

¹Ardahan University, Faculty of Engineering, Food Engineering Department, Ardahan, Turkey

²Çukurova University, Faculty of Agriculture, Department of Horticulture, Adana, Turkey

³İnönü University, Faculty of Agriculture, Department of Horticulture, Malatya, Turkey

Determination of S alleles in Paviot × Levent apricot progenies by PCR and controlled pollination

Zehra Tugba Murathan^{1*}, Salih Kafkas², Bayram Murat Asma³

(Received July 31, 2015)

Summary

In this study, the sexual incompatibility of Paviot and Levent apricot parents and 89 F₁ (Paviot × Levent) progenies was determined by self-pollination experiments and S-allele-specific polymerase chain reaction (PCR) technique. According to the self-pollination and isolation analyses under field conditions, it was found that the Paviot genotype is self-compatible (SC), whereas the Levent genotype is self-incompatible (SI). It was determined that, of all the progenies, 55 had a fruit set below 5% and were self-incompatible, whereas 34 had a fruit set over 5% and were self-compatible. The PCR-based techniques showed that, in parallel to the data obtained from the field studies, 55 F₁ progenies did not have S_c allele, whereas 34 progenies involved S_c allele. There were S_cS₂ alleles in the Paviot genotype and S₂₀S₅₂ alleles in the Levent genotype. It was determined that there were S₂S₂₀, S₂S₅₂, S_cS₂₀, and S_cS₅₂ alleles in 89 F₁ progenies and the distribution of the four alleles in the progenies was found to be as follows: 35.9% S₂S₂₀, 25.8% S₂S₅₂, 23.6% S_cS₂₀, and 14.6% S_cS₅₂. F₁ progenies nos. 41, 46, 86, and 89 should be used as pollinators in further breeding studies.

Keywords: Apricot, Paviot, Levent, Self-incompatibility, PCR

Introduction

Apricot is one of the most important fruit types grown under mild temperature conditions in the world. It is a delicious fruit owing to its strong flavor and sugar-organic acid balance (GURRIERI et al., 2001). Some European and Mediterranean countries such as Turkey, Spain, Italy, France, and Greece have many local types of apricots, and these countries contribute to more than 75% of the total apricot production in the world (LECCESE et al., 2010).

Apricot belongs to *Prunus* species of the Rosaceae family (OZBEK, 1978). It was reported that there are two genes that control the gametophytic self-incompatibility in *Prunus* species. One of these genes is S-ribonuclease (S-RNase) related to the stylus and the other one is the S-haplotype-specific F-box protein gene (SFB) related to pollen (KAO and TSUKAMOTO, 2004; QIAO et al., 2004; MCCLURE, 2006).

Similar to the incompatibility, the functional capability of pollen is ascribed by a series of genes (S₁, S₂, S₃, S₄, ..., S_n [multiple allele series]). The diploid stylus typically involves two different S genes, and each pollen grain carries one of the two genes. These gene regions code S-RNase protein, which makes the incompatible species to reject their own pollen (EBERT et al., 1986; MCCLURE et al., 1989). The glycoproteins that have this ribonuclease activity define S specificity in the pistil. If the pistil has the same gene as that of the pollen, S-RNase shows a cytotoxic effect on the pollen tubes and prevents the growth of the tubes, which leads to incompatibility (ROALSON and MCCUBIN, 2003; GOLDRAJ et al., 2006). In this case, either the tip of the pollen tube that progresses through the stylus swells or the tube end explodes (HESLOP-HARRISON, 1975). Some

physiological studies indicated that RNA degrades within 12-45 h followed by an incompatible pollination (MCCLURE et al., 1990).

The breeding experiments are divided into the following two main groups: conventional and biotechnological. The biotechnological breeding includes molecular marker-assisted selection and genetic transformation methods. This breeding yields the results more rapidly than the conventional one (BASSI, 2006). In the apricot species obtained from North America and Spain, initially, seven S alleles (S₁₋₇) were defined by using molecular techniques (ALBURQUERQUE et al., 2002). Later, nine more alleles (S₈₋₁₆) were defined through NEpHGE and polymerase chain reaction (PCR) methods (HALASZ et al., 2005). The existing S alleles were then detected in the apricot species obtained from China, North America, Europe, Turkey, and Tunisia (EGEA and BURGOS, 1996; HALASZ et al., 2005; ZHANG et al., 2008; MILATOVIC et al., 2010; HALASZ et al., 2010; LACHKAR et al., 2013).

The main objective of all breeding experiments is to improve productivity. The productivity depends on the factors such as environmental adaptability and self-incompatibility. Most of the self-incompatible apricot varieties cannot be used in breeding programs as they result in irregular fruit set and require a pollinator (ZHEBENTYAYEVA et al., 2012). Therefore, it is very important to know the incompatibility among the species that are used as parents in breeding experiments. Sexual incompatibility can be found in many commercially cultivated fruit species. In order to ensure the fruit set in these species, there is a necessity for cross-pollination by the wind or insects and pollinator species (BADANES et al., 2000). The present study aimed to determine the sexual incompatibility and to reveal the heredity of sexual incompatibility in 89 F₁ (Paviot × Levent) progenies by the field and laboratory experiments.

Materials and methods

Plant Material

The research materials were obtained from the Apricot Collection Orchard affiliated to İnönü University. This area has a continental climate with latitude: altitude 977 m, 38°20'20.23 N and longitude 38°26'26.56 E. In this study, 89 F₁ progenies (Paviot × Levent) were used. F₁ progenies were obtained through the artificial pollination performed under the scope of the TUBITAK-TOGTAG Project in 2003. The hybridization was carried out to obtain the progenies having the desired characteristics such as Paviot's big fruits, orange color of fruit peel, and resistance against Plum Pox: Levent's late blooming features. The leaf samples of each plant were stored at 4 °C after lyophilization. Fruit yield was determined as the mean fruit quantity of per apricot tree (kg/tree). For each genotype, weighting was done for every 10 fruits using a 0.05-g digital balance. The Brix° degree of the fruit juice from 10 fruits was determined by digital refractometry (ASMA and OZTURK, 2005).

Pollination tests

In the pollination tests, conducted during 2009-2011, from each progeny, three different branches, each of which having approxi-

* Corresponding author

mately 300 flowers, were selected. The first branch was labeled and left to open pollination. The second branch was bagged using double-layered cheese cloth to prevent cross-pollination and to ensure self-pollination a week before the anthesis. The third branch was emasculated and left open, after that, they were artificially pollinated for two or three times with their own pollen, which had been collected and dried a day before. Approximately after 70-80 days, the fruit set rates were determined. At the end of the isolation and artificial pollination, the progenies having the fruit set less than 5% were evaluated to be self-incompatible, and the others having more than 5% were considered self-compatible (FAUST, 1998).

DNA extraction, S allele PCR analysis, and DNA sequencing

For DNA isolation from the leaf samples, the CTAB (Cetyl Trimethyl Ammonium Bromide) protocol developed by DOYLE and DOYLE (1987) was used with minor modifications (KAFKAS and PERL-TREVES, 2001). The concentration of DNA in the samples was determined by comparing with λ -DNA that was quantified by the gel electrophoresis.

To determine S alleles, PCR was conducted using the primer combinations designed for the first and second introns of S-RNase genes and developed by TAO et al. (1999), ROMERO et al. (2004) and VILANOVA et al. (2005) as listed in Tab. 1.

Each PCR reaction in 25 μ L contained 75 mM Tris-HCl (pH 8.8), 20 mM $(\text{NH}_4)_2\text{SO}_4$, 2 mM MgCl_2 , 0.1% Tween 20, 100 μ M dATP, 100 μ M dTTP, 100 μ M dGTP, 100 μ M dCTP, 0.2 μ M of each primer, 1.0 unit of Taq DNA polymerase, and 50 ng of DNA. For PCR amplification, the samples were pre-denatured at 94 °C for 3 min, followed by 35 cycles with denaturation for 45 s at 94 °C, annealing for 45 s at 54 °C or 58 °C, and extension for 60 s at 72 °C. For the final extension step, the samples were kept at 72 °C for 10 min. The PCR products were separated by electrophoresis on a 2% or 3% agarose gel with 0.5 \times TBE (Tris-Borate-EDTA) depending on the band size and were visualized under UV light by staining after with ethidium bromide. At the same time, the amplification products were analyzed by capillary electrophoresis using an ABI prism 3130xl automatic DNA sequencer (Applied Biosystems).

The DNA sequencing of the PCR products was commercially performed following Sanger's method at Medsantek, Istanbul, Turkey. The S alleles of the parents were determined by comparing the sequences using BLAST with those available at the National Center for Biotechnology Information (NCBI) database.

Tab. 1: Primers used to determine S-alleles of genotypes

Primers	Primer Design	Base number	Reference
SRc-R	GGC CAT TGT TGC ACA AAT TG	20	Vilanova et al., 2005
SRc-F	CTC GCT TTC CTT GTT CTT GC	20	Romero et al., 2004
PruT2F	GTT CTT GCT TTT GCT TTC TTC	21	Tao et al., 1999
PruC4R	GGA TGT GGT ACG ATT GAA GCG	21	Tao et al., 1999
PruC2F	CTT TGG CCA AGT AAT TAT TCA AAC	24	Tao et al., 1999

Statistical analysis

The data are presented as means ($n=3$) \pm standard deviations (s.d.). All statistical analyses were performed using SPSS 15.0 software.

DUNCAN's test (1955) was used for the significance control ($p < 0.05$) following variance analysis (ANOVA).

Results and discussion

Pollination Tests

The fruit set was 70% for the Paviot genotype and 45% for the Levent genotype left open to the pollination. The fruit set of 51% was observed in the Paviot genotype, and no fruit set was found for the Levent genotype left to closed pollination during the harvest period in 2009. Similarly, in the self-pollination branches, the fruit set rate was detected to be 52% in the Paviot genotype and 1.5% in the Levent genotype. In the isolation and self-pollination experiments, the fruit set rate in the 56 F_1 genotypes was below 5% (Tab. 2). FAUST (1998) suggested that the varieties having a fruit set rate less than 5%, where self-pollination has been conducted, can be defined as self-incompatible, whereas those having a fruit set more than 5% can be defined as self-compatible. ASMA (2008) conducted the isolation and self-pollination experiments and reported that the fruit set rate of the Levent apricot genotype was below 5% and this genotype was self-incompatible. Thus considering the results of our study, it can be affirmed that Paviot genotype is self-compatible and the Levent genotype is self-incompatible; 55 F_1 progenies are self-incompatible while 34 are self-compatible (Tab. 2). Similar results were obtained from the field studies of different cultivars in recent years. ASKIN (1989) reported that fruit set in Tokaloglu and Sam apricot cultivars that do not yield fruit regularly in the Aegean Region was 0.46% and 0.65%, respectively, and these species were self-incompatible. BOLAT and GULERYUZ (1994) reported that the fruit set rate was higher in the case of cross-pollination than self-pollination in Hasanbey cultivars. PAYDAS et al. (2001) determined that 25 of the 62 apricot cultivars cultivated in the Malatya province were self-compatible, while GULCAN et al. (2006) determined that 32 of the 70 apricot genotypes cultivated in Adana and Malatya provinces were self-compatible. According to self-pollination studies conducted on Katy, Harcot, and Jiguang hybrids by WU et al. (2011), fruit set rates were determined to be as follows: 19.68% for Katy \times Harcot, 15.45% for Harcot \times Katy, 7.78% for Katy \times Jiguang, and 16.75% for Jiguang \times Katy. In self-pollination studies of Harcot and Chuanzhihong cultivars, fruit set rates were 0.57% and 0%; these rates were 11.29% and 22.87% in cross-pollination experiments (Harcot \times Chuanzhihong and Chuanzhihong \times Harcot), and both cultivars were reported to be self-incompatible (GU et al., 2013).

S allele PCR analyses and DNA sequencing

At the end of PCR studies conducted with the PruT2, Src-F, and Src-R primer combinations for the amplification of the first intron region of the apricot S-RNase, a band of 353-bp was found in the Paviot genotype (Fig. 1). In previous studies, the cultivars that showed the 353-bp band were reported to be self-compatible when this primer combination was used (VILANOVA et al., 2005). In addition, a band of 328-bp in the Paviot genotype and 420-bp in the Levent genotype were found.

In the PCR experiment, conducted with PruC2F and PruC4R primer combination for the amplification of the second intron region of S-RNase, no band was amplified for the Paviot genotype, and two bands of approximately 1400 and 2100 bp were detected for the Levent genotype. The analysis of the alleles included in F_1 genotypes showed that the 420-bp band in the gel obtained through PruT2-SrcF-SrcR combination in the Levent genotype was the same to the 1400-bp band found in PruC2F-C4R combination.

The comparison of the nucleotide sequence obtained from the SrcF-SrcR primer combination in parents and the current apricot S allele sequences in the NCBI database indicated that S allele sequences of

Tab. 2: Mean comparison of fruit set percentage after open, isolated and self-pollination in F₁ progenies

Progenies	Open Pollination (%)	Isolated Pollination (%)	Self Pollination (%)	Progenies	Open Pollination (%)	Isolated Pollination (%)	Self Pollination (%)
Pavlot	70 ^a	51 ^a	52 ^a	PxL 45	16.9 ^d	0.9 ^d	2.5 ^{cd}
Levent	45 ^{bc}	0	0	PxL 46	24.2 ^{cd}	40.5 ^{ab}	34.6 ^b
PxL 01	30 ^{cd}	0	0	PxL 47	47.5 ^b	10.4 ^c	10.9 ^c
PxL 02	25 ^{cd}	12.2 ^c	25.3 ^b	PxL 48	28.5 ^{cd}	20 ^{bc}	40.2 ^{ab}
PxL 03	52.7 ^b	1.2 ^d	0	PxL 49	15.5 ^d	0	3.2 ^{cd}
PxL 04	50 ^b	1.1 ^d	0	PxL 50	17.4 ^d	0	1.2 ^d
PxL 05	27.1 ^{cd}	0	0	PxL 51	38.5 ^{bc}	6.3 ^{cd}	14.6 ^{bc}
PxL 06	30.7 ^{cd}	23 ^{bc}	17 ^{bc}	PxL 52	26.5 ^{cd}	16 ^{bc}	16.1 ^{bc}
PxL 07	20 ^{cd}	0	0	PxL 53	32.1 ^c	6 ^{cd}	1 ^d
PxL 08	38 ^c	3 ^{cd}	2 ^d	PxL 54	52.2 ^b	0	4.1 ^{cd}
PxL 09	45 ^{bc}	0	2 ^d	PxL 55	25 ^{cd}	12.3 ^c	10.8 ^{cv}
PxL 10	13.9 ^d	20 ^{bc}	30 ^b	PxL 56	15.9 ^d	0	0
PxL 11	25 ^{cd}	25.9 ^b	19.1 ^{bc}	PxL 57	39.6 ^{bc}	12 ^c	19.7 ^{bc}
PxL 12	36.9 ^c	0	1.5 ^d	PxL 58	15.4 ^d	14.2 ^{bc}	40.6 ^{ab}
PxL 13	46.7 ^{bc}	45 ^{ab}	29.1 ^b	PxL 59	15 ^d	1 ^d	0
PxL 14	41.6 ^{bc}	15.2 ^{bc}	35 ^{ab}	PxL 60	21 ^{cd}	0	0
PxL 15	34.5 ^c	2.3 ^{cd}	0	PxL 61	20.1 ^{cd}	0	0
PxL 16	59.5 ^{ab}	16.7 ^{bc}	46.5 ^{ab}	PxL 62	14.5 ^d	6.7 ^{cd}	4.9 ^{cd}
PxL 17	30.4 ^{cd}	2.7 ^{cd}	1.6 ^d	PxL 63	11 ^d	5.8 ^{cd}	5 ^{cd}
PxL 18	7.4 ^{de}	1.9 ^d	0	PxL 64	11.8 ^d	0	0
PxL 19	13.3 ^d	17.5 ^{bc}	24.4 ^b	PxL 65	14.1 ^d	15.2 ^{bc}	14.3 ^{bc}
PxL 20	37.1 ^c	1.3 ^d	0	PxL 66	28.9 ^{cd}	0	0
PxL 21	31.4 ^{cd}	0	0	PxL 67	21.6 ^{cd}	0	0
PxL 22	26.7 ^{cd}	1.5 ^d	1.4 ^d	PxL 68	11 ^d	0	0
PxL 23	30 ^{cd}	1.5 ^d	0	PxL 69	17.6 ^d	0	0
PxL 24	42.6 ^{bc}	6.9 ^{cd}	17.5 ^{bc}	PxL 70	13.4 ^d	5 ^{cd}	6.7 ^{cd}
PxL 25	21.3 ^{cd}	16.7 ^{bc}	10 ^c	PxL 71	17.1 ^d	6.2 ^{cd}	6.4 ^{cd}
PxL 26	10.7 ^d	16 ^{bc}	14.7 ^{bc}	PxL 72	13.2 ^d	0	0
PxL 27	20.8 ^{cd}	13.8 ^c	20.5 ^{bc}	PxL 73	9.9 ^{de}	0	2.2 ^{cd}
PxL 28	50.6 ^b	12.9 ^c	16.5 ^{bc}	PxL 74	11.3 ^d	1 ^d	0
PxL 29	11.3 ^d	0	1.1 ^d	PxL 75	10.9 ^d	0	0
PxL 30	11.9 ^d	3.2 ^{cd}	1.7 ^d	PxL 76	7 ^{de}	0	0
PxL 31	25 ^{cd}	0	0	PxL 77	8.9 ^{de}	0	0
PxL 32	22 ^{cd}	0	1 ^d	PxL 78	12.1 ^d	7.6 ^c	6.5 ^{cd}
PxL 33	29.1 ^{cd}	0	1.7 ^d	PxL 79	9.1 ^{de}	0	3 ^{cd}
PxL 34	16.4 ^d	5.8 ^{cd}	6.7 ^{cd}	PxL 80	11.4 ^d	1 ^d	1 ^d
PxL 35	50 ^b	0	0	PxL 81	15 ^d	0	1 ^d
PxL 36	15.5 ^d	0	0	PxL 82	9.9 ^{de}	7.2 ^c	6.6 ^{cd}
PxL 37	15.6 ^d	9.4 ^c	20.9 ^{bc}	PxL 83	10.1 ^d	0	0
PxL 38	34.7 ^c	0	0	PxL 84	12.5 ^d	0	0
PxL 39	10.9 ^d	4.7 ^{cd}	4.4 ^{cd}	PxL 85	15.9 ^d	0	0
PxL 40	36.2 ^{bc}	20.5 ^{bc}	19.9 ^{bc}	PxL 86	19.8 ^{cd}	4.5 ^{cd}	5.7 ^{cd}
PxL 41	54.3 ^b	46.3 ^{ab}	34.2 ^b	PxL 87	5.7 ^e	0	0
PxL 42	41.7 ^{bc}	0	0	PxL 88	12.3 ^d	0	0
PxL 43	17.8 ^d	1.1 ^d	0	PxL 89	9.1 ^{de}	7.2 ^c	6.9 ^{cd}
PxL 44	34 ^c	12.9 ^c	11.8 ^c				

Data followed by different letters are significantly different from each other ($P < 0.05$) according to Duncan's test.

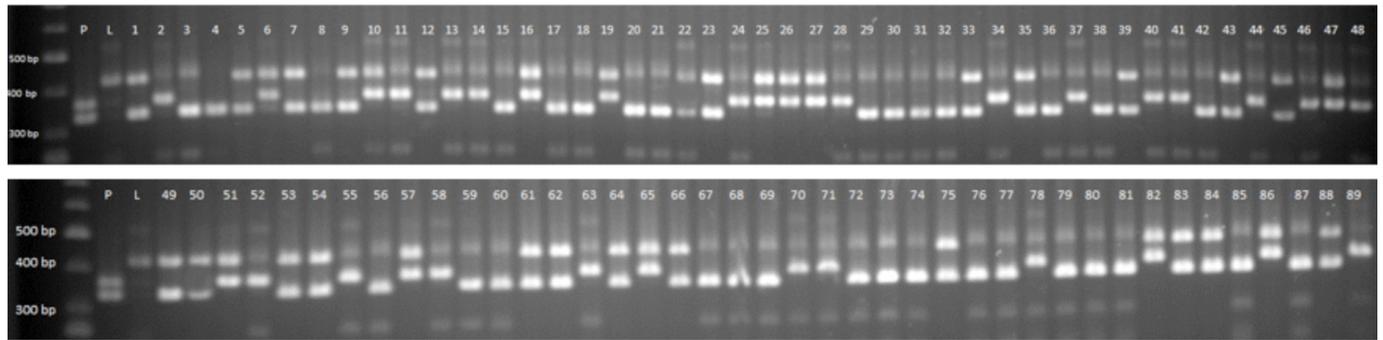


Fig. 1: S alleles determined through the use of Pru T2, SrcF and SrcR primer combination in parents and F₁ progenies

Pavlot and Levent genotypes show homology with the S_c (353 bp), S₂ (328 bp), S₅₂ (1400 bp), and S₂₀ (2100 bp) allele sequences of *Prunus armeniaca* available at GenBank (ROMERO et al., 2004; VILANOVA et al., 2006; ZHANG et al., 2008; JIANG et al., 2010). HALASZ et al. (2010) reported that there are S₆S₁₉ alleles in the Levent genotype so this apricot genotype is self-incompatible. In the present study, the PCR bands obtained for the Levent genotype were sequenced bidirectionally using the primers designed with the first and second intron regions and the obtained DNA sequences were compared with the allele sequences available in GenBank. At the end of this study, the presence of S₂₀S₅₂ allele was found in the Levent genotype. Similarly, YILMAZ et al. (2013) reported that there was no S_c allele in the Levent genotype and this genotype was self-incompati-

ble. In a study conducted with 63 wild apricot genotypes in Erzinçan, it was reported that the local apricot cultivars cultivated in the eastern region of Turkey mostly do not carry S_c allele (HALASZ et al., 2013).

In parallel with the results obtained under field conditions, it was found that 55 of the 89 F₁ progenies did not carry the S_c allele, and these progenies were self-incompatible (Tab. 3). It was found that 55 F₁ progenies carried S₂S₅₂ and S₂S₂₀ allele pairs, and these plants were self-incompatible. The distribution in F₁ progenies of the alleles detected in Pavlot (S_cS₂) and Levent (S₂₀S₅₂) parents was as follows: 35.9% for S₂S₂₀, 25.8% for S₂S₅₂, 23.6% for S_cS₂₀, and 14.6% for S_cS₅₂. BURGOS et al. (1997) reported that self-compatibility alleles are dominant over incompatible alleles. In the present study, one

Tab. 3: S genotypes of Pavlot × Levent F₁ progenies

Progenies	Alleles	Progenies	Alleles	Progenies	Alleles	Progenies	Alleles
Pavlot	S _c S ₂	PxL 22	S ₂ S ₂₀	PxL 45	S ₂ S ₅₂	PxL 68	S ₂ S ₂₀
Levent	S ₂₀ S ₅₂	PxL 23	S ₂ S ₅₂	PxL 46	S _c S ₂₀	PxL 69	S ₂ S ₂₀
PxL 01	S ₂ S ₅₂	PxL 24	S _c S ₂₀	PxL 47	S _c S ₅₂	PxL 70	S _c S ₂₀
PxL 02	S _c S ₂₀	PxL 25	S _c S ₅₂	PxL 48	S _c S ₂₀	PxL 71	S _c S ₂₀
PxL 03	S ₂ S ₂₀	PxL 26	S _c S ₅₂	PxL 49	S ₂ S ₅₂	PxL 72	S ₂ S ₂₀
PxL 04	S ₂ S ₂₀	PxL 27	S _c S ₅₂	PxL 50	S ₂ S ₅₂	PxL 73	S ₂ S ₂₀
PxL 05	S ₂ S ₅₂	PxL 28	S _c S ₂₀	PxL 51	S _c S ₅₂	PxL 74	S ₂ S ₂₀
PxL 06	S _c S ₅₂	PxL 29	S ₂ S ₂₀	PxL 52	S _c S ₂₀	PxL 75	S ₂ S ₅₂
PxL 07	S ₂ S ₅₂	PxL 30	S ₂ S ₂₀	PxL 53	S ₂ S ₅₂	PxL 76	S ₂ S ₂₀
PxL 08	S ₂ S ₂₀	PxL 31	S ₂ S ₂₀	PxL 54	S ₂ S ₅₂	PxL 77	S ₂ S ₂₀
PxL 09	S ₂ S ₅₂	PxL 32	S ₂ S ₂₀	PxL 55	S _c S ₂₀	PxL 78	S _c S ₂₀
PxL 10	S _c S ₅₂	PxL 33	S ₂ S ₅₂	PxL 56	S ₂ S ₂₀	PxL 79	S ₂ S ₂₀
PxL 11	S _c S ₂₀	PxL 34	S _c S ₂₀	PxL 57	S _c S ₅₂	PxL 80	S ₂ S ₂₀
PxL 12	S ₂ S ₅₂	PxL 35	S ₂ S ₅₂	PxL 58	S _c S ₂₀	PxL 81	S ₂ S ₂₀
PxL 13	S _c S ₂₀	PxL 36	S ₂ S ₂₀	PxL 59	S ₂ S ₂₀	PxL 82	S _c S ₅₂
PxL 14	S _c S ₂₀	PxL 37	S _c S ₂₀	PxL 60	S ₂ S ₂₀	PxL 83	S ₂ S ₅₂
PxL 15	S ₂ S ₂₀	PxL 38	S ₂ S ₂₀	PxL 61	S ₂ S ₅₂	PxL 84	S ₂ S ₅₂
PxL 16	S _c S ₅₂	PxL 39	S ₂ S ₅₂	PxL 62	S ₂ S ₅₂	PxL 85	S ₂ S ₂₀
PxL 17	S ₂ S ₂₀	PxL 40	S _c S ₂₀	PxL 63	S _c S ₂₀	PxL 86	S _c S ₅₂
PxL 18	S ₂ S ₂₀	PxL 41	S _c S ₂₀	PxL 64	S ₂ S ₅₂	PxL 87	S ₂ S ₂₀
PxL 19	S _c S ₅₂	PxL 42	S ₂ S ₂₀	PxL 65	S _c S ₅₂	PxL 88	S ₂ S ₅₂
PxL 20	S ₂ S ₂₀	PxL 43	S ₂ S ₅₂	PxL 66	S ₂ S ₅₂	PxL 89	S _c S ₂₀
PxL 21	S ₂ S ₂₀	PxL 44	S _c S ₂₀	PxL 67	S ₂ S ₂₀		

Tab. 4: Fruit characteristics of F₁ genotypes

Pro-genies	Fruit Weight (g)	Kernel Weight (g)	Brix° (%)	Pro-genies	Fruit Weight (g)	Kernel Weight (g)	Brix° (%)	Pro-genies	Fruit Weight (g)	Kernel Weight (g)	Brix° (%)
PxL 01	27.6± 2.5 ^c	3.1± 0.2 ^b	16.0± 1.1 ^b	PxL 31	31.6± 2.4 ^{bc}	3.3± 0.2 ^b	18.0± 1.0 ^a	PxL 61	24.9± 3.1 ^c	3.3± 0.2 ^b	18.5± 1.2 ^{ab}
PxL 02*	23.1± 2.2 ^c	2.6± 0.2 ^b	16.0± 1.0 ^b	PxL 32	18.2± 1.9 ^d	2.4± 0.2 ^b	16.0± 1.4 ^b	PxL 62*	24.9± 3.1 ^c	3.3± 0.2 ^b	18.5± 1.2 ^{ab}
PxL 03*	24.0± 2.8 ^c	2.2± 0.2 ^b	18.0± 0.9 ^{ab}	PxL 33	33.5± 2.4 ^{bc}	3.5± 0.3 ^b	17.0± 1.0 ^b	PxL 63*	29.9± 2.5 ^{bc}	2.8± 0.2 ^b	14.0± 0.5 ^c
PxL 04	30.2± 2.3 ^{bc}	4.3± 0.3 ^a	18.0± 0.6 ^{ab}	PxL 34*	39.4± 2.7 ^b	3.4± 0.2 ^b	18.0± 1.2 ^{ab}	PxL 64	22.9± 2.0 ^c	2.7± 0.2 ^b	23.0± 1.2 ^a
PxL 05	44.2± 4.5 ^b	3.3± 0.3 ^b	19.0± 1.2 ^a	PxL 35	31.5± 2.8 ^{bc}	3.0± 0.3 ^b	18.0± 1.4 ^{ab}	PxL 65	35.7± 2.9 ^{bc}	4.1± 0.3 ^a	20.0± 1.5 ^a
PxL 06	20.0± 2.0 ^c	2.7± 0.2 ^b	18.0± 1.5 ^{ab}	PxL 36	28.3± 2.1 ^c	3.0± 0.2 ^b	18.0± 1.1 ^{ab}	PxL 66	26.9± 2.2 ^c	2.9± 0.3 ^b	19.0± 1.3 ^a
PxL 07	18.8± 1.6 ^d	3.4± 0.3 ^b	18.0± 1.2 ^{ab}	PxL 37	44.9± 2.8 ^b	4.1± 0.3 ^a	21.0± 0.8 ^a	PxL 67	34.5± 2.5 ^{bc}	3.2± 0.3 ^b	15.0± 1.1 ^b
PxL 08	25.4± 2.3 ^c	3.1± 0.2 ^b	21.0± 1.1 ^a	PxL 38	35.8± 2.2 ^{bc}	3.5± 0.2 ^b	20.0± 0.6 ^a	PxL 68*	24.9± 2.6 ^c	2.8± 0.4 ^b	16.0± 1.1 ^b
PxL 09	24.8± 2.1 ^c	2.5± 0.3 ^b	19.0± 1.3 ^a	PxL 39	32.7± 2.1 ^{bc}	3.7± 0.2 ^{ab}	19.0± 0.9 ^a	PxL 69*	18.1± 1.8 ^d	2.4± 0.6 ^b	12.0± 1.0 ^d
PxL 10	20.2± 2.0 ^c	2.4± 0.1 ^b	18.0± 1.1 ^{ab}	PxL 40	18.4± 1.9 ^d	2.6± 0.2 ^b	14.0± 0.5 ^c	PxL 70	39.3± 3.9 ^{bc}	3.8± 0.3 ^{ab}	21.0± 1.1 ^a
PxL 11	49.8± 3.7 ^b	3.4± 0.1 ^b	20.0± 1.4 ^a	PxL 41**	51.8± 2.2 ^{ab}	4.3± 0.4 ^a	20.0± 0.8 ^a	PxL 71	40.1± 3.0 ^b	3.8± 0.3 ^{ab}	18.0± 1.0 ^{ab}
PxL 12	22.3± 2.5 ^c	2.1± 0.1 ^b	22.0± 1.2 ^a	PxL 42	40.6± 2.4 ^b	2.5± 0.1 ^b	19.0± 0.6 ^a	PxL 72*	20.6± 1.5 ^c	2.3± 0.2 ^b	16.0± 1.0 ^b
PxL 13	21.5± 3.1 ^c	2.6± 0.1 ^b	14.0± 0.6 ^c	PxL 43	30.4± 2.1 ^{bc}	3.1± 0.2 ^b	13.0± 0.5 ^c	PxL 73	27.4± 2.6 ^c	2.8± 0.2 ^b	18.0± 1.1 ^{ab}
PxL 14	23.5± 2.4 ^c	2.1± 0.1 ^b	16.0± 0.7 ^b	PxL 44	39.4± 3.9 ^b	3.0± 0.2 ^b	19.0± 0.9 ^a	PxL 74	47.3± 3.8 ^b	4.3± 0.3 ^a	19.0± 1.0 ^a
PxL 15*	30.6± 2.8 ^{bc}	3.9± 0.3 ^{ab}	20.0± 1.3 ^a	PxL 45	36.9± 2.5 ^{bc}	3.1± 0.1 ^b	20.0± 0.6 ^a	PxL 75	23.6± 2.5 ^c	2.7± 0.2 ^b	17.0± 1.4 ^b
PxL 16	18.1± 1.9 ^d	3.0± 0.2 ^b	21.0± 1.2 ^a	PxL 46*	59.3± 4.8 ^{ab}	2.5± 0.2 ^b	18.0± 0.8 ^{ab}	PxL 76	33.6± 3.7 ^{bc}	3.4± 0.3 ^b	17.0± 1.5 ^b
PxL 17**	33.5± 2.5 ^{bc}	3.8± 0.3 ^{ab}	22.0± 1.4 ^a	PxL 47	27.8± 2.9 ^c	3.2± 0.2 ^b	18.0± 0.5 ^{ab}	PxL 77	25.7± 2.5 ^c	2.1± 0.2 ^b	15.0± 1.3 ^b
PxL 18*	31.1± 2.3 ^{bc}	3.2± 0.2 ^b	17.0± 1.4 ^b	PxL 48	70.5± 4.7 ^a	4.6± 0.3 ^a	14.0± 0.6 ^c	PxL 78	21.2± 2.6 ^c	2.6± 0.2 ^b	16.0± 0.8 ^b
PxL 19	42.6± 3.6 ^b	3.7± 0.2 ^{ab}	22.0± 1.2 ^a	PxL 49	32.9± 2.5 ^{bc}	3.7± 0.2 ^{ab}	16.5± 0.8 ^{bc}	PxL 79	23.6± 2.2 ^c	2.8± 0.2 ^b	18.0± 0.6 ^{ab}
PxL 20*	30.5± 2.9 ^{bc}	3.5± 0.2 ^b	19.0± 1.1 ^a	PxL 50	31.4± 2.2 ^{bc}	3.2± 0.2 ^b	19.0± 1.5 ^a	PxL 80	34.8± 2.1 ^{bc}	3.0± 0.2 ^b	17.5± 0.9 ^{ab}
PxL 21*	34.8± 2.4 ^{bc}	3.4± 0.2 ^b	22.0± 1.0 ^a	PxL 51*	21.6± 2.1 ^c	2.9± 0.2 ^b	16.0± 0.6 ^b	PxL 81	45.6± 4.9 ^b	4.3± 0.3 ^a	18.0± 1.0 ^{ab}
PxL 22*	22.2± 2.1 ^c	2.5± 0.1 ^b	19.0± 1.5 ^a	PxL 52*	23.5± 2.9 ^c	3.4± 0.2 ^b	17.0± 0.5 ^b	PxL 82	30.6± 2.8 ^{bc}	2.5± 0.1 ^b	16.0± 1.2 ^b
PxL 23*	14.6± 1.9 ^d	1.6± 0.1 ^c	20.0± 1.5 ^a	PxL 53*	40.3± 2.1 ^b	3.4± 0.3 ^b	22.0± 0.9 ^a	PxL 83*	65.0± 5.5 ^a	4.6± 0.3 ^a	14.0± 1.2 ^c
PxL 24	35.7± 2.5 ^{bc}	3.9± 0.2 ^{ab}	17.5± 1.1 ^{ab}	PxL 54*	19.0± 1.4 ^d	2.1± 0.1 ^b	19.0± 0.9 ^a	PxL 84	59.0± 4.9 ^{ab}	4.4± 0.3 ^a	19.0± 1.1 ^a
PxL 25	29.4± 1.4 ^{bc}	2.7± 0.2 ^b	18.0± 1.4 ^{ab}	PxL 55	24.1± 1.6 ^c	2.1± 0.1 ^b	17.0± 0.5 ^b	PxL 85	37.0± 2.5 ^{bc}	3.4± 0.2 ^b	16.0± 1.0 ^b
PxL 26	40.3± 3.8 ^b	3.9± 0.2 ^{ab}	17.0± 1.6 ^b	PxL 56*	48.2± 1.5 ^b	3.0± 0.1 ^b	20.0± 1.1 ^a	PxL 86*	62.0± 5.5 ^a	4.5± 0.3 ^a	20.0± 1.2 ^a
PxL 27	32.9± 2.3 ^{bc}	2.9± 0.2 ^b	17.0± 1.2 ^b	PxL 57	46.3± 3.6 ^b	3.9± 0.1 ^{ab}	19.0± 1.0 ^a	PxL 87	43.0± 5.2 ^b	3.8± 0.2 ^{ab}	15.0± 0.8 ^b
PxL 28	38.1± 3.6 ^{bc}	3.9± 0.2 ^{ab}	19.0± 1.5 ^a	PxL 58	34.0± 3.2 ^{bc}	3.3± 0.3 ^b	15.0± 1.4 ^b	PxL 88	33.3± 2.6 ^{bc}	3.3± 0.2 ^b	16.0± 0.7 ^b
PxL 29	18.1± 1.8 ^d	2.2± 0.2 ^b	21.0± 1.5 ^a	PxL 59	31.6± 3.5 ^{bc}	3.0± 0.3 ^b	16.5± 1.2 ^b	PxL 89**	55.7± 4.5 ^{ab}	4.2± 0.3 ^a	19.0± 1.8 ^a
PxL 30	39.1± 2.6 ^b	4.1± 0.3 ^a	23.0± 1.6 ^a	PxL 60	23.8± 3.2 ^c	2.2± 0.1 ^b	20.0± 1.5 ^a				

*: High yield; **: Very high yield

Values are means ± standard deviation (SD) of three replications. Data followed by different letters are significantly different from each other ($P < 0.05$) according to Duncan's test.

Conclusion

incompatible allele and one S_c allele were found in 34 F₁ progenies, but they were self-compatible; in other words, S_c allele was found dominant over the incompatible allele.

Tab. 4 shows the pomological features of F₁ progenies observed in 2011. F₁ progenies nos. 2, 3, 15, 17, 18, 20, 21, 22, 23, 34, 41, 46, 51, 52, 53, 54, 56, 62, 63, 68, 69, 72, 83, 86, and 89 had high fruit yield. But the fruit weight and the total soluble solid content of some of these progenies were low. The genotypes that can be used in breeding experiments should be self-compatible and have good pomological properties. F₁ progenies nos. 41, 46, 86, and 89 had both high fruit yield, fruit weight, and total soluble solid content and they were also found to be self-compatible. The F₁ progeny no. 84 was self-incompatible although the quality was high in pomological characters.

The sexual incompatibility of Paviot and Levent apricot parents and 89 F₁ (Paviot × Levent) progenies was determined by self-pollination studies and S-allele-specific polymerase chain reaction (PCR). The fruit set rate was high as cross-pollination was allowed in the branches left open to pollination. No fruit set was found due to the incompatible fertilization in the isolated and self-pollinated branches in some progenies. Of the progenies, 55 were determined to be self-incompatible. In conclusion, it is recommended that F₁ progeny nos. 41, 46, 86, and 89 should be used as pollinators in further breeding experiments, as these progenies have high quality in pomological terms and they are self-compatible. The obtained results will be useful in the selection of parents in apricot breeding studies and these results will be useful for the selection of genotype in new apricot orchards.

Acknowledgments

This research was supported by a grant (No. 2010/12) from İnönü University Scientific Research Project Unit.

References

- ALBURQUERQUE, N., EGEA, J., PÉREZ-TORNERO, O., BURGOS, L., 2002: Genotyping apricot cultivars for self-(in)compatibility by means of RNases associated with S alleles. *Plant Breeding* 121, 343-347. doi: 10.1046/j.1439-0523.2002.725292.x.
- ASKIN, A., 1989: Biological studies in some apricot fruit varieties that are not regularly fruit set in the Aegean region, Phd thesis, Ege University, Izmir/Turkey.
- ASMA, B.M., 2008: Determination of pollen viability, germination ratios and morphology of eight apricot genotypes. *Afr. J. Biotech.* 7, 4269-4273. doi: 10.4314/ajb.v7i23.59562.
- ASMA, B.M., OZTURK, K., 2005: Analysis of morphological, pomological and yield characteristics of some apricot germplasm in Turkey. *Genet. Resour. Crop Ev.* 52, 305-313. doi: 10.1007/s10722-003-1384-5.
- BADANES, M.L., HURDATO, M.A., SANZ, F., ARCHELOS, D.M., BURGOS, L., EGEA, J., LLACER, G., 2000: Searching for molecular markers linked to male sterility and self-compatibility in apricot. *Plant Breeding*, 119, 157-160. doi: 10.1046/j.1439-0523.2000.00463.x.
- BASSI, D., BARTOLINI, S., VITI, R., 2006: Recent advances on environmental and physiological challenges in apricot growing. *Acta Hort.* 717, 23-32. doi: 10.17660/ActaHortic.2006.717.1.
- BOLAT, I., GULERYUZ, M., 1995: Selection of late maturation Wild Apricot (*Prunus armeniaca* L.) forms on Erzincan Plain. *Acta Hort.* 384, 183-188. doi: 10.17660/ActaHortic.1995.384.26.
- BURGOS, L., EGEA, J., GUERRIERO, R., VITI, R., MONTELONE, P., AUDERGON, J.M., 1997: The self-compatibility trait of the main apricot cultivars and new selections from breeding programmes. *J. Hortic. Sci. Biotech.* 72, 147-154. doi: 10.1080/14620316.1997.11515501.
- DOYLE, J.J., DOYLE, J.L., 1987: A rapid isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19, 11-15.
- DUNCAN, D.B., 1955: Multiple range and multiple F Tests. *Biometrics. International Biometric Society.* 11, 1-14. doi: 10.2307/3001478.
- EBERT, P.R., ANDERSON, M.A., BERNATZKY, R., ALTSCHULER, M., CLARKE, A.E., 1989: Genetic polymorphism of self-incompatibility in flowering plants. *Cell* 56, 255-262. doi: 10.1016/0092-8674(89)90899-4.
- EGEA, J., BURGOS, L., 1996: Detecting cross incompatibility of three North American apricot cultivars and establishing the first incompatibility group in apricot. *J. Am. Soc. Hortic. Sci.* 121, 1002-1005.
- FAUST, M., SURÁNYI, D., NYUJÓ, F., 1998: Origin and dissemination of apricot. *J. Am. Soc. Hortic. Sci.* 22, 225-266. doi: 10.1002/9780470650738.ch6.
- GOLDRAI, A., KONDO, K., LEE, C.B., HANCOCK, C.N., SIVAGURU, M., VAZQUEZ-SANTANA, S., KIM, S., PHILLIPS, T.E., CRUZ-GARCIA, F., MCCLURE, B., 2006: Compartmentalization of S-RNase and HT-B degradation in self-incompatible *Nicotiana*. *Nature* 439, 805-81. doi: 10.1038/nature04491.
- GU, C., WU, J., DU, Y.N., ZHANG, S.L., 2013: Two different prunus SFB alleles have the same function in the self-incompatibility reaction. *Plant Mol. Biol. Rep.* 31, 425-434. doi: 10.1007/s11105-012-0518-3
- GULCAN, R., MISIRLI, A., SAGLAM, H., YORGANCIOGLU, U., ERKAN, S., GUMUS, M., OLMEZ, H.A., DERIN, K., PAYDAS, S., ETI, S., DEMIR, T., 2006: Properties of Turkish apricot land races. *Acta Hort.* 701, 191-198. doi: 10.17660/ActaHortic.2006.701.28.
- GURRIERI, F., AUDERGON, J.M., ALBAGNAC, G., REICH, M., 2001: Soluble sugars and carboxylic acids in ripe apricot fruit as parameters for distinguishing different cultivars. *Euphytica* 117, 183-189. doi: 10.1023/A:1026595528044.
- HALASZ, J., FODOR, A., PEDRYC, A., HEGEDUS, A., 2010: S-genotyping of Eastern European almond cultivars: Identification and characterization of new (S₃₆-S₃₉) self-incompatibility ribonuclease alleles. *Plant Breeding* 129, 227-232. doi:10.1111/j.1439-0523.2009.01686.x.
- HALASZ, J., HEGEDUS, A., HERMAN, R., STEFANOVITS-BANYAI, E., PEDRYC, A., 2005: New selfincompatibility alleles in apricot (*Prunus armeniaca* L.) revealed by stylar ribonuclease assay and S-PCR analysis. *Euphytica* 145, 57-66. doi: 10.1007/s10681-005-0205-7.
- HALASZ, J., HEGEDUS, A., SZIKRISZT, B., ERCISLI, S., ORHAN, E., UNLU, H.M., 2013: The S-genotyping of wild-grown apricots reveals only self-incompatible accessions in the Erzincan region of Turkey. *Turk. J. Biol.* 37, 733-740. doi: 10.3906/biy-1306-27.
- HESLOP-HARRISON, J., 1975: Incompatibility and the pollen-stigma interaction. *Annu. Rev. Plant Physiol.* 26, 403-425. doi: 10.1146/annurev.pp.26.060175.002155.
- JIANG, X., WANG, D.J., FENG, J.R., ZHANG, D.H., JIANG, J.Q., LIU, Y.X., 2010: Identification of self-incompatibility genotype of apricot. Unpublished (NCBI GenBank).
- KAFKAS, S., PERL-TREVES, R., 2001: Morphological and molecular phylogeny of *Pistacia* species in Turkey. *Theor. Appl. Genet.* 102, 908-915. doi: 10.1007/s001220000526.
- KAO, T.H., TSUKAMOTO, T., 2004: The molecular and genetic bases of S-RNase-based self-incompatibility. *Plant Cell* 16, 72-83. doi: 10.1105/tpc.016154.
- LACHKAR, A., FATTOUCH, S., GHAZOUANI, T., HALASZ, J., PEDRYC, A., HEGEDUS, A., MARS, M., 2013: Identification of self-(in)compatibility S-alleles and new cross-incompatibility groups in Tunisian apricot (*Prunus armeniaca* L.) cultivars. *J. Hort. Sci. Biotech.* 88, 497-501. doi: 10.1080/14620316.2013.11512997.
- LECCESE, A., BUREAU, S., REICH, M., RENARD, M.G.C.C., AON, J.M., MENNONE, C., BARTOLINI, S., VITI, R., 2010: Pomological and nutraceutical properties in apricot fruit: cultivation systems and cold storage fruit management. *Plant Food. Hum. Nutr.* 65, 112-120. doi: 10.1007/s11130-010-0158-4.
- MCCLURE, B.A., 2006: New views of S-RNase-based self-incompatibility. *Curr. Opin. Plant Biol.* 9, 639-646. doi: 10.1016/j.pbi.2006.09.004.
- MCCLURE, B.A., EBERT, P.R., ANDERSON, M.A., SIMPSON, R.J., SAKIYAMA, F., CLARKE, A.E., 1989: Style self incompatibility gene products of *Nicotiana glauca* are ribonucleases. *Nature* 342, 955-957. doi: 10.1038/342955a0.
- MCCLURE, B.A., GRAY, J.E., ANDERSON, M.A., CLARKE, A.E., 1990: Self incompatibility in *Nicotiana glauca* involves degradation of pollen rRNA. *Nature* 347, 757-760. doi: 10.1038/347757a0.
- MILATOVIĆ, D., NIKOLIĆ, D., RAKONJAC, V., FOTIRIC-AKSIC, M., 2010: Cross-incompatibility in apricot cultivars. *J. Hort. Sci. Biotech.* 85, 394-398.
- OZBEK, S., 1978: Special fruit growing. Cukurova University Agriculture Faculty Issue, Adana/Turkey 126-134.
- PAYDAS, S., ETI, S., DERIN, K., 2001: In vitro investigation on pollen quality, production and self-incompatibility of some apricots varieties in Malatya-Turkey. *Acta Hort.* 701, 75-80.
- QIAO, H., WANG, H., ZHAO, L., ZHOU, J., HUANG, J., ZHANG, Y., XUE, Y., 2004: The F-box protein AhSLF-S2 physically interacts with S-RNases that may be inhibited by the ubiquitin/26S proteasome pathway of protein degradation during compatible pollination in *Antirrhinum*. *Plant Cell* 16, 571-581. doi: 10.1105/tpc.017673.
- ROALSON, E.H., MCCUBBIN, A.G., 2003: S-RNases and sexual incompatibility: structure, functions, and evolutionary perspectives. *Mol. Phylogenet. Evol.* 29, 490-506. doi: 10.1016/S1055-7903(03)00195-7.
- ROMERO, C., VILANOVA, S., BURGOS, L., MARTINEZ-CALVO, J., VICENTE, M., LLACER, G., BADANES, M.L., 2004: Analysis of the S locus structure in *Prunus armeniaca* L. identification of S-haplotype S-RNase and F-box genes. *Plant Mol. Biol.* 56, 145-157. doi:10.1007/s11103-004-2651-3.
- TAO, R., YAMANE, H., SUGIURA, A., MURAYAMA, H., SASSA, H., MORI, H., 1999: Molecular typing of S-alleles through identification, characterization and cDNA cloning for S-RNases in sweet cherry. *J. Am. Soc. Hort. Sci.* 124, 224-233.
- VILANOVA, S., BADANES, M.L., BURGOS, L., MARTINEZ-CALVO, J., LLACER, G., ROMERO, C., 2006: Self-compatibility of two apricot selections

- is associated with two pollen-part mutations of different nature. *Plant Physiol.* 142, 629-641. doi: 10.1104/pp.106.083865.
- VILANOVA, S., ROMERO, C., LLACER, G., BADENES, M.L., 2005: Identification of self-incompatibility alleles in apricot by PCR and sequence analysis. *J. Am. Soc. Hort. Sci.* 130, 893-898.
- WU, J., GU, C., DU, Y.H., WU, H.Q., LIU, W.S., LIU, N., LU, J., ZHANG, S.L., 2011: Selfcompatibility of 'Katy' apricot (*Prunus armeniaca* L.) is associated with pollen part mutations. *Sex. Plant Reprod.* 24, 23-35. doi: 10.1007/s00497-010-0148-6.
- YILMAZ, K.U., KAFKAS, S., PAYDAS KARGI, S., 2013: Determination of Self-(in)compatibility in Turkish Apricot Genotypes. *Fruit Sci.* 1, 34-40.
- ZHANG, L., CHEN, X., CHEN, X., ZHANG, C., LIU, X., CI, Z., ZHANG, H., WU, C., LIU, C., 2008: Identification of self-incompatibility (S-) genotypes of Chinese apricot cultivars. *Euphytica* 160, 241-248. doi:10.1007/s10681-007-9544-x.
- ZHEBENTYAYEVA, T., LEDBETTER, C., BURGOS, L., LLÁCER, G., 2012: Apricot, Chapter 12. In: Badenes, M.L., Byrne, D.H. (eds.), *Fruit Breeding, Handbook of Plant Breeding*, Springer, Berlin, Heidelberg.
- Address of the authors:
Zehra Tugba Murathan, Ardahan University, Faculty of Engineering, Food Engineering Department, Ardahan, Turkey
E-mail: ztugbaabaci@hotmail.com
Salih Kafkas, Çukurova University, Faculty of Agriculture, Department of Horticulture, Adana, Turkey
Bayram Murat Asma, İnönü University, Faculty of Agriculture, Department of Horticulture, Malatya, Turkey

© The Author(s) 2017.

 This is an Open Access article distributed under the terms of the Creative Commons Attribution Share-Alike License (<http://creativecommons.org/licenses/by-sa/4.0/>).