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Study of Compatibility Relationships Among Some Almond Cultivars and Genotypes Using of *S*Alleles Identification

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Abstract

Almond (*Prunus dulcis* L.) is one of the most important nut crops in Iran. Most almond cultivars and genotypes are self-incompatible. However, research on S-alleles indicates that it is very efficient in cultivar selection. Self-incompatibility in almond is gametophytic and controlled by a single S-locus with multiple codominant alleles. In this study, compatibility relationships among cultivars, "Tuono", "Shokofeh", "Sahand" and five improved genotypes "$A_{1.16}$", "$A_{9.7}$", "$A_{8.39}$","$A_{10.11}$" and "A_{230}," was investigated by the PCR of S-alleles. Degenerate primers (PaConsI-F, EM-PC1consRD, EM-PC2consFD, and EM-PC3consRD) were used for amplification of S-alleles. Results showed that only "$A_{10.11}$" and "$A_{8.39}$" were completely cross-incompatible, but all of the other studied cultivars and genotypes were cross-compatible. Furthermore, cultivar "Tuono" and genotype "$A_{1.16}$" had a self-fertility allele.

Key words: Almond, Degenerate primers, Incompatibility, PCR, S-alleles.

Introduction

Almond is one of the most important genus prunus. It belongs to the Rosaceae family. Most almond cultivars and genotypes are self-incompatible. Some are cross-incompatible (Socias and Alonso, 2004). Pollination, fertilization and commercial production require compatible pollen (Socias I company, 1990). Selecting cross-compatible cultivars with high quality pollen is the most important practice in almond orchard establishment (Kester *et al*., 1994). 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 I Company and Alonso, 2004; Alonso and Socias I Company, 2006), apricot (Hajilou *et al*., 2006), sweet cherry (Wunsch *et al*., 2004) and plum (Yamane *et al*., 1999 Sutherland *et al*., 2004;Tamura *et al*., 1999). In a gametophytic incompatibility system, two genotypes with similar S-alleles cannot 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; Sonneveld *et al*., 2003; Alonso and Socias I Company, 2006). Therefore, identifying cross-compatible cultivars and genotypes with favourable traits will be very beneficial for

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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 and genotype (Sanchez-Perez *et al*., 2004; Lopez *et al*., 2006). However, controlled field and laboratory pollination are needed to confirm the effects of pollens on fruit quality and for selecting suitable pollinisers of cross-compatible cultivars and genotypes identified by PCR based methods (Lopez *et al*., 2006).Identification of incompatibility alleles in almond using degenerate primers designed for different species the Prunus genus was performed. Different combinations of these primers S-alleles in almonds (Sutherland *et al*., 2004; Ortega *et al*., 2005), cherry (Sonneveld *et al*., 2001; Sutherland *et al*., 2004) and apricot (Halasz *et al*., 2005; Zhang *et al*., 2008) were identified. Pollen-pistil compatibility relationships among Iranian almond cultivars and genotypes, especially those obtained from breeding programs, have been poorly studied. Therefore, the objective of this study was to identify pollen-pistil compatibility relationships among cultivars Tuono, "Shokofeh", "Sahand" and five improved genotypes obtained from a breeding program based on their S-alleles profiles.

Materials and Methods

Plant material and Genomic DNA extraction

The plant material included among cultivar ("Sahand", "Shokofeh" and "Tuono") and five genotypes ("$A_{1.16}$", "$A_{8.39}$", "$A_{9.7}$", "$A_{10.11}$" and "A_{230}") from a breeding program at anorchad atin the Seed and Plant Improvement Institute Karaj. Cultivars and genotypes were assigned into three groups based on their overlapping blooming time. The first group included "Tuono" cultivar as the female parents, the "Sahand" cultivar with genotypes "A_{230}", "$A_{1.16}$" and "$A_{9.7}$" as male parents. The second group consisted of genotype "$A_{9.7}$" as female parent and "Tuono" with genotypes "A_{230}", "$A_{10.11}$", "$A_{8.39}$" as male parents, while third group comprised "Shokofeh" cultivar as the female parent with genotypes "Sahand", "Tuono"and"A_{230}", "$A_{1.16}$" as male parent. Genomic DNA was extracted using the procedure described by Doyle and Doyle (1987). The quantity of the DNA samples was determined using 2% agarose gel electrophoresis.

PCR Primers

A set of four specific degenerate primers were used to amplify S-alleles in the studied cultivars and genotypes. Degenerate primers Pa ConsI-F, EM-PC1consRD, EM-PC2consFD, and EM-PC3consRD were used (Table 1). For amplification, the second intron of the forward primers EM-PC2consFD and reverse primer EM-PC3consR (Sutherland *et al*., 2004) and amplification of the first intron of the forward primers PaConsI-F (Sonneveld *et al*., 2003) and backward primer EM-PC1consRD (Ortega *et al*., 2005) were used.

Table 1. View primers used

SSR primers group		Sequence	Annealing temperature
PaConsI-F	SP	5´(C/A)CTTGTTCTGT(C/G)TTT(T/C)GCTTTCTTC 3'	57°C
EM-PC1consRD	C_1	5´ GCCA(C/T)TGTTG(A/C)ACAAA(C/T)TGAA 3'	57°C
EM-PC2consFD	C_2	5´ TCAC(A/C)AT(C/T)CATGGCCTAT 3'	58°C
EM-PC3consRD	C_3	5´ A(A/T)(C/G)T(A/G)CC(A/G)TG(C/T)TTGTTCCATTC 3'	58°C

S-alleles amplification

Amplification reactions were carried out in 20 µl volumes containing; 1x PCR buffer (100mMTrisHCl, pH 8, 500 mMKCl), 0/8 µl $MgCl_2$, 2 µl dNTPs, 1/2 µl of each primer (Forward and Reverse), 0.2 U Taq polymerase and 3 µl of genomic DNA. The PCR reaction program consisted oftwo minutes at 94°C for denaturation primary, in 34 cycles with 10 seconds, temperature 94°C, two minutes at 57°C and two minutes at 68 °C, followed by a five minute extension at 72°C. After PCR, the products at 4°C (refrigerator) were stored until electrophoresis was performed (Rasouli *et al*., 2012).

Electrophoresis of PCR products

PCR products were separated in 2% agarose gel using 0.5 Tris-buffer-EDTA buffers 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 3 kbp DNA ladder plus (Rasouli *et al*., 2012; Juan *et al*., 2014).

Results

Degenerate primers used in this study were able to identify compatibility alleles (S_1, S_2, S_3, S_5, S_7, S_9, S_{12}, S_{22}, S_{24}, and S_f). The cultivars and genotypes that had the most self-incompatible alleles were S_1 and S_9 (Fig. 1).

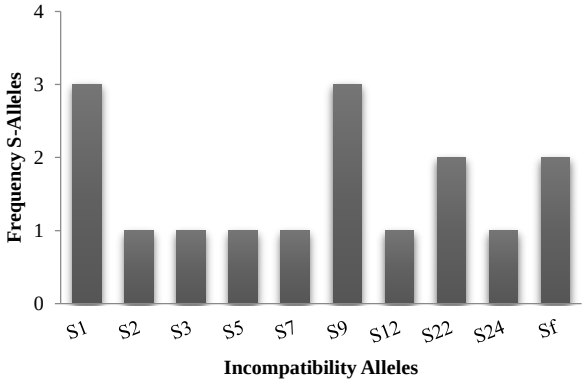

Fig 1. Frequency of allele's in-compatible in the sample studied.

Based on the results of amplification using degenerate primers alleles S in the first intron and second intron, all of bands obtained corresponded to the bands identified by similar research (Ortega *et al*., 2005; Mousavi *et al*., 2010). The new bands were not observed (Fig. 2).

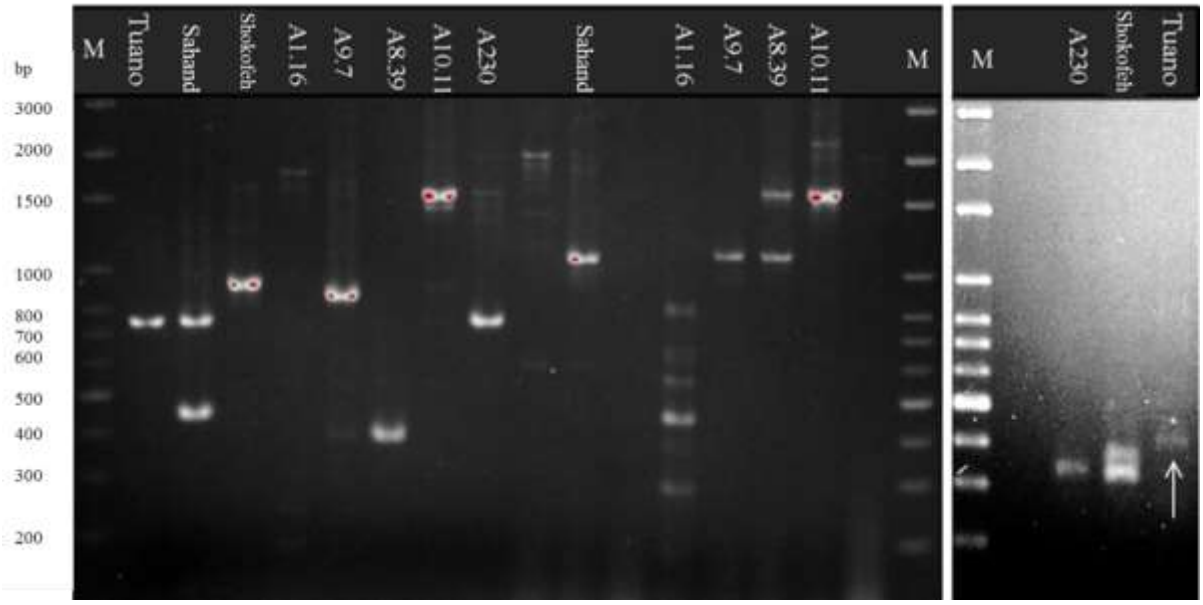

Fig.2. M indicates DNA (bp) Intron II (numbers of 1 to 8), first Intron (numbers of 9 to 16).

The comparison of the allele's size with S-alleles in gene banks revealed that the most of the fragments were in the size range of S-alleles of the gene bank (Sanchez-Perez *et al*., 2004). Results showed that the second intron, which was amplified with primers (forward primer EM-pc2consFD and backward EM-pc3consR),identified cultivars and genotypes bands as 400bp to 1720 bp, the primer for a second intron identified all varieties and genotypes "Tuono", "Shokofeh", "$A_{9.7}$", "$A_{8.39}$", "$A_{1.16}$", "$A_{10.11}$" and "A_{230}." The results from the band and cultivar "Sahand" in sizes 450 bp and 750 bp corresponded to research (Mousavi *et al*., 2010).

The results showed that the first intron that was amplified with primers (forward primer PaConsI-F and the backward primer EM-PC1consRD) had bands of 300 bp to 1560 bp. Primers for the first intron in each of the varieties and genotypes reproduced Tuono, "Shokofeh", "Sahand", "$A_{1.16}$", "$A_{9.7}$", "$A_{10.11}$" and "A_{230}." One band and in genotype "$A_{8.39}$" two band had self- incompatible alleles (Fig. 2). The band size of the first intron and second intron proliferated, which made it easy to identify different alleles. The variant on in the band sizes was similar to former findings.

Calculations of the band size reproduced to help marker 3 kilo base pairs, allele's self-incompatible varieties and genotypes were determined in this study. These included "Tuono"(S_1S_f), "Sahand" (S_2S_1), "Shokofeh" (S_3S_{10}) and genotypes $A_{10.16}$(S_fS_7), $A_{9.7}$($S_{24}S_{12}$), "$A_{8.39}$" ($S_{11}S_9$), "$A_{10.11}$" (S_9S_9) and "A_{230}" (S_1S_5) (Table 2).

Table 2. Alleles incompatibility and driver related bands in cultivars and genotypes studied.

	Cultivar or genotype	Size band(bp)	Genotype incompatible
1	Tuono	450, 750	S_1S_f
2	Sahand	450, 750 and 1130	S_1S_2
3	Shokofeh	300, 900	$S_{12}S_3$
4	$A_{1.16}$	450, 1720	S_fS_7
5	$A_{9.7}$	875, 1130	$S_{22}S_{24}$
6	$A_{8.39}$	1130,1560 and400	S_9S_{22}
7	$A_{10.11}$	1560, 1560	S_9S_9
8	A_{230}	330, 750	S_5S_1

The varieties "Sahand", "Tuono"and genotypes "A_{230}"were incompatible due to alleles S_1 joint. The genotype by "$A_{8.39}$" to "$A_{10.11}$" was incompatible due to the S_9 allele (Table 2). Genotypes "$A_{8.39}$" (S_9S_{22}) and "$A_{9.7}$" ($S_{24}S_{12}$) were compatible. (I don't know what you are trying to say here). Based on the results of amplification with primers of the second intron, the "Sahand" alleles were self-incompatible (Mousavi *et al*., 2010). Also, the results of the proliferation of primers first intron of the "Sahand" indicateda band size of 1130 bp.The results of amplification with primers for the first intron indicated self-incompatible cultivar "Shokofeh" alleles S_3S_{10}. S_3 alleles "Shokofeh" by researchers have been reported (Sheikh Alyan, 2005; Valizadeh, 2007). Alleles S_3 obtained in this study self-incompatible alleles "Shokofeh" (S_3S_4) correspond obtained frome the crosses of the "Nanparil" (S_7S_8) with the cultivar "Ai" (S_3S_4) (Chaychi *et al*., 2002). The results of amplification with primers for the first intron of the "Tuono" and genotype "$A_{1.16}$" self- compatible Sf allele size band 450 Kbp was observed(Fig. 2),It has been previously shown that "Tuono" needs sequencing bands genotype "$A_{1.16}$" or controlled crosses to be self-compatible (Zinolabedini *et al*., 2011).

Table 3. Names of Crossing Compounds and some information of them.

Groups	Crossing Compounds	Expected Genotypes
First	♀(S_1S_f)Tuono×♂(S_1S_2) Sahand	S_1S_2, S_fS_1, S_fS_2
	♀(S_1S_f)Tuono×♂($S_{22}S_{24}$)$A_{9.7}$	S_1S_{22}, S_1S_{24}, S_fS_{22}, S_fS_{24}
	♀(S_1S_f)Tuono×♂(S_5S_1)A_{230}	S_1S_5, S_fS_5, S_fS_1
	♀(S_1S_f)Tuono×♂(S_fS_7)$A_{1.16}$	S_1S_f, S_1S_7, S_fS_f, S_fS_7
Second	♀($S_{22}S_{24}$)$A_{9.7}$× ♂(S_9S_{22}) $A_{8.39}$	$S_{22}S_9$, $S_{22}S_{22}$, $S_{24}S_9$, $S_{24}S_{22}$
	♀($S_{22}S_{24}$)$A_{9.7}$ × ♂(S_9S_9) $A_{10.11}$	$S_{22}S_9$, $S_{24}S_9$
	♀($S_{22}S_{24}$)$A_{9.7}$× ♂(S_1S_f)Tuono	$S_{22}S_1$, $S_{22}S_f$, $S_{24}S_1$, $S_{24}S_f$
	♀($S_{22}S_{24}$)$A_{9.7}$× ♂(S_5S_1)A_{230}	$S_{22}S_5$, $S_{22}S_1$, $S_{24}S_5$, $S_{24}S_1$
Third	♀($S_{12}S_3$)Shokofeh × ♂(S_fS_7)$A_{1.16}$	$S_{12}S_f$, $S_{12}S_7$, S_3S_f, S_3S_7
	♀($S_{12}S_3$)Shokofeh × ♂(S_1S_f)Tuono	$S_{12}S_1$, $S_{12}S_f$, S_3S_1, S_3S_f
	♀($S_{12}S_3$)Shokofeh × ♂(S_1S_2) Sahand	$S_{12}S_1$, $S_{12}S_2$, S_3S_1, S_3S_2
	♀($S_{12}S_3$)Shokofeh × ♂(S_5S_1)A_{230}	$S_{12}S_5$, $S_{12}S_1$, S_3S_5, S_3S_1

Discussion

Three controlled crosses were used in this study. Expected progenies obtained from 14 crossing compounds in the first group showed that more than

50% of progenies were self-compatible. In the first group, genotype 9 was self-compatible and genotype 5 was self-incompatible. In cross ♀ (S_1S_f) "Tuono"× ♂

(S_fS_7) "$A_{1.16}$," a homozygous genotype, was self-compatible (S_fS_f). The self-compatibility allele (S_f) was due to the presence of the alleles "$A_{1.16}$" and Tuono. The results obtained from the second group derived from cross ♀ ($S_{22}S_{24}$) "$A_{9.7}$" × ♂ (S_1S_f) "Tuono" showed that 50% of the alleles were present and self-compatible of the 16 genotypes in the third group, 12 genotypes were self-incompatible and 4 genotypes were self-compatible. Self-compatible progeny obtained from third ♀ ($S_{12}S_3$) "Shokofeh" × ♂ (S_fS_7) "$A_{1.16}$" and ♀ ($S_{12}S_3$) "Shokofeh" × ♂ (S_1S_f) "Tuono" were the progeny, of which 50 percent wereself-alleles. The blooming time and S-allele patterns of the genotypes from first group (Tuono, Sahand, A_{230}, $A_{1.16}$, $A_{9.7}$),the second group (Tuono, $A_{9.7}$, A_{230}, $A_{10.11}$, $A_{8.39}$) and the third group (Shokofeh, Sahand, Tuono, A_{230}, $A_{1.16}$) were shown to be cross-compatible and could be used as pollinisers for each other in orchard establishment and breeding programs. However, cultivars "Sahand", "Tuono" and genotype "A_{230}" in first group and genotypes "$A_{9.7}$" and "$A_{8.39}$" in second group showed one similar S-allele. Therefore, their usetogether could prohibit the growth of 50% of pollens in the upper sections of the pistils and not fertilize the ovary.

Although the first group cultivars "Sahand", "Tuono" and genotype "A_{230}" showed one similar S allele using primers PaConsI-F and EM-PC1consRD (750 bp).Fifty percent of their pollens may stop in style. Both were fully compatible with genotype "$A_{9.7}$" and "$A_{1.16}$" because their S alleles were extensively different from each other and they could be used as a polliniser for cultivar Tuono. Also, genotypes "$A_{9.7}$" and "$A_{8.39}$" hadone similar S-allele by primers EM-PC2consFD and EM-PC3consR (1300 bp). Fifty percent of their pollens may stop in the style, but, genotypes "$A_{10.11}$", "A_{230}" and cultivar "Tuono" werefully compatible with each other. Therefore, genotypes "$A_{10.11}$", "A_{230}" and cultivar "Tuono"could be used as a polliniser. In third group, cultivars "Shokofeh" werefully compatible with cultivars "Sahand" and Tuono, genotypes "A_{230}" and "$A_{1.16}$". However, it should be mentioned that field-controlled crosses are necessary to confirm the effects of pollens on fruit traits in all genotypes

Conclusions

The results showed that the 8 studied cultivars and genotypes were clearly self-incompatible and cross-incommutability was not observed among cultivars and 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 and genotypes, which was obtained from breeding programs, was very helpful for planning future breeding and orchard establishment programs, especially for speedy selecting of a polliniser.

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