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Self-incompatibility Studies of Some Iranian Late-Blooming Almonds and Pollen Source Effect on Some Characteristics of Nuts

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Abstract: The objective of this experiment was to evaluate self-compatibility of two late blooming almond cultivars and identification and introduction of suitable pollinizers from late flowering genotypes, and evaluation of pollen source effect on fruit characteristics to earn high quality nuts for Iranian almond industry. This experiment was carried out in order to determine the best pollinizer for two commercial almond cultivars, 'Shahrood 12' and 'Shahrood 21' (♀). pollen sources were 'Shahrood 21', 'Genco', 'Tuno', '5-15' and 'Super-nova' (♂) which were applied on 'Sharood 21' mother trees, and 'Shahrood 12', 'Feilip Ceo', 'Tuno', '5-15' and 'Super-nova' (♂). The results showed no fruit formation in non-pollinated and self-pollinated flowers. However, the 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. The samples of pollinated flowers were collected for further microscopic examinations. In order to evaluate the effect of pollen sources, quantitative traits of nuts were measured. The recorded traits were length (L), width (W), thickness (T) of seeds, and W/L and T/L ratios.

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

INTRODUCTION

Almond (*Prunus amygdalus* Batsch.) is an economically important nut tree particularly in Iran.

In Iran, almond is planted in the cold and semi-cold regions and in 25 out of total provinces. Production quantity of the world was about 2,516,000 tones and Iran was third producer of almond in the world (about 158000 tones) (FAO, 2010).

As the commercial part of the almond fruit is the kernel, pollination and fruit set are essential to obtain optimal yields, and typically require the joint planting of at least two inter-compatible and simultaneously blooming cultivars, the presence of pollinating insects for pollen transfer and desirable weather during bloom and pollinators [23]. Fruit setting and pollination have been described as the most influential limiting factors for almond production [14].

Having a reliable cross method is one of the first essential steps for the progress of any breeding program. So, the crosses have done actually fertilized by the expected pollinizer (the cultivar used as the pollen donor during the crosses) are successful [9].

However, most of commercial almond cultivars have self-incompatibility (SI) of the gametophytic type [22], resulting in the arrest of pollen tube growth in the middle third of style and prevention of fertilization [8] (In cross-pollinated flowers in orchards, pollen tubes commonly grow to the base of the style and enter the ovary within 96 to 120 h

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[3]. and pollinizers are required for a commercial fruit set of 30% or higher [15].

There are numerous methods that reported the results of 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 [7, 11, 24]. Each of the methods has advantages and disadvantages but, because of the estimate of orchard performance by controlled pollination test, it is recommended for determining of the best pollinizers [18].

In almond, the majority of the important production characters of the tree like; growth, flowering and fruit characters are quantitatively inherited [12]. Kester and his colleagues (1977) observed high heritability values for nut size, kernal size and weight [16]. Dicenta and his colleagues (2001) worked out the heritability values for different characters by regression and variance component methods, which estimated heritability in a broad sense [10].

Chandrababu and Sharma (1999) studied about yield component characters like number of flowers/unit shoot length, fruit set before harvesting and number of fruits/unit shoot length, mean fruit weight and mean kernel weight [6]. Their results demonstrated comparatively higher genotypic and phenotypic coefficient of variation, heritability and genetic advance which indicated the pre-dominance of additive gene action for the control of these characters. In 2002 also, the variability and heritability of several fruit and kernel traits were studied in 22 families of Zaragoza by Artega and Socias i Company [2]. 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 and his colleagues (1998) reported heritability in almond fruit characteristics [4].

The aim of this study was to determine the best pollinizer among "Shahrood 21", "Shahrood 12", "Filip Ceo", "Genco", "Tuno", "5-15" and "Super-nova" cultivars and also determine compatibility or incompatibility of these cultivars with "Shahrood 21" and "Shahrood 12" using pollen tube growth by fluorescence microscope and investigate the effects of pollen source 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 parent (♂) and Shahrood 21 and Shahrood 12 as seed parent (♀), which were grown in the Research Orchard of Agricultural and Natural Sources College of Tehran University /Karaj, Iran.

Pollen Collection

Pollens were collected from the flower buds at balloon stage, 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 [19].

Viability Test

To estimate *in vitro* pollen germination, pollen was placed in Petri dishes with artificial medium (1% agar-agar+15% sucrose+20 mg/l boric acid), which was then kept at room temperature (20 ºC) for 24 h. [5].

Controlled Pollination

Pollinations among the cultivars being studied were carried out, using the tree’s own pollen (‘Shahrood 21’ and ‘Shahrood 12’) and pollen of other cultivars ("Genco", 'Tuno', '5-15', 'Filip Ceo' and 'Super-nova'). In the field, branches were chosen that had an average of 100–150 flowers in the

balloon stage. The flowers were emasculated to prevent self-pollination.

Hand pollination was carried out using a camel hair brush in anthesis time according to effective pollination period (EPP) [1], and then bagged using an insect-proof bag with a mesh thick enough to avoid entrance of insect. Un-opened and old flowers were removed from the selected branches and all pollinated flowers were recorded. The second pollination was carried out to obtain an acceptable fruit set level.

In order to calculate percentage of fruit set, fruit numbers were recorded at five time intervals after fertilization (15, 45, 60 and 75 days after pollination and at the time of fruit harvest).

Fluorescence Microscopy

To examine pollen tube growth in style, 10 flowers were sampled at 24, 48, 72, 96 and 120 h after pollination and fixed in FAA (18:1:1, 70% ethanol: formalin: glacial acetic acid). After 24 h in 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 [5]. After that, they were 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 [20]. Finally, if 2 or more of 12 observed pistils (>17%) showed pollen tubes reaching the ovary, the cultivars were considered to be cross-compatible [17].

Measuring Fruit Characteristics

Fruit size measurements calculated by digital caliper. Measurement characters were shell length, shell diagonal, shell diameter, shell weight, kernel length, kernel diagonal, kernel diameter and kernel weight.

Statistical Analysis

The experiment was conducted with 5 treatments and 3 replications. The treatments were arranged in a randomized complete block design (RCBD). The data were examined by the analysis of variance using the General Linear Model (GLM) procedure of SAS software [21]. The means were compared using Duncan's multiple range tests.

RESULTS

Pollen Germination Test

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

Controlled Pollination

The analysis of variance of the fruit set showed significant difference between treatments. Fruit numeration results in fourth and fifth records (75 days after pollination and at the time of fruit harvest) were equal, so they were considered as the final fruit set.

Results showed that there was significant difference between treatments. Therefore Shahrood 21 (♀) and Shahrood 12 (♀) are self-incompatible and are cross-compatible with 15-5, Genco, Tuono, Supernova and 15-5, Filip Ceo, Tuono, and Supernova respectively. Tables 1 and 2 show means fruit set in 1, 2, 3th and final records with various pollen sources for both cultivars.

Table 1. Mean fruit set in 1, 2, 3 and 4th records with various pollen grain treatments in Shahrood 21 cultivar (♀) (%)

Pollinizer	First record	Second record	Third record	Fourth record
15-5	85.43^{a}	27.16^{ab}	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}
Supernova	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 treatments in Shahrood 12 cultivar (♀) (%)

Pollinizer	First record	Second record	Third record	Fourth record
Tuono	79.50 [b]	25.57[b]	23.44[b]	21.14[b]
Supernova	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]

Also mean comparison showed that mean of fruit set on "Shahrood 21" (♀) with pollen grains of "15-5", "Genco", " Tuono ", "Supernova" and "Shahrood 21" was 19.43, 32.87, 15.2 , 25 and 0.16% and for Shahrood 12 (♀) with pollen grains of "Tuono", "Supernova", "Filip Ceo", "15-5" and "Shahrood 12", it was 21.14, 22.88, 23.29, 23.29, 32.23 and 0.19% respectively (Fig 1 and 2). According to obtained results, 'Genco' was the best pollinizer for 'Shahrood 21', and '15-5' for 'Shahrood 12' too.

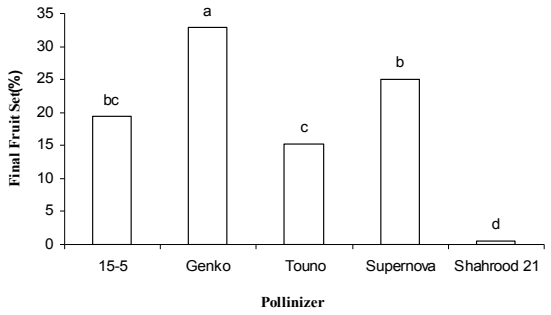

Fig 1. Mean of the final fruit set in Shahrood 21 cultivar (♀)

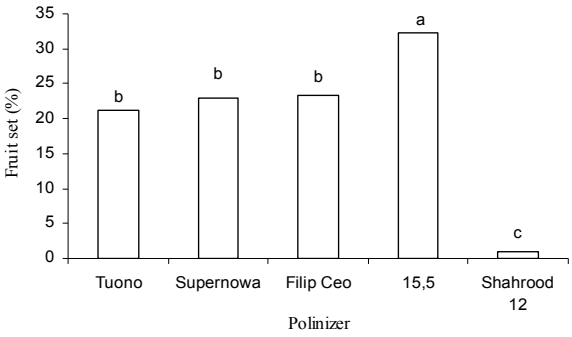

Fig 2. Mean of the final fruit set in Shahrood 12 cultivar (♀)

Pollen Tube Growth

Pollination of flowers was carried out at the temperature 25 ºC and followed by 17-25 ºC until 10 days after the pollination. Therefore, pollen germination and pollen tube growth were affected by only genotype. The result obtained from observation of pollen tube growth with Fluorescent microscopy showed that pollen tube could reach to ovary in cross-compatible cultivars (Figure 3 (A, B, C). Thus, the results showed that pollen of Shahrood 21 on owner stigma was germinated but it suppressed pollen tube growth in first 1/3 (Figure 3(D)). This observation on Shahrood 12 pollen exposure on itself stigma was the same as Shahrood 21 cultivar.

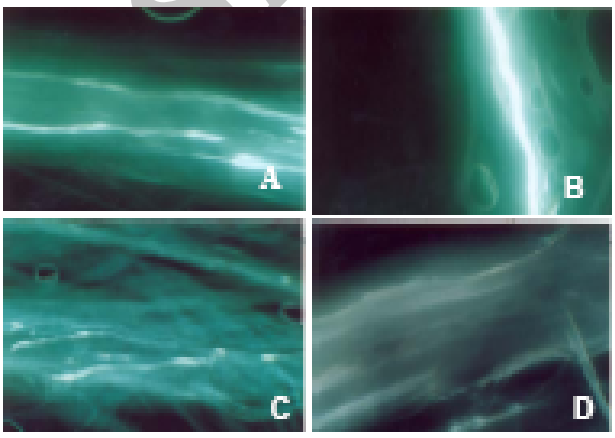

Fig 3. Pollen tube growth in different cultivars: A. Pollen tube of Genco (♂) at the end of Shahrood 21 (♀)style, B. Pollen tube of 15-5 (♂) at the end of Shahrood 21 (♀) style, C. Pollen tube of Tuono (♂) at the end of Shahrood 12 (♀) style, D. Inhibition of pollen tube growth of Shahrood 21 (♂) in the style of itself (Shahrood 21)

Fruit Measurements

The results of analysis of variance from Shahrood 21 fruits mesurments, indicated that different pollen sources had significant effect on nuts physicochemical characteristics (Table 3).

But in most cases did not follow a certain pattern. Table 4 shows these differences from Shahrood 21.

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

Measurements MS	df	Shell lenght	Shell diagonal	Shell diameter	Shell weight	Kernel lenght	Kernel diagonal	Kernel diameter	Kernel weight
Replication	2	0.28ns	0.36ns	1.35ns	0.23ns	0.25ns	0.04 ns	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
Total	44								

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

Pollinizer	15-5	Genco	Tuono	Supernova	Open pollination
Shell lenght	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 lenght	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

Figure 4 shows the mean of kernel diameter affected by different pollen parents. According to the obtained results, different pollinizers did not have a significant effect on the fruits' traits, except on kernel diameter and '15-5' pollen parent had the highest effect on kernel diameter.

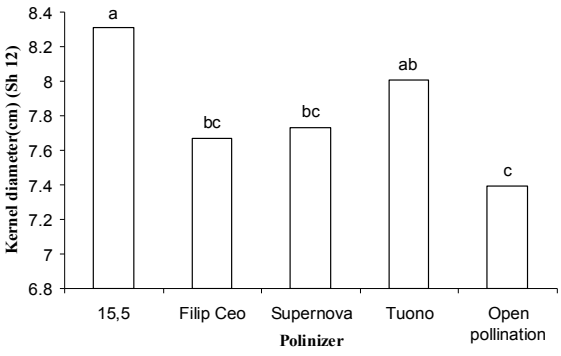

Fig 4. Kernel diameter. Shahrood 12(♀)

DISCUSSION

The large amounts of pollen grains on the stigma in nearly all pollinations showed pistils' receptivity during pollination; that the pistils were receptive when being pollinated.

Based on the present research results Shahrood 21 (♀) and 12 (♀) were self-incompatible and were cross-compatible with 15-5, Genco, Tuono, Supernova and 15-5, Genco, Tuono, Supernova cultivars with 19.43, 32.87, 15.2 and 25 % fruit sets, respectively, and for Shahrood 12 (♀) with pollen grains of Tuono, Supernova, Filip Ceo, 15-5 and Shahrood 12 were 21.14, 22.88, 23.29, 23.29, 32.23 and 0.19 were obtained, respectively.

Almond, late bloom cultivar, Shahrood 21 as shown by the results was self-incompatible and needed a compatible and suitable pollinizer for the fruit set. Also, Genco had the greatest fruit set. So, this cultivar is suitable as pollinizer for Shahrood 21 and 15-5 for Shahrood 12 cultivar. Also, overlap flowering with the main studied cultivars (Shahrood 21 and 12). Therefore these cultivars recommended as pollinizers for Shahrood 21 and 12 (♀). Pollen of Genco and 15-5 produced high fruit set possibly due to the best temperature after pollination (17-25 ºC). In the present research, the results (controlled pollination) were in agreement with the microscopic examination of pollen tube growth. Therefore pollen tube of cross-compatible cultivars reached the ovary at 96 h after pollination but self-pollination showed that pollen tube could not reach the ovary at 120 h after pollination. It has been reported that genotype, temperature and environmental conditions affect pollen tube growth

[19]. Moreover, pollen tube growth rate of various cultivars is different at the 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 which occur during flowering. Among the environmental factors, high temperature can shorten the ovule viability in different species [19]. Also, very low temperature affects ovule longevity.

There has been no research on grouping almond cultivars in Iran.

In summary, it can be concluded that almond cv. Shahrood 21 and 12 are self-incompatible. For producing commercial crops, compatible and suitable pollinizers are required; thus, in this study Genco, 15-5 and Supernova were proposed as pollinizers for the tested almonds and growers may use these cultivars in combination with Shahrood 21 and 12.

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

In this research, fruit dimension analysis showed different results. So in Shahrood 21 (♀) the most of treatments were significant but an often fruit from an open pollinated treatment was higher than others. In shahrood 12 (♀) only one treatment was significant. In fact fruit kernel diameter, 15-5 pollinizer had the most effect and, after that, Filip Ceo, Supernova and Tuono had a suitable rate. Open pollinated fruits were the lowest. In addition, based on the present research, some fruit characteristics can be inherited. And, the results of this study were adapted by Artega and Company (2002) and Bahmani *et al* (1998) [2, 4].

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