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RESEARCH PAPER

Promotion of the seedling growth of *Nitraria schoberi* L. in cultivating trays and seedling bags with seed priming

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Abstract
Nitraria schoberi plays an important role in restoration projects in arid areas of Iran. Improving the vegetative properties of this plant is critical to the success of these projects. This study was designed to achieve the best chemical, biological, and mechanical treatments to improve vegetative properties. The experiment was performed in a completely randomized design in two sections of cultivating trays and bags. Chemical treatments were 1000, 2000, and 3000 ppm salicylic acid and gibberellic acid. Biological treatments included *Azotobacter chroococcum*, *Azospirillum lipoferum*, *Bacillus megaterium*, *Flavobacterium* sp. and *Pseudomonas flourescens*. The 24 kHz wavelength of the ultrasonic device (for 5 minutes) formed a mechanical treatment. Germination percent, seed vigor index, root and shoot length, seedling growth, fresh and dry weight of root, shoot and leaf number in both cultivating trays and seedling bags, and leaf area in cultivation trays and shoot diameter in seedling bags measured. According to the results, all traits were significantly different in cultivating trays ($p \leq 0.01$) except for germination percent and shoot length. In seedling bags, seed vigor index, root length, seedling growth, root and shoot fresh weight, shoot dry weight, leaf fresh and dry weight, shoot diameter, number of leaves, and root to shoot ratio showed significant differences. There was no statistically significant difference between germination percent, shoot length, and root dry weight. Maximum seed vigor index, root length, and leaf dry weight in both of the bags and trays were seen in 2000 ppm salicylic acid treatment. Also, fresh weight of root and shoot, root dry and fresh weight, and the number of leaves in cultivating tray and in seedling bag root to shoot ratio, shoot diameter, and seedling growth in salicylic acid treatment 2000 ppm was seen. The most leaf area was in 1000 ppm salicylic acid treatment. *Azospirillum* increased root, shoot and leaf fresh weight, leaf number and shoot dry weight in the seedling bag. Results indicated that salicylic acid priming and seed inoculation with *Azospirillum* are suitable for produce seedlings with better properties.

Keywords: Biological priming, Gibberellic acid, Germination percent, Vegetative traits, *Nitraria schoberi*.

Introduction

Biological restoration projects such as seedling planting have been implemented to prevent further damage to arid lands. *Nitraria schoberi* is resistant to withstand unsuitable conditions and salinity stress (Noble and Whalley, 1978; Ranjbar and Dehghani, 2016). It is suitable for carbon sequestration (Naseri, 2014) sand dune fixation, reducing erosion, and increase of soil organic matter (Toranjzar *et al.*, 2015).

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Nitraria species are halophyte shrubs, growing in arid and semi-arid regions, with saline soils. This plant belongs to the family Nitrariaceae and has 12 species worldwide (Temirbayeva *et al.*, 2017). *Nitraria schoberi* is a large, woody, broad, and highly branched shrub. It is about one and a half meters high. Its geographical range in Iran extends from the Baluchestan, Khorasan, and desert salinities of Mighan desert, Salt lake desert, and Aran desert (Juri and Mahdavi, 2010). It has medicinal properties, biologically active constituents such as flavonoid, rutin, narcissine, diosmin, etc. In the leaves of cormorant (Turghun *et al.*, 2019) and alkaloids in all organs (Tulyaganov *et al.*, 2006) and (Tulyaganov and Allaberdiev, 2003) has been reported. The germination stage of desert plants such as *Nitraria* spp. is the most sensitive and effective vegetative stage and consequently effective establishment, Breaking seed dormancy is essential (Paunescu, 2003).

The use of different pre-treatments (seed priming), in addition to, increasing seed germination, can increase drought tolerance, increase yield and reduce enough time for plant regrowth (Nawaz *et al.*, 2013). Fast and uniform seed germination helps to increase plant tolerance to harsh environmental conditions and problems (Rafi *et al.*, 2015). The effect of hot water at different times and concentrations of potassium nitrate and different gibberellic acid concentrations on seed germination characteristics of *Cycas revoluta* L. was investigated. Ullah *et al.*, 2019 indicated that 500 ppm gibberellic acid concentration caused the highest germination rate and germination power. Among different treatments (KNO_3, NaCl, $CaSO_4$, and water) priming of *Sorghum bicolor*, *Pennisetum glaucum,* and *Zea mays*, $CaSO_4$ application was the best (Olorunmaiye and Olatunji, 2018). The effect of cold, gibberellic acid treatment on germination of *Ferula ovina* and *Ferula gummosa* seeds were investigated, the combination of cold and 1500 ppm gibberellic resulted in the most germination percentage (Keshtkar *et al.*, 2008). Tabassum *et al* in 2018 investigated the effects of osmopriming, biopriming, and hydro priming on *Hordeum vulgare* seed under salinity stress. Their results indicated that osmopriming was more effective than biopriming and hydropriming but biopriming was more effective than hydropriming. In the present study, the effect of biopriming of *Azospirillum* and phosphobacteria on maize seed was investigated. Azospirillum caused the highest germination rate, germination, root length, shoot length, dry matter production, total dry matter production index, and seed vigor (Karthika and Vanangamudi, 2013). According to Ghorbanpour and Hatami, 2014 different strains of 159 strains) and *Pseudomonas putida* (PP- 41, 108, and P. *fluorescens* had a different effect on seed germination traits of *Salvia officinalis* L. PP-41 strain *Pseudomonas putida* had a more positive effect on studied traits shows. According to researches by Bakony *et al.*, 2013, the effect of *Bacillus megaterun* and *Azotobacter choorococcum* on different seed traits of *Zea mays* is positive. According to the results of Kokila and Bhaskaran (2016), the use of *Azospirillum* inoculation fluid for 12 hours increases the vigor index of rice seed. Bio-priming of *Trichoderma viride* and *Pseudomonas fluorescens* improves germination and vegetative traits of *Capsicum annuum* seed (Ananthi *et al.*, 2014). In a study (Bayan, 2012), the effects of different priming methods (leaching, mechanical and chemical scraping, and salicylic acid application on germination and vegetative traits of cumin hot seed under drought stress conditions were investigated. According to the results of this study, salicylic acid treatment reduced root growth in this seedling while increasing carotenoid and peroxidase activity. In a study (2008), the effect of gibberellic acid concentrations on seed germination percentage of *Nitraria retusa* was investigated by Suleiman et al. All three concentrations of gibberellic acid on the germination of these seeds significantly increased. Salehi *et al.*, 2015, investigated the effects of gamma irradiation under NaCl and $CaCl_2$ salinity treatments on the vegetative traits of cormorant seeds, the radiation and salinity on shoot length, and leaf number had a significant effect. In all the above-mentioned studies, using priming methods improved the germination and vegetative traits of the seeds; considering the results of the past researchers, a study was conducted to investigate the effects of priming (mechanical, chemical, and biological) on *Nitraria schoberi* seeds, to achieve the best chemical, biological and mechanical priming treatments to improving the vegetative traits and germination.

Materials and Methods

The seeds of *Nitraria schoberi* were collected in the summer of 2018 from the Haji-Abad area (Southwest of Semnan). The seeds were refrigerated until the experiments were performed. Seeds were sterilized with 70% ethanol (30 seconds) and sodium hypochlorite for 15 minutes (Khatibzadeh *et al*., 2013). This study was performed in a completely randomized design in two sections of cultivating trays (fig. 1) and grow bags (fig. 2). The levels of 1000 (SA1 and GA1), 2000 (SA2 and GA2) and 3000 (SA3 and GA3) ppm gibberellic and salicylic acid were considered as chemical priming treatment for 24 hours. The 24 kHz wavelength ultrasonic device (for 5 minutes) was used for as mechanical treatment. Biological priming treatments included *Azotobacter chroococcum* (AZ), *Azospirillum lipoferum* (AS), *Bacillus megaterium* (BA), *Flavobacterium sp.* (FL), and *Pseudomonas fluorescent* (So). The seeds were inoculated with a population of 5 * 10^7 cfu of bacteria for 15 minutes. Root and shoot length, seedling growth, fresh and dry weight of root, shoot and leaf and leaf number, germination percent were measured in two mediums. The leaf area was evaluated in the transplant tray and shoot diameter in the transplant bag.

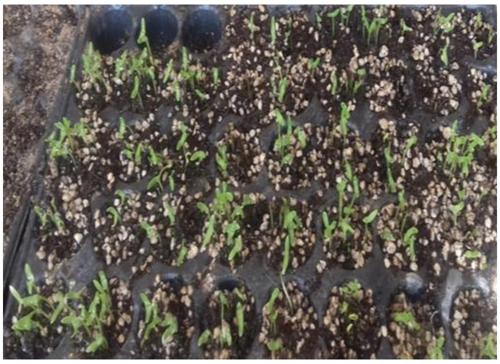

Figure 1. Planting *Nitraria schoberi* in transplant tray

Figure 2. Planting *Nitraria schoberi* in grow bag

Planting in transplant tray

In this section, a circular house tray (45 cells with 3.4 cm diameter) was used. Tray cells filled with culture medium (10% vermicompost, 30% perlite, 30% cocopeat, and 30% peat moss). Treatments were applied to the seeds and 10 replicates (each replicate with two primed seeds) were considered. Seeds were covered with a thin layer of sand. The cultivating trays were kept at 25 °C until the end of the experiment and were irrigated with 371 dS salinity.

Cultivation in the seedling grow bag

UV grow bag with a height of 35 cm and a diameter of 9 cm was used. For each treatment 4 replicates and 5 seeds per replicate were considered. Seedling bags were filled with a combination of 50% field soil, 40% sand, and 10% cattle manure. The pots after applying were kept in the research field of Semnan University for three months and were irrigated with 371 dS salinity. Root, Shoot, and Seedling growth were estimated. The fresh and dry weights of roots, shoots, and leaves were estimated with 0.001 g accuracy.

The leaf surface was measured by Axio Vision SE64 Rel. 4.9.1. The collar diameter was estimated using a caliper in centimeters.
Data were analyzed using the SAS software version 9.1 and the Tukey test was used to compare the mean data.

Results

Cultivating tray environment

Results showed that the different treatments on the seed of Nitraria in the cultivating tray medium had a significant ($p<1\%$) effect on seed vigor index, root length, seedling growth, root fresh weight, shoot, and leaf dry weight, leaf area and leaf number. There was no significant difference in germination percent and shoot dry weight (Table 1).

Comparing the mean of different treatments in transplant tray (Table2) showed that SA2 treatment had the highest seed vigor index (1281.8) and root length (10.99) compared to control and other treatments. SA1 (14/39cm) and SA2 (14/69cm) treatments produced the highest seedling growth. The highest root fresh weight (6.71g), shoot fresh (9.60g), dry root (4.50g), leaf fresh (37.94g), and leaf dry (21.10g) occurred in SA2 treatment. The leaf area in SA1 treatment (3.42) and leaf number in SA2 treatment (6.30) was higher than control and other treatments.

Table 1. one-way Analysis of variance of the *N. schoberi* seed germination components in cultivating tray

Characteristics	variation	Df	Mean Square
Germination percent	Between treatments	12	897.40ns
	Error	117	148000.00
Seed Vigor Index	Between treatments	12	506181.70**
	Error	117	170491.70
Root length (cm)	Between treatments	12	29.07**
	Error	117	5.65
Shoot length (cm)	Between treatments	12	2.71*
	Error	117	0.83
Root to shoot ratio	Between treatments	12	3.75**
	Error	117	1.05
Seedling growth (cm)	Between treatments	12	37.82**
	Error	117	7.69
Root fresh weight (g)	Between treatments	12	19.70**
	Error	117	0.03
Shoot fresh weight (g)	Between treatments	12	14.40**
	Error	117	0.04
Root dry weight (g)	Between treatments	12	1.14**
	Error	117	0.01
Shoot dry weight (g)	Between treatments	12	0.30ns
	Error	117	0.02
Leaf fresh weight (g)	Between treatments	12	58.71**
	Error	117	6.69
Leaf dry weight (g)	Between treatments	12	18.00**
	Error	117	0.12
Leaf Area (Square centimeter)	Between treatments	12	2.99**
	Error	117	0.63
Number of leaves	Between treatments	12	6.41**
	Error	117	1.35

*: significant difference 0.05, **: significant difference 0.01, ns: no significant difference

Table 2. Comparison of the average effect of different priming treatments on seed germination of N. *schoberi* in cultivating tray

Mean Square												
Sources Change Characteristics	Seed Vigor Index	Root length (cm)	Shoot length (cm)	Root to shoot Ratio	Seedling growth (cm)	Root fresh weight (g)	Shoot fresh weight (g)	Root dry weight (g)	Leaf fresh weight (g)	Leaf dry weight (g)	Leaf Area (Square centimeter)	Number of leaves
Control	763.8abc	6.99cd	3.97a	1.83ab	10.96abc	5.30ab	7.90ab	3.40bcd	33.5abcd	12.1b	2.45abcd	5.2abcd
AZ	957.5abc	9.23abcd	3.69ab	2.67ab	12.91abc	2.02c	6.46b	2.90d	23.3def	8.7cd	2.39abcd	4.6abcd
AS	796.0abc	8.34abcd	2.79ab	3.21a	11.13abc	1.82c	5.80b	1.50e	14.6f	5.3e	2.14cd	4.2bcd
FL	831.7abc	9.97abc	3.54ab	2.95ab	13.51ab	3.28bc	5.58b	2.50de	15.5ef	7.5d	2.09d	4.3bcd
BA	1170.3ab	8.69abcd	3.77a	0.34ab	12.46ab	3.81bc	6.97ab	2.20de	19.2ef	7.8d	2.09d	4.4bcd
SO	900.0abc	9.81abc	3.24ab	3.25a	13.05ab	2.82bc	6.89ab	2.50de	25.1abcde	8.2cd	3.36ab	4.4bcd
UL	720.0abc	5.86d	3.69ab	1.57b	9.55bc	3.46bc	7.77ab	1.40e	34.9abc	13.0b	3.31abc	4.1cd
SA1	938.5abc	10.99a	3.40ab	3.32a	14.39a	5.45ab	7.98b	3.20cd	35.4ab	13.5b	3.42a	5.9ab
SA2	1281.8a	10.70ab	3.99a	2.78ab	14.69a	6.71a	9.60a	4.50a	37.9a	21.1a	3.19abcd	6.3a
SA3	681.1abc	8.28abcd	2.91ab	3.04ab	11.19abc	5.07ab	7.28b	1.50e	27.5abcde	9.1c	2.66abcd	5.8abc
GA1	607.7bc	7.16bcd	2.34b	1.58b	9.50bc	3.39bc	6.04b	2.50de	24.9bcdef	7.5c	2.10d	3.7d
GA2	563.9bc	5.96d	2.84ab	2.31ab	8.80c	4.91abc	6.38b	4.30abc	21.2def	5.5e	2.16cd	4.2bcd
GA3	522.2c	7.09cd	2.86ab	2.61ab	9.95bc	3.57bc	5.23b	2.40de	22.9cdef	10.9bc	2.09d	4.5bcd

Common letters denote no significant difference between treatments

Seedling bag environment

The influence of different treatments (chemical, biological, and mechanical) on the yield of cormorant seed in the transplant bag environment were evaluated by ANOVA and results (Table 3). Seed vigor index, root length, seedling growth, root fresh weight, shoot, and leaf dry matter, shoot and leaf dry weight, shoot diameter and leaf number were significantly different at 1% level. The ratio of root to shoot was significant at the 5 % level. There were no significant difference between germination percent, shoot length, and root dry weight.

Comparison of the means of the effect of different priming treatments (Table 4) showed the highest seed vigor index (4615.6), root length (34.93), root to shoot ratio (1.99) ,and seedling growth (52.7) in SA2 treatment. The highest root weight (7/31), the shoot (55/72), and dried shoot (15/16) were created by *Azospirillum* bacteria. *Azospirillum*, SA1 (25.80), and SA2 (28.5) treatments caused the highest fresh weight of leaves. Leaf dry weight (13.83) and collar diameter (3.82) were induced in SA2 treatment. *Azospirillum* treatment caused the highest number of leaves (52.50).

Table 3. Analysis of variance (ANOVA) of the *N. schoberi* seed germination components in Seedling bag

Characteristics	variation	Df	Mean Square
Germination percent	Between treatments	12	1063.70ns
	Error	39	761.20
Seed Vigor Index	Between treatments	12	4411546.30**
	Error	39	6868255.00
Root length (cm)	Between treatments	12	224.6**
	Error	39	43.60
Shoot length (cm)	Between treatments	12	64.90ns
	Error	39	35.00
Root to shoot ratio	Between treatments	12	0.63*
	Error	39	0.27
Seedling growth (cm)	Between treatments	12	456.60**
	Error	39	153.70
Root fresh weight (g)	Between treatments	12	3.07**
	Error	39	0.84
Shoot fresh weight (g)	Between treatments	12	16.99**
	Error	39	2.23
Root dry weight (g)	Between treatments	12	1.91ns
	Error	39	1.17
Shoot dry weight (g)	Between treatments	12	0.84**
	Error	39	0.15
Leaf fresh weight (g)	Between treatments	12	30.9.50**
	Error	39	30.88
Leaf dry weight (g)	Between treatments	12	134.60**
	Error	39	39.14
Shoot diameter	Between treatments	12	3.10**
	Error	39	0.39
Number of leaves	Between treatments	12	831.60**
	Error	39	208.70

*: significant difference 0. 05, **: significant difference0. 01, ns: no significant difference

Table 4. Comparison of the average effect of different priming treatments on seed germination of N. *schoberi* in Seedling bag

Mean Square											
Sources Change characteristics	seed vigor index	Root length (cm)	Root to shoot Ratio	Seedling growth (cm)	Root fresh weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)	Leaf fresh weight (g)	Leaf dry weight (g)	Shoot diameter	Number of leaves
Control	879.4b	6.85b	0.81ab	13.23b	2.24b	17.40bc	3.28bc	7.60b	7.35b	0.73b	9.50c
AZ	1551.3b	13.93b	1.12ab	23.28ab	18. 88ab	21.15bc	5.98bc	13.60ab	8.59b	1.46b	28.25abc
AS	1987.5b	13.40b	0.73ab	31.86ab	31.78a	72.55a	16.15a	25.80a	9.48b	1.95b	52.50a
FL	2068.8b	17.03b	0.89ab	31.23ab	17.75ab	46.85ab	14.15ab	13.60ab	8.00b	1.63b	48.00ab
BA	952.5b	8.95b	1.24ab	14.35b	13.98ab	28.53bc	6.98bc	13.10ab	8.85b	1.09b	27.25abc
SO	1526.9b	11.23b	0.71ab	2313.00ab	13.28ab	28.80bc	6.93bc	8.50b	4.64b	0.74b	23.00abc
UL	758.8b	6.70b	0.59b	15.18b	2.22b	16.35bc	3.05bc	9.70b	6.04b	0.77b	7.75c
SA1	448.8b	5.18b	0.38b	11.90b	12.12ab	20.85bc	4.88bc	6.25b	4.66b	0.68b	14.25bc
SA2	4615.6a	34.93a	1.99a	52.70a	12.15ab	7.73c	1.92c	28.50a	13.83a	3.82a	23.00abc
SA3	1121.3b	9.13b	0.67b	19.43b	2.92b	2.00c	0.59cd	8.25b	6.35b	0.84b	14.25abc
GA1	1084.4b	11.43b	1.12ab	21.65b	3.90b	1.50c	0.59cd	9.53b	6.49b	1.50b	38.00abc
GA2	1067.5b	11.43b	0.87ab	21.33b	3.30b	2.35c	0.46d	6.85b	4.77b	0.58b	24.50bc
GA3	1143.8b	13.23b	1.03ab	22.88ab	5.82b	2.08c	0.60cd	5.50b	3.52b	0.76b	12.00bc

Common letters denote no significant difference between treatments

Discussion

1000 and 2000 ppm salicylic acid produced the highest seedling growth in the cultivating tray. Salicylic acid at 1000 ppm caused the highest leaf area. The highest seed vigor index, root length, and leaf dry weight under both of the bags and trays caused by salicylic acid treatment at 2000 ppm. The highest seed root and shoot fresh weight, root dry weight, fresh weight, and the number of leaves were observed in the transplant tray and the highest root to shoot length ratio, steam diameter, and seedling growth were observed in 2000 ppm salicylic acid treatment. Finally, the effect of salicylic acid was evaluated at positive concentrations of 1000 and 2000 ppm. Many sources have pointed to the positive impact of salicylic acid;

In the study, the effects of four levels of salicylic acid and four levels of drought stress on *Echinacea angustifolia* seed were investigated; Concentration of 1 mM salicylic acid increased germination percentage and rate, seedling length and dry weight, proline content, and catalase activity under drought stress conditions (Paravar *et al.*, 2019). The effect of different concentrations of salicylic acid on seed priming of *Solanum melongena* was investigated;

The concentration of 0.5 mM salicylic acid increased seed germination and seed vigor compared to control (Mahesh *et al.*, 2017). Using a 100 ppm concentration of salicylic acid, citric acid, and proline as priming of *Trigonella foenum* seed significantly increased its growth and germination traits (Behairy *et al.*, 2017). Using salicylic acid in addition to reducing the effect of salinity stress improves vegetative traits in *Vicia faba* seed (Anaya *et al.*, 2018). According to Mabrouk *et al*. (2019), using salicylic acid increases seed germination percentage, better seedling growth, and resistance to *Trigonella foenum-graecum* L. plant diseases. Salicylic acid has enzymatic and non-enzymatic effects on antioxidant activity, and modulating the production of reactive oxygen species reduces the need to produce high amounts of osmotic protectants and adaptive solutions (Bayan, 2012); It also plays an important role in regulating physiological processes such as chlorophyll synthesis, protein, and photosynthesis (Keshavarz *et al.*, 2012).

Azospirillum increased root, shoot and leaf fresh weight, leaf number, and shoot dry weight in the seedling bag. Similar to the results of this study, many studies have pointed to the positive effect of bacteria on the vegetative properties of plants. The influence of the biological priming of *Pseudomonas fluorescens* and *Azospirillum lipoferum* on germination percentage and survival index of *Curcuma longa* seed investigated. The results showed that the two bacteria increased the germination percentage and survival index (Boominathan and Sivakumar, 2012). According to a report by Mirmozaffari and Mirshekari (2013), the biological priming of *Cuminum cyminum* seeds with the aid of *Azospirillum* strains improved essential oil and seed yield. Ansari *et al.*, 2015 investigated the effects of wheat seed bio-priming with different *Pseudomonas fluorescens* strains to improve the yield and growth of this plant. Biopriming of two maize cultivars with *Azospirillum* increased germination rate and root emergence, root length, and photosynthesis (Rozier *et al.*, 2019). *Azospirillum* plays an important role in the growth of many plants due to its ability to stabilize nitrogen and produce plant hormones (Mehnaz, 2015). Inoculation of seeds with fungi or bacteria increases soluble protein and antioxidant enzymes in seeds (Piri *et al.*, 2019).

Conclusion

Nitraria schoberi is one of the useful species in restoring of arid regions, but, its poor germination and the seedling growth are the main problem in the establishment of this species. The results indicated that some biological and chemical priming treatments like *Azospirillum* (inoculated for 15 minutes with a population of $5 * 10^{7}$ cfu of bacteria) and salicylic acid (1000 and 2000 ppm) seeds improved vegetative properties of *N. schoberi*. Salicylic acid with a concentration of 2000 ppm was the most effective treatment in increasing vigor indices like root length, seedling growth, root fresh weight, shoot, and leaf dry weight, leaf area and leaf in tray environment. Our results indicated that *Azospirillum* bacteria improved the root and shoot weight in seedling bag environment. Generally the results of this study indicated that using chemical and biological treatments can improve the vegetative properties of *N. schoberi* which this factor increases the probability of *N. schoberi* establishment in arid area. Also results illustrated that using both chemical and biological treatments increased the germination power of plant therefore, using of these treatments in practical work and in nurseries is recommended to improve germination.

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