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Full Length Research Paper

Compatibility among early, medium and late bloom almond genotypes revealed by pollen tube growth and fruit set

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Most of the almond (*PRUNUS DULCIS*, Batch) cultivars and genotypes are self-incompatible and some of them are cross-incompatible, which protect high yield production. In the present study, eighteen Maragheh indigenous almond genotypes with favorable traits were selected for the investigation of compatibility relationships among them, by means of pollen tube penetration to ovary and fruit setting methods. Genotypes were divided into three groups based on their blooming time and pollinated by the pollens of synchronized-bloom genotypes. Fruit set was studied in the field, and pollen tube penetration was studied in the laboratory with florescence microscopy. Experiment was carried out based on completely randomized design (CRD) and data were analyzed with SAS software. Results showed that all of the genotypes were cross compatible and they could be selected to pollinate each others in almond breeding and orchards establishment programs.

Key words: Almond, cross-compatibility, pollen tube growth, fruit set.

INTRODUCTION

Almond is one of the most important nut crops of Maragheh, Iran. In fruit production industry, self-incompatible cultivars are undesirable because they cannot be grown in single-cultivar orchards, and their fruit set depends on the abundance of pollen transfer from other trees, which finally produce lower yields (Sharafi, 2011; Sharafi and Bahmani, 2011; Sharafi et al., 2010). Therefore, pollination and fertilization with suitable pollinizers are efficient factors in almond production because high yields in almond need to plant at least two cross-compatible cultivars with overlapping blooming time and favorable effects on each other (Kester et al., 1994; Kodad and Socias., 2008; Oukabli et al., 2000, 2002). Traditional field and laboratory controlled pollination, florescence microscopy studies and evaluation of pollen tub growth have been used in order to identify the self-and cross-(in) compatibility of cultivars/genotypes in many fruit trees (Sharafi et al., 2010; Socias i Company and Felipe, 1987), obtaining the effective pollination period (EPP) of cultivars (Ortega and Dicenta., 2004) and studding the effects of pollen types on the fruit set, fruit and seed quality (Kodad and Oukabli et al., 2000, 2002; Socias i Company and Felipe, 1987). Socias i Company et al. (1987) studied the effects of pollen type on fruit set in 'Tuono' self-compatible almond cultivar and resulted that 'Tuono' had higher fruit set following cross-pollination than self pollination. Dicenta et al. (2002) studied several fruit characteristics after self- and cross-pollination in several self-compatible almond cultivars and showed no differences between both pollination types for any of the studied fruit traits.

Sharafi et al. (2010) studied self and cross-compatibility relationships among 10 late bloom almond selections with florescent microscope and fruit setting methods. They concluded that all of the studied genotypes were self-incompatible but cross-incompatibility was not observed among genotypes and so, all of the genotypes could be used in almond breeding programs or orchard establishment for pollinating each other based on the objectives. In another research, Sharafi et al. (2010) investigated self and cross-compatibility relationships among six almond genotypes with the mentioned methods and reported similar results.

The objective of this research was to study the pollen-pistil compatibility relationships among eighteen Maragheh indigenous almond genotypes by means of

Table 1. Analysis of variances for the pollen germination percentage and pollen tube length (based on micrometer) in eighteen almond genotypes tested in the *in vitro* medium separately based on synchronized-bloom time.

Genotypes group	Source of variation	DF	Pollen germination (%)	Pollen tube length (µm)
Early bloom	Genotypes	5	1614.5**	11756.9**
	Experimental error	20	67.2	301.7
	Coefficient value		18.6	15.4
Medium bloom	Genotypes	5	1356.3**	11981.4**
	Experimental error	20	72	189.8
	Coefficient value		11.2	19.1
Late bloom	Genotypes	5	1102.6**	12782.7**
	Experimental error	20	54.6	215.8
	Coefficient value		12.3	13.4

** Significant in P<0.01% level.

pollen tube penetration to ovary and fruit setting methods for use in almond orchards establishment and breeding programs.

MATERIALS AND METHODS

This study was carried out using eighteen indigenous early, medium and late bloom genotypes of Maragheh almonds. The genotypes were divided into three groups based on their synchronized-blooming time. Early bloom genotypes were '300', '301', '302', '303', '304' and '305'; medium genotypes were '200', '201', '202', '203', '204' and '205'; and late bloom genotypes were '100', '101', '102', '103', '104' and '105'.

Pollens collected from the flower buds and pollen germination was carried out in an *in vitro* medium with 1.5% agar and 15% sucrose, and after 24 h, their growth was protected with chloroform. Seven microscopic areas were counted randomly for evaluation of germinated pollens percentage and length of pollen tubes using a light microscope. In spring 2009, genotypes pollinated by the pollens of synchronized-blooming genotypes and for each cross 4 replications were regarded. In each replication, at least 2 branches with 60 to 100 flower buds at 'D' stage were bagged. Consequently, 15 crosses were programmed in each group as follow: '100'×'101', '100'×'102', '100'×'103', '100'×'104', '100'×'105', '101'×'102', '101'×'103', '101'×'104', '101'×'105', '102'×'103', '102'×'104', '102'×'105', '103'×'104', '103'×'105' and '104'x'105' for late bloom genotypes; '200'×'201', '200'×'202', '200'×'203', '200'×'204', '200'×'205', '201'×'202', '201'×'203', '201'×'204', '201'×'205', '202'×'203', '202'×'204', '202'×'205', '203'×'204', '203'×'205' and '204'x'105' for medium bloom genotypes and '300'×'301', '300'×'302', '300'×'303', '300'×'304', '300'×'305', '301'×'302', '301'×'303', '301'×'304', '301'×'305', '302'×'303', '302'x'304', '302'×'305', for early bloom genotypes respectively. '303'×'304','303'×'305' and '304'×'305' for early bloom genotypes.

Flowers were pollinated when the pistils were acceptable for pollens. Branches on each tree were labeled and the percentages of initial and final fruit set were noted 4 and 8 weeks after pollination, respectively. After 5 to 6 days of pollination, pistils were collected and fixed in FAA (formaldehid, alcole and acid acetic) solution and prepared for fluorescence microscopy observation as indicated in Ortega and Dicenta (2006). The experimental design was completely randomized (CRD) in different treatments (crosses) with 4 replications. Data were analyzed separately for each group following SAS software (Version 9.1), while the mean values were analyzed by Duncan's multiple range test.

RESULTS

Pollen tube growth and germination in vitro and inside the pistils after controlled pollination

Analysis of variance indicated the significant differences for percentage of pollen germination and pollen tube growth in the eighteen studied almond genotypes in the in *vitro* condition (Table 1). Means of pollen germination percentage was ranged among 32.3 and 91% in late bloom, 23.5 and 75.4% in medium bloom and 26.4 and 85.1% in early bloom genotypes in the in *vitro* medium respectively (Table 2). However, the means of pollen tube length were ranged among 385.1 and 864.2 µm in late bloom, 196.1 and 707.8 µm in medium bloom and 183.5 and 546.3 µm in early bloom genotypes respectively (Table 2). Means of germinated pollens percentage in the stigma of cross-pollinated pistils were 49.4% to 93.6% and all of the crosses in each group, showed significantly differences in pollen grain germination percentage on the stigma, pollen tube number in the first, second and third section of the style and pollen tube number in the ovary (data not shown). The number of pollen tubes in the ovary had significant differences in all of the 45 crosses (Tables 3, 4 and 5). Means of the pollen tube reached to ovary were 2.4 to 8.3 in late bloom, 2.6 to 8.7 in medium bloom and 1.4 to

6.8 in early bloom genotypes crosses respectively (Tables 3, 4 and 5). However, pollen germination on the stigma had not shown correlation with pollen tube number reaching the ovary.

Fruit set

Analysis of variances and comparison of the means were

Table 2. Comparison of means for the pollen germination percentage and pollen tube growth (based on micrometer) in eighteen almond genotypes carried out separately based on synchronized-bloom time in the in vitro condition.

Genotypes group	Genotypes	Pollen germination (%)	Pollen tube length (µm)
Late bloom	'100'	51.7^{d}	698 ^{b}
	'101'	59.4^{b}	385.1^{bc}
	'102'	32.3 ^{c}	497.8^{c}
	'103'	91^{a}	765.9^{ab}
	'104'	43.5^{e}	543.7^{bc}
	'105'	67.5^{ab}	864.2^{a}
Medium bloom	'200'	57.6^{b}	493.2 ^{c}
	'201'	75.4^{a}	707.8^{a}
	'202'	23.5 ^{e}	363.2^{d}
	'203'	48.4^{c}	514.4^{b}
	'204'	39.7^{e}	196.1^{e}
	'205'	27.3^{d}	276.3^{ed}
Early bloom	'300'	26.4^{d}	279.6^{dc}
	'301'	51.7^{c}	546.3^{a}
	'302'	35.8^{cd}	387^{c}
	'303'	63^{b}	359.1^{bc}
	'304'	85.1^{a}	473.4^{b}
	'305'	43.7^{bc}	183.5^{d}

Same letters in each column show no significant difference among the means at 5% level.

Table 3. Comparison of means for fruit set percentage and pollen tubes number in the ovary of late-bloom group crosses.

Late-bloom group crosses	Final fruit set (%)	Pollen tube number in the ovary
'100'×'101'	26.2^{c}	3.8^{d}
'100'×'102'	34.5^{b}	5.3^{b}
'100'×'103'	17.3^{d}	4.2^{c}
'100'×'104'	19.2^{cd}	2^{d}
'100'×'105'	12.1^{f}	3.6^{d}
'101'×'102'	18.5f^{d}	2.4^{d}
'101'×'103'	14.1^{e}	4.1^{c}
'101'×'104'	21.7^{d}	5.3^{b}
'101'×'105'	18.8^{cd}	6.4^{a}
'102'×'103'	9.1^{f}	1.7^{e}
'102'×'104'	16^{e}	3.1^{d}
'102'×'105'	23^{c}	3^{d}
'103'×'104'	22.3^{c}	6.7^{a}
'103'×'105'	37.1^{a}	7.9^{a}
'104'×'105'	29.3^{b}	3.1^{d}

Same letters in each column show no significant difference among the means at 5% level.

Table 4. Comparison of means for fruit set percentage and pollen tubes number in the ovary of medium-bloom genotypes crosses.

Medium-bloom genotypes crosses	Final fruit set (%)	Pollen tube number in the ovary
'200'×'201'	24.1^{ab}	5.1^{bc}
'200'×'202'	21.2^{b}	5.6^{bc}
'200'×'203'	19.1^{b}	4.7^{c}
'200'×'204'	7.8^{e}	8.7^{a}
'200'×'205'	17^{c}	6.7^{ab}
'201'×'202'	11.3^{e}	3^{d}
'201'×'203'	29.4^{a}	7.6^{a}
'201'×'204'	27.2^{a}	4.8^{c}
'201'×'205'	22^{b}	6.1^{b}
'202'×'203'	14.1^{d}	2.6^{d}
'202'×'204'	13.9^{d}	5.4^{bc}
'202'×'205'	7.4^{e}	2.7^{d}
'203'×'204'	9.7^{e}	2^{d}
'203'×'205'	10.9^{e}	7.3^{a}
'204'×'205'	18.4^{c}	3.4^{cd}

Same letters in each column show no significant difference among the means at 5% level.

Table 5. Comparison of means for fruit set percentage and pollen tubes number in the ovary of early-bloom genotypes crosses.

Early-bloom genotypes crosses	Final fruit set (%)	Pollen tube number in the ovary
'300'×'301'	10.6^{d}	2.7^{c}
'300'×'302'	16.4^{cd}	6^{a}
'300'×'303'	23.1^{a}	6.8^{a}
'300'×'304'	14.7^{d}	4.3^{b}
'300'×'305'	9.8^{d}	1.4^{d}
301×302	15.3^{cd}	5.2^{b}
'301'×'303'	21.4^{a}	5.4^{b}
'301'×'304'	12.9^{d}	3.7^{c}
'301'×'305'	7.2^{e}	4.7^{c}
'302'×'303'	22.8^{a}	4.3^{c}
'302'×'304'	8.1^{e}	1.9^{d}
'302'×'305'	17.4^{c}	3.2^{c}
'303'×'304'	19.1^{b}	2.4^{cd}
'303'×'305'	4.7^{e}	1.9^{d}
'304'×'305'	18.3^{b}	3.1^{c}

Same letters in each column show no significant difference among the means at 5% level.

carried out among the crosses of each group separately. Results showed that the final fruit set means 9.1 to 37.1% in the late bloom, 7.4 to 29.4% in the medium bloom and 4.7 to 23.1% in the early bloom genotypes crosses respectively (Tables 3, 4 and 5). Highest fruit set mean was observed in crosses '103'×'105', '201'×'203' and '300'×'303' for late bloom, medium bloom and early bloom genotypes crosses, respectively (Tables 3, 4 and 5). All of the crosses with the highest final fruit set showed the lowest fruit abscission too. Regarding the genotypes that were pollinated by different pollen types, the final fruit set was significantly affected by the pollen type in all of the crosses.

DISCUSSION

Results obtained from the pollen tube growth pattern and fruit set in the crosses of eighteen genotypes demonstrated that all the genotypes were cross- compatible.

Percentage of germinated pollens on the stigma was high when compared with the *in vitro* medium. This might have been caused by the ideal condition on the stigma versos to the in *vitro* conditions especially existence of proteins, amino acids and enzymes in the stigma. All the crosses showed significant differences in the pollen tubes number in the ovary and the final fruit set was affected by the pollen type. Means of the pollen tubes in the ovaries were 1.4 to 8.7 in all the 45 crosses among the 18 genotypes (Tables 3, 4 and 5). The high number of tubes in the ovaries and the high fruit set indicated the good compatibility of genotypes; therefore, they could be introduced for pollination in almond breeding programs and orchard establishments.

Many researchers studied the self- and cross-(in) compatibility of cultivars/genotypes using fruit set and fluorescence microscopy methods, and reported self-(in) compatible and cross-(in) compatible cultivars/genotypes in almond species with different effects on fruit taints (Alonso and Socias i Company, 2005; L´opez et al., 2006; Socias i Company and Felipe, 1987; Sharafi et al., 2010). For instance, Ortega et al. (2004), following field studies, showed that although 'Marcona' cultivar and 'S_{5133}' genotype had similar pollen tubes in the style, the fruit set of 'Marcona' was higher than 'S_{5133}'. This phenomenon expresses the interference of other factors (of pollen tube number) in the fruit setting processes. Dicenta et al. (2002c), following self and cross pollination of 6 self-compatible almond cultivars ('Antoneta', 'Laurann', 'Marta', 'Guara', 'S2332' and 'S4017'), found that self or cross pollination of cultivars did not show significant differences for pollen germination, pollen tubes number penetration to the ovary, fruit set, fruit traits and nut traits. Furthermore, Ortega et al. (2006) demonstrated that some of the selections among 26 self-compatible almond genotypes and two cultivars ('Lauranne' and 'Marta') had differences in some of the fruit traits following cross or self pollination. Consequently, in this work, pollen tube number in the ovary, initial and final fruit set of cross-pollination groups showed that all the genotypes were cross-compatible and could pollinate each other with the overlapping time of blooming.

Sharafi et al. (2010) investigated self and cross-(in) compatibility relationships among 10 favorable almond genotypes obtained from a breeding program using fruit set and pollen tube penetration to the ovary by fluorescent microscope and revealed that all of them were self-incompatible, but cross-incompatibility was not shown among them. As such, they demonstrated these results with amplification of the self-incompatibility alleles (S-alleles) in the other research (Sharafi et al., 2010). However, Sharafi et al. (2010) studied self and cross-(in) compatibility among six Maragheh indigenous almond genotypes using fruit set and pollen tube penetration to the ovary by fluorescent microscope and revealed that all of them were self-incompatible, but cross-incompatibility was not shown among them. It should be stated that in their first study, spring cold damaged fruits and they only focused on the initial fruit set; but in their recent study, the final fruit set was evaluated.

Conclusion

This research concluded that the eighteen almond genotypes for the late, medium and early bloom genotype groups were cross-compatible; therefore, all of them could be used in the almond breeding programs and orchard establishment for pollinating each other based on the study's objectives.

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