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Identification of *S*-genotypes of sweet cherry cultivars from Central and Eastern Europe

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Abstract

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Sweet cherry is a species that is characterized by self-incompatibility, which causes difficulties in obtaining high yields of sweet cherry fruit. Determination of the composition of the *S*-alleles of sweet cherry cultivars is useful both to growers producing the fruit and breeders when selecting cultivars for cross-fertilizations. In this work, *S*-alleles have been identified for 47 sweet cherry cultivars from Central and Eastern Europe, mostly from Ukraine and the Czech Republic, with *S*-genotypes of 43 cultivars identified for the first time. To identify the *S*-alleles, PCR-based methods were used. In each cultivar, two of the following eight *S*-alleles were identified: S_1, S_2, S_3, S_4, S_5, S_6, S_9 and S_{13}. In the cultivars from the Czech Republic, the most frequently occurring were the alleles S_3, S_1 and S_4. In the cultivars originating in Ukraine, the most frequently found were the alleles S_5 and S_9, which makes them differ considerably from the sweet cherry cultivars from other regions of Europe. The tested sweet cherry cultivars were assigned to 20 of the existing incompatibility groups.

Keywords: *Prunus avium* L.; self-incompatibility; *S*-RNase; incompatibility groups; allele frequency

The production of sweet cherry fruit (*Prunus avium* L.) is an important part of agriculture in Poland as almost 40 thousand tonnes of the fruit is harvested annually (FAOSTAT 2012). Due to the phenomenon of self-incompatibility, which occurs in sweet cherry, it is necessary to select suitable pollinating cultivars to enable the setting of fruit in the orchard and achieve high fruit yields. The knowledge of cross(-in-)compatibility between cultivars is also important for the breeders because it allows correct selection of cultivars for cross-fertilization.

The phenomenon of self-incompatibility is a system developed by plants for the purpose of preventing self-pollination to ensure constant genetic exchange within the population (De Nettancourt 1984). Self-incompatibility in sweet cherry is determined by a single multi-allelic locus (*S*-locus), and includes two genes coding for the synthesis of proteins responsible for the incompatibility response. The *S*-RNase gene determines the incompatibility response of the pistil, while the *S*-haplotype-specific F-box gene (SFB) is responsible for the incompatibility response of the pollen (Ushijima et al. 2003; Yamane et al. 2003). Fertilization occurs when the *S*-allele in the genome of the haploid pollen grain is different from the two *S*-alleles of the diploid genome of the pistil (Wünsch, Hormaza 2004). Knowledge of the *S*-alleles of cultivars and selections gathered in collections enables correct selection of pollinating cultivars for production orchards and allows proper selection of cultivars for cross-fertilizations.

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In the beginning, *S*-alleles were identified using biochemical methods based on analysis of stylar proteins (Bošković et al. 1997). The development of molecular techniques enabled identification of the *S*-alleles of sweet cherry cultivars based on PCR of the *S*-RNase gene (Wiersma et al. 2001; Sonneveld et al. 2001, 2003, 2006; Choi et al. 2002; Wünsch, Hormaza 2004; Marchese et al. 2007; Gisbert et al. 2008). The *S*-RNase gene contains several conservative domains and two introns of variable size depending on the *S*-haplotype (Sonneveld et al. 2001; Gisbert et al. 2008). Identification of the *S*-alleles of sweet cherry using the techniques based on PCR is possible by amplifying the introns I and II of the *S*-RNase gene by means of consensus primers, and by amplifying the *S*-RNase gene using specific primers. Specific primers based on the *S*-RNase gene were developed for the alleles S_1–S_{16} (Sonneveld et al. 2001, 2003), and for alleles S_{17}–S_{19}, S_{21}–S_{25}, S_{34} and S_{37} (Szikriszt et al. 2013). The effectiveness of the use of consensus primers and specific primers in the identification of the *S*-alleles of sweet cherry was confirmed in the studies by Békefi et al. (2003); De Cuyper et al. (2005); Schuster et al. (2007); Ipek et al. (2011); Ercisli et al. (2012); Szikriszt et al. (2013).

By analysing the *S*-RNase gene with methods based on PCR, the six most frequently occurring alleles, S_1–S_6, were identified (Sonneveld et al. 2001; Wiersma et al. 2001). Subsequently, the next *S*-alleles to be identified were S_7–S_{16} (Sonneveld et al. 2003). During the analyses, it was found that the alleles S_8, S_{11} and S_{15} are identical to the alleles S_3, S_7 and S_5 (Sonneveld et al. 2001, 2003; Wünsch, Hormaza 2004). In subsequent years, more *S*-alleles were identified in wild cherry and local cultivars of sweet cherry from different regions of Europe. In Belgian wild cherries, 6 new alleles were identified, designated from S_{17} to S_{22} (De Cuyper et al. 2005). The alleles S_{19} and S_{22} were also identified in the local sweet cherry cultivars from south-western Germany, gathered in a gene bank (Schuster et al. 2007). In addition, the S_{22} allele was identified in the local sweet cherry cultivars from a south-eastern region of Spain (Gisbert et al. 2008). Next, the presence of a further 6 alleles, which were given the numbers from S_{27} to S_{32}, was revealed in wild cherry cultivars growing in an ancient woodland in the county of Kent in England (Vaughan et al. 2008). Recently, a new allele, S_{37}, was identified in the local sweet cherry cultivars from a Black Sea region, and also the S_{34} allele, which was identified in sour cherry before (Szikriszt et al. 2013).

Cultivars with the same *S*-alleles are placed in the same cross-incompatibility group (CIG). At first, 26 incompatibility groups were created (Tobutt et al. 2004). As the composition of the *S*-alleles in other cultivars of sweet cherry was determined, new groups were created (Békefi et al. 2003, 2010; Sonneveld et al. 2003; Marchese et al. 2007; Schuster et al. 2007; Gisbert et al. 2008; Ipek et al. 2011; Schuster 2012; Szikriszt et al. 2013). Currently, the cultivars of sweet cherry are assigned to 47 incompatibility groups I–XLVII, to the group of self-compatible cultivars and to the group '0' of unique *S*-genotypes as universal pollen donors (Schuster 2012).

Identification of *S*-alleles was performed for sweet cherry cultivars from Western Europe, for Belgian and British wild cherries, for sweet cherry cultivars and selections gathered in the Swedish gene bank, and for the local Sicilian, Spanish, Greek, Turkish and Croatian cultivars (Bošković, Tobutt 2001; Tobutt et al. 2004; De Cuyper et al. 2005; Marchese et al. 2007; Gisbert et al. 2008; Lacis et al. 2008; Vaughan et al. 2008; Ganopoulos et al. 2010; Ipek et al. 2011; Ercisli et al. 2012; Szikriszt et al. 2013; Cachi, Wünsch 2014). *S*-alleles were also identified in sweet cherry cultivars originating in several countries of Central and Eastern Europe, mostly in the cultivars and selections gathered in the Latvian collection, and also in the Hungarian cultivars, but in only a few of the Czech and Ukrainian cultivars (Békefi et al. 2003, 2010; Schuster et al. 2007; Lacis et al. 2008; Schuster 2012). However, there is still no information on the composition of *S*-alleles for a large group of sweet cherry cultivars from the central and eastern regions of Europe, particularly from Ukraine. Since the existing results indicate an uneven distribution of *S*-alleles in the European cultivars of sweet cherry (Ercisli et al. 2012), identification of *S*-alleles in the cultivars from Central and Eastern Europe will help expand our knowledge on this subject.

Due to the high importance of sweet cherry cultivation in Poland, the Research Institute of Horticulture in Skierniewice maintains a collection consisting of nearly 300 accessions of sweet cherry. In this work, we present the results of the identification of incompatibility alleles obtained for the first time for 43 sweet cherry cultivars and selections from Central and Eastern Europe, mostly from Ukraine and the

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Czech Republic, gathered in that collection. We have assigned the tested cultivars to the existing incompatibility groups. The results will be of use in breeding work when selecting cultivars for cross-fertilizations, and will find application in the production of sweet cherry fruit by facilitating the proper selection of pollinators for commercial orchards.

MATERIAL AND METHODS

Plant material. The analyses were conducted for 47 cultivars and selections of the sweet cherry (*Prunus avium* L.) growing in the collection maintained at the Research Institute of Horticulture in Skierniewice (Poland). Cultivars with known *S*-alleles were used in the analyses as reference cultivars: Bianca S_1S_5, Karina S_3S_4, Rivan S_1S_2, Octavia S_1S_3, Merchant S_4S_9, Sam S_2S_4. For the analyses, young leaves were collected from trees and then frozen at –80°C until DNA isolation.

DNA extraction. DNA was isolated from a 100 mg sample of leaves using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The DNA obtained was purified further using an Anty-Inhibitor Kit (A&A Biotechnology, Gdynia, Poland). DNA concentration was measured spectrophotometrically at a wavelength of 260 nm. For analyses, 10 ng/µl dilutions of DNA were prepared.

PCR procedure. PCR reactions were conducted with primers amplifying the conserved regions of the gene which codes for the synthesis of *S*-RNase. For this purpose, primers amplifying the introns I and II were used: PaCons IF/PaCons IR, PaCons IIF/PaCons IIR (Sonneveld et al. 2003). To confirm the identification, reactions were carried out with 13 pairs of primers specific for the S_1–S_{16} alleles of the *S*-RNase gene (Sonneveld et al. 2001, 2003). The reactions were conducted in a total volume of 20 µl, containing 1× reaction buffer, 0.2mM dNTPs, 0.4µM of each primer, 0.5U of Taq DNA polymerase (DreamTaq™ Green; Thermo Scientific, Waltham, USA) and 10 ng of template DNA. The reactions were carried out in an DNA Engine Dyad (Bio-Rad, Hercules, USA) DNA thermocycler under the thermal conditions described by Sonneveld et al. (2003). The PCR products obtained by the amplification of intron I were separated in a 2.0% agarose gel, whereas the products of the amplification of intron II and the PCR products obtained in the reactions with specific primers were separated in a 1.4% aga-

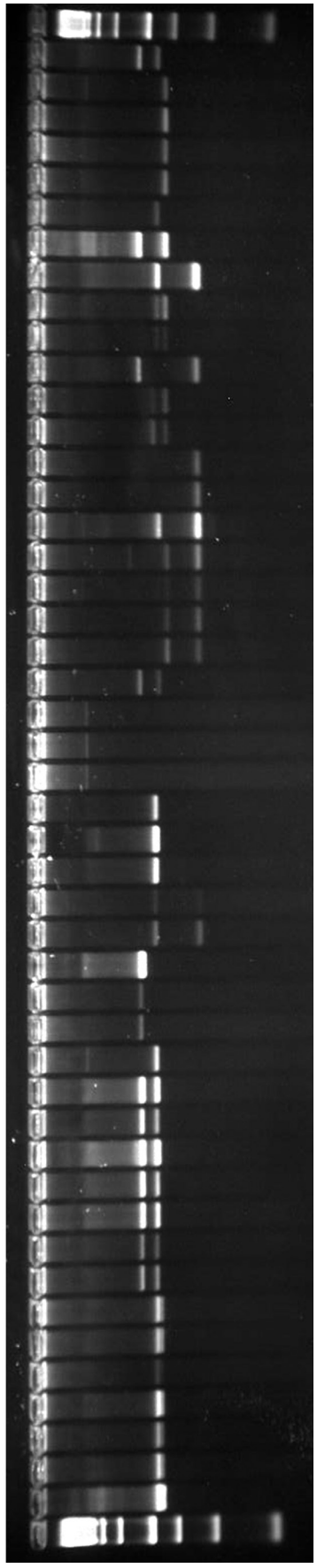

Fig. 1. Amplification of intron II of the *S*-RNase gene in sweet cherry genotypes with the PaConsIIF/PaConsIIR consensus primers
(M) 1 kb ladder, genotypes: (lane 1) Helga, (2) HL 10072, (3) HL 10726, (4) Justyna, (5) Ljubawa, (6) Kasandra,(7) Marta, (8) Amid, (9) Annuszka, (10) Early Korvik, (11) Jacinta, (12) Pola, (13) Ruksandra, (14) Sandra, (15) HL 15361, (16) D 44-1, (17) Nieznost, (18) Tim, (19) Leningradzka Slodka, (20) Wspleska, (21) D 49-1, (22) Jaroslawna, (23) Rannaja Rozowinka, (24) Alienuszka, (25) D 48-52, (26) Kitajewskaja, (27) Wahtanka, (28) Kijewlianka, (29) Kitajewskaja Cziornaja, (30) Krasawica Kijewa, (31) Priusadiebnaja, (32) Kanarkowa, (33) Nektarnaja, (34) Samocwiet, (35) Donieckaja Krasawica, (36) Wasilisa, (37) Debora, (38) Skazka, (39) Tamara, (40) Fabiola, (41) HL 16165, (42) Vospominanje, (43) Anons, (44) Prestiznaja, (45) Donieckij Ugolek, (46) Proszczalna, (47) Bladoróżowa

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Table 1. *S*-allele composition and incompatibility groups of sweet cherry cultivars and selections from Central and Eastern Europe

Genotype	Origin	*S*-genotyping with consensus primers		*S*-allele results based on specific primers								*S*-allele composition	Incompatibility group
		1st intron	2nd intron	S_1	S_2	S_3	S_4	S_5	S_6	S_9	S_{13}		
Helga*	Czech Republic	S_1S_5/S_3	$S_1S_3S_{13}$	+		+						S_1S_3	II
HL 10072	Czech Republic	S_1S_5/S_3	$S_1S_3S_{13}$	+		+						S_1S_3	II
HL 10726	Czech Republic	S_1S_5/S_3	$S_1S_3S_{13}$	+		+						S_1S_3	II
Justyna	Czech Republic	S_1S_5/S_3	$S_1S_3S_{13}$	+		+						S_1S_3	II
Kasandra*	Czech Republic	S_1S_5/S_3	$S_1S_3S_{13}$	+		+						S_1S_3	II
Ljubawa	Ukraine	S_1S_5/S_3	$S_1S_3S_{13}$	+		+						S_1S_3	II
Marta	Czech Republic	S_1S_5/S_3	$S_1S_3S_{13}$	+		+						S_1S_3	II
Amid	Czech Republic	S_3/S_4S_6	$S_1S_3S_{13}/S_4$			+	+					S_3S_4	III
Annuszka	Ukraine	S_3/S_4S_6	$S_1S_3S_{13}/S_4$			+	+					S_3S_4	III
Early Korvik	Czech Republic	S_3/S_4S_6	$S_1S_3S_{13}/S_4$			+	+					S_3S_4	III
Jacinta*	Czech Republic	S_3/S_4S_6	$S_1S_3S_{13}/S_4$			+	+					S_3S_4	III
Pola	Poland	S_3/S_4S_6	$S_1S_3S_{13}/S_4$			+	+					S_3S_4	III
Ruksandra	Ukraine	S_3/S_4S_6	$S_1S_3S_{13}/S_4$			+	+					S_3S_4	III
Sandra	Czech Republic	S_3/S_4S_6	$S_1S_3S_{13}/S_4$			+	+					S_3S_4	III
HL 15361	Czech Republic	S_2S_9/S_3	S_3/n.a.		+	+						S_2S_3	IV
D 44-1	Ukraine	S_4S_6/S_1S_5	S_4/n.a.				+	+				S_4S_5	V
Nieznost	Ukraine	S_4S_6/S_1S_5	S_4/n.a.				+	+				S_4S_5	V
Tim	Czech Republic	S_4S_6/S_1S_5	S_4/na				+	+				S_4S_5	V
Leningradzka Slodka	Russia	S_3/S_4S_6	$S_1S_3S_{13}/S_6$			+			+			S_3S_6	VI
Wspleska	Ukraine	S_3/S_4S_6	$S_1S_3S_{13}/S_6$			+			+			S_3S_6	VI
D 49-1	Ukraine	S_1S_5/S_3	$S_1S_3S_{13}$/n.a			+		+				S_3S_5	VII
Jaroslawna	Ukraine	S_1S_5/S_3	$S_1S_3S_{13}$/n.a.			+		+				S_3S_5	VII
Rannaja Rozowinka	Ukraine	S_1S_5/S_3	$S_1S_3S_{13}$/n.a.			+		+				S_3S_5	VII
Alienuszka	Ukraine	S_1S_5/S_2S_9	n.a.		+			+				S_2S_5	VIII
D 48-52	Ukraine	S_1S_5/S_2S_9	n.a.		+			+				S_2S_5	VIII
Kitajewskaja	Ukraine	S_1S_5/S_2S_9	n.a.		+			+				S_2S_5	VIII
Wahtanka	Ukraine	S_1S_5/S_4S_6	$S_1S_3S_{13}/S_4$	+			+					S_1S_4	IX
Kijewlianka	Ukraine	S_2S_9/S_4S_6	S_6/S_9						+	+		S_6S_9	X

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Table 1 to be continued

Genotype	Origin	*S*-genotyping with consensus primers		*S*-allele results based on specific primers								*S*-allele composition	Incompatibility group
		1st intron	2nd intron	S_1	S_2	S_3	S_4	S_5	S_6	S_9	S_{13}		
Kitajewskaja Cziornaja	Ukraine	S_2S_9/S_4S_6	S_6/S_9						+	+		S_6S_9	X
Krasawica Kijewa	Ukraine	S_2S_9/S_4S_6	S_6/S_9						+	+		S_6S_9	X
Priusadiebnaja	Ukraine	S_2S_9/S_4S_6	S_6/S_9						+	+		S_6S_9	X
Kanarkowa	Poland	S_4S_6/n.a.	$S_1S_3S_{13}/S_6$						+		+	S_6S_{13}	XII
Nektarnaja	Ukraine	S_1S_5/S_4S_6	S_6/n.a.					+	+			S_5S_6	XV
Samocwiet	Ukraine	S_1S_5/S_4S_6	S_6/n.a.					+	+			S_5S_6	XV
Donieckaja Krasawica	Ukraine	S_2S_9/S_3	$S_1S_3S_{13}/S_9$			+				+		S_3S_9	XVI
Wasilisa	Ukraine	S_2S_9/S_3	$S_1S_3S_{13}/S_9$			+				+		S_3S_9	XVI
Debora	Czech Republic	S_4S_6	S_4/S_6				+		+			S_4S_6	XVII
Skazka	Ukraine	S_1S_5/S_2S_9	$S_1S_3S_{13}/S_9$	+						+		S_1S_9	XVIII
Tamara*	Czech Republic	S_1S_5/S_2S_9	$S_1S_3S_{13}/S_9$	+						+		S_1S_9	XVIII
Fabiola	Czech Republic	S_1S_5/S_4S_6	$S_1S_3S_{13}/S_6$	+					+			S_1S_6	XX
HL 16165	Czech Republic	S_2S_9/S_4S_6	$S_1S_3S_{13}/S_4$				+			+		S_4S_9	XXI
Vospominanje	Ukraine	S_1S_5/n.a.	$S_1S_3S_{13}$/n.a.					+			+	S_5S_{13}	XXVI
Anons	Ukraine	S_1S_5/S_2S_9	S_9/n.a.					+		+		S_5S_9	XXXVII
Prestiznaja	Ukraine	S_1S_5/S_2S_9	S_9/n.a.					+		+		S_5S_9	XXXVII
Donieckij Ugolek	Ukraine	S_1S_5/S_2S_9	S_9/n.a.					+		+		S_5S_9	XXXVII
Proszczalna	Ukraine	S_2S_9	S_9/n.a.		+			+		+		S_2S_9	XLIII
Bladoróżowa	Poland	S_4S_6/n.a.	$S_1S_3S_{13}/S_4$				+				+	S_4S_{13}	XLVI

*cultivars for which *S*-alleles were identified in the work by Schuster (2012); n.a. – lack of amplification of S_{13} allele (intron I) and lack of amplification or weak band of S_2/S_5 allele (intron II)

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rose gel. The agarose gels were stained with ethidium bromide and visualized under UV light (GelDoc-It® Imaging System; UVP, Upland, USA).

RESULTS

For the majority of cultivars, the amplification of intron I or intron II of the *S*-RNase gene using consensus primers resulted in two DNA fragments characterizing the *S*-alleles of the sweet cherry cultivars (Fig. 1). The size of the resulting products was determined in the range from 300 bp to 520 bp for intron I, and from 580 to 1,060 bp for intron II.

The identification of *S*-alleles with consensus primers was, however, difficult because the PCR products obtained were of a size very similar to that of different *S*-alleles. Using the PaConsIF/PaConsIR primers, it was impossible to distinguish the S_4 allele from S_6, the S_1 allele from S_5, and the S_2 allele from S_9, and, moreover, amplification of the S_{13} allele was not possible. Using the PaConsIIF/PaConsIIR primers, the resulting PCR products did not allow us to distinguish the alleles S_1, S_3 and S_{13}, while the fragments characteristic of the S_2 and S_5 alleles, 2,204 bp and 2,159 bp in size, respectively, were absent or barely visible. After the reactions with the primers specific for the S_1–S_{16} alleles of the *S*-RNase gene, the resulting DNA fragments ranged in size from 300 to 960 bp. The analyses identified the *S*-alleles in all of the 47 genotypes tested. In total, eight different *S*-alleles were identified: S_1, S_2, S_3, S_4, S_5, S_6, S_9 and S_{13} (Table 1).

In the group of 16 cultivars and selections from the Czech Republic, the alleles S_1, S_2, S_3, S_4, S_5, S_6, S_9 occurred with frequency of 25.0, 3.1, 34.4, 21.9, 3.1, 6.2 and 6.2%, respectively. In the group of 27 cultivars and selections from Ukraine, the alleles S_1, S_2, S_3, S_4, S_5, S_6, S_9, S_{13} were identified with frequency of 5.5, 7.4, 16.6, 11.1, 25.9, 12.9, 20.4 and 3.7%, respectively. The results of the identification of *S*-alleles obtained in the reactions with consensus and specific primers allowed 47 cultivars to be classified into 20 incompatibility groups (Table 1).

DISCUSSION

The identification of *S*-alleles was performed in two stages. In the first stage, the introns I and II of the *S*-RNase gene were amplified using consensus primers. In the next stage, reactions were conducted with primers specific for the S_1–S_{16} alleles of the *S*-RNase gene. The *S*-alleles were identified after analysing all the results. The size of the DNA fragments obtained with the consensus primers and specific primers was consistent with the size of the fragments, characterizing the *S*-alleles, described by Sonneveld et al. (2003) and Schuster et al. (2007).

The use of consensus primers enabled identification of the majority of the *S*-alleles. However, identification of some *S*-alleles was difficult because the resulting products were of a size similar to that of different *S*-alleles, or there were no amplification products. Following the use of the PaConsIF/PaConsIR primers, no product of the amplification of the S_{13} allele was obtained, like in the analyses by Sonneveld et al. (2003) and Schuster et al. (2007). After amplifying intron I, it was impossible to distinguish the alleles S_4 from S_6, S_1 from S_5, and S_2 from S_9, which confirms the results obtained for sweet cherry cultivars from Turkey and Croatia (Ipek et al. 2011; Ercisli et al. 2012). Amplification of intron II did not make it possible to identify the alleles S_1, S_3 and S_{13}, which is consistent with the results obtained by other authors (Sonneveld et al. 2003; Schuster et al. 2007; Ipek et al. 2011). Similarity in the size of the products of amplification of intron I or intron II was also observed for the alleles S_{16} and S_{32}, S_2 and S_7, S_9 and S_{10} (Szikriszt et al. 2013), for the alleles S_2, S_7, S_9 and S_{12} (Ipek et al. 2011; Ercisli et al. 2012), and for the alleles S_{10} and S_{14} (Ipek et al. 2011).

After using the PaConsIIF/PaConsIIR primers, the 2,159 bp fragment, which corresponds to the S_5 allele, was absent or barely visible. Sonneveld et al. (2003) obtained that fragment in the reaction with the PaConsIIF/PaConsIIR primers, but it was seen in the form of a weak band. In our analyses, there were similar difficulties with the amplification of the S_2 allele with the PaConsIIF/PaConsIIR primers.

In order to confirm and verify the results of the amplifications of intron I and intron II of the *S*-RNase gene, the *S*-RNase gene was amplified using the primers specific for each *S*-allele, which made it possible to verify the results obtained with the consensus primers and to identify conclusively the *S*-alleles in all the sweet cherry genotypes tested. In each cultivar, two *S*-alleles were identified, indicating that all of the cultivars were diploid.

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Comparison of the *S*-genotypes of 222 self-incompatible and 25 self-compatible cultivars of sweet cherry has shown that the alleles S_1, S_2, S_3, S_4, S_5, S_6 and S_9 occurred with frequency of 21, 13, 29, 15, 7, 11 and 6%, respectively (Tobutt et al. 2004; Lacis et al. 2008; Ipek et al. 2011). In our study, the most strongly represented were the sweet cherry cultivars from Ukraine (27) and the Czech Republic (16), and for these groups the frequency of the occurrence of *S*-alleles was determined. In comparison with the cultivars listed by Tobutt et al. (2004), the alleles S_1 (25%), S_3 (34.4%) and S_4 (21.9%) occurred in the cultivars from the Czech Republic with slightly higher frequency, while the S_9 allele occurred with similar frequency (6.2%). The alleles S_2 (3.1%), S_5 (3.1%) and S_6 (6.2%) occurred less frequently in the Czech cultivars than in the group being compared.

In the Ukrainian cultivars of sweet cherry, the alleles S_4 (11.1%) and S_6 (12.9%) occurred with similar frequency as in the group of 247 cultivars published by Tobutt et al. (2004), while the alleles S_1 (5.5%), S_2 (7.4%) and S_3 (16.6%) were less frequent. The alleles S_5 (25.9%) and S_9 (20.4%) occurred in the Ukrainian sweet cherry cultivars with much greater frequency than in the group of cultivars being compared. Studies by other authors indicate that the S_5 allele occurred with low frequency (2.5% to 7%) in Sicilian, Swedish, Hungarian, Greek, Turkish, and Croatian cultivars (Marchese et al. 2007; Lacis et al. 2008; Békefi et al. 2010; Ganopoulos et al. 2010; Ipek et al. 2011; Ercisli et al. 2012). The S_5 allele was absent in the Belgian wild cherries and in the Spanish local cultivars of sweet cherry (De Cuyper et al. 2005; Gisbert et al. 2008; Cachi, Wünsch 2014). The S_5 allele, however, was frequently found (22%) in Latvian sweet cherry taxa gathered in the Latvian germplasm bank (Lacis et al. 2008). These results indicate that the sweet cherry cultivars from Eastern Europe are characterized by high frequency of the occurrence of the S_5 allele.

The S_9 allele is rather rarely seen among the European cultivars of sweet cherry; it occurred with frequency ranging from 2% to 10% in the Belgian wild cherries and in the Sicilian, Hungarian, Turkish, Spanish, Croatian, Sicilian and Greek cultivars (De Cuyper et al. 2005; Marchese et al. 2007; Gisbert et al. 2008; Békefi et al. 2010; Ganopoulos et al. 2010; Ipek et al. 2011; Ercisli et al. 2012; Cachi, Wünsch 2014). The high frequency of the occurrence of S_5 and S_9 alleles in the Ukrainian sweet cherries (20.4%) greatly differentiates these cultivars from the sweet cherry cultivars originating in other regions of Europe. Furthermore, a comparison of the Czech and Ukrainian cultivars shows that the alleles S_1, S_3 and S_4 are much more frequently found in the Czech cultivars, while the alleles S_2, S_5, S_6 and S_9 occur with much higher frequency in the Ukrainian cultivars.

Of all the identified *S*-alleles, the most rarely seen was the S_{13} allele, which was found in two cultivars from Poland and two cultivars from Ukraine. The frequency of the occurrence of the S_{13} allele in the cultivars from Central and Eastern Europe is similar to that in the other European cultivars of sweet cherry. The S_{13} allele was seen with frequency of 2% to 5% in sweet cherry cultivars from Western Europe and in the Sicilian, Hungarian, and Greek cultivars (Bošković, Tobutt 2001; Marchese et al. 2007; Békefi et al. 2010; Ganopoulos et al. 2010). The S_{13} allele was not found in the local Spanish, Turkish, and Croatian cultivars of sweet cherry (Gisbert et al. 2008; Ipek et al. 2011; Ercisli et al. 2012).

In our analyses, despite the use of primers specific for the S_1–S_{16} alleles, the presence of the alleles S_7, S_{10}, S_{12}, S_{14} and S_{16} was not detected in sweet cherries from Central and Eastern Europe. Likewise, there was no positive result for the presence of some *S*-alleles, such as S_{13}, S_{14} and S_{16}, in Turkish and Croatian sweet cherry cultivars (Ipek et al. 2011; Ercisli et al. 2012). This unequal distribution of the *S*-alleles among the European cultivars of sweet cherry was described by Ercisli et al. (2012) and also by Cachi and Wünsch (2014), and the results of our studies on the identification of the *S*-alleles in sweet cherries from Central and Eastern Europe confirm this phenomenon. The reason for the differences in the distribution of *S*-haplotypes in different regions of Europe may be the common origin of the cultivars in isolated areas, or the connection between specific *S*-haplotypes with adaptive traits and the climatic conditions of the different areas (Cachi, Wünsch 2014).

As a result of the analyses, *S*-alleles for 43 sweet cherry cultivars from Central and Eastern Europe were identified for the first time. The tested cultivars were assigned to 20 of the existing incompatibility groups. Sixteen cultivars from the Czech Republic were assigned to 8 groups, with groups II and III being the most strongly represented, numbering 6 and 4 cultivars, respectively. This indicates only a slight variation in S-alleles among the sweet cherry cultivars from the Czech Republic. Twenty-

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seven cultivars originating in Ukraine were more varied in the composition of *S*-alleles and were assigned to 14 incompatibility groups. The most strongly represented group was Group X, consisting of 4 cultivars, while each of the groups VII, VIII and XXXVII comprised 3 cultivars.

Pola is a new sweet cherry cultivar, increasingly introduced into cultivation in Poland. The knowledge of the composition of its *S*-alleles will facilitate establishment of new orchards and help obtaining high yields by planting suitable pollinators for this cultivar.

The identification of *S*-alleles for sweet cherry cultivars from Central and Eastern Europe conducted in our study extends the existing knowledge on the frequency of the occurrence of *S*-alleles in the European cultivars of sweet cherry. Determination of the composition of the *S*-alleles in the tested cultivars and classification of them into incompatibility groups will find application in the production of fruit by enabling proper selection of pollinators for commercial sweet cherry orchards. In addition, the knowledge of the incompatibility alleles in the sweet cherry accessions gathered in the gene bank will enable full characterization of the collected cultivars and allow them to be used effectively in breeding work when selecting cultivars for cross-fertilizations.

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