



Identification of the pollinizer for a new almond genotype 'Karaj 33'

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ABSTRACT

Purpose: Most almond cultivars grown in Iran exhibit gametophytic self-incompatibility. Therefore, they need to be pollinated by cross-compatible cultivars that bloom in the same time to produce commercial crop. One of the new almond genotypes is Karaj 33, which has very late flowering, high productivity, paper shell, high percentage of kernel to fruit, no double and twin kernel, and self-incompatible. **Research method:** In order to select the suitable pollinizer for 'Karaj 33', an experiment was conducted using fruit set under field conditions and fluorescence microscopy methods, in which Tuono, Shekofeh and H were evaluated as pollen donors. **Findings:** All almond cultivars/genotypes had high (80-85%) pollen germination. Tuono and H bloomed earlier (from 24th to 29th March), whereas Shekofeh and then Karaj 33 bloomed later (from 26th March to 1st April and 30th March to 3rd April, respectively). Cross-compatibility was confirmed in all the three cultivars/genotype by both methods. Shekofeh had almost overlapping flowering time with Karaj 33. The penetration rate of Shekofeh pollen tube was faster so that 96 h after cross-pollination, it reached the base of Karaj 33 style, while the other two reached 120 h later. The highest final fruit set was observed with the cross of Karaj 33 × Shekofeh. **Research limitations:** No limitations were found. **Originality/Value:** we can consider Shekofeh as a suitable pollinizer for Karaj 33.

INTRODUCTION

Almond, *Prunus dulcis* (Mill.) D. A. Webb. [syn. *Prunus amygdalus* (L.) Batsch] is an economically important nut crop in warm temperate regions. It is thought to have originated in the arid mountainous regions of Central Asia (Gharaghani et al., 2017). Iran with 119972 tons in production in 2019 is the third largest producer of almond in the world after USA and Spain (FAOSTAT, 2021).

Although almond is one of the most important perennial nut crops in the arid and semi-arid regions of Iran, its production in Iran is based on locally adapted clones (Gharaghani et al., 2017). A lot of efforts have been made for breeding and improvement of new almond cultivars in Iran. One of the new almond genotypes obtained by open pollination of 'Tardy Nonpariel' is Karaj 33 (K-33), which has the following characteristics: very late flowering, high productivity (1.5 t/ha), paper shell, high percentage of kernel to fruit (60-65%), no double and twin kernel, and self-incompatible (Esmailpour et al., 2017).

Almond pollination is affected by several factors such as bloom overlap between cultivars and compatibility of pollen with the maternal parent cultivar (Connel, 2000). Most almond cultivars are self-incompatible requiring a pollinizer cultivar to encourage pollination for a commercial production. Self-incompatibility in almond is gametophytic and controlled by a single *S*-locus with multiple codominant alleles (Gharaghani et al., 2017; Imani et al., 2014; Najafi et al., 2015). In addition to self-incompatibility, almond cultivars also show cross-incompatibility. Therefore, identification of compatible and incompatible groups is of great importance for the establishment of almond orchards (Gharaghani et al., 2017).

Incompatibility is usually determined by: I) monitoring fruit set in pollinated and isolated flowers under field conditions, II) pollination of flowers and observation of pollen tube growth in the style under a fluorescent microscope, III) detection of stelar ribonucleases (S-RNases), and IV) DNA amplification and identification by PCR analysis (Khoshharf Motlagh et al., 2019; Nikolić et al., 2012).

The purpose of this work was to determine a suitable pollinizer for the almond genotype Karaj 33 by fruit set under field conditions and fluorescence microscopy methods to recommend a potential pollinizer to be used in commercial production of Karaj 33 genotype.

MATERIALS AND METHODS

Plant material

The plant material used in this study was taken from 8-year-old almond trees from the experimental orchard of Horticultural Science Research Institute (35° 74' 58" N and 50° 95' 11" E and altitude 1235 m), Karaj, Iran. Three almond cultivars/genotype with favorable traits (Tuono, Shekofeh and H) was investigated as pollinizers for Karaj 33. The monthly mean temperature and precipitation during the flowering period until fruit ripening are presented in Figure 1.

Flowering time

The flowering time of recipient and donor of the studied cultivars/genotypes was recorded. The opening of 1% and 80% of flowers, and fall petal were considered as the initial blooming, full bloom and the end of flowering stages, respectively.

Pollen collection

To collect the pollen, shoots with flower buds of all investigated cultivars/genotypes were taken and placed in jars with 50 g L⁻¹ sucrose solution at room temperature. After 24 h, the anthers from unopened flowers at balloon stage were taken and left in open petri-dishes to desiccate for 12-18 h at room temperature. The collected pollens were kept at 4 °C in the refrigerator.

Pollen viability test

The applied method for pollen quality was *in vitro* germination on a substrate containing 10 g L⁻¹ sucrose, 0.2 g L⁻¹ Ca(NO₃)₂, 0.1 g L⁻¹ KNO₃, 0.1 g L⁻¹ MgSO₄, 0.1 g L⁻¹ H₃BO₃, and 20 g L⁻¹ agar. Pollen germination was determined after 8 h incubation at 24 °C. A germinated pollen grain was considered to be the one whose pollen tube was longer than the diameter of the pollen grain itself (Čolić et al., 2010).

Controlled pollination and fruit set

For fruit set monitoring shoots were randomly selected and all open flowers and closed buds were removed. Emasculation and hand cross-pollination was applied for a total of 100 flowers at the late balloon stage. To avoid possible open pollination, the shoots were bagged. Fruits were counted after 20 and 90 days after cross-pollination and at ripening time.

Pollen tube growth

To observe pollen tube growth, the cross-pollinated flowers were taken 24, 48, 72, 96 and 120 h after pollination and fixation of the pistils was done with FAA fixative (70% ethanol, glacial acetic acid and formaldehyde in a ratio 90:5:5). Fixed materials were immersed overnight in 8 M NaOH to soften their tissues and then rinsed in the running water five times. The pistils were stained for 24 h with 0.1 N K₃PO₄ containing dissolved 0.1% aniline blue. Pollen tube growth was observed under a fluorescence microscope. At least 15 pistils were analyzed from each cross.

Statistical Analysis

The experiment was conducted in a randomized complete block design (RCBD). The data were examined by the analysis of variance by SAS software. The means were compared using Duncan's multiple range tests.

RESULTS AND DISCUSSION

Incidence of bloom between compatible cultivars is essential for cross-pollination (Connel, 2000). The flowering period of the studied cultivars/genotypes is presented in Figure 2. Tuono and H bloomed earlier (from 24th to 29th March), whereas Shekofeh and then Karaj 33 bloomed later (from 26th March to 1st April and 30th March to 3rd April, respectively). The longest (7 days) and shortest (5 days) flowering period were also dedicated to Shekofeh and Karaj 33, respectively. Of the three pollinizers studied, only the flowering time of Shekofeh overlaps with Karaj 33. Bloom overlap refers to the coincidence of bloom time between cultivars in the orchard. The maximum opportunity for nut set results when cultivars are in the same stage of bloom at approximately the same time (Connel, 2000).

Knowledge of pollen functional ability is very important, because sterility and poor fertilization can be explained by pollen low viability. Pollen germination depends on a wide range of factors: species, cultivar, nutrition and environment factors (Čolić et al., 2010). All

analyzed almond cultivars/genotype had high (80-85%) pollen germination (Table 1), which is a prerequisite for successful fertilization.

Results obtained from fruit set and pollen tube growth pattern in the crosses of three cultivars/genotype demonstrated that all of them were cross-compatible with Karaj 33. No fruit set occurred in self-pollinated Karaj 33, while significant differences in fruit set were found between the studied cultivars/genotype in all three measurements 30 and 90 days after cross-pollination and at ripening time (Table 2). The highest fruit set in 20 and 90 days after cross-pollination was observed with open pollination (90.0 and 54.4%, respectively) and Shekofeh as pollinizer (89.1 and 53.4%, respectively), while the lowest value was for Karaj 33 × H (78.7 and 33.3%, respectively). At the time of fruit ripening, the crosses of Karaj 33 × Shekofeh accounted for the highest fruit set (32.8%), while the lowest amount was found with Karaj 33 × Tuono (22.6%). This is similar to results of Agajanlo et al. (2011), who reported Ferragness fruit set rate of 38.8, 32.1 and 22.6% at open pollination, Ferragness × Shekofeh and Ferragness × Tuono crosses, respectively. Alizadeh-Salteh et al. (2012) also observed that the final fruit set rate in the crosses of Shahrood 21 × Tuono and Shahrood 12 × Tuono is 15.2 and 21.1%, respectively. Positive effects of pollinators on fruit set of other almond cultivars have also been reported (Cunningham et al., 2019; Yaman & Uzun, 2021). Yaman and Uzun (2021) investigated the effects of different pollinators on fruit set and quality characteristics of Texas cultivar, and found that fruit set varied between 8.0 to 13.2% based on pollinators.

Table 1. Pollen germination of studied cultivars/genotypes of almond

Trait	Tuono	Shekofeh	H	Karaj 33
Pollen germination (%)	82 ab	85 a	80 b	83 ab

Means with at least one common letter do not differ significantly according to Duncan's Multiple Range Test at $P \leq 0.05$.

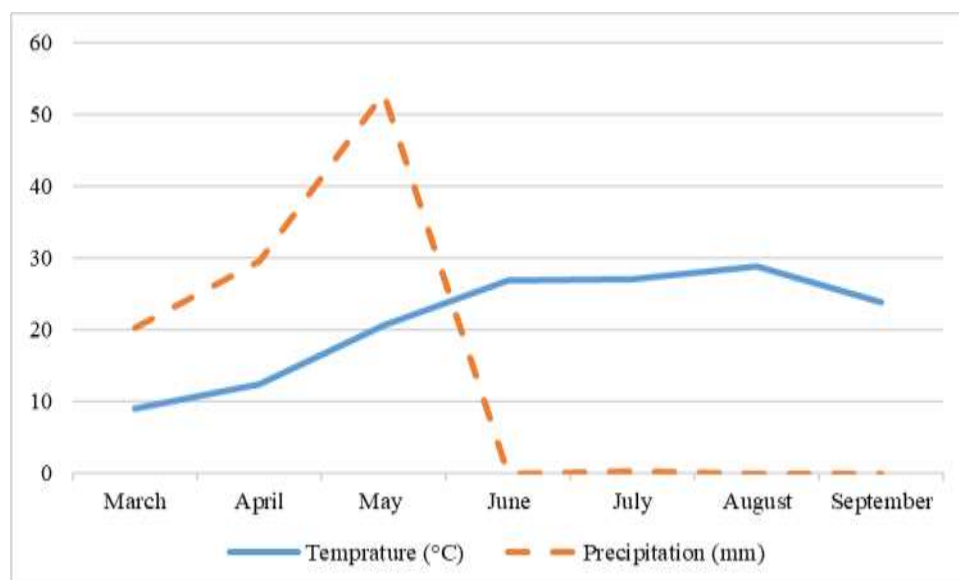


Fig. 1. The monthly mean temperature and precipitation during the study period in Karaj.

After pollen adhesion on the stigmatic surface, the pollen grains germinate. During growth, the pollen tubes compete for space and nutrients and only a genetically compatible pollen grain can reach the base of the ovary and penetrate the ovule (Čolić et al., 2010). In cross-pollinated almond flowers under orchard conditions, pollen tubes typically grow to the base of the style and enter the upper portion of the ovary within 96 to 120 h (Connel, 2000). Observation of pollen tube growth by fluorescence microscopy (Fig. 3) showed germinated pollen grains on the stigma, as well as pollen tubes in the different levels of style. A reduction in the number of pollen tubes from the stigma to the base of the style was noticeable (Fig. 3a & b). The growth rate of the Shekofeh pollen tube was faster than the other two, reaching to the style base 96 h after cross-pollination, while Tuono and H penetrated 120 h later (Fig. 4). Furthermore, the growth of Tuono and H pollen tubes were slow in the upper third of the style, but then increased to the base of the style, which is probably due to the higher density and the competition of the pollen tubes in the upper part of the style.

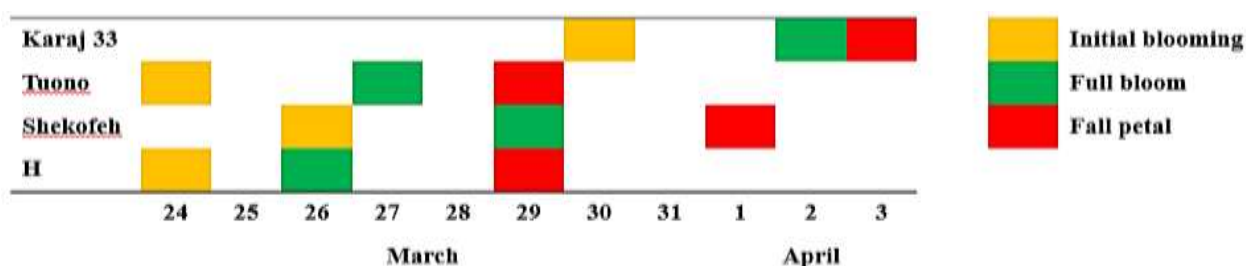


Fig. 2. The flowering period of almond cultivars/genotypes.

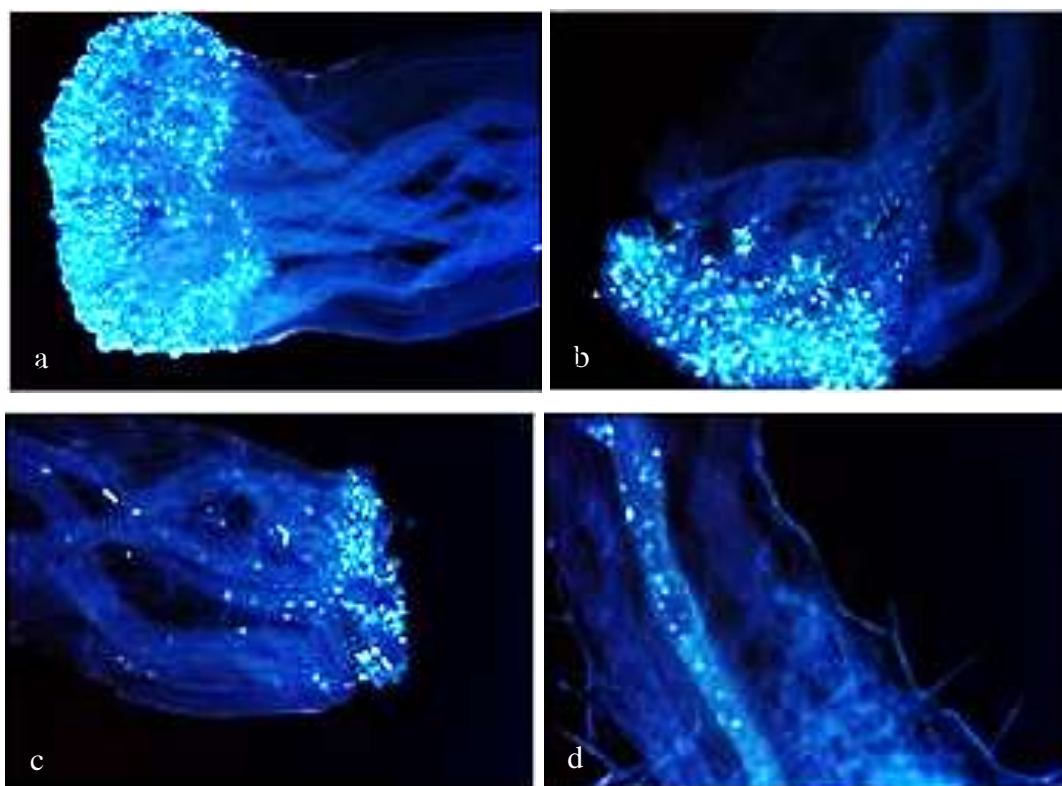


Fig. 3. Germination of pollen grains on stigma (a); pollen tubes reached the upper (b), middle (c) and base (d) of Karaj 33 style.

Table 2. Fruit set of pollinated Karaj 33 with studied cultivars/genotype of almond

Pollinizer	Fruit set after 20 days (%)	Fruit set after 90 days (%)	Fruit set at ripening time (%)
Open pollination	90.0 a	55.4 a	28.3 b
Tuono	84.5 b	50.5 b	22.6 c
Shekofeh	89.1 a	53.4 a	32.8 a
H	78.7 c	33.3 c	27.7 b

Means with at least one common letter in each column do not differ significantly according to Duncan's Multiple Range Test at $P \leq 0.05$.

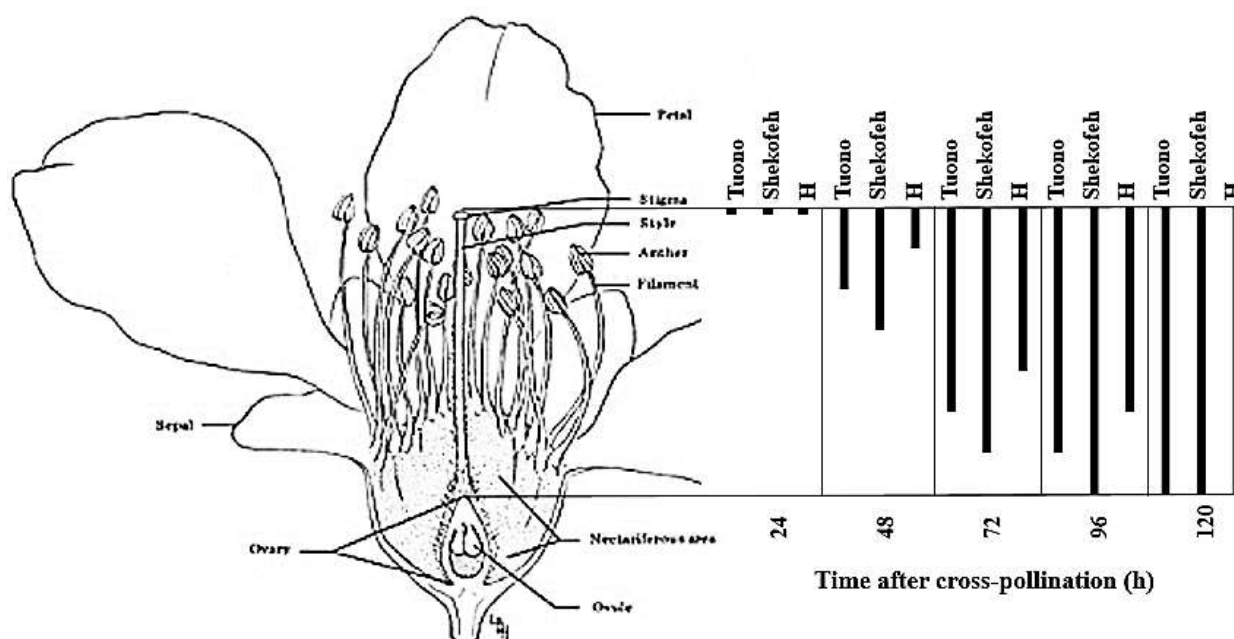


Fig. 4. Growth of pollen tube (thick line) in style of Karaj 33 cross-pollinated with Tuono, Shekofeh and H during 24 to 120 h after pollination.

CONCLUSION

Based on the present study, cross-pollination in Karaj 33 is critical and necessary for producing adequate fruit set. Cross-(in) compatibility studies through fruit set and fluorescence microscopy methods confirmed the compatibility of all three selected cultivars/genotype. However, due to the overlap of Shekofeh flowering time with Karaj 33 and high final fruit set, it can be introduced as a suitable pollinizer for Karaj 33.

Conflict of interest

The authors have no conflict of interest to report.

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