

## S-Genotype Profiles of Turkish Apricot Germplasm

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### Abstract

In flowering plants, gametophytic self-incompatibility, controlled by a single locus with several allelic variants, is one of the major problems preventing self-fertilization. Among fruit trees, apricots show to a high degree self-incompatibility, especially in Middle-Asian and Iranian-Caucasian eco-geographical groups. In the present study, self-(in)compatibility characteristics of a total of 236 apricot genotypes (218 Turkish and 18 foreign) found within the National Apricot Germplasms of Apricot Research Institute in Malatya, Turkey was studied. Analyses were carried out by using four primer pairs (SRc-F and SRc-R, EM-PC2consFD and EM-PC3consRD, AprSC8-R and PaConsI-F, AprFBC8-F and AprFBC8-R). A total of 11 S-RNase alleles ( $S_2$ ,  $S_3$ ,  $S_6$ ,  $S_7$ ,  $S_8$ ,  $S_9$ ,  $S_{11}$ ,  $S_{12}$ ,  $S_{13}$ ,  $S_{20}$  and  $S_c$ ) were determined in the 236 apricot genotypes. As Turkish and foreign apricot genotypes are determined mostly self-incompatible, the data obtained hereby might be of good use for apricot breeding programs and more practically, for apricot new plantations; thus pollinator cultivars should be considered when self-incompatible apricot cultivars are being used.

**Keywords:** alleles, *Prunus armeniaca*, primers, self-(in)compatibility

### Introduction

The world apricot production is 3.95 million t/year and Mediterranean countries provide a great majority of this production. Turkey is the most important country in terms of apricot production in the world, with nearly 800.000 t per year (FAO, 2012). In Turkey 28.489 t of table fruit and 90.321 t of dried apricots have been exported (FAO, 2011). Although Turkey is not the homeland of apricot, the specie has been adapted very well to Anatolia conditions and is successfully grown on this land for centuries. Previously, apricot production in Turkey was based on seedlings obtained from seeds; however, in the last 50-60 years this figure changed and grafting with true to name cultivars on seedlings has been more common. Grafting technique increased the quality and quantity of apricot production in Turkey and enhances the importance of apricots both in domestic and foreign markets (Yilmaz *et al.*, 2013).

In Rosaceae, many fruit species such as Japanese pear (*Pyrus pyrifolia*), apple (*Malus × domestica*), sweet cherry (*Prunus avium*), almond (*Prunus dulcis*) and apricot (*Prunus armeniaca*) exhibit self-incompatibility (SI) and require pollination with pollen from compatible SI genotypes for stable fruit production. Aside from this practical importance, SI of Rosaceae is interesting from an

evolutionary point of view, because the common ancestor of Asterid and Rosid is thought to exhibit S-RNase-based gametophytic self-incompatibility (Igc and Kohn, 2001).

In new apricot plantations, self-incompatibility is one of the important problems and now days, in order to solve this issue, molecular techniques are being used to determine self-(in)compatibility in apricot cultivars (Burgos *et al.*, 1998; Halasz *et al.*, 2005, 2007; Yilmaz, 2008; Yilmaz *et al.*, 2013). Genetically, SI of Rosaceae is controlled by a single S locus with multiple alleles (Sonneveld *et al.*, 2003). The S-gene product is a ribonuclease enzyme, while the pollen product is an F-box protein (Entani *et al.*, 2003; Romero *et al.*, 2004).

The conventional methods to determine self-(in)compatibility are time consuming and can be effected by environmental factors (Zhang *et al.*, 2003). Even more, molecular markers have been developed in recent years to determine the self-incompatibility of genotypes (Yaegaki *et al.*, 2001). The Sc-haplotype was long suspected to be a pollen-part mutant of the S<sub>8</sub>-haplotype (Halasz *et al.*, 2007) with a 353-bp insertion in the SFB<sub>C</sub> gene (Vilanova *et al.*, 2005). Although most apricot cultivars are self-compatible, self-incompatibility is present in some interesting cultivars (Hormaza *et al.*, 2007). Up to 2010, a total of 20 SI (self-incompatible) alleles and one SC (self-compatible) allele were determined among European

eco-geographical group of apricot (Burgos *et al.*, 1998; Halasz *et al.*, 2005, 2007, 2010) and studies undertaken to determine new SI alleles in apricot have been continuing (Halasz *et al.*, 2013).

The aim of this study was to identify *S*-allele constitution of several apricot genotypes from apricot germplasm in Malatya-Turkey using polymerase chain reaction (PCR) with specific primer pairs.

## Materials and Methods

### Materials

A total of 236 apricot genotypes were used in this study from the Apricot Research Institute in Malatya, Turkey. From all genotypes studied, 18 were foreign, originated from different countries, while the rest of the 218 genotypes had Turkish origin (Table 1).

### DNA Isolation

Genomic DNA was extracted from full-expanded young apricot leaf samples, using the Cetyltrimethyl Ammonium Bromide (CTAB) method (Doyle and Doyle, 1987).

### PCR studies with *S*-RNase and *SFB*-specific primers

For first intron region, SRc-R (Vilanova *et al.*, 2005) and SRc-F (Romero *et al.*, 2004) primer pair were used to determine *Sc* allele, which yielded bands at 353 bp at apricot cultivars (Vilanova *et al.*, 2005). PCR products were separated on an ABI 3500 capillary electrophoresis instrument (Applied Biosystems, Foster City, CA, USA) at the core laboratory of the Genome and Stem Cell Centre (GENKOK) in Erciyes University, Kayseri, Turkey. For the identification of the *SC*-haplotype, a 2-step approach was used. An allele-specific reverse primer, AprSC8R (Halasz *et al.*, 2010), was designed to selectively amplify the *Sc*/*S<sub>8</sub>*-RNase allele and used in combination with PaConsI F (Sonneveld *et al.*, 2003). AprFBC8-F (5'-CAT GGA AAA AGC TGA CTT ATG G -3') and AprFBC8-R (5'-GCC TCT AAT GTC ATC TAC TCT TAG -3') were used for detecting *SFB<sub>C/8</sub>* allele (Halasz *et al.*, 2007). The amplification was carried out using a temperature profile according to Halasz *et al.* (2010).

For the second intron, PCR was conducted according to Sutherland *et al.* (2004) using the degenerate primers EM-PC2consFD and EM-PC3consRD. For PCR amplification in a 20-mL reaction volume, containing 1X PCR buffer (Thermo) with the final concentrations of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.0 mM MgCl<sub>2</sub>, 0.2 mM of dNTPs, 0.3 mM of each primer, and 1.0 U of Taq DNA polymerase (Thermo). The PCR products were electrophoresed in 1.5% (w/v) agarose gel, stained with ethidium bromide (0.5 µg/mL) using 1×TAE buffer, at 110 V for 2 h and visualized under UV light. Molecular size of the amplified fragments was estimated using a 100-bp ladder (Thermo). PCR's were repeated three times to determine the clear band size from apricot DNA.

### Evaluation of data

To determine the exact size of the *S*-RNase first intron region fragments under 100 bp DNA ladder (Invitrogen), the fluorescently labelled products were run on an automated sequencer ABI Prism 3500 Genetic Analyzer. For the determination of size (genotyping), GENEMAPER software and the GS600 LIZ size standard (Applied Biosystems) were

used.

The second intron PCR products were separated by electrophoresis in 1.2% TAE agarose gels for 2 h at 100 V, whereas DNA bands were visualised by ethidium bromide staining. Fragment lengths were estimated by comparison with the 1-kb DNA ladder (Promega, Madison, WI, USA). In the case of unknown alleles, PCR products were cloned and sequenced in an automated sequencer and analysed as described by Halasz *et al.* (2010).

## Results and Discussion

The determination of the *S*-genotypes of 236 Turkish and foreign apricot genotypes was carried out using the SRc-F and SRc-R consensus primers (Vilanova *et al.*, 2005) for the first intron and EM-PC2consFD / EM-PC3consRD primers (Sutherland *et al.*, 2004) for the second intron analysis of the *S*-RNase gene (Table 1). AprFBC8 F and R primers were used for discrimination of *SFB<sub>C/8</sub>* allele (Halasz *et al.*, 2007). The size of the PCR products was compared with those previously published by other researchers (Vilanova *et al.*, 2005; Halasz *et al.*, 2007, 2010). For *S<sub>8</sub>* and *Sc* alleles, although Vilanova *et al.* (2005) and Halasz *et al.* (2010) reported as 353 and also, Halasz *et al.* (2013) reported as 355 bp, the hereby result obtained was 354 bp band size. These differences might be explained by the genetic analyzers that affect the sensitivity of the method (Cachi and Wünsch, 2014).

A total 11 *S*-RNase alleles (*S<sub>2</sub>*, *S<sub>3</sub>*, *S<sub>6</sub>*, *S<sub>7</sub>*, *S<sub>8</sub>*, *S<sub>9</sub>*, *S<sub>11</sub>*, *S<sub>12</sub>*, *S<sub>13</sub>*, *S<sub>20</sub>* and *Sc*) were determined in the 236 apricots genotypes, while a total of 8 (*S<sub>2</sub>*, *S<sub>6</sub>*, *S<sub>7</sub>*, *S<sub>8</sub>*, *S<sub>9</sub>*, *S<sub>11</sub>*, *S<sub>13</sub>* and *Sc*) were determined within the foreign apricot genotypes, which were used as control (Table 1). Halasz *et al.* (2010) conducted a study to determine the *S*-genotypes of a set of Turkish and Hungarian apricot cultivars by amplification of their *S*-RNase intron regions. A specific primer (AprSC8) for *Sc* and *S<sub>8</sub>* was designed to anneal within the second intron region of the *Sc*- and *S<sub>8</sub>*-RNase alleles. This primer pair amplified a fragment in the case of *S<sub>8</sub>/Sc*-alleles. They reported that the presence of *S<sub>8</sub>/Sc*-alleles was confirmed among the tested 18 cultivars. Some of them ('Canakkale', 'Ethembey', 'Kayisi Erigi', 'Mektep', 'Sam' and 'Yerli Izmir') were proved as self-compatible (*ScSc*). Two Turkish cultivars shared the *ScS<sub>8</sub>*-genotype ('Ethembey' and 'Mektep'). Also, it was reported in their study that AprSC8 primer could distinguish between the *SI* and *SC* cultivars. Twelve previously described *S*-alleles were identified among the Turkish cultivars. *S<sub>9</sub>* was the most frequent *S*-allele in the tested Turkish germplasm (occurring in 72 cultivars), followed by *S<sub>8</sub>* (51), *S<sub>6</sub>* (43), *S<sub>2</sub>*, *S<sub>13</sub>* (34 each), *S<sub>19</sub>* (17), *S<sub>7</sub>* (43), *Sc* (52), *S<sub>3</sub>* (25), *S<sub>11</sub>* (18), *S<sub>12</sub>* (25), while *S<sub>20</sub>*-allele was only found in seven cultivars. Also, *ScS<sub>8</sub>* allele combination was found in 16 genotypes.

In the present study, 'Artvin PA' (*S<sub>2</sub>S<sub>7</sub>*), 'Hasanbey' (*S<sub>2</sub>S<sub>9</sub>*), 'Cataloglu' (*S<sub>6</sub>S<sub>9</sub>*), 'Ozal' (*S<sub>6</sub>S<sub>9</sub>*), 'Soganci' (*S<sub>6</sub>S<sub>9</sub>*), 'Gec Aprikoz' (*S<sub>6</sub>S<sub>11</sub>*), 'Ziraat Okulu' (*S<sub>6</sub>S<sub>12</sub>*), 'X1 Zerdali' (*S<sub>6</sub>S<sub>12</sub>*), 'Ordubat' (*S<sub>7</sub>S<sub>12</sub>*), 'X2 Zerdali' (*S<sub>7</sub>S<sub>12</sub>*), 'Agerik' (*S<sub>7</sub>S<sub>13</sub>*), 'Mektep' (*S<sub>8</sub>S<sub>C</sub>*), 'Yegen' (*S<sub>8</sub>S<sub>11</sub>*), 'Adilcevaz-5' (*S<sub>8</sub>S<sub>13</sub>*), 'Hacihaliloglu' (*S<sub>9</sub>S<sub>13</sub>*), 'Kabaasi' (*S<sub>9</sub>S<sub>13</sub>*), 'Kamelya' (*S<sub>9</sub>S<sub>13</sub>*), 'No.2 Zerdali' (*S<sub>9</sub>S<sub>13</sub>*), 'X3 Zerdali' (*S<sub>9</sub>S<sub>20</sub>*), 'Aprikoz' (*S<sub>11</sub>S<sub>13</sub>*), 'Kayisi Erigi' (*S<sub>11</sub>S<sub>plant?</sub>*), 'Alyanak' (*S<sub>2</sub>S<sub>8</sub>*), 'Sam' (*S<sub>2</sub>S<sub>C</sub>*), 'Dortyol-4' (*S<sub>2</sub>S<sub>19</sub>*), 'Sebbiyki' (*S<sub>2</sub>S<sub>19</sub>*), 'Hacikiz' (*S<sub>6</sub>S<sub>8</sub>*), 'Sakit-1' (*S<sub>7</sub>S<sub>8</sub>*), 'Turfanda Izmir' (*S<sub>7</sub>S<sub>8</sub>*), 'Yerli Izmir' (*S<sub>7</sub>S<sub>C</sub>*), 'Akcadag Gunay' (*S<sub>9</sub>S<sub>3</sub>*), 'Ismailaga' (*S<sub>9</sub>S<sub>11</sub>*), 'Kadioglu' (*S<sub>9</sub>S<sub>8</sub>*), 'Seftalioglu' (*S<sub>9</sub>S<sub>8</sub>*), 'Alioglu-49' (*S<sub>12</sub>S<sub>8</sub>*), 'Adilcevaz-3' (*S<sub>13</sub>S<sub>19</sub>*), 'Guz

Table 1. S-genotype profiles of Turkish Apricot Germplasm in Malatya Apricot Research Institute, Turkey

1 intron	2 intron	Sc/Ss-Rnase	SFbc/8	S-genotype	Genotypes
354, 354	2800, 2800	+	Sc	ScSc	2, 5, 1343, 1860, 12-Kadoğlu, M1343, M1345, M1346, Mehmet Yüksel 1860, Y9, Ambrosia
332, 354	900, 2800	+	Sc	S <sub>2</sub> Sc	7, 17, 23 A, 01-K-13, Canino, Karacabey, Pavior, Rakowsky, Sefer Çoban, Şam, Tokaloğlu Yalova,
332, 332	900, 900	-	-	S <sub>2</sub> S <sub>2</sub>	65 K, 31-K-04, K 5002, Roxana
332, 424	900, 1300	-	-	S <sub>2</sub> S <sub>6</sub>	K 0618
332, 402	900, 820	-	-	S <sub>2</sub> S <sub>7</sub>	11, 2216, 11/1-2P, Arrvin PA, Y1
332, 354	900, 2800	+	S <sub>8</sub>	S <sub>2</sub> S <sub>8</sub>	62 K, 693 K, K 0616, K 0621, Alyanak, Ziraat Okulu
332, 204	900, 500	-	-	S <sub>2</sub> S <sub>9</sub>	De Rona, Hasanbey, M 2254,
332, 304	900, 1700	-	-	S <sub>2</sub> S <sub>11</sub>	K 0617, K 3811
332, 378	900, 1250	-	-	S <sub>2</sub> S <sub>13</sub>	2639, Y3
332, 424	900, 1980	-	-	S <sub>2</sub> S <sub>19</sub>	Dörtöl-4, Şebbiyik, Y11
332, 222	900, 500	-	-	S <sub>2</sub> S <sub>20</sub>	K 0613
332, 236	900, 1270	-	-	S <sub>2</sub> S <sub>21</sub>	1295
268, 424	310, 2800	+	Sc	S <sub>3</sub> Sc	5103
268, 268	310, 310	-	-	S <sub>3</sub> S <sub>3</sub>	M 1299, M 1302, M 1342, M 2251
268, 424	310, 1300	-	-	S <sub>3</sub> S <sub>6</sub>	Şekerpare, Şekerpare İğdir
268, 402	310, 820	-	-	S <sub>3</sub> S <sub>7</sub>	M 1364
268, 268	310, 2800	+	S <sub>8</sub>	S <sub>3</sub> S <sub>8</sub>	Kayseri PA, K 3812
268, 204	310, 500	-	-	S <sub>3</sub> S <sub>9</sub>	Akçadağ Güney
268, 262	310, 370	-	-	S <sub>3</sub> S <sub>12</sub>	Adilcevaz-2, K 3816
268, 378	310, 1250	-	-	S <sub>3</sub> S <sub>13</sub>	Erken Ağırık
268, 268	310, 1250	-	-	S <sub>3</sub> S <sub>13</sub>	M 2234
---, ---	310, 1980	-	-	S <sub>3</sub> S <sub>19</sub>	5101
268, ---	310, 1980	-	-	S <sub>3</sub> S <sub>19</sub>	1342
268, 424	310, 1980	-	-	S <sub>3</sub> S <sub>19</sub>	3803, Sakat-3, Tokaloğlu İzmir
268, 222	310, 500	-	-	S <sub>3</sub> S <sub>20</sub>	Köfte Kayısı
268, 402	310, ---	-	-	S <sub>3</sub> S <sub>21</sub>	M 2243, 1364
268, 424	310, ---	-	-	S <sub>3</sub> S <sub>21</sub>	Casna Drenova, İmrahor
424, 354	1300, 2800	+	Sc	ScSc	614, 07-K-01, 07-K-09, 07-K-14, 07-K-15
424, 424	1300, 1300	-	-	S <sub>6</sub> S <sub>6</sub>	Adilcevaz-4, Dörtöl-1, Güz Ereği, M 2250, Sakat-4
424, 354	1300, 820	-	-	S <sub>6</sub> S <sub>7</sub>	K 5106
424, 402	1300, 820	-	-	S <sub>6</sub> S <sub>7</sub>	01-K-12, K 4207
424, 354	1300, 2800	+	S <sub>8</sub>	S <sub>6</sub> S <sub>8</sub>	4201, Hacıkız
424, 204	1300, 500	-	-	S <sub>6</sub> S <sub>9</sub>	Çataloğlu, Dörtöl-2, K 4206, K 5105, M 2245, No.1 Zerdali, Özal, Precoce de Boulbon, Soğanca
424, 304	1300, 1700	-	-	S <sub>6</sub> S <sub>11</sub>	13, Geç Aprikoz, K 3860, Güz Aprikozu
424, 262	1300, 370	-	-	S <sub>6</sub> S <sub>12</sub>	23, GÜ-103, M 2236, No.1 Zerdali (Ziraat Okulu), X1 Zerdali
424, 378	1300, 1250	-	-	S <sub>6</sub> S <sub>13</sub>	Mahmudun Ereği, Silistre de Rona, XX Zerdali
---, ---	1300, 1250	-	-	S <sub>6</sub> S <sub>13</sub>	K 3814
424, 424	1300, 1980	-	-	S <sub>6</sub> S <sub>19</sub>	Sakat-1
424, ---	1300, 1980	-	-	S <sub>6</sub> S <sub>19</sub>	Sakat-2, Ordlubat Benzeri
424, ---	1300, ---	-	-	S <sub>6</sub> S <sub>21</sub>	Levent
402, 354	820, 2800	+	Sc	ScSc	6, 1344, 66 K, M 1344, Yerli İzmir
402, 424	820, 2800	+	Sc	ScSc	10
402, 424	820, 2800	+	S <sub>1</sub>	S <sub>6</sub> S <sub>6</sub>	Cafona
402, 402	820, 820	-	-	S <sub>6</sub> S <sub>7</sub>	3, 1292, 1294, 1296, M 1294, Proyma, Tokaloğlu Konya Ereği
402, 354	820, 2800	+	S <sub>8</sub>	S <sub>6</sub> S <sub>8</sub>	Abuzer Gülen, Turfanda Eski Malatya, Turfanda İzmir
---, 354	820, 2800	+	Sc	ScSc	Kırmızı
402, 204	820, 500	-	-	S <sub>6</sub> S <sub>7</sub>	Kurukabuk
402, 304	820, 1700	-	-	S <sub>6</sub> S <sub>11</sub>	M 2241
402, 262	820, 370	-	-	S <sub>6</sub> S <sub>12</sub>	615, Ordlubat, X2 Zerdali
402, 378	820, 1250	-	-	S <sub>6</sub> S <sub>13</sub>	Ağırık, M 2252, Sakat-7
402, 402	820, 1980	-	-	S <sub>6</sub> S <sub>19</sub>	M 1296
402, 424	820, 1980	-	-	S <sub>6</sub> S <sub>19</sub>	Sivas PA
402, 236	820, 1270	-	-	S <sub>6</sub> S <sub>21</sub>	GÜ-2, M 2620, Tokaloğlu Erzincan
304, 236	820, 1270	-	-	S <sub>6</sub> S <sub>21</sub>	K 0614
332, 354	820, ---	-	-	S <sub>6</sub> S <sub>21</sub>	1293
354, ---	820, ---	-	-	S <sub>6</sub> S <sub>21</sub>	1346
354, 354	2800, 2800	+	Sc/Sc	ScSc	Precoce de Colomer, 18, 2239, 3808, 4202, Çanakdale, K 4205, M 1277, M 1298, M 2437, Mektap, Precoce de Tyrinthe, Tokaloğlu 1295, Y5, Y8, Y10
354, 354	2800, 2800	+	S <sub>6</sub>	S <sub>6</sub> S <sub>6</sub>	20, 2249, Royal, Erivan
354, 204	2800, 500	+	S <sub>8</sub>	S <sub>6</sub> S <sub>7</sub>	Çöloğlu, Kadoğlu, Şeftalioğlu, GÜ-13, GÜ-50, M 2256, No.8 Zerdali, Perfection, Y7
354, 304	2800, 1700	+	S <sub>8</sub>	S <sub>6</sub> S <sub>11</sub>	2213, Yeğen
354, 304	---, 1700	+	S <sub>8</sub>	S <sub>6</sub> S <sub>11</sub>	31-K-05
354, 378	2800, 1250	+	S <sub>8</sub>	S <sub>6</sub> S <sub>13</sub>	M 2246, 69 K
---, ---	2800, 1980	+	S <sub>8</sub>	S <sub>6</sub> S <sub>19</sub>	61 K
354, 222	2800, 500	+	S <sub>8</sub>	S <sub>6</sub> S <sub>20</sub>	92-58-01, 92-58-02
204, 204	500, 500	-	-	S <sub>6</sub> S <sub>7</sub>	12, 31-Hachalioğlu, GÜ-52, M 2257, M 2235
204, 304	500, 1700	-	-	S <sub>6</sub> S <sub>11</sub>	İsmailağa
204, 262	500, 370	-	-	S <sub>6</sub> S <sub>12</sub>	8, 691, 92-23-01, M 2242
204, 354	500, 370	-	-	S <sub>6</sub> S <sub>12</sub>	Alkaya
---, ---	500, 370	-	-	S <sub>6</sub> S <sub>12</sub>	K 5104
204, 378	500, 1250	-	-	S <sub>6</sub> S <sub>13</sub>	Adilcevaz-5, Hachalioğlu, Kabaşı, Karnelya, Mahmut Ölmez, No.2 Zerdali
204, 424	500, 1250	-	-	S <sub>6</sub> S <sub>13</sub>	4203
204, 222	500, 500	-	-	S <sub>6</sub> S <sub>20</sub>	X3 Zerdali
204, 236	500, 1270	-	-	S <sub>6</sub> S <sub>21</sub>	92-58-03
304, 354	1700, 2800	+	Sc	S <sub>11</sub> Sc	Polonais
304, 262	1700, 370	-	-	S <sub>11</sub> S <sub>12</sub>	68 K, K 0620
304, 378	1700, 1250	-	-	S <sub>11</sub> S <sub>13</sub>	Aprikoz, EB, K 3809

Table 1 (continued).

1 intron	2 intron	Sc/Ss-Rnase	SFBc/8	S-genotype	Genotypes
304,354	1700,1700	-	-	S <sub>11</sub> S <sub>11mz</sub>	Kayst Erigi
362,354	370,2800	+	S <sub>8</sub>	S <sub>2</sub> S <sub>8</sub>	49-Alioglu
262,262	370,370	-	-	S <sub>12</sub> S <sub>12</sub>	92-23-02,K 5001,Tevfik Yildirim
262,378	370,1250	-	-	S <sub>12</sub> S <sub>13</sub>	31-K-03,GÜ-8,K 4204,M2244
354,354	370,—	-	-	S <sub>12</sub> S <sub>7</sub>	K 3813
378,354	1250,2800	+	Sc	S <sub>12</sub> Sc	269,63K,64K,67K
378,378	1250,1250	-	-	S <sub>15</sub> S <sub>15</sub>	692K,M2435
378,424	1250,1980	-	-	S <sub>15</sub> S <sub>9</sub>	Adilcevaz-3
354,354	1980,1980	-	-	S <sub>18</sub> S <sub>19</sub>	M2240
454,222	1980,500	-	-	S <sub>20</sub> S <sub>20</sub>	Tekeker
222,222	500,500	-	-	S <sub>20</sub> S <sub>20</sub>	Hirmanlı

Aprikozu' ( $S_6S_{11}$ ), 'Kayseri PA' ( $S_3S_8$ ), 'Sakit-3' ( $S_3S_{19}$ ), 'Tokaloglu Izmir' ( $S_3S_{19}$ ), 'Imrahor' ( $S_3S_7$ ), 'Sekerpare' ( $S_3S_6$ ) had the same alleles with those of Halasz *et al.* (2010).

Never the less, there were some differences at three apricot cultivars from the results of Halasz *et al.* (2010) such as 'Karacabey' ( $S_2Sc / S_2S_8$ ), 'Ziraat Okulu' ( $S_2S_8 / S_2Sc$ ) and 'Canakkale' ( $S_8Sc / ScSc$ ). Also, the second allele at 'Levent' ( $S_6S_7 / S_6S_{19}$ ) was not obtained in the current experiment. Otherwise, although Halasz *et al.* (2010) could not determine second allele for 'Dortyol-2' ( $S_6S_7 / S_6S_7$ ), 'Mahmudun Erigi' ( $S_6S_{13} / S_6S_{13}$ ) and 'Cologlu' ( $S_5S_8 / S_5S_7$ ), the hereby study determined second alleles for the mentioned genotypes (Table 1). These differences should come because of gel images or PCR conditions.

Mehlenbacher *et al.* (1991) reported that the European group of apricot (Europe, North America, South Africa and Australia are included) may be described as self-compatible. It was reported by Halasz *et al.* (2013) to support the *S*-genotype determinations, as first intron lengths were also determined for all genotypes using fluorescently labelled primers and automated sizing on a capillary sequencer. Analysis of the first intron in 63 wild-grown apricot accessions from Turkey showed that 17 of 63 apricot accessions had 355 bp fragment. This fragment size was previously attributed to both the *Sc*- and *S*<sub>8</sub>-*RNase* alleles (Halasz *et al.*, 2007).

Vilanova *et al.* (2005) used SRC-R and SRC-F primer pair for 10 apricot cultivars to determine their *S* alleles. Six of 10 apricot genotypes were obtained via reciprocal crossing. They determined apricot genotypes that had *Sc* allele, which yielded at 353 bp. It was reported with previous studies that most of the European cultivars had *Sc* allele, whereas old Turkish cultivars were self-incompatible (Yılmaz, 2008; Halasz *et al.*, 2010).

Since coding regions of the *S*<sub>8</sub>- and *Sc*-*RNase* alleles are identical, discrimination between the 2 alleles was not possible. In apricot, self-compatibility is attributed to a pollen-part mutation: a 353 bp insertion in the *SFB* gene. To distinguish between the self-incompatible (SI) and self-compatible (SC) accessions, a previously designed specific primer pair (AprFBC8) can be used (Halasz *et al.*, 2010), which amplifies a fragment of approximately 500 bp in the case of *SFB*<sub>c</sub>-allele, while genotypes carrying the *SFB*<sub>s</sub>-allele show a fragment of approximately 150 bp (Halasz *et al.*, 2013). Thus, Halasz *et al.* (2013) determined 17 apricot accessions carrying *SFB*<sub>s</sub>-allele among 63 apricots from Turkey using AprFBC8 primer pair and they were stated as self-incompatible.

Based on the structure of *S*-*RNase*, many pairs of primers have been developed for *Prunus* species, such as Pru-C2 and PCE-R (Tao *et al.*, 1999a; Yamane *et al.*, 2001), SRC-F and EM-PC5consRD, SRC-F and PM-C5 (Vilanova *et al.*, 2005; Sutherland *et al.*, 2004; Habu *et al.*, 2008), ASIII and AmyC5R (Tamura *et al.*, 2000), EM-PC2consFD and ED-PC3cons-RD

(Sutherland *et al.*, 2004), PaConsI-F and PaConsI-R, PaConsII-F and PaConsII-R (Sonneveld *et al.*, 2003). Yaegaki *et al.* (2001) first determined *S*-*RNase* genotypes using the primer pair Pru-C2 and Pru-C5. Tao *et al.* (2002) cloned novel *S*<sub>8</sub>-*RNase* and *Sc*-*RNase* using Pru-C2 and PCE-R. Recently, the *S*-genotypes of 14 Japanese apricot cultivars native to Japan were determined using Pru-C2 and PCE-R, SRC-F and EM-PC5consRD, SRC-F and PM-C5 (Habu *et al.*, 2008). The primer pair Pru-C2 and PCE-R was developed from C2 and C3 in *Prunus* by Tao *et al.* (1999) and Yamane *et al.* (2001) and is considered as the universal primer pair for determining the *S*-genotypes in Japanese apricot (Habu *et al.*, 2008).

Halasz *et al.* (2013) carried out a study to determine *S*-genotypes of wild-growing Turkish apricots by PCR amplification of the *S*-*RNase* intron regions and *SFB* gene, in order to characterize their sexual (in) compatibility phenotype. The authors determined the complete *S*-genotype of 63 wild-grown apricot accessions that originated in the Erzincan region. Ten previously described and 2 new *S*-alleles (provisionally labeled *S*<sub>X</sub> and *S*<sub>Y</sub>) were identified in the studied genotypes. *S*<sub>2</sub> was the most frequent *S*-allele in the tested germplasm (occurred in 19 accessions), followed by *S*<sub>8</sub> (17), *S*<sub>19</sub> (16), *S*<sub>3</sub> (13), *S*<sub>12</sub> (11), *S*<sub>6</sub> (10) and *S*<sub>7</sub> (10), while *S*<sub>7</sub>, *S*<sub>11</sub>- and *S*<sub>13</sub>-alleles were found in 8 accessions each. A total of 36 different *S*-genotypes were assigned to the tested accessions. The *Sc*-allele responsible for self-compatibility in apricot was not present, indicating that all accessions were self-incompatible. The analysis of *S*-allele frequencies allowed to conclude the close relationship of wild-grown and cultivated apricots in Turkey and helped to raise hypotheses that may explain the high occurrences of *S*<sub>2</sub>- and *S*<sub>8</sub>-alleles.

One of the most important factors in apricot crop evolution was the emergence of self-compatibility, which has resulted in a serious loss of genetic diversity in Europe and the Mediterranean Basin (Pedryc *et al.*, 2009; Bourguiba *et al.*, 2012). In a previous study, Halasz *et al.* (2010) detected an uneven distribution of the *Sc*-allele in Turkish apricot cultivars: no self-compatible cultivar was found among 11 tested genotypes in the Eastern Region, while 7 out of 14 tested cultivars from the Western part of the country were self-compatible. Although the 55 cultivars analyzed in their study did not reveal a sound conclusion regarding the place of the origin of self-compatibility in apricot, the increasing number of *Sc* cultivars from East to West was suggestive.

## Conclusion

Turkey is a main producer of apricot. Also, there are broad genetic variation apricot cultivated areas. Determination of *S*-allele constitution of apricot germplasm is very important for

orchard management and breeding programs. Within the current study it was determined *S*-allele constitution of apricot germplasm in Turkey and the results showed that there were big variations among apricot genetic material studied with regard to *S* allele constitution.

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