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DETERMINATION OF SELF-(IN) COMPATIBILITY USING MOLECULAR MARKERS OF SOME APRICOT CULTIVARS WHICH CULTIVATED IN TURKEY

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Abstract
In fruit trees, gametophytic self-incompatibility is one of the major problem because of preventing self-fertilization controlled by a single locus with some allelic variants. Among the fruits, apricots also show self-incompatibility especially originated from Middle-Asian and Iranian-Caucasian. In present study, it was determined self-compatible/incompatible of some apricot cultivars which cultivated in Turkey. 17 Turkish and 42 foreign apricot cultivars were used in this study. Analyses were carried out using three primer pairs (SRc-R-SRc-F, EM-PC2consFD / EM-PC3consRD and AprFBC8). A total 5 S-RNase alleles (S2, S3, S6, S9 and S11) were determined in the 11 new Turkish cultivated apricots and a total 8 (S3, S7, S8, S9, S11, S12, S13 and SC) were determined in old Turkish apricot cultivars. A total 11 S-RNase alleles (S2, S3, S4, S6, S7, S8, S9, S11, S12, S19 and SC) were determined in foreign 42 apricot cultivars. It was determined self (in)compatibility alleles of 11 new Turkish and 38 foreign apricot cultivars first time with this study. These results can be used for plantation new orchards and breeding programs. Also, pollinator cultivars should be considered for plantation new orchards for these self-incompatible cultivars because of some Turkish and foreign apricot are mostly self-incompatible.

Keywords: Prunus armeniaca, S-genotype, Self-(In)Compatibility

1. INTRODUCTION

The world apricot production is 4.11 million ton/year and majority of the total production have provided from Mediterranean countries. Turkey is the most important country in the world in apricot production with nearly 800.000 ton/year production (FAO, 2019). 28.489 ton table and 90.321 ton dried of this production have been exported (FAO, 2019). Apricot has been produced throughout Anatolia especially Eastern Region since ancient times for its edible fruit.

Fresh and dried apricots have been grown in different regions of Turkey for a long time, so Turkey is one of the most important apricot genetic resource centers. Malatya is an important region for dried apricots, where approximately 51% of Turkey's production is produced. On the other hand, the Mediterranean Region of Turkey (the provinces of Hatay, Mersin and Antalya) has great potential due to the continuous and early harvest of apricots devoted to fresh apricot fruit. (Anonymous, 1991).

Apricot is very important economically for the grower. Therefore, good quality, disease resistance (especially PPV resistance), post-harvest applications, early harvest and self-adaptation, etc. breeding programs are in place. Gametophytic self-incompatibility (GSI) is a common mechanism

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in flowering plants, usually controlled by a single multiallelic locus, a so-called S-locus, which prevents inbreeding and promotes crossing over (De Nettancourt, 2001).
Species which belong to Rosaceae family show gametophytic self-incompatibility (GSI) in order to avoid inbreeding depression. This feature is controlled by a single multiallelic locus, named as the S-locus (De Nettancourt, 1977). A ribonuclease enzyme (S-Rnase) is the S-gene product in styles tissue (McClure et al., 1989), while an F-box protein is recently identified pollen product (Entani et al., 2003; Romero et al., 2004). Successful fertilization may only occur when the S-allele carried by the haploid pollen grain does not match any of the two alleles reside in the pistil. On the other hand, pollen tube growth will be arrested towards the ovary (Halász et al. 2005).
Although it has been considered traditionally a self compatibility species (Halasz, 2007), especially Middle-Asian and Iranian-Caucasian apricot genotypes shows self-incompatibility. Althought the Irano-Caucasian apricots were reported as predominantly self-incompatible (SI), whereas most European apricots are self-compatible (SC) (Halasz et al. 2005; Kostina, 1970). Almost all the European apricot cultivars have been traditionally considered self-compatible (Kostina, 1977); nevertheless more and more exceptions were found (Nyujtó et al., 1985; Burgos et al., 1997). One S-allele for SC and several alleles for self-incompatibility were described in Mediterranean and American cultivars (S1-S7; Burgos et al., 1998) and in Hungarian and Central Asian genotypes (S8-S16).
Traditional methods of self-determination (in-compatibility) are time consuming and environmental factors can effect(Zhang et al., 2003). In recent years, it has developed molecular markers to determine the self-incompatibility in plant genotypes (Yaegaki et al., 2001).
Recent studies have identified 21 S-RNase alleles in European apricots, of which 20 (S1-S20) allow for self-incompatibility and one (SC) allows for self-compatibility(Burgos et al., 1998; Halasz, 2005; 2007). The SC haplotype has long been suspected and was recently confirmed to be a pollen-part mutant of the S8 haplotype with a 358 bp insertion in the SFBC gene (Vilanova et al., 2005; Halasz et al., 2007). Although most of the apricot cultivars have known as self-compatible, some interesting apricot cultivars used at breeding programs are self-incompatible (Hormaza, 2007).
The aim of present study is to determine the self-compatibility allele in some apricot cultivars which have cultivated in Turkey using molecular markers.

2. MATERIALS AND METHODS

Fifty-nine apricot cultivars and genotypes, 17 of which were Turkish cultivars or breeding material, and 42 foreign cultivars, were used in this study. All varieties obtained from Alata Horticultural Research Institute and Eğirdir Fruit Research Institute fruit germplasm (Table 1). Genomic DNA was extracted from fully expanded apricot leaf samples by the Doyle and Doyle method (1987). SRc-R and SRc-F primer pairs(Romero et al., 2004; Vilanova et al., 2005) were used to determine Sc allele at 353 bp at apricot cultivars (Vilanova et al., 2005). AprFBC8-F and AprFBC8-R were used for SFBC/8 alleles (Halász et al. 2007). Amplification was performed using termal cycle profile according to Halász et al. (2010). For EM-PC2consFD and EM-PC3consRD, PCR protocol was followed according to Sutherland et al. (2004) using the degenerate primers for the amplification of the second intron region of the S-RNase gene. All PCR products were electrophoresed in 1.5% (w/v) agarose gel, stained using ethidium bromide (0.5 lg/mL) with 1xTAE buffer, at 110 V for 2 h and visualized under UV light for AprFBC8 and EM-PC2consFD-EM-PC3consRD primer pairs. However, the SRc PCR product was electrophoresed in metaphor

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agarose. The molecular size of the amplified fragment was estimated using a 100-bp ladder(Thermo).

3. RESULTS AND DISCUSSIONS

S-genotypes of a total of 59 Turkish and foreign apricot genotypes were determimed Identification using SRc-F and SRc-R primers for the first intron (Vilanova et al., 2005) and also, for second intron EM-PC2consFD/EM-PC3consRD primers were used (Table 1). Also, AprFBC8 was used for the SFBC/8 allele.

A total 5 S-RNase alleles (S2, S3, S6, S9, and S11) were determined in the 11 new Turkish cultivated apricots and a total 8 (S3, S7, S8, S9, S11, S12, S13 and SC) were identified in old Turkish apricot cultivars which used as control (Table 1). Among new Turkish apricot cultivars the most widely allele was SC (occurred in 7) and followed by S6(5) and S9(4), S3(4), S2(2) and S19(2) respectively. S9 was the most frequent S-allele among old Turkish cultivars in the tested Turkish germplasm (occurred in 3 cultivars). Also, S3, S7, S8, S9, S11, S12 and S13 were found in one cultivar. In this study, Ismailaga yielded 500 bp band and determined S9 using EM-PC2consFD / EM-PC3consRD primer pairs but didn't yield second band. Also, Sekerpare yielded a band at 250 bp and named as S3. These results were different from Halasz et al. (2010) because they determined two alleles for these three cultivars. Otherwise Cologlu (S8S9), Ordubat(S7S12) and Soganci (S6S9) where grown Igdir and Malatya regions were consistent with Halasz et al. (2010). Eight of 11 new Turkish apricot cultivars were developed at Alata Horticulture Research Station in Mersin-Turkey. They are Cagataybey (obtained from Sakit-2 x Precoce de Colomer crossing), Dr. Kaska (Precoce de Colomer x 07-K-11), Cagribey (Sakit-6 x P. de Colomer), Alata Yildizi (Sakit-6 x Precoce de Colomer), Sahinbey (Sakit-6 x Joubert Faulon), 2-89 (Precoce de Colomer x 07-K-11), 7-89 (Sakit-6 x Joubert Faulon) and 33-89 (Sakit-1 x Cafona). Alata Yildizi and Cagribey have same parents (Sakit-6 x Precoce de Colomer) which have carried S3SC allels. Also, Cagataybey, Dr. Kaska, Cagribey and Alata Yildizi obtained from Precoce de Colomer crossing and they have carried SC allele like Precoce de Colomer. But 2-89 didn't have SC allele, although one of its parents is Precoce de Colomer. Maybe, 2-89 carries two S allels of 07-K-11 apricot accession which one of 2-89 of parents.

Although 8 of 11 new Turkish cultivars were developed using crossing at Alata Horticultural Research Institute, 3 of them (Sakit cultivars) which selected from Sakit Valley where located south part of Turkey grown under Mediterranean climate in Turkey. Especially, they have used fresh consumption and export in Turkey. One of them (Sakit-7) was carrying Sc allele, but 2 of them (Sakit-2 and Sakit-6) didn't have Sc allel. An apricot breeding program at Alata Horticultural Research Station, some European apricot cultivars were used as parent. Most of new developed apricot cultivars had Sc allele except 33-89. Report of some researchers supports to these results (Mehlenbacher et al., 1991).

It was reported that although the European apricot group (including North America, Europe, Australia and South Africa) has been described as self-compatible, many commonly grown apricot cultivars have been reported to be self-incompatible (Mehlenbacher et al., 1991). Pollen grain alleles for self-compatibility will allow pollen tube growth in any style, but self-incompatibility alleles will arrest pollen tube growth if the same allele is present in the pistil in apricot. (Burgos et al., 2004). In present study, a total 11 S-RNase alleles (2, 3, 4, S6, 7, 8, 9, 11, 12, 19 and C) were identified in foreign 42 apricot cultivars which most of them have used genetic material for breeding in Turkey. Among foreign the apricot cultivars the most widely allele was SC (occurred in

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28 apricot cultivars) and followed by S2(6), S3(4), S7(8), S8(3), S9(5) and S12(5) respectively. But among foreign the apricot cultivars the most rarely alleles were S6(2), S4(2), S19(1) and S11(1).
Burgos et al. (1998) determined and reported for Beliana (SCS7), Harcot(S1S4) and Priana (S2S7). In this study, we determined for Beliana same alleles, for Harcot one allele (S4) and for Priana one allele (S7). Same Turkish apricot cultivars yielded one band using EM-PC2consFD / EM-PC3consRD primer pairs. Some of them are Sakit-7, Alata Yildizi, Sahinbey, Dr. Kaska, Cagribey, Cagataybey and 7-89. But most of them had SC allel (SRc primer pairs). There was same situation among foreign cultivars.
Halasz et al. (2013) checked to 355 bp fragments in 63 wild apricot entries from Turkey showed that 17 of the 63 apricot entries in the first intron. It was tested SRc-R and SRc-F primer pair using 10 apricot cultivars to determine their S alleles. They determined Sc allele which yielded at 353 bp at all apricot genotypes(Vilanova et al. 2005). In present study, some apricot cultivars and genotypes were determined via linked Sc allele showed self compatibility in apricot using SRc-F and SRc-R primer pairs. Although some Turkish apricot genotypes which grown middle of Turkey hadn't Sc allele, 7 of 11 Turkish apricot cultivars yielded band at 353 bp using SRc-F and SRc-R primer pairs. These apricot cultivars were developed with using SC allele carrying apricot cultivars. Also, most of foreign cultivars had Sc allele for self compatibility. While most of the European cultivars have the Sc allele, self-incompatibility of the old Turkish cultivars has been reported in previous studies (Yilmaz, 2008; Halasz et al. 2010). Our results for all foreign apricot cultivars were consistent with previous studies such as Beliana (S7Sc), Harcot (S1S4) and Priana (S2S7) (Burgos et al., 1998).
Because the coding regions of the S8- and SC-RNase alleles are the same, it was not possible to distinguish between the 2 alleles. Self-compatibility is adjectived to pollen fragment mutation with a 358 bp insertion in the SFB gene in apricot. Formerly designed specific primer pair (AprFBC8) can be used (approximately 500 bp) to differenciate between the self-incompatible (SI) and self-compatible (SC) accessions(Halasz et al., 2010). When SFBC allele were used, genotypes have the SFB8 allele give a fragment of approximately 150 bp(Halasz et al., 2013). According to Halasz et al. (2013) identified 17 apricot accessions carrying the SFB8- allele(AprFBC8 primer pairs) from 63 apricots in Turkey, and these apricot accesions were stated as self-incompatible.
In this study, a specific primer (AprSC8) was used to determine SC- and S8-RNase alleles. Results showed that 31 of Turkish and foreign apricot cultivars had Sc allele, one of them (Cologlu) had S8 allele and 3 of them (Hungarian Best, Canino, Wilson Delicious) had SC/8 alleles.
Halasz et al. (2010) reported the S-RNase intron regions by polymerase chain reaction (PCR) amplification to determine the S-genotypes of a number of Turkish and Hungarian apricot (*Prunus armeniaca* L.) cultivars. AprSC8 primer was designed for SC and S8 to anneal in the second intron region of the SC- and S8-RNase alleles. Allel spesific primer pair amplified a fragment in the case of the S8/SC alleles. It was reported by Halasz et al. (2010) confirming the presence of S8/SC alleles among the 18 cultivars tested. Some of these (Çanakkale, Ethembey, Apricot Erigi, Mektep, Paşa Mismisi, Sam and Yerli İzmir) have proven to be self-compatible (ScSc). Three Turkish cultivars shared the SCS8 genotype (Ethembey, Mektep and Paşa Mismisi). In addition, it was reported in this study that the primer (AprSC8) was able to distinguish between SI and SC variants. Twelve previously identified S-alleles were identified among Turkish varieties. S9 was the most common S allele in the used Turkish germplasm (occurring in 18 cultivars), followed by S8 (14), S6 (12), S2, S13 and S19 (9), S7 (8), SC (7). , S3 (6), S11 and S12 (5), S20 allele was found in only two cultivars.

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Based on the structure of S-RNase in Prunus species, many primer pairs have been developed (Tao et al., 1999a; Tamura et al., 2000; Yamane et al., 2001; Sonneveld et al., 2003; Sutherland et al., 2004; Vilanova et al., 2005; Habu et al., 2008). Recently, it was determined to the S-genotypes of 14 Japanese apricot cultivars native to Japan using Pru-C2 and PCE-R, SRc-F and EM-PC5consRD, SRc-F and PM-C5(Habu et al., 2008).

In apricot crop evolution, one of the most important factors were the occurence of self-compatibility, which has resulted in a serious loss of genetic diversity in Europe and the Mediterranean Region (Pedryc et al., 2009; Bourguiba et al., 2012). In a previous study, it was reported an unequal distribution of the SC-allele in Turkish apricot cultivars and there was'nt self-compatible cultivar was found among 11 cultivars which tested in the Eastern Region of Turkey, whereas 7 cultivars out of 14 tested cultivars were self-compatible in the west of the country (Halasz et al. 2010). Although the 55 varieties of apricot examined in their studies did not reveal a sound result about the origin of self-adaptation, the number of SC varieties increasing from east to west is suggestive.

4. CONCLUSIONS

In conclusion, with this study, it was determined self-(in)compatibility alleles of 11 new Turkish and 38 foreign apricot cultivars first time. These results can be used for plantation new orchards and breeding programs. In addition, pollinator varieties should be considered when planting new orchards with self-incompatible varieties, since some Turkish and foreign apricots are self-incompatible.

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Table 1. Label, sizes of the first and second intron regions of the S-RNase gene; specific PCR for the SC/8-RNase and SFBC/8 alleles; and S-genotypes of the tested some Turkish and foreign apricots

No	Genotypes	Origin	Self (in)compability	EM-PC2consFD / EM-PC3consRD				AprFBC8-F / R	SRc - F / R	*S*-genotype
				2nd intron (bp)		1st allele	2nd allele	SFBc/SFB8	SC/S*8*-RNase (353 bp)	
1	Sakit-2	TC	Unknown	900	500	S_2	S_9	-	-	S_2S_9
2	Sakit-6	TC	Unknown	1900	900	S_{19}	S_2	-	-	$S_{19}S_2$
3	Sakit-7	TC	Unknown	500	-	S_9	-	S_C	+	S_9S_C
4	Alata Yildizi	TC	Unknown	250	-	S_3	-	S_C	+	S_3S_C
5	Sahinbey	TC	Unknown	250	-	S_3	-	S_C	+	S_3S_C
5	Dr. Kaska	TC	Unknown	1400	-	S_{13} or S_6	-	S_C	+	$S_{13}S_C$
7	Cagribey	TC	Unknown	250	-	S_3	-	S_C	+	S_3S_C
8	Cagataybey	TC	Unknown	1400	-	S_{13} or S_6	-	S_C	+	$S_{13}S_C$
9	2-89	TC	Unknown	1700	1300	S_{11}	S_6	-	-	$S_{11}S_6$
10	7-89	TC	Unknown	250	-	S_3	-	S_C	+	S_3S_C
11	33-89	TC	Unknown	900	500	S_2	S_9	-	-	S_2S_9
12	Ismailaga	TC	SI[a,c]	500	-	S_9	-	-	-	S_9-
13	Aprikoz	TC	SI[a,c]	1800	1400	S_{11}	S_{13}	-	-	$S_{11}S_{13}$
14	Cologlu	TC	SI[a,c]	500	-	S_9	-	S_8	+	S_8S_9
15	Ordubat	TC	SI[a,c]	800	350	S_7	S_{12}	-	-	S_7S_{12}
16	Sekerpare	TC	SI[a,c]	250	-	S_3	-	-	-	S_3-
17	Soganci	TC	SI	500	-	S_9	-	-	-	S_9-
18	Fracasso	FC	SC[f]	-	-	-	-	S_C	+	S_C S_C
19	Ninfa	FC	SC[g]	800	-	S_7	-	S_C	+	S_7S_C
20	Beliana	FC	SC[b,e]	800	-	S_7	-	S_C	+	S_7S_C
21	Precoce de Tyrinthe	FC	SC[e]	-	-	-	-	S_C	+	S_C S_C
22	Aurora	FC	SI[e]	1980	1300	S_{19}	S_6	-	-	$S_{19}S_6$
23	Canino	FC	SC[b,e]	-	-	-	-	S_C /S_8	+	S_CS_8
24	Bebeco	FC	SC[e]	1300	-	S_6	-	S_C	+	S_6S_C
25	Precoce de Colomer	FC	Unknown	1700	-	S_{11}	-	S_C	+	$S_{11}S_C$
26	Castelbrite	FC	SC[h]	950	800	S_2	S_7	-	-	S_2S_7
27	Roxana	FC	Unknown	800	-	S_7	-	S_C	+	S_7S_C
28	Harcot	FC	SI [c,i]	300	-	S_4	-	-	-	S_4-
39	Priana	FC	SI[e]	800	-	S_7	-	-	-	S_7-
30	Vitillo	FC	Unknown	500	-	S_9	-	-	-	S_9-
31	Markulesti	FC	SC[e]	800	-	S_7	-	S_C	+	S_7S_C

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Table 1. Continued

No	Genotypes	Origin	Self (in)compatibility	EM-PC2consFD / EM-PC3consRD				AprFBC8-F / R	SRc - F / R	*S*-genotype
				2nd intron (bp)		1st allele	2nd allele	SFBc/SFB8	SC/S*8*-RNase (353 bp)	
32	Rougge de Rousillion	FC	SC[j]	350	-	S_{12}	-	S_C	+	$S_{12}S_C$
33	Early Gold	FC	Unknown	250	-	S_3	-	-	-	S_3-
34	Pisana	FC	SC[e]	900	-	S_2	-	S_C	+	S_2S_C
35	Re Umberto	FC	Unknown	900	-	S_2	-	S_C	+	S_2S_C
36	Early Kishinevskiy	FC	Unknown	800	-	S_7	-	S_C	+	S_7S_C
37	Harlayne	FC	SC[e]	250	-	S_3	-	-	-	S_3-
38	Kechkemeti Rozsa	FC	SC[e]	-	-	S_2	-	S_C	+	S_2S_C
39	CNEF-C	FC	Unknown	250	-	S_3	-	-	-	S_3-
40	Feriana	FC	Unknown	350	-	S_4	-	S_C	+	S_4S_C
41	Palstein	FC	SC[e]	350	-	S_{12}	-	S_C	+	$S_{12}S_C$
42	Proyma	FC	Unknown	900	500	S_2	S_9	-	-	S_2S_9
43	Harglow	FC	Unknown	350	-	S_{12}	-	-	-	S_{12}-
44	Joubert Foulon	FC	Unknown	-	-	-	-	S_C	+	S_C S_C
45	Palummella	FC	SC[f]	-	-	-	-	S_C	+	S_C S_C
46	Boccuccia	FC	SC[e]	-	-	-	-	S_C	+	S_C S_C
47	Hungarian Best	FC	Unknown	-	-	-	-	S_C /S_8	+	S_8S_C
48	Zard	FC	SI[d]	900	350	S_{12}	S_2	-	-	$S_{12}S_2$
49	Silistre de Rona	FC	Unknown	-	-	-	-	S_C	+	S_C S_C
50	Goldcot	FC	SC[k]	250	-	S_3	-	-	-	S_3-
51	Portici	FC	SC[e]	800	500	S_7	S_9	-	-	S_7S_9
52	Rougge de Sernhac	FC	SC[j]	500	-	S_9	-	S_C	+	S_9S_C
53	Wilson Delicious	FC	Unknown	-	-	-	-	S_C /S_8	+	S_8S_C
54	San Castrese	FC	SC[k]	-	-	-	-	S_C	+	S_C S_C
55	Early Gold	FC	Unknown	-	-	-	-	S_C	+	S_C S_C
56	Ivonne Liverani	FC	Unknown	-	-	-	-	S_C	+	S_C S_C
57	Hinta	FC	Unknown	-	-	-	-	S_C	+	S_C S_C
58	Orenzhevo Krasny	FC	Unknown	350	-	S_{12}	-	-	-	S_{12}-
59	Harogem	FC	Unknown	500	-	S_9	-	S_C	+	S_9 S_C

SI: Self-incompatible, SC: Self-compatible; Self-(in)compatibility phenotypes determined by a: Yilmaz, 2008; b: Halasz, 2007; c: Halasz et al. 2010; d: Halazs et al., 2005; e: Burgos et al., 1997; f: Cappellini and Limongelli, 1981; g: Bassi et al., 1995; h: Ramming and Tanner, 1978; i: Egea and Burgos, 1996; j: Audergon et al., 1988; k= Lamb and Stiles, 1983; +: yielded SRc band; -: didn't yield SRc band; FC: Foreign Cultivars; TC: Turkish Cultivars

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