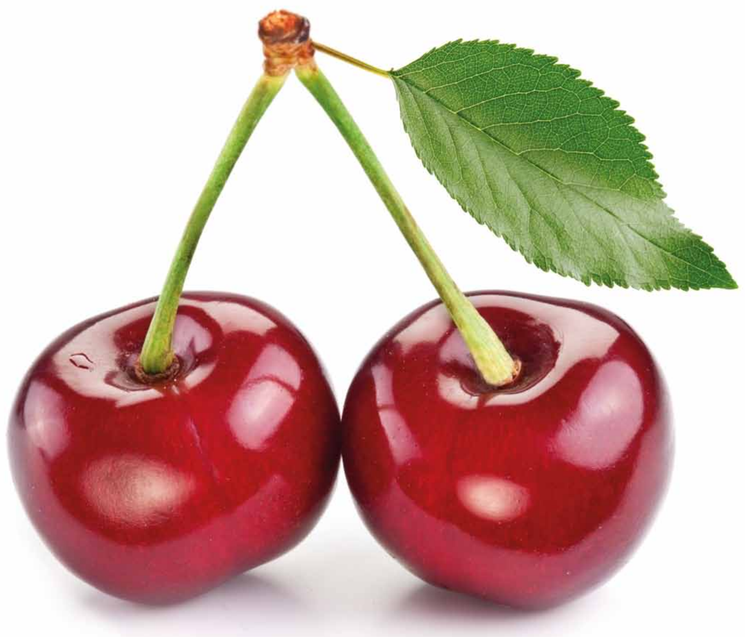

DETERMINATION OF SELF-INCOMPATIBLE GENOTYPES IN SWEET CHERRY ACCESSIONS OF CZECH GENETIC RESOURCES

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Sweet cherries (*Prunus avium L.*) are self-incompatible determined by a gametophytic self-incompatibility system (GSI). The self-incompatibility is controlled by a multi-allelic S-locus and includes two genes coding for the synthesis of pro¬teins responsible for the incompatibility response. Recently, Schuster (2012) compiled the S-genotype of 734 sweet cherry accessions and reported 18 different S--alleles (S1 to S7, S9, S10, S12 to S14, S16, S17, S19, S21, S22, S24), 47 incompatibility groups. The knowledge about the S-allele constitution of cultivars is very important for fruit growers and breeders. Recently, molecular methods have been developed to distinguish the S-alleles in sweet cherries and different molecular primers for the PCR-based identification of all S-alleles were designed during the last years (Wiersma et al., 2001; Sonneveld et al., 2006; Schuster et al., 2007; Iezzoni et al., 2008). In our work, we analyzed S-locus genotypes by 10 universal and specific PCR primer combinations within 82 current, old and local sweet cherry cultivars from Czech genetic resources of Research and Breeding Institute of Pomology in Holovousy. Overall results were summarized in Table 1 and example analyses of amplification products of S-alleles showed Figure 1. Totally, we detected 12 different S-alleles in 21 S-locus combinations for individual incompatibility group. The most frequent S-alleles were S3 (55 genotypes), S4 (26 genotypes) and S1 (25 genotypes), followed by S2 (18 genotypes) and S6 (17 genotypes) (Figure 2). Rare alleles were S7, S13, S14 and S19 (Table 1, Figure2). These results were in accordance to published data for European germplasm (De Cuyper et al., 2005; Schuster, 2012; Cachi and Wünsch, 2014). The most frequent incompatibility groups were III (S3S4), IV (S2S3), VI (S3S6) and II (S1S3) with 13, 11, 9 and 8 genotypes, respectively.

We confirmed previous S-genotyping for 46 accessions excepted Droganova, Hedelfingen, Erika, Frühe Meckenheimer, Huldra, Rivan, Vanda and Winkler. It can be due to mislabelling or mistakes in our or previous analyses. Newly, S-genotyping was determined for 36 accessions when we found 4 new S-loci combinations S4 (Těchlovická), S19 (Droganova), S2S13 (Szwecija) and S2S3S4 in cultivars Leopoldova and Baltavarská.

Materials and methods

In our experiment, we totally used 82 cherries accessions of current, old and local cultivars (Table 1) from cherry genetic resources of Research and Breeding Institute of Pomology in Holovousy (CR). Collected 1 g of young green leaves were powdered with liquid nitrogen and used for DNA isolation by SDS isolation method according to Goulão et al. (2001). Isolated DNAs were afterwards cleaned by ChargeSwitch® gDNA Plant Kit (Invitrogen, ThermoFisher Scientific, Waltham, MA, USA). For identification of S locus sequence, we used universal primer pairs for Intron 1 and 2 and specific allele primer pairs S2, S3, S4, S7, S9, S9a, S10, S12, S13, S14 and S16 (Sonneveld et al., 2003; Iezzoni et al., 2008) for molecular analyses. 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 TGradient thermocycler (Biometra, Goettingen, FRG). Annealing temperatures were used according to references. Amplification products were resolved via electrophoresis in horizontal 1 - 2 % agarose gels and visualized by ethidium bromide staining according to Patzak (2001). The products were scored for the presence or absence in each sample, based on size measured with pGEM DNA marker and 100 bp ladder (Promega, Madison, WI, USA).

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Table 1. Identification of S-alleles and incompatibility groups (IG) within 82 current, old and local sweet cherry cultivars.

Cultivar	Origin	Detected S-alleles	Known S-alleles	IG[1]
Raná Černá Edra	BGR	S1S2		I
Raná Laskovská	BGR	S1S5		XIV
Sam	CAN	S2S4	S2S4	XIII
Stella	CAN	S3S4	S3S4	III
Summit	CAN	S1S2	S1S2	I
Sunburst	CAN	S3S4	S3S4	III
Vega	CAN	S2S3	S2S3	IV
Velvet	CAN	S2S3	S2S3	IV
Viva 221	CAN	S2S3	S2S3	IV
Alfa	CHE	S3S4	S1S6	III, XX
Beta	CHE	S1S5	S1S5	XIV
Beta 8 VF	CHE	S1S5	S1S5	XIV
Delta	CHE	S5S6	S5S6	XV
Zweitfrühe	CHE	S5S6	S5S6	XV
Černá špička	CZE	S1S3		II
Černá z Hořan	CZE	S4S12		XXVII
H 21/40 Černá	CZE	S1S6		XX
Holovouská chrupka	CZE	S1S3		II
Karešova	CZE	S1S3	S1S3	II
Kordia	CZE	S3S6	S3S6	VI
Ladeho pozdní	CZE	S1S3	S1S3	II
Libějovická raná	CZE	S3S4		III
Moravská rychlice	CZE	S2S3		IV
Pivka	CZE	S1S3		II
Pivovka	CZE	S3S7		XLIV
Plavečský granát	CZE	S3S6	S3S6	VI
Pumra	CZE	S3S12		XXII
Šakvická	CZE	S1S6		XX
Semenáč č.13	CZE	S4S12		XXVII
Srdcovka přeúrodná	CZE	S3S6		VI
Těchlovan	CZE	S3S6	S3S6	VI
Těchlovická	CZE	S4		0
Vanda	CZE	S1S6	S9, S6	XX
Vlachova	CZE	S1S2		I
Vosenka	CZE	S2S4		XIII
Boppardská raná	DEU	S3S6		VI
Büttners späte Knorpelkirsche	DEU	S3S4	S3S4	III
Dönissenova SE 5043	DEU	S3S6	S3S6	VI
Droganova	DEU	S19	S1S5	0
Erika	DEU	S4S6	S1S3	XVI
Frühe Meckenheimer	DEU	S3S4	S1S4	III, IX
Germersdorfer	DEU	S3S12	S3S12	XXII
Grollova	DEU	S3S4		III
Hedelfingenská	DEU	S1S3	S3S5	II, VII
Hildesheim	DEU	S1S3		II
Kassins Frühe	DEU	S2S3	S2S3	IV
Leopoldova	DEU	S2S3S4		0
Muncheberská	DEU	S3S4	S3S4	III
Napoleonova	DEU	S3S4	S3S4	III
Německá rychlice	DEU	S2S3		IV
Querfurter Königskirsche	DEU	S3S4	S3S4	III
Rebekka	DEU	S1S3	S1S3	II
Regina	DEU	S1S3	S1S3	II
Spitze Braune	DEU	S3S14		XXXI
Troprichterova	DEU	S1S3	S1S3	II
Valeska	DEU	S1S3	S1S3	II
Winklerova	DEU	S2S3	S1S3, S3S9	IV
Zeisbergova	DEU	S2S4		XIII
Bigarreau de la Charmes	FRA	S3S9		XVI
Burlat	FRA	S3S9	S3S9	XVI
Lyonska	FRA	S6S9		X
Early Rivers	GBR	S1S2	S1S2	I
Merchant	GBR	S4S9	S4S9	XXI
Merla	GBR	S1S6	S1S6	XX
Mermat	GBR	S3S6		VI
Merton Favourite	GBR	S3S6		VI
Merton Glory	GBR	S4S6	S4S6	XVII
Merton Premier	GBR	S2S3	S2S3	IV
Baltavarská	HUN	S2S3S4		0
Kišiněvskaja	MDA	S1S3		II
Skierniewice 1	POL	S2S3		IV
Skierniewice 3	POL	S2S3		IV
Skorospielka	RUS	S2S3		IV
Asenova raná	SRB	S3S9		XVI
Huldra	SWE	S3S6	S1S3	VI
Rivan	SWE	S2S4	S1S2	XIII
Szwecija	SWE	S2S13		0
Valerij Tschkalov	UKR	S3S9	S1S9	XVI
Hudson	USA	S1S4	S1S4	IX
Kristin	USA	S3S4	S3S4	III
Lapins	USA	S3S4	S3S4, S1S4	III
Mona Cherry	USA	S3S4		III

[1]Incompatibility group, according to SCHUSTER (2012).

Figure 1. Example analyses of PCR amplified products of S-alleles loci S2, S3, S4, S9 and S12 in cherry cultivars on 1,5% agarose gels. Lanes: 1 – Plavečský granát, 2 – Spitze Braune, 3 – Rebekka, 4 – Grollova, 5 – Merchant, 6 – Burlat, 7 – Zweitfrühe, 8 – Šakvická, 9 – Beta, 10 – Delta, 11 – Mona Cherry, 12 – Mermat, 13 – Černá z Hořan, 14 – Kassins Frühe, 15 – Merton Glory, 16 – Raná Černá Edra, 17 – Erika, 18 – Baltavarská, M1 - pGEM DNA marker M2 - 100 bp Ladder, (Promega, USA)

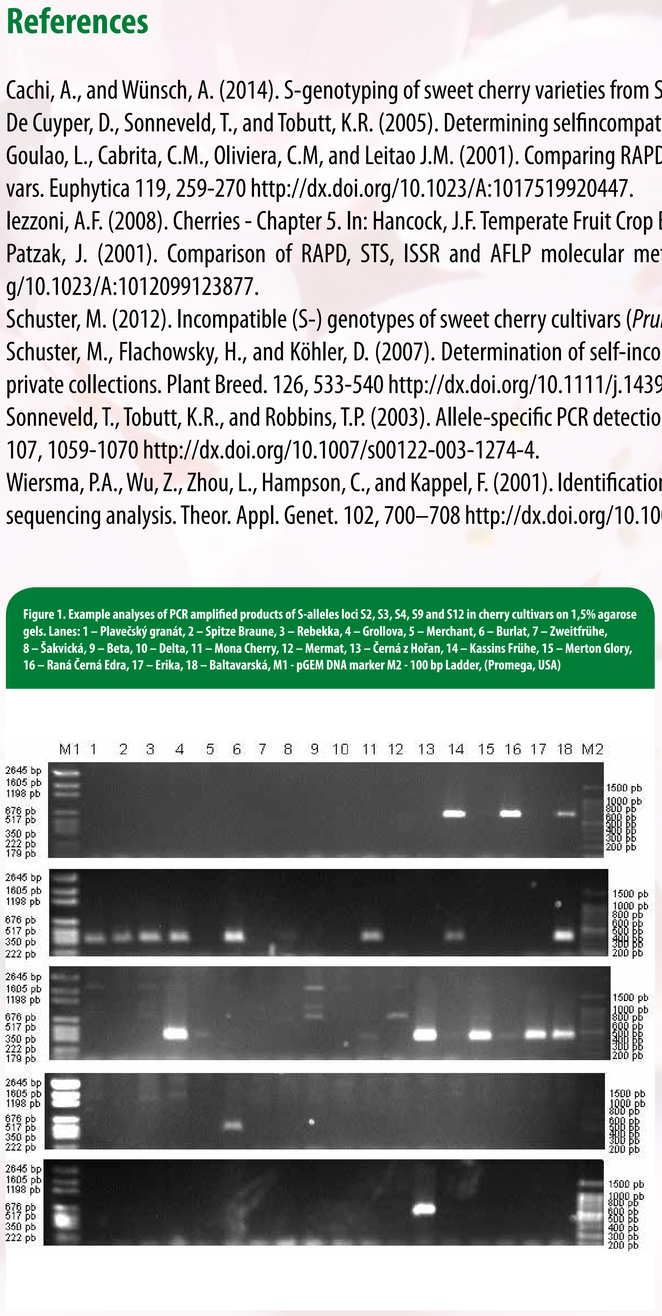
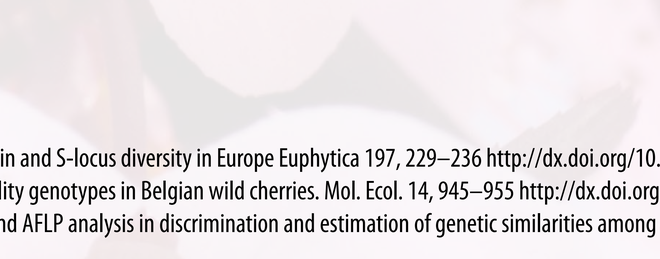


Figure 2. S-allleles frequencies within studied cherry cultivars.



Těchlovická (Pilnáčkova, Ziklova) - dark bigarreau

Origin: chance seedling found in Těchlovice near Hradec Králové at the end of the 19th century

Fruit: fruits are bigger, globular, dark red with substantial light dots. The flesh is firm, dark red, subacidic sweet with spicy flavour. The juice has intense coloration ability. It is suitable cultivar for consumption and fruit stew. It ripens in the 5th sweet cherry week. Fruits are relatively susceptible to cracking. The stalk is medium long, the stone is smaller up to medium size.

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