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Full Length Research Paper

Pollen-pistil compatibility relationships in some Iranian almond (*Prunus dulcis*, Batch) genotypes as revealed by PCR analysis

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The identification of pollen-pistil compatibility relationships among almond cultivars and genotypes is very important for breeders and growers. In the present study, PCR based technique was used to identify S-alleles in 10 late blooming almond genotypes. In total, 19 alleles were amplified by five primer pairs in the studied genotypes. The size of bands ranged between 480 - 2000 bp. Seven S-alleles were amplified using AS1II/AMYC5R primer pair, whereas each of the Alsc11/AMYC5R, Pru-C2/Pru-C4R, Pru-C2/Pru-C5R and Pru-C2/Pru-C6R primer pairs amplified nine different S-alleles. Based on S-allele patterns, all of the studied genotypes were identified as self-incompatible. However, some of the genotypes had only one similar S-allele, all of the genotypes could be used in establishment of commercial orchards based on their blooming times.

Key words: Almond, self-incompatibility, cross-incompatibility, specific primers, polymerase chain reaction.

INTRODUCTION

Almond (*Prunus dulcis,* Batch) is one of the most important nut crops in the world and most of the almond cultivars and genotypes are known to be gametophitically self-incompatible with pollination, fertilization and fruit setting problems (Channuntapipat et al., 2001; Sedgly and Collins, 2003; Sanchez-Perez et al., 2004). However, cross incompatibility was also observed among almond cultivars/genotypes (Barckley et al., 2006; Lopez et al., 2006). This trait is controlled by a single locus with multiple alleles and expressed within the styles of flowers as S-RNAs glycoproteins (Wiersma et al., 2001; Halasz et al., 2007). These glycoproteins are responsible for the inactivation of self-pollen tube growth in most species of genus Prunus including almond (Socias and Alonso, 2004, Alonso and Socias, 2006), apricot (Hajilou et al., 2006), sweet cherry (Wunsch et al., 2004) and plum (Yamane et al., 1999 Sutherland et al 2004 and Tamura et al 1999). In gametophytic incompatibility system, two genotypes with similar S-alleles can not fertilize each other, but the presence of different S-alleles in two genotypes will result in successful fertilization. Cross-incompatibility will take place when two similar S-alleles are presented in pollen and style (Yamane et al., 1999; Sonncvald et al., 2003; Alonso and Socias, 2006). Therefore, identifying cross-compatible cultivars/ genotypes with favorable traits will be very plentiful for growers; in addition, the identification of S-alleles is essential for almond breeding programs to maximize the efficiency of crosses (Alonso and Socias I Company, 2006).

Recently, methods based on DNA techniques to identify S-alleles pattern are incorporated into fruit breeding programs in order to accelerate and optimize the determination of pollen-pistil compatibility relationships between cultivar/genotype (Sanchez-Perez et al., 2004; Lopez et al., 2006). However, controlled field and laboratory pollination are needed to confirm the effects of

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Abbreviations: PCR, Polymerase chain reaction; **dNTPs,** deoxynucleoside 5'-triphosphate; **DNA,** deoxyriboucleic acid; **Sf,** self-compatibility allele.

Table 1. S-alleles size detected by primer pairs in the studied genotypes, divided to 3 groups based on blooming time.

Genotypes	AS1II/ AMYC5R	Alsc1/AMYC5R	Pru-C2 /Pru-C4 R	Pru-C2 /Pru-C5 R	Pru-C2 / Pru-C6R
Group 1					
D	1250/ -	1900/ -	1100/ -	900/ -	1250/ 900
F	1100/ -	-/ -	1000/ -	1000/ 650	1050/ -
Q	-/ -	700 -	1900/ 1200	1900/850	2000/1000
Group 2					
I	1600/ 1300	1300/ 480	1500/ 1100	1400/ 900	1200/ 900
L	1300/ -	-/ -	1200/ -	900/ -	1000/ -
O	1100/ 580	800/ 700	1000/ -	800/ -	850/ -
Group 3					
E	2000/ 1300	1000/ -	2000/ 1300	-/ -	2000/ -
G	-/ -	-/ -	1000/ 800	-/ -	1100/ -
K	1300/ -	1000/ 650	1250/-	1000/ -	1000/ -
P	1200/ -	1400/ 1100	-/ -	700/ -	-/ -

Genotypes assigned into three groups based on their overlapping blooming time.

pollens on fruit quality in selecting suitable pollinizers of cross-compatible cultivars/genotypes identified by PCR based methods (Lopez et al., 2006). Pollen-pistil compatibility relationships among Iranian almond cultivars/ genotypes, especially, those obtained from breeding programs have been poorly characterized. Therefore, the objective of this work was to identify pollen-pistil compatibility relationships among 10 superior late blooming almond genotypes obtained from a breeding program based on their S-alleles profiles.

MATERIALS AND METHODS

Plant materials and genomic DNA extraction

Ten (10) late blooming genotypes (designated as D, E, F, I, L, K, G, O, P and Q) obtained from a breeding program in Sahand Horticultural Research Station of Agriculture and Natural Resource Research Center, East Azarbaijan, Iran were used. Genotypes were assigned into three groups based on their overlapping blooming time; Group I included D, F and Q, group II consisted of I, L and O while group III comprised E, G, K and P genotypes. 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 amplification

A set of six specific primers were used to amplify S-alleles in the studied genotypes. The sequence of primers was as follows (Tamura et al., 2000; Sanchez-Perez et al., 2004; Wunsch and Hormoza, 2004):

AS1II: TATTTTCAATTTGTGCAACAATGG (Forward),
AMYC5R: CAAAATACCACTTCATGTAACAAC (Reverse),
Alsc1: CAGACACTTAATCAATTCCAG (Forward),
Pru-C2: AATTATTCAAACCCTTTGGCCAAGT (Forward),
Pru-C4R: TGTGGTACGATTGAAGGG (Reverse),
Pru-C5R: TACCACTTCATGTAACAACTGAG (Reverse)
and Pru-C6R: CATTGCCACTTTCCACGT (Reverse).

The primers were used in five primer combinations: AS1II (F)/AMYC5R (R), Alsc1/AMYC5R, Pru-C2/Pru-C4R, Pru-C2/Pru-C5 and Pru-C2-Pru-C6R. The two first primer pairs represent common sequences in the almond S-RNase and others are common in genus *Prunus*. Polymerase chain reaction (PCR) was performed in volume of 25 µl containing 1 µl-PCR buffer, 2 mM $MgCl_2$, 0.1 mM dNTPs, 0.2 µM of each primer, 0.7 unit Taq DNA polymerase and 50 ng of total genomic DNA. PCR was 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 followed by final extension at 72°C for 10 min. PCR products were separated in 1.5% agarose gel using 0.5 Tris-Boric acid-EDTA buffer and were stained with ethidium bromide. The gels were photographed using UV light with UVitec gel documentation. The molecular sizes of the PCR products were estimated based on 1 kbp DNA ladder plus (Fermentas).

RESULTS

In total, 19 S-alleles with approximate size of 2000, 1900, 1600, 1500, 1400, 1300, 1250, 1200, 1100, 1050, 1000, 900, 850, 800, 700, 620, 650, 580 and 480 bp were amplified using five primer combinations (Table 1). Each primer combination amplified different S-alleles in the studied genotypes. AS1II/AMYC5R combination (which generally amplifies first intron of S-allele) amplified seven S-alleles (2000, 1600, 1300, 1250, 1200, 1100 and 580 bp) and others shown in Table 1. The use of AS1II/ AMYC5R primer pair resulted in amplification of two alleles in genotypes E (2000/1300), I (1600/1300) and O (1100/580) and one in D (1250), F (1100), K (1300), L

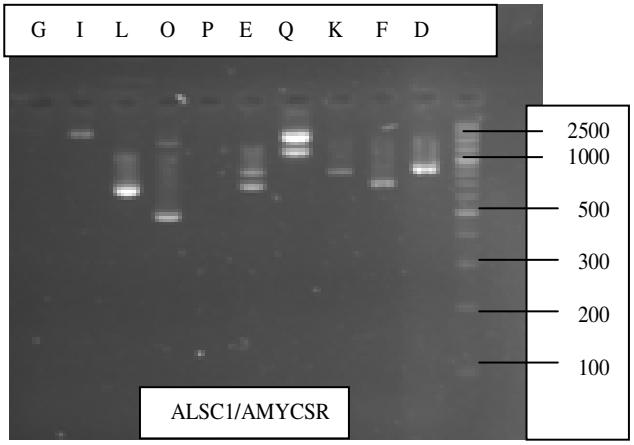

Figure 1. PCR analysis of S-alleles in studied genotypes using ALSC1/AMYC5R. PCR products were separated in 1.5% agarose gel electrophoresis. Lanes from left to right G, I, L, O, P, E, Q, K, F, D and 1 kb plus ladder in panel A.

(1300) and P (1200). No fragment was amplified in Q and G genotypes. Two alleles were ampified in K (1000/620), I (1300/480), O (800/700) and P (1400/1100) and one band in E (1000), D (1900) and Q (700) genotypes using Alsc11/AMYC5R combination, whereas no band was observed in F, G and L genotypes. Furthermore, PCR using specific primer pair of Pru-C2/Pru-C4R to amplify the second intron resulted in two bands at E (2000/1300), I (1500/1100), G (1100/800) as well as Q (1900/1200) and one band in D (1100), F (1000), K (1250), L (1200) and O (1000) genotypes, where no band was produced in P genotype. Based on Pru-C2/Pru-C5 R, two alleles were amplified in F (1000/650), I (1400/900) and Q (1900/850), one in D, (900) K (1000), L (900), P (700) and O (800) genotypes. No band was observed in genotypes E and G. Finally, using Pru-C2/Pru-C6R primer pair two alleles were identified in D (1250/900), I (1200/900) and Q (2000/1000), one in E (2000), F (1050), G (1100), K (1000), L (1000) and O (850) and no band in P. Pru-C2/Pru-C4R combination was amplified maximum number of S alleles (Table 1).

DISCUSSION

Based on fragments size, 19 different S-alleles were detected in the studied genotypes showing high frequency of S-alleles in the genotypes (Table 1). Although none of the primer pairs could amplify all of the S-alleles in the studied genotypes, detected alleles indicated the self-incompatibility of the genotypes and no cross-incompatibility could be identified based on S-allele pattern. All of the primer pairs amplified maximally two bands per genotype which was in agreement with diploid constitution of almond but, in genotype L and only one band was amplified using all of the primer pairs. The amplification of only one band in genotype L using all primer pairs must be due to preferential amplification of one of the alleles, as primers for the first intron and second intron, respectively, amplified two different bands in other studied genotypes. The comparison of the allele's size with S-alleles in Gene banks revealed that most of the fragments were in the size range of S-alleles of Gene bank (Sanchez-Perez et al., 2004).

Based on blooming time and S-allele patterns of the genotypes, from group I (D, F and Q), L, I and O from group II E, K, G and P from group III were identified as cross-compatible and could be used as pollinizer, for each other in orchard establishment and breeding programs. However, genotypes L and I from group II and E and K from group III showed one similar S-allele; therefore, their use in same programs together could prohibit the growth of 50% of pollens in the upper sections of the pistils and not fertilize the ovary.

Although In group II, genotypes L and I showed one similar S allele using AS1II/AMYC5R (1300 bp) and Pru-C2/Pru-C5R (900 bp) and 50% of their pollens may stop in style, both of them were fully compatible with genotype O because their S alleles were extensively different from each other (Table 1) and they could be used as pollinizer for genotype O. Also in group III, genotypes K and E with one similar S-allele by AS1II/AMYC5R (1300 bp) and Alsc11/AMYC5R (1000 bp) and 50% of their pollens may stop in the style, but, genotypes E, K, G and P are fully compatible with each other; therefore, genotypes E, K, G and P could be used as pollinizer. However, it should be mentioned that field-controlled crosses are necessary to confirm the effects of pollens on fruit traits in all genotypes for introducing studied genotypes as suitable pollinizer, of each other. Sanchez-Perez et al. (2004) amplified S-alleles with sizes of 2000,1600, 1400, 1200, 1100, 800, 700 and 600 bp using AS1II/AMYC5R, Alsc11/AMYC5R and multiplex PCR with three primers; AS1II/CEBASf/AMYC5R for the amplification of self-compatibility allele (Sf) in almond cultivars. Sequencing of PCR products demonstrated that these bands represented S7, S12, S13, S3, S1, S2, S11, and S5, respectively. Martinez-Gomez et al. (2004) also 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 (Tammura et al. 2000). 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. Hadjilou et al. (2006) studied self-and cross-(in) compatibility relationships among five commercial apricot cultivars using SRC-F/SRC-R and pruc2/pruc4R and reported that three and two cultivars were self-incompatible and cross incompatible based on amplified S alleles, respectively (Figures 1 and 2). Barkley et al. (2006) showed 15 cross-incompatible groups in California almond cultivars using AS1II/AMYC5R, AlSc1/AMYC5R and AlSd21/ AMYC5R.

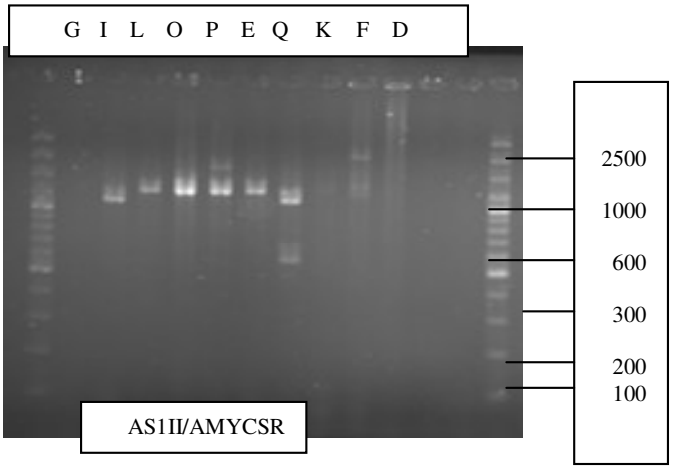

Figure 2. PCR analysis of S-alleles in studied genotypes using AS1II/AMYC5R. PCR products were separated in 1.5% agarose gel electrophoresis. Lanes from left to right. -, G, I, L, O, P, E, Q, K, F, D, - and 1 kb plus ladder.

Analysis of S-alleles pattern and determination of selfand cross-(in)compatibility relationships of cultivars/ genotypes in almond, apricot, sweet cherry and other species of genus *Prunus* were carried out by several groups, using specific and non specific primers and different results were reported (Ma and Oliveira 2001;Ortega et al., 2005; Sedgly and Collins 2003; Sonncvald et al., 2003 Channuntapipat et al 2002 and 2003).

Conclusion

The results revealed that the 10 studied genotypes were clearly self-incompatible and cross-incommutability was not observed among genotypes; therefore, all of them could be used in breeding programs or orchard establishment for pollination of each other. 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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