1986; Vincent

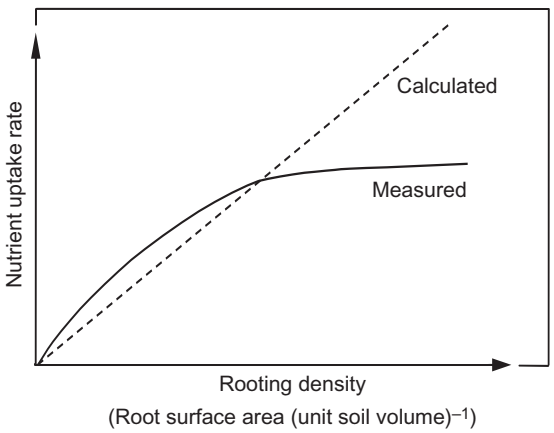


FIGURE 12.7 Relationship between root density and uptake rate of nutrients supplied by diffusion.

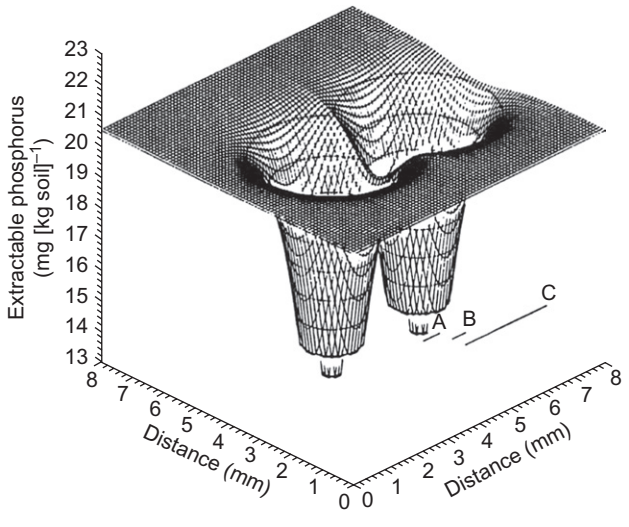


FIGURE 12.8 Profile of extractable P around two maize roots with overlapping depletion zones. Root cylinder (A), root hair cylinder (B) and maximal depletion zone (C). *From Fusseder and Kraus (1986) with permission from Elsevier.*

TABLE 12.10 Root length distribution of maize at flowering in a luvisol

	Root length	
Soil depth (cm)	Density ($cm\,cm^{-3}$)	Total ($km\,m^{-1}$)
0–15	6.2	9.3
15–30	3.1	4.6
30–45	1.2	1.7
45–60	0.5	0.7
60–75	0.4	0.6
75–90	0.3	0.4
90–135	0.2	0.3

Horlacher (1991).

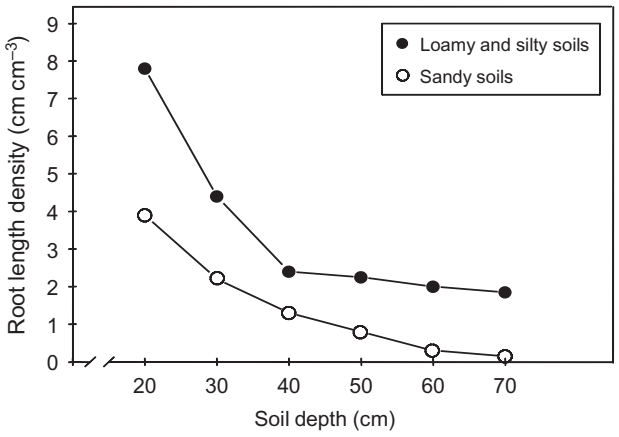


FIGURE 12.9 Root length densities at heading of cereal crops in different soils as a function of soil depth. *From Gäth* et al. *(1989). Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.*

and Gregory, 1989) as the topsoil is depleted of nutrients and, in drier climate, of water (e.g., Tang *et al.*, 2002). An example of the average root densities of cereal crops at heading is shown in Fig. 12.9.

Despite the lower root density, nutrient uptake from the subsoil can be considerable. The importance of subsoil nitrate for N nutrition of crop plants is widely established (N_{min}) (also Dunbabin *et al.*, 2003). For cereal crops such as winter wheat growing in deep loess soils, on average, 30% of the total N uptake by the crop can be derived from the subsoil (Kuhlmann *et al.*, 1989).

Uptake from the subsoil is important also for other nutrients such as Mg, K and P. The relative importance of subsoil supply depends not only on root density in the subsoil (Fig. 12.9), but also on root density and nutrient availability in the topsoil (Barber and Mackay, 1986; Kuhlmann and Baumgärtel, 1991). Roots in the subsoil can be important in preventing nutrient leaching and maximizing nutrient capture. While root proliferation in the top soil early in the season is important in reducing total nitrate leached, producing deep roots is important for capturing leached nitrate (Dunbabin *et al*., 2003). Accessibility of nutrients in the subsoil can also depend on the activity of the soil fauna, earthworms in particular; in barley and sugar beet between 20 and 40% of the roots in the subsoil (>65 cm) were found to follow earthworm channels (Meuser, 1991).

12.5 NUTRIENT AVAILABILITY AND DISTRIBUTION OF WATER IN SOILS

In dry climates, nutrient availability in the topsoil declines during the growing season because the low soil water content becomes a limiting factor for nutrient delivery to the root surface. Nutrient uptake is further decreased by impaired root growth in dry soil.

The effect of water supply on root distribution was demonstrated for spring barley in two successive years (Scott-Russell, 1977). In the first year, with high rainfall (82 mm) occurring a month after planting, more than 70% of the total root mass was found in the topsoil (<0.13 m) two months after planting and only ~10% of the roots had penetrated >0.23 m. In contrast, in the following year, with inadequate rainfall (24 mm) during the first month after planting, 40% of the root mass was in the topsoil and 30% in the subsoil. This shift in root distribution has important consequences for nutrient uptake from different soil horizons.

Depending on the rainfall during the spring wheat growing season, the percentage of the total K taken up from the subsoil may vary between ~60% in a dry year and ~30% in a wet year (Fleige *et al.*, 1983). It is important to keep in mind that hydraulic lift of water from moist subsoil to dry topsoil can facilitate uptake of K from dry topsoil, at least for example in oilseed rape (Rose *et al.*, 2008). However, in 10 duplex soils (sand over clay) in Western Australia, subsoil K was not important for wheat growth (Wong *et al*., 2000). Using a combination of large-scale field experiments on root length distribution in various cereal crops (Fig. 12.9), measurements of exchangeable K and consideration of the climatic water balance at a given location, models have been established which predict K delivery to roots of cereal crops (Gäth *et al.*, 1989). However, such models fail to adequately account for mobilization of K in the rhizosphere (e.g., of sugar beet) (El-Dessougi *et al*., 2002) because of inadequate knowledge of the underlying mechanisms (Rengel and Damon, 2008).

Similarly to K, the uptake of P from different soil horizons is affected by differences in soil water content within the soil profile. Under low or erratic rainfall during the growing season (Table 12.11), despite a higher concentration of extractable ('available') P in the topsoil, between 30 and 40% of the total P taken up by spring wheat in the later

TABLE 12.11 Phosphorus uptake by spring wheat and P delivery from different soil depths during plant development

		Developmental stage		
		Booting stage	Anthesis	Milk stage
		P uptake ($kg\,ha^{-1}\,d^{-1}$)		
		0.35	0.27	0.15
Available P[a] ($mg\,kg^{-1}$)	**Soil depth (cm)**	**Delivery of P from different soil depths (%)**		
115	0–30	83	59	67
45	30–50	8	18	16
25	50–75	6	16	12
20	75–90	3	7	5

Based on Fleige *et al.* (1981).
[a]*Extraction with calcium ammonium lactate.*

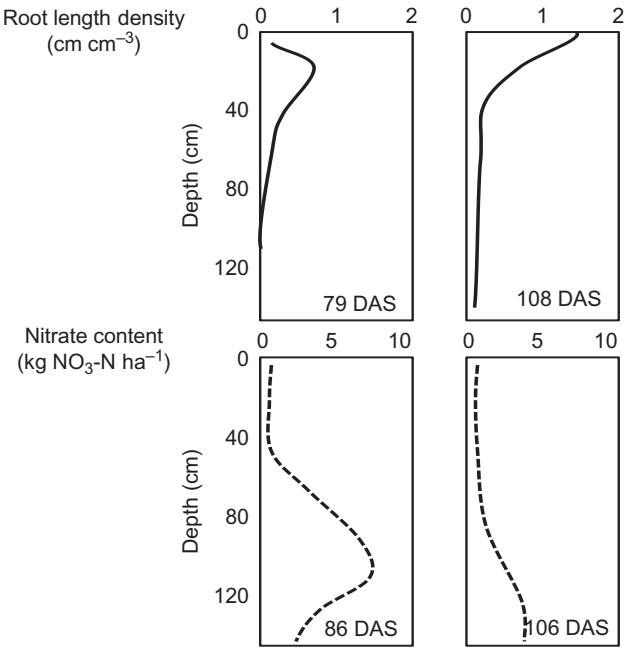


FIGURE 12.10 Root length density of *Lupinus angustifolius* and nitrate concentrations in the soil profile at different times after sowing (DAS). *Based on Dunbabin* et al. *(2003).*

stages of growth came from the subsoil. Nevertheless, plants can compensate for reduced diffusion caused by low moisture content in the topsoil by exuding low- and high-molecular-weight (particularly mucilage) exudates to enhance P (but not K) uptake (Liebersbach *et al*., 2004). In addition, hydraulic lift of water to the P-containing topsoil may increase P uptake by wheat (Valizadeh *et al*., 2003).

The prediction of the N supply in the form of nitrate from the topsoil and subsoil is different from that for K and P. Transport of nitrate by mass flow contributes substantially to the total nitrate delivery to the root surface (Strebel *et al.*, 1983; Strebel and Duynisveld, 1989). However, unless a high nitrate concentration is maintained in the soil solution by fertilizer applications during the growing season, the relative proportions of nitrate supplied by mass flow and diffusion may shift considerably because the soil buffer power for nitrate is low, except in soils with a high amount of readily mineralizable organic N. A decrease in nitrate concentration in the soil solution results in a decline in the amount of nitrate supplied to spring wheat by mass flow and an increase in supply via diffusion, which may then supply more than 50% of the total nitrate (Strebel *et al.*, 1980). The soil depth from which nitrate is taken up also changes during the growing season (Dunbabin *et al*., 2003). In the early stages of growth, nitrate is mainly taken up from the top soil. However, after depletion of nitrate there, roots grow deeper and subsoil nitrate is depleted (Fig. 12.10).

During the entire growing season for sugar beet, the supply of nitrate by mass flow was low (an average of 32 kg nitrate-N) compared with diffusion (181 kg) (Strebel *et al.*, 1983). A time-course study (Fig. 12.11) demonstrated that the supply by mass flow was restricted to the early growing period and to the topsoil that had relatively high nitrate concentration in the soil solution. Upon depletion of nitrate in the topsoil and root proliferation into the subsoil, nitrate was supplied exclusively by diffusion. Hence, the average contribution of mass flow and diffusion (as well as of different soil horizons) to total nitrate supply may be misleading. Therefore, simulation models for predicting nutrient uptake in field-grown plants have to take into account soil non-uniformity, water and nutrient dynamics in space and time, root plasticity in terms of structure and function as well as differences among plant species and genotypes within a species (Dunbabin *et al*., 2004). Considerable genotypic differences were found in the extent of root growth and nitrate depletion in the subsoil among maize (Wiesler and Horst, 1993) and wheat cultivars (Liao *et al*., 2004).

12.6 ROLE OF SOIL STRUCTURE

Soil structure plays an important role in determining the amounts of nutrients that are available for uptake by roots. Location of roots and the surrounding micro-environment influence root uptake. Various techniques have been used for characterizing root properties and soil structure, for example (i) X-ray computed micro-tomography for determining properties of soil

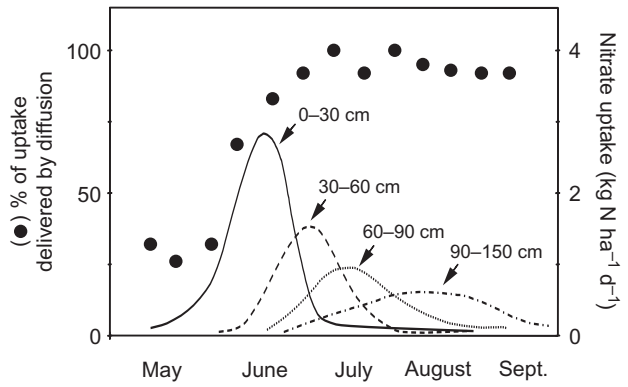


FIGURE 12.11 Nitrate uptake rate and delivery by diffusion to sugar beet plants as a function of soil depth and time. *Based on Strebel* et al. *(1983).*

aggregates and mesopores in the 27–67 µm range (Gryze *et al*., 2006), for characterizing macro- and mesoporosity down to 19 µm pore resolution at interfaces of texture-contrast soils (Jassogne *et al*., 2007) and for imaging roots in 3-D in undisturbed soil columns (Tracy *et al*., 2010), (ii) a combination of X-ray absorption and phase contrast imaging (Moran *et al*., 2000), or (iii) high-resolution 2-D X-ray imaging (Pierret *et al*., 2003) to characterize root properties and the soil structure in intact soil cores, which provided a good fit with simulation models (cf. Doussan *et al*., 2006). Recently, X-ray microscopy is becoming the method of choice because of its capacity to image particles in the nanometre size range with sub-micrometre spatial resolution and the option of combining with high spectral resolution for spectromicroscopy studies (Thieme *et al*., 2010).

In structured soils not all roots have complete contact with the soil matrix, and in non-mycorrhizal plants the degree of root–soil contact at various positions along the root axis may vary from 0 to 100% (Van Noordwijk *et al*., 1992). Soil–root contact can be improved by mucilage (Read *et al*., 1999).

Increasing bulk density increases root–soil contact, but reduces root elongation (Table 12.12). This reduction in root elongation is in part compensated for by higher uptake rates per unit root length, for example of nitrate and water (Table 12.12), as well as of P, particularly in soils high in available P (Cornish *et al*., 1984). In soils with high bulk density, limitation of root penetration through the soil matrix is accompanied by increased proportion of roots in macropores that have increased organic matter content, enhanced nutrient availability and increased abundance of microorganisms (Pankhurst *et al*., 2002).

High bulk density and aggregation of roots in certain zones can also lead to O_2 depletion. In experiments in which soil bulk density was increased and the average soil–root contact was increased from 25 to 75%, root

TABLE 12.12 Soil porosity (macropores >30 µm), root length, estimated root–soil contact, and uptake rate of nitrate and water per unit root length of maize at different soil bulk density

	Bulk Density (g cm^{-3})		
	1.1	1.3	1.5
Soil porosity (%)	60	51	44
Root length (m pot^{-1})	114	83	50
Root surface with soil contact (%)	60	72	87
Uptake (mmol m^{-1} root length)			
Nitrate	14	15	19
Water	18	21	24

Compiled data from Van Noordwijk *et al.* (1992), Kooistra *et al.* (1992) and Veen *et al.* (1992).

aggregation in certain zones resulted in localized high O_2 demand (Asady and Smucker, 1989). For maintenance of root respiration at such sites of high root density, the required external O_2 concentrations increased more than three-fold. The degree of soil–root contact and soil bulk density for optimal nutrient uptake and plant growth thus depends not only on soil fertility, but also on aeration (Van Noordwijk *et al.*, 1992).

The conventional methods for determination of available nutrients in soil use soil samples that are mixed and sieved prior to extraction. Hence, they not only ignore the importance of spatial non-uniformity in availability of nutrients (as discussed above), but also destroy the soil structure and thereby gradients that occur in cation exchange capacity and base saturation between the external and internal surfaces of soil aggregates (Horn, 1987, 1989; Kaupenjohann and Hantschel, 1989). These aspects are particularly important in acid forest soils where such gradients in soil solution chemistry are distinct, in addition to the spatial non-uniformity related to the distance from the stem.

More realistic data on nutrient availability in the soil can be obtained by collecting soil solution from lysimeters or suction cups in the field (e.g., Liedgens *et al*., 2000), or from undisturbed soil cores. Soil solution can be obtained from such cores either by circulation of a percolating solution (Hildebrand, 1986) or by centrifuging after adjustment to field capacity. Cation concentrations differ between equilibrium soil solution from homogenized soil and percolation solution of the same but undisturbed acid soil (Table 12.13). The concentrations of cations (except H^+) are usually higher in the homogenized samples as

TABLE 12.13 Cation concentrations in soil equilibrium solutions and in soil percolation solution of a Brown earth, pH ($CaCl_2$) 3.1

	Concentration in the soil solution (μM)				
	K	Ca	Mg	Al	Fe
Equilibrium solution	55	41	39	104	39
Percolation solution	13	15	17	52	17

Based on Hantschel *et al.* (1988) and Kaupenjohann and Hantschel (1989).

a result of the destruction of aggregates and exposure of internal surfaces to the extractant. Accordingly, concentrations of K and Mg in the needles of Norway spruce correlated poorly with the concentrations of the two nutrients in the soil extraction solution, but correlated strongly with the concentrations of two nutrients in the undisturbed soil (Kaupenjohann and Hantschel, 1989).

12.7 INTENSITY/QUANTITY RATIO, PLANT FACTORS AND CONSEQUENCES FOR SOIL TESTING

Routine soil testing methods determine the fraction of 'chemically available' nutrients. In terms of an intensity/quantity concept, depending on the extraction method, this mainly characterizes the intensity (e.g., water extraction) or a variable amount of the quantity, represented by the labile pool (Fig. 12.12). Soil testing for P in water extracts (at a 1:60 (v/v) soil:water ratio shaken for 22 h) is a reasonable compromise between measuring intensity and capacity of P supply in soil (Van Noordwijk *et al.*, 1990). Mild extractants such as sodium bicarbonate (Olsen-method) primarily characterize the P fraction adsorbed onto aluminium at clay surfaces (Kuo, 1990). More detailed information concerning binding strengths, rate of replenishment and the intensity/quantity ratios for different nutrients can be obtained with the electro-ultrafiltration method (EUF), which involves the use of different electrical field strengths and temperatures in an aqueous soil suspension (Nemeth, 1982; Nemeth *et al.*, 1987). However, for routine soil testing the EUF method is not necessarily superior or technically simpler than conventional extraction methods (e.g., with $CaCl_2$) in the prediction of fertilizer requirement (Houba *et al.*, 1986; Rao *et al.*, 2000) or in characterizing organic N mineralization (Mengel *et al.*, 1999).

There is a large number of extraction methods used in routine soil testing for micronutrients that, as a rule, mainly characterize the quantity component (Fig. 12.12) and predict fertilizer requirement well only when the extracted amounts are considerably different from those considered adequate (Sims and Johnson, 1991). Predictions can sometimes be improved by consideration of other soil properties such as pH, redox potential, and clay and organic matter content (Moraghan and Mascagni, 1991; Brennan, 1992c).

Ion-exchange resins can be used to determine not only the ion concentrations in the soil solution but also the rates of replenishment of these ions, for example for P (Marschner *et al.*, 2007; Wang *et al.*, 2007b), K (Shenker and Huang, 2001) or simultaneously for various cations and anions (e.g., Blank *et al.*, 2007; Castle and Neff, 2009). In experiments with bean and maize under field conditions, prediction of Zn uptake was more precise with ion exchange resins than with the routine DTPA extraction (Hamilton and Westermann, 1991).

There is a voluminous literature on sequential extraction of various nutrients, for example P (Hedley *et al.*, 1982) with various modifications (e.g., Wang *et al.*, 2007b), K (Moody and Bell, 2006) and zinc (Alvarez and Gonzales, 2006). In principle, sequential extraction quantifies the distribution of a nutrient among fractions of different chemical or binding characteristics, as defined by properties of selected extractants. However, relating different fractions to plant availability remains a difficult and unresolved task (e.g., Frossard *et al.*, 2002; Moody and Bell, 2006; Wang *et al.*, 2007b; Herencia *et al.*, 2008; Li *et al.*, 2010b).

Recommendations of N fertilization for various agricultural and horticultural field crops have been improved by the N_{min} method that measures the amount of mineralized N, mainly nitrate, in the soil profile at the beginning of the growing season, thus taking into account various components of availability, such as the high mobility of nitrate in the soil profile (mass flow) and N uptake from the subsoil (root growth). Depending on plant species and rooting depth, N_{min} is determined up to 0.9 m soil depth (Wehrmann and Scharpf, 1986; Schenk *et al.*, 1991). The N_{min} method can improve fertilizer recommendations in rain-fed agriculture under dryland conditions (Soltanpour *et al.*, 1989), as well as in a variety of other plant systems (e.g., Khayyo *et al.*, 2004; Liu *et al.*, 2005b; Cui *et al.*,

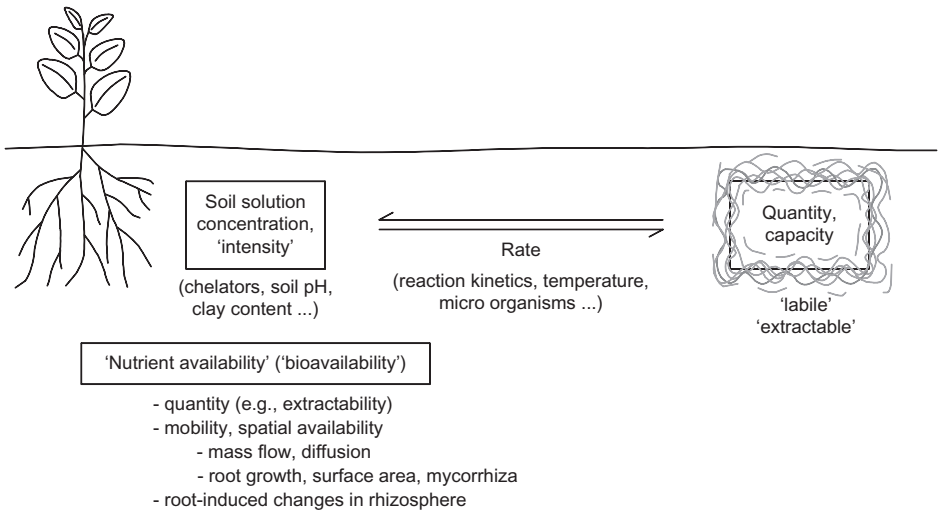


FIGURE 12.12 Intensity/quantity ratio of nutrient availability and factors determining the 'bioavailability' of nutrients. *From Marschner (1993).*

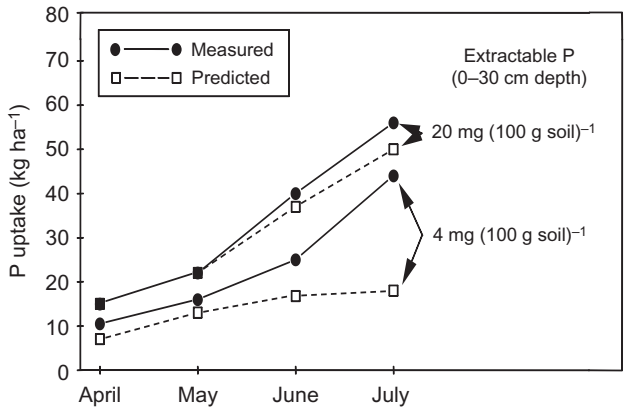


FIGURE 12.13 Measured P uptake and P uptake predicted by the Claassen-Barber model in winter wheat in a field experiment after long-term application of either 100 kg P ha^{-1} per year (high P soil) or without P fertilizer application (low P soil). *Jungk and Claassen (1986) with permission from Elsevier.*

2008), and may be as good as the EUF method in cereal cropping systems (Mengel *et al*., 2006).

In humid and semi-humid climates, most of the nitrate in the subsoil originates from mineralization of organically bound nitrogen (N_{org}) and nitrification of ammonium-N in the topsoil. Various attempts have been made to characterize this mineralizable N_{org} fraction in the topsoil prior to nitrate leaching into the subsoil, for example by the EUF method or $CaCl_2$ extraction (Mengel *et al*., 1999). For cereals, both EUF (N_{org}) and $CaCl_2$ extraction appear to be suitable alternatives to the N_{min} method (Appel and Mengel, 1992).

The principal limitation of the soil testing methods is that they only characterize some of the factors that determine nutrient supply to the roots of field-grown plants. Improving the reliability of fertilizer recommendations based on chemical soil analysis does not depend primarily on the extraction method used, but rather on the systematic consideration of the root and environmental factors such as soil water content. Current models for predicting nutrient availability and nutrient uptake under field conditions are therefore based on both soil and plant factors (Fig. 12.12) in which root parameters are the key element (e.g., Dunbabin *et al*., 2002; Doussan *et al*., 2006; Leitner *et al*., 2010). These models have been refined in recent years, and predictions of nutrient uptake are often, but not always, in good agreement with actual uptake by crops, for example for P (Fig. 12.13) or K (Ali Roshani *et al*., 2009). As shown in Fig. 12.13 for P, both predicted and measured uptake were closely related in the soil with high P concentration. However, in the soil with low P concentration, predicted uptake was lower than the measured uptake. Similar results were obtained for P uptake by maize (Mollier *et al*., 2008), indicating that the plants in the low P soil had access to soil P sources that were not considered in the model because of mycorrhizal colonization and/or root-induced changes in the rhizosphere (discussed in detail in Chapters 14 and 15).

For K, on the other hand, predictions were in close agreement with the measured uptake of the wheat crop only in K-deficient soils, whereas in the K-sufficient soil the models over-predicted K uptake by as much as four-fold (Seward *et al.*, 1990). This over-prediction may be the result of poor characterization of the plant demand and thus an underestimation of the role of negative feedback regulation of K uptake by the roots at high internal content (Chapter 2) or due to overestimation of K release from non-exchangeable pools (Ali Roshani *et al*., 2009). Using a different model, under-prediction of K uptake was found

for a range of crops under poor K supply. However, prediction could be improved by increasing soil K concentration or soil K buffer power (Samal *et al*., 2010), suggesting that release of K from non-exchangeable pools may have been underestimated in that particular model.

In conclusion, mechanistic simulation models are instrumental in increasing our understanding of the dynamics at the soil–root interface, but continuous improvements in order to better account for the relevant processes are needed. Also, strict validation of models against independently produced experimental data is crucial to maintain confidence in simulation and modelling data.

Chapter 13

Effect of Internal and External Factors on Root Growth and Development

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SUMMARY

Soils are highly complex and non-uniform in space and time with respect to chemical, physical and biological properties. Due to the high metabolic costs of root growth and maintenance, plants have to be efficient in allocating root growth as well as energy for nutrient uptake to soil domains with the greatest availability of limiting resources. After outlining the internal factors affecting root growth such as carbohydrate supply, phytohormones and genotype, this chapter describes the effects of soil chemistry, physics and biology. Among soil chemical factors, soil nutrient availability, pH, aeration and low molecular weight organic solutes are discussed. The stimulating or depressing effect of soil organisms on root growth are outlined, followed by a description of physical factors affecting root growth such as mechanical impedance, soil water content and temperature. The chapter concludes with a discussion of the ratio of shoot to root growth and how this is modulated by external and internal factors.

13.1 GENERAL

The soil environment is extremely complex and non-uniform in space and time. Nutrients occur in patches that may move over time and there are strong vertical gradients in soil. Physical, chemical and biological properties influence root growth, and many roots are lost to herbivores and pathogens. Below ground competition from neighbouring plants creates additional resource patchiness. The metabolic costs of root growth and function are significant limitations to plant growth, which means that plants must be efficient in allocating root growth as well as energy for nutrient uptake to soil domains with the greatest availability of limiting resources. The exploration and exploitation of the soil by roots is therefore a primary challenge in plant biology, and plants have evolved a range of adaptations to optimize root growth and development for specific soil environments. Root traits are under genetic control, resulting in substantial phenotypic variation within and among species. Moreover, root phenotypes may change in response to environmental conditions, either as an adaptation or simply because of external growth constraints. This chapter discusses several of the most important factors influencing root growth and development in the context of nutrient acquisition.

13.2 CARBOHYDRATE SUPPLY

The metabolic costs of soil exploration by root systems are substantial. Depending on plant species and environmental conditions, between 15 and 50% of daily photosynthate production is allocated to roots for growth, uptake and assimilation of nutrients, and maintenance respiration (Lambers *et al*., 2002a). The release of organic compounds into the rhizosphere – 'rhizodeposition' (see Chapter 15) accounts for about another 17% of daily net photosynthesis (Nguyen, 2003). The carbon costs of mycorrhizal symbiosis in various herbaceous and woody species range from 4 to 20% of daily net photosynthesis (Lynch and Ho, 2005) (Fig. 13.1). Low nutrient availability often increases root carbon costs (Fig. 13.1) by increasing the root-to-shoot ratio, and also by increasing root exudation (see Chapter 14). In addition, root losses to herbivores and pathogens increase the total carbohydrate costs of soil exploration (Fisher *et al*., 2002).

Because of these significant carbohydrate requirements, root growth and development depend upon an adequate supply of carbohydrates from current photosynthesis or stored reserves. Therefore, environmental conditions that affect photosynthesis, including water availability, temperature, light intensity and nutrient availability, may influence root growth by affecting carbohydrate supply to the roots. For example, there is often a close relationship between root growth and light intensity (Fig. 13.2).

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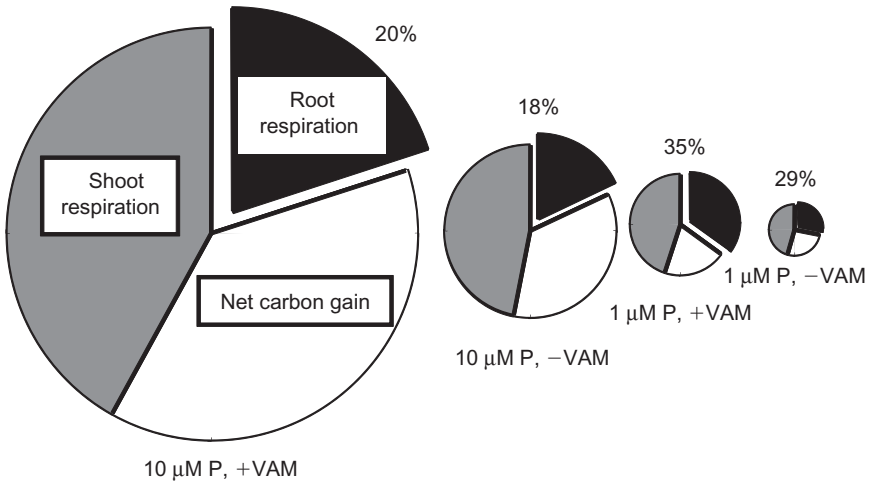

FIGURE 13.1 Allocation of carbon assimilation in common bean plants at 35 days after planting, as affected by P availability and mycorrhizal status. The size of each pie represents total carbon fixed in a 24-hour period, and subdivisions represent amount of carbon used in root (+ rhizosphere) respiration, shoot respiration and net carbon gain. *Redrawn from Nielsen* et al. *(1998).*

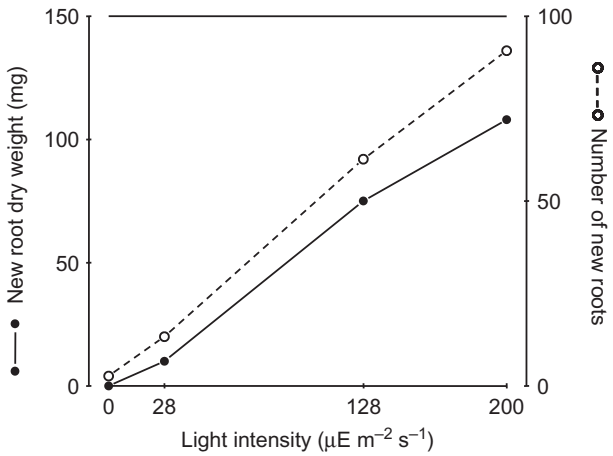

FIGURE 13.2 Production of new roots in 2-year-old Douglas fir seedling transplants after 24 days of growth at different light intensities. *Redrawn from Van den Driessche (1987).*

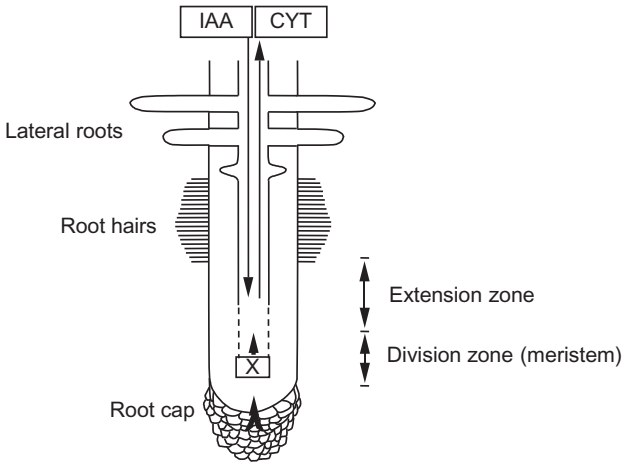

FIGURE 13.3 Main features of root morphology and some aspects of interactions in a growing root (IAA = indolylacetic acid, CYT = cytokinins, X = unidentified growth modulating compound).

In addition to their effects on photosynthesis, temperature and water availability have direct effects on root growth and development, as discussed below. Plants balance their biomass allocation between roots and shoots to attain a 'functional equilibrium' between shoots as a source of carbohydrates and roots as a source of water and nutrients (Farrar and Jones, 2000). Conditions which limit photosynthesis can be viewed as limitations on shoot processes, resulting in reduced relative allocation to roots to maintain a functional equilibrium. The signalling processes which regulate this balance are poorly understood, but appear to involve sugars, phytohormones and small RNAs (Farrar and Jones, 2000).

13.3 ROOT DEVELOPMENT

Root growth and development consist of the initiation, elongation and development of new root axes. The anatomical organization of root axes varies somewhat among plant species, but follows a general pattern, as shown in Fig. 13.3. The root cap protects the root apical meristem and facilitates root penetration through soil by production of mucilage. In many species, specialized root cap cells called 'border cells' detach from the cap and enter the rhizosphere, where they have multiple functions, including diverting pathogens from the root axis and secretion of enzymes and compounds that enhance nutrient availability (Miyasaka and Hawes, 2001). The root apical meristem

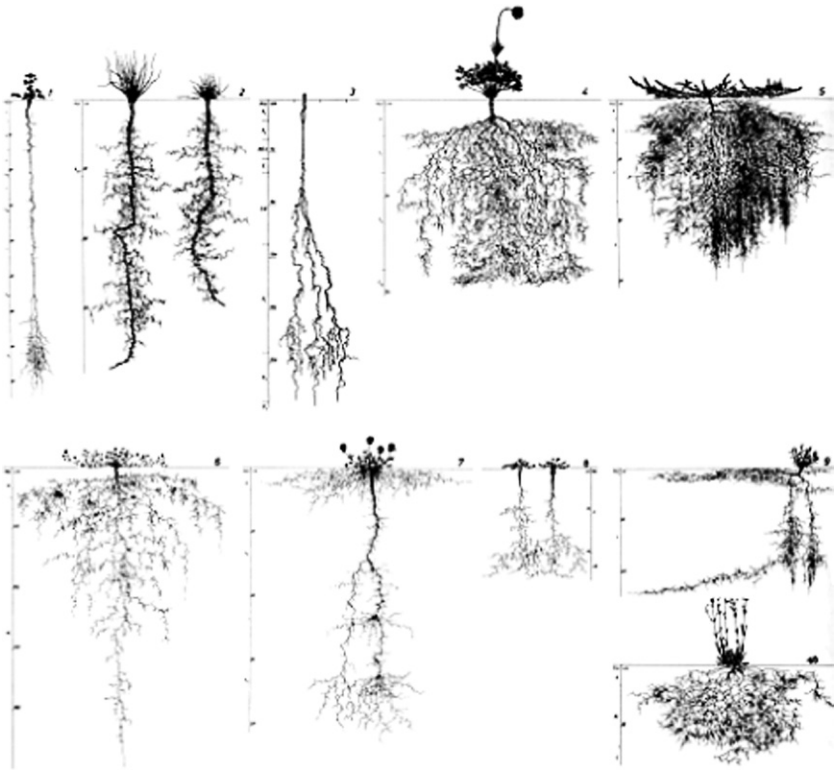

FIGURE 13.4 Root architecture of several dicotyledonous species observed in the field. *From Kutschera and Lichtenegger (1992). With permission from Gustav Fischer Verlag*.

produces cells that differentiate to form the primary tissues of the root axis, the epidermis, cortex, endodermis and the various tissues in the stele, including xylem, phloem, parenchyma, sclerenchyma and collenchyma, with their associated specialized cell types (Evert and Eichorn, 2007). In plants capable of secondary development such as dicotyledonous angiosperms and gymnosperms, root development continues through production of new cells from the vascular cambium, leading to radial thickening and tough, woody roots. The production of lateral branches from the pericycle and from stem tissue (adventitious rooting) leads to a fully developed root system, which in most plants consists of many thousands of individual root axes. The spatial arrangement of groups of roots or the entire root system is known as 'root architecture' (Lynch, 1995).

The anatomy, morphology (i.e., surface features of roots such as root hairs), and especially the architecture of root systems varies within and among species (Figs 13.4 and 13.5). Variation among species reflects the evolution of root systems from holdfasts in algae, to simple rhizoids in primitive land plants, to the increasingly complex and sophisticated root systems of ferns, gymnosperms and angiosperms (Lynch, 2005). Genetic variation for root traits among genotypes of a given species probably reflects adaptation to diverse soil environments because root traits have a strong influence on water and nutrient acquisition. For example, genotypes with shallow root growth angles (to the left side of Fig. 13.5) have better topsoil exploration and superior P acquisition because this nutrient is immobile and is found primarily in surface soil layers, whereas genotypes with deeper root growth angles (to the right side of Fig. 13.5) have better subsoil exploration and hence greater water acquisition under drought (Ho *et al.*, 2005). Genetic variation in root hair length and density is important for P uptake (Foehse *et al.*, 1991). The genetic basis of root phenotypic variation in several crop species has been examined for basal root growth angles, adventitious rooting, basal root whorl number, root hair length and density, and rooting depth. In general, these are quantitative traits under complex genetic control, but several of these traits are used in crop breeding programmes to enhance nutrient and water acquisition (Lynch, 2007).

Plant hormones regulate growth and development of roots, as they do in shoots (see also Chapter 5). Root development varies among species and genotypes, and is highly plastic, i.e. root architecture responds to the soil environment by changing growth and branching patterns. Differences in plant hormone synthesis and response probably mediate these plastic responses to the environment as well as contribute to genetic differences in root architecture and plasticity (Basu *et al.*, 2007; Nibau *et al.*, 2008; Schmidt, 2001). For example, when soils become

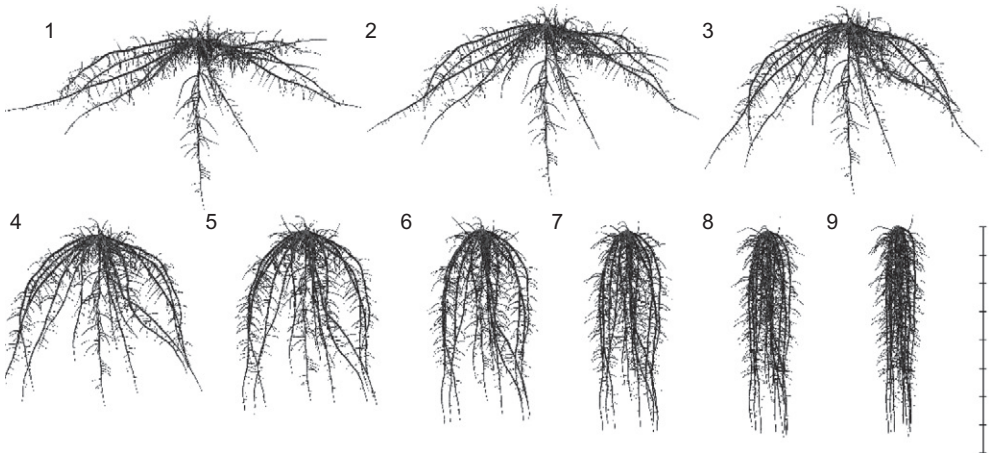

FIGURE 13.5 Simulated common bean root systems differing in basal root gravitropism (gravitropic set point angle, or GSA) but identical in other regards. *From Ge* et al. *(2000). With kind permission from Springer Science & Business Media.*

TABLE 13.1 Shoot and root growth of potato at different nitrate concentrations in the nutrient solution

N supply	Dry weight ($g\,plant^{-1}$)		Root/ shoot ratio	Root surface area ($m^2\,plant^{-1}$)	Root length ($m\,plant^{-1}$)
mM	Shoot	Root			
0.05	0.8	0.45	0.56	0.63	67
0.5	3.5	1.39	0.40	3.14	277
5.0	9.2	1.82	0.20	5.77	502

From Sattelmacher *et al.* (1990a).

waterlogged, plants respond to the resulting hypoxia with a variety of metabolic and growth responses that help the root system to continue functioning under low oxygen stress. Given that gases such as oxygen, CO_2 and ethylene diffuse more slowly in water than in air, oxygen becomes depleted in the respiring plant tissue, while ethylene and CO_2 accumulate. This increased ethylene induces a variety of growth and development responses such as aerenchyma formation, development of adventitious roots and enhanced shoot elongation (Bailey-Serres and Voesenek, 2008) (see also Section 17.4).

13.4 SOIL CHEMICAL FACTORS

13.4.1 Nutrient Supply

Nutrient supply can strongly affect root growth, morphology and distribution of root systems in the substrate (e.g., soil profile). This effect is particularly marked with N, less distinct with P, and usually absent with other nutrients, except Mg (Section 6.5). In the responsive range (i.e., concentration range where nutrients limit plant growth), increasing N supply enhances both shoot and root growth, but usually shoot growth more than root growth, leading to a reduction in root/shoot ratio with increasing N supply (Table 13.1). However, this comparison is somewhat misleading in terms of nutrient acquisition as the roots become finer (higher branching) and the surface area increases so that, despite a decrease in root/shoot ratio, the sink–source relationship, shoot weight (demand) and root surface (source, supply) for nutrient and water acquisition may remain similar. In soil-grown plants this effect of N in increasing root surface area is usually more distinct with supply of ammonium as N source than with nitrate (Marschner *et al.*, 1986b).

The reasons for the differential effect of the two N forms on root morphology are unclear, but differences in pathways of assimilation in the roots and in plant hormonal balance (Chapter 5) are likely to be involved. Hormonal effects are probably also responsible for an increase in the formation of aerenchyma in the cortex of maize roots even in well-aerated solutions when the N supply is low (Konings and Verschuren, 1980). An increase in aerenchyma formation is a typical root response to low oxygen supply to the roots

TABLE 13.2 Shoot and root growth of 15-week-old field-grown maize with or without N supply (NH_4NO_3)

	Dry weight ($g\,plant^{-1}$)		Roots		
N supply ($kg\,ha^{-1}$)	Shoot	Grain	Length ($m\,plant^{-1}$)	Dry weight ($g\,plant^{-1}$)	Root/ shoot ratio
0	186	54	2,189	42	0.23
180	352	138	2,521	38	0.11

Based on Anderson (1988).

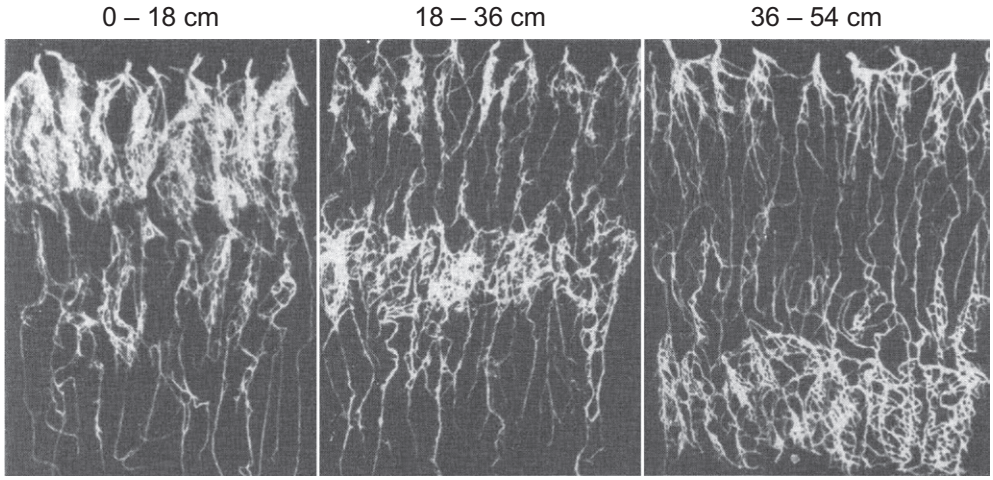

FIGURE 13.6 Root distribution of barley growing in sandy soil with N fertilizer placement at different depth. *From Gliemeroth (1953). With permission from Wiley & Sons.*

(e.g., waterlogged soils; Section 17.4) or to elevated concentrations of ethylene; Section 17.4.

Under field conditions, the enhancing effect of N supply on root growth is usually less distinct (Table 13.2), but in principle the same pattern occurs as in nutrient solutions, namely an increase in total root length and a decline in root/shoot ratio. In field-grown plants, however, data on root dry weight and root length in particular often underestimate true values because of considerable losses of fine roots during collection and preparation (Grzebisz *et al.*, 1989). Furthermore, the turnover rate of roots is presumably much higher in plants adequately supplied with N than those that are N deficient. In broadleaf and coniferous forest stands, the turnover rate of fine roots per year increased from 50% in low N soils to up to 200% in high N soils (Aber *et al.*, 1985).

Root growth is enhanced at sites of high as compared to low nutrient supply. This effect of increased root growth can be demonstrated in split-root experiments, by differential fertilizer placement in soils, or by localized nutrient supply to only part of the root system. In long-term split-root experiments with Sitka spruce, root growth rate and total dry weight increased more strongly on the side where nutrients were supplied. Nitrogen is often the most effective nutrient in terms of localized stimulation of root growth, with P showing some effect and K none (Coutts and Philipson, 1977; Philipson and Coutts, 1977; Brouder and Cassman, 1994). Responsiveness of crops to P fertilizer placement differed among species, with canola and wheat allocating relatively more root biomass and root length to the high P zone than narrow-leaf lupin (Rose *et al.*, 2009). Root proliferation on the nutrient-rich side was accompanied by reduced root growth on the side without nutrient supply, possibly, but not necessarily, reflecting source limitation. The distribution of roots in soils can thus be modified by the placement of fertilizers. In annual species, rooting density rapidly increases several-fold in zones of higher nutrient concentrations, especially of N (Fig. 13.6). This also demonstrates the potential risk of having high N availability only in the topsoil as roots concentrate there at the expense of subsoil penetration. Deep placement of N or P fertilizer, therefore, enhances plant growth

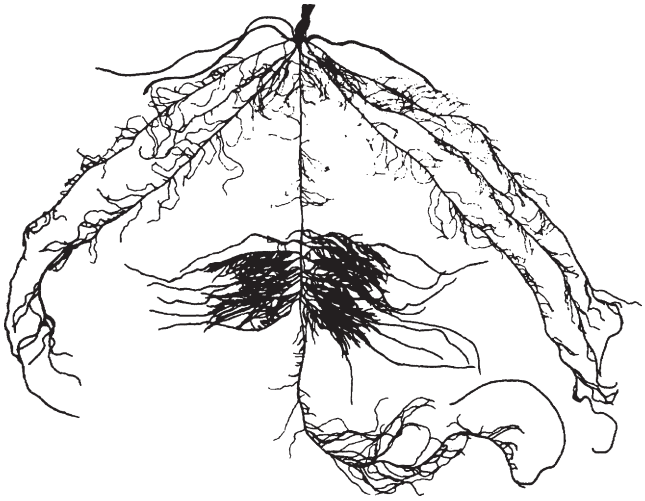

FIGURE 13.7 Modification of the root system of barley by providing 1 mM nitrate to the middle part of one root axis for 15 days, the remainder of the root systems received 0.01 mM nitrate. *From Drew and Saker (1975) by permission of Oxford University Press.*

TABLE 13.3 Lateral root length and dry weight of barley (21 days) with uniform or localized P supply (to middle section only)

	Uniform supply		Localized supply	
	Lateral roots			
Root zone	Length (m)	Dry weight (mg)	Length (m)	Dry weight (mg)
A (basal)	40	9	14	4
B (middle)	27	4	332	38
C (apical)	18	10	11	5

Based on Drew and Saker (1978).
P was applied to the 4 cm section in zone B (middle) to a single seminal root axis.

under drought stress conditions when the water potential of the topsoil decreases, but water is available in the subsoil (Garwood and Williams, 1967; Ma *et al.*, 2009).

Localized nutrient supply to only one part of the root system also affects root morphology. As shown in Fig. 13.7, lateral root growth was strongly enhanced in the root zone where nitrate concentration was high. For N, it has been shown that root proliferation occurs when patches supply >10% of the total plant N requirement (Hodge, 2004).

Localized supply of P can also lead to enhanced lateral root growth (Table 13.3). Over the 21-day period, the total length of the lateral roots increased more than 10-fold over that of the controls with uniform P supply. The increase in lateral root length and dry weight in the zone with P was at the expense of the remaining root zones, where no P was supplied. In soils low in readily available P, placement of P fertilizers is a common and effective practice to ensure an adequate nutrient supply to the roots, especially in the early growth stages. In a study with pearl millet, Valluru *et al*. (2010) showed that even very small amounts of P placed next to the seedling (0.25, 0.50 and 1.0 mg soluble P per seedling, equivalent to 125–500 g P/ha) can enhance growth.

Not only root density but also nutrient uptake rates may also be altered when growing in nutrient-rich patches. *Lupinus angustifolius* had 1.5–2.5 times greater nitrate uptake rates per unit of root length in high nitrate patches compared to areas with low nitrate concentration, whereas *Lupinus pilosus* did not show such increased uptake capacity (Dunbabin *et al*., 2001a, b). However, root density in the nitrate patches was 1.7 times greater in *L. pilosus* (Dunbabin *et al.*, 2001a). This suggests that the two plant species had different strategies to acquire nutrients from nitrate patches: increased N uptake rates in *L. angustifolius* and increased root density in *L. pilosus*.

The question arises by which mechanisms nutrients induce morphological changes in the development of root systems. In maize roots with localized nitrate supply, phloem unloading of photosynthates was enhanced at the zone of supply already two days after the treatment began, with increased cell division rate after four days (Thoms and Sattelmacher, 1990). Respiration rates also increase at the sites of nitrate supply, but not in the total root system (Granato and Raper, 1989), suggesting alteration in photosynthate partitioning within the root system in favour of sites with high nutrient supply. The enhanced initiation of lateral roots at sites of high nutrient supply is presumably not caused by higher unloading of photosynthates *per se*, or higher respiration rates, but rather by phloem unloading of IAA together with the photosynthates (Thoms and Sattelmacher, 1990).

Nutrient deficiency also influences root morphology, for example decreasing nitrate concentrations, with increased root hair density and root hair length in oilseed rape (Bhat *et al.*, 1979; Robinson and Rorison, 1987).

In oilseed rape, spinach and tomato, root hair formation is strongly reduced at high P concentrations (>100 µM), whereas root hair length is increased at low concentrations (<10 µM) (Föhse and Jungk, 1983). In soil-grown maize, a reduction in P availability had no effect on root hair length, but increased root hair density per unit root length (Fig. 13.8).

Phosphorus deficiency, like N deficiency, leads to an increase in the root/shoot ratio (Table 13.4). Increasing the duration of P starvation increased root dry weight, particularly root length, hence the roots became finer. The increase in root surface area in P-deficient plants is a strategy for enhancing P acquisition from soils (Section 13.3).

TABLE 13.4 Shoot and root growth of maize seedlings grown for 1–6 days without P supply

	Shoot		Root		
Days without P	Dry weight ($g\,pot^{-1}$)	P concentration ($mg\,kg^{-1}$)	Dry weight ($g\,pot^{-1}$)	Length ($m\,pot^{-1}$)	Radius (mm)
1	2.10	9.5	0.27	46	2.3
2	2.34	6.5	0.31	58	2.2
4	1.93	3.2	0.40	76	2.0
6	1.65	2.7	0.43	91	1.8

Based on Anghinoni and Barber (1980).

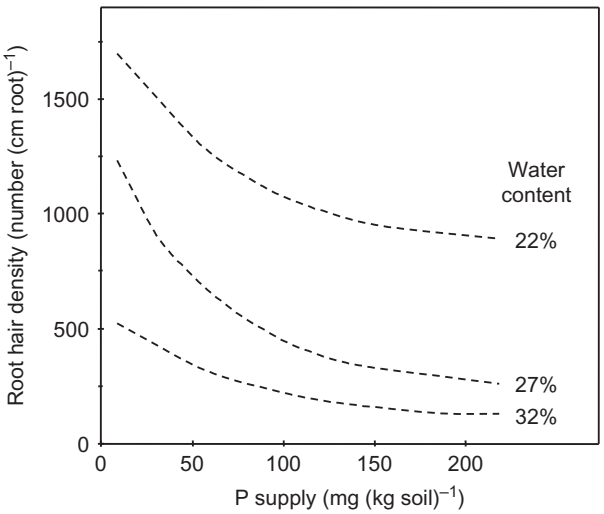

FIGURE 13.8 Root hair density in 21-day-old maize plants grown in soils with different P supply and water content. *From Mackay and Barber (1985). With kind permission from Springer Science & Business Media.*

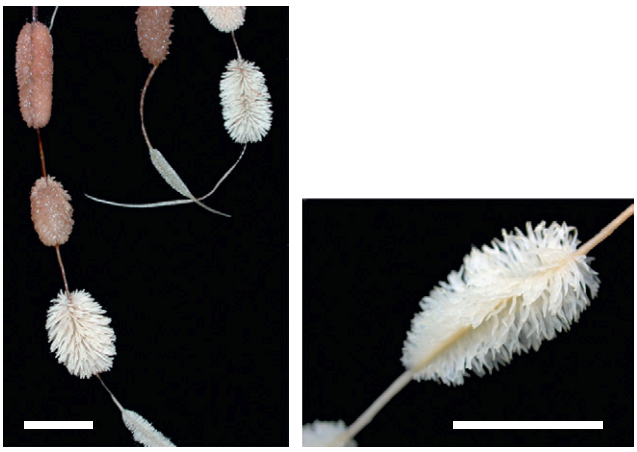

FIGURE 13.9 Proteoid root morphology (simple type) induced in species of hydroponically grown Proteaceae by a low (≤1 μM) P supply in nutrient solution. (A) *Hakea petiolaris* and (B) *Hakea prostrata*. Both species are well adapted to soils of extremely low P concentrations and endemic to the South West Botanical Province of Western Australia. White bar represents 20 mm. *Courtesy of Michael Shane*.

Phosphorus availability is a key regulator of many aspects of root growth and development, including root hair length and density, cortical organization, cortical aerenchyma formation, elongation, secondary development, branching, adventitious rooting and gravitropism (Lynch and Brown, 2008). These changes are adaptive by enhancing the 'topsoil foraging' because P availability is typically greatest in surface soil layers (Lynch and Brown, 2001).

In certain plant species, formation of root clusters is a response to P deficiency (Lambers *et al*., 2006). The best known example are the cluster roots (formerly also called proteoid roots) in *Proteaceae* (Lamont, 1982; Vorster and Jooste, 1986; Fig. 13.9), although they also occur in species of *Myricaceae* (Louis *et al.*, 1990), leguminous trees such as *Casuarinaceae* (Racette *et al.*, 1990), and annual legumes such as white lupin. Cluster roots consist of clusters of short laterals which have long root hairs. In infertile soils, cluster roots may make up as much as 80% of the total root dry weight (Lamont, 1982), in white lupin it was about 50%. A similar morphological change can be seen in many sedges (*Cyperaceae*); they form dauciform roots which are carrot shaped with long root hairs (Lambers *et al*., 2006).

In some plant species cluster root formation is also enhanced under N (Lamont, 1972; Dennis and Prasad, 1986) or Fe deficiency (White and Robson, 1989), but P deficiency has the most pronounced effect. Cluster roots are characterized by high respiration rates and, thus, high oxygen demand (Vorster and Jooste, 1986). Their capacity to mobilize sparingly soluble P is due to release of organic acid anions, phenolics and phosphatases (Lambers *et al.*, 2006). Hence, the limited soil volume in the immediate vicinity of the cluster root zones is subjected to intense chemical extraction (see also Chapter 14).

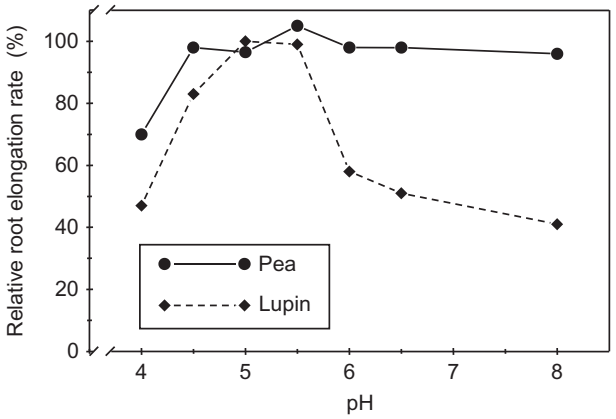

FIGURE 13.10 Root elongation of *Lupinus angustifolius* and *Pisum sativum* grown in nutrient solution at different pH for 60 h. *From Tang* et al. *(1992b). With permission from Elsevier.*

TABLE 13.5 Elongation of cotton roots in an unlimed or limed acid subsoil with the amount of lime added was the same in all treatments

Subsoil mass limed (%)	Distance between limed layers (cm)	Relative root length (%)
Unlimed	–	32
10	4.5	38
20	4.0	57
40	3.0	57
60	2.0	70
100	–	100

Based on Pearson *et al.* (1973).

13.4.2 Soil pH, Calcium/Total Cation Ratio

Root growth is little influenced by external pH in the range of 5.0–7.5. In contrast to low pH (<5) stress, relatively little information is available on root growth at high pH. Root growth can be inhibited by high pH either directly or indirectly. Direct effects of high pH are to be expected in relation to establishment and maintenance of the transmembrane pH and electrochemical gradients, and proton–anion cotransport across the plasma membrane (Chapter 2). Another well-known direct effect linked to high pH is ammonia toxicity. Root elongation is severely inhibited by ammonia concentrations as low as 0.05 mM (Schenk and Wehrmann, 1979). Ammonia toxicity is probably also the reason for the inhibition of root growth in neutral or alkaline soils after application of ammonium phosphate (Bennett and Adams, 1970) or the band-application of urea (Creamer and Fox, 1980). At high soil pH, root growth, particularly of calcifuge plant species (Lee and Woolhouse, 1969a) and lowland rice (Dogar and van Hai, 1980), may also be inhibited by elevated bicarbonate concentrations.

Root growth of *Lupinus angustifolius* is particularly sensitive to high pH and is depressed even at pH 6.0 (Fig. 13.10). This growth inhibition is caused by a decrease in the rate of cell elongation occurring within 1 h after exposure to high pH and leading to an increase in root diameter. The effect is reversible, with cell elongation rate restored upon lowering the pH. The inhibitory effect of high pH may be related to the paucity of protons in the apoplasm that are needed for growth ('acid growth theory'). These findings are in accordance with the poor field performance of *L. angustifolius* in alkaline soils (Tang *et al.*, 1992b).

Inhibition of root elongation at pH <5 (Fig. 13.10) is a common feature in many plant species and is caused by various factors such as impairment of H^+ efflux (Schubert *et al.*, 1990b) and related processes as discussed in Section 2.4. In soil-grown plants, inhibition of root elongation at these low pH values is often due to high activities of monomeric Al and, thus, Al toxicity (Section 17.3).

Aluminium toxicity is one of the major growth-limiting factors in crop production on acid mineral soils (Section 17.3). However, the Al concentration in the soil solution is not the only important parameter; instead, toxicity is modulated by Al speciation and presence of Ca. Calcium plays a key role in protecting root growth against low pH stress. For a given species, the Ca requirement for root growth is a function of both pH and the concentrations of other cations, including Al. For example, in cotton at pH 5.6, even ~1 µM Ca in the external solution is sufficient for maximal root growth, whereas more than 50 µM Ca is required at pH 4.5 (Lund, 1970).

In sensitive species such as cotton, root elongation rate was severely inhibited when Al/Ca molar activity ratios were greater than 0.02 (Lund, 1970), whereas in mungbean grown in nutrient solution much greater ratios (Al/Ca of up to 4) were not inhibitory to root elongation (Yang *et al.*, 2001). In soils, this ratio may vary widely, depending on soil properties, Al speciation and root-induced changes in the rhizosphere (Chapter 14).

On average, a molar ratio in the soil solution of Ca to total cations of ~0.15 is needed for maximal root growth (Fig. 13.11). In acid mineral soils, ratios lower than 0.15 often occur, resulting in root growth inhibition. Liming such soils enhances root extension and also root hair length (Sartain and Kamprath, 1975; Table 13.5), but this effect is due to the increase in pH and Ca supply. In contrast, gypsum applied to acidic soils does not alter the pH, but enhances root growth by providing Ca and decreasing Al toxicity, for example by formation of non-toxic $AlSO_4^+$ ions (Caires *et al.*, 2002).

The Ca/total cation ratio is also important for root growth under saline conditions (Kafkafi, 1991; Section

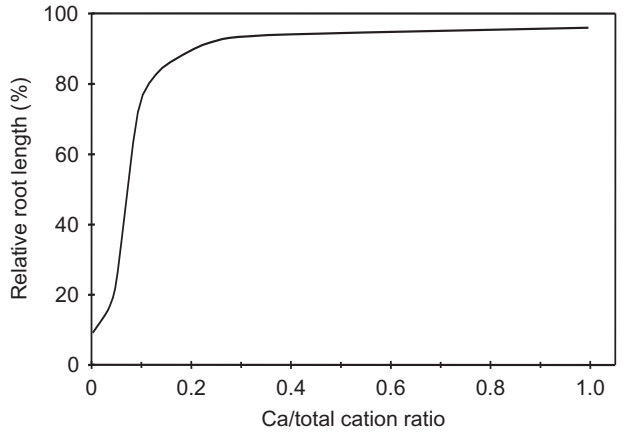

FIGURE 13.11 Growth of seminal roots of cotton seedlings at different Ca/total cation molar ratios of the soil solution. *From Adams (1966). With permission from Elsevier.*

17.6) and in relation to the application of ammonium phosphate fertilizer. In acid soils with a low cation-exchange capacity, ammonium phosphate can severely inhibit root growth by inducing NH_3 toxicity (Bennett and Adams, 1970) and Ca deficiency (a low Ca/total cation ratio) (Bennett and Adams, 1970; Moody *et al*., 1995; Zhang and Rengel, 2000); both NH_3 toxicity and Ca deficiency can be alleviated by addition of gypsum (Zhang and Rengel, 2000).

In addition to poor aeration and high mechanical impedance, low subsoil pH is an important factor in restricting subsoil penetration by roots (Jentschke *et al*., 2001; Tang *et al*., 2002, 2003). Because Ca is phloem immobile, the Ca required for root growth must be taken up from the external solution by the apical zones, which is particularly problematic in acidic subsoils because of (i) poor physiological status of roots due to acidity and Al toxicity and (ii) relatively low Ca concentration in the soil solution. Roots may be severely inhibited in their capacity to penetrate acid subsoils even when an adequate amount of lime was mixed into the topsoil because of poor solubility and thus limited leaching of lime into subsoil horizons (Whitten *et al*., 2000). There is a close correlation between the increasing proportion of soil mass mixed with lime and root elongation in the soil. In the field, incorporation of lime to 0.2 rather than 0.1 m improved crop yields in two out of four seasons, with particularly large increases in the dry seasons (Scott *et al*., 1997).

Increased penetration of roots into acidic subsoils can be achieved by (i) amelioration of subsoil acidity, for example by surface placement of relatively large amounts of lime and allowing years to pass to allow sufficient leaching of dissolved lime into subsoil layers (Scott *et al*., 1997; Whitten *et al*., 2000; Tang *et al*., 2003), (ii) applying gypsum that increases concentration of Ca in subsoil (leaching of gypsum is greater than of lime because of its higher solubility; Caires *et al*., 2002), (iii) using Al-resistant genotypes, for example of wheat (Tang *et al*., 2002), or (iv) the combination of liming and Al-resistant genotypes (Tang *et al*., 2002). In a field with acidic subsoil in Western Australia, the Al-resistant wheat genotype had up to 50% higher root density in the 0.1–0.4 m layer compared with the Al-sensitive genotype, which resulted in a greater depletion of water in the subsurface layers and 51% higher yield of the resistant compared with the sensitive genotype (Tang *et al*., 2002).

For heavy metal toxicity, inhibition of root elongation is in many instances the most sensitive parameter, for example for lead (Pb) (Breckle, 1991) or nickel (Ni) and copper (Cu) (Gonnelli *et al*., 2001). However, seed germination rate or root necrosis can also be very sensitive to heavy metal toxicity (Valerio *et al*., 2007). Probably due to binding of heavy metal cations to biomolecules (resulting in displacement of essential metals and blocking of active sites), the order of toxicity usually conforms to the stability of the metal–organic complexes, for example $Cu > Ni > Cd > Zn > Al > Fe$ (Wong and Bradshaw, 1982), even though toxicity mechanisms other than metal binding to biomolecules (e.g., induction of the oxidative stress) are also important (Schutzendubel and Polle, 2002).

13.4.3 Aeration

Because of their high rates of respiration (Section 13.1), roots have a high demand for oxygen (O_2). In a dense crop stand, O_2 consumption and CO_2 evolution may be as high as $17\,L\,m^{-2}\,day^{-1}$ (Cannell, 1977). The transfer of gases between soil and atmosphere occurs mainly in air-filled pores because gas diffusion is about 100 times more rapid in air than water. In many species adapted to waterlogging (e.g., wetland rice), sufficient internal diffusion of O_2 from leaves to roots takes place in the aerenchyma (Section 17.4). However, in mesophytic (non-wetland) species this internal transfer is either unimportant or insufficient to meet the requirement of large root systems.

The critical soil O_2 concentrations that affect root growth differ for different plants. In most mesophytic species, root growth is not affected even when O_2 concentrations in the soil gaseous phase decrease to about 15 to 10% (v/v) (Geisler, 1967). However, in maize, lowering the O_2 concentration from 21 to about 10% severely impaired root extension, even though root respiration was unaffected, indicating that, at least in this concentration range, processes other than respiration are responsible for the root growth inhibition by poor aeration (Saglio *et al*., 1984). Sensitivity to waterlogging (poor aeration) may be affected by plant developmental stage. The ranking of winter wheat genotypes for waterlogging tolerance differed at the seedling stage (measured as root growth) and at maturity (grain yield) (Dickin *et al*., 2009).

A decrease in soil O_2 concentration is usually associated with an increase in CO_2, and often also with an increase in ethylene concentration (Jackson, 1990a,b). Compared with the ambient CO_2 concentration (~0.037 % v/v), the soil atmosphere CO_2 concentration increases with soil depth and is maximal in summer when the respiration rates by roots and soil microorganisms are high. Soil CO_2 concentrations are in the range of 2–4% (v/v) at the 10–20 cm depth and may increase to 10–15% (v/v) at the 40–60 cm depth in summer (Nakayama and Kimball, 1988). Depending on concentration, CO_2 has either stimulatory (~1–2% v/v) or inhibitory effects (>5% v/v) on root growth (Geisler, 1968). In contrast to most other species, desert succulents are very sensitive to elevated CO_2 concentrations. Even at CO_2 concentrations as low as 0.5% CO_2 (v/v) in the soil atmosphere, root growth rates may be severely inhibited (e.g., in *Agave deserti*), explaining the confinement of these species to coarse-textured, well-aerated soils (Nobel, 1990). In a forest stand with CO_2 concentrations of 0.5% (v/v) in the soil atmosphere, enhancement of aeration was accompanied by an increase in root growth of Norway spruce throughout the soil profile, and particularly at 40 cm depth (Murach *et al.*, 1993). However, it remains to be elucidated whether enhanced root growth upon increased aeration may be due to decreases in the concentration of gases other than CO_2 (e.g., ethylene).

Generally, inhibition of root growth in poorly aerated soils is caused by elevated concentrations of ethylene (Jackson, 1990a,b). In poorly aerated soils, ethylene production in the roots is often enhanced, which is coupled with a reduced loss of ethylene from roots by radial diffusion as a result of water around roots. Ethylene stimulates auxin biosynthesis and modulates the transcription of several components of the basipetal auxin transport toward the elongation zone (see also Chapter 5). Additionally, ethylene activates a local auxin response leading to inhibition of cell elongation (Ružička *et al.*, 2007). Interestingly, the presence of a root cap is crucial for the function of ethylene in regulating root growth in the elongation zone (Hahn *et al*., 2008).

13.4.4 Low-Molecular-Weight Organic Solutes

Root growth is affected in various ways by the water-soluble fraction of soil organic matter. Humic acids have been shown to increase shoot and root growth as well as root ATPase activity (Canellas *et al.*, 2009). Root initiation and elongation may be enhanced by low concentrations of high-molecular-weight fraction, especially fulvic acid (Mylonas and McCants, 1980) and also by some phenolics in the low-molecular-weight fraction (Pingel, 1976; Wilson and Van Staden, 1990). However, at higher concentrations, the low-molecular-weight fraction inhibits root growth. This is particularly true for phenolic and short-chain fatty acids, which often accumulate in poorly aerated or waterlogged soils during decomposition of organic material (e.g., straw or green manure). In well-structured soils, clumps of organic matter may cause the formation of anaerobic microhabitats. Poor germination and emergence of plants on these soils is often not caused by oxygen limitation or elevated ethylene levels, but by high concentrations of phenolics and short-chain fatty acids. Evidence for this comes from the similar inhibitory effects on germination and emergence (Hicks *et al.*, 1989) or root respiration, root growth and root hair formation (Patrick, 1971) that can be achieved with water extracts from these soils, particularly 3–4 weeks after incorporation of organic matter (Patrick, 1971).

During the decomposition of organic materials with a high lignin concentration (e.g., straw), phytotoxic substances, including phenolic acids such as *p*-coumaric and *p*-hydroxybenzoic acid, may accumulate and severely inhibit root elongation in sensitive species, such as rye and wheat, even at concentrations between about 7 and 70 µM (Börner, 1957) and in tolerant species such as sugarcane at about 750 µM (Wang *et al.*, 1967).

In paddy soils after incorporation of straw, inhibition of rice root elongation is caused by phenylpropionic acids even at concentrations below 50 µM (Tanaka *et al.*, 1990). In waterlogged soils, other phytotoxic substances, primarily acetic acid and other volatile (short-chain) fatty acids, may reach concentrations that are phytotoxic (Harper and Lynch, 1982). These acids are detrimental to root elongation (Lynch, 1978), and inhibit root and shoot growth, even in plant species adapted to waterlogging.

In acids, phytotoxicity increases with chain length and with decreasing substrate pH (Jackson and St John, 1980). At low pH, the acids are undissociated and therefore more easily permeate the plasma membrane. The inhibitory effect of decomposition products (e.g., of straw) on root growth therefore depends not only on the concentration of volatile fatty acids but also on the pH of the rooting medium.

13.5 SOIL ORGANISMS

13.5.1 General

Soil organisms, which include microorganisms, mesofauna and macrofauna, can stimulate, inhibit, or have no effect on root growth, depending on the type of organism, plant species and environmental conditions (Bonkovski, 2004; Scheu, 2003; Bowen and Rovira, 1991). Soil microorganisms play a critical role in nutrient cycling by mineralizing organic compounds into inorganic nutrients or transforming nutrients which can then be taken up by plants. Mineralization is primarily carried out by microorganisms

such as bacteria and fungi. The larger organisms stimulate microbial activity by fragmenting organic matter and mixing these fragments with soil particles and microorganisms (Killham, 1994). Additionally, soil organisms may affect root growth by release of stimulating or inhibiting compounds, root damage or, in case of larger soil organisms such as earthworms, by creating pores, thereby improving drainage and aeration as well as providing pathways for roots.

13.5.2 Pathogens and Pests

Traditionally, in studies of negative soil microbe–plant interactions, the main interest has been focused on soilborne pathogens such as *Gaeumannomyces graminis*, cyst nematodes (Chapter 10) or pathogens which impair specific root functions such as cytokinin production (Cahill *et al.*, 1986). However, 'minor pathogens' or deleterious microorganisms, which do not induce clear symptoms, but can also reduce plant growth, are increasingly studied. They inhibit root growth by production of phytotoxins (e.g., cyanide), competition for nutrients or inhibition of mycorrhizal function (Schippers *et al.*, 1990; Bolton *et al.*, 1989; Nehl *et al.*, 1997).

Harmful rhizosphere microorganisms belong to various genera of bacteria and fungi. They are often responsible for depression of growth and yield of crop plants in short rotations or monocultures (de Weger *et al.*, 1987). In fruit trees or grapevine, this situation is often described as 'soil sickness' or 'replant disease'. Typically, in case of soil sickness, different types of deleterious microorganisms (and also pests such as nematodes) are involved. These microorganisms can be eliminated by soil sterilization (Pankhurst *et al.*, 2005). On the other hand, long-term monoculture of wheat or barley can also lead to a decline in pathogens such as *Gaeumannomyces graminis* var. *tritici* which has been associated with an increase in microorganisms such as pseudomonads or actinomycetes (Raajimakers *et al.*, 1999) which produce antibiotics that inhibit the growth of the pathogen (Thomashow and Weller, 1988). Although the majority of nematodes are beneficial, some are plant pathogens, for example root-knot nematodes (*Meloidogyne* spp.) or cyst nematodes (*Globodera* and *Heterodera* spp.), the larvae of which penetrate the roots by production of cellulases and pectinases and then induce the formation of giant cells to use host assimilates for growth. Root growth is reduced by the C drain and destruction of root tissue; phytohormones produced by the nematodes may also play a role (Gregory, 2006a). Plant resistance mechanisms involve phytohormones as well as phenolics which may be directly toxic to the nematodes (e.g., phytoalexins), regulate defence reactions and modify tissue development (Mateille, 1994).

13.5.3 Beneficial Rhizosphere Bacteria

Certain microorganisms can stimulate root growth considerably and are often referred to as plant growth-promoting rhizosphere microorganisms (PGPR). They influence root growth mainly by improving nutrient availability, producing phytohormones and inhibiting pathogens (Lugtenberg *et al.*, 1991; Dutta and Podile, 2010). Many of these are diazotrophic bacteria (e.g., *Azospirillum*, *Azotobacter*, or *Pseudomonas* ssp.), which improve N uptake (Rodrigues *et al.*, 2008). Others improve P availability by P solubilization (Wahid and Mehana, 2000). Furthermore, improved nutrient uptake can also be due to stimulation of colonization by arbuscular mycorrhizal fungi (Dwivedi *et al.*, 2009).

Many rhizosphere bacteria enhance root growth directly by production of phytohormones, IAA in particular (Barazani and Freidman, 1999). Inoculation of soil-grown wheat plants with *A. brasilense* Cd stimulated root growth in general and the formation of lateral roots and root hairs in particular (Table 13.6). Similar stimulating effects on root growth could be obtained by IAA application to soil-grown wheat plants (Martin *et al.*, 1989). These effects of *A. brasilense* and other diazotrophic rhizosphere

TABLE 13.6 Root, root hair and shoot growth of soil-grown wheat without or with inoculation with *Azospirillum brasilense*

	Total root length (m plant^{-1})	Lateral roots (no. plant^{-1})	Root hair		Shoot fw (g plant^{-1})
			Density (no. mm^{-1})	Length (mm)	
Control	0.25	5	24	1.2	0.8
Inoculated	0.4	21	36	1.8	1.0

Based on Martin *et al.* (1989).

bacteria in enhancing root growth and development improve nutrient acquisition. Phytohormone production of rhizosphere microorganisms is not confined to IAA. Depending on the availability of phytohormone precursors, the production, for example, of CYT by *Azotobacter* can be quite high, leading to a strong increase in root and shoot growth of radish plants (Nieto and Frankenberger, 1990). However, PGPR do not necessarily have to produce phytohormones themselves, they may also influence phytohormone synthesis by the plant (Ryu *et al*., 2005).

Plant growth-promoting rhizosphere microorganisms may also affect root growth indirectly by suppression of pathogens, for example *Fusarium oxysporum* in potato (Beauchamp *et al.*, 1991), *Alternaria* in sunflower (Hebbar *et al.*, 1991) or cyst nematodes in soybean and tomato (Kloepper *et al.*, 1992a; Siddiqui and Shaukuat, 2003). Antibiotics are the main mechanism by which PGPRs suppress pathogens, but siderophores which limit Fe availability to the pathogen may also be involved (de Weger *et al*., 1986). Moreover, PGPRs can induce systemic resistance in plants: they reduce the negative effect of the pathogen, while remaining physically separated from it (van Loon *et al*., 1998).

By changing root growth, PGPR can increase drought tolerance and recovery after drought stress. Of particular interest in this respect are PGPR which produce ACC deaminase. 1-Aminocyclopropane-1-carboxylate (ACC) is a precursor of ethylene which reduces membrane fluidity, increases leakage of solutes and suppresses root elongation. Hence, release of ACC deaminase reduces the synthesis of ethylene and thereby stimulates root growth which can increase drought tolerance in plants (Mayak *et al*., 2004, Shahzad *et al*., 2010).

13.5.4 Mesofauna and Earthworms

Protozoa and bacteria-feeding nematodes indirectly influence root growth by grazing bacteria in the rhizosphere. Due to the similarity in C/N ratios between bacteria and protozoa and the low N assimilation efficiency of protozoa, about 60% of the ingested N is released (Ferris *et al*., 1997; Bonkowski, 2004). Protozoa grazing may also stimulate auxin-producing bacteria (Bonkowski and Brandt, 2002).

Earthworms generally increase root growth, although there are also reports showing a negative effect (Scheu, 2003). Earthworms can increase nutrient availability, probably by stimulating mineralization of organic material by microorganisms through fragmentation and mixing (Tuffen *et al*., 2002). Compared to the surrounding soil, earthworm casts and burrows are characterized by greater soil structural stability (Marashi and Scullion, 2003), higher concentrations of total and available nutrients and higher microbial activity (Le Bayon and Binet, 2006); all of which stimulate root proliferation. Additionally, roots often grow in earthworm burrows because they represent pathways of low resistance (Springett and Gray, 1997). Moreover, it has been proposed that earthworms may influence root growth by production of phytohormones and dispersal of plant growth-promoting and anti-pathogenic microorganisms (Scheu, 2003).

13.6 SOIL PHYSICAL FACTORS

13.6.1 Mechanical Impedance

As roots grow through soil, they must either follow pores or channels (see below) or penetrate and displace the soil matrix. Mechanical impedance refers to the resistance offered by the soil matrix against deformation, and has substantial effects on root growth (Bengough *et al*., 2011). As soil impedance increases because of inherently high bulk density, soil drying, or soil compaction (commonly caused by vehicle traffic and cultivation in agricultural soils) root elongation is progressively retarded (Figs 13.12 and 13.13). Root elongation is driven by cell turgor in the elongation zone. This turgor must overcome the impedance of the soil as well as frictional forces along the outside of the root (Bengough *et al*., 1997). These frictional forces are generally low because of the lubricating effect of mucilage released from the root tip, and shearing of border cells from the root cap. As the root encounters hard soil, cell elongation is decreased and radial expansion increases, resulting in a greater root diameter and a build-up of solutes that decreases the osmotic potential in the elongation zone. The rate of cell production slows and cell walls are stiffened in the direction of growth. These responses increase the penetrating force of the root. Additionally, increased sloughing off of border cells and

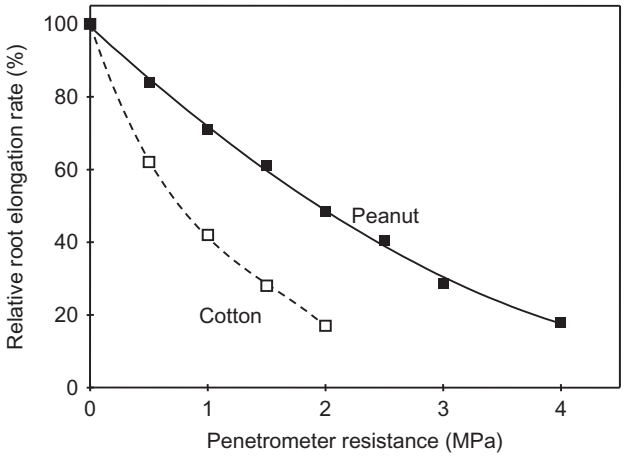

FIGURE 13.12 Root elongation rates of peanut and cotton as a function of soil strength (measured as penetrometer resistance). *From Taylor and Ratcliff (1969). With kind permission from Springer Business and Media.*

root exudation decrease the lateral friction (Bengough *et al*., 2011). These responses of an individual root axis to high soil impedance appear to be adaptive. Soil impedance is typically non-uniform in space, and often increases with soil depth, especially in tilled soils. As some roots encounter hard soil and slow their elongation, while other roots continue to elongate normally, the architecture of the root system is changed, often resulting in a shallower, less dispersed root architecture (Tardieu, 1994). These architectural changes may cause additional growth effects, for example by decreasing water acquisition from deeper soil strata. In compacted soils, shoot growth is also often more depressed than root growth, suggesting root-derived hormonal signals in response to soil compaction (Fig. 13.14).

In hard soils, a significant fraction of roots elongate through low-resistance pathways created by cracks between soil structural units and in biopores formed by soil fauna and the previous growth of roots. For example, in an extremely hard soil in Australia, 30–40% of wheat roots at <60 cm depth were growing in pores or soil cracks, increasing to 85–100% of roots at >60 cm depth (White and Kirkegaard, 2010). Nutrient, water, and oxygen availability in these channels, as well as microbial populations, are different than those in bulk soil. Clustering of root growth in macropores may have important consequences for root competition and water acquisition from drying soil (Passioura, 2002; Smucker and Aiken, 1992).

FIGURE 13.13 Root systems of young barley plants grown in the field in soils with different bulk densities: 1.35 g cm^{-3} (*left*) and 1.50 g cm^{-3} (*right*). *From Scott-Russell and Goss (1974). Reproduced with kind permission from KLV Wageningen Alumni.*

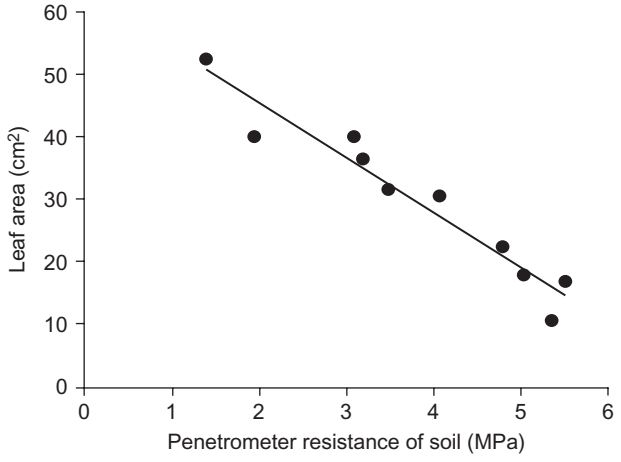

FIGURE 13.14 Leaf area in young wheat plants at different soil impedances. *Based on Masle and Passioura (1987). Reproduced with permission from the Australian Journal of Plant Physiology.*

13.6.2 Water Content

Soil water content has a dominant influence on root growth, through direct effects of water availability on root growth, effects of water on photosynthesis and therefore carbohydrate availability, effects of water on oxygen availability in wet soils, and effects of soil impedance on root growth because dry soils tend to be hard. The direct effects of soil water content on root growth have been intensively studied because of the obvious importance of this topic for global agriculture and ecology. A general response to suboptimal water availability is increased biomass allocation to roots at the expense of shoots, which increases water capture and decreases water use, consistent with the 'functional equilibrium' model (Fig. 13.15).

Root responses to low soil water content improve water capture by increasing exploration of soil domains with the greatest water content. In arid environments, the rainfall may not completely wet the soil profile, and water tends to be more available in surface soil layers. In such environments, some plants are capable of rapidly proliferating shallow roots in response to rain in order to take advantage of the brief availability of water (and nutrients) in the surface soil. These so-called 'rain roots' have specific features that allow them to exploit the water resource at a minimal overall carbon investment by the plant (Rundel and Nobel, 1991). However, as soils dry, water usually remains in the deep soil layers, and therefore a more general response of (herbaceous) plants to low soil water content in the topsoil is to maintain root growth at the expense of shoot growth, and to emphasize root growth processes that extend the depth of soil exploration. Part of the effect of soil water content on rooting depth may be the increased impedance of dry soil, so that roots in moist soil, such as in the subsoil, may continue elongating while roots in dryer and therefore harder surface soils may slow their elongation, resulting in an overall root architecture that is deeper.

The direct effects of water availability on the elongation of individual root axes has been intensively studied by Sharp and colleagues, who have focused on the primary root of maize seedlings growing in vermiculite with varying water content, thereby avoiding confounding effects of

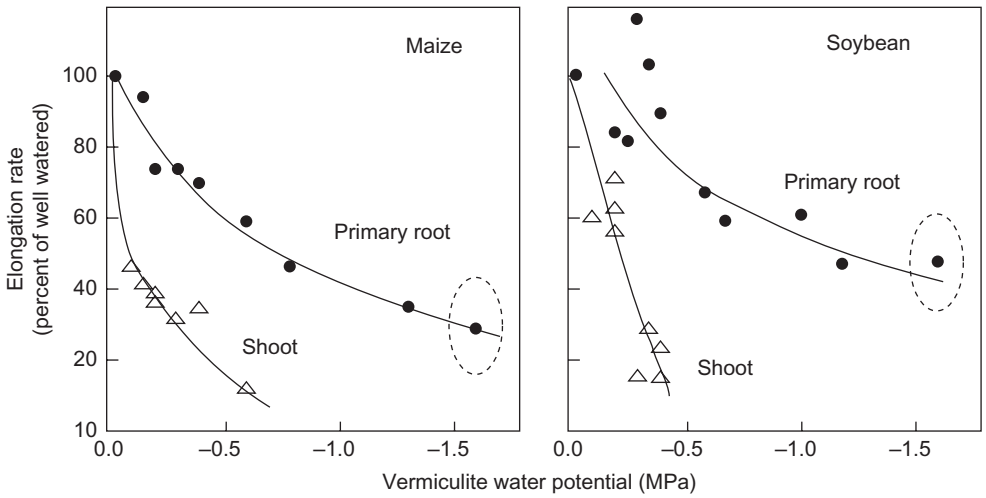

FIGURE 13.15 Root and shoot growth in maize and soybean at different soil water potentials. *From Yamaguchi and Sharp (2010). With permission from Wiley Blackwell.*

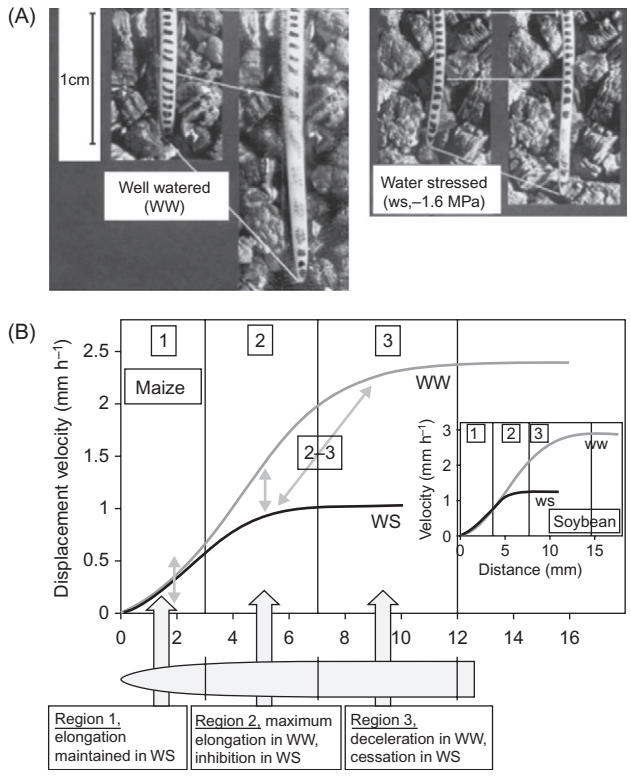

FIGURE 13.16 Root elongation maize in at different soil water potentials. *From Yamaguchi and Sharp (2010). With permission from Wiley Blackwell.*

soil impedance (Yamaguchi and Sharp, 2010). In this system, shoot growth is more sensitive to soil water content than root elongation (Fig. 13.15). Kinematic analysis of root elongation showed that the growth zone more than 3 mm from the root apex is particularly sensitive to the inhibitory effects of low soil water content (Fig. 13.16). Continued elongation of the tip region is associated with osmotic adjustment from increased deposition of proline, greater emphasis on elongation growth rather than radial thickening, and greater extensibility of cell walls along the axis of elongation (Sharp *et al.*, 2004). The molecular basis of these responses is quite complex, and includes (i) increased production of wall-loosening proteins, (ii) regulation of reactive oxygen species (ROS) metabolism, which is important for the mechanical properties of cell walls and other processes, and (iii) regulation of phenylpropanoid metabolism, which is involved in ROS metabolism, wall biosynthesis and auxin transport. ABA is an important regulator of root growth at low soil water content, through direct effects and also by interactions with ethylene (Yamaguchi and Sharp, 2010).

13.6.3 Temperature

In many soils, temperature varies greatly with depth and over time. Temperature in the surface soil layers fluctuates substantially in response to changes in air temperature, irradiation and radiant heat transfers, whereas the temperature is more stable in deeper soil layers. Soil temperature has a substantial influence on root growth. The optimum temperature for root growth is under genetic control (Fig. 13.17, Table 13.7). Also, the temperature optimum varies among species and tends to be lower for root growth than for shoot growth (Fig. 13.18).

Although in many parts of the lowland tropics soil temperatures of ≥40°C at 15 cm depth are common, relatively little information is available on root growth and functioning at supraoptimal temperatures (Liu and Huang, 2005). Within a given species, considerable genotypic differences exist in tolerance to supraoptimal root zone temperatures,

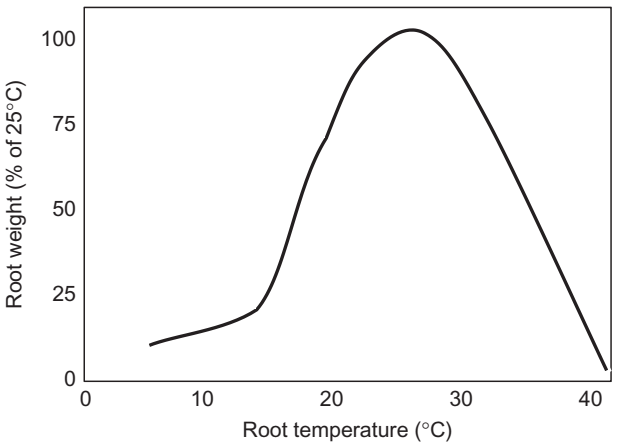

FIGURE 13.17 Root biomass of 24-day-old maize seedlings at different temperatures. *Based on Kaspar and Bland (1992). With permission from Soil Science*.

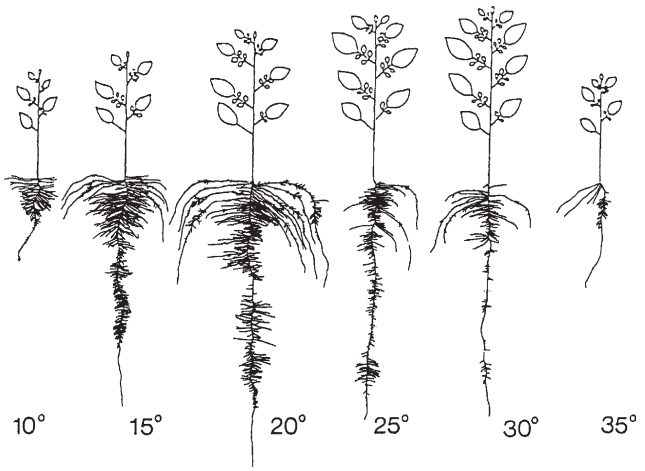

FIGURE 13.18 Root morphology and shoot growth of potato seedlings at different root zone temperatures. *From Sattelmacher* et al. *(1990c). With permission from Oxford University Press*.

TABLE 13.7 Root temperature range for root growth rates ≥50% of the maximum

	Temperature (°C)	
	Low	High
Flax (*Linum usitatissimum* L.)	10	31
Peas (*Pisum sativum* L.)	9	33
Common bean (*Phaseolus vulgaris* L.)	12	33
Maize (*Zea mays* L.)	17	37
Strawberry (*Fragaria* sp.)	5	31
Broad bean (*Vicia faba*)	12	32
Rape (*Brassica napus* L.)	16	32
Oat (*Avena sativa* L.)	9	32

Based on Klepper (1987).

for example in potato (Sattelmacher *et al.*, 1990c). In wheat, supraoptimal temperature decreased the diameter of metaxylem vessels, thereby reducing the root's ability to conduct water (Huang *et al*., 1991). Reduced CYT content may be an early signal of high temperature stress (Liu and Huang, 2005).

Low temperature reduces both root elongation (Pahlavanian and Silk, 1988) and branching (Gladish and Rost, 1993). Low temperatures generally inhibit shoot growth more than root growth, leading to a high root/shoot dry weight ratio (Table 13.8). Root respiration slows in cold soil due to the inhibitory effect of low temperature on enzyme activity (Covey-Crump *et al*., 2002). Sink limitation caused by cold soils has been proposed as a primary limitation to root growth in spring in temperate latitudes and at high elevations (Alvarez-Uria and Korner, 2007).

A reduction in the elongation rates of roots at low temperatures may be caused by a decrease in cell wall extensibility of the cells in the extension zone, not a loss in turgor. By lowering the temperature from 30 to 15°C, cell wall extensibility in the extension zone of maize roots decreased to 25% of its original value (Pritchard *et al.*, 1990). Low temperatures also alter root anatomy. In wheat, lignification of late metaxylem vessels is delayed and axial hydraulic conductivity is higher in roots grown at low compared with high temperatures (Huang *et al*., 1991).

Cooling of roots inhibits shoot and leaf elongation rates without affecting leaf water potential (Milligan and Dale, 1988) and is associated with an increase in ABA concentration in the leaves (Smith and Dale, 1988). In maize, a decrease in root zone temperature from 28 to 8°C increased the ABA concentration in the xylem exudate about two-fold (Atkin *et al*., 1973). This is another example of root-to-shoot communication upon stress in the root environment. This signal upon low temperature stress appears to be dependent on the plant nutritional status, as it is particularly effective in P-deficient plants (Radin, 1990). At low root zone temperatures, CYT production in roots and its export is depressed; in maize at 18°C to about 15% of that at 28°C (Atkin *et al*., 1973). In grapevine roots at a temperature of 12°C, the concentration of CYT in the xylem sap is only about 50% of that at 25°C, and the CYT spectrum changes qualitatively (Zelleke and Kliewer, 1980).

13.7 SHOOT/ROOT RATIO

The size of the root system, and also the root/shoot ratio, required for supply of nutrients, water and growth regulators mainly depend on the concentration of nutrients in the root environment, and the physical, chemical and

TABLE 13.8 Growth and development of roots of winter wheat grown for 20 days at different temperatures

Temperature (°C)	Length of seminal roots ($m plant^{-1}$)	Primary lateral roots		Specific root length ($m g^{-1} dw$)	Root/ shoot ratio
		(No. $plant^{-1}$)	($m plant^{-1}$)		
10	77	56	63	45	2.2
20	98	167	463	125	0.5
25	275	556	1,536	160	0.6
30	138	389	352	125	0.6

Compiled data from Huang *et al*. (1991).

biological properties of the substrate which affect root activity and formation of new roots. For example, within a given crop species, the root/shoot ratio is considerably higher in dryland areas than in temperate climates (Gregory *et al.*, 1984). The root/shoot ratio is proportional to nutrient supply/fertilization, with a greater ratio at low nutrient supply (Chapin, 1988; Kang and Van Iersel, 2004). On the other hand, when there is a large and continuous supply of water and nutrients, a small root system may be sufficient (Greenwood, 1983), as shown by plant production in water culture (e.g., the 'Nutrient Film Technique') in commercial horticulture.

The ratio of shoot-to-root growth varies widely among plant species, during ontogenesis of plants, and is strongly modified by a variety of external factors. There is a general tendency both among and within species to maintain a characteristic relationship between root and shoot dry weight (e.g., graminaceous species $>>$ trees). When parts of the shoot are removed, plants tend to compensate by lowering root growth and returning to a ratio characteristic for the species (e.g., Hansen *et al*., 1995). However, it is unclear whether this reflects 'functional equilibrium' between roots and shoots (Klepper, 1991). Although this hypothesis may adequately describe relative root and shoot growth as influenced by some environmental factors (Farrar and Jones, 2000), a sound physiological basis for the hypothesis is yet to be established because there is little evidence of fine control of phloem loading in response to sink demand for photosynthates (Minchin *et al*., 2002).

There are various feedback mechanisms regulating the root/shoot ratio, some of which are under hormonal control. Examples are retardation or cessation of shoot growth when roots are exposed to drought stress, soil compaction or poor soil aeration). Similarly to the low root zone temperatures (Atkins *et al.*, 1973), waterlogging also decreases root export of CYT (Burrows and Carr, 1969) and gibberellins (Reid *et al.*, 1969) within 1–2 days, reduces shoot elongation and enhances leaf senescence (Chapter 17.4). Foliar sprays with CYT can counteract at least some of the negative effects of waterlogging on shoot growth (Reid and Railton, 1974). Under nutrient deficiency, inhibition of shoot growth accompanied with continuation, or even enhancement, of root growth (e.g., Cakmak *et al*., 2000; Nigussie *et al*., 2003) which may reflect alteration in photosynthate allocation (Liu *et al.*, 2005), but is at least in some instances under direct hormonal control as has been shown in experiments in which shoot growth has been restored by CYT supply to nutrient-starved plants.

Root/shoot ratios were strongly correlated with root amines/nitrate ratios, with values of >1 being characteristic of high N status. It has been proposed that the amine/nitrate ratio interacts with the gibberelic acid signalling and respiratory pathways to regulate the partitioning of biomass between shoots and roots (Pellny *et al*., 2008).

The competition for photosynthates between shoot and roots is the dominant factor limiting root growth and activity during reproductive growth (French and Buirchell, 2005). This is of particular interest in perennial species, for example in Scots pine stands in Sweden where the proportion of photosynthates allocated belowground and used for fine root production was more than 50% (Persson, 1979). However, during reproductive phase, the fine root biomass formation is usually depressed by sink competition of the shoot (Buwalda and Lenz, 1992).

Chapter 14

Rhizosphere Chemistry in Relation to Plant Nutrition

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SUMMARY

This chapter describes the physicochemical processes determining the rhizosphere as soil compartment influenced by the activity of plant roots and the consequences for plant nutrition. An introductory section discusses properties and the spatial extent of the rhizosphere as well as the temporal variability of rhizosphere processes. The physiological mechanisms determining root-induced modifications of the pH and the redox conditions in the rhizosphere are discussed with respect to the consequences for the solubility and plant availability of nutrients and toxic elements in soils and the impact on plant–microbe interactions. A final section describes the composition, quantity and the release mechanisms of organic compounds by plant roots (rhizodeposition) and their role in rhizosphere processes, covering passive release, associated with turnover of fine root structures and mycorrhizal hyphae and diffusion-mediated losses of organic substances from root cells but also the controlled release of root border cells, mucilage, ecto-enzymes and various low-molecular-weight compounds with specific adaptive functions for nutrient acquisition and stress tolerance. This includes also the current knowledge on the underlying mechanisms at the physiological and molecular level, as well as the impact of environmental factors on release of organic compounds from plant roots.

14.1 GENERAL

In 1904, the German phytopathologist Lorenz Hiltner defined the soil–root interface or rhizosphere as the volume of soil surrounding the roots, which is influenced by root activity. Hiltner described the so-called 'rhizosphere effect' with stimulation of microbial growth in the soil near the root surface, as well as plant interactions with beneficial or pathogenic microorganisms and potential implications for nutrient cycling in soils, plant nutrition and plant health (Hiltner, 1904). The stimulation of microbial activity and density in the rhizosphere is mainly due to the release of easily decomposable root exudates. The effects of root exudates on rhizosphere microorganisms are discussed in Chapter 15.

Plant roots can modify the rhizosphere chemistry in a number of ways: (i) by release and uptake of organic compounds, (ii) by gas exchange (CO_2/O_2) related with respiration of roots and rhizosphere microorganisms, and (iii) by root uptake as well as release of water and nutrients, which may be associated with uptake or extrusion of protons and modifications of the redox potential. Roots also modify the physical properties of the rhizosphere soil, such as aggregate stability, hydrophobicity and numbers and size of micropores by their growth through the soil as well as presence of polymeric substances. These root-induced changes also determine availability of nutrients. Therefore it is appropriate to state that life on earth is sustained by the small volume of soil surrounding roots and influenced by living roots, called the rhizosphere (Hinsinger *et al*., 2009). Typical features of the rhizosphere as compared to the bulk soil influenced by root activity are compiled in Table 14.1. The underlying processes are discussed in greater detail in the following sections.

Determining the properties of the rhizosphere is challenging. Rhizosphere soil can be obtained by carefully separating roots from loosely adhering soil (*rhizosphere soil*) and closely adhering soil (*rhizoplane soil*). However, the amount of rhizosphere soil will depend on root morphology (particularly root hairs) and physiology (release of binding agents such as mucilage) as well as on soil properties (texture, water content, organic matter content). In a more elegant manner, root and bulk soil compartments can be separated by mesh (Fig. 14.1) in vertical or horizontal orientation (Engels *et al*., 2000). After formation of a root mat on the mesh of the rhizosphere compartment, soil analysis in the bulk soil compartment is possible at defined distances from the root surface, for example after microtome slicing of frozen soil.

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TABLE 14.1 Changes in selected rhizosphere processes compared to the bulk soil

Rhizosphere process	Extent of change in the rhizosphere compared with bulk soil	Plant species (conditions)	References
Rhizosphere pH	Decrease or increase by 2 pH units	Wheat, maize (different forms of N)	Römheld (1990)
Redox processes	Decrease by 3 pH units	White lupin (low P soil)	Dinkelaker *et al*. (1989)
Root exudates	Reductase activity enhanced by factor 100	E.g. peanut (Fe deficiency)	Römheld (1990)
LMW compounds in the rhizosphere	2–3-fold increase in sugars	Average values for various plant species	Jones *et al*. (2003a)
Phosphatase activity	10-fold increase in amino acids	Average values for various plant species	Jones *et al*. (2003a)
Depletion of extractable P and K	60-fold increase in citrate	White lupin (low P soil)	Dinkelaker *et al*. (1989)
Accumulation of Ca	2–8-fold increase	Red clover, wheat, oilseed rape (low P soil)	Tarafdar and Jungk (1987)
Accumulation of NaCl	5–7-fold decrease up to 10 fold increase with precipitation	Oilseed rape, wheat, maize (young seedlings)	Jungk (2002), Vetterlein and Jahn (2004)
Bacteria (colony-forming units)	6-fold increase	Trees, azalea	Jungk (2002)
	3–24-fold increase	Barley, maize (saline soils)	Schleiff (1986), Vetterlein and Jahn (2004)
		Various plant species	Rouatt and Katznelson (1961)

Modified after Römheld and Neumann (2006).

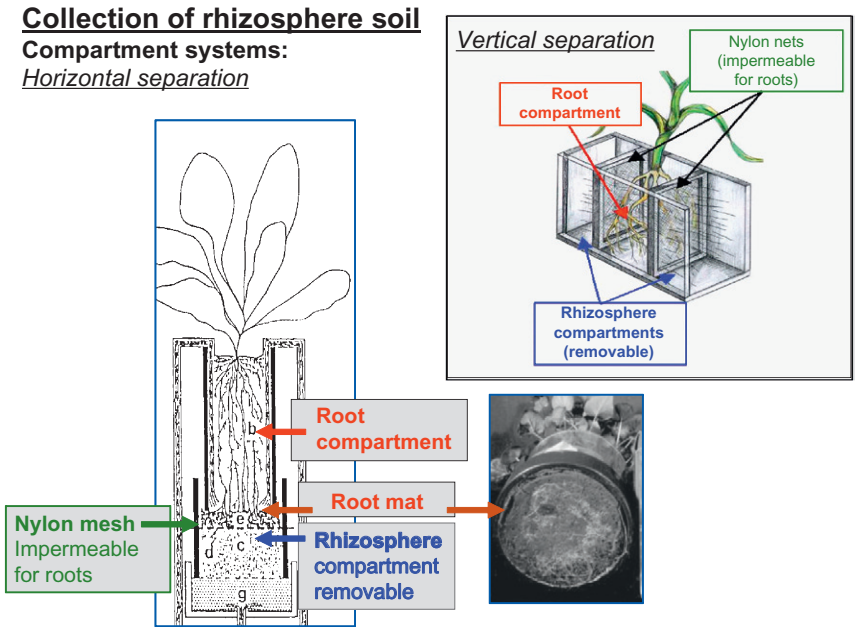

FIGURE 14.1 Compartment systems for rhizosphere soil sampling. *Adapted from Engels et al. (2000) and Kuchenbuch and Jungk (1982); courtesy of E. Neumann.*

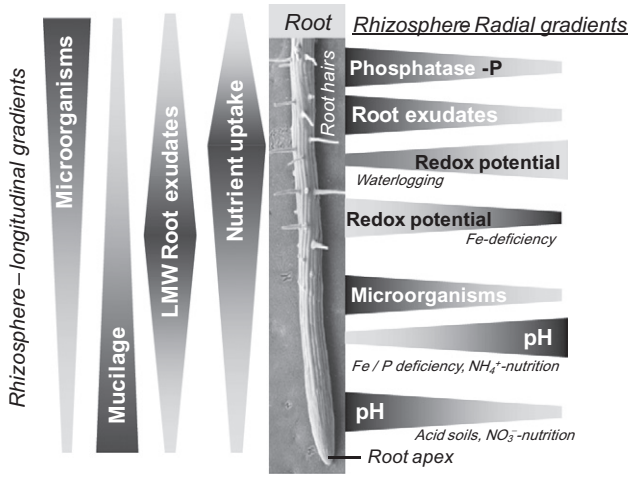

FIGURE 14.2 Physico-chemical and biological gradients in the rhizosphere.

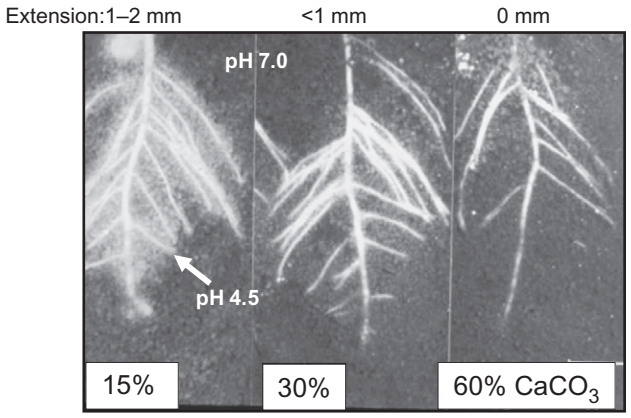

FIGURE 14.3 Extension of ammonium-induced rhizosphere acidification in chickpea at different soil buffering capacity (detected by embedding the roots of soil-grown plants in agar with pH indicator). *Adapted from Römheld, 1986.*

Although this method can provide more accurate data on gradients in the rhizosphere, the root-induced changes may be overestimated due to the high root density on the mesh separating the root and the bulk soil compartment.

Other methods include isotope techniques using radioactive nutrient tracers mixed with the soil substrate in combination with autoradiography, and, more recently, ion-sensitive microelectrodes and computer assisted tomography (CAT) to study the distribution of ions and water in the rhizosphere of single roots (Neumann *et al*., 2009).

14.2 SPATIAL EXTENT OF THE RHIZOSPHERE

The extent of the rhizosphere in space and time is highly variable. Gradients exist both in radial direction towards the bulk soil and in longitudinal orientation along the roots which are also influenced by temporal changes in root activity (Fig. 14.2).

14.2.1 Radial Gradients

The rhizosphere can be subdivided into three compartments: (i) the apoplasm within the cell walls of the rhizodermis and outer root cortex cells represent the so-called *endo-rhizosphere*; (ii) the outer surface of the rhizodermis, the *rhizoplane*, which is surrounded by (iii) the *outer rhizosphere*. Depending on the rhizosphere processes considered (exudation of reactive compounds, respiration, uptake of more or less mobile nutrients and water), the radial extent of the rhizosphere can range from scales of less than 1 μm to several centimetres (Hinsinger *et al*., 2005; Gregory, 2006b).

The concentration of organic compounds released from plant roots declines with increasing distance from the roots. The distance of diffusion largely depends on soil properties and adsorption characteristics of the respective compounds. Whereas polar compounds such as uncharged low-molecular-weight sugars or simple amino acids can diffuse several millimetres from the root surface (Gisi, 1997), di- and tri-carboxylates, such as malate, citrate or oxalate may be adsorbed to positively charged sorption sites of the soil matrix (Jones *et al*., 2003) and are only detectable close to the rhizoplane. This holds true also for root-secretory proteins, polygalacturonic acids and phenolic compounds (Jones *et al*., 1994, 2003; Gisi, 1997).

The radial gradients of nutrients in the rhizosphere are determined by their solubility and mobility, and the uptake capacity of the roots. Depending on the mobility of these nutrients, gradients can extend over less than 1 mm up to several cm distance from the root surface (Hinsinger *et al*., 2009). Poorly mobile nutrients such a P, K, ammonium and micronutrients with low concentrations in the soil solution are frequently depleted in the rhizosphere by rapid root uptake, whereas soluble nutrients, for example Ca and Mg, may accumulate close to the roots (see also Chapter 12).

Nutrient uptake is closely coupled to uptake or release of protons and therefore frequently associated with root-induced changes in rhizosphere pH (see below). In some plant species, increased rhizosphere acidification is also an adaptive response to improve acquisition of Fe and P (Neumann and Römheld, 2002). However, the spatial extent of these pH changes into the rhizosphere strongly depends on the buffering capacity of the soil. Figure 14.3 shows that the extent of the acidification by chickpea decreases with increasing concentrations $CaCO_3$ in the soil. Under field conditions, localized patches of organic matter and the distribution of $CaCO_3$ particles may contribute to the variablity of soil pH and are not easily distinguished from root-induced pH changes (Schöttelndreier and Falkengren-Grerup, 1999).

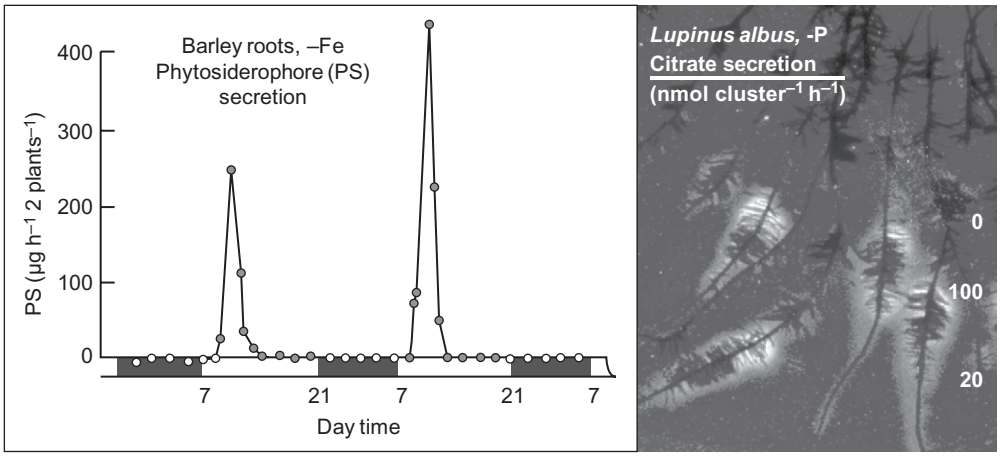

FIGURE 14.4 Temporal variability of rhizosphere processes: diurnal pulses of phytosiderophore secretion in Fe-deficient barley (*left*) and secretion of citrate and protons in different developmental stages of cluster roots in P-deficient white lupin (*right*). *Adapted from Tagaki (1984) and Neumann* et al. *(2000).*

Gas exchange due to root and microbial respiration also leads to formation of gradients in the rhizosphere. While CO_2 dissolved in the soil solution leads to the formation of H^+ and HCO_3^- and therefore can contribute to rhizosphere acidification, respiratory O_2 consumption leads to depletion of O_2 close to the root surface, particularly in young root tissues characterized by high respiration rates (Bidel *et al*., 2000). However, at low O_2 concentrations in the soil (e.g., in waterlogged soils), adapted plant species can also release O_2, transported from aerial plant parts with gradients extending 0.4–4.0 mm from the root surface (Revsbech *et al*., 1999).

14.2.2 Longitudinal Gradients

Along single roots, gradients are formed between apical root zones (root meristem, elongation zone, zone of root hair formation) and the older, more basal parts of the root. Water and nutrient uptake are usually highest in the apical root zones (Häussling *et al*., 1988; Clarkson, 1991; Reidenbach and Horst, 1997). This has been attributed to incomplete development of the endodermis and exodermis in these root zones, facilitating the uptake of water and dissolved nutrients via the apoplasmic pathway (Chapter 2). The presence of root hairs in the sub-apical root zones increases the surface available for nutrient absorption and may also be responsible for an increased release of protons and organic compounds in this zone. Additionally, apical root zones are frequently characterized by lower rhizosphere microbial densities as compared with older roots (von Wirén et al., 1995) which may reduce the rate of microbial degradation of organic compounds in apical root zones (von Wirén et al., 1995; see Chapter 15). On the other hand, mucilage (mainly galacturonic acid polymers with high viscosity) covering the surface of apical root zones, may limit the diffusion of low-molecular-weight organic compounds into the surrounding soil, which in turn can stimulate microbial colonization of the mucilage layer. The cluster gradients and their orientation in the rhizosphere are summarized in Fig. 14.2.

14.2.3 Temporal Variability

Apart from spatial variation, rhizosphere processes also exhibit a temporal variability (Hinsinger *et al*., 2009). Diurnal variations have been demonstrated, for example, for the release of root exudates involved in Fe (Fig. 14.4) and P mobilization (Nishizawa and Mori, 1987; Watt and Evans, 1999) and root-induced rhizosphere acidification (Blossfeld and Gansert, 2007). In *Lupinus albus*, root exudation of organic acid anions induced by P limitation is strongly influenced by the developmental status of the roots (Neumann and Martinoia, 2002; Fig. 14.4).

14.3 INORGANIC ELEMENTS IN THE RHIZOSPHERE

Although the total soil content of nutrients frequently exceeds the plant requirements by several orders of magnitude, plant availability is often limited by low solubility of nutrients such as P, K, ammonium, Fe, Zn, Mn, Cu and Mo. Therefore, these nutrients reach the root surface mainly by diffusion. As described in Chapter 12, the rate of diffusion may be too low to meet plant demand. Therefore, plants have developed several strategies for acquisition of nutrients with limited solubility, comprising:

1. adaptations to exploit a larger soil volume for improved acquisition of the available nutrient fraction in the soil solution by root and root hair growth (see Chapter 12) or association with mycorrhizal fungi (see Chapter 15)

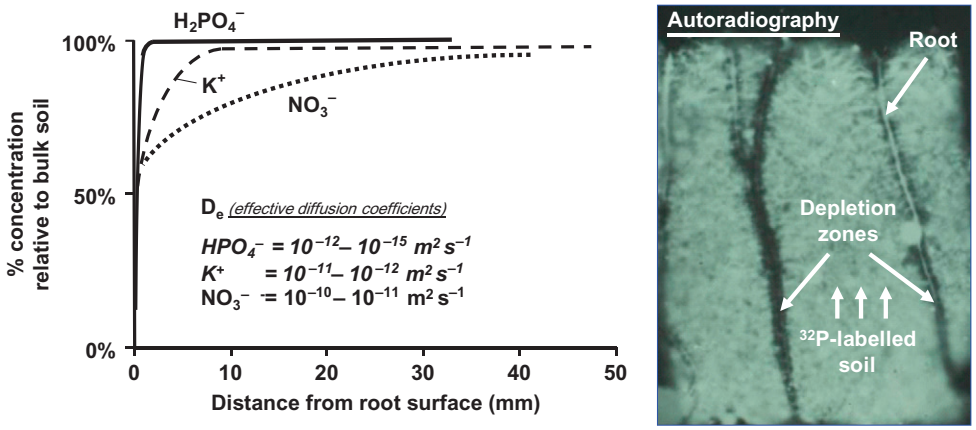

FIGURE 14.5 Root-induced depletion of nutrients in the rhizosphere depending on the effective soil diffusion coefficients (D_e) (*left*) and auto-radiographic visualization of ^{32}P depletion in the rhizosphere of soil-grown barley roots (*right*). *Adapted from Hinsinger (2004) and Jungk (2002).*

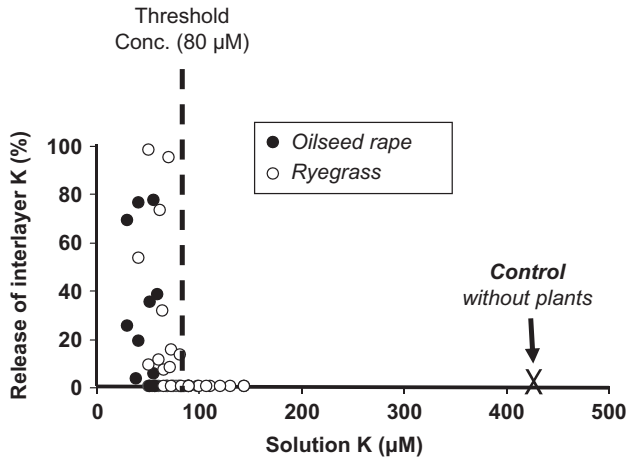

FIGURE 14.6 Release of interlayer K^+ from clay minerals and transformation of mica to vermiculite by root-induced depletion of K^+ in the rhizosphere of oilseed rape and ryegrass. *Adapted from Hinsinger and Jaillard (1993).*

2. modifications of the rhizosphere chemistry to increase the solubility of sparingly available nutrients.

The latter is the focus of the following sections.

The concentration of a particular ion in the rhizosphere can be lower, higher, or similar to that in the bulk soil, depending on the concentration in the bulk soil solution, the rate of delivery of the ion to the root surface, and its rate of uptake by the root (see also Chapter 12). When the flow of nutrients transferred to the root surface is lower than root uptake, their concentration decreases in the rhizosphere (Lorenz *et al*., 1994; Barber, 1995; Jungk, 2002; Hinsinger, 2004). This typically occurs for nutrients with low concentrations in the soil solution which are slowly delivered by diffusion to the soil solution, such as P, Fe, Zn, Mn, NH_4^+ and K, and generates a diffusion gradient towards the root surface (Fig. 14.5). In soils low in available K, this can lead to the disaggregation of shale particles and the accumulation of amorphous Fe and Al oxyhydrates, indicative of enhanced weathering of minerals at the soil–root interface (Sarkar *et al*., 1979; Kong and Steffens, 1989). In ryegrass (*Lolium multiflorum*), the K concentration in the rhizosphere soil solution can decrease below 80 µM, which, within a few days, enhances the release of interlayer K (Fig. 14.6) and concomitant transformation of trioctahedric mica into vermiculite in the rhizosphere (Hinsinger and Jaillard, 1993). In the rhizosphere of oilseed rape (*Brassica napus*), depletion of both K and Mg, together with a decrease in pH to about 4, increases not only the release of interlayer K, but also of octahedral Mg and, thus, induces irreversible transformations of the mica (Hinsinger *et al*., 1993).

On the other hand, a greater uptake of water than of ions leads to ion accumulation in the rhizosphere. This can be predicted from calculations based on models of solute transport by diffusion and mass flow to the root surface for those ions which are present in high concentrations in the soil solution. After two months' growth in a sandy loam soil, the concentration of Ca and Mg in the rhizosphere was increased 2–3-fold compared to the bulk soil (Fig. 14.7).

Plant species differ in mass flow (transpiration) to the roots and rate of uptake by the roots. For example, in ryegrass and lupin grown in the same soil, Ca supply by mass flow was 2.8 and 8 mg Ca, respectively, but Ca uptake was 0.8 mg in ryegrass and 9.0 mg in lupin. Thus, despite the higher supply, Ca was depleted in the rhizosphere of lupin but accumulated in ryegrass (Barber and Ozanne, 1970). At high Ca^{2+} and SO_4^{2-} concentrations in the soil solution, $CaSO_4$ may precipitate at the root surface (Jungk, 1991) and, over a long period, form a solid mantle around the roots (*pedotubules*) with diameters of a few millimetres to more than 1 cm (Barber, 1984).

In calcareous soils, calcified roots of herbaceous plants may occur (Fig. 14.7), in which the calcite elements retain the structure of the original cortex cells (Jaillard, 1985).

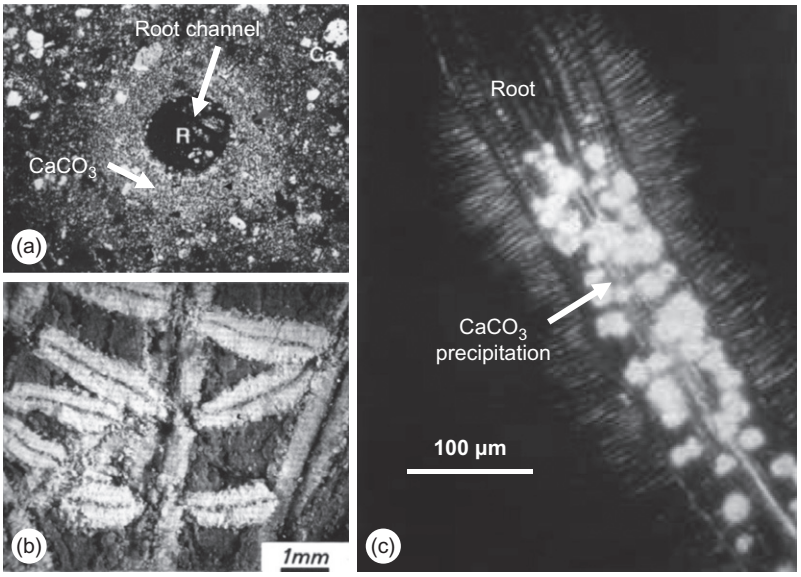

FIGURE 14.7 Nutrient accumulation in the rhizosphere: (a) accumulation of $CaCO_3$ in the rhizosphere around a peach tree root channel; (b) $CaCO_3$ precipitation on the root surface of oilseed rape; (c) root calcification by calcite precipitation in root cortex cells. *Adapted from Jaillard* et al. *(1991) and Chalot* et al. *(1992).*

These cytomorphic calcite elements (~60–80 µm) are formed by root activity and cycles of rhizosphere acidification and precipitation of $CaCO_3$ within root cells. In agreement with this, calcified roots are surrounded by a decalcified rhizocylinder with a silico-aluminium matrix (Jaillard, 1985; Jaillard *et al*., 1991). This is an example of the role of root-induced changes in the rhizosphere which can be of importance in pedogenesis, since in certain locations the cytomorphic calcite fraction may represent up to a quarter of the soil mass (Jaillard *et al*., 1991).

Accumulation of salts of low solubility in the rhizosphere (e.g., $CaCO_3$; $CaSO_4$) may not be very harmful to plants. This is different, however, in saline soils with high concentrations of water-soluble salts such as NaCl where Cl and Na can accumulate in the rhizosphere creating a concentration gradient to the bulk soil, and this gradient becomes steeper as the transpiration rate increases. Hence, the electrical conductivity of the soil increases near the root surface, especially at high transpiration rates.

Increasing the salt concentration and osmotic potential of the soil solution decreases water availability to plants and can severely impair plant water relations (see also Section 17.6). In non-halophytes ('salt excluders') grown in saline soils for four days, the salt concentrations in the rhizosphere soil solution can increase from 50 to 300 mM (Schleiff, 1986). At high salt concentrations, the relationship between transpiration rate and salt accumulation in the rhizosphere is not linear, indicating some back-diffusion of solutes from the root surface back into the surrounding soil, counteracting, in part, the salt accumulation (Hamza and Aylmore, 1991).

Accumulation of soluble salts at the root surface is important for plant growth and irrigation in saline soils. Estimations of expected growth reduction of plants growing in saline soils are usually based on calculations of salt concentrations in saturated soil extracts. Due to the lower water content, the salt concentration in the soil solution under field conditions is estimated to be about two to four times higher than that in the saturation extract. This, however, does not necessarily reflect the actual conditions in the rhizosphere, where the water content may be lower as a consequence of water uptake. Hence, water may not be available to plants before the critical conductivity levels (see Section 17.6) are reached in the bulk soil (Schleiff, 1986, 1987).

Gradients in ion uptake rates along the root axis are also important for ion competition and selectivity in uptake (see also Chapter 2). The strong reduction of Mg uptake by K, which can be readily demonstrated in nutrient solution culture, occurs in soil-grown plants only if the rhizosphere K concentration is high. Depletion of K in the rhizosphere soil solution below 20 µM increases the uptake rate of Mg by ryegrass two-fold. The increasing extent of the depletion zone of K from apical to basal zones allows higher uptake rates of Mg in the basal zones. Thus, the spatial separation of ions in the rhizosphere along the root axis of soil-grown plants can overcome limitations in nutrition of plants caused by ion competition for uptake sites. However, in saline soil with high Na concentrations, the preferential K uptake in apical zones may also increase Na uptake rates in basal zones and, thus, decrease the overall selectivity in K/Na uptake (Hinsinger and Jaillard, 1993; Hinsinger *et al*., 1993).

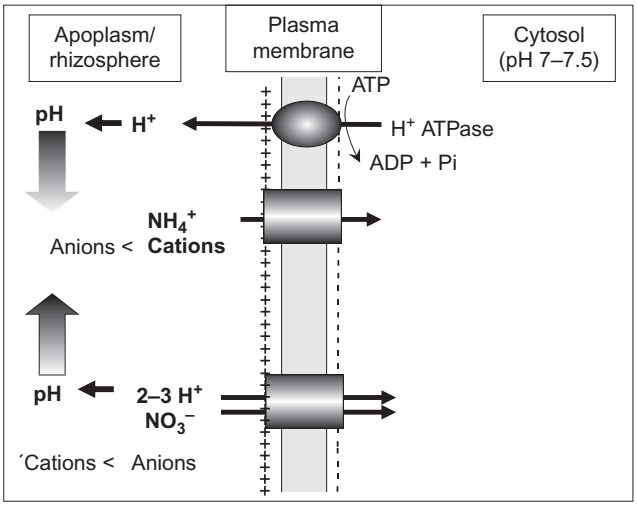

FIGURE 14.8 Impact of uptake of ammonium or nitrate on root-induced changes in rhizosphere pH.

14.4 RHIZOSPHERE pH

The rhizosphere pH may differ from the bulk soil pH by up to two units, depending on plant and soil factors, with important consequences for the pH-dependent solubility of nutrients and toxic elements in the soil solution (see also Chapter 2). The most important factor for root-induced changes in rhizosphere pH is the uptake of nutrients, which is coupled with proton (H^+) transport in higher plants. The driving force for nutrient uptake by root cells is H^+ extrusion, mediated by the activity of a plasma membrane-bound H^+ pumping ATPase (PM-ATPse), which creates an outward positive gradient in electropotential and pH between the cytosol (pH 7–7.5) and the apoplasm (pH 5–6). This electrochemical potential gradient provides the energy for anion uptake by proton–anion co-transport (H^+ uptake) and for cation uptake via uniport or proton–cation countertransport (H^+ release). Due to differences in plant requirements and also in the availability of nutrients, uptake of cations and anions is often not balanced. Excess uptake of anions over cations leads to net removal of protons in the rhizosphere and to an increase in rhizosphere pH. In contrast, excessive uptake of cations is balanced by a net release of protons and consequently leads to rhizosphere acidification (Fig. 14.8). Proton extrusion via the PM-ATPase is also an important component of the system for stabilization of the intracellular pH which is modified by metabolic reactions involving H^+ production or consumption.

Rhizosphere pH may be also influenced by release and uptake of HCO_3^-, respiratory CO_2 production of roots and rhizosphere microorganisms, and release of low-molecular-weight organic compounds which may also be coupled with proton transport. In aerated soils, CO_2 is of minor importance for rhizosphere pH, because it rapidly diffuses away from the roots through air-filled pores (Nye, 1986). It is mainly the CO_2 dissolved in the soil solution (forming H^+ and HCO_3^-), which affects rhizosphere pH as the mobility of H^+, HCO_3^- is relatively low in the soil solution.

The pH buffering capacity of the soil and the initial soil pH are the main soil factors determining the extent to which plant roots can change the rhizosphere pH (Fig. 14.3). The pH buffering capacity of soils depends primarily on initial pH and organic matter content, but also on clay content; the pH buffering capacity is lowest at about pH 6, and increases to both lower and higher pH values (Schaller and Fischer, 1985; Nye, 1986). However, a lack of significant pH change in a soil with high pH buffering capacity does not necessarily mean the absence of proton flux in the rhizosphere. Indeed, the protons may replace other cations from the cation exchange sites of the soil and thereby affect the mobilization/immobilization of nutrients (Hinsinger *et al*., 2009).

14.4.1 Source of N Supply and Rhizosphere pH

Nitrogen is plant available both in cationic (ammonium, NH_4^+) and anionic (nitrate, NO_3^-) forms and can comprise up to 80% of the total ion uptake. Therefore, the form of N supply determines the cation/anion uptake ratio and thus also the rhizosphere pH (Fig. 14.9) both in annual (Marschner and Römheld, 1983) and perennial plant species (Rollwagen and Zasoski, 1988). Nitrate is the major form of inorganic N available for plant growth in many well-aerated agricultural soils. Nitrate uptake results in excess uptake of anions over cations, net uptake of protons and thus an increase in rhizosphere pH. Furthermore, nitrate assimilation in the root tissue is associated with production of OH^-, and may therefore contribute to some extent to rhizosphere alkalinization by release of OH^- into the rhizosphere for intracellular pH stabilization.

In acid soils, the pH increase induced by nitrate supply enhances P uptake by exchange of phosphate adsorbed to Fe and Al by HCO_3^- (Gahoonia *et al*., 1992) or by stimulation of microbial P mineralization (Fig. 14.10; Alvey *et al*., 2000; Bagayoko *et al*., 2000). For pasture grasses grown in P-deficient acid soils, the increase in rhizosphere pH results in P depletion in the rhizosphere (Armstrong and Helyar, 1992). Rhizosphere alkalinization may also alleviate the negative effects of soil acidity on plant growth (see Section 17.3) by increasing the availability of Ca and Mg, but reducing the concentration of toxic Al species in the rhizosphere soil solution (Fig. 14.10; Degenhardt *et al*., 1998; Bagayoko *et al*., 2000, Pineros *et al*., 2005). In acidic mineral soils, nitrate supply may also increase the availability of molybdate by reduced adsorption to sesquioxide surfaces (Trobisch, 1966).

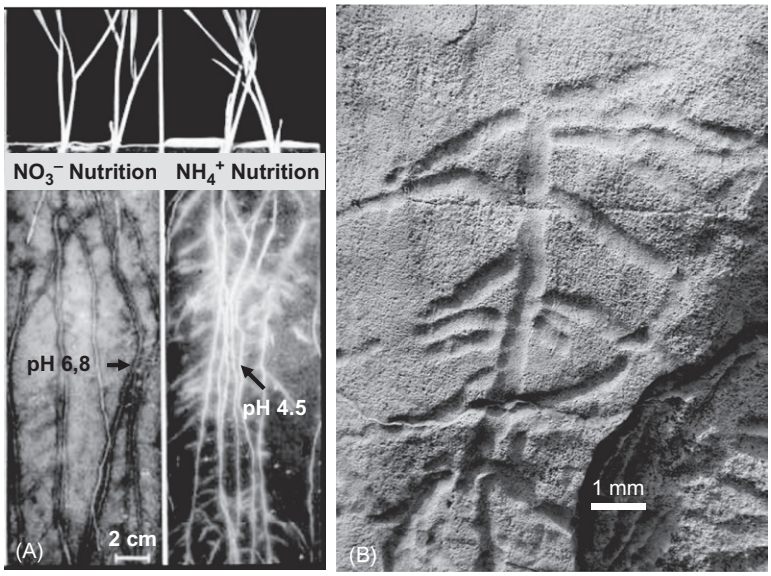

FIGURE 14.9 (A) Root-induced alterations in rhizosphere pH as affected by the form of nitrogen supply, detected by embedding of soil-grown roots in pH indicator agar. (B) Root-imprints in limestone as a consequence of root-induced rhizosphere acidification. *Adapted from Roemheld (1986) and Jaillard and Hinsinger (1993).*

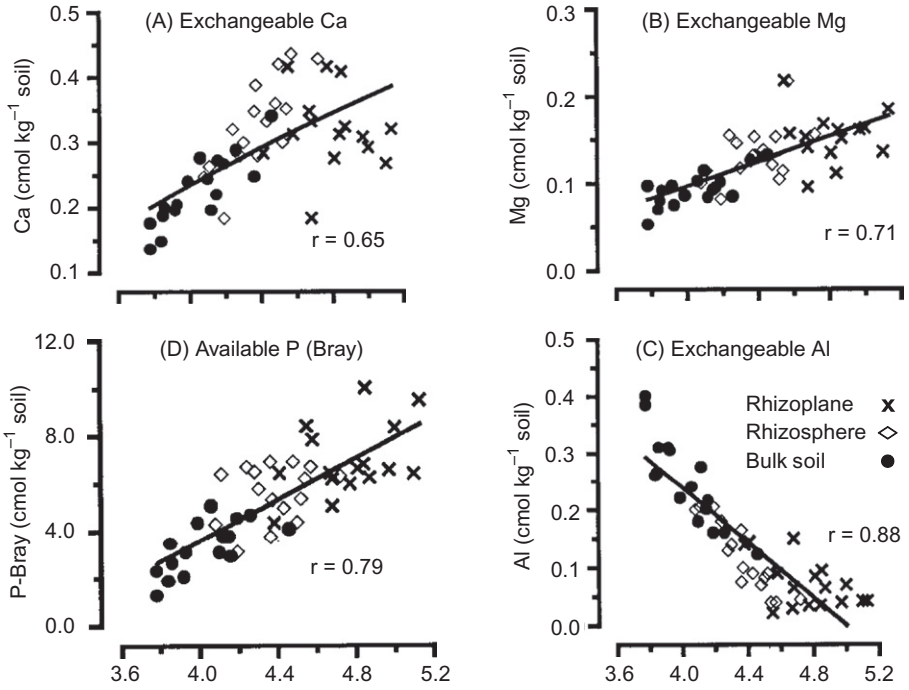

FIGURE 14.10 pH in the rhizoplane, rhizosphere and in the bulk soil and the availability of nutrients (P, Ca, Mg) and toxic elements (Al) in field-grown pearl millet in an acidic sandy soil in West Africa. *Adapted from Bagayoko* et al. *(2000).*

On the other hand, ammonium uptake and H^+ production during ammonium assimilation in the root tissues ($3NH_4^+ \rightarrow 4H^+$; see also Chapter 2, Section 6.1) results in enhanced net extrusion of H^+ and rhizosphere acidification (Fig. 14.9). Preferential uptake of ammonium occurs when nitrification is inhibited or delayed particularly in wetland soils, acid soils and in soils of arctic tundras (Chapin *et al.*, 1993; Marschner, 1995) or shortly after application of ammonium fertilizers, organic fertilizers and nitrification inhibitors. In neutral or alkaline soils, rhizosphere acidification with ammonium supply can enhance mobilization of sparingly soluble Ca phosphates and thereby increase P uptake (Gahoonia

TABLE 14.2 Rhizosphere pH and concentration of nutrients in shoots of bean (*Phaseolus vulgaris* L.) plants grown in a Luvisol (pH 6.8) with nitrate or ammonium

		Concentration in shoot				
		K	P	Fe	Mn	Zn
N form	Rhizosphere pH	(mg g^{-1} dw)		(μg g^{-1} dw)		
NO_3	7.3	13.6	1.5	130	60	34
NH_4	5.4	14.0	2.9	200	70	49

Adapted from Thomson et al. (1993).

TABLE 14.3 Rhizosphere pH and Cd concentration in *Lolium perenne* grown with different forms of N

		Cd concentration in shoot (mg kg^{-1} dw)		
N form	Rhizosphere pH	1st cut	2nd cut	3rd cut
NO_3	6.8	6.5	5.5	4.2
NH_4NO_3	6.8	9.2	8.2	7.6
NH_4	5.5	12.4	12.8	12.2

Adapted from Wu et al. (1989).

et al., 1992), as well as the uptake of micronutrients such as B (Reynolds *et al*., 1987), Fe, Mn and Zn (Table 14.2).

Enhanced resistance to plant diseases, such as take-all (*Gaeumannomyces graminis*) and powdery mildew (*Erysiphe graminis*) with ammonium supply in wheat, may be related to improved micronutrient uptake as some micronutrients are co-factors for enzymes involved in defence reactions, such as diaminoxidase (Cu), polyphenol oxidase (Cu), ascorbate oxidase (Cu), peroxidase (Mn) and lipoxigenase (Fe) (see also Chapters 7 and 8). Rhizosphere acidification due to ammonium uptake may also enhance the mechanical resistance of the cell walls due to higher incorporation of SiO_2 (Leusch and Buchenauer, 1988; Graham and Webb, 1991; see also Section 8.3). On the other hand, soil acidification promotes some diseases, such as club rot in cabbage and Fusarium wilt in cotton (Huber and Wilhelm, 1988).

Ammonium-induced rhizosphere acidification may also increase the availability of toxic elements such as Cd (Table 14.3; Wu *et al*., 1989) and has been proposed as a bioremediation strategy to improve the solubility and thus uptake of heavy metals in neutral and alkaline soils by accumulator plants (phyto-extraction), while the pH remains high in the bulk soil which prevents metal leaching (Zaccheo *et al*., 2006).

On acid soils, however, a pH decrease will not enhance mobilization of nutrients and may even cause adverse effects on plant growth as a consequence of enhanced P adsorption to Fe and Al oxides, solubilization of toxic Al species or even acid-induced root injury (see also Section 17.3).

In waterlogged soils, the inhibition of nitrification results in ammonium uptake and thus low rhizosphere pH. Moreover, in flooded soils, rhizosphere oxidation by release of O_2 from plant roots is an essential adaptation to prevent the accumulation of Fe^{2+}, Mn^{2+}, H_2S and monocarboxylic acids to phytotoxic levels (see Section 17.4). Oxidation of Fe^{2+} further promotes rhizosphere acidification according to the reaction: $4Fe^{2+} + O_2 + 10H_2O \rightarrow 4Fe(OH)_3 + 8H^+$, which can enhance (i) mobilization of Zn adsorbed to Fe^{III} hydroxides (Kirk and Bajita, 1995), (ii) solubilization of acid-soluble soil P fractions (Saleque and Kirk, 1995), and (iii) release of fixed NH_4^+ (Schneider and Scherer, 1998).

For rhizosphere pH measurements, average values integrated over the whole root system can be misleading and may result in erroneous conclusions about nutrient relationships in the rhizosphere. For example, within the root system of an individual plant, pH differences of more than two pH units may occur between primary and lateral roots or along the root axis (Marschner and Römheld, 1983; Marschner *et al*., 1986a). In Norway spruce in acid soil, the pH is high at the root apex and decreases in the subapical (extension) zone, irrespective of the form of N in the soil solution. In contrast, in the more basal root zones, the expected pH changes occur, namely a pH increase with nitrate supply only, and a pH decrease with ammonium (Häussling *et al*., 1988). When ammonium and nitrate are present at similar concentrations, ammonium is taken up preferentially (Arnold, 1992). Thus, rhizosphere acidification takes place despite the presence of high nitrate concentrations, particularly at high soil water content which facilitates diffusion of ammonium (Gijsman, 1991). A higher pH at the root apex is a common feature of plants grown in acid soils and may be related to the release of root exudates (see below) or in nitrate-fed plants, to high nitrate reductase activity in root apical zones (Klotz and Horst, 1988a).

Large differences in the rhizosphere pH exist between plant species growing in the same soil and supplied with nitrate. Buckwheat (Raij and van Diest, 1979) and chickpea (Marschner and Römheld, 1983) have a very low rhizosphere pH compared to, for example, wheat or maize. These genotypical differences reflect differences in cation/anion uptake ratios (Bekele *et al*., 1983) which are related to differential Ca demand for cell wall biosynthesis in monocotyledonous and dicotyledonous plant species (see also Chapter 2 and Section 6.5). In chickpea, white lupin and other large-seeded legumes, rhizosphere acidification is

TABLE 14.4 Yield, P uptake from rock phosphate and acidity and alkalinity generated by roots of lucerne plants and soil pH with different forms of N supply

Treatment		Yield	P uptake	Acidity	Alkalinity	
N source	Rock P	(g dw pot^{-1})	(mg pot^{-1})	(meq g^{-1} dw)		Soil pH
NO_3	–	2.5	1	–	1.1	6.3
NO_3	+	18.8	23	–	0.8	7.3
N_2	–	4.7	4	0.5	–	6.2
N_2	+	26.9	49	1.4	–	5.3

From Aguilar and van Diest (1981).

characteristic for early plant growth and related with low rates of nitrate uptake due to utilization of N reserves in the seed (Neumann and Römheld, 1999).

Legumes and actinorhizal plants which meet their N requirement by symbiotic N_2 fixation take up more cations than anions since uncharged N_2 enters the roots and most other macronutrients (K, Ca, Mg) are cations (see also Chapter 16). The high cation/anion uptake ratio of N_2-fixing plants results in net release of H^+, although per unit assimilated N, it is less than in ammonium-fed plants (Raven *et al*., 1991). The capacity of plants to utilize P from rock phosphate is therefore higher in N_2-fixing plants than in nitrate-fed plants. In soybean, Fe and Mn concentrations in N_2-fixing plants were higher than in nitrate-fed plants and they did not show Fe deficiency symptoms (Wallace, 1982) (Table 14.4).

On severely P-deficient soils, utilization of rock phosphate as a P source for legumes can be low when nodulation is limited by P deficiency. Thus, a starter supply of soluble P can enhance nodulation, N_2 fixation and rhizosphere acidification and thereby utilization of rock phosphate (Swart and Van Diest, 1987). Therefore, simulation models predicting P uptake by N_2-fixing legumes, particularly when supplied with rock phosphate, or grown at high soil pH, should consider this P mobilization by rhizosphere acidification, otherwise actual uptake by far exceeds the predicted uptake (Gillespie and Pope, 1990; Li and Barber, 1991). When N_2-fixing legumes are grown together with non-legumes (e.g., intercropping), rhizosphere acidification of legumes can increase P uptake from rock phosphate by non-legumes (Fig. 14.11), for example in black walnut tree seedlings by two-fold when interplanted with lucerne (Gillespie and Pope, 1989).

In the long run, symbiotic N_2 fixation also affects the acidification of the bulk soil and thus the lime requirement. A lucerne crop fixing N_2 with an annual shoot dry matter production of 10 tons per hectare produces soil acidity equivalent to 600 kg $CaCO_3$ ha^{-1} (Nyatsanaga and Pierre, 1973). In legume pastures which are not limed, there is a negative correlation between age of the pasture and soil pH

FIGURE 14.11 Phosphorus uptake of maize intercropped with N_2 fixing faba bean under field conditions when roots of the two crops were either separated or allowed to intermingle. *Adapted from Li* et al. *(1999).*

(Haynes, 1983). In soils in which legumes are continuously grown, exchangeable Mn can thus be released into the soil solution and may induce Mn toxicity in plants (Bromfield *et al*., 1983a, b). In humid climate, the loss of symbiotically fixed N through leaching of nitrate and an equivalent amount of cations such as Ca and Mg contribute to soil acidification under leguminous pastures. A similar impact on the long-term soil acidification by N_2 fixation can be observed in forest ecosystems where the pH under the actinorhizal red alder is lower than under Douglas fir (Van Miegroet and Cole, 1984), and in crop rotations with a high proportion of legumes (Coventry and Slattery, 1991).

TABLE 14.5 Ratio of cation/anion uptake, uptake of nitrate and net extrusion of protons, PEP carboxylase (PEPC) activity and carboxylate accumulation in roots of different plant species with or without P

Plant species	P supply	Cation/anion uptake ratio	Nitrate uptake (% change)	Δ pH in growth medium	PEPC activity (nmol NADH min^{-1} mg $protein^{-1}$)	Carboxylates (μmol g^{-1} root fw)
Tomato						
	+P	0.78			90	8.5
	0P	1.33	−83	−1.4	375	12.0
Chickpea						
	+P	1.17			144	8.5
	0P	1.26	−48	−0.6	302	17.1
White lupin						
	+P	nd			120	8.7
	0P	1.38	−56	−1.1	270	22.0
Wheat						
	+P	0.39			426	1.2
	0P	0.29	nd	+1.6	703	5.4

Compiled data from Dinkelaker *et al.* (1989); Le Bot *et al.* (1990); Heuwinkel *et al.* (1993); Pilbeam *et al.* (1993); Neumann *et al.* (1999).
nd = not determined.

14.4.2 Nutritional Status of Plants and Rhizosphere pH

Irrespective of the N source, root-induced changes in rhizosphere pH are also related to the nutritional status of plants (Römheld, 1990; Cakmak and Marschner, 1990). Examples are rhizosphere acidification in cotton and other dicotyledons under Zn and P deficiency (Hoffland *et al.*, 1989; Cakmak and Marschner, 1990; Neumann and Römheld, 1999), and in non-graminaceous species under Fe deficiency (Römheld, 1987). In P- and Zn-deficient plants, uptake of nitrate is inhibited due to nutrient stress which results a high cation/anion uptake ratio and thus increased net release of H^+ (Cakmak and Marschner, 1990; Rufty *et al.*, 1990; Gniazdowska *et al.*, 1999) (Table 14.5).

A more direct stimulation of rhizosphere acidification in response to nutrient limitation may occur in non-graminaceous plant species (so-called Strategy I plants, see also Chapter 2) under Fe deficiency, where a strong local acidification occurs in the sub-apical root zones. This reaction is part of a coordinated response to Fe deficiency, including up-regulation of Fe transporters and plasma membrane reductase, formation of root epidermal transfer cells and proliferation of root hairs in the respective root zones (Fig. 14.12, see also Chapter 2). This response facilitates Fe^{3+} solubilization, transport to the root surface, reduction and uptake as Fe^{2+}, particularly in neutral and alkaline soils. Analysis of the tomato *fer* mutant revealed that the FER gene encodes a transcription factor involved in the coordinated regulation of the Strategy I responses (Schmidt, 2003; Yeong and Guerinot, 2009).

For the root system as a whole, the rates of Fe deficiency-induced net H^+ release per unit root weight are in a similar order of magnitude as in Fe-sufficient, ammonium-fed plants. However, average values are misleading as under Fe deficiency, enhanced net release of H^+ is confined to the apical root zones where the actual rates are nearly eight times higher than in the ammonium-fed plants. This highly localized acidification may enable the roots to decrease the rhizosphere pH in apical zones even in well-buffered calcareous soils to enhance Fe mobilization.

Protons released into the rhizosphere are replaced by up-regulation of phophoenolpyruvate carboxylase (PEPC) (Table 14.5) and glycolysis for pH stabilization in the cytosol of the root cells (Sakano, 1998). The PEPC reaction results in biosynthesis of carboxylates (oxaloacetate, malate), since PEPC catalyses the carboxylation of PEP via non-photosynthetic CO_2 fixation. Accordingly, PEPC activity usually increases in response to Fe and P limitation (Hoffland *et al.*, 1992; Rabotti *et al.*, 1995; Johnson *et al.*, 1996; Neumann and Römheld, 1999) and also with ammonium uptake. The remaining carboxylate anions may be (i) further metabolized (with ammonium uptake), (ii) stored in the vacuoles of the root tissue (Table 14.5), or (iii) translocated to the shoot or (iv) released

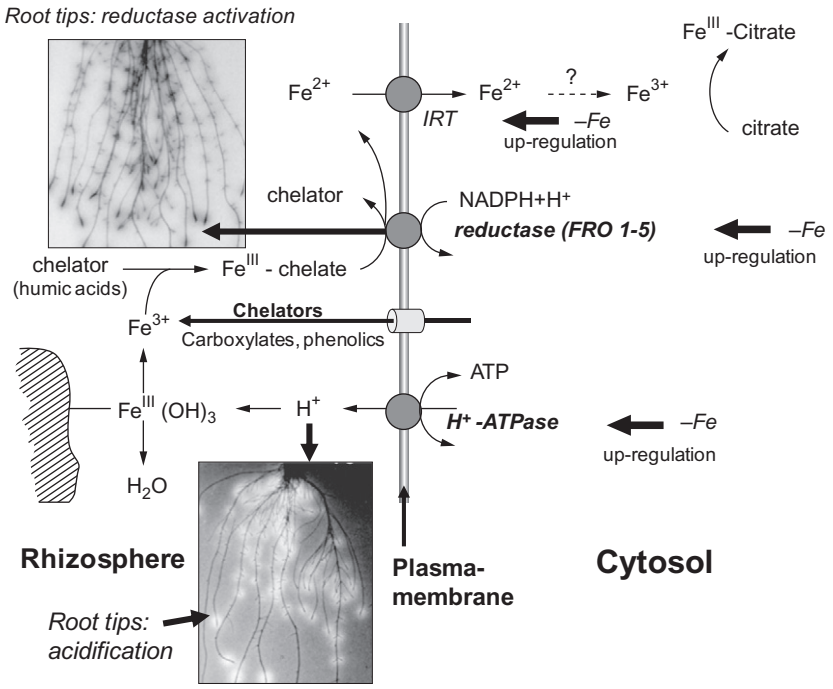

FIGURE 14.12 Model for Fe deficiency-induced changes in root physiology and rhizosphere chemistry associated with Fe acquisition in Strategy I plants. *Adapted from Marschner* et al. *(1986b).*

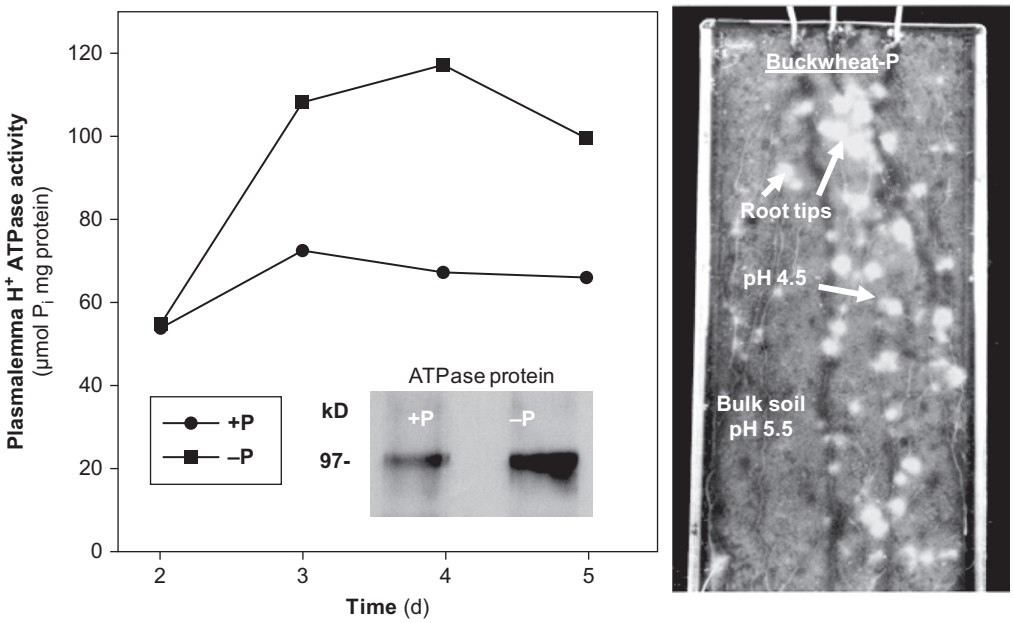

FIGURE 14.13 Phosphorus deficiency-induced up-regulation of the plasma membrane H^+ ATPase in white lupin and localized rhizosphere acidification in apical root zones of P-deficient buckwheat detected by embedding the roots of soil-grown plants in agar with pH indicator. *Adapted from Römheld (1987).*

into the rhizosphere (see also Chapters 2 and 3). In some plant species such as *Lupinus albus* and members of the Proteaceae and Cyperaceae, a very strong release of carboxylates and the concomitant release of H^+ or K^+ as counter ions is part of a strategy for mobilization of sparingly soluble P forms (Neumann and Römheld, 2007). The enhanced extrusion of protons is mediated by up-regulation of the PM H^+-ATPase in the respective root cells at the transcriptional, translational and post-translational level (Fig. 14.13; Rabotti and Zocchi, 1994; Yan *et al*., 2002; Tomasi *et al*., 2009). Moreover,

external factors may modulate the activity of the PM H^+-ATPase; for example, humic substances can stimulate root extrusion of H^+ (Pinton *et al*., 1997). Effects of humic substances on ion uptake may, at least partly, be explained by interactions of phenolics such as humic substances with the root plasma membrane H^+ATPase (Varanini *et al*., 1993; Pinton *et al*., 1999a).

14.4.3 pH Effects on Nutrient Uptake

Changes in rhizosphere pH not only affect nutrient solubility in soils, but also nutrient uptake. Generally, cation uptake decreases with declining pH, whereas anion uptake is inhibited when the pH of the external medium increases. This can be attributed to (i) competition between H^+ and OH^- (HCO_3^-) with cations or anions; (ii) external pH effects on the electrochemical potential gradient providing energy supply for nutrient uptake, and (iii) pH-induced alterations of root metabolism. However, positive pH effects on nutrient availability may counteract negative pH effects on nutrient uptake. For example, in hydroponics or sand culture, a low pH of the growth medium inhibits uptake of Mn^{2+} and other cations (Islam *et al*., 1980; Elamin and Wilcox, 1986). In contrast, on alkaline soils, ammonium-induced rhizosphere acidification results in Mn solubilization, which increases Mn^{2+} availability and uptake (Friedrichsen, 1967). Rhizosphere pH can also affect P uptake by altering the dissociation equilibrium of the phosphate anions, favouring P uptake at low external pH due to increased formation of the monovalent $H_2PO_4^-$ anion, which is the preferential form for P uptake in higher plants.

14.5 REDOX POTENTIAL AND REDUCING PROCESSES

14.5.1 Effect of Waterlogging

As soil water content increases, redox potentials decrease until in submerged soils, negative values are reached (see also Section 17.4). The decrease in redox potential is correlated with a range of changes in the solubility of nutrients (e.g., Mn, Fe and, occasionally, P). Low-molecular-weight organic acids as products of microbial fermentation processes and Fe^{2+}, Mn^{2+} and H_2S can accumulate in phytotoxic concentrations. Moreover, in poorly aerated soils with low pH, a high microbial activity in response to a high supply of root-borne carbohydrates can promote a decline in rhizosphere redox potential which may result in increased Mn solubility and Mn toxicity in plants.

As redox potential and O_2 concentration decrease, nitrate is used by microorganisms as an alternative electron acceptor, followed by Mn oxides. Due to the greater O_2 consumption in the rhizosphere compared to the bulk soil, the risk of N losses by denitrification or incomplete

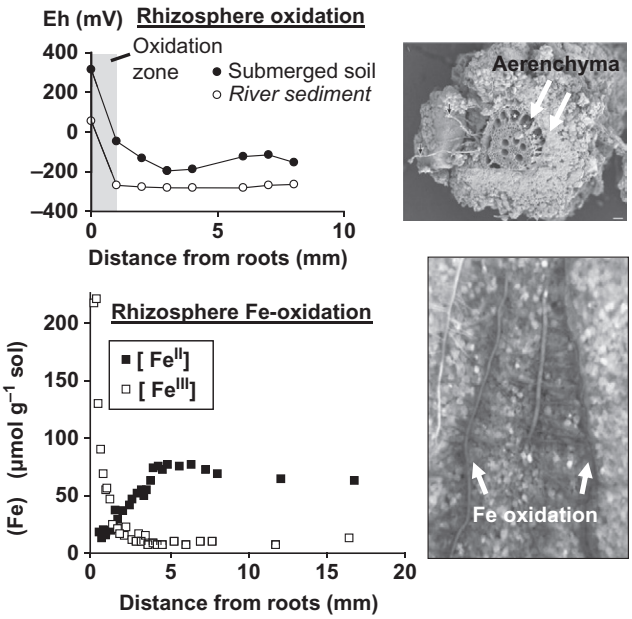

FIGURE 14.14 Rhizosphere oxidation via aerenchyma in submerged rice plants associated with oxidation of Fe^{2+} in the rhizosphere. *Adapted from Flessa and Fischer (1992), Begg* et al. *(1994) and Watt* et al. *(2006).*

nitrification (Klemedtsson *et al*., 1988; Papen *et al*., 1989) is higher in a planted than in unplanted soil. Rhizosphere denitrification is further promoted by input of organic carbon from the roots into the rhizosphere (Bakken, 1988), particularly in K-deficient plants (von Rheinbaben and Trolldenier, 1984; Trolldenier, 1989).

Plants adapted to waterlogging and submerged soils (e.g., lowland rice) maintain high redox potentials in the rhizosphere by the transport of O_2 from the shoot through aerenchyma in the roots and release O_2 into the rhizosphere (Fig. 14.14, see also Section 17.4). Aerenchyma are formed by autolytic processes of the cortex cells, induced by increased ethylene concentrations not only under conditions of oxygen shortage but also in response to N and P deficiency (Lynch and Brown, 1997). The oxidation of the rhizosphere is essential for avoiding phytotoxic concentrations of organic solutes and Fe^{2+} and Mn^{2+} present in the bulk soil solution of poorly aerated or submerged soils. Oxygen transport within the roots and the rate of O_2 consumption in the roots and particularly in the rhizosphere are strongly affected by nutrition.

The oxidation zone extends between 0.4 and 4 mm from the rhizoplane into the bulk soil, depending on O_2 supply and O_2 consumption, and on the redox buffer capacity of the soil. The distance also varies along the axis of individual roots (Flessa and Fischer, 1992; Revsbech *et al*., 1999). In flooded rice, the redox potential strongly increases behind the root apex, for example from −250 mV to about +100 mV, is low in more basal zones, and is high again at sites where lateral roots penetrate the cortex. This pattern in

redox potential along the root axis may be related to the pattern in density of rhizosphere microorganisms (as main O_2 consumers) which is low at the apex and increases in basal zones prior to the emergence of lateral roots (Murakami *et al*., 1990).

Oxygen released from the roots of wetland plants such as *Typha latipholia* L. can be even used for respiration by neighbouring plants that would otherwise not withstand the low ambient O_2 (Callaway and King, 1996).

In aerated soils average redox potentials are in the range of 500–700 mV. However, aerated soils are non-uniform, and hypoxic microsites may occur. Such microsites are most likely more abundant in the rhizosphere than in the bulk soil (Fischer *et al*., 1989), and are particularly important for the acquisition of Mn and Fe, and for gaseous N losses (e.g., N_2, N_2O).

14.5.2 Mn Mobilization

Since Mn is plant available only in the reduced form (Mn^{2+}), in aerated soils, root-induced reduction of Mn oxides may be a mechanism for Mn acquisition. Reduction is mediated by combined effects of (i) enzymatic reduction at the root surface, (ii) chemical reduction by release of reductants, such as phenolics and malate, and (iii) Mn reduction by Mn-reducing rhizosphere microorganisms (Godo and Reisenauer, 1980). The activity of rhizosphere microorganisms is of particular significance for Mn nutrition of plants, since microorganisms can mediate Mn immobilization by oxidation reactions in soils as well as Mn solubilization by Mn reduction. Thus, the balance of Mn oxidizing bacteria (e.g., *Arthrobacter* spp.) to Mn reducers (e.g., fluorescent pseudomonads) strongly influences Mn availability in the rhizosphere (Posta *et al*., 1994; Rengel, 1997, see also Chapter 15).

14.5.3 Fe Mobilization

Enhanced reducing activity at the root surface of sub-apical root zones is a typical feature of roots of Fe-deficient dicotyledons and non-graminaceous monocots. The reductive capacity is increased by expression of a PM-bound reductase oxidase system with a low pH optimum (Brüggemann *et al*., 1991; Holden *et al*., 1991) encoded by the FRO2 gene identified in *Arabidopsis* (Robinson *et al*., 1997). The PM reductase-oxidase is further activated by rhizosphere acidification (Römheld and Kramer, 1983) which is the result of increased expression of PM H^+-ATPase in the sub-apical root zones (Fig. 14.12).

In Strategy I plants, increased reduction capacity and rhizosphere acidification is associated with enhanced release of Fe-chelating and Fe-reducing compounds, such as phenolics and carboxylates (Olsen and Brown, 1980; Römheld, 1987). Mobilization and uptake of Fe occurs in several steps: (i) solubilization of Fe^{3+} mediated by rhizosphere acidification, (ii) complexation with chelating compounds, and (iii) complex splitting by reduction to Fe^{2+} and subsequent uptake via the IRT1 Fe transporter, which is up-regulated under Fe limitation (Römheld, 1987a; Guerinot, 2000). Phenolic compounds released from Fe-deficient plant roots have been implicated in the remobilization of Fe precipitated on the root surface and in the root apoplast (Jin *et al*., 2007). Considerable genotypic variation exists in the level of expression of Strategy I responses, which is positively correlated with the resistance of plant species and cultivars to Fe deficiency under field conditions (Römheld, 1987a).

Complexation of Fe^{3+} with soluble humic acids and subsequent complex splitting by Fe^{3+} reduction may also contribute to Fe acquisition of Strategy I plants (Pinton *et al*., 1998). Additionally, humic substances may enhance the root-induced responses to Fe deficiency (Pinton *et al*., 1999b).

14.6 RHIZODEPOSITION AND ROOT EXUDATES

In higher plants, a substantial proportion (20–60%) of photosynthetic C is allocated below-ground (Grayston *et al*., 1996; Kuzyakov and Domanski, 2000). Depending on root activity, 15–60% of this carbon fraction is used for root respiration and is released as CO_2 (Lambers *et al*., 2002a). However, a substantial proportion of the assimilates reaches the rhizosphere as organic carbon, as *rhizodeposition* (Fig. 14.15). Amount and composition of the released compounds is highly variable and affected by multiple factors. Estimates of rhizodeposition range from 800 to 4,500 kg C ha^{-1} $year^{-1}$ (Kuzyakov and Domanski, 2000; Lynch and Whipps, 1990) and can comprise up to 70% of the C translocated below-ground in perennials and up to 40% in annual plants. This is associated with an input of N ranging between 15 and 60 kg ha^{-1} $year^{-1}$ (Hooker *et al*., 2000). Free amino acids and proteins usually make up only a minor fraction of organic compounds released from undamaged plant roots (typically 1–2% of released C; Kraffczyk *et al*., 1984; Jones and Darrah, 1993), therefore it is assumed that N rhizodeposition may be related to root turnover or efflux of inorganic N forms such as ammonium and/or nitrate (Feng *et al*., 1994; Scheurwater *et al*., 1999; Jones *et al*., 2009).

In contrast to the bulk soil, where microbial growth is C-limited (Wardle, 1992), microbial growth in the rhizosphere is N-limited. Therefore, rhizodeposition by growing roots enhances the microbial turnover of soil organic carbon in the rhizosphere ('priming effect'; Helal and Sauerbeck, 1989, Kuzyakov, 2002), particularly in plants well supplied with N (Liljeroth *et al*., 1990b).

Nitrogen temporarily immobilized in the rhizosphere microbial biomass can be partially released and mineralized via excretions of protozoa and nematodes grazing on

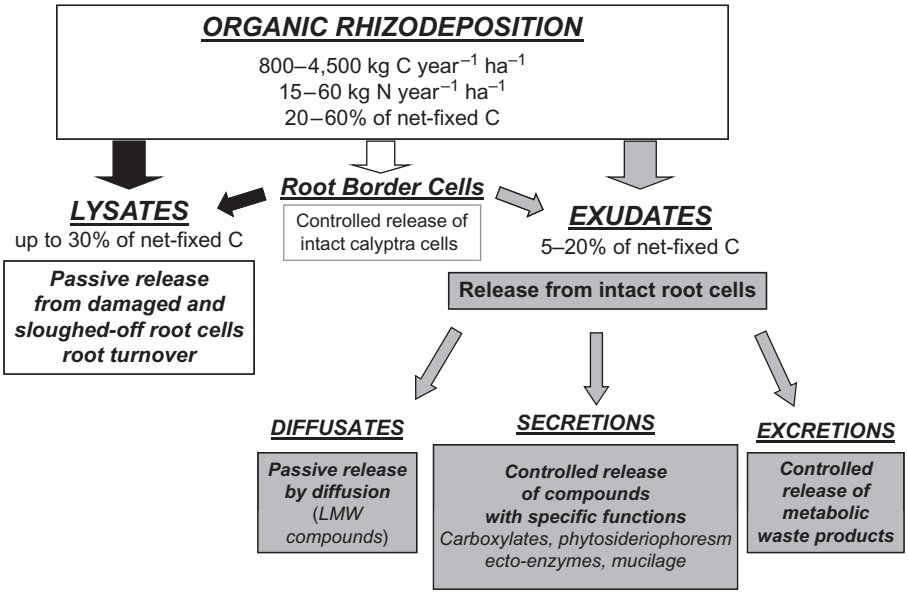

FIGURE 14.15 Classification and quantities of organic rhizodeposition. *Adapted from Neumann (2007).*

rhizosphere microbial populations, the so-called 'microbial loop' (Bonkowski, 2004; Bonkowski *et al*., 2009, see also Chapter 15). The importance of the rhizosphere for cycling of C and nutrients in soils is further illustrated by the fact that organic rhizodeposition, which can account for 30–40% of the total soil organic matter input, is released into the rhizosphere soil, which comprises only 2–3% of the total soil volume (Grayston *et al*., 1996).

A wide range of internal and external factors determine amount and composition of rhizodeposition. Rhizodeposition can be stimulated by increased mechanical impedance of the growth substrate (Boeuf-Tremblay *et al*., 1995; Groleau-Renaud *et al*., 1998), by toxic elements and low pH in the soil solution (Römheld and Marschner, 1983; Kochian, 1995; Costa *et al*., 1997), limitation of nutrients (Marschner, 1998; Neumann and Römheld, 2007), high light intensity (Rovira, 1959; Cakmak *et al*., 1998), elevated atmospheric CO_2 concentrations (Haase *et al*., 2007), temperature extremes (Rovira, 1959; Vancura, 1967), and the presence of microorganisms (Meharg and Kilham, 1995).

Depending on origin and release mechanisms, rhizodeposition may be subdivided into two main fractions: (i) lysates of sloughed-off cells and tissues originating from root turnover which can comprise up to 50% of the belowground C translocation (Grayston *et al*., 1996), and (ii) organic compounds released from intact root cells as so-called *root exudates*. The root exudate fraction may be further subdivided into (a) low-molecular-weight organic compounds permanently lost from root cells by diffusion (*diffusates*), (b) *root secretions* with special functions in nutrient mobilization, detoxification, defence reactions or as root signals, released by controlled mechanisms via membrane channels or transport proteins, and (c) metabolic waste products released as *root excretions* (Fig. 14.15). From a methodological point of view, however, it is not always easy to differentiate between these fractions.

Carbon input into soils via root exudation may comprise 5–10% of the net fixed carbon in soil-grown plants (Jones *et al*., 2004). A significant proportion of C also reaches the soil via mycorrhizal hyphae which may be comparable with that of fine roots (Johnson *et al*., 2002). In case of ectomycorrhizal fungi, hyphal C input may even be the dominant pathway of C transfer into the soil (Godbold *et al*., 2006).

Organic rhizodeposition also includes nutrients previously taken up by the plant and bound in organic molecules. In young wheat plants, for example, this contributes 1–5% of plant P (McLaughlin *et al*., 1987) and in wheat plants over the whole growing period, it makes up 18% of the total N in plants of low N status and 33% in plants of high N status (Janzen, 1990).

14.6.1 Sloughed off Cells and Tissues

In soil-grown plants, parts of the rhizodermis including root hairs and cortical cells may degenerate and release their content into the rhizosphere (Fusseder, 1984; McCully, 1999). During the growing season, approximately 25% of the roots turn over each month (Jones *et al*., 2009) with the diameter and lifespan of fine roots being positively correlated (Gill and Jackson, 2000). The lifespan of arbuscular mycorrhizal hyphae has been estimated to be 5–6 days (Staddon *et al*., 2003).

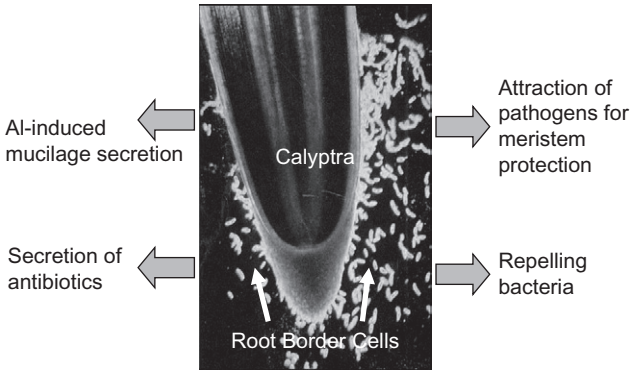

FIGURE 14.16 Liberation and potential functions of root border cells. *Adapted from Hawes* et al. *(2000).*

The release of sloughed-off root cells may be a genetically controlled process. The so-called *root border cells* are produced and released from peripheral cells of the root cap (Fig. 14.16). The number of released border cells exhibits genotypic variation and ranges between approximately 100 cells day^{-1} for root caps of tobacco (*Nicotiana tabaccum*) to up to 10,000 cells day^{-1} in cotton (*Gossypium hirsutum*). The production and release is stimulated by various environmental factors, including water, elevated CO_2 concentrations and mechanical impedance, but is also regulated by hormonal signals, such as auxins and ethylene (Driouich *et al*., 2007). The release of root border cells starts with increased expression of pectolytic enzymes, such as polygalacturonidase and pectin methyl esterase, responsible for the hydrolysis of the pectin matrix of the cell wall. This is associated with the production of protons; the resulting acidification may further activate other cell wall-degrading enzymes (Driouich *et al*., 2007). After detachment, a yet unknown feedback signal released by the root border cells leads to down-regulation of the hydrolytic processes in the cell walls of the root cap. Embedded into a layer of mucilage polysaccharides, the cells are viable after detachment from the root cap for up to one week, and can be transported during root growth to more basal parts of the root (McCully, 1999; Hawes *et al*., 2000).

Border cells can produce antibiotics. Also specific attraction of root pathogens, such as parasitic nematodes, fungal zoospores and pathogenic bacteria has been reported, which may serve as distraction and reduce infection of the apical root meristem. Secretion of a certain set of proteins during detachment of the root border cells seems to be involved in this process (Hawes *et al*., 2000; Wen *et al*., 2007). In response to toxic Al concentrations, root border cells of Al-resistant plants secrete large amounts of mucilage, which may be involved in Al detoxification due to complexation with the galacturonates of the mucilage layer (Hawes *et al*., 2000; Miyasaka and Hawes, 2001).

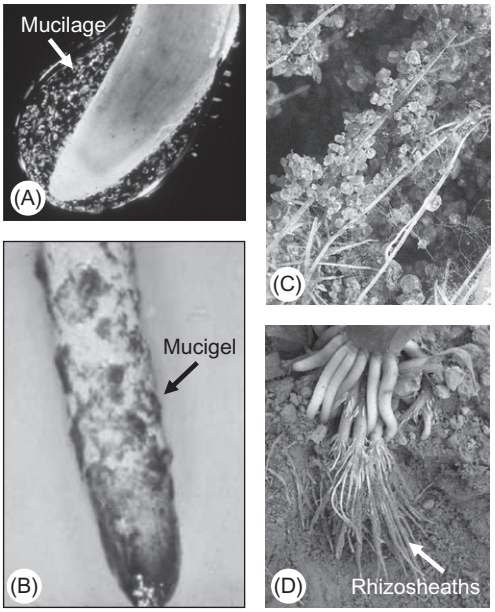

FIGURE 14.17 (A) Swelling of the mucilage layer covering the calyptra by rapid water uptake; (B) formation of mucigel by mucilage with enclosed soil particles; (C) mucilage-mediated binding of soil particles in the rhizosphere of *Thlaspi caerulescens*; (D) mucilage-mediated formation of soil rhizosheaths around roots of field-grown maize. *Adapted from Ingwersen* et al. *(2006) and Neumann (2007).*

14.6.2 Root Exudates – High-Molecular-Weight (HMW) Compounds

High-molecular-weight (HMW) secretions, such as mucilage polysaccharides and ectoenzymes, are released from roots by vesicle transport via exocytosis (Battey and Blackbourn, 1993; Battey *et al*., 1999).

14.6.2.1 Mucilage and Mucigel

Mucilage is mainly secreted via the Golgi apparatus of hypersecretory cells of the root cap as a gelatinous polygalacturonic acid polysaccharide (Fig. 14.17), and is subsequently transferred during root elongation to older root zones, but epidermal cells are also able to secrete mucilage (Vermeer and McCully, 1981). In non-sterile media, mucilage also includes substances produced by microbial degradation of the cell walls (Rovira *et al*., 1983). In soil-grown plants, the mucilage is usually invaded by microorganisms, and both organic and inorganic soil particles are embedded in it. This mixture of gelatinous material, microorganisms and soil particles is termed *mucigel* (Bowen and Rovira, 1991).

Mucilage and mucigel may protect the root meristem and improve the root–soil contact by inclusion and aggregation of soil particles (McCully, 1999). A putative function as lubricant, suggested in earlier studies, seems to be unlikely, since at water potentials lower than zero mucigel

TABLE 14.6 Root growth and Al concentration and content in roots and mucilage of cowpea grown in nutrient with or without Al in presence or absence of mucilage (removed 3 times day^{-1})

			Al in root tips			
			Content (μg (25 tips)$^{-1}$)		Concentration (mg g^{-1} dw)	
Al treatment	Mucilage	Root growth (cm d^{-1})	Roots	Mucilage	Roots	Mucilage
0 Al	+	6.3	–	–	–	–
	–	5.9	–	–	–	–
5 mg Al L^{-1}	+	4.8	12.4	16.6	2.1	16.6
	–	2.1	20.6	3.6	3.2	14.5

From Horst et al. (1982).

does not retain water or swell (McCully, 1999). However, mucilage translocated during root elongation to more basal parts of the root can form so-called rhizosheaths by inclusion of adhering soil particles (McCully, 1999). Shrinking of mucilage with declining water potentials leads to a tighter association of soil particles within the rhizosheaths (Fig. 14.17). This contributes to improved waterholding capacity of the rhizosphere soil and increases the proportion of water-stable soil aggregates from about 2% to nearly 40% (Morel *et al*., 1991). Accordingly, in desert plants (*Opuntia*), formation of rhizosheaths under drought stress can reduce water loss from roots by approximately 30% (Huang *et al*., 1993).

The close contact between soil particles and root surface via mucilage can be of considerable importance for the uptake of nutrients. This applies particularly to micronutrients and P. In the transition zone at the soil/root interface, processes may be different from those occurring in the free solution ('two-phase-effect'; Matar *et al*., 1967). In P-deficient soil, plants take up P which is not in equilibrium with the soil solution, but is mobilized at the root/soil interface presumably via P desorption from clay surfaces by the polygalacturonate component of mucilage (Nagarajah *et al*., 1970). Possible functions of phosphatidylcholine surfactants within root mucilages for P mobilization, inhibition of nitrification and modification of soil water retention have been discussed by Read *et al*. (2003). Two-phase effects supply only a minor fraction of the total demand for macronutrients such as P, but can have greater importance for uptake of micronutrients. In dry soils, stimulation of mucilage secretion in response to increased soil mechanical impedance can contribute to the maintenance of Zn^{2+} uptake by facilitating Zn^{2+} transport from embedded soil particles to the root surface (Nambiar, 1976a, b). Zinc acquisition may be further promoted by transfer of water within the roots from the subsoil and subsequent release into the dry top soil layer, the so-called *hydraulic lift* (Vetterlein and Marschner, 1993).

By complexation with galacturonates mainly in exchange with Ca^{2+}, mucilage may also contribute to exclusion of toxic elements such as Al (Table 14.6; Horst *et al*., 1982) and heavy metals (Cd, Pb; Morel *et al*., 1986). In roots of cowpea (*Vigna ungiulata*) exposed to Al toxicity, a high proportion of the Al is bound to the mucilage (see also Section 17.3). On a dry weight basis, the mucilage contains about eight times more Al than the root tissue and removal of the mucilage leads to an increase in the Al concentration of the root tissue and inhibition of root extension. The enhancement of mucilage production by mechanical impedance is therefore a major contributing factor to the higher Al tolerance of roots growing in solid substrates compared with nutrient solution culture.

14.6.2.2 Secretory Proteins

Plant roots release a wide range of proteins including various enzymes. Secretory proteins are synthesized by polysomes attached to the endoplasmic reticulum (ER) and are segregated into the ER lumen already during the translation process. During the passage through the Golgi apparatus, transfer vesicles containing the secretory proteins are separated from vesicles with vacuolar destination. After reaching the plasma membrane, the proteins in the vesicles are released into the apoplasm via exocytosis (Chrispeels, 1991; Chrispeels and Raikhel, 1992). All processes linked with exocytosis strongly depend on Ca^{2+} supply (Battey and Blackbourn, 1993; Battey *et al*., 1999).

A wide range of enzyme activities involved in the hydrolysis of organic P esters, such as phytase, nuclease, pyrophosphatase, apyrase and alkaline phosphatise, have been detected in the rhizosphere (Neumann and Römheld,

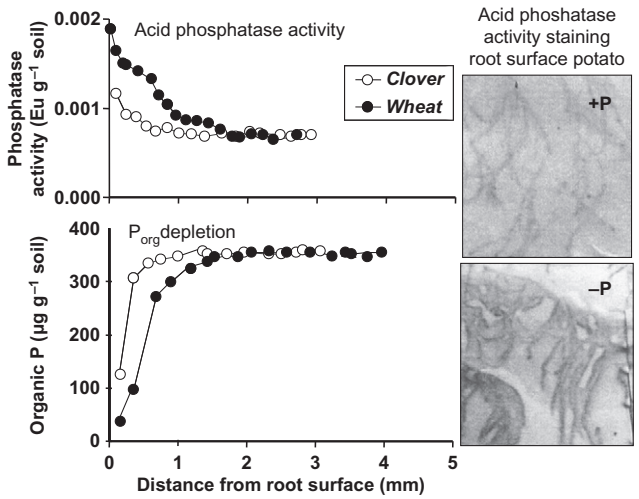

FIGURE 14.18 Phosphatase activity and depletion of organic soil P in the rhizosphere of clover and wheat and activity staining of root-secretory acid phosphatase in P-sufficient and P-deficient potato. *Adapted from Tarafdar and Jungk (1987) and Dinkelaker and Marschner (1992).*

TABLE 14.7 Dry matter and shoot P concentration of potato grown in hydroponics and in soil culture with organic P (phytate) or soluble inorganic P (KH_2PO_4)

	Dry matter (g plant^{-1})		Shoot P concentration (mg g^{-1} dw)	
	Nutrient solution	Soil	Nutrient solution	Soil
0 P	6.5		1.6	
Phytate	14.1	8.5	4.3	1.8
KH_2PO_4	22.5	12.5	6.9	4.5

Adapted from Neumann *et al*. (2001).

2007). These enzymes may originate from plant roots but also from rhizosphere microorganisms. However, Wasaki *et al*. (2005) demonstrated that acid phosphatase activity in the rhizosphere of cluster roots in *Lupinus albus* was predominantly of plant origin. In most agricultural soils, between 30 and 70% of the total soil P is present in organic form. In forest soils this proportion may rise to 80–90% (Häussling and Marschner, 1989). Organic P may comprise phytate (myo-inositolhexaphosphate), the least soluble, and therefore frequently the dominant organic P fraction in many soils, whereas sugar, lipid or nucleotide phosphates exhibit higher solubility and thus higher rates of mineralization (Richardson *et al*., 2005).

Phosphorus limitation frequently leads to a stimulation of phosphatase secretion from plant roots (Fig. 14.18), but considerable variation exists within plant species and cultivars (Römer *et al*., 1995; Li *et al*., 1997). Phosphorus deficiency-induced root secretion of acid phosphatases is probably regulated at the transcriptional level (Wasaki *et al*., 1997; Neumann *et al*., 2000) and may involve sensing of external P concentrations in the growth medium (Wasaki *et al*., 1999) and differential induction of iso-enzymes (Gilbert *et al*., 1999).

Plants grown in nutrient solution or sand culture can use organic P forms to a similar extent as inorganic P. However, in many soils, enzymatic hydrolysis by root-secretory phosphatases is limited by the low solubility of organic P forms in soils (Table 14.7; Adams and Pate, 1992; Hübel and Beck, 1993), due to adsorption and precipitation processes similar to those for inorganic P by formation of sparingly soluble salts and complexes with Ca, Fe and Al. Furthermore, the hydrolysis of organic P esters in the rhizosphere may be limited by immobilization of the secretory phosphatases (i) in the root cell wall or the mucilage layer (Dinkelaker *et al*., 1997), and (ii) adsorption and inactivation on clay minerals and organo-mineral complexes (Rao *et al*., 1996). Accordingly, attempts to increase the acquisition of phytate P by transgenic expression of a secretory phytase gene from *Aspergillus niger* in *Arabidopsis* was successful in agar media, but largely failed in soil culture (Richardson *et al*., 2005).

At least in some plant species, root secretion of carboxylates, such as oxalate and citrate, may enhance the solubility of organic P forms, making them available for hydrolysis by phosphohydrolases in the rhizosphere (see Fig. 14.20; Beissner, 1997; Otani and Ae, 1999). Root secretory acid phosphatases may also contribute to P retrieval by hydrolysis of organic P lost into the rhizosphere from sloughed off and damaged root cells (Lefebvre *et al*., 1990).

Many other enzymes are located in the root apoplasm, particularly in the epidermal cells of apical root zones. These include enzymes potentially involved in defence reactions such as chitinase, glucanase, peroxidase and phenoloxidase, as well as those needed for cell wall biosynthesis and C supply to mycorrhizal fungi (e.g., invertase). Their role in nutrient dynamics in the rhizosphere and nutrient acquisition is not clear. Whereas the role of proteases released from plant roots in N cycling in the rhizosphere is unclear, proteases excreted by some ericoid and ECM fungi have been shown to increase access of the host plant to complex organic sources of N such as protein (see also Chapter 15). Since the host plants themselves have little or no access to these resources, their fungal associate may play a crucial role for host plant growth on substrates with complex organic N (Hutchison, 1990).

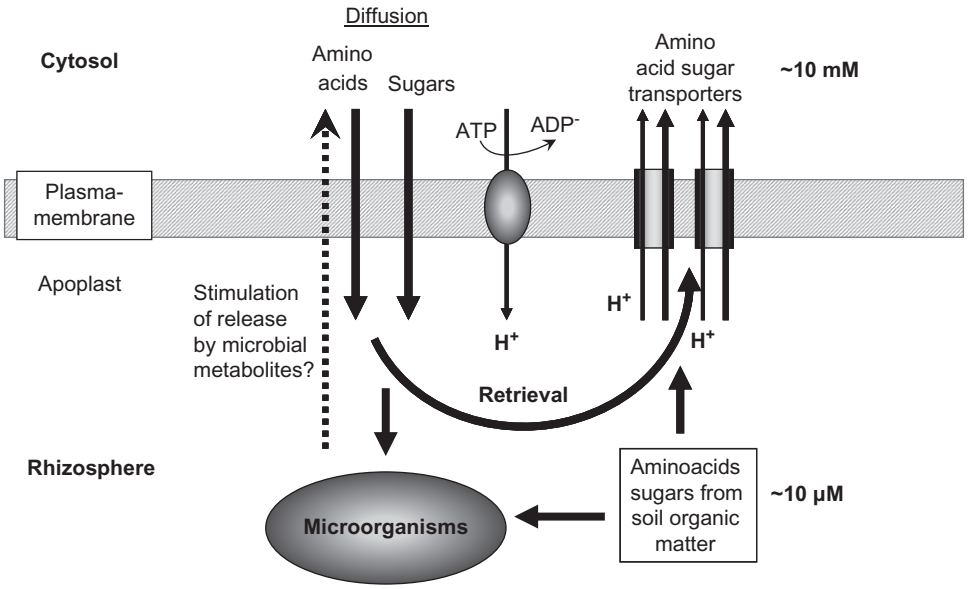

FIGURE 14.19 Model for uptake and retrieval of LMW sugars and amino acids by plant roots. *Adapted from Jones* et al. *(2005).*

14.6.3 Low-Molecular-Weight (LMW) Root Exudates

14.6.3.1 Diffusion-mediated Release of LMW Compounds

Even in intact root cells, any soluble LMW compound present in the cytosol may be lost into the rhizosphere. The large concentration gradient of LMW solutes usually existing between the cytosol (millimolar concentrations) and the rhizosphere (micromolar concentrations as a consequence of microbial degradation), promotes outward diffusion of LMW compounds. Moreover, the outward positive electrochemical potential gradient created by proton extrusion via PM H^+ ATPases further promotes outward diffusion of LMW compounds which are negatively charged at the pH of the cytosol (7.0–7.5), such as organic acids and amino acids (Neumann, 2007). In the rhizosphere, these compounds may contribute to nutrient cycling as C and N sources for rhizosphere microorganisms.

Major fractions of LMW compounds detected in root exudates include sugars, organic acid anions, amino acids and various phenolics. Due to rapid microbial decomposition, the half-life of many LMW compounds in the rhizosphere is only 1–5 hours (Jones *et al*., 2005). However, immobilization of exudates on the soil matrix by adsorption and complexation can protect against biodegradation and substantially increase their residence time in soils (Boudot, 1992).

14.6.3.2 Retrieval Mechanisms

For sugars and particularly for N-containing compounds, such as amino acids and small peptides, efficient uptake systems have been identified in roots of a wide range of different plant species (Xia and Saglio, 1988; Steiner *et al*., 1994; Fischer *et al*., 1998) and there is even some evidence for root uptake for high-molecular-weight (HMW) compounds via endocytosis (Samaj *et al*., 2005).

Up to 90% retrieval of amino acids and sugars lost by plant roots via diffusion has been demonstrated in hydroponic culture (Jones and Darrah, 1993; Darrah, 1993). Uptake is mediated by transporters and involves an active mechanism with H^+ co-transport. Amino acid and peptide transporters frequently show enhanced expression under limited N supply (Persson and Nashölm, 2002; Nazoa *et al*., 2003). In natural ecosystems, N is a major limiting nutrient and rhizosphere microorganisms are responsible not only for N mineralization but also for N competition with plant roots by N immobilization in the microbial biomass (Bonkowski *et al*., 2009, see also Chapter 15). Therefore, efficient re-uptake of amino acids lost from plant roots via diffusion may be a successful strategy for N competition with rhizosphere microorganisms (Fig. 14.19). These transporters are located in the plasma membrane, and may therefore retrieve compounds from the root apoplasm, before amino acids reach the rhizoplane. This view is in agreement with K_m determinations for amino acid uptake by plant roots, which are in the millimolar range, which is the concentrations of LMW compounds in the apoplasmic fluid. In contrast, only micromolar concentrations of amino acids occur in the rhizosphere soil solution, suggesting a rather low efficiency of plant roots for competition in amino acid uptake with microorganisms in the rhizosphere (Jones *et al*., 2005). Nevertheless, in forest ecosystems, arctic tundra, waterlogged and acidic soils characterized by high concentrations of dissolved organic N in the soil solution, root uptake of organic N forms may also significantly contribute to the N supply (Chapin *et al*., 1993).

Under certain stress conditions, such as nutrient deficiency, drought or oxidative damage, sugar supply may be a limiting factor for plant growth due to reduced photosynthesis. Thus, re-uptake of sugars lost by diffusion may be a strategy to minimize C losses. The expression of retrieval mechanisms for LMW sugars in plant roots (Xia and Saglio, 1988) may also enable plants to control microbial colonization at the rhizoplane and in the rhizosphere by modifying the supply of easily available carbohydrates to rhizosphere microorganisms (Fig. 14.19; Jones *et al*., 2004b).

14.6.3.3 Controlled Release of LMW Compounds

Apart from continuous passive outward diffusion of LMW compounds and the release from damaged root cells, there is also evidence for a controlled excretion of metabolic waste products and secretion of specific compounds into the rhizosphere. For example, strong release of lactate from root tips of maize seedlings adapted to low oxygen environments can prevent excessive intracellular accumulation of lactic acid as a product of fermentation induced by shortage of oxygen (Xia and Roberts, 1994). Another example is the strong excretion of malate and citrate which occurs in apical root zones of rice cultivars tolerant to bicarbonate toxicity, which may occur in calcareous soils at high soil water content. High concentrations of bicarbonate in the soil solution induce intracellular accumulation of organic acid anions in the root tissue that may inhibit root growth (Lee, 1998). Adapted cultivars are able to avoid this by secretion of organic acid anions into the rhizosphere (Hajiboland *et al*., 2005). The secretion of organic acid anions into the rhizosphere may also increase Zn availability in calcareous soils, thereby improving the Zn efficiency of bicarbonate-tolerant rice cultivars (Yang *et al*., 1994).

Secretion of organic acid anions is also a mechanism to mobilize sparingly available P adsorbed to Fe and Al oxides/hydroxides or as Fe, Al and Ca phosphates via solubilization and chelation of metal cations (Fig. 14.20; Jones *et al*., 2003a). Citrate, oxalate, malonate and malate are the most efficient organic acid anions with respect to P mobilization in soils (Neumann and Römheld, 2007). However, the effectiveness of this mechanism has been questioned because the concentration of organic acid anions in the rhizosphere of most plants is low. To mobilize significant amounts of P, organic acid anion concentrations in the soil solution in the millimolar range are required (Jones, 1998; Gerke *et al*., 2000). Such concentrations have only been measured in the rhizosphere of cluster-rooted plant species (Neumann and Martinoia, 2002; see also Section 6.3 and Chapter 13).

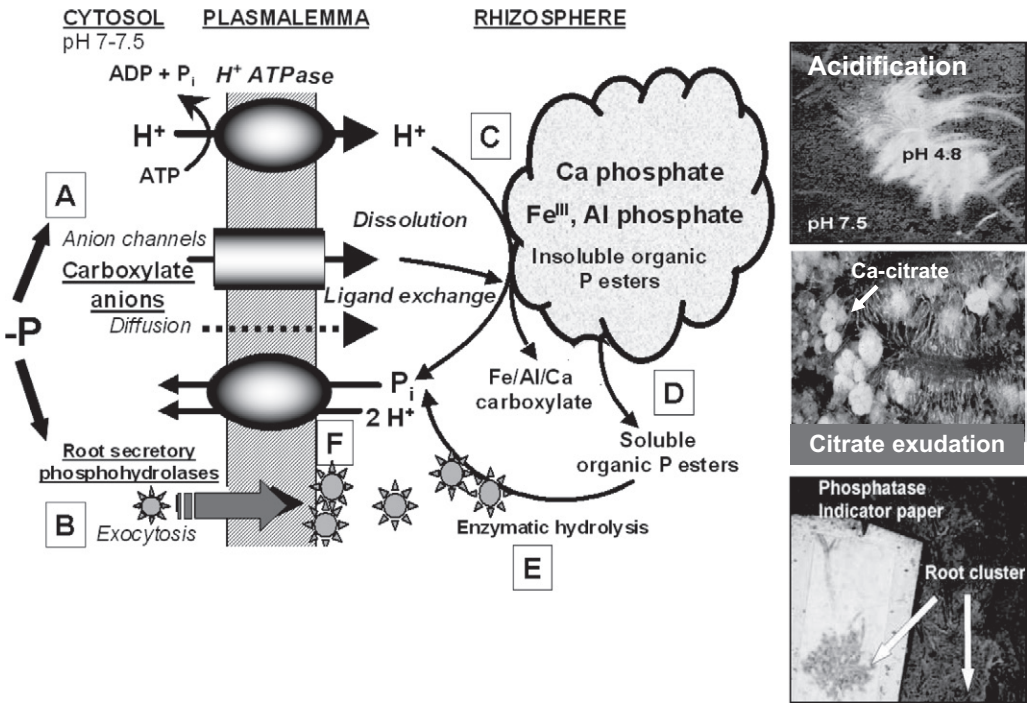

FIGURE 14.20 Model for the role of root exudates in P mobilization in the rhizosphere. Phosphorus deficiency-induced secretion of carboxylates by anion channels with concomitant H^+ extrusion (A) and of root-secretory acid phosphatase (B); dissolution of acid soluble Ca-P byroot-induced H^+ release and displacement of phosphate anions from anion sorption sites (Fe/Al/Ca) on the soil matrix by carboxylates (C); displacement of organic P esters from anion sorption site on the soil matrix by carboxylates (D); enzymatic hydrolysis of organic P esters in the soil solution by the activity of phosphatases released by roots and microorganisms (E); root uptake of mobilized inorganic P via H^+ co-transport by P transporters (F). *Adapted from Dinkelaker* et al. *(1989), Neumann and Martinoia (2002) and Neumann (2007).*

Compared with normal lateral roots, the formation of the bottlebrush-like cluster roots with densely spaced, second order lateral rootlets, covered with root hairs, strongly increases the root surface area available for secretion of organic acid anions. Organic acid anions secretion is further increased by up-regulation of biosynthetic pathways. In cluster roots of white lupin (*Lupinus albus*), the inhibition of citrate turnover in the roots contributes to preferential accumulation of citrate in the cluster root tissue (Neumann and Martinoia, 2002). In white lupin cluster roots, citrate secretion is very high over a period of 2–3 days and appears to be mediated by activation of a citrate channel (Zhang *et al*., 2004), charge-balanced by a concomitant extrusion of protons via activation of the PM H^+-ATPase (Yan *et al*., 2002). The resulting acidification of the rhizosphere can inhibit microbial growth, thereby minimizing microbial degradation of carboxylates. Chitinases, glucanases and flavonoids, which are also secreted from cluster roots may have a similar anti-microbial effect (Weisskopf *et al*., 2006).

In graminaceous plant species, secretion of *mugineic acids* or *phytosiderophores* (*PS*) (Fig. 14.21) is induced by limitation of Fe and Zn (Neumann and Römheld, 2007; see also Chapter 2 and Section 7.1). Derived from nicotianamine which is an ubiquitous intracellular metal chelator in higher plants, PS are synthesized at high rates in the roots of Fe-deficient graminaceous plants (Ma and Nomoto, 1996). The release mechanism is yet unknown. In barley, diurnal pulses of very high PS secretion in the morning, restricted to the young tissues in apical root zones with limited microbial colonization is considered as a mechanism to minimize microbial degradation of PS (Nishizawa and Mori, 1987; von Wirén *et al*., 1995). In the rhizosphere, PS mobilize Fe^{3+}, but also other micronutrients, such as Zn, Mn and Cu, by formation of stable complexes even at high soil pH (Treeby *et al*., 1989). The soluble Fe^{3+}–PS complex is subsequently taken up by H^+ co-transport via the ZmYs1 transporter (Curie *et al*., 2001; Schaaf *et al*., 2004). Up-regulation of ZmYS1 in maize is induced by Fe deficiency, but not by deficiency of other micronutrients such as Zn, Mn or Cu (Roberts *et al*., 2004; Schaaf *et al*., 2004), suggesting that Fe mobilization is the primary function of PS release. Large genotypic differences in the capacity for PS secretion occur between plant species and cultivars. Graminaceae originating from the humid tropics with abundance of acid soils where Fe availability is high are usually less efficient in PS secretion and more susceptible to Fe deficiency chlorosis. Phytosiderophore secretion and tolerance to Fe limitation in upland rice was improved in pot experiments and under field conditions by transgenic expression of the nicotianamine aminotransferase (NAAT) gene of barley as key enzyme for PS biosynthesis (Takahashi *et al*., 2000).

Root secretion of carboxylates is an important component determining Al resistance in higher plants (Fig. 14.22; see also Section 17.3). At soil pH below 5, solubilization

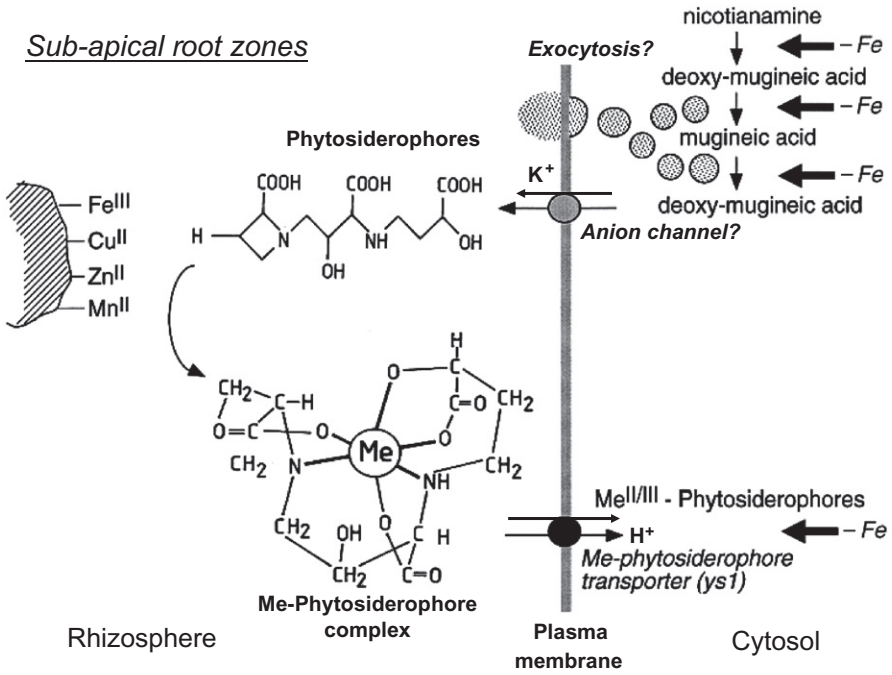

FIGURE 14.21 Model for root-induced mobilization of Fe and other micronutrients (Zn, Mn, Cu) in the rhizosphere of graminaceous (Strategy II) plants, mediated by release of phytosiderophores (PS) and uptake of PS–metal complexes. *Adapted from Neumann (2007).*

of mononuclear Al species limits root growth. In many Al-tolerant plant species and cultivars, Al-induced root secretion of carboxylates (particularly malate, citrate and oxalate) is an important factor for Al^{3+} detoxification by external complexation in the root apoplast (Kochian *et al*., 2004). Malate citrate and oxalate are among the most efficient. The carboxylates are released in response to Al toxicity, particularly in the zone of transition between cell division and cell elongation in root apices which is the most Al-sensitive part of the root (Kollmeier *et al*., 2000). In Al-resistant wheat, malate is released immediately after exposure to high Al concentrations via an anion channel (ALMT1) expressed in the root tips (Zhang *et al*., 2004; Sasaki *et al*., 2004). Transgenic expression of ALMT1 confers Al resistance to Al-sensitive barley (Delhaize *et al*., 2004); hence, ALMT1 may be a tool to increase Al resistance in transgenic plants. In contrast, Al-mediated citrate release in Al-resistant genotypes of sorghum and barley seems to be mediated by MATE transporters (Magalhaes *et al*., 2007; Wang *et al*., 2007). Some members of the ALMT and MATE families mediate carboxylate transport independently of Al stress and may therefore provide candidate genes for a more general manipulation of carboxylate exudation (Ryan *et al*., 2009b).

Low-molecular-weight phenolics, flavonoids and strigolactones and other yet unidentified compounds released by plant roots are important signals for the establishment of plant–microbial interactions such as symbiosis with N_2 fixing microorganisms, mycorrhiza but also in interactions with bacterial communication systems (quorum sensing) and in parasitic intereactions (Martin *et al*., 2001; Bauer *et al*., 2005; Akiyama *et al*., 2006; Werner, 2007; see also Chapter 15). Particularly in the rhizobium symbiosis with leguminous plants, the molecular events involved in the infection process of the rhizobial microsymbiont are well characterized (Werner, 2007; see also Chapter 16). However, surprisingly little is known concerning the release mechanisms of these signals, which obviously require a highly coordinated regulation in space and time (Neumann, 2007).

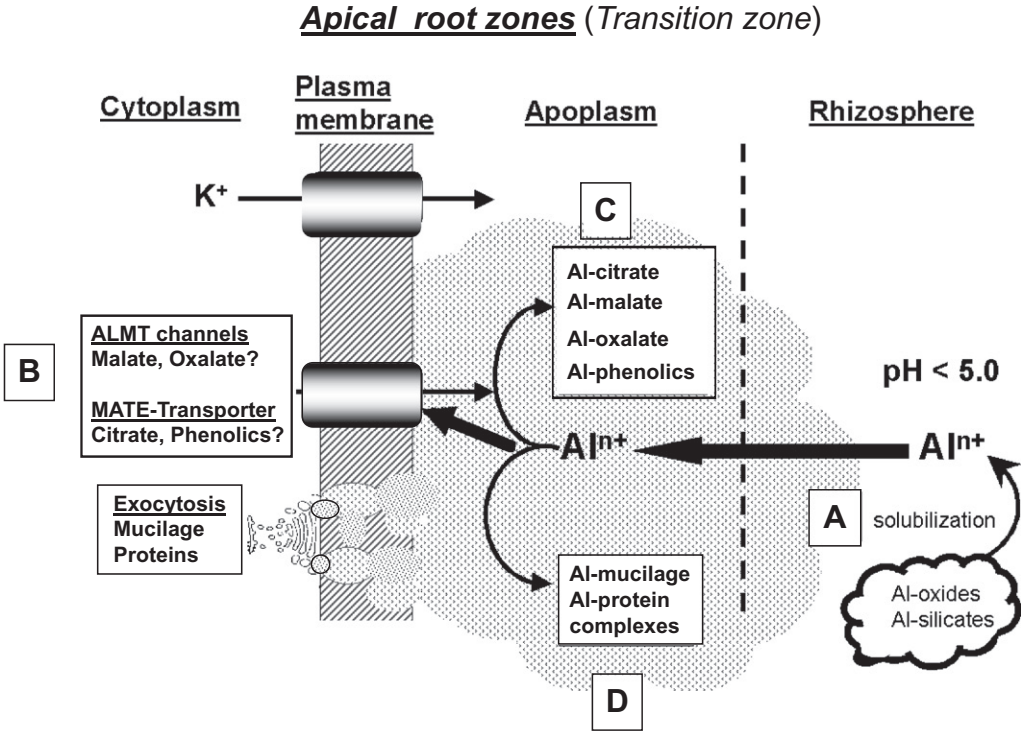

FIGURE 14.22 Model for root-induced Al detoxification by secretion of organic Al chelators. Solubilization of toxic Al^{n+} species in acid mineral soils at pH $<$ 5.0 (A), Al-induced activation of anion channels in the Al-sensitive apical root zones and release of organic Al chelators (B); detoxification of Al^{3+} by complexation with low-molecular-weight (LMW) chelators (C) with proteins and mucilage (D). *Adapted from Neumann (2007).*

Chapter 15

Rhizosphere Biology

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SUMMARY

The release of easily decomposable root exudates by roots leads to higher microbial density and activity in the rhizosphere compared to the bulk soil. In this chapter, the colonization of the roots by microorganisms is outlined and it is discussed how these microorganisms may enhance nutrient availability to plants but also reduce nutrient availability. Another important group of microorganisms with respect to plant nutrition are mycorrhizal fungi, which improve plant uptake of poorly mobile nutrients such as P and Zn via the network of hyphae extending into the surrounding soil. This chapter also discusses other beneficial effects such as increased heavy metal and drought tolerance, and disease resistance. Furthermore, the reasons for differential responsiveness of plants to mycorrhizal colonization are outlined. The chapter ends with an outlook on the use of mycorrhiza in plant production.

15.1 GENERAL

The release of easily decomposable low-molecular-weight exudates by roots attracts soil microorganisms into the rhizosphere where they proliferate to densities which can be several orders of magnitude higher than in the bulk soil. The high density of microorganisms, in turn, attracts predators such as nematodes and protozoa. Soil microbes play a pivotal role in nutrient turnover and thus nutrition of plants by decomposing and mineralizing organic material and releasing as well as transforming inorganic nutrients by solubilization, chelation and oxidation/reduction. Grazing by predators releases nutrients from the microbial biomass and enhances microbial growth rates. Rhizosphere microorganisms may also affect plant nutrient uptake indirectly by enhancing root growth (See also Chapter 13).

Almost all plant species form an association with mycorrhizal fungi which improve plant uptake of poorly mobile nutrients such as P and Zn. The improved uptake of P and Zn is due to the extensive network of external hyphae accessing nutrients beyond the rhizosphere. Other benefits of mycorrhizal colonization include direct access to organic N, increased heavy metal and Al tolerance, decreased disease susceptibility, and, in some cases, improved water uptake. However, mycorrhizal colonization comes at a cost: plants have to supply the fungus with carbon. Under conditions where the fungus provides little or no benefit (e.g., high soil nutrient availability), the cost of the symbiosis may outweigh its benefit, and mycorrhizal colonization can result in growth depression.

Another symbiosis which plays a key role in plant nutrition is that between certain plant species and N_2-fixing microorganisms. This will be discussed in Chapter 16.

15.2 RHIZOSPHERE MICROORGANISMS

15.2.1 Root Colonization

In soil, growth and activity of microorganisms is mainly limited by carbon availability (De Nobili *et al.*, 2001; Demoling *et al.*, 2007) because of the complex nature and thus poor decomposability of the soil organic matter. Root exudates are generally of low molecular weight and thus easily decomposable, therefore the population density of microorganisms is considerably higher in the rhizosphere than in the bulk soil. The relative increase in the number of microorganisms is expressed as the *R/S* ratio, *R* being the numbers per gram of soil in the rhizosphere and *S* in the bulk soil. The ratios vary greatly, between 5 and 50, depending on, for example, microbial species, plant age, plant species and nutritional status of plants. However, only a proportion of the root surface is covered by microorganisms. For example, of the total root surface area of maize, bacteria surface cover is about 4% in apical zones, 7% in the root hair zone and up to 20% in basal zones (Schönwitz and Ziegler, 1986b).

Root colonization by microorganisms is not confined to the rhizoplane, but may also occur to a varying degree

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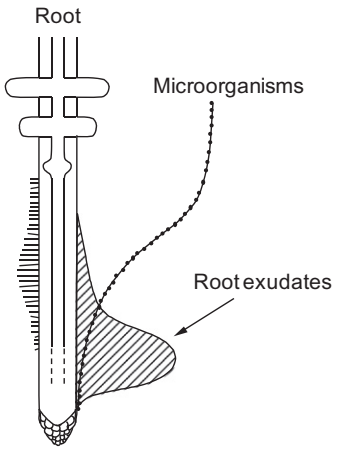


FIGURE 15.1 Schematic diagram of release of LMW root exudates and microbial activity in the rhizosphere of soil-grown plants.

also in the apoplasm of the cortex (e.g., *Azospirillum brasilense*). These so-called endophytes can be bacteria, actinobacteria or fungi which may affect plant growth and nutrient uptake (Kloepper *et al.*, 1992b; Schardl *et al.*, 2004; Rahman and Saiga, 2007; Rodriguez *et al.*, 2009).

As the root grows through the soil, the new root surface just behind the meristematic tissue is colonized by microorganisms that are attracted to the root surface. Root exudates released in the zone immediately behind the root tip and in the distal elongation zone stimulate microbial growth and attract more soil microorganisms to the root surface. Further from the root tip, in the root hair zone and adjacent zone, root exudation is lower, leading to lower microbial growth rates (Nguyen and Guckert, 2001; Trivedi *et al.*, 2008). Along the more mature root parts exudation is even lower and the primary substrates are cellulose and other recalcitrant cell wall materials, thus microbial growth rates and activity are low (Nguyen and Guckert, 2001) (Fig. 15.1).

Hence, in fast growing roots there is usually a steep gradient of rhizoplane and rhizosphere microorganisms from apical to basal zones along the root axis (Bowen and Rovira, 1991). The differences in type and quantity of carbon available in different root zones also lead to distinct rhizosphere community structures (Chiarini *et al.*, 2000; Yang and Crowley, 2000; Baudoin *et al.*, 2001; Marschner *et al.*, 2001). The changes in microbial density along the root axis are important for nutrient turnover within the microbial biomass (Marschner *et al.*, 2011). An increase in microbial biomass may result in net immobilization of nutrients, whereas a decrease in biomass can cause a net release of nutrients.

Equally important as microbial density for root growth, physiology of the roots and nutrient dynamics in the rhizosphere is the microbial community composition (i.e., which genotypes (species, strains) are present at which abundance and in which proportion), because each genotype has certain physiological characteristics; they may be, for example, ammonifiers and nitrifiers, producers of phytohormones, N_2 fixers, minor pathogens or antagonists. Different plant species have a different rhizosphere microflora in terms of abundance and physiological characteristics (Kloepper *et al.*, 1991; Marschner *et al.*, 2001, Miethling *et al.*, 2003; Marschner and Timonen, 2004) which can be further modified by the properties of the soil (Miethling *et al.*, 2000; Marschner *et al.*, 2001), plant age (Gomes *et al.*, 2001; Smalla *et al.*, 2001) and plant nutritional status (Yang and Crowley, 2000; Marschner *et al.*, 2004; Solaiman *et al.*, 2007b).

For a given plant species, the amount and form of N fertilizer supply may also alter the rhizosphere microflora. For example, increasing N supply inhibits both the number and proportion of diazotrophic bacteria at the rhizoplane of various grasses, whereas the total number of bacteria increases (Kolb and Martin, 1988). In wheat, depending on whether N is supplied as ammonium or nitrate, there is a considerable shift in the proportion of pathogen (*Gaeumannomyces graminis*) and antagonists (*Pseudomonas* spp.) in the rhizosphere (Sarniguet *et al.*, 1992a, b). Bacterial community composition is also altered by the amount and form of P fertilization (Marschner *et al.*, 2004).

Microorganisms decompose root exudates and may therefore decrease the efficiency of the exudates in nutrient mobilization. The gradient in microbial populations along the root axis and the rapid decomposition of exudates by rhizosphere microorganisms have important implications for the efficiency of root exudates released in response to nutrient deficiency. The half-life of organic acid anions (such as citrate and malate) or amino acids in soils is 6–12 h (Jones *et al.*, 1994; Jones, 1998). Consequently, Jones (1998) questioned the role of organic acid anions in mobilization of P and other nutrients (see Chapter 14). However, high exudation rates immediately behind the root tip where microbial density is low maximize their effectiveness. An example is grasses under Fe deficiency, where phytosiderophore release is confined to the zone immediately behind the root tips (See also Chapters 2 and 14). In addition, phytosiderophores are released in a short period between 2 and 8 h after onset of light. This pulse of phytosiderophore release minimizes microbial decomposition and thereby maximizes their effictiveness (Römheld, 1991; Crowley and Gries, 1994). Similarly, under P deficiency, release of organic acid anions is highest immediately behind the root tip or specialized root structures such as cluster roots (Chapter 14). Moreover, decomposition of root exudates is reduced by sorption to soil particles where they may still be effective in nutrient mobilization (Jones and Edwards, 1998). On the other hand, factors that favour

a more uniform distribution of rhizosphere microorganisms along the roots, such as reduced root growth (Watt *et al*., 2003), decrease the effectiveness of root-released phytosiderophores (von Wirén *et al.*, 1993). Model calculations of effectiveness of root exudates in nutrient acquisition have to consider this spatial separation of root exudation and microbial activity (Darrah, 1991, 1993).

15.2.2 Role in Nutrition of Plants

Rhizosphere microorganisms may affect nutrition of plants through their influence on (i) growth, morphology and physiology of roots (Chapter 13), (ii) physiology and development of plants, (iii) availability of nutrients, and (iv) nutrient uptake processes.

Rhizosphere microorganisms are the main drivers of turnover of organic C, N and P and thus recycling of organically bound nutrients, for example by ammonification and nitrification, but may also increase N loss via denitrification (Sylvia *et al*., 1999). The mineralization of organic N, which represents more than 90% of the total N in soils, to ammonium and nitrate by soil microorganisms is critical for plant N uptake because most plants have a very limited ability to take up organic N (Dunn *et al.*, 2006; Xu *et al.*, 2008). Another important source of N for plants is atmospheric N; the role of N_2 fixing microorganisms for plant nutrition is discussed in Chapter 16.

Microorganisms can mobilize P by solubilization of poorly soluble inorganic P such as apatite (Banik and Dey, 1983; Jorquera *et al*., 2008) and mineralization of organic P (Tarafdar and Jungk, 1987; Richardson and Hadobas, 1998; George *et al*., 2007; Jorquera *et al*., 2008) and, in alkaline soils, by decreasing the pH. There has been considerable speculation as to whether inoculation with such microorganisms may allow increased utilization of soil and fertilizer P. Although these bacteria are capable of mobilizing P *in vitro*, it is unlikely that this mechanism operates to any great extent in the rhizosphere. When introduced into the soil as 'biofertilizer', microbial density rapidly declines because (i) they were not adapted to the soil conditions and (ii) because of competition with other rhizosphere microorganisms for organic carbon as an energy source (Postma *et al*., 1990; Gyaneshwar *et al*., 2002). Hence, effectiveness of 'biofertilizers' in the field is highly variable, but there are some examples where inoculation increased plant growth and yield (e.g., Bajpai and Sundara-Rao, 1971; Dhillion, 1992; Zahir *et al*., 2009).

Microorganisms release organic acid anions or siderophores that chelate and thus mobilize Fe^{3+} (Neilands, 1984). However, Fe bound to bacterial siderophores is usually a poor Fe source for both monocotyledonous and dicotyledonous plants (Bar-Ness *et al*., 1991; Crowley *et al*., 1992). Moreover, the higher affinity to Fe^{3+} of siderophores compared to phytosiderophores (Yehuda *et al*., 1996) (Table 15.1) may cause ligand exchange with Fe moving from phytosiderophores to siderophores. There are, however, some microbial siderophores with relatively low affinity to Fe, such as rhizoferrin, a siderophore produced by the fungus *Rhizopus arrhizus*. Rhizoferrin can increase Fe uptake and growth of monocotyledonous and dicotyledonous plants in solution culture (Yehuda *et al*., 1996, 2000). It remains to be seen if inoculation with *Rhizopus arrhizus* can improve plant Fe acquisition in the field.

TABLE 15.1 Apparent stability constants for the complexes between Fe^{3+} and various chelators (the bacterial siderophore DFOB, the synthetic chelator EDTA, the phytosiderophore mugineic acid and the siderophore from *Rhizopus arrhizus* rhizoferrin)

Chelate	Apparent stability constant with Fe^{3+}
DFOB (bacterial siderophore)	21.2
EDTA (synthetic chelator)	20.3
Mugeneic acid (phytosiderophore)	15.3
Rhizoferrin (Rhizopus siderophore)	17.1

Based on Yehuda *et al.* (1996).

Reduction and oxidation of Mn by microorganisms are important for Mn availability in soil. Whereas reduction ($Mn^{3+} \rightarrow Mn^{2+}$) increases Mn availability, oxidation ($Mn^{2+} \rightarrow Mn^{3+}$) decreases it. Interestingly, Mn reducers appear to be more abundant in the rhizosphere of some Mn-efficient compared with -inefficient wheat (*Triticum aestivum*) genotypes (Rengel, 1997). Colonization by arbuscular mycorrhizal (AM) fungi decreased the density of Mn reducers in the rhizosphere of maize, which may explain the lower Mn concentration in mycorrhizal plants (Kothari *et al*., 1990c). The root pathogen *Gaeumannomyces graminis* var. *tritici* (Ggt) is a strong Mn oxidizer (Marschner *et al*., 1991), thereby reducing Mn availability to plants. The lower Mn availability could also facilitate the infection of roots by the fungus as Mn is required for biosynthesis of phenolic compounds and lignin, which are involved in the defence reaction by plants (Section 7.2). Consequently, Mn fertilization can reduce susceptibility to Ggt because the plants are able to produce sufficient phenolics and lignin to limit the spread of the fungus in the roots (Rengel *et al*., 1993).

Due to the differential availability of root exudates (Chapter 14), Marschner *et al.* (2011) proposed that the role of rhizosphere microorganisms for nutrient uptake by plants changes along the root (Fig. 15.2). Just behind the root tip in the distal elongation zone, exudation rates are

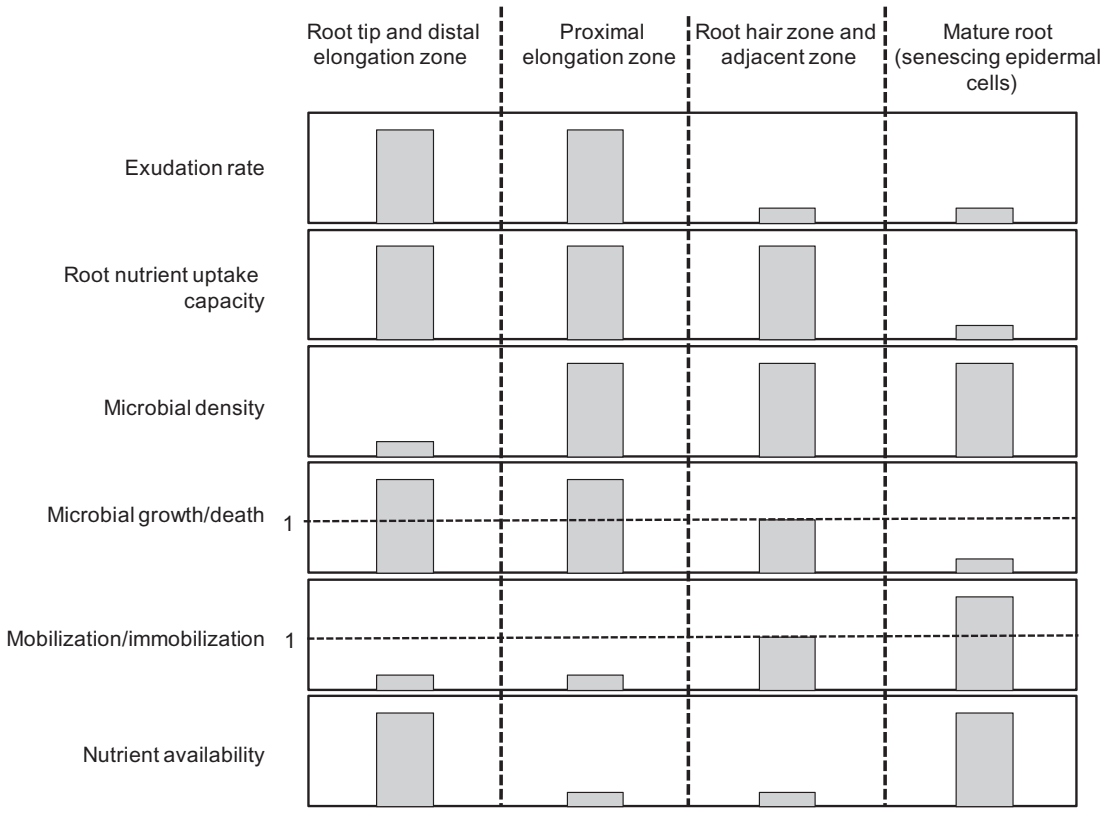


FIGURE 15.2 Model of plant–microorganism interactions in the rhizosphere in relation to nutrient availability in different root zones. It should be noted that the boundaries between the different root zones are gradual. *From Marschner* et al. *(2011), with permission from Elsevier*.

high and microbial density in the rhizosphere is relatively low. Therefore root exudates can mobilize nutrients without strong competition from microorganisms or substantial decomposition of exudates by microorganisms. The high rate of exudation just behind the root tip stimulates growth of rhizosphere microorganisms, resulting in high microbial density in the proximal elongation zone, accompanied by strong nutrient mobilization. However, most of the mobilized nutrients will be taken up by the rapidly multiplying microorganisms, leading to net immobilization in the microbial biomass. Compared to the root zone immediately behind the root tip, root exudation is lower in the root hair zone and the adjacent root zone. Therefore, microbial activity and growth will be reduced and nutrient mobilization may be equal or less than immobilization, and some of the nutrients mobilized by root exudates can be taken up by the plant. In the mature root zones where epidermal cells start to senesce, the concentration of easily decomposable C sources is low, resulting in very low microbial growth rates and thus nutrient demand will be low; part of the microbial biomass may die, releasing previously immobilized nutrients. However, plant uptake of these nutrients may be relatively low because of the low capacity of root tissues in these root zones to take up nutrients (Häussling *et al.*, 1988; Colmer and Bloom, 1998; Fang *et al.*, 2007).

15.2.3 Root Exudates as Signals and Phytohormone Precursors

Root exudates may also act as signals for microbial recognition. For example, some flavonoids (released by legume roots) attract rhizobia (Chapter 16), whereas other flavonoids may be suppressors of certain pathogenic fungi (Hartwig *et al.*, 1991b). Quercetin and strigolactone act as signals for spore germination and hyphal growth of AM mycorrhizal fungi (Phillips and Tsai, 1992; Akiyama *et al.*, 2005; Xie *et al.*, 2010), although hyphal growth may also be influenced by the elevated CO_2 concentrations in the rhizosphere (Bécard and Piché, 1989). Specific root exudates, however, act as a signal not only for establishment of symbiotic interactions, but also for parasitic flowering plants. A hydroquinone (sorgolactone) in root exudates of *Sorghum bicolor* strongly stimulates germination of *Striga asiatica*, and thus the formation of the parasitic interaction (Fate *et al.*, 1990; Hauck *et al.*, 1992). Root cap cells and the root cap mucilage also appear to play a role in establishment of specific root–microbial interactions. Root cap mucilage of maize elicits a chemotactic response in strains of *Azospirillum lipoferum* isolated from maize rhizoplane, but not by strains isolated from rice rhizoplane (Mandimba *et al.*, 1986). Root cap cells, also called 'border cells' when

detached from the roots, carry host-specific traits into the rhizosphere and contribute to establishment of a characteristic rhizosphere bacterial flora, or suppress certain soil-borne root pathogens (Hawes, 1990; Gochnauer *et al.*, 1990; Hawes *et al*., 2002). In *Eucalyptus*, border cells act chemotactically to the ectomycorrhizal fungus *Pisolithus tinctorius* (Horan and Chilvers, 1990).

A relatively large number of rhizosphere bacteria are producers of phytohormones such as IAA and CYT. However, in the absence of the appropriate precursors, synthesis of phytohormones by microorganisms is low. Several precursors for phytohormone production are components of root exudates or of lysates from decaying root tissues, which thus play an important role in phytohormone synthesis by rhizosphere microorganisms. The CYT and IAA production by *Azotobacter chroococcum* is enhanced when supplied either with maize root exudates (Gonzáles-Lopez *et al.*, 1991) or with adenine (Nieto and Frankenberger, 1990). Root exudates also contain amino acids such as tryptophan and L-methionine which are required as precursor for IAA and ethylene (C_2H_4) production by rhizosphere microorganisms (Arshad and Frankenberger, 1991; 1993; Ahmed *et al*., 2010; Ali *et al*., 2009).

Release of phytohormones is most likely the reason for changes in root and shoot morphology and growth that are observed after inoculating plants with so-called plant growth-promoting rhizobacteria (Martin *et al*., 1989; Ahmed *et al*., 2010; Ali *et al*., 2009), although other mechanisms such as nutrient mobilization or N_2 fixation are also involved (Bashan and de Bashan, 2010).

15.3 MYCORRHIZA

15.3.1 General

Mycorrhiza is the most widespread association between microorganisms and higher plants (Smith and Read, 2008). The roots of most soil-grown plants are mycorrhizal. On a global basis, mycorrhiza occur in about 83% of dicotyledonous and monocotyledonous plants, and all Gymnosperms are mycorrhizal (Smith and Read, 2008). Non-mycorrhizal plants occur in habitats where the soils are either very dry or saline or waterlogged, severely disturbed (e.g., mining activities), or where soil fertility is extremely high or extremely low (Brundrett, 1991). Mycorrhiza are absent in Cruciferae and Chenopodiaceae (Smith and Read, 2008), and also quite rare or absent in many members of the Proteaceae or other typical cluster root-forming plant species (Brundrett and Abbott, 1991).

Generally, the fungus is strongly or wholly dependent on the higher plant, whereas the plant may or may not benefit. Only in some plants (orchids) is mycorrhizal colonization essential. For plants, mycorrhizal associations are therefore either mutualistic, neutral or parasitic (Johnson *et al*., 1997), depending on a range of factors, for example fungal and host species, P availability and light intensity. This suggests that there is a delicate balance between the benefits for the host in terms of nutrient acquisition and the cost associated with supporting the fungus. For a comprehensive review on mycorrhiza in natural ecosystems the reader is referred to Alexander (1989), Brundrett (1991), Fitter (1991), and Smith and Read (2008).

15.3.2 Mycorrhizal Groups, Morphology and Structures

There are two major mycorrhizal groups based on differential morphology and physiology: endomycorrhiza and ectomycorrhiza (Fig. 15.3).

Endomycorrhiza. The fungi form structures within the cortical cells and also grow intercellularly. Hence, at the fungus–plant interface, the membranes of the fungus and the plant are in direct contact with each other. There are several types of endomycorrhiza, the best known being *arbuscular mycorrhiza* (AM, formerly called vesicular-arbuscular mycorrhia (VAM)), *ericoid* and *orchid mycorrhiza*.

Arbuscular mycorrhiza is by far the most abundant of the endomycorrhiza (Smith and Read, 2008). The AM fungi are now classified as a separate phylum, *Glomeromycota*. They belong mainly to four genera, *Acaulospora*, *Gigaspora*, *Glomus* and *Sclerocystis.* The AM is characterized by the formation of (i) intracellular structures (arbuscules or hyphal coils) within the cortex cells, (ii) intercellular hyphae in the cortex, and (iii) a mycelium that extends well into the surrounding soil (external mycelium; Fig. 15.4). It is now recognized that there are two types of AM with respect to the structures formed in the cortex cells: *Arum* type mycorrhiza characterized by arbuscules and *Paris* type that form hyphal coils (Smith and Read, 2008). Interestingly, a given AM fungus can form either arbuscules or hyphal coils depending on the host plant (Dickson, 2004). The arbuscules and coils are the main sites of solute exchange with the host, but they are short lived, being active for about 7 days. Many, but not all, AM fungi form vesicles as lipid-rich storage organs (Fig. 15.3).

Ericoid mycorrhiza occur in Ericales; they form coils of hyphae within rhizodermal (epidermal) cells and individual hyphae extending into the soil as in the case of AM (Smith and Read, 2008).

Orchid mycorrhiza are formed between plants of the family Orchidaceae and a variety of fungi. All orchids are myco-heterotrophic at some point in their lifecycle. The colonization by mycorrhizal fungi is critically important during seedling development. Orchid seeds have virtually no energy reserve and seedlings obtain their carbon from the fungal symbiont. Hence, during this stage of the

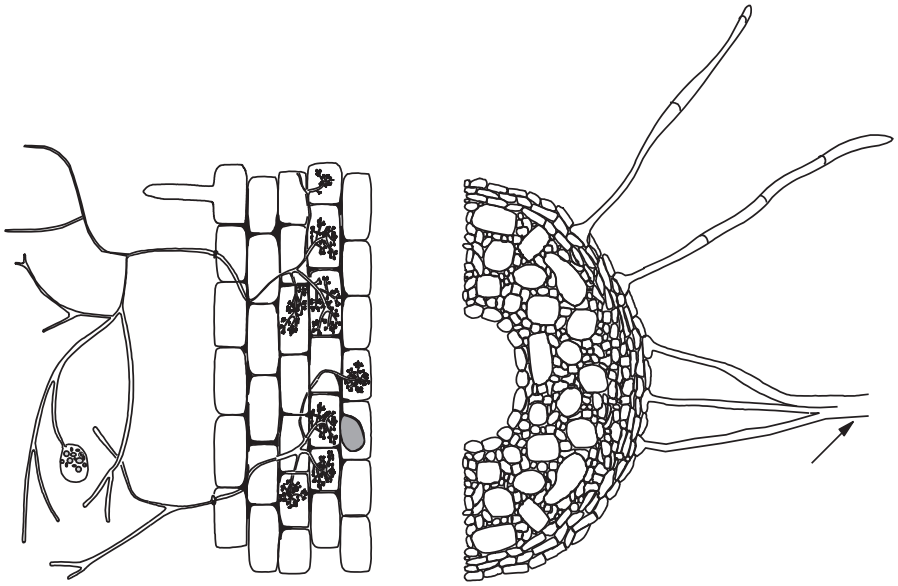


FIGURE 15.3 Schematic diagram of the main structural features of AM mycorrhiza (*left*) and ectomycorrhiza (*right*). Arrow indicates rhizomorphs.

FIGURE 15.4 Mycorrhizal root systems. Root of soil-grown potato with external hyphae of *Glomus mosseae* (*top*), ectomycorrhizal short roots of soil-grown Norway spruce (*bottom*). *Courtesy of G. Hahn*.

symbiosis, the flow of C is from the fungus to the host which is distinctly different from the other mycorrhiza where C is supplied by the host plant to the fungus. Many adult orchids retain their fungal symbionts, although the benefits to the adult photosynthetic orchid and the fungus remain largely unexplored (Smith and Read, 2008).

Ectomycorrhiza (ECM). These occur mainly on roots of woody plants and only occasionally on herbaceous and graminaceous perennial plants (Smith and Read, 2008). Ectomycorrhiza are most common in the northern hemisphere, especially in Pinaceae, Betulaceae, Fagaceae and Salicaceae. However, ECM may also occur in some tropical and subtropical forests (Högberg, 1986). They are characterized by (Figs 15.3, 15.4) (i) an interwoven mantle of hyphae (fungal sheath) around the roots, (ii) hyphae that penetrate the root intercellular space of the cortex to form a network of fungal mycelium, the *Hartig net*, which surrounds the cortex cells and increases the surface area at the fungus–root interface, and (iii) an extensive network of eternal hyphae. However, the hyphae of the Hartig net remain intercellular, they do not penetrate the host cells. Since the fungus remains intercellularly, nutrient transfer across the fungus–plant interface has to occur through the cell walls and membranes of both partners, which is quite different from the interface in AM, where nutrients only have to be transferred across the membranes of the two partners. ECM fungi are Basidiomycetes or Ascomycetes. Some ECM produce hyphal strands or *rhizomorphs* which extend far into the surrounding soil (Fig. 15.13). The rhizomorphs are differentiated multi-hyphal organs with

a diameter of up to 200 µm, and are important for solute transport over large distances.

In some tree species both AM and ECM occur simultaneously, for example in *Salix* and *Populus* (Lodge, 1989) or *Eucalypt* (Gardner and Malajczuk, 1988), and the proportion of both types appears to depend on external factors such as soil water content and aeration, and internal factors such as age of the trees.

On a global scale, ECM are more abundant in boreal and temperate forests with a distinct surface humus horizon, and in N-limited ecosystems. On the other hand, AM are more abundant in warmer climates with drier soils, in pastures and deciduous forests with high turnover of organic material, and where P supply is limited (Read, 1991). AM are usually the only form of mycorrhiza in crop plants, pastures and fruit trees.

Besides the differences in distribution and in morphology and structure (Fig. 15.3) there is another principal difference between ECM and AM. Whereas most ECM fungi can be grown in pure culture (*in vitro*), this is not possible for AM fungi. Therefore, knowledge on physiology of AM fungi is based on studies of fungal structures and fungal functions associated with the host roots (Smith and Gianinazzi-Pearson, 1988).

15.3.3 Root Colonization, Photosynthate Demand, and Host Plant Growth

15.3.3.1 Root Colonization

Root colonization by mycorrhizal fungi is initiated either from soil-borne propagules (spores, colonized root residues) or from neighbouring roots of the same or different plants and plant species. Colonization is enhanced by a pre-existing network in the soil. Therefore, severe soil disturbance, for example clear-cut logging or rigorous soil mixing (Jasper *et al.*, 1989b), as well as tillage compared with no-till (Miller and McGonigle, 1992; Garcia *et al.*, 2007), may severely depress and delay mycorrhizal colonization. Tillage can also alter the community composition of AM fungi, suggesting that AM fungal species differ in sensitivity to soil disturbance (Jansa *et al.*, 2003).

Root exudates of host plants, particularly flavonoids, elicit a strong chemotactic response in ECM (Horan and Chilvers, 1990) and AM (Gianinazzi-Pearson *et al.*, 1989) fungi, and their effectiveness appears to be enhanced by elevated CO_2 concentrations (Bécard *et al.*, 1992). Rhizosphere bacteria may enhance or suppress mycorrhizal colonization. The former are referred to as 'mycorrhiza helper bacteria' and have been shown to stimulate AM (Pacovsky *et al.*, 1985; Duponnois and Plenchette, 2003) and ECM colonization (Duponnois and Garbaye, 1991).

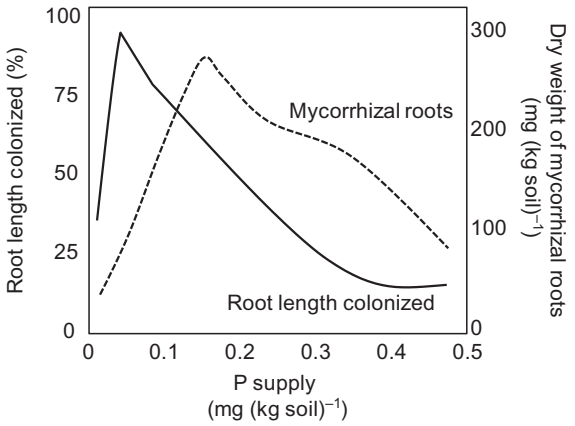


FIGURE 15.5 Percentage root length colonized and dry weight of mycorrhizal roots of subterranean clover at different P supply. *Redrawn from Bolan* et al. *(1984).*

In plants that are not hosts to AM mycorrhiza, for example members of the Chenopodiaceae and Cruciferae, incompatibility may be caused by the composition of root exudates, toxins, or enhanced defence reactions of the host against infection, similar to the response to pathogens (Anderson, 1988; Parra-Garcia *et al.*, 1992; Akiyama *et al.*, 2010). Different levels of host plant responses may be involved in the large genotypic differences in root colonization by AM that have been found to vary between zero and 18–30% in wheat and cowpea cultivars(Mercy *et al.*, 1990; Vierheilig and Ocampo, 1991).

Soil nutrient supply may enhance or suppress mycorrhizal colonization. At extremely low soil P concentrations, root colonization by AM is low (Bolan *et al.*, 1984) as P may limit the growth of the fungi itself (Fig. 15.5). With increasing P supply, root growth and the proportion of colonized root length increase until an optimum supply of P is attained; beyond this level colonization rate is depressed to a varying degree, depending on AM (Bolan *et al.*, 1984) or ECM species (Jones *et al.*, 1990), and the host species (Davis *et al.*, 1984) or cultivar (Baon *et al.*, 1993). It should be noted, however, that at moderate P supply, where the percentage of colonized root is reduced, the weight (or length) of the mycorrhizal roots may still be high due to the greater root growth.

High N supply also depresses AM and ECM colonization, particularly in combination with high P concentrations and when N is supplied as NH_4^+ (Baath and Spokes, 1988). In ECM particularly, the mass of the mycelium decreases at high N supply (Wallander and Nylund, 1991). A decrease in AM-colonized root length or the proportion of ECM root tips at high supply of P or N is, however, not necessarily an expression of a specific regulation mechanism; it may be the result of enhanced root growth outpacing the fungus.

The negative effect of high P (or N) availability can be explained by a number of factors (i) reduced colonization due to suppression of hyphal growth in the soil, (ii) reduced carbohydrate supply to the fungus, and (iii) increased root growth (Jasper *et al*., 1979; Bruce *et al*., 1994; Smith and Read, 2008).

Total AM-colonized root length or total number of ECM root tips is often a more appropriate parameter, but for evaluation of effectiveness in nutrient acquisition, quantification of the external mycelium would be the most important parameter (see below).

15.3.3.2 Photosynthate Demand

In mycorrhizal roots, a substantial proportion of the photosynthates allocated to the roots is required for fungal growth and maintenance. In AM plants, root + fungus respiration may be 20–30% higher than in roots of non-mycorrhizal plants, and 87% of the higher respiration can be attributed to the fungus (Baas *et al.*, 1989). This agrees well with the estimates by Lambers *et al*. (2002a) that, generally, the C cost of the AM symbiosis ranges between 4 and 20% of the C fixed, the majority of which is respired by the root + fungus. In cucumber, 20% of the net photosynthates were allocated below ground in non-mycorrhizal plants, whereas it was 43% in AM plants (Jakobsen and Rosendahl, 1990). In highly colonized plants, AM fungal biomass may reach 20% of the root biomass; however, typically it is about 10% (Fitter, 1991). Unfavourable environmental conditions such as shading and defoliation depress mycorrhizal development (Same *et al.*, 1983; Son and Smith, 1988), but to a lesser degree than host root growth and, in nodulated legumes, nodule weight (Bayne *et al.*, 1984).

These costs in terms of photosynthates are not relevant when the mycorrhizal plants can compensate the higher demand by an increase in rate of photosynthesis per unit leaf area (Wright *et al*., 1998; Mortimer *et al*., 2008). Enhanced rates of photosynthesis in mycorrhizal plants are therefore often an expression of a higher sink activity (Dosskey *et al.*, 1990) rather than a specific stimulatory effect of the mycorrhizal association. The costs of photosynthates also have to be compared with the benefits such as enhanced uptake of nutrients like P when they limit photosynthesis and growth in non-mycorrhizal plants. However, despite the beneficial effect on plant growth, as a rule in mycorrhizal plants, root growth (dry weight) is less enhanced or even depressed (Dosskey *et al.*, 1991) compared with shoot growth, resulting in a decreased root/shoot ratio.

In ECM Douglas fir ecosystems, about 60–70% of the net photosynthates are allocated below-ground for growth of roots, mycorrhiza and for respiration (Fogel, 1988). Estimates on the proportions of carbon flow to the ECM in forest stands vary between 5 and 30% of the net photosynthates (Söderström, 1992; Hobbie, 2006). Thus, ECM fungi play an important role in carbon import into the soil via the external mycelium, particularly in view of the high turnover rate of the fungal carbon which is about five times higher than that of litter fall (Fogel and Hunt, 1979).

TABLE 15.2 Net carbon transfer from the donor plant (*Betula papyrifera*) to the receiver plant (*Pseudotsuga menziesii*) that was grown in full light, partial shade or full shade. Net carbon transfer is expressed in percentage of total isotope in the donor

Light treatment of receiver plant	Net C transfer (% of total isotope in the donor plant)
Full light	2.7
Partial shade	4.3
Full shade	9.5

Based on Simard *et al*. (1997).

In forest stands, ECM hyphae can act as a conduit for photosynthate transfer from over-storey plants to seedlings shaded by these plants (Griffiths *et al.*, 1991). The amount of C transported to the seedling is small (<10% of C assimilated by the donor), with more being transferred to shaded receiver plants than those in full light (Table 15.2). Only 13% of received C was transported into the shoots of the receiver plant (Simard *et al*., 1997). Nevertheless, this C transfer (the so-called 'nurse plant effect') could be important for the establishment of seedlings in forests.

Compared to non-mycorrhizal plants, the soil/root interface is altered in mycorrhizal plants and an additional, or new, '*mycorrhizosphere*' is formed (Fogel, 1988; Linderman, 1988; Timonen and Marschner, 2005). As most soil-grown plants are mycorrhizal, this mycorrhizosphere might be the rule, rather than the exception.

Mycorrhizal colonization alters not only the density of rhizosphere microorganisms (Table 15.3) but also their composition (Kothari *et al.*, 1991; Marschner *et al*., 2001); this effect appears to be mediated by the plant because the rhizosphere microbial community composition of AM plants is affected not only for roots colonized by the fungus, but also for non-mycorrhizal roots (Marschner and Baumann, 2003). It is not only the mycorrhizal colonization *per se* which is important, but also which mycorrhizal species colonize the roots as they may have a differential effect on the rhizosphere microflora (Marschner *et al.*, 2001). Depending on the AM species, the densities of total bacteria, diazotroph bacteria and actinomycetes were affected to a different degree (Table 15.3). The low numbers of rhizosphere bacteria in the non-mycorrhizal plants

TABLE 15.3 Density of total bacteria, N_2 fixers and actinomycetes in the rhizosphere of *Panicum maximum* without AM or colonized by different AM fungi

	Rhizosphere population density (colony-forming units g^{-1} soil)		
	Bacteria ($\times 10^6$)	N_2 fixers ($\times 10^5$)	Actinomycetes ($\times 10^4$)
Control (non-AM)	14.7	12.4	13.4
Glomus fasciculatum	41.9	42.0	26.1
Gigaspora margarita	34.0	87.9	17.6
Acaulospora laevis	8.1	10.6	28.6

Based on Secilia and Bagyaraj (1987).

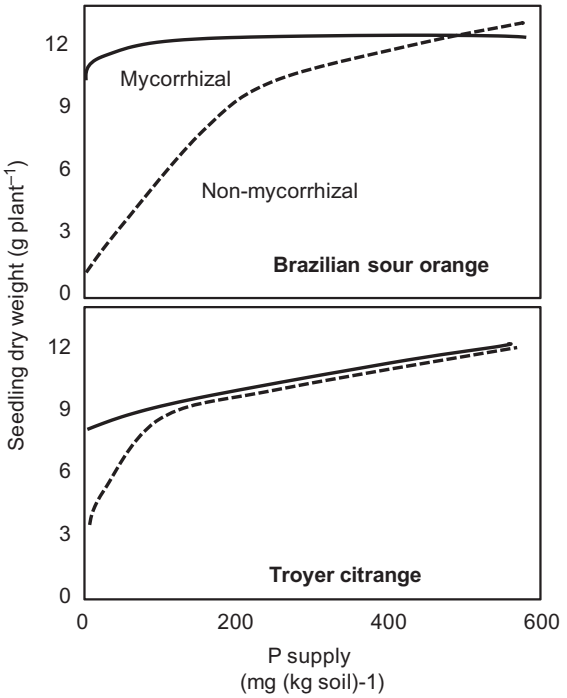


FIGURE 15.6 Dry weight of Brazilian sour orange and Troyer citrange without or with inoculation with *Glomus fasciculatus* at different P supply. *Redrawn from Bolan* et al. *(1984).*

in the study shown in Table 15.3 were caused by P limitation which resulted in poor plant growth.

In view of the effect of rhizosphere microorganisms on root morphology and nutrient availability, this alteration may affect nutrient acquisition and root and shoot growth.

Mycorrhiza not only affect the microorganism in the immediate vicinity of the roots. The density and activity of microorganisms at greater distance from the rhizoplane may be enhanced because carbon is provided by the external mycelium, especially in ECM plants. This interface between external hyphae and the soil has similarities to the rhizosphere and has been termed the 'hyphosphere' (Linderman, 1988).

15.3.3.3 Host Plant Root and Shoot Growth

Mycorrhizal colonization affects root and shoot growth differently. In a nutrient-poor substrate, the external mycelium increases surface area; hence, compared with non-mycorrhizal plants, mycorrhizal plants have greater access to growth-limiting nutrients, for example P and N (Fig. 15.6). As a typical plant response to higher nutrient supply, shoot growth is enhanced more than root growth, leading to a decrease in root/shoot ratio (Oliver *et al*., 1983). At a given nutritional status of the host plant, this shift is more pronounced in mycorrhizal plants (Bell *et al.*, 1989) as the fungus competes with the roots for photosynthates. In legumes colonized by AM or Rhizobium or both, C allocation to mycorrhiza can be greater (Mortimer *et al*., 2008), similar (Piccini *et al.*, 1988) or smaller (Kucey and Paul, 1982) than C allocation to Rhizobium. Hence, the relative sink strength of the two symbionts varies and may depend on P availability or light intensity.

If the mycorrhiza are either ineffective in delivering nutrients, or nutrients are not growth-limiting factors in non-mycorrhizal plants, mycorrhizal colonization depresses root growth primarily by sink competition. Mycorrhizal fungi are a strong sink for photosynthates irrespective of their contribution to host plant growth (Douds *et al.*, 1988; Lambers *et al.* 2002). In principle, growth depression can be predicted when root colonization remains high at high P supply and limited photosynthetic source capacity to compensate for the extra costs of mycorrhizal colonization (Gerdemann, 1975; Sanders, 1993).

In addition to root growth depression caused by competition for photosynthates, phytohormones may also be involved in root growth reduction in mycorrhizal plants. In ECM plants, elongation of the short lateral roots is inhibited by IAA production of the fungi. In AM plants, total root length is decreased, but branching and number of lateral roots per unit root length, or per plant are increased (Berta *et al.*, 1990; Barker *et al*., 1998).

A decrease in root surface area and root activity as well as in root/shoot dry weight ratios is, however, not necessarily harmful for shoot growth and plant yield as long as the external mycelium of the mycorrhizal fungi can fully compensate for root function in uptake of nutrients and water.

TABLE 15.4 Shoot dry weight and shoot nutrient concentrations in non-mycorrhizal (NM) and mycorrhizal (*Glomus fasciculatum*) (M) soybean at different P supply

P supply (mg kg^{-1} soil)	Shoot dw (g plant^{-1})		Shoot concentration							
			P (g kg^{-1} dw)		Cu		Zn		Mn	
					(mg kg^{-1} dw)					
	NM	M	NM	M	NM	M	NM	M	NM	M
0	1.3	2.8	0.6	1.7	3.3	10.3	21	44	366	111
60	1.6	3.2	0.8	2.1	3.7	7.9	27	35	515	109
150	1.9	3.4	0.8	2.1	2.9	6.3	30	36	412	115
270	2.8	3.8	1.4	1.8	3.5	4.6	29	33	556	123

Based on Lambert and Weidensaul (1991).

15.3.4 Role of Mycorrhiza in Nutrition of their Host Plant

15.3.4.1 Arbuscular Mycorrhiza

The most distinct effect of AM on plant growth is the improved supply of nutrients of low mobility in the soil solution, particularly P (Fig. 15.6). External hyphae can absorb and transfer P to the host from soil beyond the rhizosphere depletion zone (Tinker *et al.*, 1992; Smith and Read, 2008). Given the key importance of the root hair length on the P depletion zone and P acquisition (see also Chapter 12), such an enhancing effect of AM is to be expected. In mycorrhizal plants, the P uptake rate per unit root length is 2–3 times higher than in non-mycorrhizal plants (Tinker *et al.*, 1992). Moreover, the small diameter of the hyphae (1–12 µm) allows them to enter soil pores not accessible to roots. Figure 15.6 also demonstrates that the beneficial effect of AM on plant growth diminishes with increasing P supply as the plants no longer require the fungal hyphae for sufficient P uptake (see also Table 15.4).

An example of the differential extension of the P depletion zones in mycorrhizal and non-mycorrhizal roots is shown in Fig. 15.7. By restricting root extension by a net, and hyphal extension by a membrane, the P depletion could be measured at the root/soil interface, in the hyphal compartment, and at the hyphae/soil interface. In non-mycorrhizal plants, the depletion zone extended about 1 cm from the rhizoplane, whereas in the mycorrhizal plants P was uniformly depleted in the hyphal compartment (2 cm from the rhizoplane). At the hyphae/soil interface, a new depletion zone was formed, extending several millimetres into the bulk soil. In the mycorrhizal plants, the hyphae contributed between 70 and 80% of total P uptake (Li *et al.*, 1991c). In mycorrhizal white clover with larger hyphal compartments, P was uniformly depleted more than 11 cm from the rhizoplane (Li *et al.*, 1991a).

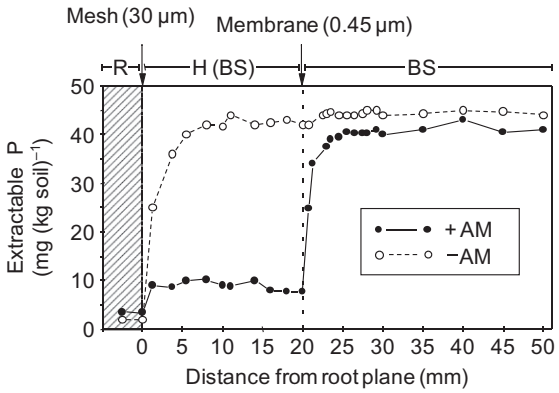


FIGURE 15.7 Concentration of water-extractable P in the root (R), hyphal (H) and bulk soil (BS) compartment of non-mycorrhizal (−AM) and mycorrhizal (*Glomus mosseae*, +AM) white clover. *From Li* et al. *(1991c) with permission from Wiley and Sons.*

AM fungi access the same labile inorganic P pools as non-mycorrhizal roots (Bolan, 1991). Similarly to the host roots, the external hyphae of AM fungi release acid phosphatase (Fig. 15.8) and, thus, also have access to organic P (Tarafdar and Marschner, 1993). This suggests that AM plants have access to similar soil P pools as non-AM plants, but AM plants are able to acquire P from a greater soil volume.

In the hyphae, P is transported as poly-phosphate (poly-P) most likely in motile vacuoles (Uetake *et al.*, 2002) rather than by cytoplasmic streaming as suggested earlier (Smith and Gianinazzi-Pearson, 1988). The poly-P is hydrolysed by fungal phosphatases at the fungus/root interface of arbuscules and hyphal coils (van Aarle *et al.*, 2005)

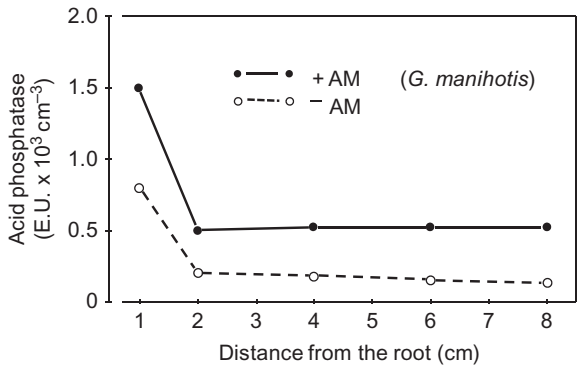


FIGURE 15.8 Acid phosphatase activity in the rhizosphere of non-mycorrhizal and mycorrhizal (*Glomus manihotis*) wheat. *From Tarafdar and Marschner (1994) with permission from Elsevier.*

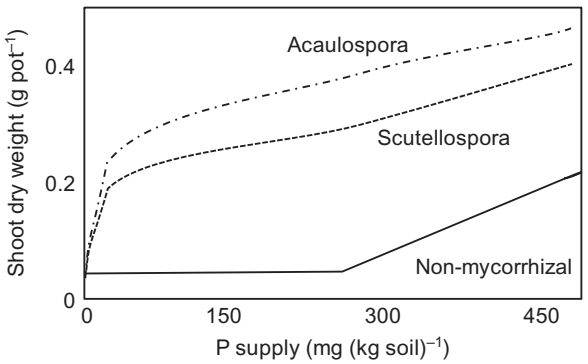
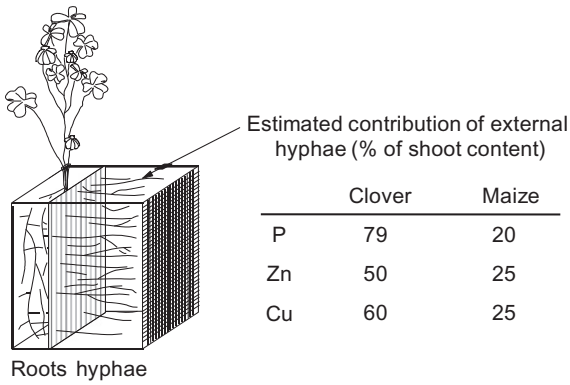


FIGURE 15.9 Shoot dry weight of subterranean white clover non-mycorrhizal or mycorrhizal with *Acaulospora laevis* or *Scutellospora calospora* at different P supply. *Redrawn from Schweiger* et al. *(2007).*

and transported as inorganic P across the plasma membrane of the host root cell (Smith and Gianinazzi-Pearson, 1988). Poly-P may also aid the transfer of cations to the host/fungus interface. Poly-P is a strongly negative poly-anion which can bind cations such as Mg, K and basic amino acids such as arginine and glutamine (Jennings, 1989). The solute transport in the hyphae is bidirectional, with carbohydrates being transported towards the tips of the hyphae whereas P and other elements move towards the host/fungus interface (Smith and Gianinazzi-Pearson, 1988; Gianinazzi-Pearson *et al*., 2000).

The effectiveness of AM fungi in providing P to the host plants depends on the AM species. Per cent colonization may be positively correlated with P uptake by the host plant (Raju *et al*., 1990), but this is not always the case (Smith *et al*., 2003). It appears that the extent of the external mycelium is a better indicator for the capacity of AM fungi to improve P uptake by plants. In subterranean clover, shoot dry weight at low P availability was more strongly enhanced by *Acaulospora* than by *Scutellospora* (Fig. 15.9). The stronger growth increase by *Acaulospora* was not related to colonization rate which was similar to that of *Scutellospora* (Schweiger *et al*., 2007). However, both distance and rate of spread of the external hyphae are about three-fold greater in *Acaulospora* than in *Scutellospora* (Jacobsen *et al*., 1992).

There is now clear evidence of mycorrhiza-induced P transporters at the fungus/host interface which transfer P across the host membrane (Smith and Read, 2008). Mycorrhiza-induced P transporters have been identified in many plant species, for example barley (Glassop *et al*., 2005) and potato (Rausch *et al*., 2001). These transporters are not expressed in non-mycorrhizal plants, and they are distinct from the P transporters in the root epidermis. Interestingly, AM colonization leads to a down-regulation of the P transporters in the root epidermis, suggesting that in mycorrhizal plants, most P is taken up via the fungal



	Clover	Maize
P	79	20
Zn	50	25
Cu	60	25

FIGURE 15.10 Contribution of external hyphae (*Glomus mosseae*) to the uptake of P, Zn and Cu in white clover and maize grown in a Luvisol in compartmented boxes. *Compiled data of Kothari* et al. *(1991) and Li* et al. *(1991b).*

pathway (Glassop *et al*., 2005), even if there is no positive growth response (Smith *et al*., 2003).

In AM plants, the uptake and concentrations of Zn and Cu are also usually higher than in non-mycorrhizal plants (Kothari *et al.*, 1990a; Lambert and Weidensaul, 1991). The uptake of Cu and Zn by the external hyphae may account for about 50–60% of the total uptake in white clover and 25% in maize (Fig. 15.10). By changing the P supply in the hyphal compartment, the molar ratio of P/Cu transport in the hyphae could be varied by a factor of about 25, indicating that hyphal uptake and/or transport of both nutrients are regulated separately (Li *et al.*, 1991b).

Increasing P availability in the soil is associated with a decrease in AM colonization of the roots, or of hyphal length and activity, and is usually compensated for by higher root uptake of P. This is not necessarily the case for Zn and Cu in soils with low concentrations of these micronutrients. Hence, the often-reported negative effects of P fertilizer application on plant Zn and Cu concentrations,

TABLE 15.5 Dry weight, water relations and shoot nutrient concentrations in non-mycorrhizal and mycorrhizal (*Glomus mosseae*) maize grown in calcareous soil

Growth and water relations

	Dry weight ($g\,plant^{-1}$)		Root length	Root hair		Transpiration	Water uptake
	Shoot	Root	($m\,plant^{-1}$)	Density (# mm^{-1})	Length (μm)	(L (plant 42 d)$^{-1}$)	($mL\,cm^{-1}\,s^{-1}$) $\times 10^7$
NM	20.0	4.8	619	35	347	3.40	0.61
M	22.8	4.6	367	25	235	4.08	1.34

Concentrations in shoot dry matter

	K	P	Mg	Ca	Zn	Cu	Mn	Fe	B	Mn reducers
	($g\,kg^{-1}$)				($mg\,kg^{-1}$)					($\times 10^5\,g^{-1}$ soil)
NM	17	2.1	4.0	9.0	10	5.6	139	88	46	44.1
M	12	3.7	4.1	5.3	36	7.1	95	58	35	1.7

Compiled from Kothari *et al.* (1990b, c).

which by far exceed the 'dilution effect' by growth, indicate the importance of AM in acquisition of Zn and Cu from soils (Table 15.4).

In contrast to Zn and Cu, the shoot concentrations of Mn are often lower in AM than in non-mycorrhizal plants (Table 15.4). In red clover, there is a negative correlation between percentage of root colonization with AM and Mn concentration in roots and shoots (Arines *et al.*, 1989). The decrease in Mn uptake in mycorrhizal plants could be due to low uptake and transport of Mn in the external hyphae, but may also be explained by reduced Mn acquisition by AM roots. AM colonization affects rhizosphere microbial community composition and reduces the density of Mn reducers compared with non-mycorrhizal plants. In maize, AM plants had lower shoot and root Mn concentrations and lower density of Mn-reducing bacteria and amount of exchangeable manganese (Mn^{2+}) in the rhizosphere soil (Kothari *et al.*, 1991). In red clover, lower Mn concentrations in roots and shoots were associated with higher numbers of Mn-oxidizing bacteria in the rhizosphere (Arines *et al.*, 1992), which would cause a similar reduction in Mn availability as a decrease in abundance of Mn-reducing bacteria.

Little is known about the role of AM in uptake of K, Mg and S. In *Agropyron repens*, about 10% of the total K in mycorrhizal plants was attributed to hyphal uptake and delivery (George *et al.*, 1992). Although hyphal transport has been demonstrated for S and Ca by using radioisotopes, the amounts transported are probably small, at least for Ca as indicated by the frequently reported lower Ca concentrations in shoots of mycorrhizal compared with non-mycorrhizal plants (Table 15.5) (Kothari *et al.*, 1990b, c; Azcon and Barea, 1992). Lower Ca concentrations in mycorrhizal plants are probably related to changes in root morphology and differentiation, for example enhanced lignification and suberization of the endodermis upon AM infection (Dehne and Schönbeck, 1979a, b). Greater lignification and suberization would restrict water uptake and thus particularly uptake of elements delivered to the roots by mass flow. In agreement with this, in mycorrhizal maize plants shoot Si concentrations were lower than in non-mycorrhizal plants (Kothari *et al.*, 1990c).

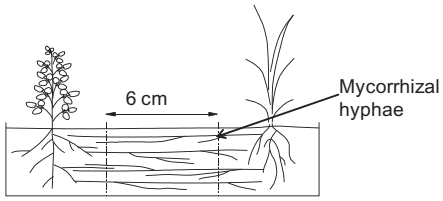
Although both natural and agricultural ecosystems are often limited by N, the role of AM in N acquisition is unclear. In celery, about 20% of total N uptake was attributed to hyphal uptake (Ames *et al.*, 1983), whereas in *Agropyron repens* this proportion was about 31% (George *et al.*, 1992). High transport rates of N in AM hyphae (Ames *et al.*, 1983) and transfer of N from hyphae to the plant (Johansen *et al.*, 1992) suggest that AM could be important in N nutrition of plants. On the other hand, AM colonization had no effect on N uptake from various organic and inorganic N sources in a number of grassland perennials (Reynolds *et al.*, 2005). Moreover, even at similar capacity for uptake and delivery – on a molar basis – of N, P and K by AM hyphae, because of the higher total demand by the host plant, the proportion of K and N contributed by external hyphae would be relatively low compared to P.

Due to the high P requirement for nodulation, a high AM dependency in legumes is to be expected, but the interactions between N_2-fixing Rhizobium and AM are complex (Bethlenfalvay, 1992). At low P availability, AM increased nodulation in soybean (Table 15.6). In bean, AM

TABLE 15.6 Plant dry weight, shoot P content, number of nodules and nitrogenase activity (ARA) in nodules of soybean, non-mycorrhizal at high or low P supply or mycorrhizal (*Glomus mosseae*) at low P supply

	NM		AM
	Low P	High P	Low P
Shoot dry weight ($g\,plant^{-1}$)	2.8	3.8	5.6
Root dry weight ($g\,plant^{-1}$)	1.7	1.9	2.0
P content ($mg\,plant^{-1}$)	2.9	6.0	5.8
Nodules (# $plant^{-1}$)	33	30	97
ARA ($\mu mol\ C_2H_4\ plant^{-1}\,h^{-1}$)	4.6	22.8	9.0

Brown *et al.* (1988).



	Dry weight ($g\,plant^{-1}$)		N content ($mg\,plant^{-1}$)	
N supply to soybean	Soybean	Maize	Soybean	Maize
-N	3.9	7.2	30	33
$+NH_4NO_3$	21.8	8.6	351	55
$+N_2$ fix	25.1	6.9	419	40

FIGURE 15.11 Dry weight and N uptake of soil-grown mycorrhizal (*Glomus mosseae*) maize and soybean grown either without N (−N), with ammonium nitrate ($+NH_4NO_3$) or nodulated (N_2 fix). *Based on Bethlenfalvay* et al. *(1991).*

increased respiration by about 10% and N_2 fixation by up to 40%, but also increased root respiration (root + symbionts) by 30% (Mortimer *et al.*, 2008) and delayed nodulation. Kucey and Paul (1982) showed that AM colonization increased nodulation, which resulted in doubling of the C allocation into the nodules. In their study, C allocation to roots in the plants colonized by Rhizobium and AM was greater (12% of recently fixed C) than for plants colonized only with mycorrhiza (4%).

A major problem in evaluation and quantification of the role of AM in plant nutrition arises from the changes in growth, and particularly root morphology and physiology, induced by mycorrhizal colonization. As summarized in Table 15.5 for maize, at similar shoot and root dry weight, root surface area was lower in mycorrhizal plants compared with non-mycorrhizal plants. The mycorrhizal plants had a larger leaf area (Kothari *et al.*, 1990b) and also a higher photosynthate demand (and thus lower stomatal resistance), therefore transpiration rates as well as water uptake rates per unit root length and the rates of mass flow to the root surface were higher. Hence, in terms of water uptake, the smaller root surface area in mycorrhizal plants was compensated for by increased uptake per unit root length. The lower K concentration in the shoot of mycorrhizal plants is in accordance with the reduction in root surface area and the relatively low hyphal transport of K. The concentrations of Fe and B are lower in mycorrhizal plants, suggesting that hyphal uptake and transport of these two micronutrients is small or absent.

The existence of external AM hyphal bridges between individual plants of the same species, or among different plant species in mixed stands, is a potential pathway of nutrient transfer between plants. In principle, such transfer is also possible for N between legumes and non-legumes in a mixed stand, or in intercropping, as shown in Fig. 15.11 for soybean and maize. However, a substantial amount of N was transferred from the legume to maize only when the legume was supplied with mineral N, but not when it was relying on N_2 fixation. Due to the high carbon costs for N_2 fixation, it is not surprising that legumes have mechanisms to prevent the drain of fixed N via AM hyphae to the non-legume. In field-grown soybean intercropped with maize, direct transfer of fixed N from the soybean to maize via AM hyphae was negligible (Hamel and Smith, 1992). In another study, N and P transfer between barley and pea was also very compared to the requirements of the plants (Johansen and Jensen, 1996).

So far, most of the stimulating effects of AM on host plant growth and nutrient uptake have been obtained under controlled conditions which are usually optimized for the fungus, i.e. low P concentrations, sterilized soil, high light intensity to maximize C assimilation by the plant. The results demonstrate the potential of mycorrhiza for improving host plant growth. Under field conditions, however, the realization of this potential may be restricted, for example, by collembola grazing on the external mycelium and thus decreasing the absorbing surface area (McGonigle and Fitter, 1988). Another limitation is shown in Table 15.7. High colonization increased shoot dry weight at flowering and maturity, but reduced seed yield and therefore harvest index. Additional measurements suggested that in highly colonized plants, the more vigorous vegetative shoot growth in combination with a shallower root system and a lower root length/shoot weight ratio caused a more severe drought stress during reproductive growth and thus limited seed production.

15.3.4.2 Ectomycorrhiza

With respect to their role in nutrition of their host plant, ECM fungi have many common features with AM.

TABLE 15.7 Shoot dry weight, P content, seed yield and harvest index of chickpea without or with re-inoculation with indigenous AM grown in fumigated field soil in northern Syria at flowering and maturity

	Flowering		Maturity		
	Shoot dw ($g\,plant^{-1}$)	Shoot P ($mg\,plant^{-1}$)	Shoot dw ($g\,plant^{-1}$)	Seed yield ($g\,plant^{-1}$)	Harvest index (%)
Low AM (fumigated)	2.7	4.4	6.0	2.5	41
High AM (fumigated and re-inoculated)	4.9	9.3	6.9	1.9	27

Weber *et al.* (1993).

TABLE 15.8 Growth, P content and ectomycorrhizal root length in *Eucalyptus diversicolor* seedlings without (NM) or with inoculation with *Laccaria laccata* (ECM) at different P supply

P addition ($mg\,kg^{-1}$ soil)		Dry weight ($g\,plant^{-1}$)	P content ($mg\,plant^{-1}$)	P uptake ($mg\,g^{-1}$ fine root)	ECM root length ($m\,plant^{-1}$)
0	NM	0.1	0.02	0.4	0
	ECM	0.2	0.07	0.7	0.25
8	NM	0.3	1.73	0.6	0
	ECM	2.2	2.41	2.2	4.10
16	NM	2.5	2.03	1.4	0
	ECM	3.5	4.26	2.1	4.71
32	NM	8.6	10.56	3.8	0
	ECM	8.7	11.57	3.6	0.90

Based on Bougher *et al.* (1990).

However, there are some principal differences in terms of structural arrangements with the roots and mechanisms of nutrient acquisition. In ECM plants such as Norway spruce, more than 90% of the root apices may be enclosed by a fungal sheath, whereas in some broad-leafed species, such as *Eucalypt*, this proportion may not exceed 40–50%. Thus, depending on the tree species, as well as on root growth rate and season of the year, a varying proportion of plant nutrients may be taken up via the fungal hyphae of the external mycelium and the sheath. However, ECM fungi differ substantially in thickness of the sheath (Agerer, 1987; Smith and Read, 2008) and hydraulic resistance to solute flow. The fungal sheath may be more or less sealed and prevent an apoplasmic route of solute and water flux into the root cortex, for example in *Eucalypt* with *Pisolithus tinctorius* (Ashford *et al.*, 1989), whereas it provides a relatively unrestricted apoplasmic route in others, for example *Pinus sylvestris* with *Suillus bovinus* (Behrmann and Heyser, 1992).

The extent of the external mycelium varies substantially between ECM species, with $300\,m\,m^{-1}$ colonized root length in *Salix* seedlings (Jones *et al.*, 1990) and $500\,m\,m^{-1}$ in *Pinus taeda* (Rousseau *et al.*, 1994). In contrast to AM, many ECM fungal species form rhizomorphs (Agerer, 1992), which can be the main routes for bidirectional solute transport. As in individual hyphae, solute transport in rhizomorphs is driven by cytoplasmic streaming and concentration gradients. However, their large diameter (~100 µm) and hollow centre may also allow rapid apoplasmic solute transport (Jennings, 1987; Cairney, 1992). Similarly to AM, ECM hyphae contain poly-P (Orlovich *et al.*, 1989; Bucking and Heyser, 1999) and ECM increase P uptake (Table 15.8), with nearly three-fold greater P influx in mycorrhizal *Salix* compared

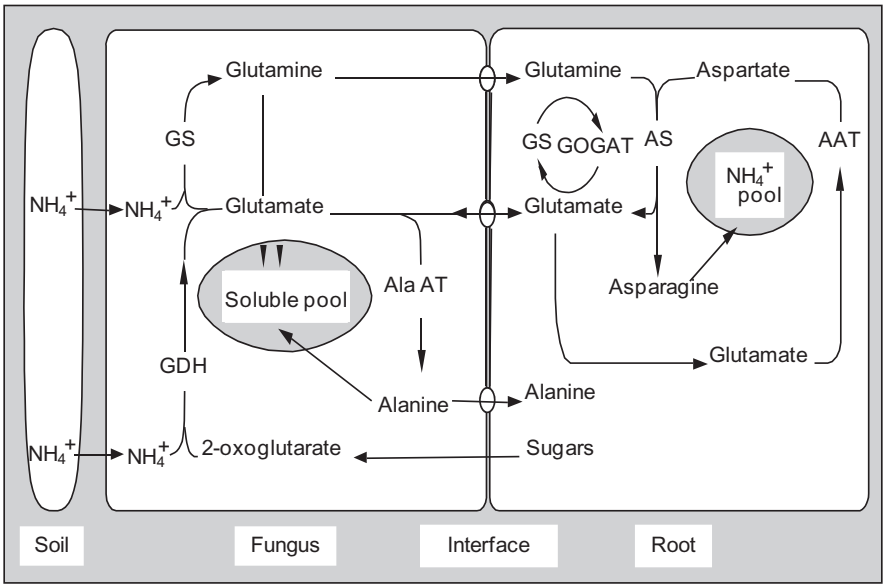


FIGURE 15.12 Proposed schematic diagram of N assimilation in Norway spruce ectomycorrhiza and localization of N-assimilating enzymes in the fungus and host cells. GDH: glutamate dehydrogenase, GS: glutamine synthase, GOGAT: glutamate synthase. *From Chalot* et al. *(1991) with permission from Wiley and Sons.*

to the non-mycorrhizal plants (Jones *et al*., 1991). In eucalypt which forms ECM and AM, P inflow compared to the non-mycorrhizal control was four-fold greater in ECM plants and 2–3-fold greater in AM plants, indicating that ECM may be more effective in delivering P to plants than AM. ECM may also increase plant growth when supplied with the poorly soluble apatite; however, there is considerable variation among ECM fungi which may be related to their capacity to release oxalate (Wallander, 2000).

ECM hyphae can transport K and have been shown to increase plant K (Jentschke *et al*., 2001) and Mg concentrations (van Schoell, 2006), the latter possibly due to the weathering of minerals by organic acid anions released by EMC hyphae (see below).

The release of acid phosphatase by ECM fungi is well established, its activity being high in the entire external mycelium (Dinkelaker and Marschner, 1992) and at the surface of mycorrhizal roots (Gourp and Pargney, 1991). Some ericoid fungi such as *Hymenoscyphus ericae* produce siderophores (Schuler and Haselwandter, 1988), which may explain the greater Fe acquisition and shoot Fe concentrations of mycorrhizal *Calluna vulgaris* when grown on substrates with low Fe availability such as calcareous soils (Shaw *et al.*, 1990). In contrast to AM fungi, ECM fungi have considerable capacity to produce and excrete organic acid anions. These organic acid anions, and perhaps also siderophores, are involved in the enhanced weathering of micas by ECM as compared with non-mycorrhizal pine (Leyval and Berthelin, 1991). This so-called 'ectomycorrhizal weathering' or 'rock eating' has been confirmed in several recent studies (e.g., Landeweert *et al*., 2001; Wallander, 2006). Some ECM fungi such as *Paxillus involutus* release large amounts of oxalic acid, particularly when supplied with nitrate (Lapeyrie *et al.*, 1987). Oxalic acid dissolves sparingly soluble Ca phosphates, but also forms Ca oxalate crystals which may cover the external mycelium and the hyphal sheath of mycorrhizal roots (Lapeyrie *et al.*, 1990).

Similarly to many plants, ECM fungi prefer ammonium compared with nitrate as N source (Plassard *et al.*, 1991). Accordingly, when both ammonium and nitrate are supplied (e.g., as ammonium nitrate), ECM fungi take up ammonium preferentially and therefore acidify their substrate similarly to the host roots (See also Section 6.1 and Chapter 14). After uptake of ammonium or nitrate reduction in the cells of the external mycelium and the fungal sheath, ammonium is incorporated into glutamate and glutamine by the action of glutamate dehydrogenase (GDH) and glutamine synthase (GS), respectively (Fig. 15.12). The key role of GDH in ECM fungi is in contrast to higher plants where ammonium assimilation occurs via the glutamate synthase cycle involving the sequential action of GS and glutamate synthase (GOGAT), whereas GDH plays a minor role (Section 6.1). Glutamine is transported to the sheath via the external hyphae (Fig. 15.12). In the sheath and the Hartig net, GOGAT may also become important in ammonium assimilation in some ECM (Martin *et al.*, 1992). Later, Martin *et al.* (1994) found that the relative contribution of GS and GDH in assimilation of ammonium by *Laccaria bicolor* varies with growth stage, with GS being the main enzyme

in the early stages of growth, whereas later, GS and GDH contribute equally to assimilation.

The extent to which inorganic N is either assimilated in the fungal cells or passes the sheath to be assimilated in the host root cells is unclear and may depend on the relative enzyme activities, carbohydrate supply, and thickness of the sheath.

Some ericoid and ECM fungi release proteinases and thereby provide the host plant with an access to complex organic sources of N such as proteins. Because the host plants themselves have little or no access to these N sources, mycorrhiza may play a crucial role for host plant growth on substrates with complex organic N. As shown in Table 15.9, in contrast to non-mycorrhizal pine seedlings, seedlings colonized by the ECM fungus *Suillus bovinus* can readily utilize N from protein sources, similarly to plants provided with ammonium. However, this table also shows varying capacity among ECM fungi to utilize protein N. The capacity to directly utilize organic N minimizes leaching and gaseous N losses from the soil, and simultaneously decreases competition for N by other soil microorganisms (Vogt *et al.*, 1991). It may also increase the competitiveness of mycorrhizal plants compared to non-mycorrhizal plants in ecosystems with high organic matter and thus organic N content (Northup *et al.*, 1995).

TABLE 15.9 Nitrogen content in *Pinus contorta* seedlings either non-mycorrhizal or mycorrhizal with *Suillus bovinus* or *Pisolithus tinctorius* and supplied with ammonium or protein as source of N

	Ammonium-N	Protein-N
	N content ($mg\,plant^{-1}$)	
Non-mycorrhizal	3.66	1.14
Suillus bovinus	4.05	3.20
Pisolithus tinctorius	3.27	1.30

Based on Abuzinadah *et al.* (1986).

15.3.5 Role of Mycorrhiza in Heavy Metal Tolerance

A large number of ECM fungi increase heavy metal tolerance of host plants (Wilkins, 1991; Colpaert and van Assche, 1993). For example, in birch seedlings, tolerance to high Ni concentrations in the substrate was increased by inoculation with the ECM fungi *Lactarius rufus* or *Scleroderma flavidum* (Jones and Hutchinson, 1988), whereas tolerance to high Zn concentrations in the substrate was enhanced by inoculation with *Paxillus involutus* (Denny and Wilkins, 1987). In *Pinus banksiana* seedlings, tolerance to various heavy metals (Pb, Ni, Zn) can be increased by *Suillus luteus* at low and intermediate but not at high external concentrations which are directly harmful to the fungus (Jones and Hutchinson 1986; Dixon and Buchena, 1988).

Heavy metal tolerance can be due to several processes (Bellion *et al.*, 2006): (i) extracellular binding on the external mycelium or the fungal mantle by excreted ligands, (ii) surface sequestration by binding to the fungal cell wall, (iii) enhanced metal efflux from the fungal cell, (iv) binding to methallothionein or glutathione in the fungal cytoplasm, and (v) sequestration of the glutathione–metal complex in the vacuole. These processes decrease the concentration of the heavy metals in the soil solution in the mycorhizosphere, in the roots, and particularly in the shoot tissue. Most heavy metals, and also Al, exert their toxic influence by damaging root apical zones; therefore, preventing heavy metals and Al from reaching the root tips increases host tolerance.

The specific heavy metal binding capacity of the external mycelium, and its mass, are therefore important for the effectiveness of heavy metal retention in the ECM (Colpaert and van Assche, 1992). As shown in Table 15.10,

TABLE 15.10 Shoot and root Zn concentrations in *Pinus sylvestris* seedlings non-mycorrhizal or mycorrhizal with *Paxillus involutus* or *Thelephora terrestris* at high Zn supply

	Shoot dw ($g\,plant^{-1}$)	Shoot Zn		Fungal biomass (% of short roots)	Short root Zn concentration ($mg\,kg^{-1}$ dw)
		($mg\,kg^{-1}$ dw)	($mg\,plant^{-1}$)		
Non-mycorrhizal	16.2	197	3.19	0	273
Paxillus involutus	14.3	106	1.52	54	708
Thelephora terrestris	16.2	240	3.89	66	309

Based on Colpaert and Van Assche (1992).

Paxillus involutus has a high Zn retention capacity in its mycelium and thereby effectively decreases plant Zn concentration compared with the non-mycorrhizal plants. In contrast, despite of a similar fungal biomass, *Thelephora terrestris* retains little Zn in its structures, and even increases the Zn concentration in the host plant. This and many other examples in the literature demonstrate that the effect of ECM on heavy metal tolerance of host plants cannot be generalized.

The retention capacity of the fungus can also be exceeded over time. In Norway spruce grown at 800 μM Al, *Paxillus involutus* decreased Al toxicity (as indicated by increased chlorophyll content compared to the non-mycorrhizal plants) after 5 weeks, but had no ameliorating effect after 10 weeks (Hentschel *et al*., 1993).

In contrast to ECM, there are only a few reports on the effect of AM on heavy metal tolerance of the host plant. This is not surprising given the importance of the ECM sheath in retaining heavy metals. Indirect effects may also occur, for example, by improving P nutritional status and growth of the host plant on a P deficient soil high in heavy metals or Al, i.e. by a dilution effect. A more specific effect is the alleviation of Mn toxicity by reducing Mn uptake (see above). In contrast, the effect of AM on Zn tolerance is controversial. Under Zn deficiency, AM fungi can enhance Zn uptake (e.g., Manjunath and Habte, 1988; Faber *et al*., 1990). In many studies high effectiveness of AM in the acquisition and delivery of Zn to the host plant was also retained when plants are grown at high external Zn supply, thus increasing Zn toxicity (Schuepp *et al.*, 1987; Symeonidis, 1990). On the other hand, there is also substantial evidence that AM reduce Zn accumulation and thereby toxicity at high Zn concentrations (Li and Christie, 2001; Burleigh *et al*., 2003). The increase in Cu tolerance in AM plants (Gildon and Tinker, 1983a, b) is probably related to a high retention of Cu in the fungal mycelium within the host roots (Li *et al.*, 1991b).

15.3.6 Mycorrhizal Responsiveness

A major beneficial effect of mycorrhizal colonization on host plant growth is due to the increase in below-ground surface area (roots and mycorrhizal hyphae) for acquisition of nutrients. The beneficial effect of mycorrhiza is therefore of particular importance for plants with a coarse and poorly branched root system (Hetrick, 1991), and which lack the capacity to mobilize P by root exudates (Chapter 14). The beneficial effect of mycorrhiza on host plant growth is referred to as *mycorrhizal responsiveness* (Alexander, 1989; Smith and Read, 2008).

Due to the abundance of soils with low P availability and large number of agriculturally important AM plants, most studies of mycorrhizal dependency have been focused on AM and P. In most soils, roots are colonized by indigenous AM, thus studies on mycorrhizal responsiveness require soil sterilization (fumigation, steaming) and re-inoculation with the indigenous soil microflora but not AM in contrast to re-inoculation with both indigenous soil microflora and AM. Plant growth response is then used as the parameter of mycorrhizal responsiveness. The results shown in Table 15.11 represent the range of mycorrhizal responsiveness of crop species grown in soil low in P. Elimination of AM by soil fumigation elicited three types of growth responses: (i) carrot and leek grew very poorly; growth was restored to about the level of growth in non-fumigated soil after re-inoculation with AM; (ii) tomato and wheat exhibited small or negligible growth responses to AM inoculation despite high colonization rates; and (iii) cabbage as a non-mycorrhizal plant species (member of Cruciferae) grew better in fumigated soil, with inoculation with AM having no further effect. The growth enhancement in cabbage by fumigation was presumably due to elimination of soil-borne pathogens. In citrus, Menge *et al.* (1978) found distinct differences in mycorrhizal responsiveness between Brazilian sour orange (responsive) and Troyer citrange (non-responsive) (Fig. 15.6). Similarly, Klironomos (2003) showed a high variability in mycorrhizal responsiveness (from a strongly positive to a strongly negative effect) among a large number of plant species.

The data in Table 15.11 also suggest that one should not expect large growth stimulation by inoculation of field-grown plants unless indigenous AM fungi have been damaged, for example, by fungicides (Hale and Sanders, 1982; Khasa *et al.*, 1992). Coarse root systems are found in many woody species, and among crop species in cassava (*Manihot esculentum*). In non-mycorrhizal cassava plants, the critical deficiency level of extractable soil P is 190 mg kg^{-1} soil, compared with only 15 mg kg^{-1} soil in mycorrhizal plants.

The importance of root morphology for mycorrhizal responsiveness in different plant species is shown in Fig. 15.13. The grass with the large root surface area, does not respond to Am inoculation even at very low soil P levels. In contrast, in the legume with short roots and short root hairs, mycorrhizal responsiveness is high. Schweiger *et al.* (1994) showed that there is an inverse relationship between root hair length and mycorrhizal dependency. In general, for a given plant species, the wild relatives tend to be less responsive to AM than the cultivars, for example in oat (Koide *et al.*, 1988), tomato (Bryla and Koide, 1990a, b) and wheat (Zhu *et al*., 2001). In wheat, modern cultivars are less responsive to AM colonization than land races (Manske, 1989; Zhu *et al.*, 2001), suggesting that modern breeding, often carried out at high P supply, may have reduced AM responsiveness. This is partly due to

TABLE 15.11 Growth and AM colonization of different plants in soil non-fumigated (native AM), fumigated (AM suppressed), or fumigated and re-inoculated with native AM

	Dry weight (g pot^{-1})			AM colonization (% root length)		
Plant species	Non-fumigated	Fumigated	Fumigated + re-inoculated	Non-Fumigated	Fumigated	Fumigated + re-inoculated
Carrot	8.5	0.4	7.4	61	0	60
Leek	4.4	0.4	4.0	50	0	67
Tomato	4.1	2.5	5.1	61	0	90
Wheat	2.0	1.7	2.1	63	0	79
Cabbage	11.9	14.2	13.6	0	0	0

Plenchette *et al*. (1983).

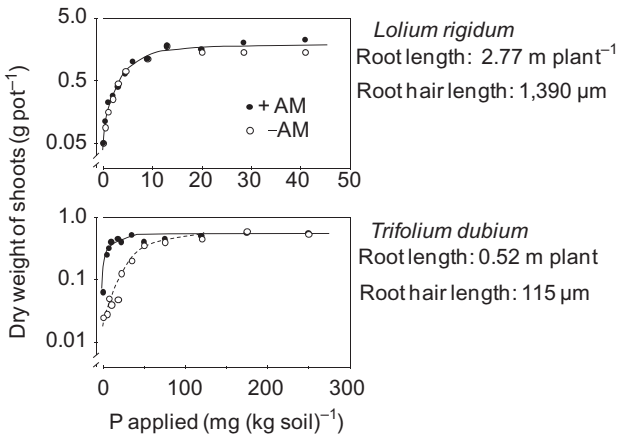


FIGURE 15.13 Relationship between root morphology and mycorrhizal benefits to P acquisition in *Lolium rigidum* and *Trifolium dubium*. *Based on Schweiger* et al. *(1994).*

differences in root morphology and root/shoot dry weight ratio, but also in growth rate, growth potential and P-use efficiency. Inherent differences in the latter parameters are often overlooked in comparisons between plant species in AM responsiveness. Seed size, and thus seed reserves of P, as well as other nutrients, is another important factor for AM responsiveness. In a comparison of 15 wild species grown in a P-deficient soil, there was a negative correlation was found between AM responsiveness and seed size (Allsopp and Stock, 1992).

Orchids have an absolute mycorrhizal dependency, and many woody and forest tree species are highly responsive to mycorrhiza (Table 15.8). The same holds true for ericoid mycorrhizal plants such as *Calluna*. In many natural ecosystems, the dependency on ECM or ericoid mycorrhiza may mainly be related to N and not so much P availability, but systematic studies on this topic are scarce.

15.3.7 Other Mycorrhizal Effects

15.3.7.1 Hormonal Effects and Plant Water Relations

Mycorrhiza may alter host plant growth and development through direct and indirect effects on hormonal balance and plant water relations. The IAA production by ECM fungi may be responsible for the typical morphological changes of ECM short roots (reduced elongation or increased branching) and the enhanced shoot elongation (Frankenberger and Poth, 1987). However, not all ECM fungi produce hormones, and quite often there is a poor or no correlation between fungal hormone production *in vitro* and the enhancing effect of the fungus on host plant growth. For example, in *Pinus taeda*, inoculation with *Pisolithus tinctorius* enhanced shoot growth and increased CYT concentration in the needles, although this fungus does not produce CYT, whereas inoculation with the CYT producer *Suillus punctipes* did not affect shoot growth and CYT concentration in the needles (Wullschleger and Reid, 1990). Thus, depending on the ECM fungal species, hormones produced by these species, or their effects on hormone synthesis by the plant, the altered hormonal balance in plants may, or may not, play a role in the effects of ECM fungi on host plant growth.

There are also reports of higher CYT concentration in plants inoculated with AM, for example in flax (Drüge and Schönbeck, 1992) and citrus (Dixon *et al.*, 1988). In maize, inoculation with AM increased ABA concentrations in roots and shoots two-fold compared with non-mycorrhizal plants (Danneberg *et al.*, 1992). However, in all these studies, the increase in hormone concentrations in the plants was associated with a strong growth increase by AM inoculation, most probably via improved P nutritional status of the host plants. Hormone concentrations are affected by the P nutritional status in non-mycorrhizal

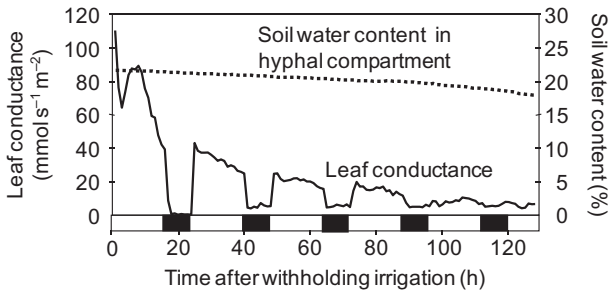


FIGURE 15.14 Leaf stomatal conductance and soil water content in the hyphal compartment over time after withholding water supply in mycorrhizal *Agropyron repens*. *Based on George* et al. *(1992a).*

plants (Chapter 5). Therefore, it is likely that AM inoculation affects plant hormone concentrations indirectly.

Mycorrhizal colonization may affect plant water relations directly or indirectly (Auge, 2001). Increased water supply to the host plant has been shown for ECM fungi which form extended extensive mycelium and rhizomorphs (Brownlee *et al.*, 1983). Due to their large diameter and hollow centre, rhizomorphs are suited for rapid and substantial water transport to the host plant (Lamhamedi and Fortin, 1991). Hence in *Pinus pinaster*, there was a positive relationship between diameter of the fungal rhizomorphs, xylem water potential and speed of recovery of the host plant after drought stress (Lamhamedi *et al.*, 1992).

An increase in drought stress tolerance has also been observed in AM plants compared with non-mycorrhizal plants. AM colonization can increase transpiration and stomatal conductance during water stress and recovery (Safir *et al*., 1972; Fitter, 1988). Differences in plant P nutritional status may in part account for this effect because P-deficient plants have low stomatal conductance (Koide, 1985; Fitter, 1988). The improved water uptake may also be due to increased root branching induced by AM colonization (Allen *et al*., 1981). Moreover, improved soil structure by binding microaggregates into stable macroaggregates (Davies *et al*., 1992) by hyphae or production of extracellular polysaccharides (Tisdall, 1991; Degens, 1997) could enhance water flux to the root (Fitter, 1985; Davies *et al.*, 1992; Lamhamedi *et al.*, 1992). Glomalin, a glycoprotein associated with AM hyphae (Wright and Jawson, 2001), may also increase aggregate stability, but its importance remains unclear (Smith and Read, 2008).

Water transport in the external AM hyphae to the host plant has been observed (Faber *et al.*, 1991). However, due to the small diameter of the hyphae (~1–12 µm), the contribution of hyphal water transport to water uptake by the host is likely to be small (Kothari *et al*., 1990b). Despite severe drought stress of the host plant, there was no substantial water transport from the hyphal compartment through the hyphae to the host plant when direct soil contact at the root–hyphal compartment interface is prevented (Fig 15.14). On the other hand, hyphal uptake from the outer compartment accounted for 49% of the total P and 35% of the total N taken up by the myorrhizal plants (George *et al.*, 1992a).

Hence, the effects of mycorrhiza on water uptake are complex, but are mainly related to the enhanced plant nutritional status and improved soil structure. Water transport by ECM hyphae (particularly rhizomorphs) is likely, whereas this can be ruled out for the thin AM hyphae.

15.3.7.2 Suppression of Root Pathogens

There are many examples of suppression of soil-borne fungal and bacterial root pathogens by inoculation with mycorrhiza, AM in particular. For a recent overview, see St-Arnaud and Vujanovic (2006).

For example, inoculation with AM fungi increased resistance of tomato to *Fusarium oxysporum* (Dehne and Schönbeck, 1979a) and *Pseudomonas syringae* (Garcia-Garrido and Ocampo, 1989), of casuarina to *Fusarium vesicubesum* (Gunjal and Paril, 1992) and of barley against *Gaeumannomyces graminis* var. *tritici* (Khaosaad *et al*., 2007). This suppressing effect of AM is also evident in cases of ‘soil sickness’, or ‘replant disease’, where minor pathogens or deleterious soil microorganisms may reduce root growth and activity. An example of such a suppressing effect is shown in Table 15.12. The growth of grapevine seedlings was poor on soil with replant disease, but could be considerably improved by inoculation with AM, which also increased AM root colonization. Suppression of *Pseudomonas fluorescens* by AM inoculation was presumably a key factor for improvement of plant growth in the soil with replant disease. Soil sterilization was, however, more effective than AM inoculation as it restored plant growth to the level in the control soil (Waschkies *et al.*, 1993).

Protection of the host plant from root pathogens is also well documented for ECM fungi, an example being shown in Table 15.13 for *Paxillus involutus*. The ECM fungus suppressed the harmful effects of *Fusarium oxysporum* on *Pinus resinosa* seedling growth.

The main mechanisms for disease suppression are (i) competition for colonization sites (Perrin, 1990), (ii) mobilization of plant defence reaction by mycorrhizal colonization (Khaosaad *et al*., 2007), and (iii) improved nutritional status which enhances the capacity of the plant to compensate for damage by increased growth (Dehne und Schönbeck, 1979b; Smith and Read, 2008). Direct inhibitory effects of mycorrhizal fungi on pathogens have also been reported (Kope and Fortin, 1990; Benhamou *et al*., 1994; Duchesne *et al*., 1989), but appear to be the exception rather than the rule.

TABLE 15.12 Growth and density of rhizoplane bacteria in grapevine (*Vitis vinifera*) without or with inoculation with AM fungi growing in soil without (control) or with replant disease (RPD)

	Shoot dw ($g\,plant^{-1}$)	Root fw ($g\,plant^{-1}$)	AM colonization (% root length)	Total bacteria $\times 10^7$ (# g^{-1} root fw)	Pseudomonads $\times 10^5$ (# g^{-1} root fw)
Control					
−AM	6.3	10.1	33	3.2	0.18
+AM	6.2	12.5	39	3.7	0.16
RPD					
−AM	1.3	3.6	21	4.4	5.88
+AM	2.3	7.8	34	3.2	0.71

Waschkies *et al.* (1994).

TABLE 15.13 Shoot and root length and seedling mortality in *Pinus resinosa* seedlings non-inoculated or inoculated with *Fusarium oxysporum* (pathogen) and/or *Paxillus involutus* (ECM fungus)

	Seedling mortality (%)	Shoot length ($cm\,plant^{-1}$)	Root length ($cm\,plant^{-1}$)
Control	0	3.0	2.3
+*Paxillus involutus*	0	3.0	2.5
+*Fusarium oxysporum*	50	1.5	0.6
+*P. involutus* +*F. oxysporum*	20	2.5	1.5

Based on Chakravarty *et al.* (1991).

Mycorrhizal colonization can also induce systemic resistance, that is, even roots not colonized by the fungus may be resistant to pathogen attack. Khaosaad *et al.* (2007) grew barley in a split-root system where one half of the root system was inoculated with AM, while the other, non-mycorrhizal, root half was challenged with the root pathogen *Gaeumannomyces graminis* var. *tritici* (Ggt). Infection of roots by Ggt was reduced and plant growth enhanced when the other half of the root system was inoculated with AM 14 days prior to inoculation with Ggt. However, simultaneous inoculation of AM and Ggt did not result in improved resistance. This suggests that AM colonization induced a defence reaction in the host, which then reduced colonization by the pathogen. The improved resistance may be due to the higher P concentration of the AM plants, but involvement of salicylic acid, which often acts as a signal for systemic resistance, was ruled out because its concentration was not affected by AM.

15.3.8 Mycorrhiza: Practical Implications

Although higher plants may benefit from mycorrhiza mainly by improved nutritional status, P in particular, other beneficial effects may occur and should not be overlooked. Diverse beneficial mycorrhizal effects can readily be demonstrated under controlled environmental conditions, but may vary with fungal species or strains. However, our knowledge of the functioning of mycorrhiza under field conditions is poor. Moreover, the presence of an indigenous mycorrhizal community may limit the effect of inoculation with 'effective' mycorrhizal species (Abbott and Robson, 1982; Hall, 1988). However, there are certain situations where mycorrhizal inoculation may be effective, for example in horticulture, where seedbeds are often fumigated to reduce plant pathogens. Tree seedlings are also often grown in fumigated nursery substrate. Inoculation of forest trees with ECM in nurseries can substantially improve transplantation success and increase survival and growth rate in the field (Guehl and Garbaye, 1990; Villeneuve *et al.*, 1991; Grove and Le Tacon, 1993). The same is true for AM in reforestation of mining sites (Jasper *et al.*, 1989a) or for production fruit tree seedlings in nurseries (Menge, 1983).

Inoculation with ECM fungi is not very difficult as many of them can readily be multiplied in pure culture. This is different, however, for AM fungi, where a major constraint is the production of a pathogen-free inoculum in sufficient quantities (Menge, 1984; Gianinazzi and Vosatka, 2004).

Chapter 16

Nitrogen Fixation

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SUMMARY

The chapter begins with an assessment of the contribution of biological N_2 fixation to the N economy of terrestrial ecosystems, followed by a description of the diversity of N_2-fixing systems. Subsequent sections address the biochemistry of nitrogenase and the significance of symbiotic microorganisms as suppliers of N to higher plants. Legume–rhizobia symbioses are treated in detail from several viewpoints: range of partnerships; interactive signalling and root infection; formation and structure of root nodules; bacteroid metabolism; amounts of N_2 fixed; and host plant sanctions on non-fixing strains. The influence of mineral N on fixation in legumes and the effects of additions or deficiencies of other nutrients and trace elements are discussed, as are responses of legumes to variations in soil pH, salinity, temperature and water stress. A brief review of prospects for improving N_2 fixation in legumes, transferring fixation to non-legume crops and developing a practical non-biological N_2 fixation process that operates at ambient temperature and pressure concludes the chapter.

16.1 GENERAL

Biological nitrogen fixation (BNF) is an important N supply route for both natural vegetation and crop plants (Vance, 2002), but quantifying its contribution on a global scale is problematic, mainly due to inaccurate statistics on areas under legume cultivation and the sparseness of fixation data for non-legume crops and most natural ecosystems (Cleveland *et al*., 1999; Herridge *et al*., 2008). The contribution of BNF to natural terrestrial ecosystems has been estimated at $107\,Tg\,N\,y^{-1}$ by Galloway *et al.* (2004) and $195\,Tg\,N\,y^{-1}$ by Cleveland *et al*. (1999) while figures for BNF inputs into agricultural systems range from 40 (Galloway *et al*., 2008) to $50–70\,Tg\,N\,y^{-1}$ (Herridge *et al*., 2008). In comparison, current world fertilizer N usage in the form of ammonia and its compounds is ca. $100\,Tg\,N\,y^{-1}$ (FAO, 2008). This N is produced by the catalytic reduction of inert N_2 to NH_3 under conditions of high temperature (350–550°C) and pressure (150–350 atm) in the industrial Haber-Bosch process ($N_2 + 3H_2 \rightarrow 2NH_3$). This process, brought into commercial operation in 1913 and based on the reaction discovered by the German chemist Fritz Haber in 1908 (see Stern, 1999), revolutionized the production of N fertilizers and has had profound impact on world food supply (Smil, 2001). Energy requirements for the reaction are high, with fossil fuels being used for both hydrogen and heat/pressure generation. In stark contrast, the biological reduction of N_2 to NH_3, which the Haber-Bosch reaction mimics, takes place at ambient temperature and subambient pressure and can be performed by only a few prokaryotes: those that possess the nitrogenase enzyme.

16.2 BIOLOGICAL NITROGEN-FIXING SYSTEMS

The ability to fix atmospheric N_2 to NH_3 is restricted to a small subset of taxonomically diverse organisms within the prokaryotes. They may be symbiotic, associative or free-living in relation to higher plants.

Symbionts are the most significant N fixers for plants; they are represented primarily by rhizobia (Protobacteria), *Frankia* (Actinomycetes) and *Nostoc/Anabaena* (Cyanobacteria). These organisms reside in specialized structures where they have access to a plentiful energy source in the form of photosynthates and an environment that is conducive both to nitrogenase activity (e.g., through the provision of an oxygen protection mechanism) and the translocation of fixed N directly to the host plant. Legumes nodulated by rhizobia, such as lucerne and

Marschner's Mineral Nutrition of Higher Plants. DOI: 10.1016/B978-0-12-384905-2.00016-9

soybean, are among the most prominent N_2-fixing systems in agriculture. Tree legumes (e.g., *Leucaena leucocephala* and *Robinia pseudoacacia*) also benefit from N_2-fixing root nodule symbioses with rhizobia as does one non-legume, the tropical tree *Parasponia*. However, in some forest and woodland ecosystems most of the N input from BNF comes from non-rhizobial symbionts in root nodules of non-leguminous tree species: actinomycetes from the genus *Frankia* and woody perennials such as *Alnus*, *Casuarina* and *Caenothus*. Approximately 200 species of woody shrubs and trees, mostly from temperate regions, form actinorhizal symbioses with *Frankia* (Vessey *et al*., 2005). Symbioses between diazotrophic cyanobacteria and photolithotrophic eukaryotes are widespread (Raven, 2002). Members of the gymnosperm order Cycadales form cyanobacterial N_2-fixing root symbioses, mainly with filamentous *Nostoc* species (Rasmussen and Nilsson, 2002). *Nostoc* can also enter into a stem/petiole symbiosis with a tropical flowering angiosperm, *Gunnera*. A symbiosis between *Nostoc* and the feather moss, *Pleurozium schreberi*, may contribute significant quantities of fixed N to the soils of boreal forests (DeLuca *et al.*, 2002). In aquatic ecosystems, for example rice paddies, an important source of BNF is the symbiosis between the heterocyst-forming cyanobacterium, *Anabaena azollae*, and a pteridophyte, *Azolla*.

The roots of higher plants are colonized by other bacteria, which do not reside in specialized organs but may, in some cases, invade the plant cortex as endophytes (Rosenbleuth and Martinez-Romero, 2006). The majority of these associations, however, involve free-living bacteria with relatively low rates of N_2 fixation growing on the rhizoplane or in the rhizosphere. Many studies report plant growth-promoting properties for associative bacteria but the mechanisms responsible for this effect are often unclear (see also Chapter 15). There are numerous examples of the isolation of associative bacteria from the rhizosphere with N_2-fixing capabilities and growth-promoting activity; however, in many cases increased plant growth cannot be solely attributed to fixed N, other factors such as phytohormone production or improved availability of various nutrients are also involved (Vessey, 2003). One exception appears to be *Gluconacetobacter diazotrophicus*, which is reported to contribute part of the N requirement of sugar cane via fixation. Some evidence also points to associative N_2 fixation as a factor in plant growth stimulation by, for example, *Azospirillum* with maize, rice and wheat; *Azoarcus* with Kallar grass; *Burkholderia* with rice and *Herbaspirillum* with rice, sorghum and sugar cane (see reviews by Vessey, 2003 and Kennedy *et al*., 2004).

Free-living N_2 fixers are widely distributed in soils but in the case of heterotrophic bacteria, they are usually restricted in their fixation capacity by lack of organic substrates for energy generation. Examples can be found among aerobes (*Azotobacter*), anaerobes (*Clostridium*) and facultative anaerobes (*Klebsiella*). A few N_2 fixers occur among chemolithotrophic (e.g., *Acidothiobacillus ferrooxidans*, *Leptospirillum ferrooxidans*, *Mycobacterium flavum*) and photolithotrophic (e.g., *Chlorobium*, *Chromatium*, *Rhodospirillum*) bacteria. Some heterocyst-forming cyanobacteria (e.g., *Anabaena*, *Nostoc*, *Calothrix*, *Cylindrospermum*) can also fix N_2 without entering into symbiosis with a eukaryotic host.

16.3 BIOCHEMISTRY OF NITROGEN FIXATION

The mechanism of BNF is of great interest from both the agricultural and chemical viewpoints; not only is it the means by which a substantial portion of the N demand of some crop plants is satisfied, it is also an efficient catalytic system which accomplishes the equivalent of the Haber-Bosch reaction at ambient temperature and subatmospheric pressure. Biological reduction of N_2 to NH_3 is a highly energy-demanding process with a minimum energy requirement of ca. 960 kJ mol^{-1} N fixed (Sprent and Raven, 1985). In all N_2-fixing microorganisms the principal steps of the reaction are the same (Fig. 16.1). The key enzyme, nitrogenase, is unique to N_2-fixing microorganisms. Three genetically distinct oxygen (O_2)-sensitive nitrogenase systems are currently recognized: molybdenum nitrogenase (Nif), vanadium nitrogenase (Vnf) and iron-only nitrogenase (Anf) (Zhao *et al*., 2006). Of the three, the molybdenum variant is the best studied and the most widely distributed in nature (Rees and Howard, 2000). A fourth, O_2-insensitive nitrogenase, has been reported in one bacterium, *Streptomyces thermoautotrophicus* (Ribbe *et al.*, 1997).

Molybdenum nitrogenase consists of two non-heme Fe proteins: dinitrogenase (Component I) and dinitrogenase reductase (Component II). Component I, which contains the active site for N_2 reduction, is the larger of the two with a molecular mass of approximately 240 kDa. It is an MoFe-protein comprising an $\alpha_2\beta_2$ tetramer which is associated with two copies each of two metalloclusters termed the FeMo-cofactor and the P-cluster. The former contains the site of substrate (N_2) reduction and the latter is thought to be the initial acceptor of electrons from Component II (Rees *et al.*, 2005). Component II is a Fe-protein in the form of a homodimer that contains one metallocluster per dimer. It has a molecular mass ca. 60 kDa and couples ATP hydrolysis to inter-protein electron transfer (Zehr *et al*., 2003).

The nitrogenase reaction requires energy (ATP) and reducing equivalents (electrons), or flavodoxin. A basic four-stage mechanism for Mo nitrogenase at the protein level has been proposed: (i) formation of a complex between the reduced Fe protein with two ATP molecules and the MoFe protein; (ii) electron transfer between the two proteins coupled with the hydrolysis of ATP; (iii) dissociation of the Fe protein accompanied by re-reduction and exchange of ATP

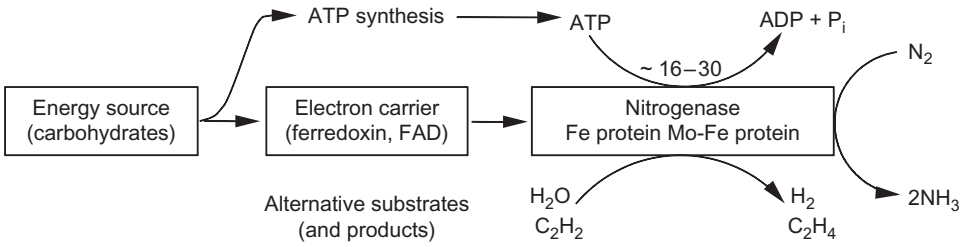

FIGURE 16.1 Scheme illustrating the energy supply and principal reactions of the nitrogenase system. *Based on Evans and Barber (1977). Reprinted with permission of the American Association for the Advancement of Science.*

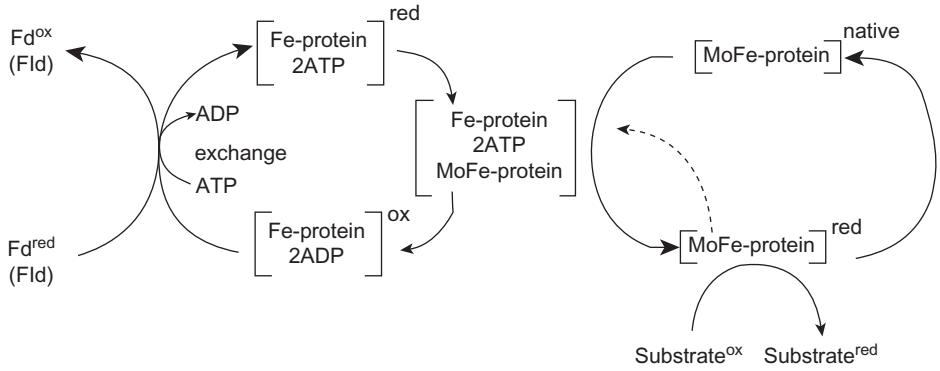

FIGURE 16.2 Scheme for turnover cycle of nitrogenase, with (from left to right) flow of electrons from ferredoxin (Fd) or flavodoxin (Fld) to the Fe-protein, transfer of electrons to the Mo-Fe protein coupled to hydrolysis of ATP and reduction of substrates with return of the Mo-Fe protein to its native redox state. *Based on Rees* et al. *(2005)*.

for ADP; (iv) repetition of this cycle until sufficient numbers of electrons and protons have accumulated to reduce available substrates (Rees and Howard, 2000). This sequence is shown in Fig. 16.2 and the overall stoichiometry of the reaction catalysed by Mo nitrogenase is:

$$N_2 + 8e^- + 8H^+ + 16\text{MgATP} \rightarrow 2NH_3 + H_2 + 16\text{MgADP} + 16\text{Pi}$$

The question of exactly where N_2 binds during catalysis remains unresolved (Seefeldt *et al.*, 2004), as does the mechanistic detail of its reduction (Seefeldt *et al*., 2009). Several reviews have considered recent progress in this field: Rees *et al.* (2005); Howard and Rees (2006); Dance (2007); Hoffman *et al*. (2009).

Nitrogenase also catalyses the reduction of other substrates, such as acetylene, which is converted to ethylene. Ethylene can be detected at low concentrations and in the past was used extensively in the acetylene reduction assay (ARA) for measuring N_2 fixation (Bothe *et al.*, 1983). Nowadays, ^{15}N dilution and natural abundance methodology are the preferred choices, particularly if quantification, as opposed to obtaining data on relative rates of fixation, is the objective (e.g., Carranca *et al.*, 1999; James, 2000; Asis *et al.*, 2002).

As noted above, nitrogenase is very sensitive to O_2 and various means are employed by diazotrophic microorganisms to protect the enzyme from irreversible inactivation by O_2 *in vivo* (Becana and Rodriguez-Barrueco, 1989). These include:

1. Living and fixing N_2 exclusively under anaerobic conditions (e.g., *Clostridium*).
2. Living under aerobic or anaerobic conditions but fixing N_2 only under the latter (e.g., *Klebsiella*).
3. Providing microaerophilic conditions at the enzyme site, in an otherwise aerobic environment, by consumption of most of the O_2 through excessive respiration, i.e. respiratory protection (e.g., *Azotobacter*).
4. Living in colonies covered by slime sheets, which restrict O_2 diffusion.
5. Spatial separation of nitrogenase and sites of photosynthesis/O_2 evolution (e.g., N_2 fixation in heterocysts of cyanobacteria such as *Anabaena*).
6. Controlling O_2 diffusion through physical barriers, and by binding to leghemoglobin (e.g., in root nodules of legumes).

7. Synthesis of scavenging enzymes for toxic oxygen species and H_2O_2 (e.g., ascorbate peroxidase in root nodules of legumes).

The high demand for ATP, which can be provided in large amounts only by aerobic catabolism of carbohydrates, coupled with the need to protect nitrogenase from O_2, necessitates that BNF is highly regulated at the transcriptional level by networks that respond to changes in various environmental parameters (Dixon and Kahn, 2004). This can be achieved in symbioses with higher plants which therefore have a higher potential for N_2 fixation than other systems.

16.4 SYMBIOTIC SYSTEMS

16.4.1 General

Two broad categories of symbiotic system can be identified, based chiefly on the type and location of the structure that houses the microsymbiont:

I. Nodulated legumes and non-legumes;
II. Symbioses with cyanobacteria.

In Category I, the N_2-fixing microorganisms are either rhizobia (in legumes) or actinomycetes of the genus *Frankia* (in non-legumes). Nodules are usually located on the roots of the host plant (some exceptions occur among legumes, for example *Sesbania rostrata* where rhizobia form stem nodules). Photosynthesis by the host plant is the source of substrates used for ATP generation by the microsymbiont through aerobic respiration.

In Category II, N_2-fixing cyanobacteria are found in various locations on a diverse (relative to those of rhizobia), but somewhat restricted, range of hosts (Usher *et al.*, 2007). They occur extracellularly on fungal hyphae in lichens, in cavities on the leaves of *Azolla* and the thalli of bryophytes, and in the coralloid roots of cycads. In *Gunnera,* on the other hand, they are found intracellularly in stem glands. In some cyanobacterial symbioses (e.g., in cycad roots where light is excluded) the cyanobiont must switch from photoautotrophy to chemoheterotrophy in order to be symbiotically competent (Rasmussen and Johansson, 2002; Vessey *et al.*, 2005). In others, where the cyanobiont receives illumination and remains photosynthetically active, the photosynthetic host (e.g., *Azolla*, *Gunnera*) may still be the major provider of carbohydrate for the ATP requirement of N_2 fixation (Raven, 2002).

In agricultural production systems *Anabaena azolla*, in symbiosis with the freshwater fern *Azolla*, has long been recognized for its contribution to the N balance of paddy soils. Average values are 50–80 kg fixed N $ha^{-1}y^{-1}$ (Bothe *et al.*, 1983) but higher figures have been reported from long-term field studies (79–103 kg N $ha^{-1}y^{-1}$; App *et al.*, 1984).

The main suppliers of fixed N to crop plants are rhizobia. Amounts fixed are variable (ca. 25–250 kg N ha^{-1} per season), depending on rhizobial strain, host genotype and environmental factors (see Table 16.8), but higher values have been reported. Inoculation of legume seed with selected strains of rhizobia prior to sowing is sometimes practised, where indigenous soil strains are either absent or considered to be inadequate fixers; however, inoculation success is variable. The root nodule symbiosis between rhizobia and legumes, where knowledge of the functioning of a system is most advanced, is the focus of the following discussion. For further information on root-based symbioses involving *Frankia* or cyanobacteria, see Vessey *et al.* (2005), Pawlowski and Newton (2008) and Franche *et al*. (2009).

16.4.2 Range of Legume–Rhizobia Symbioses

Legumes hold a position of special significance within, agricultural plants: their ability to capture (fix) atmospheric N_2 through the presence of rhizobia in root nodules makes their growth theoretically independent both of soil N status and extraneous fertilizer N addition. Among them are important grain and forage crops that grow in tropical or temperate climatic zones, including soybean, beans, peas, lucerne, clovers, trefoils and lupin (Sprent, 2009). Nitrogen fixation occurs when these plants are in the symbiotic state and the agents of fixation are soil bacteria from the genera *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Azorhizobium* and *Allorhizobium* (the traditional rhizobia from the alpha-Proteobacteria) as well as some more recently discovered genera that lie phylogenetically outside this group. These include *Methylobacterium*, *Devosia*, *Ochrabactrum* and *Phyllobacterium* in the alpha-Proteobacteria and *Burkholderia*, *Ralstonia and Cupriavidus* from the beta-Proteobacteria (Willems, 2006; Sprent, 2009). Strictly speaking, the term 'rhizobia' should be limited to the traditional group of alpha-Proteobacteria listed above plus, possibly, *Agrobacterium* (Young *et al.*, 2001; Willems, 2006). However, for the sake of simplicity all currently recognized legume root nodule bacteria are hereafter referred to as rhizobia. More examples are likely to be discovered in future, complicating their classification and nomenclature that are even now the subjects of much debate and controversy.

Rhizobia exhibit varying degrees of specificity towards their hosts. With the exception of the non-legume *Parasponia*, infection is confined to members of the *Leguminosae*. Some rhizobial species or biovar, are specific for individual or small groups of legume genera. For example, *Rhizobium leguminosarum* bv. *phaseoli* and *R. leguminosarum* bv. *trifolii* form root nodules only on *Phaseolus* and *Trifolium* species, respectively. In contrast, symbiotic promiscuity can be found in some other rhizobia and is perhaps more widespread than previously thought,

especially among isolates from the tropics (Perret *et al.*, 2000). A strain from New Guinea with a very broad legume host range is *Rhizobium* sp. NGR234; it nodulates at least 112 genera (Pueppke and Broughton, 1999). From the host plant perspective, some legumes (e.g., *Vigna*, *Phaseolus*) are considered to be non-selective for rhizobia, whereas others (e.g., *Pisum*, *Trifolium*) are nodulated by a single rhizobial species or biovar. Examples of rhizobia and their legume hosts are given in Table 16.1.

16.4.3 Legume Root Infection by Rhizobia

Much is now known, at the biochemical and molecular genetic level, about the interactions between rhizobia and their host plants that lead to the formation of root nodules.

TABLE 16.1 Some species of rhizobia and their legume hosts

Species[a]	Examples of hosts nodulated
Rhizobium leguminosarum	
biovar phaseoli	*Phaseolus*
biovar trifolii	*Trifolium*
biovar viciae	*Pisum, Lens, Vicia*
Rhizobium etli	*Phaseolus*
Rhizobium galegae	*Galega*
Rhizobium lupinii	*Lupinus, Ornithopus*
Rhizobium tropici	*Phaseolus, Leucaena*
Sinorhizobium fredii	*Glycine, Vigna, Cajanus*
Sinorhizobium meliloti	*Medicago, Melilotus, Trigonella*
Mesorhizobium loti	*Lotus, Lupinus, Anthyllis, Leucaena*
Mesorhizobium huakuii	*Astragalus*
Bradyrhizobium japonicum	*Glycine, Macroptilium, Vigna*
Bradyrhizobium sp.[b]	*Aeschynomene*
Azorhizobium caulinodans[c]	*Sesbania*
Rhizobium spp.[d]	*Vigna, Arachis, Desmodium, Lotus*
Burkholderia caribensis	*Mimosa*
Burkholderia tuberum[c]	*Cyclopia* spp.
Methylobacterium nodulans	*Crotalaria*

[a]Rhizobium *and* Sinorhizobium *species are relatively fast growing in laboratory culture media.* Bradyrhizobium *species grow more slowly and* Mesorhizobium *species display an intermediate growth rate.*
[b]*Photosynthetic and lacks* nod *genes.*
[c]*Unusual ability to fix* N_2 *in free-living state.*
[d]*Includes strain NGR234, which can nodulate at least 112 legume genera. Sometimes referred to as* Sinorhizobium *sp. strain NGR234.*

The symbiotic state is reached via a 'molecular dialogue' (Dénarié *et al.*, 1993), involving the generation, transmission, recognition and processing of signals by both partners.

Plant secondary metabolites, the flavonoids, are responsible for initiating symbiotic interaction. They are released from legume seed coats or roots in micromolar or even nanomolar amounts and are sensed by free-living rhizobia in the legume rhizosphere and on root hair surfaces. Their principal function is to act, together with NodD proteins, as co-inducers of the nodulation genes of rhizobia in the following manner. In the rhizobia, a flavonoid–protein complex is formed through combination with a constitutively expressed regulatory *nodD* gene product, NodD, which is already bound to conserved DNA sequences (*nod* boxes) in the promoter regions of structural nodulation genes (*nod*, *noe* and *nol*; collectively known as *nod* genes). The presence of a flavonoid at the NodD binding site in the *nod* box activates transcription of these genes. The various proteins produced from the induced *nod* genes of the bacterium act in concert to synthesize and release sensed reciprocal signal molecules – the lipochito-oligosaccharide Nod factors – that are by the plant root. These compounds elicit a number of changes in root metabolism and morphogenesis and they are essential signals for the entry of rhizobia into legume roots (Relić *et al.*, 1994).

The first *nod* gene-inducing flavonoids to be discovered were flavones: luteolin from *Medicago sativa* seed coats (Peters *et al.*, 1986) and 7,4′-dihydroxyflavone from the roots of *Trifolium repens* (Redmond *et al.*, 1986). Subsequently, nearly 30 more compounds have been identified in nine legume genera; they are either glycones or aglycones from a variety of flavonoid subgroups such as chalcones, flavones, flavanones, isoflavones and coumestans (Cooper, 2004); some examples are shown in Fig. 16.3. Most of the legumes analysed release several compounds, and in the case of *Phaseolus vulgaris* 13 *nod* gene inducers have been isolated from aseptically grown plants. The mode of action of flavonoids as co-inducers of *nod* gene transcription has not been completely resolved. There is a strong indication though, that the presence of an appropriate flavonoid alters the angle of bend in the DNA at the points where a NodD protein is bound to the *nod* box site in a *nod* gene promoter region, thereby allowing the RNA polymerase to initiate gene transcription (Chen *et al.*, 2005). Different rhizobia respond to different sets of flavonoid inducers and while in some cases this aspect of interaction has a bearing on host specificity, there is no consistent correlation between the host range of a bacterium and the number of flavonoid inducers to which it is responsive. The *nod* genes of *Rhizobium* sp. NGR234 are induced by many flavonoids and this bacterium has a very broad host range, but *R. leguminosarum* bv. *viciae*, which also responds to many inducers, has a narrow host range.

A few non-flavonoid compounds of plant origin can also induce *nod* gene expression but usually at higher, millimolar, concentrations, and none has been shown to fully substitute for flavonoids in this function. Examples are some betaines from *Medicago* (Phillips *et al.*, 1992) and aldonic acids from *Lupinus* (Gagnon and Ibrahim, 1998). Plant flavonoids not only induce *nod* genes. Transcriptional and proteomic studies have shown a wide range of new and varied regulatory and structural gene expression that is flavonoid inducible (e.g., Kobayashi *et al.*, 2004; Süß *et al.*, 2006; Zhang and Cheng, 2006; Lang *et al.*, 2008). The extensive range of rhizobial responses to flavonoids has been reviewed by Cooper (2004).

Nod factors are lipo-chitooligosaccharides composed of β-1,4 linked *N*-acetyl-D-glucosamine residues with a fatty acyl chain at the non-reducing terminus. The first such compound to be characterized was isolated from *Rhizobium* (now *Sinorhizobium*) *meliloti* by Lerouge *et al.* (1990). Variations on the basic structure arise from several sources: (i) the number of acetylglucosamine residues in the oligosaccharide (chitin) backbone (between 2 and 6); (ii) the type of fatty acid at the non-reducing end (common saturated/monounsaturated or specific highly unsaturated); and (iii) the number and types of substituent groups carried by the molecule (acetyl, arabinosyl, fucosyl, mannosyl, sulphate, etc.). A Nod factor structure is shown in Fig. 16.4 and the *nod* gene products that are needed for Nod factor synthesis are listed in Table 16.2. Extra gene products, NodI and NodJ, are required for secretion of Nod factors from the bacterium.

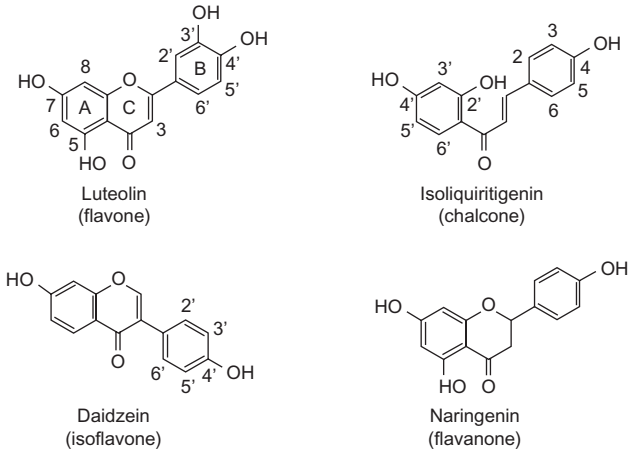

FIGURE 16.3 Rhizobial *nod* gene inducers from four flavonoid sub-classes.

When applied to legume roots in nanomolar or femtomolar concentrations, appropriate Nod factors elicit a number of responses, including: (i) deformation and plasma membrane depolarization of root hairs; (ii) rapid increases then oscillations in intracellular-free Ca (so-called calcium spiking) in root hairs; (iii) pre-infection thread formation in deformed root hairs; (iv) cytokinin-stimulated cortical cell division at incipient nodule sites, and (v) inhibition of the reactive oxygen-generating system. Nod factors, even in the absence of the bacteria that produce them, can induce some of the many plant nodulin genes that are expressed in the pre-infection, infection, nodule development and nodule function phases of the symbiosis (see Colebatch *et al.*, 2004; Barnett and Fisher, 2006; Küster *et al.*, 2007). The interaction between Nod factors and legumes is involved in manifestation of host specificity. For some Nod factors certain features (e.g., sulphation in those produced by *S. meliloti* and an arabinosyl substitution in Nod factors of *Azorhizobium caulinodans*) are needed for nodule formation on the hosts (*Medicago* and *Sesbania*, respectively) (Lerouge *et al.*, 1990; D'Haeze *et al.*, 2000). Substitutions and other aspects of structure, such as the length of the oligosaccharide backbone and the size and degree of saturation of

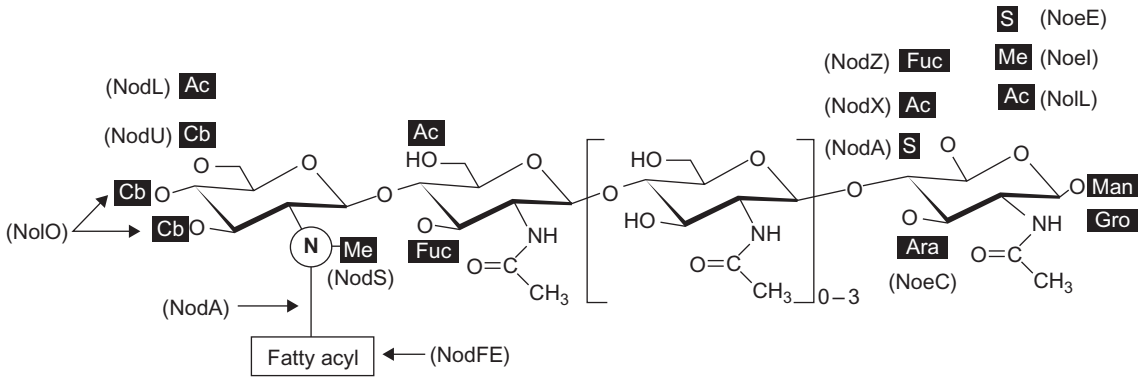

FIGURE 16.4 Composite Nod factor structure showing possible substitutions on the oligosaccharide spine of the molecule (Ac, acetyl; Ara, arabinosyl; Cb, carbamoyl; Fuc, fucosyl; Gro, glycerol; Man, manosyl; Me, methyl; S, sulphate). Nod proteins responsible for structural modifications (e.g. NodA, NoeC, NolO) are indicated where known.

its acyl chain decorations, may also be host range determinants (D'Haeze and Holsters, 2002; Radutoiu *et al.*, 2007; Oldroyd and Downie, 2008). Nevertheless, for many Nod factors there appears to be no correlation between their structure and nodulation of a particular host or group of hosts (Kannenberg and Carlson, 2005). Rhizobia may synthesize more Nod factor variants (>50 in the case of *R. tropici*, which does not have a broad legume host range) than originally thought (Morón *et al.*, 2005). Although the reason for this has not been established, it is unlikely that such a large number of variants of Nod factors can be understood solely in terms of host specificity. Until recently it was assumed that all rhizobia harboured *nod* genes and produced Nod factors, but Giraud *et al.* (2007) identified two unusual strains of photosynthetic bradyrhizobia in which both these properties are absent. They can, nevertheless, nodulate the aquatic legume *Aeschynomene*, perhaps by means of a cytokinin-type signal (Masson-Boivin *et al*., 2009).

TABLE 16.2 Nodulation gene products required for synthesis of Nod factors

Protein	Function
Biosynthesis of glucosamine (chitin) oligosaccharide backbone	
NodM	Glucosamine synthase
NodC	N-acetyl-glucosamine transferase
NodB	Deacetylase, acting at the non-reducing end of glucosamine oligosaccharide
Biosynthesis and transfer of fatty acid moiety at non-reducing terminus	
NodF	Acyl carrier protein
NodE	β-Ketoacyl synthase
NodA	Acyl transferase involved in N-acylation of deacetylated non-reducing terminus of glucosamine oligosaccharide
Modification of non-reducing terminus	
NodS	S-adenosyl methionine methyltransferase
NodU	Carbamoyl transferase
NolO	Carbamoyl transferase
NodL	O-acetyl transferase, O-acetylates at 6-C position
Modification of reducing terminus	
NodP,Q	ATP sulphurylase and APS kinase, provide activated sulphur for sulphated Nod factors
NodH	Sulphotransferase
NoeE	Sulphotransferase involved in sulphation of fucose
NolK	GDP fucose synthesis?
NodZ	Fucosyl transferase
NolL	O-acetyltransferase, involved in acetyl-fucose formation
NodX	O-acetyltransferase, specifically O-acetylates the 6-C of the terminal reducing sugar of the penta-N-acetylglucosamine of R. leguminosarum TOM from Afghanistan pea
NoeI	2-O-methyltransferase involved in 2-O-methylation of fucose

In those partnerships where nodulation is dependent on Nod factors (i.e., all important crop legumes), the nature of the plant receptors for these molecules and the signal-transduction pathways leading to symbiosis-related plant gene activation have been intensively investigated (see Geurts *et al.*, 2005; Mulder *et al.*, 2005; Oldroyd and Downie, 2008; Limpens and Bisseling, 2009) as have genes encoding a symbiosis receptor-like kinase (SYMRK) in *Lotus* (Stracke *et al.*, 2002) and a nodulation receptor kinase (NORK) in *Medicago* (Endre *et al.*, 2002). SYMRK has since been found to be a common element in legumes and non-legumes, whether they form root endosymbioses with rhizobia, *Frankia* or arbuscular mycorrhizal fungi (Gherbi *et al.*, 2008). Genes encoding LysM receptor-like kinases that function upstream of SYMRK, and which could be direct receptors for Nod factors, occur in *Lotus japonicus* (Madsen *et al.*, 2003; Radutoiu *et al.*, 2003, 2007). Likewise in *Medicago truncatula* has receptor-like kinase genes that are encoders of potential Nod factor receptors (Limpens *et al.*, 2003; Arrighi *et al.*, 2006), as well as other genes required for transduction of rhizobial Nod factor signals, but not for mycorrhizal colonization (Amor *et al.*, 2003; Oldroyd and Long, 2003).

Nod factors are not the only rhizobial compounds participating in the molecular dialogue with legumes (Fig. 16.5). Other signal molecules influence, the successful progression to a functioning nodule (Gibson *et al*., 2008). Particularly important are the surface polysaccharides that are found in all rhizobia: extracellular (EPS), lipo (LPS), capsular (KPS) and cyclic glucans. They are involved in various phases of symbiotic development, including root colonization, host recognition, infection thread formation and nodule invasion (Fraysse *et al.*, 2003; Simsek *et al.*, 2007). Additionally, numerous proteins are released by rhizobia which also affect the course of a symbiotic infection (Fauvart and Michiels, 2008). For example, the proteins released a type III secretion system influence legume host range (Bartsev *et al.*, 2004). Signal molecules produced by rhizobia and their functions during root infection have been reviewed by Cooper (2007).

With regard to plant factors other than flavonoids and non-flavonoid *nod* gene inducers, for over 30 years the carbohydrate-binding lectin proteins, found on legume root

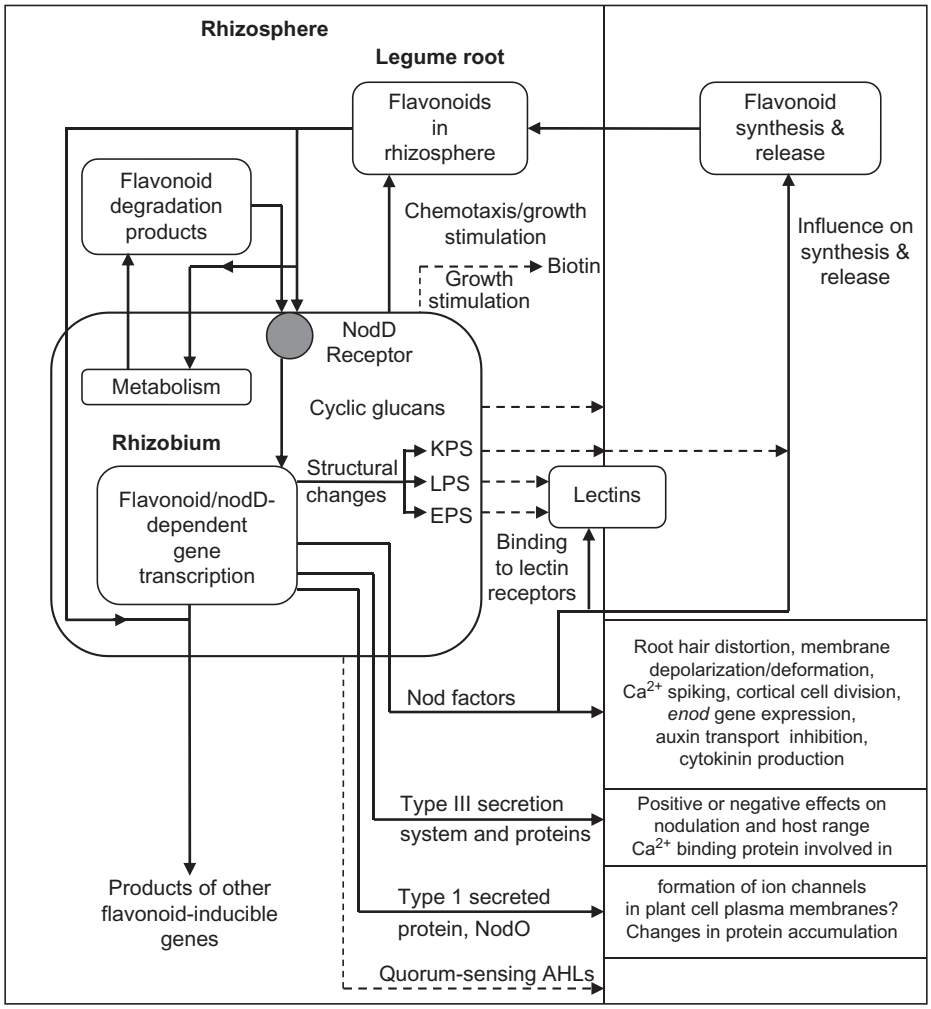

FIGURE 16.5 Early interactions between legumes and rhizobia. Flavonoid-induced rhizobial responses are indicated by a solid line. Some signals (e.g. type III and NodO secretion) are not found in all symbioses. *Modified from Cooper (2004)*.

hair surfaces, have been proposed as determinants of host specificity. However, despite much research on their interaction with rhizobial surface polysaccharides (especially EPS), the precise role of lectins in the infection process has not yet been elucidated (see Hirsch, 1999). Another category of plant proteins, the flotillins, has recently been shown to play a critical role in legume infection by rhizobia (Haney and Long, 2010).

16.4.4 Nodule Formation and Function in Legumes

The mode of infection by rhizobia may be inter- or intracellular or a combination of both (Sprent, 2001; Vessey *et al.*, 2005; Sprent and James, 2007). In many cases, including crop plants such as beans, peas, soybean, lucerne and clover, intracellular invasion involves rhizobia entering via infection threads in root hairs. Coordinated with bacterial infection is a nodule morphogenesis (Oldroyd and Downie, 2008), beginning with root cortical cell division at the sites of nodule primordia and the initiation of a meristem. Following attachment around the tip of a root hair, rhizobia become entrapped in a pocket when the tip curls backwards. Hollow, cylindrical infection threads, constructed by the plant in response to Nod factor develop along the length of the root hair and terminate at nodule primordia in the root cortex (Lhuissier *et al.*, 2001; Gage, 2004) and are colonized by the invading rhizobia. Branches of infection threads penetrate cells of the nodule primordium and release rhizobia into them (with some plant cells in the nodule remaining free of rhizobia throughout the life of the nodule). Once inside a host cell, rhizobia differentiate from Gram-negative motile bacteria into non-motile bacteroids within an organelle-like entity,

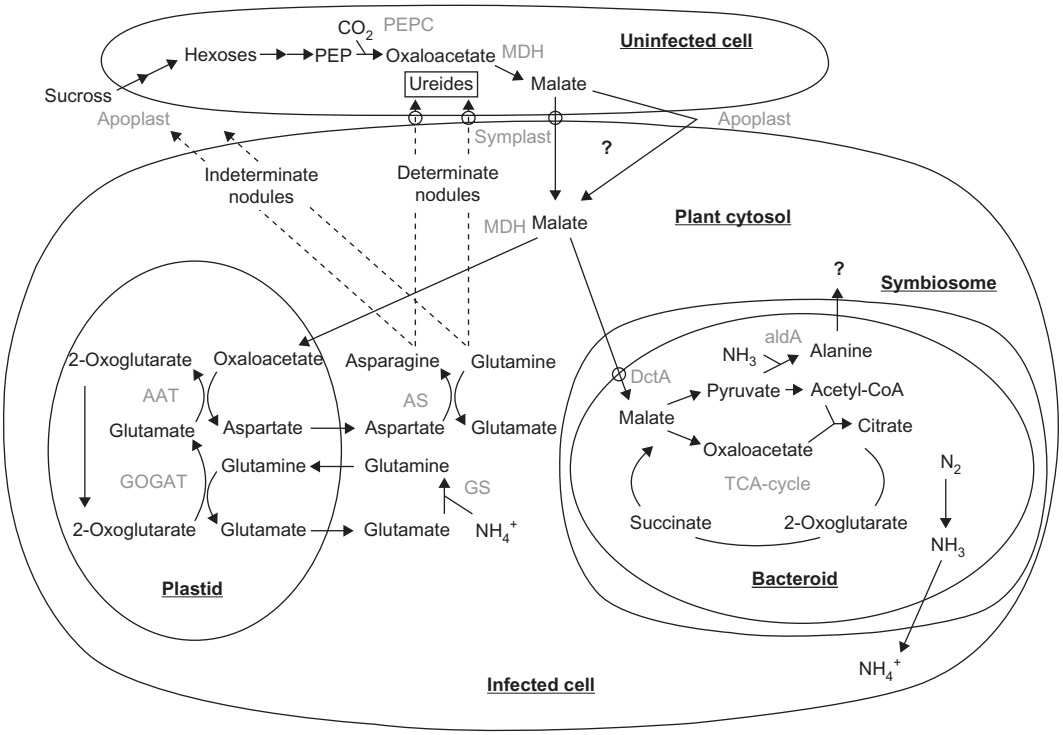

FIGURE 16.6 Proposed scheme for carbohydrate metabolism and export of fixed nitrogen by legume root nodules. *From White* et al. *(2007c). Reproduced with permission of the American Society of Plant Biologists.*

the symbiosome. A symbiosome is surrounded by a symbiosome membrane (derived from endocytosis of bacteroids by the plant plasma membrane) and may contain one or several bacteroids each enclosed in a peribacteroid membrane and each infected nodule cell can be filled with several thousand symbiosomes (Werner, 2007).

The region between the peribacteroid membrane(s) and the symbiosome membrane is termed the symbiosome (or peribacteroid) space (see Fig. 16.6).

Depending on the host plant, nodules may be initiated in the inner or outer root cortex. In crop legumes such as *Medicago*, *Lens*, *Trifolium*, *Pisum* and *Vicia*, nodules are initiated in the inner cortex and are of the indeterminate type, maintaining an active apical meristem and distinct developmental zones, from bacteroid differentiation at the growing tip through mature bacteroids, where N_2 fixation is located, to a senescent zone at the base (see Vessey *et al.*, 2005). Nodules originating in the outer root cortex, as in *Glycine*, *Lotus*, *Phaseolus* and *Vigna*, are of the determinate type; they do not maintain an active meristem and have a more limited lifespan. Another difference between the two types is that bacteroids from determinate nodules can regenerate the free-living form of the bacterium, whereas those from indeterminate nodules are not (Zhou *et al.*, 1985). Host plants supporting indeterminate nodules control bacteroid differentiation by blocking bacterial cell division and force rhizobia towards a terminally differentiated bacteroid state; thus it is the host that dominates this symbiosis (Mergaert *et al.*, 2006; Den Herder and Parniske, 2009).

The root system of a single plant, and perhaps also individual nodules, can be infected by more than one strain of a rhizobium species or biovar (Hagen and Hamrick, 1996). Since not all strains have a high capacity for N_2 fixation, this is a problematic feature of the symbiosis in agricultural applications, as it can cause variability in legume crop yields.

The great potential of nodulated legumes for N_2 fixation is based on three main factors: (i) direct supply of photosynthetically fixed C to bacteroids in the nodules; (ii) effective maintenance of very low O_2 concentrations in the nodule interior to protect nitrogenase; and (iii) rapid export of fixed N via the xylem. A scheme for nutrient exchange in root nodules is shown in Fig. 16.6.

In all legume–rhizobia symbioses, energy substrate for N_2 fixation is derived from photosynthates transported to the nodule cytosol as sucrose in the phloem. After entering uninfected nodule cells, sucrose synthase converts it to monosaccharides; some of these undergo glycolysis to produce phosphoenolpyruvate (PEP) which is carboxylated to oxaloacetate by PEP carboxylase followed by reduction to the C_4-dicarboxylic acid malate by malate dehydrogenase.

Monosaccharides that do not enter the glycolytic pathway are channelled into cellulose or starch synthesis. Malate, together with other C_4-dicarboxylic acids such as succinate and fumarate, is transported from uninfected nodule cells into the cytosol of infected cells and then across the symbiosome and peribacteroid membranes into bacteroids by a dicarboxylic acid transport (Dct) system. In the bacteroids, oxidation in the TCA cycle generates the reducing equivalents and ATP needed for nitrogenase function. Via metabolism outside the bacteroid, malate also provides carbon skeletons required for the assimilation of fixed N in the nodule cytosol. The supply of carbon to nodules and its metabolism in bacteroids have been reviewed by Lodwig and Poole (2003) and White *et al.* (2007c).

Nodules have to be equipped to deal with two seemingly incompatible physiological requisites for N_2 fixation in bacteroids – ensuring a plentiful supply of O_2 for oxidative phosphorylation in order to provide energy for nitrogenase activity, while at the same time, and in more or less the same location, protecting nitrogenase from the damaging effect of O_2. Low O_2 concentrations are achieved in two ways: (i) an O_2 diffusion barrier in densely packed cells in the inner nodule cortex, and (ii) high respiration rates of the bacteroids. The precise mode of action of the diffusion barrier is not known but its permeability to O_2 rapidly adjusts to changes in external O_2 concentration or internal O_2 demand (Vessey *et al.*, 2005). In this barrier of one to five cell layers in thickness, the intercellular spaces can be filled with air or water; since the diffusion coefficient for O_2 in air is about 10^4 times higher than in water, a water barrier is an effective means of limiting O_2 diffusion to the interior of nodules (Blevins, 1989). Oxygen diffusion rates may further be influenced by the path length of intercellular water (Denison, 1992) and intercellular glycoprotein (James *et al.*, 1994, 2000). In this O_2-limited environment, leghemoglobin plays an important role to ensure sufficient O_2 supply to bacteroids. Leghemoglobin is encoded by at least four *lb* genes and constitutes (in mature soybean nodules) about 5% of total nodule protein (Cvitanich *et al.*, 2000). This protein, with a central Fe atom in a porphyrin ring (identical to cytochromes), acts by binding O_2 from intercellular spaces in the infected zone of the nodule and delivering it by diffusion along an oxyleghemoglobin concentration gradient to a high affinity cbb_3-type cytochrome oxidase in the bacteroids (Preisig *et al.*, 1996; Denison and Okano, 2003). The high O_2 consumption of nodules, necessary for provision of energy, can also lead to production of reactive oxygen species (ROS). For protection against their toxicity, rhizobia employ a variety of anti-oxidant defence mechanisms, including ROS scavengers and reductants, which appear to be essential for normal nodule development (Tavares *et al.*, 2007).

Nitrogen fixed in bacteroids diffuses as NH_3 into the acidic symbiosome space. Here it is protonated to ammonium and prevented from being recycled back into the bacteroid by suppression of an ammonium-transporting system (Amt) which otherwise operates in free-living rhizobia (Tate *et al.*, 1998). A channel for monovalent cations transports ammonium across the symbiosome membrane into the host cytosol where it is assimilated into glutamine via the glutamine synthetase (GS)/glutamate synthase (GOGAT) pathway (Vance and Gantt, 1992). Another amino acid, alanine, may be synthesized in bacteroids and then transported to the host cytosol. This is disputed (Li *et al.*, 2002; White *et al.*, 2007), but evidence exists for the exchange (cycling) of some amino acids between the bacteroid and the plant (see Prell and Poole, 2006). Fixed N is delivered via the xylem to the shoots mainly as asparagine in legumes with indeterminate nodules, or as ureides from determinate nodules. Ureides are synthesized in uninfected cells, from asparagine and glutamine received from the cytosol of adjacent infected cells, before being exported in the xylem.

Many attempts have been made to calculate the carbon costs to plants of N_2 fixation versus uptake of mineral N from the rhizosphere. Although the energy costs of fixation may exceed those of uptake by $> 10\%$ d^{-1} (Ryle *et al.*, 1979), such comparisons may be misleading because they do not take into account the economic cost of fertilizer N production, its low use efficiency in agriculture (frequently less than 50% of the amount applied to soil is taken up by the crop) and the environmental costs due to N leaching.

Not all rhiziobia fix high amounts of N_2 once they have nodulated their host legume. N_2-fixing capacities vary substantially between strains of a rhizobium species or biovar ranging from zero (ineffective) to high (effective). For one species, *Rhizobium giardinii*, that nodulates *Phaseolus vulgaris* Depret and Laguerre (2008) noted that all strains so far described were ineffective. For a given rhizobium strain, its effectiveness may vary depending on the genus, species or variety of the host it is nodulating. Therefore high N_2 fixation and yield is frequently not realized even when other conditions for plant growth (e.g., soil pH, availability of water and nutrients other than N) are favourable. Seed inoculation with selected rhizobium strains is sometimes employed in an attempt to ensure effective nodulation of plants in soils whose indigenous strains are poor N_2 fixers. Unfortunately, knowledge of the mechanisms governing inter-strain competition for root infection and nodulation is lacking and inoculant strains chosen for their high N_2-fixing properties will not necessarily outcompete the indigenous rhizobia.

The benefits to a legume from an effective root nodule symbiosis with rhizobium are obvious: a supply of N regardless of soil N status. The benefits to an effective

rhizobium strain, however, are not immediately evident. It can exist and multiply as a free-living heterotroph in soil for decades, even in the absence of a suitable host legume, and it does not have to fix N to survive. In symbiosis it sacrifices a significant percentage of its own respiratory potential to supply its host with N, whereas an ineffective strain can nodulate a host plant without having such demand placed on its own metabolism. Furthermore, the bacteroids of indeterminate nodules lose the ability to replicate and to be released from senescent nodules as free-living bacteria. Indeed, the questions have been posed – 'why do rhizobia fix nitrogen?' (West *et al.*, 2002) and 'why are most rhizobia beneficial to their host plants, rather than parasitic?' (Denison and Kiers, 2004). A possible answer is that the host plant imposes metabolic sanctions on nodules containing weakly effective or ineffective strains of rhizobia. One strategy (particularly in determinate nodules containing replicable bacteroids) could involve restricting O_2 supply to non-fixing nodules, thereby limiting the multiplication of rhizobia within them and reducing the number of viable cells eventually released from senescing nodules (Kiers *et al.*, 2003). It has been argued that such restrictions could counter the evolution of parasitism and stabilize an N_2-fixing symbiosis by favouring the effective rhizobium strains (Denison, 2000; Oono *et al*., 2009). At present, the bulk of the literature on restrictions is of a theoretical nature (studies of pea nodulation (Depret and Laguerre, 2008) and partner choice in the *Medicago truncatula–Sinorhizobium* symbiosis (Gubry-Rangin *et al*., 2010) are recent exceptions) and some of the complexities of nodulation have not yet been taken into account. Among these are: (i) a rhizobium strain may be ineffective on one host but effective on another; (ii) individual root nodules that harbour both ineffective and effective strains exist but their frequency of occurrence is unknown; (iii) ineffective strains sometimes produce far more nodules on a root system than effective ones, thus potentially compensating for host sanctions; and (iv) a host legume may influence rhizobium strain selection without employing restrictions (Gubry-Rangin *et al*., 2010).

16.4.5 Effects of Nutrients other than Nitrogen

Nutrient deficiencies can adversely affect legume root nodule symbioses at a very early stage of development, including multiplication of the microsymbiont in the host plant rhizosphere, its capacity to detect nodulation signals and its ability to produce and excrete Nod factors (McKay and Djordjevic, 1993). The essential nutrients required by rhizobia are those with a direct involvement in the structure and metabolic functioning of the microbial cell (O'Hara, 2001). Subsequently, these elements influence N_2 fixation at various stages of symbiotic interaction: infection and nodule development, nodule function and growth of the host plant. Furthermore, the relative requirement for a given nutrient for plant growth on the one hand and establishment and functioning of the symbiotic apparatus on the other, may differ. For reviews on this topic the reader is referred to Martin (1990), Robson and Bottomley (1991) and O'Hara (2001).

As mentioned earlier in this chapter, chemical signalling between rhizobia and legumes is of fundamental importance for the nodulation process. Phosphorus plays a key role in signalling systems through non-kinase and kinase receptors, and in other metabolic processes requiring phosphorylation steps such as the regulation of intracellular enzymes and the binding of protein to DNA for gene regulation (O'Hara, 2001). Under P stress, rhizobia respond in the same way as the host plant: induction of the expression of genes that are involved in acquisition of P (Sadowsky, 2005).

Phosphorus. Phosphorus has an essential function in the energy metabolism of plants and thus plays an important role in N_2 fixation due to the high ATP demand from the nitrogenase reaction. P deficiency, has a negative impact on the energy status of legume nodules (Olivera *et al.*, 2004). Transformation of ammonium in the GS/GOGAT cycle and further transformations into amino acids or ureides are also energy-consuming processes.

The synthesis of nucleic acids and phospholipids is also dependent on P (O'Hara, 2001). Compared with plants receiving mineral N fertilization, N_2-fixing plants need more P due to the development of nodules and associated signal transduction pathways, and to phospholipids in bacteroids (Graham and Vance, 2000). This requirement may be greater than for root or shoot growth of the host plant. The minimum P concentration for nodulation is about $0.5\,\mu g\,P\,l^{-1}$ in the external solution. An increase from 200 to $500\,\mu g\,P\,l^{-1}$ results in a greater increase in nodule dry weight relative to shoot and root dry weight (Cassman *et al*., 1980) (Table 16.3).

Nodules are a strong sink for P; the P concentration is usually considerably higher (up to three-fold) in nodules than in roots and shoots, particularly when external P supply is low (Adu-Gyamfi *et al.*, 1989; Hart, 1989). The ability of developing nodules to compete with other vegetative sinks (root and shoot meristems) for P when external supply is limited differs among legume species. This may be partly responsible for disagreements concerning the amounts of P needed for nodulation (Jakobsen, 1985; Robson and Bottomley, 1991). Rhizobia have high-affinity active uptake systems for P for acquiring phosphate from the external environment (Al-Niemi *et al.*, 1997; O'Hara, 2001). High P consumption during nodulation can, to some extent, be satisfied by mycorrhizal colonisation of the legume (Reinhard *et al.*, 1993). Tripartite symbioses

TABLE 16.3 Relationship between P supply and dry weight of roots, nodules and shoots in soybean

	Dry weight (g plant^{-1})		
P supply (μg l^{-1})	Roots	Nodules	Shoots
0.5	0.60	0.07	1.21
20	0.76	0.10	1.55
50	0.79	0.16	1.86
200	1.23	0.35	4.22
500	1.35	0.64	6.57

Based on Cassman *et al.* (1980).

TABLE 16.4 Number of nodules and nodule dry weight in three soybean genotypes at different P supply

Genotype	P rate (mg P kg^{-1} soil)			
	0	30	60	90
	Nodule number plant^{-1}			
Chippewa	32.1	33.2	35.0	38.4
Bragg	73.4	81.3	86.0	74.1
nts 382	387.3	350.2	449.2	661.1
	Nodule dry weight (mg plant^{-1})			
Chippewa	93.0	118.3	139.0	144.0
Bragg	185.3	234.7	266.3	278.3
nts 382	529.0	573.0	697.3	780.3

Based on Gunawardena *et al.* (1993).

between legumes, rhizobia and mycorrhizal fungi are common (and perhaps the norm). Mycorrhizal colonization increases the acquisition of P by plants grown in low P soils (Chapter 15) Nodulation can also be improved either by application of P fertilizer (Abbasi *et al.*, 2010).

Shoot N and P concentration of legumes are positively correlated (Kuang *et al*., 2005). Therefore, when legumes that are dependent on N_2 fixation receive an inadequate supply of P, they may also suffer from N deficiency. At low P availability, P addition increases root nodule number (Abbasi *et al.*, 2010), nodule mass (Gunawardena *et al*., 1993) (Table 16.4), nodule size (Kuang *et al*., 2005) and yield (Dadson and Acquaah, 1984). However, P application mainly affects the total N uptake rather than the percentage derived from the atmosphere (%N dfa) Somado *et al*. (2006).

Calcium. Calcium is particularly important for early infection events (Munns, 1970). A low nodule Ca concentration reduces N_2 fixation due to inadequate Ca for nodule structure (Banath *et al.*, 1996). High Ca supply increases the number of nodules (Lowther and Loneragan, 1968) and the amount of *nod* gene-inducing compounds in root exudates (Richardson *et al*., 1988a). In the nodulation signalling pathway, Ca plays an essential role as a secondary messenger, via a unique Ca-activated kinase (Oldroyd and Downie, 2006). Some of the negative effects of soil acidity on legume nodulation are linked to restricted Ca availability.

Sulphur. Deficiency of the S-containing amino acids cysteine and methionine may restrict the nutritional value of the seeds for food and forage (Sexton *et al*., 1998). Sulphur deficiency may also decrease N_2 fixation by affecting nodule development and function (Pacyna *et al*., 2006), reduce leghemoglobin concentration in nodules (Singh and Raj, 1988; Pacyna, 2005) and lower ATP concentrations in bacteroids as well as in mitochondria of root nodules (Scherer *et al*., 2008) (Fig. 16.8) The latter may be caused by low carbohydrate supply to nodules by S-starved legumes (Scherer *et al*., 2006). Sulphur is important for nitrogenase activity because the smaller of the two oxygen-sensitive non-heme iron proteins contains a single Fe_4S_4 unit (Jeong and Jang, 2006). Besides nitrogenase, the activities of other important enzymes involved in N_2 fixation, such as PEP-carboxylase, malate dehydrogenase or glutamate synthase, are also reduced when S supply is inadequate (Lange, 1998).

Sulphur application on S-deficient soils may not only increase the quality of grain legumes, but also the number of root nodules and nodule weight (Scherer *et al*., 2006) (Fig. 16.7) and nitrogenase activity (Lange, 1998).

Molybdenum. Since Mo is a metal component of nitrogenase, legume–rhizobia symbioses have a high Mo

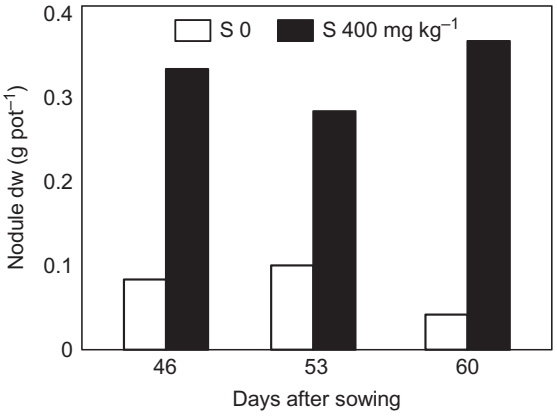

FIGURE 16.7 Nodule dry matter without and with S supply. *Based on Scherer* et al. *(2006).*

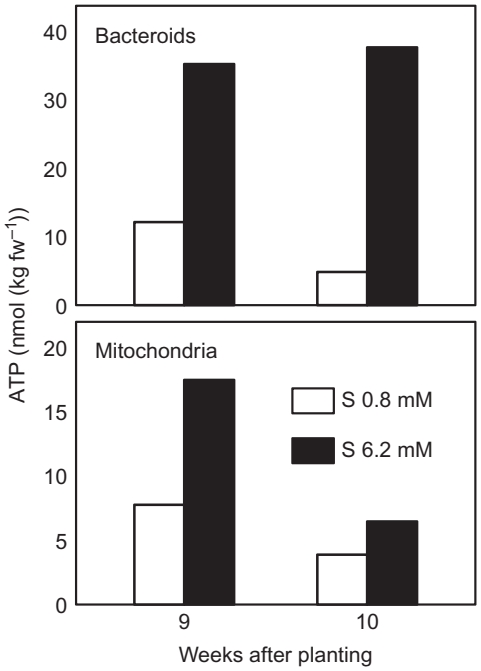

FIGURE 16.8 ATP concentrations of bacteroids and mitochondria. *Based on Scherer* et al. *(2008). With kind permission from Springer Science and Business Media.*

requirement (Bambara and Ndakidemi, 2010). Although Mo is not specifically needed for nodulation, Mo deficiency affects nodule development by reducing bacteroid multiplication (O'Hara, 2001), even though, at low supply, this element is preferentially transported into the nodules (Brodrick and Giller, 1991a). Molybdenum deficiency-induced N deficiency in legumes relying on N_2 fixation is widespread, particularly in acid mineral soils of the humid and subhumid tropics. Under acidic conditions, seed pelleting or treatment of soil with Mo can increase of N_2 fixation rates in legumes, as shown in Table 16.5. Seed pelleting with 100 g Mo ha^{-1} increased nitrogenase activity, leaf N content and, particularly, nodule dry weight, whereas mineral N addition decreased nodule dry weight and suppressed nitrogenase activity compared with plants supplied with P only. In acidi soils, application of lime and Mo can enhance the formation of root nodules and seed yield by 370% (De Oliveira et al., 1998). At maturity, beans receiving mineral N had a higher shoot but lower pod dry weight compared with N_2-fixing plants supplied with Mo. This lower harvest index in the plants fertilized with mineral N was most likely the result of higher water consumption and a more severe drought stress during early pod-filling (Hafner et al., 1992). Thus, under certain ecological conditions, supplying as little as 100 g Mo ha^{-1} may not only enhance N_2 fixation, total N uptake and drought tolerance but also increase pod yield more than an application of 60 kg N ha^{-1} as mineral fertilizer (Table 16.5).

Iron. Iron is an essential nutrient for both legume and its root nodules. In N_2 fixation it is a component of several enzymes such as nitrogenase, the electron carrier ferredoxin, leghemoglobin and several hydrogenases (Abdelmajid *et al*., 2008). The heme component of leghemoglobin has a particularly high Fe requirement. Therefore, Fe is needed in greater amounts for nodule formation (Table 16.6) than for host plant growth (Abdelmajid *et al*., 2008). Although Fe deficiency does not significantly affect shoot growth in peanut (*Arachis hypogaea*), it severely decreases nodule mass, leghemoglobin concentration, number of bacteroids and

TABLE 16.5 Nodulation, nitrogenase activity, dry weight and N content of groundnut (*Arachis hypogaea*) grown in an acid sandy soil with or without N fertilizer (2 × 30 kg N ha^{-1} as NH_4NO_3) and molybdenum seed pelleting (100 g Mo ha^{-1} as MoO_3)

	Early podfilling		Maturity			
				Dry wt (kg ha^{-1})		
Treatment	Nodule dry weight (mg plant^{-1})	Nitrogenase (μmol C_2H_4 g^{-1} nodule fw)	Leaf N content (mg (g dw^{-1}))	Shoots	Pods	N uptake (kg ha^{-1})
+P[a]	80	50	25	861	1,570	77
+P + N	70	43	33	1,817	1,783	110
+ P + Mo	180	60	37	1,380	1,948	119

Based on Hafner *et al.* (1992).
[a] *16 kg P ha^{-1} as single superphosphate.*

nitrogenase activity, compared with plants treated with foliar Fe. Unlike peanut, in lupin (*Lupinus angustifolius*) Fe is not transported from the leaves to the nodules after a foliar spray, and direct Fe supply at the infection sites on roots is required for satisfactory nodulation (Tang *et al*., 1990, 1992c). Iron deficiency does not impair infection *per se* but the further division of cortical cells, i.e. the early stages of nodule development and the proliferation of invading rhizobia in root tissue (Tang *et al*., 1992a). High bicarbonate concentrations induce visual symptoms of Fe deficiency (chlorosis) and decrease net photosynthesis in many dicotyledonous plants. Legumes are further adversely affected by depressed nodulation and N_2 fixation (Tang *et al.*, 1991). For example, in peanuts grown on alkaline soils Fe deficiency arrested nodule development and delayed or even prevented nitrogenase synthesis (O'Hara *et al*., 1988b).

TABLE 16.6 Nodule dry weight and symbiotic N_2 fixation in two common bean (*Phaseolus vulgaris* L.) varieties (Coco blanc and ARA 14) grown in a soil with low Fe availability with and without Fe supply

	Nodules (g plant^{-1})	Symbiotic N_2 fixation (mmol plant^{-1})
Coco blanc		
+ Fe	0.34	1.04
− Fe	0.17	0.55
ARA 14		
+ Fe	0.45	1.08
− Fe	0.36	0.82

Based on Abdelmajid *et al.* (2008).

Boron. Legumes require B in relatively high concentrations for nodule development. The B concentration in nodules is about four to five times higher than in roots (Carpena *et al*., 2000) and an absence of B in the rooting medium leads to decreased nodulation and altered nodule development (Bolanos *et al*., 1994). Boron is required for the development of infection threads and nodule cell invasion. In absence of B, the binding of rhizobial cell surfaces to the infection thread wall is inhibited, bacteria cannot progress through the infection thread and are unable to reach the endophytic environment. Boron deficiency also causes abortion of infection threads as well as degeneration of cell walls and the membranes surrounding the intracellular bacteroids (Bolanos *et al*., 1996). These impairments to nodule development result in decreased N_2 fixation and necrosis.

Cobalt. Severe Co deficiency reduces infection and retards nodule formation whereas nodule growth rate is not affected by Co supply (O'Hara *et al*., 1988a). Moreover, Co is required for the synthesis of leghemoglobin. Cobalt deficiency affects nodule development and function in various ways (Table 16.7). For example, in lupins relying on symbiotic N_2 fixation, Co deficiency depresses host plant growth but not nodule mass, which even increases (Riley and Dilworth, 1985). The most sensitive indicator of Co deficiency is the bacteroid content of nodules. Whereas the synthesis of leghemoglobin is enhanced by Co supply, the increase in activity of nitrogenase per unit of leghemoglobin is only relatively small.

Nickel. Although Ni is a constituent of a number of uptake hydrogenases, and lower hydrogenase activity has been found in bacteroids isolated from Ni-deficient soybean plants (Klucas *et al.*, 1983), evidence is lacking that under field conditions N_2 fixation is impaired by Ni deficiency.

TABLE 16.7 Nodule development and function in *Lupinus angustifolius at different* Co supply

	Co supply (mg $CoSO_4 \cdot 7H_2O$ (6 kg^{-1} soil))				
Parameter	0	0.01	0.05	0.10	0.50
Foliage mass (g fw plant^{-1})	5.0	6.1	7.5	9.6	14.0
Nodule mass (g fw plant^{-1})	2.9	2.8	2.5	2.3	1.1
Bacteroid content (no. × 10^9 per nodule)	6.0	12.0	12.5	20.5	22.5
Leghemoglobin content (nmol g^{-1}lateral root fresh weight)	–	1	11	20	120
Nitrogenase activity (nmol C_2H_2 reduced g^{-1} nodule fresh wt min^{-1})	10	21	58	104	172
nmol C_2H_2 reduced (nmol^{-1} leghemoglobin min^{-1})	1.1	2.5	3.7	3.8	3.2

Recalculated from Riley and Dilworth (1985).

16.4.6 Effect of Mineral Nitrogen

The effect of soil mineral N (from the soil or fertilizers) on BNF is well documented (Peoples and Baldock, 2001). In legumes (and other symbiotic N_2-fixing systems), mineral N can enhance or depress N_2 fixation, depending on a range of factors and the rate of N supply in particular. As shown schematically in Fig. 16.9 increasing the supply of combined N (soil + fertilizer N) results in an asymptotic increase in total N per plant. The enhancing effect of low levels of combined N on N_2 fixation in legumes is related to the lag phase between root infection and the onset of N_2 fixation. Nitrogen deficiency during this phase is detrimental to the formation of a source leaf area that is sufficiently large to supply the photosynthates needed for nodule growth and activity. At zero or very low levels of combined N, the enhancing effect of fertilizer N (Fig. 16.9) depends on the N reserves in seeds. As a rule, the highest nodulation and nodule activity (N_2 fixation) is therefore obtained when the seed N reserves and mineral N, either from the soil or fertilizers, are available in amounts that are sufficient for vigorous plant growth during the first weeks of legume establishment. Low rates of mineral fertilizer, supplied as starter-N, increased nodulation of soybeans and total amount of N derived from N_2 fixation, but high rates drastically decreased nodulation and inhibited N_2 fixation (George *et al.*, 1992b). However, according to Hungria *et al*. (2005), starter-N rates as low as 20–40 kg of N ha^{-1} may decrease both nodulation and N_2 fixation under Brazilian conditions, with no benefits to yield.

When the concentrations of combined N increase, nitrogenase activity and nodule numbers decrease (Scherer and Danzeisen, 1980). Shoot growth, on the other hand, continues to increase, indicating a shift from symbiotic to inorganic N nutrition. The highest N content in the shoot coincides with the highest nitrogenase activity but not with the highest dry weight of the plants. This suggests that at maximum N_2 fixation dry matter production was source-limited (photosynthate supply) and the diversion of some photosynthates to N_2 fixation may be can restrict plant growth. The extent to which nodulation and nodule activity are reduced at high levels of combined N is dependent on the plant genotype and form of N supplied. It is generally agreed that infection and nodule development are more sensitive to nitrate than ammonium. In pea, continuous supply of moderately high levels of ammonium-N (1 mM) may not only increase nodulation and N_2 fixation, but even stimulate the proliferation of small nodules, (Waterer *et al*., 1992). Although a high nitrate supply generally depresses nodulation, marked genotypic differences in nitrate sensitivity exist. As shown by Harper and Gibson (1984), high nitrate supply inhibits nodulation much more in soybean than in chickpea or lupins, whereas nitrogenase activity is severely inhibited in chickpea and lupins but only slightly affected in subterranean clover. In common bean, differences exist even between cultivars with regard to inhibition of nodulation by high nitrate supply (Martin, 1990). In actinorhizal plants, genotypical differences among, species in nitrate sensitivity are partly related to the mode of root infection. Infections may occur in root hairs (e.g., in *Alnus glutinosa*) or at sites of lateral root emergence (e.g., in *Elaeagnus angustifolia*). High nitrate concentrations depressed nodulation in species with root hair infections, but not in those displaying the other type of infection (Kohls and Barker, 1989).

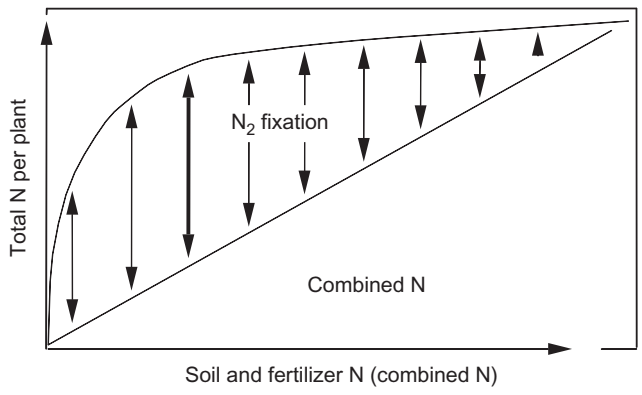

FIGURE 16.9 Simplified scheme of the relationship between N_2 fixation and N uptake from soil and fertilizer in nodulated legumes.

The regulation of root metabolism to ensure that plants are supplied with adequate amounts of N is known to involve feedback systems, such that the N status of the whole plant influences root growth, transport activity and, in the case of legumes, nodule growth and activity (Parsons and Sunley, 2001, see at Chapter 6.1). Although the precise signals that transmit plant N status to the root nodules are still unknown, that N-rich amino acids are transported from the shoot play a role in regulating nodule formation and function (Baker *et al*., 1997). However, knowledge of the exact mechanisms of plant sensing and signalling in relation to N status remains rather poor. Sensing may occur in cells in the shoots and signals could be communicated to the roots and nodules via the phloem (Parsons and Sunley, 2001). These signals appear to be operating in a quantitative manner, allowing N uptake and N_2 fixation to be matched accurately to demand.

The inhibitory effect of nitrate on nitrogenase activity in established nodules may operate at several levels: (i) a reduction of nitrate in the nodules could lead to competition for reducing equivalents and malate (Heckmann *et al.*, 1989), (ii) nitrite toxicity (Becana *et al.*, 1985), and (iii) O_2 deficiency (Vessey *et al.*, 1988). The last two factors are considered to be particularly relevant. Nitrate supply induces nitrate reductase activity in bacteroids fairly rapidly, but in some strains of rhizobia nitrite reductase

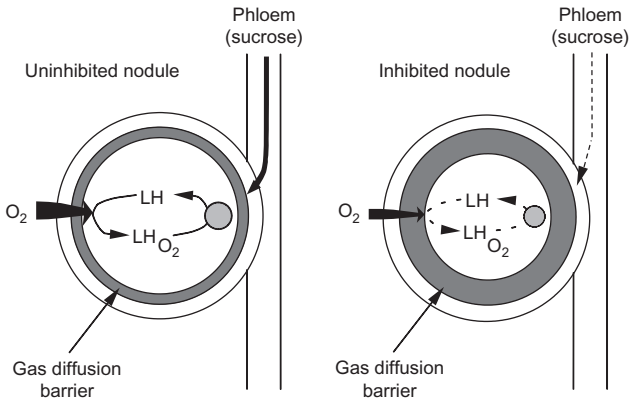

FIGURE 16.10 Model of possible mechanisms of inhibition of N_2 fixation rate by O_2 limitation of nitrogenase activity. *Modified from Vessey and Waterer (1992).*

is induced after a considerable delay, leading to the accumulation of nitrite (Arrese-Igor *et al.*, 1990), which can directly inactivate leghemoglobin through the formation of nitrosylleghemoglobin (Kanayama and Yamamoto, 1991). Inhibition of nitrogenase by nitrate may also be linked to O_2 deficiency since the effect can be alleviated by increasing the O_2 partial pressure in the rhizosphere (Vessey *et al.*, 1988). Similarly to stem girdling and defoliation, nitrate also increases by several fold the resistance of nodules to O_2 diffusion (Fig. 16.10) (Vessey *et al.*, 1988). This is probably due to a reduction in phloem import of photosynthates and other solutes and, consequently, a decrease in osmotic pressure in the nodules (Vessey and Waterer, 1992).

16.4.7 Environmental Effects

The rhizosphere environment strongly affects the symbiotic interaction between rhizobia and their host legumes. Favourable conditions for plant growth and the establishment of bacterial populations enhance inoculum success and promote the development of infection sites on root hairs (El-Hamdaoui *et al*., 2003). Therefore, soil factors that influence plant and rhizobial growth, such as acidity, alkalinity, salinity, temperature, moisture, fertility (including nutrient deficiencies) and physical structure (Slattery *et al.*, 2001) will also affect infection and nodulation.

Salinity. Most legumes are classified as salt-sensitive crops (El-Hamdaoui *et al*., 2003) and the effects of salt stress on N_2 fixation in these plants have been widely reported. For a review on this topic the reader is referred to Zaran (1999). Salinity can affect the symbiosis directly by reducing the growth of the host plant or indirectly by impairing interactions between rhizobium and host leading to inhibition of nodule formation (Anthraper and DuBois, 2003). Under saline conditions, bacterial attachment to the roots is reduced and root hairs do not show the characteristic response to Nod factor, i.e. root hair deformation and curling (El-Hamdaoui *et al*., 2003). Salt stress also inhibits bacterial invasion and proliferation inside the host cells, which can be alleviated by supplementing inoculated legumes with B and Ca (Bolanos *et al.*, 2006). However, these effects vary among legume species, with common bean, for example, being more salt sensitive than soybean and alfalfa (Serraj *et al*., 1998).

Soil water content. In arid regions, poor nodulation of legumes is most likely due to death of rhizobia during the dry season. Soil water content also influences the growth of rhizobia by altering plant growth, root architecture and root exudation (Sadowsky, 2005). The timing of the drought stress relative to growth stage of the plant has an important effect on nodulation and N_2 fixation and an extended period of stress during the vegetative stage retards both processes. Once nodules are established, drought reduces N_2 fixation (Pena-Cabriales and Castellanos, 1993). The effect of drought on nodule activity is mainly due to an increased resistance to O_2 diffusion into the bacteroids (Durand *et al*., 1987).

To equilibrate external and internal osmotic concentrations, salt-tolerant rhizobia accumulate compatible organic or inorganic solutes. *S. meliloti* overcomes water stress-induced growth inhibition by accumulating solutes such as glutamate or proline (Smith *et al*., 1988). In *Glycine max* L., decreased N_2 fixation at low water availability is associated with increased concentrations of ureides and free amino acids in plant tissue, indicating a potential feedback inhibition by these compounds in response to drought (King and Purcell, 2005).

On the other hand, the impact of anaerobic conditions on N_2 fixation seems to be less pronounced: in a periodically flooded forest of the central Amazon floodplain no differences in N_2 fixation by various legumes were found between the dry and flooded phase (Kreibich *et al*., 2006).

Temperature. High temperatures impair the survival and persistence of rhizobial strains in soils and also root infection (Sadowsky, 2005). Furthermore, elevated temperatures may affect the production or release of *nod* gene inducers (Hungria and Stacey, 1997), alter the functioning of nodules through changes to leghemoglobin synthesis and nitrogenase activity, and accelerate nodule senescence (Hungria and Vargas, 2000). However, there are high-temperature (40°C) tolerant rhizobia (Hungria *et al*., 1993). In *Trifolium repens*, N_2 fixation decreased with decreasing root temperature (Bouchart *et al.*, 1998), being four-fold and two-fold higher at 12°C than at 6 and 9°C, respectively. Sadowsky (2005) suggested that every legume/rhizobium combination has a specific optimum temperature, which is between 25 and 30°C for common bean, about 30°C for peas and clover and between 35 and 40°C for cowpea, peanut and soybean.

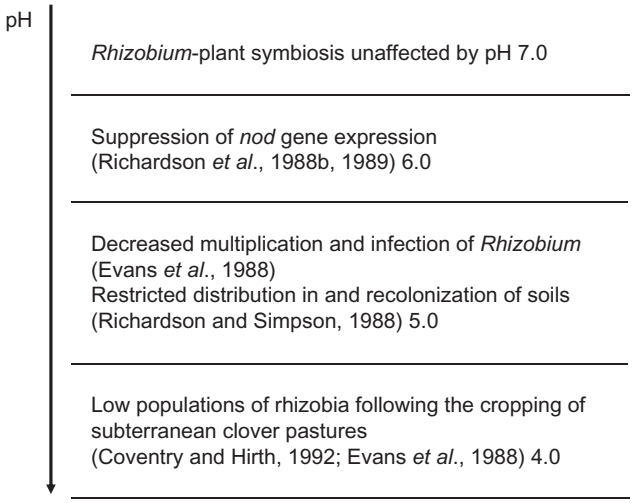

FIGURE 16.11 Activity of *Rhizobium leguminosarum* bv. *trifolii* in association with subterranean clover at different pH. *Modified from Slattery* et al. *(2001)*.

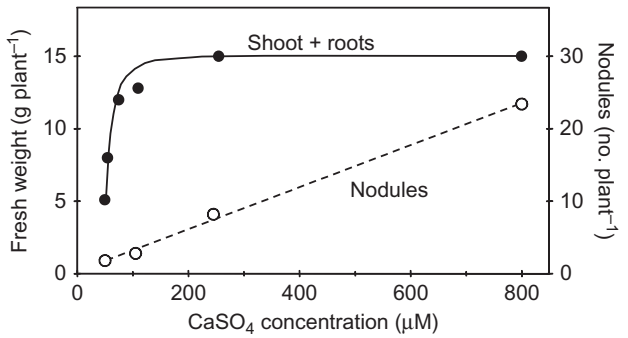

FIGURE 16.12 Fresh weight and nodule number in subterranean clover at different Ca concentrations in nutrient solution (pH 5.0). *Based on Lowther and Loneragan (1968)*.

Acidity. Acidity affects free-living rhizobia and N_2 fixation (Fig. 16.11) (Slattery *et al.*, 2001). In acid soils, rhizobia density can be low (Coventry and Hirth, 1992; Schubert *et al*., 1990a). Increasing soil pH by liming is therefore very effective in increasing nodule number, for example in common bean (Buerkert *et al*., 1990), lucerne (Pijnenberg and Lie, 1990) and peanut (Angelini *et al*., 2005). In acid soils, various factors such as high concentrations of H^+, monomeric Al (Alva *et al.*, 1990) and sometimes micronutrients (Campillo *et al.*, 2005) that become more soluble at low pH, may contribute to poor nodulation and inhibition of plant growth (Hungria and Vargas, 2000). Moreover, Ca and P availability are adversely affected by low soil pH and may also influence the growth and survival of rhizobia (Sadowsky, 2005). As shown in Fig. 16.12 nodule formation has a greater requirement for Ca than root and shoot growth of the host plant. However after nodule initiation, further nodule growth was not affected by a decrease in Ca concentration (Lowther and Loneragan, 1968a,b), suggesting that only the first step of infection is highly sensitive to Ca supply. At low Ca concentrations, particularly in combination with high proton concentrations, the attachment of rhizobia to the host root surface is impaired. In contrast to acid-sensitive species, the root exudates released by tolerant *Medicago* species at low pH and low Ca concentrations are effective in inducing *nod* gene expression (Howieson *et al.*, 1992).

Poor nodulation in acid soils can be caused by low survival of rhizobial strains (Howieson *et al.*, 1988), as well as effects on root morphology. Inhibition of root hair formation by low concentrations of Ca (Ewens and Leigh, 1985) and high concentrations of Al and H^+ (Franco and Munns, 1982) may explain impaired nodulation in species where root hairs are the dominant infection sites.

In acid soils, the net release of H^+, i.e. rhizosphere acidification, inherently coupled with N_2 fixation of legumes and nodulated non-legumes (see chapters $$ and 14), may exacerbate the negative impact of acidity. Depending on the legume species between 37 and 49 mg H^+ are formed per gram of fixed N, amounting to an annual production per hectare of 4.6 kg H^+ in sweet clover (*Melilotus alba*) and 15.2 kg H^+ in lucerne (Lui *et al.*, 1989). Soil pH was lowered from 6.0 to 5.6 by faba beans during a cultivation period of 45 days (Yan *et al.*, 1996). Only in soils with high $CaCO_3$ concentrations will this acidifying process not be harmful, because the H^+ released by roots will be immediately neutralized (Mengel, 1994a). Otherwise, on average an equivalent of 80 to 96 kg of lime ($CaCO_3$) is required to neutralize the acidity formed in the production of one ton of legume dry matter (Jarvis and Hatch, 1985).

16.5 AMOUNTS OF N FIXED BY LEGUMES, AND ITS TRANSFER TO OTHER PLANTS IN MIXED STANDS

BNF is a renewable resource, which may reduce requirement for N fertilizers. In agricultural systems, legumes are commonly used as a source of N for a following, non-legume crop and for maintaining soil N levels (Glasener *et al*., 2002). This type of application is particularly important in the humid tropics where use of N fertilizers is not economically feasible due to poor market and infrastructure development (Palm and Sanchez, 1991). However, both the total amount of N_2 fixed and the proportion of plant N from to fixation vary greatly. In some cases nearly all the N recovered in plants appears to be derived from atmospheric N_2. According to Werner (2005) N_2 fixation by legumes is in the range 24–250 kg N ha^{-1} per season

TABLE 16.8 Average N_2 fixation of different legumes

Species	Fixation (kg N ha^{-1} per season)
Arachis hypogaea	100
Cajanus cajan	91
Calliandra calothyrsus	24
Cicer arietinum	135
Crotolaria grahamina	142
Glycine max	100
Lens culinaris	80
Lupinus sp.	150
Macroptilium atropurpureum	64
Medicago sativa	250
Pisum sp.	150
Trifolium sp.	250
Vicia faba	200

Based on Werner (2005).

TABLE 16.9 N Transfer from *Trifolium alexandrinum* to *Zea mays* by mycorrhiza (AM)

	N content (mg $plant^{-1}$)	
	T. alexandrinum	*Z. mays*
Z. mays without AM	–	13.1
Mycorrhizal *T. alexandrinum;* AM colonizing *Z. mays*	50.0	15.8

Based on Frey and Schüepp (1992).

(Table 16.8), while Braun et al. (2010) reported values up to 340 kg N ha^{-1} per season. Inherent differences in biomass production, effectiveness of rhizobial strains and environmental factors such as variable soil fertility are responsible for this wide spectrum of values. On average, between 20 and 25 kg of shoot N are fixed per tonne of legume herbage dry matter produced across a broad range of environments (Peoples and Baldock, 2001).

As sole crops, legumes usually contain a lower and more variable proportion of fixed N than when grown in mixtures with non-legumes (provided the mixture is dominated by a legume) (Mallarino *et al.*, 1990a). As mentioned above, high concentrations of mineral N can depress N_2 fixation in legumes. Nitrogen uptake by an intercropped non-legume can reduce the mineral N content of soil to the extent that symbiotic N_2 fixation can be higher than it would be for a legume grown in monoculture (Waterer *et al*., 1994; Xiao *et al*., 2004; Hauggaard-Nielsen *et al.*, 2009). In various *Phaseolus vulgaris* genotypes, N derived from N_2 fixation may vary between 5.6 and 21.1% in monoculture, and 18.2 and 56.6% when intercropped with maize (Tsai *et al.*, 1993). In temperate climate in mixed stands of *Trifolium* spp. with *Lolium* spp., annual N_2 fixation has been estimated to be in the order of 232 to 308 kg N ha^{-1} (ca. 75–86% of the total plant N) and may even reach 390 kg N $(ha\,y)^{-1}$ in mixed stands of *Trifolium* spp. and *Festuca arundinacea* (Mallarino *et al.*, 1990b). In addition to soil nutrient concentrations, environmental factors and rhizobium strain parameters, the amount of N fixed depends on the species, morphology and density of the legume component in the mixture (Ofori and Stern, 1987).

Nitrogen fixed by the legume in a mixed crop may be available to the associated non-legume in the current growing season or as residual N for the succeeding crop (Ofori and Stern, 1987). Both possibilities are important for enhancing the N economy of various legume-based systems (Ofori and Stern, 1987). The degree to which N from a legume benefits an associated crop depends primarily on the amount of N fixed, and also on the decomposition rate, its residues and the amounts of mineral N released from them. In mixed stands of legumes and non-legumes, direct transfer of fixed N to the non-legune may occur during the growing season. In a cowpea–maize combination planted without N fertilizer, Remison (1978) attributed a 72% increase in intercropped maize grain yield (over that of a sole maize crop) to the transfer of N from cowpea. A network of arbuscular mycorrhiza hyphae can link the roots of neighbouring plants and may be involved in the transfer of N from legumes to non-legumes (Frey and Schüepp, 1992) (Table 16.9). However, the amounts transferred may be relatively small, most likely $<10\%$ of the total N fixed (Morris *et al.*, 1990). There was no direct N transfer from pigeon pea to sorghum (Tobita *et al*., 1994) or to non-mycorrhizal mustard (Waterer *et al*., 1994).

Part of the N fixed by legumes remains in the soil as root residues and nodules or is returned as litter fall. In annual species, some fixed N becomes available for the next crop. In field-grown lupin more than 80% of the N requirement can be derived from N_2 fixation (Herridge and Doyle, 1988) with amounts fixed exceeding the N in harvested seeds. Therefore the increased soil N concentrations after growing this legume contribute to the higher yields of cereals grown in rotation with it. Incorporation of legume residues into soil initially causes faster and more complete decomposition and release of N in comparison with surface placement (Varco *et al*., 1993), but Ladd *et al*. (1981) found that wheat recovered, on average, only 14% of the N in residues of *Medicago littoralis* that were incorporated

into three different soil layers under field conditions. Yaacob and Blair (1980) noted that recovery of forage legume N increased from 13.4 to 55.5% as the number of years the soil was previously cropped with legumes increased from one to six. The release of mineral N from plant material in the soil can be increased by C rhizodeposition from plant roots due to stimulation of microbial activity (Ayres *et al*., 2007). Another benefit of legume cultivation is increasing soil organic N content which conserves N for use by subsequently planted crops.

16.6 SIGNIFICANCE OF FREE-LIVING AND ASSOCIATIVE NITROGEN FIXATION

Free-living N_2-fixing microorganisms are ubiquitous in soils. However, because of carbon limitation, especially in non-rhizosphere soil, amounts of N_2 fixed by the chemoheterotrophs among them are usually very small ($<1\,kg\,N\,ha^{-1}\,y^{-1}$), even if the process is otherwise favoured by low concentrations of mineral N. Transfer of fixed N to plants occurs mainly, after mineralization of organic N in dead microbial cells. Adding plant residues with high C/N ratios to soils may temporarily encourage higher rates of N_2 fixation, but these are still very low compared with those in symbiotic systems. Carbon supply is greater in the vicinity of roots due to rhizodeposition (Chapter 14). Using a mathematical model, Jones *et al.* (2003b) estimated that rhizodeposition could support the fixation of between 0.2 and $4\,kg\,N\,ha^{-1}\,y^{-1}$ in natural ecosystems and this may increase to $20\,kg\,ha^{-1}\,y^{-1}$ under optimal conditions. These values are in agreement with experimentally derived values (Bremer *et al.*, 1995).

The situation is different for photosynthetic cyanobacterial diazotrophs living on the soil surface. In soils in temperate zones, fixation rates between 13 and $38\,kg\,N\,ha^{-1}\,y^{-1}$ have been recorded (Witty *et al.*, 1979), slightly higher than in cyanobacteria–rhizosphere interactions in rice (10–30 kg) but lower than estimates for the *Azolla–Anabaena* symbiosis (20–100 kg; Roger and Ladha, 1992). Cyanobacteria are also found on leaf surfaces and estimates of fixation rates in forest trees in the range from 10–$20\,kg\,N\,ha^{-1}\,y^{-1}$ in temperate zone forests (Favilli and Messini, 1990) and up to $90\,kg\,N\,ha^{-1}\,y^{-1}$ in tropical rain forests.

Other diazotrophs form a more intimate relationship with plants: preferential colonization of the rhizosphere and/or root surface, or the occupation of intercellular (apoplasmic) spaces of various plant organs as endophytes. The frequently reported detection of diazotrophs in the rhizosphere of various non-legumes from the 1960s through to the 1980s, coupled with evidence of yield increases when these organisms were applied as inoculants, created enthusiasm among researchers for the possibilities of associative N_2 fixation. Subsequently, the same high level of interest was directed towards the endophytic diazotrophs. However, despite extensive experimentation, conclusive evidence that N_2 fixation is the main cause of improved plant growth in most rhizospheric and endophytic associations is scarce, and recent reviews reflect an increasingly sceptical outlook among researchers in this field (e.g., James, 2000; Dobbelaere *et al.*, 2003; Giller and Merckx, 2003; Vessey, 2003; Miller and Cramer, 2004). Associative diazotrophs are now often placed into the broader category of 'plant growth-promoting rhizobacteria' (PGPR) in recognition of the likelihood that their beneficial effects can be due to a number of factors other than, or in addition to, N_2 fixation. These include: the production of phytohormones and vitamins; inhibition of plant ethylene synthesis; improvement of nutrient uptake and P supply; anti-pathogen activity (Dobbelaere *et al.*, 2003; Vessey, 2003) (see also Chapter 15). The following discussion is limited to PGPR for which good evidence exists that their ability to stimulate plant growth is attributable, at least partly, to their N_2-fixing activity.

The endophyte *Gluconacetobacter diazotrophicus* can contribute substantial amounts of N to sugar cane under controlled conditions (Sevilla *et al.*, 2001) and it has been estimated that it could supply 20–60% of sugar cane N requirements (Boddey *et al.*, 2001). Experiments using $^{15}N_2$ demonstrated significant uptake of fixed N by sugar cane in association with *G. diazotrophicus* but the mutant lacking N_2 fixation ability (*nif*2) also increased plant growth over the non-inoculated control (Sevilla *et al.*, 1998), indicating the involvement of additional factors in growth promotion. James (2000) cautioned that not all varieties of sugar cane show evidence of BNF and noted that a great variety of bacteria, diazotrophs and non-diazotrophs can be isolated from sugar cane rhizospheres and endorhizospheres. It is therefore not possible, without further investigation, to attribute N_2 fixation to a particular bacterium. These arguments also apply to other plants for which associative N_2 fixation has been claimed.

Azoarcus is an endophyte of Kallar grass, *Leptochloa fusca* (Reinhold-Hurek and Hurek, 1998). It expresses *nif* genes and nitrogenase proteins *in planta* and may supply up to 26% of plant N via fixation (Malik *et al.*, 1997). Further evidence for a contribution of fixed N has come from experiments with gnotobiotically grown plants inoculated with either wildtype or *nif* $^-$ mutant *Azoarcus* strains (Hurek *et al.*, 1998).

Herbaspirillum (on rice, sorghum and sugar cane) is another endophyte that has been reported to supply significant amounts of fixed N to its hosts, particularly rice (see Kennedy *et al.*, 2004 for references). The rhizospheric diazotroph *Azospirillum*, sometimes also classified as an endophyte (Baldani *et al.*, 1997), can increase growth of maize, rice and wheat. The growth-promoting effect is attributed to a combination of phytohormone production

and N_2 fixation (but predominantly the former) (Vessey, 2003; Kennedy *et al.*, 2004; Rodrigues *et al.*, 2008).

16.7 OUTLOOK

In agriculture, consistency of performance of legumes through reliable N_2 fixation rates by effective rhizobium strains is crucial. It has been suggested that the later steps in symbiotic interaction, such as bacteroid differentiation and bacterial hydrogenase expression, are likely to have the greatest effect on fixation capacity and should therefore be taken into account in any strategy for increasing the contribution of N_2 fixation to primary agricultural production. These steps are controlled by the plant genome and they display a greater diversity of molecular mechanisms than the initial recognition and infection events (Den Herder and Parniske, 2009). This approach to enhancing legume performance assumes any rhizobia gaining entry to a legume to be potentially capable of high N_2 fixation rates, but, as already noted, this is not the case. Therefore, a better understanding of inter-strain competition in populations of rhizobia and the mechanisms employed by host plants for selecting strains from those populations are needed so that the infection by indigenous rhizobia with low N_2 fixation capacity can be prevented. With advances in both these areas it should eventually be possible to precisely match the legume host and rhizobium strain so as to ensure consistently high rates of N_2 fixation under field conditions. The prospect of transferring nodulation to non-legume crop plants, such as rice, has been brought somewhat closer by recent progress in deciphering the signalling pathways governing nodule morphogenesis in legume roots. For example, Gleason *et al.* (2006) found that the removal of an auto-inhibition domain from a Ca^{2+}/calmodulin-dependent protein kinase (CcaMK) causes autoactivation of the nodulation signalling pathway in a legume root and the formation of nodules in the absence of rhizobia or their Nod factors. Thus, a single regulation event was shown to be sufficient to activate nodule morphogenesis. Plants that fix N in absence of N_2 fixing procaryotes are not known to exist; nevertheless Cheng (2008) has advanced the concept of a yet to be discovered light-utilizing, oxygen-independent nitrogenase that could free them from their dependency on microorganisms for supplies of fixed N_2. Finally, research continues for a non-biological N_2 fixation reaction that operates at ambient temperature and pressure (Shilov, 2003; Yandulov and Schrock, 2003; Schrock, 2006) which could revolutionize N fertilizer production and save much of the energy presently consumed by the Haber-Bosch process.

Chapter 17

Adaptation of Plants to Adverse Chemical Soil Conditions

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SUMMARY

In this chapter, the constraints of plants in adverse soil conditions such as low nutrient availability, acidity, poor aeration, alkanity and salinity are described, as well as mechanisms of adaptation with particular emphasis on plant nutrition. After a brief introduction into growth strategies of plants in the natural vegetation on nutrient-poor soils, the concept of nutrient efficiency is discussed. Nutrient efficiency can be based on either low nutrient demand for metabolism and growth (nutrient use efficiency) and/or high nutrient acquisition (nutrient acquisition efficiency). This is followed by a description of constraints to plant growth in acid soils, such as Al, Mn and proton toxicity and mechanisms of adaptation (prevention of uptake, external or internal detoxification). The main constraints to plant growth in flooded or submerged soils are the low oxygen availability, Fe and Mn toxicity and toxic metabolites of anaerobic decomposition. Adaptation to flooded soils includes aerenchyma formation to transport oxygen to the roots, efficient generation and use of energy and carbohydrate conservation. Plant growth in alkaline or calcareous soils is inhibited by low availability of Fe, Zn, Mn and B. Tolerance to such soils is achieved by mobilization of Fe and Zn via exudation of chelating compounds such as organic acid anions in Strategy I plants and phytosiderophores in Strategy II plants. The main constraints to plant growth in saline soils are low osmotic potential and ion toxicity (Na, Cl, sulphate) as well as ion imbalances (low K/Na ratio). Salt tolerance mechanisms can be divided into salt exclusion (reduced uptake, increased efflux) and salt inclusion (compartmentation into the vacuole, release of salts via salt glands, salt-tolerant enzymes). To avoid water loss, plants accumulate osmotically active compounds to retain water in the cells.

17.1 NATURAL VEGETATION

Soil chemical factors such as pH, salinity, and nutrient availability determine the distribution and composition of natural vegetation. Species and ecotypes can be classified in ecophysiological terms according to their relatonship to soil properties. Some examples of groupings are acidophobes and acidophiles; calcifuges and calcicoles; halophytes and glycophytes; and metallophytes (adapted to metalliferous soils). Reviews have been published on ecophysiological aspects of plant responses with increasing focus on the molecular understanding of adaptation mechanisms to soil pH (Kinzel, 1983; Kochian *et al.*, 2004), salinity (Flowers and Colmer, 2008; Munns and Tester, 2008), heavy metals (Woolhouse, 1983; Baker, 1987; Clemens, 2006; Maestri *et al.*, 2010) and nutrient availability (Chapin, 1980; Aerts and Chapin, 2000; Lambers and Poorter, 2004).

Crop species are usually selected for growth at high soil fertility and their nutrient-use characteristics may be quite different from those of the natural vegetation grown on soils of low fertility (Chapin, 1988). But also within the natural vegetation, there are species adapted to high soil fertility (fast growing, ruderal species) which show similar nutritional characteristics as crop species (Table 17.1). The most striking property of wild plants of naturally nutrient-poor habitats is their low maximal potential growth rate; they grow slowly even at high nutrient supply (Hommels *et al.*, 1989b). They are not necessarily more efficient in nutrient uptake although they partition more assimilates to the roots. But on soils with very low nutrients availability, some plants have evolved very efficient mechanisms of nutrient mobilization (Chapter 14). A main strategy of efficient use of resources is the high leaf longevity of slow growing plants which invest more assimilates in protective compounds and structures. The high productivity of fast growing species on nutrient-rich soils, on the otherhand mainly depends on a high specific leaf area and thus photosynthesis per unit of leaf weight, and high nutrient-utilization efficiency, particularly photosynthetic N-use efficiency.

In adaptation to N-limited ecosystems in cold climates, plants have developed the capacity to utilize the organic soil N, either by hydrolysis of proteins in ectomycorrhizal forest trees or preferential uptake of amino acids by the

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TABLE 17.1 Characteristics of wild plants in adaptation to soils of their natural habitat with low or high nutrient supply and their response to increasing or decreasing nutrient supply

	Slow growing		Fast growing	
Characteristic	Low nutrient supply	High nutrient supply	Low nutrient supply	High nutrient supply
Growth rate	Low	Slightly enhanced	Decreased	High
Nutrient uptake rate	Low	Slightly enhanced	Low	High
Root/shoot ratio	High	Slightly decreased	Enhanced	Low
Nutrient mobilization	High	Decreased	Slightly enhanced	Low
Specific leaf area	Low	Slightly enhanced	Decreased	High
Nutrient efficiency	Low	Low	High	High
Photosynthetic N-use efficiency	Low	Low	High	High
Leaf longevity	High	High	Low	Low

Based on Chapin (1980, 1988), Aerts and Chapin (2000), Lambers and Poorter (2004).

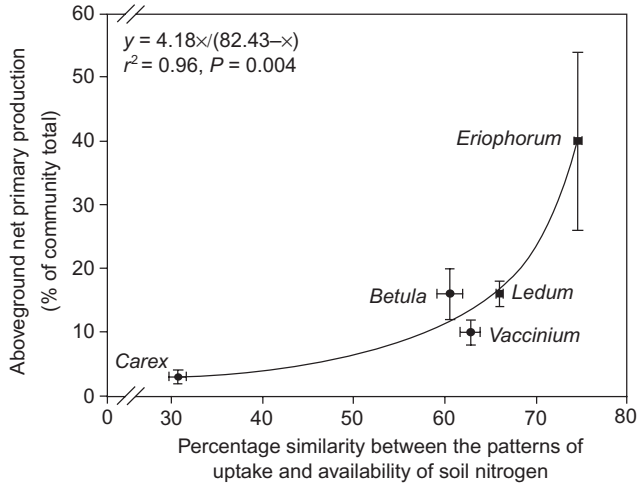


FIGURE 17.1 Above-ground net primary production (mean ± standard error) of the five most common species in tussock tundra. *From McKane* et al. *(2002) with permission from Nature.*

non-mycorrhizal artic sedge (*Eriophorum vaginatum*). In its natural environment, at least 60% of the N demand of this sedge may be met by amino acid uptake (Chapin *et al.*, 1993). Since the main available soil N form in the arctic tundra is organic N, this capacity to utilize organic N directly gives the sedge a competitive advantage compared to the other members of the plant community who rely on the mineralization of organic N by soil microorganisms (McKane *et al.*, 2002; Fig. 17.1).

Many natural ecosystems are also P-limited. Although mycorrhiza play a particularly important role in the acquisition of P at low soil-P availability (see also Chapter 15), there are no principal differences between wild species and their related crop species in AM dependency for P acquisition (Bryla and Koide, 1990a, b).

17.2 HIGH-INPUT VERSUS LOW-INPUT APPROACH

17.2.1 General

In the past, the approach to soil fertility problems in crop production emphasized the importance of changing the soil to fit the crop's demand. Soil fertility factors, such as pH and nutrient availability, were adjusted to optimum levels for a given crop. This approach of high external input, including high rates of chemical fertilizers, has been and still is successful in increasing food production under favourable crop-product/fertilizer price/cost relationships, for example in the industrialized world in the past and in parts of Southeast Asia at present. However, the extensive use of fertilizer in inorganic or organic form has led to substantial ecological problems such as pollution of waters and emission of gases contributing to global warming and the destruction of the stratospheric ozone layer. Also, this approach failed in regions with less favourable economic and socio-cultural conditions such as commonly found in Sub-Saharan Africa, parts of South Asia and South and Latin America (Vitousek *et al.*, 2009). Moreover, in many of these regions soil conditions cannot easily be ameliorated because of their extent, the cost of improving the soils, or both (Vose, 1983). In tropical America, for example, ~70% of the soils are acid and infertile (Sanchez and Salinas, 1981). In subtropical and semi-arid regions, soil salinity and alkalinity and related nutritional problems such as Fe and Zn deficiency are widespread. About 25% of the world's area of cultivable soil has acute chemical problems (Vose, 1983).

The awareness of the difficulties or even failure of the high-input approach in most tropical and subtropical soils led to a shift towards a greater emphasis on fitting plants to soils over the last 40 years. This approach requires genotypes better adapted to given ecological conditions, as well

as selection and breeding programmes for high nutrient efficiency and high tolerance to constraints such as Al and Mn toxicity, waterlogging and salinity.

This low-external input high-efficiency approach using adapted genotypes with a more efficient use of nutrients from soil reserves and fertilizer may lead to yields that are only 10–20% below the maximum. This approach involves addressing extreme soil chemical conditions (e.g., salinity) but also selection and breeding of genotypes that are highly efficient in using soil and fertilizer nutrients.

In the past, progress in selection and breeding for higher yield was achieved to a large extent by increasing the harvest index, i.e. the proportion of biomass allocated to seeds and storage organs in relation to total biomass (Dambroth and El Bassam, 1990). In recent years, greater emphasis has been on combining high crop yields with high efficiency in acquisition and utilization of nutrients. Modern cultivars, for example of wheat or potato, tend to have lower root/shoot dry weight ratios than old, traditional cultivars, but the efficiency in nutrient acquisition is often similar, or even higher. This is presumably because of a finer and more active root system in the modern cultivars (Sattelmacher *et al.*, 1990b), often in combination with a more efficient internal utilization (retranslocation) as has been shown for P in wheat (Horst *et al.*, 1993).

TABLE 17.2 Grain yield of different cereal cultivars grown without or with Zn in Zn-deficient soil

	Grain yield		
	+Zn	−Zn	
Cereal/cultivar	($kg\,ha^{-1}$)		Relative yield (% of +Zn)
S. cereale			
Aslim	2,404	2,588	108
Triticale			
Presto	3,556	2,032	57
T. aestivum			
Bezostaja-1	3,630	1,240	34
Atay-85	1,906	366	19
T. durum			
Kunduru-1149	2,164	316	15
C-1252	1,366	152	11

Data compiled from Cakmak et al. (1997c).

17.2.2 Genetic Basis of Plant Nutrition

The nutrition of plants is under genetic control. In crop plants, this is indicated by the numerous examples of nutritional differences between cultivars or genotypes. More specific evidence comes from inheritance studies involving cultivars and genotypes differing in nutritional requirements. Since the early 1960s, there has been an increasing interest in the genetic basis of plant nutrition. This has led to impressive progress in both breeding programmes for improving the adaptation of crop species to problem soils and in research on physiological and increasingly molecular mechanisms involved in, adaptation. In some cases, major nutritional features are under the control of single genes. However, in the majority of cases, more complex genetic control systems are involved in acquisition and utilization of nutrients or in control of salt tolerance and aluminium resistance.

Genotypic differences particularly in micronutrient efficiencies in cereals highlight the genetic control of plant nutrition, and the possible progress in understanding of the mechanisms involved by applying molecular biological approaches. Durum wheat and bread wheat are generally sensitive to low Cu supply whereas in contrast, rye and triticale are relatively tolerant (Graham, 1978). Important differences also exist in Cu efficiency of wheat cultivars (Owuoche *et al.*, 1996). The genes controlling Cu efficiency are carried on the long arm (L) of chromosome 5R of rye (Graham *et al.*, 1987a). Wheat cultivars carrying the 5RL chromosome of rye have, therefore, a high Cu efficiency (Graham *et al.*, 1987a). Similar inter- and intraspecific differences in cereals exist in Zn efficiency (Cakmak *et al.*, 1997a; Table 17.2). Oat and durum wheat are particularly sensitive to low Zn supply. Zinc efficiency in rye is controlled by genes on the chromosomes 1R and 7R. Transfer of these genes to triticale and wheat increases their Zn efficiency (Cakmak *et al.*, 1997b).

The 5R chromosome from rye is also the carrier of genes which encode enzymes operative in Strategy II plants in Fe acquisition (see also Chapter 2). These enzymes regulate the synthesis of the phytosiderophore mugineic acid (MA) from deoxymugineic acid (DMA), and hydroxymugineic acid (HMA) from MA (Mori *et al.*, 1990). In barley, the gene which encodes the synthesis of MA from DMA is on chromosome 4; introducing this chromosome into wheat leads to synthesis of MA in wheat (Mori and Nishizawa, 1989). Transgenic rice expressing the barley nicotianamine aminotransferase gene, one of the genes coding for enzymes involved in the biosynthesis of MA, was more tolerant to low Fe availability when grown in a calcareous soil than its wildtype (Takahashi, 2003), demonstrating the prospects of biotechnology in breeding crops with improved adaptation to adverse soil chemical properties.

In recent years, exploiting genotypic differences and breeding for higher micronutrient concentration in seeds has attracted particular interest in order to overcome micronutrient malnutrition which affects more than half of the world population, particularly in developing countries (Mayer *et al.*, 2008; White and Broadley, 2009; Cakmak *et al.*, 2010a,b; see also Chapters 7 and 9).

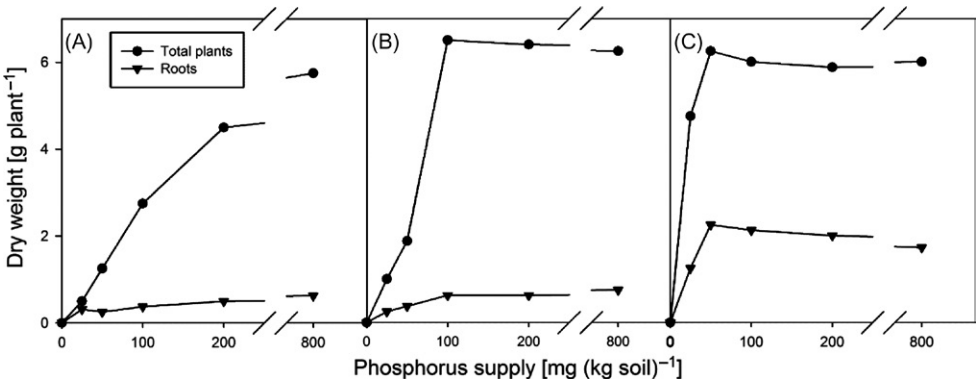


FIGURE 17.2 Growth response of three pasture species to P fertilizer applied to a P-deficient soil. (A) *Trifolium cherleri*; (B) *Trifolium subterraneum*; (C) *Lolium*. *Based on Ozanne* et al. *(1969).*

17.2.3 Nutrient Efficiency

There are many definitions of nutrient efficiency or nutrient use efficiency (for an overview see Fageria and Baligar, 2008) which creates confusion rather than contributing to a conceptual and in-depth understanding of the underlying mechanisms in plants. However, from an agronomical point of view and in an operational sense, genotypic differences in nutrient efficiency of crop plants need to be defined as differences in growth or in yield of crops when grown in a nutrient-deficient soil. A nutrient-efficient plant is able to produce a higher yield in a soil that is limiting in one or more nutrients compared to a standard genotype, as originally defined by Graham (1984) and further specified by Sattelmacher *et al.* (1994). This definition can be applied to comparisons of genotypes within a species or of plant species.

There have been a large number of reports in recent years on nutrient efficiency, comparing the yield, or the percentage of yield reduction, in genotypes supplied with insufficient amounts of nutrients (e.g., Graham *et al.*, 1982; Randall *et al.*, 1993; Yan *et al.*, 2006; Fageria and Baligar, 2008). A typical example of differences in P efficiency between three pasture species is given in Fig. 17.2 (Ozanne *et al.*, 1969). Despite a similar final dry weight at the highest P supply, the growth of the three species at a given P supply increased from *Trifolium cherleri* to *T. subterraneum* and *Lolium rigidum*. The minimum rates of applied P required for 90% of maximum yield in the three species were 302, 87 and 26 mg P kg^{-1} soil, respectively. As one would expect from the role of root growth and root surface area in P acquisition (see also Section 6.3 and Chapter 13), there is a close correlation between efficiency of fertilizer P utilization and root dry weight.

The generally greater P and K efficiency of grasses compared to legumes results in dominance of grasses in mixed pastures on P- or K-deficient soils (Steffens and Mengel, 1980).

The plant characteristics contributing to nutrient efficiency are shown in Fig. 17.3. As mentioned above, one of the main adaptation mechanisms of plants in natural vegetation to soils low in available nutrients is a reduction in growth rate. Also, the storage of high amounts of nutrients in the seeds either through high nutrient concentrations or large seeds is a common strategy of wild plants but also of crops (Bolland and Baker, 1989; Riley *et al.*, 1993) to ensure seedling establishment in nutrient-poor soils. Therefore, the effects of differences in seed nutrient concentrations on growth and yields should be considered in screening of genotypes for nutrient efficiency at seedling stage (Shen *et al.*, 2002; Naegle *et al.*, 2005).

Two components may contribute to overall nutrient efficiency: *uptake efficiency*, which is the amount of nutrient absorbed, and *utilization efficiency*, which characterizes the efficiency with which the absorbed nutrients are utilized to produce yield (Fig. 17.3). The relative importance of uptake efficiency and utilization efficiency primarily depends on the nutrient supply by the soil and fertilizers. Generally, the lower the nutrient supply the more important becomes uptake efficiency. Also, plant strategies for nutrient uptake efficiency depend on the nutrient supply of the soil. In soils with medium supply of plant-available nutrients, a more efficient acquisition of these nutrients may be sufficient. In soils with low supply of plant-available nutrients, increasing the supply by mobilization of sparingly soluble nutrients may be necessary.

The nutrient uptake system can be characterized using Michaelis Menton kinetics (see also Chapter 2). A common response of plant roots to nutrient starvation is the up-regulation of genes coding for high-affinity transporters (Bucher, 2007). However, this may not be effective for nutrients with low concentration in the soil solution and low mobility in the soil (such as K^+, NH_4^+ and particularly $H_2PO_4^-$) where the transport to the root is the main factor limiting nutrient uptake (see also Chapter 2). More promising are changes in root morphology aiming at a better spatial exploration of the soil profile and facilitating nutrient diffusion (Abel *et al.*, 2002). Among the morphological characteristics, increasing

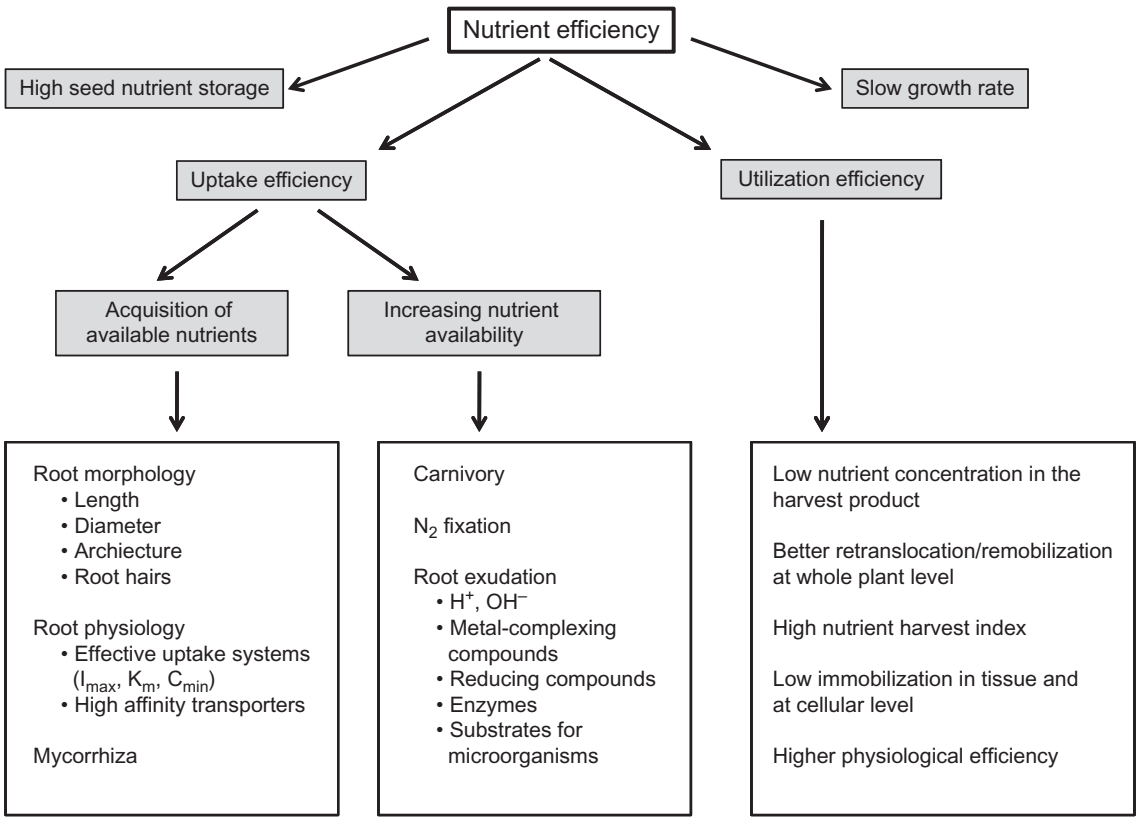


FIGURE 17.3 Mechanisms of adaptation of plants to low nutrient supply.

the root length while reducing the root diameter, thus increasing the root surface area per assimilate investment in root growth, appears to be a successful strategy in response to low P supply and a characteristic of P efficient genotypes (Lynch and Ho, 2005, Lambers *et al.*, 2006). Not the absolute root growth but rather the root length/shoot weight ratio is an indicator of P efficiency (Föhse *et al.*, 1988). This ratio is not only constitutively different between genotypes, but also the response of this ratio to P deficiency may be genotype specific (Whiteaker *et al.*, 1976; Table 17.3). Under P deficiency, growth of genotype 6 was strongly reduced, but the root/shoot ratio was approximately the same as with adequate P supply. In contrast, root growth and root/shoot ratio of genotype 11 nearly doubled. The capacity to distribute a higher proportion of photosynthates to the roots is obviously under genetic control and is an important aspect of P efficiency for plants grown in P-deficient soils.

TABLE 17.3 Root and shoot growth of two genotypes of common bean with adequate or inadequate P supply

Genotype	P supply	Root dry weight (mg dw plant^{-1})	Shoot dry weight (mg dw plant^{-1})	Root/shoot ratio
6	Adequate	242	1,465	0.17
11		181	1,233	0.15
6	Inadequate	124	77	0.16
11		365	1,141	0.31

Based on Whiteaker *et al.* (1976).

In most soils, plant-available P is higher in the surface soil, thus, changing root architecture allowing the better foraging of this soil layer is a major determinant of genotypic P efficiency in common bean (Lynch and Brown, 2001) and soybean (Ao *et al.*, 2010). Even more effective is the formation of long root hairs which allows the plant to increase root length/root surface at low carbon cost (Lynch and Ho, 2005). The increase in root hair length and density is in response to low P supply under genetic control (Gahoonia and Nielsen, 2004; Zhu *et al.*, 2010).

An alternative effective plant strategy to cope with low P supply is the formation of a symbiosis with mycorrhizal fungi (Smith *et al.*, 1992; see also Chapter 15). An example for the role of arbuscular mycorrhizal fungi (AM) in genotypical differences in P acquisition by alfalfa was shown by Lambert *et al.* (1980) (Table 17.4). In the absence of AM all

TABLE 17.4 Shoot dry weight of three lucerne cultivars with or without arbuscular mycorrhiza (AM) at different levels of P supply. Plants were grown in a P-deficient soil, pH 7.2

Soil treatment		Shoot dry weight (mg plant^{-1})		
mg P kg^{-1}	AM	Buffalo	Cherokee	Du Puits
0	–	22	18	32
20	–	114	235	375
80	–	2,389	2,058	2,115
0	+	1,113	1,740	2,177

From Lambert *et al.* (1980).

three cultivars responded similarly to the increase in P supply. With AM, growth increased nearly 100-fold in all three cultivars, but there were obvious differences in the efficiency of the AM association with the host plants. In cultivar Du Puits, AM completely replaced P application but in Buffalo AM colonization resulted in only 50% of the growth with the high P rate. These differences in mycorrhizal response may be related to differences in root colonization with AM which not only differs markedly between species but also between genotypes of a species (Smith *et al.*, 1992). However, not only colonization rate but also length of external mycelium and P transport rate within the hyphae may explain the differential effect of AM on plant growth. There is little indication that mycorrhizal fungi can mobilize P which is not available to plants. Thus the main function of mycorrhiza is the efficient uptake and transfer to the plants of P which is otherwise spatially unavailable to the roots.

For mobile nutrients such as nitrate, the efficiency of the uptake system and up-regulation of genes coding for high-affinity transporters may be of greater importance (Garnet *et al.*, 2009). This assumption is supported by the close relationship between soil nitrate uptake and root length density of 10 maize cultivars (Wiesler and Horst, 1994), suggesting that the uptake capacity per unit of root length was limiting for plant N uptake. However, this relationship existed only in deeper soil layers which supports the conclusion that the recovery of nitrate from the subsoil is a major factor of plant N uptake efficiency (Kristensen and Thorup-Kristensen, 2004a,b).

If the acquisition of available nutrients in the rooting zone is not sufficient to meet nutrient demand, plant strategies aiming at increasing nutrient availability become increasingly important. In severely N-deficient environments fixation of atmospheric N_2 through symbiosis or associations with N_2-fixing bacteria (see also Chapter 16) or trapping insects (carnivory) are widespread adaptation mechanisms (Fig. 17.3).

Sparingly soluble nutrients may be mobilized at the soil/root interface through root exudates (Fig. 17.3; see also Chapter 14). The release of H^+ into the rhizosphere will increase availability of micronutrients (except Mo) and Ca phosphates, whereas the release of OH^- will improve availability of P bound to Fe and Al oxides in acid soils (Gahoonia *et al.*, 1992). Even more effective is the exudation of chelators such as organic acid anions for P, Zn and of phytosiderophores for Fe and Zn solubilization, particularly if combined with morphological adaptations aimed at concentrating the root exudates in a small soil volume. For this the formation of cluster roots in response to P deficiency is an excellent example (Lambers *et al.*, 2006). Root exudates with reducing properties may be important for the solubilization of Fe and Mn where the reduced metal is much more soluble than the oxidized form. The release of phosphatase improves the ability of the plant to use organic P in the soil solution. The role of root-derived phosphatases in genotypic P efficiency of crops is not yet fully clarified (McLachlan, 1980; Kamh *et al.*, 1999; George *et al.*, 2005). Root exudates are the main substrate for rhizosphere microorganisms which may also increase nutrient availability. Additional rhizosphere microorganisms may increase plant-inherent nutrient acquisition capacity by enhancing root or root hair growth (Martin *et al.*, 1989; see also Chapter 15).

The relative importance of the different plant mechanisms involved in nutrient uptake efficiency is difficult to assess because 'small causes may have big effects'(Wissuwa, 2003). Large genotypic differences in P uptake from a P-deficient soil can be caused by rather small changes in efficiency mechanisms. For example, high seed P concentration is an important mechanism of plant adaptation to P-limited soils although the seed P concentration represents only a small portion of the plant P requirement.

Nutrient utilization efficiency becomes equally or even more important under conditions of mild nutrient-deficiency stress (Wang *et al.*, 2010b). Nutrient utilization efficiency can be enhanced by reducing the nutrient concentration in the harvested product, thus in cereals the grain (Fig. 17.3). However, this strategy may lead to lower nutritional quality (protein, P) and seedling emergence under conditions of low soil nutrient supply (P). Breeding crops for high harvest index (HI, biomass of the harvested organ relative to the total plant biomass) has been a very successful strategy in enhancing crop yielding capacity. Generally, there is a close correlation between HI and nutrient harvest index (NHI) which is defined accordingly. For NHI, not only biomass partitioning but particularly high retranslocation/remobilization efficiency from the vegetative to the reproductive plant organs is important. A more efficient mobilization and retranslocation of N from leaves involving cytosolic glutamin synthetase (GS) to the grain has been implicated in genotypic differences in N efficiency of maize

(Hirel *et al.*, 2007). Hence, breeding for high cytosolic GS activity may represent a promising target to improving N utilization efficiency (Bernard *et al.*, 2008). Mobilization of Zn from senescing leaves under the control of a senescence-accelerating NAC transcription factor contributes to enhancing Zn and Fe concentrations of seeds in wheat (Uauy *et al.*, 2006). However, enhanced senescence during the reproductive stage may lead to lower yield because it shortens green leaf duration and leaf photosynthesis; in maize and oilseed rape delayed leaf senescence was an important component of genotypic N efficiency (Schulte Auf'm Erley *et al.*, 2007a, b). Thus, timing of the initiation and the development of leaf senescence and nutrient remobilization are important for nutrient utilization efficiency. Additionally, efficient retranslocation between shoot organs may contribute to nutrient utilization efficiency, for example in sorghum for higher N utilization efficiency (Alagarswamy *et al.*, 1988), and in bean (*Phaseolus vulgaris*) for higher P (Youngdahl, 1990; Kouas, 2009) or Mo utilization efficiency (Brodrick and Giller, 1991b). In maize, Mg utilization efficiency was due to a combination of differences in the rates of both Mg uptake and transport to the shoot (Clark, 1975). Genotypic differences in translocation of Ca^{2+} to low transpiring meristematic apical shoot meristems (Behling *et al.*, 1989) and particularly storage organs are important in sensitivity to Ca deficiency-induced disorders (Volz *et al.*, 2006).

There are several examples of genotypic differences in the short-distance transport of nutrients within roots or in long-distance transport from the roots to the shoots (Läuchli, 1976b). Low Fe efficiency of soybean cultivar PI can be explained by a slow rate of transport of Fe from roots to shoot (Brown *et al.*, 1967). Impaired root to shoot transport of Fe may often be due to sequestering of Fe (precipitation) at the rhizoplane and in the apoplasm of rhizodermal cells. Genotypic differences in Mg efficiency in sorghum seem to be related to differences in K^+ uptake rates: Mg-efficient genotypes may have lower K/Mg ratios in the shoots (Keisling *et al.*, 1990).

At the tissue and cellular level, the 'physiological activity' of a nutrient is dependent on its binding stage and compartmentation. The former is particularly important for Ca and micronutrients. Differences in susceptibility to Ca deficiency between tobacco cultivars was related to differential inactivation of Ca as Ca oxalate (Brumagen and Hiatt, 1966). The concentration of easily extractable Fe and Zn is more closely related to the occurrence of deficiency symptoms than the total leaf concentrations, suggesting that differences in inactivation of Fe and Zn in the plant tissue are important for nutrient utilization efficiency.

Efficient transport of ions stored in the vacuole may also lead to higher physiological use efficiency. This appears to be particularly important for P. A higher utilization efficiency of some white clover genotypes was related to a better use of stored P_i (Caradus and Snaydon, 1987; Hart and Colville, 1988). From their study on the P efficiency of soybean overexpressing an APase gene, Wang *et al.* (2010a) concluded that the higher P efficiency of the transformant was primarily due to the improvement of the P utilization efficiency. A more efficient mobilization of nitrate from vacuoles, where nitrate is important to establish the necessary turgor potential driving cell expansion and thus leaf growth, through replacement by chloride, may contribute to reducing the N requirement of leafy vegetables such as spinach (Hähndel, 1984). Exploiting genotypic differences or genetic engineering of vacuolar chloride accumulation may be an attractive approach increasing N utilization efficiency in crops harvested at the vegetative growth stage.

The best example of differences in physiological N efficiency at the cellular level is the comparison of C3 and C4 plant species. C4 species achieve maximum carbon exchange rates at lower leaf N concentrations than C3 plants (see also Chapter 5). In C3 species RuBP may make up to 50% of the total leaf protein, thus it has been argued that RuBP is produced in excess of the requirement for photosynthesis, and reducing the RuBP concentration may lead to a higher N utilization efficiency. Transformation of rice using an antisense rbcS sequence leading to lower concentrations of RuBP in leaves confirmed that reducing the RuBP concentration increased photosynthetic N efficiency (photosynthesis per unit of leaf N) under saturating CO_2 and light (Makino *et al.*, 1997). However, overall there is little support for the assumption of RuBP luxury concentration particularly under N-limiting conditions (Mae, 1997; Parry *et al.*, 2003). Another approach to increase N utilization efficiency may be to decrease the allocation of leaf N from the cell walls to RuBP (Onoda *et al.*, 2004); (Harrison *et al.*, 2009).

Further examples of genotypic differences in physiological efficiency of nutrients contributing to nutrient utilization efficiency are differences in the Ca and B requirements between monocotyledonous and dicotyledonous plants (see also Sections 6.5 and 7.7). Within a given species, nutrient efficiency, for example for Ca, may differ between cultivars depending on differential functional requirement within the tissue (English and Barker, 1987; Horst *et al.*, 1992a). The magnitude of such genotypic differences is demonstrated in Fig. 17.4 and Table 17.5. In bread wheat, maintaining the functioning of Zn-requiring enzymes under low Zn conditions, thus biochemical Zn utilization, was suggested to be an important component of Zn efficiency (Hacisalihoglu *et al.*, 2003).

The efficiency in acquisition and internal utilization also depends on the level of nutrient supply (Godwin and Blair, 1991; Smith *et al.*, 1990) and on plant age (Schjorring and Nielsen, 1987; Brouder and Cassman, 1990). To evaluate genotypic differences in nutrient efficiency, dose–response curves to increasing nutrient supply need to be obtained and sequential harvests taken. When grown in deficient soils,

FIGURE 17.4 Growth of two cowpea cultivars at 1.25 or 0.005 mM Ca in full nutrient solution.

TABLE 17.5 Dry weight and Ca concentrations in roots and shoots of two cowpea (*Vigna unguiculata* L. Walp) cultivars supplied with different Ca concentrations

			Ca concentration (μmol g^{-1} dw)		
Ca supply (μM)	Cultivar	Dry weight (g $plant^{-1}$)	Roots	Stem	Leaves
10	Solojo	0.75	34	40	35
	TVu 354	1.75	25	46	34
50	Solojo	2.10	37	58	62
	TVu 354	1.80	32	70	57

From Horst *et al.* (1992).

P-efficient barley genotypes (Schjorring and Nielsen, 1987) and K-efficient cotton genotypes (Brouder and Cassman, 1990) were characterized by higher uptake rates of these nutrients after ear emergence and flowering due to maintenance of high root growth and activity. For the N efficiency of maize (Worku *et al.*, 2007) and oilseed rape (Wiesler, 2001), N uptake during the reproductive stage was more important than that during the vegetative stage.

17.2.4 Resistance to Excessive Supply of Elements

Adaptation to adverse chemical soil conditions may require resistance to excessive levels of elements such as Al and Mn in acid mineral soils, Mn in waterlogged soils, and NaCl in saline soils. Thus, *multiple stress resistance* is often necessary for adaptation. In analogy to the definition of nutrient efficiency, element resistance is defined as a plant property which allows a genotype to grow and yield better than the population mean when grown in a soil with excessive supply of elements. The plant characteristics contributing to element resistance are shown in Fig. 17.5. Element resistance is an important part of the adaptation of plants to acid mineral soils (Section 17.3), waterlogged and flooded soils (Section 17.4), alkalnine soils (Section 17.5), and saline soil (Section 17.6), thus these mechanisms will be described in detail in these sections.

Element resistance may be achieved through exclusion of the toxic element from uptake through modification of the root anatomy/morphology or root physiology/biochemistry. The formation of a suberized exodermis may restrict the flow of solutes into the root cortex, further movement of solutes into the stele can be prevented by formation of a Casparian strip.

Low membrane transport or efficient efflux pumps can restrict the uptake of ions (particularly Na^+ and Cl^-) present in high concentrations in the soil solution. The CEC of the root apoplasm can be reduced by a lower pectin concentration and/or high degree of methylation of the pectin which reduces the binding of di- and trivalent cations such as Al^{3+} in the root apoplasm and Al uptake. A particularly effective way of detoxification of metals in the root apoplasm and reduction of their uptake is the release of metal chelating ligands from the roots, particularly organic acid anions which play a major role in Al resistance. A high oxidation capacity of the roots contributes to the inactivation of Fe^{2+} in waterlogged soils.

When potentially toxic elements are taken up (inclusion) plants may avoid toxicity in the shoots (avoidance) by restricting the translocation from roots, effective retranslocation of the element from the shoot back to the roots

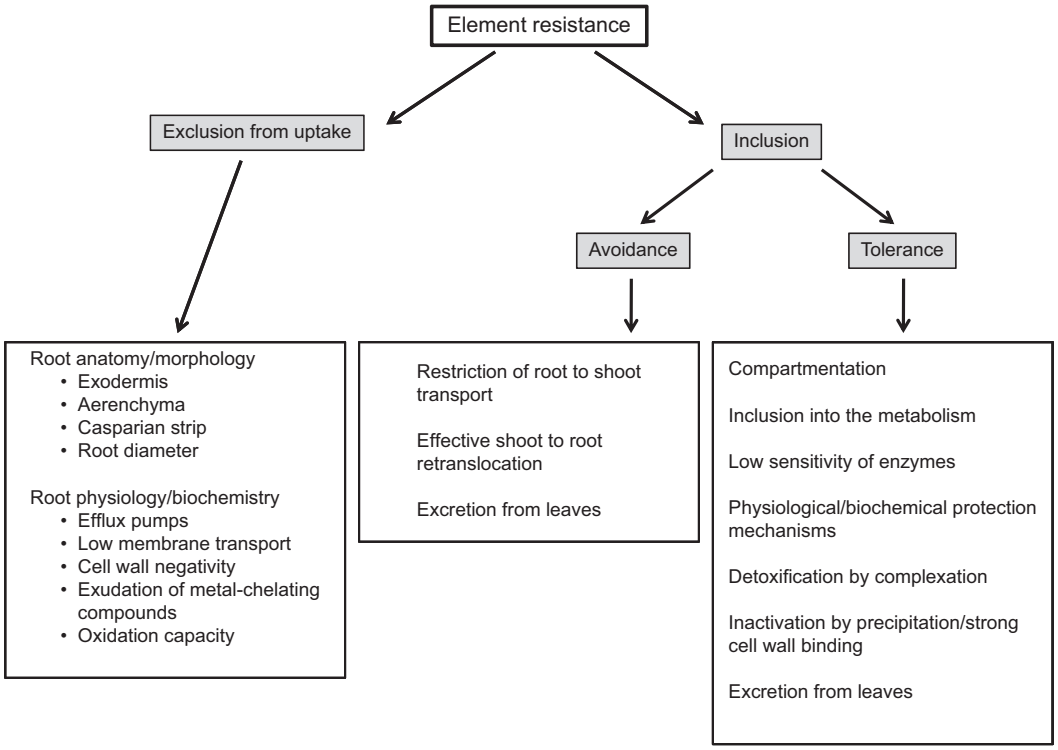


FIGURE 17.5 Mechanism of adaptation of plants to excessive supply of elements.

and excretion from the roots as has been shown for Na^+ in natrophobic glycophytes. Excessive concentrations in the shoots can be avoided by excretion of the element through specialized leaf cells (salt glands) in halophytes.

Element tolerance of the shoot tissue requires sequestration of the toxic ions in plant compartments where they no longer affect the metabolism such as the vacuole which plays a major role in Na^+ and heavy metal tolerance (e.g., Mn). Natrophilic glycophytes and halophytes have evolved several strategies to deal with high shoot Na concentrations: (i) Na can replace K in certain functions, (ii) Na has metabolic functions, and (iii) Na-tolerant enzymes. Element toxicity is often related to oxidative stress, therefore a more efficient avoidance strategy and detoxification/scavenging of reactive oxygen intermediates may contribute particularly to Fe and Mn tolerance. For Al and heavy metal tolerance, detoxification by complexation with organic ligands, particularly organic acids, may even allow hyperaccumulation in the shoots. Alternatively, strong binding in the cell walls and precipitation particularly in vacuoles may also confer enhanced heavy metal tolerance.

17.3 ACID MINERAL SOILS

17.3.1 Major Constraints

Acid soils, which are defined by a pH lower than 5.5 in their surface layers, comprise about 30% of the total ice-free land (von Uexküll and Mutert, 1995), primarily in humid climates. Plant growth inhibition and yield reduction on acid soils results from a variety of specific chemical factors and their interactions (Marschner, 1991b). In acid mineral soils the major constraints to plant growth are toxicity of protons, Al and Mn and deficiency of Mg, Ca, P and Mo. The relative importance of these constraints depends upon plant species and genotype, soil type and horizon, parent material, soil pH, concentration and species of Al, soil structure and aeration, and climate. The N concentrations in acid mineral soils are generally low except in areas with high atmospheric input by air pollution (Schulze, 1989). Aluminium toxicity and Ca and Mg deficiencies occur in more than 70% of the acid soils of tropical America, and nearly all of these soils are P deficient or have a high P-fixing capacity (Sanchez and Salinas, 1981). Subsoil acidity is a potential growth limiting factor throughout many areas of the USA (Foy *et al.*, 1974) and of the tropics (Van Raij, 1991).

Forest soils in many regions of the world are typically acidic. Concern has been expressed about the increasing acidification of forest soils by atmospheric emissions of SO_2 and nitrogen oxides ('acid rain') being a major contributor to forest damage (forest decline), particularly in Europe and North America. Although the emission of SO_2 has been substantially reduced during the last 30 years, the emission of acid-producing NH_3 mainly in areas of

intensive livestock production continues to contribute to further acidification of natural ecosystems. There is still controversy about the importance of soil acidification in forest decline. Forest damage may also be related to (i) an increase in Al solubility and thus Al toxicity (Murach and Ulrich, 1988), and (ii) a decrease in uptake of nutrients, particularly Mg, and thus Mg deficiency (Zöttl and Huettl, 1986; Kaupenjohann *et al.*, 1987; Liu and Huettl, 1991), and (iii) an increase in Mg and Ca deficiency due to high atmospheric N input (Schulze, 1989; Aber *et al.*, 1989).

It is not possible to generalize, however, as the importance of soil acidity stress without considering site-specific conditions. The role of atmospheric N deposition depends not only on the amounts of N, but also on the cropping history of forest sites (Zöttl, 1990). In European beech (*Fagus sylvatica* L.), root growth is more sensitive to high concentrations of H^+ than to Al, whereas in Norway spruce (*Picea abies* (L.) Karst.) the reverse appears to be true (Murach and Ulrich, 1988). For a given plant species, the location and distribution of roots within the soil profile may be an important factor in determining the form of expression of soil acidity. In the topsoil, where the organic matter content is higher, H^+ toxicity may dominate, but in the subsoil root growth may be depressed by Al toxicity. Whether Mg deficiency becomes a dominant factor in stress induced by soil acidity depends mainly on the parent material (Zöttl and Huettl, 1986) and the atmospheric input of Mg (e.g., distance from the open sea). In soils high in Mn reserves and in exchangeable Mn^{2+}, for example after continuous cultivation of legumes, Mn toxicity may become a major factor in soil acidity stress (Bromfield *et al.*, 1983a, b).

Given the different ways in which soil acidity can restrict plant growth, plants adapted to acid mineral soils require a variety of mechanisms to cope with the adverse soil chemical factors (Howeler, 1991). On a worldwide scale, high concentrations of Al, H^+ for some plant species, and in some locations also of Mn, are key factors of soil acidity stress, therefore high resistance to these three factors is required for adaptation particularly of crop plants to acid soils.

17.3.2 Proton Toxicity

Proton toxicity is primarily expressed as inhibition of root elongation and root death (Koyama *et al.*, 1995) but the pH at which H^+ toxicity occurs differs between plant species (Islam *et al.*, 1980). The physiological and molecular mechanisms of H^+ toxicity are not yet fully understood, but there are principally three mechanisms: (i) disruption of cell wall integrity, (ii) interference with the maintenance of the cytosolic pH, and (iii) inhibition of the uptake of cations. High H^+ concentrations in the root apoplasm disturb the stability in the pectic polysaccharide network by displacing Ca^{2+} which plays a key role in the maintenance of the network (Koyama *et al.*, 2001). At high apoplasmic H^+ concentrations, the plasma membrane H^+-ATPase is unable to maintain the cytosolic pH (Yan *et al.*, 1992). Increased tolerance to high H^+ concentrations has been explained by a higher ATPase H^+ pumping capacity (Yan *et al.*, 1998). Studies with an H^+-hypersensitive *Arabidopsis* mutant suggested that the Zn finger protein STOP1 is involved in metabolic pathways controlling the cytosolic pH (Sawaki *et al.*, 2009). High proton concentrations inhibit the uptake of cations by depolarization of the plasma membrane (Shabala *et al.*, 1997) and decrease loading of polyvalent cations (Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}) in the apoplasm of root cortical cells which then reduces their uptake into the symplasm (see also Chapter 2). For legumes, high H^+ concentrations in combination with low Ca concentrations negatively affect nodulation and N_2 fixation (Alva *et al.*, 1987; see also Chapter 16).

17.3.3 Aluminium Toxicity

17.3.3.1 Aluminium Solution Chemistry and Aluminium Toxicity

In acid mineral soils below pH 5.5, an increasing proportion of the cation exchange sites of clay minerals is occupied by Al^{3+} where it replaces the divalent cations Mg^{2+} and Ca^{2+}. Thus, with decreasing soil pH, the percentage of exchangeable Al (Al saturation) increases prior to the soil solution Al concentration (Haynes and Swift, 1986). Therefore, Al saturation is often used to predict Al excess and its reduction as a target for liming soils (Kamprath, 1970). The total Al concentration in the soil extract or soil solution is often not well correlated with the inhibition of root growth, the most sensitive parameter for Al toxicity.

The phytotoxicity of Al primarily depends on the Al speciation in solution. Aluminium released from soil minerals into the soil solution under acid conditions, or the Al in nutrient solutions of pH $\leq$4.0, is mainly $Al(H_2O)_6^{3+}$ (referred to as Al^{3+}). As the pH increases, the total Al concentration of the solution decreases, but mononuclear hydrolysis products such as $Al(OH)^{2+}$ and $Al(OH)_2^{+}$ are formed as intermediates in the precipitation of solid $Al(OH)^0_3$. Above pH 7, the solution Al concentration increases again due to the formation of the aluminate ion $Al(OH)_4^-$ (Kinraide, 1990). At elevated OH^-/Al ratios in solution, polynuclear hydroxyl aluminium species such as $AlO_4Al_{12}(OH)_{24}(H_2O)_{12}^{7+}$ (referred to as Al_{13}) may form (Parker *et al.*, 1988).

There have been contradicting results regarding the relative phytotoxicity of the various mononuclear cationic Al species (Kinraide and Parker, 1990) at low pH, but Al^{3+} is considered to be the most phytotoxic Al mononuclear species. In nutrient solution experiments, a high phytotoxicity

has also been attributed to Al_{13} which may form at pH 4.5 (Parker *et al.*, 1989). However, the role of Al_{13} in Al toxicity in acid soils is unclear. Aluminium toxicity may also occur in alkaline soils amended with fly ash (Jones, 1961; Rees and Sidrak, 1955) and bauxite residue (Fuller and Richardson, 1986), Al rhizotoxicity has clearly been demonstrated in hydroponic culture with pH values adjusted to >8.0 (Kinraide, 1990; Eleftheriou *et al.*, 1993; Ma *et al.*, 2003). But it is unlikely that the aluminate ion is the toxic Al species at alkaline pH. Kinraide (1990) and Kopittke *et al.* (2004) hypothesized that aluminate is not toxic and that the inhibition of root elongation by Al is attributable to the formation of Al_{13} in the apolasm of the roots. Stass *et al.* (2006) provided evidence that at pH 4.3, Al^{3+} inhibits root growth of maize through binding to sensitive binding sites in the apoplasm of the epidermis and the outer cortex, whereas at pH 8.0 with $Al(OH)_4^-$ as dominant Al species, a strong decrease of the apoplasmic pH leads to $Al(OH)_3$ precipitation in the epidermis causing a mechanical barrier which impairs its functioning.

Some mononuclear aluminium species associated with inorganic ligands such as AlF^{2+}, AlF_2^+ or $AlSO_4^+$ are less or not phytotoxic compared to Al^{3+} (Kinraide, 1991, 1997). The low phytotoxicity of $AlSO_4^+$ is of particular practical importance because it explains the amelioration of Al phytotoxicity by application of gypsum ($CaSO_4$) (Wright *et al.*, 1989b; Fig. 17.6). Because of their sulphate component and higher water solubility compared with lime ($CaCO_3$), $CaSO_4$, or gypsum-containing P fertilizer (e.g., single superphosphate compared with triple superphosphate), are more suitable for amelioration of subsoil acidity (Ritchey *et al.*, 1980; Alva and Sumner, 1990).

Aluminium readily forms complexes with organic ligands, particularly with organic acid anions, which reduce phytotoxicity of Al. Their detoxifying capacity decreases in the order citric > oxalic > malic > succinic acid due to the differential stability of the Al complex (Hue *et al.*, 1986). Because of a high c stability with citrate, a 1:1 Al:ligand ratio nearly completely eliminates Al rhizotoxicity, whereas for malate a 1:8 ratio is necessary (Li *et al.*, 2002; Fig. 17.7). Fulvic acid (Suthipradit *et al.*, 1990) and phenols may also detoxify of Al (Barceló and Poschenrieder, 2002). Detoxification of plant-available Al in the soil is one of the reasons for the amelioration of Al toxicity by soil organic

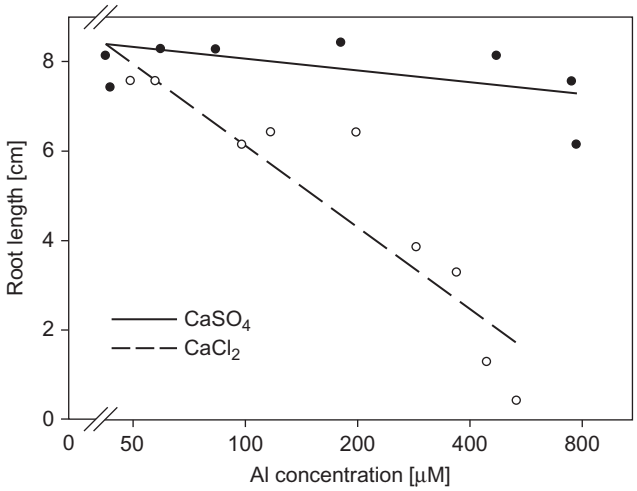


FIGURE 17.6 Root length of wheat as a function of the Al concentration in soil solution from soils treated with $CaSO_4$ or $CaCl_2$. *Modified from Wright* et al. *(1989b).*

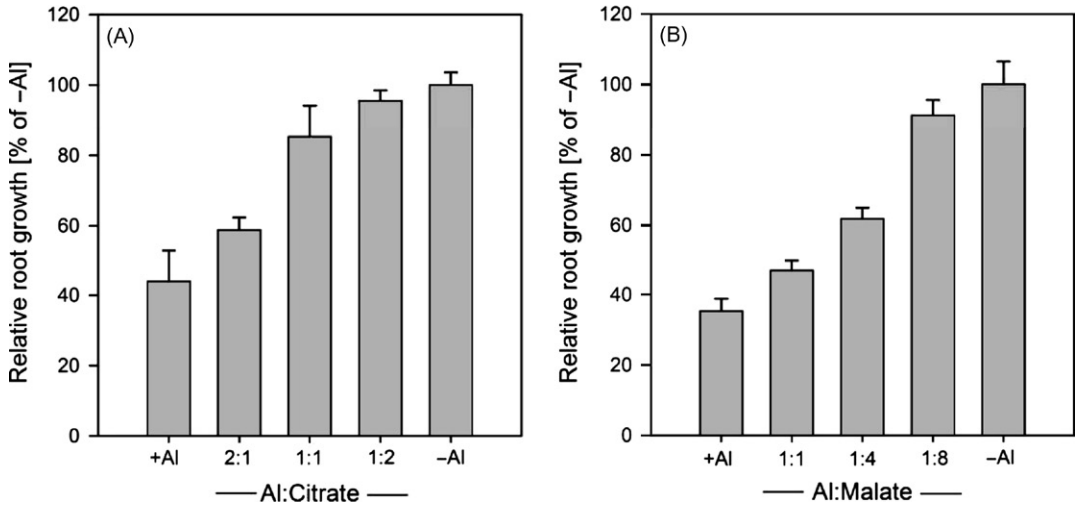


FIGURE 17.7 Relative root growth of wheat (cv Scout-66) as affected by 50 µM Al supply for nine hours without or in the presence of citrate or malate at different Al:organic anion ratios. *From Li* et al. *(2002) with kind permission from Springer Science and Media.*

matter (Haynes and Mokolobate, 2001). As demonstrated by Adams and Moore (1983), root growth of soybean was reduced by lower Al concentrations in the soil solution from the subsoil (low in organic matter) than from the top soil (high in organic matter). Application of mulch (Duong and Diep, 1986) or green manure (Hue and Amien, 1989) may therefore ameliorate Al toxicity in acid soils. A soil organic matter extract was as effective as citrate in detoxifying Al in the nutrient solution (Bartlett and Riego, 1972).

Because of the complex chemistry of Al in solution, the determination of the phytotoxic Al concentration is essential in all studies on Al toxicity. The mononuclear Al (Al_{mono}) has been shown to be a reliable measure of the potentially phytotoxic Al (Kerven *et al.*, 1989). Alternatively, or better additionally, the Al^{3+} activity should be calculated which, however, requires the knowledge of the composition of the solution (Parker *et al.*, 1987; Shaff *et al.*, 2010).

The phytotoxicity of Al not only depends on its speciation and solution concentration, but also on the ionic strength of the solution (Blamey *et al.*, 1991) and, particularly, the Al^{3+}:Ca^{2+} (Lund, 1970) and Al^{3+}:H^+ ratios. The ameliorating effect of high H^+ concentrations (i.e., very low pH) on Al toxicity has been explained by the higher competitiveness of H^+ for apoplasmic binding sites with Al^{3+} as compared to Ca^{2+} (Grauer and Horst, 1992) and/or the reduction of the cell surface negativity (Kinraide *et al.*, 1992). Proton amelioration of Al toxicity is also assumed to be the responsible factor for the lower Al concentration in apical root zones and less severe inhibition of root elongation in plants supplied with ammonium compared with nitrate (Klotz and Horst, 1988b; Grauer and Horst, 1990) since ammonium uptake results in proton release (see also Chapter 2). Proton amelioration of Al toxicity is, however, confined to plant species of high H^+ tolerance (Kinraide and Parker, 1990). Proton amelioration of Al toxicity is also expected to be less relevant for plants grown in acid soils because protons enhance the release of Al^{3+} from the solid phase, and can reduce Ca^{2+} and Mg^{2+} uptake.

17.3.3.2 Inhibition of Root Growth

Inhibition of root elongation is the primary response of plants to excess phytotoxic Al (Fig. 17.8). It can be measured within hours after the roots have been exposed to Al (Llugany *et al.*, 1995; Blamey *et al.*, 2004). Ryan *et al.* (1993) were the first to unequivocally demonstrate the role of the root apex in the perception of Al toxicity in maize. Sivaguru and Horst (1998) showed that the distal part of the transition zone (DTZ, 1–2 mm) is the most Al-sensitive apical root zone in maize. Application of Al only to the DTZ reduced cell elongation in the elongation zone (EZ) to the same extent as application to the entire 10 mm root apex (Kollmeier *et al.*, 2000; Fig. 17.9). However, application of Al only to the EZ did not inhibit

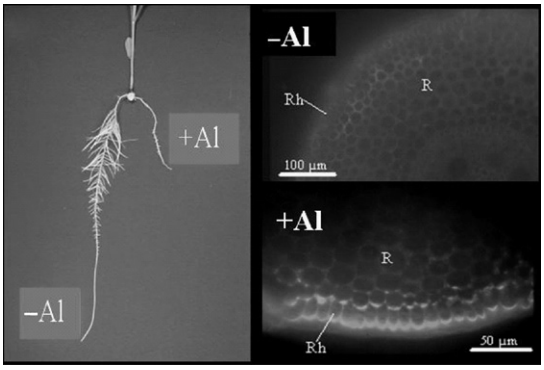


FIGURE 17.8 Inhibition of root elongation by 25 µM Al applied to one half of the root system of maize (left). Al-induced callose formation (fluorescence) in the rhizodermis (RH) and outer cortex (R) of a maize root tip (right). *Courtesy of L. Collet and A. Stass.*

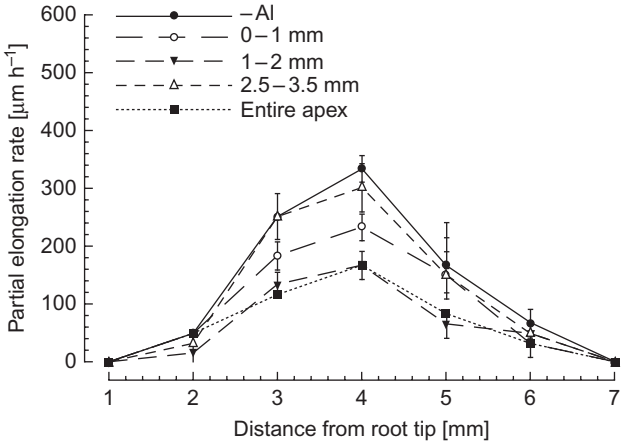


FIGURE 17.9 Partial elongation rates of 1 mm root segments of the primary roots of the maize (cv Lixis) with Al supply to the entire root apex or to specific 1 mm root zones. *From Kollmeier* et al. *(2000) with permission from the American Society of Plant Biologists.*

root elongation. This indicates that signal transduction as proposed by Bennet *et al.* (1985) between the DTZ and the EZ is involved in the inhibition of root growth by Al, possibly via basipetal auxin transport (Kollmeier *et al.*, 2000). Aluminium-induced inhibition of root elongation may also involve the inhibition of polar auxin transport by ethylene (Sun *et al.*, 2010a). The importance of the DTZ (1–2 mm) as a main target of Al was also confirmed in common bean (*Phaseolus vulgaris*) by Rangel *et al.* (2007). However, in contrast to maize, in common bean Al also reduced root elongation when applied only to the EZ, which is also the most Al sensitive root zone in mungbean (*Vigna radiata*) (Blamey *et al.*, 2004).

Treatment with Al as well as with other metals results in the development of transverse ruptures in sub-apical regions of the root through the breaking and separation of the rhizodermis and outer cortical from inner cortical cell layers (Blamey *et al.*, 2004; Kopittke *et al.*, 2008; Fig. 17.13).

It was proposed that these ruptures are due to the binding of Al to the cell wall which increases cell wall rigidity and decreases elasticity. However, the relationship between these ruptures and inhibition of root elongation is not well understood (Ryan *et al.*, 1993; Kopittke *et al.*, 2008).

Another sensitive indicator of Al injury in roots is the induction of callose synthesis (Stass and Horst, 2009; Fig. 17.8), particularly in the root apex (Wissemeier and Horst, 1995; Sivaguru *et al.*, 2006). Aluminium-induced callose formation is an indicator of Al sensitivity and a reliable parameter for classification of genotypes of different plant species for Al resistance (Wissemeier *et al.*, 1992).

The primary target site of Al phytotoxicity leading to inhibition of root elongation is still unclear. Indeed, the relative importance of symplasmic versus apoplasmic lesions in Al toxicity remains a matter of debate, and the role of cell wall properties in Al resistance is not widely acknowledged. Horst *et al.* (2010) stressed the role of the root apoplasm in Al toxicity and Al resistance based on the following observations (for references see Horst *et al.*, 2010):

1. Most of the cationic Al, which rapidly leads to inhibition of root elongation, is bound in the root apoplasmby the pectic matrix of the cell wall with its negatively charged carboxylic groups having a particularly high affinity for Al^{3+}.
2. Anionic Al which is not bound in the cell wall is not phytotoxic.
3. Lowering of the negativity of the apoplasm by reducing the pectin concentration or increasing its degree of methylation decreases Al binding and Al toxicity.
4. Inhibition of root elongation is correlated with cell wall-bound Al, but not with the symplasmic Al concentration.
5. Detoxification of Al in the root apoplasm by root-released organic anions leading to decreased apoplasmic Al binding has a pivotal role in Al resistance.

Cell elongation requires (i) cell turgor pressure which drives expansion, (ii) the release of cell wall components from the symplasm to the apoplasm for cell wall synthesis, and (iii) the formation and cleavage of Ca bonds with the pectic matrix which controls cell wall extensibility (Boyer, 2009). It has been shown that Al treatment reduces root cell wall extensibility (Ma *et al.*, 2004). Strong binding of Al to the pectic matrix may prevent cell wall extension physically and/or physiologically by decreasing the effectiveness of cell wall-loosening enzymes (Wehr *et al.*, 2004).

Aluminium not only rapidly affects properties of the cell wall but also those of the plasma membrane. Interaction of Al with membrane lipids and proteins induces modifications of its structural properties such as fluidity and permeability (Wagatsuma *et al.*, 2005a; Khan *et al.*, 2009). Such structural change in membrane properties is one of the prerequisites, in addition to an increase in the cytosolic Ca^{2+} activity, for the induction of callose synthesis. Binding of Al to the plasma membrane alters its surface negativity (Kinraide, 2006) as shown by Ahn *et al.* (2001, 2004) in squash (*Cucurbita pepo*) and wheat. Additionally, Al rapidly induced membrane depolarization specifically in the most Al-sensitive root zone (DTZ) (Sivaguru *et al.*, 1999a). This may be related to inhibition of the H^+-ATPase activity (Ahn *et al.*, 2001) which in turn may lead to a disturbance of the H^+ homeostasis in the cytosol (Plieth *et al.*, 1999). These changes in plasma membrane properties by Al affect its ion transport properties. In soybean, Al treatment led to a rapid decrease of K^+ efflux without changing K^+ influx (Horst *et al.*, 1992b; Stass and Horst, 1995).

Aluminium-induced impairment of membrane functions may be related to Al-enhanced oxidative stress through the formation of reactive oxygen species (ROS) leading to lipid peroxidation (Yamamoto *et al.*, 1997; Jones *et al.*, 2006) and protein oxidation (Boscolo *et al.*, 2003). Oxidative stress genes are among the genes that are strongly expressed after Al treatment (Ezaki *et al.*, 2005). Transformation of *Arabidopsis thaliana* with such genes conferred Al resistance (Ezaki *et al.*, 2001). However, oxidative stress in roots may not be the primary cause for Al-induced inhibition of root elongation (Yamamoto *et al.*, 2001); in most cases oxidative stress occurs only after prolonged Al treatment (Cakmak and Horst, 1991; Liu *et al.*, 2008). Nevertheless, sustained Al resistance may require protection mechanisms against oxidative stress.

Despite of these changes in plasma-membrane structure and function it should be noted that there is no indication that a severe disruption of plasma-membrane functions is a prerequisite for Al-induced inhibition of root elongation and callose formation (Horst *et al.*, 1992b). It appears that Al triggers signal transduction pathways leading to the observed symplasmic physiological disorders. In this regard the effect of Al on cytosolic Ca concentrations seems to be particularly important (Rengel and Zhang, 2003; Jones *et al.*, 2006). An increase in cytosolic Ca concentrations is an immediate response to Al treatment in a range of plant species (Jones *et al.*, 1998; Zhang and Rengel, 1999; Ma *et al.*, 2002). Increasing cytosolic Ca concentrations can explain callose formation and the disorganization of the cytoskeleton (Rengel and Zhang, 2003). Enhanced callose deposition in the cell wall may be responsible for Al-induced blockage of cell-to-cell translocation via the plasmodesmata (Sivaguru *et al.*, 2000). Aluminium-induced alterations of the cytoskeleton have been reported (Blancaflor *et al.*, 1998; Sivaguru *et al.*, 1999b). Although a direct effect of cytosolic Al on the cytoskeleton cannot be ruled out, an interaction of apoplasmic Al with the cell wall–plasma membrane–cytoskeleton continuum appears more likely (Horst *et al.*, 1999).

TABLE 17.6 Element concentrations in roots and needles of 2-year-old norway spruce (*Picea abies* (L.) Karst.) grown in sand culture and percolated with nutrient solution at different substrate pH with or without Mn and Al

Treatment		Element concentrations (mmol kg^{-1} dw)							
		Fine roots				Needles			
pH		Ca	Mg	Mn	Al	Ca	Mg	Mn	Al
6	–	132	115	0.9	n.d.*	205	74	n.d.	n.d.
3	–	100	82	n.d.	n.d.	77	37	n.d.	n.d.
3	1.5 mM Al	20	33	0.6	30	37	21	0.05	0.01
3	1.5 mM Mn	70	25	35	n.d.	67	15	25	n.d.

Based on Stienen and Bauch (1988).
**n.d. = not determined.*

17.3.3.3 Inhibited Nutrient and Water Uptake and Induced Deficiencies

The primary and specific toxic effect of Al is on root growth, longer-term Al treatment may also affect the nutrient and water supply of the whole plant and may induce water stress and deficiency of nutrients in the shoot. The influence of Al on the uptake of nutrients and water may be direct or indirect through the inhibition of root growth and thus changing root/shoot ratio. Cationic Al strongly competes with other cations such as Ca and Mg for binding sites in the apoplasm (Godbold *et al.*, 1988; Marschner, 1991b), which in turn reduces the accumulation of cations in the root apoplasm and their uptake (Stienen and Bauch, 1988; Table 17.6). Aluminium may also inhibit Ca uptake by blocking Ca channels in the plasma membrane (Huang *et al.*, 1992b), and Mg^{2+} uptake by blocking binding sites of transport proteins (Rengel and Robinson, 1989a).

In contrast to Ca and Mg, the uptake of K is usually not negatively affected by Al supply (Jorns and Hecht-Buchholz, 1985; Wheeler *et al.*, 1992a), leading to an increase in K/Ca + Mg ratio in the shoots. This increases the risk of Ca or Mg deficiency in plants as well as that of grass tetany in ruminants using grass as forage (Rengel and Robinson, 1989b).

The strong competing effect of Al on Ca and Mg uptake explains why the molar ratios of Ca/Al or Mg/Al in the soil or nutrient solution are sometimes better parameters for predicting the risk of Al-induced Ca and Mg deficiencies than the concentrations of any of the individual elements (Kruger and Sucoff, 1989). An example of this is given in Fig. 17.10 for Al-induced Mg deficiency in soybean (Grimme, 1984). Increasing the external concentrations of Al decreased shoot Mg concentration at both low and high Mg supply. However, the Al-induced growth depression was prevented at high Mg supply (i.e., high Mg/Al ratio) as the Mg concentration in the plant tissue remained above the critical deficiency level. Similar results have been shown for the Al/Ca interaction in maize (Rhue and Grogan, 1977).

In addition to the nutrient status, the relative importance of Al-induced Ca or Mg deficiency depends on the plant species (Keltjens and Tan, 1993). Aluminium-induced Ca deficiency occurs mainly in legumes (Foy *et al.*, 1972), whereas in grasses (Tan and Kjeltens, 1995) and forest trees (de Wit *et al.*, 2010) Mg deficiency is more common. To avoid Mg deficiency as a secondary effect of Al toxicity, Mg-containing limestone should be used for amelioration of acid soils (Kaupenjohann *et al.*, 1987; Huettl, 1989).

Clearly, Al/Ca and Al/Mg interactions are ecologically important and have soil fertility management implications. However, Al-induced inhibition of root growth cannot be simply explained by Ca and Mg deficiencies (Kinraide, 2003). Indeed, root growth was inhibited without indication of Ca and Mg deficiencies (Horst *et al.*, 1983; Ryan *et al.*, 1994).

Binding of Al in the root apoplasm may reduce cell wall porosity and thus the mobility of higher molecular solutes. This has led to the hypothesis that Al may directly affect the root hydraulic conductivity; however, this has yet to be proven convincingly (Kruger and Sucoff, 1989; Sivaguru *et al.*, 2006).

Aluminium also indirectly affects water uptake, via inhibited root growth (Fig. 17.11). Root growth and root hair length are crucial factors of P efficiency (see also Chapter 13), thus inhibition of root and root hair growth by Al will strongly reduce the P acquisition of the plants. Enhancing Al resistance of transgenic barley (*Hordeum vulgare* L.) expressing the wheat Al resistance gene *TaALMT1* positively affected P acquisition efficiency on an acid, Al-toxic soil compared to the wildtype

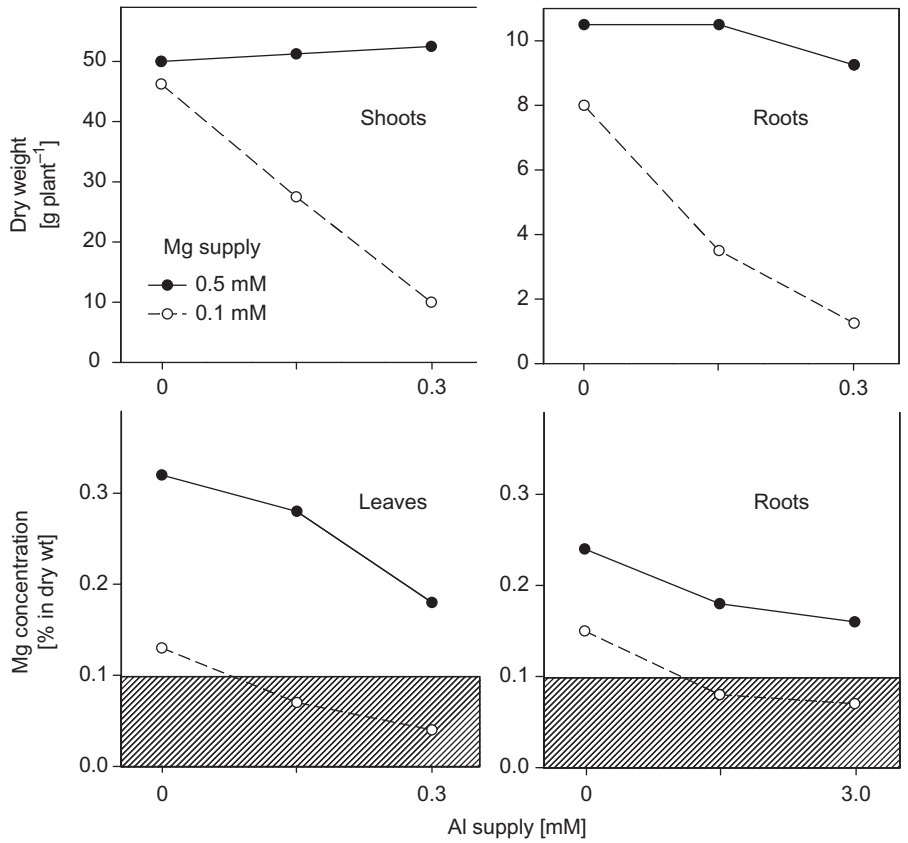


FIGURE 17.10 Relationship between supply of Mg and Al in the nutrient solution and dry weight and Mg concentrations in leaves and roots of soybean. Hatched areas indicate critical Mg deficiency concentrations. *Based on Grimme (1984).*

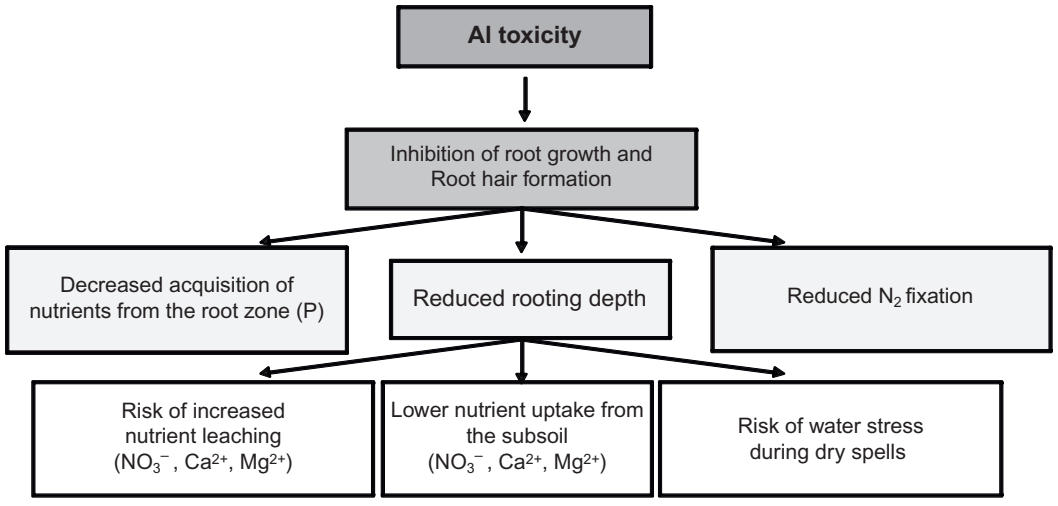


FIGURE 17.11 Consequences of root growth inhibition by Al on nutrient and water uptake.

(Delhaize *et al.*, 2009). Root hair length is even more sensitive to Al that root growth (Care, 1995). Rhizobia infect roots via root hairs (see also Chapter 16), therefore suppression of root hair growth is the main reason for reduced nodulation and N_2 fixation of legumes in acid soils (Sartain and Kamprath, 1975; Wood *et al.*, 1984). Thus, adaptation of legumes to acid soils requires Al-resistant rhizobia (Keyser *et al.*, 1979) in addition to Al resistance of the legume host.

Soil Al particularly restricts root penetration into the subsoil where Al toxicity is frequently greater than in the topsoil due to a lower Al_{mono} concentration and Ca/Al ratio. This may lead to a shallower root system (Marschner, 1991b) with a correspondingly lower uptake of nutrients and water from the subsoil which increases the risk of enhanced nutrient losses by leaching and drought stress (Goldman *et al.*, 1989). Therefore, enhanced crop Al resistance is a prerequisite for sustainable cropping on acid soils aimed at efficient use of nutrients and water.

17.3.4 Manganese Toxicity

With decreasing pH, the concentration of exchangeable Mn increases in many soils. The increase in exchangeable Mn^{2+} is also a function of the redox potential ($MnO_2 + 4H^+ + 2e^- \leftrightarrows Mn^{2+} + 2H_2O$). High concentrations of Mn^{2+} at the exchange sites and in the soil solution are, therefore, to be expected only in acid soils with large amounts of readily reducible Mn in combination with a large concentration of organic matter, high microbial activity (due to oxygen consumption during decomposition of organic matter by soil microorganisms), and anaerobiosis, either temporarily (e.g., short-term flooding) or permanently (see also Section 17.4). On soils with high concentrations of readily reducible Mn, soil acidification by N_2-fixing legumes can strongly increase the amount of exchangeable Mn^{2+} (Bromfield *et al.*, 1983a) and the risk of Mn toxicity in permanent pastures. As the soil pH decreases, amounts of exchangeable Mn^{2+} as well as concentrations of Mn^{2+} in the soil solution increase without change in the ratio Mn^{2+}/total Mn in the soil solution (Sanders, 1983). However, many acid soils in the tropics are highly weathered, and their total Mn concentration is often low because of enhanced mobilization and leaching. Thus, in these soils there is less risk of Mn toxicity than of Al toxicity and even Mn deficiency is frequently observed when these soils are limed to pH > 5.0.

In contrast to Al, Mn is readily transported from the roots to shoots; therefore, symptoms of Mn toxicity first occur in the shoots. The effects of excessive Mn supply on the uptake of other nutrients, metabolism, and phytohormone balance has been summarized by Horst (1988) and Horst *et al.* (1999) and were discussed in Section 7.2. Of particular importance for plant growth in acid mineral soils is the inhibition of Ca and Mg uptake by high Mn concentrations (Table 17.6). Crinkle leaf and chlorosis in young leaves may be related to induced deficiency of Ca and Fe, respectively, and chlorotic or brown speckling in mature leaves are symptoms of Mn toxicity in dicotyledonous plant species in acid soils. Under these conditions, visible symptoms of Mn toxicity are observed even at concentrations which may decrease growth only slightly. This is in contrast to Al toxicity, which severely inhibits growth without producing clearly identifiable symptoms in the shoot. Hence in acid mineral soils with high concentrations of exchangeable levels of both Al and Mn, the growth depression observed may be erroneously attributed to Mn toxicity when in fact Al toxicity is the more important of the two factors (Foy *et al.*, 1978). Mn depresses Mg uptake by blocking binding sites of Mg in the roots (Le Bot *et al.*, 1990; see also Chapter 2). Therefore, high Mn concentrations in the rooting medium may inhibit root and shoot growth by induced Mg deficiency (Langheinrich *et al.*, 1992). Therefore, in soils with toxic Mn concentrations, growth inhibition may be overcome by increasing Mg supply (Goss and Carvalho, 1992).

With soil acidification, Mn concentrations in the soil solution increase more strongly than Mn uptake and concentration in the shoots (Marschner, 1988). This effect can mainly be attributed to the strong inhibitory effects of high H^+ concentrations on uptake of Mn^{2+}.

The occurrence of Mn toxicity is not only a function of soil pH, concentrations of Mn^{2+} and other polyvalent cations in the soil solution, plant species and genotype and microbial activity in the rhizosphere (Section 17.3.4), but also of the availability of Si. Silicon strongly increases the tolerance of the shoot tissue to high Mn concentrations (see also Section 8.3). Thus, on acid mineral soils the harmful effects of excessive Mn concentrations on plant growth may also depend on solubility and uptake of Si.

In legumes, Mn toxicity also depends on the form of N nutrition. With high supply of Mn to common bean, the shoot N concentration decreases to a greater extent in plants depending on N_2 fixation than in plants fed with mineral N (Döbereiner, 1966). Nodulation (i.e., formation of the symbiosis) seems to be particularly sensitive to Mn toxicity in a number of legume species (Foy *et al.*, 1978; Evans *et al.*, 1987), although, at least in isolated culture, most *Rhizobium* strains are more sensitive to Al than to Mn (Keyser and Munns, 1979). In conclusion, nodulation is a very critical step for legumes in acid mineral soils; it is adversely affected by a combination of high Al or Mn or both, low Ca concentrations and low P availability.

17.3.5 Mechanisms of Adaptation to Acid Mineral Soils

17.3.5.1 General

Plants adapted to acid mineral soils utilize a variety of mechanisms to cope with the adverse chemical soil factors. These mechanisms are regulated separately (e.g., those of Al and Mn resistance) or are interrelated (e.g., those of Al resistance and efficiency in P acquisition). From the agronomic viewpoint, for crop plants the sum of the individual mechanisms is important because it determines the requirement of inputs for amelioration of acid soils

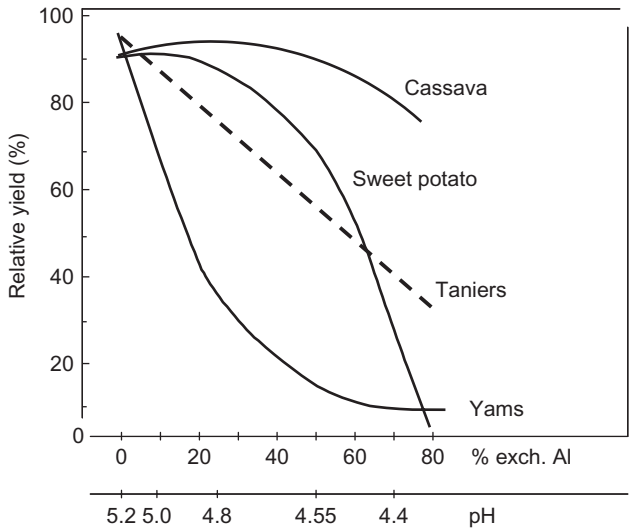


FIGURE 17.12 Relationship between exchangeable Al (Al saturation), soil pH and yield of four tropical root crops. *Redrawn from Abruna-Rodriguez* et al. *(1982).*

(fertilizers and lime in particular). In large areas of the tropics and subtropics, P deficiency is the most important nutritional factor limiting the growth of crop plants (Sanchez and Salinas, 1981).

Large differences occur between crop species in their adaptation to acid soils. For example, among the annual root crop species, cassava (*Manihot esculenta* Crantz) is known for its high tolerance to acid soils, compared to, for example, sweet potatoes, taniers and yams (Abruna-Rodriguez *et al*., 1982; Fig. 17.12). Other acid soil-tolerant crop species are rye, yellow lupin, rice, cowpea, peanut and potato, whereas barley, faba bean, maize, common bean and wheat are non-tolerant species (Sanchez and Salinas, 1981; Horst and Göppel, 1986a, b). As shown in Fig. 17.12, despite the large yield differences in response to alteration in soil pH by liming in three of the four root crops, the macronutrient and Mn concentrations of the leaves were hardly affected, except for Ca (Abruna-Rodriguez *et al.*, 1982). Here, foliar analysis would be of limited value in determining the mechanisms of adaptation and the nutritional status of plants.

Differences in acid soil tolerance between cultivars of a given species can be quite large. For example, in an unlimed soil of pH 4.5 and 80% Al saturation, a traditional, adapted dryland rice cultivar produced ~2.3 tons of grain ha^{-1}, compared with an introduced non-adapted cultivar, which produced only 1 ton; the latter required ~6 tons ha^{-1} lime and a corresponding decrease in Al saturation to 15% to achieve the grain yield of the traditional, adapted cultivar in the unlimed soil (Spain *et al.*, 1975).

Aluminium resistance is the most important individual factor required for adaptation of plants to acid mineral soils. Growth inhibition by increasing Al concentration in a nutrient solution is, therefore, a suitable parameter for the assessment of such adaptation in plants. In a large-scale screening for Al resistance of 34 plant species, Al concentrations needed for reduction of shoot dry weight by 50% varied from less than 1 µM Al in the most Al sensitive to more than 30 µM Al in resistant species (Wheeler *et al.*, 1992c). Using inhibition of root elongation growth as a parameter, the critical Al concentrations in the nutrient solution varied between 1.8 µM in barley and 150 µM in rye and yellow lupin (Horst and Göppel, 1986a). Large differences in Al resistance also exist within a given species, and in crop plants some of this genetic variability appears to have been introduced unintentionally by breeding the same species in different regions with high or low soil pH, as in the case of wheat (Foy *et al.*, 1974; Mugwira *et al.*, 1981) or soybean (Lafever *et al.*, 1977).

17.3.5.2 Aluminium Resistance

Aluminium Detoxification by Root Exudates

It is generally agreed that the Al-activated release of Al complexing solutes, particularly organic acid anions, in the Al-sensitive apical root zone is the most effective way to reduce Al uptake into the root apoplasm (Al exclusion), the impact of Al on apoplasmic functions, and thus inhibition of root elongation (Delhaize *et al.*, 2007a). Ma *et al.* (2001b) described two patterns of organic acid secretion: pattern I plants release organic anions immediately after the onset of Al treatment while in pattern II plants, organic acid anion release starts after a lag phase of several hours. This suggests that in pattern I plants the organic acid anion release mechanism is constitutively expressed, whereas in pattern II plants the induction of the resistance mechanism involves gene expression and new protein synthesis. The Al-induced release of organic acid anions is mediated by plasma-membrane anion channels (Ryan *et al.*, 1997; Kollmeier *et al.*, 2000). The genes encoding these channel proteins have been identified and characterized in several plant species; they belong to two families, *ALMT* and *MATE*.

The *ALMT* (Al-activated malate transporter) facilitates malate efflux in plant species that depend on malate exudation as Al resistance mechanism (Sasaki *et al.*, 2004; Hoekenga *et al.*, 2006). The *MATE* (multidrug and toxin extrusion) proteins are citrate transporters which play an important role in Al-induced citrate exudation (Magalhaes *et al.*, 2007; Furukawa *et al.*, 2007). Convincing evidence of the decisive role of organic acid anion transporters is the enhanced Al resistance of transgenic barley expressing ALMT1 (Fig. 17.13).

The role of the metabolism of organic acids in Al resistance is still a matter of discussion. Most studies have shown no clear relationship between the root concentration and release of organic acid anions and the activities of enzymes involved in the synthesis of organic acids (Ryan *et al.*, 2001).

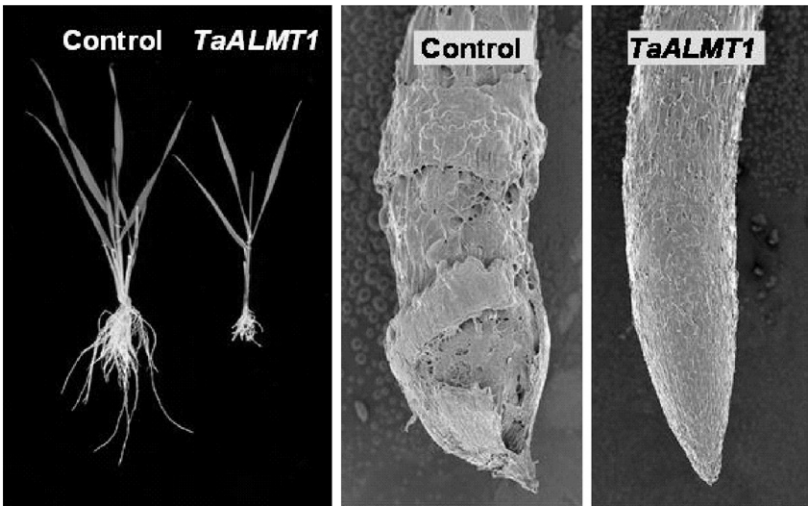


FIGURE 17.13 Growth (left) and morphology of the root apex (right) of the control (empty vector) line and a *TaALMT1* grown in nutrient solution with 3 µM aluminium over 10 days. *From Delhaize* et al. *(2004) with permission by the Natonal Academy of Science.*

These findings and others led Ryan and Delhaize (2010) to suggest convergent evolution of Al resistance in Al-excluder plant species through mutation of transport proteins to organic acid anion permeases. However, sustained recovery from Al stress through citrate exudation in the Al-resistant common bean genotype Quimbaya after 24 h Al treatment relied on restoring the internal citrate pool and the constitutively high activity of citrate synthase (CS) fuelled by high PEPC activity (Rangel *et al.*, 2009b; Fig. 17.14). In the Al-sensitive genotype VAX-1, citrate exudation and, thus, Al exclusion and root elongation could not be maintained, coinciding with exhaustion of the internal citrate pool and decreased CS activity. Similar results have been reported for soybean where Al treatment enhanced the gene expression as well as enzyme activity of mitochondrial CS and reduced the activity of citrate-degrading aconitase (Xu *et al.*, 2010).

There was no difference between the genotypes in the up-regulation of MATE genes coding for citrate permeases (Eticha *et al*., 2010; Fig. 17.14). The delay in MATE gene expression clearly classifies common bean as pattern II plant species. Further evidence for an involvement of enhanced organic acid synthesis and reduced degradation in Al resistance comes from studies using transgenic plants with modified organic acid metabolism. Aluminium-activated citrate exudation driven by Al-inducible expression of mitochondrial CS has been demonstrated in *Paraserianthes falcaria* (Osawa and Kojima, 2006), *Nicotiana benthaminana* (Deng *et al.*, 2006) and tobacco (*Nicotiana tabacum*) (Han *et al.*, 2009). Not only the overexpression of CS but also that of MDH (Tesfaye *et al.*, 2001) and PEPC (Osaki *et al.*, 2001; Ermolayev *et al.*, 2003) enhanced Al resistance of plants. It thus appears that the maintenance of cytosolic organic acid anion concentrations and their release into the root tip apoplasm through activation of anion permeases are both key factors for Al resistance in some plant species.

In addition to organic acid anions, the release into the apoplasm of polypeptides (Basu *et al.*, 1999) and phenols (Heim *et al.*, 1999; Kidd *et al.*, 2001) may be involved in genotypic Al resistance in wheat and maize, respectively. For Al exclusion, mucilage may play a key role. Mucilage is mainly secreted at the root cap and root apical zones and has a high capacity for Al binding and complexation (Horst *et al.*, 1982; Li *et al.*, 2000). In natural grassland on acid soils, the dominance of the unpalatable grass *Aristida juniformis* is most likely related to its high Al resistance due to an unusually high production of root cap mucilage (Johnson and Bennet, 1991).

Enhanced release of organic acid anions under P deficiency is a typical feature in many dicotyledonous plants and may be an important component in the strategies of plant adaptation to acid mineral soils for both increasing efficiency in nutrient acquisition and avoidance of Al toxicity. A similar mechanism is assumed to operate in certain *Eucalyptus* species adapted to extremely acid, P-deficient soils (Mulette *et al.*, 1974). The formation of complexes of Al with polyphenols or organic acids leached from leaves or litter may offer an indirect way for certain *Eucalyptus* species to achieve both high Al resistance and acquisition of P from extremely P-deficient soils (Ellis, 1971).

Aluminium Accumulation and Tolerance

In contrast to Al excluder species, Al includer species, which are among the most Al-tolerant plant species, can contain more than 1 mg $(g\,dw)^{-1}$ Al in their leaves (Jansen *et al.*, 2002). Aluminium accumulators seem to

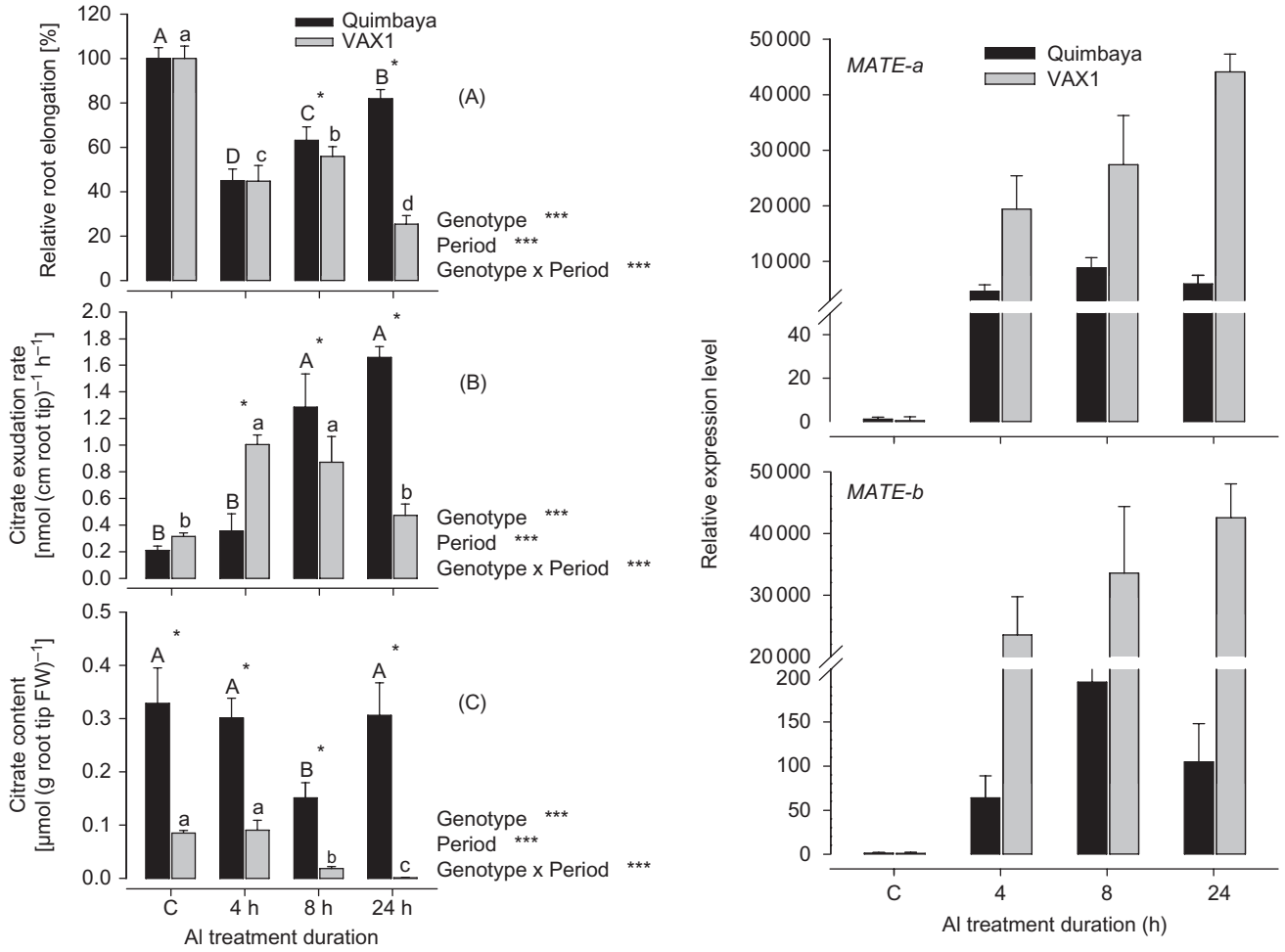


FIGURE 17.14 Root growth (A), citrate exudation rate (B), citrate concentration (C) and expression of two *MATE* genes (right) of 1 cm root tips of two common bean genotypes Quimbaya (Al-resistant) and VAX 1 (Al-sensitive) without (control C) or with 20 µM Al at pH 4.5 for up to 24 h. *From Eticha* et al. *(2010) with permission from Oxford University press.*

be particularly common in those plant families that were present in the early fossil history, for example *Proteaceae* (Chenery and Sporne, 1976). In tropical rain forests, Al includer and excluder coexist at the same site, varying in Al concentrations in the leaf press sap between less than 10 mg and 4,780 mg L^{-1} (Cuenca *et al.*, 1990). Only a few cultivated species are Al includers/accumulators, such as tea (*Carmellia sinensis*), buckwheat (*Fagopyrum esculentum*) and hortensia (*Hortensia macrophylla*). Tea plants not only tolerate high Al concentrations but also their growth is enhanced by Al supply (Konishi *et al.*, 1985). There are also several reports on stimulatory effects of low Al concentrations on growth of other Al-resistant plant species (Foy, 1983), but the mechanism of this stimulation is not clear; it may be related to amelioration of H^+ toxicity by Al (Kinraide and Parker, 1990).

Aluminium tolerance is attributed to symplasmic complexation of Al by organic ligands, particularly organic acid anions (Ma *et al.*, 1998; Morita *et al.*, 2008). Rapid transfer of Al into the symplasm may contribute to maintaining low Al activity in the apoplasm. However, also in Al accumulators Al will strongly interact with the negative binding sites of the apoplasm. Thus when exposed to Al, Al accumulators not only complex Al in the symplasm, but also release organic acid anions from the Al-sensitive root tips and complex Al in the root apoplasm. This has been shown for buckwheat (Zheng *et al.*, 1998), tea (Morita *et al.*, 2001) and hortensia (Naumann, 2001). In buckwheat, there was a close relationship between Al and oxalate concentrations not only in the symplasm but also in the apoplasm of root tips (Klug and Horst, 2010). These authors conclude from their results that the formation of a 1:1 oxalate:Al complex in the root apoplasm protects root apoplasmic binding sites from interaction with Al and is a prerequisite for rapid transport of Al into the symplasm.

Reduced Aluminium Binding in the Root Apoplast

As described above, Al binds readily to negative binding sites of the cell wall and the plasma membrane in the most Al-sensitive zones of the root apex. Since this may lead to enhanced transport of Al into the symplasm and/or to impaired of root growth and functions, reduced binding of Al in the apoplasm should be a prerequisite for Al resistance. Kinraide *et al.* (1992) were able to explain inhibition of root elongation by Al^{3+} in the presence of competing cations, including protons, on the basis of the computed cation distribution on a negatively charged root membrane surface. Blamey *et al.* (1992) and Grauer and Horst (1992) came to comparable conclusions based on similar but conceptually different approaches. A lower root cation exchange capacity as a measure of cell wall negativity has been reported in plant species adapted to acid soils with high Al supply (Blamey *et al.*, 1990; Büscher *et al.*, 1990). However, across a large range of plant species, there is no clear relationship between root CEC and Al resistance (Blamey *et al.*, 1992; Grauer, 1993).

The negativity of the cell wall depends mainly on the pectin concentration and its degree of methylation (DM). Across all plant species studied so far, there is no clear relationship between constitutive pectin concentrations and Al resistance. However, in rice, the pectin concentration of the root apex in the Al-resistant cultivar was lower than in the Al-sensitive cultivar (Yang *et al.*, 2008). In common bean, the initially high Al sensitivity and Al accumulation by roots of the Al-resistant cultivar Quimbaya (Fig. 17.14) was related to a higher concentration of unmethy-lated pectin in the 5 mm root tip (Rangel *et al.*, 2009a).

A role of the DM of root cell walls in Al resistance is supported by the comparison of potato transformants differing in the expression of pectin methylesterase (PME) from *Petunia inflata* (Schmohl *et al.*, 2000, Horst *et al.*, 2007): transformants with higher PME expression (higher DM) accumulated more Al, produced more callose and were more inhibited in root growth when exposed to Al than the wildtype. Applying a pectin immunolocalization method to root tips, Eticha *et al.* (2005) and Yang *et al.* (2008) demonstrated the importance of the DM of cell wall pectin for differential Al resistance of two maize and rice cultivars, respectively. The Al-sensitive cultivars had a lower DM and consequently accumulated more Al and experienced more severe Al injury compared to the Al-resistant maize cultivars.

There is also increasing molecular evidence showing that the modification of the binding properties of the root apoplasm contributes to Al resistance in some plant species for which the Al-induced release of organic acid anions cannot fully explain Al resistance such as maize (Maron *et al.*, 2008) and rice (Huang *et al.*, 2009).

Besides the cell wall, the plasma membrane contributes to the negativity of the apoplasm and may affect the toxicity of metals (Kinraide, 2006). Wagatsuma *et al.* (2005b) related differences in Al resistance between plant species to the plasma membrane negativity of protoplasts, and Yermiyahu *et al.* (1997) ascribed the higher Al sensitivity of the wheat cultivar Scout to its higher plasma membrane negativity compared to the Al-resistant cultivar Atlas. Recently, Khan *et al.* (2009) showed that genotypic Al tolerance in rice was related to a lower ratio of phospholipids to $\Delta 5$ sterols in the plasma membrane leading to a lower negativity and permeability compared to Al-sensitive cultivars. A role of the plasma membrane in Al resistance is also indicated by studies showing that the transformation of yeast and plants by a $\Delta 8$ sphingolipid desaturase from higher plant modulated Al resistance (Da Silva *et al.*, 2006; Ryan *et al.*, 2007).

Rhizosphere pH

At pH 4–4.5, even an increase in rhizosphere pH by 0.1–0.2 units should strongly decrease the concentration of Al^{3+}, but may simultaneously decrease competition for binding sites with H^+ and increase the negativity of the apoplasm enhancing Al phytotoxicity. An increase in rhizosphere pH was proposed as Al exclusion and detoxification mechanism (Mugwira and Patel, 1977; Foy and Fleming, 1982; Degenhardt *et al.*, 1998). However, studies with different forms of N (ammonium versus nitrate with nitrate uptake resulting in an increase in rhizosphere pH; see also Chapter 2) indicated that an increase in rhizosphere pH is of minor importance for higher Al resistance in cultivars of wheat (Taylor, 1988b) and soybean (Klotz and Horst, 1988a). Also, a higher rhizosphere pH at the root tip may be the consequence rather than the cause of Al resistance (Kollmeier *et al.*, 2000). However, the findings from nutrient solution experiments have to be interpreted with care in relation to Al resistance of soil-grown plants where an increase in rhizosphere pH may decrease the release of Al from the solid phase into the rhizosphere soil solution.

Mycorrhiza

Root colonization with mycorrhiza is another important component in adaptation to acid mineral soils with inherent low P availability and high Al concentrations (see also Chapter 15). The role of arbuscular mycorrhiza (AM) is particularly evident for AM-dependent plant species with coarse root systems such as cassava (Howeler *et al.*, 1987; Sieverding, 1991). It is also important in plant species or genotypes where P deficiency-induced root responses such as enlargement of the root system are impaired by Al toxicity (low or moderate Al resistance). In addition to compensation for Al-induced inhibition of root growth, there are indications that some AM fungi detoxify Al in the rhizosphere through exudation of organic acid anions (Klugh-Steward and Cumming, 2009).

In forest trees grown on acid soils in temperate climates, ectomycorrhiza is important not only for P acquisition, but

may also play a role in protection of roots from Al toxicity. In *Pinus rigida* grown in nutrient solution, Al supply reduced root and shoot growth and P concentration in the needles of non-mycorrhizal plants, but growth inhibition was prevented by mycorrhizal colonization (Cumming and Weinstein, 1990). Also in Norway spruce seedlings grown in sand culture, root colonization with ectomycorrhiza increased the Al resistance (Hentschel *et al.*, 1993) which may be due to higher oxalate concentrations in the rhizosphere of mycorrhizal plants (Eldhuset *et al.*, 2007).

Screening for Aluminium Resistance

Field screening of genotypes in acid soils is a labour-intensive process, requiring several months for completion, and is often influenced by secondary factors such as genotypic differences in resistance to diseases and pests. Since Al toxicity is the main factor limiting plant growth in most acid mineral soils, screening for Al resistance in nutrient solution could be a cost-effective alternative. Rapid screening methods have been developed based on inhibition of root elongation (Mugwira *et al.*, 1978), recovery from Al-inhibited root growth (Aniol, 1990), Al accumulation in the root apices using the hematoxylin or eriochrome cyanine R staining methods (Polle *et al.*, 1978; Ma *et al.*, 1997), Al-induced callose formation (Horst *et al.*, 1997) and Al-induced exudation from excised root apices (Delhaize *et al.*, 1993). The main problem in screening for Al resistance is the potential confounding effect with H^+ toxicity in H^+-sensitive plant species such as *Arabidopsis* (Bose *et al.*, 2010) and common bean (Rangel *et al.*, 2005).

In some cases, the classification of genotypes based on their Al resistance in nutrient solution culture correlates well with their Al resistance in acid substrates and soils. However, quite often the correlations are poor (Horst, 1985; Villagarcia *et al.*, 2001; Narasimhamoorthy *et al.*, 2007). This may be related to the plant-induced creation of specific conditions at the plant root–soil interface affecting the rhizotoxicity of Al which do not occur in nutrient solution culture. Of special importance may also be the release of mucilage which may be increased more than 10-fold by mechanical impedance such as that in soils. Also the presence of Si in soils may reduce Al toxicity (Wang *et al.*, 2004a).

Even more important, but also more demanding, is the correct classification of the genotypes using quick screening techniques for Al resistance, for yielding capacity on acid soils. A close correlation can only be expected if among all other edaphic factors limiting yield on acid soils, Al toxicity is the most important. Eticha *et al.* (2005) showed that Al-induced callose concentration in Al-treated root apices was negatively correlated with the relative grain yield (limed soil = 100%) of maize genotypes evaluated across five tropical environments. In addition, the diallel analysis revealed a strong genetic correlation between callose formation in nutrient solution and yield on acid soils (Fig. 17.15). These findings suggest that Al-induced callose formation is a powerful tool to enhance the breeding of maize cultivars adapted to acid soils.

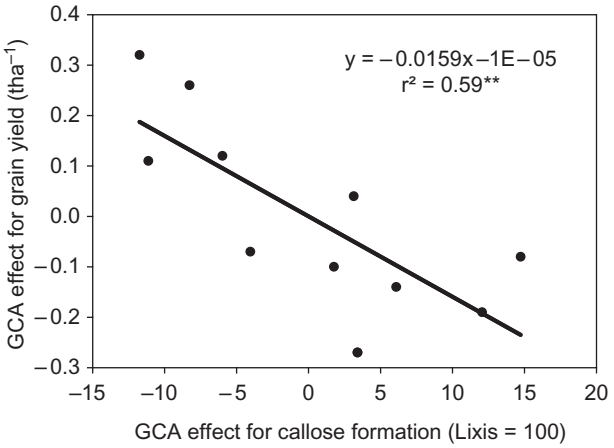


FIGURE 17.15 Relationship between general combining ability (GCA) effects for grain yields on acid soils (field experiments) and for relative Al-induced callose formation (nutrient solution experiments) of 11 maize cultivars. *From Eticha* et al. *(2005) with permission from Elsevier.*

Selection of Al-resistant genotypes in nutrient solution for improved root development in acid subsoils under field conditions may be inadequate because even Al-resistant species may avoid the Al-toxic subsoil by proliferation of roots in the less Al-toxic and P-rich topsoil (Hairiah *et al.*, 1992).

In legumes, impaired growth in acid mineral soils can be due to failure of one of the symbiotic partners, or the symbiosis is more sensitive than either host or bacterium independently (Munns, 1986). There is promising progress showing that it is possible to select Al-resistant host plant germplasm and combine it with acid and Al-resistant *Rhizobium meliloti* strains to increase plant growth under field conditions in acid soils (Hartel and Bouton, 1991).

17.3.5.3 Manganese Tolerance

Plant species, and genotypes within plant species, may differ considerably in resistance to excess Mn (Foy *et al.*, 1988). Differences in Mn resistance between two Douglas fir (*Pseudotsuga menziesii*) varieties were based on differences in Mn uptake and translocation to the shoots (Dučić and Polle, 2006). However, in most plant species, the mechanisms of Mn resistance are located in the shoots, as indicated, for example, by reciprocal root stock-scion grafts of Mn-resistant and -sensitive genotypes (Heenan *et al.*, 1981). Therefore, plants rely mainly on Mn tolerance of the shoot tissue, particularly the leaves. Presently, there are two major lines of evidence for the regulation of Mn tolerance. Based on the assumption that cytosolic Mn activity has to be kept low to avoid Mn interfering with essential metabolic functions, pumping Mn^{2+} from the cytosol into other cell

compartments has been postulated to confer Mn tolerance (Pittman, 2005). It has been reported that in Mn hyperaccumulators, Mn tolerance is mainly due to the sequestration of Mn in the vacuoles (Dou *et al.*, 2009). Molecular studies on cation/Mn transporters confirmed that sequestration of Mn in the vacuoles and ER/Golgi is important for Mn tolerance in yeast (Schaaf *et al.*, 2002) and a number of plant species (Hirschi *et al.*, 2000; Delhaize *et al.*, 2007; Peiter *et al.*, 2007).

The second line of evidence for the mechanism of Mn tolerance in plants is based mainly on experimental work with cowpea. In Mn-tolerant leaf tissue, local accumulation of Mn is prevented by a more uniform distribution (Horst, 1988), indicating that compartmentation of Mn is important for leaf tissue Mn tolerance in this plant species. Manganese toxicity symptoms are located in the cell wall and upon excess Mn supply the apoplasmic activity of H_2O_2-producing and H_2O_2-consuming peroxidases is strongly enhanced in Mn-sensitive but not in Mn-tolerant leaf tissue (Fecht-Christoffer *et al.*, 2006). Therefore, it has been proposed that the leaf apoplasm is the crucial compartment for the avoidance of Mn toxicity in cowpea (Fecht Christoffers *et al.*, 2007). It is remarkable that the molecular and the physiological mechanism of Mn tolerance in rice, one of the most Mn-tolerant crop species, are still unclear (Führs *et al.*, 2010).

In lettuce, restricted translocation of Mn to young leaves may also be involved in Mn tolerance (Blatt and Diest, 1981). An alternative explanation for higher Mn tolerance may be that Ca transport to apical meristems and young leaves is less impaired (Horst, 1988).

Manganese tolerance is not necessarily correlated with Al resistance. Separate screening for Mn tolerance and Al resistance is, therefore, necessary for soils with toxic Mn and Al concentrations. The application of Mn to the petioles of leaves provides a simple, rapid and non-destructive method for screening cowpea for Mn tolerance during vegetative growth (Horst, 1982; Wissemeier *et al.*, 1992).

Manganese tolerance during vegetative growth may not be correlated with tolerance during reproductive growth (Horst, 1988). Particularly in legumes, Mn toxicity may reduce grain yield more than vegetative growth. Under field conditions some cowpea genotypes growing in a soil with high Mn concentration produced little or no grain despite vigorous vegetative growth (Kang and Fox, 1980). The application of Mn to the peduncle seems promising as a technique for screening for Mn tolerance during reproductive growth (Horst, 1982).

17.3.5.4 Nutrient Efficiency

Adaptation of plants to acid soils requires highly efficient uptake and utilization of nutrients or both, especially for P, Ca, Mg and Mo. Many plant species considered to be adapted to acid mineral soils are usually heavily colonized by mycorrhizal fungi (Sanchez and Salinas, 1981; Howeler *et al.*, 1987). Aluminium resistance is combined with high P efficiency coexist in tropical root crops such as cassava (Howeler *et al.*, 1987) and in certain cultivars of wheat and dryland rice (Sanchez and Salinas, 1981).

A high Ca efficiency is usually related to better utilization within plants. In various cowpea cultivars a positive relationship has been observed between Al resistance and Ca efficiency (Horst, 1987). Selection for Al resistance should, therefore, also include adaptation to low Ca supply.

Marked differences in the inhibition of Mg uptake by Al have been found among sorghum genotypes, suggesting that genotypical differences in binding of Mg in the root apoplasm in the presence of Al may be a contributing factor to Al resistance in this species (Tan *et al.*, 1993), and in ryegrass (Rengel and Robinson, 1989a).

In acid mineral soils, Mo availability is very low (Section 7.6). Thus, Mo efficiency may be involved in adaptation to acid mineral soils. This was demonstrated by Brown and Clark (1974) in a comparison of two maize inbred lines grown in an acid soil (pH 4.3). The poorer growth of one genotype was caused by insufficient Mo uptake. Thus, low Mo efficiency may limit the overall adaptation of this genotype to acid mineral soils, despite its high P uptake efficiency even in the presence of Al.

17.4 WATERLOGGED AND FLOODED SOILS

17.4.1 Soil Chemical Factors

Waterlogged and submerged (or flooded) soils are soils with excessive water levels. This often occurs in temperate climates during the winter and spring and also, temporarily, during summer following heavy rainfall or excessive irrigation on slowly draining or poorly structured soils. In current global change scenarios, precipitation is projected to be concentrated in more intense events. Therefore, flooding events may become more frequent (Bates *et al.*, 2008). The length of time during which soils are flooded ranges from a few days to months, with the longest period in soils located below the water table. Waterlogging of soils constitutes a major abiotic stress to plants, affecting plant growth, productivity and species distribution in many areas of the world (Jackson and Colmer, 2005).

Paddy soils are the most well-known agricultural example of flooded soils. Oxygen diffuses in air about 10^4 times more rapidly than in water, and oxygen concentrations are lower in water than in air (Armstrong and Drew, 2002), therefore oxygen is depleted rapidly by the respiration of soil microorganisms and plant roots in waterlogged soils. Various degrees of oxygen depletion (*hypoxia*) and

anoxia (the absence of molecular oxygen) occur, and low oxygen concentrations are often accompanied by high CO_2 concentrations (Greenway *et al.*, 2006). Once free oxygen has been consumed by respiration, various types of microorganisms utilize other terminal electron acceptors for respiration. A sequence of reduction takes place at specific redox potentials and is shown for nutrients in Table 17.7. As soils are non-uniform and characterized by microsites differing in pore size, water content and microbial activity, redox potentials often vary widely over short distances. A change from oxygen sufficiency to deficiency can occur within a few millimetres, and even in aerobic soils, the interior of soil aggregates may be hypoxic (Renault and Stengel, 1994).

TABLE 17.7 Sequence of redox reactions in soil in relation to declines in soil redox potential

Redox reaction	Redox potential *Eh* (mV)
Reduction of O_2	812
$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$	
Nitrate reduction (denitrification)	747
$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$	
Mn^{2+} formation	526
$MnO_2 + 4H^+ + 2e^- \rightarrow Mn_2^+ + 2H_2O$	
Fe^{2+} formation	−47
$Fe(OH)_3 + 3H^+ + e^- \rightarrow Fe_2^+ + 3H_2O$	
Sulfate reduction (H_2S formation)	−221
$SO_4^{2-} + 10H^+ + 8e^- \rightarrow H_2S + 4H_2O$	
Reduction of CO_2 to CH_4	−244
$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O$	

From Chapin *et al.* (2002).

When free oxygen is depleted, nitrate is used by some soil microorganisms as an alternative electron acceptor in respiration (Table 17.7). Nitrate is reduced to nitrite (NO_2^-), various nitrous oxides (e.g., N_2O) and molecular nitrogen (N_2) in the process of denitrification (Fig. 17.16). Nitrite and various nitrous oxides are also formed during heterotrophic nitrification ($NH_4^+ \rightarrow NO_3^-$) by a wide phylogenetic range of fungi and bacteria (Hayatsu *et al.*, 2008). Denitrification may be enhanced in the rhizosphere (Mahmood *et al.*, 1997) due to the lower redox potentials in this zone induced by oxygen consumption by roots and rhizosphere microorganisms. Plants adapted to submerged conditions can release oxygen from their roots, which may stimulate nitrification and subsequent denitrification after diffusion of nitrate into the reduced zone in some distance from the root (Philippot *et al.*, 2009).

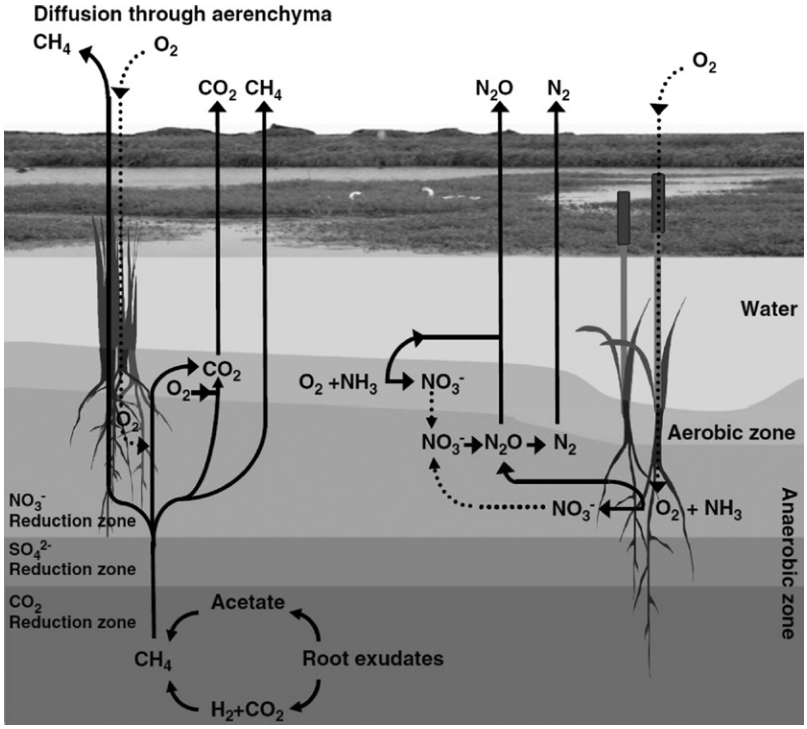


FIGURE 17.16 Production and consumption of N_2O and CH_4 in the rhizosphere of wetland plants. *From Philippot* et al. *(2009) with kind permission from Springer Science and Business Media.*

Manganese oxides (mainly Mn^{4+}) are the next electron acceptors (Table 17.7). In acid soils high in Mn oxides and organic matter but low in nitrate, high concentrations of water-soluble and exchangeable Mn^{2+} can build up within a few days. After prolonged waterlogging, Fe^{3+} is reduced to Fe^{2+} which may accumulate to toxic concentrations, particularly after repeated oxidation and reduction of soils. In acid soils, Fe reduction is associated with a marked increase in soil pH (McKee and McKevlin, 1993). In submerged soils with $FePO_4$, Fe reduction increases P solubility and availability.

Sulphate reduction (Table 17.7) is carried out by microorganisms that are strictly anaerobic. The reduction of sulphate to H_2S in submerged soils may decrease the solubility of Fe, Zn, Cu and Cd and hence their toxicity by the formation of sparingly soluble sulphides (McKee and McKevlin, 1993). Sulphide itself, however, can damage the root, by inhibiting elongation and by suberization of outer layers of the roots (Armstrong and Armstrong, 2005a). Zinc deficiency is widespread in rice. The causes are not entirely clear; however, formation of Zn sulphide in flooded soils is not one of the main reasons. Instead, a high soil pH, high bicarbonate concentrations (Wissuwa *et al.*, 2006) and the formation of sparingly soluble Zn compounds in the oxidized rhizosphere (Shuman and Wang, 1997) contribute to low Zn supply in rice.

Various products of microbial carbon metabolism, such as ethylene, accumulate in waterlogged soils. During prolonged waterlogging, volatile fatty acids and phenolics accumulate in soils high in readily decomposable organic matter (e.g., after application of green manure or straw), which may have a detrimental effect on root metabolism and growth. In submerged soils at very low redox potential, large amounts of methane (CH_4) may be formed, for example from acetic acid (Table 17.7). Indeed, wetland rice fields are a major source of CH_4 emission (Fig. 17.16) and therefore of concern in relation to global change. The global CH_4 emission from paddy fields was estimated to be 20–40 Tg $year^{-1}$, equivalent to about 11% of the total global CH_4 emissions (Qin *et al.*, 2010). Methane emissions are higher in planted than in unplanted paddy fields, as root exudates may increase methane production in the rhizosphere (Philippot *et al.*, 2009) and transport of CH_4 from the roots via the aerenchyma to the shoots where it is subsequently released into the atmosphere (Colmer, 2003).

Changes in agricultural management offer possibilities for substantial mitigation of CH_4 from paddy soils (Qin *et al.*, 2010). The development of molecular approaches has allowed some understanding of the microbial communities involved in greenhouse gas emissions from waterlogged soils. The abundance and the diversity of nitrifiers, denitrifiers, methanogens and methanotrophs are influenced by the presence of plant roots (Philippot *et al.*, 2009). The plant nutrient status may influence rhizosphere gas production and exchange processes. For example, K deficiency may increase denitrification, presumably because of enhanced root exudation and oxygen consumption by rhizosphere microorganisms in K-deficient plants (Prade and Trolldenier, 1990a).

17.4.2 Waterlogging Injury

Plant gene expression changes quickly in response to oxygen deprivation (van Dongen *et al.*, 2009). Under prolonged waterlogging, plant species not adapted to waterlogging (non-wetland, mesophytic species) develop injury symptoms sequentially over a period of several days. Wilting, leaf senescence and, in herbaceous species, epinasty (downward bending of leaves) are often the first symptoms (Drew, 1990; Jackson, 2002). Wilting and epinasty are due to a decrease in hydraulic conductivity of the roots and accumulation of ethylene in the shoots, respectively (Bradford *et al.*, 1982; Drew, 1990). The decrease in hydraulic conductivity is, at least partly, related to a rapid reduction in aquaporin synthesis in roots under hypoxia, which is preceded by a decrease in cytosolic pH (Törnroth-Horsefield *et al.*, 2006; Bramley and Tyerman, 2010). Reduction or cessation of shoot extension growth is another typical symptom in some plant species, followed, after several days of waterlogging, by enhanced senescence of the lower leaves, indicating N deficiency or lack of root-borne cytokinins. Most legumes are particularly sensitive to waterlogging, because hypoxia can interfere with nodulation and nodule function (Roberts *et al.*, 2010). Hypoxia-tolerant legumes may form secondary aerenchyma, which transport oxygen to the nodules (Shimamura *et al.*, 2010).

The severity of the effects of waterlogging on growth and yield depends on the plant species, developmental stage of the plants, soil properties (e.g., pH, organic matter content) and soil temperature. Tolerance to waterlogging among legumes decreases in the following order: faba bean > yellow lupin > narrow-leafed lupin > chickpea > lentil > field pea (Solaiman *et al.*, 2007a). In pea, waterlogging restricts leaf expansion and internode extension, leaves often senesce prematurely and respiration is decreased very quickly upon hypoxia to save oxygen (Zabalza *et al.*, 2009).

Because high soil temperatures increase respiration rates and thus oxygen consumption, the redox potential may decline more quickly, and thus injury from waterlogging may be more severe at high soil temperatures. For example, flooding Kentucky bluegrass for five days decreased leaf elongation rate at soil temperatures of 20 and 35°C by about 25 and 90%, respectively (Table 17.8). However, in oilseed rape, low temperature (10°C) and hypoxic conditions had additive effects on the reduction of leaf area (Franklin *et al.*, 2005).

Waterlogging injuries are related to oxygen deficiency in the root environment, but the actual cause of

TABLE 17.8 Leaf elongation rate, shoot dry mass, root water-soluble carbohydrate concentration, activities of root alcohol dehydrogenase and lactate dehydrogenase in Kentucky bluegrass grown under well-drained or waterlogged conditions for 3 or 5 days at different day/night temperatures

		Day/night temperature (°C)			
		20/15		35/30	
Days	Parameter	Well-drained	Waterlogged	Well-drained	Waterlogged
3	Leaf elongation rate (mm day^{-1})	11.9	11.9	9.2	4.3
	Shoot dry mass (g dw)	2.97	2.58	2.71	2.22
	Water-soluble carbohydrate concentration (mg g^{-1} dw)	119	108	114	65
	Alcohol dehydrogenase (μmol min^{-1} mg^{-1} protein)	0.06	0.92	0.15	1.50
	LDH lactate dehydrogenase (μmol min^{-1} mg^{-1} protein)	0.01	0.15	0.01	0.41
5	Leaf elongation rate (mm day^{-1})	14.0	10.4	8.9	0.6
	Shoot dry mass (g dw)	3.10	2.68	2.74	1.57
	Water-soluble carbohydrate concentration (mg g^{-1} dw)	101	111	105	54
	Alcohol dehydrogenase (μmol min^{-1} mg^{-1} protein)	0.06	0.85	0.15	0.63
	LDH lactate dehydrogenase (μmol min^{-1} mg^{-1} protein)	0.01	0.20	0.01	0.55

Based on Wang *et al.* (2009a).

TABLE 17.9 Possible causes of plant injury by hypoxia and anoxia and mechanisms of adaptation

Causes of injury	Mechanisms of adaptation
Production of toxins in the plant metabolism (fermentation end-products such as ethanol)	Avoidance of fermentation end-product accumulation
High sensitivity of root cells to oxygen deficiency	Avoidance of root anaerobiosis due to oxygen translocation from shoots by: – Aerenchyma formation – Fast under-water shoot elongation – Adventitious rooting
Cytoplasmic acidosis	Cytoplasmic pH stabilization
Impedance to the supply of water, mineral nutrients and plant hormones from roots to shoots	– Lower shoot growth rate – Root and rhizosphere processes for efficient nutrient and water uptake – Modified root-shoot relationships to decrease shoot damage
Energy starvation	Avoidance of energy starvation by efficient substrate provision and ATP production
Post-anoxic damage from free radicals	Detoxification of free radicals

Based on Vartapetian and Jackson (1997).

injury may differ depending on the circumstances (Table 17.9). Possible causes of injury are discussed in the following sections, with emphasis on plant metabolism and nutrition. For comprehensive reviews of the subject the reader is referred to Armstrong *et al.* (1994), Vartapetian and Jackson (1997), Bailey-Serres and Chang (2005), Bailey-Serres and Voesenek (2008, 2010) and Colmer and Voesenek (2009).

17.4.3 Nutrient Deficiency and Toxicity under Waterlogging

Short-term responses of plants to anaerobic soil conditions can be readily demonstrated by waterlogging of a previously well-aerated soil. In some species, the growth of existing roots ceases immediately (Fig. 17.17), and they may die within a few days. In contrast, shoot growth continues for several days at a similar or even higher rate than before waterlogging, although visible symptoms of waterlogging injury (transient wilting, inhibition of leaf extension and chlorosis) are observed in many plant species within a few days (Trought and Drew, 1980a).

Hypoxia strongly inhibits ion uptake by roots and ion transport to the shoot (Armstrong and Drew, 2002). Within a few days of waterlogging, cessation of root growth and root respiration leads to a strong reduction of uptake and transport of nutrients to the shoot (Table 17.10). The lower uptake rate can be explained by the lack of energy for active ion uptake because anaerobic metabolism generates less energy than aerobic pathways. Even mild oxygen deficiency leads to a general decrease in many ATP-dependent biosynthetic processes in the plant (van Dongen *et al.*, 2009). Because shoot growth continues, the nutrient concentration in the shoot declines by dilution. If waterlogging continues, inhibited nutrient uptake and thus nutrient deficiency lead to enhanced leaf senescence and cessation of shoot growth (Trought and Drew, 1980a). For example, N deficiency is a major factor in waterlogging-induced yield loss in soybean (Board, 2008). Nutrient transporter genes may be down-regulated and the abundance of transporter proteins is decreased under hypoxia (Kreuzwieser and Gessler, 2010). In maize, a lack of root aeration resulted in lower N, P and K concentrations in the shoot elongation zone and a decline in shoot elongation growth (Atwell and Steer, 1990). Thus, alleviating nutrient deficiency may reduce the negative effect of waterlogging on plants. In waterlogged wheat, symptoms of enhanced leaf senescence induced by waterlogging can be prevented by daily application of N (nitrate or ammonium) to the soil surface close to newly formed roots (Trought and Drew, 1980b). In long-term experiments, high N fertilizer application alleviated waterlogging injury in cereals (Watson *et al.*, 1976) by compensating for losses by denitrification and impaired uptake from poorly aerated soils. In studies with several rice genotypes, high leaf sheath, culm and leaf N concentrations were related to higher submergence tolerance (Jackson and Ram, 2003).

However, the beneficial effect of additional N application on shoot growth under hypoxia should not be overestimated and generalized, because (i) the uptake of other nutrients may also be impaired (Table 17.10), and (ii) impaired root uptake does not always result in decreased element translocation to the shoot. In a study with tomato under hypoxia, nitrate transport via the xylem to the shoots

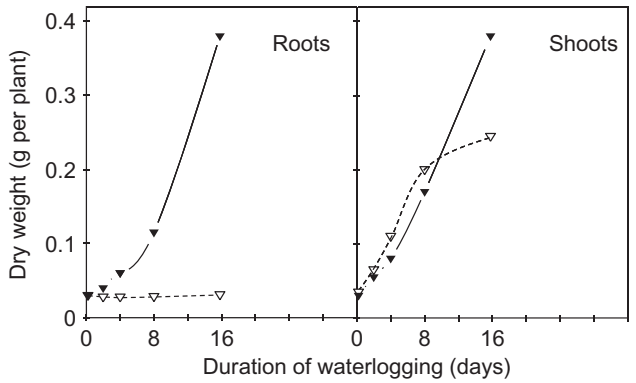


FIGURE 17.17 Dry weight of seminal roots and shoots of winter wheat seedlings in aerated or waterlogged conditions (▼, control; ▽, waterlogged). *Based on Trought and Drew (1980a).*

TABLE 17.10 Growth and shoot nutrient concentrations of barley seedlings after 2 and 6 days of growth in aerated or waterlogged conditions

	2 days		5 days		Net uptake 2–6 days (% aerated)
	Aerated	Waterlogging	Aerated	Waterlogging	
Extension youngest leaf (cm)	6.4	4.2	12.3	5.2	
Shoot dw (mg dw plant^{-1})	170	170	380	360	
Shoot concentration (μmol g^{-1} dw)					
Nitrate	390	139	470	14.3	9.9
P	217	149	210	71	2.9
K	1,540	1,190	1,420	615	9.6

From Drew and Sisworo (1979).

was inhibited, whereas P transport was enhanced. The additional P may originate from injured cells in the root or stem base (Else *et al.*, 1995). Furthermore, nutrient deficiency is only one aspect of waterlogging injury (Table 17.9). In soils high in organic matter and nitrate, sudden waterlogging may lead to accumulation of nitrite in the soil solution to concentrations which are toxic to the roots of sensitive plant species. Tobacco, for example, is injured by nitrite concentrations as low as $5\,mg\,L^{-1}$; values 10 times higher than this are often found in waterlogged soils high in organic matter (Hamilton and Lowe, 1981).

Element toxicity is often a major constraint in waterlogged environments. During waterlogging, toxic concentrations of Mn, Fe, Na, Al and B may occur (Setter *et al.*, 2009). Also, arsenic (As) (Hartley *et al.*, 2010) or iodine (I) (causing the Akagare reclamation disease of plants; Sheppard and Motycka, 1997) can become more available in waterlogged soil. Heavy metal toxicities in flooded soils may lead to contamination of the food chain, for example in peri-urban areas when previously contaminated sites are used for irrigation horticulture. Waterlogging injury caused primarily by Mn toxicity occurs in plant species with low tolerance to Mn such as alfalfa (Table 17.11), particularly in acid soils with high concentrations of Mn oxides. At pH ~5, in combination with low nitrate concentrations (nitrate can act as an alternative electron acceptor), even a 3-day period of waterlogging leads to toxic Mn concentrations in alfalfa leaves. Although liming cannot prevent Mn toxicity induced by short-term waterlogging, it can considerably increase the pH buffering capacity of the soil and thus reduce Mn oxide solubilization and the detrimental effects on growth (see also Section 7.2). Uptake of Mn under waterlogged conditions can also be reduced by high Fe concentrations (Khabaz-Saberi and Rengel, 2010).

However, high Fe availability is not always beneficial. In wetland species, excessive Fe uptake may cause Fe toxicity. 'Bronzing' of leaves is a typical nutritional disorder in wetland rice (Dufey *et al.*, 2009), but also in plant species such as wheat (Khabaz-Saberi *et al.*, 2010). Bronzing is due to Fe toxicity and occurs at leaf concentrations of $\geq 700\,mg\,Fe\,kg^{-1}$ (Yamauchi, 1989) which increase the activity of peroxidases and induce high concentrations of oxidized polyphenols (Peng and Yamauchi, 1993) as is the case with 'brown speckles' caused by Mn toxicity (see also Section 7.2.). The high peroxidase activity can be explained by the formation of oxygen radicals:

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{\cdot -}$$

$$O_2^{\cdot -} + O_2^{\cdot -} \xrightarrow{SOD} H_2O_2$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\cdot} + OH^{\cdot}$$

Reactive oxygen species such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and free radicals (superoxide radical, $O_2^{\cdot -}$; hydroxyl radical, $HO^{\cdot}$) are produced in a number of cellular reactions, including the Fe-catalysed Fenton reaction (Blokhina *et al.*, 2003). Concentrations of H_2O_2 and superoxide radicals are increased, for example, in apple roots under hypoxia (Bai *et al.*, 2010). Hydrogen peroxide accumulation is also a response of wheat roots to re-aeration after hypoxia (Biemelt *et al.*, 2000). Hydroxyl radicals are highly phytotoxic and cause peroxidation of membrane lipids and protein degradation. The enzyme responsible for the dismutation of superoxide anions to H_2O_2 is superoxide dismutase (SOD). Enhanced Fe uptake and superoxide radical formation result in increased activity of SOD and the formation of H_2O_2 which has to be detoxified by peroxidases or catalase (see also Section 7.1)

In general, waterlogging under saline conditions, for example after irrigation with saline water, causes increased Na and Cl shoot concentrations, and rapid leaf senescence (Barrett-Lennard, 2003). At low O_2 concentration in the rooting medium, the selectivity of K^+/Na^+ uptake by roots decreases in favour of Na which reduces the transport of K to the shoots (see also Chapter 2). As a result of the enhanced Na transport to the shoots, root Na concentrations

TABLE 17.11 Shoot dry weight and Mn concentration in the shoots of lucerne grown in a soil with a high organic matter content with or without lime application under aerated or waterlogged conditions for 3 days

Lime application ($g\,kg^{-1}$ soil)	Waterlogging	Soil pH	Shoot dry weight ($g\,pot^{-1}$)	Mn concentration ($mg\,kg^{-1}$ dw)
0	–	4.8	3.1	426
	+	5.2	1.2	6,067
2.5	–	6.4	5.7	99
	+	6.7	3.0	957

Based on Graven *et al.* (1965).

TABLE 17.12 Sodium and Cl concentrations in tomato leaves grown at different temperatures in soil treated for 15 days with saline solution (90 mM NaCl)

Temperature (°C)	Root zone conditions	Leaf concentration ($g\,kg^{-1}$ dw)	
		Na	Cl
10	Drained	15	26
	Waterlogged	18	30
20	Drained	30	33
	Waterlogged	58	95

From West and Taylor (1980).

may be decreased in waterlogged plants (Smethurst *et al.*, 2005). The enhanced shoot transport of Na even after short-term (1 h) oxygen deficiency may remain as a 'memory-effect' for several days, possibly due to protein modifications in the root upon waterlogging (Brauer *et al.*, 1987). In most crop species, salt tolerance is based on mechanisms which prevent or at least restrict salt accumulation in the shoots (exclusion mechanisms, Section 16.6). These mechanisms rely on a high metabolic activity in the roots and thus, in non-wetland species, on soil aeration. At a given salinity level in the substrate, leaf Na and Cl concentrations are increased both by an increase in temperature and by waterlogging (Table 17.12). Because of the higher oxygen requirement at 20°C than at 10°C, waterlogging increased leaf Na and Cl concentrations more strongly at 20°C. Salt injury is therefore more likely at 20°C than at 10°C. In sunflower, waterlogging increased leaf Na concentration more than Cl concentration, indicating the impairment of Na^+ efflux pumps in the roots (Kriedemann and Sands, 1984). This interaction between salinity and soil aeration should be considered in irrigation when saline water has to be used, especially on poorly structured soils, where anaerobic conditions are more likely. Crop species or cultivars with better root aeration under hypoxia may also have a greater tolerance to combined salt and waterlogging stress, as shown with different *Lotus* accessions (Teakle *et al.*, 2010). Some halophyte plant species typically inhabiting flooded saline soils can withstand combined waterlogging and salinity despite increased shoot Na concentrations (Colmer and Flowers, 2008).

As described in Chapter 15, mycorrhizal fungi can enhance nutrient uptake in plants in aerated soils. However, the role of mycorrhizal fungi in plant nutrient acquisition under hypoxic conditions has rarely been studied. Established mycorrhizal mycelium may survive short-term flooding events, presumably by becoming dormant. If the mycelium cannot survive, re-colonization by arbuscular mycorrhizal (AM) fungi may occur either from spores or from the intraradical mycelium (Helgason and Fitter, 2009). Wetland species and even aquatic plants are colonized by AM fungi, and it is possible that the fungi in these soils obtain not only carbon from their host but also oxygen. The benefit for the plants from mycorrhizal colonization under these conditions is still unclear, as P uptake by extraradical hyphae should not be of prime importance for plant survival on waterlogged soils.

17.4.4 Phytotoxic Metabolites under Waterlogging

Even in aerated substrates, the high oxygen consumption in root apical meristems in particular may lead to hypoxic conditions and consequently a proportion of cells can exhibit anaerobic metabolism (Armstrong and Drew, 2002). Low oxygen concentrations without waterlogging can occur not only in root tips, but also in developing seeds, the phloem tissue, or in potato tubers (Geigenberger, 2003). In air, the oxygen concentration is 21% at 20°C, but it is 1–7% in the centre of dense plant organs. Within the root, oxygen concentrations vary spatially and are lowest in meristematic cell tissue (Bailey-Serres and Voesenek, 2008). Under oxygen deficiency, cytochrome oxidase activity becomes oxygen limited and ATP has to be generated by fermentation. Pyruvate decarboxylase (PDC) converts pyruvate to acetaldehyde, which is metabolized by alcohol dehydrogenase (ADH) to ethanol (Table 17.8). NAD^+ is regenerated to sustain glycolysis. Ethanol is not detrimental because of rapid diffusion out of cells, but acetaldehyde is toxic. Acetaldehyde dehydrogenase (ALDH) catalyses the conversion of acetaldehyde to acetate, together with the concomitant reduction of NAD^+ to NADH (Bailey-Serres and Voesenek, 2008). In addition to ethanol, lactate is also produced in plant cells under oxygen deficiency. Lactate is produced from pyruvate by the action of lactate dehydrogenase (LDH; Table 17.8). The pH of the cytosol declines, for example in maize root tips, from pH 7.5 to 6.8. The transition from lactic to ethanol fermentation is controlled by cytosol pH; the pH decline in the cytosol may limit lactate and favour ethanol production. According to the Davies-Roberts pH stat hypothesis, prevention of acidification of the cytosol (e.g., by transport of malate and lactate into the vacuole) is a key factor in resistance to anaerobiosis (Drew, 1990; Kennedy *et al.*, 1992).

The energy shortage in plant cells under hypoxia requires optimizing ATP production as well as restricting energy consuming processes. Mitochondrial respiration is decreased and NADH produced by the glycolytic pathway is re-oxidized in the fermentative pathway (Licausi and Perata, 2009). Anaerobic metabolism is thus enhanced in the roots of most plant species under waterlogging, regardless of their flooding tolerance, and ethanol and lactate

formation is increased at the expense of the carbohydrate degradation in the tricarboxylic acid cycle (TCA).

Anaerobic metabolism results in a severe stress for the plant due to (i) reduced efficiency of ATP formation, (ii) a decrease in cytosolic pH, and (iii) accumulation of toxic fermentation products. Fermentation to ethanol is inefficient carbon utilization, 2 mol ATP per mol hexose are produced compared with 36 mol ATP per mol hexose in the TCA cycle. The enhanced rate of glycolysis under anaerobiosis may be considered as compensatory reaction in terms of energy charge. Minor metabolic end products further contribute to NAD^+ and $NAD(P)^+$ regeneration (Bailey-Serres and Voesenek, 2008). Waterlogging may also result in the accumulation of alanine and succinate, and production of additional ATP (Rocha *et al.*, 2010).

Waterlogging caused, for example, increased ethanol concentrations in the xylem sap of ash seedlings up to concentrations of 3.5 mM (Jaeger *et al.*, 2009). Poor soil aeration induced by excessive irrigation of apple trees may even increase the ethanol concentrations in the fruits and reduce quality during storage (Gur and Meir, 1987). Accumulation of ethanol in roots has been suggested as the main factor responsible for flooding injury to non-wetland species (Crawford and Zochowski, 1984), but this seems unlikely (Vartapetian and Jackson, 1997). Plant cells and tissues are able to tolerate high concentrations of ethanol, and although positive correlations have often been found between ethanol concentrations and flooding injury, the detrimental effects on plant growth are probably caused by the highly toxic acetaldehyde. Acetaldehyde is toxic because it (i) can bind to proteins and DNA (Licausi and Perata, 2009), and (ii) provides electrons for the formation of reactive oxygen species via xanthine oxidase (Mustroph *et al.*, 2006).

There is no universal cause of hypoxia injury. The injury from hypoxia varies greatly between species, tissues and experimental conditions; the time taken for tissues or plants to die can range from a few hours to months. When death is rapid, cellular malfunctions may be due to decline in ATP concentrations leading to impairment of the H^+ efflux pump and acidification of the cytosol. In tissues and plants where damage develops more slowly and ATP concentrations are maintained, carbohydrate shortage may limit survival under anaerobiosis. In some cases, induction of amylases promotes the conversion of starch to glucose. The slow consumption of starch in rhizomes of the flood-tolerant marsh plant *Acorus calamus* allows survival of submergence by maintaining a low level of metabolism (Arpagaus and Braendle, 2000). In agreement with this, under experimental anaerobic conditions, exogenous supply of glucose prolongs root tip viability (Webb and Armstrong, 1983), allows the germination of wheat seeds under anoxia (Perata *et al.*, 1992) and delays loss of elongation potential of roots (Waters *et al.*, 1991).

A possible aggravating factor in hypoxia injury is the resupply of air, which typically occurs under temporary flooding conditions. This 'post-hypoxia stress' is due to re-entry of oxygen into anoxic plant tissue (Blokhina *et al.*, 2003). Oxidative damage can result from increased production of reactive oxygen species or a reduced capacity to detoxify them. In wheat, cycles of hypoxia and high oxygen supply increase oxidative stress (Goggin and Colmer, 2005). After transfer from anaerobic to aerobic nutrient solution, the ratio of reduced-to-oxidized glutathione may decrease, indicating potentially greater oxidative stress in the roots (Colmer and Voesenek, 2009). Even intact roots of some wetland species with aerenchyma can suffer oxidative damage upon resupply of oxygen to the roots (Chen and Qualls, 2003). Pre-treatment with ascorbic acid as an antioxidant may increase tolerance not only during hypoxia (Bai *et al.*, 2009) but also during 'post-anoxia stress'.

Decomposition of plant residues under anaerobic conditions may produce highly phytotoxic compounds (Bonanomi *et al.*, 2006). In soils with high concentrations of organic matter and thus high microbial activity, prolonged periods of waterlogging lead to the accumulation of volatile fatty acids and phenolics in the soil, which are additional stress factors affecting root metabolism, nutrient uptake and growth (Pang *et al.*, 2007), especially at low soil pH.

The relative importance of toxic substances that accumulate in the soil and of root-borne toxins under waterlogging depends on particular circumstances. For example, in non-wetland species, sudden waterlogging at high soil temperature primarily affects root metabolism via anaerobiosis. After prolonged periods of waterlogging of soils high in organic matter, however, accumulation of soil-borne toxins may become an increasingly important cause of injury.

17.4.5 Phytohormones, Root/Shoot Signals

Shoot elongation is a possible escape mechanism for plants under waterlogging. The signal from flooded roots for enhanced shoot elongation is the gaseous phytohormone ethylene. The accumulation of ethylene in soils as well as in roots under waterlogging is well documented. In soils, ethylene concentrations strongly increase at oxygen concentrations in the soil atmosphere below 9% (Hunt *et al.*, 1981). Because of the lower rates of diffusion of gases in water as compared with air, the water film around roots entraps ethylene within the root tissue. The resulting increase in ethylene concentration in the root tissue has a number of effects on root growth and morphology, triggering anatomical changes in the root tissue and the export of ethylene, or its precursor 1-aminocyclopropane-1-carboxylate (ACC). Ethylene also acts as a root signal, inducing epinastic responses to flooding in the leaves of herbaceous

plants (Jackson, 2002). Genes of ethylene biosynthesis, for example genes encoding ACC synthase and ACC oxidase in roots, are up-regulated under flooding (Bailey-Serres and Voesenek, 2008) with expression of ACC oxidase increased after a few hours of hypoxia (Geisler-Lee *et al.*, 2010). Ethylene is transported from the roots to the shoot in the xylem. Flooding-induced ethylene responsive factors have been identified in rice and characterized in *Arabidopsis* (Licausi *et al.*, 2010).

Hypoxia inhibits the synthesis and shoot export of cytokinins (Smit *et al.*, 1990) and gibberellins. Correspondingly, in some species foliar application of cytokinins and gibberellins may counteract, at least temporarily, the inhibition of shoot elongation and enhanced leaf senescence by waterlogging (Jackson and Campbell, 1979). The rapid reduction in leaf elongation rate and stomatal aperture in these species in response to flooding, however, is not caused by lower cytokinin export from the roots (Neuman *et al.*, 1990), but by elevated concentrations of abscisic acid in the leaves. In *Phaseolus vulgaris* under hypoxia, leaf elongation rate decreases within 3–4 h from 0.94 to 0.18 mm h^{-1}, stomatal conductance from 0.94 to 0.25 cm s^{-1}, and the abscisic acid concentration increases from 0.77 to 3.99 nmol g^{-1} leaf dw (Neuman and Smit, 1991). The interplay between ethylene, cytokinins, abscisic acid and gibberellic acid in different plant species during flooding is not yet fully understood. Hormones, oxygen availability and specific metabolites (for example, ATP, sugars and pyruvate) may all be involved in growth and metabolic responses to hypoxia in rice. Ethylene can, for example, decrease abscisic acid concentrations in plants (Hoffmann-Benning and Kende, 1992). Ethylene, gibberellic acid and indole-3-acetic acid (IAA) together affect shoot morphology in flooded *Rumex palustris* (Cox *et al.*, 2004). The concentration of ethylene-regulated abscisic acid in petioles, and the responsiveness to gibberellic acid of these petioles explained the difference in shoot elongation upon submergence among *Rumex palustris* accessions (Chen *et al.*, 2010). Also, adventitious root formation is under control of a synergism between ethylene and IAA (Visser *et al.*, 1996).

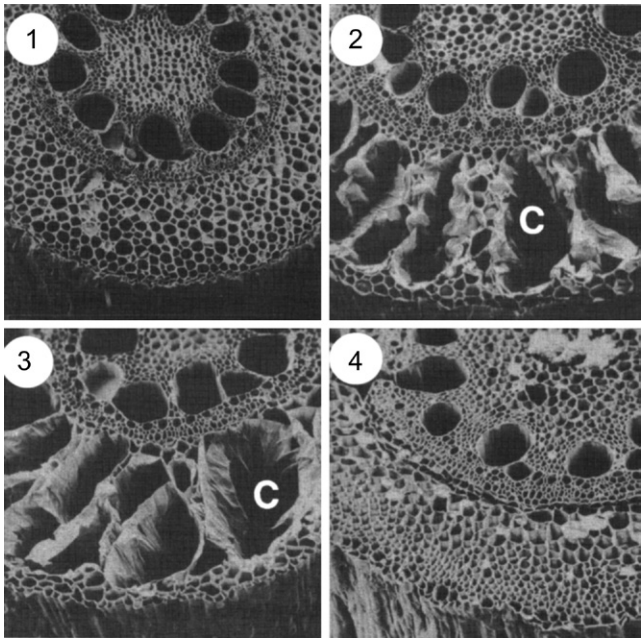


FIGURE 17.18 Transverse sections of maize roots under a scanning electron microscope. (1) control grown in well-aerated solution; (2) root receiving 5 µl ethylene l^{-1} in the air; (3) root from non-aerated solution; (4) root receiving nitrogen gas (anoxic treatment). C, Cortical air space. *From Drew* et al. *(1979) with kind permission from Springer Science and Business Media.*

The formation of aerenchyma, the prominent air spaces in the root cortex which are formed upon waterlogging or growth of roots in non-aerated solutions (Fig. 17.18) is also a consequence of the accumulation of ethylene. Aerenchyma are formed either by (i) cell wall separation and cell wall collapse (*lysigeny*), (ii) separation without collapse (*schizogeny*), or (iii) a combination of variations in cell expansion and division (Seago *et al.*, 2005). Ethylene triggers the induction of lysigenous aerenchyma in roots and a subsequent cascade leading to programmed cell death (Shiono *et al.*, 2008), which is accompanied by enhanced expression of genes involved in cell wall breakdown (Lasanthi-Kudahettige *et al.*, 2007). Even when only the apical root portion is in waterlogged soil, functional aerenchyma is formed all along the root (Malik *et al.*, 2003). This response to elevated ethylene concentrations is not restricted to the roots, but is also observed at the shoot base and basal parts of the stem where a secondary (schizogenous) aerenchyma can be formed by cell separation (Shimamura *et al.*, 2010). The shoot aerenchyma is essential for oxygen supply of roots growing in anaerobic environments.

Enhanced aerenchyma formation in roots is not confined to waterlogging or hypoxia, but also occurs in response to nutrient deficiency, N deficiency in particular, despite low rates of ethylene formation (Table 17.13). Remobilization of nutrients such as P from dying cells during aerenchyma formation may help to sustain shoot growth rates (Postma and Lynch, 2010). The increase in aerenchyma formation under nutrient deficiency may be due to higher tissue sensitivity to ethylene (Schmelz *et al.*, 2003).

17.4.6 Tolerance versus Avoidance

The oxygen deficit in flooded soils is an important driver during evolution (Jackson and Colmer, 2005). Plant species differ widely in their capacity to adapt to flooding, as is apparent from the differences between non-wetland and wetland species. Well-known are the flooding sensitivity of barley and the flooding tolerance of wetland rice,

but differences in adaptation also exist among cultivars of cereal species (Setter and Waters, 2003). Genotypic differences also exist among forage species (Gibberd and Cocks, 1997) or genotypes within a forage species (Gibberd *at al.*, 2001). According to the general stress concept of Levitt (1980), adaptation to oxygen deficiency can be achieved by avoidance of the stress factor (escape) or tolerance of the stress (quiescence) or both (Fig. 17.19).

TABLE 17.13 Ethylene production and aerenchyma formation in roots of maize seedlings grown for 4 days in oxygenated solution without nutrients (control), without N (−N) or with N (+N)

Treatment	Ethylene production ($pmol\,g^{-1}\,fw\,h^{-1}$)	Aerenchyma (% area of root cortex)
Control	~200	6
−N	~165	34
+N	~120	10

Compiled and recalculated data from Drew *et al.* (1989).

The central phenotypical plant adaptation mechanism to oxygen deficiency is internal O_2 movement from shoots to roots within aerenchyma. Other traits, however, are also important (Fig. 17.19). Plant adaptation to complete submergence has been classified into two main strategies (Bailey-Serres and Voesenek, 2008; Colmer and Voesenek, 2009): the Low Oxygen Quiescence Syndrome (LOQS) and the Low Oxygen Escape Syndrome (LOES). Plants with the LOQS are characterized by traits that (i) allow plants to use ATP economically, (ii) increase the abundance of enzymes required to produce ATP without molecular oxygen, and (iii) increase cell components that act against harmful cellular changes associated with flooding. In plants with the LOQS, shoots do not elongate when flooded and shoot growth is arrested. Examples are rice genotypes used for rain-fed rice production. In contrast, LOES plants can adjust the growth direction and increase the rate of growth of shoot organs, to emerge above the water level. Additionally, they invest more resources into aerenchyma or other structures that improve internal gas

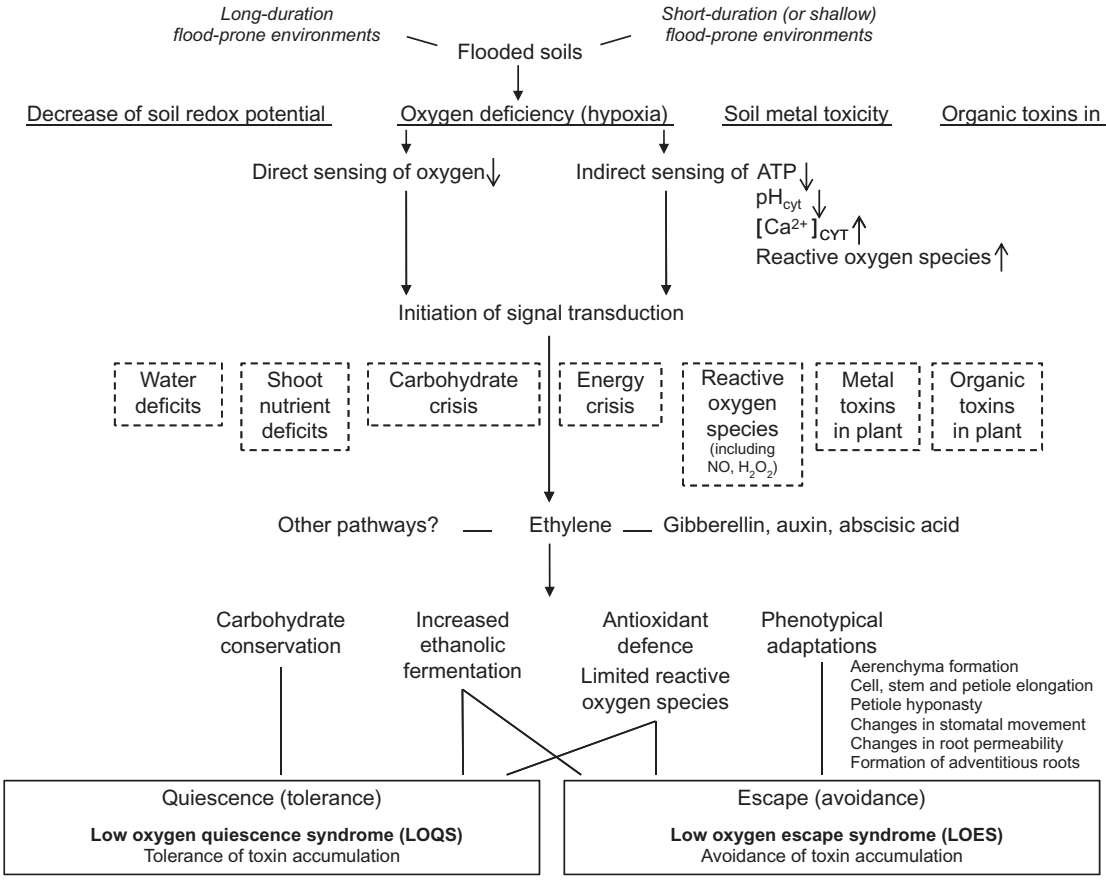


FIGURE 17.19 Adaptation traits of plants to oxygen deficiency in flooded soil, see text for details. *Modified after Bailey-Serres and Chang (2005) and Colmer and Voesenek (2009).*

TABLE 17.14 Overview of the hypothesized importance of various traits associated with plant tolerance of soil waterlogging and/or submergence, for five contrasting types of wet environments inhabited by some terrestrial plant species

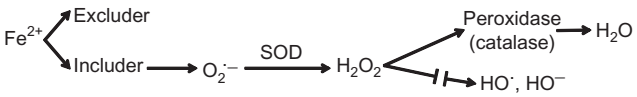
	Waterlogged		Submerged		
Traits	Short	Long	Short	Long-shallow	Long-deep
Adventitious roots (sediment)	*	***	*	***	*
Adventitious roots (water)	na	na	na	**	***
Aerenchyma	**	***	**	***	***
Radial O_2 loss barrier	*	***	*	***	*
Anaerobic energy production	***	*	**	*	***
Energy conservation	**	*	***	*	***
Prevention of ROS formation/ROS defence system	***	***	***	***	**
Tolerance to toxic soil constituents	na	***	*	***	***
Nastic movements	*	**	*	***	**
Shoot elongation	na	na	−ve	***	*
Aquatic leaf traits	na	na	na	***	***
Leaf gas films	na	na	***	***	**
Convective gas movement	*	***	na	***	na

Based on Colmer and Voesenek (2009).
, little importance; **, moderate importance; *, high importance; −ve, costs outweigh benefits; response can decrease fitness in the specific environment; na, not applicable; short duration, <2 weeks; prolonged duration, >2 weeks; shallow, <1 m (i.e., water levels that plants are capable of 'outgrowing'); deep, >1 m. Only biophysical leaf traits are considered. ROS: reactive oxygen species.*

transport or gas exchange between plants and the flooded soil (Colmer and Voesenek, 2009). Extensively studied examples of plants with the LOES syndrome are *Rumex palustris* Sm. and deep-water rice genotypes.

The relative benefit of the two strategies (tolerance versus avoidance) depends on various factors, the duration of oxygen deficit in particular (Table 17.14). For short-term oxygen deficit (e.g., after heavy rains) stress tolerance is required, whereas for long-term oxygen deficit stress avoidance is also needed. Stress avoidance becomes the key factor in the adaptation of plants to permanently waterlogged soils. Stress avoidance may start with direct or indirect sensing of low oxygen supply in some plant tissues, followed by low oxygen signalling within the plant, and a corresponding pattern of gene expression (Fig. 17.19; Licausi and Perata, 2009).

However, flooded or waterlogged soils are not only characterized by oxygen deficit, but also by a number of other features that may be detrimental to the plant (see above). For example, to cope with the high concentrations of Fe^{2+} in submerged soils, wetland species may require particular mechanisms to detoxify Fe either by exclusion from uptake (oxygenated rhizosphere) or uptake (includer) and detoxification of the reactive oxygen species produced in the cells:



17.4.7 Metabolic Adaptation

In both LQES and LOES plants, cellular adaptation to oxygen deficiency requires (i) strict regulation of ATP production and consumption, (ii) limitation of the pH decline in the cytosol, and (iii) detoxification of reactive oxygen species (Bailey-Serres and Voesenek, 2008). In addition to the major fermentation end products, lactate and pyruvate, oxygen deficiency is associated with high concentrations of alanine and γ-aminobutyric acid (GABA; Kreuzwieser and Gessler, 2010), and occasionally succinate and malate. After the end of the waterlogging period, alanine can be recycled back to pyruvate, and GABA can be converted to succinate. Amino acid oxidation can limit the decline in

cytosolic pH and reduce carbon loss via ethanol or lactate (Bailey-Serres and Voesenek, 2008).

Nitrate reduction is an alternative respiratory pathway that may be important for the maintenance of the redox and energy balance of the cell under hypoxia. In the absence of oxygen, nitrite may become an electron acceptor, yielding nitric oxide. The main function of cytosolic hemoglobins, which can be induced by hypoxia, may be the removal of nitric oxide by conversion into nitrate during oxygen deficiency in plants (Dordas *et al.*, 2003; Bailey-Serres and Voesenek, 2008).

In flooding-tolerant species, increased SOD activity under anoxia may be an important protection mechanism in preventing oxidative damage during recovery from hypoxia stress, for example after transient flooding (Vartapetian and Jackson, 1997). However, some regulation and adaptation mechanisms are triggered by reactive oxygen species. Hence, scavenging of reactive oxygen species during hypoxia could be detrimental, because it may interfere with adaptation (Licausi and Perata, 2009). Pre-treatment of roots of wheat or maize for several hours with mild hypoxia (1–6% oxygen) results in several-fold increases in alcohol dehydrogenase activity, and in the subsequent period of strong hypoxia, in increased ATP levels and survival rates (Johnson *et al.*, 1989; Waters *et al.*, 1991).

It is still unknown how plants sense oxygen concentrations. Indirect sensing, mediated by signalling compounds that accumulate during hypoxia, may also be involved. Signals from roots under hypoxia may be mediated by reactive oxygen species or by Ca. Also, hemoglobins could act as oxygen sensors in plants (Licausi and Perata, 2009). Moreover, it is not clear whether all plant cells can perceive the oxygen supply level, or whether this is restricted to certain parts of the tissue (Gibbs and Greenway, 2003). Under oxygen deficiency, special proteins ('anaerobic' polypeptides (ANPs)) are synthesized; in maize, several of these proteins have been identified as enzymes of glycolysis and fermentation (Bailey-Serres *et al.*, 1988).

For adaptation to waterlogging, a high Mn tolerance of the shoot tissue is important, as in wetland rice compared to the flooding-sensitive barley (Table 17.15). Whereas less than 200 mg Mn kg^{-1} leaf are toxic to barley, a ten-fold higher concentration is tolerated by rice without growth depression. Therefore, in barley grown in soils high in Mn, sensitivity to waterlogging may be related to the low Mn tolerance of the shoots. Tolerance mechanisms may include, for example, complexing of Mn with oxalic acid. In some wetland species such as rice, tissue tolerance to Fe is also considerably higher than in non-wetland species. However, many other wetland species are sensitive to high tissue Fe concentration. In wheat, there is considerable genetic variation in tolerance to Fe, Al and Mn toxicity, and high tolerance improves performance of wheat in waterlogged acid soils (Khabaz-Saberi and Rengel, 2010). On the other hand, regardless of the flooding tolerance, Fe toxicity may occur at similar Fe concentrations in the leaves of flooding tolerant and sensitive species (~1,100–1,600 mg Fe kg^{-1} dw), as has been shown for *Rumex* (Laan *et al.*, 1991b).

TABLE 17.15 Growth and Mn concentration of mature leaves of barley and wetland rice at different Mn supply

Mn supply (mg L^{-1})	Shoot dry weight (g $plant^{-1}$)		Mn concentration (mg kg^{-1} dw)	
	Barley	Rice	Barley	Rice
0.2	14	15	70	100
0.5	12	16	190	400
2.0	7	15	310	2,200
5.0	6	12	960	5,300

Data recalculated from Vlamis and Williams (1964).

17.4.8 Phenotypic Adaptation

Wetland plants exhibit a range of phenotypical traits that contribute to flooding tolerance. Some plant species respond to submergence by shoot elongation (an avoidance mechanism; Fig. 17.19). In rice shoots, ethylene responsive DNA binding proteins act downstream of ethylene and modulate gibberellin-mediated shoot growth (Bailey-Serres and Voesenek, 2010). In response to high water levels, deep-water rice genotypes can survive by elongating leaves and internodes (Fig. 17.20 top). The elongated shoot keeps the top leaves above the water. Increases in shoot height of 20–25 cm day^{-1} have been observed under these conditions; the plants can reach a maximum height of up to 7 m (Nagai *et al.*, 2010). On the other hand, if non-deep-water rice varieties which are generally planted in aerated soils or shallow water (Nagai *et al.*, 2010), are subjected to prolonged deep water, the plants drown and die because of oxygen starvation (Fig. 17.20 top).

In rice, two multigenic loci have been characterized that control the capacity to endure complete submergence (Submergence 1, Sub1) or the rapid shoot growth under partial submergence (Baily-Serres and Voesenek, 2010). The elongation of deep-water rice under flooding is stimulated by the ethylene-regulated genes, SK1 and SK2 (Fig. 17.20 top). In contrast, some upland rice cultivars can tolerate short periods of flooding by conserving substrates during flooding which are then used for growth after the water has receded (Fig. 17.20 bottom; Colmer and Voesenek, 2009). Genes at the Sub1 locus confer submergence tolerance in this case (Nagai *et al.*, 2010). Thus, rice seedlings without

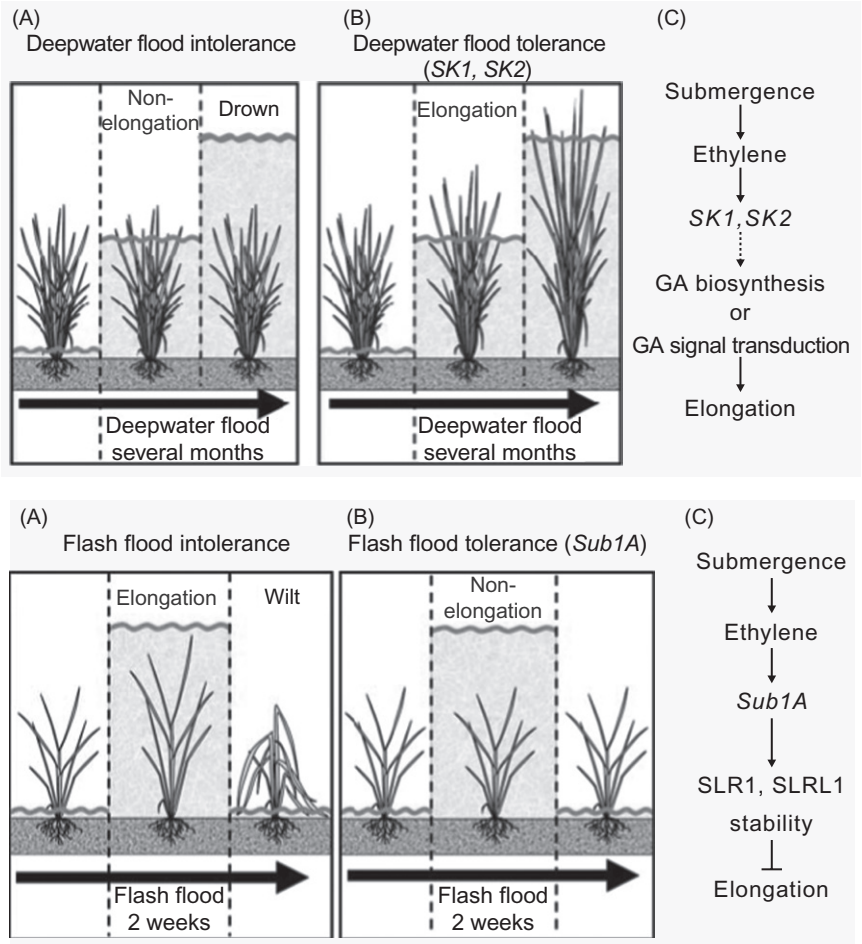


FIGURE 17.20 *Top*: Model of deep-water tolerance and plant hormones in rice: (A) strategy of non-deep-water rice in a deep-water flood; (B) strategy of deep-water rice in a deep-water flood; (C) metabolic regulation of deep-water tolerance in deep-water rice. *Bottom*: Model of flash flood tolerance and plant hormones in rice: (A) behaviour of flash flood intolerant rice; (B) strategy of flash flood tolerance at the rice seedling stage; (C) metabolic regulation of flash flood tolerance. *From Nagai* et al. *(2010) with kind permission from Springer Science and Business Media.*

or with the *Sub1A-1* allele respond differently to short-term flooding.

Irrespective of such differences in shoot growth response, the possibility to transport oxygen from the shoots to the roots and into the rhizosphere is the basis of most avoidance strategies in response to flooding or waterlogging. This transport is readily demonstrated in both wetland and non-wetland species (Greenwood, 1967), and may provide a substantial proportion of the oxygen demand of roots also of non-wetland species grown in aerated soils (Willigen and Noordwijk, 1989). Oxygen transport takes place to a limited extent in air-filled intercellular spaces; the main pathway, however, is the aerenchyma in the root cortex (Fig. 17.18). The proportion of air-filled intercellular spaces of the total root volume is an expression of root porosity. Root porosity differs between plant species and is also adaptive (Fig. 17.18; Table 17.13). For wetland rice, maize and barley grown in an aerated nutrient solution, the relative values for root porosity are 1.0, 0.25 and 0.10, respectively (Jensen *et al.*, 1967). To a certain extent, the root system of non-wetland species has the capacity to adapt to waterlogging (Table 17.16). When plants were grown in well-drained soil for 2 weeks, and thereafter were exposed to flooding or left aerated, the root porosity of most plant genotypes tested (with the exception of barley) was higher under flooding than non-flooding (Table 17.16). Maize and the wheat cultivar Pato showed the greatest degree of adaptation. The differences in the root porosity of wheat cultivars corresponded well with the higher tolerance of Pato to waterlogging compared to Inia under field conditions (Yu *et al.*, 1969).

Many flooding-tolerant species develop aerenchyma not only in the roots but also in the rhizomes (Laan *et al.*, 1989), and in most cases there is a positive correlation between flooding tolerance and size of the aerenchyma (Laan *et al.*, 1990). In *Rumex* species, root porosity was

TABLE 17.16 Rooting depth and root porosity of non-wetland plant species grown under drained and flooded conditions in a loam soil

Species/ cultivar	Root porosity (%)		Root depth (cm)	
	Drained	Flooded	Drained	Flooded
Maize	6.5	15.5	47	17
Sunflower	5.5	11.0	33	15
Wheat				
cv Pato	5.5	14.5	10	5
cv Inia	3.0	7.5	23	10
Barley	3.5	2.0	32	15

Data recalculated from Yu *et al.* (1969).

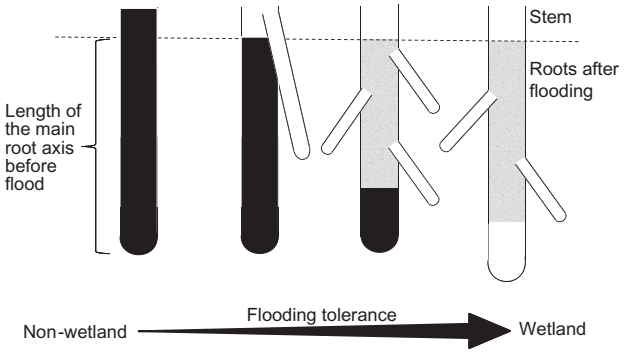


FIGURE 17.21 Suggested relationship between the responses of roots of non-wetland and wetland species to a limited period of soil flooding. Black areas, dead tissue; grey areas, surviving tissue; white areas, regrowth. *Based on Armstrong (1979).*

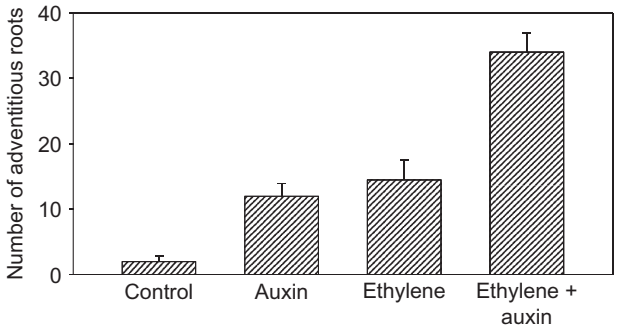


FIGURE 17.22 Number of adventitious roots in *Rumex palustris* after application of auxin, ethylene and a combination of both hormones. Control is a well-aerated nutrient solution. *Based on Blom (1999).*

10% in the sensitive, 35% in the intermediate and 50% in the highly flooding-tolerant species (Laan *et al.*, 1989). Although in many wetland species aerenchyma formation is constitutive, flooding enhances aerenchyma formation further, with ethylene involved in this effect. Aerenchyma formation in the basal part of the stem connects the root aerenchyma with hypertrophic lenticels on the stem just above the water surface which may serve as oxygen entry points (Shimamura *et al.*, 2010).

Changes in root anatomy in response to flooding are accompanied by changes in root morphology. After waterlogging many old roots die, but numerous adventitious roots with well-developed aerenchyma emerge from the base of the stem and grow to a limited extent into the anaerobic soil. Whether existing roots die upon sudden waterlogging and new (adapted) roots have to be formed, or whether the development of aerenchyma is enhanced in the existing roots, depends mainly on the plant species and the flooding tolerance of the species (Laan *et al.*, 1991a). The principal differences between species are shown schematically in Fig. 17.21. The formation of adventitious roots is regulated by hormones. Auxin that cannot be transported from the shoot to the root after flooding may accumulate at the shoot–root junction and trigger the formation of adventitious roots (Blom, 1999). In addition, ethylene is also involved in adventitious root formation in many species. In *Rumex*, a combination of ethylene and auxin is responsible for a significant increase in the number of adventitious roots (Fig. 17.22). Stem hypertrophy and adventitious root formation are thus phenotypic characteristics of plants under hypoxia.

Another plant trait that improves submergence tolerance of, for example, rice is the formation of leaf gas films (Colmer and Voesenek, 2009). A thin layer of gas is retained on submerged leaves of some species, depending on leaf surface traits (Colmer and Pedersen, 2008). The gas films enlarge the water–gas interface, thus improving gas exchange between submerged shoots and the surrounding water. Disturbance of such leaf gas films on submerged plants reduced underwater net photosynthesis and internal aeration of roots (Pedersen *et al.*, 2009).

Oxygen transport to submerged roots by diffusion is, however, not very effective over long distances, such as in trees or large shrubs. For efficient long-distance transport in the aerenchyma from shoots to roots other mechanisms are required. In some wetland species, where the roots arise from rhizomes deep below the water surface and where the shoot system may also be partially submerged, aeration can be enhanced by pressurized (convective) internal gas flows (Afreen *et al.*, 2007). In addition, internal oxygen generation in stems by chlorophyll-rich photosynthesizing cells that utilize respiratory carbon dioxide may also be important for pressurized ventilation (Armstrong and Armstrong, 2005b).

In common reed (*Phragmites australis*), in addition to pressurized gas transport from shoots to roots, the gas flow rate is enhanced by wind which sucks air into the belowground system via dead culms (Armstrong *et al.*, 1992).

Accordingly, high wind speed can considerably enhance rhizosphere aeration in common reed.

Long-distance transport of oxygen in the aerenchyma to the apical zones of roots growing in flooded soils requires restriction of oxygen loss by diffusion into the rhizosphere along the transport pathway (Armstrong and Beckett, 1987). In many wetland species, the basal zones of roots have a barrier to radial oxygen loss (Colmer and Voesenek, 2009), for example in rice under flooded conditions, while in other species this barrier may be constitutive (Colmer and Voesenek, 2009). Oxygen released at the root tip creates an aerobic rhizosphere. The oxygenation of the rhizosphere ('oxidation power' of roots) is readily apparent from $Fe(OOH)_x$ precipitated on rice roots when grown in flooded soil. It has been calculated that at maturity ~500 kg $Fe(OOH)_x$ per hectare may be present as root coating ('plaque') each season (Chen *et al.*, 1980). Other observations suggest that 'plaques' are limited to young roots that oxidize the rhizosphere and disappear in older roots due to prevailing anoxic conditions (Nanzyo *et al.*, 2010). Microbial Fe oxidation may contribute to plaque formation. The presence of the Fe-oxidizing bacterium, *Sideroxydans paludicola*, in monaxenic microcosms grown with *Juncus effusus* increased Fe^{2+} oxidation rates 1.3 to 1.7 times and increased Fe plaque formation (Neubauer *et al.*, 2007). A high oxidation power of roots and plaque formation may lead to the formation of sparingly soluble $ZnFe_2O_4$ (Sajwan and Lindsay, 1988) or Fe phosphate (vivianite) crystals (Nanzyo *et al*., 2010) and thus may induce Zn or P deficiency in rice. Iron plaques around rice roots also modify the uptake of elements such as arsenic (Chen *et al.*, 2005). Permanently flooded soils (e.g., mangrove swamps) often have high concentrations of both Fe^{2+} and hydrogen sulphide (H_2S). In addition to internal ventilation, tannins at the rhizoplane may play a role in oxidation of Fe^{2+} and H_2S and in the formation of the sparingly soluble FeS (Kimura and Wada, 1989).

In rice and probably other wetland species, the formation and stability of the aerenchyma are dependent on Si supply. With Si supply, the length of the oxidation zone along adventitious rice roots is decreased (Fig. 17.23) which may reduce uptake of Fe and particularly Mn (Ma and Takahashi, 1990). Nutrient supply may also affect 'oxidation power' indirectly: nutrient deficiencies that increase the exudation of photosynthates from the roots may simultaneously enhance microbial activity and oxygen consumption in the rhizosphere.

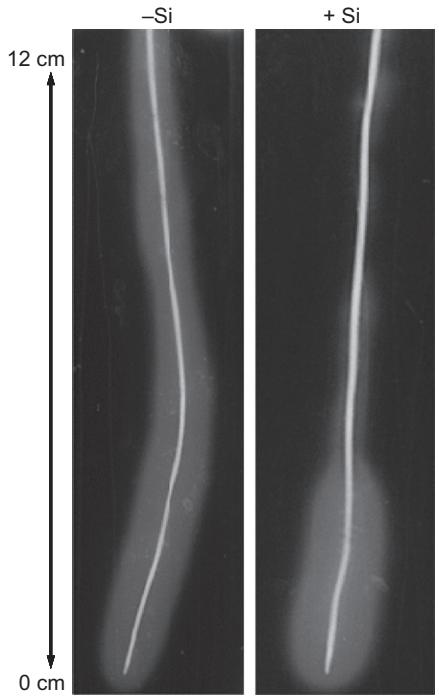


FIGURE 17.23 Oxidation power of adventitious rice roots as affected by Si supply 24 h after embedding in FeS-agar medium. Plants were grown for 28 d in nutrient solution without Si or with 1.78 mM Si. *From Fleck* et al. *(2011) with permission from Oxford University Press.*

17.5 CALCAREOUS AND ALKALINE SOILS

17.5.1 Soil Characteristics

The pH is one of the most important factors determining nutrient sorption and dissolution processes in soil (Comerford, 2005). Soils with a pH >7 are very common in semi-arid and arid climates. These soils may be grouped into calcareous and alkaline soils, with alkaline soils having a pH >8.5 (Fig. 17.24), but other authors consider calcareous and alkaline soils as synonyms. High pH soils cover more than 30% of the earth's surface (Chen and Barak, 1982). Their concentration of free $CaCO_3$ in the upper horizon varies from a few percent to 95%. Important high pH soils are the Rendzinas or Leptosols in the FAO soil classification system (FAO, 2001, 2006b), which are shallow soils with an organic layer overlying calcareous material. Typical other calcareous soils are Calcic Luvisols, Calcaric Phaeozems, Chernozems or Kastanozems. Luvisols are important calcareous soils of the humid zone in which calcite is present in subsurface horizons (Chesworth, 2008). The pH of calcareous soils is determined by the presence of $CaCO_3$, which buffers the soils in the pH range 7.5–8.5 (Table 17.17). Typical alkaline soils are Calcisols and Gypsisols. Calcisols are common in less arid grassland areas, while Gypsisols ('desert soils') are found in very dry climates (Chesworth, 2008).

When salts are present in the parent material or the groundwater, arid conditions lead to capillary rise of water from depths which will bring this salt into the top soil. Solonetz is an important soil unit under these conditions. Solonetz (also called sodic soils or alkali soils) are characterized by a pH >8 and by a natric horizon within 100 cm of the soil surface. A natric horizon

has an exchangeable sodium percentage (ESP) greater than 15 within the upper 40 cm or more exchangeable Mg + Na than Ca + exchange acidity (at pH 8.2) (FAO, 2001). The ESP is the percentage of the cation exchange capacity (CEC) occupied by Na ions (ESP = exchangeable Na × 100/CEC). The Solonetz group comprises soils with a dense clay horizon which has a high proportion of adsorbed Na and/or Mg ions (FAO, 2001). Solonetz that contain free soda (Na_2CO_3) are strongly alkaline (pH >8.5).

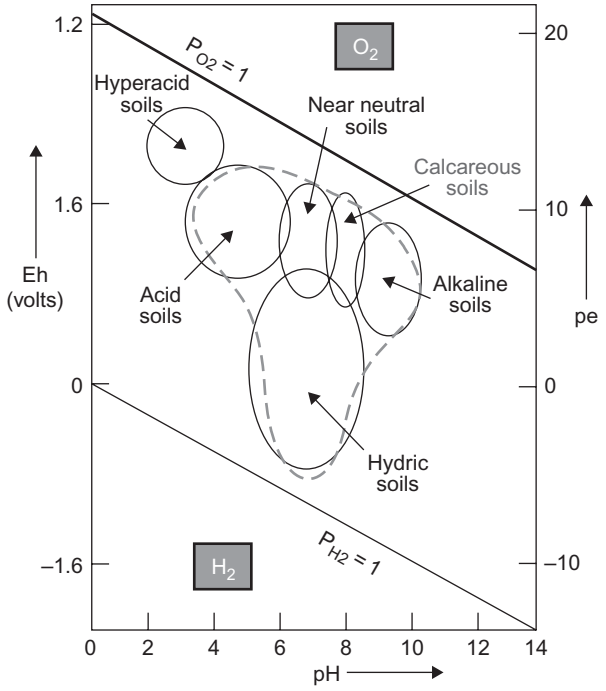


FIGURE 17.24 Properties of calcareous and alkaline soils. The dashed line encloses the conditions in most mineral soils. *From Chesworth (2008) with kind permission from Springer Science and Business Media.*

Sodic soils usually occur in association with saline soils (salt-affected soils or Solonchaks), and saline–sodic soils are more abundant than purely sodic soils. Solonchaks have a high concentration of soluble salts for at least some time of the year (FAO, 2001). In the context of constraints on plant growth it is necessary, however, to make a clear distinction between salinity and sodicity. Saline soils are not necessarily alkaline, and plant growth on saline soils is affected mainly by high concentrations of NaCl (ion toxicity, ion imbalance) and impairment of water balance (Section 17.6). Sodic soils, on the other hand, are alkaline and plant growth is impaired mainly by high pH, high bicarbonate and often poor aeration.

The major nutritional constraints in calcareous soils differ from those in sodic soils (Table 17.17). The differences are related to soil chemical factors such as bicarbonate concentration or to soil physical factors. In sodic soils, poor soil structure and correspondingly poor soil aeration are the major constraints which are often associated with Na and B toxicity.

Nitrogen is a growth-limiting factor for most crop species growing in alkaline soils. More than 90% of the soil N is organic N in the soil organic matter which becomes

TABLE 17.17 Relative abundance of high pH soils and major constraints on plant growth in these soils

	Soil pH		
	7	8	9
	Calcareous soils		Alkaline soils
Examples of soil groups	Leptosols (rendzinas), Luvisols, Chernozems		Solonetz (sodic soils) Solonchaks (saline soils)
Relative abundance			
Major nutritional constraints	Deficiency: Fe, Zn, P (Mn)[a]		Toxicity: Na, B Deficiency: Zn, Fe, P (Ca, K, Mg)
Other constraints	Excess of HCO_3^- Water deficit Mechanical impedance		Poor aeration Excess of HCO_3^- Water deficit Mechanical impedance

[a]*Parantheses indicate less frequent occurrence, or only in certain situations.*

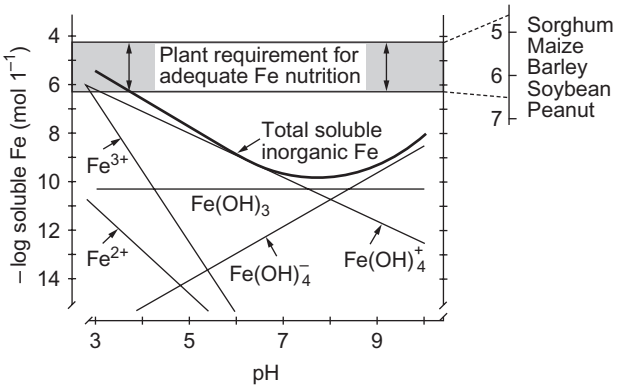


FIGURE 17.25 Solubility of inorganic Fe species in equilibrium with Fe oxides at different pH in well-aerated soils in comparison to the requirement of soluble Fe at the root surface of various plant species. *After Römheld and Marschner (1986).*

TABLE 17.18 Iron concentration in equilibrium solutions of selected iron oxides

Oxide	Fe concentration at pH 8 (M)
Ferrihydrite	10^{-9}
Goethite α-FeOOH	10^{-12}
Hematite α-Fe_2O_3	10^{-13}

Based on Kraemer (2004).

available to plants after mineralization by soil microorganisms. Both the total amount of soil N and its availability to plants are therefore closely related to the soil organic matter content and conditions for mineralization (soil moisture, temperature, aeration). The soil pH is only of minor importance for the concentration and turnover of N in high pH soils.

17.5.1.1 Iron

Mineral soils have, on average, a total Fe concentration of 20 to 40 g kg^{-1}. Most crop species remove only between 1 and 2 kg Fe ha^{-1} annually. In well-aerated soils with a high pH, however, the concentration of Fe^{2+} and Fe^{3+} in the soil solution is very low, and the total concentration of inorganic Fe species (between pH 7 and 9 mainly $Fe(OH)_2^+$, $Fe(OH)_3$ and $Fe(OH)_4^-$) in the soil solution is only around 10^{-10} M (Boukhalfa and Crumbliss, 2002; Fig. 17.25). Iron solubility is particularly low in calcareous soils. The total soluble Fe in soil expressed as the sum of all Fe species in equilibrium with the Fe oxide goethite is negligible above pH 8 (Robin *et al.*, 2008). Other Fe oxides also have very low solubility at pH 8 (Table 17.18; Kraemer, 2004). The concentration of chelated iron required for optimal growth is thought to be 10^{-6} to 10^{-5} M (Fig. 17.25). It should be noted, however, that these values are based on the supply of synthetic forms of Fe, for example iron chelates such as Fe EDTA which are utilized relatively poorly, at least by grasses.

Iron is a transition metal with two stable oxidation states, Fe^{2+} and Fe^{3+} with Fe^{2+} being more soluble in water compared to Fe^{3+}; however, Fe^{2+} exists mainly under anoxic conditions (see also Section 7.1). In aerated soils the solubility of Fe is largely controlled by Fe^{3+} oxides, especially ferrihydrite (5 Fe_2O_3.9 H_2O) and amorphous ferric hydroxide ($Fe(OH)_3$), and the formation of Fe chelates with compounds derived from soil organic matter (humic acids, organic acids, phenolics) or microbial siderophores (Lindsay, 1991). Iron chelates can be very important; for example, in a soil of pH 7.9, more than 35,000 times the concentration of soluble Fe was found than predicted from inorganic equilibrium constants (O'Connor *et al.*, 1971). In alkaline soils with a high organic matter content the concentration of Fe chelates in the soil solution can reach concentrations of 10^{-4} to 10^{-3} M (Mashhady and Rowell, 1978). Some Fe–humic complexes do not have a high Fe bioavailability (García-Mina *et al.*, 2004). Nevertheless, the application of farmyard manure to calcareous soils low in organic matter has been recommended as an effective strategy for increasing Fe solubility (Bar-Ness and Chen, 1991), and Fe uptake by crop species with low Fe efficiency such as sorghum (Mathers *et al.*, 1980).

Microbial siderophores are major Fe^{3+} chelating compounds. Under Fe deficiency, siderophores are produced by almost all microorganisms. Important groups of siderophores comprise ferrichromes, produced by fungi, and enterobactin, pyoverdine and ferrioxamines, produced by bacteria (Marschner *et al.*, 2011). The concentrations of siderophores are considerably higher in the rhizosphere than in the bulk soil (Reid *et al.*, 1984), and it has been suggested that the concentration of Fe-siderophore chelates may exceed that of soluble non-chelated Fe by several orders of magnitude (Crowley *et al.*, 1987). However, the actual concentrations of soluble Fe in the rhizosphere of soil-grown plants are difficult to estimate as siderophores are strongly adsorbed by the soil matrix at higher pH (Cline *et al.*, 1983). Bacterial siderophores are in general poor Fe sources for plants (Marschner *et al.*, 2011). In the rhizosphere, Fe solubility is mainly governed by root-induced changes in the rhizosphere, particularly under Fe deficiency (see also Chapter 2 and Section 7.1).

In sodic soils (pH >8.5), Na carbonate ($NaHCO_3$) disperses organic matter, and low-molecular-weight organic substances (mainly Na humates) form soluble complexes with Fe and Mn. Increasing the $NaHCO_3$ concentration from 12 to 75 mM (pH 8.0→8.8), increased the

concentration of Fe and Mn in the soil solution by a factor of 18 and 2.3, respectively (Mashhady and Rowell, 1978). This humate effect was also demonstrated in solution culture, where the addition of Na humates prevented Fe deficiency in tomato plants grown at high pH in the presence of high bicarbonate concentrations (Badurowa *et al.*, 1967).

17.5.1.2 Zinc

The solubility of uncomplexed Zn, like that of uncomplexed Fe, decreases with increasing pH. In the range of 5.5 to 7.0, the equilibrium concentration of Zn may decrease 30- to 45-fold for each unit increase in soil pH (Moraghan and Mascagni, 1991). Diffusion coefficients for Zn in calcareous soils are therefore about 50-fold lower than in acid soils (Melton *et al.*, 1973). The Zn concentration in the soil solution is also determined by adsorption and desorption processes occurring in the soil matrix, therefore the concentration of Zn at a given soil pH may also depend on other solute components as well as on soil organic matter content and microbial activity. Similarly to Fe, the application of farmyard manure to alkaline soils low in organic matter may increase the solubility and plant uptake of Zn (Srivastava and Sethi, 1981). Application $NaHCO_3$ increases alkalinity and therefore enhances the risk of Zn deficiency in plants because of a decrease in Zn extractability in the soil (Mehrotra *et al.*, 1986) and direct impairment of root growth (see below).

17.5.1.3 Manganese

The chemistry of Mn in soils and soil solutions is governed by pH and the redox status (Marschner, 1988; see also Section 7.2). Although Mn may form organic complexes, Mn^{2+} is the major species in the soil solution (Norvell, 1988). In well-aerated calcareous soils, the solubility of Mn decreases with increasing concentrations of both $CaCO_3$ and MnO_2 due to the adsorption of Mn on $CaCO_3$ and its oxidation on MnO_2 surfaces and, probably, to precipitation of Mn calcite (Jauregui and Reisenauer, 1982). Therefore addition of $CaCO_3$ (lime) to acidic soils low in Mn may induce Mn deficiency. In calcareous soils, Mn availability to plants is mainly determined by oxidation-reduction and root-induced changes in the rhizosphere (see also Chapter 14).

17.5.1.4 Boron and Phosphorus

Organic matter plays an important role in controlling B concentration in the soil solution, with additions of composted material reducing plant availability of B (Yermiyahu *et al.*, 2001). However, B is also adsorbed by clay; B adsorption to clay minerals increases strongly above pH 6.5 and is maximal at pH ~9 (Goldberg, 1997). In alkaline soils, the low B solubility resulting from B adsorption to clay minerals is usually compensated for, however, by a lack of leaching or by B supplied by irrigation water. Boron toxicity is thus much more likely, particularly in sodic soils, than B deficiency.

In alkaline soils (except Chernozems), P availability is generally low. The concentration of P in the soil solution is determined primarily by desorption and adsorption of P, particularly in soils with more than 1% organic matter and not by dissolution or precipitation of definite inorganic compounds such as tricalcium phosphate (see also Section 6.3). At pH 6 to 8, the P concentration in the soil solution may increase (Welp *et al.*, 1983), as P in organic matter tends to be less stable at high pH than at low soil pH (Troeh and Thompson, 2005). In alkaline soils with higher pH and low soil organic matter content, the equilibrium constants of inorganic P forms become increasingly important for the concentration of P in the soil solution. In general, however, P deficiency in crop plants growing in alkaline soils is caused primarily by very low concentrations of total P and low soil water content, which limits root growth and the mobility of P in the soil (see also Chapters 12 and 13).

17.5.2 Major Chemical Constraints to Plant Growth

17.5.2.1 Iron Deficiency

The most prominent nutritional disorder of crop plants grown in soils with more than 20% $CaCO_3$ is Fe deficiency or so-called 'lime-induced chlorosis' (Schinas and Rowell, 1977). Plant species that are mainly affected include apple, peach, citrus, grapevine, peanut, soybean, sorghum and upland rice. It is the major problem in sorghum and soybean production in the Great Plains of the United States (Clark, 1982a). For reviews on this topic the reader is referred to Chen and Barak (1982) and Hansen *et al.* (2006).

The solubility of Fe in soil is decreased with increasing pH and increasing bicarbonate concentrations. Soil pH and bicarbonate are interrelated through pH buffering by equilibria among H_2CO_3, HCO_3^- and CO_3^{-2} (Rogovska *et al.*, 2007). Iron deficiency in calcareous soils is often enhanced by poor soil aeration caused by soil compaction or high water content (Zuo *et al.*, 2007), and low soil temperatures which prolong the time in which the soil is wet (Römheld, 1985). It is, however, not oxygen deficiency which enhances chlorosis, but elevated concentrations of bicarbonate (HCO_3^-). In soils with free $CaCO_3$, an increase in CO_2 concentration (e.g., by impaired gas exchange or amendments of organic matter) leads to formation of $Ca(HCO_3)_2$. At 1–5% CO_2, HCO_3^- concentrations are predicted to be 4–20 mM (Chaney, 1984). The importance of high HCO_3^- concentrations in the soil in Fe deficiency on calcareous soils depends upon the plant

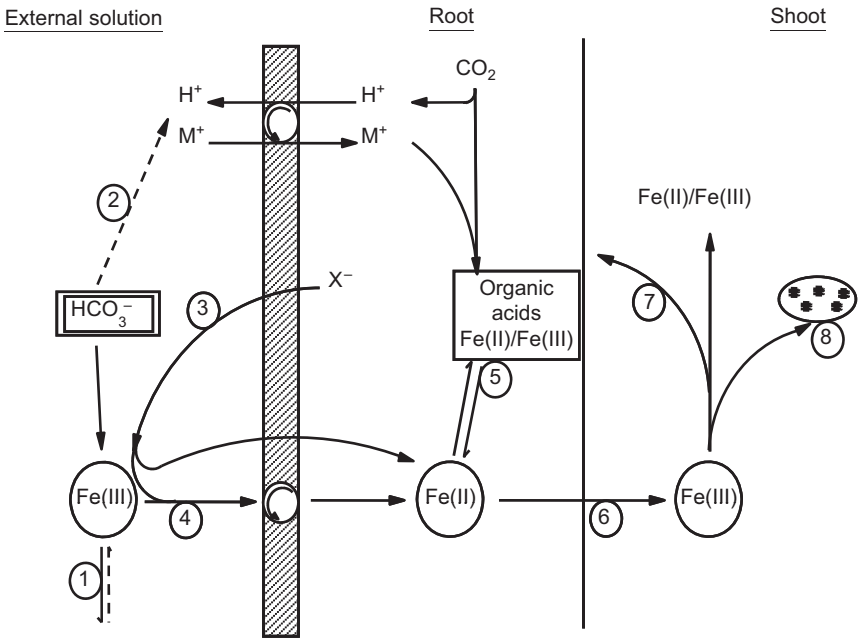


FIGURE 17.26 Possible effects of a high bicarbonate concentration in the substrate on uptake, transport and availability of Fe for chlorophyll formation in leaves. For a description of mechanisms (1)–(8), see text.

species and their root response to Fe deficiency. In graminaceous species (Strategy II, see also Chapters 2 and 14) such as sorghum and maize, Fe deficiency is not affected by elevated HCO_3^- concentrations in the soil (Chaney, 1984; Yen *et al.*, 1988), but occurs at low concentrations of poorly crystalline or amorphous Fe oxides (Loeppert and Hallmark, 1985). In contrast, in non-graminaceous species (Strategy I), the severity of Fe deficiency increases with increasing HCO_3^- concentrations, for example in peanut (Zuo *et al.*, 2007), soybean (Chaney, 1984; Inskeep and Bloom, 1986, 1987), grapevine (Römheld, 1985) and apple (Ao *et al.*, 1987). Bicarbonate can induce Fe chlorosis by pH-related effects, but also by inhibiting the expression of ferric reductase, iron transporters and H^+-ATPase genes in Strategy I plant species such as *Arabidopsis*, pea, tomato and cucumber (Lucena *et al.*, 2007). Based on this key role of bicarbonate in Strategy I plants, bicarbonate-buffered nutrient solutions containing low concentrations of soluble Fe can be used in screening for susceptibility to Fe deficiency chlorosis, for example in soybean, chickpea and citrus (Chaney *et al.*, 1992a). The relative susceptibility observed in these solutions is highly correlated with the relative susceptibility observed in wet calcareous soils.

In chlorotic leaves of Strategy I plants suffering from lime-induced chlorosis, the Fe concentration is often lower (Häussling *et al.*, 1985; Ao *et al.*, 1987; Dockendorf and Höfner, 1990), but may also be similar or even higher (Chen *et al.*, 2004; Jiménez *et al.*, 2009; Nikolic and Römheld, 2002) than in green leaves. Similar or higher Fe concentrations in chlorotic leaves indicate 'physiological inactivation' of Fe (see also Section 7.1).

Figure 17.26 summarizes some of the major mechanisms by which high HCO_3^- concentration may affect the uptake, translocation and utilization of Fe in plants. A high HCO_3^- concentration in the soil solution increases, but also buffers the pH and thus further reduces the concentration of soluble inorganic Fe (mechanism (1)). This leads to inhibition of root responses to Fe deficiency in Strategy I plants, including impairment of the H^+-efflux pump by neutralization of H^+ (mechanism (2)), reduced release of phenolics (mechanism (3)) and Fe^{3+} reduction at the plasma membrane (mechanism (4)) (Römheld and Marschner, 1986). In agreement with this, high HCO_3^- concentrations strongly decrease Fe uptake and transport to the shoot (Kolesch *et al.*, 1984; Dockendorf and Höfner, 1990). At least in short-term studies, this inhibitory effect of high HCO_3^- concentrations can be simulated by an organic pH buffer (HEPES) which demonstrates the importance of acidification of the rhizoplane and the apoplasm of the rhizodermis cells for Fe acquisition of Strategy I plants (Table 17.19).

At high HCO_3^- concentration in the rhizosphere soil solution, CO_2 fixation and organic acid synthesis in the roots are increased (Lee and Woolhouse, 1969b). It is not clear to what extent sequestering of Fe in vacuoles by certain organic acids (mechanism (5)) contributes to the inhibition of Fe transport to the shoot (mechanism (6)). The transport of Fe to expanding leaves is impaired (Rutland

TABLE 17.19 Rate of Fe^{3+} reduction and ^{59}Fe uptake in Fe-deficient peanut plants supplied with ^{59}Fe EDDHA as affected by the buffering capacity of the nutrient solution at pH 8.5. HEPES = N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid

	Fe reduction	^{59}Fe uptake
Treatment	($nmol\,g^{-1}$ root $dw\,h^{-1}$)	
Unbuffered	4,208	658
+10 mM HCO_3^-	1,592	95
+10 mM HEPES	1,513	72

Based on Marschner *et al.* (1989).

and Bukovac, 1971), and the distribution of Fe within the leaf tissue may be uneven (mechanism (7)) (Rutland, 1971). Bicarbonate-induced chlorosis was also thought to lead to alkalinization of the leaf tissue and thus Fe immobilization (Mengel and Malissiovas, 1981), but other studies showed that high HCO_3^- concentrations in the soil solution did not decrease Fe availability in the leaf apoplasm (Nikolic and Römheld, 2002).

High HCO_3^- concentrations in the rooting medium may indirectly affect Fe concentration and utilization in leaves. In many plant species, chlorosis-sensitive species in particular, high HCO_3^- concentrations inhibit root growth (Lee and Woolhouse, 1969a), root respiration, root pressure-driven solute export into the xylem (Wallace *et al.*, 1971) and the rate of cytokinin export to the shoot. Cytokinins are necessary for protein synthesis and chloroplast development (Parthier, 1979; Werner and Schmülling, 2009). In agreement with this, high HCO_3^- concentrations may inhibit shoot growth prior to the occurrence of Fe deficiency chlorosis, for example in sorghum (McCray and Matocha, 1992) and peach trees (Shi *et al.*, 1993). High Fe concentrations in leaves of plants suffering from lime-induced chlorosis may therefore also be the consequence of a limitation of factors required for leaf expansion growth, chloroplast development and chlorophyll formation (mechanism (8)).

The role of P in lime-induced chlorosis is complex. It may impair Fe nutrition at various levels, for example by decreasing the rate of dissolution of Fe from Fe oxides in the bulk soil and in the rhizosphere (root exudates). High P supply may also suppress P deficiency-induced root exudation of organic acids (see also Section 6.3 and Chapter 14) and thus chelation and solubility of Fe in the rhizosphere. In the Proteaceae, high P supply suppresses cluster root formation and thereby induces Fe deficiency chlorosis (Handreck, 1991) or decreased leaf concentrations of other micronutrients (Shane and Lambers, 2005). The P concentration in the plants also affects Fe availability with inactivation of Fe by high P concentrations. (Cumbus *et al.*, 1977; Ladouceur *et al.*, 2006) or higher Fe availability in low P plants (Zheng *et al.*, 2009), but this is unlikely to play a role in the field. High P concentrations in chlorotic leaves are probably the result of growth inhibition (concentration effect) and are thus the consequence and not the cause of Fe chlorosis. Many laboratory and greenhouse studies on P–Fe interactions have been conducted with P concentrations that are orders of magnitude higher than those typical of soil solutions in calcareous soils. Although Fe deficiency can be induced in crop plants growing in calcareous soils supplied with very high levels of fertilizer P, there is substantial doubt that P is responsible for the occurrence of lime-induced chlorosis under field conditions (Kovanci *et al.*, 1978; Mengel *et al.*, 1979). In the field, Fe deficiency chlorosis is more likely to be the consequence of high concentrations of bicarbonate or soil compaction (Hansen *et al.*, 2006).

Lime-induced chlorosis is of minor importance in sodic soils, mainly for two reasons: (i) the increase in Fe solubility by low-molecular-weight organic compounds, and (ii) growth inhibition by soil constraints other than Fe deficiency (Mashhady and Rowell, 1978).

17.5.2.2 Zinc and Manganese Deficiency

Increasing the pH of a soil by liming usually decreases the plant availability of Zn and Mn to a greater extent than of any other nutrients, including P (Table 17.20). Therefore, the risk of Zn deficiency is high in soils after liming or on calcareous soils in general. In cereals, Zn deficiency is probably the most widespread micronutrient deficiency on calcareous soils (Graham *et al.*, 1992), and thus also in food crops (Graham, 2008). The risk of Zn deficiency is further enhanced by high clay content, high P supply and low soil temperature (Fig. 17.27).

The low Zn concentrations in the shoots of plants grown with high rates of P fertilizers may be the result of growth enhancement and thus 'dilution' by growth, inhibition of arbuscular mycorrhiza (AM) and suppression of P deficiency-induced changes in the rhizosphere. The role of mycorrhiza in Zn acquisition (Cavagnaro *et al.*, 2010) is further discussed in Chapter 15. Low soil temperatures often enhance the incidence and severity of Zn deficiency symptoms with high P supply increasing the likelihood of low temperature-induced Zn deficiency in calcareous soils (Moraghan, 1980). At low temperatures, Zn uptake from soils is not specifically impaired, but root activity and mycorrhizal root colonization can be lower. Of the plant factors, rhizosphere acidification via enhanced net excretion of protons (Fig. 17.27) is of particular importance for acquisition of Zn and Mn in calcareous soils (see also Chapter 14).

Zinc deficiency is the most widespread micronutrient disorder in rice (Wissuwa *et al.*, 2006) and occurs

TABLE 17.20 Element concentration in leaves of peanut at different pH in a sandy soil

	Concentration in the dry matter				
	($mg\,kg^{-1}$)		($g\,kg^{-1}$)		
Soil pH	Zn	Mn	P	K	Mg
5.2	200	310	1.8	18.5	4.5
6.0	54	66	1.9	17.5	3.8
6.8	20	19	1.9	19.0	3.9

Parker and Walker (1986).

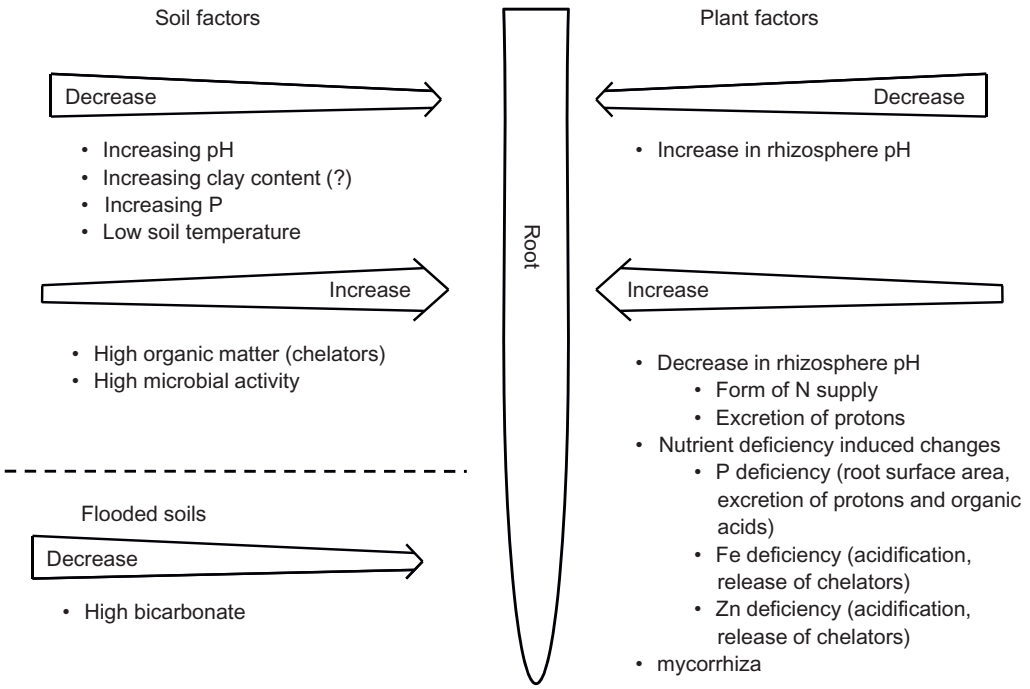


FIGURE 17.27 Schematic presentation of main soil and plant factors decreasing or increasing Zn availability and uptake by soil-grown plants. *Based on Marschner (1993).*

particularly in flooded rice on soils with high pH and high organic matter content (Moraghan and Mascagni, 1991). Thus, after salinity and Fe toxicity, the next most important nutritional limitation on yield in flooded rice is Zn deficiency (Ikehashi and Ponnamperuma, 1978). In neutral and alkaline soils, there is a negative correlation between soil pH and rice yield when no Zn fertilizer is applied (Table 17.21). The concentration of DTPA-extractable (plant available) Zn in paddy soils decreases only slightly as the pH increases from 6.5 to 8.0. Hence, the strong decrease in Zn uptake in plants without Zn fertilizer supply is mainly caused by elevated HCO_3^- concentrations. In paddy soils 3–6 weeks after planting, HCO_3^- concentrations higher than 10 mM are common; these concentrations of HCO_3^- inhibit Zn transport to the shoots (Forno *et al.*, 1975) and also uptake into the roots and may injure rice roots directly (Dogar and van Hai, 1980).

Up to soil pH 8, soil Mn availability is low, which may lead to Mn deficiency in plants. However, organic acid anions released from roots can increase release of Mn from Mn oxides by chelation (Ryan *et al.*, 2001). Low Mn supply can increase organic acid anion exudation (Gherardi and Rengel, 2004). In white lupin grown on alkaline soils either Mn deficiency or toxicity may occur, depending on the source of N supply and the P level (Moraghan, 1992). Manganese concentrations in the shoot were higher in plants depending on N_2 fixation compared

TABLE 17.21 Grain yield and leaf Zn concentration (mid-tillering) in flooded rice at different soil pH and with and without Zn supply

Treatment		Grain yield ($kg\,ha^{-1}$)	Zn concentration in leaves ($mg\,kg^{-1}$ dw)
Soil pH	$kg\,Zn\,ha^{-1}$		
6.8	0	5,934	9
	1.9	7,212	17
7.3	0	5,265	9
	1.9	6,171	18
7.7	0	2,788	10
	1.9	6,637	14

Based on Sedberry *et al.* (1988).

with nitrate-fed plants. Application of P decreased Mn concentrations in the shoots towards the deficiency range, presumably by inhibition of cluster root formation.

17.5.2.3 Other Constraints

In many calcareous upland soils, high pH and low soil water contents are the main environmental factors, impairing nutrient mobility in the soil and root growth. Boron availability is particularly impaired by low soil water content (Moraghan and Mascagni, 1991). The risk of B deficiency in crop plants is therefore increased in calcareous soils in dryland areas (Section 7.7).

17.5.3 Mechanisms of Adaptation

17.5.3.1 Calcicoles versus Calcifuges

Plant species and populations within species (ecotypes) of the natural vegetation that preferentially grow on calcareous soils (*calcicoles*) possess adaptive mechanisms for coping with constraints on growth and nutrition such as low Fe and Zn availability and often high Ca and bicarbonate concentrations in the soil solution. For example, calcicoles have a higher capacity for Fe acquisition than *calcifuges*, i.e. species and ecotypes which are adapted to acid soils (Gries and Runge, 1995) and develop chlorosis symptoms when grown on calcareous soil (Zohlen and Tyler, 2000). Calcicoles are also often highly efficient in P uptake (Musick, 1978), at least in some cases because of high root colonization by AM (Kianmehr, 1978). In agreement with this, the inability to mobilize P in calcareous soils is considered a key factor in the calcifuge behaviour of many plant species such as *Rumex acetosella* and *Silene rupestris* (Tyler, 1992). Exudation of organic acid anions from roots mobilizes P, Fe and Zn from calcareous soils and thus is also a feature of calcicole plant species (Ström, 1997). Moreover, in calcicoles high HCO_3^- concentrations have only negligible inhibitory effects on root growth (Lee and Woolhouse, 1969a). Calcifuge and calcicole behaviour can be found not only in vascular plants, but also in lichens where the distinction is also based on differences in Fe and P acquisition (Paul *et al.*, 2009).

The role of high Ca concentrations in adaptation of plants to calcareous soils is rather complex. In calcicoles, avoidance and tolerance of high Ca concentrations in the plant tissue occur. For example, many members of the Brassicaceae accumulate large amounts of soluble Ca in their vacuoles (calciotrophic types), which may have advantages in terms of osmoregulation on dry limestone habitats (Kinzel and Lechner, 1992). On the other hand, in certain calcicole species Ca uptake is more restricted than in calcifuge species (Bousquet *et al.*, 1981), presumably due to a lower affinity of root plasma membranes for Ca^{2+} (Monestiez *et al.*, 1982). The mechanisms of Ca toxicity in general and in calcifuges in particular are poorly understood. It is likely that in calcifuges, the strict compartmentation of Ca^{2+} at cellular level and maintenance of low Ca^{2+} concentrations in the cytosol (see also Section 6.5) are less effective than in calcicoles.

In many herbaceous plants grown in calcareous soils, calcified roots are abundant which are formed by solubilization of $CaCO_3$ in the rhizosphere and precipitation of $CaCO_3$ in their cortex cells (Jaillard, 1985; Jaillard *et al.*, 1991). This may reflect enhanced mobilization of sparingly soluble nutrients (P, Fe, Zn) in the rhizosphere and simultaneous protection of the shoot tissue from excessive Ca concentrations by precipitation of $CaCO_3$ in the root tissue. The deposition of $CaCO_3$ in soil is commonly considered to be an inorganic process, but organisms can also be involved in this process (Chesworth, 2008).

Certain ecto- and ericoid mycorrhizal fungi may also play a role in adaptation of perennial plant species to calcareous soils. This may be due to (i) the release of siderophores and enhanced Fe acquisition, and (ii) the production of oxalic acid (Plassard and Dell, 2010) which dissolves sparingly soluble Ca phosphates and protects the host plant from excessive Ca uptake by precipitation of Ca oxalate around the fungal hyphae. The ectomycorrhizal fungus *Paxillus involutus* is very effective in dissolving Ca phosphates by oxalic acid excretion, particularly when supplied with nitrate as N source (Lapeyrie *et al.*, 1991). However, there are marked differences between strains: those from calcareous soils accumulate less Ca in their hyphae than those from acid soils (Lapeyrie and Bruchet, 1986).

17.5.3.2 Iron Efficiency and Chlorosis Resistance

In terms of an ecological classification, crop species or cultivars within species which grow in alkaline soils

without developing symptoms of chlorosis are called iron efficient whereas those which become chlorotic are called iron inefficient (Brown and Jones, 1976). Large differences occur between crop species and genotypes within a species in Fe efficiency. The responsible mechanisms have been reviewed elsewhere (Römheld and Marschner, 1986; Römheld, 1987a, b; Curie and Briat, 2003; Hell and Stephan, 2003; Schmidt, 2003; Kim and Guerinot, 2007). For a review on cropping strategies to prevent Fe deficiency in the field see Zuo and Zhang (2011).

Mobilization of Fe in the rhizosphere of plants grown on calcareous soils can be due to non-specific (not related to Fe nutritional status) and specific mechanisms. Non-specific mechanisms include (i) root-induced decrease in pH (Blossfeld *et al.*, 2010), as a consequence of, for example, preferential cation uptake (e.g., induced by ammonium sulphate; Kafkafi and Ganmore-Neumann, 1985), or N_2 fixation in legumes (Soerensen *et al.*, 1989); (ii) release of organic acids by the roots (e.g., in response to P deficiency; Hoffland, 1992; Rengel and Marschner, 2005); (iii) release of photosynthates as substrate for rhizosphere microorganisms, which in turn affect pH, redox potential and chelator concentration (e.g., siderophores) in the rhizosphere (see also Chapters 14 and 15).

In terms of specific mechanisms, there are at least two distinct root response mechanisms (strategies) to iron deficiency in higher plants; Strategy I in dicotyledonous and monocotyledonous plants except of grasses, and Strategy II in grasses (see also Chapters 2 and 14). The root responses of Strategy I are not found in crop plants, and also indigenous shrubs and forbs of alkaline soils (Nelson, 1992). There is a positive correlation between the extent to which Fe deficiency induces enhanced reducing capacity of roots and net excretion of protons on the one hand, and the resistance of plants to Fe deficiency on calcareous soils (chlorosis resistance) on the other. This is also true for different genotypes within a species such as tomato (Olsen and Brown, 1980), sunflower (Alcantara and de la Guardia, 1991) or grapevine (Bavaresco *et al.*, 1991).

The differences in resistance to chlorosis between soybean cultivars when grown on calcareous soils provide a classical example of genetically controlled nutrition in general and Fe nutrition in particular (Weiss, 1943). In a given species, there is a large genetic potential from which to select for high resistance to chlorosis. In soybean, yield reduction in calcareous soils may vary between 6 and 82% for adapted and non-adapted cultivars, respectively (Froehlich and Fehr, 1981). Screening with $Mg(HCO_3)_2$ (as a substitute for $NaHCO_3$) solutions can provide quick ranking of chlorosis susceptibility in soybean cultivars (Norvell and Adams, 2006). Another example of the genetic potential is shown in Table 17.22 for peanut. The non-adapted cultivar Congo Red, originating from acid soils, became severely chlorotic when grown in a calcareous soil and Fe chelates had to be applied to overcome chlorosis and to obtain a reasonable yield. In contrast, in the adapted cultivar 71-238 chlorosis was absent, the yield was higher and application of Fe chelates had only a slightly beneficial effect. The differential chlorosis susceptibility of the two cultivars may, in part, be due to the large genotypical differences that occur among peanut genotypes in root capacity for Fe reduction (Gao and Shi, 2007).

TABLE 17.22 Pod yield of peanut grown in a calcareous Soil (23% $CaCO_3$, pH 7.8) with and without application of Fe chelate (10 kg Fe ha^{-1} as Fe-EDDHA)

Cultivar	Fe chelate application	Pod yield ($kg\,ha^{-1}$)	Yield increase + Fe (%)
Congo Red	0	833	
	+	2,583	210
Shulamit	0	3,305	
	+	4,749	44
71-238	0	4,388	
	+	4,777	9

Based on Hartzook *et al.* (1974).

Thus, the main factors responsible for high resistance to chlorosis of Strategy I plants are known (high reducing capacity and proton excretion, tolerance to high HCO_3^- concentrations). Therefore effective screening programmes can be designed based on nutrient solutions using either intact plants (Hintz *et al.*, 1987; Gao and Shi, 2007) or tissue cultures (Graham *et al.*, 1992). Combined with recurrent selection, such programmes can produce genotypes with high chlorosis resistance. Such programmes may in future also benefit from the fact that transcriptional responses to Fe deficiency are now described in the Strategy I species *Arabidopsis* (Buckhout *et al.*, 2009; Long *et al.*, 2010).

When comparing species of Strategy I plants which differ in chlorosis resistance, it may be difficult to attribute chlorosis resistance to a single component such as root reducing capacity, for example in citrus species (Treeby and Uren, 1993), or effects of bicarbonate on Fe uptake in *Lupinus* and *Pisum* (White and Robson, 1990). This may be due to qualitative differences in root reducing capacity, release to phenolics, or seed reserves and Fe redistribution within the plants. It should also be kept in mind that growth conditions modify the plant response to Fe deficiency. For example, increased atmospheric carbon dioxide concentrations can lead to stronger Fe deficiency induced plant responses and improved Fe nutrition in Strategy I plants such as tomato (Jin *et al.*, 2009).

In Strategy II plants, there is a close positive correlation between the release of phytosiderophores and the resistance of plants to Fe deficiency when grown on calcareous soils (Römheld, 1987a, b; Römheld and Marschner, 1990). In a study on genotypical variation between Indian cereal species, Fe deficiency-induced phytosiderophore release increased in the following order: wheat > barley > rye, oat > > maize > > > sorghum (Singh *et al.*, 1993). The release of phytosiderophores under Fe deficiency can be relatively low in flooded rice (Mori *et al.*, 1991) which is another explanation for the calcifuge behaviour of this species. Rice releases some 2′-deoxymugineic acid under conditions of low Fe supply in aerated solutions (Takagi, 1976; Nozoye *et al.*, 2011), but unlike most graminaceous crop species, it is well adapted for growth under submerged conditions and possesses an Fe uptake system for Fe^{2+} (Ishimaru *et al.*, 2006). Transcription factors regulating the response of rice to Fe deficiency have recently been described (Kobayashi *et al.*, 2009). Higher tolerance of rice to low Fe availability on calcareous soils can be obtained by introducing barley genes involved in synthesis of phytosiderophores (Suzuki *et al.*, 2008a).

Because of fast microbial decomposition of phytosiderophores (von Wirén *et al.*, 1993; Shane *et al.*, 2008), genotypical differences in the amounts of phytosiderophores recovered in nutrient solutions or by soil-based collection techniques have to be interpreted with care (see also Chapter 15; Römheld, 1991). Nevertheless, the relatively low recovery rates of phytosiderophores in maize and sorghum raise the question if these species can be considered to be typical Strategy II plants (Brown *et al.*, 1991; Lytle and Jolley, 1991), although at least in maize a ferric-phytosiderophore transporter has been characterized (Ueno *et al.*, 2009). Sorghum is more susceptible to Fe deficiency than other cereal species. This may be related to low rates of phytosiderophore release in sorghum, but also to inefficient internal Fe use for maintaining photosynthesis at low Fe supply (Mikami *et al.*, 2011).

Root responses and the pattern of Fe uptake by Strategy II plants under Fe deficiency have many common features with the microbial siderophore system (Crowley *et al.*, 1987; Marschner *et al.*, 2011). Complexes of Fe with phytosiderophores appear to be a good Fe source for bacteria (Marschner and Crowley, 1998; Marschner and Rengel, 2007). On the other hand, bacterial siderophores are usually poor iron sources for Strategy I and II plants (Bar-Ness *et al.*, 1992; Walter *et al.*, 1994a; Marschner, 2007). Table 17.23 shows that the uptake rates of Fe from Fe-siderophores (ferrioxamine B = Desferal) are very low as compared with the plant-borne phytosiderophores such as hydroxymugineic acid. Some studies, however, show that microbial siderophores may help to overcome Fe-deficiency chlorosis under certain environmental conditions (Hördt *et al.*, 2000; Yehuda *et al.*, 2000). Certain microorganims such as the growth-promoting bacterium *Bacillus subtilis* may activate the plants' Fe deficiency adaptation mechanisms (Zhang *et al.*, 2009).

TABLE 17.23 Iron mobilization from a calcareous soil by Fe^{3+} chelators and uptake of Fe supplied as $^{59}Fe^{3+}$ chelates by Fe-deficient barley plants

Chelator (10^{-5} M)	Mobilization (nmol Fe g^{-1} soil (12 h)$^{-1}$)	Uptake (nmol Fe g^{-1} root dw (4 h)$^{-1}$)
Phytosiderophore (HMA)	24	3,456.0
Siderophore (Desferal)	19	1.2
Synthetic chelate (DTPA)	2	0.5

From Römheld and Marschner (1990).

In contrast to the limited effectivity of microbial siderophores of the hydroxamate and catecholate type in providing Fe to higher plants in short-term studies, in the long-term they may be of considerable ecological importance by providing soluble Fe to the root surface and the plasma membrane of root cortical cells of plants growing on calcareous soils (Jurkevitch *et al.*, 1986, 1988). Higher concentrations of siderophores in the rhizosphere soil compared with the bulk soil (Nelson *et al.*, 1988) suggest such an ecological role. In red clover grown on calcareous soil, plant Fe deficiency changed the composition of siderophore-releasing microbes in the rhizosphere, which could lead to increased microbial siderophore release (Jin *et al.*, 2010). However, no strong evidence exists either in Strategy I or Strategy II plants that genotypical differences in efficiency of Fe acquisition and in chlorosis resistance when grown on calcareous soils are related to differences in siderophore production by rhizosphere microorganisms. The spatial and temporal variability of plant–microbe interactions in the rhizosphere along the root axis (Marschner *et al.*, 2011; see also Chapter 15) is high. Hence, microbial effects on plant performance under nutrient limitation are also variable and not always consistent. There are also no clear relationships between AM colonization of roots and chlorosis resistance.

17.5.3.3 Zn Efficiency

Differences in Zn efficiency of crop species are well documented. For example, when grown on alkaline soils, sensitivity to Zn deficiency is high in bean, maize, cotton and apple compared to wheat, oat or pea. These differences between species in Zn acquisition from soils are

TABLE 17.24 Zinc concentration of leaves at maturity and grain yield of pigeon pea cultivars grown in a zinc-deficient soil (pH 7.8) without Zn fertilization or supplied with 5 and 50 mg Zn kg^{-1} (as $ZnSO_4$)

	Zn concentration in leaves (mg kg^{-1} dw)			Grain yield (g pot^{-1})		
Cultivar	0	5	50	0	5	50
T 21	15	20	37	3.8	8.5	10.4
A-3	21	31	91	6.7	10.1	10.0

From Shukla and Raj (1980).

probably related to inherent differences in rhizosphere pH, root exudation, or root colonization with AM (Thompson, 1990; Marschner, 1993; Hacisalihoglu and Kochian, 2003; Cavagnaro, 2008). Also within a given species, there are differences among cultivars in Zn efficiency, with efficient cultivars having higher Zn uptake rates when grown in Zn-deficient alkaline soils. Examples for differential Zn efficiency among cultivars have been shown in, for example, maize (Shukla and Raj, 1976), wheat (Shukla and Raj, 1974; Graham *et al.*, 1992), barley (Graham *et al.*, 1992; Sadeghzadeh *et al.*, 2009) and soybean (Hartwig *et al.*, 1991a) and are shown for pigeon pea in Table 17.24. The Zn concentration in the leaves of the efficient cultivar was higher, and only small amounts of Zn fertilizer were necessary to obtain maximal grain yields. Screening programmes may also select for genotypes with high seed Zn concentration, to increase micronutrient intake of consumers (White and Broadley, 2009; see also Chapter 9).

The mechanisms responsible for higher Zn acquisition in efficient genotypes of a species are still not fully understood. A high production of fine roots may be a prerequisite for efficient Zn uptake from soil (Holloway *et al.*, 2010). In dry climates, the capacity to take up Zn from the subsoil may also be important (Holloway *et al.*, 2010). In soybean only a few genes seem to control uptake efficiency, or inefficiency (Hartwig *et al.*, 1991a), but other studies show Zn efficiency as a complex physiological plant trait (Gao *et al.*, 2005). In the Zn-inefficient common bean cultivar Sanilac, typical Fe deficiency-induced root responses were enhanced under Zn deficiency and the Fe concentration in the leaves increased (Jolley and Brown, 1991b). This was not observed in the Zn-efficient bean cultivar Saginaw. In graminaceous species such as wheat and barley, release of phytosiderophores may also be enhanced under Zn deficiency (Zhang *et al.*, 1989, 1991a). It is controversial whether this enhanced release is an expression of a separate regulation of phytosiderophore biosynthesis by Fe and Zn. Some studies showed that Zn-related disturbance of Fe transport can be involved in the Zn efficiency response of wheat genotypes (Walter *et al.*, 1994b; Rengel and Graham, 1996). Other evidence suggests that in barley, increased biosynthesis and secretion phytosiderophores were a response to Zn deficiency and not due to an induced Fe deficiency (Suzuki *et al.*, 2006).

In wheat cultivars, differences in Zn efficiency found on calcareous soils may relate to differences in release of phytosiderophores under Zn deficiency in nutrient solution culture (Cakmak *et al.*, 1994c). Other studies, however, indicated that plant Zn efficiency in soil and in solution culture are unrelated (Holloway *et al.*, 2010).

Both lowland and upland rice cultivars also differ strongly in Zn efficiency (Gao *et al.*, 2005), particularly when grown in high pH soils. In Zn-efficient cultivars, high bicarbonate concentrations as well as low root zone temperatures have little effect on growth and shoot concentrations of Zn, Fe and Mn, but strongly decrease the concentrations of these micronutrients in Zn-inefficient cultivars (Yang *et al.*, 1993). The bicarbonate-mediated inhibition of fine root elongation in Zn-inefficient rice genotypes may also be due to excessive accumulation of organic acids such as malate in root elongation zones (Hajiboland *et al.*, 2005). Thus, Zn efficiency in rice seems to be related to high bicarbonate tolerance of the roots, which is also reflected in the slight stimulation of root growth by bicarbonate and a better control of organic acid accumulation in the roots as compared with the Zn-inefficient cultivar (Yang *et al.*, 1994).

17.5.3.4 Mn Efficiency

Plant species differ considerably in susceptibility to Mn deficiency when grown on soils low in available Mn (see also Section 7.2). Differential Mn efficiency among a species is highly heritable, and major dominant genes appear to be involved (Graham, 1988). In Mn-deficient calcareous soils, the Mn-efficient barley cultivar Weeah, which is derived from old English landraces, achieved grain yields of 3.3 t ha^{-1} both without and with Mn fertilization, whereas the grain yield the inefficient cultivar Galleon decreased from 3.2 with Mn to 1.8 t ha^{-1} without Mn fertilization (Ralph, 1986). In wheat grown on an Mn-deficient calcareous soil, differences between cultivars in Mn efficiency were related to differential Mn acquisition and not internal utilization (Marcar and Graham, 1987).

The mechanisms responsible for cultivar differences in Mn uptake and efficiency are poorly understood. A high-affinity transport system mediating Mn^{2+} influx has been described in barley (Pedas *et al.*, 2005). The transport capacity of the Mn-efficient barely genotype Vanessa was almost four times higher than in the Mn-inefficient genotype Antonia. A gene encoding a plasma membrane-localized metal transport protein able to transport Mn^{2+} and other metal ions has recently been identified in barley, and the expression level of this gene was highest in the Mn-efficient genotype (Pedas *et al.*, 2008). Expression of this gene was regulated by the plant Mn status, but also by the plant Fe status. In addition to such differences in plant Mn uptake systems, Mn efficiency could also be related to increased Mn availability in the rhizosphere which may be related to the abundance of Mn-reducing and -oxidizing rhizosphere microorganisms (Rengel and Marschner, 2005; see also Chapter 15). For example, Mn depletion was more pronounced in the rhizosphere of Mn-efficient than Mn-inefficient wheat genotypes (Marschner *et al.*, 2003). Extractable Mn concentrations were up to two orders of magnitude greater in the rhizosphere of three Banksia species than in bulk soil (Marschner *et al.*, 2005), which may be due to the presence of Mn-reducing microorganisms in the rhizosphere.

In Strategy I plants, Mn acquisition from calcareous soils may be dependent on the Fe nutritional status of the plants. In flax, the Mn concentration in the shoots was poorly related to concentration of extractable Mn in the soil, but negatively correlated to the concentration of extractable Fe (Moraghan and Freeman, 1978). In agreement with this, Mn toxicity could be eliminated by the application of Fe-EDDHA, which strongly decreased the Mn concentration in the plants (Table 17.25). On the other hand, high Fe efficiency may prevent Mn deficiency in Strategy I plants growing in calcareous soils; it may even increase the risk of Mn toxicity, as shown for flax (Table 17.25) and for an Fe-efficient genotype of soybean (Brown and Jones, 1977).

In cluster root-forming species such as white lupin (*Lupinus albus*), Mn acquisition and concentrations in the shoots are related to the cluster root formation and not the Fe nutritional status (Moraghan, 1991b). Mature cluster roots of white lupin can accumulate high concentrations of Mn, but Mn is also transported to the shoot via the xylem (Page *et al.*, 2006). Phosphorus deficiency increases cluster root formation, increases the Mn concentrations in the shoots and may even lead to Mn toxicity when grown on calcareous soils. In contrast, the shoot Fe concentrations are generally low in white lupin. The sensitivity of white lupin to Fe deficiency when grown on calcareous soils probably reflects an inherent restricted Fe transport from roots to the shoot in this species in order to prevent Fe toxicity, despite the very high Fe concentrations in the cluster roots.

TABLE 17.25 Shoot dry weight and concentrations of Mn, Fe and P in flax grown in a calcareous soil of pH 8.0 with or without Fe addition (2 mg Fe pot^{-1} as Fe-EDDHA)

		Shoot concentrations		
		Mn	Fe	P
Treatment	Shoot weight (g dw pot^{-1})	(mg kg^{-1})		(g kg^{-1})
−Fe	3.6	881	83	3.2
+Fe	5.6	64	174	3.2

From Moraghan (1979).

17.6 SALINE SOILS

17.6.1 General

The saline areas of the world consist of salt marshes of the temperate zones, mangrove swamps of the subtropics and tropics, and their interior salt marshes adjacent to salt lakes. Saline soils are abundant in semi-arid and arid regions where the amount of rainfall is insufficient for substantial leaching. Salt enters soils mainly via rainfall, irrigation water and rising groundwater. The water is lost by evaporation or transpiration, therefore salts may accumulate on the soil surface or within the solum. Anthropogenic soil salinization is the result of inappropriate irrigation and drainage practices since ancient times, and has led to the destruction of formerly successful agrarian societies, for example in Mesopotamia and the Tigris-Euphrates valley (Gelburd, 1985). Currently out of the 230 Mio ha of irrigated agricultural land worldwide, around 45 Mio ha are salt affected (Athar and Ashraf, 2009). The use of poor quality irrigation water is one reason for an increasing salinization of agricultural land, mainly in arid or semi-arid areas. Even good quality water may contain from 100 to 1,000 g salt m^{-3}. With an annual application of 10,000 m^3 ha^{-1}, between 1 and 10 tons of salt are added to the soil. To prevent salinization, the accumulated salts have to be removed periodically by leaching and drainage.

In some areas, rising groundwater tables in response to excessive irrigation water supply, leakage from canals or removal of perennial vegetation are the cause of soil salinization. For example in India, 20 to 40% of irrigated land along the canal projects ‘Sharda Sahayak’ and ‘Indira Gandhi Nahar’ have become unproductive due to rising saline groundwater within only 30 years (Singh, 2009).

Salt tolerance of most crop species is relatively low, and in the face of a growing world population, strategies to maintain or increase plant production on saline soils are required. Progress in the utilization of genetic variability

between plant cultivars for the breeding of particularly salt-tolerant lines has been relatively slow (Flowers, 2004). Molecular biological studies have recently shed light on some mechanisms involved in plant salt tolerance, and this may translate into more rapid selection of salt-tolerant genotypes or even the development of suitable transgenic cultivars in the future (Munns, 2005). Some halophytes are currently explored for their potential to be used as crop plants, for example in the production of animal fodder or biofuel (Rozema and Flowers, 2008). Halophytes may also be used in the phytoremediation of saline soils (Ravindran *et al.*, 2007). For conventional crop plants, precise knowledge of the complex mechanisms behind salt tolerance are required to achieve progress in genotype selection and the development of appropriate agricultural production practices for salt-affected soils.

17.6.2 Soil Characteristics and Classification

Salt-affected soils are characterized by high concentrations of soluble salts in the solution phase (saline soils), and/or a considerable fraction of the cation exchange sites being occupied by Na^+ (sodic soils). The electrical conductivity of the soil saturation extract (EC_e) is commonly used as a measure of soil salinity, i.e. the concentration of soluble salts (Fig. 17.28). The saturation extract comprises the soil solution extracted from a soil at its saturation water content. According to the *Glossary of Soil Science Terms* published by the Soil Science Society of America (2008, based on the criteria published by the US Salinity Laboratory Staff, 1954), a soil is considered saline when the EC_e is above $4\,dS\,m^{-1}$ which is equivalent to approximately 40 mM NaCl (Fig. 17.29). It is assumed that the growth of most crop plant species will be negatively affected at $EC_e > 4\,dS\,m^{-1}$. However, the effects of soil salinity also depend on the soil texture, its water content and the composition of the salts. The sodium absorption ratio (SAR), which provides information on the concentration of Na^+ in relation to Ca^{2+} and Mg^{2+} in the soil solution which is in equilibrium with the adsorbed fraction of these ions, can be used to further describe ion relations in salt-affected soils. Plant growth can be negatively affected by a high SAR even if the EC_e is below 4. Non-saline soils with an SAR above 13 are termed 'sodic soils' (SSSA, 2008). The extent by which the cation exchange fraction in a soil is occupied by Na^+ is reflected in the exchangeable sodium percentage (ESP). Saline soils with an ESP greater than 15% are termed 'saline–sodic' soils (SSSA, 2008). A high ESP or SAR causes clay minerals and organic matter to disperse, therefore saline–sodic and sodic soils are often characterized by a poor soil structure which can result in high soil density, clogging of pores and surface crusts, making the soils impermeable to air and water. Although the pH of saline soils can vary over a wide range, it is usually around neutrality, with a tendency toward slight alkalinity. Saline–sodic soils commonly have pH values between 7 and 8.5, whereas the pH of sodic soils exceeds 8.5. Soils that are saline over at least a certain period of the year are classified as 'Solonchaks', while saline–sodic and sodic soils commonly represent the 'Solonetz' (FAO *World Reference Base for Soil Resources*, 2006; see also Section 17.5). Approximately 950 Mio ha of land are covered by salt-affected soils worldwide, representing 8% of the land surface. Out of these, around 260 Mio ha are Solonchaks (FAO Map of World Soil Resources, 2003).

In most saline soils, Na^+, Ca^{2+}, Mg^{2+} and to a lesser extent K^+ and Fe^{2+} are the main cations. The most abundant anions are Cl^-, SO_4^{2-}, HCO_3^-/CO_3^{2-} and NO_3^- (Szabolcs, 1989). All salts with solubility greater than that of gypsum ($15\,mmol\,l^{-1}$) contribute to the osmotic potential of the soil solution. In addition to the ion composition of

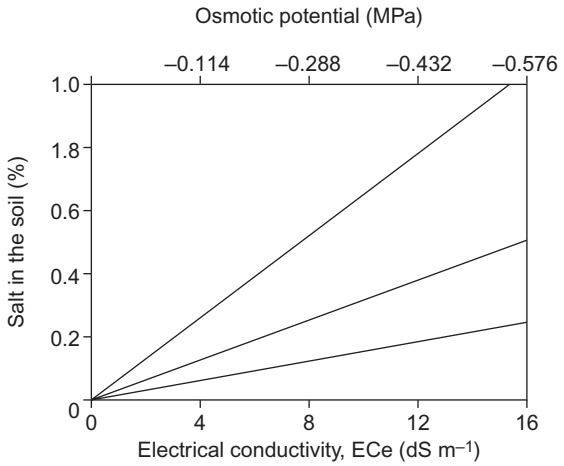


FIGURE 17.28 Relationship between the salt concentration in the soil and the electrical conductivity (dS = deci Siemens) of the extract at 25ºC for different amounts of water in the paste (% of saturation extract). *Based on US Salinity Laboratory Staff (1954).*

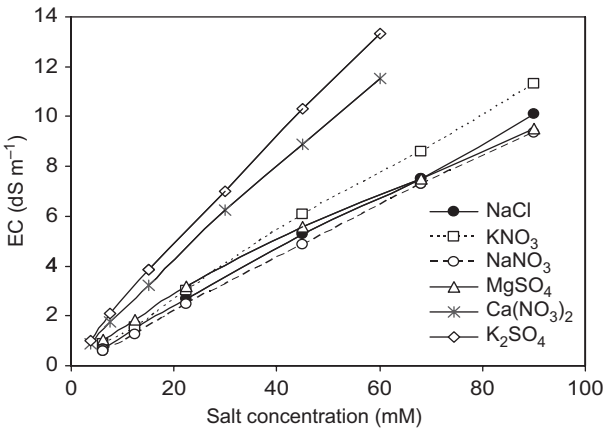


FIGURE 17.29 EC_e at different concentrations of various salts. *Based on Sonneveld* et al. *(1966).*

the soil solution, the EC_e is an important determinant for the suitability of a saline soil for crop production. The osmotic potential of the saturation extract can be calculated from the EC_e:

$$\text{Osmotic potential (MPa)} = EC_e \times -0.036$$

Measurement of the EC_e value is an easy and very commonly used tool to characterize the salt concentration in a soil, nutrient solution or irrigation water. However, it needs to be considered that the soil water content under field conditions is usually far below that of the saturation paste (the value 100 in Fig. 17.28). The salt concentration in the soil solution at field capacity will be about twice that of the saturation extract and correspondingly higher when the soil water content declines below field capacity. It is further possible that salts accumulate in the rhizosphere when their transport towards the root surface via mass flow exceeds plant uptake.

Thus, although it offers some valuable information, measurement of the EC_e value alone is not sufficient to assess the effect of a saline soil on plant growth. This is not only because it may underestimate the actual salinity level the plant is exposed to, but also because it does not provide any information on the identity of the ions present in the soil solution. Furthermore, plants growing on saline soils are often exposed to additional environmental stress, such as shallow groundwater tables or excessive concentrations of available B in the soil. The sensitivity of many plant species towards soil salinity increases significantly upon exposure of their roots to waterlogging or oxygen deficiency (Barrett-Lennard, 2003). Soil salinity and excessive B availability do not appear to have additive effects in most plant species investigated so far (Tripler *et al.*, 2007). However, high B concentrations may be more limiting for the growth of sensitive plant species than the high salt concentrations *per se*: irrigation water with more than $0.5\,mg\,B\,L^{-1}$ may injure sensitive species such as citrus and walnut, and more than $2.0\,mg\,L^{-1}$ is harmful to most crop species.

17.6.3 Salinity and Plant Growth

17.6.3.1 Genotypic Differences in Growth Response to Salinity

Plant species differ greatly in their growth response to salinity, as shown schematically in Fig. 17.30. The growth of halophytes is optimal at relatively high concentrations of NaCl, a response which can be explained in part by the role of Na as a nutrient in these species (see also Section 8.2). Among crops, very few species are slightly stimulated by low salinity levels. These belong to the relatively small group of agricultural plants classified as salt-tolerant (T). Most crops are non-halophytes (glycophytes), and either their salt tolerance is moderate (M), or their growth is severely inhibited even at low salinity (sensitive, S).

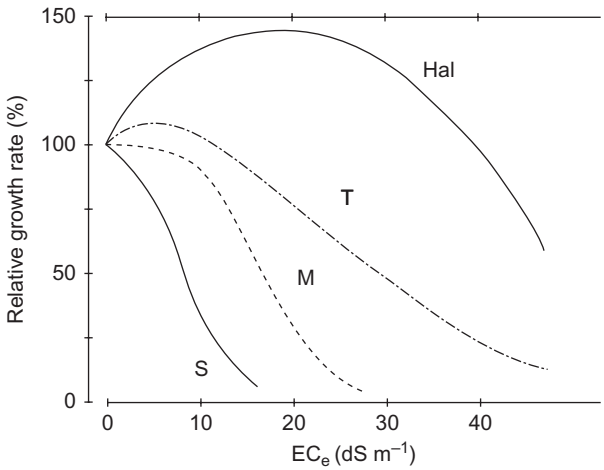


FIGURE 17.30 Typical growth responses of plant species of different salt tolerance to increasing soil salinity. Hal: halophytes; T: salt-tolerant crop species; M: moderately salt-tolerant crop species; S: salt-sensitive crop species. *Modified after Greenway and Munns (1980).*

Generally, classification of the salt tolerance (or sensitivity) of crops, forage species and fruit trees is based on two parameters: the threshold EC_e and the slope, i.e. percentage of yield decrease beyond the threshold. Some examples of crop plants of different sensitivity towards soil salinity are given in Table 17.26.

Considerable differences in salt tolerance among cultivars of the same species or between cultivated species and their wild ancestors suggest a great potential for improvement of plant salt tolerance by breeding. Examples of differences in salt tolerance between cultivars of the same plant species are given in Table 17.27.

Due to the complex physiology of salt tolerance mechanisms in plants, progress in the breeding of salt tolerant cultivars, has proven difficult for most crops; for many plant species only relatively little progress has been achieved so far. Genetically, plant salt tolerance is a quantitative trait that is determined by a relatively large number of genes. Chromosome sections with genes relevant for different aspects of salt tolerance (quantitative trait loci, QTL) were first identified in tomato (Breto *et al.*, 1994). More recently, QTLs for salt tolerance have been identified in several other plants, such as rice (Lin *et al.*, 2004) or citrus (Tozlu *et al.*, 1999). However, their use in marker-assisted plant breeding approaches is limited, as they seem to be dependent on environmental conditions and plant developmental stage (Flowers and Flowers, 2005). Plants appear to express different genes for salt adaptation depending on their age, the soil salinity level or other factors.

This is in agreement with the common observation that the sensitivity of a given species or cultivar towards

soil salinity changes during ontogeny. Depending on the plant species, cultivar, or environmental factors, salt tolerance may be controlled by different mechanisms and thus increase or decrease during plant growth (Foolad and Lin, 1997). Sugar beet, for example, is highly tolerant to salinity during most of its life cycle, but sensitive during germination. In contrast, the salt sensitivity of rice, tomato, wheat and barley usually increases after germination (Maas and Hoffman, 1977). In rice, generative development appears to be more affected by salinity than vegetative growth (Khatun and Flowers, 1995).

TABLE 17.26 Tolerance of crop species to soil salinity

Crop species	EC_e Threshold ($dS\,m^{-1}$)	EC_e Slope (% per $dS\,m^{-1}$)	Rating
Bean (*P. vulgaris*)	1.0	19.0	S
Carrot (*D. carota*)	1.0	14.0	
Apricot (*P. armeniaca*)	1.6	24.0	
Grapevine (*Vitis* sp.)	1.5	9.6	MS
Corn (*Z. mays*)	1.7	12.0	
Tomato (*L. lycopersicum*)	2.5	9.9	
Soybean (*G. max*)	5.0	20	MT
Perennial ryegrass (*L. perenne*)	5.6	7.8	
Wheat (*T. aestivum*)	6.0	7.1	
Date palm (*P. dactylifera*)	4.0	3.6	T
Sugar beet (*B. vulgaris*)	7.0	5.9	
Barley (*H. vulgare*)	8.0	5.0	

Based on Maas and Hoffman (1977).
Threshold EC_e (saturation extract) = maximum soil salinity that does not reduce yield; slope = yield reduction per unit increase in EC beyond threshold[a]; rating = classification of the plant as either sensitive (S), moderately sensitive (MS), moderately tolerant (MT) or tolerant (T) towards soil salinity.

17.6.3.2 Major Constraints

There are three major constraints for plant growth on saline substrates (Fig. 17.31): (i) water deficit ('osmotic stress') arising from the low (strongly negative) water potential of the rooting medium; (ii) ion toxicity resulting from excessive uptake mainly of Cl^- and Na^+; and (iii) nutrient imbalance by depression in uptake and/or shoot transport and impaired internal distribution of nutrients.

It is often not possible to assess the relative contribution of these three major constraints to growth inhibition at high substrate salinity, as this may be different for individual plant organs, and shift depending on the duration of the exposure to salinity and the developmental stage of the plant. Plant genotype and other environmental conditions also determine to which extent individual components of salinity stress affect plants.

Long-term exposure of a plant to salinity may, for example, result mainly in ion toxicity in the older leaves and water deficit and shortage of carbohydrates in the younger leaves. The following examples illustrate the possible role of the three major constraints, and also the difficulties of generalizations concerning the effects of salinity.

TABLE 17.27 Range of growth depressions (% of non-saline control) of cultivars within crop species in response to salinity

Species	Treatment (mM NaCl)	Growth parameter	Growth depression of cultivars	Reference
Wheat	200	Grain yield	64–23	Rahnama *et al.* (2010)
Wheat	100	Shoot length	100–12	Ali *et al.* (2007)
Sugar beet	150	Total dry weight	92–49	Marschner *et al.* (1981a)
Pepper	150	Shoot dry weight	86–42	Aktas *et al.* (2006)
Olive	100	Shoot length	70–16	Marin *et al.* (1995)
Tobacco	500	Surviving plants	100–15	Nabors *et al.* (1980)

17.6.3.3 Water Deficit

Plant water availability is determined by the water potential of the soil, which is the sum of matric and osmotic potential, in relation to potential of the root tissues. As soil salinity increases, plants have to overcome an increasing gradient in water potential between the soil (more negative) and their roots. The threshold value for sufficient water extraction for most plants lies at a soil water potential of around −1,500 kPa. The water content at which this critical value is reached is lower for saline soils because of the greater contribution of osmotic potential to water potential (Fig. 17.32). Moreover, at a given EC_e, the water potential decreases with decreasing soil water content (i.e., matric potential becoming more negative).

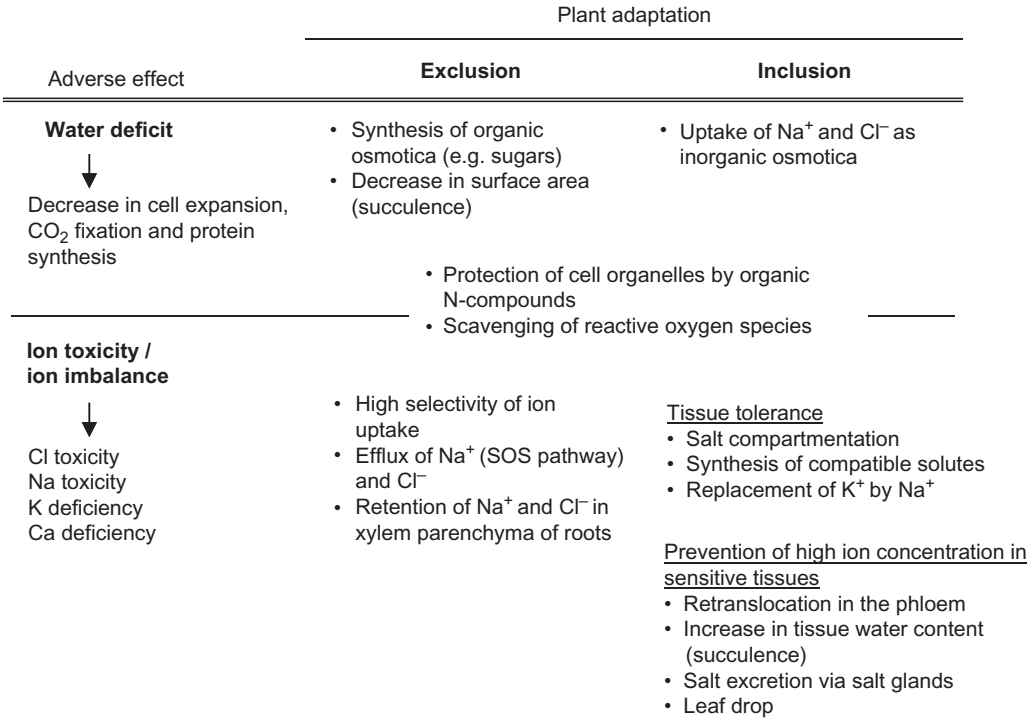


FIGURE 17.31 Adverse effects of salinity and possible mechanisms of adaptation. *Modified after Greenway and Munns (1980).*

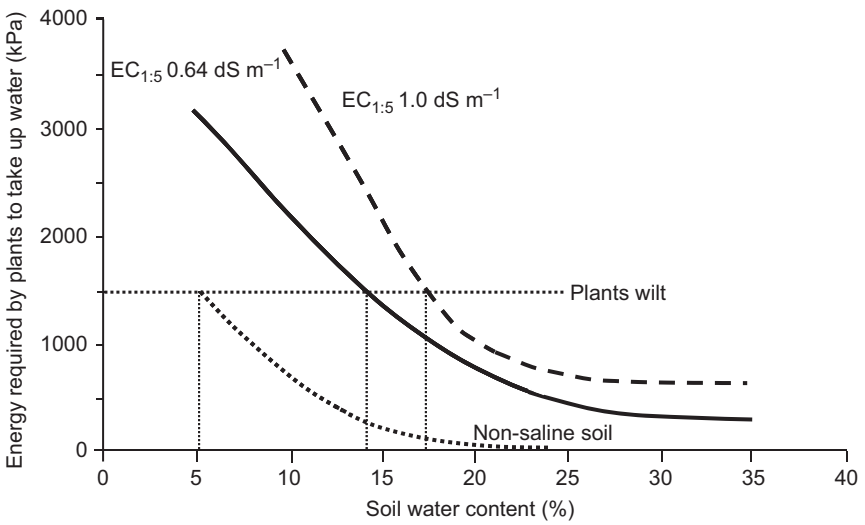


FIGURE 17.32 Energy required by plants to take up water (=soil matric + osmotic potential) from a sandy loam soil at different $EC_{1:5}$ (EC measured in a 1:5 water extract) and soil water content. *Based on Rengasamy (2006).*

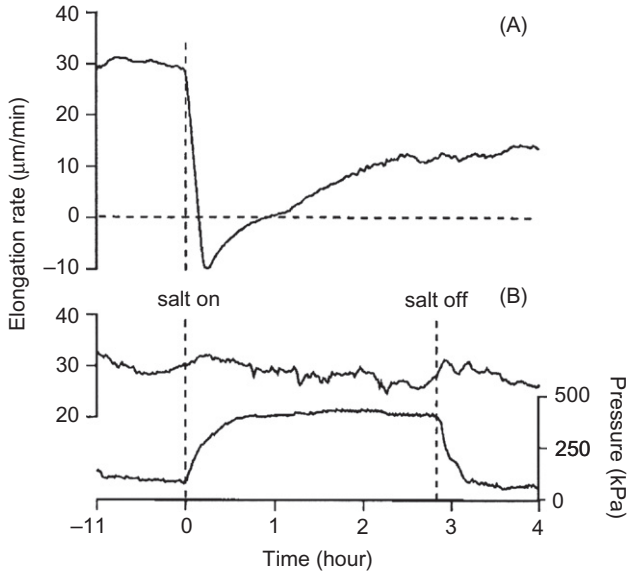


FIGURE 17.33 Effect of salinity (80 mM NaCl) on the leaf elongation rate of maize seedlings (A) without balancing pressure and (B) with balancing pressure to maintain leaf water status. Vertical broken lines indicate times at which the roots were flushed with saline or non-saline solution. *From Munns* et al. *(2000a) with permission from Oxford University Press.*

Reduction in growth rate is the most commonly observed response of plants to increasing substrate salinity. A rapid decrease in leaf and root expansion is observed in many plant species upon sudden exposure of the roots to salinity (Neumann, 1993; Frensch, 1997). The rapid responses in leaf elongation rate to substrate salinity are mainly due to changes in leaf water status. Upon removal of root-zone salinity within a few hours after onset of salinity, leaf extension rate immediately reverts to the pre-salinity level, suggesting that water deficit is the main reason for growth reduction by root zone salinity rather than salt toxicity. Following an initial strong decrease, leaf expansion rates of plants suddenly exposed to salinity recover gradually to a certain level, and reach a new steady state (Fig. 17.33). In roots, growth rates recover to a greater extent than shoot tissues, and for many plants decreases in dry weight in response to salinity are smaller for roots than for shoot tissues (Hsiao and Xu, 2000).

When the leaf water status of plants exposed to salinity is maintained by a pressurization technique, the early growth reduction in response to exposure to salinity is prevented (Fig. 17.33). However, pressurization was not able to restore leaf expansion rates in plants exposed to salinity for more than 24 h (Termaat *et al.*, 1985; Munns *et al.*, 2000b). This suggests that, after a certain time of exposure to salinity, factors other than the cell turgor govern tissue expansion. In some studies, cell expansion was decreased even though tissue Na^+ and Cl^- concentrations were in a non-toxic range (Hu and Schmidhalter, 1998), and thus it is likely that this effect is not salt-specific. Hormonal signals similar to those in roots in response to a decreased influx of water at low soil moisture (e.g., ABA) have been proposed as an explanation (Munns, 2002). However, it also needs to be considered that under salinity, the root pressure-driven flux of water and solutes into the xylem is impaired. In tomato and pepper plants 27 days after exposure to the salt stress (50 mM NaCl), the xylem exudation flow was decreased by a factor of 17 to 20 compared with the control plants (no salt stress), and ion concentrations in the xylem sap increased only by a factor of 2 to 3 (Kafkafi, 1991). Thus it cannot be completely ruled out that even in the short term, decreased nutrient concentrations or unfavourable nutrient ratios (e.g., Na^+/Ca^{2+}) in the leaf elongation zones contribute to impaired leaf elongation rates (Lynch *et al.*, 1988; Munns *et al.*, 1989).

17.6.3.4 Sodium and Chloride Uptake and Toxicity in Plants

In saline substrates, Na^+ and Cl^- are usually the dominant ions. Despite the essentiality of Cl as a micronutrient for all higher plants and of Na as nutrient for many halophytes and some C4 species (see also Sections 7.8 and 8.2), the concentrations of both ions in saline substrates by far exceeds this demand. Sodium and Cl^- are toxic to plants when accumulated in the cytoplasm at high concentrations. They may, for example, displace other ions from binding sites of enzymes, and thus impair cellular functioning.

In salinity-sensitive plants, growth inhibition and injury of the foliage (chlorosis and necrosis on the margins of mature leaves) occur even at low concentrations of NaCl (Sykes, 1992; Maas, 1993). Under such conditions, water deficit is not limiting plant growth (Greenway and Munns, 1980) and instead high Cl sensitivity and thus Cl toxicity impair plant growth (Maas, 1993). In many plants, particularly legumes such as *Trifolium* (Winter, 1982a, b) or *Medicago* (Sibole *et al.*, 2003), and many fruit trees such as citrus (Moya *et al*., 2003) or *Vitis* (Alexander and Groot-Obbink, 1971), there is a positive correlation between the ability to exclude Cl from the shoot and root tissues, and growth under salinity. This suggests that Cl toxicity is the major limitation for these plants when grown in a saline substrate. In some plants, however, growth on saline soils appears to be mainly limited by Na (e.g., in Medicago, Aydi *et al*., 2008). Root growth of rice seedlings also appeared to be limited by increasing Na rather than Cl concentrations in the growth medium (Lin and Kao, 2001). In *Sorghum*, salinity induced by Na_2SO_4 can decrease growth to a similar extent as NaCl at low and moderate salinity and even more at high substrate salinity. This decrease in growth is in part due to depression in shoot concentrations of K and Mg at concentrations of Na_2SO_4 (Boursier and Läuchli, 1990).

As plants generate a negative electrochemical potential gradient across their plasma membranes, uptake of Cl^- and other anions must be mediated by active carriers

in co-transport with H^+ or cations. To date, little is known about the identity of transporters mediating active Cl^- uptake at the plasma membranes of root cells. Cation-chloride co-transporters (CCCs) that transport Cl^- together with K^+ and Na^+ appear to play an important role in Cl uptake by *Arabidopsis* root tissues (Colmenero-Flores *et al*., 2007). The *At*CCC gene is expressed mainly in the xylem parenchyma, suggesting that this transporter retrieves Cl^- from the xylem sap (Colmenero-Flores *et al*., 2007).

Channels for passive uptake of anions also exist in the plasma membrane (Skerrett and Tyerman, 1994), and thus passive influx of Cl into plant cells would be possible if the plasma membrane is sufficiently depolarized. This may occur over short periods of time, either when Cl concentrations in the outer medium suddenly increase, or when cells rapidly take up considerable amounts of cations, such as Na^+. The extent to which membrane depolarization and passive influx of Cl play a role in Cl uptake by roots is currently discussed controversially (Teakle and Tyerman, 2010).

In contrast to Cl, Na is transported through the plasma membrane only passively. High Na concentrations in the absence of Cl or other anions in the outer medium may reduce the electrochemical potential across the plasma membrane, and increase the driving force for Na import (Blumwald *et al.*, 2000). As K and Na are similar in their ionic properties, they may enter the cytoplasm through the same ion channels, particularly when these are non-selective cation channels (NSCCs, Schachtman and Liu, 1999).

The net influx of Cl and Na into the cells depends not only on the respective uptake, but also on the efflux rates. Under salinity, Na and Cl influx and efflux occur simultaneously. The energy requirements for these 'futile cycles' are most likely similar, with energy spent mainly for efflux in Na, and for influx in Cl (Teakle and Tyerman, 2010).

Efflux channels for anions in the plasma membranes of root cells are activated by membrane depolarization, pH shifts and protein phosphorylation (Franchisse *et al*., 2000; Diatloff *et al*., 2004). The active efflux of Na from the cytoplasm into the apoplasm is mediated by the plasma membrane Na^+/H^+ antiporter SOS1 (Wu *et al.*, 1996). This transporter is expressed in the plasma membrane of root tips and vascular tissues. Its ability to pump Na out of the cytoplasm back into the rhizosphere may explain why its expression is positively correlated with salinity tolerance in *Arabidopsis* (Shi *et al.*, 2002). The transporter may, however, also function in xylem loading of Na, for example in the halophyte *Thellungiella halophila*, where the SOS1 gene is also expressed in shoots and roots in the presence and absence of salinity (Taji *et al*., 2004).

Long-distance distribution of Na and Cl within the shoot is mainly a function of the transpiration stream. Leaves accumulate increasing amounts of both ions the longer they transpire. Thus, toxicity symptoms specific to Na and Cl usually become visible as necrosis in older leaves, starting from the leaf tip and the margins. When salinity-induced leaf loss is considerable, the decreased photosynthetic capacity of the plant may contribute to growth depressions and a low yield commonly observed in plants exposed to salinity.

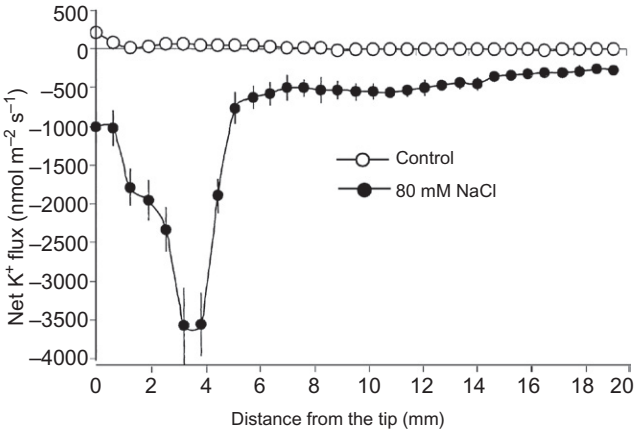


FIGURE 17.34 K^+ flux profiles along the root axis of barley seedlings without or with 80 mM NaCl. Positive values indicate uptake and negative values release from the root. *Based on Chen* et al. *(2005b).*

17.6.3.5 Ion Imbalances

Sodium toxicity is based mainly on its competition with K, therefore the cytosolic K/Na ratio, rather than the Na concentrations alone, causes the deleterious effects of elevated plant Na uptake. The measurement of ion concentrations in the cytoplasm is not easy to perform, thus, cytosolic K homeostasis in response to salinity has rarely been studied (Shabala and Cuin, 2007).

Potassium homeostasis can be achieved by a high selectivity of the cation uptake systems for K or efflux of Na from the cytoplasm, either into the vacuoles or into the apoplasm. The ability of plants to maintain K within the cytoplasm under salinity stress may play a crucial role in K homeostasis. Already 1 hour after exposure to salinity, considerable efflux of K from roots may occur (Fig. 17.34) which may also be due to decreased membrane integrity under salinity. In 62 out of 69 wheat cultivars investigated, salinity tolerance was negatively correlated with the K efflux rate upon salt exposure (Chen *et al.*, 2007).

Calcium, which has long been known for its role in alleviating plant salinity stress, appears to be involved in the maintenance of the K/Na homeostasis. The conductance of non-selective cation channels which are most likely major sites of Na entry into the cell, is strongly inhibited by Ca (Demidchik *et al*., 2002).

In its function as a secondary messenger (see also Section 6.5), Ca also appears to be involved in the salinity

perception and induction of physiological responses. Cytosolic Ca concentrations are usually in the range of 10–200 nM, but may quickly increase to millimolar range upon salinity stress. Cytosolic Ca concentrations may then remain high, decrease after a short while or oscillate at certain amplitude (Knight *et al.*, 1997). These fluctuations in cytosolic Ca concentration are likely to be signals for cellular responses to salinity (Kader and Lindberg, 2010). They are created by transport of Ca between the apoplasm or the vacuole and the cytoplasm. Plant species, cell types and even cell organelles appear to differ in their particular Ca signals upon perception of salinity (Kiegle *et al.*, 2000; Kader *et al.*, 2007). These Ca signals are induced by osmotic stress and elevated levels of Na or Cl within and outside the cell (Zhu, 2003), with osmotic and ionic stress resulting in different Ca^{2+} signals (Tracy *et al.*, 2008). However, the corresponding salinity sensors have not yet been identified. In *Arabidopsis*, the salinity-induced Ca signal is perceived by the Ca sensor SOS3, which then interacts with the protein kinase SOS2 to activate the Na^+/H^+ antiporter SOS1 (Mahjan *et al.*, 2008). This mechanism is named the 'Salt Overly Sensitive' (SOS) pathway.

Calcium has important functions in membrane and cell wall stabilization and may therefore maintain tissue integrity under salinity, contribute to exclusion of Na and decrease K efflux. However, a decreased uptake of Ca in response to salinity is a common observation (Table 17.28). One reason could be that movement of Ca through the apoplasm of root tissues is inhibited by the presence of positively charged Na. Both ions may also compete for plant uptake via non-selective cation channels. Active transport of Ca from the endodermis or xylem parenchyma into the xylem has been shown to be impaired at elevated Na levels (Halperin *et al.*, 1997). Furthermore, decreased root pressure due a low osmotic potential of saline soils may reduce Ca supply to plant parts that have a low transpiration rate (Ehret *et al.*, 1990).

Increasing the ratio of plant available Ca/Na in the soil can promote plant growth and Na/K homeostasis in saline substrates (Tables 17.29, 17.30). For example, in coastal saline–sodic soils, rolling and bleaching of young leaves of rice may occur resembling Ca deficiency which may be explained by the high Na/Ca ratios (~150) in these soils. These symptoms can be prevented by application of gypsum (Muhammed *et al.*, 1987). Thus, at high Na concentrations, increasing Ca concentrations can strongly enhance growth and prevent Na-induced Ca deficiency.

Manganese supply is usually not impaired in response to salinity, but in barley supplied with low concentrations of Mn, high NaCl concentrations in the substrate depressed growth mainly by inhibiting Mn uptake and inducing Mn deficiency (Cramer and Nowak, 1992).

TABLE 17.28 Calcium concentration and Ca deficiency symptoms in artichokes (*Cynara scolymus* L.) at different root zone salinity achieved by NaCl + $CaCl_2$ (1:1) in the irrigation water

Root zone salinity ($dS\,m^{-1}$)	Ca concentration ($mmol\,kg^{-1}$ dry wt)		Percentage of moderately to extremely damaged buds
	Leaf blades	Inner bracts	
4.6	306	25.1	11
7.4	336	16.4	22
10.6	362	13.7	42

Compiled data from Francois *et al.* (1991).

TABLE 17.29 Total dry weight of plum rootstocks differing in salinity tolerance grown in a sandy substrate supplied with nutrient solution containing NaCl without or with $CaSO_4$

Treatment	Total dry weight ($g\,plant^{-1}$)		
	Marianna GF 8-1	Myrobolan B	Pixy
Control	8.34	5.69	4.04
40 mM NaCl	4.05	3.89	3.72
40 mM NaCl + 5 mM $CaSO_4$	5.43	4.67	4.36

From Bolat *et al.* (2006).

A salinity-induced decreased uptake of anions, for example in response to competition with Cl for uptake sites, may occur in some plant species. For example, in grapevines nitrate and Cl uptake is negatively correlated, hence increasing nitrate supply can reduce Cl uptake and vice versa (Miklós *et al.*, 2000). However, Cl-induced N deficiency is not likely to be an important factor in growth depression caused by salinity. In contrast, salinity may induce P deficiency; in cotton grown at low P concentrations (10–30 µM), high NaCl concentrations (150 mM) reduced P uptake and translocation (Martinez and Läuchli, 1991). Competition between Cl^- and PO_4^{3-} for uptake was apparently not the reason for decreased P uptake into melon plants grown under salinity (Navarro *et al.*, 2001). Instead, NaCl may impair the transfer of PO_4^{3-} into the xylem. Additionally, salinity may depress P utilization efficiency in the leaves. In tomato with increasing NaCl concentrations in the substrate from 10 to 50 and 100 mM, the P concentrations in the youngest mature leaf required

TABLE 17.30 Cation concentrations and electrolyte leakage in leaves of plum rootstocks differing in salinity tolerance grown in a sandy substrate supplied with nutrient solution containing NaCl without or with $CaSO_4$

Treatment	Leaf element concentrations ($mM\,kg^{-1}$ dw)				Electrolyte leakage (%)
	Na	K	Na:K	Ca	
			Marianna GF 8-1		
Control	34	605	0.6	242	20
40 mM NaCl	1,969	433	4.55	154	52
40 mM NaCl + 5 mM $CaSO_4$	1,243	530	2.35	187	42
			Myrobolan B		
Control	46	623	0.08	330	17
40 mM NaCl	1,559	492	3.17	250	50
40 mM NaCl + 5 mM $CaSO_4$	1,176	564	2.09	295	27
			Pixi		
Control	24	564	0.04	380	21
40 mM NaCl	407	545	0.75	345	25
40 mM NaCl + 5 mM $CaSO_4$	200	516	0.39	397	16

From Bolat *et al.* (2006).

TABLE 17.31 Leaf area, CO_2 fixation, evolution and net assimilation of cotton at different salinity imposed by NaCl

Salinity (Mpa)	Leaf area ($dm^2\,plant^{-1}$)	CO_2 fluxes (mg CO_2 dm^{-2} (24 hr)$^{-1}$		
		Net fixation light period	Evolution dark period	Net assimilation
−0.04	30	57	11	46
−0.64	24	44	16	29
−1.24	18	41	19	23

Based on Hoffmann and Phene (1971).

to obtain 50% yield were increased from 1.8 to 2.4 and 3.0 g P kg^{-1} dm (Awad *et al.*, 1990).

17.6.3.6 Photosynthesis and Respiration

Water loss per plant by transpiration decreases with increasing salinity, which may, in part, be due to the negative relationship between salinity level and leaf area. With salinity, total leaf area and also net CO_2 fixation per unit photosynthetic tissue may decline, whereas respiration (dark respiration) increases, leading to a reduction in net CO_2 assimilation per unit leaf area per day (Table 17.31). Lower rates of net CO_2 fixation during the light period may be caused by water deficit and partial stomatal closure, loss of turgor of mesophyll cells due to salt accumulation in the apoplasm, or direct toxic effects of ions. A negative feedback on photosynthesis by a decreased demand of sink tissues for assimilates may also contribute to lower photosynthetic activity (Iyengar and Reddy, 1996).

Low rates of photosynthesis may be due to a decrease of intercellular CO_2 concentrations under salinity (Kurban *et al*., 1999). Hence, plants that are able to fix CO_2 even at low intercellular CO_2 concentrations via the C4 or CAM pathway often have higher growth rates when grown in saline soil than C3 plants (Katerji *et al.*, 1996). Some salt-tolerant plants are even able to shift from C3 to C4 or CAM metabolism under salinity (e.g., *Atriplex lentiformis*;

Zhu and Meinzer, 1999). Low CO_2 concentration and osmotic potential in leaf tissues seem to trigger the shift from C3 to CAM in salt-exposed *Mesembryanthemum crystallinum* (Kholodova *et al.*, 2002).

Elevated CO_2 concentrations, the negative effect of salinity on CO_2 fixation in tomato, demonstrating the importance of CO_2 supply to photosynthetic active cells under salt stress (Meiri and Plaut, 1985). However, this may only apply to plants that are able to maintain a functional photosynthetic apparatus under salinity. Avocado plants exposed to salinity showed increased intercellular CO_2 concentrations compared with control plants, most likely due to negative effects of Cl on photosynthesis (Musyimi *et al.*, 2007). In mangroves exposed to high salinity, K^+/Na^+ imbalance strongly inhibited photosystem II and decreased O_2 evolution (Ball *et al.*, 1987). Chloroplasts of salt-stressed plants often become disorganized, swollen and show increased starch accumulation (Bruns and Hecht-Buchholz, 1990; Hernández *et al.*, 1995). In many plant species, salinity decreases the concentration of photosynthetic pigments (Khavarinejad and Mostofi, 1998), possibly due to the negative effect of high Na^+/K^+ ratios on protein synthesis.

It needs to be considered, however, that photosynthesis of the whole shoot is often not informative for elucidating the mechanisms of salt injury, as salts primarily accumulate in mature leaves. In rice at low substrate salinity, net photosynthesis in the whole shoot was not affected but it was in the older leaves, where net photosynthesis was negatively related to the Na concentration in the leaves, (Yeo *et al.*, 1985).

Moderate salinity increases the carbohydrate requirement for maintenance respiration and respiration rates (Schwarz and Gale, 1981), which is most likely due to the energy costs of the compartmentation of ions, ion secretion (e.g., Na efflux transporters), or the repair of cellular damage. However, when salinity levels exceed a certain threshold, root respiration may also decrease because ion toxicity impairs cell metabolism (Epron *et al.*, 1999).

17.6.3.7 Protein Synthesis

The decline in protein synthesis in the leaves of plants growing in saline substrates may either be due to a water deficit or to a specific ion excess. When a low substrate water potential was imposed either by Carbowax (a high-molecular-weight organic solute) or NaCl, protein synthesis in the leaves of bean was inhibited, but inhibition was stronger with salinity stress than with water deficit alone (Frota and Tucker, 1978). The effects of NaCl salinity on protein synthesis may be due to Cl toxicity in sensitive species (e.g., soybean), whereas in the more salt-tolerant barley, Na/K imbalance in the leaves is probably the responsible factor (Tables 17.32, 17.33). Salinity may have adverse effects on the activity of nitrate reductase (Flores *et al.*, 2000) and ferredoxine-dependent glutamate synthase (Popova *et al.*, 2002); however, the synthesis of amino acids does not appear to be a major limitation to protein synthesis under salinity. Instead protein synthesis may be reduced in favour of the accumulation of a number of amino acids and other N-containing organic compounds (e.g., glycine betaine or proline) that are involved in osmotic adjustment, protection of enzymes or detoxification of oxygen radicals. It has also been suggested that Na^+ in the cytoplasm impairs ribosomal attachment to rRNA by competing with K for binding sites (Tester and Davenport, 2003). In legumes, salinity can reduce symbiotic N_2 fixation (Serraz *et al.*, 1998), and thereby reduce N supply and protein biosynthesis.

In barley, the adverse effect of high NaCl concentrations on K concentrations and protein synthesis can be counterbalanced by KCl, despite the further decrease in the osmotic potential and increase in Cl concentration of the substrate (Table 17.34), suggesting that increased K uptake Ke may allow osmotic adjustment in expanded leaves. However except in the case of a few halophytes, Na cannot replace K in its function in protein synthesis even in salt-tolerant cultivars of crops, for example wheat (Gibson *et al.*, 1984).

TABLE 17.32 Growth, K and Na concentration and protein synthesis in barley at different salt treatments

Treatment	Shoot biomass (mg plant^{-1})	Concentration (mmol (100 g dw)$^{-1}$)		^{15}N concentration (% of total ^{15}N)*	
		K	Na	Protein N	Inorganic N
Control	371	126	14	44	3
80 mM NaCl	286	80	208	29	20
80 mM NaCl + 10 mM KCl	323	136	160	49	1

Based on Helal and Mengel (1979).
**After supply of $^{15}NH_4{}^{15}NO_3$ for 24 h.*

TABLE 17.33 Dry weight and shoot concentrations of Na, Cl, K and Ca in sugar beet, maize and bean grown at different NaCl concentrations

Species	Concentration of NaCl (mM)	Dry weight (relative)	Concentration (meq g^{-1} dw)			
			Na	Cl	K	Ca
Sugar beet	0	100	0.1	0.1	3.3	1.6
	25	108	1.7	1.0	2.2	0.5
	50	115	2.1	1.2	2.0	0.4
	100	101	2.6	1.5	1.9	0.3
Maize	0	100	0.0	0.0	1.6	0.5
	25	90	0.2	0.5	1.8	0.3
	50	70	0.2	0.6	2.0	0.3
	100	62	0.3	0.8	2.0	0.3
Bean	0	100	0.0	0.0	1.7	2.9
	25	64	0.0	1.0	2.2	3.7
	50	47	0.2	1.4	1.9	3.4
	100	37	0.4	1.5	2.2	3.6

From Lessani and Marschner (1978) and H. Marschner (unpublished data).

TABLE 17.34 Concentrations of Na and Cl in vacuoles of the epidermis and 1st mesophyll layer in the leaves of two barley cultivars after 1 day exposure to 100 mM NaCl

Cultivar	Organ	Tissue	Concentration in vacuoles (mM)	
			Na^+	Cl^-
California Mariout (salt tolerant)	Blade	epidermis	35	110
		1st mesophyll	42	4
	Sheath	epidermis	134	204
		1st mesophyll	72	223
Clipper (salt sensitive)	Blade	epidermis	41	170
		1st mesophyll	58	44
	Sheath	epidermis	171	238
		1st mesophyll	157	191

From Huang and Van Stevenick (1989c).

17.6.3.8 Phytohormones

In response to salinity, cytokinin and auxin concentrations decrease, whereas those of ABA and ethylene increase, in a similar way as under drought stress (Fig. 17.35). High ABA concentrations are important for rapid osmotic adjustment to salinity, both of individual cells (LaRosa *et al.*, 1985) and whole plants and induce the transcription of genes involved in salt tolerance (Gupta *et al.*, 1998). Application of ABA may therefore increase salt tolerance by enhancement of mechanisms for rapid adaptation to salinity (Fig. 17.36), for example by increasing leaf PEP carboxylase activity which may enhance CO_2 fixation rate despite reduced stomata aperture (Amzallag *et al.*, 1990). Pre-treatment with ABA prior to exposure to elevated NaCl may improve tolerance of plants to salt stress (Noaman *et al.*, 2002; Parida and Das, 2005).

Leaf senescence in response to salt stress is most likely the result of decreasing concentrations of cytokinin and increasing concentrations of ethylene, rather than ABA (Ghanem *et al.*, 2008). In citrus, ABA reduced ethylene release and leaf senescence, most likely due to activation of mechanisms that exclude Cl from the cytoplasm (Gómez-Cadenas *et al.*, 2002). There are also several reports of applications of cytokinin counteracting salinity-induced leaf senescence (Katz *et al.*, 1978; Bejaoui, 1985). In *Sorghum* growth at high NaCl salinity could be improved by supplying cytokinins, particularly combined with gibberellin (Amzallag *et al.*, 1992).

The polyamine growth regulators putrescine or spermidine accumulate in plants under salinity stress. They may stabilize plant cell membranes and enhance protein synthesis. They may also play an important role in ion homeostasis under salinity by blocking non-selective cation channels (Shabala *et al.*, 2007). Application of polyamines

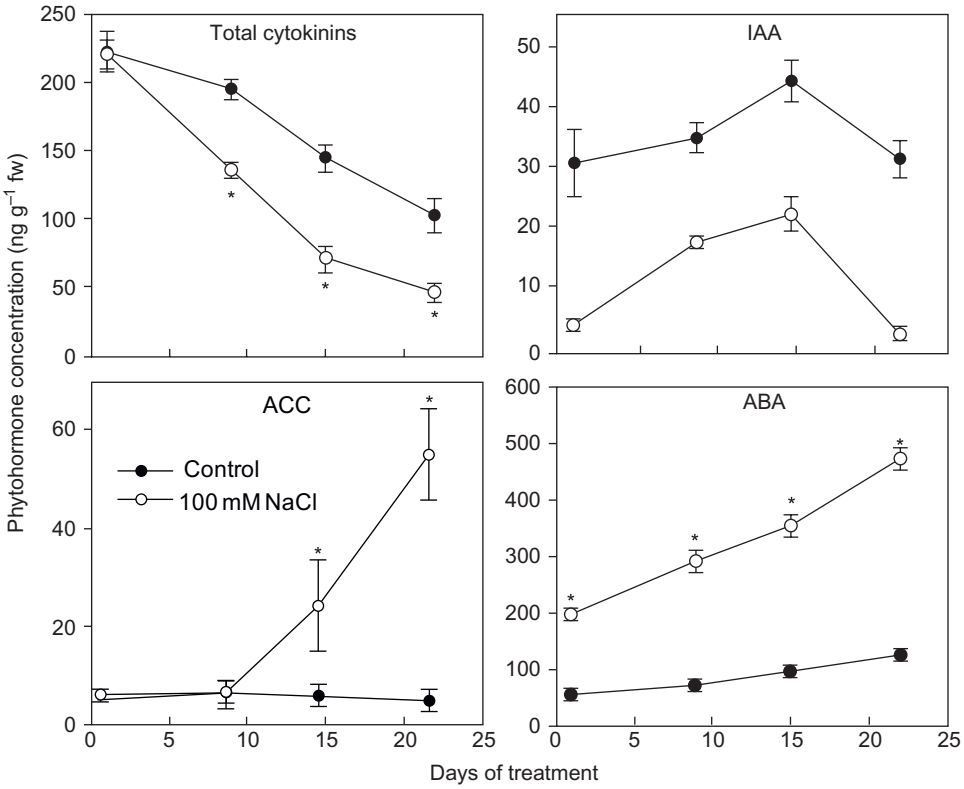


FIGURE 17.35 Phytohormone concentrations in a young expanding leaf of tomato plants grown in a nutrient solution with or without 100 µM NaCl. Total cytokinins: zeatin + zeatin-riboside; IAA: indole-3-acetic acid (auxin); ACC: 1-aminocyclopropane-1-carboxylic acid (ethylene precursor); ABA: abscisic acid. Asterisks indicate significant differences between control and saline treatment. *Based on Ghanem* et al. *(2008).*

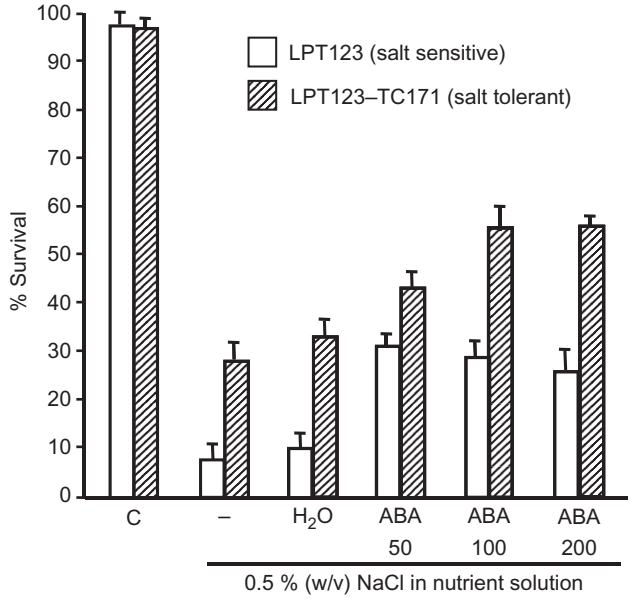


FIGURE 17.36 Survival rate of plants of a salt-sensitive and a salt-tolerant rice line after 4 weeks in either non-saline (C) or saline growth substrate. The salt-stressed plants remained either non-treated (−) or were sprayed daily with water (H_2O) or a 50, 100 or 200 µM ABA solution. *From Sripinyowanich* et al. *(2010) with permission from Elsevier.*

can increase plant growth under salinity. For example, in rice foliar application of putrescine had no effect on growth of non-saline control plants, but ameliorated the salt-induced depression in growth rate, chlorophyll, RNA and DNA concentrations in plants grown under salinity (Krishnamurthy, 1991).

Although these examples show interactions between salinity and phytohormones, the precise action of endogenous phytohormone levels on salt tolerance mechanisms is highly complex, and is not yet fully understood. Moreover, it should be noted that the observed effects may depend not only on the activity of the hormone itself, but also on the presence of corresponding receptors.

17.6.4 Mechanisms of Adaptation to Saline Substrates

17.6.4.1 Salt Exclusion versus Salt Inclusion

Salt tolerance can be achieved by salt exclusion or salt inclusion (Fig. 17.31). Adaptation by salt exclusion requires mechanisms for avoidance of an internal water deficit. Adaptation by salt inclusion requires either high tissue tolerance to Na^+ and Cl^-, or avoidance of high

tissue concentrations. A clear distinction is often made between salt excluders and salt includers; however, in reality there is a continuum of different degrees of exclusion and inclusion, differing between Na^+ and Cl^-, and parts and organs of plants.

Terrestrial halophytes, representing around 1% of all land plants, belong mainly to the Chenopodiaceae and Poaceae. Particularly in halophytes of the Chenopodiaceae, high salt tolerance is based mainly on inclusion of salts and their utilization for turgor maintenance or for replacement of K by Na in various metabolic functions. Across 32 species of the Chenopodiacea, Na and Cl accounted for around 70% of the solute concentration in the shoot water (Albert *et al.*, 2000). Within the monocotyledonous plants, the highly salt tolerant kallar grass (*Leptochloa fusca*) is also a salt includer (Gorham, 1987), although it also shows components of excluders, such as intensive retranslocation from the shoot to the roots and root release of Na and Cl (Bhatti and Wieneke, 1984). The ability to tightly regulate influx as well as efflux of Na and Cl is most likely crucial for all halophytes (Hasegawa *et al*., 2000). In highly salt tolerant *Casuarina* species (Aswathappa and Bachelard, 1986) and in the halophytic monocotyledonous *Puccinellia peisonis* (Stelzer and Läuchli, 1977) and *Festuca rubra* (Khan and Marshall, 1981), exclusion also contributes to salt tolerance.

In glycophytes, which comprise most crop species, there is generally an negative relationship between salt uptake and salt tolerance; that is, exclusion is the predominant strategy (Greenway and Munns, 1980; Gorham *et al.*, 1985). Their salt uptake is substantially lower than that of includers.

Typical differences between crop species in response to NaCl salinity in terms of growth and the element content of the shoots are shown in Table 17.33. Sugar beet shows the typical features of a salt-tolerant halophytic includer. Growth is enhanced by NaCl and the concentrations of Cl and especially Na in the shoot increase with increasing external supply. On the other hand, the K and Ca concentrations decline due to cation competition. Maize is less salt tolerant than sugar beet, its growth is inhibited although the concentrations of Cl and especially Na in the shoot remain relatively low. Of the three species shown in Table 17.33, bean has the lowest salt tolerance, with Cl toxicity the main reason for growth depression at the low salinity. In contrast to Cl, the shoot transport of Na is effectively restricted in bean. Thus bean, like many other salt-sensitive crop species, is an effective excluder of Na but not of Cl.

Differences in the capacity for Na and Cl exclusion also exist among cultivars of species. For example, the higher salt tolerance of certain cultivars of wheat (Munns and James, 2003), barley (Shavrukov *et al*., 2010) and citrus (Maas, 1993) is related to a more effective restriction of shoot transport of Na and/or Cl. In grapevine, differential salt tolerance is related to the capacity of rootstocks for Na and particularly Cl exclusion from the shoots (Downton, 1985). In wheat, two gene loci confer salinity tolerance (Nax1 and Nax2; Munns *et al.*, 2003). The two genes encode for Na^+ transporters of the HKT gene family (Huang *et al*., 2006). They are most likely expressed in the xylem parenchyma and retrieve Na from the xylem sap of the root (Nax1 and Nax2) and the leaf sheath (Nax2), thus reducing the amount of Na entering the shoot and the leaf blades (James *et al.*, 2011).

The capacity of Cl exclusion seems to be based on a major dominant gene and independent of the ability of Na exclusion from the shoot (Sykes, 1992). Among grapevine cultivars differing in their ability to exclude Cl from the shoot, uptake rates of Cl were similar, but transfer of Cl into the xylem was lower in the roots of the efficient excluders (Tregeagle *et al*., 2010). This may suggest that a similar mechanism of retrieval exists for Cl as for Na. In *Arabidopsis*, cation-Cl cotransporters have been proposed to function in Cl retrieval from the xylem (Colmenero-Flores *et al*., 2007).

Retranslocation of Na from the shoots to the roots can contribute to low Na concentrations in the shoots of salt-sensitive species such as bean, *Phaseolus vulgaris*, and salt-tolerant species such as common reed, *Phragmites communis* (Matsushita and Matoh, 1992) and berseem, *Trifolium alexandrinum* (Winter, 1982a,b). However, the proportion of Na that is translocated from leaves back to the roots seems to be higher for salt-sensitive than for salt-tolerant plant species (Jeschke and Wolf, 1993).

When halophytes such as the mangrove *Avicennia marina* are exposed to salinity, about 80% of the salts delivered by mass flow to the root surface are excluded from uptake (Waisel *et al.*, 1986). In many halophytes barriers are particularly developed in the roots against passive influx of salts. For example, the width of the Casparian band is 2 to 3 times greater than in glycophytes (Poljakoff-Mayber, 1975), and the inner cortex cell layer may be differentiated into a second endodermis (Inan *et al*., 2004).

17.6.4.2 Salt Distribution in Shoot Tissue

In includers, Na and Cl have to be effectively partitioned between old and young leaves, leaf sheath and leaf blades, cell types within leaf blades, and vegetative and reproductive organs. Restricted import of Na and Cl into young leaves is characteristic for salt-tolerant species. In *Kosteletzkya virginica*, a dicotyledonous halophyte, the optimum substrate concentration for growth is 85 mM NaCl. At this concentration, the Na concentration in the leaf water decreased from 230 to 25 mM from the oldest to the youngest leaf, whereas the K^+ concentration increased from 100 to 320 mM (Blits and Gallagher, 1990). Effective restriction of Na and Cl import into young leaves compared to old leaves was also typical for a clone of *Agrostis stolonifera*

from salt marshes compared with a clone from inland (Robertson and Wainwright, 1987).

For salt tolerance of crop species the total salt concentration in the shoot is less important than the capacity to restrict the import into young leaves, inflorescences and seeds, and maintaining a steep concentration gradient of Na and Cl between old and young leaves as has been shown for wheat (Gorham *et al.*, 1986) and maize (Hajibagheri *et al.*, 1987). In sugar beet as a salt-tolerant crop species, and also in halophytes, steep inverse Na^+/K^+ gradients between old and young leaves are maintained. High K^+ but low Na^+ concentrations in young leaves and reproductive organs are achieved by a general low xylem import of both K and Na, but high phloem import of K from mature leaves (Wolf *et al.*, 1991).

The importance of Cl partitioning within individual leaves for salt tolerance has been demonstrated for sorghum (Boursier and Läuchli, 1989) and barley (Table 17.34). In both species, Cl is particularly accumulated in the leaf sheath and in the epidermal cells of blades, whereas concentrations are low in the mesophyll (barley, sorghum) and bundle sheath cells (sorghum). The maintenance of lower Cl concentrations in the mesophyll cells of leaf blades of the salt-tolerant cultivar California Mariout (Table 17.34) may be important for protecting photosynthetic tissues from salt stress. These examples demonstrate how misleading average values for the shoots are in terms of interpreting mechanisms of salt tolerance.

17.6.4.3 Osmotic Adjustment

With a sudden increase in salinity, osmotic adjustment is achieved initially by a decrease in tissue water content (partial dehydration). In Fig. 17.33, negative growth rates of maize seedlings during the first 30 min after onset of the salt treatment are due to dehydration and shrinking of cells. Salt tolerance and further growth in a saline substrate require a net increase in the concentration of osmotically active solutes in the tissue (Gorham *et al.*, 1985). In genotypes in which salt exclusion is the predominant mechanism of salt tolerance, either the synthesis of organic solutes such as sugars and amino acids or the uptake rate of, for example, K, Ca, or nitrate are increased. Accumulation of these solutes decreases the osmotic potential in the cells and therefore allows uptake of water by compensating for the low osmotic potential in the surrounding solution. This is a very energy-demanding mechanism and growth rates of such genotypes under salinity are therefore rather low.

In genotypes in which salt inclusion is the predominant strategy, osmotic adjustment is achieved by the accumulation of salts (mainly NaCl) in the leaf tissue (Flowers, 1988). In natrophilic species Na can replace K not only in its function as an osmotically active solute in the vacuoles, but to some extent also in specific functions in cell metabolism (see also Section 7.2). Among these are protein synthesis (Flowers and Dalmond, 1992) and charge equalization during photosynthesis (Preston and Critchley, 1986). Its ability to replace K in enzyme functioning, however, is most likely rather limited.

When halophytes from the Chenopodiacea are exposed to salinity (40–500 mM), Na concentrations in the cytosol are commonly in the range of 150 to 220 mM (Flowers and Colmer, 2008); the cytosolic Cl concentration in *Salicornia maritima* was 86 mM (Flowers, 1988). In most glycophytes, cytosolic Na concentrations above 10 mM have negative effects on cell functioning and plant growth.

The physiological basis behind the tolerance of halophytes towards high cytosolic Na and Cl concentrations is not yet completely clear. Amino acid patterns of various enzymes isolated from halophytes and glycophytes did not differ (Huchzermeyer *et al.*, 2004), suggesting that enzymes in halophytes may not have enzymes with greater salt tolerance than those of glycophytes. Indeed, malate dehydrogenase and aspartate transaminase of halophytes such as *Atriplex spongiosa* and glycophytes like *Phaseolus vulgaris* showed similar sensitivity towards high NaCl concentrations *in vitro* (Greenway and Osmond, 1972). Nevertheless, halophytes may be better able to protect enzymes from adverse effects of Na or Cl, for example through the synthesis of organic compounds such as polyols that can stabilize the structure of membranes and macromolecules (Galinski, 1993; Bohnert and Jensen, 1996).

Despite differences in cytosolic salt tolerance, Na and Cl taken up by glycophytes and halophytes under salinity would rapidly accumulate to toxic concentrations in the cytoplasm if there were not mechanisms for their transfer to compartments where they do not interfere with the metabolism, such as the vacuole or the apoplasm. In addition, particularly the succulent halophytes are able to considerably increase the water content of their tissues, thus diluting their salt concentration.

In roots, accumulation of Na^+ and Cl^- in the apoplasm can occur as a result of exclusion of these ions from uptake. Accumulation may, however, also occur in the apoplasm of leaves, when leaf cells pump out excessive Na or Cl. It has been suggested that increasing accumulation of Na and/or Cl in the apoplasm may cause dehydration of the cytoplasm and eventually death of leaf tissues commonly observed under salinity (Oertli, 1968; Volkmar *et al.*, 1998). In rice exposed to 50 mM NaCl in the growth medium, NaCl concentrations as high as 600 mM in the leaf apoplasm solution may occur (Flowers *et al.*, 1991). However, in studies with different maize cultivars and cotton (Lohaus *et al.*, 2000; Mühling and Läuchli, 2002) Na or Cl did not accumulate in the apoplasm to concentrations that would affect cell turgor. In leaf apoplasms of the halophyte *Sarcobatus vermiculatus*, Na concentrations

TABLE 17.35 Solute concentration in isolated chloroplasts and in leaf extracts of spinach plants grown without (control) or with 300 mM NaCl[a]

	Control		+300 mM NaCl	
Solute	Chloroplast	Leaf	Chloroplast	Leaf
Meq L^{-1}				
Na^+	7	2	22	405
K^+	180	318	108	191
Mg^{2+}	18	32	13	39
Cl^-	1	21	25	335
HPO_4^{2-}	30	31	16	51
mmol L^{-1}				
Quarternary ammonium compounds (e.g., glycine betaine)	57	21	181	47

Based on Schröppel-Meier and Kaiser (1988).

TABLE 17.36 Examples of taxonomic distribution of compatible solutes

Solute	
D-Sorbitol	Rosaceae, Plantaginaceae
D-Pinitol	Leguminoseae, Caryophyllaceae
Glycine betaine	Chenopodiaceae, Gramineae, Solanaceae
Proline	Asteraceae, Gramineae
3-dimethylsulphonio-propionate	Asteraceae, Gramineae

Based on Gorham *et al.* (1985).

of 80–250 mM were measured. It has been suggested that in halophytes, apoplasmic ion accumulation may help to avoid cell turgor increases above a critical level during the night when transpiration is low (James *et al*., 2006).

17.6.4.4 Vacuolar Compartmentation and Compatible Solutes

In saline substrates, osmotic adjustment in includers requires salt concentrations in the symplasm of 300 to 500 mM of both Cl and Na (Gorham *et al.*, 1985). This implies transfer of considerable amounts of these ions into the vacuole to avoid toxic concentration in the cytoplasm. In some halophytes, Na/K ratios can be 20 times higher in vacuoles compared with the cytoplasm (Koyro and Stelzer, 1988). High Na/K ratios are also often observed in chloroplasts of salt-tolerant plants (Zhao *et al*., 2005; Table 17.35).

Sodium enters the vacuole via Na/H^+ antiporters using the proton motive force generated by the vacuolar H^+ ATPase (V-ATPase) and an H^+ pyrophosphatase (V-PPase). Exposure to salinity increases V-ATPase activity in glycophytes such as *Vigna unguiculata* (Otoch *et al.*, 2001), as well as in halophytes (Wang *et al*., 2001; Vera-Estrella *et al.*, 2005). In *Arabidopsis*, the vacuolar Na/H^+ transporter is encoded by the *At*NHX1 gene (Gaxiola *et al*., 1999). Homologous genes have been identified in several plant species, such as rice (Fukuda *et al*., 2004), wheat (Brini *et al*., 2005) and the halophyte *Atriplex gmelini* (Hamada *et al.*, 2001). The level of expression of the NHX1 gene increased in response to salinity in all plants investigated so far. In *Arabidopsis*, not only NaCl but also KCl and ABA increased the expression of NHX1 (Shi and Zhu, 2002). Over-expression of the *Os*NHX1 gene from rice in maize (Chen *et al*., 2007) or poplar (Wang *et al*., 2005b) resulted in increased Na accumulation and improved growth of the transformants under salinity. Transgenic rice plants over-expressing the *Cg*NHX1 gene from the halophyte *Chenopodium glaucum* or the homologue from a glycophyte did not differ in their salt tolerance, suggesting that the Na/H^+ antiporters encoded by NHX1 do not differ in their properties between halophytes and glycophytes (Li *et al*., 2008a).

It has been suggested that efficient compartmentation of Na and Cl in vacuoles may also depend on prevention of leakage of these ions through the tonoplast back into the cytoplasm. Leakage of ions through the tonoplast may be reduced by certain amino acids or polyols that function in membrane stabilization.

For osmotic adjustment of the cytoplasm and its organelles, organic solutes have to be synthesized upon accumulation of ions in the vacuole. These '*compatible solutes*' do not interfere with plant metabolic processes in the cytoplasm. The amino acid proline, glycine betaine and several polyols are the most common compatible solutes found in glycophytes and halophytes (Parida and Das, 2005; Tipirdamaz *et al*., 2006), with the chemical nature of compatible solutes varying among taxonomic groups (Tipirdamaz *et al.*, 2006; Table 17.36).

Apart from maintaining osmotic balance, some compatible solutes also protect membranes and macromolecules. For example, in chloroplasts, dissociation of intrinsic proteins from the O_2 evolving system caused by high Cl concentrations is prevented by glycine betaine (Papageorgiou *et al.*, 1991). When spinach was exposed to 200 mM NaCl, the concentrations of glycine betaine in the leaf tissue increased from 2.5 to 16.4 mM and in the

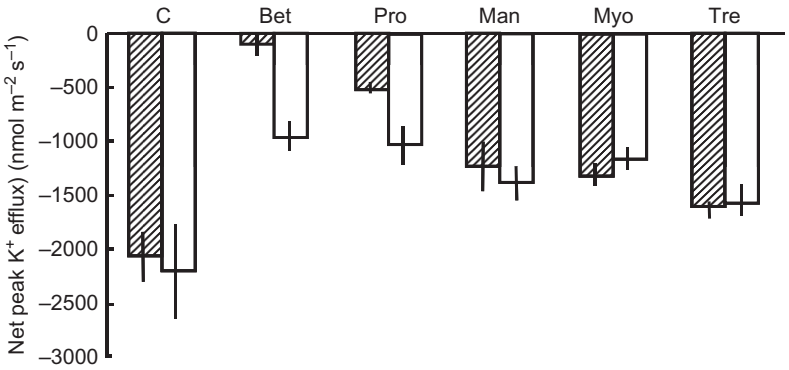


FIGURE 17.37 Net peak K^+ efflux from *Arabidopsis thaliana* roots after exposure to either a 50 mM NaCl or a 1 mM solution of OH-generating Cu^{2+}/ascorbate mixture for 1 hour with exogenous supply of compatible solutes. C: control; Bet: glycine betaine; Pro: proline; Man: mannitol; Myo: Myo-inositol; Tre: trehalose. *Based on Cuin and Shabala (2008).*

chloroplasts from 26 to 289 mM (Robinson and Jones, 1986). At least 30–40% of the total leaf glycine betaine was located in the chloroplasts of the salt-stressed plants. Most of the remaining glycine betaine is usually located in the cytosol, whereas the concentrations in the vacuoles are very low.

Glycine betaine is a very effective compatible solute because it is highly water soluble and does not carry a net charge, hence has no effect on the charge balance of the cytoplasm. Glycine betaine protects activity of pyruvate kinase isolated from halophytes such as *Atriplex gmelini*, and also reduces the K requirement for enzyme activation. The K_m value for K decreased from 5.6 mM in the absence of glycine betaine to 3.2 and 1.3 mM in the presence of 0.5 and 1M glycine betaine (Matoh *et al.*, 1988). Thus, in halophytes glycine betaine may reduce the demand of K in the cytosol by more than half, at least for pyruvate kinase. Compatible solutes may also decrease the leakage of K from roots exposed to salinity (Fig. 17.37) by either improving membrane integrity, or increasing ion efflux selectivity.

The importance of compatible solute synthesis for plant salt tolerance has recently been demonstrated in transgenic *Arabidopsis* plants with the mannose-6-phosphate reductase gene from celery with constitutive expression under the CaMV35S promoter (Zhifang and Loescher, 2003) which had the ability to synthesize and accumulate mannitol. Growth of the transformants did not differ from the wildtype in the absence of salinity, but had greater biomass under saline conditions with the difference to the wildtype increasing with increasing salt concentrations (Fig. 17.38).

D-pinitol, another polyol, is an important compatible solute in *Mesembrianthenum crystallinum*, where it is located in the cytosol and chloroplasts (Paul and Cockburn, 1989), whereas in *Viscum album* it may also contribute to the osmotic potential in the vacuoles (Richter and Popp, 1992). Proline accumulation is a well-known response to water deficit and to salt stress in glycophytes and halophytes

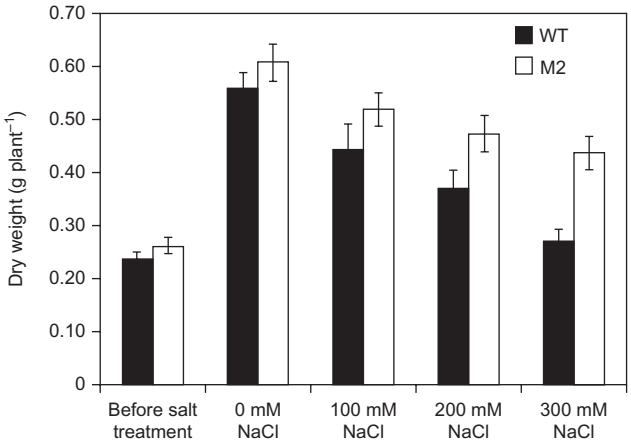


FIGURE 17.38 Dry weights of wildtype (WT) and transgenic (M2, ability to synthesize mannitol by insertion of the M6PR gene from celery) *Arabidopsis thaliana* plants grown under different salinity levels. Plants were grown for 10 days in the absence of salinity before treatments were established and maintained for 15 days. *From Zhifang and Loescher (2003) with permission from Wiley and Sons.*

(Rabe, 1990; Khatkar and Kuhad, 2000). Proline not only plays a role in osmotic adjustment, but most likely also in the detoxification of radical oxygen species (Radyukina *et al.*, 2008).

Osmotic adjustment in plants via salt inclusion or exclusion has important implications for the energy balance. Since NaCl and other soluble salts are abundant in saline substrates, they can be regarded as potentially 'cheap', although dangerous, osmotica. According to Wyn Jones (1981) the approximate energy cost of accumulating 1 osmol of solute for osmoregulation is 0.54, 13 and 54 mole ATP for NaCl uptake, synthesis of K-malate and accumulation of C_6 sugars, respectively.

Hence, osmotic adjustment via synthesis of organic osmotica is energetically very expensive, reducing the energy available for growth. However, the form of osmotic adjustment may vary between organs of the same plant

species. For example, in *Aster tipolium*, osmotic adjustment in the leaves is mediated by Na and Cl, whereas that in the flowers is mediated by K, glycine betaine and sugars (Gorham *et al.*, 1980). Even within a given leaf, the role of solutes may vary; in young leaves of sorghum, glycine betaine is important for osmotic adjustment in the leaf blades, but not in the leaf sheaths (Grieve and Maas, 1984). In barley grown on saline substrates, sugars are not important compared with Na^+ and Cl^- in osmotic adjustment in mature leaves, whereas they contribute more than 20% to the osmotic adjustment in expanding leaves (De Lane *et al.*, 1982).

17.6.4.5 Detoxification of Reactive Oxygen Species

In plant tissues, reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide (O_2^-) or hydroxyl radicals ($OH^\cdot$) are continuously formed in the cytosol, chloroplasts and mitochondria by various metabolic processes (McCord, 2000). ROS play a key role in signal transduction, but may also damage cells, for example by membrane peroxidation, protein degradation and DNA mutation. Plants therefore scavenge excessive amounts of ROS by enzymes such as superoxide dismutase, catalase or glutathione peroxidase. Antioxidants such as ascorbic acid, tocopherol or glutathione also contribute to the detoxification of ROS.

Salinity and drought stress cause a rapid increase in cellular ROS concentrations in plants (Moran *et al*., 1994; Fadzilla *et al.*, 1997; Mittler, 2002) which is most likely due to a limited CO_2 reduction by the Calvin cycle during periods of osmotic stress. This causes a decrease in the amount of reduced $NADP^+$ as an electron acceptor in the light reaction, resulting in electrons from PS1/ferredoxine being transferred to O_2 instead of $NADP^+$ which leads to the formation of O_2^- (Hsu and Kao, 2003). Chloroplasts and mitochondria are thus primary sites of ROS formation under salinity. The enzyme superoxide dismutase calayses the conversion of superoxide to H_2O_2 which is broken down by the enzymes catalase and peroxidases. When H_2O_2 remains within the stroma of chloroplasts, it can inhibit the Calvin cycle, contributing further to decreasing rates of CO_2 assimilation often observed in salt-stressed plants (Shen *et al*., 1997; Table 17.31).

The transcription of genes coding for antioxidative enzymes and their activity in plant tissues are usually increased in response to salinity stress. In many plant species, salinity tolerance is positively correlated with the ability to up-regulate the cellular ROS scavenging system. For example, in pea and tomato, protection of chloroplasts from salinity-induced oxidative damage and maintenance of photosynthetic activity was correlated with superoxide dismutase and peroxidase activities in the chloroplasts (Hernández *et al*., 1995; Mittova *et al*., 2002). Cell cultures of a salt-tolerant cotton cultivar had higher antioxidative activities and grew better on a saline medium compared with those from a sensitive cultivar (Garratt *et al*., 2002). When exposed to salinity, the antioxidant scavenging system in C4 plants appears to be more effective than that in C3 plants (Stepien and Klobus, 2005).

The important role of ROS scavenging in plant salinity tolerance was also confirmed by studies with transgenic plants. For example, rice over-expressing the *Escherichia coli* catalase gene 'KatE' was more salt-tolerant than the wildtype (Moriwaki *et al.*, 2008). Similarly, salinity tolerance could be improved in *Arabidopsis* by over-expression of superoxide dismutase (Wang *et al*., 2004b).

External supply of the antioxidant ascorbic acid improved the survival of tomato seedlings exposed to high salinity by decreasing oxidative stress (Shalata and Neumann, 2001). Among the compatible solutes, proline and polyols are able to scavenge ROS (Smirnoff and Cumbes, 1989), suggesting that the positive effects of compatible solutes on membrane integrity and K homeostasis are in part due to their ability to protect membranes from peroxidation (Fig. 17.37).

The precise mechanisms leading to the activation of antioxidant responses in plants upon salinity or drought stress are still unclear, but ABA and Ca^{2+} signals are likely to be involved (Agarwal *et al*., 2005). Hydrogen peroxide itself is a trigger for antioxidant synthesis, and pre-treatment of plants with a low concentration of H_2O_2 can improve their salinity tolerance (Dias de Azevedo Neto *et al*., 2005).

17.6.4.6 Salt Excretion

Haloyphytes may reduce the salt concentration of the photosynthetic active tissue by various mechanisms: accumulation in bladder cells, excretion by salt glands, shedding of salt-saturated leaves, and retranslocation to other organs (Waisel *et al.*, 1986). Bladder cells are modified trichomes that swell to a bladder of up to 0.2 mm diameter while accumulating high concentrations of NaCl. Salt glands vary strongly in anatomy and efficiency. They may be multicellular organs of highly specialized cells, for example in *Avicennia marina*, or simple glands comprising only two cells, for example in kallar grass, *Leptochloa fusca* (Wieneke *et al.*, 1987). Salt glands are highly selective, but selectivity may be reduced at low substrate salinity (Sobrado and Greaves, 2000). Salt glands can remove large amounts of salt by excretion to the leaf surface, where they can be washed off by rain or dew. In *A. germinans*, daily excretion increased with increasing salinity of the growth substrate, and reached a maximum of around $47\,mM\,m^{-2}$ at $865\,mol\,m^{-3}$ NaCl (Sobrado and Greaves, 2000). Excretion of Na^+ is probably mediated by a homologue of the Na/H^+ antiporter SOS1, and driven by plasma membrane ATPases (Chen *et al*., 2010).

The importance of salt excretion to the salt tolerance of many halophytes is indicated by the fact that the salt tolerance of intact plants (*Sueda* or *Artiplex*) cannot be reproduced in callus cultures (Smith and McComb, 1981). In four grass species of the genus *Zoysia* salinity tolerance was positively correlated with salt gland density and leaf Na^+ excretion (Marcum *et al.*, 1998).

In the mangrove *A. marina*, between 40% (Waisel *et al.*, 1986) and 90% (Ball, 1988) of the salts transported in the xylem to the shoot are excreted by salt glands. However, salt excretion in *A. marina* or *L. fusca* is considered to be a secondary mechanism of salt tolerance, the exclusion at the roots (i.e., avoidance) being the major mechanism (Waisel *et al*., 1986; Gorham, 1987).

17.6.5 Outlook

Strategies of tolerence to salinity are highly diverse, and may vary depending on the plant genotype, its developmental stage and environmental factors. Understanding of mechanisms underlying salinity tolerance is important, not only to allow for the selection of adapted crop plant genotypes, but also to understand how soil salinity affects natural plant communities and ecosystem functioning. For example, Feldman *et al.* (2008) found that the abundance of C4 species among halophytes and non-halophytes was strongly increased with increasing soil salinity (Table 17.37), illustrating the effect changes in soil salinity may have on ecosystems.

Anthropogenic activities may not only affect salinity of agricultural soils, but also that of natural ecosystems, for example due to rising saline groundwater (Rengasamy, 2006) or to flow regulation and channelling of rivers (Ohmart *et al.*, 1988). Glenn *et al.* (1998) have shown that along channelled rivers in the south-western United States, increasing soil salinity has led to the replacement of native riparian plant species by salt-tolerant invasive species.

Given the projected increase in salt-affected areas in the future, it is important to develop management strategies for these soils. Amelioration (e.g., gypsum application or drainage) may be one option; the other is cultivation of salt-tolerant plants. To date, non-cultivated plants with a particularly high salinity tolerance have not been explored for their potential in production of biofuel, animal fodder or pharmaceutics on saline soils or in areas where non-saline irrigation water is scarce. Future research will have to address, for example, how salinity tolerance in halophytes is correlated with oil content of seeds, biomass production or palatability of the vegetative tissues. Plant physiologists and agronomists will jointly have to develop cultivation practices that allow for long-term use of saline soils for halophyte cultivation. For this purpose it will also be necessary to better assess the long-term impact of halophytes on soil chemical and physical properties.

Concerning the use of saline soils for the cultivation of conventional crops, further progress will have to be made in screening and breeding programmes designed to improve crop yield under salinity. This may involve the use of gene transfer as well as interspecific hybridization. So far, the developed genotypes have been insufficiently tested for their yield potential under field conditions, even though the laboratory data are promising (Flowers, 2004).

In the past, salt tolerance was often measured as the relative growth decrease in response to salinity compared with a non-saline control. This may not necessarily lead to the selection of the plant genotype with the highest yield under salinity. For example, among the genotypes shown in Table 17.29, the genotype with the highest relative decrease in growth (Marianna GF 8-1) still produces the highest biomass under saline conditions. Future research will not only have to further unravel physiological mechanisms of Na and Cl exclusion or inclusion in plants, but also characterize these in terms of carbon allocation costs.

TABLE 17.37 Mean cover–abundance percentages of C3 and C4 + CAM species in halophyte and non-halophyte plant communities of three phytogeographical provinces of Santa Fe/Argentina differing in soil salinity

		Phytogeographical provinces of Santa Fe		
Communities		Pampean (low salinity)	Espinal (medium salinity)	Chaquenian (high salinity)
C3	Non-halophytes	78	36	8
	Halophytes	28	8	6
C4 + CAM	Non-halophytes	22	64	92
	Halophytes	72	92	95

After Feldman *et al.* (2008).

Chapter 18

Nutrient and Carbon Fluxes in Terrestrial Agro-Ecosystems

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SUMMARY

This chapter summarizes the biological, biochemical and physical factors determining the turnover of N, P, K and C in soils as well as the effects of soil amendments such as plant residues, manure and composts in agro-ecosystems. Topics discussed include the role of bacterial and fungal decomposer communities in soils, the importance of pH and other soil properties, the possible effects of global warming and the quality of organic substrates such as determined by the C/N ratio and secondary plant compounds. Further, the role of animal husbandry systems in nutrient cycling at different scales is described. Lastly, the importance and limitations of current modelling approaches are discussed and current research gaps identified.

18.1 MICROBIOLOGICAL FACTORS DETERMINING CARBON AND NITROGEN EMISSIONS

Carbon is emitted from soil mainly as CO_2 and CH_4 (IPCC, 2007), although a range of other volatile organic components, such as terpenes, can also be released from soil (Scheller, 2001; Ludley *et al.*, 2009). Currently, the CO_2 concentration in the atmosphere is 388 ppm, which equates to 800 Gt C (IPCC, 2007). It is increasing by 3 ppm annually, contributing considerably to global warming and subsequent climate change (IPCC, 2007). Photosynthetic organisms such as plants, algae and cyanobacteria convert approximately 120 Gt C into their biomass per year (Field *et al.*, 1998), i.e. 20% of the atmospheric CO_2. An increase in CO_2 concentration increases plant biomass production (Manderscheid *et al.*, 2009, 2010) and increases their water use efficiency (Qiao *et al.*, 2010; Chun *et al.*, 2011). Nitrogen is an important nutrient for plants, therefore gaseous N emissions from agricultural ecosystems are economically relevant. Nitrogen is emitted mainly as N_2 which is the end product of denitrification, but also as N oxides, especially N_2O and NH_3 (Adviento-Borbe *et al.*, 2010). N_2O oxide has become the third most important anthropogenic greenhouse gas (IPCC, 2007), and plays an important role in ozone depletion (Ravishankara *et al.*, 2009); its global warming potential is 300 times that of CO_2 (IPCC, 2007). On the other hand, in the vadose zone below the subsoil and also in waste water treatment plants, denitrification can remove NO_3 thereby protecting groundwater and aquatic surface ecosystems (Fryar *et al.*, 2000; Deurer *et al.*, 2008; Leu *et al.*, 2010).

18.1.1 Carbon Dioxide Emission

Gaseous emissions of carbon (C) from soil consist mainly of CO_2, derived from respiration by plant roots and soil microorganisms. The contribution by autotrophic plants to the CO_2 emission from soils shows diurnal and seasonal variations, depending on photosynthetic activity, plant development and species. Root respiration of N_2-fixing legumes is particularly high to meet the energy demand of rhizobia (Merbach *et al.*, 1999; Wichern *et al.*, 2004a). The C input of plants into the soil is in the form of residues (Poll *et al.*, 2010) and rhizodeposition which are decomposed microorganisms (Wichern *et al.*, 2007). The contribution of microbial respiration to soil CO_2 emissions depends on soil temperature, soil moisture and C availability (Wichern *et al.*, 2004a). The latter depends primarily on the quality of the organic C input, but also on soil properties such as pH (Anderson and Domsch, 1993), clay content (Müller and Höper, 2004), soil structure (Farquharson and Baldock, 2008) and other factors controlling microbial activity (Joergensen and Emmerling, 2006), and on gas diffusion within the soil (Šimůnek and Suarez, 1993; Müller

Marschner's Mineral Nutrition of Higher Plants. DOI: 10.1016/B978-0-12-384905-2.00018-2

et al., 2011). Microbial CO_2 production from the decomposition of soil organic matter and organic residues is an important driver of global warming (Kump, 2002). The decomposition of soil organic matter by soil microorganisms also results in the release of plant available nutrients such as N, S and P, but to some extent also Ca and Mg (Rottmann *et al.*, 2011).

18.1.2 Fungal and Bacterial Contributions to CO_2 Emissions

Soil microorganisms encompass archaea, bacteria, fungi and protozoa. They are responsible for the majority of enzymatic processes in soil and store energy and nutrients in their biomass (Jenkinson and Ladd, 1981). The diversity of soil microorganisms is enormous (Torsvik *et al.*, 1990) and the majority of species are still unknown (Torsvik and Øvreås, 2007). Soil ecological concepts, for example describing the interactions of soil animals and soil microorganisms in food webs, often separate the microbial community into fungi and bacteria (Hedlund *et al.*, 2004; van der Putten *et al.*, 2004; Coleman, 2008; Holtkamp *et al.*, 2008), which are the two largest functional microbial subgroups in the soil. Archaea and protozoa contribute only approximately 1 and 2%, respectively, to the soil microbial biomass (Gattinger *et al.*, 2002; Bardgett and Griffiths, 1997). The reason for separating the microbial community into fungi and bacteria is their different roles in the soil.

Fungal energy channels are considered to be slow cycles. Fungi have, compared to bacteria, relatively long generation times and are abundant in soils with high C/N ratio, low pH and low nutrient availability and high concentrations of recalcitrant organic materials; and they are favoured by a reduction in tillage intensity (Blagodatskaya and Anderson, 1998; Högberg *et al.*, 2007; Beare, 1997; Frey *et al.*, 1999; Thiet *et al.*, 2006). Soil fungi have been shown to use organic substrates more efficiently than bacteria; they form more biomass per unit substrate (Holland and Coleman, 1987; Sakamoto and Oba, 1994). Fungal hyphae are more resistant against microbial decomposition than bacterial cells (Webley and Jones, 1971; Guggenberger *et al.*, 1999). Therefore, the promotion of fungi may be an important tool for C sequestration in soils (Bailey *et al.*, 2002; Jastrow *et al.*, 2007) although this has recently been questioned (Scheller and Joergensen, 2008; Heinze *et al.*, 2010).

Bacteria are promoted by increasing land use intensity, for example fertilization (Högberg *et al.*, 2003), tillage (Beare, 1997; Frey *et al.*, 1999) and livestock grazing of the aboveground biomass (Bardgett *et al.*, 1993).

18.1.3 Methane Emissions

Methane (CH_4) is one of the main hydrocarbons in the atmosphere and responsible for approximately 20% of global warming (IPCC, 2007). It has a global warming potential 25 times that of CO_2. Currently, the atmospheric CH_4 concentration is 1.78 ppm (Forster *et al.*, 2007). CH_4 is produced under anaerobic conditions by prokaryotic archaea (Chaban *et al.*, 2006), yeasts (Botha, 2011) and bacteria such as *Clostridium* sp. (Liu *et al.*, 2009b). Most biogenic methane is the result of autotrophic CO_2 reduction (Noll *et al.*, 2010). Methanogenic archaea play a vital ecological role by removing excess hydrogen and fermentation products from soil and typically grow in strictly anaerobic environments in which all electron acceptors other than CO_2 (O_2, NO_3^-, SO_4^{2+}, and Fe-III) have been depleted (Dubey, 2005) (See also Section 17.4). Their activity strongly increases with soil temperature (Chin *et al.*, 1999; Bergman *et al.*, 2000) and is particularly high in water-saturated soils, such as fens, bogs, swamps, marshland and paddy rice fields (Liu *et al.*, 2010). However, CH_4 is also produced in anaerobic microsites after heavy rainfall events (Sey *et al.*, 2008, Kamman *et al.*, 2009) and after incorporation of easily decomposable substrates (Gregorich *et al.*, 2006).

18.1.4 N_2 and N_2O Emissions

Denitrification is the microbial process of dissimilatory nitrate reduction that produces N_2 via a series of intermediate gaseous nitrogen oxide products. The electron acceptors in order of decreasing energy yield are $NO_3^- > NO_2^- > NO > N_2O$. Denitrification completes the N cycle by returning N_2 to the atmosphere and occurs mainly in poorly aerated soil, i.e. where O_2 consumption exceeds the rate of O_2 supply, such as in wetlands or in the detritusphere around crop residues in the soil where anoxic microsites are induced by enhanced microbial respiratory O_2 consumption (Parkin, 1987; Flessa and Beese, 1995; Chang *et al.*, 1998; Velthof *et al.*, 2003). N_2O is the third most important anthropogenic greenhouse gas, the single most important ozone-depleting gas (Ravishankara *et al.*, 2009) and soils are its major source (IPCC, 2007). In soils, N_2O production is primarily from denitrification, and, to a lesser extent, from nitrification (Bremner, 1997; Webster and Hopkins, 1996; Baggs, 2008; Kool *et al.*, 2011).

Although N_2O production is commonly attributed to bacterial activity (heterotrophs and autotrophs), it can also be produced by eukaryotes (Laughlin and Stevens, 2002; Crenshaw *et al.*, 2008). Shoun *et al.* (1992) demonstrated that the ability to produce N_2O during the dissimilatory reduction of NO_2^- and NO_3^- was relatively widespread among filamentous fungi. Most of these fungi lack the capability to reduce N_2O to N_2, thus in contrast to bacterial denitrification (which may also produce N_2), fungal NO_3^- reduction only yields N_2O.

Increased C and N sequestration may result in increased N_2O emissions. which is in agreement with

modelling results (Li *et al.*, 2005; Qiu *et al.*, 2009). On the other hand, long-term application of manure did not increase N_2O emissions compared to application of mineral fertilizers, despite C and N additions with the manure (Meng *et al.*, 2005). These contrasting effects of C sequestration on N_2O emissions may be partly due to the different experimental conditions and site-dependent differences in the extent of organic matter accumulation.

18.2 EFFECTS OF ORGANIC SOIL AMENDMENTS ON EMISSIONS

The quality and composition of organic amendments has a strong impact on emission, on the one hand, and on organic matter sequestration in soils, on the other. Higher nutrient concentrations in plant residues usually lead to higher decomposition rates, especially during the initial phases of decomposition (Swift *et al.*, 1979). The most important quality index for organic amendments is the N concentration, often expressed as C/N ratio, although P (Cleveland *et al.*, 2002), S, Ca, Mg and K concentrations may also affect decomposition rates (Tyler, 2005; Cleveland *et al.*, 2006; Salamanca *et al.*, 2006).

Organic amendments with low C/N ratio are usually considered to be more easily decomposable than those with high C/N ratio (Swift *et al.*, 1979; Henriksen and Breland, 1999a, b; Potthoff *et al.*, 2005) and supply more inorganic N to plants but also result in greater CO_2 and N_2O emissions. High initial N concentrations of plant residues increase the production and activity of microbial exo-cellulases, endo-cellulases and xylanases (Henriksen and Breland, 1999a) and consequently cellulose degradation (Recous *et al.*, 1995; Berg, 2000; Henriksen and Breland, 1999b). Net N immobilization in the microbial biomass usually occurs after incorporation of organic amendments with a C/N >25, whereas net N mobilization occurs with C/N <15 unless other stabilizing molecules such as lignin and cellulose are present (Powlson *et al.*, 2001). On the other hand, excessive supply of inorganic N in temperate humid forests may depress litter decomposition by inhibition of lignolytic white-rot fungi (Berg, 2000). High Zn and Cu concentrations, such as in sewage sludge, compost, farmyard manure and animal dung, may also inhibit decomposition (Khan and Joergensen, 2006).

The types of carbon compounds in the organic material also affect decomposition rates, particularly the concentrations of lipids, carbohydrates, protein and lignin. Carbohydrates are usually subdivided into a soluble fraction, starch and the structural components hemi-cellulose and cellulose. Many plants contain polyphenols which inhibit N mineralization (Fig. 18.1) (Quarmby and Allen, 1989; Hättenschwiler and Vitousek, 2000).

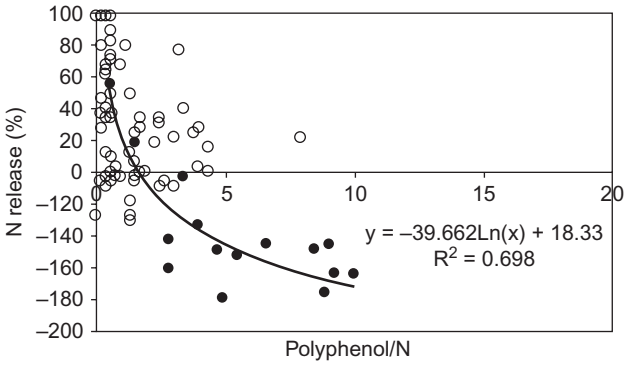

FIGURE 18.1 Effect of the polyphenol/nitrogen (N) ratio on N release of plant residues (data from 11 studies). ● plant residues with N concentrations of <1%, ○ plant residues with N concentrations of 11%. *From Seneviratne (2000) with kind permission from Springer Science and Business Media.*

In tropical and subtropical agro-ecosystems termites often play a major role in decomposing recalcitrant plant material thereby contributing to the recycling and redistribution of plant nutrients (Tian *et al.*, 1993; Mando and Brussaard, 1999; Buerkert *et al.*, 2000; Esse *et al.*, 2001), but may also contribute to CO_2 and CH_4 emissions from savannahs and humid rainforests (Martius *et al.*, 1993, 1996).

The mineralization of litter not only depends on the quality of organic components, but also on amount and composition of the litter colonizing microbial community (Dilly and Munch, 2004; Flessa *et al.*, 2002; Potthoff *et al.*, 2008). Only the more readily available fractions are decomposed at low temperatures, whereas decomposition of the more recalcitrant fractions occurs predominantly at higher temperatures (Nicolardot *et al.*, 1994; Azmal *et al.*, 1996; Gu *et al.*, 2004). Therefore, the decomposition rates of recalcitrant fractions may increase more strongly with increasing temperature than that of the labile fractions (Fierer *et al.*, 2005; Bol *et al.*, 2003); but this is not always the case (Bååth and Wallander, 2003; Fang *et al.*, 2005, 2006).

18.3 EFFECTS OF PH, SOIL WATER CONTENT AND TEMPERATURE ON MATTER TURNOVER

Increasing soil pH, for example by application of lime, may enhance mineralization and release of N and C (Singh and Beauchamp, 1986; Lyngstad, 1992; Clay *et al.*, 1993) by increasing the availability of labile organic matter, although the effect may be restricted to the first days after application of lime (Curtin *et al.*, 1998).

Soil water content affects organic matter decomposition and CO_2 release. Decomposition rates are low in very dry and very wet soil and strongly increase after rewetting of dry soil (Formowitz *et al.*, 2007; Wichern *et al.*, 2004a, b).

TABLE 18.1 Carbon (C) storage of grasslands, forests and agro-ecosystems

Ecosystem	Vegetation	(10^9 t ha^{-1}) Soils	Total	C storage
Grasslands				
high-latitude	14–48	281	295–329	271–303
mid-latitude	17–56	140	158–197	79–98
low-latitude	40–126	158	197–284	91–131
Total	71–231	579	650–810	123–154
Forests	132–457	481	613–938	211–324
Agro-ecosystems	49–142	264	313–405	122–159
Other[a]	16–72	160	177– 232	46–60
Global total	268–901	1484	1752–2,385	120–164

Modified after White *et al.* (2000).
[a]*Includes wetlands, barren areas and human settlements.*

At high soil water content, soils become increasingly anaerobic. Soil respiration strongly declines with redox-potential (Eh) when microbial activity turns from oxidation of C sources to facultative and subsequently anaerobic fermentation (Patrick and Jugsujinda, 1992; Salomons, 1995) (see also Section 17.4). With increasingly lower O_2 availability the following processes predominate: nitrification and denitrification (Eh $\geq$ 300 mV, $NH_3 \rightarrow NO_2^- \rightarrow NO_3^- \rightarrow N_2$), Mn^{4+}-reduction (Eh = 300 to 100 mV), Fe^{3+}-reduction (Eh = 100 to $-$ 100 mV)· SO_4^{2-}-reduction (Eh = -100 to $-$ 200 mV) and finally methanogenesis (Eh < -200 mV). These anaerobic processes yield less energy than aerobic decomposition, hence decomposition rates are low (Kögel-Knabner *et al.*, 2010).

Microbial activity and thus C and N mineralization are also strongly temperature dependent (De Neve *et al.*, 1996). The long-term accumulation of C in soils of arctic ecosystems (Rodionow *et al.*, 2006) indicates that despite the adaptation of some bacterial decomposer communities to below-freezing point conditions (Steven *et al.*, 2006; Mikan *et al.*, 2002), mineralization is overall more sensitive to low temperature than plant growth (Schulze *et al.*, 2000).

It may thus be summarized that C storage in soils is influenced by a combination of factors such as composition of the organic amendments, temperature, soil water content and soil chemistry. These factors affect microbial activity not only directly, but also indirectly via their effects on plant growth and nutrient uptake. The soil C content of a given ecosystem is a function of the interactions of plant input, on the one hand, and decomposition rate, on the other (Table 18.1).

18.4 GLOBAL WARMING EFFECTS

The 0–100 cm soil layer of temperate, boreal and arctic ecosystems, which together occupy 43% of the world's surface area, store an estimated 64% of global soil organic carbon (SOC) and 53% of the soil N (Batjes, 1996, 1997; Nieder and Benbi, 2008). Of the 1,462,000 Mio t C globally stored in soils, cool and temperate zone, peatlands alone contain about 450,000 Mio t organic C and fix 200–400 kg C (ha year)$^{-1}$ (Gorham, 1991; Tolonen and Turunen, 1996). Even small increases in annual temperature in these regions can therefore strongly increase C and N emissions (Jahn *et al.*, 2010). The thawing of permafrost soil layers increases microbial turnover processes (Marchand *et al.*, 2004; Lawrence and Slater, 2005; Steven *et al.*, 2007) which may enhance the release of CO_2 (and under anaerobic conditions of CH_4) from the stored organic matter in northern Histosols that were so far protected by cooler conditions (McGuire *et al.*, 2006; Field *et al.*, 2007; Table 18.2).

18.5 PLANT–ANIMAL INTERACTIONS AFFECTING NUTRIENT FLUXES AT DIFFERENT SCALES

18.5.1 Species-specific Relationship between Feed Intake and Excreta Quality

Amount and quality of livestock excreta (faeces and urine) are primarily determined by the amount and quality of the feed ingested. When feed is abundant, the voluntary

TABLE 18.2 Estimated stocks of soil organic carbon (SOC) in the northern high latitudes and on the global scale

Ecosystem	Estimated SOC stock (10^9 t C)
Wetlands	
Global	120–460
Northern	202–535
Tundra ecosystems	43–200
Boreal ecosystems	200–750
Northern high latitudes >45°N	1,400–1,850

From Jahn *et al.* (2010).

feed intake of an animal depends on its requirements for energy and nutrients and the so-called 'palatability' of the feed – which is a function of qualitative characteristics of the feed, as well as other physico-chemical characteristics such as odour and taste (van Soest, 1994; Provenza, 1995). Energy and nutrient requirements depend on animal species and breed, physiological stage of the animal, its production level, health status, as well as on environmental variables (CISRO, 2007).

General estimates of the quantity of excreta from different livestock species in different regions of the world have been published by FAO (2006c). However, given the large number of variables and their interaction that are modulating feed intake and location-specific aspects of livestock farming, such generalized values are often of only limited use to predict the quantity of excreta per animal unit as well as its spatio-temporal variation.

In weaned ruminants, ingested feeds are at first fermented in the rumen by its microflora (van Soest, 1994). The rumen microflora breaks down crude fibre (cellulose, hemicellulose, lignin) and other non-starch polysaccharides (oligo-saccharides, pectin, beta-glucan) which cannot be broken down by the ruminant animal's own digestive enzymes. Sugars and starch as well as lipids, protein and non-protein N compounds are also broken down in the rumen (van Soest, 1994). The microbial fermentation processes yield varying proportions of the short chain fatty acids (SCFA) acetate, propionate and butyrate, and also NH_3, which are absorbed through the rumen wall and used in the animal's metabolism. Growth and turnover of the rumen microbial population yields microbial protein, which can be utilized by the host's metabolism after post-ruminal digestion in the abomasum and small intestine (van Soest, 1994). Feed constituents, microbial debris as well as epithelial cells and mucus shed from the lumen of the gastrointestinal tract (GIT) that escape digestion in the small intestine may undergo fermentative microbial breakdown in the colon ('hindgut fermentation'), the principles of which are similar to the processes in the rumen (van Soest, 1994). However, in the colon the host animal can only absorb the released SCFA, amides, NH_3 and water; microbial protein synthesized in the hindgut is excreted along with any unfermented feed residues and endogenous N (Breves *et al.*, 2009).

In both pigs and poultry, microbial fermentation of undigested feed components only takes place in the hindgut (pig: colon; poultry: caecum) from where resulting SCFA, NH_3 and possibly non-protein N can be absorbed, while other products of fermentation remain unavailable to the animal (Mead, 1989; van Soest, 1994).

The degradability and actual degradation of N-containing feed constituents in the rumen, protein digestion in the lower GIT, and the extent of hindgut fermentation determine the form and proportion of N excretion in faeces and urine which may then be available for plant uptake. Urea accounts for >70% of urine N (Bristow *et al.*, 1992; FAO, 2006c), and depending on ambient temperature, the enzyme urease in the urine will quickly break down urea to NH_3 and CO_2, leading to large volatilization losses. Low ruminal and post-ruminal degradability of feed N and high microbial activity in the hindgut increases the proportion of N excreted with faeces. If faeces dry quickly, the N is largely preserved: at a temperature of 60°C and air humidity <30%, goat faeces dried for 48 hours contained only 2% less N than the fresh material (unpublished data).

Due to the greater stability of N in faeces compared to urine, diverting N excretion from urine to faeces seems advantageous from a plant nutrition point of view. The degradability of nitrogenous feed compounds is low if animals are consuming mature and thus strongly lignified grasses, or if their diet contains tannins, which are prevalent in many tropical ligneous and legume feeds (Makkar, 2003). Polyphenols bind proteins and can inhibit protein degradation in the rumen as well as in the post-ruminal GIT. The affinity of tannins to proteins depends on the type of tannin as well as on the type of protein. Tannins in ruminant diets can also reduce the speed of N release from faeces after their application to the soil (Somda *et al.*, 1995; Powell *et al.*, 1999).

Fresh chicken excreta contain on average 1.6% N, which consist of 60% uric acid, 2% urea, 6% ammonium-N and 32% decomposition products of protein. The dominance of uric acid over urea in poultry excreta does not affect the release of ammonium from poultry manure (Rothrock *et al.*, 2010), the acidity of poultry litter may decrease soil pH if applied regularly. In pigs, the partitioning of N excretion between faeces and urine depends on the amino acid pattern of the diet, the structure of feed proteins, the presence of secondary plant metabolites such as protease inhibitors or tannins, pre-treatment

of protein-rich feeds, and on the concentration of structural carbohydrates in the diet (Kirchgessner *et al.*, 2008). In feeds of high digestibility, high protein availability, and high biological value of the protein, about 78% of N excreted by a growing pig may be in the urine, while with lower digestibility, lower protein availability, and/or lower biological value of the feed protein, the urinary N excretion may be only 59% (de Wit *et al.*, 1997).

The majority of organic C contained in ruminant (and non-ruminant) faeces originates from undigested cell wall constituents. However, coarsely milled grain particles, particularly from maize may be small enough to escape rumen fermentation and also withstand enzymatic breakdown in the small intestine and colon; faeces may also contain a considerable proportion of starch (Kirchgessner *et al.*, 2008). Strongly lignified cell walls such as those of mature C4 grasses and legume stems can withstand digestion by the enzymatic systems of mammals and their intestinal microflora (van Soest, 1994). Whereas undigested sugars, starch and non-lignified cellulose and hemicellulose can be easily degraded by soil microbes after faecal excretion (leading to CO_2 and CH_4 emissions), the lignified cell wall constituents may contribute to (temporary) soil organic matter build-up.

As in ruminants, organic C in pig and poultry faeces originates from undigested cell wall constituents plus non-starch polysaccharides that cannot be digested by the animal's own enzymes and have escaped hindgut fermentation. This may lead to high C concentrations in faeces of pigs and poultry fed with diets rich in non-starch polysaccharides (Hadorn, 1994) which are typical for sows in organic farming (Abel and Breves, 2005) and in many tropical smallholder production systems.

In ruminants, large quantities of P are secreted with the saliva, especially when roughage-rich diets are fed. With increasing roughage concentration of the diet, the partitioning of absorbed P in the gastro-intestines changes: relatively more P is excreted with the saliva and consequently with the faeces and less via urine (Table 18.3). Urinary P excretion is negligible unless energy-rich pelleted diets are fed or if P is oversupplied (Boeser *et al.*, 2002). Faecal P concentration therefore mainly depends on total dry matter intake, feed P concentration, P availability and the animal's P requirements (Underwood and Suttle, 2001).

Phytate is the main storage form of P in cereal grains (see also Section 6.3). In ruminants, rumen microbes secrete phytases and thus mineralize phytate-bound P which can be utilized by the animal (Underwood and Suttle, 2001). Since birds as well as mammals do not secrete phytases, undigested phytate-P is excreted in the faeces unless the non-ruminant animal's diet is supplemented with phytase (Kirchgessner *et al.*, 2008). An increasing concentration of grain-bound phytate-P in the diet will thus increase faecal P excretion and may also lead to decreased Zn availability because Zn is bound to phytate. Depending on feeding practices, fresh matter P concentrations may be 0.36–0.39% in the slurry of pigs (5.5–7.5% dry matter) (de Wit *et al.*, 1997). In poultry manure, fresh matter concentration of P varies from 0.5 to 1.0% (de Wit *et al.*, 1997).

Potassium excretion is mainly determined by the animal's K requirements (Underwood and Suttle, 2001). In ruminants, 85% of total K taken up is excreted in the urine and 15% in faeces (Lhoste *et al.*, 1993). Typical concentrations of K in slurry fresh matter are 0.4–0.7% for pigs, while the poultry manure may contain 0.5–1.1% K in fresh matter.

18.5.2 Livestock-mediated Nutrient Fluxes

Currently >55% of the world's pigs, 60% of laying hens and 72% of broilers and other meat-providing birds are kept in industrial systems (Steinfeld *et al.*, 2006). These typically store excreta in slurry tanks, lagoons and pits near the confinement area; where excreta and bedding materials are mixed, dung heaps may also be found. The length of storage in such collection systems will largely determine nutrient losses and potential negative environmental effects (Ju *et al.*, 2005; Mendoza Huaitalla *et al.*, 2010).

Globally, over 70% of beef, >85% of dairy cattle and >65% of sheep and goats are kept in mixed crop-livestock systems (Steinfeld *et al.*, 2006), where animals are stall-fed (zero-grazing systems) or graze grassland, rangeland or harvested fields on a daily or seasonal basis. In zero-grazing systems, slurry or dung is often stored near the animal stables as described for the industrial systems. In grazing systems, where excreta are voided in the grazed areas, excretion frequencies differ between species, with small ruminants urinating and defecating about twice as often as cattle (Schlecht *et al.*, 2006).

TABLE 18.3 Weight, dry matter excretion and nitrogen (N) and phosphorus (P) concentration in urine and feces (slurry) of different livestock species

Species	Animal weight (kg)	Dry matter (kg year^{-1})	Water content (%)	N (%)	P (%)
Cattle	230	860	87	3.8	0.7
Pig	90	249	88	6.0	2.0
Sheep, goat	45	165	75	4.2	0.6
Chicken	1.8	9	75	5.3	2.0

From Eshenaur (1984).

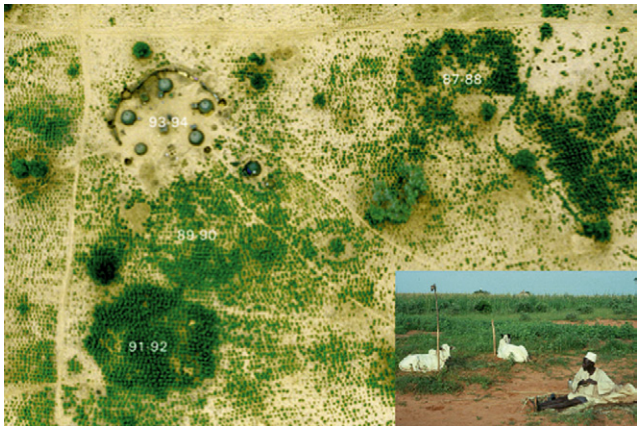

FIGURE 18.2 Aerial photograph showing residual effects of changes in soil productivity due to human activities and tethering of animals (see insert lower right). Numbers indicate the years during which the settlement remained at a particular site. The picture was taken 75 days after sowing from an altitude of about 300 m above ground. Hardpans (indicated by lacking plant growth) within the boundaries of former settlement areas are the result of clay applications to the foundations of the five houses. *From Buerkert* et al. *(1996) with kind permission from Springer Science and Business Media.*

Carbon and N are easily lost from stored livestock excreta through gaseous emissions (Sommer, 2001; Predotova *et al.*, 2010a, b), and on sandy soils through leaching from the upper soil horizons. Losses of K and P are smaller due to rapid absorption by clay particles or other ligands. Leaching of urine K and P bound to (dissolved) organic matter to deeper soil layers may, however, occur if excreta are stored unprotected or are applied in the field in large quantities. For sandy subtropical soils, Brouwer and Powell (1998) and Siegfried *et al.* (2011) reported losses of C-bound P to deeper soil layers at high application rates. Of the N in urine, a large proportion, if not all, can be lost via N leaching and NH_3 emission. In faeces, however, initial gaseous N emissions (as NH_3 or N_2O) are negligible, and more substantial volatilization only occurs after microbial degradation of N compounds, which depends on environmental and storage conditions (FAO, 2006c).

Excretion of faeces and urine show a considerable diurnal variation, but are more frequent at the start of a meal, during drinking and after resting (Schlecht *et al.*, 2006). This implies that in grazing systems, excreted C and nutrients are usually concentrated around resting places and watering points. Apart from these particular events, however, excretions are more or less equally distributed across the day and are therefore proportional to the time spent per land unit.

The grazing itinerary and behaviour (Moreau *et al.*, 2009) are modulated by the location of salt licks and shading trees, grazing and watering regimes, supplement feeding and herding practices (Turner *et al.*, 2005; Fig. 18.2). Allocation of grazing and resting time to individual land units leads to distinct spatial patterns of nutrient off-take and deposit, and eventually to the build-up of nutrient gradients along livestock routes (Turner, 1998; Cech *et al.*, 2008, 2010; Fig. 18.3). To concentrate manure on a field scheduled for cultivation, livestock can also be corralled or tethered overnight. Across five village territories in Western Niger, herds of 25 to 60 animals spend between 15 and 46 nights on one field, with depositing 3.4 to 15.5 t ha^{-1} of faecal dry matter and small ruminants 1.3 to 7.2 t ha^{-1} (Schlecht *et al.*, 2004; Table 18.4).

Since Stoorvogel and Smaling (1994) presented their frequently cited, but at the local level rarely verified, modelling results on large nutrient losses in selected African countries, a number of studies using higher resolution have

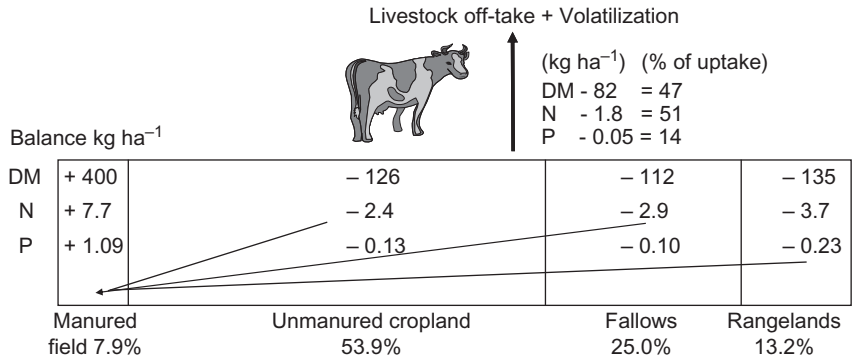

FIGURE 18.3 Livestock-mediated organic matter and nutrient transfers over a period of 1 year within village lands of Kodey, Niger (area of observation 75 km^2). Dry matter (DM), N and P stored in animal tissue and lost by the animal (*top*), or transferred via intake and excretion from grazed rangelands, fallows and unmanured croplands to the manured fields. Values in the bottom line indicate the proportion of the land use types. For the calculation of animal-related transfers the respective relative surface areas indicated in the figure were taken into account. Mean annual stocking rate is 12 tropical livestock units km^{-2}, average total rainfall is about 510 mm. *From Buerkert and Hiernaux (1998) with permission from Wiley VCH Verlag.*

TABLE 18.4 Average N and P availability after crop harvest and aggregated yearly rates of N and P intake and faecal excretion by two village herds of grazing cattle, sheep and goats on different land use types in SW niger (weighted annual averages). N and P availability determined at the end of the rainy season (September for fallows) and after crop harvest (October for fields), respectively

Parameter	Land use type	Banizoumbou	Kodey
		N	
Availability (kg ha^{-1})	Rangeland	9.6	7.4
	Fallow	10.5	11.5
	Cropland	19.7	19.2
	Weighted average per site	17.9	17.1
Intake (kg ha^{-1} yr^{-1})	Rangeland	3.7	5.3
	Fallow	3.2	4.0
	Cropland	2.6	3.9
	Weighted average per site	3.0	3.7
Excretion (kg ha^{-1} yr^{-1})	Rangeland	1.1	1.5
	Fallow	0.8	0.8
	Cropland	0.7	0.8
	Weighted average per site	0.8	0.8
		P	
Availability (kg ha^{-1})	Rangeland	0.70	0.54
	Fallow	0.77	0.85
	Cropland	1.13	1.10
	Weighted average per site	1.26	1.15
Intake (kg ha^{-1} yr^{-1})	Rangeland	0.26	0.37
	Fallow	0.23	0.28
	Cropland	0.11	0.16
	Weighted average per site	0.19	0.21
Excretion (kg ha^{-1} yr^{-1})	Rangeland	0.13	0.17
	Fallow	0.09	0.10
	Cropland	0.08	0.10
	Weighted average per site	0.09	0.11

Modified after Schlecht *et al.* (2004).

been conducted to quantify C and nutrient flows at the field level (Fig. 18.4). These ranged from detailed horizontal balances of crop rotations (Bationo *et al.*, 1998; Buerkert *et al.*, 2005; Table 18.5) to farm balances (Hiernaux *et al.*, 1997; de Jager *et al.*, 1998; Haas *et al.*, 2007) and the measurement of matter fluxes in agro-ecosystems (Hoffmann *et al.*, 2008; Titlyanova, 2007). Modelling of turnover processes and losses at different scales became increasingly important (Fig. 18.5). When current scaling problems and uncertainties about the use of transfer functions for unmeasured flux components in agro-ecosystem models are resolved, this will allow overcoming the limitations imposed by the often questionable standard values for feed digestibility, leaching, volatilization or nutrient deposition that simple tool boxes such as NUTMON or FARMSIM depend on (van den Bosch *et al.*, 1998; Rufino *et al.*, 2007; van Wijk *et al.*, 2007, 2009).

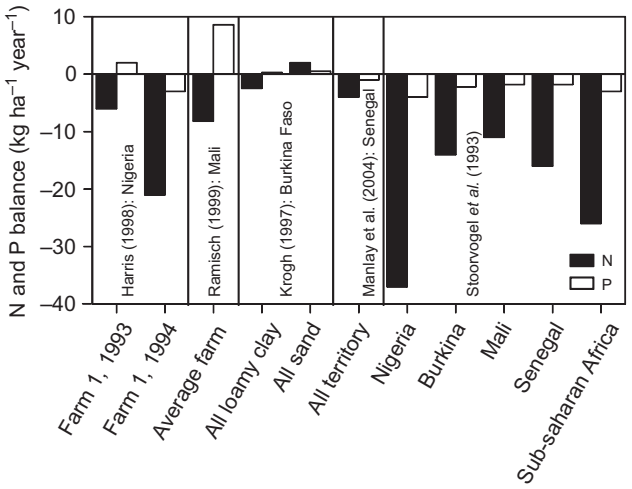

FIGURE 18.4 N and P balances determined at different scales in different locations of sub-Saharan West Africa. *Modified after Schlecht and Hiernaux (2004).*

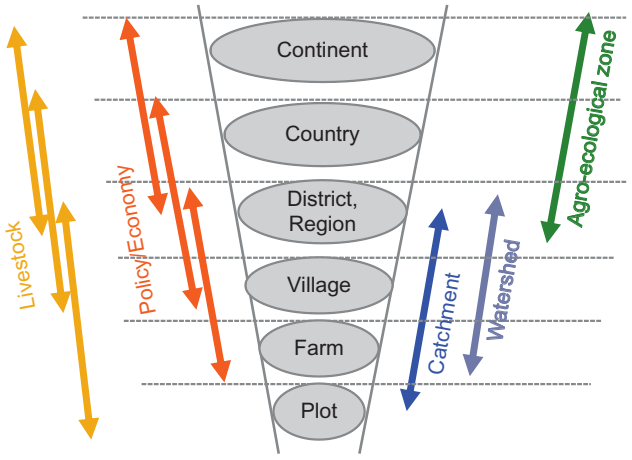

FIGURE 18.5 Diagram indicating the different scales of nutrient cycling research. *Modified after Schlecht and Hiernaux (2004).*

TABLE 18.5 Annual inputs and outputs per ha (kg ha^{-1} year^{-1}) and per area (kg year^{-1}) of nitrogen (N), phosphorus (P) and potassium (K) in cropland and palm groves at balad seet (Oman). Data represent annual averages of a 24-month measurement period from October 2000 to October 2002

Land use		Input and output[a]					
		kg (ha year)$^{-1}$			kg year^{-1}		
	Source/Process	N	P	K	N	P	K
Cropland (4.6 ha)	Synthetic fertilizer	143	24	45	658	120	207
	Animal manure	180	40	267	828	185	1,228
	Irrigation water[b]	10	5	17	46	24	78
	Symbiotic N_2-fixation	63	–	–	290	–	–
	Crop harvest	−265	−33	−245	−1,219	−151	−1,127
	Cumulative partial balance	131	37	84	603	178	386
Palm groves (8.8 ha)	Synthetic fertilizer	59	2	4	519	16	35
	Animal manure + ashes	141	8	289	1,241	70	2,543
	Irrigation water	10	5	17	88	46	150
	Human faeces	170	37	50	1,496	326	440
	Date harvest[c]	−63	−13	−176	−554	−112	−1,549
	Harvested understory fodder	−14	−2	−11	−123	−16	−97
	Cumulative partial balance	303	38	173	2,632	324	1,469
Oasis (13.4 ha)	**Oasis partial balance**	244	37	142	3,235	502	1,855

[a] *Positive values indicate gains and negative ones losses.*
[b] *Total amount of irrigation water (228,587 m^3) was multiplied by nutrient concentrations (mg l^{-1}) 0.57 N, 0.30 P and 1.0 K and adjusted to the respective irrigated surface area.*
[c] *Dates + stems + leaves.*

18.6 MODELLING APPROACHES IN MATTER FLUXES

A number of models to estimate C and N turnover in surface soils have been developed. Most models assume spatial homogeneity and calculate turnover processes for one point up to a certain soil depth such as the Ap horizon. Examples are the Rothamsted Carbon (RothC) model, CENTURY, CANDY, DNDC and ExpertN (Metherell *et al.*, 1993; Coleman and Jenkinson, 1996; Coleman *et al.*, 1997; Franko *et al.*, 1997; Kaharabata *et al.*, 2003; Ludwig *et al.*, 2007; Li, 2009). These models calculate C dynamics and, except RothC, also consider N dynamics and crop growth.

Most modelling studies emphasize the importance of temperature, moisture, soil cover, clay content and amounts and quality of C inputs on soil organic C stocks in arable soils. Several studies, however, criticized that the existing models are too simplistic and that future models need to consider soil structure and microbial kinetics (Arah and Gaunt, 2001). A comparison of the RothC model with a conceptual model derived from a number of experimental fractionation procedures and the use of ^{13}C and ^{14}C (von Lützow *et al.*, 2008) revealed that black C dynamics and the interactions of soil organic carbon with mineral surfaces need to be considered (Ludwig *et al.*, 2008).

In contrast to the large number of models which calculate C and N dynamics in surface soils, there are only very few modelling studies which specifically address rhizosphere processes. For example, Kuzyakov and Domanski (2002) used a chamber experiment with ^{14}C pulse labelling of ryegrass to parameterize a rhizodeposition model. The model includes shoots and roots, the soil and the atmosphere and is able to separate CO_2 evolved by root respiration and rhizo-microbial respiration after a $^{14}CO_2$ pulse labelling of plants. The model described well the $^{14}CO_2$ efflux from the soil and ^{14}C dynamics in shoots, roots and soil, but the prediction of the ^{14}C content in the microbial biomass and in dissolved organic carbon was unsatisfactory. A spatially explicit model of plant root–bacteria interaction in the rhizosphere has been developed by Raynaud *et al.* (2006). The model considers diffusion of solutes in the soil, nutrient uptake by plants, bacterial activity and bacterial predation. The model provided a quantitative explanation of how plants may benefit from liberating low molecular organic matter and the subsequent stimulation of the microbial loop and N mineralization.

Although there have been attempts to better implement spatial non-uniformity in models of soil organic matter turnover (Kuka *et al.*, 2007), more efforts are required to reduce the gap between conceptual models based on experimental findings and quantitative models which often have the advantage that they need only few easily obtainable inputs.

To up-scale matter fluxes from the plot, field, farm or village level to the regional scale, and from the weekly or seasonal scale to one or several years, the restrictions and interdependencies used have to be translated into mathematical relationships. Empirical models are direct reflections of available data, which are set at a defined level of organization and described mathematically (Dijkstra and France, 1995). Mechanistic models instead depict processes occurring within a system and account for organizational hierarchy (France and Thornley, 1984). Numerous models exist to evaluate nutrient balances, soil, crop and livestock management strategies, farm economics and regional land use (e.g., de Jaeger *et al.*, 1998; van den Bosch, 1998; Titonell *et al.*, 2005; Rufino *et al.*, 2007). The choice of model depends on the aim of the work and the level of detail required for the modelling approach, or the available data in time and space. The extrapolation or interpolation of processes between scales must consider the degree of non-uniformity specific to each scale which is generally increasing from the micro- to the macroscale. Flows and budgets calculated at larger scales such as administrative districts or agro-ecological zones may be useful for policy decision at higher levels (Schlecht and Hiernaux, 2004) or for global environmental assessments such as carbon sequestration.

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Index

D

E

F

G

Q

R

S

T

U

V