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Investigating the effect of hydropriming on germination components of Atriplex plant (*Atriplex canescens*) under salinity stress

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ABSTRACT

Salinity stands as one of the major abiotic stresses impacting seed germination and plant establishment in arid and semiarid regions. To explore the effects of hydropriming on Atriplex seed germination components under salinity stress, an experiment was conducted following a completely randomized factorial design with three replications. The experiment involved hydropriming at four levels (0, 1, 2, and 4 days) and salinity at five levels (0, 100, 200, 400, and 600 mM). The findings indicate that salinity stress significantly reduced Atriplex seed germination. However, several germination components, such as germination percentage and rate, seed vigor index, and root length, did not significantly differ compared to the control treatment at the salinity level of 100 mM. The treatment with 600 mM salinity yielded the lowest percentage (1.2%) and rate (0.04 germinations/day) of germination, as well as the shortest root length (0.0083 cm) and shoot length (0.025 cm), alongside the lowest seed vigor index. Hydropriming treatment notably enhanced Atriplex seed germination, with increasing durations of hydropriming correlating to higher germination percentages and rates. The treatments with 0 and 4 days of hydropriming yielded the minimum and maximum germination percentages, respectively. Given that untreated seeds and short-term hydropriming led to limited germination, it appears that applying this treatment is essential for Atriplex seed germination under salinity stress conditions.

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1. Introduction

Atriplex spp. is one of the genera of the Amaranthaceae family, with over 200 species growing in dry and semi-dry regions worldwide. This genus is known as a halophyte plant due to its ability to survive in saline environments (Bueno *et al*., 2017). In addition to its salt tolerance, it is used as a suitable species in desertification projects due to its forage production and evergreen nature for most of the year. Therefore, the cultivation of this plant in desert regions is of special importance (Gul *et al*., 2013). Soil salinity is one of the most important environmental stresses in dry and semi-dry areas, which affects seed germination, plant growth, and production (Gupta and Huang, 2014). Thus, only a small group of plants, known as halophytes, have the ability to survive in these areas (Gul *et al*., 2013). Saline soils make up more than 800 million hectares of the world's land (Munns, 2005). Soil salinity affects seed germination and plant growth by reducing water potential in the root growth environment and the toxicity of ions such as Na^+ and Cl^- (Li *et al*., 2010). Although halophytes are salt-tolerant, seed germination and initial growth stages are the most vulnerable stages in their life cycle and are the most critical stages for the establishment, growth, and expansion of salt-tolerant species (Malcolm *et al*., 2003). Reduced germination and establishment of seedlings under salinity conditions have been observed in many halophyte plants such as *Haloxylon ammodendron* (Huang *et al*., 2003), *Limonium stocksii* and *Suaeda fruticose* (Hameeda *et al*., 2014), *Atriplex stocksii* and *Suaeda fruticose* (Ajmal Khan *et al*., 2003), and *Atriplex prostrata* and *A. patula* (Katembe *et al*., 1998). Therefore, the first step in the restoration of vegetation in these areas is seed germination and rapid establishment of plants.

Hydropriming is a simple, economical, and non-hazardous method for enhancing seed capacity for germination, seedling establishment, and crop production under stress conditions (Kaur *et al*., 2002). In this type of priming, seeds are soaked in sterile distilled water for a certain period of time under suitable temperature conditions before the radical emergence stage (Kaya, 2006). It has been shown that hydro primed seed protoplasts have lower viscosity, higher water and nutrient permeability, and greater resistance to water loss (Thomas, 2000). Therefore, this treatment accelerates seed germination and plant establishment (Kaur *et al*., 2002). Improvement in germination components through priming has been reported in seeds of plants such as sunflower (Kaya *et al*., 2006). Factors such as priming duration, temperature, seed structure, plant species, and seed storage conditions affect the response of seeds to priming (Maiti and Pramanik, 2013). Therefore, optimizing the priming technique is crucial to achieve the best results. Considering that seed germination and seedling establishment are two vital stages in rangeland restoration, especially in dry and semi-dry areas with saline soils, this study was conducted to optimize the duration of hydropriming and investigate the effect of this treatment on improving the germination components of Atriplex seeds under salinity stress..

2. Materials and Methods

In order to optimize the duration and investigate the effect of hydropriming on germination and seedling growth components of *Atriplex canescens* under salinity conditions, an experiment was conducted in a factorial design with three replications at the laboratory of the Department of Horticultural Sciences, Arak University. The experimental factors included hydropriming at four levels (0, 1, 2, and 4 days) and salinity at five levels (0, 100, 200, 400, and 600 mM). The seeds used in this experiment were obtained from Atriplex plants located in the Miqan wetland realm in Arak. For hydropriming, the Atriplex seeds were soaked in sterile distilled water at a temperature of 25°C for the specified durations after removing their bracteoles, and then they were dried by placing them in an open environment. In this stage,

after surface disinfection of the seeds with 1% sodium hypochlorite for 5 min, they were rinsed three times with sterile distilled water.

Then, for germination testing, a total of 20 seeds were placed inside each petri dish on two layers of filter paper, and 10 mL of the desired salt concentration was added to each dish, and a layer of filter paper was placed on top of the seeds. Salt solutions were prepared using sodium chloride in sterile distilled water. Then, the petri dishes were transferred to a germinator at a temperature of 24 ± 2 °C and under dark conditions, and daily counting of germinated seeds continued until the eighth day when no further changes in the number of germinated seeds were observed (ISTA, 2008). In this stage, seeds whose radicles reached a length of 2 mm were considered as germinated seeds (Ellis and Robert, 1981). Measurements of radicle and hypocotyl length were performed using a digital caliper. The percentage of germination (GP) was calculated using formula (1) (Nicols and Heydecker, 1968):

1) $GP= S/T\times100$

In which GP represents the percentage of germination, S is the number of germinated seeds, and T is the total number of seeds. Germination rate was determined using formula (2) (Ellis and Robert, 1981):

2) $GR=\sum Ni/Ti$

In which GR is the germination rate (in terms of the number of germinated seeds per day), Ni is the number of germinated seeds on the i^{th} day, and Ti is the number of days until the i^{th} count. The mean germination time was also determined using the Ellis and Roberts equation (1981) (formula 3):

3) $MGT=\sum(nd)/\sum n$

Where nd is the number of germinated seeds during d days, d is the number of days from the start of germination, and $\sum n$ is the total number of germinated seeds. The seed vigor index was calculated using formula (4) (Abdul-Baki and Anderson, 1973):

4) $SV= (PL+RL) \times GP$

Where SV is the seed vigor index, PL is the hypocotyl length, RL is the radicle length, and GP is the percentage of germination.

2.1. Statistical analysis

The experiment was carried out as factorial in a completely randomized design with three replications. Significant differences among means were estimated at the 5% ($P < 0.05$) level, using Duncan test. All statistical analysis was performed using the SAS software (v. 9.1.3.).

3. Results and discussion

The analysis of variance showed that there was no significant interaction between hydropriming treatment and salinity on the evaluated traits. However, the main effect of salinity had a significant effect on all traits, and the main effect of hydropriming had a significant effect on germination percentage, seed vigor index, mean germination time, and hypocotyl length (Table 1).

Based on the mean comparison results (Table 2), an increase in salinity level led to a significant decrease in germination percentage and rate. The highest and lowest germination percentages were obtained in the control treatment (35.7%) and the 600 mM salinity treatment (1.2%), respectively. Similarly, the highest and lowest germination rates were observed in the

control treatment (1.65 seed d^{-1}) and the 600 mM salinity treatment (0.04 seeds d^{-1}), respectively. The results showed that the 100 mM salinity treatment did not have a significant difference with the control treatment in both germination percentage and rate, indicating the absence of stress in this treatment. On the other hand, the highest stress was observed in the 400 and 600 mM salinity treatments, which severely reduced seed germination in these treatments. According to these results, increasing salinity level had an inhibitory effect on seed germination in halophytic plants such as *Atriplex stocksii* and *Suaeda fruticose* (Ajmal Khan *et al.,* 2003), *Atriplex prostrata*, and *Plantago coronopus* (Bueno *et al.,* 2017).

Table 1 Analysis of variance for the effect of hydropriming on germination components of *Atriplex canescens* seeds under salinity stress

S.O.V.	Degree of freedom	Mean square					
		Percentage of germination	Germination rate	Seed vigor index	Mean germination time	Radicle length	Plumule length
Hydropriming (H)	3	1641.5 *	1.711 ns	1.396 *	5.696 **	1.841 ns	1.86 *
Salinity (S)	4	3239.7 **	6.136 **	4.356 **	8.315 **	6.977 **	8.271 **
H × S	12	403.01 ns	0.525 ns	0.49 ns	1.701 ns	0.768 ns	0.442 ns
Error	40	418.31	0.813	0.601	1.026	1.13	0.575
CV (%)		10.37	11.57	10.96	8.97	10.32	8.35

ns: nonsignificant; *: Significant at 0.05 probability; **: Significant at 0.01 probability.

Table 2 The effect of different salinity levels on germination components of *Atriplex canescens* seeds

Salinity (mM)	Seed vigor index	Radicle length (cm)	Plumule length (cm)	Mean germination time (day)	Germination rate (seed d^{-1})	Percentage of germination
0	1.4658 a	2.0891 a	1.9286 a	1.6183 ab	1.65 a	35.8 a
100	1.1067 ab	1.0743 b	1.2847 ab	2.1667 a	1.35 ab	35.8 a
200	0.7250 bc	1.1483 b	1.4122 ab	1.2775 b	0.69 cb	17.85 b
400	0.2342 cd	0.2028 c	0.5167 bc	0.4167 c	0.15 c	4.80 c
600	0.0050 d	0.0250 c	0.0083 c	0.1667 c	0.04 c	1.20 c

The same letters indicate there is no significant difference in levels $p \leq 0.05$.

In this regard, Jamil *et al*. (2005), Patade *et al*. (2011) and Rouhi *et al*. (2011) stated that increasing salinity level leads to a decrease in germination percentage and rate. The severe reduction in germination percentage and rate under salinity stress in this *Atriplex* species indicates the sensitivity of this plant species to salinity during the germination stage. In addition to causing osmotic stress, salinity leads to an increase in reactive oxygen species (ROS) levels (Munns, 2005). The accumulation of ROS causes oxidative damage to cellular components such as enzymes, nucleic acids, membrane lipids, and other macromolecules (Ashraf and Foolad, 2005). Under salinity stress, lipid peroxidation may be one of the most important factors inhibiting germination (Yang *et al.,* 2010). Moreover, salinity stress alters the balance of plant hormones. Increasing salinity is associated with a decrease in auxins, cytokinins, gibberellins, and salicylic acid, and an increase in abscisic acid and jasmonates (Miransari and Smith, 2014). All of these factors collectively contribute to the reduction of seed germination under stress conditions, including salinity.

According to the results (Table 2), the average germination time significantly decreased with increasing salinity level, and the maximum and minimum average germination times were obtained in the control treatment (1.6 days) and the 600 mM salinity treatment (0.16 days), respectively. These results differ from the findings of Fathee *et al*. (2012), who reported a 33.98% increase in the average germination time at a salinity level of 248 mM compared to the control in black cumin seeds. These results may be due to the higher salinity levels used in this experiment. Contrary to the low salinity level and the control treatment, germination completely stopped in the subsequent days after germination in the high salinity

levels. In other words, before the manifestation of salinity's undesirable effects, germination occurred in a few seeds in the initial days, but the detrimental and inhibitory effects of high salinity levels completely prevented germination from occurring. In general, low salt concentration induces dormancy or delays the onset of germination, while high salt concentrations inhibit germination and reduce germination percentage (Khan and Weber, 2008).

The longest hypocotyl (1.19 cm) and epicotyl (2.08 cm) lengths were observed in the control treatment, and the hypocotyl and epicotyl lengths significantly decreased with increasing salinity level. The minimum hypocotyl and epicotyl lengths were obtained in the 600 mM salinity treatment, which showed a decrease of 99.5% and 98.8%, respectively, compared to the control. In line with these results, Chauhan *et al*. (2012) reported a reduction in seed germination and seedling growth in all sorghum varieties with increasing salinity level, and salinity stress significantly reduced the hypocotyl and epicotyl lengths of sorghum varieties. Under stress conditions, the amount of cell wall proteins involved in cell elongation and growth decreases. In environments with high salinity levels, in addition to the decrease in water potential of the environment and the lack of embryo imbibition, toxic elements such as Na^+ and Cl^- severely affect embryo survival and germination (Daszkowska-Golec, 2011).

Additionally, with increasing salinity level, the seed vigor index also decreased. The minimum seed vigor index was observed in the 600 mM salinity treatment, which had a 99% reduction compared to the control. The concentrations of 600 and 400 mM had the greatest effect on reducing seed vigor. Reduction in seed vigor has also been reported in halophytic plants (Kafi & Rahimi, 2010). Since salinity stress has a negative effect on the length of the shoot, root, and germination percentage, the seed vigor, which is the product of these factors, also decreased with increasing salinity level. On the other hand, the results indicate the insensitivity of seed germination components of *Atriplex* seeds, especially germination percentage, germination rate, and seed vigor index, to low salinity levels (100 mM), while the germination of non-halophytic plants decreases at this stress level. Safarnejad *et al*. (2007) reported that in *Nigella sativa*, the seed vigor index decreased with increasing salinity, and this decrease was 98.84% in the 100 mM sodium chloride treatment compared to the control.

The results showed that seed hydropriming improves germination percentage, seed vigor index, and average germination time. With an increase in the duration of hydropriming, the germination percentage and seed vigor index increased, and the maximum germination percentage (32.4%) was obtained in the 4-day hydropriming treatment, showing an increase of 73.4%, 58.9%, and 32.4% compared to the 0, 1, and 2-day hydropriming treatments, respectively. On the other hand, with an increase in the duration of hydropriming, the average germination time decreased. Consistent with these results, hydropriming improved seed germination percentage in seeds of cactus (Dubrovsky, 1996), onion (Caseiro *et al.*, 2004), cabbage (Fujikura *et al.*, 1993), and mustard (Srivastava *et al.*, 2010). Seed priming, by inducing physiological, biochemical, cellular, and molecular changes, enhances the percentage, speed, and uniformity of germination. These changes include cell division and elongation, plasma membrane fluidity, induction of stress-responsive proteins (such as heat shock proteins), changes in transcription, increased H+-ATPase enzyme activity, and increased antioxidant system activity (Ashraf and Foolad, 2005; Siri *et al.*, 2013). Furthermore, it is believed that seed priming increases the activity of many enzymes involved in carbohydrate metabolism (such as α and β-amylases), proteins (proteases), and enzymes such as isocitrate lyase involved in the transfer of stored food. These changes improve seed vigor during germination and the emergence of seedlings under salinity stress (Di Girolamo and Barbanti., 2012). Additionally, when primed seeds are sown, the imbibition phase and the lag phase of seed germination are shortened, resulting in an increase in germination speed (Khan *et al.*, 2009).

The longest shoot length was obtained in the 2-day and 4-day hydropriming treatments, which did not have a significant difference between them. However, the results indicated a significant increase in shoot length in the 2-day hydropriming treatment compared to the control treatment, with a 22% growth increase in root length compared to the control. Similar results have been reported for the highest shoot length in chickpea in the hydropriming treatment compared to unprimed seeds (Kaur *et al.*, 2002). T

Table 3 The effect of hydropriming on germination components of *Atriplex canescens* seeds

Hydropriming (day)	Mean germination time (day)	Radicle length (cm)	Seed vigor index	Percentage of germination
0	0.3727 ab	0.998 ab	0.378 b	8.6 b
1	1.8887 a	0.433 b	0.542 ab	13.3 b
2	0.6220 b	1.279 a	0.848 ab	21.9 ab
4	0.6333 b	0.920 ab	0.060 a	32.4 a

The same letters indicate there is no significant difference in levels p≤0.05.

his result is due to the faster emergence of the root and shoot, higher seedling vigor, and better resistance to environmental stresses (Kaur *et al.*, 2002). Therefore, seed priming, by inducing physiological, biochemical, cellular, and molecular changes, improves seedling establishment under salinity stress (Ashraf and Foolad, 2005). Seed priming stimulates metabolic processes during germination and prepares seeds for root emergence (Farooq *et al.*, 2006). Nakaune *et al*. (2012) also reported that the observed effects of seed priming in tomato are due to the activation of genes involved in gibberellin synthesis, which are hormones that affect growth. It has been reported that gibberellic acid induces appropriate metabolic responses in basil seeds and improves germination and establishment of seedlings under salinity stress (Sedghi *et al.*, 2010). Similarly, a significant increase in seedling length in chickpea has been reported in the hydropriming treatment compared to unprimed seeds (Kaur *et al.*, 2002). Furthermore, Sung and Chiu (1995) reported that hydropriming greatly enhances the force of emergence and growth of seedlings in watermelon seeds.

4. Conclusion

In general, it can be concluded that *Atriplex* seeds are sensitive to high levels of salinity during the germination stage, and under these conditions, seed germination is significantly reduced. On the other hand, hydropriming successfully improves germination, and the best treatment for this purpose is 4-day hydropriming. Since germination was minimal in the other hydropriming treatments, it appears that applying these treatments is necessary for seed germination in Atriplex seeds.

Conflict of interest

The authors have no conflicts of interest.

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