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IDENTIFICATION OF SELF INCOMPATIBILITY GENOTYPES IN SWEET CHERRY COMMERCIAL CULTIVARS

SUMMARY

Sweet cherry exhibits RNase based gametophytic self-incompatibility system which is controlled by a single multi-allelic locus S. The identification of the self-(in) compatibility genotypes facilitates orchard management and is important for future breeding programmes. However, to ensure an effective pollination and fruit set compatible cultivars should have overlapping flowering time. The PCR-based detection of S-alleles was carried out in seventeen commercial sweet cherry cultivars of foreign origin by using two consensus primers for the RNase gene and two specific primers for the confirmation of the S_3 and S_4 allele. Totally, eight different S-alleles in ten S locus combinations were detected. 35.3 % of cultivars were self-compatible, whereas 59 % were assigned into seven individual self-incompatibility groups. S-alleles distribution linked with flowering and fruit ripening time was also analyzed. Most S-alleles (7) were identified in the mid-early blooming cultivars where the most frequent were S_3; S_4 and S_1.

On the other hand, late blooming cherry cultivars had the S_3S_{12} genotype, where the S_{12} allele was specific to these cultivars. The most frequent allele in the early fruit ripening cultivars was the allele S_4. The S_{14} allele was specific of cultivars with medium fruit ripening time while the alleles S_6 and S_{12} were detected only in the late fruit ripening cultivars. The synchronization of harvesting time within a commercial orchard would benefit to growers. Thus, identifying S genotype compatible cultivars, with the overlapping flowering and fruit ripening time would enable better orchard management.

Keywords: S-alleles, Sweet cherry, Self-incompatibility, flowering time, Albania

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INTRODUCTION

Sweet cherry (*Prunus avium* L.) is an economically important fruit species cultivated for its edible fruit used mainly for fresh consumption, jam, liqueur production and as ingredient in different processed foods. The cultivar structure in Albanian orchards had changed over years, affected by the consummator and trade preferences, leading to the introduction of several foreign cultivars. Successful pollination of the flowers is a requirement for a high yield in sweet cherry production. Many other factors, such as the same blooming time, climate conditions, the presence of pollinators and the right pollen donor are crucial factors for fertilization (Schuster *et al*., 2007).

Prunus avium L. exhibits an RNase gametophytic SI system (GSI), controlled by a single multi-allelic locus called S-locus (Tao *et al.,* 1999). Fertilization can only occur when the S-haplotype expressed by pollen and pistil are different (McCubbin and Kao, 2000). Cultivars showing the same S-genotype belong to the same incompatibility group, they cannot pollinate each other as they are cross-incompatible. In self-incompatible cultivars as sweet cherry, a self-incompatible cultivar must be planed with a pollinator one to ensure fruit set (Ikeda *et al*., 2004) and two to three cross-compatible cultivars must be planted to achieve effective cross-pollination (Schuster *et al.,* 2007). Therefore, the knowledge of the cross-incompatibility groups between sweet cherry cultivars is useful for growers, and breeders especially when specific crosses between two cultivars are desired.

S-allele genotyping employing molecular PCR methods has become a useful tool for the rapid determination of the incompatibility groups in sweet cherry cultivars (Patzak *et al*., 2019a). To identify S-RNase alleles several specific and consensus primers that amplify two S-RNase introns were designed (Tao *et al.,* 1999; Wiersma *et al*., 2001; Sonneveld *et al*., 2003; Sharma *et al*., 2014). In addition, Sonneveld et al. (2006) designed florescent primers that amplify first intron alleles enabling precise identification of alleles and accurate S-incompatibility group assignment. Several studies have addressed S-incompatibility evaluation and S-allele identification in sweet cherry cultivars grown in different countries employing PCR based S allele typing (Sonneveld *et al*., 2003; Ershadi and Moghadam, 2009, Ipek *et al*., 2011, Ganopoulos *et al,*. 2012, Sharma *et al*., 2014, Cachi and Wunsch, 2014). A total of 22 different S-alleles, 63 incompatibility groups and 91 self-compatible sweet cherries have been defined so far and reported in a summarized list of 1483 cultivars by Schuster (2012, updated 2020).

S-allele constitution in commercial sweet cherry cultivars in Albania have not been previously defined. The present study aims the characterization of S-allele combination, the identification of self-incompatibility groups of sweet cherry cultivars that constitute most of the commercial orchards by employing PCR based method. In addition, the distribution of the S-alleles linked with the flowering and fruit ripening time will be investigated to provide valuable insights into the compatible cultivars with overlapping flowering and fruit ripening time.

MATERIAL AND METHODS

Seventeen accessions of sweet cherries, *Prunus avium* L., of foreign origin well adapted and widely grown in local orchards were collected, their list and origin are given in table 1. The genomic DNA was isolated from 100-120mg fresh leaves using CTAB method described by Kump and Javornik (1996). The amplification was carried out using the consensus primers for the first and second intron, PaConsI (Sonneveld *et al*., 2003, 2006) and PaConsII (Sonneveld *et al*., 2003), respectively. The presence of the allele S3 and S4 were confirmed by using the allelic specific primers PasS3 and PaS4 (Sharma *et al*., 2014)) The PCR reaction was performed in a total volume of 15µl containing 1xPCR buffer, 2.5mM $MgCl_2$, 0.2mM dNTPs, 0.2mM each primer, 0.4U Taq polymerase (New England BioLabs). The reaction conditions for 1st intron consensus primer PaConsI were as follow: denaturation 94°C for 2 min, 10 cycles of 94°C for 10 sec, 58°C for 2 min, 68°C for 2 min, followed by 25 cycles of and 72°C for 5 min (Sonveld *et al.*, 2006), while for the 2nd intron consensus primer PaConsII, the amplification was carried out in 94°C for 2 min, 10 cycles of 94°C for 10s and 68°C for 2 min, followed by 25 cycles of 94°C for 10 sec, 58°C for 2 min and the final extension at 68°C for 2 min increasing 10 sec for each cycle (Sonneveld *et al.*, 2003). The thermal conditions for specific primers PaS4 and PaS3 were initial denaturation 95°C for 2 min, 36 cycles of 30 sec at 94°C, 45 sec at 58-61°C, 2 min at 72°C and final elongation 72°C 10 min (Sharma *et al.*, 2014). The PCR products were run in different concentration of agarose gel in 1x TAE, stained with ethidium bromide at 9 v/cm for 40 to 60 min; 2% agarose gel for the PaConsI-F/PaConsI-R2, 1.3% agarose gel for PaConsII-F/PaConsII-R, while the PCR amplification products of specific primers PaS3 and PaS4were differentiated in 1.2 % agarose gel. The images were captured with GeneSmart UV gel documentation system and the size estimation of fragments was performed against 1kb standard using PyElph version 1.4 software.

The correlation between distribution of the S alleles and ripening time of the cherry fruit was investigated. The ripening period data about fourteen cherry cultivars used in this study was previously studied (Lazaj and Ferraj, 2020) and included a time span of five weeks, from April to June.

RESULTS AND DISCUSSION

The analysis of 17 sweet cherry cultivars with S locus consensus and specific primers for RNase first and second intron, identified in total eight different S-alleles in ten locus combinations (Table 1).

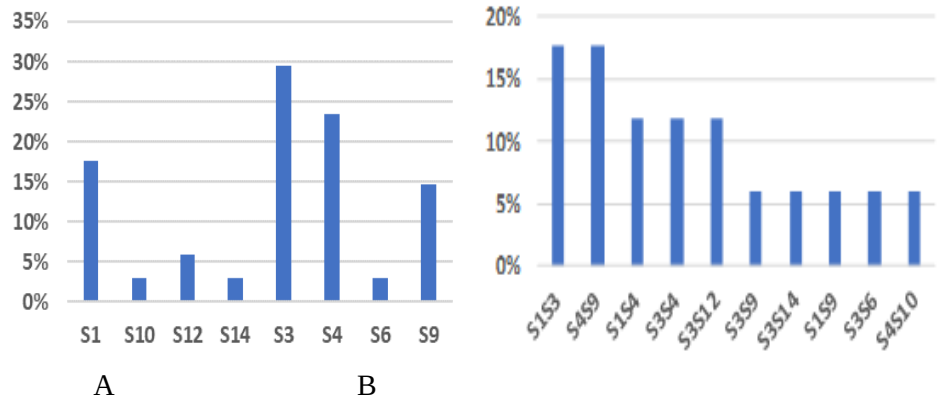
The size of S alleles generated by the amplification of our sample set with first intron consensus PaConsI primer ranged from 230-450 bp. The low difference between fragments some alleles had similar size, hence their accurate identification was difficult. However, the PCR products obtained using the second intron consensus PaConsII primer ranged from 570 - 2300bp, herein the S-allele identification was easier compared with the first intron amplified fragments, since the fragments run distantly from each other in the agarose gel

enabling accurate discrimination. Furthermore, the specific PaS3 and PaS4 primers were used to confirm the presence of these two specific alleles, the estimated size of obtained S3 and S4 alleles was 325bp, and 390bp, respectively. The allele had the expected sizes as reported by Sharma *et al.* (2014) confirming their presence in studied cherries genotypes.

Table 1. S genotypes and self-in compatibility groups of sweet cherry cultivars

Cultivar	Origin	S locus genotype determined with consensus primers	S3 and S4 specific primers confirmation		(In)compatibility group
			S_3	S_4	
Celeste	Canada	$S_1S_{4'}$		+	SC
Grace Star	Italy	$S_{4'}S_9$		+	SC
Mora di Cazzano	Italy	S_3S_{14}	+		LIV
Ferrovia	Italy	S_3S_{12}	+		XXII
Lala Star	Italy	S_1S_3	+		II
Regina	Germany	S_1S_3	+		II
Lapins	Canada	$S_1S_{4'}$		+	SC
Black Star	Italy	S_1S_3			II
New Star	Canada	$S_3S_{4'}$	+	+	SC
Burlat	France	S_3S_9	+		XVI
Kordia	Czech Republic	S_3S_6	+		VI
Feu 5	France	S_4S_{10}		+	-
Sweet Heart	Canada	S_3S_4	+		SC
Schneider	Germany	S_3S_{12}	+		XXII
Sweet Early	Italy	S_1S_9			XVIII
Big Star	Spain	$S_{4'}S_9$		+	SC
Crazy Star	Italy	S_4S_9		+	XXI

The analysis identified a total of eight different alleles, the most frequent were S_3 (29.4%), S_4 (23.4%), S_1 (17.6%) and the allele S_9 (14.7%), followed by S_{12} (5.9%). The rarest alleles were the alleles S_6, S_{10}, $S_{14,}$ which were identified only once in three different cultivars 'Kordia', 'Feu 5' and 'Mora di Cazzano', respectively (Figure 1). The number of detected S alleles is considered high considering the small number of genotypes analyzed (17 sweet cherry genotypes). However, high diversity of S alleles observed in present study might be because of the different origin of the analyzed cherry genotypes.



A B

Figure 1. Frequency of S alleles is sweet cherry cultivars (A), and S genotypes in sweet cherry cultivars (B)

Across 17 sweet cherry cultivars there were identified ten allele combinations (Table 1, Figure 1). Genotypes S_1S_3 and S_4S_9 had a frequency of 17.6%, followed by S_1S_4, S_3S_4 and S_3S_{12} with a frequency of 12%. The genotypes S_3S_9, S_3S_{14}, S_1S_9, S_3S_6 and S_4S_{10} were detected only in once in 'Burlat', 'Mora di Cazzano', 'Sweet Early', 'Kordia', 'Feu 5', respectively. A total of eight groups of incompatibility were found from 47 groups reported by Schuster M (2012). Six cultivars were classified as self-compatible (SC), 'Celeste', 'Grace Star', 'Lapins', 'New Star', 'Sweet Heart' and 'Big Star', ten cultivars resulted incompatible, while Feu5 was not assigned to any group presented by Schuster M. (2012, updated 2020) (Table 1).

The most frequent incompatibility groups were II (S_1S_3) and XXII (S_3S_{12}) with 18.8% and 12.5% percent, respectively. Whereas the other groups of incompatibility groups had a frequency of 6.3% each.

To obtain a successful pollination the S-alleles constitution of sweet cherry cultivars within production orchards is crucial, however the overlapping of blooming periods of the cultivars within the orchards is also relevant to obtain a high rate of pollination (Patzak *et al*., 2019b).

Therefore, the data obtained in a previous study on the time of flowering and fruit ripening time (Lazaj and Ferraj, 2020) on fourteen cultivars included in our study were used as a basis to investigate the distribution of S alleles linked to blooming and fruit ripening time.

The classification of 14 out of our 17 sample set of sweet cherry cultivars based on the data published by Lazaj and Ferraj (2020), according to the flowering onset and ripening time was given in the table 2.

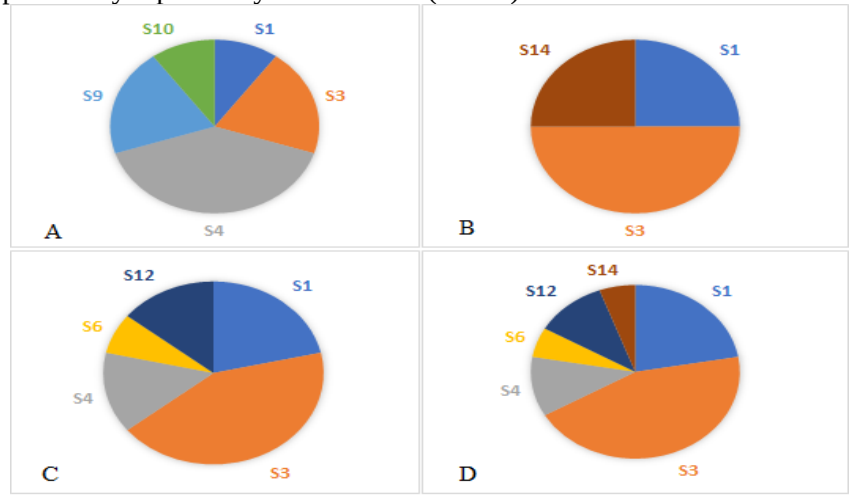
The correlation between distribution of the S alleles and ripening time of the cherry fruit was investigated. The ripening period data about the cherry cultivars was previously studied (Lazaj and Ferraj, 2020) and included a time span of five weeks, from from April 28th to June 6th.

Table 2. Ripening period of cultivars

Cultivars		
Early ripening	**Medium ripening**	**Late ripening**
Burlat	Black Star	Lala Star
Feu 5	Mora di Cazzano	Lapins
Celeste		Kordia
Grace Star		Ferrovia
New Star		Sweet Heart
		Regina
		Schneiders
Cultivars		
Early flowering	**Mid flowering**	**Late flowering**
Burlat	Lapins	Schneiders
Black Star	Mora di Cazzano	Ferrovia
Feu 5	Celeste	
Grace Star	New Star	
Regina	Kordia	
Sweet Heart		
Lala Star		

**data on fruit ripening and flowering time of sweet cherry culticars were obtained by Lazaj and Ferraj (2020)*

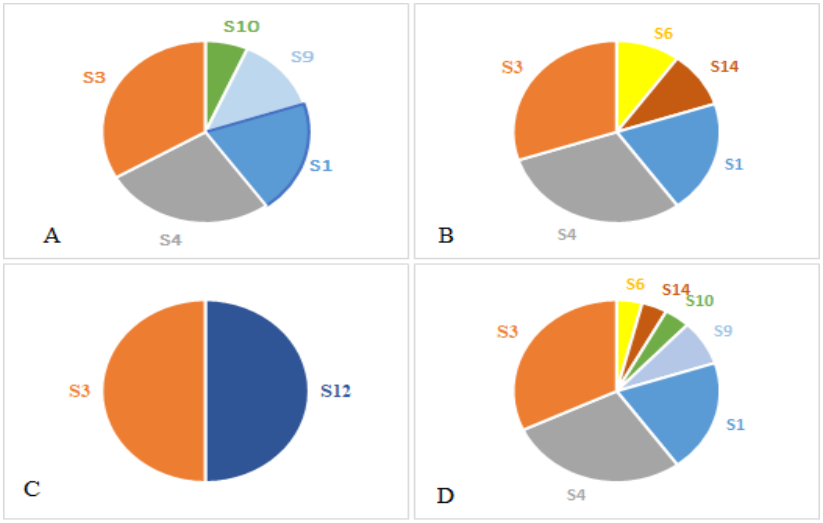
The most frequent allele in the early season ripening cultivars was S_4 while the allele S_9 and S_{10} were detected only in cultivars with early ripening in the 1st, 2nd and 3rd week. The allele S_{14} was detected only in cultivars with medium ripening period in the 3rd to 4th week. The alleles S6 and S12 were detected only is late ripening cultivars from the 3rd to 5th week (Figure 2). The occurrence of S_9 in the 2nd and 3rd week of cherry ripening and of the S_{12} allele in the 3rd to 6th was also previously reported by Patzak *et al*. (2019b).



**A-early; B-medium; C-late; D-medium-late fruit ripening*

Figure 2. S-alleles distribution of cherry cultivars linked with ripening time

The most S-alleles (7) were found in the mid-early blooming cultivars in which the flowering time overlap. Overlapping in flowering period is a critical element in the cultivar composition of cherry orchards as these cultivars are considered suitable for crosses if their S-genotype is compatible (Radičević *et. al*., 2015). In these cultivars the most frequent S-alleles were S_3; S_4 and S_1 (Figure 3).



**A-early; B-medium; C-late; D-early-mid flowering time*

Figure 3. S-alleles distribution of cherry cultivars linked with flowering time

The allele S_{10} was detected only in early blooming cultivars (‘Feu 5’) while S_{14} and S_6 allele were detected only in mid-flowering period cultivars ‘Mora di Cazzano’ and ‘Kordia’, respectively. On the other hand, late blooming cherry cultivars had S_3S_{12} genotype and the S12 allele was found only in these cultivars (‘Shneiders’ and ‘Ferrovia’).

CONCLUSIONS

Sweet cherry is an economically important fruit tree species, widely cultivated for its edible fruits. The high rate of successful pollination would provide greater production of fruits and benefits for growers. The harmonization of the S-compatibility cultivars with the flowering time would provide better management of the orchards. Therefore, 17 sweet cherry cultivars were analyzed by S locus consensus and specific primers for the S-RNase gene to determine their composition of the S-allele and identifying self-incompatibility genotypes. Herein, we report the identification of ten S-allele and seven groups of self-incompatibility identified across 17 analyzed cultivars. The distribution of the s-alleles linked with the flowering and fruit ripening time for 14 of the analyzed cultivars has provided valuable insights into the compatible cultivars with overlapping flowering and fruit ripening time. This study represents the first S-genotype characterization of sweet cherry cultivars in Albania which will be of

use as reference for future wider studies in S-allele detection as well as in future selection of individuals for cross -fertilization in breeding programmes.

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