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Xenia in Almonds: Pollen Source Effect on Characteristics of Some Iranian Late-Blooming Almonds and their self-Incompatibility

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Abstract

The objective of this experiment was evaluation of self-compatibility and identification, introduction and selection of late flowering genotypes as cultivars. This experiment was carried out in order to determine the best pollinator for two commercial almond cultivars, 'Shahrood 12' and 'Shahrood 21'. Applied pollinator cultivars were included pollen from 'Shahrood 21', 'Genco', 'Tuno', '5-15' and 'Super-nova' that were applied on 'Shahrood 21' mother trees, and 'Shahrood 12', 'FeilipCeo', 'Tuno', '5-15' and 'Super-nova'. Results showed no fruit formation in non-pollinated and self-pollinated flowers. Although, results from cross pollination using pollen from other cultivars showed higher fruit set using pollen of 'Genco' for 'Shahrood 21' , and '15-5' for 'Shahrood 12' cultivars. Samples were collected using pollinated flowers for further microscopic examinations. Field and microscopic examination of pollen tube growth in the style confirmed above results. The quantitative traits of fruits obtained of some known almond hybrids were assessed in order to evaluation of this phenomenon which known as xenia. The recorded traits were length (L), width (W), thickness (T) of fruits and seeds, and W/L and T/L ratios.

Keywords*:* Almond, Controlled pollination, Pollen tube growth, Self-incompatibility, Xenia.

Introduction

Almond (*Prunus amygdalus* Batsch. Rosaceae, 2n=16) is an economically important nut tree, particularly in the Mediterranean basin. Due to its low chilling requirement and early flowering in spring, almost all of almond cultivars are damaged by delayed spring frost damage. So identification, introduction and selection of late flowering genotypes and the latter, as cultivars are very important for the Iranian almond industry. In addition, most commercial almond cultivars exhibit self-incompatibility (SI) (Socias I Company and Alonso, 2004), resulting in the arrest of pollen tube growth in the middle third of the style (De Nettancourt, 1977), and for a commercial fruit set of 30% or higher, pollinators are required (Kester and Griggs, 1959). The effect of self and cross pollination on pollen tube growth and embryo sac development has been studied by Pimienta and Polito (1983). SI is a property of many plants and in most cases is determined by a single multi allelic locus, the s locus. If the alleles expressed at this locus in the pollen grain both match the corresponding alleles in the pistil, the pollen grain will be recognized as incompatible. In Iran, almond trees are planted in the cold and semi-cold regions and in 25 of the 31 total provinces.

In order to make an almond cross, one of the processes followed is emasculating flowers before anthesis (removing stamens, petals and part of the sepals, using the finger-nails or a small pair of scissors) and subsequently pollinating the stigma. Although the flower is damaged, very acceptable fruit set can be achieved, but usually less than the fruit set obtained by natural pollination (De Nettancourt, 1977).

In almond, the majority of the important production characteristics of the tree viz. growth, flowering and fruit characters are quantitatively inherited (Grasselly and Crossa-Raynaud, 1983. Kester *et al*., (1977) observed high heritability values for nut size, kernel size and weight. Dicenta *et al*. (2001) worked out the heritability values for different characteristics by regression and variance component methods which estimate heritability in a broad sense. There are numerous methods that reported results for compatibility or incompatibility tests between and within cultivars, including controlled pollination tests, pollen tube growth tests, pedigree examination, stylar ribonuclease detection on isoelectric focusing (IEF) gels and PCR based S-allele. Each of the methods has an advantage and disadvantage but because it provides the best estimate of orchard performance, the controlled pollination test is recommended for determination of the best pollinators (Ortega and Dicenta, 2004).

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Chandrababu and Sharma (1999) studied yield component characteristics such as number of flowers/unit shoot length, fruit set before harvesting, and number of fruits/unit shoot length, mean fruit weight and mean kernel weight and reported comparatively higher genotypic and phenotypic coefficients of variation, heritability and genetic advance, indicating pre-dominance of additive gene action for the control of these characters. Also, in 2002, the variability and heritability of several fruit and kernel traits were studied in 22 families of Zaragoza by Artega and Sociasi Company (2002). Their results showed a correlation of heritability of the same traits for fruit and kernel and that the shape and size of the fruit and kernel were highly heritable. In addition, Bahmani *et al*. (2002) reported heritability in almond fruit characteristics, especially in their bitterness.

The objective of this study was to determine the best pollinator among the "Shahrood 21", "Shahrood 12", "Filip Ceo", "Genco", "Tuno", "5-15" and "Super-nova" cultivars and also to confirm the compatibility or incompatibility these cultivars with "Shahrood 21" and "Shahrood 12" using pollen tube growth by fluorescence microscopy and investigation of pollinator effects on some fruit characteristics.

Materials and Methods

Plant material

This experiment was carried out using five almond (*Prunus amigdalus* Batch) cultivars, including "Shahrood 21", "Genco", 'Tuno', '5-15', 'Shahrood 12', 'Filip Ceo' and 'Super-nova' as pollen parents and Shahrood 21 and Shahrood 12 as seed parents which are grown in theresearch orchard of the Agricultural and Natural Sources college University of Tehran/Karaj, Iran.

Pollen collection

Pollen was prepared using the flowers at the balloon stage. Twigs 0.5–1.5 m long from all of the treatments were collected and placed in water (20 l bucket) (Arzani and Khalighi, 1997). Pollen was collected by forcing cut branches indoor at room temperature and rubbing flowers over 2 mm mesh screen to separate anthers, which were then allowed to dehisce by overnight exposure to light and then placed in small glass bottles and stored at 4 ºC until pollination (Ortega *et al*., 2004).

Viability test

For estimation of *in vitro* pollen germination, pollen grains were placed in Petri dishes with artificial medium (1% agar-agar+15% sucrose+20 mg/l boric acid) which were then kept at room temperature (20ºC) for 24 hours.

Emasculating and bagging branches, and controlled pollination and measurement

For each treatment (pollen grain), two branches with 100–150 closed floral buds were emasculated. Hand pollination was carried out using a camel hair brush, two days after anthesis base of effective pollination period (EPP) (Alizadeh-Salteh *et al.*, 2009) on shoots located on experimental trees and then bagged using an insect-proof bag with a mesh thick enough to avoid entrance of insects into the bag. Un-opened and old flowers were removed from the selected shoots and all pollinated flowers were recorded. A second pollination was carried to obtain an acceptable fruit set level.

In order to calculate percentage of fruit set, amounts of fruits on the selected shoots were recorded at five time intervals after hybridization (15, 45, 60 and 75 days after pollination and at the time of fruit harvest).

Fluorescence microscopy

For examining pollen tube growth after each treatment, five flowers were sampled at 24, 48, 72, 96 and 120 h after pollination and fixed in FAA (70% ethanol: formalin: glacial acetic acid, 18:1:1). After 24 h in the fixative, pistils were washed several times and were transferred to 70% ethanol where they were stored for up to 3 months. Then the pistils were washed several times with distilled water and incubated in 8 N NaOH for 24 h to soften the tissues (Beppu *et al.*, 2003). Then they was rinsed in distilled water and stored for 3 h prior to transfer to 0.1% (w/v) aniline blue in 0.1 N K_3PO_4 for 3 h. The stigma and style were placed on a microscope slide in a drop of stain, squashed under a cover slip, and observed using an Olympus BX-60 microscope (Olympus America, Melville, N.Y.) equipped with a UV lamp (Reed, 2004). The number of pistils compatible with pollen from the male parent was divided by the total number of pistils tested, and if two or more of 12 pistils observed (>17%) showed pollen tubes reaching the ovary, the cultivars were considered to be cross-compatible (Lopez *et al*., 2004)

Fruit characteristics measurements

Fruit dimensions were measured by digital caliper. Measured characteristics were: shell length, shell diagonal, shell diameter, shell weight, kernel length, kernel diagonal, and kernel diameter and kernel weight.

Statistical analysis

The experiment was conducted with 5 treatments and 3 replications. Treatments were arranged in a Randomized Complete Block Design (RCBD). Two branches in two sides of the tree (North and South) for each treatment were assumed. The data were examined by analysis of variance using the General Linear Model (GLM) procedure of SAS software Means were compared using the Duncan multiple range test.

Results

Pollen germination test

The result of pollen viability showed that all pollens of cultivars had a suitable viability and can be used for controlled pollination (the mean for pollen germination was 65%).

Controlled pollination

Analysis of variance of the fruit set showed significant difference with various pollen grains. The

fruit set rates in the fourth and fifth records were equal, so they were considered as the final fruit set. The analysis of variance of the fruit set in fifth record showed significant differences with various pollen grains. In addition, mean comparison by the Duncan test showed that the mean fruit set on "Shahrood 21" with pollen grains of "15-5", "Genco", "Touno", "Supernowa" and "Shahrood 21 were 19.43, 32.87, 15.2, 25 and 0.16 percent, respectively and for Shahrood 12 with pollen grains of "Tuono", "Supernowa", "Filip Ceo" , "15-5" and "Shahrood 12," they were 21.14, 22.88, 23.29, 23.29, 32.23, 0.19 percent, respectively.

Therefore Shahrood 21 and Shahrood 12 are self-incompatible, but are cross-compatible with 15-5, Genco, Touno, Supernowa and 15-5, Filip Ceo, Touno and Supernowa. The means fruit set in 1st, 2nd, 3rd and 4th records with various pollen grain treatments for both cultivars show in tables 1 and 2. The mean of final fruit sets is showed in figures 1 and 2.

Table 1.Mean fruit set in 1, 2, 3 and 4th records with various pollen grain treatment in Shahrood 21 cultivar.

Fruit set percent treatment	First record	Second record	Third record	Fourth record
15-5	85.43a	27.16ab	20.5^{bc}	19.43^{bc}
Genco	79.66^{a}	36.33^{a}	33.7^{a}	32.87^{a}
Tuono	84.16^{a}	20.86^{b}	16.5^{c}	15.2^{c}
Supernowa	87.13^{a}	27.83^{ab}	26.2^{ab}	25^{b}
Shahrood 21	79^{a}	0.33^{c}	0.16^{d}	0.16^{d}

Table 2. Mean fruit set in 1, 2, 3 and 4th records with various pollen grain treatment in Shahrood 12 cultivar.

Fruit set percent treatment	First record	Second record	Third record	Fourth record
Tuono	79.50 b	25.57^{b}	23.44^{b}	21.14^{b}
Supernowa	88.22^{a}	30.33^{ab}	25.15^{b}	22.88^{b}
Filip Ceo	87.12^{ab}	27.99^{b}	25.002^{b}	23.29^{b}
15-5	86.57^{ab}	37.88^{a}	34.98^{a}	32.23^{a}
Shahrood 12	88.54^{a}	$.28^{c}$	$.125^{c}$	$.09^{c}$

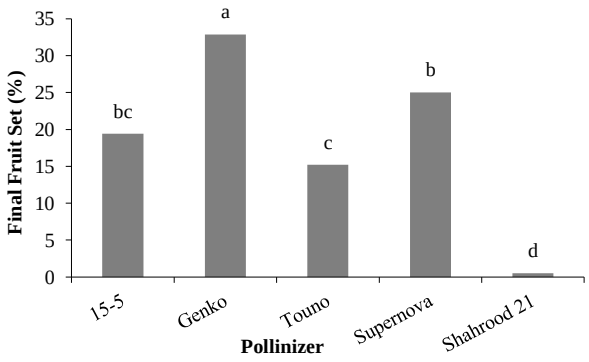

Fig. 1. Mean of final fruit set in Shahrood 21 cultivar

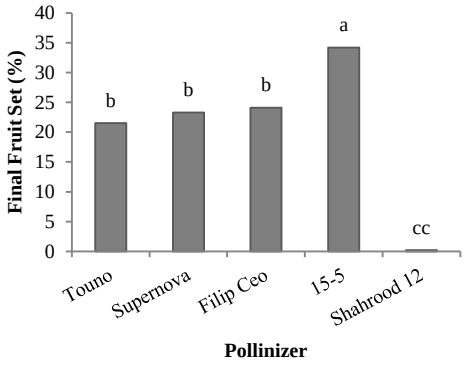

Fig. 2.Mean values of final fruit set in Shahrood 12 cultivar

Pollen tube growth

Pollination of flowers was carried out at the optimal temperature (25 ºC), after which they were kept at 17-25 ºC until 10 days after pollination. Therefore pollen germination and pollen tube growth was affected only by genotype. The results obtained from these samples showed the amount of pollen tube growth in cross-compatible cultivars.

Table 3. Analysis of variance of fruit characteristics of Shahrood 21

Measurements MS	df	Shell length	Shell diagonal	Shell diameter	Shell weight	Kernel length	Kernel diagonal	Kernel diameter	Kernel weight
Replication	2	0.28ns	0.36ns	1.35ns	0.23ns	0.25ns	0.04	0.37ns	0.03ns
Treatment	4	9.90**	26.41**	23.51**	1.038**	4.67**	6.09**	15.18**	0.67**
T×R	8	1.01ns	0.26ns	0.52ns	0.07ns	0.12ns	0.16ns	0.91ns	0.08ns
Error	30	1.41	1.75	1.85	0.24	0.99	0.87	2.85	2.85

Table 4. The effect of different pollinizers on characteristics of Shahrood 21 fruits.

Treatment measurement	15-5	Genco	Tuono	Supernowa	Open pollination
Shell length	29 b	31.3 a	29.1 b	29.15 b	30.52 ab
Shell diagonal	19.5 cd	21.5 b	20.2 c	19 d	23.2 a
Shell diameter	16.3 b	16.8 b	16.7 b	14.8 c	19.2 a
Shell weight	2.35 bc	2.48 b	2.4 bc	2.1 c	4.7 a
Kernel length	21.8 c	22.2 bc	21.54 c	22.51 b	23.45 a
Kernel diagonal	12.5 bc	13 b	12 c	12.2 bc	14 a
Kernel diameter	10 b	9.5 b	9 b	8.4 b	12 a
Kernel weight	1.22 b	1.19 b	1.5 bc	1.1 c	1.71 a

The results also showed that the pollen of Shahrood 21 on the owner stigma germinated but that pollen tube growth was suppressed in first 1/3. Tables 3 and 4 show the results of fruits measurements and show that there are differences between Shahrood 21 and 12 fruits. The mean of kernel diameter was affected b**y** different pollen parents (Fig.3).

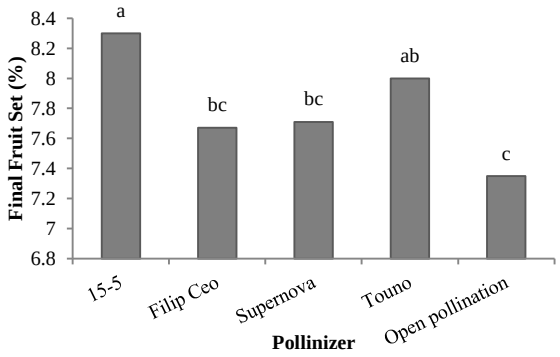

Fig 3. Kernel diameter. Shahrood 12

Discussion

The large amounts of pollen grains on the stigma in nearly all pollinations shows that the pistils were receptive when pollinated.

Based on our research results; Shahrood 21 and 12 are self-incompatible, but are cross-compatible with the 15-5, Genco, Touno, Supernowa. For Shahrood 21, the fruit set for 15-5, Genco, Touno, Supernowa cultivars is 19.43, 32.87, 15.2 and 25%, respectively; for Shahrood 12 with pollen grains of Tuono, Supernowa, Filip Ceo , 15-5 the fruit set is 21.14, 22.88, 23.29, 23.29, 32.23, 0.19 respectively .

The late blooming almond cultivar, Shahrood 21, as shown by the results is self-incompatible and needs compatible and suitable pollinator for fruit set. Genco had the greatest fruit set. So, the following cultivars are optimal as pollinators: Genco for Shahrood 21 and 15-5 for Shahrood 12. This cultivar also has flowering times that overlap with the main studied cultivars (Shahrood 21 and 12). Therefore these cultivars are recommended as pollinator for Shahrood 21 and 12. The pollens of Genco and 15-5 produce a high fruit set possibly due to the best temperature after pollination (17-25ºC). The present results (controlled pollination) were in agreement with microscopic examination of pollen tube growth. Therefore pollen tube of cross-compatible cultivars reached to the ovary at 96 h after pollination but in the case of self-pollination the pollen tube could not reach the ovary even 120 h after pollination. It has been reported that genotype, temperature and environmental conditions all affect pollen tube growth (Ortega *et al*., 2004). It is considered that the pollen tube growth rate of various cultivars is different at same temperature and environmental conditions.In addition, ovule longevity had an effect on effective pollination period (EPP) and fruit set. Ovule longevity is determined by several genetic, physiological and environmental conditions occurring during flowering. Among the environmental factors, high temperature can shorten the ovule viability in different species (Ortega *et al*., 2004). Also very low temperature effects on ovule longevity.

There is no research about grouping of almond cultivars in Iran.

In summary, we conclude that almond cultivars Shahrood 21 and 12 are self-incompatible, and to produce commercial crops one needs compatible and suitable pollinator. We propose Genco, 15-5 and Supernowa as pollinators for the almonds tested in this study. Growers may use these cultivars in combination with Shahrood 21 and 12.

In conclusion, we suggest that further research on local and imported almond cultivars is necessary to identify compatibility or incompatibility groups by the PCR method that followed by controlled pollination to determine best pollinator for each commercial cultivar.

In this research, fruit dimension analysis showed varying results. In the case of Shahrood 21 most of treatments were significant but an often fruit from open pollinated treatment was higher than others. In Shahrood 12 only one treatment was significant. In terms of fruit kernel diameter, 15-5 pollinatorr was the most effective, followed by Filip Ceo, Supernowa and Tuono, Open pollinated fruits were the lowest. In addition based on our research, some fruit characteristics can be inherited. Our results in agreement with those of Artega and Company (2002) and Bahmani *et al*., (2002).

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