International Journal of Horticultural Science and Technology
Journal homepage: https://ijhst.ut.ac.ir

Evaluation of Pollen Incompatibility in Iranian Almond Cultivars using Classical and Molecular Methods

Mousa Rasouli[1]*, Ali Jafari -Taemeh[2], Mostafa Rahmati-Joneidabad[2], Zeynab Koulivand[3], Pedro Martínez-Gomez[4]

1 Department of Horticultural Sciences Engineering, Faculty of Agriculture and Natural Resources, Imam Khomeini International University, Qazvin, Iran
2 Department of Horticultural Science, Faculty of Agriculture, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran
3 Horticulture Science and Landscape Department, Faculty of Agriculture, Malayer University, Malayer, Hamedan, Iran
4 Department of Plant Breeding CEBAS-CSIC, PO Box 164, E-30100 Murcia, Spain

ARTICLE INFO

Article history:

Received: 24 July 2025,
Received in revised form: 9 November 2025,
Accepted: 19 November 2025,

Article type:

Research paper

Keywords:

Almond,
Controlled pollination,
Fatty acids,
Gametophytic self-incompatibility,
S-alleles,
'Shahrood12' cultivar

*Corresponding author's email: m.rasouli@eng.ikiu.ac.ir

ABSTRACT

One reason for yield reductions in almond orchards is the existence of self-incompatibility and cross-incompatibility between different cultivars, which reduces fruit set. In this study, pollen compatibility and self-incompatibility of alleles were evaluated in selected cultivars and genotypes, including 'MSK81', 'MSK82', 'MSK83', 'MSK84', 'Shahrood12' cultivar and 'Shokufeh' as paternal cultivars. The 'Shahrood12' cultivar was considered as a parent with two controlled pollination methods. Assessments were made through the polymerase chain reaction. Degenerate primers, CEBAS*f* and AmyC5R determined possible incompatibility alleles. Also, some morphological and biochemical traits, elements, oil percentage and amount of dry fatty acids of fruits and kernels of fruits obtained from controlled pollination of 'Shahrood12' cultivar with selected pollinators were evaluated. The results of controlled pollination under orchard conditions showed that the highest percentage of fruit set (14.39%) was obtained at the crossing of 'Shahrood12' and 'Shokufeh' cultivars. The lowest percentage of fruit set (0%) was obtained in the self-pollination of 'Shahrood12' cultivar, which showed complete self-incompatibility of this cultivar. The degenerate primers used in this study identified 8 incompatibility alleles including *S1, S2, S3, S9, S21, S24, S27* and an unknown *SA* allele with a band size of 550 bp in the studied cultivars and genotypes. The *S21* and *SA* self-incompatibility alleles were the most abundant. Identified self-incompatibility alleles included selected genotypes 'MSK81' (*SAS9*), 'MSK82' (*S2S21*), 'MSK83' (*S2S24*), and 'MSK84' without any band amplification, 'Shahrood12' cultivar (*S1S3*), and 'Shokufeh' cultivar (*S24S27*/*S21SA*). The combination of CEBAS*f* and AmyC5R primers did not identify any bands indicating self-compatibility in the genotypes and cultivars studied.

Introduction

Almond (*Prunus dulcis* (Mill.) DAWebb; syn. *P. amygdalus* Batsch) is one of the oldest fruit trees in Southwest Asia and Central Asia and has a high economic value. Almond cultivars are commercially distributed in large parts of the world. Also, the kernels of this crop contain valuable compounds such as fats, proteins, carbohydrates (soluble fiber and sugars), minerals, vitamins and oil content between 50-65% of dry kernel weight (Socias in Company, 2007). Almond kernels are an important source of unsaturated fats that may help significantly lower blood cholesterol levels (Hamasaki and

COPYRIGHT
© 2027 The author(s). This is an openaccess article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other medium is permitted, provided the original author(s) and source are cited, in accordance with accepted academic practice. No permission is required from the authors or the publishers.

Hamasaki, 2017; Csakvari et al., 2019) and reduces the risk of cardiovascular disease (Fodor et al., 2018; Abdel-Daim et al., 2018). Most almond cultivars are incompatible, so for fertilization and fruit set of each cultivar requires the transfer of pollen from flowers of other cultivars. The selection of pollen compatible with the main cultivar can be effective in the production of products with high quantity and quality (Kester et al., 1994). Self-incompatibility prevents self-fertility, which is an advantage in evolution because it increases interspecific crosses (Herrera, 2018). It has been observed in other plants as well that cross-pollination offers advantages.

Incompatibility in almonds is one of the main problems in the production of this valuable product and many efforts have been made to solve it over the years. Self-incompatibility in different almond cultivars is of gametophytic type which is controlled by seed haploid genotype and female diploid (Mc Cubbin and Kao, 2000). The gametophytic incompatibility system is controlled by a gene locus called S in females and the SFB gene locus in pollen grains. They stop the growth of pollen tubes with the same genotype at location S (Gómez et al., 2019). This trait in almonds is controlled by a multivalent gene locus (S-locus). The presence of similar alleles in pollen and females prevents the pollen tube from reaching the ovary and stopping it during creaming. This locus is located on the G6 ligation group in the almond genetic map (Ballester et al., 1998). The interaction of the two genes *S*-Rnases and SFB causes the expression of incompatibility in cream (Sutherland et al., 2008). Prunus incompatible alleles have two introns (Martinez- Gomez et al., 2003b). By obtaining molecular primers, a large number of specific and general primers are designed based on conserved sequences in almond incompatibility alleles and are available for genetic testing of fruit trees (Ortega and Dicenta, 2004; Sanchez-perez et al., 2004). This method is fast, relatively easy, safe and applicable to young seedlings and has a high ability to identify incompatible genotypes in fruit trees such as almonds. Molecular research on Gametophytic Self-Incompatibility (GSI) in the Rosaceae family indicates that this mechanism is controlled by a gene locus called the S-locus, which plays a key role in recognizing and preventing self-pollination. Identifying and studying the diversity of S-alleles in different almond varieties, as shown in this research, can contribute to a better understanding of GSI mechanisms and improve breeding programs for more compatible and higher-yielding varieties. This knowledge is particularly important in regions with climatic limitations and the need for resistant varieties (Coulibaly et al., 2024). Sharma et al. (2025) also found that pollen diversity among apple cultivars is important for fruit set and quality. The research findings can help farmers select compatible varieties to increase yields (Sharma et al., 2025). Climate change can lead to a reduction in almond yields, but continuous innovation can offset these negative effects. Increased minimum temperatures and humidity during the flowering and pollination period, as well as heat stress during the growing season, are major factors contributing to yield decline (Wu et al., 2025). One of the suitable methods for identifying the autoimmune allele in almonds is the use of degenerate primers designed for different species of the genus Prunus. Using different combinations of these primers, the S alleles in almonds (Ortega et al., 2005; Sutherland et al., 2008), cherries (Sonneveld et al., 2003; Sutherland et al., 2008) and apricot (Halasz et al., 2005) were identified. Forward primers EM-PC2cons FD based on the conserved and fixed sequence of protected region C2 and C3 of location S in the genus Prunus by Sutherland et al. (2008) were designed and are able to amplify the second introns of almond incompatibility alleles. The CEBAS*f* primer is used as a specific primer to identify the almond self-compatible allele, which is *Sf* (Sanchez-Perez et al., 2004). Researchers have used various methods to determine the compatibility of pollen with females, such as field-controlled pollination and calculation of fruit formation percentage, controlled crosses and then tracked pollen tube growth with fluorescent microscopy, style protein electrophoresis and S-specific PCR (Ortega et al., 2002; Martinez-Gomez et al., 2003a; Sanchez-Perez et al., 2004).

In pollination with different pollen sources, the possibility of xenia should be considered. Therefore, it is very important to study the effects of different pollinating parents on the dry properties of fruit and nuts. In several studies, the effects of different sources of pollen on the dry characteristics of fruits and nuts have been studied, which can be examined in studies on pistachios (Afshari et al., 2008), hazelnut (McKay and Crane, 1939) and pecan (Romberg and Smith, 1946) pointed out. In a study conducted in Kashmir, India, to study xenia in almonds, it was found that traits related to the dry fruit and nucleus of the offspring resulting from crossbreeding were significantly influenced by pollinating parents (Majid et al., 2020). In addition, Fonti Forcada et al. (2011) and Alizadeh-Salte et al. (2018) reported that the biochemical composition of almond kernels is affected by pollen type. Rasouli and Imani (2016) explain that the amount of some fatty acids in the oil composition was affected by cross-pollination. The effect of pollinating parent (xenia effect) on the kernel and characteristics of several fruits such as almonds, chestnuts and pecans has also been observed (Kunar and Dos, 1996).

Ercik et al. (2023) reported that the F1 hybrids resulting from the crossbreeding of ‘Gulcan 2’ and ‘Lauranne’ almond varieties (‘Gulcan 2’ x

'Lauranne') had higher levels of oleic acid and lower levels of linoleic acid compared to the F1 hybrids resulting from crosses between 'Guara' and 'Nurlu' almond varieties and their respective parents ('Guara' x 'Nurlu'). The study also revealed a significant amount of variation in the fatty acid composition of the F1 populations, highlighting the potential for breeding new almond varieties with desirable fatty acid profiles.

The main purpose of this study was to investigate incompatibility in almonds on orchard condition by hand pollination and PCR amplification to determine self-incompatibility alleles in selected late flowering cultivars and genotypes for use in controlled almond crosses in order to achieve target crosses and produce superior offspring. In this study, we also pursued another goal, which was to investigate the effect of different sources of pollen on morphological characteristics, the amount of fatty acids, oil content and some mineral elements in the kernel as a result of controlled crosses.

Materials and Methods

Plant materials and experimental design

This research was conducted in the years 2017-2020 in the orchard of Malayer University research station of Hamedan province, Iran. This research station is located 25 km from Malayer on the Malayer-Boroujerd road with has a longitude of 48°47'80.56", a latitude of 34°16'38.89" N, an altitude of 1733 meters and an average rainfall of 242 mm per year. After the necessary studies and considering the quality of the product, fruit weight, overlap in terms of flowering and other factors, the maternal parent and genotypes and pollinizers cultivars were selected as paternal parents. Treatments including crossbreeding of 'Shahrood 12' cultivar as seed parents and genotypes 'MSK81', 'MSK82', 'MSK83', 'MSK84', 'Shokufeh' cultivar, pollen of 'Shahrood 12' cultivar were considered as pollinizers. Selected late flowering genotypes with superior fruit and nut dry characteristics included 'MSK81', 'MSK82', 'MSK83', 'MSK84' genotypes, which were selected from one thousand almond seed genotypes at Malayer University research station.

Evaluation of pollen germination and percentage of fruit set and fruit characteristics

In order to prepare and collect pollen, before opening the flowers, branches were cut to a length of 1-1.5 m that had enough flower buds, and then the branches were transferred to the laboratory. In the bud swelling stage and a few days before the flowers of selected cultivars opened, the branches that had sufficient flower buds on both north and south sides of 'Shahrood12' parent trees were selected as pollen. In addition to labeling to prevent free pollination, the branches were covered with 50 × 70 cm bags before opening the flowers. To ensure the germination capacity of the collected pollens were planted in solid culture medium. For this purpose, solid culture medium containing 15% sucrose, 1% agar and 20 ppm boric acid was used. After culturing the pollen, the culture dishes were transferred to a growth chamber at a temperature of 25 °C. After 6 h, pollen cultured with binoculars (10X) were examined and counted and then their germination percentage was determined (Rasouli and Imani, 2016).

PCR identification of self-incompatibility alleles

In May 2018, leaf samples were taken from young shoots of the studied genotypes and cultivars and transferred to the Genomics Laboratory of the Iranian Agricultural Biotechnology Research Institute affiliated to the Ministry of Jihad Agriculture in a tank containing dry ice. Extraction of genomic DNA from young leaf samples developed using the optimized method for this plant was modified by Doyle and Doyle (1987) methods, DAN extraction was performed from the studied samples. Quantification and quality of DNA was performed using DNA electrophoresis method in agarose gel prepared at a concentration of 0.8% in TBE buffer and also spectrophotometric method at 260 and 280 nm wavelengths using a spectrophotometer and the same concentration of them (5 ng μL^{-1}) was prepared. Finally, the DNA of the samples was stored at -20 °C until PCR.

The method Ortega et al. (2005) and Sutherland et al. (2008) were used for polymerase chain reaction. The primer combination of CEBAS*f* and AmyC5R was also used to investigate the possibility of self-compatible alleles in cultivars and genotypes used in this study (Tamura et al., 2000; Sanchez-Perez et al., 2004) (Table 1).

Electrophoresis of PCR products was performed in TAE buffer. In this method, 5 µL of PCR product was combined with 2 µL of d solution and loaded in 2% agarose gel wells. Electrophoresis was performed for 2.5 h at a voltage of 75 (Rasouli, 2017).

The gel was stained in ethidium bromide solution at a concentration of 0.8 cc L^{-1} for 3 min and then placed in pure distilled water for 20 min and separated bands were observed and after that, the amplified bands were photographed by a gel doc (Syngene Company, England). The size of amplified bands in the studied cultivars and genotypes was determined using the marker (M) the size of one kilo bp (1 kb) of Motagen Company and based on the size of these bands, the compatibility between genotypes and cultivars was evaluated.

Table 1. Sequences of degenerate primers, CEBASf and AmyC5R used in this study.

Row	Primer name	Primer code	Primer sequence	References
1	PaConsI-F	SP	5′ (C/A)CTTGTTCTGT(C/G)TTT(T/C)GCTTTCTTC 3′	(Sonneveld et al., 2003)
2	EM-PC1consRD	C1	5′GCCA(C/T)TGTTG(A/C)ACAAA(C/T)TGAA 3′	(Ortega et al., 2005)
3	EM-PC2consFD	C2	5′TCAC(A/C)AT(C/T)CATGGCCTAT 3′	(Sutherland et al., 2008)
4	EM-PC3consRD	C3	5′A(A/T)(C/G)T(A/G)CC(A/G)TG(C/T)TTGTTCCATTC 3′	(Sutherland et al., 2008)
5	CEBAS*f*	C4	5′ AGATCTATCTATATCTTAAGTCTG3′	(Sanchez-Perez et al., 2004)
6	AmyC5R	C5	CAAAATACCACTTCATGTAACAAC3′ 5′	(Tamura et al., 2000)

Evaluation of fruit characteristics, percentage of oil, fatty acids and elements

To study the characteristics of nut and kernel morphological traits, at least 20 fruits were harvested from each cross combination and dried at room temperature for 4 weeks according to the method presented (Dicenta et al., 2002). Some important fruit traits that are commonly evaluated in the almond breeding program were analyzed according to almond descriptor (Gulcan, 1985) and proposed methods by Ortega et al. (2006). To evaluate the characteristics of fruit and nuts, at least 10 almonds from each crossing with 4 replications were examined. The method (Golzari et al., 2016) was used to measure the oil content of the samples. The amount of fatty acids was measured by gas chromatography (GC) (Golzari et al., 2016). The method (Chapman and Pratt, 1961) was also used to measure the amount of copper, iron and manganese.

Statistical analysis

Experimental data related to controlled pollination with 7 treatments in four replications and experiments related to measured biochemical traits (fatty acids, oil content and some nutrients) and traits related to dried fruit and almond kernels with 11 treatments in four repetition was analyzed in a randomized complete block design in Excel (Version, 2019) and with SAS (Version 9.1) software**.** Also, the mean data were compared using Duncan's multiple range test at the level of 5 and 1% error probability.

Results

Evaluation of pollen germination

In order to ensure the viability of the tested pollen, pollen grains of pollinizers and genotypes were studied several times. The results showed that the germination percent of pollen of different cultivars and genotypes was between 55 and 78%. The results of pollen germination in this study were close to Rasouli and Imani (2016) experiments. They reported the percentage of pollen germination (65-78%).

Percentage of fruit set and fruit characteristics

In order to determine the percentage of fruit set and also the percentage of shedding of pollinated flowers in three times (15,45,105) d after pollination, the results were recorded. The results of comparing the means showed that the crossing of ‘Shahrood 12’ and ‘Shokufeh’ parents with 14.39% fruit set and the crossing of ‘Shahrood12’ with ‘MSK81’ genotype with 9.80% fruit set had the highest percentage of fruit set among the crosses. On the other hand, the lowest percentage of fruit set was related to the crossing of ‘Shahrood12’ parent with self-pollen with 0% fruit set (Table 2).

Table 2. Comparison means of the effect of crass-pollination on percentage of fruit set of ‘Shahrood12’ parent at different stages of enumeration.

Treatment	Maternal parent	Pollinizer	Count 1	Count 2	Count 3
1	‘Shahrood 12’	‘MSK81’	93.58 ^{a}	15.19^{ab}	9.80^{ab}
2	‘Shahrood 12’	‘MSK82’	91.98^{a}	11.58^{ab}	8.71^{ab}
3	‘Shahrood 12’	‘MSK83’	75.05^{a}	12.29^{ab}	8.83^{ab}
4	‘Shahrood 12’	‘MSK84’	72.91^{b}	13.96^{ab}	7.80^{ab}
5	‘Shahrood 12’	‘Shokufeh’	87.21^{ab}	18.80^{a}	14.39^{a}
6	‘Shahrood 12’	Open-pollination	78.73^{ab}	3.24^{b}	0.93^{b}
7	‘Shahrood 12’	‘Shahrood12’	73.95^{b}	4.75^{ab}	0.00^{b}

Means in each column with the same letter are not significantly different at the 5% level using Duncan Test.

Identification of S allele by using PCR

In unknown cultivars and genotypes used to construct almond orchards, the study of compatibility relationships is one of the most important orchard practices. Therefore, in this study, the bands related to incompatibility alleles of some cultivars and promising selected almond genotypes were investigated using degenerate primers and polymerase chain reaction (PCR) method. Using degenerate primers is a good way to identify incompatible genotypes. Also, CEBAS*f* and AmyC5R primers were used to detect the possibility of self-compatibility allele in promising genotypes. The degenerate primers used in this study were able to identify 8 incompatibility alleles (S_1, S_2, S_3, S_9, S_{21}, S_{24}, S_{27} and an unknown S_A allele with a band size of 550 bp in the studied cultivars and genotypes. The S_{21} and S_A self-incompatibility alleles were the most abundant (Table 3). Based on the results of amplification of *S* alleles using first intron and second intron degenerate primers in all cultivars and genotypes studied in this study, all band sizes obtained with band sizes identified by Ortega et al. (2005), Mousavi et al. (2011), Fallah et al. (2014) and Rasouli (2017) were consistent, and an unknown new SA band with a size of 550 bp was observed (Table 3). Results of amplification with first intron primers (PaConsI-F forward primers and EM-PC1consRD forward primers) and second intron primers (EM-PC2consFD forward primers and EM-PC3consR forward primers) in the studied cultivars and genotypes bands in the range of 450-2000 bp showed (Table 3).

Table 3. Incompatibility alleles and band sizes related to these alleles in studied almond cultivars and genotypes.

Row	Cultivar or genotype	Size (bp)	Incompatibility Genotype S-allele
1	Selective genotype ('MSK81')	500[2]/1560[1]-550	S_AS_9
2	Selective genotype ('MSK82')	450-500	S_2S_{21}
3	Selective genotype ('MSK83')	450-875	S_2S_{24}
4	Selective genotype ('MSK84')	?-?[4]	-
5	'Shahrood12'	1100-1200/500-1360	$S_1S_3/S_{21}S_{27}$
6	'Shokufeh'	875-2000/550-500	$S_{24}S_{27}/S_{21}S_A$

1 = The band size is determined by the first intron primers, 2 = Band size is determined by the second intron primers, 3 = Alleles are new with the letters A, 4 = No bands were reproduced.

The first intron primers were able to detect 550-1560 bp in the specific 'MSK81' genotype and 1 specific band, which was somewhat consistent with the results of Mousavi et al. (2011), Fallah et al. (2014) and Rasouli (2017). The results of amplification with the first intron primers (PaConsI-F forward primers and EM-PC1consRD reverse primers) showed a band of 450 bp. Intron II primers were also able to amplify bands of 500-2000 bp. Hamzaoui et al. (2015) reported in a study to identify S alleles in 70 Moroccan almond genotypes that amplified bands between 200-590 bp using the first intron primers (PaConsI-F and EM-PC1consRD). Using the second intron primers (EM-PC2consFD and EM-PC3consRD), they detected bands in the range of 250-4500 bp. They also stated that most alleles were between 400-2000 bp, which was largely in line with the findings of the present study. The size of the bands resulting from the amplification of the first and second introns varied greatly and made it possible to identify different alleles. This variation in band size was consistent with the results of Ortega et al. (2005), Mousavi et al. (2011), Fallah et al. (2014) and Rasouli (2017) (Table 3).

By calculating the size of amplified bands using a 1 kb marker, self-incompatibility alleles of cultivars and genotypes were determined (Table 3). The self-incompatibility alleles were identified including selected genotypes 'MSK81' (S_AS_9), 'MSK82' (S_2S_{21}), 'MSK83' (S_2S_{24}), selective genotype of 'MSK84' without amplification of any band, 'Shahrood12' cultivar (S_1S_3), 'Shokufeh' cultivar ($S_{24}S_{27}/S_{21}S_A$) (Table 3). In order to definitively introduce alleles related to cultivars and especially genotypes used in this study, the complete sequence of bands related to them should be determined.

Some morphological characteristics of fruit, percentage of oil, elements and composition of fatty acids

According to the results, the weight of fruit peel was observed between 2.93 to 5.84 g, which results from open pollination had the highest weight of fruit peel. The highest amount of kernel weight (2.43 g) was

obtained from the offspring of open- pollination. Significant differences in the kernel weight of the offspring resulting from crossing where the seed parent of all crosses was the same show that this difference is affected by the change of pollinizer (Table 4). The highest percentage of kernel weight with 11.46% was related to the offspring obtained from the crossing of 'Shahrood 12' with the 'Shokufeh' parent and in this trait showed a significant difference with the rest of the offspring from the crosses. This percentage represents the ratio of the weight of the kernel to the total weight of the fruit, so that the higher the percentage of kernel weight, the higher the weight of the kernel compared to the total weight of the fruit (green shell, wood husk) (Table 4). These results indicate that due to the change of pollen parent, the characteristics related to dry and fruit and kernel change and confirm the effect of pollen parent on characteristics of nut and kernel, this result were consistent with (Kunar and Dos, 1996) and (Wallace, 2003) founding.

The results showed that there was a significant difference between the different treatments in the level of 1% probability in terms of the effect of pollen on the percentage of kernel oil. The highest amount of kernel oil with 54.42% was observed in the offspring resulting from the crossing of 'Shahrood12' as a seed parent with 'MSK82' pollinizer and the lowest amount at 'MSK83' paternal parent at 38.32%.

Also, the results showed that among the parents and the offspring of the crosses, the amount of iron elements (85.48-138.53 mg 1000 g^{-1}), copper (20.32-47.48 mg 1000 g^{-1}) and manganese (18.04- 44.67 mg 1000 g^{-1}). Investigation of mineral compounds in walnut genotypes ('Sebin-Type-I', 'Korcegoz', 'Karabodur', 'Tozam' and 'Guvenli') cultivated in Turkey, average mineral content including phosphorus (316 mg 100 g^{-1}), potassium (270 mg 100 g^{-1}), calcium (85.0 mg 100 g^{-1}), magnesium (90.0 mg 100 g^{-1}), zinc (2.01 mg 100 g^{-1})) showed manganese (2.46 mg 100 g^{-1}), copper (1.01 mg 100 g^{-1}), iron (2.90 mg 100 g^{-1}) and boron (1.03 mg 100 g^{-1}) (Çağlarırmak, 2003). The results showed that there was a significant difference between the different treatments in terms of the effect of pollen on the amount of fatty acid composition of the offspring from the crossing of 'Shahrood12' parent with different parents. Mean comparison results showed that oleic acid (18:1) is the predominant fatty acid in almond oil. The highest amount of oleic acid (18:1) was observed in 'MSK84' paternal parent (75.51%) and the lowest amount was observed in open pollination progeny (66.13%). In the offspring of the cross, the highest amount of oleic acid was obtained in the offspring of the cross between the 'Shahrood 12' and 'Shokufeh' (72%) (Table 5).

On the other hand, in the offspring of the cross, the highest amount of linoleic acid was obtained in the offspring of the cross between the 'Shahrood 12' and the 'MSK84' at the rate of 18.40% (Table 5). The highest amount of palmitic acid (16:0) was observed in the offspring of open pollination at 9.50% and the lowest amount with 7.25% was observed in the offspring of the crossing of the parent 'Shahrood 12' with the parent 'MSK81' (Table 5). The results related to the fatty acid content of the offspring resulting from the crossing of 'Shahrood12' with different pollinizer are listed in Table 5. Due to the variable composition of fatty acids in cultivars and almond genotypes, these compounds can also be used as traits in the selection of parents and offspring.

Discussion

Evaluation of pollen germination

The results of pollen germination in this study were less than the self-incompatible growth reported from Murcia in Spain (68-94%) (Dicenta al., 2002). In a study by Sharafi et al. (2011) reported the percentage of pollen grain germination in 6 native almond genotypes (35-82%) which was close to the experimental results. In fruit trees, the percentage of pollen germination may vary depending on the concentration of chemicals and the type of culture medium. Therefore, a suitable culture medium of any species or cultivar must be found. Pollen germination percentage of almond cultivars in-vitro crops has been reported in the range of 12-95%, which is higher than apricots, peaches and cherries (Bolat and Pirlak, 1999).

Percentage of fruit set and fruit characteristics

In almonds, the percentage of fruit formation in incompatible crosses is usually less than 3% and in compatible crosses is more than 5% (Choi et al., 2002). If the percentage of fruit formation in almond pollination is between zero and 1% as self-incompatible, between 2 to 5% semi-self-compatible, between 6 to 10% self-compatible and above 10% completely self-compatible (Ortega and Dicenta, 2004). Therefore, in this experiment, the crossing of the parent 'Shahrood12' with the paternal parents 'MSK81', 'MSK82', 'MSK83', 'MSK84', whose final fruit set percentage was between 6 and 10%, have cross-compatibility between the pollinizers and the maternal parent. Also, the crossing of 'Shahrood12' parent with 'Shokufeh' parent, in which the fruit set percentage was 14.39%, is completely compatible, but the crossing of 'Shahrood12' parent with its own pollen, in which the fruit set percentage was 0%, is incompatible (Table 2).

Table 4. Comparison of the average measured traits in progeny, parent and maternal parent 'Shahrood12'.

Treatment	Pollinizers and progeny	Weight of wooden shell (g)	Kernel weight (g)	Kernel percentage weight (ratio)	Nut weight (g)	Green shell weight (g)	Kernel diameter (mm)	Ratio of length to width of kernel	Kernel width (mm)	Kernel length (mm)
1	'MSK81'	5.77^{b}	2.07^{cd}	13.07^{d}	8.04^{c}	7.92^{h}	10.09^{e}	1.85^{e}	15.19^{d}	28.21^{d}
2	'MSK82'	3.34^{h}	1.17^{h}	14.23^{b}	4.52^{k}	3.61^{j}	8.12^{j}	1.77^{g}	12.39^{k}	22.18^{j}
3	'MSK83'	3.44^{g}	1.37^{g}	15.71^{a}	5.03^{j}	3.74^{j}	9.83^{g}	1.43^{j}	14.10^{h}	20.18^{k}
4	'MSK84'	4.91^{c}	2.04^{d}	14.12^{c}	7.11^{f}	7.37^{i}	9.42^{i}	2.05^{b}	14.48^{g}	30.01^{b}
5	'Shokufeh'	5.81^{ab}	2.61^{a}	11.72^{e}	8.74^{a}	13.52^{b}	9.84^{g}	2.11^{a}	14.95^{e}	31.85^{a}
6	F1 of 'Shahrood12' × 'MSK81'	3.72^{f}	1.65^{f}	11.41^{g}	5.62^{i}	8.25^{g}	9.46^{h}	1.82^{f}	13.62^{i}	24.49^{i}
7	F1 of 'Shahrood12' × 'MSK82'	3.08i	2.11^{c}	10.27^{j}	7.67^{d}	11.93^{d}	11.01^{a}	1.63^{i}	16.04^{a}	26.03^{f}
8	F1 of 'Shahrood12' × 'MSK83'	4.59^{d}	2.05^{d}	11.28^{h}	6.80^{g}	11.01^{e}	10.41^{d}	1.74^{h}	14.82^{f}	25.60^{h}
9	F1 of 'Shahrood12' × 'MSK84'	2.93^{j}	2.03^{d}	10.04^{k}	7.22^{e}	12.85^{c}	10.80^{b}	1.75^{gh}	15.80^{b}	27.41^{e}
10	F1 of 'Shahrood 12' × 'Shokufeh'	4.22^{e}	1.78^{e}	11.46^{f}	6.31^{h}	8.86^{f}	10.01^{f}	1.94^{c}	13.48^{j}	25.89^{g}
11	Open-pollination of 'Shahrood 12'	5.84^{a}	2.43^{b}	10.49^{i}	8.62^{b}	14.05^{a}	10.76^{c}	1.89^{d}	15.45^{c}	29.53^{c}

The numbers of each column with non-similar characters at the 1% probability level are significantly different from the Duncan.

Table 5. Comparison of the average fatty acid, oil and nutritional elements in progeny, parent and maternal parent 'Shahrood12'.

Treatment	Pollinizers and progeny	Cu	Mn	Fe	Oil	Linolenic 18:3 (%)	Linoleic 18:2 (%)	Oleic acid 18:1 (%)	Stearic 18:0 (%)	Palmitoleic16:1 (%)	Palmitic 16:0 (%)	Myristic 14:0 (%)
1	'MSK81'	35.02^{f}	31.03^{d}	134.51^{b}	51.31^{c}	0.10^{b}	15.44^{g}	70/32^{e}	2.60^{j}	0.77^{b}	8.50^{c}	2.09^{d}
2	'MSK82'	25.51^{i}	18.04^{k}	88.26^{i}	51.51^{b}	0.13^{b}	10.59^{k}	74.76^{b}	3.35^{b}	0.73^{cd}	7.73^{g}	2.44^{a}
3	'MSK83'	39.31^{d}	28.64^{e}	115.92^{d}	38.32^{j}	0.15^{b}	15.20^{h}	70.85^{d}	2.93^{e}	0.74^{c}	7.80^{fg}	2.11^{f}
4	'MSK84'	25.15^{j}	20.87^{i}	99.83^{h}	39.33^{i}	0.15^{b}	12.57^{j}	75.51^{a}	1.72^{k}	0.79^{b}	7.85^{fg}	1.12^{f}
5	'Shokufeh'	20.32^{k}	22.85^{g}	102.85^{g}	45.91^{f}	0.12^{b}	19/20^{a}	66.62^{h}	2.77^{h}	0.91^{a}	8.03^{e}	2.17^{c}
6	'Shahrood12' × 'MSK81'	36.93^{e}	25.33^{f}	106.41^{f}	42.88^{h}	0.11^{b}	17.08^{d}	69.45^{f}	3.01^{d}	0.53^{f}	7.25^{h}	1.51^{e}
7	'Shahrood12' × 'MSK82'	30.68^{g}	21.08^{h}	85.48^{k}	54.42^{a}	0.45^{a}	17.02^{e}	68.26^{g}	3.16^{c}	0.52^{f}	8.33^{d}	2.11^{d}
8	'Shahrood12' × 'MSK83'	47.48^{a}	44.67^{a}	138.53^{a}	51.31^{c}	0.16^{b}	15.10^{i}	69.44^{f}	4.03^{a}	0.32^{g}	7.81f^{g}	2.43^{a}
9	'Shahrood12' × 'MSK84'	26.46^{h}	19.18^{j}	87.08^{j}	48.11^{d}	0.10^{b}	18.40^{b}	68.01^{g}	2.85^{f}	0.71^{d}	9.05^{b}	0.61^{h}
10	'Shahrood12' × 'Shokufeh'	45.72^{b}	42.95^{b}	126.31^{c}	47.41^{e}	0.08^{b}	15.65^{f}	72.00^{c}	2.64^{i}	0.53^{f}	7.94^{fc}	0.97^{g}
11	Open-pollination	41.83^{c}	37.29^{c}	112.86^{e}	45.03^{g}	0.13^{b}	18.30^{c}	66.13^{i}	2.81^{g}	0.67^{e}	9.50^{a}	2.28^{b}

The numbers of each column with non-similar characters at the 1% probability level are significantly different from the Duncan.

In another experiment (Mousavi et al., 2014) they examined the effect of several different pollinating parents on the cultivar 'Mamaei' and reported that the highest percentage of fruit set (21.15%) was related to pollination with the paternal parent of 'Sefid' cultivar. This rate of fruit formation was somewhat close to the results of the present study. On the other hand, studies conducted by (Rasouli and Imani, 2016) showed that in the initial time after taking notes, which was done 17 d after the cross, the percentage of fruit set between different compounds was not statistically significant and over time, in the last stages of fruit counting measured at 46 and 103 d later, the first note-taking stage took place only two weeks after pollination. Two weeks seems to be a very short time to observe differences in fruit set. The results of this study showed that the effect of the first note taken 15 d after pollination on fruit set was significant but the fruit set percentage of different crosses was close to each other. The results of research have shown that in 30 d after pollination, some small fruits formed due to lack of proper fertilization and lack of embryo formation fall, which self-incompatibility and cross-incompatibility is the most important reason for shedding at this stage. In this study, the fruits showed a sharp drop in the count that was done 46 d after the cross. In this study, the percentage of fruit set was relatively low. The maximum fruit set in this study was 14.39%. However (Kodad et al., 2021) reported a minimum fruit set rate of 30% for a commercial product. Regarding the reason for the unusual fall of flowers and fruits, Ortega and Dicenta (2004) stated that many factors in almonds cause the fall of flowers and fruits and affect the yield. Also, fruit loss in the second and third stages can be due to fetal underdevelopment or fruit competition for nutrients. On the other hand, in a controlled crossing, a large amount of pollen is poured on the stigma, and therefore the exact distinction between fully compatible and semi-compatible composition is difficult and sometimes erroneous. In addition, for controlled cross-breeding experiments, the plants need to enter the flowering stage and the offspring are greatly affected by the environmental and physiological factors of the tree (Boskovic et al., 2003; Certal et al., 2002). In addition to growth conditions, technical manipulation and self-incompatibility characteristics in yield evaluation, other factors such as the effect of inbreeding between cultivars and genotypes should be considered (Kodad et al., 2021). Therefore, a combination of both classical and molecular methods is necessary to study and evaluate self-incompatibility in almonds (Mousavi et al., 2014). In a study by Alizadeh Salteh and Arzani (2014), examined the effect of a number of pollinators on two almond cultivars, late flowers 'Shahrood 12' and 'Shahrood21'. Their results showed that these two cultivars were completely self-incompatible, which was completely consistent with our results. They also stated that the highest percentage of fruit set (32.23%) was obtained from the crossing of the parent of 'Shahrood12' with pollen of cultivar '15-5', which was higher than the maximum fruit set in our study. The results of the present study showed us that late flowers cultivar 'Shahrood12' is completely self-incompatible and in order to form the desired fruit, it needs pollination with a compatible and suitable pollinizer, which is consistent with the findings of Alizadeh Salteh and Arzani (2014). Our results showed that 'Shokufeh' cultivar had the highest percentage of fruit set with 'Shahrood12' cultivar, so it can be considered as a suitable pollinizer with 'Shahrood12' cultivar. Also, their flowering time completely overlapped. Brukenta et al. (2024) findings indicated that using wild species can be a valuable source for traits such as self-compatibility, flower and fruit size, and high yield. In another study, Nakao et al. (2025) reported that the pollination method in kiwifruit can significantly affect the flower fertility ratio, fruit growth, seed number and distribution, but may not necessarily affect chemical parameters such as TSS and acidity.

Identification of S allele by using PCR

Based on the results of determining self-incompatibility alleles, it is possible to pollinate 'Shahrood12' and 'Shokufeh' cultivars with selected genotypes and also the mentioned cross to obtain superior offspring based on the purpose of almond breeding because there was at least one different self-incompatibility allele in them. Therefore, it will not be a problem to form fruit. This is provable because of the almond gametophytic incompatibility system. Zeinalabedini et al. (2012) using specific primers AmyC5R _AS1II, CEBAS*f* _AmyC5R, Alsc1 _ AmyC5R and AmyC5R _Alsd2 were able to achieve 14 self-incompatibility alleles S_1, S_2, S_3, S_5, S_6, S_7, S_8, S_{11}, S_9, S_{11}, S_9, S_{10}, Identify S_{21}, *Sc* and *Sd* in 70 Iranian and foreign almond cultivars, 22 other *Prunus* species and some interspecific crosses. Also, they stated that the frequency of S_1 and S_5 self-incompatibility alleles was higher in the studied cultivars than other alleles. Mousavi et al. (2011) showed 70 Iranian cultivars and genotypes and 17 foreign almond cultivars using specific primers paCons1-f, EM-pC1consRD, EM-pC3consRD, EM-pC5consRD and EM-pC2consFD the second intron degeneration primers EM-pC3consRD and EM-pC2consFD are capable of amplifying 28 alleles out of a total of 32 known self-incompatibility alleles in almonds. Only the S_{29} allele is amplified by the first intron paCons1-f and EM-pC1consRD, and the primers paCons1-f and EM-pC5consRD were used to amplify both introns.

In addition, they showed that among Iranian almond cultivars and genotypes, self-incompatibility alleles

S1, *S22*, *S4*, *S7*, *S12*, and *S24* had the highest frequency, respectively. Martinez-Gomez et al. (2003a) using the specific primer pair AS1II_ AmyC5R were able to detect 8 incompatibility alleles *S1*, *S5*, *S6*, *S7*, *S9*, *S10*, *S13*, and *S18* in 18 cultivars and 12 species of almond relatives. Abouei et al. (2021) conducted a study to identify and determine alleles related to self-incompatibility in almond genotypes in Yazd province and reported that alleles *S13* and *S23*, *S11*, *S10*, *S5*, *S1*, *S2*, *S3*, *Sf* were identified using the AS1II-AmyC5R primer pair and alleles *S1*, *S2*, *S3*, *S10*, *S11*, *S23*, and *S31* were obtained in the samples using the ConF-ConR primer pair. They also stated that alleles *S1*, *S2*, *S3*, *S11* showed the highest frequency. Kamareh et al. (2015) examined 37 cultivars and genotypes of Iranian almonds for the identification of S alleles and reported that using degenerate primers, they were able to identify to alleles (*S1*, *S2*, *S3*, *S5*, *S7*, *S24*, *S22*, *S12*, *S9*, and *Sf*). Hamzaoui et al. (2015) were able to identify the S_f allele, which is the self-compatibility allele in almonds, using a combination of primers (AS1II, CEBAS*f* and AmyC5R). Identification of S alleles in local almond germplasm is very important for breeding programs and helps in selecting parents to optimize crosses (Hamzaoui et al., 2015). Identification of incompatibility alleles in the samples used in this study, especially identification of incompatibility alleles in late-flowering offspring obtained from crossing, makes it possible to provide compatible cultivars and genotypes for orchard establishment, in order to achieve higher yields after more accurate evaluations.

Some morphological characteristics of fruit, percentage of oil, elements and composition of fatty acids

Wallace (2003) in his research on some tropical trees showed that the type of pollen could change the quality of macadamia and citrus fruits. The effect of xenia can affect various fruit parameters, such as fruit ripening, shape, size and color of fruit, fruit flavor, factors such as sugars and acids, as well as the quality of nutrients such as anthocyanins. The internal quality of the fruit is very remarkable (Yang et al., 2020). Acar et al. (2016) studied the effect of xenia on almonds and reported that the nut properties of the fruit and kernel were affected by different sources of pollen. Alizadeh Salteh and Arzani (2014) studied the effect of xenia on two cultivars of 'Shahrood12' and 'Shahrood21'. Their results showed that some of the nut and kernel are heritable. Kodad et al. (2011) reported that almond kernel oil content was between 50 and 65% of dry kernel weight, which was consistent with the results of this study. In addition to genetic differences related to cultivars, genotypes and offspring, other factors can affect the amount and quality of oil, including geographical location, climatic effects, fruit ripening rate, harvesting and their maintenance noted (Moayedi et al., 2011). Some observations confirm that the amount of oil is affected by pollen and eggs. Alizadeh Salteh et al. (2018) in their study reported that the percentage of almond kernel oil was not affected by pollen and concluded that the effect of pollen on different almond cultivars could be variable. The effect of different pollinizers on the amount of kernel oil in hazelnuts (Balik and Beyhan, 2020), walnut (Golzari et al., 2016) and chestnut (Xuhui et al., 2016) has also been reported.

The predominant fatty acids in almond oil are oleic acid and then linoleic acid. The fatty acids in almond oil are mainly unsaturated fatty acids with a simple double bond, especially oleic acid, unsaturated fatty acids with several double bonds, especially linoleic acid, and saturated fatty acids such as stearic acid (Jaceldo-Siegl et al., 2011; Ercik et al., 2023), which is consistent with the results of this study. In a study of six Romanian almond species, oleic acid (66-77%) and linoleic acid (19-28%) were reported (Csakvari et al., 2019), which is consistent with the present study. In recent years, genes have been discovered that are responsible for producing enzymes that control the biosynthesis of fatty acids in fruits. It is the enzyme desaturase that affects simple and unsaturated compounds.

Alizadeh Salteh et al. (2018) reported that the fatty acid composition of almond oil in 'Shahrood12' and 'Shahrood21' cultivars was significantly affected by the type of pollen source and oleic acid and linoleic acid were the predominant fatty acids in almond oil. Brittain et al. (2014) stated that the fatty acid composition in almonds is strongly affected by pollination and self-pollinating trees have a lower ratio of oleic acid to linoleic acid in the kernel compared to other pollinizers. The effect of pollen source on the fatty acid composition of the kernel in hazelnuts (Balik and Beyhan, 2020), walnut (Golzari et al., 2016) and chestnut (Xuhui et al., 2016) has also been reported. Edlund et al. (2004) stated that the reason for the effect of pollen type on the percentage of fatty acids in the fruit kernels, as well as the effect on the percentage of fat, may be the fatty acid compounds in the pollen wall. The presence of different amounts of volatiles, isoprenoids, fats, fatty acids, benzoids, etc. in the pollen of different plants can be one of the possible reasons for the effect of pollen on the fat content of fruit derived from plants (Aslan, 2002). In another study, Sarami et al. (2025) investigated the phenomenon of Xenia, examining the direct influence of pollen on seed traits such as color, size, and nutritional content. Their studies indicated that different pollen types can significantly affect seed appearance; for instance, pollination with 'Mamaee' pollen results in brighter seeds, whereas 'Orientalis' pollen produces darker seeds (Sarami et al., 2025).

Conclusion

The results of the present study showed that all pollinizers used were compatible with 'Shahrood12' cultivar. Also, due to 'Shahrood 12' self-incompatibility, no fruits were obtained. The highest percentage of fruit set was obtained from the crossing of 'Shahrood12' and 'Shokufeh' cultivar. Molecular observations and determination of alleles were inconsistent with field results. Based on the results of determining self-incompatibility alleles, degenerate primers used in this study included 8 incompatibility alleles, i.e., S_1, S_2, S_3, S_9, S_{21}, S_{24}, S_{27} and an unknown S_A allele with a band size of 550 bp in cultivars and genotypes. The subjects identified that the S_{21} and S_A self-incompatibility alleles were the most abundant. The identified self-incompatibility alleles included selected genotypes 'MSK81' (S_AS_9), 'MSK82' (S_2S_{21}), 'MSK83' (S_2S_{24}), and 'MSK84' without amplification of any band, 'Shahrood12' cultivar (S_1S_3), and 'Shokufeh' cultivar ($S_{24}S_{27}/S_{21}S_A$). These results showed that it is possible to pollinate 'Shahrood12' and 'Shokufeh' cultivars with selected genotypes to obtain superior offspring based on the purpose of almond breeding because there was at least one different self-incompatibility allele in them and therefore fruit formation was not a problem. The results of this study also showed that pollen type had an effect on nut and kernel traits, oil percentage and almond kernel fatty acid content. The highest percentage of kernel weight (11.46%) and the highest amount of oleic acid (72%) of the kernel was related to the offspring of the crossing of 'Shahrood12' with 'Shokufeh'. The highest amount of kernel oil was obtained from the crossing of 'Shahrood12' parent with 'MSK82' (54.42%). Finally, according to the results of the present study, it is suggested that almonds of late-flowering 'Shokufeh' cultivar be used as a suitable pollinizer for 'Shahrood12' to achieve the desired yield in almond orchards in western Iran, which have had problems with spring frosts.

Acknowledgments

The authors thank Agricultural Sciences and Natural Resources University of Khuzestan and Malayer University for research support.

Author Contributions

Conceptualization, Methodology, Writing-Original draft, AJT; Methodology, Writing - Review and Editing, and Supervision, MR; Validation and Formal analysis and Methodology, MRJ; Resources, Visualization, Investigation, ZK; Resources, Writing- Review and Editing, PMG. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Conflict of Interest

The authors indicate no conflict of interest in this work.

References

Abdel-Daim MM, Zakhary NI, Aleya L, Bungău SG, Bohara RA, Siddiqi NJ .2018. Aging, Metabolic, and Degenerative Disorders: Biomedical Value of AntiAntioxidants. Oxid Med Cell 1-2. https://doi.org/ 10.1155/2018/2098123

Abouei M, Kamali Aliabad K, Soluki M, Fahmideh L, Imani A .2021. Identification of self-incompatibility alleles in some of Yazd province almond genotypes by using PCR method. Iranian J of Hort Sci 51(4): 797-808. https://doi.org/ 10.22059/IJHS.2019.283959.1670

Acar I, Yilmaz A, Ak BE .2016. Paternal effects on fruit characteristics of some almond cultivars. CIHEAM Options Mediterraneennes 119: 29-32. http://om.ciheam.org/article.php.

Afshari H, Tajabadipour A, Mohamadi Moghadam M, Hokmabadi H, Laee G .2008. Studying some compounds existing in pistachio fruits and the effect of pollen grains of different male genotypes on the changes in their quantity. World Appl. Sci. J. 5(1):105–110.

Alizadeh Salteh S, Arzani K (2014) Xenia in almonds: Pollen source effect on characteristics of some Iranian late-blooming almonds and their self-incompatibility. J. Nuts 5(01): 33-38. . https://doi.org/ 10.22034/JON.2014.515695

Alizadeh Salteh S, Farhadi N, Arzani K, Khoshghalb H .2018. Almond oil quality as related to the type of pollen source in Iranian self-incompatible cultivars. Inter J of Fruit Sci 18(1):29-36. https://doi.org/10.1080/15538362.2017.1367983

Aslan M, Orhan I, Şener B .2002. Comparison of the seed oils of *Pistacia vera* L. of different origins with respect to fatty acids. Int. J. Food Sci. Technol 37(3):333-335. https://doi.org/10.1046/j.1365-2621.2002.00594.x

Balik HI, Beyhan N .2020. Xenia and metaxenia affects bioactive compounds of hazelnut. Turkish J of Food and Agriculture Sciences 2(2):42-49. https://doi.org/ 10.22069/JOPP.2021.18900.2788

Ballester J, Boskovic R, Batlle I, Arus P, Vargas F, MC de Vicente .1998. Location of the self-incompatibility gene on the almond linkage map. Plant Breeding 117:69-72. https://doi.org/10.1111/j.1439-0523.1998.tb01450.x

Bolat I, Pirlak L .1999. An investigation on pollen

viability, germination and tube growth in some stone fruits. Turk. J. Agr. Forestry 23:383–388.

Boskovic R, Tobutt KR, Battle I, Duval H, Mart´ınez-G´omez P, Gradziel TM .2003. Stylar ribonucleases in almond: correlation with and prediction of incompatibility genotypes. Plant Breed 122:70–76. https://doi.org/10.1046/j.1439-0523.2003.00744.x

Brittain C, Kremen C, Garber A, Klein AM .2014. Pollination and plant resources change the nutritional quality of almonds for human health. Plos One 9(2): e90082.
https://doi.org/10.1371/journal.pone.0090082

Brukental H, Doron-Faigenboim A, Bar-Ya'akov I, Harel-Beja R, Trainin T, Hatib K, Holland D. 2024. Exploring the wild almond, *Prunus arabica* (Olivier), as a genetic source for almond breeding. Tree Genetic Genom 20(5), 37. https://doi.org/10.1007/s11295-024-01668-4

Çağlarırmak N .2003. Biochemical and physical properties of some walnut genotypes (*Juglans regia* L.). Nahrung 47(1):28-32. https://doi.org/ 10.1002/food.200390004

Certal AC, Almeida RB, Boskovic R, Oliveira MM, Feijo JA .2002. Structural and molecular analysis of self-incompatibility in almond (*Prunus dulcis*). Sex Plant Reprod 15:13–20. https://doi.org/10.1007/s00497-002-0138-4

Chapman HD, Pratt PF .1961. Methods of Analysis for Soils, Plants and Waters. Priced Publication 4034. Division of Agriculture Sciences. University of California, Berkeley 5-350.

Choi C, Livermore K, Lersen R .2002. Identification of self-incompatibility alleles and pollen incompatibility groups in sweet cherry by PCR based S-alleles typing and controlled pollination. Euphytica 123:9-20. https://doi.org/10.1023/A:1014403802677

Coulibaly D, Gao F, Bai Y, Ouma K O, Antwi-Boasiako A, Zhou P, Gao Z. 2024. Molecular research progress on gametophytic self-incompatibility in Rosaceae species. Horticulturae 10(10),
1101. https://doi.org/10.3390/horticulturae10101101

Csakvari AC, Lupitu A, Bungău S, Gitea MA, Gitea D, Ţiţ DM, Copolovici D .2019. Fatty acids profile and antioxidant activity of almond oils obtained from six Romanian varieties. Farmacia 67:882-887. https://doi.org/10.31925/farmacia.2019.5.19

Dicenta F, Ortega E, Canovas JA, Egea J .2002. Self-pollination vs. cross-pollination in almond: pollen tube growth, fruit set and fruit characteristics. Plant Breed 121:163–167. https://doi.org/10.1046/j.1439-0523.2002.00689.x

Doyle JJ, Doyle JL .1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11-15.

Duke J .2001. Almond (*Prunus dulcis*). In: Handbook of Nuts. (Second Pub), CRC press, Boca Raton, Florida, USA, pp 249-252. https://doi.org/10.1201/9781351071130

Edlund AF, Swanson R, Preuss D .2004. Pollen and stigma structure and function: the role of diversity in pollination. The Plant Cell 16(suppl 1): S84-S97. https://doi.org/ 10.1105/tpc.015800

Ercik K, Comlekcioglu S, Kafkas NE, Ercisli S, Sagbas HI .2023. Fatty Acid Characterization of "Gulcan 2× Lauranne" and "Guara× Nurlu" F1 Hybrid Almond Population. Erwerbs-Obstbau 1-9. https://doi.org/10.1007/s10341-023-00887-8

Fallah M, Rasouli M, Sharaf Y, Imani A .2014. Study of Compatibility Relationships Among Some Almond Cultivars and Genotypes Using of SAlleles Identification. J. Nuts 05(02):49-56. https://doi.org/10.22034/jon.2014.515689

Fodor K, Ţiţ DM, Paşca B, Buştea C, Uivaroşan D, Endres L, Iovan C, Abdel-Daim M, Bungău S .2018. Long term resveratrol supplementation as a secondary prophylaxis for stroke. Oxid Med Cell Longev 1-10. https://doi.org/10.1155/2018/4147320

Font I Forcada C, Kodad O, Juan T, Estopañan G, Socias I Company R .2011. Genetic variability and pollen effect on the transmission of the chemical components of the almond kernel. Span J Agric Res 9(3):781–
789. https://doi.org/10.5424/sjar/20110903-423-10

Golzari M, Hassani D, Rahemi M, Vahdati K .2016. Xenia and metaxenia in Persian walnut (*Juglans regia* L.). J Nuts 7:101-108. https://doi.org/10.22034/jon.2016.527095

Gómez EM, Buti M, Sargent DJ, Dicenta F, Ortega E .2019. Transcriptomic analysis of pollen-pistil interactions in almond (*Prunus dulcis*) identifies candidate genes for components of gametophytic self-incompatibility. Tree Genetics Genom 15(53):1-13. https://doi.org/10.1007/s11295-019-1360-7

Gulcan R .1985. Descriptor list for almond (*Prunus amygdalus*), International Board for Plant Genetic Resources, Rome.

Halasz J, Hegedus A, Herman R, Stefanovits-Banyai E, Pedryc A .2005. New self-incompatibility alleles in apricot (*Prunus armeniaca* L.) revealed by stylar ribonuclease assay and S-PCR analysis. Euphytica 145:57–66.
https://doi.org/10.17660/ACTAHORTIC.2019.1231

.29

Hamasaki H, Hamasaki Y .2017. Nuts for physical health and fitness: A review. AIMS Med Sci 4(4):441-455. https://doi.org/10.3934/medsci.2017.4.441

Hamzaoui AE, Oukabli A, Moumni M .2015.Identification of self-incompatibility S alleles in Moroccan almond (Prunus dulcis Miller) germplasm using PCR. J Hortic Sci Biotechnol 90(3):337-343. https://doi.org/10.22059/IJHS.2019.283959.1670

Herrera S, Rodrigo J, Hormaza JI, Lora J .2018. Identification of self-incompatibility alleles by specific PCR analysis and S-RNase sequencing in apricot. Int. J Mol Sci 19(11):3612. https://doi.org/10.3390/ijms19113612

Jaceldo-Siegl K, Sabaté J, Batech M, Fraser GE .2011. Influence of body mass index and serum lipids on the cholesterol-lowering effects of almonds in free-living individuals. Nutrition, Metabolism and Cardiovascular Diseases 21: S7-S13. https://doi.org/ 10.1016/j.numecd.2011.03.007

Kamareh A, Sharafi Y, Ghanbari A .2015. Identification of Self-incompatibility Alleles in Some Almond Genotypes by Degenerate S-RNase Primers. J Nuts 6(02):165-172. https://doi.org/ 10.22034/JON.2015.516329

Kaseb F, Rashidi M, Afkhami-Ardekani M, Fallahzadeh H .2013. Effect of olive, almond and walnut oil on cardiovascular risk factors in type 2 diabetic patients. Int J Diabetes Dev Ctries 33(2):115-119. https://doi.org/ 10.1007/s13410-012-0108-9

Kaur P, Cowan M, De Faveri J, Alam M, Topp B. 2024. Evaluating Self-Pollination Methods: Their Impact on Nut Set and Nutlet Abscission in Macadamia. Plants 13(24), 3456. https://doi.org/10.3390/plants13243456

Kester DE, Gradziel TM, Micke WC .1994. Identifying pollen incompatibility groups in California almond cultivars. J Am Soc Hortic Sci 119:106-109.

Kodad O, Alonso JM, Espiau MT, Estopañán G, Juan T .2011. Chemometric characterization of almond germplasm: compositional aspects involved in quality and breeding. J Am Soc Hortic Sci 136(4):273-281. https://doi.org/10.3390/foods10010153

Kodad O, Nahli SE, El Baji M, Martinez Gomez P, Garcia PJM .2021. Low fruitfulness in local almond orchards could be due to the inbreeding depression effect. Moroccan J of Agri Sci 2 (1):1-8. https://doi.org/ 10.5281/zenodo.15466118

Kodad O, Socias I Company R, Alonso JM .2015. Unilateral recognition of the Sf allele in almond. Sci Hortic 1:1-6. https://doi.org/ 10.22059/IJHS.2019.283959.1670

Kunar K, Dos B .1996. Studies on xenia and methaxenia in almond. J Hortic Sci 71(4):545 – 549.

Majid S, Kumar A, Bashir S, Amin A, Nagoo SA, Rashid Z, Paul S .2020. Xenia studies in exotic and indigenous almond (*Prunus amygdalus* L.) varieties of Kashmir, India. j appl na. sci 12(2):244-251. https://doi.org/10.31018/jans.vi.2266

Martinez Gomez P, Alonso JM, Lopez M, Battle I, Ortega E, Sanchez-perez R, Disenta F .2003a. Identification of self-incompatibility alleles in almond & related Prunus species using PCR. Theor Appl Genet 123:397-401.

Martinez-Gomez P, Ortega E, Sanchez-Perez R, Dicenta F, Dandekar AM, Alonso J M, Socias i Company R, Lopez M, Batlle I, Gradziel TM .2003b. Identification of self-incompatibility alleles in almond and related Prunus species using PCR. Acta Hortic 622:397-401.

Mc Cubbin AG, Kao TH .2000. Molecular recognition and response in pollen and pistil interactions. Annu. Rev. Cell Dev Biol 16: 333-364. https://doi.org/doi: 10.1146/annurev.cellbio.16.1.333.

McKay JW, Crane HL .1939. The immediate effect of pollen on the fruit of the chestnut. Proc. J Am Soc Hortic Sci 36:293–298.

Moayedi A, Rezaei K, Moini S, Keshavarz B .2011. Chemical compositions of oils from several wild almond species. J Am Oil Chem Soc 88(4),503-508.https://doi.org/10.1007/s11746-010-1701-z

Mousavi Ghahfarokhi SA, Babadaei R, Fatahi R, Zamani Z, Dicenta F, Ortega E .2014. Self-incompatibility in the Iranian almond cultivar 'Mamaei' using pollen tube growth, fruit set and PCR technique. J Nuts 5(2):1-10. https://doi.org/10.22034/jon.2014.515685

Nakao Y, Haruki T, Murase K, Morita Y, Morita T. 2025. Effects of Pollination of Some Stigmas in Kiwifruit Flowers on Seed Distribution and Fruit Quality. The Horticulture J 94(2): 184-189. https://doi.org/10.2503/hortj.SZD-009

Ortega E, Dicenta F .2004. Suitability of four different methods to identify self-compatible Seedlings in an almond breeding program. J Hortic Sci Biotechnol 79(5):747-753. https://doi.org/10.1080/14620316.2004.11511837

Ortega E, Egea J, Cánovas JA, Dicenta F .2002.

Pollen tube dynamics following half- and fullycompatible pollinations in self-compatible almond cultivars. Sex Plant Reprod 15:47-51. https://doi.org/10.1007/s00497-002-0137-5.

Ortega E, Egea J, Dicenta F .2006. Self-fertilization in homozygous and heterozygous self-compatible almonds. Sci Hortic 109:288–292. https://doi.org/10.1016/j.scienta.2006.04.017

Ortega E, Sutherland BG, Dicenta F, Boskovic R, Tobutt KR .2005. Determination of incompatibility genotypes in almond using first and second intron consensus primers detection of new S alleles and correction of reported S genotypes. Plant Breeding 124:188-196. https://doi.org/10.1111/j.1439-0523.2004.01058.x.

Rasouli M .2017. 'The Study of Morphological Traits and Identification of Self-incompatibility Alleles in Almond Cultivars and Genotypes'. J Nuts 8(2):137-150. https://doi.org/10.22034/jon.2017.536244

Rasouli M, Imani A .2016. Effect of supplementary pollination by different pollinizers on fruit set and nut physicochemical traits of 'Supernova', a self-compatible almond. Fruits 71:299-306. https://doi.org/10.1051/fruits/2016021

Romberg LD, Smith CL .1946. Effects of cross-pollination, self-pollination and sib pollination on the dropping, the volume and the kernel development of pecan nuts and on the vigor of the seedling. Proc. J Am Soc Hortic Sci 47:130–133.

Sanchez-Perez R, Dicenta F, martinez-Gomez P .2004. Identification of S-alleles in almond using multiplex PCR. Euphytica 138:263-269. https://doi.org/10.1023/B:EUPH.0000047097.96271.bf

Sarami F, Shiran B, Rabiei G, Jafari M. 2025. Morpho-physiological and molecular investigations of the effect of pollen grains on the browning of almond kernels. Heliyon 11(6). https://doi.org/10.1016/j.heliyon.2025.e42975

Sathe SK, Seeram NP, Kshirsagar HH, Heber D, Lapsley KA .2008. Fatty acid composition of California grown almonds. J Food Sci 73(9):C607-C614. https://doi.org/10.1111/j.1750-3841.2008.00936.x

Schirra M (1997) Postharvest technology and utilization of almonds. Hortic Rev 20:267-311.

Sharma S, Sundouri AS, Kumar A, Sharma G, Singh J, Singh D, Choudhary R. 2025 Pollination compatibility studies between red and green colored cultivars of apple (*Malus*× *domestica*). https://doi.org/10.33545/26174693.2025.v9.i4h.4172

Socias i Company R, Kodad O, Alonso JM, Gradziel TM .2007. Almond quality: a breeding perspective. Hortic Rev 34:197-238. https://10.1002/9780470380147.ch3

Sonneveld T, Tobutt KR, Robbins TP .2003. Allele-specific PCR detection of sweet cherry self-incompatibility (S) alleles S1 to S16 using consensus and allele-specific primers. Theor Appl Genet 107:1059-1070. https://10.1007/s00122-003-1274-4

Sutherland BG, Tobutt KR, Robbins TP .2008. Trans-specific S-RNase and SFB alleles in prunus self-incompatibility haplotypes. Mol Genet Genom 279:95-106. https://10.1007/s00438-007-0300-7

Tamura M, Ushijima K, Sassa H, Hirano H, Tao R, Gradziel TM, Dandekar AM .2000. Identification of self-incompatibility genotypes of almond by allele-specific PCR analysis. Theor Appl Genet 101:344-349. https://doi.org/10.1007/s001220051489

Wallace H M .2003. Genetic and environmental control of quality in subtropical fruit and nut crops. XXVIth International horticulture symposium on citrus and other subtropical and tropical fruit crops 1120 – 1140.

Wang C, Zhao H, Zhang H, Sun S, Xue Y. 2025. PSIA: A Comprehensive Knowledgebase of Plant Self-Incompatibility. Genomics Proteomics Bioinformatics, qzaf046. https://doi.org/10.1093/gpbjnl/qzaf046

Wu S, Zikalala PG, Alba S, Jarvis-Shean KS, Kisekka I, Segaran M, Monier E. 2025. Advancing the modeling of future climate and innovation impacts on perennial crops to support adaptation: A case study of California almonds. Earth's Future 13(4): e2024EF005033. https://doi.org/10.1029/2024EF005033

Xuhui Z, Deyi Y, Feng Z, Xiaoming F, Jing T, Zhoujun Z .2016. A study on the xenia effect in Castanea henryi. Hortic. Plant J 2(6):301- 308. https://doi.org/ 10.14067/j.cnki.1673-923x.2023.12.005

Yang Q, Fu Y, Liu Y, Zhang T, Peng S, Deng J .2020. Novel Classification Forms for Xenia. HortScience 55(7):980-987. https://doi.org/10.21273/HORTSCI14939-20

Zeinalabedini M, Khayamnekue M, Imani A, Majidian P .2012. Identification of self-compatibility and self-incompatibility genotypes in almond and some *Prunus* species using molecular markers. Iranian J of Seed and Plant Improvement 28(2): 227-238. https://doi.org/10.22092/spij.2017.111104