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Genetic variation of *S*-alleles in wild almonds and their related *Prunus* species

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Abstract

Wild almond genotypes are a rich source of desirable characteristics which can be useful to almond breeding programs. However, almonds express self-incompatibility which affects breeding parent selection. Self-incompatibility is controlled by a multi-allelic, single gene (*S*-locus). Here, the *S*-alleles were studied in 96 wild almonds and related *Prunus* species from 10 taxonomic groups. Polymerase chain reactions (PCR) were carried out using six sets of primers including: three degenerate primer pairs (PaConsI-F(FAM)/EM-PC1consRD, PaConsI-F(FAM)/EM-PC3consRD, EM-PC2consFD/EM-PC3consRD), one general primer pair AS1II/AmyC5R, one allele specific primer pair (CEBASf/AmyC5R), and one set of multiplex primers (AS1II/CEBASf/AmyC5R). The number of amplified bands (155) and their size ranges were higher than in previous reports. The primers, including the allele specific (CEBASf/AmyC5R), did not amplify any self-compatibility allele (*Sf*) among samples evaluated. Sizes of amplified alleles were compared with previous reports in almond and labeled accordingly. Alleles *S*9, *S*2, *S*13, and *S*25 had the highest frequencies (12.26, 8.39, 7.74, and 7.74 percent respectively). Alleles *S*16, *S*17, *S*18, *S*19, *S*22, and *S*28 were not observed in examined samples and alleles *S*15 and *S*26 had a low frequency (0.65). Presumably, the geographical distribution of these species had influenced their *S*-allele frequencies. The taxonomic groups were clustered by using amplified allele sizes from the first degenerate primers (PaConsI-F(FAM)/EM-PC1consRD). The dendrogram revealed that *S*-alleles were more similar within a taxonomic group than among groups.

Keywords: Geographical distribution, *S*-allele, Self-compatibility, Self-incompatibility, *S*-RNase, Taxonomic groups

Introduction

Almonds are primarily self-incompatible (SI) (Tufts, 1919; Gregory, 2004). The self-incompatibility prevents self-fertilization (Socias i Company and Felipe, 1992) which can be an advantage in evolution as it increases out-crossing (Ortega and Dicenta, 2003) and prevents inbreeding depression (de Nettancourt, 1977; Halasz et al., 2005). The out-crossings increase almond diversity, distribution and adaptation to different geographical locations (Kester and Gradziel, 1996; Woolley et al., 2000). This high diversity and rich genetic pool is useful in almond breeding, as valuable characteristics can be found in almond germplasm (Popov et al., 1929). Knowledge of self-incompatibility status of almonds and their related species is very important in breeding programs (Vezvaei, 1994). *S*-alleles identity is particularly important for designing crosses and choosing parents for breeding self-compatible cultivars suitable for monoculture orchards with reduced need for honeybee pollinators (Batlle et al., 1997; Channuntapipat et al., 2003; Martinez-Gomez et al., 2003; Lopez et al., 2006; Ortega et al., 2006). These studies can also help determine the origin of cultivated and wild almonds (Martinez-Gomez et al., 2003; Zeinalabedini et al., 2007a).

In almond, incompatibility is controlled by a single multi-allelic *S*-locus (Gagnard, 1954; Channuntapipat et al., 2001; Halasz et al., 2008). Incompatibility loci have five conserved regions (C1-C5), a hypervariable region (RHV) and two introns (Ushijima et al., 1998). While *S*-alleles of almond can be determined by several approaches, molecular methods can determine *S*-alleles faster, and more precisely. This technique is being routinely used for the identification of cross-incompatibility groupings for current almond cultivars (Gradziel et al., 2001a; Ortega and Dicenta, 2003; Sanchez-Perez et al., 2004). To date, 44 *S*-alleles have been detected in cultivated almonds (Kodad et al., 2008 and Ortega et al., 2009). Tamura et al. (2000) initially used the general primers (AS1II and AmyC5R) for amplification of *S*-alleles in almond. Ma and Oliviera (2001) and Channuntapipat et al. (2001, 2002, 2003) subsequently designed other primers for amplification of new *S*-alleles. Sanchez-Perez et al. (2004) introduced the allele

specific primer CEBASf for studying self-compatibility (Sf) alleles. They also used multiplex PCR by simultaneous application of AS1II, CEBASf and AmyC5R, to detect 10 incompatible and one self-compatible *S*-alleles in almond. Application of primers based on the conserved sequences of the first and second introns have been shown to amplify several *S*-alleles (Channuntapipat et al., 2001, 2003). Ortega et al. (2005) have used two degenerate primers (EM-PC2consFD and EM-PC3consRD) to obtain the sequences of 24 self-incompatible alleles (*S*1-*S*24) and one self-compatible allele from European and American almond cultivars. They also increased the number of identified *S*-alleles in almond to 29 (Ortega et al., 2006). Boskovic et al. (2007) introduced *S*30 as a wild type allele (St) of self-compatible *P. webbii* and Halasz et al. (2008) reported *S*31 in a Hungarian almond cultivar. Kodad et al. (2008) studied the diversity of *S*-RNases in Spanish cultivars and introduced 5 new alleles (*S*32-*S*35), while Ortega et al. (2009) identified another nine alleles (*S*36-*S*44) in seven Iranian almond cultivars.

In wild almond relatives, Martinez-Gomez et al. (2003) studied *S*-alleles in 12 related almond species. They identified six alleles from wild species (*P. tangutica*, *P. bucharica*, *P. argentea*, *P. webbii*, *P. kuramica,* and *P. pentunikowii*). However, in some species (*P. scoparia*, *P. mira*, *P. kasuensis*, *P. tenella*, and *P. glandulosa*) no band was detected. They reported a close genetic distance between cultivars and wild almonds supporting earlier reports by Kester et al. (1990). These results also supported the possibility of transferring *S*-alleles conferring self-compatibility from wild almonds to cultivated ones, as proposed by Gradziel et al. (2001b). There have been several studies on self-compatibility in *P. webbii* (Gradziel et al., 2001b; Channuntapipat et al., 2003; Socias i Company et al., 2004; Sanchez and Oliviera, 2005; Boskovic et al., 2007; Banovic et al., 2009). Sanchez and Oliviera (2005) revealed that *P. webbii* is the source of *S*f allele in the self-compatible Italian cultivar 'Tuono'. In characterizing *S*-alleles in wild almond species, Zeinalabedini et al. (2007b) detected 13 self-incompatible alleles in *P. elaegnifolia, P. hauskunechtii, P. scoparia,* and *P. lycioides*, and one *S*f allele in *P. elaegnifolia*. However, Elahi et al. (2008) could not amplify any self-compatible alleles in *Prunus* species (*P. elaegnifolia, P. hauskunechtii, P. scoparia, P. lycioides, P. orientalis,* and *P. communis*) native to Iran using allele specific primers (CEBASf/AmyC5R).

The aims of this study were a) to determine presence of self-compatible and incompatible alleles in selected wild almonds and their related *Prunus* species by PCR method using well specified primers, and b) to study the genetic relationship of the species based on their amplified *S*-alleles.

Materials and Methods

Plant material

Leaves from 75 plants (15 wild almond species) collected from different parts of Iran along with 21 samples from related *Prunus* species (15 samples from University of California, Davis; two samples from University of Georgia and four samples from University of Florida) were dried by silica gel or lyophylisation. The 96 collected samples (Table 1) were from 10 different taxonomic groups (*Amygdalus*, *Orientalis*, *Spartioides*, *Dodecandra* (*Lycioides*), *Chameamygdalus*, *Leptopus*, *Almond spp.*, peach × almond hybrid, peach and plum).

DNA extraction

Total genomic DNA was extracted from dried leaves by CTAB protocol based on Doyle and Doyle (1987) as described in Ortega and Dicenta (2003). DNA quantity and quality were determined by spectrophotometer and agarose gel electrophoresis.

Polymerase Chain Reactions

Six sets of primers including three pairs of degenerate primers (PaConsI-F(FAM)/EM-PC1consRD, PaConsI-F(FAM)/EM-PC 3consRD, and EM-PC2consFD/EM-PC3consRD), one pair of general incompatibility primers (AS1II/AmyC5R), one pair of self specific primers (CEBASf/AmyC5R) and one set of multiplex primers (AS1II/CEBASf/AmyC5R) were used for polymerase chain reactions (Table 2).

The forward primer PaConsI-F was designed from the signal peptide region of cherry *S*-RNases (Sonneveld et al., 2003) and primer EM-PC1consRD was from the first conserved region, both flanking the first intron. Primers EM-PC2consFD and EM-PC3consRD were designed based on the second and third conserved regions of *Prunus S*-alleles at the East Malling research station of England (Ushijima et al., 1998; Sutherland et al., 2004) to amplify across the second intron, which is variable among genotypes. Primers PaConsI-F and EMPC3consRD amplify from the signal peptide region to conserved region 3 (Ortega et al., 2006). AS1II and AmyC5R were designed based on the C1 and C5 conserved regions and have common sequences in Rosaceae *S*-RNases (Tamura et al., 2000). The allele specific primer CEBASf was designed from the intron sequence of the Tuono S*f* allele (Sanchez-Perez et al., 2004).
The primers were prepared using kits from MWG (Biotech, Germany) and Eurofins (US). Reactions were done in Eppendorf 5341 Mastercycler®epgradient thermocycler with optimized PCR conditions (Table 3 and 4).

Gel electrophoresis

The PCR products (6 µL) and 1 µL loading dye (Blue/Orange 6X-Promega) were electrophoresed on 1.5 % agarose gel (GPG/LE, American Bioanalytical), containing 0.05 % Ethidium Bromide, in 1× TAE buffer with 100 V for 6 hours. Six micro-litters of 2 Log DNA (Biolab, 1 ng DNA) were used as the size marker. The gels were stained with 0.0005 % Ethidium Bromide solution for 1 h, exposed to the UV light and photographed with PcImage program (Foto/Analyst®PcImage) in Photoanalyser (Foto/UV® 300). All the procedures were run twice for null or faint bands.

The bands of all amplified alleles were sized by Quantity One software (4.6.6, Basic, BioRad for gel analysis). As the software cannot predict the band sizes very accurately, all bands amplified with first intron primers (PaConsI-F(FAM)/EM-PC1consRD) were sized with an automated sequencer (ABI 3730XL) because of their small sizes (Ortega et al., 2005). To do this, the fluorescent forward primer (PaConsI-F(FAM)) was used and band sizes were coded with Gene Marker software (GeneMarker®, Version 1.80). The alleles were labeled as candidate *S*-alleles, by comparing their sizes (for each primer sets) with available reports on almond *S*-alleles (de Cuyper et

Table1. *Prunus* species evaluated for *S*-allele variation, their origin and taxonomic group

Accession No.	Species	Taxonomic group	Country	State/ Province	City
128	*P. argentea*	*Orientalis*	USA.	California	Davis
31	*P. brahuica*	*Dodecandra*	IRAN	West Azarbaijan	Mohabad
129	*P. bucharica*	*Amygdalus*	USA.	California	Davis
115	*P. carduchorum*	*Orientalis*	IRAN	West Azarbaijan	Urmieh
116	*P. carduchorum*	*Orientalis*	IRAN	West Azarbaijan	Urmieh
120	*P. carduchorum*	*Orientalis*	IRAN	Kordestan	Saghez
33	*P. communis*	*Amygdalus*	IRAN	West Azarbaijan	Sardasht
47	*P. communis*	*Amygdalus*	IRAN	Kordestan	Saghez
48	*P. communis*	*Amygdalus*	IRAN	Kordestan	Saghez
66	*P. dulcis*	*Amygdalus*	IRAN	West Azarbaijan	Soufian
67	*P. dulcis*	*Amygdalus*	IRAN	West Azarbaijan	Gharehbag
68	*P. dulcis*	*Amygdalus*	IRAN	Fars	Estahban
69	*P. dulcis*	*Amygdalus*	IRAN	Fars	Niriz
89	*P. dulcis*	*Amygdalus*	IRAN	Tehran	Kordan
90	*P. dulcis*	*Amygdalus*	IRAN	Tehran	Hashtgerd
126	*P. dulcis* (cv.Carmel)	*Amygdalus*	USA.	California	Davis
125	*P. dulcis* (cv.Nonpareil)	*Amygdalus*	USA.	California	Davis
127	*P. dulcis* (cv.Texas, Mission)	*Amygdalus*	USA.	California	Davis
143	*P. dulcis* × *P. persica* (Nonpareil ×Florida King)(1444)	Peach×Almond	USA.	Florida	Gainesville
124	*P. dulcis* × *P. persica* (Tardy Nonpareil×97-47C)	Peach×Almond	USA.	Florida	Gainesville
58	*P. eburnea*	*Dodecandra*	IRAN	Fars	Niriz
15	*P. elaeagnifolia*	*Orientalis*	IRAN	Fars	Darab
43	*P. elaeagnifolia*	*Orientalis*	IRAN	Kordestan	Kamyaran
44	*P. elaeagnifolia*	*Orientalis*	IRAN	Kordestan	Kamyaran
64	*P. elaeagnifolia*	*Orientalis*	IRAN	Fars	Darab
98	*P. elaeagnifolia*	*Orientalis*	IRAN	Chaharmahal va Bakhtiari	Lordegan
106	*P. elaeagnifolia*	*Orientalis*	IRAN	Fars	Fasa
108	*P. elaeagnifolia*	*Orientalis*	IRAN	Fars	Fasa
60	*P. elaegnifolia*	*Orientalis*	IRAN	Fars	Niriz
61	*P. elaegnifolia*	*Orientalis*	IRAN	Fars	Niriz
109	*P. erioclada*	*Dodecandra*	IRAN	Fars	Fasa
102	*P. erioclada*	*Dodecandra*	IRAN	Chaharmahal va Bakhtiari	Farsan
111	*P. erioclada*	*Dodecandra*	IRAN	Fars	Fasa
27	*P. fenzliana*	*Amygdalus*	IRAN	West Azarbaijan	Urmieh
28	*P. fenzliana*	*Amygdalus*	IRAN	West Azarbaijan	Urmieh
53	*P. fenzliana*	*Amygdalus*	IRAN	West Azarbaijan	Makoo
118	*P. fenzliana*	*Amygdalus*	IRAN	West Azarbaijan	Urmieh
103	*P. fenzliana*	*Amygdalus*	IRAN	Fars	Fasa
130	*P. fenzliana*	*Amygdalus*	USA.	California	Davis
141	*P. geniculata*	Plum	USA.	Florida	Gainesville
136	*P. glandulosa*	Plum	USA.	California	Davis
112	*P. glauca*	*Spartioides*	IRAN	Fars	Fasa
62	*P. hauskonechtii*	*Amygdalus*	IRAN	Kordestan	Marivan
63	*P. hauskonechtii*	*Amygdalus*	IRAN	Kordestan	Marivan
40	*P. hauskonechtii* (var. pubescence)	*Amygdalus*	IRAN	Kordestan	Sanandaj
142	*P. kansuensis*	Peach	USA.	Georgia	Attapulgus
79	*P. keredjensis*	*Spartioides*	IRAN	Tehran	Tehran
16	*P. korshinskyi*	*Amygdalus*	IRAN	Fars	Darab
21	*P. korshinskyi*	*Amygdalus*	IRAN	West Azarbaijan	Urmieh
22	*P. korshinskyi*	*Amygdalus*	IRAN	West Azarbaijan	Urmieh
23	*P. korshinskyi*	*Amygdalus*	IRAN	West Azarbaijan	Urmieh
24	*P. korshinskyi*	*Amygdalus*	IRAN	West Azarbaijan	Urmieh
25	*P. korshinskyi*	*Amygdalus*	IRAN	West Azarbaijan	Urmieh
26	*P. korshinskyi*	*Amygdalus*	IRAN	West Azarbaijan	Urmieh
105	*P. korshinskyi*	*Amygdalus*	IRAN	Fars	Fasa
107	*P. korshinskyi*	*Amygdalus*	IRAN	Fars	Fasa
110	*P. korshinskyi*	*Amygdalus*	IRAN	Fars	Fasa
29	*P. kotschii*	*Orientalis*	IRAN	West Azarbaijan	Urmieh
30	*P. kotschii*	*Orientalis*	IRAN	West Azarbaijan	Urmieh
32	*P. kotschii*	*Orientalis*	IRAN	West Azarbaijan	Sardasht
49	*P. kotschii*	*Orientalis*	IRAN	Kordestan	Baneh
119	*P. kotschii*	*Orientalis*	IRAN	Kordestan	Saghez
131	*P. kuramica*	*Amygdalus*	USA.	California	Davis
55	*P. lycioides*	*Dodecandra*	IRAN	Fars	Niriz
13	*P. lycioides (var.horrida)*	*Dodecandra*	IRAN	Fars	Niriz
18	*P. lycioides (var.horrida)*	*Dodecandra*	IRAN	Fars	Darab
56	*P. lycioides var.horrida*	*Dodecandra*	IRAN	Fars	Darab
34	*P. nairica*	*Dodecandra*	IRAN	West Azarbaijan	Oshnavieh
38	*P. nairica*	*Dodecandra*	IRAN	Kordestan	Sanandaj
39	*P. nairica*	*Dodecandra*	IRAN	Kordestan	Sanandaj
121	*P. orientalis*	*Orientalis*	IRAN	Kordestan	Saghez
45	*P. pabotti*	*Orientalis*	IRAN	Kordestan	Kamyaran
137	*P. pedunculata*	*Leptopus*	USA.	California	Davis

Table1. *Prunus* species evaluated for *S*-allele variation, their origin and taxonomic group

Accession No.	Species	Taxonomic group	Country	State/ Province	City
123	*P. persica* (cv.Okinawa)	Peach	USA.	Florida	Gainesville
132	*P. petunnikowii*	*Chameamygdalus*	USA.	California	Davis
104	*P. reticulata*	*Orientalis*	IRAN	Fars	Fasa
140	*P. salicina* (cv. Gulf rose)	Plum	USA.	Florida	Gainesville
19	*P. scoparia*	*Spartioides*	IRAN	Fars	Darab
57	*P. scoparia*	*Spartioides*	IRAN	Kerman	Orzoeieh
92	*P. scoparia*	*Spartioides*	IRAN	Tehran	Karaj
101	*P. scoparia*	*Spartioides*	IRAN	Chaharmahal va Bakhtiari	Lordegan
113	*P. scoparia*	*Spartioides*	IRAN	Fars	Fasa
78	*P. spartioides*	*Spartioides*	IRAN	Tehran	Tehran
91	*P. spartioides*	*Spartioides*	IRAN	Tehran	Karaj
99	*P. spartioides*	*Spartioides*	IRAN	Chaharmahal va Bakhtiari	Shahrekord
100	*P. spartioides*	*Spartioides*	IRAN	Chaharmahal va Bakhtiari	Shahrekord
54	*P. spp*	Almond *spp.*	IRAN	Fars	Shiraz
59	*P. spp*	Almond *spp.*	IRAN	Fars	Shiraz
139	*P. spp*	*Leptopus*	USA.	California	Davis
133	*P. tangutica* (*P. dehiscens*)	*Amygdalus*	USA.	California	Davis
134	*P. tennella* (*P. nana*)	*Chameamygdalus*	USA.	California	Davis
11	*P. trichamygdalus*	*Amygdalus*	IRAN	Fars	Darab
117	*P. trichamygdalus*	*Amygdalus*	IRAN	West Azarbaijan	Urmieh
138	*P. triloba*, (*P. ulmifolia*)	Plum	USA.	California	Davis
122	*P. webbii*	*Amygdalus*	USA.	Georgia	Attapulgus
135	*P. webbii*	*Amygdalus*	USA.	California	Davis

al., 2005; Stanys et al., 2008). The *S*-allele results, as determined by the six primer sets, were compared and the two most frequent types were used to determine final *S*-alleles identity (Table 5). Allele frequencies (Fig. 1) were determined by calculating the ratio of every allele to total number of *S*-alleles from all accessions (de Cuyper et al., 2005; Schueler et al., 2006; Stanys et al., 2008).

Data analysis

The data obtained by the Gene Marker software (using first degenerate primers, PaConsI-F(FAM) and EM-PC1consRD) were transferred to Power Marker software as an Excel file. Genetic distances of putative taxonomic groups were calculated (Table 6) based on their amplified *S*-allele sizes, and the phylogenetic dendrogram of groups was prepared with Tree view software (Fig. 2).

Results and Discussion

***S*-allele variation**

The six primer sets amplified a very diverse range of incompatibility alleles in the samples studied (Table 5). The allele sizes (estimated by Quantity One software) amplified by the degenerate primers (PaConsI-F(FAM)/EM-PC1consRD), ranged between 196 bp in almond (*P. dulcis,* accession 68) to 1148 bp in plum (*P. geniculata,* accession 141). Most allele sizes were between 200 to 400 bp and only three samples (cv. Texas, *P. bucharica* and *P. geniculata*) had larger allele sizes (949, 1076 and 1148 bp respectively). Ortega et al. (2006) also reported most *S*-allele sizes between 122 to 346 bp but detected two larger sized alleles (799 bp for *S*1 and 1064 bp for *S*14) using these primers. Based on their results, the larger amplified allele was labeled *S*1 for cv. Texas and *S*14 for both *P. bucharica* (accession 68) and *P. geniculata* (accession 141).
The PaConsI-F(FAM)/EM-PC3consRD primers, based on the first and second introns, amplified alleles from 608 (*P. korshinskyi*, accession 23) to 2630 bp (*P. orientalis*, accession 121). Ortega et al. (2006) used these primers for discriminating *S*26 in almond (cv. Avellanera Gruesa) from other amplified

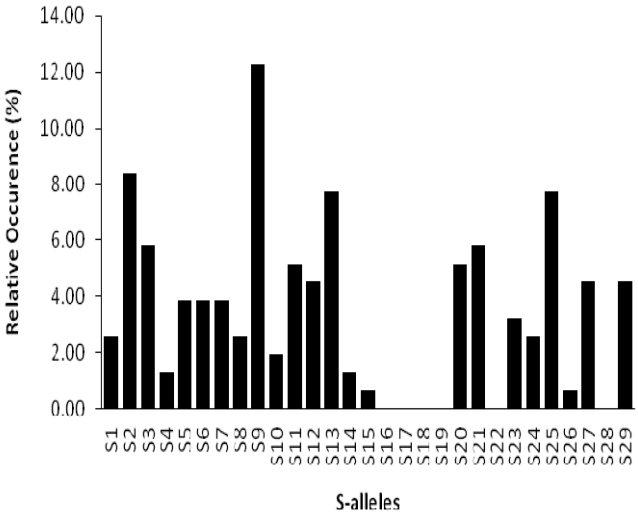

Fig 1. Comparison of the relative occurrence of *S*-alleles amplified by five sets of primers in wild almonds and related *Prunus* species

alleles. This is the first use of these primers (PaConsI-F(FAM)/EM-PC3consRD) for characterizing *S*-RNase in related almonds species.

The size of amplified alleles using primers EM-PC2consFD/EM-PC3consRD ranged from 243 (*P. korshinskyi*, accession 21) to 2066 (*P. elaeagnifolia*, accession 108). Ortega et al. (2006) reported a size range of 80-2872 bp for these primers in their almond cultivars studied. They reported a smaller size range (80-196 bp) for *S*10, *S*15, *S*28, *S*18 and *S*11. The sizes of amplified alleles in our samples were higher than 200 bp, which explain the lack of small *S*-alleles (*S*28 and *S*18) or their low frequencies (*S*10 and *S*15) in the studied genotypes (Fig. 1).

AS1II/AmyC5R primers, amplified sections of the first and fifth conserved regions of *S*-locus and were not able to discriminate *S*3 from *S*f allele. Both of these alleles showed a size of 1200 bp. These primers amplified allele sizes from 555

Table 2. Characteristics of six sets of primers (references, sequences and annealing temperatures) used for *S* allele discimination

	S-allele Primers	References	Molecular description	Ta (annealing temperature)
Degenerate Primers (First Intron)	PaConsI-F(FAM) EM-PC1consRD	Ortega et al. (2005)	5'-(C/A)CT TGT TCT TG(C/G) TTT (T/C)GC TTT CTT C-3' 5'-GCC A(C/T)T GTT G(A/C)A CAA A(C/T)T GAA-3'	54.7
Degenerate Primers (First and Second Introns)	PaConsI-F(FAM) EM-PC3consRD	Ortega et al. (2006)	5'-(C/A)CT TGT TCT TG(C/G) TTT (T/C)GC TTT CTT C-3', 5'-AWS-TRC-CRT-GYT-TGT-TCC-ATT-C-3'	58
Degenerate Primers (Second Intron)	EM-PC2consFD EM-PC3consRD	Sutherland et al. (2004)	5'-TCA-CMA-TYC-ATG-GCC-TAT-GG-3' 5'-AWS-TRC-CRT-GYT-TGT-TCC-ATT-C-3'	58
General S alleles primers	AS1II-F AmyC5R	Tamura et al. (2000)	5'-TATTTTCAATTTGTGCAACAATGG-3' 5'-CAAAATACCACTTCATGTAACAAC-3'	57
Specific Primers	CEBASf AmyC5R	Zeinalabedini et al. (2007b)	5'-AGATCTATCTATATCTTAAGTCTG-3' 5'-CAAAATACCACTTCATGTAACAAC-3'	57
Multiplex Primers	AS1II-F CEBASf AmyC5R	Sanchez-Perez et al. (2004)	5'-TATTTTCAATTTGTGCAACAATGG-3' 5'-AGATCTATCTATATCTTAAGTCTG-3' 5'-CAAAATACCACTTCATGTAACAAC-3'	57

Table 3. The PCR ingredients, their final concentrations and primer sets used for *S* allele dicrimination

Materials	(PaCons I-FD/EM-PC1ConsRD)	(PaCons I-FD/EM-PC3ConsRD) (EM-PC2ConsFD/EM-PC3ConsRD)	(AS1II/AmyC5R) (CEBASf/AmyC5R)	(AS1II / CEBASf / AmyC5R)
PCR Buffer	1x	1x	1x	1x
Forward Primer*	0.3µM	0.3µM	0.2µM	0.15µM
Forward Primer2*	-	-	-	0.15µM
Reverse Primer*	0.3µM	0.3µM	0.2µM	0.3µM
dNTPs	0.2mM	0.2mM	0.2mM	0.2mM
Taq polymerase	1U	1U	1U	1U
MgCl2	1mM	1mM	1.75mM	1.75mM
Q – solution	-	0.5x	-	-
DI H_2O	-	-	-	-
Total Master mix	-	-	-	-
DNA	5ng/µl	5ng/µl	5ng/µl	5ng/µl
Total volume	-	-	-	-

(*P. communis*, accession 48) to 2323 bp (*P. elaeagnifolia*, accession 108) and detected 94 alleles in 63 genotypes. Similarly Tamura et al. (2000) reported an amplified size range of 600-2019 bp in five almond cultivars and explained the differences in allele sizes as due to differences in their second introns which are located in the hypervariable region. This region also appears to have an important role in determining *S* specifity of pollen (Ushijima et al., 1998). However the allele size range in *Prunus* genotypes has been reported between 500-1200 bp (Martinez-Gomez et al., 2003; Zeinalabedini et al., 2007a) which is much lower than the size range in the present samples. The former report introduced six new alleles and the later amplified 14 new alleles among four wild almond genotypes. Martinez-Gomez et al. (2003) reported that the sequences of these primers (AS1IIF/AmyC5R) were highly conserved in wild almonds. Zeinalabedini et al. (2007a) reported fewer alleles in this region for *P. scoparia* relative to other species (one *S*-allele from one *P. scoparia* out of four accessions). Martinez-Gomez et al. (2003) also could not amplify any allele by using these primers in *P. scoparia* accessions. In this research, *S*-alleles have been amplified only in two out of five *P. scoparia* genotypes (two alleles in accession 19 and one allele in accession 101). These findings confirmed the reports of Zeinalabedini et al. (2007a) for this species. *S*-alleles failed to amplify in *P. kansuensis, P. tenella*, and *P. glandulosa* using these primers as previously reported by Martinez-Gomez et al. (2003). However, two alleles were amplified in *P. kansuensis* in our study.

The allele specific primers (CEBASf/AmyC5R), which was designed to detect the *Sf* allele, failed to amplify any self-compatible allele in the 96 samples. However, Zeinalabedini et al. (2007b) amplified one *Sf* allele in *P. elaegnifolia*. This could be due either to the lack of *Sf* allele or its different sequence in the current samples. Also, primers which were designed for cultivated almond may not be useful to amplify *S*-locus in wild genotypes. Boskovic et al. (2007) proposed that *Sf* in almond may be the result of a mutation of the *S*-allele in self-incompatible genotypes, or it could have been transferred from *P. webbii* in Apulia region of Italy as first proposed by Godini et al., (2002). Self-compatibility alleles in peach were not amplified by using CEBASf/AmyC5R primers, possibly due to sequence differences (Tao et al., 2007). Similarly, these primers did not amplify any *Sf* allele in hybrids of peach × almond or in *P. webbii*. Although Godini et al., (2002) reported that self-compatibility originated from *P. webbii*, Boskovic et al. (2007) could not find self-compatibility alleles in populations of this species. They believed that *P. webbii* possessed both self-compatible and self-incompatible types and *P. dulcis* mostly self-incompatible alleles, while *P. persica* may utilize a different pollen-based self-compatibility mechanism. Channun-

Table 4. Termocycler conditions for amplifying S alleles by different primer sets

		Initial denaturation	Denaturation	Annealing	Extension	Denaturation	Annealing	Extension	Final extension
(PaCons I-FD/EM-PC1ConsRD)	Temperature (°C)	94	94	54.7	72	-	-	-	72
	Time (min)	2	1	1	1	-	-	-	5
	Cycles	-		35		-	-	-	-
(PaCons I-FD/EM-PC3ConsRD) (EM-PC2ConsFD/EM-PC3ConsRD)	Temperature (°C)	94	94	58	68	94	58	68	-
	Time (min)	2	10 S'	2	2	10 S'	2	2+10S' per each cycle	-
	Cycles	-		10			25		-
(AS1II/AmyC5R) (CEBASf/AmyC5R) (AS1II /CEBASf/AmyC5R)	Temperature (°C)	95	94	57	72	-	-	-	72
	Time (min)	3	1	1	2	-	-	-	10
	Cycles	-		35		-	-	-	-

Table 5. Allele sizes (bp) amplified by the primer sets used for *S*-alleles discrimination in 96 wild almonds and their related *Prunus* species.

Primer sets			PaConsI-F(FAM) / EM-PC1consRD		Pacons1F(FAM)/ EM-PC3consRD		EM-PC2consFD/ EM-PC3consRD		AS1IIF/ AmyC5R		AS1II/ CEBASf//AmyC5R		
Species	Accession No.	Sizing by sequencer	Sizing by Quantity One	Candidate S-alleles*	Sizing by Quantity One	Candidate S-alleles	Sizing by Quantity One	Candidate S-alleles	Sizing by Quantity One	Candidate S-alleles	Sizing by Quantity One	Candidate S-alleles	Conclusion
P. argentea	128	325/401	380/455	S25/S20	1176/?	?/?	858/?	S25, Sf/?	1191/?	S3/?	1213/?	S3/?	S20/S25
P. brahuica	31	327/367	306/339	S25/S26, S13,S19	1138/2112	?/?	431/772	S2,S11,S21/S1,S16	1080/2190	S1/S7	1057/1930	S1/S7	S13/S25
P. bucharica	129	287/?	333/1076	S3/S14	1322/1484	?/?	506/1052	S21,S14	825/1377	S31/S13	808/1387	S31/S13	S3/S14
P. carduchorum	115	371/387	?/430	S26/S13,S19,Sf	1635/?	?/?	718/1387	S1,S16,S17/S27	1237/1766	Sf/-	?/1716	?/-	S25/S27
P. carduchorum	116	337/419	395/477	S3/S11,Sf	1105/1259	?/?	716/1004	S1,S16,S17/S14	1046/1315	S1/S13	1028/1297	S1/-	S1/S3
P. carduchorum	120	325/380	373/431	S25/S23,Sf	875/1203	?/?	479/848	S21/S25, Sf	841/1203	S32/S3,Sf	837/1213	S32/S3	S21/S25
P. communis	33	?/?	?/?	?/?	693/?	?/?	429/?	S2/?	730/?	S2,S11/?	743/?	S2/?	S2/?
P. communis	47	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?
P. communis	48	389/399	358/?	S13,S19/S20	678/1013	?/?	254/554	S10/S6	555/862	-/S32	556/867	-/S32	S5/S6
P. dulcis	66	346/433	329/405	S9/S21	?/?	?/?	?/?	?/?	612/848	S5/S32	573/664	-/S11	S9/S21
P. dulcis	67	275/346	273/336	S2/S9	716/825	?/?	422/1337	S11/S27	759/?	S2/?	731/931	S2/-	S2/S9
P. dulcis	68	191/371	196/353	S10/S12	693/?	?/?	?/?	?/?	?/?	?/?	761/?	S2/?	S10/S12
P. dulcis	69	275/325	268/314	S2/S25	734/1024	?/?	421/?	?/?	?/?	?/?	767/?	S2/?	S2/S25
P. dulcis	89	191/346	200/333	Sk/S9	709/1719	?/?	468/1356	S2/S27	778/?	S2/?	782/?	S2/?	S9/S12
P. dulcis	90	191/346	?/347	-/S9	716/1725	?/?	499/1380	S21/S27	789/?	S2/?	789/1671	S2/S34,S35	S9/S12
P. dulcis (cv.Carmel)	126	411/?	475/?	S8/?	?/?	?/?	316/?	S5/?	655/?	S5/?	633/?	S5/?	S5/S8
P. dulcis (cv.Nonpareil)	125	360/410	413/468	S7/S8	1988/?	?/?	799/1747	-/S7	2128/?	S7/?	2177/?	S7/?	S7/S8
P. dulcis (cv.Texas)	127	?/?	623/949	-/-	1594/?	?/?	315/786	S5/S1	642/1121	S5/S1	624/1148	S5/S1	S1/S5
P. dulcis×*P. persica* (Nonpareil×Flo.King)	143	360/406	353/399	S7/S8	964/2041	?/?	511/1701	S21/S7	839/1011	S32/S1	843/?	S32/?	S7/S8
P. dulcis × *P. persica* (Tardy Nonpareil)	124	205/360	248/411	S12/S7	1462/1966	?/?	1275/1719	S12/S7	1645/2114	S12/S7	1655/2143	S12/S7	S7/S12
P. eburnea	58	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?
P. elaeagnifolia	15	411/?	389/?	S8/?	?/?	?/?	498/?	S21,S2/?	752/?	S2/?	786/?	S2/?	S2/S8
P. elaeagnifolia	43	326/367	314/352	S25/S26,S13	1057/?	?/?	649/?	S4/?	922/?	S32/?	908/1369	S32/S13	S13/S25
P. elaeagnifolia	44	363/367	339/?	S29/S26, S13,S19	1020/?	?/?	382/604	S11/S20	884/?	S32/?	897/?	-/?	S13/S29
P. elaeagnifolia	64	346/401	?/?	S9/S20	870/?	?/?	?/?	?/?	?/?	?/?	588/773	-/S2	S9/S20
P. elaeagnifolia	98	251/401	292/433	-/S20	688/?	?/?	407/?	S11/?	746/939	-/-	724/910	S11/-	S11/S20
P. elaeagnifolia	106	370/395	417/?	S26/-	833/1972	?/?	397/1295	S11/S27	724/1599	S11/S12	724/1980	S11/S7	S11/S27
P. elaeagnifolia	108	330/375	?/?	S3,S25/S27	?/?	?/?	2066/?	-/?	931/2323	-/-	?/?	?/?	S3/S7
P. elaegnifolia	60	?/?	?/?	?/?	713/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	S9/?
P. elaegnifolia	61	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?
P. erioclada	109	364/431	412/485	S29/S21	910/1026	?/?	504/?	S21/?	869/?	S32/?	867/?	S32/?	S21/S29
P. erioclada	102	385/430	434/486	S13,S19/S21	1037/1699	?/?	568/1410	S6/S27	926/?	-/?	908/?	-/?	S21/S27
P. erioclada	111	?/?	?/?	?/?	?/?	?/?	480/544	S21/S6	851/?	S32/?	?/?	?/?	S6/S21
P. fenzliana	27	?/?	?/?	?/?	884/?	?/?	396/453	S11/S2	776/?	S2/?	?/?	?/?	S2/S11
P. fenzliana	28	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?
P. fenzliana	53	?/?	348/?	S13,S19,24/?	1029/1275	?/?	?/?	?/?	?/?	?/?	1148/?	S33/?	S24/?
P. fenzliana	118	191/367	237/424	-/S7	733/2169	?/?	542/?	S6/?	871/?	S32/?	852/?	S32/?	S12/S13

Table 5. Allele sizes (bp) amplified by the primer sets used for *S*-alleles discrimination in 96 wild almonds and their related *Prunus* species.

Primer sets			PaConsI-F(FAM) / EM-PC1consRD		Pacons1F(FAM)/ EM-PC3consRD		EM-PC2consFD/ EM-PC3consRD		AS1IIF/ AmyC5R		AS1II/ CEBASf//AmyC5R		
Species	Accession No.	Sizing by sequencer	Sizing by Quantity One	Candidate S-alleles*	Sizing by Quantity One	Candidate S-alleles	Sizing by Quantity One	Candidate S-alleles	Sizing by Quantity One	Candidate S-alleles	Sizing by Quantity One	Candidate S-alleles	Conclusion
P. fenzliana	103	395/411	?/408	-/S8,S27	1582/?	?/?	1323/?	S12/?	?/?	?/?	1187/?	S3/?	S3/S27
P. fenzliana	130	361/380	414/?	S6/S23	856/969	?/?	473/593	S2,S21/S6,S20	817/914	S31/S32	797/903	S2,S31/S32	S6/S23
P. geniculata	141	390/?	405/1148	S20/S14	1667/2165	?/?	1074/1291	S19/S12	1432/1612	S13/S12	1435/1637	S13/S12	S14/S20
P. glandulosa	136	322/390	366/439	S25/S21	1221/?	?/?	785/?	S1,S16,S17/?	?/?	?/?	607/1187	S5,S10/S3	S13/S25
P. glauca	112	358/?	406/?	S7/?	961/?	?/?	589/?	S6/?	911/?	-/?	907/?	-/?	S6/S7
P. hauskonechtii	62	324/346	325/385	S25/S9	1039/1619	?/?	590/1204	S6/-	908/1508	S32/S12	696/786	S11/S2	S9/S25
P. hauskonechtii	63	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?
P. hauskonechtii (var. pubescence)	40	325/346	310/?	S25/S9	1744/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	S9/S25
P. kansuensis	142	341/380	337/382	S3/S23	907/?	?/?	417/519	S11/S21	777/856	S2/S32	760/844	S2/S32	S21/S23
P. keredjensis	79	325/374	309/356	S25/S27	1025/1720	?/?	410/650	S11/S4,S20,S23	?/?	?/?	1620/?	S12/?	S1/S25
P. korshinskyi	16	381/?	359/?	S24/?	?/?	?/?	486/?	S21,S2/?	759/?	S2/?	759/1731	S2/-	S2/S24
P. korshinskyi	21	343/?	?/?	S3/?	?/?	?/?	243/?	S10,S5,S15/?	?/?	?/?	603/?	S10/?	S3/S10
P. korshinskyi	22	346/?	?/?	S9/?	?/?	?/?	741/?	S1,S16,S17/?	780/?	S2/?	771/?	S2/?	S2/S9
P. korshinskyi	23	363/?	344/?	S29/?	608/?	?/?	269/?	S15/?	600/?	S10/?	575/?	-/?	S15/S29
P. korshinskyi	24	?/?	?/?	?/?	?/?	?/?	668/?	S23/?	?/?	?/?	?/?	?/?	S23/?
P. korshinskyi	25	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?
P. korshinskyi	26	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?
P. korshinskyi	105	399/?	428/?	S20/?	?/?	?/?	298/?	S10	639/?	-/?	613/?	S5,S10/?	S10/S20
P. korshinskyi	107	362/395	398/440	S6,S29/-S13,S19	965/1447	?/?	572/1068	S6/S13	893/1385	-/S13	888/1387	-/S13	S13/S29
P. korshinskyi	110	346/364	408/?	S9/S29	1720/1873	?/?	1464/1621	-/S9	1729/1876	-/S9	1757/1913	-/-	S9/S27
P. kotschii	29	381/390	353/?	S24/S13,S19,S20	1092/?	?/?	649/?	S4,S20,S23/?	906/?	S32/?	948/?	-/?	S20/S24
P. kotschii	30	388/?	357/?	S13,S19/?	2112/?	?/?	?/?	?/?	?/?	?/?	1280/?	-/?	S13/?
P. kotschii	32	?/?	339/?	S3,S6,S13,S19,S26/?	?/?	?/?	1025/?	S14, S13, S19/?	?/?	?/?	?/?	?/?	S13/?
P. kotschii	49	351/362	331/?	S9/S6,S29	706/1854	?/?	614/1456	S4,S20/S9	1853/?	S9/?	?/?	?/?	S6/S9
P. kotschii	119	380/?	436/?	S23,S24,S27/?	853/?	?/?	316/488	S5/S21	651/841	?/?	624/831	S5/S32	S5/S23
P. kuramica	131	339/425	374/460	S3/S21	851/1003	?/?	412/640	S11/S4,S20	727/1007	S11/S1	706/1014	S11/S1	S4/S21
P. lycioides	55	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?
P. lycioides (var.horrida)	13	?/?	?/?	?/?	653/?	?/?	?/?	?/?	?/1132	?/S33	682/?	S11/?	S11/S23
P. lycioides (var.horrida)	18	275/375	265/354	S2/S27	657/743	?/?	321/?	S5,S15/?	613/?	S5/?	579/780	-/S2	S2/S5
P. lycioides (var.horrida)	56	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?
P. nairica	34	324/437	304/397	S28/S11	805/1013	?/?	361/645	S18/S23	667/?	S11/?	652/?	S11/?	S1/S11
P. nairica	38	384/409	363/?	S24,S13,S19/S4,S8	1056/1618	?/?	588/1247	S6/S12	860/?	S32/?	865/?	S32/?	S4/S13
P. nairica	39	383/437	348/396	S13,S19,24/S21	818/1258	?/?	363/839	S18/S25	652/1137	S11/S33	656/1136	S11/S33	S5/S24
P. orientalis	121	350/388	396/444	S9/S13,S19	1553/2630	?/?	1265/?	S12/?	1563/?	S12/?	1575/?	S12/?	S9/S12
P. pabotti	45	?/?	?/?	?/?	1026/?	-/?	?/?	?/?	?/?	?/?	?/?	?/?	S13/?
P. pedunculata	137	?/?	388/435	S13,S19/S21	884/959	?/?	405/537	S11/S6,S21	731/869	-/S32	707/849	S11/S32	S11/S21
P. persica (cv.Okinawa)	123	205/?	247/?	S1/?S12/?	1449/?	?/?	1255/?	S12/?	1635/?	S12/?	1675/?	S12/?	S12/?
P. petunnikowii	132	?/?	?/?	?/?	864/1263	?/?	450/?	S2/?	754/?	S2/?	?/?	?/?	S2/S9
P. reticulate	104	346/?	358/?	S9/?	880/1020	?/?	566/701	S6/S23	872/1060	S32/S1	824/1010	S31,S32/S1	S6/S9
P. salicina (cv. Gulf rose)	140	340/367	382/?	S3/S26	1492/1823	?/?	625/1202	S4,S20/-	1554/1869	S12/S9	1538/1900	S12/S9	S3/S13
P. scoparia	19	367/383	345/?	S26/S24	1201/1591	?/?	583/1236	S6/S12	1156/1528	S33/-	1160/1539	S3/S12	S3/S13
P. scoparia	57	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?
P. scoparia	92	370/395	?/?	S26/-	?/?	?/?	?/?	?/?	?/?	?/?	613/?	S5,S10/?	S26/S27
P. scoparia	101	337/395	385/449	S3/-	845/?	?/?	?/?	?/?	715/?	S11/?	740/945	S2,S11/-	S3/S27
P. scoparia	113	?/?	?/?	?/?	873/?	?/?	477/?	S2,S21/?	?/?	?/?	845/?	S32/?	S2/?
P. spartioides	78	?/?	?/?	?/?	650/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	S9/?
P. spartioides	91	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?
P. spartioides	99	363/423	397/463	S29/S21	2223/?	?/?	821/1865	S25/-	727/2156	-/-	2134/?	-/?	S7/S29
P. spartioides	100	275/363	321/409	S3/S29S2/S29	802/1002	?/?	621/1168	S4/S14,S19	942/1454	-/-	849/?	S32/?	S3/S29
P. spp	54	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?
P. spp	59	275/364	268/360	S2/S29	748/887	?/?	472/?	S2/?	?/?	?/?	694/786	S11/S2	S2/S29
P. spp	139	227/351	380/?	-/S9	974/1397	?/?	440/?	S2/?	?/?	?/?	?/?	?/?	S2/S9

Table 5. Allele sizes (bp) amplified by the primer sets used for *S*-alleles discrimination in 96 wild almonds and their related *Prunus* species.

Primer sets			PaConsI-F(FAM) / EM-PC1consRD		Pacons1F(FAM)/ EM-PC3consRD		EM-PC2consFD/ EM-PC3consRD		AS1IIF/ AmyC5R		AS1II/ CEBASf//AmyC5R		
Species	Accession No.	Sizing by sequencer	Sizing by Quantity One	Candidate S-alleles*	Sizing by Quantity One	Candidate S-alleles	Sizing by Quantity One	Candidate S-alleles	Sizing by Quantity One	Candidate S-alleles	Sizing by Quantity One	Candidate S-alleles	Conclusion
P. tangutica (*P. dehiscens*)	133	345/387	362/409	*S*9/S13,S19	822/?	?/?	401/1038	S11/S14	806/?	*S*2/?	793/?	*S*2/?	*S*9/S11
P. tennella (*P. nana*)	134	?/?	?/?	?/?	?/?	?/?	520/?	S21/?	?/?	?/?	?/?	?/?	S21/?
P. trichamygdalus	11	346/421	336/400	*S*9/S11	701/1719	?/?	?/?	?/?	569/?	S10/?	564/1626	S10/S12	*S*9/S11
P. trichamygdalus	117	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?
P. triloba, (*P. ulmifolia*)	138	349/?	?/?	*S*9/?	892/1248	?/?	629/?	S4,S20/?	814/1106	S31/S1	837/1160	S32/S33	S1/S9
P. webbii	122	325/401	382/457	S25/S20,Sf	1051/1183	?/?	664/868	S23/S24,S25,Sf	1006/1216	-/S20,Sf	1006/1213	-/?	S20/S25
P. webbii	135	325/401	329/403	S25/S20, Sf	1020/1156	?/?	590/802	S6,S4,S20/S25, Sf	950/1144	-/S33,Sf	937/1134	-/S33	S20/S25

*-:The allele could not be labeled

Table 6. Frequency based distance of amplified *S*-alleles in wild almonds and related *Prunus* species (Nei and Takezaki, 1983), (OTUs: Observed Taxonomy Units).

OUT	*Almond spp.*	*Amygdalus*	*Chameamygdalus*	*Dodecandra*	*Leptopus*	*Orientalis*	Peach	Peach×Almond	Plum	*Spartioides*
Almond spp.	0.0000									
Amygdalus	0.7442	0.0000								
Chameamygdalus	1.0000	0.9209	0.0000							
Dodecandra	0.8232	0.8882	1.0000	0.0000						
Leptopus	1.0000	1.0000	1.0000	1.0000	0.0000					
Orientalis	0.8174	0.6296	1.0000	0.8442	1.0000	0.0000				
Peach	1.0000	0.8882	1.0000	0.8232	1.0000	0.8709	0.0000			
Peach×Almond	0.7500	0.7840	1.0000	0.6464	1.0000	0.8174	0.7500	0.0000		
Plum	1.0000	0.9209	0.7500	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	
Spartioides	0.8557	0.8067	0.8557	0.7536	1.0000	0.6311	0.7959	1.0000	0.8557	0.0000

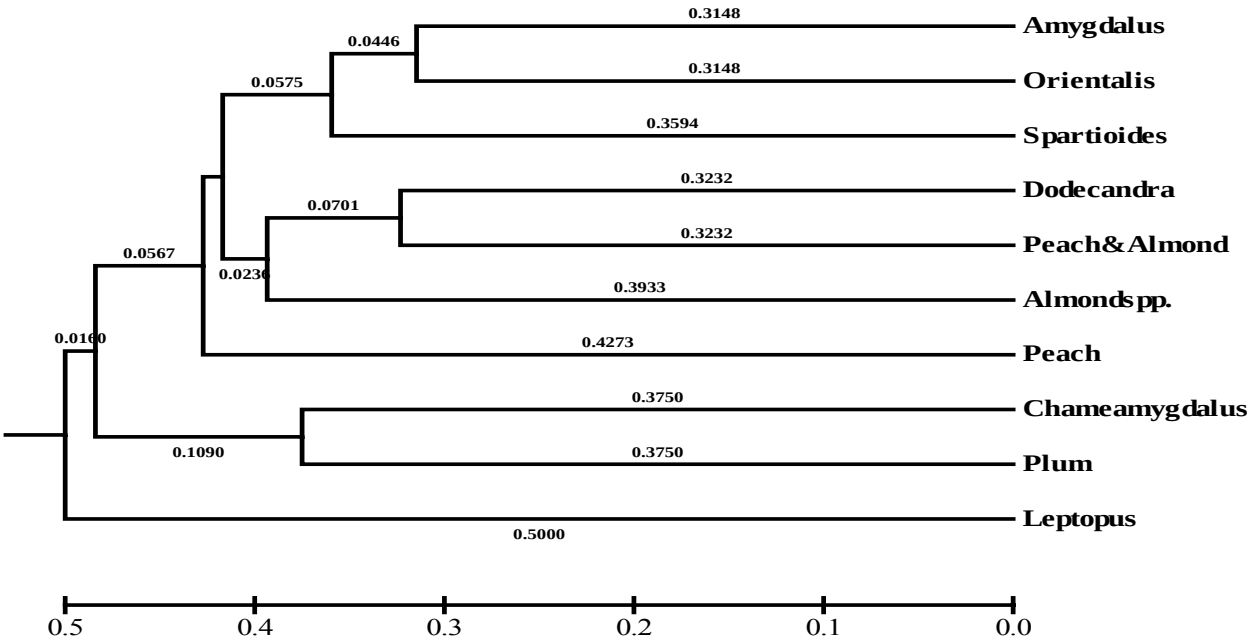

Fig 2. Dendrogram of 10 *Prunus* taxonomic groups using Nei and Takezaki (1983) UPGMA method based on the genetic distances obtained by *S*-allele sizes amplified by PaConsI-F(FAM)/EM-PC1consRD measured by ABI 3730XL sequencer.

tapipat et al. (2003) could not observe any *Sf* allele in *P. webbii*, using (*Sf*F/*Sf*R) primers. They concluded that self-compatibility in *P. webbii* is mostly due to low *S*-RNase activity for normal self-incompatibility alleles. Absence of any *Sf* allele has also been reported when applying other *S*-allele primers designed for *Sf* amplification. Boskovic et al. (2007) could not amplify *Sf* allele in one sample of *P. webbii* when using primers 2 and 8 designed by Ma and Oliviera (2001). These primers and *Sf*F/*Sf*R primers designed by Channuntapipat et al. (2003) also could not amplify any *Sf* allele in wild species (*P. elaegnifolia, P. hauskunechtii, P. scoparia, P. lycioides, P. orientalis,* and *P. communis*) as reported by Elahi et al. (2008).

To confirm results obtained by specific primers (CEBASf/AmyC5R), multiplex primers (AS1II/CEBASf/Amy-C5R) were also used. The multiplex primers could amplify band sizes of 400 and 1200 bp for *Sf* and *S*3 alleles respectively. Sanchez-Perez et al. (2004) reported size ranges from 400 to 2019 bp for this primer set in almond. The band size of 400 bp, which corresponded to *Sf* alleles in their study, was not amplified in this study, supporting the lack of *Sf* allele among our genotypes. The genotypes exhibited alleles with range sizes from 556 (*P. communis*, accession 48) to 2177 bp (cv. 'Nonpareil', accession 125).

The 'Nonpareil', 'Texas' and 'Carmel' cultivars were included as standards. The *S*-alleles amplified in these cultivars were the same as in previous reports. Cultivar 'Nonpareil' amplified *S*7 and *S*8 as previously reported (Tamura et al., 2000; Boskovic et al., 2003; Sanchez-Perez et al., 2004). 'Texas' amplified *S*1 and *S*5 alleles (Boskovic et al., 1997; Tamura et al., 2000; and Sutherland et al., 2004) and 'Carmel' (a progeny of a 'Nonpareil'×''Texas' cross), amplified *S*5 and *S*8 alleles (Martinez-Gomez et al., 2003; Lopez et al., 2004).

Among the 96 genotypes studied, no alleles were amplified in 13 genotypes (*P. korshinskyi* (accessions 25 and 26) *P. fenzliana* #28, *P. communis* #47, *P. spp* #54, *P. lycioides* #55, *P. lycioides* var. horrida #56, *P. scoparia* #57, *P. eburnea* #58, *P. elaegnifolia* #61, *P. hauskonechtii* #63, *P. spartioides* #91 and *P. trichamygdalus* #117) when using any of the six primer sets. In some genotypes (such as *P. nairica* accessions 38 and 39) more than two alleles were amplified, which could be due to polyploidy or heterduplex of the bands.

_S_- allele frequency

When using the five primer sets designed for amplification of *S*-alleles, 155 incompatibility alleles were amplified among the 96 *Prunus* species tested. Their sizes were determined with Quantity One software on agarose gel, or an automated sequencer (in case of primers PaConsI-F(FAM)/EM-PC1consRD), then labeled based on the similarity with sizes of previously reported *S*-alleles (Table 5) and their frequencies determined (Fig. 1). Alleles *S*9, *S*2, *S*13 and *S*25 had the highest frequencies (12.26, 8.39, 7.74 and 7.74 % respectively). Alleles *S*16, *S*17, *S*18, *S*19, *S*22, and *S*28 were not detected in the studied genotypes and alleles *S*15 and *S*26 had the lowest frequencies (0.65 %). Lopez et al. (2006) reported *S*1, *S*5, *S*7 and *S*8 as the most frequent alleles among 115 European and American almond cultivars studied. However, Mousavi et al. (2010) reported another set of *S*-alleles (*S*4, *S*1, *S*24, *S*7, *S*12 and *S*2) with higher frequencies among 70 Iranian almond cultivars analyzed. This supports the proposal of Lopez et al. (2006) that *S*-alleles are most diverse in almonds originating from different geographical regions.

Cluster analysis

The accurate sizes obtained by the automated sequencer for alleles amplified by the first degenerate primers (PaConsI-

F(FAM) and EM-PC1consRD) were used to make a dendrogram for comparing genetic distances (Fig. 2). The genotypes were previously grouped in 10 taxonomic groups (Table 1). The cluster analysis results (Fig. 2) showed good agreement with the taxonomic classification introduced by Socias i Company (1998). It revealed that *S*-alleles available within each taxonomic group are similar to each other, possibly having common origins. In contrast, common *S*-alleles were found to be rare among different taxonomic groups. Browicz (1974) and Socias i Company (1998) separated the *Dodecandra* (*Lycioides*) from *Icosandrae* series based on their morphological characteristics (prefoliation in bud, hypantium shapes, number of stamens and existence of spins). In contrast to their findings, , *Dodecandra* located relatively close to the *Icosandrae* series (containing *Amygdalus, Orinetalis*, *Spartiodes*, *Chameamygdalus* and *Leptopus*) when based on the *S*-allele sizes.

Genetic distances of putative groups

The lowest genetic distance (0.6269) was between the *Amygdalus* and *Orientalis* sections indicating that they were the most closely related (Table 6). Their close relation had previously been established by grouping both in *Euamygdalus* section by Socias i Company, (1998) based on morphological characteristics. *Leptopus*, and *Chameamygdalus* and the plum species *P. geniculata, P. salicina, P. triloba* and *P. glandulosa* showed high genetic distances (0.8557 to 1) from other groups. This supports earlier proposals on: a) the distinctness of *Leptopus* from almonds by Socias i Company (1998), b) the separation of plum from almond and peach early in their evolution (Watkins 1995), and c) the difficulty of hybridization between *Chameamygdalus* species and cultivated almonds (Kester and Gradziel, 1996). The *Chameamygdalus* section has been proposed as a separate subgenus in *Prunus* by Focke (1894) and more recently Lee and Wen (2001).

Conclusion

The *S*-alleles sizes which were amplified by five set of primers showed a high variability among the 96 samples of wild almonds and their related *Prunus* species representing 10 taxonomic groups. Neither the five primer sets, nor *Sf* allele specific primer pairs (CEBASf/Amyc5R) successfully amplified *Sf* alleles in any wild almonds and related species evaluated. The allele sizes amplified by PaConsI-F(FAM)/EM-PC1consRD clustered into 10 distinct groups which were in agreement with previously established taxonomic groups. *Amygdalus* and *Orientalis* groups clustered close together. In contrast to previous reports, *Dodecandra* clustered close to *Icosandrae* (including *Amygdalus, Orientalis, Spartiodes, Chameamygdalus*, and *Leptopus*), however *Leptopus* was distinct from other groups based on the calculated genetic distances.

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