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Full Length Research Paper

Self and cross incompatibility traits analysis in some diseases tolerant almond genotypes by PCR

Yavar Sharafi[1]*, Magsood Kafshnochi[2] and Amir Mohammad Naji[3]

[1]Department of Horticulture, Faculty of Agriculture, Shahed University- Opposite Holy Shrine of Imam Khomeini- Khalij Fars Expressway- Tehran- Iran.
[2]Departement of Basic Sciences, science Paradise, University of Tehran, Tehran- Iran
[3]Department of Plant Breeding and Agronomy, Faculty of Agriculture Shahed University- Opposite Holy Shrine of Imam Khomeini- Khalij Fars Expressway- Tehran- Iran.

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Almond is one of the oldest nut crops in the world and has a main problem called self-incompatibility. Identification of S alleles in almond cultivars and genotypes is essential for breeders and growers. The aim of this study was to identify S alleles pattern in 30 late bloom and resistant to aphid, mite, *Monilia laxa* and "*Polystigma occharaceum"* fungus almond genotypes from different geographical regions of East-Azerbaijan Iran. Most of the S alleles were amplified using Pru-C_2/Pru-C_5R primer pair and size of alleles ranged between 2000- 220 bp in genotypes. Considering all of the S alleles amplified using 5 primer pairs in thirty numbers of the genotypes two S alleles were detected and showed their fully self-incompatibility pattern, in 10 genotypes only one of the S alleles were.

Key words: Almond, S allele, specific primers, self-incompatibly, inter-incompatibility.

INTRODUCTION

Prunus dulcis Batch, is one of the stone fruit trees which most of its cultivars and genotypes have monogenic-gametophitic self-incompatibility with pollination, fertilization and fruit setting problems. However, self compatibility (Channuntapipat et al., 2003; Sanchez-Perez et al., 2004) and cross–incompatibility (Lopez et al., 2006; Barckley et al., 2006) were observed in some of almond cultivars/genotypes. This trait is controlled by a single locus with multiple codominant alleles and is expressed within the styles as S-RNas glycoproteins. These glycoproteins are responsible for the inactivation of self-pollen tube growth in most species of genus *Prunus* (Wiersma et al 2001; Wunsch and Hormoze 2004; Yamane et al., 1999). Therefore, if two cultivars/ genotypes have similar S-alleles in pollens and styles, pollens could not penetrate to ovary and fertilization could not be carried out successfully. But, if they have different S-alleles, fertilization could be carried out successfully, and cross-incompatibility takes place when two S-alleles of pollen and style are been completely similar (Sonncvald et al., 2003; Alonso and Socias i company, 2006; Sonncvald et al., 2003; Tamura et al., 1999; 2000). The identification of S-alleles is essential for almond breeding programs to maximize the efficiency of crosses (Alonso and Socias i company, 2006; Sharafi et al., 2010 a and b). DNA based methods should be incorporated into fruit breeding programs in order to accelerate and optimize determination of cultivar/ genotype self-and cross-(in) compatibility relationships (Sanchez-Perez et al., 2004; Sharafi et al 2010 a and b). S alleles pattern and compatibility relationships in Iranian almond cultivars/genotypes have been poorly characterized. The objective of this work was to the iden-tify S alleles pattern and self-and cross-(in) compatibility relationships among 30 superior selected late bloom and resistant to aphid, mite, *Monilia laxa* and "*polystigma occharaceum"* fungus, almond genotypes from East- Azerbaijan

*Corresponding author. E-mail: y.sharafi@shahed.ac.ir. Tel: +982151210 or +989144200882.

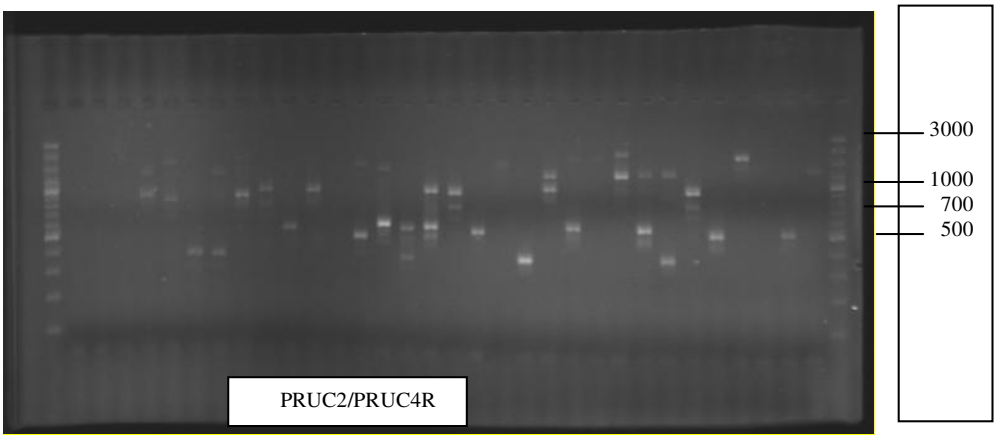

Figure 1. PCR analysis of S-alleles in studied genotypes using PRUC2/PRUC4R; PCR products were separated in 1.5% agarose gel electrophoresis. Line from right to left is 1-25 genotypes and 1kb plus ladder.

province, Iran based on specific PCR primers.

MATERIALS AND METHODS

Plant materials and genomic DNA extraction

In this study thirty late bloom and resistant to aphid, mite, "*polystigma occharaceum"* fungus, almond genotypes from East Azerbaijan, Iran were used. Genomic DNA was extracted using the procedure described by Doyle and Doyle (1987). The quality and quantity of the DNA samples were determined using spectrophotometer and 0.8% agarose gel electrophoresis. DNA samples were diluted in 25 ng/µl and used in PCR.

S alleles detection

PCR amplification to identify S-alleles was performed using specific PCR primers AS_1II, $AMYC_{5R}$, $Alsc_1$, Pru-C_2, Pru-C_4R, Pru-C_5R and Pru-C_6R. Amplification was carried out of 25 µl volumes containing 1 µl-PCR buffer, 2 mM MgCl2, 0/1 mM (dNTPs), 0/2 µM of each primers, 0.7 unit Taq DNA polymerase and 50 ng of total genomic DNA. PCR were carried out in an Eppendorf Master Cycler gradient programmed as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles of 1 min at 94°C, 1 min at 53°C and 2 min at 72°C and final one cycle of extension at 72°C to 10 min. PCR products were separated in 1.5% agarose gel electrophoresis using 0.5 Tris-Boric acid-EDTA buffer, and were stained with ethidium bromide and photographed under UV light with UVitec gel documentation. The sizes of the PCR products were estimated using 1 kbp DNA ladder plus (Figure 1) (Sanchez-Perez et al., 2004).

RESULTS AND DISCUSSION

According to the Table 1, in total, 31 S alleles were amplified in the studied genotypes. The sizes of alleles ranged between 220-2000 bp. However, S alleles fragment pattern was 2000, 1700, 1500, 1400, 1300, 1250, 1200, 1100, 1050, 1000, 950, 900, 850, 800, 750, 700, 650, 600, 550, 520, 500, 480, 450 400, 380, 350, 300, 280, 250, 230 and 220 bp, respectively (Table 1). Fragment 600 bp has maximum frequency among all of the S alleles and observed in 18 genotypes and fragments 1100 and 800 bp were observed in 16 genotypes. Each of the primer pairs amplified different S-alleles and none of them could amplify all S-alleles in all of the genotypes. In genotypes 1, 3, 4, 14, 21, 30, 33, 35, 40 and 48 only one of the S alleles were amplified (Table 1). Differences in the sizes of alleles allowed detection of S alleles. Since all of the genotypes showed two bands representing two S-alleles. Therefore, the genotypes were identified self-incompatible; which none of their alleles were amplified. Comparison of detected S-alleles with S alleles in GenBank revealed that most of the fragments were in range of GenBank alleles (Sanchez-Perez et al., 2004).

All primer pairs produced maximum two bands per genotype, in agreement with the diploid constitution of these genotypes and showed that all of the genotypes were self-incompatible except 3 of them. Cross-incompatibility was not observed among the genotypes. Therefore, based on blooming time, all of the genotypes could be cultured in orchards or breeding programs for pollination of each other's successfully.

The amplification of S-alleles in 30 studied almond genotypes identified 31 different S-alleles indicating high variability of self-incompatible alleles in the genotypes especially S allele with size of 600 bp that observed in 18 genotypes. The combination of Pru-C_2/Pru-C_5R and $Alsc_1$/$AMYC_{5R}$ primers amplified maximum (24) and minimum (13) S alleles number, respectively (Table 1). Considering all of the primer pairs, 27 of the studied genotypes showed two S-alleles demonstrating fully self-incompatibility and 10 genotypes only showed one S allele. Cross-incompatibility relationships were not observed among the genotypes. Some of the genotypes

showed only one similar S-allele, but most of them showed two different S-alleles and could be cross-

Table 1. S-alleles size detected by per primer pairs in the studied genotypes.

Genotypes	AS_1II/ $AMYC_{5R}$	$AIsc_1$/$AMYC_{5R}$	Pru-C_2 /Pru-C_4R	Pru-C_2 /Pru-C_5R	Pru-C2 /Pru-C6R
1	1050/-	-/-	1500/-	-/-	-/-
2	850/650	-/-	500/-	1300/380	1300/400
3	700/-	-/-	-/-	550/-	550/-
4	-/-	250/-	1700/-	1500/-	1500/-
5	850/-	-/-	500/400	550/350	550/400
6	700/600	250/-	1050/800	800/700	900/800
7	650/-	-/-	1100/300	1100/400	220/-
8	800/-	-/-	1100/500	1050/950	1100/400
9	600/-	-/-	1200/1100	1500/1200	1100/1000
10	-/-	-/-	1200/-	1500/400	1500/1300
11	850/-	280/-	1250/1100	1200/-	1500/950
12	-/-	-/-	1200/1050	230/-	1100/-
13	1000/600	-/-	300/-	1400/-	220/-
14	-/-	-/-	1500/-	400/-	1500/-
15	1100/1000	-/-	950/-	800/600	450/-
16	1200/-	-/-	950/750	450/-	900/600
17	900/-	250/-	950/650	400/-	500/-
18	900/700	-/-	650/350	500	450/280
19	1200/950	-/-	700/-	350/-	500/-
20	800/600	-/-	1100/400	800/550	400/-
21	650/-	-/-	1000/-	400/-	-/-
22	-/-	-/-	700/-	800/650	900/650
23	-/-	-/-	1000/800	700/-	480/-
24	-/-	-/-	900/850	280/-	850/600
25	-/-	-/-	2000/400	280/-	850/-
26	-/-	1300/400	-/-	-/-	280/-
27	-/-	400/-	600/-	-/-	280/-
28	-/-	1700/850	-/-	700/-	800/-
29	-/-	1500/900	-/-	750/-	900/-
30	-/-	-/-	900/-	-/-	-/-
31	-/-	-/-	-/-	-/-	-/-
32	-/-	-/-	-/-	1300/550	1300/600
33	-/-	1000/-	-/-	850/-	850/-
34	800/-	1300/600	-/-	1200/400	1100/400
35	-/-	1300/-	-/-	-/-	-/-
36	900/600	500/-	800/700	400/-	450/-
37	800/-	600/400	-/-	450/-	480/-
38	1000/700	-/-	-/-	-/-	-/-
39	-/-	-/-	-/-	-/-	-/-
40	-/-	-/-	-/-	1050/-	1100/-
41	-/-	-/-	-/-	-/-	-/-
42	1300/700	1000/-	1200/-	-/-	-/-
43	1700/1200	-/-	-/-	1250/1100	1200/1100
44	1000/-	1200/-	900/-	1250/1050	1200/1100
45	750/600	400/-	600/-	1100/300	350/-
46	850/600	500/-	700/-	400/-	380/-
47	1050/850	900/500	1000/700	700/400	700/380
48	-/-	600/-	-/-	450/-	450/-
49	1100/800	500/-	900/600	500/-	500/-
50	1200/600	1000/800	1200/-	1000/800	1000/520

compatible for pollination of each others, if their blooming time be synchronized.

Sanchez-Perez et al. (2004) also detected S-alleles with sizes of 2000, 1600, 1400, 1200, 1100, 800, 700 and 600 bp using $AS_1II/AMYC_{5R}$, $AIsc_1/AMYC_{5R}$ and multiplex PCR using three primers $AS_1II/CEBAS_f/AMYC_{5R}$ in almond cultivars. Sequencing of PCR products demonstrated that these bands represented S_7, S_{12}, S_{13}, S_3, S_1, S_2, S_{11}, and S_5 alleles, respectively. They reported that these primers could amplify S_f but could not amplify S_8 and S_{22}; in addition, S_{13} (1400 bp) was amplified first by the primers. In our study, PCR products were not sequenced. Martinez-Gomez et al. (2004) studied S-alleles in 18 almond cultivars and related *prunus* species using $AS_1II/AMYC_{5R}$ and reported the presence of eight and six S-alleles in almond cultivars (S_1, S_5, S_6, S_7, S_9, S_{10}, S_{13} and S_{18}), and in related *prunus* species, respectively but they could not amplify S_3 and S_8 with the same primers. Barkley et al. (2006) showed 15 cross-incompatible groups in California almond cultivars based on specific primer pairs $AS_1II/AMYC_{5R}$, $AISc_1/AMYC_{5R}$ and $AISd_2/AMYC_{5R}$. Martinez-Gomez et al. (2003) studied S-alleles of 18 almond cultivars and related prunus species using AS1II/ AMYC5R primers and reported eight S-alleles in the studied almond cultivars (S1, S5, S6, S7, S9, S10, S13 and S18) and six S-alleles in related *prunus* species. They could not amplify S3 and S8 with the same primers. Therefore, they reported unspecificity of the PCR primers AS1II and AMYC5R developed by Tammura et al. (2000) for identification of S-alleles in almond and related *prunus* species

Conclusion

Based on the results of present study, 50 genotypes of the studied genotypes were clearly self-incompatible and cross-incommutability was not observed among them. Therefore, all of the genotypes could be used in breeding programs or orchard establishment for pollination of each other based on their blooming time. The identification of S alleles of new cultivars/genotypes which was obtained from breeding programs is very helpful for planning the future breeding and orchard establishment programs especially for speedy selecting of pollinizer.

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