Comparing the effects of various halopriming treatments on some vegetative characteristics of pistachio *(Pistacia vera L.)* seeds under salinity stress

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Abstract

Introduction: Pistachio is one of the top export products of Iran,. Hence, due to groundwater depletion and as a result of salinization of soil and water used for agriculture, a solution should be sought to increase the tolerance of the plant under salinity stress .

Materials and Methods: In order to investigate the effect of various priming treatments , an experiment was conducted in the form of a factorial design with five treatments and three replications in the greenhouse of the Rafsanjan Pistachio Research Institute in 2020. Priming treatments include control (without priming), P in two concentrations of 100 (P1) and 200 (P2) mg/liter, potassium nitrate (KNO3) in two concentrations of 100 and 200 mg/liter, and the salinity stress treatments were at three levels of 6, 12, and 18 deciSiemens/meter (dS/m). The traits to be measured were diameter and stem height after stress, fresh and dry weight of shoots and roots, and germination percentage.

Results: The results of the research showed that salinity significantly reduced the germination percentage at all three levels. The highest dry weight of shoots and roots was due to the interaction of P2 and salinity level of 12 dS/m. P2 in seed priming caused an increase in all investigated traits.

Conclusion: In general, it can be concluded that priming with tested materials can reduce salinity to an acceptable and satisfactory level .

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1. Introduction

For a long time, Iran, and especially Rafsanjan, has been one of the major pistachio-producing areas in the world and has always taken first place among the world's pistachio producers. Pistachio is a plant of the Anacardiaceae family and Pistacia genus, which was named by Carl Linnaeus in 1737 AD. Since it is propagated through sowing seed, one of the important ways to produce superior and better seedlings is seed priming or pre-treatment with different materials and treatments. During seed germination, the soil environment often does not accelerate germination and seedling emergence. For example, the harmful effects of biotic and abiotic stresses, such as high and low temperatures, soil crust, too much or too little water, salinity, pathogenic diseases, and insects can reduce the rate of germination and seedling emergence or stop these processes. Salinity has a negative effect on the yield of the product, which disturbs the ionic balance of the plants and as a result leads to a decrease in the absorption of mineral elements, growth, and yield of the plant. The excessive accumulation of sodium ions leads to a decrease in the relative content of the leaf and hence, increases the leakage of electrolytes [1, 2]. In recent years, evidence of the success of priming (pre-seed treatment) under stress conditions has been collected [3]. The experiment conducted by Hosseini et al. [4] on pistachio seeds showed that the characteristics of stem length, stem, root and seedling fresh weight, and root, and seedling dry weight were significantly different from those of the control. The highest seedling dry weight and shoot length were related to KNO_3 (150) treatment. This technique is effective for increasing the percentage and uniformity of seed germination and improving seedling growth and seed vigor index [5]. Hosseini et al. reported the positive effects of priming on pistachio seeds [6]. various There are different methods of priming, one of which is halopriming in the sense of seed pretreatment in an inorganic salt solution. This treatment is especially used to plant the seed in saline soils; the length of the soaking period may depend on the hard or thin seed coat. For example, cotton seeds may need more time for priming to be effective compared to rice or wheat seeds. Moreover, the special temperature that exists during priming or the drying time after priming contributes to the effectiveness of priming [7]. Salicylic acid is also a plant growth regulator that plays a role in regulating physiological processes and the plant's response to adverse biological conditions such as salinity. Mahdavian's study [8] found that salinity causes a significant decrease in the growth of shoots and roots, a decrease in fresh and dry weight of shoots and roots, as well as decreased chlorophyll, carotenoid, and anthocyanin while in plants pretreated with salicylic acid, it moderated the effect of salinity on the mentioned parameters. By examining the above material, it can be concluded that the use of salicylic acid increases the plant's tolerance to salinity stress.

Since the mutual effect of salinity stress and priming has not been widely studied, this research aimed to investigate the resistance of pistachio seeds under the conditions of priming and applying salinity stress.

2. Materials and Methods

In order to compare the effect of different seed halopriming treatments on some vegetative characteristics of pistachio seeds of the *Badami*

Zarand cultivar under salinity stress, an experiment was conducted in the form of a factorial design with five treatments and three replications in the greenhouse of the Rafsanjan Pistachio Research Institute in 2020. The first factor: priming treatments including control (no priming and no stress), P in two concentrations of 100 (P1) and 200 (P2) mg/liter, potassium nitrate (KNO_3) with a concentration of 100, and potassium nitrate (KNO_3) with a concentration of 200 mg/liter, and the second factor: the salinity stress treatments were at three levels of 6, 12, and 18 dS/m, and the normal irrigation water with Ec 3 deciSiemens was considered as control. The traits to be measured included the diameter and height of the stem after stress, fresh and dry weight of shoot and root, as well as germination percentage. In this experiment, 96 pots were used and 2 pistachio seeds were planted in each pot. The seeds were planted in the desired pots after being placed in the mentioned solutions for 24 hours as priming. The purpose of this research was that the conditions of the experiment and the process of producing seedlings were similar to the conditions of the farm and garden; therefore, the pots were arranged in a greenhouse-like space that was only covered with 50% shade, and a certain temperature was not set for the seedlings so the conditions were quite normal. In the beginning stages, the seedlings were irrigated once every other day and then twice a week when the weather became cooler and the seedlings stabilized and established. After 6 months and when the seedlings reached sufficient and acceptable growth levels, salinity stress was applied for two months. Then the seedlings were removed from the pots for measurement: the height, diameter, and fresh weight of the root and stem were measured and after drying the seedlings, their dry weight was measured. The data analysis of this research was done with MSTAT-C software and the means of data were compared with Duncan's test.

3. Results:

Germination percentage

The results of variance analysis of the data showed that this trait was significant at the five percent level, and in the case of priming, the highest germination percentage was related to KNO_3 100, which did not show a significant difference with P1; however, it showed a very significant difference with the control (Table 1). In Table 4, the interaction effect of salinity and priming showed that salinity significantly decreased the germination percentage compared to primed treatments. Fadaei et al. [9] reported that the viability percentage of pistachio seeds has a significant correlation with salinity, and with the increase in salinity levels, the percentage of germination will decrease. However, in the present study, such an event did not happen, which indicates the superiority of the treatments used.

Table 1. Results of variance analysis of the effect of different priming and salinity treatments on the growth characteristics of pistachio seeds

Source of changes	df	Germination percentage	Stem diameter (mm)	Height (cm)	Shoot fresh weight (gr)	Shoot dry weight(gr)	Root fresh weight (gr)	Root dry weight (gr)
Cultivar	4	1988.498**	12.268**	1101.675**	69.427**	23.022**	33.435**	15.084**
Salinity stress	3	0.001 **	0.540 ns	69.281*	7.494*	3.203*	16.395*	5.266*
Salinity stress ×cultivar	12	0.001 **	0.293 ns	75.153*	7.647*	2.255*	5.313*	1.266*
Prime	1	335.671*	4.949*	0.192ns	1.540 ns	2.582*	52.211**	14.523*
Prime ×cultivar	4	241.455**	1.853*	54.223*	3.207 ns	1.293*	5.597*	2.184*
Salinity stress prime ×	3	0.001 **	0.827*	15.985 ns	0.619 ns	0.512 ns	2.960*	0.499 ns
Cultivar× Salinity stress× prime	12	0.001**	0.669*	45.639*	5.220*	1.388 ns	5.389*	2.348*
Error	78	25.766	0.592	44.389	4.080	1.443	2.849	1.225
Coefficient of variation	_-	6.13%	15.97%	30.90%	49.84%	52.44%	37.28%	40.59%

Stem diameter after tension

The results of variance analysis of the data showed that the trait of stem diameter was significant at the level of five percent (Table 1). P2 had the largest stem diameter in this study and increased the stem diameter by 14.19%. In addition, the interaction effect of P2 at the salinity level of 18 dS/m (level 3) had the largest stem diameter, which was not significantly different from P2 at the salinity level of 12. This means that pistachio seed priming will reduce or even eliminate the negative effect of salinity, even up to the level of 18 dS/m.

Table 2. Comparison of the mean of investigated traits and the effect of priming on pistachio seeds of Badami cultivar

Treatments	Germination percentage	Stem diameter (mm)	Height (cm)	Shoot fresh weight (gr)	Shoot dry weight (gr)	Root fresh weight (gr)	Root dry weight (gr)
Control	69.38 d	5.029 b	21.44 bc	3.968 b	2.180 b	4.973 b	3.181 a
P1	88.85 ab	5.129 b	24.34 b	4.229 b	2.315 b	4.598 bc	2.507 b
P2	77.73 c	5.743 a	30.81 a	6.647 a	3.842 a	6.205 a	3.788 a
P3	86.08 b	4.146 c	18.74 c	3.483 b	1.973 b	3,700 cd	2.460 b
P4	91.65 a	4.046 c	12.48 d	1.937 c	1.143 c	3.160 d	1.696 c

Means followed by the same letters in each column are not significantly different according to Duncan's multiple range test.

Stem height

According to the table of results of analysis of variance, it was found that the trait of seedling height was significant at the level of five percent (Table 1). In the comparison table of the mean and the effect of priming on this trait, the P2 treatment had the highest height and had a significant difference from the control and other treatments. P2 treatment has increased the seedling height by 43.70% compared to the control. In addition, according to the comparison table of the mean interaction effect of priming and salinity stress, it becomes clear that P2 in all three salinity levels as well as the control (without stress) caused the elimination of the adverse effects of salinity stress and as it increased the height in the priming treatment by itself, the application of tension did not affect it either (Table 4).

Table 3. Comparison of the mean of traits investigated in pistachio seeds of the Badami cultivar under salinity stress

Treatments	Germination percentage	Stem diameter (mm)	height (cm)	Shoot fresh weight (gr)	Shoot dry weight (gr)	Root fresh weight (gr)	Root dry weight (gr)
Control	82.74 a	4.655 a	19.59 a	3.415 b	1.861 b	3.745 c	2.288 b
Salt 1	82.74 a	4.892 a	22.32 a	4.005 ab	2.319 ab	4.085 bc	2.502 b
Salt 2	82.73 a	4.958 a	23.09 a	4.625 a	2.656 a	5.341 a	3.238 a
Salt 3	82.74 a	4.770 a	21.24 a	4.167 ab	2.326 ab	4.939 ab	2.878 ab

Means followed by the same letters in each column are not significantly different according to Duncan’s multiple range test.

In the tables comparing the means, the letter “a” has always indicated the maximum of that trait. Moreover, as shown in Table 3, the salinity level of 12 dS/m has caused the highest amount of damage for all traits, which has no statistically significant difference from the salinity level of 18 dS/m. However, there were significant differences between the fresh and dry weight of roots and shoots and the control, which indicates that salinity causes negative effects on the plant.

Shoot fresh and dry weight

In the analysis of the variance table (Table 1), it is clear that these traits, like other traits, were significant at the five percent level. In addition, as it can be seen in the comparison table of the mean of data related to priming, the highest fresh and dry weight is related to the P2 treatment, which had a significant difference from the control and other treatments. This treatment which is the special formula of the Kia Nahal Mehrgan company has increased the fresh weight by 67.51% and the dry weight by 76.23%. In the table of mutual effects of priming and salinity stress, it is the same, i.e. P2 treatment is still the best treatment, and there are no negative effects of salinity stress in this prime. Of course, P1 treatment has also been able to reduce the negative effects of salinity stress in terms of the fresh and dry weight of shoots and although its positive effects are less than P2, it had no significant difference with P2 treatment in the two levels of 12 and 18 dS/m.

Table 4. Comparison of the mean of the interaction effect of investigated traits

Treatments	Germination percentage	Diameter (mm)	Height (cm)	Shoot fresh weight (gr)	Shoot dry weight (gr)	Root fresh weight (gr)	Root dry weight (gr).
Control×salt 1	69.38 c	4.970 abcde	16.08 efgh	2.101 ef	1.214 efg	3.713 defg	2.328 cdefg
Control×salt 2	69.38 c	5.055 abcd	23.67 bcde	3.846 cde	2.048 cdefg	4.477 bcdefg	2.913 bcdefg
Control×salt 3	69.38 c	5.190 abc	25.60 bcd	5.552 abc	2.995 bcd	6.418 b	4.059 ab
Control×salt 4	69.38 c	4.900 abcdef	20.42 cdef	4.372 cde	2.463 cdef	5.286 bcdef	3.423 bcd
P1 × salt 1	88.87 a	4.717 bcdefg	21.27 cdef	2.729 def	1.397 defg	3.065 fg	1.689 efg
P1 × salt 2	88.87 a	5.160 abc	22.65 bcdef	3.813 cde	2.390 cdefg	4.252 bcdefg	2.266 cdefg
P1 × salt 3	88.82 a	5.368 abc	27.93 abc	5.168 bcd	2.651 bcdef	5.114 bcdef	2.938 bcdefg
P1 × salt 4	88.87 a	5.270 abc	25.50 bcd	5.206 bcd	2.823 bcde	5.960 bcd	3.137 bcde
P2× salt 1	77.73 b	5.685 ab	28.95 abc	6.173 abc	3.516 abc	5.486 bcde	3.475 bc
P2 × salt 2	77.73 b	5.778 a	27.30 abc	5.221 bcd	3.003 bcd	4.670 bcdefg	3.056 bcdef
P2 × salt 3	77.73 b	5.813 a	35.78 a	7.861 a	4.707 a	8.484 a	4.931 a
P2 × salt 4	77.73 b	5.697 ab	31.20 ab	7.334 ab	4.141 ab	6.181 bc	3.691 abc

P3 × salt 1	86.08 a	4.000 efg	20.13 cdefg	3.863 cde	2.138 cdefg	4.023 cdefg	2.507 cdefg
P3 × salt 2	86.08 a	4.083 defg	23.07 bcdef	4.436 cde	2.518 cdef	3.575 efg	2.456 cdefg
P3 × salt 3	86.08 a	4.633 cdefg	17.28 defgh	3.509 cdef	2.157 cdefg	4.010 cdefg	2.683 bcdefg
P3 × salt 4	86.08 a	3.867 fg	14.47 fgh	2.123 ef	1.080 fg	3.193 efg	2.195 cdefg
P4 × salt 1	91.65 a	3.902 fg	11.50 gh	2.209 ef	1.039 fg	2.437 g	1.444 g
P4 × salt 2	91.65 a	4.383 cdefg	14.93 efgh	2.707 def	1.638 defg	3.452 efg	1.819 efg
P4 × salt 3	91.65 a	3.783 g	8.867 h	1.034 f	0.7707 g	2.680 g	1.578 fg
P4 × salt 4	91.65 a	4.117 defg	14.63 efgh	1.798 ef	1.124 fg	4.072 cdefg	1.944 defg

Means followed by the same letters in each column are not significantly different according to Duncan's multiple range test.

The fresh and dry weight of roots

According to the data variance analysis table, these traits were significant at the five percent level (Table 1). As can be seen in the comparison table of the mean of the studied traits, the highest root fresh weight is related to P2, which had a significant difference with both the control and other treatments, and this treatment increased the root fresh weight by 24.77%. However, in the case of root dry weight, the highest weight is related to the P2 treatment, which was not significantly different from the control, but still increased this trait by 19.08% compared to the control. Salinity stress reduced the tested traits, but the P2 treatment significantly reduced the effect of salinity, especially at the salinity level of 12 dS/m. In general, and according to the reports of Akbari et al. [10], the *Badami* species has a better and more complete root system for the elimination and detoxification of salts.

4. Discussion

The results of this experiment showed that salinity stress is a factor that can cause irreparable damage to pistachio orchards, as Karimi and Kuhbanani [15] also stated in their experiment that due to salinity, the growth rate of Pistachio trees has decreased. Furthermore, Ghanaatiyan and Sadeghi (16) reported that the resistance of the plant against stresses such as salinity occurs by increasing the production of enzymes such as proline and glycine betaine and a series of soluble sugars. Therefore, a solution should be sought that minimizes this amount of damage. In this research, despite the significant difference between the control and the materials used for priming, it was found that the priming technique was an effective factor to eliminate or minimize this damage. There is a salinity tension in most of the pistachio-growing areas of the country. Salinity means the presence of an excessive amount of soluble salts and mineral elements in the water and soil solution, which leads to the accumulation of salt in the root area, and the plant faces difficulties in absorbing enough water from the soil solution [11]. In the present study, considering the increase in the dry weight of the root, it can be concluded that since the primed seeds produce stronger roots than the control, in the same way, they help the plant in

absorbing water in adverse conditions. In fact, salinity is an important factor that endangers the production of agricultural products and the continuation of cultivation in many regions. Salinity reduces the productivity and fertility value of land and causes disturbance and reduction in water absorption by plants and causes problems in nutritional and metabolic processes [12]. About 12.5% of the agricultural lands in Iran are facing salinization. Most of the pistachios are cultivated in sodium soils with low irrigation quality, and this has reduced the yield of pistachios in recent years, especially in the southeast (Kerman) and the center of Iran [13]. In similar research, the results of measuring the fresh and dry weight of pistachio shoots and roots treated with different concentrations of salinity showed that the fresh and dry weight of the pistachio shoots under the treatment of different concentrations of salinity is significantly reduced compared to the control [14]. According to the results of the present research, the primed seeds, especially primed with P treatment, which was referred to by its nickname at the beginning, were noticeably and significantly spared from the damage caused by stress.

5. Conclusion:

According to the mentioned test results, it can be concluded that by applying priming and the materials used in halopriming, the negative effects of salinity can be significantly reduced. Moreover, by creating water-resistant bases or salty lands, it is possible to prevent the lowering of the pistachio production level in Iran, especially in the southeastern regions of the country, and of course, in Rafsanjan. As the present study showed, the dry weight of the plant mass was higher under the stress condition compared with the control, which indicates the production of a resistant seedling through the priming technique.

In general, it can be concluded that the product of Kia Nahal Mehrgan company, nicknamed P2, can neutralize the effects of salinity in pistachio seedlings significantly and to an acceptable extent, and it is better than other substances that are usually used to deal with salinity in halopriming. Considering that the pistachio production level of Iran and especially Rafsanjan as the center of pistachio production is decreasing, practical solutions should be sought. This issue is being studied and updated by the researchers of this company as well as other researchers.

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Conflict of Interest

The authors declare no conflict of interest.

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