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ORIGINAL ARTICLE

Pistacia vera and *P. integerrima* Hybrids for Pistachio Rootstock: Seed set, Seedling Survival and Early Growth Evaluation under Chilling Temperature

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K E Y W O R D S

Growth indices;
Interspecific hybridization;
P. integerrima;
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A B S T R A C T

Interspecific hybrid rootstocks have the potential to improve the productivity and resilience of pistachio orchards in response to environmental stresses. This study aimed to produce inter-specific hybrids between *P. vera* and *P. integerrima* (*In*) and compare their early growth with their parents under chilling temperatures. Controlled pollination using *In* pollen was conducted on five pistachio cultivars including 'Kaleh-Ghuchi', 'Khanjari-Ghermze', 'Akbari', 'Khanjari-Sefid', 'Ohadi' and a local variety. The fruit set in female parents through controlled pollination of interspecific crossings was significantly lower than natural open pollination, resulting in a high percentage of blank seeds. Only 'Khenjari-Ghermze' and 'Kalleh-Ghoochi' produced a sufficient number of seeds for further experiment. The germination and survival rate of hybrid seeds were lower than that of pistachio cultivars ($P<0.01$). Growth parameters of hybrid seedlings and their parents were examined at 5°C and 25°C. All rootstocks showed a significant reduction in growth at low temperatures, with chlorophyll and chlorophyll fluorescence also decreasing significantly at 5°C. Overall, *In* seedlings showed more growth at 25°C than other rootstocks, and inter-specific hybrids showed better growth characteristics than seedlings obtained from pistachio cultivars. *In* seedlings growth almost stopped at 5°C, but pistachio seedlings continued growing despite the low temperature. In most traits, hybrid cultivars were intermediate between their parents, indicating the suitable inheritance of growth characteristics from *In* and cold resistance from *P. vera*. The hybrid between 'Khanjari-Ghermze' ×*In* showed good growth in the early growing stages under chilling temperature and could be a suitable rootstock for further research on interspecific hybrid.

Introduction

Pistacia is a genus in the Anacardiaceae family that consists of 11 species (Zohary, 1952; Nazoori *et al.*, 2022). All *Pistacia* species are dioecious, wind-pollinated, and require cross-pollination for fruit production (Crane and Iwakiri, 1981; Sharifkhah *et al.*, 2020; Hosseini *et al.*, 2022). Iran, the United States, and Turkey are the top producers of pistachios worldwide (FAO, 2021). Iran is second in pistachio production in the world (Norozi et al., 2019) with the pistachio industry which is playing a significant role in the Iranian economy by providing income and employment opportunities in rural areas (Taghizadeh-Alisaraei *et al.*, 2017). However, pistachio cultivation in Iran faces challenges such as climate change and water scarcity, particularly in the main cultivation areas and central desert regions (Alipour, 2018; Bagheri and Babaeian, 2020). As a result, production has expanded to higher latitudes and desert borders

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where cultivation was less prevalent in the past (Mohammadzadeh Seddigh *et al.*, 2021). Nevertheless, lower temperatures in these areas can hinder pistachio growth, making it essential to prioritize growth in colder temperatures, especially during the early growing stages of tree establishment.

Choosing the appropriate rootstock is a crucial decision when establishing a pistachio orchard. The pistachio rootstocks can significantly affect the yield of pistachio trees and their resistance to biotic and abiotic stresses (Ferguson *et al.*, 2001; Ferguson *et al.*, 2005; Gharibiyan *et al.*, 2023). Although, Iran being one of the world's primary pistachio producers, it has not given enough attention to rootstock selection (Razavi, 2005). The majority of pistachio orchards in Iran rely on seedlings of *P. vera*, such as 'Sarakhsi', 'Badami Zarand', and 'Ghazvini', as rootstocks onto which commercial or local varieties are grafted (Karimi, 2012; Vahdati *et al.*, 2021). Whilst *P. vera* rootstocks offer desirable traits like tolerance to environmental stresses such as drought, salinity, and cold, they also have some drawbacks, including slow growth and susceptibility to soil diseases (Behzadi Rad *et al.*, 2021; Mir *et al.*, 2023; Mohit Rabari *et al.*, 2023). In America, hybrid rootstocks from different pistachio species are used in many pistachio orchards because of their desirable growth characteristics, better nutrient absorption, and resistance to certain diseases such as Verticillium wilt. (Ferguson *et al.*, 2005). For example, the famous UCB_1 rootstock in USA pistachio industry is a hybrid of *P. atlantica* and a specific male parent from *P. integerrima* (*In*) (Ferguson *et al.*, 2005). The In species has been widely used as a parent in most intergeneric hybrids of pistachio because of its fast growth and soil disease resistance (Kallsen and Parfitt, 2011; Akbari *et al.*, 2020). However, this rootstock is sensitive to environmental stresses, including salinity, drought, and cold stresses (Ferguson *et al.*, 2005; Zhang *et al.*, 2021). It appears that hybrids between *P. vera* and *In* might be suitable options for the Iranian climatic conditions, as they can possess high growth power and disease resistance from *In* and environmental stress tolerance and graft compatibility from *P. vera*.

To produce hybrid seeds, information about pollination techniques, seed set, and seedlings survival rates are of great importance (Hancock, 2008). In inter-specific hybrids, due to the incomplete match between parents, the percentage of healthy seed and their fertility greatly decreases (Dehghani Shuraki, 2001). In addition, the amount of seed production varies significantly among different parent genotypes. Furthermore, techniques for preserving young seeds in hybrids are valuable and can help improve performance and maintain hybrid seed production. The aim of this study is to evaluate the production of hybrid seeds from crosses between different domestic pistachio varieties and *In*, and to examine the initial growth of seedlings under varying temperature conditions. This research aims to develop a hybrid rootstock that is fast-growing, resistant to environmental stressors, and can be grafted in the first year.

Materials and Methods

Plant Materials and Experimental Design

In this study, hybrid seeds were generated from the female parents including 'Kaleh-Ghuchi', 'Khanjari-Ghermze', 'Akbari', 'Khanjari-Sefid', 'Ohadi' and a local variety (cultivars commonly grown in Damghan, Iran) and the male parent from an *In* genotype collected from Shamarran area located in Hajjiabad, Hormozgan, Iran. This area is located in the southern part of Iran and at lower geographical latitudes (28°18′33″N 55°54′06″E). It has a tropical climate and the flowers of the *In* trees bloom earlier. The pollination process was carried out at Salman Farsi farm in Kola Village, Damghan, Iran. Damghan is located at a longitude of 54°20'8" N and latitude of 36°9'48" E, with an elevation of 1170 meters above sea level and a dry and semi-dry climate. The chilling experiments were conducted at the Shahrood University of Technology research greenhouse located in Bastam, Iran.

The pollen collection, Bagging, and controlled pollination

Male clusters of *In* trees were collected during the color change stage to yellow and dried on paper. The pollen was then separated by shaking and sometimes by rubbing on a fine sieve. It was then placed in a desiccator containing silica gel to reduce moisture. After 24 hours, it was transferred in a refrigerated container at 4°C to the main experimental site in Damghan, Iran and kept in a -18°C freezer until pollination. Before pollination, the pollen germination percentage was tested on a pollen culture medium. The female tree flowers were bagged before blooming. Three cc of diluted pollen with wheat flour was poured into a 50 cc syringe. The syringe needle was then inserted into a bag and the piston was repeatedly pressed inside the cylinder to perform pollination by spraying with air injection. This process was repeated three times: during early flowering, and again after 12 and 24 hours. After the fruit set and observing small fruit let, the cover over the cluster was removed. Pollination was performed on four branches containing 1 to 3 clusters in each parent plant. The seeds resulting from pollination were harvested in normal harvesting time.

Germination and Seedling Growth

Prior to the germination process, both hybrid and pistachio cultivars' seeds were subjected to disinfection using a 0.2% Benomyl fungicide solution and a 10% sodium hypochlorite solution, followed by soaking in sterile water for 24 hours. The seeds were then wrapped in moist towels and placed in the laboratory at a temperature of 25°C. After 48 hours, the germinated seeds were collected and planted in polyethylene bags (10×40 cm) containing a mixture of cocopeat$^{1/2}$ and perlite$^{1/2}$. The pots were watered regularly once the soil surface was dry, on a weekly basis. After the third true leaf emerged, the seedlings were fertilized with a 5-10-5 complete fertilizer. The hybrid seedlings were identified based on leaf shape after germination to ensure successful hybridization and obtain hybrid seedlings between the pistachio and *P. integerrima* species (Fig. 1).

Fig. 1. Left image: Two-month-old seedlings in plastic bags, 'Khenjari-Ghermez' pistachio species with simple leaves on the right, the *Pistacia integerrima* species on the left with small and clustered leaflets, and a hybrid plant in the middle with larger leaflets. Right image: A sample of mature leaves from the 'Khenjari-Ghermez' pistachio species (right), *P. integerrima* species (left), and a hybrid between the two species (middle).

Temperature Treatments

Hybrid seedlings obtained from 'Kaleh-Ghuchi', 'Khanjari-Ghermze' pistachio cultivars as female parents and *In* as the male parent was used in this test. The study was performed in a factorial design. The first factor was the pistachio rootstocks consisting of five levels: 'Kaleh-Ghuchi', 'Kaleh-Ghuchi' x *In*, 'Khanjari-Ghermze', 'Khanjari-Ghermze' x *In*, and *In*. The second factor was temperature, with two levels (an ambient temperature of 25 ± 5°C and a temperature of 5 ± 2°C for two weeks in the growth chamber) replicated five times. Twenty-five uniform seedlings from each rootstock were prepared in plastic bags (Fig. 1) and placed under temperature treatment after reaching the fifth leaf. The temperature treatments were applied in a growth chamber with 16 h light, 8 h darkness, and a light intensity of 8000 lux. After the temperature treatment, the seedlings were transferred to the field for further evaluation.

Measured Traits

After harvesting hybrid seeds, the amount of healthy seeds, the percentage of bank and malformed nuts in each cross were measured (Fig. 2).

Growth Indices

To measure the growth rate of the seedlings under temperature treatments, the relative growth rate (RGR) was calculated based on stem length, and number of leaves and leaflet using the following formula: Plant height RGR: (H2-H1)/n; RGR based on the number of leaves and leaflets: (L2-L1)/w. Here, H1 and L1 refer to the height or number of leaves or leaflets at time 1, and H2 and L2 refer to the height or number of leaves or leaflets at time 2. Additionally, n refers to the number of days, and w refers to the number of weeks.

At the end of the summer, plant height, stem diameter, number of lateral shoots, and number of main shoots were measured in the seedlings grown in the field.

Chlorophyll content and chlorophyll fluorescence

Chlorophyll content was measured using a SPAD meter (Minolta SPAD-502). Initially, the device was calibrated by taking a reading without placing a leaf in the chamber. Then, three readings were taken from each mature leaf at three different points, and the average of the three points was determined using the "average" button. It should be noted that leaf sampling was not done on the midrib.

Chlorophyll fluorescence was measured in fully developed leaves of the upper part of each plant. Fluorescence parameters of chlorophyll were measured using a MINI-PAM-II fluorometer (Welz-Germany). Measurements were taken under adaptation to darkness. A part of the leaf was covered with a special clip for 30 minutes and adapted to darkness. Then, the fiber-optic tube of the PAM-II device was connected to the clip, and by setting the device to a wavelength of 695 nanometers (light act), it was irradiated onto the leaf. The photochemical quantum yield (Y_{II}) was measured. Measurements were taken twice for each seedling, and the average was used for analysis.

Leaf Nitrogen, Potassium, and Phosphorus

The concentrations of nitrogen (N), phosphorus (P), and potassium (K) were measured in the leaf tissue of each rootstock after temperature treatments. For each treatment, fully mature leaves were sampled from the middle part of the plant and oven-dried at 80°C for 48 hours. The dry samples were ground to pass through a 0.5-mm mesh sieve. 0.3 g of dry samples of leaves were soaked in 10 mL of sulfuric acid for 24 hours, and then digested in a digestion system in a fume hood, heated to 180°C for 3 hours, followed by the addition of 5 mL of hydrogen peroxide. The extracted solution was transferred to 100-mL volumetric flasks, diluted to 100 mL with deionized water for N, P, and K assays. The N was analyzed using the automatic Kjeldahl apparatus method (Vapodest 20S). P was measured using the molybdate-blue colorimetric method (Murphy and Riley, 1962). K were determined by atomic emission spectrometry using a flame photometer (Jenway, U.K).

Statistical analysis

A factorial experiment was conducted based on a completely randomized design with three replications. The treatment factors were five rootstocks and soil temperature (5 and 25°C); with each plot containing five seedlings. Statistical analysis was performed using SAS 9.1, and mean comparisons were conducted using the Duncan test.

Results

Fruit set and Blank nuts

As shown in Table 1, among the various crosses made between *In* and the six cultivars used in this experiment, the crosses of 'Kaleh-Ghuchi' × *In* and 'Khanjari-Ghermze' × *In* hybrids resulted in the highest number of fruit set, with 116 and 264 nuts, respectively. It is worth noting that in the same cultivars (female parent) in the trees of the same orchard, between 720 to 1150 nuts are normally formed. Additionally, the seed set through hybridization exhibited a high degree of deformity and blindness, as shown in Fig. 2.

Table 1. The number of healthy and blank seeds of pisachio cultivars and interspecific hybrids resulting from crosses between Iranian pistachio cultivars with the pollen of the *Pistacia integerrima*

Rootstock	Total final nut (number)	Malformed and blank nuts (%)	Healthy seed (number)
'Akbari'× *In*	14	50	7
'Akbari'	***810***	***86***	***700***
'Kaleh-Ghuchi'×*In*	116	62	43
'Kaleh-Ghuchi'	***980***	***81***	***795***
'Khanjari-Ghermze' × *In*	264	78	57
'Khanjari-Ghermze'	***1150***	***82***	***950***
'Khanjari-Sefid' × *In*	25	72	7
'Khanjari-Sefid'	***1100***	***84***	***920***
Local variety× *In*	41	95	2
Local variety	***720***	***70***	***510***
'Owhadi'× *In*	50	82	9
'Owhadi'	***970***	***80***	***780***
Total	510	75	125

In: Pistacia integerrima*. The data in ***italic* and ***bold*** font is related to pistachio cultivars in open pollination during one year

Fig. 2. Images of blank and shriveled seed samples in pistachio interspecific hybrids. A: Blank seeds in *'Kaleh-Ghuchi'* ×*In* hybrid, B: Shrivelled and adhered hull to shell in blank seeds contrast to healthy normal seeds in 'Owhadi'× *In* hybrid, C shriveled and blank seeds in Khanjari – Ghermez × *In* hybrid**.** The arrow in images A and C indicate the effect of *In* pollen on the malformation of the hard shell in pistachio nuts.

Seed germination and seedling establishment

Table 2 shows the percentage of germination and seedling establishment in interspecific hybrids of pistachio with *In* and two pistachio cultivars, 'Kaleh-Ghuchi' and 'Khanjari-Ghermze'. The results indicate that both 'Kaleh-Ghuchi' and 'Khanjari-Ghermze' had high seed germination rates of 90% and 94%, respectively, and also high seedling establishment rates of 88% and 95%, respectively. On the other hand, the hybrids of 'Kaleh-Ghuchi'×*In* and 'Khanjari-Ghermze'×*In* had lower germination rates of 58% and 56%, respectively, which could indicate some incompatibility issues between the pistachio cultivars and Pistacia integerrima. However, the 'Kaleh-Ghuchi'×*In* hybrid had a relatively good seedling

establishment rate of 72%, while the 'Khanjari-Ghermze'×*In* hybrid had a lower seedling establishment rate of only 56%. The seeds of *In* had a germination rate of 75%, but a lower seedling establishment rate of 40%.

Table 2. Percentage of Germination and Seedling establishment in interspecific hybrids of Pistachio with *Pistacia integerrima* (*In*) and two pistachio cultivars.

Rootstock	Seed germination (%)	Seedling establishment (%)
In	75	40
'Kaleh-Ghuchi'	90	88
'Kaleh-Ghuchi' ×In	58	72
'Khanjari-Ghermze'	94	95
'Khanjari-Ghermze' × In	56	56

The chilling experiments

Growth of pistachio seedlings

The successful cultivation of pistachios relies heavily on the growth of pistachio seedlings. Two months after planting, there were noticeable differences in growth among the pistachio bushes, with *In* seedlings having an average height of around 50 mm, while the pistachio cultivars and hybrids had an average height of about 100 mm (Fig. 1). Therefore, to better investigate the effect of temperature, our study evaluated the relative growth rate (RGR) index of several pistachio rootstocks. Our results show that low temperatures have a significant impact on the RGR of pistachio seedlings during the initial growth stages ($P<0.01$). This finding underscores the importance of carefully selecting planting times and locations to ensure optimal growth conditions for pistachio seedlings. Interestingly, at normal temperatures, the *In* species had a higher RGR based on height than other pistachio rootstocks (Fig. 3). However, this index decreased significantly at low temperatures. In contrast, hybrids performed better than other rootstocks in terms of leaf and leaflet number, especially at low temperatures, although the difference was not statistically significant.

Six months after being transplanted to the field, the height, diameter, and sucker count of the pistachio seedlings were assessed (Fig. 4). The 'Khanjari-Ghermze' rootstock had the tallest height, reaching 745 mm, while the Kh×*In* hybrids and 'Kaleh-Ghuchi' were similar in height to the 'Khanjari-Ghermze' rootstock. The Kal×*In* hybrids and *In* rootstock had the shortest heights. The 'Khanjari-Ghermze' and Kh×In hybrids had the largest stem diameters, with no significant difference between them. The *In* and Kal×*In* hybrids had the smallest stem diameter. The *In* rootstock produced the fewest number of suckers, showing a significant difference from other rootstocks. 'Kaleh-Ghuchi' and 'Khanjari-Ghermze' had the highest number of suckers, with their hybrid rootstocks following the *In* in ranking. These four rootstocks did not show a significant difference in the mean comparison chart (Fig. 4).

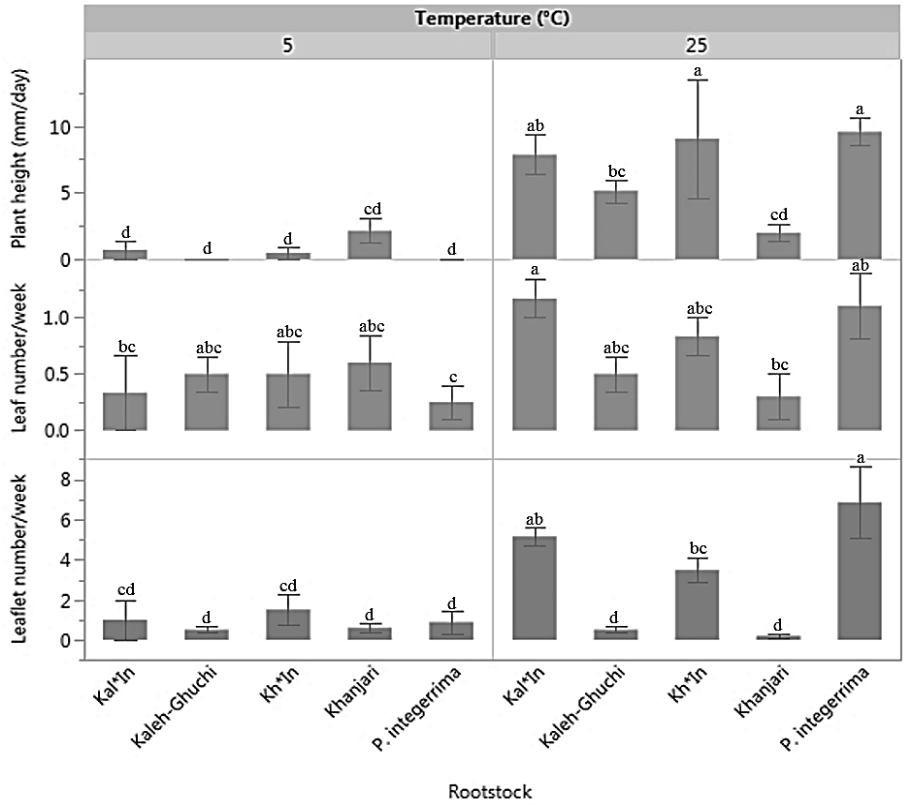

Fig. 3. The relative growth rate (RGR) based on the plant height, number of leaves, and leaflets of the *P. integerrima*, *P. vera* cultivars (Kaleh-Ghuchi and Khanjari), and thiers hybrids at 25 and 5°C in the initial stage of seedling growth. In: *Pistacia integerrima*

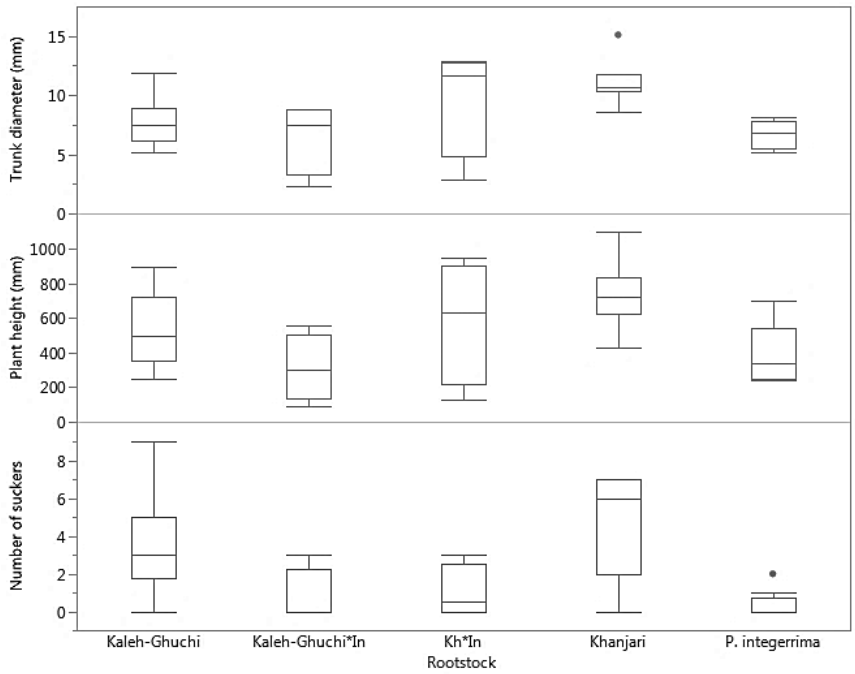

Fig. 4. The number of suckers, plant height, and diameter of pistachio seedlings were evaluated six months after transplantation in the field. In: Pistacia integerrima

Chlorophyll and Chlorophyll Fluorescence

The amount of chlorophyll is shown based on the SPAD index in the Fig. 5. In general, the *In* specie has a lower chlorophyll index than other rootstock, and this effect is also seen in hybrids. The 'Khanjari-Ghermze' and 'Kaleh-Ghuchi' rootstocks recorded the highest SPAD values of 51.5 and 50.1 respectively. *In* had the lowest SPAD value with an average of 25. In cold temperatures, chlorophyll levels in the 'Khanjari-

Ghermze' and 'Kaleh-Ghuchi' rootstocks significantly decreased, while hybrids showed a slight increase in the chlorophyll index. It is possible that the increase in greening could be due to the decrease in leaf area and cell size. The level of chlorophyll fluorescence varied from 0.61 to 0.81 with an average of 0.74. This index clearly showed the effect of chilling stress on the studied rootstocks, and photosynthetic efficiency had been significantly reduced at 5°C. The lowest level of chlorophyll fluorescence was observed in *In* with an average of 0.63 at 5°C.

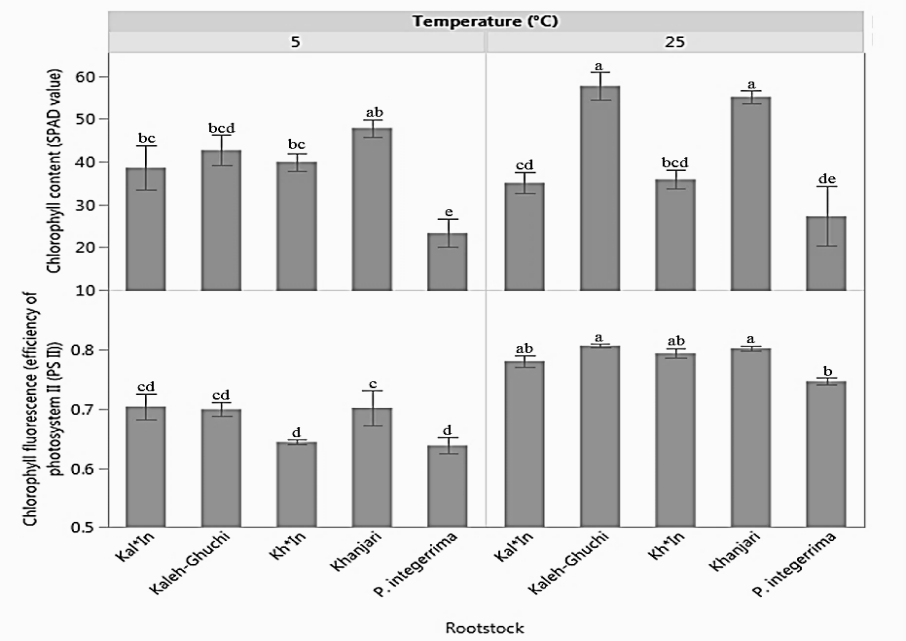

Fig. 5. Chlorophyll content and Chlorophyll Fluorescence values in the *P. integerrima*, *P. vera* cultivars (Kaleh-Ghuchi and Khanjari), and thier hybrids at 25 and 5°C. *In: Pistacia integerrima*

Laef N, P, K in rootstock

The results of measuring nutrient elements in pistachio rootstock leaves presented in Table 3. Among the studied rootstocks, the *Kh×In* hybrid had the highest percentage of N in the leaves, while the *'Khanjari-Ghermze'* rootstock had the lowest but there were no significant differences between rootstocks. *'Kaleh-Ghuchi*' had the highest percentage of P, while the *Kh×In* hybrid had the lowest significantly. The *'Khanjari-Ghermze'* rootstock had the highest percentage of K, and the *'Kaleh-Ghuchi'* rootstock had the lowest percentage of K in the leaves.

Table 3. The leaf N, P and K in the *Pistcia integerrima*, *P. vera* cultivars (Kaleh-Ghuchi and Khanjari), and thier hybrids.

Rootstock	N(% DW)	P(% DW)	K(% DW)
In	3.33 a	0.29 ab	1.05 a
'Kaleh-Ghuchi'	3.60 a	0.41 a	0.95 a
'Kaleh-Ghuchi' ×In	3.53 a	0.30 ab	1.04 a
'Khanjari-Ghermze'	3.26 a	0.30 ab	1.31 a
'Khanjari-Ghermze' × In	3.70 a	0.22 b	1.22 a

In: Pistacia integerrima

Discussion

In the results, we observed significant variations in fruit set among the different crosses involving *In* and the six pistachio cultivars under investigation. Notably, the hybrids produced by crossing 'Kaleh-Ghuchi' with *In* and 'Khanjari-Ghermze' with *In* showed the highest fruit set, and the percentage of

blank nuts varied between 50 to 95 percent in the crosses. Furthermore, the seed set resulting from hybridization displayed a notable degree of deformity and blindness, as visually represented in Figure 2. The use of pollen grains from different *Pistacia* species for pollination has been of interest to various researchers (Riazi and Rahemi, 1994; Ak, 1997, 2001, 2010). However, several studies have shown that the pollen of *Pistacia* species other than *Pistacia vera* is undesirable for pollination purposes (Riazi and Rahemi, 1994). Cross-pollination of Pistachio cultivars with species other than *Pistacia vera* can result in metaxenia, which is the effect of pollen on fruit shape (deformity and blank nuts) (Crane and Iwakiri, 1980). Pollen from non-vera species also inferences kernel weight or increases the percentage of embryo abortion and the production of blank nuts, known as xenia effects(Crane and Iwakiri, 1980). Previous studies on xenia and metaxenia in pistachio have mainly used pollen from *P. atlantica* and *P. terebinthus* for cross-pollination (Ak, 2001; Afshari *et al.*, 2008; Ak, 2010). However, it has been found that pollen from *P. integerrima* also significantly increases the percentage of deformity and embryo abortion compared to normal self-pollination. It should be noted that in some cases, fruit or embryo abortion in the crosses of this experiment may be caused by stress resulting from artificial pollination, insufficient pollination, or flower abortion resulting from excessive pollen shedding on the stigma surface. This phenomena can also be influenced by the maternal parent genotype, and pistachio cultivars and genotypes may show different percentages of blankness, even under similar nutritional and alternate bearing conditions (Rezaei *et al.*, 2019).

The results presented in Table 2 provide insights into the percentage of germination and seedling establishment among interspecific hybrids of pistachio. It is noteworthy that 'Kaleh-Ghuchi' and 'Khanjari-Ghermze' exhibited robust seed germination, demonstrating their high potential for successful seedling establishment (Table 2). Conversely, the hybrids 'Kaleh-Ghuchi'×*In* and 'Khanjari-Ghermze'×*In* showed comparatively lower germination rates. These lower germination rates may suggest potential compatibility issues between these pistachio cultivars and *In*. The seeds of *In* had lower germination rate and seedling establishment (Table2). The genetic differences or incompatibilities between the parents of an interspecific hybrid plant can result in reduced germination rates or lower seedling survival (Andersson *et al.*, 2008). Additionally, the successful formation of hybrid plants can be limited by post-fertilization barriers, such as embryo and endosperm abortion, abnormal growth and inviability of the hybrids, or their sterility. These barriers may further reduce the viability of the hybrid offspring, limiting their potential for growth and survival (Rieseberg and Carney, 1998).

Despite the initial higher growth rate of *In* seedlings observed under normal temperature conditions, this RGR index experienced a significant decline when exposed to low temperatures. Conversely, the 'Khanjari-Ghermze'×*In* performed better than other rootstocks in terms of leaf and leaflet number, especially at low temperatures, although the difference was not statistically significant. The 'Khanjari-Ghermze' is a local variety in the Damghan region (Abdoshahi *et al.*, 2011), where has shorter summers and cooler weather than other main pistachio production regions in Iran such as Rafsanjan and Kerman. This finding suggests that the 'Khanjari-Ghermze' may be a good choice for pistachio cultivation in similar climates.

The examination of both chlorophyll content and chlorophyll fluorescence, as depicted in Figure 5, offers valuable insights into how the studied pistachio rootstocks respond physiologically to varying temperature conditions. Notably, the *In* species consistently exhibited a lower chlorophyll index compared to other rootstocks, a trend also observed in the hybrids. 'Khanjari-Ghermze' and 'Kaleh-Ghuchi' rootstocks, on the other hand, registered the highest SPAD values, underlining their relatively robust

chlorophyll content. Furthermore, chlorophyll fluorescence measurements revealed a clear impact of chilling stress on the investigated rootstocks. The chlorophyll fluorescence levels ranged from 0.61 to 0.81, with an average of 0.74, underscoring the sensitivity of this index to stress conditions. Particularly at 5°C, there was a notable reduction in photosynthetic efficiency across all rootstocks. The *In* exhibiting the lowest level of chlorophyll fluorescence, averaging 0.63 at 5°C. Chlorophyll fluorescence can measure most types of plant stress (Roháček *et al.*, 2008). Chlorophyll fluorescence can be used as a plant stress indicator because non-living environmental stresses can reduce the plant's natural metabolism (Murchie and Lawson, 2013). This could mean an imbalance between chlorophyll energy absorption and energy use in photosynthesis (Roháček *et al.*, 2008; Murchie and Lawson, 2013). However, since photosynthetic efficiency also varies with light intensity, samples should be compared at the same light intensity (Murchie and Lawson, 2013). Chlorophyll fluorescence is a suitable tool for studying photosynthetic efficiency under stress conditions and investigating the potential of different treatments to improve the photosynthesis process(Roháček *et al.*, 2008).

The analysis of nutrient elements in pistachio rootstock leaves, as presented in Table 3, provides valuable insights into the nutrient profiles of the studied rootstocks. Notably, among the rootstocks examined, the 'Khanjari-Ghermze'×*In* hybrid displayed the highest percentage of N in the leaves, whereas the 'Khanjari-Ghermze' rootstock had the lowest percentage. In terms of P content, 'Kaleh-Ghuchi' had the highest percentage, while the 'Khanjari-Ghermze'×*In* hybrid exhibited the lowest P content. Interestingly, the 'Khanjari-Ghermze' rootstock had the highest K content, while the 'Kaleh-Ghuchi' rootstock had the lowest K content in the leaves. Efficient nutrient uptake could be advantageous for pistachio trees, especially in dry climates and under dry soil conditions (Murchie and Lawson, 2013). The results indicated that the absorption of N and K was generally similar among the examined rootstocks. However, there was a significant decrease in P absorption specifically in the *'Khanjari-Ghermze'* × *In*. The hybridization process, as examined in this study, did not have a detrimental effect on the efficiency of N and K absorption.

Conclusions

The study investigated the effects of cross-pollination between *Pistacia integerrima* and five pistachio cultivars, on fruit set, seed germination, and seedling establishment. The results showed that the hybrids had a high degree of deformity and blindness, resulting in a significant percentage of blank nuts. The germination rates and seedling establishment varied among the different rootstocks. Additionally, the study evaluated the effects of chilling stress on plant growth and photosynthetic efficiency, with the hybrids showing better performance than other rootstocks in terms of leaf and leaflet number. Hybrid rootstocks demonstrated a favorable potential for mineral absorption, particularly in terms of nitrogen and potassium. However, there was a significant decrease in phosphorus absorption observed in the *'Khanjari-Ghermze'* × *In*. Overall, the study provides valuable insights into the challenges and opportunities of cross-pollination and hybridization in pistachio cultivation, and highlights the need for further research in this area.

Conflict of interest

There is no conflict of interest.

Reference

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