Greenhouse Plant Production Journal 1(1) (2024), 38-46 gppj.araku.ac.ir

Greenhouse Plant Production Journal

1972

The effect of hydropriming on germination characteristics and seedling growth of *Nitraria schoberi* seeds under salinity stress

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Original Article

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Citation: Khaleghi, Gh. R. 2024. The effect of hydropriming on germination characteristics and seedling growth of *Nitraria schoberi* seeds under salinity stress. Greenhouse Plant Production Journal, 1(1): 38-46.

https://10.61186/gppj.1.1.38

KEYWORDS

Germination rate
Nitrebush
Seedling emergence
Seedling vigor
Viability

ABSTRACT

Salinity is one the major abiotic stresses that affect seed germination and establishment of plants in arid and semiarid regions. In order to investigate the effect of seed hydropriming on seed germination and seedling growth of *Nitraria schoberi* under salinity stress, an experiment was conducted in a completely randomized factorial design with three replications. The treatments included hydropriming at four levels (0, 2, 4, and 8 days) and salinity at five levels (0, 100, 200, 400, and 600 mM). The results showed that at lower levels of hydropriming, the percentage of germination and other germination components were minimal. The highest percentage of germination (50%) and rate germination, as well as seed vigor index, were obtained in the 8-day hydropriming treatment and 100 mM salinity level. Furthermore, with an increase in hydropriming duration, the root length and fresh and dry weight of the seedlings increased, indicating better establishment of the seedlings in the 8-day hydropriming treatment. On the other hand, based on the results, the seeds of this plant were sensitive to high salinity levels (600 mM) during the germination stage, and despite the hydropriming treatment, germination did not occur. It concluded that, the best treatment for germination and seedling growth of *N. schoberi* was the 8-day hydropriming treatment, particularly under mild salinity stress (100 mM NaCl), which improved the germination components.

ARTICLE HISTORTY

Received: 13 February 2024
Revised: 03 March 2024
Accepted: 04 March 2024

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Publisher: Arak University

1. **Introduction**

Nitraria schoberi L., from the Zygophyllaceae family, is a valuable plant that has been used in recent years for creating green spaces, producing suitable forage, and as one of the suitable species in desertification control and sand stabilization projects. Therefore, the development of cultivation of this plant in desert areas is of particular importance (Shahriari *et al.*, 2011). Water and soil salinity are among the most significant non-biological stresses that have spread extensively in dry and semi-arid regions (Munns and Tester, 2008). More than 800 million hectares of land worldwide (Munns, 2005), including 44 million hectares in Iran (Tabande *et al.*, 2015), are comprised of saline soils. Soil salinity occurs due to the accumulation of soil salts, and as the concentration of these salts increases, soil salinity also increases. Since Iran is located in the world's dry belt and most of its area consists of dry and semi-arid climates, soil salinity in these areas gradually increases due to the lack of leaching and the accumulation of salts at the soil surface caused by high evaporation (Malakouti and Homaee, 2005). These lands have poor and meager vegetative cover, but even this scanty vegetation plays a significant role in the ecological balance of these biomes. The resistance of halophytic plants in these lands depends on their tolerance to saline stress at various stages of their life cycle. In most of these plants, seed germination and seedling establishment are two stages highly sensitive to unfavorable stresses (Hubbard *et al*., 2012). Therefore, the first step in the restoration of vegetation in these areas is seed germination, followed by rapid establishment of the plants.

The rapid accumulation of salts in growing cells is one of the reasons for the plant's sensitivity to salinity during the germination stage (Farokhi and Galeshi, 2005). Additionally, seeds are usually located in the surface layer of the soil, which is much saltier than the deeper soil layers (Esechie, 1995). Salinity affects seed germination and growth by reducing water potential in the root growth environment, oxidative stress, toxicity of ions such as sodium and chloride, as well as a decrease in essential nutrient ions required by the plant, such as calcium and potassium (Kaydan and Yagmur, 2008; Khan and Gulzar , 2003; Mauromicale and Licandro, 2002). Moreover, the osmotic phenomenon has a strong inhibitory effect on the imbibition of the embryo, endosperm, and testa (Vicente *et al*., 200). Ebrahimi *et al*. (2015) investigated the effect of salinity on germination and growth of three varieties of peanuts and found that plants have low resistance to stress during the germination stage but exhibit higher resistance in later stages. Therefore, the high germination capacity of seeds under stressful conditions will increase the chances of plant establishment.

Seed priming, as an easy and cost-effective technique, is one of the solutions to overcome the cultivation challenges of plant species in saline areas. Priming refers to the controlled hydration process in which a portion of the seeds undergo biochemical processes, hydrolysis of carbohydrates, and the metabolism of inhibitory substances during the first and second stages of germination before radicle emergence. Such treatment improves and accelerates seed germination and facilitates the establishment of vegetation (Farooq *et al*., 2006; Kaur *et al*., 2002). Faster germination leads to increased plant establishment, enabling better utilization of resources (Massarat *et al*., 2014). Hydropriming is one of the most common seed priming methods. In this method, seeds are treated with pure water, and the water absorption is controlled by the duration of seed-water contact (Farooq *et al*., 2006). Various studies have been conducted on the effects of priming on seed germination under stress conditions. It has been shown that priming improves germination components of cotton seeds under drought stress (Soltani *et al*., 2008) and salinity stress (Toselli and Casenave, 2005). Massarat *et al*. (2014) examined the effect of priming on seed germination and initial growth of maize hybrid SC704 under salinity and drought stress and reported that seed priming improved germination and seedling growth under these stresses. It has been reported that hydropriming of sunflower seeds increases the percentage of germination and seedling growth under drought and salinity stresses (Demir Kaya *et al*., 2006). Seyedi *et al*. (2013) also reported that hydropriming enhances germination characteristics and drought tolerance in safflower during the germination stage. Factors such as priming duration, temperature, seed structure, plant species, and seed storage conditions influence the response of seeds to priming. Therefore, optimizing the priming technique is crucial to achieve the best results (Maiti and Pramanik, 2013). Based on the fact that seed germination and seedling establishment are two vital stages in rangeland restoration, especially in dry and semi-arid areas with saline soils, this study was conducted to optimize the duration of hydropriming and investigate its effect on improving germination components of Nitrebush seeds under salinity stress.

2. Material and methods

In order to optimize the duration and investigate the effect of hydropriming on the germination and seedling growth components of Nitrebush (*Nitraria schoberi*) under saline conditions, a factorial experiment was conducted in a completely randomized design with three replications in the laboratory of the Department of Horticultural Sciences at Arak University. The experimental factors included seed hydropriming at four levels (0, 2, 4, and 8 days) and salinity at five levels (0, 100, 200, 400, and 600 millimolar). The seeds used in this experiment were obtained from Nitrebush plants located in the Miqan desert near Arak. For hydropriming, the seeds were soaked in sterile distilled water at a temperature of 25 °C for the specified durations and then 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, 20 seeds were placed inside a 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. Salinity treatments 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 darkness, and the germinated seeds were counted daily until the tenth day, when no further changes in the number of germinated seeds were observed (ISTA, 2008). In this stage, seeds with a radicle length of 2 mm were considered as germinated seeds (Ellis and Robert, 1981). The length of the radicle and the shoot were measured using a digital caliper, and to determine the dry weight of the seedlings, the samples were first dried in an oven at 75 °C for 72 hours and then weighed using a digital balance with an accuracy of 0.0001 g. The percentage of germination was calculated using equation (1) (Nicols and Heydecker, 1968).

1) $GP = S/T \times 100$

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 ∑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) × 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 v. 9.1.3. software.

3. Results and discussion

The analysis of variance showed that there was a significant interaction between priming treatment and salinity on the percentage and rate of germination, seed vigor index, and shoot length of Nitrebush (Table 1).

Based on the mean comparison results (Table 2), the maximum percentage of germination, germination speed, and seed vigor index were obtained in the treatment with 8 days of seed hydropriming and a salinity level of 100 mm. According to the results, seed hydropriming had a positive and significant effect on the germination percentage of Nitrebush seeds and the establishment of seedlings. Increasing the duration of hydropriming from zero to eight days resulted in a significant increase in germination percentage and speed. On the other hand, increasing salinity levels up to 600 mm reduced germination to a minimum, and even seed priming could not overcome the inhibitory effects of salinity on germination. Additionally, according to the obtained results, seed priming is essential for successful germination.

Table 1 Analysis of variance of the hydropriming effects on germination characteristics of *Nitraria schoberi* seeds under salinity stress

(S.O.V)	Degree of freedom	Mean square						
		Germination percentage	Rate of germination	Seed vigor index	Radicle length	Plumule length	Seedling fresh weigh	Seedling dry weight
Hydropriming	3	1042.2^{**}	0.460^{**}	3.61^{**}	11.94^{**}	3.34^{**}	0.00102^{ns}	0.000025^{*}
Salinity	4	655.8^{**}	0.248^{**}	2.10^{**}	7.32^{**}	2.82^{**}	0.00095^{ns}	0.000017^{*}
Salinity×Hydropriming	12	315.8^{**}	0.145^{**}	1.11^{**}	2.60^{ns}	0.91^{*}	0.00043^{ns}	0.000008^{ns}
Error	38	66.05	0.031	0.37	1.85	0.44	0.0003	0.000006
CV (%)		14.34	16.10	18.66	17.53	15.37	28.35	25.89

**,* and ns show significant differences at levels of 1% , 5% and not difference, respectively.

In this regard, Jamil *et al*. (2005), Patade *et al*. (2011), and Rouhi *et al*. (2011) have stated that increasing salinity levels lead to a decrease in germination percentage and rate. The negative effect of salinity on germination has been demonstrated in many plants, including *Silybum marianum* L. (Zavariyan *et al*., 2012), A*belmoschus esculentus* L. (Dkhil *et al*., 2014), *Haloxylon phyllum*, *Seidlitzia rosmarinus*, *Haloxylon persicum*, *Pteropyrom aucheri*, *Zygophllum euryypterm*, *Sueda fruticosa*, and *Atriplex lentiformis* (Anvari *et al*., 2009). Consistent with these results, salinity stress in Nitrebush (Table 2) caused a reduction in germination percentage to the extent that even primed seeds showed zero germination percentage at a salinity level of 600 mm. Salinity, in addition to creating osmotic stress, increases the levels of reactive oxygen species (ROS) (Munns, 2005). The accumulation of ROS leads to oxidative damage in cellular components such as enzymes, nucleic acids, membrane lipids, and other macromolecules (Ashraf and Foolad, 2005). Lipid peroxidation may be one of the most important factors inhibiting germination under saline conditions (Yang *et al*., 2010). Furthermore, salinity stress alters the balance of plant hormones. Increasing salinity is associated with a decrease in hormones such as auxin, cytokinin, gibberellin, and salicylic acid, and an increase in abscisic acid and jasmonates (Miransari and Smith, 2014).

Table 2 The mean comparisons of hydropriming and salinity interaction effects on germination characteristics of *Nitraria schoberi* seeds

Time of hydropriming (day)	(mM) Levels of salinity (mM)	Germination percentage	Seed vigor index	Rate of germination (seed/day)	Plumule length (cm)
0	0	0 c	0b	0 c	0c
	100	6.67 c	0.49 b	0.095 c	0.42 c
	200	0 c	0 b	0 c	0 c
	400	0 c	0 b	0 c	0 c
	600	0 c	0 b	0 c	0 c
2	0	0 c	0 b	0 c	0 c
	100	0 c	0 b	0 c	0 c
	200	0 c	0 b	0 c	0 c
	400	0 c	0 b	0 c	0 c
	600	0 c	0 b	0 c	0 c
4	0	0 c	0 b	0 c	0 c
	100	10 c	0.30 b	0.148 c	1.083 abc
	200	6.67 c	0.48 b	0. 095 c	2.27 a
	400	0 c	0 b	0 c	0 c
	600	0 c	0 b	0 c	0 c
8	0	3.33 c	0.15 b	0.048 c	0.33 c
	100	50 a	2.82 a	1.065 a	1.69 ab
	200	33.33 b	2.09 a	0.65 b	2.12 a
	400	3.33 c	0.22 b	0.083 c	0.76 bc
	600	0 c	0 b	0 c	0 c

-Values sharing a common letter are not significantly different at $p < 0.01$.

Table 3 The mean comparisons of hydropriming effect on germination characteristics of *Nitraria schoberi* seeds

Time of hydropriming (day)	Radicle length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)
0	0.4067 b	0.002 b	0.0003867 b
2	0 b	0 b	0 b
4	0.6433 b	0.007447 b	0.0005467 b
8	2.0541a	0.01848 a	0.0028667 a

-Values sharing a common letter are not significantly different at $p < 0.01$.

Table 4 The mean comparisons of salinity effect on germination characteristics of *Nitraria schoberi* seeds

Levels of salinity (mM)	Radicle length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)
0	0.2917 b	0.000575 b	0.000067 b
100	1.6444 a	0.012208 ab	0.002325 a
200	1.5940 a	0.020208 a	0.0022 ab
400	0.350 b	0.001917 b	0.000158 b
600	0 b	0 b	0 b

-Values sharing a common letter are not significantly different at $p < 0.01$.

In the treatment of 8-day of seed priming and with a salinity stress of 100 mm, the maximum germination was achieved with 50%. Previously, Naseri *et al.* (2011) observed the highest germination rate of 34% under non-salinity stress conditions. Consistent with these results, seed priming has been shown to improve the percentage and rate of germination in cactus (Dubrovsky, 1996), onion (Caseiro *et al.*, 2004), cauliflower (Fujikura *et al.*, 1993), and mustard (Srivastava *et al*., 2011). Seed priming induces physiological, biochemical, cellular, and molecular changes that enhance the speed and uniformity of germination. These changes include cell division and elongation, increased 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). Additionally, 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 involved in nutrient transport. These changes improve seed vigor index during germination and the emergence of seedlings under salinity stress (Girolamo and Barbanti, 2012). Furthermore, when primed seeds are sown, the imbibition phase and the lag phase of seed germination are shortened, resulting in an increased germination rate (Khan *et al*., 2009).

The maximum shoot length (2.27 cm) was obtained in the treatment of primed seeds for 4-day with a salinity level of 200 mm, although this treatment did not have a significant difference in shoot length compared to the treatment of primed seeds for 8-day with a salinity level of 100 mm, which had the maximum percentage and rate of germination and seed vigor index. According to these results, the highest shoot length in chickpea has been reported to increase 3 to 4 times in the priming treatment compared to non-primed seeds (Kaur *et al*., 2002). This 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).

Based on the results of the analysis of variance (Table 1), the main effect of hydropriming treatment and the main effect of salinity stress had a significant effect on the root length, fresh weight, and dry weight of the seedlings. According to the mean comparison results (Table 3), the maximum root length and fresh and dry weight of the seedlings were obtained in the 8-day hydropriming treatment. These results indicate that the establishment of Nitrebush seedlings improves with an increase in the duration of hydropriming. Seed priming improves the establishment of seedlings under salinity stress by inducing physiological, biochemical, cellular, and molecular changes (Ashraf and Foolad, 2005). Seed priming stimulates the metabolic processes of seed germination and prepares the seeds for radicle emergence (Farooq *et al*., 2006). Nakaune *et al*. (2012) also reported that the observed effects of seed priming in tomato are attributed to the activation of genes involved in gibberellin biosynthesis, which are growth-regulating hormones. It has been reported that gibberellic acid induces favorable metabolic responses in basil seeds and improves germination and establishment of seedlings under salinity stress (Sedghi *et al*., 2010). Consistent with these results, a significant increase in root length and dry weight has been reported in chickpea and basil, respectively, in the hydropriming treatment compared to non-primed seeds (Farahani and Maroufi, 2011; Kaur *et al*., 2002). Furthermore, Sung and Chiu (1995) reported that the force of radicle emergence and seedling growth is greatly enhanced by hydropriming in watermelon seeds.

The results showed that low levels of salinity, especially the 100 mM treatment, can have a stimulatory effect on germination and establishment of Nitrebush seedlings, resulting in the maximum root length and dry weight of seedlings in the 100 mM salinity treatment. Additionally, the maximum shoot weight of seedlings was obtained in the 200 mM salinity treatment, which did not have a significant difference compared to the 100 mM salinity treatment. However, with an increase in salinity levels, the mentioned traits significantly decreased, and no germination and growth occurred at the 600 mM salinity level. It seems that the 100 and 200 mM salinity levels may induce osmotic adjustment by accumulating nonionic solutes or synthesizing organic solutes such as proline, glycine betaine, and free amino acids in response to the decrease in external water potential, thereby overcoming the osmotic effects of salinity. Moreover, this stress increases the activity of antioxidant enzymes such as catalase, peroxidase, and superoxide dismutase, as well as non-enzymatic antioxidant compounds like ascorbic acid. According to Zhu *et al*. (2011), similar results were reported in four species of Brassica, where salinity stress at levels of 34, 68, and 102 mM during germination and early seedling growth stages increased the activity of superoxide dismutase enzyme and carotenoid content, resulting in increased salt tolerance. However, these traits decreased at higher salinity levels.

4. Conclusion

In general, it can be concluded that hydropriming successfully improves germination, and the optimal treatment for this purpose is an 8-day hydropriming. On the other hand, due to the very low or lack of germination in other priming treatments, the application of this treatment for germination of Nitrebush seeds seems necessary. Furthermore, since the highest germination percentage was achieved in the 8-day hydropriming treatment and the 100 mM salinity level, it appears that the application of osmopriming treatments can also be beneficial and effective in improving germination components of Nitrebush seeds, which requires further investigation in this area.

Conflict of interest

The authors have no conflicts of interest.

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