Voćarstvo, 57, 217–218 (2023), 93–103
Journal of Pomology, 57, 217–218 (2023), 93–103
https://doi.org/10.18485/pomology.2023.57.217_218.2

UDC: 634.232:575.22
Original scientific paper
Originalni naučni rad

An overview of *S*-genotype diversity in sweet cherry landraces grown in the central region of the Republic of Serbia

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Received: 06 November 2023; Accepted: 13 November 2023

Abstract. Identification of the *S*-genotypes in landraces is a crucial step in the molecular characterization of Serbian autochthonous sweet cherry germplasm. It is also of enormous significance for breeders and growers, as this fruit species exhibits a gametophytic self-incompatibility, controlled by the multi-allelic two genes of the *S*-locus. The aim of this study was to summarize known information and reveal new data on the *S*-alleles in 23 sweet cherry landraces originating in the Republic of Serbia. The use of polymerase chain reaction (PCR) with consensus primers for the second intron of *S-RNase*, primers specific for *S-RNase* and certain *SFB* alleles, along with DNA fragment analysis using fluorescently labelled forward primers to amplify both the *S-RNase* first intron and the *SFB* intron, revealed 10 alleles (S_1 to S_6, S_9, S_{12}, S_{13} and S_{22}) that generated the following 13 *S*-genotypes: S_1S_2 (one landrace), S_1S_4 (one landrace), S_1S_5 (one landrace), S_2S_3 (four landraces), S_3S_4 (two landraces), S_3S_5 (two landraces), S_3S_6 (three landraces), S_3S_9 (two landraces), S_3S_{12} (two landraces), S_4S_5 (one landrace), S_4S_{13} (one landrace), S_5S_{22} (one landrace) and S_6S_9 (two landraces). The most frequent *S*-allele and *S*-genotype in this sweet cherry material were S_3 and S_2S_3, with occurrence frequencies of 32.6% and 17.4%, respectively. Based on the obtained results, the sweet cherry landraces were assigned to 12 incompatibility groups and one group of universal donors ('0'). These results provide important information about their cross-compatibility and the diversity of the *S*-locus in Serbian sweet cherry germplasm.

Key words: *Prunus avium* L., autochthonous genotype, *S-RNase*, *SFB*, *S*-haplotype, gametophytic self-incompatibility

Introduction

Sweet cherry (*Prunus avium* L.) is an economically important perennial fruit crop in the Republic of Serbia (RS), with an average annual production of 19,900 tonnes in the period 2012–2021, according to the Food and Agriculture Organization of the United Nations. Also, a wide range of local and foreign sweet cherry cultivars are grown in the RS. This fruit species is one of the most popular table fruits and represents the domesticated form of the wild cherry. In general, sweet cherry fruits are highly valued by consumers for the-

ir taste, attractiveness, nutritional value and health benefits.

The natural occurrence of the sweet cherry in Europe, from Sweden to Greece, Italy and Spain, has been documented (Faust & Surányi, 1997). As a result of centuries of natural and human selection, hundreds of local landraces have been raised under different environmental conditions and cultivated for local or family consumption (Marchese et al., 2017). The ubiquitous problem is that uncertainties regarding the origin and names of these genotypes, including the frequent occurrence of homonyms and synonyms, need to be solved. Thus, accurate identification of sweet cherry landraces is required in many countries worldwide.

The richness of Serbian sweet cherry landraces was observed, including the high risk of genetic erosion, as many of them exist as *in situ* individual specimens (Radičević et al., 2019; Marić et al., 2019, 2021). The same authors mentioned that this germplasm is adapted to different environments and potentially provides useful genetic variability in terms of agro-pomological traits, primarily fruit quality and resistance. An example of this is the recently released early-ripening sweet cherry cultivar 'Canetova' (Fotirić-Akšić et al., 2016), which was selected as a spontaneous seedling and is currently being studied in detail within the ongoing project entitled '*Genetic potential of Serbian autochthonous cherry genotypes for temperature-adaptable reproductive behaviour and nutraceutical value*' (CherryScRB), which is supported by the Science Fund of the Republic of Serbia – Program IDEAS (2022–2025). Besides, Serbian sweet cherry germplasm is still rather underutilized, which limits its potential as a source of useful traits for both commercial and breeding purposes.

Sweet cherry, like other *Prunus* species, exhibits gametophytic self-incompatibility (GSI), which is controlled by two multi-allelic linked genes of the *S*-locus (Bošković & Tobutt, 1996; Yamane et al., 2003). In this mechanism, the *S-RNase*, produced in the style, interacts in an allele specific manner with the SFB, a product of the pollen. With a few exceptions, most sweet cherries are self-incompatible, and certain pairs are cross-incompatible, either reciprocally or unilaterally. To date, more than 30 *S*-alleles have been revealed in cultivated and wild sweet cherry genotypes: S_1–S_{16} (Sonneveld et al., 2001, 2003), S_{17}–S_{22} (De Cuyper et al., 2005), S_{23}–S_{25} (Wünsch & Hormaza, 2004), S_{27}–S_{32} (Vaughan et al., 2008), and S_{34} and S_{37} (Szikriszt et al., 2013). Several *S*-alleles, were found to be indistinguishable from each other; therefore, a total of 22 unique *S*-alleles were described in cultivated sweet cherries (Schuster, 2020). In the latest update, the same author reported a total of 63 incompatibility groups (IGs) in 1,483 genotypes, then a group '0', described as universal donors, comprising 26 genotypes, and a group 'SC' consisting of 91 self-compatible sweet cherries. In addition, Kivistik et al. (2022) proposed four new IGs (64–67) for the *S*-genotypes S_3S_{17}, S_4S_{17}, S_5S_{17} and S_6S_{17}, detected in sweet cherry cultivars grown in Estonia. The cause of self-compatibility in sweet cherry is attributed to the artificial pollen-part mutation of SFB_3' and SFB_4' (Sonneveld et al., 2005), the natural pollen-part mutation of SFB_5' (Marchese et al., 2007), and the mutation of a non *S*-locus EMPaS02, which also leads to a loss of pollen function (Cachi & Wünsch, 2011). *S*-alleles revealed in wild sweet cherries, such as S_{10}, S_{13}, S_{16}, S_{17}, S_{19}, S_{21}, S_{22}, S_{30} (De Cuyper et al., 2005; Vaughan et al., 2008; Schuster, 2020), are rare, while others, like S_{27}–S_{29} and S_{31}–S_{32}, have not been found in cultivated genotypes so far (Vaughan et al., 2008; Schuster, 2020). Knowledge of the sweet cherry *S*-alleles and the correct assignment of cultivars to IGs are essential for both sweet cherry breeders and growers.

The first DNA markers for cross-(in)compatibility and self-compatibility, made available to breeders, were based on known base pair and insertion/deletion differences in the sequences of both genes of the *S*-locus (Quero-García et al., 2019). Therefore, consensus/allele-specific polymerase chain reaction (PCR)-based methods enabled the use of the *S*-locus as a genetic marker for genotyping and identification of domestic (released cultivars and landraces) and foreign sweet cherry cultivars at the Fruit Research Institute, Čačak (FRI) (Marić & Radičević, 2014; Radičević et al., 2015; Marić et al., 2017, 2019, 2021). The polymorphism of the *S*-locus has also been used for assessment of local sweet cherry germplasms in different countries of Europe and North and West Asia, where a high genetic diversity among landraces was found (Ipek et al., 2011; Ercisli et al., 2012; Cachi & Wünsch, 2014a; Lisek et al., 2015; Marchese et al., 2017; Schuster, 2020; Kivistik et al. 2022). In addition, the Italian sweet cherry landrace 'Kronio' and the Spanish 'Cristobalina' and 'Talegal Ahin' have so far been identified as sources of self-compatibility (Marchese et al., 2007; Cachi & Wünsch, 2014b). However, Mariette et al. (2010) pointed out that domestica-

tion and breeding have significantly reduced the diversity from wild cherries to landraces and further to modern sweet cherry cultivars.

Recently, interest in the collection and evaluation of sweet cherry landraces with good agronomic traits and their molecular characterization at the FRI has increased. Therefore, the aim of this study was to summarize all known data on the *S*-genotypes of Serbian sweet cherry landraces and to present new, previously unpublished genotype data.

Materials and Methods

Plant material and DNA extraction. Twenty-three sweet cherry landraces (refer to Table 1) were used in this study and were sampled on several occasions from orchards of individual growers in the regions of Čačak and Belgrade. Fresh young leaves of the three as-yet-unpublished or uncompleted landraces were collected in spring 2022, frozen in liquid nitrogen and stored at

Table 1. *S*-genotypes and incompatibility groups of sweet cherry landraces
Tabela 1. S-genotipovi i grupe inkompatibilnosti autohtonih genotipova trešnje

Location *Lokacija*	Landrace designation *Oznaka genotipa*	*S*-genotype *S-genotip*	IG	Reference for S-genotype *Referenca za S-genotip*
Čačak (town)/*Čačak (grad)*	'GT-1'	S_1S_5	XIV	Marić et al. (2019a)
Čačak (Jezdina)	'GT-2'	S_6S_9	X	Marić et al. (2019a)
Čačak (Jezdina)	'GT-3'	S_3S_5	VII	Marić et al. (2021)
Čačak (Trbušani)	'GT-4'	S_3S_6	VI	Marić et al. (2019a)
Čačak (Trbušani)	'GT-5'	S_3S_{12}	XXII	Marić et al. (2019a)
Čačak (Trbušani)	'GT-6'	S_3S_{12}	XXII	Marić et al. (2019a)
Čačak (Jezdina)	'GT-7'	S_2S_3	IV	Marić et al. (2019a)
Čačak (Prislonica)	'GT-8'	S_3S_9	XVI	Marić et al. (2019a)
Čačak (Prislonica)	'GT-9'	S_5S_{22}	0	This study
Čačak (Prislonica)	'GT-10'	S_3S_4	III	Marić et al. (2019a)
Čačak (Trbušani)	'GT-11'	S_6S_9	X	Marić et al. (2019a)
Belgrade (Grocka)	'GT-13'	S_3S_6	VI	Marić et al. (2019a)
Belgrade (Grocka)	'GT-14'	S_2S_3	IV	Marić et al. (2019a)
Belgrade (Grocka)	'GT-15'	S_2S_3	IV	Marić et al. (2019a)
Belgrade (Grocka)	'GT-16'	S_2S_3	IV	Marić et al. (2019a)
Čačak (Prislonica)	'GT-17'	S_3S_9	XVI	Marić et al. (2021)
Čačak (Prislonica)	'GT-18'	S_1S_2	I	Marić et al. (2021)
Čačak (Prislonica)	'GT-19'	S_4S_{13}	XLV	Marić et al. (2021)
Čačak (Prislonica)	'GT-20'	S_1S_4	IX	Marić et al. (2021)
Čačak (Prislonica)	'GT-21'	S_3S_6	VI	Marić et al. (2021)
Čačak (Ljubić)	'GT-23'	S_4S_5	V	Marić et al. (2021)
Čačak (Trbušani)	'GT-24'	S_3S_4	III	This study
Čačak (Prislonica)	'GT-25'	S_3S_5	VII	This study

-80°C. Genomic DNA was then isolated according to the method of Doyle & Doyle (1987). The extracted DNA was dissolved in TE buffer, treated with RNase A (Invitrogen, Groningen, the Netherlands), and kept at -20°C until used for PCRs.

PCR analysis for S-RNase genotyping. The determination of *S-RNase* alleles in sweet cherry landraces was based on the method described by Sonneveld et al. (2001, 2003). For PCRs, consensus primer pairs specific to the second intron of the *S-RNase* (PaConsII-F/R, Sonneveld et al., 2003) and specific primers for S_1*-RNase* to S_5*-RNase* and S_7*-RNase* alleles (Sonneveld et al., 2001, 2003) were used. Annealing temperatures for these *S-RNase* alleles were reported in the study by Marić and Radičević (2014). Sweet cherry cultivars with known *S-RNase* alleles were used as reference genotypes.

PCR analysis for SFB_4 *and* SFB_4*' genotyping*. Five sweet cherry landraces possessing S_4*-RNase* (Marić et al., 2019, 2021) were further analysed to distinguish the functional variant SFB_4 from the non-functional variant SFB_4', which has a 4 bp deletion (Zhu et al., 2004). Amplification of SFB_4 was performed using PaSFB4-F and PaSFB4-R specific primers (Ikeda et al., 2005) with PCR reactions and conditions according to the study by Sebolt et al. (2017). Compared to Zhu et al. (2004), the PCR reaction for amplification of the SFB_4' allele was modified as follows: ~100 ng of genomic DNA was used in a 25 µl reaction, containing 1× PCR reaction buffer, 3 mM $MgCl_2$, 200 µM dNTPs, 0.8 µM of BFP200 and BFP201 primers, and 0.625 U *Taq* DNA polymerase (Qiagen GmbH, Hilden, Germany). PCR conditions for the amplification in Mastercycler® nexus gradient (Eppendorf AG, Hamburg, Germany) were reported in the study by Sebolt et al. (2017), with an annealing temperature of 52°C and an extension time of 75 seconds.

Detection and visualization of DNA fragments. The PCR products obtained with the consensus primer pairs were separated on a 2% agarose gel (70 V cm^{-1} for 4 h), whereas products of the allele-specific PCRs for both genes of the *S*-locus were separated on a 1.5% agarose gel (70 V cm^{-1} for 2–3 h) using the Biometra Horizon 11.14 system (Analytik Jena GmbH, Jena, Germany). Visualization of the DNA bands was performed by ethidium bromide staining and under ultraviolet light using the BIO-PRINT-1500/26M imaging system (Vilber Lourmat, Collégien, France). A 1 Kb plus DNA ladder (Invitrogen, Groningen, the Netherlands) was used to determine the size of the DNA fragments.

Fragment analysis to identify the S-haplotype. To complete the identification of the *S*-haplotype in the landrace 'GT-9', fragments analysis using fluorescently labelled forward primers was performed to amplify both the *S-RNase* first intron (PaConsI-F, labelled with Yakima Yellow, and PaConsI-R2; Sonneveld et al., 2006) and the *SFB* intron (F-BOX5'A, labelled with 6-FAM, and F-BOX intronR; Vaughan et al., 2006). The first intron of *S-RNase* was amplified using the PCR reaction and conditions as reported by Sonneveld et al. (2006). The PCR reaction and amplification conditions for the *SFB* intron were carried out according to Cachi & Wünsch (2014a). Amplifications of both genes were checked on a 1.5% agarose gel, and PCR fragments were sized by comparison to 500 LIZ dye size standard (Thermo Fisher Scientific) using the Applied Biosystems SeqStudio™ Genetic Analyzer with SeqStudio™ Data Collection Software (Thermo Fisher Scientific Oy, Vantaa, Finland) and GeneMapper 6.1. (Thermo Fisher Scientific).

Results and Discussion

Identification of S-RNase genotypes in unpublished or uncompleted landraces. The *S-RNase* alleles in three sweet cherry landraces ('GT-9', 'GT-24' and 'GT-25') were identified in two steps: first, by amplification with consensus primers specific for the second intron, followed by using of allele-specific primer pairs in the subsequent step.

In the first step, the amplification of the second intron of *S-RNase* resulted in two PCR products, except for landrace 'GT-9', which gave two typical weak bands in the S_5*-RNase* position (~2,160 bp and ~1,650 bp). For this landrace, the assumption was made that the additional strong band (~2,200 bp) corresponded to the position of the second allele, possibly S_2*-RNase,* S_7*-RNase*, or S_{22}*-RNase*. However, the size of the PCR product for the second intron ranged from ~880 bp (S_1*-RNase* or S_3*-RNase*) to ~2,200 bp [S_2*-RNase,* S_5*-RNase* (top band), S_7*-RNase*, or S_{22}*-RNase*] (Table 2). This amplification allowed the identification of S_4*-RNase* and S_5*-RNase*, but discriminating between S_1*-RNase* and S_3*-RNase*, as well as distinguis-

Table 2. *S-RNase* alleles identification in the sweet cherry landraces with consensus and allele-specific primer pairs
Tabela 2. Identifikovanje alela S-RNaze primenom konsenzus i alel-specifičnih prajmera kod autohtonih genotipova trešnje

Landrace *Genotip*	Amplification using PaConsII-F and PaConsII-R primers *Amplifikacija sa PaConsII-F i PaConsII-R prajmerima*		Amplification using allele-specific primers *Amplifikacija sa alel-specifičnim prajmerima*					
	Allele 1 *Alel 1*	Allele 2 *Alel 2*	S_1 (820 bp)	S_2 (640 bp)	S_3 (960 bp)	S_4 (820 bp)	S_5 (300 bp)	S_7 (584 bp)
'GT-9'	S_5 (~2,160 + 1,650 bp)	S_2/S-/S_{22} (~2,300 bp)		–			+	–
'GT-24'	S_1/S_3 (~880 bp + 1,650 bp)	S_4 (~1,060 bp)	–		+	+		
'GT-25'	S_1/S_3 (~880 bp)	S_5 (~2,160 + 1,650 bp)	–		+		+	

hing *S_2-RNase* from *S_7-RNase* and *S_{22}-RNase,* required additional analysis. Difficulties in identifying these alleles using PCRs with consensus primers specific for the second intron of *S-RNase* were also stated by Sonneveld et al. (2003), Ipek et al. (2011), Ercisli et al. (2012), Lisek et al. (2015), and Marić et al. (2017). In addition, Marić et al. (2019) pointed out the need to use allele-specific primers, particularly for landraces whose *S*-genotypes have not yet been published.

To confirm the *S-RNase* alleles in the sweet cherry landraces studied, the genomic fragments were amplified using specific primers for *S_1-, S_2-, S_3-, S_4-, S_5-*, and *S_7-RNase* alleles. After the PCRs with allele-specific primers, the resulting DNA fragments ranged in size from 300 bp (*S_5-RNase* allele) to 960 bp (*S_3-RNase* allele) (Table 2). The absence of amplification with *S_1-RNase* specific primers confirmed the presence of *S_3-RNase* in the sweet cherries analysed. In the landrace 'GT-9', the lack of amplification with *S_2-RNase* and *S_7-RNase* specific primers indicated that this landrace possesses the *S_{22}-RNase* allele, which was later confirmed in this study through fragment analysis since a specific primer pair for this allele is not available. The size of the PCR products corresponding to the identified *S-RNase* alleles in the sweet cherry landraces studied was in agreement with the results reported by Sonneveld et al. (2001).

The *S-RNase* genotypes of two sweet cherries were determined by combining the results obtained with consensus and allele-specific primers and are published in this paper for the first time. The genotypes are as follows: S_3S_4 ('GT-24') and S_3S_5 ('GT-25'). Based on this analysis of the 'GT-9' landrace, the *S*-genotype remained S_5S_x, as reported by Marić et al. (2019), and the second allele was not revealed.

Identification of the S_4-haplotype in sweet cherry landraces. The predominant self-compatible mutation used in sweet cherry breeding and production is the pollen-part mutant *SFB_4'* allele. To ensure that sweet cherries possessing the *S_4-RNase* were correctly assigned to proper IGs, amplifications of *SFB_4* and *SFB_4'* alleles were conducted in five landraces ('GT-10', 'GT-19', 'GT-20', 'GT-23' and 'GT-24').

The use of PaSFB4-F and PaSFB4-R primers specific for the *SFB_4'* allele allowed the amplification of a fragment of 780 bp in all five sweet cherry landraces. On the other hand, the fragment of 453 bp, obtained upon amplification with BFP200 and BFP201 primer pair, corresponding to the *SFB_4'* allele, was only detected in the control cultivars ('Lapins' – *S_1B_4'* and 'Sunburst' – S_3S_4'), showing that these sweet cherry landraces possess the *SFB_4* functional variant. Therefore, these landraces are properly assigned to the following IGs previously reported by Schuster (2020): III ('GT-10' and 'GT-24'), V ('GT-23'), IX ('GT-20') and XLV ('GT-19'). The sizes of the PCR products corresponding to the *SFB_4* and *SFB_4'* alleles in the sweet cherry landraces and control cultivars studied were consistent with the results of Sebolt et al. (2017) and Zhu et al. (2004), respectively.

Fragment analysis for the identification of the S-haplotype in the landrace 'GT-9'. Fluorescent amplification products and the use of the automated sequencer

revealed two defined peaks for both genes of the *S*-locus (*S-RNase* and *SFB*) in the landrace 'GT-9'.

This study revealed fragments of 395 bp and 424 bp corresponding to the S_5-*RNase* and S_{22}-*RNase* (Figure 1), respectively, as well as fragments of 192 bp and 177 bp associated with the SFB_5 and SFB_{22} alleles. The size of the obtained fragments was the same as the fragment sizes reported for these alleles by Cachi & Wünsch (2014a) using the ABI PRISM 3130xl genetic analyzer (Applied Biosystems). However, the data obtained in this study detected a difference of +2 bp compared to the data generated using the ABI PRISM 310 genetic analyzer (Applied Biosystems), as reported by Vaughan et al. (2006) and Cachi & Wünsch (2014a). Specifically, Cachi & Wünsch (2014a) found that differences in equipment usage (comparing the ABI PRISM 310 and ABI PRISM 3130xl genetic analyzers) also resulted in size discrepancies among the various *S-RNase* and *SFB* alleles in sweet cherry.

The combination of the results obtained from the amplification of *S-RNase* with consensus and allele-specific primers, together with fragment analysis using fluorescently labelled forward primers for both the *S-RNase* first intron and the *SFB* intron, revealed the S_5S_{22} haplotype in the landrace 'GT-9'. Based on the identified *S*-haplotype, 'GT-9' is assigned to the group '0', described as universal donors. According to Schuster (2020), this haplotype has so far only been identified in the Hungarian cultivar 'Rita'.

Frequency of S-allele occurrence. This study presents an overview of *S*-alleles identification in 23 landraces sampled from the main sweet cherry growing regions of RS (Table 1). Among the assessed landraces, a total of 10 alleles (S_1, S_2, S_3, S_4, S_5, S_6, S_9, S_{12}, S_{13} and S_{22}) were found in this germplasm survey, resulting in the following 13 *S*-genotypes: S_1S_2 ('GT-18'), S_1S_4 ('GT-20'), S_1S_5 ('GT-1'), S_2S_3 ('GT-7', 'GT-14', 'GT-15' and 'GT-16'), S_3S_4 ('GT-10' and 'GT-24'), S_3S_5 ('GT-3' and 'GT-25'), S_3S_6 ('GT-4', 'GT-13' and 'GT-21'), S_3S_9 ('GT-8' and 'GT-17'), S_3S_{12} ('GT-5' and ?GT-6'), S_4S_5 ('GT-23'), S_4S_{13} ('GT-19'), S_5S_{22} ('GT-9') and S_6S_9 ('GT-2' and 'GT-11'). Considering the results obtained, the sweet cherry landraces were assigned to 12 IGs (I, III, IV, V, VI, VII, IX, X, XIV, XVI, XXII and XLV) and a group of universal donors ('0'), as previously reported by Schuster (2020); with the inclusion of the Serbian landraces, these IGs are extended. Group IV was the most common IG, comprising 17.4% of the assessed landraces.

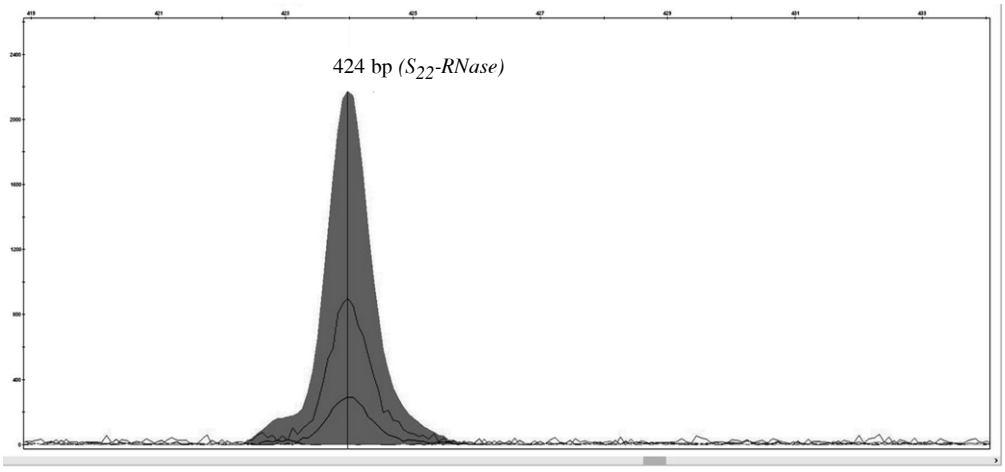

Figure 1. S_{22}-*RNase* fragment size identified in the sweet cherry landrace 'GT-9' by capillary electrophoresis with the Applied Biosystems SeqStudio™ Genetic Analyzer

Slika 1. Dužina fragmenta alela S_{22}-RNaze identifikovana kapilarnom elektroforezom na automatskom sekvenceru (Applied Biosystems SeqStudio™ Genetic Analyzer) kod genotipa trešnje GT-9

Table 3. The frequency of the *S-RNase* alleles identified in the sweet cherry landraces presented in Table 1
Tabela 3. Frekvencija alela S-RNaze identifikovanih kod genotipova trešnje prikazanih u Tabeli 1

S-RNase allele *Alel S-RNaze*	Number of allele identification times *Broj identifikacija alela*	*S-RNase* allele frequency (%) *Frekvencija alela S-RNaze*
S_1	3	6.5
S_2	5	10.9
S_3	15	32.6
S_4	5	10.9
S_5	5	10.9
S_6	5	10.9
S_9	4	8.6
S_{12}	2	4.3
S_{13}	1	2.2
S_{22}	1	2.2
Total:	46	100.0

The number of allele identification times and the frequency of *S-RNase* alleles in the assessed sweet cherry landraces are shown in Table 3. Among the 10 alleles identified, the most frequent allele in this material was S_3 (32.6%), and a relatively higher frequency of occurrence (> 10%) was observed for alleles S_2, S_4, S_5 and S_6 (with a frequency of 10.9%). Similarly, Ercisli et al. (2012) reported that S_3 (39%) was the highly frequent allele in 37 Croatian sweet cherry genotypes. Cachi & Wünsch (2014a) speculated that the geographical distribution of *S*-alleles could indicate a common origin or genetic relationship among genotypes in closer areas. Alternatively, the same authors suggested a possible link between specific *S*-alleles and adaptive traits related to diverse climatic conditions in different regions. In addition, the S_3 allele was the most frequent sweet cherry allele in Turkey, Czech Republic, Italy and Spain with a frequency of 29.6%, 34.4%, 25% and 38%, respectively (Ipek et al., 2011; Cachi & Wünsch, 2014a; Lisek et al., 2015; Marchese et al., 2017).

The occurrence of the S_2 allele in our study (10.9%) was slightly lower compared to the Turkish germplasm (14.8%) reported by Ipek et al. (2011), while the frequency of this allele in Croatian genotypes was 8% (Ercisli et al., 2012). In Italian sweet cherry germplasm, Marchese et al. (2017) found the rarity of the S_2, S_4 and S_5 alleles (1% frequency) in 186 local sweet cherry accessions from 12 different regions. Additionally, Cachi & Wünsch (2014a) reported the rarity of the S_2 allele in western Spain (1%) and noted that it was not found in the genotypes originating from the eastern and northern parts of this country. Regarding the S_4 allele, which occurred at a frequency of 10.9% in our study, this allele was more frequent in Spanish genotypes (from the northern part; 23%; Cachi & Wünsch, 2014a), Czech genotypes (21.9%; Lisek et al., 2015) and Turkish genotypes (13.6%; Ipek et al., 2011). In contrast, Ercisli et al. (2012) reported that the S_4 allele (2.5%) is relatively rare in Croatian genotypes. Our study revealed a higher frequency of allele S_5 (10.9%) compared to its occurrence in Turkish (4.9%) and Croatian (7%) germplasms (Ipek et al., 2011; Ercisli et al., 2012). A high frequency of S_5 (25.9%) was reported in Ukrainian germplasm (Lisek et al., 2015), which clearly distinguishes these cultivars from those from other regions of Europe. In addition to Italian genotypes (Marchese et al., 2017), the S_5 allele (1%) was also extremely rare in Spanish genotypes from the western part of the country, and was not found in the northern and eastern parts (Cachi & Wünsch, 2014a). The allele S_6, which occurred with a frequency of 10.9% in our study, was also common in Ukrainian (12.9%; Lisek et al., 2015), Turkish (11.1%; Ipek et al., 2011) and Croatian (8%; Ercisli et al., 2012) sweet cherry genotypes. A relatively high incidence of the allele S_6 was observed in landraces from Spain (26%) and Italy (19%) (Cachi & Wünsch, 2014a; Marchese et al., 2017).

In our study, the S_9 allele occurred with a frequency of 8.6%. A similar frequency of this allele was observed in Croatian (8%; Ercisli et al., 2012), Spanish (8% in the northern part of the country; Cachi & Wünsch, 2014a) and Turkish (7.5%; Ipek et al., 2011)

sweet cherry landraces, and less frequently in Italian germplasm (4%; Marchese et al., 2017). A relatively high incidence of the S_9 allele (20.4%) was observed in genotypes from Ukraine (Lisek et al., 2015). The incidence of the S_1 allele in our study (6.5%) was slightly higher than in Turkish and Croatian landraces (2.5%) reported by Ipek et al. (2011) and Ercisli et al. (2012), respectively, and in Italian germplasm (3%) reported by Marchese et al. (2017). The higher frequency of the S_1 allele was observed in genotypes from Czech Republic (25%) and Northern Spain (12%), as noted by Lisek et al. (2015) and Cachi & Wünsch (2014a), respectively. The rarity of the S_{12} allele in European cultivars was reported by Cachi & Wünsch (2014a). In our study, this allele occurred with a frequency of 4.3%, which was slightly higher than in Italian landraces (3%; Marchese et al., 2017) but lower than the frequency in Croatian (19%; Ercisli et al., 2012) and Turkish (7.4%; Ipek et al., 2011) sweet cherry genotypes. In our study, the frequency of alleles S_{13} and S_{22} was 2.2%. These two alleles were differently represented in Spanish and Italian sweet cherry landraces. Specifically, the frequencies of S_{13} and S_{22} were 19% and 0.3% in Italian landraces (Marchese et al., 2017) and 2% and 12% in Spanish germplasm (Cachi & Wünsch, 2014a).

As already mentioned, ten *S*-alleles (S_1 to S_6, S_9, S_{12}, S_{13} and S_{22}), were identified in our study. Ipek et al. (2011) and Cachi & Wünsch (2014a) also reported the same number of alleles in Turkish and Spanish landraces, respectively. It is noteworthy that, S_7 and S_{10} alleles were found instead of S_{13} and S_{22} in the Turkish landraces, while S_{16} was found instead of S_{12} in the Spanish landraces. Eight *S*-alleles were found in Croatian landraces (S_1 to S_6, S_9 and S_{12}; Ercisli et al., 2012), as well as in Czech and Ukrainian genotypes (S_1 to S_6, S_9 and S_{13}; Lisek et al., 2015). Six *S*-alleles (S_3 to S_6, S_{13} and S_{17}) were identified in Estonian sweet cherry cultivars (Kivistik et al., 2022). The most striking difference in Estonian germplasm was the occurrence of the S_{17} allele, which was found with a frequency of 53%. The highest polymorphism of *S*-loci was observed in Italian landraces; Marchese et al. (2017) reported 17 alleles, including S_5', S_7, S_{10}, S_{14}, S_{16}, S_{17} and S_{19}, in addition to the ten alleles identified in our study. However, the literature data do not yet provide an explanation for why certain alleles are frequent in specific sweet cherry germplasms while others are rare.

Conclusion

The overview of *S*-genotypes presented in this study expands the existing knowledge on the genetic diversity and frequency of occurrence of *S*-alleles in Serbian sweet cherry germplasm. Therefore, *S*-genotyping and assignment of sweet cherry landraces into incompatibility groups will allow them to be used for planning crosses and further improvement by breeders, as well as for direct use by growers for efficient and high-yielding fruit production. To avoid the loss of genetic richness of Serbian sweet cherry germplasm, which is characterized by high variability in terms of useful agronomic traits, it is important to collect and evaluate these landraces to enable their conservation in on-farm and *ex situ* collections. This work represents an important step in the conservation and characterization of sweet cherry germplasm, which is essential for the valorisation of genetic resources linked to the history and traditions of a territory.

Acknowledgement

This study was supported by the Ministry of Science, Technological Development and Innovation of the RS (Contract numbers: 451-03-47/2023-01/200215 and 451-03-47/2023-01/200042) and the Science Fund of the RS, GRANT No7739716: Genetic potential of Serbian autochthonous cherry genotypes for temperature-adaptable reproductive behaviour and nutraceutical value – CherrySeRB.

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DIVERZITET *S*-GENOTIPA AUTOHTONOG MATERIJALA TREŠNJE GAJENE NA PROSTORU CENTRALNE SRBIJE

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Rezime

Trešnja (*Prunus avium* L.) je samobesplodna vrsta voćaka, čija je auto-inkompatibilnost gametofitnog tipa, regulisana ekspresijom dva gena *S*-lokusa odgovornih za sintezu proteina *S*-RNaze i SFB, odnosno ženske i muške komponente specifičnosti prepoznavanja sopstvenog i nesopstvenog polena. Stoga je ekspresija gena *S*-lokusa od esencijalnog značaja za regulisanje genetičke kompatibilnosti sorti trešnje, koje su međusobno inkompatibilne ukoliko su istog *S*-haplotipa. Za trešnju je karakteristično da postoji određeni broj samooplodnih sorti koje mogu zametati plodove pri oprašivanju sopstvenim polenom i istovremeno, zahvaljujući mutaciji u *SFB* genu, predstavljaju univerzalne oprašivače za ostale sorte. Na osnovu *S*-genotipa, sorte trešnje su klasifikovane u do sada poznate 63 grupe inkompatibilnosti (označene od I do LXIII), kao i grupu samoplodnih sorti i grupu „0", kojoj pripadaju sorte retkog *S*-genotipa kojii im omogućava da budu potencijalno dobri oprašiuvači sortama drugih grupa. Identifikacija *S*-genotipova kod autohtonih genotipova trešnje je ključan korak u molekularnoj karakterizaciji materijala kolekcionisanog na prostoru Republike Srbije, i od ogromnog je značaja kako za oplemenjivače, tako i za proizvođače zainteresovane za proizvodnju kvalitetnih genotipova adaptiranih na određene agroekološke uslove. Cilj ovog istraživanja bio je da sažme poznate informacije i otkrije nove podatke o *S*-alelima kod 23 autohtona, odnosno genotipa trešnje nepoznatog porekla gajenih na različitim lokalitetima centralne Srbije. Genomska DNK ispitivanih genotipova trešnje je izolovana iz uzoraka mladog lista, primenom modifikovane CTAB mini prep metode. Upotreba lančane reakcije polimeraze (PCR) sa konsenzus prajmerima za drugi intron *S-RNaze*, prajmerima specifičnim za *S-RNazu* i određene alele *SFB* gena, zajedno sa DNK fragment analizom sa fluorescentno obeleženim prajmerima za amplifikaciju prvog introna *S-RNaze* i *SFB* introna, otkrila je 10 alela (S_1 do S_6, S_9, S_{12}, S_{13} i S_{22}) koji su generisali 13 *S*-genotipova: S_1S_2 (jedan genotip), S_1S_4 (jedan genotip), S_1S_5 (jedan genotip), S_2S_3 (četiri genotipa), S_3S_4 (dva genotipa), S_3S_5 (dva genotipa), S_3S_6 (tri genotipa), S_3S_9 (dva genotipa), S_3S_{12} (dva genotipa), S_4S_5 (jedan genotip), S_4S_{13} (jedan genotip), S_5S_{22} (jedan genotip) i S_6S_9 (dva genotipa). U ispitivanom autohtonom materijalu ustanovljeno je da je S_3 najzastupljeniji alel, sa frekvencijom od 32,6%, dok je S_2S_3 najčešći *S*-genotip, sa učestalošću od 17,4%. Na osnovu dobijenih rezultata, autohtoni genotipovi trešnje su svrstani u 12 grupa inkompatibilnosti i grupu univerzalnih donora („0"). Rezultati ovog rada pružaju važne informacije o njihovoj međusobnoj kompatibilnosti i diverzitetu *S*-lokusa kod genotipova trešnje decenijama gajenih na prostoru naše zemlje.

Ključne reči: *Prunus avium* L., autohtoni genotip, *S-RNaza*, *SFB*, *S*-haplotip, gametofitna samo-inkompatibilnost