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**ORIGINAL ARTICLE**

# Evaluation of Xenia and Metaxenia in Pistachio (*Pistacia vera* L.) Following Pollination with Different Pollen Sources

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K E Y W O R D S

Dioecious;
Fruitset;
Metaxenia;
Pistachio;
Pollinizer;
Xenia

A B S T R A C T

Pistachio is a dioecious and cross-pollination species and suitable male genotypes is essential for its adequate fruit production. The quality of the produced nuts is also directly affected by pollen sources. In the present study, the female pistachio trees of Akbari commercial cultivar, in their ON-year cycle, were selected and hand-pollinated with three different pollen sources (T5, T17, T18) and compared to open-pollinated trees (T0). This research was undertaken as a completely randomized block design with three replications. Artificial pollination had a positive effect on the fruit set percentage. Furthermore, fruit fresh and dry weights, fruit dimensions, nut as well as kernel characteristics were affected corresponding to each pollen source. Though all 3 pollen sources had a positive effect on measured fruit characteristics, the effect of T17 was more prominent. Owing to xenia and metaxenia effects, longer nuts were observed following pollination with T5 pollen grains. Also, utilization of T17 pollen grains led to production of weightier fruits with higher splitting rate (93.33 % as compared to control, open pollinated trees (63.78%)). The results of the present study approved the imperative role of pollen source for pistachio fruit production. The results also recommended T17 genotype as a suitable pollinizer for Akbari pistachio cultivar.

## Introduction

Pistachio is a dioecious tree that is an economically important product (Eslami *et al*., 2019; Hosseini *et al*., 2022). The presence of sufficient male tree genotypes and appropriate pollination overlap is considered as important factors that directly affect commercial pistachio fruit production (Sharifkhah *et al.*, 2020). A suitable pollinizer tree for pistachio is of great importance and the characteristics of pollinizer

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genotypes have a great effect on the quantitative and qualitative characteristics of the nut and kernel of the pollinated female varieties (Ghareyazie *et al*., 1995; Kester and Gradziel, 1996; Hosseini *et al*., 2022). These traits as a final point, determine pistachio nut marketability. Such phenomena are called xenia and metaxenia which already reported in different types of fruit and nut trees (Golzari *et al.*, 2010). The term 'Xenia' was coined by Wilhem Olbers Focke in 1881, in an article entitled "Mixing Plants", and he expressed this phenomenon as the pollen-induced changes in mother tissues. Then in 1928, Swingle realized in his experiments that pollen grains cause changes in the shape, size, weight and ripening time of the date palm fruit. He called the effect of pollen grain type on the physical and chemical characteristics of date palm fruit as "metaxenia" (Swingle, 1928). However, the definitions of these two words, xenia and metaxenia, are frequently ambiguous and numerous xenia and metaxenia definitions directly contradict one another. There are numerous definitions of xenia provided by scientists and experts. In his studies, Swingle (1992) divided xenia and metaxenia on the basis of the effect on the synchronous tissues (embryo and endosperm) and maternal tissues. So, the effect of pollen grains on the physical and chemical characteristics of the fruit tissue was called metaxenia and the effect of pollen grains on the physical and chemical properties of the seed or fruit core, was called xenia.

The idea of xenia can be understood easily through the process of double fertilization, in which the male and female gametes combine to produce the diploid zygote, and the male and female gametes combine to form the endosperm. Consequently, xenia is the result of the genes from the male parent influencing the endosperm and embryonic development. The direct effect of pollen on the seed may be explained by double fertilization, however this does not account for pollen's effects on the fruit outside of the embryo and endosperm (Saini *et al*., 2022).

The simplest and most likely theory to explain metaxenia, according to Swingle (1928), is that the embryo or endosperm, or both, secrete hormones or soluble substances analogous to them. These substances diffuse out into the mother plant's tissues that constitute the seed and fruit and they exert a specific effect on these tissues, varying depending on the specific male parent used to fecundate the embryo and endosperm.

The impact of xenia on the production of fruits and seeds, as well as applications in plant breeding and increasing grain yield or fruit size and quality, is of significant agronomic importance (Pozzi *et al*., 2019). The effects of xenia and metaxenia in products such as pistachios can be attributed to the effect of different pollen sources on the total dry weight of nut and blankness (Afshari *et al*., 2017), nut diameter, sphericity, fruitset, blankness and nut falling percentage, splitting percentage (Alhajjar *et al*., 2015), length and dry weight of kernel and nut (Neyshaburi *et al*., 2021). In date palm, the effect of different pollen sources on ripening time, color, weight, size and other qualities of fruit (Shafique *et al*., 2011; Farag, 2012) were reported. Furthermore, increasing production, fruit quality and early fruit ripening (Omar *et al*., 2014), fruit set percentage, bunch weight, pulp weight, fruit moisture content and TSS content were already cited for date palm (Munir *et al.*, 2020). The average fruit weight, fruit biochemical characteristics and seed weight, number and protein content were reported in kiwifruit following pollination with different pollen grains (Stasiak *et al*., 2019). Similarly, in hazelnut, final fruit setting, kernel and nut weight, kernel percentage, number of blank nuts (Balik and Beyhan, 2019) were reported. Application of an aerial pollination system in a walnut orchard was nut successful that much (Mazinani *et al.*, 2023).

Therefore, in fruit crops, especially pistachio, that fruits and seeds are the main purpose of harvesting; the influence of pollen grains on the quantitative and qualitative characteristics of nuts would be

remarkable. Also, choosing the right pollen for pollination is of particular importance. The purpose of the present research is to investigate the effect of pollen source (three male genotypes) on the quantitative and qualitative characteristics of "Akbari" commercial pistachio cultivar.

## Materials and Methods

The female pistachio trees, Akbari commercial cultivar, from 36-year-old trees of the Agricultural Station, Rafsanjan Pistachio Research Institute, were randomly selected and properly labeled. The trees were in their ON-year cycle and had enough flower buds (about 4-5 flower buds per branch). The number of three fruitful branches, positioned in different directions, were selected in such a way that the flower buds were accessible for further workings. This research was implemented as a completely randomized block design with three replications. Each tree was considered as a replication and three branches were selected in each tree. The female cluster inflorescences were covered with a double-layer, pre-sterilized cloth bags (50x50 cm dimensions, sprayed with ethanol) one week before commencing pollination (Fig. 2). In order to prevent open pollination, cloth was fully stretched over the unopened buds of female flowers. Similarly, three uncovered branches also were labeled as open pollination (T0).

**Fig 1.** The pistachio male cluster, detached flowers and mixing pollen with wheat flour (top row). The properly mixed pollen: wheat flour (1:1) and injection inside the pockets (mid row). The pocket cloth was replaced with net and displaying the fruitset (bottom row).

**Fig 2.** The selection and preparation of flower clusters of Akbari pistachio for artificial pollination (top first row). The different stages of fruit growth following pollination (mid and bottom rows).

Pollen grains of the three male genotypes (T5, T17, T18) were selected from the pistachio collection of the same institute. These three genotypes were selected among 20 different male genotypes following a morphological, phenological and molecular evaluation program (Ranjbar Kabootarkhani *et al*., 2023). Furthermore, these were seed originated trees (chance seedling) and their average age was already recorded to be about 36 years. As soon as the male flower clusters were changed from red to green color, pollen grains were collected. The male clusters were harvested and kept on white paper at room temperature. Four to five hours later, the pollen grains of each cluster were collected. The pollen grains were stored in small plastic containers containing silica gel and were kept in the freezer at -4°C awaiting hand pollination.

When the stigma of most flowers was bright green and revealed a prominent and sticky surface, the hand pollination process was performed. A mixture of wheat flour and pollen (1:1) (Fig.1) was used to inject pollen grains into the bags. The hand pollination process was done four times, with one to two day intervals (according to weather conditions), so that the first and fourth injections were undertaken in a six days' period. In the first injection, 1.0 cc (0.5 g) of the mixture of flour-pollen was loaded in a medical syringe and then injected into each bag. As the all flowers in each cluster may not be opened at this time, the low amount of mixture was injected. In the second and third stages, 1.5 cc of pollen mixture was used. The amount of 1 cc of pollen mixture was also loaded and injected in the last stage. Therefore, the first stage of hand pollination was done on 19$^{th}$ April, and the next stages were undertaken on 21, 23 and 25$^{th}$ April (at 9:00 to 12:00 a.m.) The open pollinated branches were also considered as a control. One week after hand pollination, cloth bags were opened and were replaced with net bags (50 x 50 cm).

In order to determine the percentage of fruitset, three clusters were selected from each female tree in four different geographical directions, few days before pollination, and the total number of flowers per cluster was recorded. Then, 14 days after pollination, the developing nuts were counted to ascertain initial fruitset. The total number of nuts were again recorded just before harvest. As the total number of flowers in each cluster was already calculated, the percentage of

fruitset was calculated with the help of the following formula:

Initial fruitset (%) = (Total number of developing nuts 14 DAP / Total number of flowers) ×100

Stabilized fruitset (%) = (Total number of nuts before harvest / Total number of flowers) ×100

Final fruitset (%) = (stabilized fruitset /initial fruitset) ×100

To measure the length and width of nut, 10 nuts were randomly selected from each replication and their dimensions were measured in millimeters using a caliper, then the average size of a nut was calculated. Ten nuts were also randomly selected from each replication and then their color quality was measured through observation. These were scored as 1 (high greenness) to 5 (high purple or redness). Similarly, the fresh and dry weights were measured in both nut and kernel again in 10 fruits. Then the average fresh and dry weights of a nut and kernel was calculated. When the samples were completely dried (oven 55°C for 72 hours), their dry weights were measured and recorded. The percentage of splitting, non-splitting and blankness ratio were also measured in a batch of 10 randomly selected nuts per each replication.

## Results

### *The fruitset percentage*

According to the results of the present experiment, artificial pollination had a positive effect on the fruitset percentage. The fruitset observed in hand pollinated shoots was significantly higher than open pollinated ones. The amount of fruits produced following pollination with T17 genotype was found to be 13.16% higher than open pollinated shoots (Fig. 3).

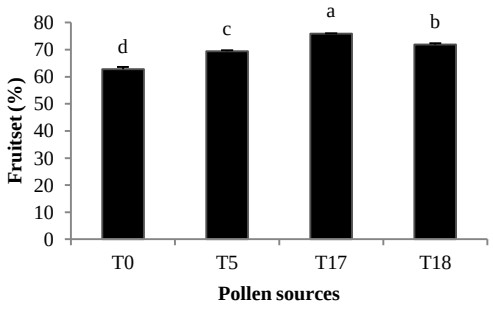


**Fig 3.** The effect of different pollen sources on the fruitset of "Akbari" pistachio cultivar.
(T0: Open pollination; T5, T17 and T18: Trees pollinated with T5, T17 and T18 pollinizers).

### *Fruit length and width*

The results revealed that artificial pollination increased the fruit length as compared to open pollination (control). No significant difference was observed in fruit length produced after pollination with either T5 or T18 pollen grains. However, the greatest metaxenia effect was observed when T5 pollen grains were used for pollination. The fruit length produced following pollination with T5 genotype increased by 19.21% as compared to the control open-pollinated trees (Fig. 4).

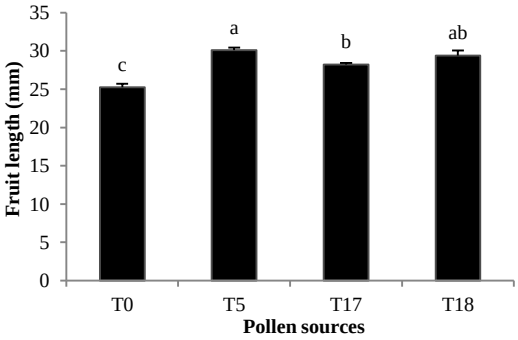


**Fig. 4.** The effect of different pollen sources on the fruit length of "Akbari" pistachio cultivar.
(T0: Open pollination; T5, T17 and T18: Trees pollinated with T5, T17 and T18 pollinizers)

Though pollination with T17 pollen grains led to production of wider fruits, but there was not any significant difference among pollen sources or open pollination for pistachio fruit width.

***Fruit color***

The fruit color is changed during different stages of fruit growth (Fig. 2). However, in our experiment, fruit color was not affected by the type of pollen grain. Even though pollination improved nut color of Akbari cultivar, it was not significantly different as compared to the open pollination (data not shown).

***Fruit fresh and dry weights***

The different pollen sources had noticeable effect on fruit fresh and dry weights. All three pollen sources produced significant fruit fresh and dry weights as compared to open pollination. However, the effect of pollen grains of T17 was more obvious. Hence, when the trees pollinated with T17, the fruit fresh and dry weights were increased by 19.63% and 20.20%, respectively, as compared to the control (Fig. 5).

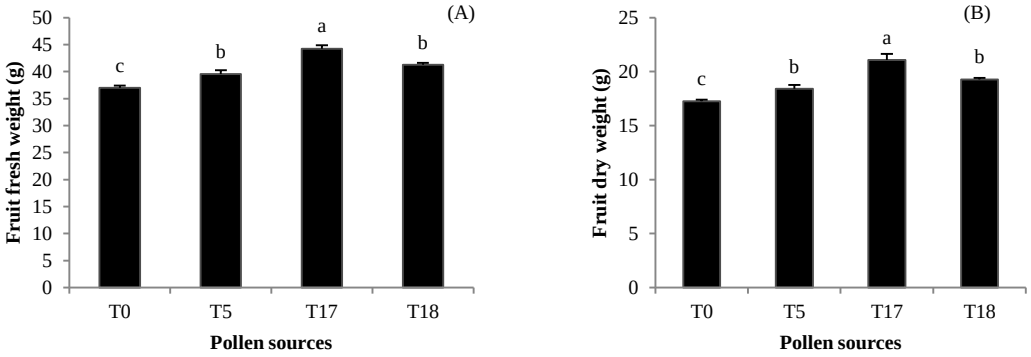


**Fig. 5.** The effect of different pollen sources on fruit fresh and dry weights of Akbari pistachio cultivar.
(T0: Open pollination; T5, T17 and T18: Trees pollinated with T5, T17 and T18 pollinizers).

***Nut fresh and dry weights***

The trend observed for changes in nut fresh and dry weights was similar to whole fruit. The different pollen sources had visible effect on nut fresh and dry weights. The influence of T17 pollens was more clear (Fig. 6).

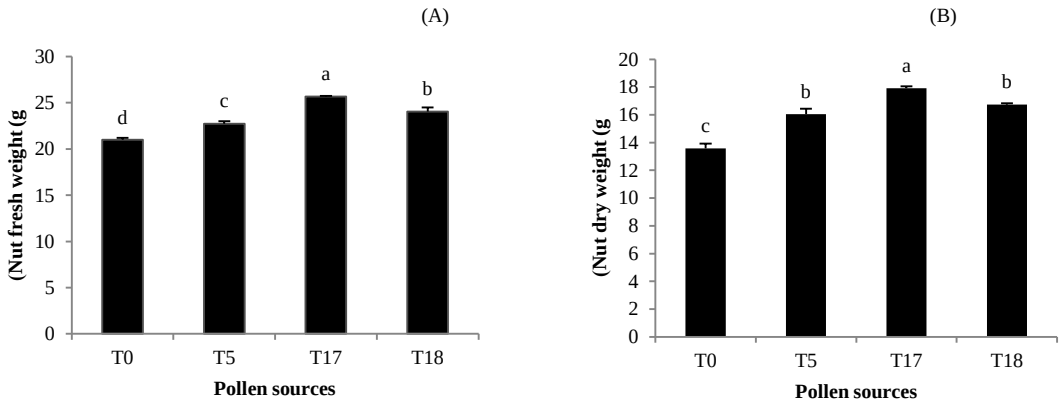


**Fig. 6.** The effect of different pollen sources on nut fresh and dry weights of Akbari pistachio cultivar.
(T0: Open pollination; T5, T17 and T18: Trees pollinated with T5, T17 and T18 pollinizers).

***Kernel fresh and dry weights***

The kernel weight was also affected by pollen type already used for pollination. The change in kernel fresh and dry weights was shown in Fig. 7. This xenia effect would be important from economical point of view. When T17 pollens were used for pollination, the kernel weight was significantly increased. (Fig. 6).

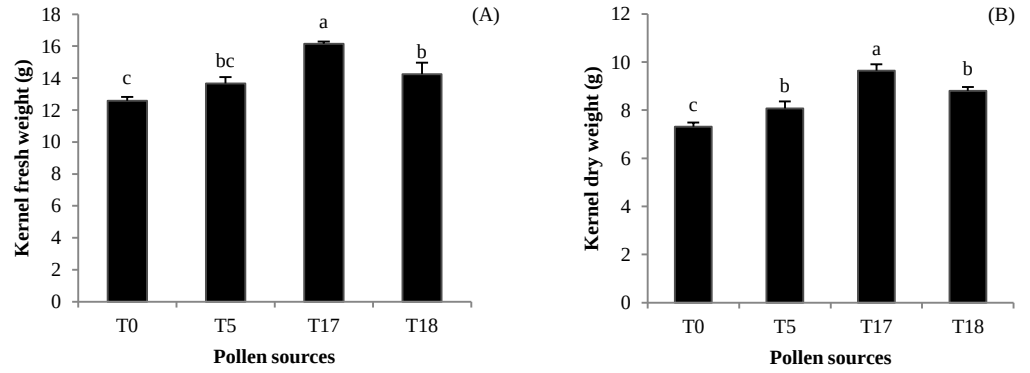


**Fig. 7.** The effect of different pollen sources on kernel fresh and dry weights of Akbari pistachio cultivar.
(T0: Open pollination; T5, T17 and T18: Trees pollinated with T5, T17 and T18 pollinizers).

***Nut Splitting percentage***

The pollen sources had direct effect on While the splitting was increased following pollination with all three pollen sources, but more split fruits (93.33%) were observed in trees pollinated with T17 pollen grains. In open pollinated trees, splitting rate was 63.78% only (Fig. 8). It means that whenever the T17 pollens were used, the nut splitting can be increased to about 29.25 % more, which is very important in commercial pistachio production.

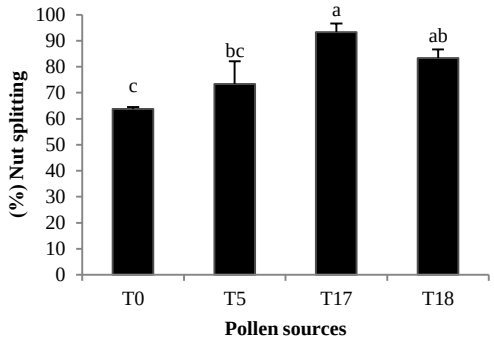


**Fig. 8.** The effect of different pollen sources on nut splitting rate in Akbari pistachio cultivar.
(T0: Open pollination; T5, T17 and T18: Trees pollinated with T5, T17 and T18 pollinizers).

***Non-split, closed nuts***

The trend for non-split nuts was exactly contrary to splitting rate. As it is clear in Fig. 9, only 6.67% of nuts produced by T17 pollinated trees were non-split. Conversely, 36.22% of nuts obtained from control trees had non-split nuts. It is noteworthy that non-split fruits are usually blank. However, in this experiment with Akbari cultivar, there was no significant difference between control trees and those pollinated with different pollen sources with respect to blankness percentage (data not shown).

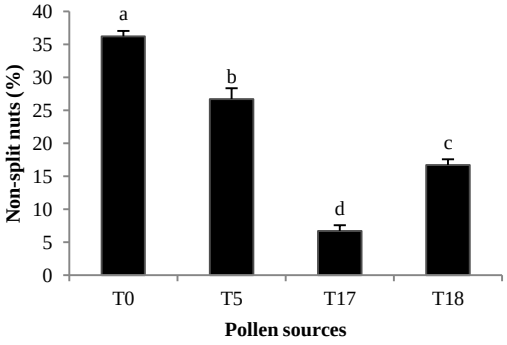


**Fig. 9.** The effect of different pollen sources on production of non-split closed shell nuts in Akbari pistachio cultivar.
(T0: Open pollination; T5, T17 and T18: Trees pollinated with T5, T17 and T18 pollinizers).

## Discussion

Pistachio is a dioecious and cross-pollination species. The pollinizers, male genotypes have a key and essential role on fruitset. The quality of the produced nuts is also directly affected by pollen sources.

***The fruitset percentage***

As it is clear in Fig. 3, the fruitset percentage was improved following pollination with different pollens. Hokmabadi and Awaz Abadian (2014) have also evaluated the influence of some pollinizers in pistachio. They observed that the highest number of fruits per cluster (31.83) was recorded when pollinizer number 6 was used for pollination. They also recorded the lowest number of fruits per cluster (9.16) following pollination with pollinizer number 1. Another research for manual pollination of the commercial cultivars of pistachio was previously undertaken by Kardoush *et al*. (2009). They pollinated Ashouri, Olaimi and Baturi cultivars using the pollen of the male genotypes of *P.vera* and also the pollen of some other species of *Pistacia* including *P.atlantica, P.palestina, P.khinjuk.* Their data has also clearly shown that the fruitset is definitely influenced by the pollen source. Their results showed that the

combination of *P. atlantica* (male) × Ashouri (female) created the highest percentage of fruitset (79.9%). So, it is obvious that the selection of pollen type has prominent effect on fruitset in commercial orchards. Hence, whenever the superior pollinizer genotypes were already determined in a region, their utilization would be a simple way to improve the pistachio yield. The degree of viability of pollen grains is considered as one of the most significant factors in successful pollination and fruit set in fruit crops (Munir *et al*., 2020). Insufficient viability and germination ability of some pollen grains may cause a low yield in trees (Romano, 2006). Working with date palm, it was demonstrated that application of pollen grains with high germination power in 'Barhi' and 'Piarom' cultivars can increase fruit production. Some males have higher fertility than others, and the difference in the fruit set percentage is due to the difference in the amount of pollen grain viability or the difference in the amount of mutual compatibility (Zargari *et al*., 2023). In a research, Bahmani *et al*. (2002) studied the effect of pollinizer parent on nut dimensions and some taste characteristics of almond kernels. Traits such as length, width, thickness, the weight of nut and kernel, and the ratio of weight of kernel to nut were measured. their results showed clear differences due to the change of parents. A significant difference was observed between pollinizer parents in terms of the effect on quantitative traits. However, no direct effect on the taste of the kernel in terms of bitterness or sweetness that can be recognized in terms of taste was observed on the maternal parent. The results of the research of Mustafa *et al*. (2014) showed that the physical characteristics of date fruit, including weight, volume, length and diameter of the fruit, as well as the biochemical characteristics of the fruit such as organic acid, soluble solids and total sugar are affected by the type of pollen. It seems that this effect is related to metaxenia. Although the reason for metaxenia and such effects of pollen grains on fruit traits is still not completely clear. Omar *et al*. (2014) investigated the effect of xenia in dates and reported that the length, diameter and weight of the kernel were affected by the type of pollen which is in agreement with the results of the present experiment.

According to Shafique *et al*. (2011), pollen increases the length and weight of the fruit by affecting the growth of the ovary and embryo. Neyshaburi *et al*. (2021) also reported the fruit and kernel length changes as affected by different pollen sources.

***Kernel fresh and dry weights***

The kernel weight was affected by pollen type in pistachio (Fig. 6). In the research of Ak (2010) utilization of *Pistacia* species as pollinizer was studied. He considered the pollen source to be important and effective on the kernel characteristics of "Siirt" and "Bilgen" cultivars. The results of the present study confirm the results of Ak (2010) as well as Kardoush *et al*. (2009). As pistachio is a dioecious species, hand pollination of female trees with pollen grains collected from other pistachio species is also feasible. However, there may be some unwanted xenia effects. For example, Gouta *et al*. (2002) investigated the effect of pollen from domestic species, *P. atlantica* and *P. terebintus* on two domestic female cultivars, Mateur and Gl-guitar, and concluded that for both cultivars, the exploitation of *P. atlantica* pollen was better.

***Nut Splitting percentage***

When the pistachio fruit ripens, it changes from green to an autumnal yellow/red and shell abruptly splits partly open. This is known as dehiscence, and happens with a clear pop. The splitting open is a trait that has been selected by humans and it is considered as a valuable commercial trait critical for nut marketability and is the first important characteristic in selecting new cultivars (Afshari and Hokmabadi, 2008; Tsantili *et al*., 2010; Panahi and Khezri, 2011). Splitting is influenced by the type of cultivar, pollinizer (Ozeker *et al*., 2005), water supply during kernel development (Kaska, 1990) and the blossoming

of the pericarp when the nut ripens (due to the enlargement of the green kernel and physical pressure on the pericarp) (Nazouri *et al*., 2013). Ferguson (2005) stated that there is always a positive correlation between the volume of the green kernel and splitting and the larger the diameter of the green kernel, the higher the percentage of splitting. The effects of pollen on the growth and development of pistachio nut are mostly related to the effect of metaxenia. The pollen sources have direct effect on splitting rate in pistachio (Ranjbar Kabootarkhani, 2023). In the present study, nut splitting, as an interesting metaxenia effect, was observed. Riazi *et al*. (1996) studied the effect of Xenia and Metaxenia in three commercial Iranian pistachio cultivars (Owhadi, Kalleh-ghuchi and Mumtaz). They reported that the pollen of *P. mutica* and *P. atlantica* species reduced the growth of kernels and the percentage of splitting. While the pollen of domesticated cultivars *i.e.* Momtaz and Soltani increased the size and number of split nuts.

***Non-split nuts***

Non-split nut is also one of the unwanted traits in pistachio nut, which reduces its economic value. Among the important factors that play an effective role in Non-split shells are cultivar, type of pollen, high yield, inappropriate harvest time, tree age, biennial bearing, climatic conditions at the time of inoculation, irrigation stress and proper nutrition management during the period (Ranjbar Kabootarkhani, 2023). The blankness induced by pollen sources were previously reported by Alhajjar *et al*., 2015 as well as Afshari *et al*., 2017.

The xenia and metaxenia have been also studied in other fruit crops. Golzari *et al*. (2016 and 2010) reported xenia and metaxenia in persian walnut. In another research work undertaken by Balik and Beyhan (2019), in hazelnut, the effects of the pollinizer cultivars on the nut and kernel characteristics were studied. They utilized Tombul, Palaz, Çakıldak, Foşa, Allahverdi, Sivri, Kalınkara and Yassı Badem cultivars as pollen sources to cross Ombul, Palaz, Çakıldak, Foşa and Allahverdi cultivars. They observed that the pollinizer cultivars caused changes in some nut and kernel characteristics with a clear xenia and metaxenia effects. The effects of cross pollination on cluster set and number of nuts per clusters were quite striking. They also found that cross-pollination increased the percentage of kernels and their quality. Furthermore, they observed that the pollinizer cultivars may cause changes in nut shape, so whenever pollen grains of Palaz used for pollination, it reduced the index of nut shape in the pollinated cultivars. Ajamgard et al. (2017) also reported the effect of various pollinizers on pecans and concluded that pollination in pecan orchards is necessary for adequate production.

Similarly, Rasouli *et al*. (2008) has conducted an experiment with almond. They studied the compatibility and effect of supplemental pollination of Supernova variety with the pollen of different almond cultivars. The highest percentage of nut formation was achieved when Shahroud cultivar 21 was used as pollinizer. The lowest percentage of nut formation was related to the self-pollinated flowers of Supernova cultivar.

Working with date palm, another dioecious tree, the effect of different pollen sources on the quantitative and qualitative characteristics of Zahedi cultivar, an experiment was conducted with three pollinizer genotypes (M001, M002 and M003). The results showed that the length, diameter, weight of fruit and seed, pH, titratable acidity, soluble solids, total sugars and yield were affected by pollinizer genotype. It was found that the M003 male genotype improved the fruit traits and finally increased the yield as compared to the other male genotypes (Siahsar *et al*., 2018). Rahnama (2013) also investigated the effect of three pollen sources on the date of Majul cultivar. He stated that Verdi pollen causes a significant increase in fruitset and date yield, while the fruit quality parameters such as sugar, acidity and

soluble solids were not affected by this type of pollen. The positive effect of pollen on the percentage of fruit formation and yield of dates has been reported by other researchers (Shafique *et al*., 2011; Omaima *et al*., 2015).

The results of the present research, corroborated with the results of Riazi *et al*. (1996), Gouta *et al*. (2002), Abu-Zahra and Al-Abbadi (2007), Kardoush *et al*. (2009), AK (2010) and Hokmabadi and Awaz Abadian (2014) already reported in different pistachio cultivars and species. Furthermore, the xenia and metaxenia reported in the literature such as Balik and Beyhan (2019) on hazelnut, Rasouli *et al*. (2018) on almond, Rahnama (2013); Siahsar *et al*. (2018); Shafique *et al*. (2011) and Omaima *et al*. (2015) on date palms are closely corresponding to our results.

The results obtained from the present research confirm that the xenia and metaxenia phenomena exist in pistachios and the type of pollen grains can affect some physical and qualitative characteristics of pistachio nut. It was found that, pollen grains have a significant effect on the percentage of fruitset, fruit length, nut as well as kernel weights, nut shell splitting and non-split nuts. However, pollen sources had no significant effect on the width and color of nuts. Furthermore, it was found that the T17 pollinizer genotype had the greatest effect in fruitset, kernel and nut weights, the percentage of splitting nuts and production of low number of closed, non-split nuts. It is suggested that the T17 male genotype may be further analyzed for some other traits such as flowering synchrony with commercial female pistachios of Kerman province. Then, it may be cloned and released as a promising pollinizer genotype for pistachio commercial orchards.

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**Conflict of interests**

There is no conflict of interest among authors.

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