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Effects of dormancy-breaking treatments on seed germination and seedling growth of *Pistacia khinjuk* Stocks using as rootstock for pistachio trees

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Summary

This study was carried out to determine the effects of different dormancy-breaking treatments including stratification, sulphuric acid scarification, dehulling (removing the mesocarp and exocarp from the nut) and gibberellic acid (GA_3) on seed germination and seedling development of two different *Pistacia khinjuk* genotypes (A and B) using as rootstock for pistachio cultivars. Seed dormancy-breaking treatments were shelled (control), shelled + GA_3, dehulled, dehulled + GA_3, sulphuric acid scarification and sulphuric acid scarification + GA_3 applications in the present experiment. The seeds of both genotypes were stratified at 4 ºC for 50 days after the dormancy-breaking treatments. Stratified seeds were sown in the vials filled with peat in the greenhouse to determine the germination percentage. Plantlets were transplanted to plastic containers to determine the vegetative growth. The highest germination rate was obtained from sulphuric acid scarification in both *P. khinjuk* genotypes. In *P. khinjuk*-A seedlings, the highest stem growth was obtained from scarification and dehulled applications, whereas the poorest development was observed from dehulled + GA_3 application. The best growth in the *P. khinjuk*-B seedlings was obtained from scarification + GA_3 application. The effect of the dehulled application on the root development of *P. khinjuk*-A seedlings was better than the other applications; however the effect of dormancy-breaking applications on root development of *P. khinjuk*-B seedlings was found to unsteady. Scarification increased the number of leaves in both genotypes. As a result, dormancy-breaking applications have been found to be effective on seed germination and seedling growth of *P. khinjuk*. It was determined that GA_3 applications negatively affected both seed germination and root, stem and leaf growth of *P. khinjuk*-A.

Keywords: *Pistacia khinjuk*, stratification, scarification, GA_3, seed germination, seedling growth

Introduction

Horticultural plants including pistachio have been an indispensable part of human life for ages. Ever since ancient times, their fruits, seeds, even roots and branches have been used to meet personal and social needs such as severing food, curing diseases and beautifying the planet (Sahin et al., 2002; Ercisli, 2009; Erturk et al., 2010; Hricova et al., 2016; Yazici and Sahin, 2016; Zorenc et al., 2016). *Pistacia* genus is a member of the *Anacardiaceae* family and consists of at least eleven species (Zohary, 1952). Except for *P. mexicana* and *P. texana*, which originated in the USA and Mexico, all other species are distributed mainly within the Mediterranean region, Western and Central Asia and the Middle East (Esmail-Pour, 2001). Turkey has a large population of *Pistacia* species and seven species, namely *P. vera*, *P. terebinthus*, *P. khinjuk, P. atlantica, P. mutica, P. palaestina* and *P. lentiscus* are present and distributed in different regions of Turkey (Atli et al., 2001). The main pistachio rootstock used in Turkey is *P. vera*, and followed by *P. khinjuk, P. terebinthus* and *P. atlantica*.

Pistacia khinjuk Stocks. is an Irano-Turanian species and widely distributed in Iran, Iraq, Syria, Turkey, Afghanistan and Pakistan. Its leaves vary greatly in shape, size and leaflet number not only from tree to tree, but even on the same tree and even on the same branch (Karimi et al., 2012).

It has been postulated that *P. khinjuk* has four varieties, var. *populifolia* Boiss., var. *glabra* Engl., var. *microphylla* Boiss. and var. *macrocarpa* Zoh. (Zohary, 1952). *P. khinjuk* trees are distributed at Siirt, Hakkari, Gaziantep, Adıyaman and Bitlis provinces in Turkey and their trees may grow up to 10 m in height. (Bilgen, 1973; Tekin et al., 2001). Although able to withstand some of the harshest weather conditions, it is sensitive to the fungus *Phytophthora* spp. (Banihashemi, 1995) but has moderate resistance to root-knot nematodes (Farivar-Mehin, 1995).

There are two types of *P. khinjuk* in nature having large fruits (A) and small fruits (B). Fruits of *Pistacia* species consist of a nutmeat (kernel) enclosed in a thin, hard shell (endocarp) surrounded by a fleshy hull (mesocarp and exocarp) (Ferguson et al., 2005). The hulls of *P. khinjuk* (A) fruits are oily and are mainly used for soap making in Siirt province of Turkey.

The seedlings of *P. khinjuk* form a smooth body. Although the *P. vera* and *P. atlantica* seedlings are taller, the seedling diameters at the site of budding do not grow as much as *P. khinjuk*. The base of the seedling and its budding point grow faster than the other rootstocks, thus they can reach to suitable budding thickness more quickly (Tekin et al., 2001). It was determined that *P. atlantica* and *P. khinjuk* rootstocks were better than *P. vera* rootstock in terms of tree vigour and crown formation of budded cultivars in dry conditions (Ulusarac, 1992). Among the *Pistacia* species used as rootstocks for pistachio, it was determined that *P. khinjuk* is the best beneficiary of the soil nitrogen. Graft compatibility is well with pistachio cultivars, and no swelling or growth differences at the budding area (Atli et al., 2001). Pistachio trees have significant potential for arid and semi-arid areas having suitable climatical conditions in the world. Areas suitable for pistachio production have long, hot, dry summers and moderate winters (Ferguson et al., 2005). Pistachio cultivars are extremely difficult to propagate clonally on their own roots, and therefore rootstocks offer a simple method for propagate the pistachio cultivars.

Rootstocks have been used for propagating temperate fruit trees for more than 2000 years. Rootstocks can influence scion vigour, cropping, fruit quality, climatic adaptability, and susceptibility to pests and diseases. Until the mid 19th Century, almost all of deciduous fruit tree rootstocks were raised from seed of fruits collected from indigenous wild populations. Usually, the seedling rootstocks and the fruiting clones grafted on them were of the same botanical genus and species (Webster, 1995).

Because the seeds of *Pistacia* species are surrounded by a hard scle-

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rotic endocarp that makes it difficult to germinate, the germination rate in these species is low (Isfendiyaroglu and Ozeker, 2002). Various chemical solutions are used to stimulate seed germination. Gibberellic acid (GA) is one of the growth regulators which can be used to partially or fully replace the required period of cold moist stratification in a number of plant species (Baskin and Baskin, 1998). Scarification and cold stratification were found to improve the seed germination in *Pistacia* spp. (Ak et al., 1995). Seed scarification favours significantly the germination process, therefore it involved the fast inhibition of the tegument of seeds and the entry of water in the reserves that allows the fast exit of the root and the starting of the metabolic reactions of the embryo and the cotyledons (Ahoton et al., 2009; Chebouti-Meziou et al., 2014).

There are many researches dealing with stratification, acid scarification and GA_3 application on seed germination of different *Pistacia* species (Abu-Qaoud, 2005; Ak, 1988 and 1990; Ak et al., 1995; Chebouti-Meziou et al., 2014; Crane and Forde, 1974; Kafkas and Kaska, 1997; Piotto, 1995; Rahemi and Baninasab, 2000). But, no detailed study has so far been reported about the effects of dormancy-breaking treatments on seed germination and seedling growth of *Pistacia khinjuk*. The objective of this study was to determine the effect of dormancy-breaking treatments on seed germination and seedling growth of *Pistacia khinjuk* stocks using as rootstock for pistachio trees.

So the study is testing two types of dormancy: physical dormancy where seeds are impermeable to water (hence the need for scarification) and physiological dormancy where the embryo is prevented from growing (hence GA_3), and a combination of the two types.

Materials and methods

Plant material

This experiment was carried out in the greenhouse of the Harran University, Faculty of Agriculture, Department of Horticulture located in the Şanlıurfa province of Turkey. Dried seeds of both *Pistacia khinjuk* genotypes having large fruits (A) and small fruits (B) were obtained from Siirt province of Turkey in 2015. Seeds were collected from the trees of *Pistacia khinjuk* (A) and (B) genotypes at maturity, dried for 7 days in room conditions and stored in refrigerator (+4 °C, 70-80% relative humidity) for 4 months. The seeds were used to determine the seed germination and seedling development in respect to dormancy-breaking treatments in the research.

Treatments

Six different dormancy-breaking treatments were applied to the seeds. Treatments were a) shelled (seeds with hull), b) shelled + GA_3 application, c) dehulled (seeds without hull), d) dehulled + GA_3 application, e) sulphuric acid scarification and f) sulphuric acid scarification + GA_3 applications. Treatments were;

- Shelled (control): Seeds (with hull) soaked for 24 h in the tap water and then stratified in perlite for 50 days in refrigerator (dark conditions, +4 °C, 70-80% relative humidity).
- Shelled + GA_3: Seeds (with hull) soaked in the 500 ppm GA_3 for 24 h and then stratified in perlite for 50 days in refrigerator (dark conditions, +4 °C, 70-80% relative humidity).
- Dehulled: Seeds soaked for 24 h in the tap water and then removed their hulls (mesocarp and exocarp). Dehulled seeds (without hull) were washed and stratified in perlite for 50 days in refrigerator (dark conditions, +4 °C, 70-80% relative humidity).
- Dehulled + GA_3: Seeds soaked for 24 h in the tap water and then removed their hulls. Dehulled seeds soaked in the 500 ppm GA_3 for 24 h (Ak, 1990; Kose, 2001), and then stratified in perlite for 50 days in refrigerator (dark conditions, +4 °C, 70-80% relative humidity).
- Sulphuric acid scarification: Seeds were immersed in sulphuric acid for 90 min and then washed and soaked for 24 h in the tap water. Scarified seeds were stratified in perlite for 50 days in refrigerator (dark conditions, +4 °C, 70-80% relative humidity).
- Sulphuric acid scarification + GA_3: Seeds were immersed in sulphuric acid for 90 min and then washed and soaked for 24 h in the tap water. Scarified seeds soaked in the 500 ppm GA_3 for 24 h and then stratified in perlite for 50 days in refrigerator (dark conditions, +4 °C, 70-80% relative humidity). (Ak, 1988; Baninasab and Rahemi, 2001).

Stratified seeds were sown in the vials filled with peat in the greenhouse conditions (temperature 25-35 and 15-20 °C during day and night respectively) to determine the germination percentage. Then, plantlets were transplanted from vials to plastic containers (20 cm × 30 cm in size) filled with 2:1:1, peat, soil, and perlite mixture and irrigated at 4-day irrigation intervals to determine the vegetative growth. Vegetative properties were determined in terms of stem and root growth, and some leaf characteristics of the seedling. For this purpose, seedling diameter, seedling height, stem fresh and dry weight; the number of lateral roots, primary root length, fresh and dry root weight; and number of leaves per plant and leaf chlorophyll contents were measured. Leaf chlorophyll contents were determined as chlorophyll content index (CCI) by using portable chlorophyll meter (CCM-200 Plus, Apogee Instruments, Inc., Logan, UT) (Van Den Berg and Perkins, 2004).

Statistical analyses

The experimental design was completely randomized design with 3 replications, and 25 plants were used for each dormancy-breaking treatment in each replication of both *Pistacia khinjuk* A and B genotypes. Data were analysed using Minitab 17 software (PA, USA, Minitab Inc.). Means was separated by Duncan's Multiple Range Test at $p \leq 0.05$. The relationship between morphological characteristics of the seedlings was evaluated through a simple Pearson correlation analysis.

Results and discussion

The results obtained from the present study confirmed that dormancy-breaking treatments had an important effect on seed germination and seedling growth of *P. khinjuk* genotypes.

Seed germination

In the present study, there were significant differences among the treatments for germination rates of both *P. khinjuk* genotypes (Fig. 1). It was known that the exogenous application of various stimulants and inhibitors could affect the plant growth regulators levels and rates in the seeds and thus increases the germination rates (Mehanna et al., 1985; Baninasab and Rahemi, 2001). Scarified seeds were reached the highest germination, and followed by dehulled and scarification+GA_3 applications in *P. khinjuk*-A and B, respectively. Shelled + GA_3 had the lowest germination percentage in both genotypes (Fig. 1). Data obtained from all treatments were tested for Pearson correlations, and the interaction of seed germination and dormancy-breaking treatments were presented in Tab. 3. There was a significant positive relationship between the seed germination and dormancy-breaking treatments and number of leaves ($p \leq 0.01$); and between the seed germination and seedling diameter and primary root length ($p \leq 0.05$) (Tab. 3). Kafkas and Kaska (1997) were obtained the highest seed germination from the stratification and the lowest germination from the directly sown treatment in *P. khinjuk* types. It was stated that there were not significant differences between the scarified and scarified + prechilled seeds of *P. lentiscus* (Piotto,

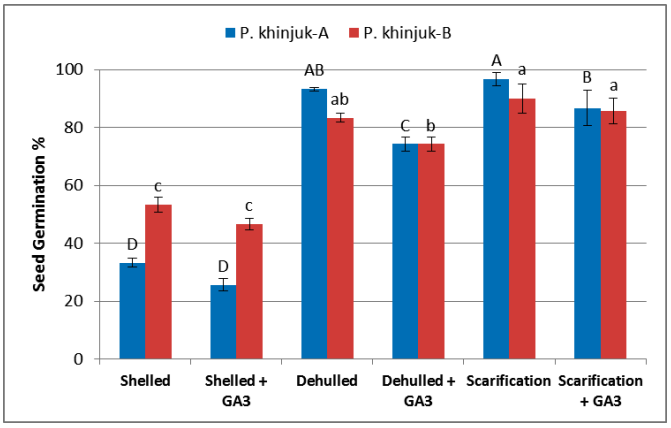

Fig. 1: Effects of dormancy-breaking treatments on seed germination of *Pistacia khinjuk* seedlings. The letters over the columns indicate different groups determined by Duncan's test ($p \leq 0.05$). Capital letters indicate *P. khinjuk*-A, and small letter indicate *P. khinjuk*-B.

1995). Besides, seeds soaking with 1000 ppm GA_3 for 24 h did not increase the germination percentages in *P. atlantica* and *P. vera* (AK et al., 1995). On the other hand, AK (1990), mentioned that the increases in stratification periods were found positively correlated with the germination percentages of *P. vera* and *P. khinjuk* seeds. PIPINIS et al. (2014) reported that, in non-stratified seeds of *Cotinus coggygria*, acid-scarified+GA_3 treatment improved germination significantly, whereas in stratified seeds, no significant differences were observed in the germination percentages of GA_3 treated and untreated seeds. Furthermore, the concentration of GA_3 was not found to affect germination. ABU-QAOUD (2005) obtained the highest germination rate from *P. palaestina* acid scarified+cold stratified seeds as 60%, on the other hand the germination rate of scarified seeds and scarified+GA_3 applied seeds were 13.3% and 34% respectively in *P. lentiscus*. GUNES et al. (2013) reported that mechanical scarification and sulfuric acid treatment were the most successful treatments for enhancing seed germination in carob.

Stem growth of *P. khinjuk* seedlings

Obtained results showed that seedling stem growth, number of leaves per seedling and leaf chlorophyll content index (CCI) was significantly different between *P. khinjuk* genotypes and dormancy-breaking treatments (Tab. 1). Simple correlations between the genotypes, dormancy-breaking treatments and morphological properties of *P. khinjuk* A and B seedlings were presented in Tab. 3.

In *P. khinjuk*-A seedlings, the best seedling growth was obtained from acid scarification and dehulled applications, whereas the poor development was from dehulled + GA_3 application. The highest seedling diameters, seedling height, stem fresh and dry weights, number of leaves and chlorophyll content were determined for scarification. Although high values were obtained from the acid scarification, it was observed that these values decreased when GA_3 application was added. The lowest results from seedling diameters, seedling height, stem fresh and dry weights, number of leaves and chlorophyll content were achieved in dehulled + GA_3 application (Tab. 1). According to KAFKAS and KASKA (1997), GA_3 treated seedlings of *P. khinjuk* types had the biggest stem diameter and followed by stratification treatment, and 92% of B-56A1 and 88% of B-5602 types seedlings were reached to budding stage 6 month after the sowing. QURESHI et al. (2016) reported that 30 days cold stratification and 100 ppm GA3 was feasible procedure for forcing seed germination and to extend the period of propagation in walnut. RAHEMI and BANINASAB (2000) reported that GA_3 application during and after stratification significantly increased the length, trunk diameter, internode length, leaf area and fresh and dry weight of seedlings of both *Pistacia mutica* and *Pistacia khinjuk* species. However, application of GA_3 after stratification was more effective on seedling growth of *P. mutica*. GA_3 applied at higher concentrations (500 and 750 ppm) increased the rate of growth, but growth malformations were clearly evident in seedlings of *P. khinjuk*. GA_3 at 250 ppm enhanced seedling growth

Tab. 1: Effects of dormancy-breaking treatments on stem growth, leaf number and leaf chlorophyll content index of *Pistacia khinjuk* seedlings.

Treatments	Seedling diameter (mm)	Seedling height (cm)	Stem fresh weight (g)	Stem dry weight (g)	Number of leaves	Chlorophyll content index (CCI)
Pistacia khinjuk-A Seedlings						
Shelled (Control)	6.59 b*	19.83 bc	3.60 b	2.56 b	11.61 bc*	54.49 a
Shelled+GA_3	6.32 bc	14.61 d	2.57 c	1.82 b	9.50 cd	49.03 a
Dehulled	7.26 a	21.11 b	5.02 a	3.59 a	13.55 b	57.53 a
Dehulled+GA_3	4.75 d	11.97 e	1.33 d	0.92 c	8.95 d	40.16 b
Scarification	7.36 a	23.61 a	4.93 a	3.60 a	17.78 a	55.79 a
Scarification+GA_3	5.79 c	18.53 c	3.16 bc	2.10 b	11.45 bc	54.81 a
LSD ($p \leq 0.05$)	0.67	2.38	0.98	0.74	2.03	7.82
Pistacia khinjuk-B Seedlings						
Shelled (Control)	5.54 a	15.10 b	1.63	1.10	9.33 d	50.09 bc
Shelled+GA_3	5.16 b	15.03 b	2.02	1.41	9.33 d	67.63 a
Dehulled	5.52 a	14.47 b	2.09	1.46	14.00 ab	56.75 ab
Dehulled+GA_3	4.93 b	18.20 ab	2.03	1.39	11.67 c	43.61 c
Scarification	4.37 c	17.50 ab	1.64	1.29	15.00 a	47.93 bc
Scarification+GA_3	5.21 b	21.07 a	2.23	1.53	12.67 bc	51.84 bc
LSD ($p \leq 0.05$)	0.28	3.48	ns	ns	1.45	11.03

*The letters following the numbers indicate different groups determined by Duncan's test ($p \leq 0.05$)

Tab. 2: Effect of dormancy-breaking treatments on root growth of *Pistacia khinjuk* seedlings.

Treatments	Number of lateral roots	Primary root length (cm)	Root fresh weight (g)	Root dry weight (g)
Pistacia khinjuk-A Seedlings				
Shelled (Control)	4.67 c*	35.74 b	8.51 b	6.52 b
Shelled+GA_3	6.67 b	38.95 ab	4.42 d	3.47 cd
Dehulled	8.66 a	48.19 a	12.15 a	9.21 a
Dehulled+GA_3	4.33 c	30.64 b	2.67 e	1.96 d
Scarification	7.00 b	47.95 a	9.96 b	7.52 b
Scarification+GA_3	3.00 d	46.68 a	6.40 c	4.77 c
LSD ($p \leq 0.05$)	1.31	8.78	1.49	1.63
Pistacia khinjuk-B Seedlings				
Shelled (Control)	2.67 bc	50.74 a	3.64 c	2.69 c
Shelled+GA_3	4.00 b	39.98 d	4.35 a	3.42 a
Dehulled	6.00 a	39.42 d	4.02 b	3.12 b
Dehulled+GA_3	5.67 a	43.68 c	3.12 d	2.37 d
Scarification	2.67 bc	46.87 b	3.12 d	2.30 d
Scarification+GA_3	2.33 c	49.52 a	4.04 b	3.18 ab
LSD ($p \leq 0.05$)	1.36	1.96	0.31	0.25

*The letters following the numbers indicate different groups determined by Duncan's test ($p \leq 0.05$)

Tab. 3: Pearson correlations between genotypes, dormancy-breaking treatments, seed germination and seedling growth properties of *P. khinjuk* genotypes.

Morphological characteristics	Genotypes	Treatments	Seed germination	Seedling diameter	Seedling height	Stem fresh weight	Stem dry weight	Primary root length	Number of lateral roots	Root fresh weight	Root dry weight	Number of leaves
Treatments	0.000^{ns}											
Seed germination	0.083^{ns}	0.773^{**}										
Seedling diameter	-0.618^{**}	-0.195^{ns}	-0.026^{ns}									
Seedling height	-0.197^{ns}	0.344^{*}	0.376^{*}	0.550^{**}								
Stem fresh weight	-0.584^{**}	0.057^{ns}	0.223^{ns}	0.866^{**}	0.779^{**}							
Stem dry weight	-0.584^{**}	0.056^{ns}	0.234^{ns}	0.849^{**}	0.763^{**}	0.982^{**}						
Primary root length	0.246^{ns}	0.271^{ns}	0.384^{*}	0.177^{ns}	0.491^{**}	0.279^{ns}	0.249^{ns}					
Number of lateral roots	-0.450^{**}	-0.211^{ns}	0.046^{ns}	0.651^{**}	0.182^{ns}	0.558^{**}	0.580^{**}	-0.157^{ns}				
Root fresh weight	-0.600^{**}	-0.049^{ns}	0.194^{ns}	0.842^{**}	0.667^{**}	0.923^{**}	0.930^{**}	0.216^{ns}	0.574^{**}			
Root dry weight	-0.586^{**}	-0.058^{ns}	0.182^{ns}	0.853^{**}	0.679^{**}	0.940^{**}	0.937^{**}	0.248^{ns}	0.549^{**}	0.983^{**}		
Number of leaves	-0.050^{ns}	0.457^{**}	0.628^{**}	0.372^{*}	0.630^{**}	0.517^{**}	0.545^{**}	0.340^{*}	0.321^{ns}	0.480^{**}	0.473^{**}	
Chlorophyll content	0.061^{ns}	-0.175^{ns}	-0.058^{ns}	0.375^{*}	0.306^{ns}	0.415^{*}	0.370^{*}	0.247^{ns}	0.118^{ns}	0.415^{*}	0.431^{**}	0.168^{ns}

*: $p \leq 0.05$, **: $p \leq 0.01$, ns: not significant

of *P. khinjuk*.

In *P. khinjuk*-B, the highest seedling diameters were observed for control and dehulled applications and followed by scarification+GA_3 while high in seedling height, stem fresh and dry weights in scarification+GA_3. Leaf number and chlorophyll content were high in scarification and shelled+GA_3, respectively (Tab. 1). The effect of the GA_3 application was unstable in growth of *P. khinjuk*-B seedlings.

There was a significant negative relationship ($p \leq 0.01$) between the genotypes and seedling diameter, stem fresh and dry weights. Dormancy-breaking treatments had a positive and significant correlation with seedling height ($p \leq 0.05$) and number of leaves ($p \leq 0.01$). The seedling diameter was an important factor in the budding and grafting of pistachio seedlings. Seedling diameter had a positive and significant correlation with seedling height, stem fresh and dry weight ($p \leq 0.01$), and number of leaves and leaf chlorophyll content ($p \leq 0.01$). Seedling height was highly correlated ($p \leq 0.01$) with stem fresh and dry weight and number of leaves. Also stem fresh and dry weights were correlated with number of lateral root, root fresh and dry weight, number of leaves ($p \leq 0.01$) and leaf chlorophyll content ($p \leq 0.05$) (Tab. 3).

Root growth of *P. khinjuk* seedlings

Dormancy-breaking treatments were significantly affected the root growth of both genotypes seedlings. The effect of the dehulled application on the root development of *P. khinjuk*-A seedlings was better than the other applications. In *P. khinjuk*-A, the best root growth was obtained from seedling of dehulled seeds. GA_3 addition had a negative effect on primary root length, root weights and lateral root number except for shelled application. The lowest root growth was observed for shelled and dehulled + GA_3 applications (Tab. 2).

Dormancy-breaking applications' effect on root development of *P. khinjuk*-B seedlings was found to unsteady. Number of lateral roots was higher in dehulled and dehulled + GA_3 applications than the other treatments. While maximum root length was obtained from shelled and scarification + GA_3 applications, the maximum root fresh and dry weight obtained from shelled + GA_3. The effect of the GA_3 application was also unstable in root growth of *P. khinjuk*-B seedlings (Tab. 2).

RAHEMI and BANINASAB (2000) reported that generally, application of GA_3 was more effective on the growth of shoots than on roots. The small or insignificant effect of GA_3 on root growth may be due to the synthesis of GA_3 in roots of many species of plants, and a lower level of GA_3 may be required for optimum root growth than in shoots (BUGBEE and WHITE, 1984).

There was a significant negative correlation ($p \leq 0.01$) between the genotypes and number of lateral root and root fresh and dry weights (Tab. 3). Primary root length was correlated with number of leaves ($p \leq 0.05$); number of lateral root was correlated with root fresh and dry weight ($p \leq 0.01$); root fresh and dry weights were correlated with number of leaves and leaf chlorophyll content ($p \leq 0.01$), significantly.

As a conclusion, dormancy-breaking applications have been found to be effective on seed germination and seedling growth of *P. khinjuk*. It was determined that GA_3 applications negatively affected both seed germination and root, stem and leaf growth, as known to the contrary.

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