Journal of Agricultural Sciences (Belgrade)
Vol. 69, No. 2, 2024
Pages 169-180

https://doi.org/10.2298/JAS2402169N
UDC: 634.233-152.6:575.17(55)
Original scientific paper

DETERMINATION OF S-ALLELES IN IRANIAN SOUR CHERRY (*PRUNUS CERASUS*) USING CONSENSUS PRIMERS

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Abstract: Sour cherry is a tetraploid species, and gametophytic self-incompatibility (SI) operates in this species in the same way as in other stone fruit trees. However, while self-compatibility is most common in sour cherry and self-compatibility (SC) genotypes are rarely found, both SI and self-compatible (SC) types are selected in sour cherry. In this work, S-alleles have been identified for 70 sour cherry accessions and cultivars from the Shabestar regions of Iran, with S-genotypes of 68 cultivars identified for the first time. To identify the S-alleles, PCR-based methods were used. The amplification of the different alleles using combinations of the four forward primers (PaConsI-F, PruC2, PaConsII-F, EM-PC2consFD) and the five reverse primers (PruC4R, PCE-R, PaConsI-Rnew, PaConsII-R, EM-PC5consRD) revealed that they were the most useful for the identification of the sour cherry alleles. Nine known S-haplotypes (S6, S4, S9, S6m, S6m2, S24, S26, S35, S36a) were identified. In our study, alleles S6, S9, and S6m2 had a high frequency. It was shown that the consensus primers can be used to detect incompatibility alleles in sour cherry accessions. Our study has proved that the diversity of S alleles between the studied accessions was low, indicating low genetic diversity, which could also be due to the selection of superior genotypes by farmers.

Key words: sour cherry, self-incompatibility, S-RNase, consensus primer.

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Introduction

Gametophytic self-incompatibility (GSI) is a common genetic mechanism that promotes outcrossing in flowering plants. Most rosaceous fruit trees exhibit SI which is controlled by a single locus (S-locus) with multiple alleles (Franklin-Tong and Franklin, 2003). Sour cherry (2n = 4x = 32) is an allotetraploid species produced by hybridization of the diploid sweet cherry and tetraploid ground cherry (*Prunus fruticosa* Pall.), which displays gametophytic self-incompatibility (GSI), whereby the specificity of self-pollen rejection is controlled by alleles of the stylar and pollen specificity genes, S-RNase and SFB (S haplotype-specific F-box protein gene), respectively (Tsukamoto et al., 2006). Although most sour cherries are self-compatible (SC), some sour cherry selections are self-incompatible (SI), and therefore require a pollinator cultivar to achieve fruit set.

As each S-RNase allele has two introns varying in length, it was possible to differentiate S-RNase alleles by detecting intron size polymorphisms by DNA tests. Molecular methods based on PCR now allow precise S-genotyping and are being used to S-genotype many diverse sour cherry collections and wild populations (Lansari and Iezzoni, 1990; Yamane et al., 2001; Hauck et al., 2002; Yamane et al., 2003; Boskovic et al., 2006; Tsukamoto et al., 2006, 2008a, 2010; Khadivi-Khub et al., 2014; Lisek et al., 2015; Sebolt et al., 2017). To date, 34 S-haplotypes have been reported in sweet cherry and sour cherry, numbered S_1 to S_{37} as S_8, S_{11}, S_{15}, S_{23}, S_{24} and S_{25} were discontinued when they were later found to be identical to S_3, S_5, S_7, S_{14}, S_{22} and S_{21}, respectively. In sour cherry, 12 functional S-haplotypes (S_1, S_4, S_6, S_9, S_{12}, S_{13}, S_{14}, S_{16}, S_{26}, S_{33}, S_{34}, and S_{35}) were identified. Unlike sweet cherry, most sour cherry cultivars are SC due to the presence of either stylar-part mutants (designated Sm) or pollen-part mutants (designated S′) that disrupt either the stylar S-RNase or pollen SFB, respectively. So far, 9 non-functional S-haplotypes (S_1', S_{6m}, S_{6m2}, S_{13m}, $S_{13'}$, S_{36a}, S_{36b}, S_{36b2} and S_{36b3}) were identified in sour cherry (Hauck et al., 2006a, b; Tsukamoto et al., 2006, 2008a, 2010; Yamane et al., 2003; Sebolt et al., 2017). In fact, only non-functional variants were detected for S_{36}. As predicted by Hauck et al. (2006b), the presence of at least two of these non-functional S-haplotypes in the S-genotype results in SC (Yamane et al., 2001; Tsukamoto et al., 2006, 2010; Sebolt et al., 2017). The genetic switch from SI to SC in sour cherry results from the accumulation of non-functional S-haplotypes according to the one-allele-match model (Hauck et al., 2006a). In this model, the match between a functional pollen-S in the 2x pollen and its cognate functional S-Rnase in the style results in an incompatible reaction (Tsukamoto et al., 2008b). A similar reaction would occur regardless of whether the pollen contained a single functional pollen-S gene or two different pollen-S genes. The absence of any functional match results in a compatible reaction. Thus,

for successful self-fertilization, the 2x pollen must contain two non-functional S-haplotypes.

In this study, PCR analysis was performed using primers based on the conserved sequences of *S*-RNases to characterize the S-genotype of 70 sour cherry accessions and cultivars, including 68 accessions of Iranian sour cherry cultivars whose S-allele constitution had not been previously described.

Material and Methods

Plant material

Sixty-eight local Iranian sour cherry accessions from eight different regions of Shabestar, East Azerbaijan province, were used in this study (Figure 1, Table 1). In addition, two Hungarian sour cherry cultivars ('Cigany' $S_{6m}2S_9S_{26}S_{36b2}$; 'Erdi Botermo' $S_4S_{6m}S_{35}S_{36a}$) with known S-genotype were used as S-allele size standards.

Figure 1. Geographical locations of the collection sites of the sour cherry accessions.

Table 1. Sour cherry accessions analyzed by consensus primers and their S-genotypes identified in this study.

Origin	Accession name	S-genotyping with consensus primers		S-genotype
		1st intron	2nd intron	
Ali Beyglu	A01	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A02	S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A03	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A04	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A05	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A06	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A07	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A08	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A09	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A10	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A11	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A12	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A13	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A14	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A15	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A16	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A17	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A18	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	A19	S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
Chehrgan	Ch01	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ch02	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ch03	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ch04	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ch05	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ch06	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ch07	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ch08	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ch09	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ch10	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ch11	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
Til	Ti01	S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ti02	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ti03	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ti04	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ti05	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ti06	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ti07	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ti08	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Ti09	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
Nazarlou	N01	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	N02	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	N03	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	N04	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	N05	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	N06	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	N07	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$

Continuation Table 1. Sour cherry accessions analyzed by consensus primers and their S-genotypes identified in this study.

Origin	Accession name	S-genotyping with consensus primers		S-genotype
		1st intron	2nd intron	
Qareh Tappeh	Q01	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Q02	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Q03	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Q04	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Q05	S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Q06	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Q07	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
Tasuj	T01	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	T02	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	T03	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	T04	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	T05	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	T06	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
Sharafkhaneh	Sh01	-	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Sh02	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Sh03	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Sh04	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	Sh05	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
Sis	S01	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	S02	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	S03	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
	S04	S_6/S_{6m2}, S_9	S_{6m2},S_9, S_{24}	$S_{6m2}S_9S_{24}S_?$
Hungarian cultivars	Cigany	S_9/S_{6m2}	$S_{6m2}/S_9/S_{36b2}/S_{26}$	$S_{6m2}S_9S_{26}S_{36b2}$
	Erdi botermo	S_{6m}	$S_{35}/S_{36a}/S_4$	$S_4S_{6m}S_{35}S_{36a}$

DNA isolation and consensus PCR analysis

Young, unfolded leaves were collected in the spring, frozen in liquid nitrogen, lyophilized, and stored at – 80ºC. Genomic DNA was extracted using the procedure described by Doyle and Doyle (1990). DNA concentrations and purification parameters were measured using a Picodrop 200 spectrophotometer (PicoDrop µl spectrophotometer, Cambridge, United Kingdom). PCR was conducted in a volume of 25 µL containing 50 ng of template DNA, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 3 mM MgCl2, 200 µM dNTP, 0.8 µM of each primer, and 0.625 units of Taq DNA polymerase. PCR reactions were set up on ice and the tubes were transferred to an Applied Biosystems thermal cycler (Life Technologies GmbH, Germany) and run for 4 min at 94ºC initial denaturation 10 cycles of 10 s at 94ºC, 2 min at the annealing temperature of each primer (see Table 2 for temperature) and 2 min at 68ºC, followed by 25 cycles of 10 s at 94ºC, 2 min at the annealing temperature and 2 min at 68ºC with 10 s per cycle to the 68ºC extension step. The PCR products were separated on a 1.3% agarose gel for about 2 h at 80

V. Also, for the first intron, PCR amplification was carried out for 4 min at 94ºC, 35 cycles of 1 min at 94ºC, 1 min at the annealing temperature of each primer (see Table 2 for temperature) and 1 min at 72ºC, with a 5-min final extension step at 72ºC. The products were run on a 2% agarose gel, for about 3 h at 70 V as described above. DNA bands were visualized by the ethidium bromide staining and observed and documented using the Geldoc G:box (Syngene, USA) gel imaging system. Fragment lengths were estimated by comparison with the 1 kbp DNA ladder. PCR products were purified using the NucleoSpin® gel and PCR clean-up kit (Macherey-Nagel, Germany) and sequenced by Macrogen Inc (Seoul, Korea). All PCR reactions and analyses were repeated at least twice.

Table 2. Nucleotide sequences of the consensus primers for PCR amplification of the first and the second intron of sour cherry S-RNases.

Primer	Sequence 5′ → 3′	Amplified region	Annealing temperature	References
PaConsI-F	(C/A)CTTGTTCTTG(C/G)TTT(T/C)GCTTTCTTC	1st intron	55 ºC	Sonneveld et al., 2003
PaConsI-R2 new	GCCATTGTTGCACAAATTGA			Sonneveld et al., 2006
Pru-C2	CTA TGG CCA AGT AAT TAT TCA AAC C	2nd intron	58 ºC	Tao et al., 1999
Pru-C4R	GGATGTGGTACGATTGAAGCG			
Pru-C2	CTA TGG CCA AGT AAT TAT TCA AAC C	2nd intron	58 ºC	Tao et al., 1999
PCE-R	TGTTTGTTCCATTCGCYTTCCC			
PaConsII-F	GGCCAAGTAATTATTCAAACC	2nd intron	58 ºC	Sonneveld et al., 2003
PaConsII-R	CA(T/A)AACAAA(A/G)TACCACTTCATGTAAC			
EM-PC2consFD	TCA-CMA-TYC-ATG-GCC-TAT-GG	2nd intron	55 ºC	Sutherland et al., 2004
EM-PC5consRD	CAA-AAT-ACC-ACT-TCA-TGT-AA-CAR-C			

Results and Discussion

As a result of the analyses, S-alleles were identified for the first time for 68 sour cherry accessions from the Shabestar regions. The amplification of the different alleles using combinations of the four forward primers (PaConsI-F, PruC2, PaConsII-F, EM-PC2consFD) and the five reverse primers (PruC4R, PCE-R, PaConsI-Rnew, PaConsII-R, EM-PC5consRD) proved to be the most useful for the identification of sour cherry alleles (Figure 2). In this study, the S-genotypes of all the 68 sour cherry accessions were analyzed (Table 1). Nine known S-haplotypes (S_4, S_6, S_9, S_{6m}, S_{6m2}, S_{24}, S_{26}, S_{35}, S_{36a}) previously reported in sour cherry were identified. ‘Cigany’ and ‘Erdi Botermo’ as the standard sour cherry cultivars showed S_4, S_{6m2}, S_9, S_{26}, S_{35} and $_{S36a}$ alleles, confirming the results of previous studies for these cultivars (Yamane et al., 2001; Yamane et al., 2003;

Hauck et al., 2006b; Tsukamoto et al., 2006, 2008a, 2010). In the present study, the S_6 and S_9 alleles of sour cherry could be easily distinguished in Iranian sour cherry according to other studies (Khadivi-Khub et al., 2014). Yamane et al. (2001) and Boskovic et al. (2006) also observed combinations of the S-RNases S_1, S_4, S_6, S_9 and S_{13} in sour cherry. Another study has also shown that the alleles S_1, S_2, S_3, S_4, S_5, S_6 and S_9 occurred with high frequency (Lacis et al., 2008; Ipek et al., 2011). S-allele frequency per region revealed that some S-alleles are more frequent in some regions and some only in a specific geographical area. It has also been confirmed that the Extremadura group of sour cherry has a higher frequency of alleles S_3 and S_6 (Wunsch and Hormaza, 2004), while the allele S22 is more frequent in the Mediterranean regions, as previously found in accessions from the Alicante region (Gisbert et al., 2008). The reason for the differences in the distribution of S-haplotypes in the different regions of Europe could be the common origin of the cultivars in isolated areas or the relationship between specific S-haplotypes with adaptive traits and the climatic conditions of the different areas (Cachi and Wünsch, 2014).

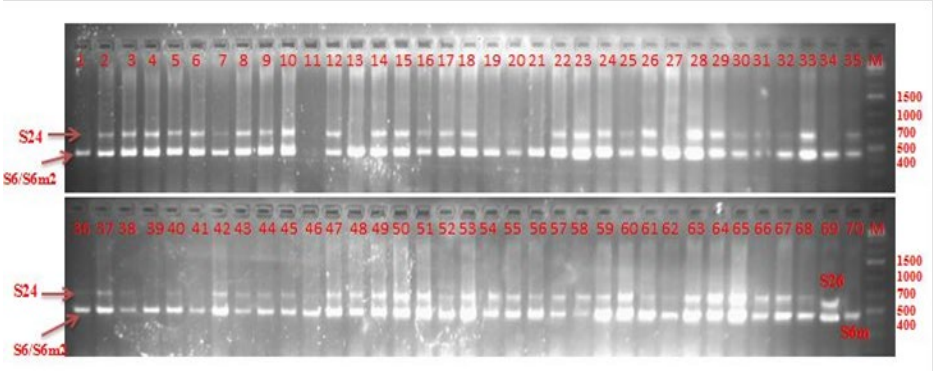

Figure 2. Amplification of intron II of the S-RNase gene in sour cherry accessions with the Pruc2 – PruC4R consensus primers. (M) 1 kb ladder, genotypes: 1 (Ch8), 2 (A12), 3 (T03), 4 (Q02), 5 (A11), 6 (N10), 7 (A16), 8 (Ti02), 9 (T07), 10 (A10), 11 (A15), 12 (Ch05), 13 (A03), 14 (A14), 15 (Q05), 16 (A17), 17 (T03), 18 (Ch12), 19 (Ch09), 20 (Ch01), 21 (Ch10), 22 (S02), 23 (S03), 24 (Ch04), 25 (Ch02), 26 (N09), 27 (Sh02), 28 (Ti08), 29 (N02), 30 (A13), 31 (Ti09), 32 (Ti02), 33 (Ti05), 34 (Ch6), 35 (A09), 36 (S01), 37 (A02), 38 (Ti04), 39 (Ch03), 40 (Ch07), 41 (T04), 42 (N03), 43 (N05), 44 (A07), 45 (N01), 46 (A04), 47 (N04), 48 (Q07), 49 (Ti10), 50 (T5), 51 (S04), 52 (A19), 53 (A01), 54 (Ti01), 55 (Q04), 56 (Sh01), 57 (Ti06), 58 (T01), 59 (A08), 60 (A05), 61 (Q06), 62 (A09), 63 (Q01), 64 (Sh03), 65 (Sh05), 67 (Q03), 68 (Sh04), 69 (Cigany), 70 (Erdi Botermo).

According to the obtained results, other methods should be used to distinguish among S_6-, S_{6m}- and S_{6m2}-RNases. For this purpose, PCR tests with the dCAPS marker (Derived Cleaved Amplified Polymorphic Sequences) and the insert-specific primer pair are required. The dCAPS markers can be used to distinguish S_{6m2}-Rnase from S_{6-} and S_{6m}-RNases. The PCR tests with the combination of dCAPS markers and insert-specific primer pairs are also required to distinguish among non-functional variants of the S_{36}-haplotype (Tsukamoto et al., 2010; Sebolt et al., 2017).

Polymerase chain reaction (PCR)-based S allele genotyping methods using consensus primers and separation of the products on agarose gels to detect length variations of the two introns have been reported (Tao et al., 1999, Wiersma et al., 2001, Sonneveld et al., 2003). Consensus primers will be especially useful for genotyping accessions of unknown parentage. As shown, consensus primers developed based on the sweet and sour cherry S-RNase sequences also lead to PCR amplification in a range of cherry species and could therefore be useful for studies of self-incompatibility in these species. S-allele identification by PCR analysis is highly reproducible and, thus, this technique can be used to complement other molecular methods in cherry cultivar identification (Wunsch and Hormaza, 2004; Khadivi-Khub et al., 2014; Lisek et al., 2015; Sebolt et al., 2017), almond (Channuntapipat et al., 2003; Tamura et al., 2000), apricot (Yaegaki et al., 2001; Hajilou et al., 2006, Muñoz-Sanz et al., 2017). The use of consensus primers enabled the identification of the majority of the S-alleles. However, the identification of some S-alleles was difficult because the resulting products were similar in size to those of other S-alleles, or no amplification products were present. 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 purified and sequenced, verifying the results obtained with the consensus primers and unambiguously identifying the S-alleles in all the sour cherry genotypes tested.

However, in some accessions, only two alleles could be amplified, and some alleles could only be amplified with one of the primer pairs. One of the reasons for this is gaps or mismatches in the primer sequence, which can lead to inefficient annealing and, therefore, no amplification (Sonneveld et al., 2004; Cachi et al., 2017). Even if two alleles are very similar in size, the bands obtained after PCR amplification could be difficult to distinguish under standard agarose gel electrophoresis (Vaughan et al., 2006). This is the case for the S_6 and S_{24} alleles, which differ in 20 bp and cannot be differentiated after amplification with PruC2–PruC4R.

Knowledge of the S-genotypes of different accessions further enables the determination of relationships within the cultivated fruit species. The center of origin of cherry was described in Asia Minor including all of Transcaucasia, Iran and Turkmenistan. The diversity of the S-genotypes of the investigated cultivars

from parts of Turkey and Iran is lower than that of the European cherry genotypes. This could be due to the extensive fruit growing conditions and the absence of cherry breeding activities in the past. Furthermore, the number of studied cherry genotypes is lower compared to the data from Europe (Schuster, 2012).

Conclusion

Our study has proved that the diversity of S alleles between the studied accessions was low, indicating low genetic diversity, which could also be due to the selection of superior genotypes by farmers. Therefore, S-allele analysis is recommended for the local sour cherry cultivars of other regions of Iran, as well as for wild cherry, which is native to Iran. Our results demonstrate the limitation of using exclusively consensus primers for the reliable determination of S-genotypes.

Acknowledgements

We thank the Agriculture Biotechnology Research Institute of Iran and the Center of Excellence for Temperate Fruit Research in University of Tabriz for financial assistance.

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Received: February 11, 2023
Accepted: May 13, 2024

ODREĐIVANJE S-ALELA U IRANSKOJ VIŠNJI (*PRUNUS CERASUS*) POMOĆU KONSENZUSNIH PRAJMERA

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R e z i m e

Višnja je tetraploidna vrsta, koju karakterise gametofitna inkopatibilnost (SI) koja kod ove vrste deluje na isti način kao i kod ostalih koštičavih vrsta voćaka. Iako je samooplodnost (SO) najčešća kod višnje, auto-inkompatibilni (SI) genotipovi se retko nalaze, kod višnje su selekcionisani i SO i SI tipovi. U ovom radu, S-aleli su identifikovani za 70 genotipova i sorti višnje iz regiona Šabestara u Iranu, pri čemu je 68 genotipova identifikovano po prvi put. Za identifikaciju S-alela korišćene su metode zasnovane na lančanoj reakciji polimeraze. Amplifikacija različitih alela korišćenjem kombinacije četiri prednja prajmera (PaConsI-F, PruC2, PaConsII-F, EM-PC2consFD) i pet obrnutih prajmera (PruC4R, PCE-R, PaConsI-Rnew, PaConsII-R, EM-PC5consRD) je pokazala da su oni najkorisniji za identifikaciju alela višnje. Identifikovano je devet poznatih S-haplotipova (S6, S4, S9, S6m, S6m2, S24, S26, S35, S36a). U našem istraživanju aleli S6, S9 i S6m2 su imali visoku učestalost. Pokazalo se da se konsenzusni prajmeri mogu koristiti za detekciju alela inkompatibilnosti kod genotipova višnje. Naše istraživanje je dokazalo da je raznovrsnost S-alela među proučavanim genotipovima niska, ukazujući na nisku genetičku raznovrsnost, što takođe može biti rezultat odabira superiornih genotipova od strane proizvođača.

Ključne reči: višnja, auto-inkompatibilnost, S-RNaza, konsenzusni prajmer.

Primljeno: 11. februara 2023.
Odobreno: 13. maja 2024.

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