Journal of Plant Physiology and Breeding
2023, 13(2): 169-179 ISSN: 2008-5168

**Research Paper**

# Effects of pollen source on pollen tube dynamics and fruit set in two almond promising genotypes

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Received: May 24, 2023 Accepted: December 4, 2023
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**Abstract**
In this study, a pollination experiment on almond species was conducted about the pollen tube penetration in the pistil with a fluorescence microscope and the rate of fruit formation by the controlled pollination in the orchard. These species included two new almond genotypes of KD 13 and K11-8 as pollen receptors, and two almond cultivars Tuono (self-compatible) and D5 (self-incompatible), a peach cultivar HolouYazdi, and GN15 (hybrid between almond and peach) as donor pollen sources. This experiment was conducted in an almond orchard with six-year-old trees at Kamal-Shahr Horticulture Research Station in Karaj, Iran. The results showed a significant difference among pollen sources for in vitro pollen grain germination. The pollen germination percentage ranged from 45 to 86% in the cultivars and genotypes under study. There were significant differences among crosses for the rate of pollen tube penetration in different parts of the style. KD13 × D5 (self-incompatible) with 9.6% and K11-8 × D5 (self-incompatible) with 7.63% showed the highest percentage of pollen tube penetration but KD13 × Tuono and K11-8 × Tuono with 1% and 2.12%, respectively, had the lowest pollen tube insertion into the style. Ninety days after pollination, the crosses of KD13 × KD5 and K11-8 × D5 showed the highest percentage of fruit set (48.90 and 42.45%, respectively) as compared to other pollinizers. It was determined that the fruit formation was mainly influenced by the pollen source and therefore the source of pollen had a decisive role in the final fruit set. As a result, it is important to choose the right pollen source in the breeding programs before recommending the cultivation of new almond varieties.

**Keywords:** almond, fertilization, hybrid, *Prunus dulcis*, self-incompatibility

**How to cite:** Imani A. 2023. Effects of pollen source on pollen tube dynamics and fruit set in two almond promising genotypes. J Plant Physiol and Breed. 13(2): 169-179.

## Introduction

Almond (*Prunus dulcis*) is native to Central and Western Asia, especially Iran. This species is one of the most important nuts that grow worldwide in hot and dry Mediterranean climates (Casas-Agustench *et al.* 2011; Socias i Company and Gradziel 2017).

The growing and trading of almonds is important due to its high nutritional value and use as a raw material in many sectors, including food, cosmetic, and pharmaceutical industries (Taş and Gökmen 2017; Socias i Company and Gradziel 2017).

Most commercial almond cultivars are self-incompatible and should be used in orchard establishment with cultivars that have

pollination compatibility and overlapping flowering time (Dicenta *et al.* 2002; Rasouli and Imani 2016). For this reason, in addition to determining the pollination compatibility of cultivars before establishing the orchard, planting at least two compatible pollinizer cultivars and overlapping the flowering time with the main cultivar in the orchard is of great importance to obtain nuts with higher quantity and quality (Martinez Gomez *et al.* 2003; Fallah *et al.* 2016).

Adequate knowledge of compatibility between pollen and pistil of different fruit tree cultivars is one of the most important aspects in fruiting. which from a fruit-growing point of view is also of special importance in the selection of compatible sources of pollen for new cultivars introduced from breeding programs (Dicenta *et al.* 2002; Rasouli *et al*. 2021). Researchers have determined the compatibility of pollen with pistil by various methods such as field-controlled pollination and calculation of fruiting percentage, laboratory-controlled pollination, and then, have tracked pollen tube growth by fluorescent microscopy, electrophoresis of style proteins, and different molecular markers (Gómez *et al.* 2019; Herrera *et al.* 2020; Gómez *et al.* 2022).

The most common method for evaluating the compatibility of pollen with pistil is controlled pollination in the field and calculating the percentage of fruit. This method has low accuracy due to the effect of the environment. Conversely, having controlled conditions in the greenhouse for temperature, humidity, and photoperiod, and also by tracking the penetration of the pollen tube into the style using a fluorescent microscope, the compatibility of pollen with pistil in different cultivars can be determined. This method is less affected by the environmental conditions, and, therefore, is more accurate than the field conditions and requires a shorter period (Sharafi *et al.* 2010). However, the use of both methods in assessing the compatibility of pollination can be much more useful than using either of these methods alone. Rasouli *et al.* (2009) by investigating the effect of pollen of Shahroud 21, Shahroud 12, Fragiulio, 10-4, 5-11, and Supernova cultivars on the Supernova cultivar reported that all cultivars are compatible with the Supernova cultivar. The effect of pollinizers on the percentage of fruit formation 46 days and 103 days after flowering was significantly different. The lowest percentage of fruit formation was related to the self-pollinated flowers of Supernova.

So far, there have been no reports on the intra-species and inter-species pollination compatibility of almonds. Therefore, the pollination compatibility of some self-incompatible cultivars of almond with self-compatible cultivars and cultivars of other species, and a hybrid of almond and peach was investigated to make a recommendation for

establishing orchards and using the results of this experiment in future almond breeding programs.

## Material and Methods

### *Plant materials*

The plant material included two new almond genotypes of KD-13 and K11-8 (selections from the native almond germplasm of Iran) as pollen receptors, and two almond cultivars Tuono (self-compatible) and D5 (self-incompatible)), a Yazdi peach cultivar, and GN15 (a hybrid between almond and peach) as donor pollen sources.

### *Selection of pollinizers*

At the bud swelling stage and a few days before the opening of flowers, the branches of the mother trees, which had sufficient flower buds, were selected as pollen recipients. To prevent the interference of unwanted pollen in the flowers of the branches to be pollinated, the branches were covered with muslin bags before the opening of flowers and after emasculating and pollination.

After collecting the pollen of the pollinizers at the time the stigma of the mother plants was ready to receive the pollens (surface secretions of the stigma), they were pollinated with a special pollinator brush. This research work was done in the form of a randomized complete block design with three replications in two different trials.

### *Field and laboratory sampling*

The pollen germination test was performed in petri dishes before using pollen grains collected from the varieties to ensure the viability of collected pollen grains. For this purpose, a culture medium containing 10% sucrose, 100 ppm boric acid, and 2% agar was used. To prevent pathogens, the Petri dishes were closed with parafilm and transferred to the growth chamber at a temperature of 24 °C. After 12 hours, circles were randomly drawn on the petri dishes and the number of germinated pollen grains was counted by a light microscope with a magnification of 40X. The following formula was used to determine the viability of pollen:

$$\text{Percentage of pollen germination} = \frac{\text{Number of germinated pollen}}{\text{Total number of pollen grains}} \times 100$$

To measure the length of pollen tubes and to identify compatible and incompatible cultivars, every 24 hours after pollination of the female flowers (10 flowers for each treatment), their pistils were prepared and fixed in the fixative solution (10:7:2:1 ethanol (95%): H2O: formalin: acetic acid) and stored at 4 °C for 24 h before softening of the tissue (Socias I Company 1990). In the laboratory, the pistils were removed from the fixative and washed three times with distilled water for 45 minutes. Then, the pistils were placed in sodium sulfite and transferred to the incubator to observe the change from hard to soft tissue. After that, they were washed five times with

distilled water and kept in the refrigerator for 24 hours in a 1% aniline blue solution for staining. After taking out the pistils from aniline blue, they were depilated with tweezers and a scalpel, and then, mounted on the slides to observe the growth of pollen tubes by a fluorescent microscope (Axio Imager 373, Qimaging Color camera, Zeiss, Germany). Scoring included the number of germinated pollen grains on the stigma and the number of pollen tubes grown down the style to the ovary (Henselek *et al.* 2018).

Also, to determine the percentage of fruit set, the number of falling pollinated flowers was recorded three times after pollination (after petal falling at the end of June and mid-August).

Statistical analysis was performed by the SAS, version 9.1, and the graphs were drawn by Minitab 16 and Excel 2010 software.

## Results

The results of pollen germination in vitro showed a significant difference among six studied cultivars and genotypes at the 1% probability level (Table 1). The percentage of pollen grain germination varied from 45 to 86%. The highest pollen germination percentage belonged to the genotype D5 (86%) and the lowest value belonged to the Tuono cultivar (45%) (Figure 1).

Table 1. Analysis of variance of pollen grain germination of almond genotypes.

| Sources of variation | df | Mean squares |
|---|---|---|
| Genotype | 5 | 791.50** |
| Error | 12 | 6.45 |
| CV (%) | 3.56 | |

**: Significant at the 1% probability level

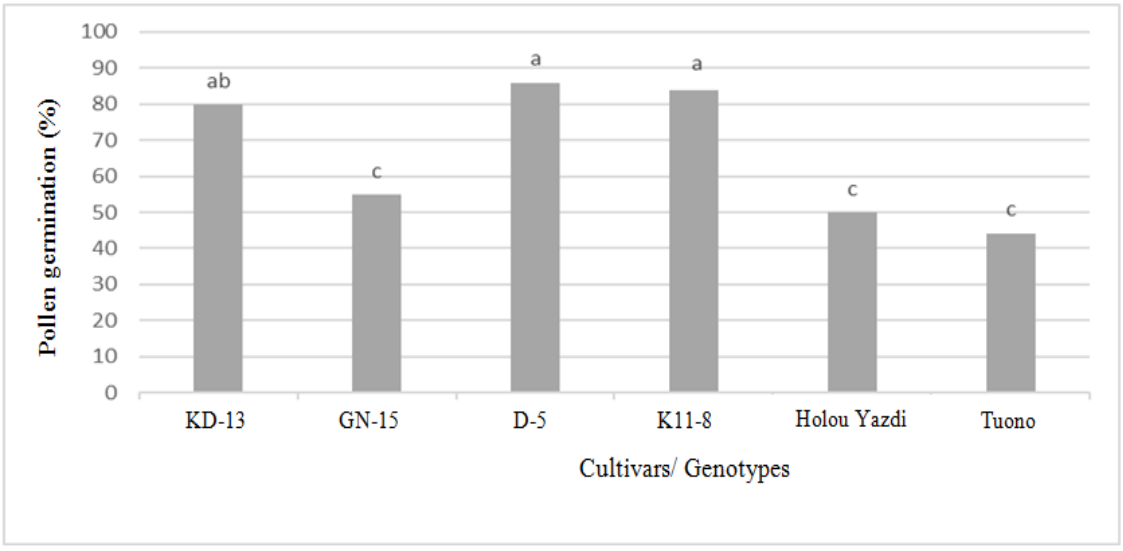


Figure 1. Pollen germination of almond genotypes; The genotypes with different letters are significantly different at the 1% probability level.

***Pollen tube growth***

The position of the pollen tube in different parts of the pistil is shown in Figure 2. Based on the analysis of variance, there was a significant difference among the pollinizer genotypes under study in terms of pollen tube penetration into the pistil of K11-8 and KD13 almonds (Tables 2 and 3). The comparison of the average percentage of pollen tube inserted into the base of style showed that the D5 (self-incompatible) × K11-8 with 7.63% (Table 4) and D5 (self-incompatible) × KD13 with 9.6% (Table 5) had the highest percentage of pollen tube penetration, while average percentage of pollen tube inserted into the base of style in the Tuono × K11-8 and Tuono × KD13 crosses was 2.12% and 1%, respectively (Tables 4 and 5). This indicates that genotype D5 has a high potential as a pollinizer of the pollen receptor cultivar.

Comparing the pollen receptor cultivars (Tables 4 and 5) indicated that the behavior of different pollen tubes in the base of the style of K11-8 and KD13 pollen receptors were almost similar. In both pollen recipient cultivars, pollen tube penetration was low in the Tuono cultivar, and it was the only pollinator that did not continue pollen tube growth at 48 hours to the middle of the style (data not shown).

There was a significant difference among pollinizers in terms of fruit formation in the K11-8 and KD13 receptors (Tables 6 and 7). So, 90 days after pollination, the crossing of D5 with K11-8 and KD13 had the highest percentage of fruit set (42.45 and 48.90%, respectively) as compared to other pollinizers, (Tables 8 and 9).

Table 2. Analysis of variance of pollen tube penetration of various pollinizers in different parts of the pistil of K11-8 almond.

| SOV | df | Mean squares | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Stigma | | | | The top third of the style | | | |
| | | 24 | 48 | 72 | 96 | 24 | 48 | 72 | 96 |
| Pollinizer | 4 | 190** | 92** | 722** | 640** | 115** | 1498** | 247** | 658** |
| Error | 10 | 2.34 | 2.52 | 3.18 | 4.81 | 0.95 | 1.11 | 1.8 | 30.78 |
| CV (%) | | 2.57 | 2.23 | 2.84 | 3.16 | 17.97 | 4.18 | 3.96 | 13.11 |

**: Significant at the 1% probability level

Table 2 continued

| SOV | df | Mean squares | | | |
|---|---|---|---|---|---|
| | | Middle of the style | | Style base | |
| | | 72 | 96 | 72 | 96 |
| Pollinizer | 4 | 2.8** | 18.6** | 0.96** | 24** |
| Error | 10 | 0.03 | 0.31 | 0.01 | 0.19 |
| CV (%) | | 21.30 | 5.23 | 16.75 | 13.87 |

**: Significant at the 1% probability level

Table 3. Analysis of variance of pollen tube penetration of various pollinizers in different parts of the pistil of KD13 almond.

| SOV | df | Mean squares | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Stigma | | | | Top third of the style | | | |
| | | 24 | 48 | 72 | 96 | 24 | 48 | 72 | 96 |
| Pollinizer | 4 | 915** | 791** | 135** | 65** | 218** | 368** | 137** | 139** |
| Error | 10 | 0.37 | 0.37 | 0.59 | 0.33 | .07 | 0.42 | 0.85 | 30.78 |
| CV (%) | | 0.91 | 0.90 | 1.11 | 0.77 | 6.85 | 1.19 | 1.58 | 1.28 |

**: Significant at the 1% probability level, respectively.

Table 3 continued

| SOV | df | Mean squares | | | |
|---|---|---|---|---|---|
| | | Middle of the style | | Style base | |
| | | 72 | 96 | 72 | 96 |
| Pollinizer | 4 | 92** | 303** | 2.56** | 42** |
| Error | 10 | 0.71 | .24 | .001 | 0.09 |
| CV (%) | | 4.82 | 2.91 | 6.25 | 8.42 |

**: Significant at the 1% probability level, respectively.

Table 4. Percentage of pollen tube penetration of different pollinizers in different parts of the pistil of K11-8 almond.

| Pollinizer | Stigma | | | | The top third of the style | | | | Middle of the style | | Style base | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 24+ | 48 | 72 | 96 | 24 | 48 | 72 | 96 | 72 | 96 | 72 | 96 |
| K11-8(Self-pollination) (♂) | 56.91c++ | 76.89a | 51.85d | 73.37b | 13.30a | 51.46a | 46.22d | 66.30a | 0.65c | 10.47b | 0.00a | 0.00d |
| D5 (self-incompatible (♂) | 70.23a | 75.37a | 81.30a | 85.11a | 10.65b | 45.86b | 40.36c | 34.20c | 1.29b | 14.49a | 1.26a | 7.63a |
| HolouYazdi (♂) | 64.77b | 63.22d | 71.10b | 75.63b | 0.00d | 16.20c | 30.11b | 45.50b | 0.00d | 9.05bd | 0.00a | 3.74b |
| GN15(♂) | 50.52d | 71.43b | 67.22c | 66.22c | 0.00d | 0.00e | 24.56a | 27.98d | 0.00d | 7.94d | 0.00a | 2.28c |
| 'Tuono (Self-incompatible) (♂) | 54.91c | 68.22c | 42.5e | 46.11d | 3.10c | 12.30d | 28.11b | 37.63c | 2.26a | 11.05b | 0.00a | 2.12c |

+Hours; ++Mean values in each column with different letters are significantly different at the 5% level of probability.

Table 5. Percentage of pollen tube penetration of different pollinizers in different parts of the pistil of KD13 almond.

| Pollinizer | Stigma | | | | The top third of the style | | | | Middle style | | Style base | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 24+ | 48 | 72 | 96 | 24 | 48 | 72 | 96 | 72 | 96 | 72 | 96 |
| KD-13 ( Self-pollination)(♂) | 87.6a++ | 86.8a | 59.7d | 78.3a | 0.23b | 36.2a | 54.7b | 49.5bc | 22.4 a | 7.8c | 0.0b | 0.0d |
| D5 (Self-incompatible)(♂) | 84.0b | 79.7b | 72.1b | 78.8a | .19.11a | 62.6a | 66.2a | 60.6a | 21.3ab | 34.1a | 2.1a | 9.6a |
| HolouYazdi (♂) | 57.0c | 67.5c | 64.4c | 67.7c | 0.00c | 63.2a | 65.3a | 49.7b | 20.2b | 12.8b | 0.0b | 3.3b |
| GN15 (♂) | 55.5d | 56.8d | 75.7a | 77.8a | 0.00c | 53.8c | 51.3c | 48.3c | 13.8c | 14.2b | 0.0b | 3.7b |
| Tuono (Self-incompatible) (♂) | 50.5e | 47.1e | 73.2b | 74.2b | 0.00c | 58.4b | 55.3b | 41.6d | 9.6d | 15.6b | 0.0b | 1.0c |

+Hours; ++ Mean values in each column with different letters are significantly different at the 5% level of probability.

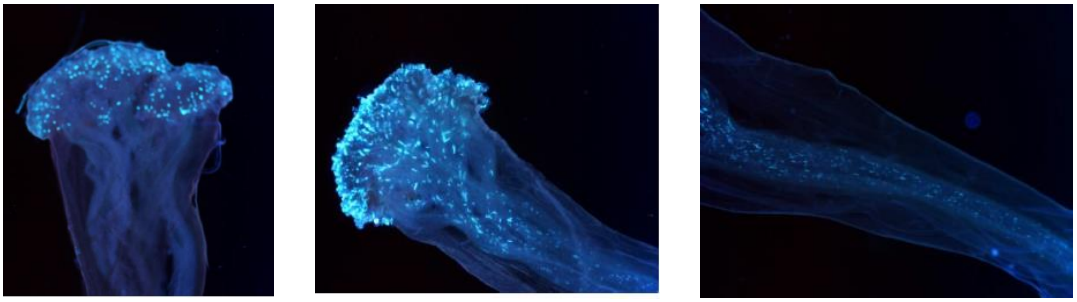

Figure 2. Pollen tube positions in different parts of the pistil: at the surface of the stigma 24 hours after pollination (right), in the middle of the style 48 hours after pollination (middle), in the base of the style 96 hours after pollination (left)

Table 6. Analysis of variance of fruit set in the K11-8 almond.

| SOV | df | Mean squares | | |
|---|---|---|---|---|
| | | The percentage of fruit formation 15 days after pollination | The percentage of fruit formation 45 days after pollination | The percentage of fruit formation 90 days after pollination |
| Pollinizer | 4 | 806.1** | 1613.0** | 578.6** |
| Error | 10 | 0.38 | 0.50 | 4.69 |
| CV(%) | | 2.19 | 1.64 | 3.37 |

**: Significant at the 1% probability level

Table 7. Analysis of variance of fruit set in the KD-13 almond.

| SOV | df | MS of fruit set | | |
|---|---|---|---|---|
| | | The percentage of fruit formation 15 days after pollination | The percentage of fruit formation 45 days after pollination | The percentage of fruit formation 90 days after pollination |
| Pollinizer | 4 | 602.0** | 1680.5** | 945.6** |
| Error | 10 | 1.29 | 1.28 | 0.97 |
| CV(%) | | 1.75 | 2.94 | 3.24 |

**: Significant at the 1% probability level

Table 8. Effects of different pollen sources on fruit set in the K11-8 almond.

| Pollinizer | The percentage of fruit formation 15 days after pollination | The percentage of fruit formation 45 days after pollination | The percentage of fruit formation 90 days after pollination |
|---|---|---|---|
| K11-8 (Self-pollination) (♂) | 47.00d | 2.03d | 0.46d |
| D5 (Self-incompatible (♂) | 81.67a | 60.73a | 42.45a |
| HolouYazdi (♂) | 53.78d | 50.45b | 36.45b |
| GN15 (♂) | 72.98b | 54.45b | 37.34b |
| Tuono (Self-incompatible) (♂) | 69.48c | 45.97c | 27.67c |

Mean values in each column with different letters are significantly different at the 5% level of probability.

Table 9. Effects of different pollen sources on fruit set in the KD-13 almond.

| Pollinizer | The percentage of fruit formation 15 days after pollination | The percentage of fruit formation 45 days after pollination | The percentage of fruit formation 90 days after pollination |
|---|---|---|---|
| KD-13 (Self-pollination) (♂) | 50.45c | 2.03d | 1.04d |
| D5 (Self-incompatible(♂) | 89.08a | 67.61a | 48.90a |
| HolouYazdi (♂) | 65.41b | 45.78b | 39.34b |
| GN15 (♂) | 66.09b | 41.45b | 35.61b |
| Tuono (Self-incompatible) (♂) | 55.34c | 35.87c | 29.16c |

Mean values in each column with different letters are significantly different at the 5% level of probability.

## Discussion

Pollen germination of the pollinizer genotypes used in the present study was high, so if the maternal parents are compatible with the paternal parents, it can be expected that a good number of pollen tubes reach the base of the style. In fruit trees, the percentage of germination of pollen grains is variable and the appropriate medium for each species or cultivar should be provided separately (Vezvaei and Jackson 1995; Imani and Talaie 1998). Radović *et al.* (2016) reported a range of pollen germination from 23.56% (Ferragnes) to 51.81% (Tuono) in almond

cultivars, which was lower compared to the results obtained by Sharafi *et al.* (2010) who reported a range of 35 to 82% in the almond cultivars grown in Iran. Pollen germination in our study was similar to the results obtained by Sharafi *et al.* (2010). These differences may be due to genetic, climatic, and nutritional variables (Radović *et al.* 2016; Socias I company and Gradziel 2017).

The rate of pollen tube movement within the style of different almond cultivars is also variable. Research shows that environmental factors such as wind, rain, and low temperature can slow down the growth of the pollen tube in the style and delay or hamper the arrival of the pollen tube to the ovary (Rasouli and Imani 2016). Temperature and especially genetic differences also affect the speed of pollen tube penetration in the style (Socias i Company *et al.* 1976).

According to reports, pollination failure in almonds interacts with the unavailability of water and nutrients. Between the pollen tube growth and the time when the ovule starts to swell, fruit development lack of success can occur. For example, the poor genetic quality of pollen or pollen limitation have been shown to constrain seed production (Socias i Company and Gradziel 2017).

The use of pollinizers and pollen receptor cultivars with simultaneous flowering and an effective pollination period significantly increases the yield of almonds. Therefore, investigating the pollination needs of almond cultivars from different aspects before planting is very important and can play an important role in increasing almond yield (Radović *et al.* 2016; Socias i Company and Gradziel 2017) .

Results showed that in the crosses with a higher rate of pollen tube penetration into the base of style, the percentage of fruit set was also higher. This result was consistent with the results of other researchers (Ortega *et al.* 2002; Socias i Company and Gradziel 2017) .

In the crosses where Tuono was the paternal parent, the lowest percentage of pollen tube penetration into the ovary and the lowest percentage of fruit formation were observed. One of the most important problems in the almond cultivation is fruit loss. There are many factors such as the amount and quality of water, soil quality, pruning, fertilizer, and other environmental conditions before and after flowering, cause flower and fruit drop in almonds and ultimately affect the yield (Socias i Company and Gradziel 2017). Due to the similarity of these factors for all pollinizers in our study, the effective factors in the fruiting of the almond genotypes can be pollen compatibility and ovule fertilization (Ortega and Dicenta 2004). According to Ortega *et al.* (2006), some selections from among 26 self-compatible almond genotypes and two varieties (Lauranne and Marta) showed different reactions in some fruit traits after cross-pollination or self-pollination.

For successful pollination and pollen tube growth, fertilization is necessary for sexual reproduction (Vezvaei and Jackson 1995). According to our results, a significant difference was found between the fruits formed in different crosses, also the percentage of fruit drop in the crosses was different. Fruit dropping can be due to embryo underdevelopment or fruit competition for nutrients. Therefore, choosing a suitable pollen source in almond cultivation is important and recommended (Alonos and Socias i Company 2005)

Since in the crosses of KD13 × D5 and K11-8 × D5, the time when the pollen tube enters the ovary was lower and their effective pollination period was longer than other crosses, these crosses can be used in future breeding programs and in the almond orchards in the areas where the effective pollination period is low due to the adverse environmental conditions such as cold (by reducing the pollen tube growth rate), and warm (by reducing the longevity of the eggs).

## Conclusion

In conclusion, D5 as the best pollinizer can be used in KD13 and K11-8 almond orchards to obtain the maximum yield.

## Acknowledgments

The author would like to thank the Iran National Science Foundation (INF) for financial support and the Temperate Fruit Research Center (TFRC), Iran, for their assistance in conducting this research.

## Conflict of interest

The author declares no conflict of interest with any organization regarding the subject of the manuscript.

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# تأثیر منبع گرده بر دینامیک لوله گرده و تشکیل میوه در دو ژنوتیپ امیدبخش بادام

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**چکیده**

در این مطالعه، آزمایش گرده‌افشانی در گونه‌های بادام با بررسی رشد لوله گرده در مادگی با استفاده ازمیکروسکوپ فلورسانس و میزان تشکیل میوه در گرده‌افشانی کنترل‌شده در مزرعه انجام شد. این گونه‌ها شامل دو ژنوتیپ جدید بادام KD13 و K11-8 به عنوان گیرنده گرده و دو رقم بادام خودسازگار (Tuono) و خود ناسازگار (D5)، یک رقم هلو به نام یزدی و GN15 (هیبرید بین بادام و هلو) به عنوان گرده دهنده بودند. این آزمایش در باغ بادام با درختان شش ساله در ایستگاه تحقیقات باغبانی کمال شهر کرج انجام شد. نتایج حاصل از جوانه زنی دانه گرده در شرایط آزمایشگاهی تفاوت معنی‌داری را بین منابع گرده دهنده نشان داد. میانگین درصد جوانه زنی گرده در ارقام و ژنوتیپ‌ها بین ۴۵ تا ۸۶ درصد متغیر بود. بین تلاقی‌های مختلف، از نظر میزان نفوذ لوله گرده در قسمت‌های مختلف خامه گل تفاوت معنی‌داری دیده شد. نتایج نشان داد که تلاقی D5 (خود ناسازگار) × KD13 با ۹٫۶٪ درصد و D5 (خود ناسازگار) × K11-8 با ۷٫۶۳٪ بیشترین درصد نفوذ لوله گرده به پایه خامه را داشتند، در حالی که درصد متوسط لوله گرده وارد شده به پایه خامه گل در تلاقی KD13 × Tuono و K11-8 × Tuono به ترتیب ۱ و ۲٫۱۲ درصد بود. نود روز پس از گرده افشانی، تلاقی‌های KD13 × D5 و K11-8 × D5 بیشترین درصد تشکیل میوه (به ترتیب ۴۸٫۹۰ و ۴۲٫۴۵ درصد) را نسبت به سایر گرده دهنده‌ها نشان دادند. در این تحقیق مشخص شد که تشکیل میوه عمدتاً تحت تأثیر نوع گرده است و بنابراین منبع گرده نقش تعیین کننده‌ای در تشکیل میوه نهایی دارد. در نتیجه، انتخاب منبع گرده مناسب در برنامه‌های اصلاحی پیش ازتوصیه کشت ارقام جدید بادام حائز اهمیت می‌باشد.

**واژه‌های کلیدی:** بادام، تلقیح، خود ناسازگاری، هیبرید