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Effects of Priming on Germination and Seedling Growth of *Zygophyllum atriplicoides* under Drought Stress

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ABSTRACT

Aims The present study aims to shed light on the effects of different treatments of gibberellic, salicylic and ascorbic acid on improving germination parameters of *Zygophyllum atriplicoides* under *in vitro* drought stress condition.

Materials & Methods The experiment was conducted in a factorial in a completely randomized design. Five levels of drought stress (0, -0.3, -0.6, -0.9, and -1.2Mpa) with and without priming treatments were used in this experiment. The priming treatments included three levels of salicylic acid (100, 200, and 300mg/L), three levels of gibberellic acid (125, 250, and 500ppm), three levels of ascorbic acid (100, 200, and 300mg/L) and simultaneously distilled water were used as control. Each level of treatments had 4 replications and the total number of replications was 200. The studied traits included germination rate, germination percentage, rootlet length, shoot length, seedling length, and seed vigor index.

Findings According to the results, priming improved germination rate, germination percentage, rootlet length, shoot length, seedling length, and seed vigor index. Among all priming levels, 250ppm gibberellic acid accounted for the highest effect on germination traits of this species under all drought stress conditions.

Conclusion priming with gibberellic acid had significantly more impact on germination parameters under drought stress. Hence, this method serves as a promising step to improve the germination parameters of this plant.

Keywords Drought Stress; Germination; *Zygophyllum atriplicoides*; Chemical Stimuli

CITATION LINKS

[1] Introduction of some important rangeland species suitable for ... [2] Seed germination at different temperatures and water stress levels, and ... [3] Pre-sowing seed treatment-a shotgun approach to improve ... [4] Germination and seedling development of drought tolerant and susceptible wheat ... [5] Effect of seed priming on seed old physiological quality under drought ... [6] Effect of GA3, kinetin and indole acetic acid on carbohydrate metabolism in chickpea ... [7] Acetyl salicylic acid (Aspirin) and salicylic acid induces multiple stress tolerance in ... [8] Seed technology and its biological ... [9] Effects of seed priming on germination and seedling growth under ... [10] Effect of seed priming on germination and seedling growth of the caper ... [11] Effects of light, hydropriming and abiotic stress on seed germination ... [12] Osmo and hydro priming improvement germination characteristics and ... [13] Effects of drought on the growth and resource use efficiency of two ... [14] Seed treatment to overcome salt and drought stress during ... [15] Impact of priming on seed germination and seedling growth of ... [16] The effects of seed priming with ascorbic acid on seed germination ... [17] Seed priming with salicylic acid improves germination and ... [18] Seed priming and tolerance to salt and water stress in ... [19] The osmotic potential of polyethylene ... [20] Seed treatments to overcome salt and drought stress ... [21] Techniques in seed ... [22] Seed ... [23] The evaluation of drought and salinity effects on germination and ... [24] Seed germination and radicle growth of a halophyte ... [25] Seed enhancements to induced salt tolerance in wheat ... [26] Application of physiological and biochemical indices as a ... [27] Physiological effects of drought stress by polyethylene glycol on ... [28] Is reduced seed germination due to water limitation ... [29] The Effect of different salicylic acid ... [30] Sorghum and salinity: I response of ... [31] Antioxidant role of glutathione associated with ... [32] Comparative study of the effects of different ... [33] The effects of light and some presoaking treatments ... [34] Comparative lipid peroxidation, antioxidant defense ... [35] Changes in the activity of the alternative oxidase ... [36] The function and metabolism of ascorbic ... [37] Effects of different drought and salinity levels on seed germination ...

Introduction

Zygophyllum atriplicoides is a perennial shrub, characterized with a height of 80 to 100cm and sometimes up to 2 meters. It has a very wide distribution range (about 1.8% of the country's total area) and inhabits arid and desert regions mostly in Iran, Touran area. It grows well on sandy hills and dunes, creating an extended horizontal and vertical root system, sometimes with a radius of up to 15 meters, as well as dense crown cover, it is able to stabilize moving dunes and prevent wind erosion [1].

Water scarcity is one of the major drawbacks of agricultural production in the world. At present, Iran is among the world's arid regions with an average of 250mm/year precipitation. The more intense drought, the less germination rate will be, by which this strategy seems to be a germination adaptation mechanism for seeds in sand dunes, and germination reduction rate depends on the plant species and drought extent [2]. Priming is one of the ways to improve germination under stress conditions [2]. Seed priming is controlled by seedlings hydration before seeding followed by seed dehydration, which is a common practice to increase the rate and uniformity of germination and emergence under stress and non-stress conditions [3]. Seed priming is used to improve germination uniformity, shorten the germination period and enhance the emergence of seedlings, improve planting and establishment, and to increase seed vigor and to reduce the damage caused by late planting [4].

The reports are full of evidence on using chemical stimuli to improve and accelerate germination, and most reports have confirmed positive results. Growth hormones that are normally used for seed priming include auxins, acetic acid, polyamines, ethylene, salicylic acid, and ascorbic acid [5].

Seed priming with gibberellic acid usually results in improved germination, growth, and extensive root system. In addition, it increases tolerance to biotic and abiotic stresses, and at the same time seedlings primed with gibberellic acid accelerates flowering and increases the yield [6] salicylic acid or ortho-hydroxybenzoic acid belongs to a group of phenolic compounds that are known as an important molecule to modulate plant responses to environmental stresses [7]. One of the ways to fight free radicals for seeds is antioxidant vitamins, including ascorbic acid (vitamin C). Vitamin C is a water-soluble vitamin that has the ability to react and eliminate the effects of super-oxide and hydroxyl-free radicals [8]. Seed priming elevates glucose and proline content, improving germination quality and index in drought conditions [9]. Much research has conducted on the effect of drought and salinity on the plants, but there is relatively little research on the effect of priming on reducing the effect of drought stress. Heydariyan *et al.* [10] indicated that acetylsalicylic acid 200mg/L had the greatest effect among of levels (three levels of gibberellic acid and ascorbic acid) used in priming on the germination of *Capparis spinosa* under drought stress. Li *et al.* [11], investigated the seed germination of *Tanacetum cinerariifolium* and they reported germination was decreased with increasing salinity, drought and light. They reported that hydro-priming improves the germination percentage and reduces mean time to germinate in all osmotic potential. Ansari and Sharifzadeh [12] mentioned osmo and hydro-priming improved germination characteristics and enzyme activity of Mountain Rye (*Secale montanum*) seeds under drought stress. Fang *et al.* [13] showed that height, base diameter and number of leaves were reduced under drought conditions in two species of *Salix paraqplesia* and *Hippophae rhamnoides* and root to shoot ratio was increased to enhance tolerance of the plants under drought stress. Kaya *et al.* [14] used seed treatment to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). Zhu *et al.* [15] mentioned that seed priming treatment with appropriate GA3 concentration and priming time could enhance seed germination and drought resistance of *Oldenlandia diffusa* in seedling stage. Alvani *et al.* [16] investigated the effects of seed priming with ascorbic acid on seed germination and morphological traits of *Taverniera cuneifolia* under drought stress. The highest specific leaf area (cm^2/g), leaf area (cm^2), and dry weight biomass were found in priming seeds in 200mM. Shatpathy *et al.* [17] reported that seed-priming with ascorbic acid of 100ppm not only increased seedling dry weight, but also reduced the mean germination time compared to the untreated seeds of *Oryza Sativa*. Seedling growth of SA-primed seeds had significantly higher root and shoot length than non-primed seeds. Seed priming and tolerance to salt and water stress in divergent grain sorghum genotypes were investigated by Pinheiro *et al.* [18]. They mentioned the seeds that underwent priming processes improved the performance of the genotypes under salt and water stress conditions, especially when using seeds of the more tolerant genotype (BRS 330) subjected to hormonal priming at 100ppm gibberellic concentration.

Given the lack of understanding of behavior of rangeland species against environmental stresses, there is an urgent need to extensive studies in this field for a better understanding of the types of resistant species to environmental stresses such as salinity, and drought to establish vegetation in arid and semi-arid areas. *Z. atriplicoides* is an important species for rehabilitation of arid region with 70-250mm precipitation. The highest seed vigor was reported 70-75% and its germination percentage was reported 70% with seed soaking treatment [1]. This species is a xerophytes and has a high tolerance to drought. The present research aims to shed light on the effects of different treatments of gibberellic, salicylic and ascorbic acid on improving germination parameters of *Z. atriplicoides* under *in vitro* drought stress condition.

Materials and Methods

This study was conducted in 2014 in the laboratory of the University of Zabol. Seeds of *Z. atriplicoides* were purchased from Pakan Bazr Isfahan. Seeds were first sterilized with 5% sodium hypochlorite solution and then washed with distilled water. After that, seeds were pretreated separately for 10 hours with salicylic acid (100, 200, and 300mg/L) and 24 hours with gibberellic acid (125, 250, and 500ppm), and 8 hours with ascorbic acid (100, 200, 300mg/L) at 25°C and simultaneously distilled water was used as a control. Four replications were used for each level of treatments. After the soaking period, all seeds were rinsed with distilled water. Once dried, the seeds were placed in Petri dishes with a diameter of 9cm on a Whatman's filter paper to be exposed to drought stress at different concentrations. Polyethylene glycol 6000 solution was used to prepare different drought concentrations. To calculate the amount of polyethylene glycol needed for osmotic pressure [19], the following formula was used:

ψs= -(1.18×10-2)C-(1.18×10-4)C2+(2.67×10-4)CT+(8.39×10-7)C2T

ψs= Osmotic pressure in bar (Bar); C= Concentration of polyethylene glycol 6000 in grams per kilogram of water (g/kg H_2O); T= Temperature in centigrade (°C)

Distilled water was used to create zero water potential (control). The experiment was done as a factorial based on a completely randomized design with four replications (25 seeds per replicate) at different concentrations (0, 3, 6, 9, 12 Megapascal) in the germinator at 25°C during 16 hours light and 8 hours darkness. The total number of replications was 200. In a period of 15 days, germinated seeds with a root length of more than 2mm were counted each day [20]. In this study, the germination percentage, germination rate [21] and seed vigor index [22] were calculated based on the following equations:

$$GP= 100\times\frac{ni}{s}$$

$$GR= \sum\frac{ni}{ti}$$

GP: Germination percentage; S: Total number of seeds; ni: Germinated seeds at time ti; GR: Germination rate; ti: Days after germination

Plant length×Final germination percentage= Seed vigor index

Data analysis was performed using MSTAT-C 2.1 software and Microsoft Excel 2013 was used to create Graphs. Before performing ANOVA, normality of data was tested using the Kolmogorov-Smirnov test and Duncan's Multiple Range test was applied to assess differences in means.

Findings

Germination percentage

Results of ANOVA showed that drought stress and chemical stimuli had a significant effect on germination percentage at the 1% level (Table 1).

The results of the Duncan test showed that application of chemical stimuli increased the germination percentage of *Z. atriplicoides* seeds compared to control in drought stress conditions. The greatest influence of priming on germination percentage was in -3MPa using 250ppm gibberellic acid followed by 200mg/L salicylic acid, 125ppm gibberellic acid, so that these stimuli at -3MPa potential increased germination percentage from 30% in control treatment at the same potential to 67.5, 60, and 60%, respectively. There was no significant difference between the two concentrations of 125ppm gibberellic acid and 200mg/L salicylic acid, in concentration -0.3MPa. The lowest germination was observed in the control treatment. There was not any germination percentage on high levels of drought stress spatially on 1.2MPa on all treatments (Diagram 1).

Germination rate

Chemical stimuli and drought concentrations had a significant effect on the germination rate of *Z. atriplicoides* species at a level of 1% (Table 2).

Table1) Analysis of variance of the effects of chemical stimuli on germination percentage of *Z. atriplicoides* seed under drought stress

CV	Pretreatment	Drought	Pretreatment ×Drought	Error
Df	9	4	36	150
Ss	12860.5	123458	5922	2075
Ms	1428.94	30839.5	164.5	13.83
F	103.29**	2229.36**	11.89**	-

**: Significant at probability level of 1%

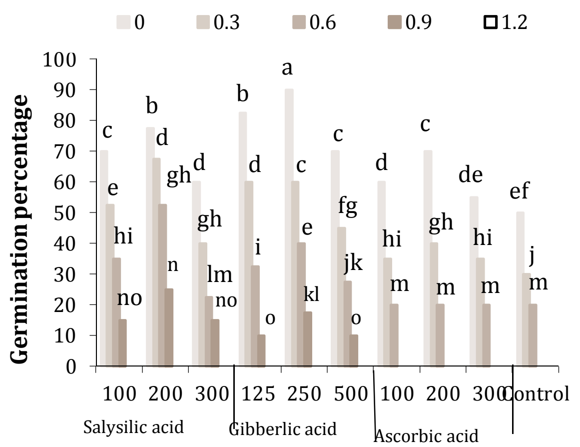

Diagram 1) Means comparison for interactions of chemical stimuli on germination percentage of *Z. atriplicoides* seed under drought stress; There was not any germination percentage on high levels of drought stress spacially on 1.2MPa.

Table 2) Analysis of variance for the effects of chemical stimuli on the germination rate of *Z. atriplicoides* seed under drought stress

CV	Pretreatment	Drought	Pretreatment× Drought	Error
Df	9	4	36	150
Ss	11.66	109.140	6.31	7.61
Ms	1.29	27.285	0.175	0.051
F	25.53**	537.78**	3.45**	-

**: Significant at 1% level

As drought increased, the germination rate decreased. In all drought priming levels, 250ppm gibberellic acid accounted for the highest germination rate followed by salicylic acid levels hand ascorbic acid. At 0, 0.3, and 0.6MPa priming with 250ppm gibberellic acid, increased germination rate of 20, 0.6, and 0.3 seeds per day increased to 20, 1.66, and 1.15 seeds per day. The germination rate was the lowest at -0.9 concentrations of gibberellic acid and salicylic acid. No germination occurred in pretreatment with ascorbic acid at -0.9MPa (Diagram 2).

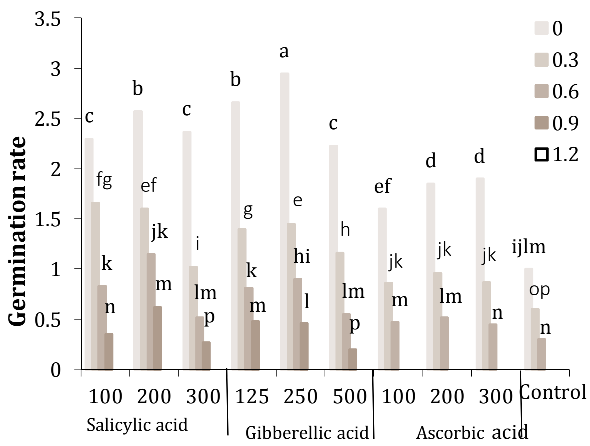

Diagram 2) Means comparison for interactions effects of chemical stimuli on germination rate of *Z. atriplicoides* seed under drought stress; There was not any germination percentage on high levels of drought stress spatially on 1.2MPa.

Rootlet length

The results of ANOVA on root length showed that the interaction of chemical stimuli and different levels of drought on root length were significant (Table 3).

The rootlet length decreased by increasing drought stress, but all chemical stimuli increased rootlet length. The highest rootlet length was observed in non-stress conditions and the application of gibberellic acid 250ppm and the lowest root length was related to control treatment. The results showed that in -0.3MPa, the highest root length was obtained by 250ppm gibberellic acid that it elongated root length from 0.8cm at -0.3 MP to 2.8cm also, all concentrations of gibberellic acid in 0.6MPa resulted in an increase in rootlet length relative to the control treatment. Salicylic acid and ascorbic acid increased rootlet length at different concentrations (Diagram 3).

Shoot length

According to the analysis of variance, effects of chemical stimuli and drought stress on shoot length are significant (Table 4).

Table 3) Analysis of variance for effects of chemical stimuli on seed germination root length of *Z. atriplicoides* under drought stress

CV	Pretreatment	Drought	Pretreatment ×Drought	Error
Df	9	4	36	150
Ss	43.31	98.58	15.70	0.158
Ms	4.81	24.64	0.43	0.001
F	4583.96**	23427.18**	415.52**	-

**: Significant at 1% level

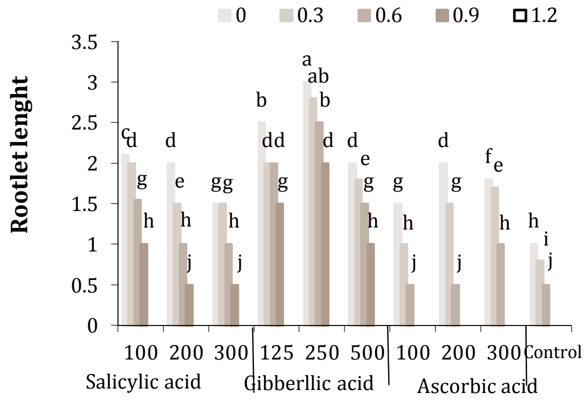

Diagram 3) mean comparison for interactions of chemical stimuli on rootlet length of *Z. atriplicoides* seed under drought stress. There was not any germination percentage on high levels of drought stress spatially on 1.2 MPa.

Table 4) Analysis of variance for the effects of chemical stimuli on shoot length of *Z. atriplicoides* seed under drought stress

CV	Pretreatment	Drought	Pretreatment ×Drought	Error
Df	9	4	36	150
Ss	18.39	58.60	7.77	0.42
Ms	2.04	14.62	0.21	0.003
F	730.29**	5235.62**	77.17**	

**: Significant at 1% level

As Duncan test showed, the more intense the drought, the shorter the shoots of *Z. atriplicoides* such reduction in primed seeds was by far much less than non-primed seeds. Among the stimuli studied, gibberellic acid showed the greatest effect on shoot length to adjust negative effects of drought stress on all levels, so that at a potential of -0.3MPa, the shoot length was 0.5cm in control seed and was 1.77cm in seeds primed with 250ppm gibberellic acid. Also, due to the application of 200mg/L salicylic acid, shoot length at -0.6MPa increased compared to control treatment about 0.85 cm. Although, ascorbic acid improved the shoot length during drought stress, its effect on shoot length was not significant compared to other acids (Diagram 4).

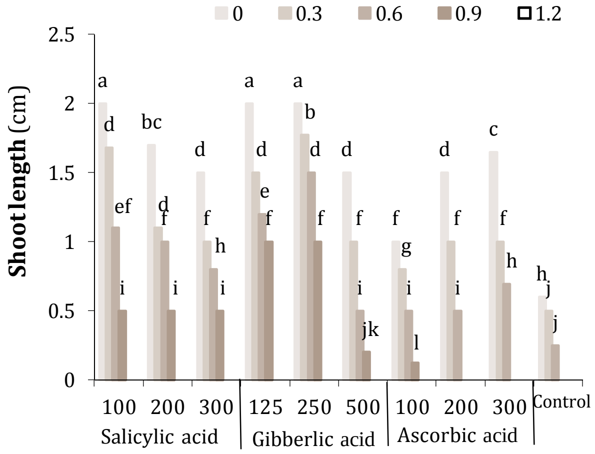

Diagram 4) mean comparison (Duncan test) for interactions of chemical stimuli on shoot length of *Z. atriplicoides* seed under drought stress. There was not any germination percentage on high levels of drought stress spacially on 1.2MPa.

Seedling length

According to Table 5, drought stress and chemical stimuli had a significant effect on seedling length. The effect of drought stress on the reduction of seedling length for both primed and non-primed seeds was significant.

Table 5) Analysis of variance of the effects chemical stimuli on seedling length of *Z. atriplicoides* under drought stress

CV	Pretreatment	Droughts	Pretreatment ×Drought	Error
Df	9	4	36	150
Ss	116.47	312.285	39.31	0.35
Ms	12.94	78.071	1.09	0.002
F	54733.49**	32777.83**	458.49**	

**: Significant at 1% level

The result showed that dry weight at -0.3MPa of seedling length of 1.3cm in the control treatment was 4.7cm in seeds primed with 250ppm gibberellic acid. At in all drought levels, gibberellic acid had the most significant effect on reducing the negative effects of drought stress and in the concentration of -0.6 MPa seedling lengths, it was increased from 0.75cm in the control treatment to 3.92cm 250ppm gibberellic acid (Diagram 5).

Seed vigor index

A significant effect between chemical stimuli and different levels of drought was observed (Table 6).

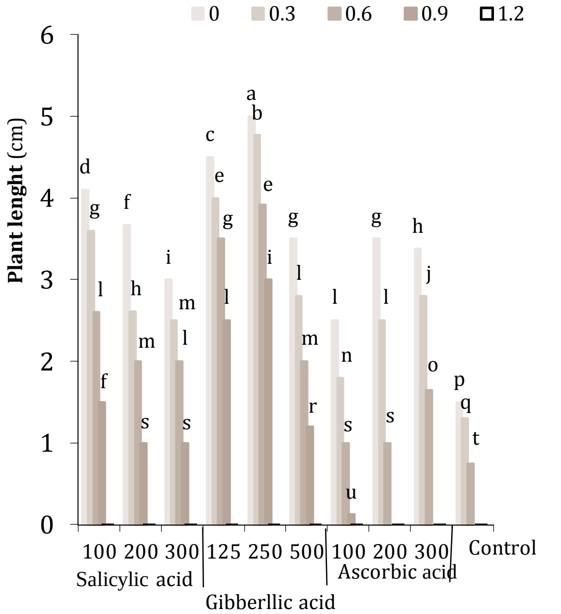

Diagram 5) Mean comparison of interactions of chemical stimuli on seedling length of *Z. atriplicoides* under drought stress; There was not any germination percentage on high levels of drought stress specially on 1.2MPa.

Table 6) Analysis of variance of the effects of chemical stimuli on seed vigor index of *Z. atriplicoides* under drought stress

CV	Pretreatment	Drought	Pretreatment ×Drought	Error
Df	9	4	36	150
Ss	455475.57	1703188.48	312041.28	15858.61
Ms	50608.39	425797.12	8667.81	105.72
F	478.68**	4027.43**	81.98**	-

**: Significant at 1% level

Duncan test showed that the chemical stimuli increased the seed vigor so that the highest increase was achieved by using a concentration of 250ppm of gibberellic acid. The interaction between chemical stimuli and different levels of drought stress was significant on the seed vigor index. The highest seed vigor index in drought stress conditions was obtained using gibberellic acid 250ppm. This level increased the acidity of the strawberry index from 39 in the control and dry matter from -0.3MPa to 286.5. After gibberellic acid, different levels of salicylic acid had a positive role in reducing the negative effects of drought stress. There was no significant difference between 100mg/L and 200mg/L of salicylic acid in terms of the effect on the seed vigor index in -0.3MPa. Also, in high levels of ascorbic acid, there was little role in increasing the index of seed vigor than control (Diagram 6).

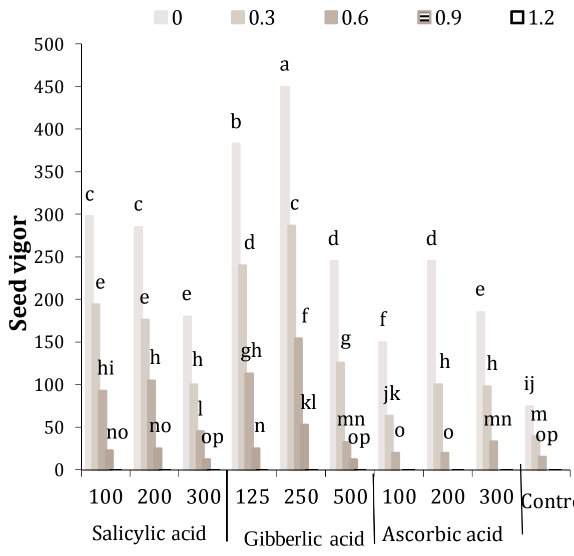

Diagram 6) Mean comparison of interactions for chemical stimuli on seed vigor index of *Z. atriplicoides* under drought stress; There was not any germination percentage on high levels of drought stress specially on 1.2MPa.

Discussion and Conclution

In the current study, drought stress reduced germination percentage and germination index, which is in line with the findings of Ramazani *et al.* [23] and Haydarian *et al.* [10]. Seeds should absorb enough water to be germinated [24]. The soluble materials such as polyethylene glycol, can reduce water absorption of seeds and subsequently can delay or stop germination. In germination under drought stress due to osmotic pressure drop, the water absorption process is impaired and the activity of the enzyme alpha-amylase is further inhibited [25]. By increasing drought stress, the length of rootlet and shoot was decreased in the treatments. Reduction of root and shoot lengths has been confirmed by other researchers [26, 11] under drought stress, which is consistent with the results of the present study. The main reason for reducing shoot length under drought stress is that it reduced non-transferring nutrients from cotyledons to embryo. In addition, decreasing water absorption by seeds under stress conditions reduces hormonal secretion and enzyme activity and thus disrupts seedlings growth. Also, with increasing drought stress, seed strain decreased [27].

Zeng *et al.* [28] while working on xerophyte seeds showed that *Caragana korshinskii* accounted for the highest germination percentage (about 15%) at the most negative osmotic potential of -2.1MPa and *Hedysarum* *scoparium* species had 6.4 germination percentage in osmotic potential of -1.8MPa and *Reaumuria soongorica* had 2.2 germination percentage at a potential of -1.8MPa. Additionally, *Artemisia sphaerocephala* had 8.3 germination percentage at a potential of -1.5MPa while *Zygophyllum xanthoxylum* had 5.2 germination percentage at a potential of -1.2MPa. It seems to be a germination adaptation mechanism for native sand dune seeds.

In general, priming improved seeds germination parameters under stress and non-stress conditions. This is consistent with the findings of Zare *et al*. and Li *et al*. [29, 11]. Seed priming accelerates germination by extending two phases from three phases of germination by shortening the metabolic timing [30]. Priming increases the antioxidant enzymes, such as glutathione in seeds, which these enzymes reduce the activity of lipid peroxidation during germination and so increase germination percentage [31]. Improving germination makes it possible to begin seed sooner germination so that they can be successful in competing with weeds. In other words, the treated seeds start seed germination sooner than their control seeds, and these seeds can be established faster under the environmental stresses [32].

The results of the present study showed that applying gibberellic acid significantly increased the germination and growth of *Z. atriplicoides* seedlings under nonstress and drought stress conditions. One of the reasons for the positive effect of chemical stimuli, such as gibberellic acid, on the early growth of *Z. atriplicoides* seedlings is probably due to the equilibrium of the hormonal ratio of seeds and the reduction of growth retardant materials, such as abscisic acid (ABA). Gibberellins increase the synthesis of hydrolytic enzymes that are beneath the aleuronic layer. The synthesized enzymes are transmitted to endosperm and break down stored food and provide energy for germination and growth [33]. Pretreatment with salicylic acid also improved the germination traits under drought stress, which is consistent with the results of Demiral and Türkan [34]. The mechanism by which salicylic acid increases seed germination is still not clear, salicylic acid can inhibit the activity of the catalase enzyme. Reducing catalase leads to an increase in H_2O_2, high oxide hydrogen is toxic to plant tissues, which is able to improve seed germination [35]. Under stress conditions, ascorbic acid acts as an effective antioxidant. Ascorbic acid, due to the removal of free radicals from stress, especially radical oxygen and its role in stimulating and expanding cells and absorbing materials into cells, prevents the oxidation of plants from environmental stresses [36]. Due to the fact that most of the country's lands are exposed to soil (primary and secondary) erosion and dehydration, chemical stimuli especially gibberellic acid as a pretreatment can increase significantly seed germination and hence can increase succession of plant recovering in biological project in arid zone like what reported by Saberi *et al*. [37]. Hence, this method serves as a promising step to improve the germination parameters of the plant in arid and also semi-arid regions.

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