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Molecular S-genotyping of sweet cherry (*Prunus avium* L.) genetic resources

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Abstract: Sweet cherries are self-incompatible, which is determined by a gametophytic self-incompatibility system (GSI). The self-incompatibility is controlled by a multi-allelic S-locus. Knowledge about the *S*-allele constitution of the cultivars is essential for fruit growers and breeders. Recently, molecular PCR-based methods have been developed to distinguish all S-alleles in sweet cherries. In our work, we analysed *S*-locus genotypes by 13 universal and allele-specific PCR primer combinations within 117 registered, old and local sweet cherry cultivars from the Czech genetic resources of the Research and Breeding Institute of Pomology in Holovousy, the Czech Republic. We confirmed the previous S-genotyping for 66 accessions except for Drogans Gelbe, Hedelfinger, Erika, Meckenheimer Frühe, Badeborner, Bing, Alfa, Gamma, Huldra, Rivan, Valerij Tschkalov, Viola and Winkler's Frühe. It could be due to either mislabelling or mistakes in the previous analyses. Newly, *S*-genotyping was determined for 51 accessions in which we found 4 new *S*-loci combinations. We detected the *S*-locus combinations in 19 incompatibility groups. The most frequent incompatibility groups were III (S_3S_4), II (S_1S_3), IV (S_2S_3), and VI (S_3S_6) with 22, 20, 12 and 12 genotypes, respectively.

Keywords: self-incompatibility; *S-RNase* alleles; incompatibility groups; cultivars

The sweet cherry (*Prunus avium* L.) is an important fruit species in the temperate zone. Sweet cherries are generally self-incompatible, which is determined by a gametophytic self-incompatibility system (GSI). The incompatible phenotype is determined by a ribonuclease called *S-RNase* (Tao et al. 1999) and the specificity of the pollen is determined by the product of the F-box gene *SFB* (Yamane et al. 2003). The *S-RNase* locus is multiallelic and the cloning and sequence characterisation of it has allowed the development of PCR and RFLP based methods (Dirlewanger et al. 2009). These *S-RNase* sequences have two introns varying in length for each different allele and, consequently, the PCR amplification with those primers enables the differentiation of the different *S*-alleles according to the size of the amplified fragments. Subsequently, other sweet cherry *S-RNases* were cloned and other PCR methods were developed using conserved sequence primers (Wiersma et al. 2001) and allele specific primers (Sonneveld et al. 2003). Recently, Schuster (2012) compiled the *S*-genotype of 734 sweet cherry accessions and reported 18 different *S*-alleles (S_1 to S_7, S_9, S_{10}, S_{12} to S_{14}, S_{16}, S_{17}, S_{19}, S_{21}, S_{22}, S_{24}), 47 incompatibility groups, a group '0' of unique *S*-genotypes (15 cultivars or universal pollen donors) and a group of 44 self-compatible cultivars. Additionally, another

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six *S*-alleles have been described in the wild cherry only, these are S_{27} to S_{32} (De Cuyper et al. 2005; Vaughan et al. 2008). The knowledge about the S-allele constitution of the cultivars is very important for fruit growers and breeders regarding the planning of new orchards, for artificial hybridisations and as a genetic marker (Schuster 2012).

The aim of this study was to identify the *S*-allele combinations within the set of the Czech and world registered, old and local cherry cultivars by PCR molecular markers.

MATERIAL AND METHODS

In our experiment, we used 117 cherry accessions of the registered, old and local cultivars in total (Table 1) from the cherry genetic resources of the Research and Breeding Institute of Pomology in Holovousy in the Czech Republic. 1 g of the collected young green leaves were powdered with liquid nitrogen and used for DNA isolation by the SDS isolation method according to Goulão et al. (2001). The isolated DNAs were afterwards cleaned by a ChargeSwitch® gDNA Plant Kit (Invitrogen, ThermoFisher Scientific, Waltham, USA). For the PCR identification of the *S*-locus sequence, we used universal primer pairs for Intron 1 and 2, and the allele-specific primer pairs S_2, S_3, S_4, S_7, S_9, S_{10}, S_{12}, S_{13}, S_{14} and S_{16} (Sonneveld et al. 2003; Iezzoni 2008; Sharma et al. 2014). In a typical PCR reaction (*Taq* PCR master mix kit, Qiagen, Hilden, FRG), we used the following amplification conditions: 2 min at 94°C, 35 cycles/ (30 s at 94°C; 60 s at 50–60°C, 90 s at 72°C); 10 min at 72°C, in a TGradient thermocycler (Biometra, Goettingen, FRG). The annealing temperatures were used according to the references. The amplification products were resolved via electrophoresis in horizontal 1–2 % agarose gels and visualised by ethidium bromide staining according to Patzak (2001). The products were scored for the presence or absence in each accession, based on the size measured with the pGEM DNA marker and a 100 bp ladder (Promega, Madison, USA).

RESULTS AND DISCUSSION

The introduction of molecular methods in sweet cherry S-allele typing has allowed for the rapid confirmation of the S-alleles and incompatibility groups of different cultivars, the identification of the S-genotype of new varieties and the identification of putative new S alleles by their correlation with the new PCR products (Dirlewanger et al. 2009). Currently, the specific allele primer pairs for the S2, S3, S4, S7, S9, S10, S12, S13, S14 and S16 alleles have been useful (Sonneveld et al. 2003; Iezzoni 2008; Sharma et al. 2014) for the identification of the S locus sequence. All of them we used for the identification of the S-genotypes within 117 cherry accessions of the registered, old and local cultivars (Table 1). Therefore, there are more than ten other S-alleles, so it was also necessary to use universal primer pairs for Intron 1 and 2 of the S-RNase gene. The sizes of the genomic amplification products of both introns corresponded with different S-alleles (Sonneveld et al. 2003; Schuster et al. 2007) for their useful identification. Overall, the results are summarised in Table 1.In total, we detected 13 different S-alleles in 24 S-locus combinations for 19 individual incompatibility groups. The most frequent S-alleles were S_3 (74 genotypes), S_1 (43 genotypes) and S_4 (39 genotypes), followed by S_2 (27 genotypes) and S_6 (24 genotypes) (Fig. 1a). The rare alleles were S_7, S_{13}, S_{14}, S_{16} and S_{19} (Table1, Fig. 1a). These results were in accordance with published data for the European germplasm (De Cuyper et al. 2005; Cachi, Wünsch 2014; Lisek et al. 2015). Among 734 sweet cherry cultivars, the frequencies were in the order S_3 (383 cultivars), S_4 (227 cultivars), S_1 (221 cultivars), S_6 (190 cultivars), S_2 (112 cultivars), S_9 (98 cultivars) and S_5 (81 cultivars) (Schuster 2012). The most frequent incompatibility groups were III (S_3S_4), II (S_1S_3), IV (S_2S_3) and VI (S_3S_6) with 22, 20, 12 and 12 genotypes, respectively (Fig. 1b).

We confirmed the previous S-genotyping for 66 accessions except for Drogans Gelbe, Hedelfinger, Erika, Meckenheimer Frühe, Badeborner, Bing, Alfa, Gamma, Huldra, Rivan, Valerij Tschkalov, Viola and Winkler's Frühe. This could be due to the mislabelling or mistakes in our previous analyses. The published results of the S-genotypes have not been based on the PCR markers only, but also on previously reported data and breeders' knowledge (Shuster 2012). Newly, S-genotyping was determined for 51 accessions when we found 4 new S-loci combinations S_4S_x (Těchlovická), $S_{19}S_x$ (Drogans Gelbe), S_2S_{13} (Szwecija) and S_1S_{16} (Buketova). It is also known that there are difficult to detect S-alleles

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Table 1. Identification of S-alleles and incompatibility groups (IG) within 117 registered, old and local sweet cherry cultivars

Cultivar	Origin[1]	Detected *S*-alleles	Published *S*-alleles	IG[2]
Raná Černá Edra	BGR	S_1S_2		I
Raná Laskovská	BGR	S_1S_5		XIV
Sam	CAN	S_2S_4	S_2S_4	XIII
Star	CAN	S_3S_4	S_3S_4	III
Stella	CAN	S_3S_4	S_3S_4	III
Sue	CAN	S_2S_3	S_2S_3	IV
Summit	CAN	S_1S_2	S_1S_2	I
Sunburst	CAN	S_3S_4	S_3S_4	III
Sweetheart	CAN	S_3S_4	S_3S_4	III
Van	CAN	S_1S_3	S_1S_3	II
Vega	CAN	S_2S_3	S_2S_3	IV
Velvet	CAN	S_2S_3	S_2S_3	IV
Venus	CAN	S_1S_3	S_1S_3	II
Vic	CAN	S_2S_4	S_2S_4	XIII
Vineland	CAN	S_3S_6		VI
Viva	CAN	S_2S_3	S_2S_3	IV
Vogue	CAN	S_2S_3	S_2S_3	IV
Buketova	CZE	S_1S_{16}		0
Černá špička	CZE	S_1S_3		II
Černá z Hořan	CZE	S_4S_{12}		XXVII
Děkanka	CZE	S_3S_6		VI
H 21/40 Černá	CZE	S_1S_6		XX
Holovouská chrupka	CZE	S_1S_3		II
Chlumecká Černá	CZE	S_1S_3		II
Karešova	CZE	S_1S_3	S_1S_3	II
Kordia	CZE	S_3S_6	S_3S_6	VI
Ladeho pozdní	CZE	S_1S_3	S_1S_3	II
Libějovická raná	CZE	S_3S_4		III
Moravská rychlice	CZE	S_2S_3		IV
Mramorovaná chrupka	CZE	S_1S_5		XIV
Pivka	CZE	S_1S_3		II
Pivovka	CZE	S_3S_7		XLIV
Plavečský granát	CZE	S_3S_6	S_3S_6	VI
Samofertilní	CZE	S_3S_4		III
Semenáč č.13	CZE	S_4S_{12}		XXVII
Srdcovka přeúrodná	CZE	S_3S_6		VI
Šakvická	CZE	S_1S_6		XX
Těchlovan	CZE	S_3S_6	S_3S_6	VI
Těchlovická	CZE	S_4S_x		0
Vanda	CZE	S_1S_6	S_1S_6	XX

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Table 1. To be continued

Cultivar	Origin[1]	Detected *S*-alleles	Published *S*-alleles	IG[2]
Vlachova	CZE	S_1S_2		I
Vosenka	CZE	S_2S_4		XIII
Žalanka	CZE	S_1S_4		IX
Alma	DEU	S_1S_5	S_1S_5	XIV
Badeborner	DEU	S_3S_4	S_3S_5	III
Büttners späte Knorpelkirsche	DEU	S_3S_4	S_3S_4	III
Dönissens Gelbe	DEU	S_3S_6	S_3S_6	VI
Drogans Gelbe	DEU	$S_{19}S_x$	S_1S_5	0
Emperor Francis	DEU	S_3S_4	S_3S_4	III
Erika	DEU	S_4S_6	S_1S_3	XVI
Frühe von Boppard	DEU	S_3S_6		VI
Germersdorfer	DEU	S_3S_{12}	S_3S_{12}	XXII
Grolls Schwarze Knorpelkirsche	DEU	S_3S_4		III
Hedelfinger	DEU	S_1S_3	S_3S_5	II
Hildesheim	DEU	S_1S_3		II
Kassins Frühe	DEU	S_2S_3	S_2S_3	IV
Knauffs Schwarze	DEU	S_2S_6	S_2S_6	XXV
Leopoldskirsche	DEU	S_2S_4		XIII
Meckenheimer Frühe	DEU	S_3S_4	S_1S_4	III
Müncheberger	DEU	S_3S_4	S_3S_4	III
Napoleon	DEU	S_3S_4	S_3S_4	III
Německá rychlice	DEU	S_2S_3		IV
Oktavia	DEU	S_1S_3	S_1S_3	II
Querfurter Königskirsche	DEU	S_3S_4	S_3S_4	III
Rebekka	DEU	S_1S_3	S_1S_3	II
Regina	DEU	S_1S_3	S_1S_3	II
Simonis	DEU	S_3S_4		III
Spitze Braune	DEU	S_3S_{14}		XXXI
Thurn Taxis	DEU	S_3S_{12}		XXII
Troprichters Schwarze Knorpelkirsche	DEU	S_1S_3	S_1S_3	II
Valeska	DEU	S_1S_3	S_1S_3	II
Velká Černá Chrupka	DEU	S_1S_6		XX
Viola	DEU	S_4S_{12}	S_3S_6	XXVII
Winkler's Frühe	DEU	S_2S_3	S_1S_3, S_3S_9	IV
Zeisberger	DEU	S_2S_4		XIII
Bigarreau Charmes	FRA	S_3S_9		XVI
Burlat	FRA	S_3S_9	S_3S_9	XVI
Ramon Oliva	FRA	S_6S_9		X
Early Rivers	GBR	S_1S_2	S_1S_2	I
Merchant	GBR	S_4S_9	S_4S_9	XXI
Merla	GBR	S_1S_6	S_1S_6	XX

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Table 1. To be continued

Cultivar	Origin[1]	Detected *S*-alleles	Published *S*-alleles	IG[2]
Mermat	GBR	S_3S_6		VI
Merton Favourite	GBR	S_3S_6		VI
Merton Glory	GBR	S_4S_6	S_4S_6	XVII
Merton Premier	GBR	S_2S_3	S_2S_3	IV
Baltavarská	HUN	S_3S_4		III
Alfa	CHE	S_3S_4	S_1S_6	III
Basler Adlerkirche	CHE	S_1S_5	S_1S_5	XIV
Basler Langstieler	CHE	S_1S_2	S_1S_2	I
Beta	CHE	S_1S_5	S_1S_5	XIV
Delta	CHE	S_5S_6	S_5S_6	XV
Gamma	CHE	S_1S_6	S_3S_5	XX
Schöne von Marien	CHE	S_1S_2		I
Zweitfrühe	CHE	S_5S_6	S_5S_6	XV
Durone Nero 1	ITA	S_1S_3		II
Nero 1	ITA	S_1S_3		II
Nero 2	ITA	S_3S_4		III
Kišiněvskaja	MDA	S_1S_3		II
Skierniewice 1	POL	S_2S_3		IV
Skierniewice 3	POL	S_2S_3		IV
Skorospielka	RUS	S_2S_3		IV
Asenova raná	SRB	S_3S_9		XVI
Ladzanská 1	SVK	S_1S_{13}		0
Medňanská	SVK	S_4S_6		XVII
Huldra	SWE	S_3S_6	S_1S_3	VI
Rivan	SWE	S_2S_4	S_1S_2	XIII
Szwecija	SWE	S_2S_{13}		0
Valerij Tschkalov	UKR	S_3S_9	S_1S_9	XVI
Bing	USA	S_1S_3	S_3S_4	II
Gil Peck	USA	S_1S_3	S_1S_3	II
Hudson	USA	S_1S_4	S_1S_4	IX
Kristin	USA	S_3S_4	S_3S_4	III
Lambert	USA	S_3S_4	S_3S_4	III
Lapins	USA	S_3S_4	S_3S_4	III
Mona Cherry	USA	S_3S_4		III
Seneca	USA	S_1S_5	S_1S_5	XIV
Starking Hardy Giant	USA	S_1S_2	S_1S_2	I

[1]three-letter country codes, according to ISO3166-1 alpha-3; [2]incompatibility group, according to Schuster (2012); IG 0 groups together different unique *S*-genotypes

by PCR markers in some S-genotype combinations (Hanada et al. 2014). Although the S-genotype must be hetero-allelic we did not detect a second allele of the S-locus in Těchlovická and Drogans Gelbe. Another disadvantage of the PCR markers is that they cannot distinguish wild type S-alleles

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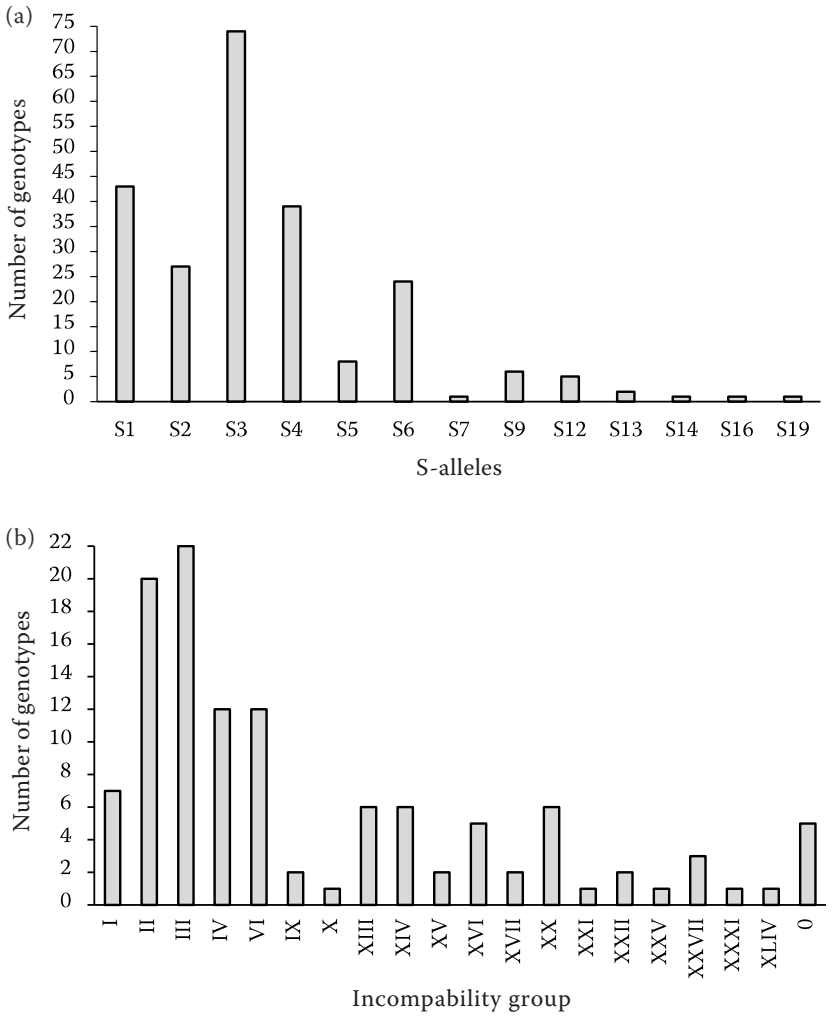

Fig. 1. The S-allele (a) and incompatibility group (b) frequencies within the studied cherry cultivars

from mutated S-alleles. Therefore, we could not exactly determine if the newly S-genotyped accessions were self-incompatible or self-compatible.

In our study, we proved that molecular PCR methods can be used for S-allele genotyping and for the rapid determination of the incompatibility groups in sweet cherry cultivars. It is mainly useful for the evaluation of old and local cherry cultivars and information for the breeders and growers.

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