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Enhancing the germination of seeds and the seedling growth and development of *Pistacia khinjuk* stocks via a seed dormancy breaking method

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Summary

The behaviour of individuals with adaptations that will ensure survival is of critical importance for the continuation of species during periods when environmental factors reach challenging levels for living beings. In seed plants, this behaviour is achieved through dormancy, in which vital functions are reduced to a minimum. Seed dormancy is not germinating of seeds despite favorable environmental conditions due to internal and external factors. The impermeable and hard seed coat prevents or delays germination in wild *Pistacia* seeds, causing problems in the production of rootstocks in the desired numbers. In the present study, dormancy-breaking methods including treatment with sulfuric acid, Gibberellic acid (GA_3), 6-Furfurylaminopurine (kinetin), 6-Benzylaminopurine (BAP) and their combination, were tested on *P. khinjuk* seeds stored at 4 ºC and 25 ºC for 6 months after harvest. The seeds were then allowed to germinate for 45 days in sand-filled seedling trays. The germinated seeds were transferred to pots, and the contents of dry and fresh weight, total soluble sugar and protein, chlorophyll a, b and carotenoid of the seedlings were measured after 28 days of the growth and development. It was found that the highest germination rate, dry and fresh weight, total soluble sugar and protein and chlorophyll a,b contents were obtained from seeds that treated with scarification+GA3+ BAP and stored at 25 ºC for 6 months.

Keywords: BAP, carotenoid, dry weight, GA_3, protein, sapling

Introduction

Pistachio (*Pistacia vera* L.) is a product with very high commercial value since it can be consumed in the daily diet, confectionery, baked foods, and ice cream or as a nuts (Tous and Ferguson, 1996). Its commercial value is gradually increasing, with an annual gross value of 6 billion dollars in the 2022-2023 season (Shahbandeh, 2023). The United States is the largest producer and exporter, followed by Iran, and Turkey (Shahbandeh, 2023). Pistachio rootstocks are conventionally propagated by seeds. However, their germination rates are very low, which significantly limits mass propagation. This situation has been identified as a major problem for pistachio producers (Labdelli, 2019). Although mass propagation of these rootstocks by cuttings is not as successful as in other tree species (Serdar and Fulbright, 2019), new propagation methods are being explored. Micropropagation is a biotechnological method that enables mass propagation of genetically superior and disease-free woody plant species in a limited time and area. Although it has been used to propagate *P. atlantica* Desf., *P. vera*, *Pistacia Lentiscus*, *P. terebinthus*, and *P. khinjuk* species (Al-Safadi and Elias, 2010), their rootstock production has not yet been widely adopted in the world or in Turkey. The reason for this is the culture conditions created for micropropagation due to high labor and production costs, low levels of the growth and development, plant loss due to *in vitro* contamination, poor rooting, and low survival rates during acclimatization, the most important stage (Kozai and Kubota, 2001).

The rapid and economical mass propagation of these species is not only for use as pistachio rootstocks. Additionally, many countries are focusing on second-generation biofuels to develop a sustainable biofuel industry due to the increasing energy demand and rising oil prices worldwide (Faaij and Londo, 2010). *P. chinensis* is a species that can grow in temperate tropical and subtropical areas and is well-adapted to arid and poor soils. *P. chinensis* also has comprehensive advantages in terms of oil yield (seed oil content, 35-42%) and conversion rate, biodiesel quality, geographical distribution, and economic benefits (Tang et al., 2012). According to Tang et al. (2012), this plant species can be used as an important source of biodiesel in China. Similarly, the oil content of *P. khinjuk* (Saraee, 2017) and *P. vera* seeds is around 35-40% (Satil et al., 2003) and 40-63% respectively. Artificial forests are being created for the second-generation biofuel sources in mountainous regions where agriculture is not possible. In China the species *P. chinensis* (Parfitt, 2003) is used, while in Iran *P. atlantica* is being used (Pourreza et al., 2008). Considering the need for pistachio rootstocks, the significance of the species as biofuel, or its ecological importance, there is a need for a large number of wild *Pistacia* species saplings for new plantations.

The most practical method to obtain the desired number of saplings quickly is by increasing seed germination and stimulating seed growth and development. Several studies have investigated the effects of dormancy-breaking factors such as scarification, cold stratification, and GA_3 treatment on the germination of *Pistacia* seeds. Nevertheless, it is necessary to conduct new studies to enhance the dormancy-breaking factors that will cause strong growth and development in these rootstock seedlings. However, no detailed study has been reported to date on the effects of dormancy-breaking methods on *P. khinjuk* seed that is stored at 4 ºC and 25 ºC after harvest, germination and sapling growth. In the current study, seed germination rate, dry and fresh weight of seedlings and total soluble sugar and protein, chlorophyll a,b, and carotenoid contents were measured to determine the effects of dormancy-breaking applications on the germination and the seedling growth and development of *P. khinjuk* seeds stored at 4 °C and 25 °C for 6 months after harvest.

Materials and methods

Plant material

Seeds were harvested randomly from the trees of Çimenli village of Hakkari province (latitude 37º29’06’’N, longitude 43º37’57’’E, altitude 1144 m) in october 2020. Seeds stored at 4 ºC in the refrigerator and 25 ºC in the greenhouse for six months after harvest and were subjected to experimental tests. After being soaked in tap water for 1 day, the fruits were removed and dormancy breaking methods were applied (except for the control of applied groups). Each treatment was carried out with three replicates of 100 seeds. The seeds were subjected to dormancy breaking methods, and then planted in sand-filled seedling trays. The seedlings were watered with 100 ml every other day with tap water for 45 days. The germinated seeds were then transfered to 2-liter pots (14 × 17 cm) containing a mixture of soil,

peat, and perlite (2:1:1 by volume) under greenhouse conditions for 28 days to promote seedling growth and development.

Pre-germination treatments

Control group; The seeds were soaked in tap water for 1 day and the fruits were removed by hand. Then seeds were washed and soaked in the tap water for 24 h.

Scarification; After fruits removed as in the control group, the seeds were submerged in sulphuric acid (95-97%) for 30 minutes and washed and soaked in tap water for 24 h (Baninasab and Rahemi, 2001).

GA3, Kinetin, BAP; After scarification, the seeds soaked in the 500 mg/L GA_3, Kinetin, BAP and their combination for 24 h, then washed and soaked in tap water for 24 h (Baninasab and Rahemi, 2001).

Germination was determined by the formation of roots, stems and leaves of the embryo.

Germination percentage; Number of seeds forming seedlings / Total number of seeds X100

Seedling fresh weight; after removing the dust and soil from the seedling, it was weighed on a digital balance which a precision sensitive to 0.001 grams.

Seedling dry wight; the sapling was wrapped in a paper bag and dried in an oven at 70 °C for 72 h and then it was weighed on a digital balance witha precision sensitive to 0.001 grams. Fresh and dry weight measurements were made on the seedlings obtained in each treatments.

Determination of biochemical parameters

The chlorophyll (chl) and carotenoid contents were extracted using 80% acetone from the fresh leaves of *P. khinjuk* seedlings. The absorbance of the extracts was measured with a spectrophotometer at 480, 663 and 645 nm and recorded. Chlorophyll a, chlorophyll b, and carotenoid contents were calculated as previously described (Arnon, 1949). The concentrations of chlorophyll a, chlorophyll b and carotenoid were calculated using the following equation:

Chlorophyll a: 12.7(A663) – 2.69(A645)

Chlorophyll b: 22.9(A645) – 4.68(A663)

Carotenoid: [A480 +(0.114(A663)-(0.638-A645)]×V/1000×W

The total soluble sugar content was measured using the phenolsulphuric acid method (Dubois et al., 1956). 100 mg of fresh leaf samples were homogenized in 2 ml of 80% ethanol in mortar. The homogenate was incubated for 30 minutes in a water bath at 75 °C and then centrifuged at 2236 g · 1 ml of the supernatant, 1 ml of 5% phenol, and 5 ml of concentrated sulphuric acid (H_2SO_4) were added to the test tubes and mixed by vortexing. The absorbance of the reaction mixture was read by a spectrophotometer at a wavelength $\lambda = 490$ nm, and the total soluble sugar content was expressed as mg g^{-1} dry weight.

To determine the total soluble protein content, 1 g of fresh leaf samples was taken. Leaf samples were homogenized with a chilled pestle and mortar in 5 mL of extraction buffer (0.1 M phosphate buffer, pH 7.0), containing 10 mM KCl, 1 mM $MgCl_2$, 10 mM Na_2EDTA, and 1% polyvinyl poly pyrrolidone (PVPP), and centrifuged. After centrifugation of the homogenate, the supernatant phase was taken for protein content determination. The protein content was found using a standard curve prepared with Bovine Serum Albumin (BSA) and expressed as µg g^{-1} fresh weight (Bradford, 1976). Of the fresh plant sample, 0.5 g was homogenized in 100 mM phosphate buffer (pH 7.0) and centrifuged at +4 °C and 17530 g for 20 minutes. 20 µl of the supernatant was taken, 480 µl of distilled water and 5000 µl of Bradford solution were added to it, respectively, and the absorbance was measured with a UV-Vis spectrophotometer at a wavelength of 595 nm.

Determination of triphenyl tetrazolium chloride test for seed viability

Seed germination and viability test in 1% 2,3,5 triphenyl tetrazolium chloride (TZ) Assay (Verma and Majee 2013) method was modified for *P. khinjuk*. For negative control seeds (approximately 100 seeds) killed with head (100 °C, 1 h) in drying oven. To prepare 1% Tetrazolium (TZ) solution, added 1 g 2,3,5 triphenyl tetrazolium chloride in 100 ml autoclaved distilled water and dissolved. The pH of TZ was adjusted 6. Firstly, approximately 100 seeds were incubated in distilled water for hydration 30 °C for 24 h. Secondly the seeds were sterilized with 20% 100 ml commercial sodium hypochlorite for 10 minutes than washed at least three times with sterile distile water to remove sodiumhypochlorite. After sterilisation, excess water was removed and the seeds incubated with 1% TZ solution at 30 °C for 24 h in dark. Viability percent was calculated according to AOSA (2005).

Data replication and statistical analysis

The data were analyzed by employing the standard analysis of variance (one-way ANOVA) procedure. The mean separation was checked by Post hoc Multiple Comparisons Duncan's multiple range test. The signifcance level was set at $P< 0.05$. The results were expressed with standard error (SE). Statistical analysis was performed by using the SPSS version 16.0 for Windows.

Results and discussion

Germination of *P. khinjuk* seeds

The present study found important differences in germination rates of *P. khinjuk* seeds depending on treatments and storage temeprature. This study clearly observed that (1) Scarification+GA_3+BAP combination reached the highest germination rate in seeds stored at both 4 ºC and 25 ºC, (2) the temperature of storage on the 25 ºC more effective than on the 4 ºC and (3) kinetin treatment was not effective for germination of *P. khinjuk* seeds (Tab. 1). It was understood that the temperature of storage conditions change endogenous plant growth regulators particularly ABA/GA_3 contents of seed (Su et al., 2016). Also, the exogenous treatment of different stimulants and inhibitors could effect the endogenous plant growth regulators levels and rates in the seeds and thus rises the germination rates (Baninasab and Rahemi, 2001). Our results are in agreement with those of Moradi and Otroshy (2012), who found that BAP was more efficient than kinetin and GA_3 in promoting seed germination. In another study, Kadhim (2023) reported that the combination of GA_3 and kinetin increased the germination rate in both cultivars of pistachio. But, contrary to Kadhim (2023), we found that kinetin application reduces the germination rate of *P. khinjuk* seeds. The combination of GA_3 and BAP was found to be more effective than GA_3 or BAP alone in promoting seed germination. Scarification with sulfuric acid was found to increase the germination percentage by thining the hard coat and improving water and gas permeability (Guo et al., 2022). The data presented in Tab. 1-2 indicate that the scarification treatment improved seed germination rate of our seeds. These results are in agreement with the findings of Acar et al. (2017), Chebouti-Meziou et al. (2014) and Guo et al. (2022) who described the positive effect of sulfuric acid scarification method on *P. khinjuk, P. atlantica* and *P. chinenesis* seed germination respectively. In our study, it is seen that scarification application were caused a significant increase in seed germination rate compared to seed of Control, GA_3, Kinetin

Tab. 1: Effect of dormancy breaking treatments on *P. khinjuk* seedlings dry and fresh weight and seeds germination

	Germination* (%)	Seedling Dry Weight* (g)	Seedling Fresh Weight* (g)
		4 °C	
Seed stored time (6 months)			
Control	15±**2.5**^{f}	3.03±0.8^{c}	4.15±**0.7**^{e}
GA_3	15±**2.3**^{f}	3.05±0.7^{c}	4.13±0.2^{e}
BAP	16±**2,6**^{f}	3.15±**0**.8^{c}	4.31±0.5^{de}
Kinetin	15±**1.1**^{f}	3.10±**0**.18^{c}	4.09±0.5^{e}
Scarification	27±**2.6**^{cde}	3.18±0.9^{c}	4.37±0.4^{de}
Scarification+ GA_3	28±**1.1**^{cde}	4.10±0.1^{b}	5.73±0.5^{c}
Scarification+BAP	29±2.08^{cde}	4.18±**0**.1^{b}	4.70±0.5^{d}
Scarification+Kinetin	15±**1.5**^{f}	3.10±**0**.2^{c}	4.39±0.5^{de}
Scarification+GA_3+BAP	34±**2.3**^{bc}	4.44±**0**.2^{ab}	6.23±0.5^{b}
Scarification+GA_3+Kinetin	28±**1.1**^{cde}	3.20±**0**.1^{c}	4.49±0.5^{de}
Scarification+GA_3+BAP+Kinetin	29±**2.3**^{cde}	3.23±**0**.2^{c}	4.63±0.5^{d}
		25 °C	
Seed stored time (6 months)			
Control	24±**1.1**^{e}	3.12±0.8^{c}	5.62±0.8^{c}
GA_3	24±**2.4**^{e}	3.20±0.1^{c}	4.70±0.2^{d}
BAP	24±**2.9**^{e}	3.15±**0.1**^{c}	5.70±0.5^{c}
Kinetin	23±**3.6**^{e}	3.14±**0.1**^{c}	5.59±0.5^{c}
Scarification	33±**1.5**^{bcd}	3.20±0.0^{c}	4.74±0.3^{d}
Scarification+ GA_3	37±**1.1**^{ab}	4.32±0.1^{b}	6.63±0.5^{ab}
Scarification+BAP	39±**2.8**^{ab}	4.45±**0**.3^{ab}	6.70±0.5^{a}
Scarification+Kinetin	23±**1.7**^{e}	3.15±**0**.0^{c}	5.50±0.5^{c}
Scarification+GA_3+BAP	42±**2.**^{a}	4.80±**0.1**^{a}	6.80±0.5^{a}
Scarification+GA_3+Kinetin	28±**1.1**^{cde}	3.12±**0**.0^{c}	5.55±0.5^{c}
Scarification+GA_3+BAP+Kinetin	26±**1.1**^{de}	3.13±**0**.1^{c}	5.67±0.5^{b}

*Means followed by the different lowercase letter in the column of each explant are significantly different at P< 0.05 according to the Duncan's Multiple Range Test.

Tab. 2: Total protein, total soluble sugar and photosynthetic pigment contents of *P. khinjuk* seedlings leaves after dormancy-breaking treatments to seeds

	Total Protein* (mg/ g FW)	Suluble Sugar* (mg/g FW)	Chlorophyll* a (mg/ g FW)	Chlorophyll* b (mg/ g FW)	Carotenoid* (µg /g FW)
		4 °C			
Seed stored time (6 months)					
Control	2.24±0.11^{h}	1.30±0.07^{j}	0.53±0.07^{c}	0.27±0.34^{abc}	10.95±0.48^{ab}
GA_3	2.36±0.18^{gh}	1.30±0.0j^{i}	0.59±0.03^{abc}	0.28±0.05^{abc}	11.05±046^{a}
BAP	2.43±**0**.47^{gh}	1.29±**0**.78^{j}	0.61±**0**.16^{abc}	0.28±**0**.36^{abc}	10.98±0.48^{ab}
Kinetin	2.24±**0**.34^{h}	1.32±**0**.67^{i}	0.58±**0**.17^{abc}	0.23±**0**.40^{bc}	10.91±0.51^{ab}
Scarification	2.49±0.02^{g}	1.42±0.02^{hi}	0.55±0.05^{bc}	0.28±0.05^{abc}	10.98±0.58^{ab}
Scarification+ GA_3	3.09±0.25^{bc}	1.81±0.04^{gh}	0.77±0.03^{abc}	0.31±0.02^{ab}	10.91±0.31^{ab}
Scarification+BAP	2.88±**0**.63^{def}	1.71±**0**.56^{hij}	0.79±**0**.56^{abc}	0.27±**0**.40^{abc}	11.03±**0**.09^{a}
Scarification+Kinetin	2.70±**0**.15^{f}	1.56±**0**.45^{hij}	0.69±**0**.52^{abc}	0.27±**0**.16^{abc}	10.95±**0**.54^{ab}
Scarification+GA_3+BAP	3.01±**0**.89de	1.87±**0**.35^{b}	0.83±**0**.25^{a}	0.31±**0**.27^{ab}	10.98±**0**.23^{ab}
Scarification+GA_3+Kinetin	2.80±**0**.76^{cd}	1.68±**0**.23^{hij}	0.69±**0**.87^{abc}	0.28±**0**.20^{abc}	11.05±**0**.61^{a}
Scarification+GA_3+BAP+Kinetin	2.85±**0**.98^{def}	1.67±**0**.19^{hij}	0.70±**0**.98^{abc}	0.27±**0**.60^{abc}	11.01±**0**.53^{a}
		25 °C			
Seed stored time (6 months)					
Control	3.13±0.13^{ab}	2.26±0.12^{def}	0.62±0.02^{abc}	0.24±0.05^{abc}	11.05±0.27^{a}
GA_3	3.14±0.36^{ab}	2.47±0.27^{bcd}	0.60±0.03 ^{abc}	0.21±0.04^{c}	10.93±0.35^{ab}
BAP	3.09±**0**.17^{bc}	2.37±**0**.16^{cde}	0.63±**0**.50^{abc}	0.22±**0**.21^{bc}	10.99±**0**.27^{ab}
Kinetin	3.01±**0**.21de	2.40±**0**.15^{bcd}	0.59±**0**.17^{abc}	0.22±**0**.45^{bc}	11.02±**0**.09^{a}
Scarification	3.01±0.25^{de}	2.18±0.12^{def}	0.55±0.05^{bc}	0.25±0.05^{abc}	11.08±0.11^{a}
Scarification+ GA_3	3.10±0.42^{bc}	2.78±0.36^{ab}	0.82±0.04^{ab}	0.30±0.06^{abc}	11.01±0.34^{a}
Scarification+BAP	3.06±**0**.52^{cd}	2.80±**0**.42^{ab}	0.80±**0**.45^{ab}	0.31±**0**.45^{ab}	11.03±**0**.24^{a}
Scarification+Kinetin	3.00±**0**87.^{de}	2.15±**0**.20^{def}	0.80±**0**.30^{ab}	0.24±**0**.36^{abc}	11.00±**0**.36^{a}
Scarification+GA_3+BAP	3.30±0.25^{a}	2.87±0.46^{a}	0.85±0.05^{a}	0.33±0.05^{a}	11.00±**0**.20^{a}
Scarification+GA_3+Kinetin	3.03±**0**.90^{ab}	2.47±**0**.30^{b}	0.79±**0**.21^{abc}	0.27±**0**.23^{abc}	10.97±**0**.19^{ab}
Scarification+GA_3+BAP+Kinetin	3.18±**0**.23^{ab}	2.59±**0**.34^{cde}	0.78±**0**.40^{abc}	0.25±**0**.42^{abc}	10.98±**0**.46^{ab}

*Means followed by the different lowercase letter in the column of each explant are significantly different at P< 0.05 according to the Duncan's Multiple Range Test.

and BAP treatment groups stored at 4 ºC and 25 ºC (Tab. 1). It is due to the hard sclerotic coat, which makes germination difficult, acts as a barrier preventing the permeation of water, oxygen, GA_3, kinetin and BAP solutions.

Dry-fresh weight and soluble protein-sugar

The data presented in Tab. 1 indicate that dry and fresh wight were increased by scarification, BAP and GA_3 treatments but the kinetin treatment had no positive effect in either tested temperature condition. GA_3 application showed similar effects with BAP application on dry and fresh wight of seedlings. The highest dry and fresh weights were obseved in scarification+GA_3+BAP combination treatment in both teperature conditions. It is known that synthetic cytokinins such as kinetin and BAP can improve plant growth by increasing cell division, breaking dormancy and promoting bud growth (Hossain et al., 2006). Our study results are consistent with the results of Azab (2018) on sugar beet (*Beta vulagaris* L.) seeds and Acar (2017) on *P. khinjuk* seeds who reported a similar response of dry and fresh weight of seedling. These studies reported that GA_3 application caused an increase in dry and fresh weight of *B. vulgaris* and *P. khinjuk* seedlings. In the present study, exogenous application of BAP and GA_3 enhanced the seedling growth and development. Since seedlings with the highest fresh and dry weight were obtained from seeds in GA_3, BAP treatments (Tab. 1), and these seedlings also had the highest sugar and protein contents (Tab. 2). Also, the synergy of BAP and GA_3 had more effective results in terms of soluble protein and sugar content. These results are also in agreement with Sonnewald (2013) who mentioned that dry weight refers to the total sugar and protein content in the tissue, and it depends on the anabolic metabolism of the seedlings.

Chlorophyll a,b and carotenoid

As shown in Tab. 2, although high levels of chlorophyll a,b content were obtained by scarification treatment, it was observed that these levels increased by addition of GA_3 and BAP and decreased by addition of GA_3, BAP and kinetin. In the study of Azab (2018) and Acar (2017) levels of chlorophyll a,b content by GA_3 treatment in leaf of *B. vulgaris* and *P. khinjuk* seedlings respectively. The synergistic effect of BAP and GA_3 together is seen in the increase in the amount of chlorophyll a,b, as in our other results. In addition, it has been observed that the chlorophyll a,b content is generally high in seedlings with high fresh and dry weight. The chlorophyll a,b content of seedlings after the germination of GA_3+BAP-treated seeds has been investigated in many species. For example, in their study performed to determine the effects of dormancy-breaking factors on the seedling growth and development in apple seeds, Górnik et al. (2018) found that GA_3+BAP treatment increased the growth of seedlings and chlorophyll a,b content. In another study conducted similarly on scotch pine seeds, salicylic acid, jasmonic acid, BAP and kinetin, indole-3-acetic acid (IAA) and 1-naphthaleneacetic acid (NAA) plant growth regulators were used together with GA_3, but the best seedling growth and development and the highest chlorophyll a,b content were obtained in GA_3-treated seeds (Nawrot-Chorabik et al., 2021). Our results are also in agreement with the results those of Górnik et al. (2018) and Nawrot-Chorabik et al. (2021) who described the positive role of BAP an GA_3 treatment on chlorphyll content of seedling leaf. The absence of a significant difference in the carotenoid content in our study, as shown in Tab. 2, indicates that the seedling growth and development occurred under similar environmental conditions and this case increases the reliability of the fresh and dry weight, sugar and protein and chlorophyll a,b content results used for the seedling growth and development. In addition, it is known that environmental conditions and genotype interactions determine the plant response (Uarrota et al., 2018), and carotenoids are usually associated with environmental stress and actively participate in the plant response required for survival strategies; carotenoid types may increase or decrease depending on the type of biotic and abiotic stress (Lemoine and Schoefs, 2010).

Ttriphenyl tetrazolium chloride (TZ) test for seed viability

According to AOSA (2005) and (Verma and Majee, 2013) triphenyl tetrazolium chloride estimating viability test reports, the estimating viability of our seeds was determined to be 72% (Fig. 1). The highest germination rate in our study was 42% in the test group of scarified+GA3+BAP stored at 25 °C. It is believed that the difference in germination rate between the result of the germination and viability tests are due to microbial degradation of the seeds in the pots. This is because seed decay in seed germination studies.

Fig. 1: A) Stained negative control of *P. khinjuk* seeds with triphenyl tetrazolium chloride. Bar: 22 mm. B) Stained seeds of *P. khinjuk* with triphenyl tetrazolium chloride. Bar: 20 mm. C) *P. khinjuk* seedlings, Bar: 40 mm.

Conclusion

According to all the results, it was found that keeping *P. khinjuk* seeds at 25 °C for 6 months, then treating them with sulphuric acid and keeping them in GA_3+BAP solution gave more efficient results in both the germination and the growth and development of seedlings. It was revealed that keeping *P. khinjuk* seeds at 4 ºC was detrimental to germination, growth and development if they were to germinate within 6 months. Scarification of the seed coat with sulphuric acid and exogenous application of GA_3 and BAP significantly increased the germination rate of *P. khinjuk* seeds. In adition, it was determined that kinetin applications were not as effective as GA_3 and BAP in the seed germination and the seedling growth and development. Additionally, the combination of GA_3 and BAP was found to have a

synergistic effect on the germination and the seedling growth and development of *P. khinjuk* seeds. However, it is necessary to conduct experiments on seeds of other *Pistacia* species to determine the effects of the combination of BAP, GA_3 and another synthetic cytokinin combinations on the seed germination and the seedling growth and development.

Conflict of interest

No potential conflict of interest was reported by the author.

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