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S-genotyping of 25 sweet cherry (*Prunus avium* L.) cultivars from the Czech Republic

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ABSTRACT

Sweet cherry (*Prunus avium* L.) is a self-incompatible species. Determination of the S-genotypes of cherry cultivars is crucial for breeding and to select appropriate cultivars for cross-fertilisation and fruit set. In this study, we characterised the S-genotypes of 25 sweet cherry cultivars, some of which had been bred at the Research and Breeding Institute of Pomology (RBIP), Holovousy, Czech Republic, and others were European cultivars in the RBIP collection. S-genotyping was carried out by polymerase chain reaction using consensus primers for the *S-RNase* and *SFB* genes, and capillary electrophoresis. Nine different known S-haplotypes (*S*₁, *S*₂, *S*₃, *S*₄, *S*₅, *S*₆, *S*₉, *S*₁₃, and *S*₁₆) were identified and the cultivars were assigned to 12 incompatibility groups. One local cultivar, 'Ptačka z Plzně', originated from a wild forest seedling and used as a pollinator, was assigned to Group 0 of universal donors. The pedigree of some cultivars was confirmed by their S-genotype. This study represents the first comprehensive S-genotype screening of sweet cherry genetic resources in the Czech Republic and will be useful for the design of crossing programmes and orchard management of these sweet cherry cultivars.

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Sweet cherry (*Prunus avium* L.) is an important fruit species in the Czech Republic (Sedlák & Paprštejn, 2008), which has a long tradition of growing sweet cherry. Over 17,000 metric tonnes (MT) were harvested in the Czech Republic in 2013 (FAOSTAT, 2012, 2013). A sweet cherry rootstock and cultivar breeding programme focused on adaptation to winter and spring frosts, a low incidence of fruit cracking, and resistance to brown rot, leaf spot, and prune dwarf virus, is being carried out at the Research and Breeding Institute of Pomology (RBIP), Holovousy, Czech Republic (Blažková, 2004; Blažková and Hlušíčková, 2001, 2002; Kloutvor & Paprštejn, 1999). Six sweet cherry rootstocks were developed between 1975 and 2003, and breeding for sweet cherry cultivars has also been initiated (Blažková, 2004). 'Karešova' and 'Kordia' were the first two sweet cherry cultivars bred in the Czech Republic. Later, other important sweet cherry cultivars such as 'Aranka', 'Halka', 'Horka', 'Marta', and 'Vilma' were developed at RBIP (Blažková, 2004) and registered in 2001.

Most sweet cherry cultivars are self-incompatible. In this species, gametophytic self-incompatibility

(GSI) is genetically controlled by a multi-allelic S-locus (Crane & Lawrence, 1929) which includes two linked genes, *S-RNase* (Bošković & Tobutt, 1996; Tao et al., 1999) and *SFB* (Yamane, Ikeda, Ushijima, Sassa, & Tao, 2003). In this species, pollen-tube growth is arrested in the style when the S-haplotype of pollen matches either of the two S-haplotypes expressed in the diploid pistil tissue. Cultivars with the same S-genotype are cross-incompatible and are classified in the same incompatibility group (IG). Information on the IG of each sweet cherry cultivar is important for breeding purposes and is essential for orchard management to ensure high fruit set.

Following the initial description of the first six cherry S-alleles (*S*₁–*S*₆; Crane & Lawrence, 1931), another 10 S-alleles have been reported using biochemical and polymerase chain reaction (PCR)-based molecular methods (Bošković, Russell, & Tobutt, 1997; Bošković & Tobutt, 2001). Subsequently, many new S-alleles (*S*₁₇–*S*₂₂ and *S*₂₇–*S*₃₂) have been identified in wild and sweet cherries in Belgium, Spain, and the UK (De Cuyper, Sonneveld, & Tobutt, 2005; Vaughan,

Bošković, Gisbert-Climent, Russell, & Tobutt, 2008; Wünsch & Hormaza, 2004).

Molecular methods, based on PCR, now allow precise S-genotyping and are being used to S-genotype many diverse sweet cherry collections and wild populations (Cachi & Wünsch, 2014; De Cuyper et al., 2005; Gu, Wang, Korban, & Han, 2015; Sonneveld, Tobutt, & Robbins, 2003; Sonneveld, Robbins, & Tobutt, 2006; Szikriszt et al., 2013; Tao et al., 1999; Vaughan et al., 2008; Wiersma, Wu, Zhou, Hampson, & Kappel, 2001; Wünsch & Hormaza, 2004). Schuster (2012) compiled information from different studies that included 734 cultivars. In that work, Schuster (2012) described 18 different S-haplotypes, 47 incompatibility groups, and 15 cultivars with unique S-genotypes. In addition, 44 cultivars were self-compatible (Schuster, 2012).

Although a large number of sweet cherry cultivars and wild accessions from Europe and North America have been S-genotyped (Schuster, 2012), only a limited number of cultivars from the Czech Republic have been characterised. Schuster, Flachowsky and Köhler (2007) S-genotyped 149 sweet cherry cultivars from Germany and other European countries, including three cultivars from the Czech Republic ('Ladeho Pozdni', 'Těchlovan', and 'Vanda'). Other cultivars that originated in the Czech Republic (e.g. 'Early Korvik', 'Helga', 'Jacinta', 'Karešova', 'Kasandra', 'Kaštanka', 'Early Rivers', 'Kordia', 'Ladeho Pozdni', 'Tamara', 'Těchlovan', and 'Vanda') were S-genotyped in later studies (Schuster et al., 2007; Schuster, 2012; Schmidt, Wolfram, & Bošković, 1999; Tobutt, Sonneveld, Bekefi, & Bošković, 2004).

The main objective of this study was to characterise the S-genotypes of 25 sweet cherry cultivars from RBIP in the Czech Republic, in order to verify IGs for planning orchards with these cultivars by selecting the correct pollinator.

Materials and methods

Twenty-five sweet cherry cultivars from the RBIP in the Czech Republic were studied (Table 1). Fifteen corresponded to cultivars from the Czech Republic, five were from the Ukraine, and the rest were from the USA and Europe, or had unknown origins and/or pedigrees.

Six cultivars from CITA de Aragon (Zaragoza, Spain) with known S-genotypes ['Blanca de Provenza' (S_1S_5), 'Brooks' (S_1S_9), 'Reverchon' (S_3S_{13}), 'Schneiders' (S_3S_{12}), 'Son Miro' (S_6S_{16}), and 'Vic' (S_2S_4); Cachi & Wünsch, 2014] were used as standards for S-allele size fragment comparisons.

Table 1. Sweet cherry cultivars analysed by their S-genotypes and the incompatibility groups (IG) identified in this work.

Origin	Cultivar name	Pedigree	S-genotype	IG*
Czech Republic	'Adélka'		'Knauffs Schwarze' × 'Granát'	S_1S_6
	XX			
	'Amid'	'Kordia' × 'Vic'	S_3S_4	III
	'Aranka'	'Kaštanka' ('Early Rivers') × 'Moreau'	S_1S_3	II
	'Chlumecká pozdní' ('Chlumecka cerna')	Random seedling	S_3S_5	VII
	'Fabiola'	'Van' × 'Kordia'	S_1S_6	XX
	'Granát'	Random seedling	S_3S_6	VI
	'Halka'	'Van' × 'Stella'	S_1S_4	IX
	'Irena'	'Kordia' × 'Merton Reward'	S_4S_6	XVII
	'Justyna'	'Kordia' × 'Starking Hardy Giant'	S_1S_3	II
	'Livia'	'Těchlovická I' ('Ziklova') o.p.**	S_2S_6	XXV
	'Marta'	'Kordia' × 'Kaštanka' ('Early Rivers')	S_1S_3	II
	'Ptačka z Plzně'	Wild random seedling	S_5S_{16}	0
	'Sandra'	'Kordia' × Seedling 13	S_3S_4	III
	'Tim'	'Krupnoplodnaja' × 'Van'	S_4S_5	V
'Vilma'	'Kordia' × 'Vic'	S_2S_6	XXV	
Ukraine	'Amazonka'	Unknown	S_4S_5	V
	'Annuška'	Unknown	S_3S_4	III
	'Donetskiiugolek'	Unknown	S_3S_5	VII
	'Donnanka'	Unknown	S_3S_6	VI
	'Sestrenka'	Unknown	S_3S_5	VII
Belgium	'Verdel v 1360'	Unknown	S_5S_{13}	XXVI
Germany	'Moser'	Random seedling	S_6S_{13}	XII
USA	'New York 200'	Unknown	S_4S_6	XVII
	'Proba' ('SIMCOE Proba')	'Stella' × 'Hollander' × 'Starking Hardy Giant'	S_1S_3	II
Unknown	'Belise Bodel'	Unknown	S_1S_9	XVIII

*Incompatibility group, according to Schuster (2012).

**By open-pollination.

Genomic DNA was extracted from 50 mg of young leaf tissue of each plant following the protocol described by Hormaza (2002). The extracted genomic DNA was diluted to 10 ng μl^{-1} . S-locus analysis was performed by PCR using the primer pairs FBOX50A/FBOXintronR for SFB genotyping (Vaughan, Russell, Sargent, & Tobutt, 2006), and PaConsI-F/PaConsIR2 for S-RNase genotyping (Sonneveld et al., 2003, 2006). The forward primers were fluorescently labelled with NEDTM (Life Technologies, Carlsbad, CA, USA) for SFB and with 6-FAMTM (Life Technologies) for S-RNase. All PCR reactions were carried out according to Cachi and Wünsch (2014). PCR fragments were detected using an ABI PRISM 3100 genetic analyser (Applied Biosystems, Foster City, CA, USA) and Peak Scanner version 1.0 software (Applied Biosystems). A standard 500-GeneScan LIZ (Applied Biosystems) was

used for sizing the PCR fragments. All PCR reactions and analyses were repeated at least twice.

Results and discussion

S-haplotypes

In this work, the S-genotypes of all the 25 sweet cherry cultivars were analysed (Table 1). Nine known S-haplotypes (S_1 , S_2 , S_3 , S_4 , S_5 , S_6 , S_9 , S_{13} , and S_{16}) were identified.

Haplotype S_{13} was identified in ‘Moser’ and ‘Verdel’ (Table 1). As reported by Marchese, Bošković, Caruso and Tobutt (2010), this S_{13} -RNase gene had a micro-satellite, and variations in allele size have been reported (Sonneveld et al., 2006; Cachi & Wünsch, 2014). In this work, S_{13} -RNase was detected as a low-intensity micro-satellite and two variants were observed. The micro-satellites were 454 basepairs (bp) in ‘Verdel’ and 456 bp in ‘Moser’. At least three variants of S_{13} were detected by Sonneveld et al. (2006) in the cultivars ‘Sir Don’ (451 bp), ‘Bigarreau Reverchon’ (434 bp), and ‘Goodnestone Black’ (452 bp). In another study, Cachi and Wünsch (2014) reported another two variants of 437 bp in ‘Reverchon’ (S_3S_{13}) and 454 bp in four local cultivars from Spain [‘Cor de Colom’ (S_3S_{13}), ‘Del Cubeto’ (S_3S_{13}), ‘Garrafal de Lérida’ (S_9S_{13}), and ‘Madrileña’ (S_3S_{13})].

The size of the S_{13} microsatellite detected in ‘Verdel’ was the same as in the local Spanish cultivars and may correspond to the same allele. However, the origin of these varieties was different, with ‘Verdel’ coming from Belgium, and it was not possible to establish a genetic relationship that could confirm this hypothesis. In addition, different analytical equipment was used in these studies. As reported earlier (Guerra, López-Corrales, & Wünsch, 2012; Cachi & Wünsch, 2014), small size differences were found when using different equipment and conditions for capillary electrophoresis. Thus, sequencing information would be required to clarify the S_{13} microsatellite alleles. Haplotype S_{13} is not common in sweet cherry cultivars, and was found in <2% of the 734 cultivars compiled by Schuster (2012).

The S_{16} -haplotype was identified only in one cultivar, ‘Ptačka z Plzně’. This cultivar came from a wild forest seedling. The use of cultivars from the same IG could lead to a low pollination efficiency in the orchard. This was a common problem in the Czech Republic, where almost all old and extensive plantations were supplemented by relatively unproductive random seedlings of wild cherries as pollinators. This was the case for ‘Ptačka z Plzně’ (which means

random *P. avium* seedling from Pilsen) that had a high pollination rate against all other cultivars. The S-genotype of ‘Ptačka z Plzně’ was S_5S_{16} , both of alleles of which are low-frequency S-haplotypes in cultivated material, but are found in wild *P. avium* from the Czech Republic. S_{16} had also been previously reported in wild cherry trees from Belgium (De Cuyper et al., 2005), France (Mariette et al., 2010; Stoeckel, Castric, Mariette, & Vekemans, 2008), the UK (Vaughan et al., 2008), and in cultivated material from northern (Sonneveld et al., 2003; Tobutt et al., 2004; Stanys, Stanytė, Stanienė, & Vinskienė, 2008), and southern Europe (Cachi & Wünsch, 2014; Marchese et al., 2007). Thus, it appeared that S_{16} was widely distributed across Europe.

S-genotypes and incompatibility groups (IG)

All accessions had two S-alleles for both genes tested, and therefore their S-genotypes could be determined unequivocally (Table 1). According to the S-genotypes detected, all 25 cultivars were assigned to their corresponding IG (Table 1). Thirteen different S-genotypes were identified among the 25 cultivars analysed. Twelve of these belonged to previously described sweet cherry IGs (Schuster, 2012) and one, ‘Ptačka z Plzně’ (S_5S_{16}), could be a universal donor as no other cultivar has been described with the same S-genotype (Schuster, 2012).

Some of the cultivars studied in this work had been analysed previously to check for the presence of the S-alleles S_3 , S_4 and S_9 using allele-specific primers (Sharma, Sedlák, Zeka, Vejl, & Soukop, 2014). The presence of S_3 was confirmed in ‘Amid’, ‘Granát’, and ‘Probla’ and the presence of S_4 was confirmed in ‘Amid’ and ‘Halka’.

The pedigree of all varieties analysed, for which pedigree information was available, was confirmed by their S-genotypes, and only one incorrect pedigree was observed, in ‘Tim’. Therefore, in the 10 breeding cultivars from the Czech Republic (‘Adélka’, ‘Amid’, ‘Aranka’, ‘Fabiola’, ‘Halka’, ‘Irena’, ‘Justyna’, ‘Marta’, ‘Sandra’, and ‘Vilma’) their pedigree was confirmed by their S-genotypes, and all had inherited one S-haplotype from each parent. Most interestingly, ‘Halka’ was probably self-compatible as it had inherited the allele S_4 from ‘Stella’, which was self-compatible due to a pollen function mutation (Sonneveld, Tobutt, Vaughan, & Robbins, 2005). Conversely, ‘Tim’ (S_4S_5) could not be a descendant from ‘Krupnoplodnaja’ and ‘Van’, as these were S_5S_9 and S_1S_3 , respectively. The inheritance of S_5 confirmed that ‘Tim’ may have descended from

‘Krupnoplodnaja’, but as it had not inherited any S-haplotype from ‘Van’, the pollen parent could not be ‘Van’.

New cultivars from RBIP

Some of the cultivars analysed (‘Sylvana’ registered in 2001; ‘Adélka’, ‘Justyna’ and ‘Sandra’ in 2006; ‘Amid’, ‘Livia’, and ‘Tim’ in 2008; and ‘Irena’ in 2014) were new cultivars bred at RBIP and no information on their S-genotypes was yet available in the literature. Therefore, we have provided the essential genetic compatibility information for these cultivars (Table 1).

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References

- Blažková, J. (2004). Resistance to abiotic and biotic stresses in sweet cherry rootstocks and cultivars from the Czech Republic. *Journal of Fruit and Ornamental Plant Research* (Special Edition), 12, 303–311.
- Blažková, J., & Hlušíčková, I. (2001). Hodnocení růstu stromů třešní na nových slabě rostoucích podnožích v Holovousích. *Vědecké Práce Ovocnářské*, 17, 109–113.
- Blažková, J., & Hlušíčková, I. (2002). Testing of wood hardiness to winter freezes in selections from progenies of *Ceropadus* × *Prunus avium* L. crosses. *HortScience*, 29, 133–142.
- Bošković, R., & Tobutt, K. R. (1996). Correlation of stylar ribonuclease zymograms with incompatibility alleles in sweet cherry. *Euphytica*, 90, 245–250.
- Bošković, R., & Tobutt, K. R. (2001). Genotyping cherry cultivars assigned to incompatibility groups, by analyzing stylar ribonucleases. *Theoretical and Applied Genetics*, 103, 475–485.
- Bošković, R., Russell, K., & Tobutt, K. R. (1997). Inheritance of stylar ribonucleases in cherry progenies, and reassignment of incompatibility alleles to two incompatibility groups. *Euphytica*, 95, 221–228.
- Cachi, A. M., & Wünsch, A. (2014). S-genotyping of sweet cherry varieties from Spain and S-locus diversity in Europe. *Euphytica*, 197, 229–236.
- Crane, M. B., & Lawrence, W. J. C. (1929). Genetical and cytological aspects of incompatibility and sterility in cultivated fruits. *Journal of Pomology and Horticultural Science*, 7, 276–301.
- Crane, M. B., & Lawrence, W. J. C. (1931). Sterility and incompatibility in diploid and polyploid fruits. *Journal of Genetics*, 24, 97–107.
- De Cuyper, B., Sonneveld, T., & Tobutt, K. R. (2005). Determining self-incompatibility genotypes in Belgian wild cherries. *Molecular Ecology*, 14, 945–955.
- FAOSTAT (2012). <http://faostat3.fao.org/home/>
- FAOSTAT (2013). <http://faostat3.fao.org/home/>
- Gu, C., Wang, L., Korban, S. S., & Han, Y. (2015). Identification and characterization of S-RNase genes and S-genotypes in *Prunus* and *Malus* species. *Canadian Journal of Plant Science*, 95, 1–13.
- Guerra, M. E., López-Corrales, M., & Wünsch, A. (2012). Improved S-genotyping and new incompatibility groups in Japanese plum. *Euphytica*, 186, 445–452.
- Hormaza, J. I. (2002). Molecular characterization and similarity relationships among apricot (*Prunus armeniaca* L.) genotypes using simple sequence repeats. *Theoretical and Applied Genetics*, 104, 321–328.
- Kloutvor, J., & Paprštejn, F. (1999). Zakrslé podnože pro třešně z České republiky. *Vědecké Práce Ovocnářské*, 16, 47–51.
- Marchese, A., Tobutt, K. R., Raimondo, A., Motisi, A., Bošković, R. I., Clarke, J., et al. (2007). Morphological characteristics, microsatellite fingerprinting and determination of incompatibility genotypes of Sicilian sweet cherry cultivars. *Journal of Horticultural Science & Biotechnology*, 82, 41–48.
- Marchese, A., Bošković, R. I., Caruso, T., & Tobutt, K. R. (2010). Intra-allelic variation in introns of the *S₁₃-RNase* allele distinguishes sweet, wild and sour cherries. *Tree Genetics & Genomes*, 6, 963–972.
- Mariette, S., Tavaud, M., Arunyawat, U., Capdeville, G., Millan, M., & Salin, F. (2010). Population structure and genetic bottleneck in sweet cherry estimated with SSRs and the gametophytic self-incompatibility locus. *BMC Genetics*, 11, 77.
- Sharma, K., Sedlák, P., Zeka, D., Vejl, P., & Soukup, J. (2014). The allele-specific PCR detection of sweet cherry self-incompatibility alleles *S₃*, *S₄* and *S₉* using consensus and allele-specific primers in the Czech Republic. *Horticultural Science* (Prague), 41, 153–159.
- Schmidt, H., Wolfram, B., & Bošković, R. (1999). Befruchtungsverhältnisse bei Suskirschen. *Erwerbsobstbau*, 41, 41–45.
- Schuster, M., Flachowsky, H., & Köhler, D. (2007). Determination of self-incompatibility genotypes in sweet cherry (*Prunus avium* L.) accessions and cultivars of the German Fruit Gene Bank and from private collections. *Plant Breeding*, 126, 533–540.
- Schuster, M. (2012). Incompatible (S-) genotypes of sweet cherry cultivars (*Prunus avium* L.). *Scientia Horticulturae*, 148, 59–73.
- Sedlák, J., & Paprštejn, F. (2008). *In vitro* shoot proliferation of sweet cherry cultivars Karešova and Rivan. *Horticultural Science* (Prague), 35, 95–98.
- Sonneveld, T., Tobutt, K. R., & Robbins, T. P. (2003). Allele-specific PCR detection of sweet cherry self-incompatibility

- (S) alleles S_1 to S_{16} using consensus and allele-specific primers. *Theoretical and Applied Genetics*, 107, 1059–1070.
- Sonneveld, T., Tobutt, K. R., Vaughan, S. P., & Robbins, T. P. (2005). Loss of pollen-S function in two self-compatible selections of *Prunus avium* is associated with deletion/mutation of an S haplotype-specific *F-Box* gene. *The Plant Cell*, 17, 37–51.
- Sonneveld, T., Robbins, T., & Tobutt, K. R. (2006). Improved discrimination of self-incompatibility S-RNase alleles in cherry and high-throughput genotyping by automated sizing of first intron polymerase chain reaction products. *Plant Breeding*, 125, 305–307.
- Stanys, V., Stanytė, R., Stanienė, G., & Vinskienė, J. (2008). S-allele identification by PCR analysis in Lithuanian sweet cherries. *Biologia*, 54, 22–26.
- Stoeckel, S., Castric, V., Mariette, S., & Vekemans, X. (2008). Unequal allelic frequencies at the self-incompatibility locus within local populations of *Prunus avium* L. – an effect of population structure? *Journal of Evolutionary Biology*, 21, 888–899.
- Szikriszt, B., Dogan, A., Ercisli, S., Akcay, M. E., Hegedus, A., & Halasz, J. (2013). Molecular typing of the self-incompatibility locus of Turkish sweet cherry genotypes reflects phylogenetic relationships among cherries and other *Prunus* species. *Tree Genetics & Genomes*, 9, 155–165.
- Tao, R., Yamane, H., Sugiura, A., Murayama, H., Sassa, H., & Mori, H. (1999). Molecular typing of S-alleles through identification, characterization and cDNA cloning for S-RNases in sweet cherry. *Journal of the American Society for Horticultural Science*, 124, 224–233.
- Tobutt, K. R., Sonneveld, T., Bekefi, Z., & Bošković, R. (2004). Cherry (in)compatibility genotypes – An updated cultivar table. *Acta Horticulturae*, 663, 667–672.
- Vaughan, S. P., Russell, K., Sargent, D. J., & Tobutt, K. R. (2006). Isolation of S locus *F-box* alleles in *Prunus avium* and their application in a novel method to determine self-incompatibility genotype. *Theoretical and Applied Genetics*, 112, 856–866.
- Vaughan, S. P., Bošković, R. I., Gisbert-Climent, A., Russell, K., & Tobutt, K. R. (2008). Characterization of novel S-alleles from cherry (*Prunus avium* L.). *Tree Genetics & Genomes*, 4, 531–541.
- Wiersma, P. A., Wu, Z., Zhou, L., Hampson, C., & Kappel, F. (2001). Identification of self-incompatibility alleles in sweet cherry (*Prunus avium* L.) and clarification of incompatibility groups by PCR and sequencing analysis. *Theoretical and Applied Genetics*, 102, 700–708.
- Wünsch, A., & Hormaza, J. I. (2004). Molecular evaluation of genetic diversity and S-allele composition of local Spanish sweet cherry (*Prunus avium* L.) cultivars. *Genetic Resources and Crop Evolution*, 51, 635–641.
- Yamane, H., Ikeda, K., Ushijima, K., Sassa, H., & Tao, R. (2003). A pollen-expressed gene for a novel protein with an F-box motif that is very tightly linked to a gene for S-RNase in two species of cherry. *Prunus cerasus* and *P. avium*. *Plant and Cell Physiology*, 44, 764–769.