Treatments for Optimization of *Salsola turcomanica* (Litv) Seed Germination and Effects of Different Drought and Salinity Levels

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Abstract. *Salsola turcomanica* (Litv) is a widespread wild plant species in Middle Eastern arid and semi-arid areas and is a relatively palatable halophyte species that has low establishment rate in the field. In winter 2017, in order to optimize the germination indices, its seeds were treated by scarification, stratification, scarification after stratification, gibberellic acid, potassium nitrate and hot water. Then, in separate trials, the effects of six levels of drought stress (0, -2, -4, -6, -8, -10 Bar) and salinity (0, 5, 10, 15, 20, and 25 ds/m) were assessed under laboratory conditions as well as the effect of four sowing depths (0, 0.5, 1, and 1.5 cm) investigated on seedling emergence under greenhouse conditions. All four experiments were carried out using completely randomized designs with four replications. A significant decrease was observed in the seed germination indices by increasing drought and salinity stress levels (p<0.05). This species had moderate to low germination percent under -2 bar drought stress and 15 ds/m salinity. Based on the obtained result, the scarification was the best treatment of seed germination, so the seeds of *S. turcomanica* were scarificated and sown (100 kg/ha) in the field at the end of autumn 2018. According to the results, the highest seed emergence was observed at the shallow depth sowing and resulted in 28.7% vegetation cover and 176.44 (g/m\(^2\)) dry matter production at the end of growing season. So, it was concluded that for dry land farming system, cultivation of this species in winter rangelands can reduce wind erosion during its growth period in summer and provide a considerable quantity of forage. Based on the results of this research, cultivation of this species can be recommended to rangeland improvement or ley-farming projects.

**Key words:** *Salsola turcomanica*, Seed germination indices, Salinity, Drought, Greenhouse, Sowing
Introduction

Insufficient freshwater, high concentration of salt, soil degradation, soil poor nutrients and environmental stresses are considered as the major problems in most of the arid and semi-arid regions of the developing countries (Hanif et al., 2018; Azimi et al., 2018).

Forage shortage is a severe problem in many of dry and semi-arid regions threatening nutritious protection of livestock and local people. The low efficiency of rangelands in the large parts of Iran is lacking forage production capacity because of low annual precipitation (Pourmeidani et al., 2017). One of sources for forage production in the arid and semi-arid regions which are facing with drought and salinity stress is using native wild plant species. Paying attention to the forage yield potential of these plant species is necessary. These plant species have instinct tolerance potential to the aforementioned stresses. In the frame of participatory action research project with local farmers and herders in Northern Iran, potentials of some native salt and drought tolerant plant species in terms of forage production and palatability are taken into consideration. According to personal experiences of local farmers and herders who have experiences with native wild plant species for feeding their livestock, *Salsola turcomanica* (Litv.) was identified as a good alternative for forage crops which are not able to grow under high salinity and drought stresses in this region.

The most diverse genus of the sub-family Salsoloideae in central and middle Asia is *Salsola* which mostly grows in typical deserts and semi desert zones (Gintzburger et al., 2003). Genus *salsola* is annual semi-dwarf to dwarf shrubs and woody tree species which is widely distributed across the arid and semi-arid areas in the world. Several features such as good fodder quality and biomass, high seed production, and high tolerance to extreme climatic stress like the drought are the reasons toward their success as a potential forage species in arid and semi-arid ecosystems. In addition to their halophyte nature, the species in this genus are classified as mostly xerophytes and halophytes which make them potential candidates for rehabilitation and reclamation of degraded saline and sodic soils and ecosystems (Gintzburger et al., 2003; Hanif et al., 2018). *Salsola turcomanica* (Litv.) along with *Plantago coronopus* L, *Halostachys caspica*, *Halocnemum strobilaceum*, and *Frankenia hirsuta* are important and relatively palatable halophyte species of Turkmen Sahara in the northern part of Iran. It has been reported that *S. turcomanica* has higher dry matter digestibility and mineral content (ash) than that for the above species (Pasandi et al., 2017).

Despite their self-seeding capability, most *Salsola* species have a low field emergence rate (Gintzburger et al., 2003). Since the germination represents a fundamental stage of plant life and is highly responsive to change of environments, the low germination percent and seedling establishment are basic problems in saline ecosystems (Ghavam, 2018). Breaking seed dormancy of *S. turcomanica* and identifying its tolerance range to salinity and drought stress in phase of germination is the first stage of domestication of this plant. Hard and impermeable seed coats as well as dormant or immature embryos are the main reasons for seed germination delay or inhibition, which is termed seed dormancy (Olmez et al., 2008; Cavanagh, 1980). Physical, physiological, morphological, morpho-physiological and combined dormancies are different types of seed dormancy (Baskin and Baskin, 2004). One of the most common types of seed dormancy among plant is physical dormancy. It has been suggested that species in all angiosperm families have this type of dormancy (Baskin et al., 2000). Mechanical scarification with sandpaper, chemical scarification with acids and soaking of seeds.
in hot water for a short period are common methods for breaking physical seed dormancy (Ali et al., 2011; Keshtkar et al., 2008).

The osmotic and matric potentials of soils along with temperature and light can control seed germination in the soil (Ajmal Khan et al., 2000): thus, drought and salinity are of specific importance among the environmental stresses. The highest plant sensitivity to salinity is observed during the germination and early seedling stages. Salinity stress may cause other secondary stresses such as oxidative stress that can lead to cell death. A reduction or delay in seed germination and seedling growth is caused by drought stress. A remarkable reduction in photosynthesis, disruption of physiological processes, growth repression and finally plant death are observed during the very high drought stress (Saberi et al., 2017; Hanci and Cebeci, 2015). Numerous studies on environmental stresses have demonstrated negative effects of salinity and drought on all germination indices of many plant species (Saberi et al., 2017). The present study describes improvement of the germination indices of S. turcomanica and the effect of salinity and drought stress on the seed germination indices of this species. The objective of this study was to assess seed dormancy of S. turcomanica and to use different seed treatments in order to increase seed germination indices and to assess the seed germination indices under different salinity and drought stresses and optimization of germination indices of S. turcomanica in both greenhouse and field.

Material and Methods

Seed collection

Seeds of S. turcomanica were collected in autumn 2017 around Gomishan [lat.:37° 10′ to 37° 18′ semiarid temperate climate with a 16.6°C annual temperature, and 343 mm of annual rainfall (Niknahad-Gharmakher et al., 2017), Iran (Fig.1) . After seed collection, the healthy, mature, intact seeds were stored at 4°C.

The viability of seed sample was tested using the tetrazolium chloride (TTC) staining technique (Esno et al., 1996). For this purpose, 100 seeds (8.4 g) were incubated at 25±1°C in the dark for 24 h. The result demonstrated that S. turcomanica seeds have 98% viability.

Seed dormancy was determined by counting germinated seeds in Petri dishes lined with filter paper moistened with distilled water at room temperature (25±1°C and 60-70% RH1) for 7 days. Seeds were considered to have germinated when the radicle was emerged. The seed germination percent was slightly higher than 50%; thus, the seeds were considered to be weakly dormant (Naredo et al., 1998).

1. Relative humidity
Germination optimizing experiment

Seeds were exposed to six treatments [scarification by sand paper, KNO₃ (0.6%) for 24 h, hot water (70°C) for 5 minutes, gibberellic acid (500 ppm) for 6 h, stratification at 4°C for two months and scarification after stratification at 4°C for two months] to optimize the seed germination indices. Petri dishes and the substrate (Whatman paper) were sterilized in an oven for 48 h at 200°C. To prevent contamination, seeds were disinfected for 2 min with a solution of 5% sodium hypochloride. After being rinsed three times with distilled water, seeds were transferred into filter paper in Petri dishes. To prevent from contamination and evaporation of the solution, all of the Petri dishes were enclosed with paraffin (Mcgee, 1988).

Salinity and drought stress experiments

The best treatment for optimizing the seed germination indices was determined for all seeds before investigating the tolerance of the seeds to different salinity and drought stresses. The seeds were exposed to six levels of salinity (0, 5, 10, 15, 20, and 25 ds/m), and sodium chloride was used to simulate salt stress. Six drought levels (Control, -2, -4, -6, -8 and -10 bars) were simulated using polyethylene glycol (PEG-6000). Non-treated seeds (distilled water) were used in both experiments as a control. The salt and drought concentrations in the Petri dishes were maintained throughout experiment by checking the Petri dishes periodically and adding solutions and distilled water whenever necessary (Ashraf et al., 1992; Michel and Kaufmann, 1973).

In each experiment, the Petri dishes were labeled and incubated in a germinator at 20°C temperature, 75% humidity, 5000 lux light intensity and 12/12 h day/night illumination. The germination test was performed using four replicates (25 mature and equally-sized seeds per Petridishes). Germinated seeds were counted each day over a period of 14 days. The radicle, plumule and seedling lengths were measured at the end of the experiment. The germination percent, germination speed, seedling length, and vigor index of the seeds were calculated based on the following equations (ISTA, 2002):

\[
GP = \frac{\sum G}{N} \times 100 \quad (\text{Equation 1})
\]

Where, \(GP\) = the germination percent, \(G\) is the number of germinated seeds, and \(N\) is number of seeds.

\[
GR = \sum_{i=1}^{n} \frac{S_i}{D_i} \quad (\text{Equation 2})
\]

Where,  
\(GR\) = Germination rate,  
\(S_i\) = the number of germinated seeds at each counting,  
\(D_i\) = days until the nth counting, and  
\(n\) is numbers of countings.
Germination optimizing: There were significant differences between treatments for germination percent and rate of germination with the highest and lowest values of germination percent and rates observed in the scarification and stratification treatments, respectively (Table 2). No seed germinated in the hot water (70 °C) treatment, so the corresponding data for this treatment were removed from statistical analysis.
analysis. Soaking the seeds in gibberellic acid or KNO₃ increased germination rate to some extent; however, there were no significant differences compared with the control (Table 2). Scarification increased the germination percent by 58.73% relative to the control. Its application increased the germination rate by nearly three times as compared with the control. A significant increase in the mean radical, plumule, and seedling length indices was observed in the scarification treatment (p<0.05) as compared with control. For Scarification treatment, the largest alteration from the control was determined for radicle length, plumule length, seedling length and vigor indicating 109.46%, 48.83%, 80.47% and 200% increase as compared to the control.

No significant differences were observed between the gibberellic acid and KNO₃ treatments while a significant decrease (p<0.05) was observed in the stratification treatment (Table 2).

The highest seed vigour index was observed in the scarification treatment. Soaking the seeds in gibberellic acid and KNO₃ did not have a significant effect on the seed vigour index. A significant decrease was observed in the stratification treatment as compared with the control (Table 3).

**Table 2. Comparisons of means for germination indices of S. turcomanica submitted to different breaking dormancy treatments**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Germination (%)</th>
<th>Germination Rate (seeds/day)</th>
<th>Radicle length (mm)</th>
<th>Plumule length (mm)</th>
<th>Seedling Length (mm)</th>
<th>Vigour index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>63.0ᵇ</td>
<td>3.43ᵇ</td>
<td>20.07ᵇ</td>
<td>17.82ᵇ</td>
<td>37.90ᵇ</td>
<td>22.79ᵇ</td>
</tr>
<tr>
<td>Scarification</td>
<td>98.0ᵇ</td>
<td>13.25ᵃ</td>
<td>42.05ᵃ</td>
<td>26.35ᵃ</td>
<td>68.40ᵃ</td>
<td>68.40ᵃ</td>
</tr>
<tr>
<td>Gibberellic acid (500 ppm)</td>
<td>83.0ᵇ</td>
<td>4.62ᵇ</td>
<td>18.87ᵇ</td>
<td>13.27ᵇ</td>
<td>32.15ᵇ</td>
<td>26.06ᵇ</td>
</tr>
<tr>
<td>KNO₃ (0.6%)</td>
<td>85.0ᵇ</td>
<td>3.45ᵇ</td>
<td>18.50ᵇ</td>
<td>12.40ᵇ</td>
<td>30.90ᵇ</td>
<td>25.86ᵇ</td>
</tr>
<tr>
<td>Stratification</td>
<td>18.6ᶜ</td>
<td>0.34ᵈ</td>
<td>8.02ᶜ</td>
<td>6.70ᶜ</td>
<td>14.72ᶜ</td>
<td>2.66ᶜ</td>
</tr>
<tr>
<td>Stratification+ Scarification</td>
<td>15ᶜ</td>
<td>2.76ᶜ</td>
<td>6.25ᶜ</td>
<td>5.20ᶜ</td>
<td>11.45ᶜ</td>
<td>1.55ᶜ</td>
</tr>
</tbody>
</table>

* Means followed by the same letters in each column are not significantly different (p<0.05)

**Salinity stress test**

The results demonstrated significant effects (p<0.05) of salinity on the germination percentage and germination rate (Table 2 and Fig.2). For both traits the highest and lowest values observed in the control and 20 ds/m salinity level, respectively. No seed germination was observed at the highest level (25 ds/m) of salinity stress. The germination percentage and rate significantly decreased with an increase in the salinity stress level from 5 to 20 ds/m. Among the salinity stress treatments, the highest germination percentage (27%) and rate (1.16 seeds/day) were observed at salinity level of 5 ds/m and the lowest germination percentage (1%) and rate (0.02 seeds/day) were observed at salinity level of 20 ds/m.

A significant decrease (p<0.05) was observed in the radicle, plumule and seedling lengths in the salinity stress treatments (Fig 2). Notably there were significant differences (P<0.05) in all three above mentioned indices between the 20 ds/m salinity stress treatment and the other treatments. The negative effect of salinity on radicle length was stronger than the negative effect on plumule (Fig. 2). There was a significant reduction (p<0.05) in the seed vigour at all salinity levels. The highest and lowest values were observed in the control and 20 ds/m treatments, respectively (Fig. 2).
Fig. 2. Effect of salinity stress on the germination percent and rate, radicle, plumule and seedling length and seed vigor of *S. turcomanica*. Means followed by the same letters in each level are not significantly different (P<0.05).

**Drought stress test**

The germination percent and germination rate decreased by increasing drought stress levels and this reduction was significant at all levels (p<0.05). For both traits, the highest and lowest values were observed in the control and -10 bars, respectively (Fig. 3). Among the drought stress treatments, the highest germination percent (33%) and rate (1.91 seeds/day) were observed at the drought level of -2 Bar, and the lowest germination percent (4%) and rate (0.24 seeds/day) were observed at the drought level of -10 Bar.

Effect of drought levels was significant (P<0.05) on the radicle, plumule, and seedling lengths. (Fig. 3). The longest and shortest radicles, plumules, and seedling were observed in the control and -10 Bar treatments, respectively. No significant difference were observed among the four treatments from -2 to -8 Bar.
The vigour index decreased significantly (P<0.05) by increasing drought stress (Fig. 3). The highest and lowest seed vigour indices were observed in the control and -10 Bar, respectively. There were no significant differences in the seed vigour index among the different drought levels.

![Bar graph showing germination percentage](image1)

![Bar graph showing germination rate](image2)

![Bar graph showing radicle length](image3)

![Bar graph showing plumule length](image4)

![Bar graph showing length of seedling](image5)

**Fig.3.** Effect of drought stress on the germination percent and rate, length of radicle, plumule and seedling, and the seed vigor of *S. turcomanica*. Means followed by the same letters in each level are not significantly different (P>0.05)

**Greenhouse experiment**

The emergence percent at 0, 0.5, 1 and 1.5 cm sowing depths was 80, 60, 45 and 0 %, respectively. The highest emergence percent was observed at the first depth (0 cm); however, it was not significantly higher than second depths (0.5 cm).
Field Measurements

According to the data (Table 4), the mean of plant density, vegetation cover and dry matter production of *S. turcomanica* under field conditions were 16.4 (plant/m²), 28.70 (%) and 176.44 (g/m²), respectively.

Table 3. Measurements of *S. turcomanica* in the sowed area

<table>
<thead>
<tr>
<th>Density (plant/m²)</th>
<th>Vegetation cover (%)</th>
<th>Dry matter production (g/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.4 ± 6.02</td>
<td>28.70 ± 17.03</td>
<td>176.44 ± 64.79</td>
</tr>
</tbody>
</table>

Discussion

Several factors including water availability, salinity, temperature and light can control the germination responses of halophytic seeds (Khan, 2002a). Some plant species have hard and water impermeable seed coats, so their seeds germinate in a delayed manner even if their embryos develop (Evren et al., 2012). In the present study, seed scarification resulted in a significant increase in all germination indices of *S. turcomanica* (Litv) that is in agreement with Saberi et al. (2018). It has been reported that mechanical scarification using sandpaper has significant effect on *Medicago scutellata*, *Medicago polymorpha*, *Prosopis koezliana*, *Prosopis juliflora*, *Capparis ovata*, *S. rigida* and *S. kotschyan* seeds germination (Saberi et al., 2018). Hard and water-impermeable seed coats inhibit water and oxygen infiltration of the seed, causing a reduction in seed germination (Pallavi et al., 2014). Moreover, the seed coat may contain growth inhibitors or may prevent the leaching of inhibitors from the embryo. Seed scarification by permeating the seed shell to water increases the seed germination (Arowosegb, 2016). Application of gibberellic acid increased seed germination percent and rate of *S. turcomanica* by 32% and 35% relative to the control that is in agreement with Nadjafi et al. (2006) and Soyler and Khawar (2007). Gibberellic acid activates various enzymes including amylase in the seed aleurone layer. This enzyme breaks down sugars and starches and converts them into usable material for the seed embryos and increases seed germination.

A higher germination percent (19%) relative to the control was recorded for Potassium nitrate treatment that is in agreement with Nadjafi et al. (2006), Cirak et al. (2007) and Faraji-Allahi (2011). Potassium nitrate stimulates seed germination by stimulating their light sensitivity and increasing the rate of moisture absorption (AshrafiZadeh et al., 2019). It has been reported that KNO₃ could overcome the dormancy of *Salsola nitraria*.
and increase its germination percent (Khajeh-Hosseini et al., 2010)

In the present study, hot water had a destructive effect on S. turcomanica seeds germination indices. Our results are in contrast with Rincon et al. (2003) who have applied hot water to increase germination of Acacia angustissima, but in agreement with Amusa (2011) who had hot water to improve germination of Afzelia africana. It can be argued that hot water treatment might have damaged the embryo and caused the embryo to die. The results of this study revealed that all germination indices of S. turcomanica were affected by salinity and drought stress. The results of present study are consistent with the results of Eskandari and Alizadeh-Amraie (2017) that reported the negative effects of drought stress on germination indices. Variation in the equilibrium of mineral and organic ions present in the cellular latex and ion imbibition (Dinari et al., 2013) lead to internal osmotic potential reduction in plants as a consequence of salt and water stress (Ebadi-Almas et al., 2013). It has been reported that discontinuity in nutrient uptake can lead to a lack of germination (Iraki et al., 1989). Prolongation of the time to reach a minimum dewatering level can lead to a reduction or prevention from germination under drought stress (Dodd et al., 1999).

Khan et al. (2002a and b) reported that increases in NaCl concentration progressively inhibit seed germination of Salsola iberica and Salicornia rubra, so few seeds of S. iberica germinated at 10 ds/m NaCl while no seeds of S. rubra germinated at this salinity.

Limited seed water content due to drought stress decreases the activity of hydrolytic enzymes that are responsible for hydrolyzing the cotyledon reserves required for supplying energy in the initial stages of seed growth by respiration (Dodd et al., 1999 and Saeedi-Goraghami et al., 2013). Due to drought stress, the movement and transfer of seed stocks in addition to seed water uptake are limited. Moreover, the direct effect of drought stress on the organic structure of the embryo and protein synthesis is also assumed to cause a reduction in germination (Saberi et al., 2017). The reduced radicle, plumule and seedling lengths under drought stress maybe due to a lack of nutrient delivery from seed storage tissues to the embryos (Takel, 2000). S. turcomanica seeds germination decreased along with increasing the levels of salinity that is consistent with the result of Saeedi-Goraghami et al. (2013) who studied the effects of salinity on germination parameters of Agropyron destorum. Kazuo-Tobe et al. (2000) reported that when the seeds of Haloxylon ammodendron and H. persicum (Chenopodiaceae) were moistened with solutions of NaCl or PEG, germination in both species after 1 and 2 days was considerably decreased. The negative effects of salinity are physiological drought effects as accumulation of minerals, and ion toxicity. Limited water absorption by seeds due to salinity stress can cause decreased germination and seedling growth (Yaniv et al., 1999). Dry matter accumulation in root storage tissues or turgescence pressure restriction may explain radicle and plumule length reductions under salinity stress (Saeedi-Goraghami et al., 2013). The negative effects of ionic toxicity on the cell membrane, and the destruction of the cytoplasmic membrane occur due to high levels of salinity which can suppress seedling growth. The activity of some enzymes is damaged at high levels of salinity; therefore, seedling growth is affected by such a level of salinity (Saberi et al., 2017).

Considering greenhouse experiment of S. turcomanica, reduction of gases exchange, CO₂ emissions from soil biological activities and decrease in seed energy storage and light quality can be considered as reasons for decreasing seed germination with increasing planting depth (Ashrafzadeh et al., 2019). Regarding field measurements of S.
turcomanica, it can be suggested that cultivation of this species can reduce wind erosion during its growth season (summer) and provide considerable quantity of forage after its maturity (late summer and early autumn). In conclusion, seeds of S. turcomanica have to be scarified before sowing. The cultivation of this species is not recommended at salinity level greater than 15 ds/m. Due to the negative effects of drought stress, sowing time of this species should coincide with rainy months. Field measurements of S. turcomanica revealed that this species has a high potential to use in rangeland improvement or dry land ley-farming projects.

Acknowledgments

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different treatments on improving seed germination and comparing morphological characteristics of two ecotypes of *Citrullus colocynthis* from Sisitan and Baluochestan. Rangeland.11 (3): 353-364 (In Persian).


تیمارهای بهینه سازی جوانه‌زنی بذر Salsola turcomanica (Litv) و بررسی اثرات سطوح مختلف شوری و خشکی بر آن

چکیده.

گونه گیاهی شورپسند نسبتاً خوش‌خوراک Salsola turcomanica (Litv) گونه‌ی گیاهی، به‌وسیلهٔ دانشمندان، به‌عنوان گونه‌ی به‌بین‌سازی جوانه‌زنی بذر و بررسی اثرات سطوح مختلف شوری و خشکی بر آن، تحقیقاتی انجام شد.

در زمستان سال 1391، بمنظور بهبود شاخص‌های جوانه‌زنی، بذر این گیاه در معرض تیمارهای سرماده‌ی، خراشده‌ی فیزیکی (کاغذ سبک)، سرماده‌ی بعلاوه خراشده‌ی فیزیکی، آب داغ 70 درجه و خراشده‌ی شیمیایی (اسید جیبرلیک، نیترات پتاسیم) قرار گرفتند و به‌طور همزمان اثرات شش سطح شوری (0، 0،5، 1، 3، 6، 7 بار) و شش سطح خشکی (7، 2، 4، 1، 8، 67 دسی‌زیمنس بر متر) در شرایط آزمایشگاهی و نیز اثر عمق کاشت بر درصد سبز شدن بذور در شرایط گلخانه با استفاده از طرح کاملاً تصادفی و با چهارتکرار انجام شد. نتایج نشان داد که تیمار "خراشده‌ی فیزیکی" موثرترین تیمار جهت بهبود درصد و نرخ جوانه‌زنی این گونه گیاهی است و بالاترین درصد تیمار "خراشده‌ی فیزیکی" موثرترین تیمار جهت بهبود درصد و نرخ جوانه‌زنی این گونه گیاهی است و بالاترین درصد سبز شدن آن در عمق کشت سطحی مشاهده شد.

نتایج نشان داد که بذرپاشی این گونه گیاهی در مراتع قشلاقی می‌تواند طی فصل رشد آن (تابستان) فرسایش بادی را کاهش داده و پس از بلوغ (اواخر تابستان به اوایل پاییز) مقدار قابل ملاحظه‌ای علوفه تولید نماید. بنابراین به‌نتایج این تحقیق، کاربرد این گونه گیاهی در پروژه‌های اصلاح مرنعو و تناوب علوفه و مرتع توصیه می‌گردد.

کلمات کلیدی: Salsola turcomanica، شاخص‌های جوانه‌زنی، بذر، گلخانه، شوری، خشکی، بذرپاشی.