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S-allele diversity in *Prunus* L. *Cerasus* subgenus from Iran

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ABSTRACT

In this study, *S*-allele diversity of eight wild and two commercial species of the *Cerasus* subgenus in Iran was investigated using two primer pairs. A high level of *S*-allele polymorphism was detected among and within the species evaluated. Furthermore, most of wild species showed 2–4 alleles based on *S*-allele primers and may be considered as tetraploid. Sweet cherry cultivars, Siah-Mashhad, Siah-Shabestar, Takdaneh-Mashhad, Siah-Daneshkadeh and Protiva showed S_3S_{12}, S_3S_{12}, S_3S_{12}, S_3S_5 and S_3S_4 combinations, respectively, allele S_3 showing the highest frequency. Three Iranian sweet cherry cultivars had the same allelic combination (S_3S_{12}) that the same ancestor in genealogy of these cultivars may explain the loss of diversity observed at the *S*-locus. Wild cherry (mazzard) accessions showed wide range of alleles such as S_1, S_2, S_7, S_{14} and S_{20} and unknown alleles, while sour cherries showed S_6, S_9, S_{13} and S_{27} alleles. In conclusion, the conservation of these highly diverse native species of Iranian wild *Cerasus* germplasm is recommended for future breeding activity.

1. Introduction

The species of *Cerasus* subgenus, belonged to *Prunus* genus, grow wild throughout the temperate climate zone on the Northern Hemisphere. Five species are distributed in the area from Europe to Western Asia (Koehne, 1912; Rehder, 1940), while two species are found in North America (Kawasaki, 1991). Distribution center of subgen. *Cerasus* is Eastern Asia. Nine species and some varieties are distributed in Japan (Kawasaki, 1991), whereas 33 species and six varieties are distributed in China (Kawasaki, 1991). Also nine wild species are found in Iran (Rechinger, 1969; Mozaffarian, 2002). These wild species can be used as sources of new genes or alleles to introduce in rootstocks or probably the cultivated cherries. These species may also be used to improve the cultivated *Prunus* species as gene donors because of their wide hybridization capability with each other (Khadivi-Khub et al., 2012).

Self-incompatibility in flowering plants prevents self-fertilization through the rejection of pollen from the same plant. This trait prevents inbreeding. In many species, molecular and classical genetic studies indicate that specificity determination in pollen and style is controlled by a single locus with multiple alleles, the *S*-locus. In gametophytic self-incompatibility (*GSI*) systems, pollen specificity is determined by the pollen's own haploid genotype. *GSI* is the most common system, and has been claimed in more than 60 families of flowering plants (Kao and Cubbin, 1996). Molecular studies of the *S*-locus in three plant families, Solanaceae, Rosaceae and Scrophulariaceae, have shown that the *S*-locus product in pistils is a basic glycoprotein

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Table 1
Studied plant materials and their produced *S*-alleles.

No.	Cultivar, accession	Species	Collection area	PaConsI-F/PaConsI-R2	FBOX5′A/FBOXintronR
1	Siah-Mashhad	*P. avium*	Iran, Karaj	236/347	204/188
2	Siah-Shabestar	*P. avium*	Iran, Karaj	236/347	204/188
3	Takdaneh-Mashhad	*P. avium*	Iran, Karaj	236/347	204/188
4	Siah-Daneshkadeh	*P. avium*	Iran, Karaj	236/395	204/193
5	Protiva	*P. avium*	Iran, Karaj	236/452	204/192
6	Ambrunes[a]	*P. avium*	Spain, Zaragoza	236/444	204/182
7	Vic[a]	*P. avium*	Spain, Zaragoza	346/452	189/192
8	Stella[a]	*P. avium*	Spain, Zaragoza	236/452	204/192
9	Mazzard1	*P. avium*	Iran, Gorgan	431/382	192/174
10	Mazzard2	*P. avium*	Iran, Gorgan	410/382	192
11	Mazzard3	*P. avium*	Iran, Gorgan	346/194	184/171
12	Mazzard4	*P. avium*	Iran, Gorgan	346/194	184/171
13	Mazzard5	*P. avium*	Iran, Gorgan	346/330	191/178
14	Sour cherry1	*P. cerasus*	Iran, Karaj	445/370/358	190/183/180
15	Sour cherry2	*P. cerasus*	Iran, Karaj	445/370/358	190/183/180
16	Montmorency[a]	*P. cerasus*	Spain, Zaragoza	444/367/297	193/189/183
17	Daneshkadeh	*P. cerasus*	Iran, Karaj	446/368/297	193/189/183
18	Mahaleb1	*P. mahaleb*	Iran, Khoramabad	395/386	192/178
19	Mahaleb2	*P. mahaleb*	Iran, Khoramabad	372/347	195
20	Mahaleb3	*P. mahaleb*	Iran, Khoramabad	372/347	195
21	Mahaleb4	*P. mahaleb*	Iran, Khoramabad	387/231	183
22	Mahaleb5	*P. mahaleb*	Iran, Khoramabad	372/346	194
23	St.Lucia64	*P. mahaleb*	Spain, Zaragoza	391/432	193/187
24	Brachy1	*P. brachypetala*	Iran, Sisakht	386/400/377/373	190/187/178/169
25	Brachy2	*P. brachypetala*	Iran, Sisakht	381/377/360/194	190/187/179
26	Brachy3	*P. brachypetala*	Iran, Sisakht	386/400/377	190/187/178/169
27	Brachy4	*P. brachypetala*	Iran, Sisakht	415/389/386/194	190/178
28	Brachy5	*P. brachypetala*	Iran, Sisakht	386/345/341	195/188/178
29	Incana1	*P. incana*	Iran, Ahar	403/350/183	192/188/163
30	Incana2	*P. incana*	Iran, Ahar	393/375/343/339	194/189
31	Incana3	*P. incana*	Iran, Ahar	393/375/343/339	194/189
32	Incana4	*P. incana*	Iran, Ahar	406/393	190/187/173
33	Incana5	*P. incana*	Iran, Ahar	350/321/288	188/172/163
34	Mic.diffusa1	*P. microcarpa* subsp. *diffusa*	Iran, Yasouj	338/230	189
35	Mic.diffusa2	*P. microcarpa* subsp. *diffusa*	Iran, Yasouj	387/371	197/188
36	Mic.diffusa3	*P. microcarpa* subsp. *diffusa*	Iran, Yasouj	390	179
37	Mic.diffusa4	*P. microcarpa* subsp. *diffusa*	Iran, Yasouj	397/362	190/179
38	Mic.diffusa5	*P. microcarpa* subsp. *diffusa*	Iran, Yasouj	390	179
39	Mic.micro1	*P. microcarpa* subsp. *Microcarpa*	Iran, Arak	342	191
40	Mic.micro2	*P. microcarpa* subsp. *microcarpa*	Iran, Arak	423/344	187/178
41	Mic.micro3	*P. microcarpa* subsp. *microcarpa*	Iran, Arak	342	191
42	Mic.micro4	*P. microcarpa* subsp. *microcarpa*	Iran, Arak	253/182	188/169
43	Mic.micro5	*P. microcarpa* subsp. *microcarpa*	Iran, Arak	344	195/178
44	Pseudo1	*P. pseudoprostrata*	Iran, Mashhad	411/391	190
45	Pseudo2	*P. pseudoprostrata*	Iran, Mashhad	413/404/345	195/193/188
46	Pseudo3	*P. pseudoprostrata*	Iran, Mashhad	404/391	195/186
47	Pseudo4	*P. pseudoprostrata*	Iran, Mashhad	411/360	192
48	Pseudo5	*P. pseudoprostrata*	Iran, Mashhad	404/363/360	193/187/173
49	Yazdiana1	*P. yazdiana*	Iran, Mehriz	313	194
50	Yazdiana2	*P. yazdiana*	Iran, Mehriz	313	194
51	Yazdiana3	*P. yazdiana*	Iran, Mehriz	313	194
52	Yazdiana4	*P. yazdiana*	Iran, Mehriz	313	194
53	Yazdiana5	*P. yazdiana*	Iran, Mehriz	313	194

[a] These cultivars with known *S*-genotype were used as control: Stella (S_3S_4'), Vic (S_2S_4), Ambrunes (S_3S_6), Montmorency ($S_6S_{13\ m}S_{27a}$).

with ribonuclease (*RNase*) activity, the *S-RNase* (Anderson et al., 1986; Sassa et al., 1996; Xue et al., 1996). Richman (2000) directly determined the putative *S*-genotypes of plants sampled from Solanaceae by sequence polymorphism at the *S-RNase* locus. Vieira and Charlesworth (2002) revealed sequence variation at the *S*-locus in Scrophulariaceae. In the Rosaceae, molecular techniques for identifying *S*-alleles by allele-specific PCR are now being developed for crossing and breeding of fruit cultivars (Sonneveld et al., 2001; Wiersma et al., 2001; Sonneveld et al., 2006; Vaughan et al., 2006).

Furthermore, polymerase chain reaction (PCR) technique employing primers designed to anneal within conserved regions flanking each of the *S*-locus introns have been employed to identify *S-RNase* alleles in cultivars of sweet cherry (Sonneveld et al., 2003; Tao et al., 1999; Wiersma et al., 2001; Wünsch and Hormaza, 2004a), populations of wild cherry (De Cuyper et al., 2005; Vaughan and Russell, 2004; Schueler et al., 2006; Vaughan et al., 2007, 2008) and sour cherry (Yamane et al., 2001; Hauck et al., 2002; Boskovic et al., 2006). This method has circumvented some of the problems associated with determining alleles from conventional controlled crosses (Tao et al., 1999; Sonneveld et al., 2003).