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Full Length Research Paper

Seed germination and viability of *Salsola imbricata* Forssk

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Salsola imbricata **is a halophytic shrub widely distributed in the coastal and inland regions of Kuwait. The effect of light, temperature, salinity, storage temperature and water potential on germination was conducted in the laboratory experiments.** ***S. imbricata*** **seeds germinated well under wide range of temperatures and in both light and dark conditions; the higher the salinity, the lower the percentage of germination. Ungerminated seeds when transferred to distilled water recovered completely. In the water potential experiment, maximum germination was obtained in distilled water (99%) and decreasing water potential inhibited seed germination, less than 15% of the seeds germinated at -2.4 MPa. Seeds stored at -18 and 4°C for 24 months had 80 – 100% germination rate compared with 0% for those stored at ambient temperature and at 50°C. Based on this result, it is concluded that** ***S. imbricata*** **seeds could establish in wide range of environmental conditions. However the water stress may reduce the establishment of seedlings in the natural population. The seeds are short lived and should be stored at lower temperatures (-18 and 4°C) to maintain viability.**

Key words: Halophyte, viability, salinity, storability, water stress.

INTRODUCTION

Kuwait is a small country situated at the northwestern corner of the Arabian Gulf. Over grazing, environmental factors, military activities and other human related activities increased the constraints and pressures on the desert ecosystem. *Salsola imbricata* Forssk. (Chenopodiaceae) is a halophytic perennial shrub distributed in the coastal and inland region of Kuwait. It usually grows in association with *Zygophyllum qatarense*, *Cressa critica and Aizoon hispanicum*. *S. imbricata* is a highly salt tolerant plant, which grows both in the coastal and inland areas under high salinity foraged by camels. The economic potential of this species is that it is a source for producing alkali and is widely used by locals (Mehrun-Nisa et al., 2007). The new shoots are bright and red in color. The leaves are reduced in surface area when exposed to high salt content in soil. The flowers are tiny and are followed by the appearance of conspicuous winged fruits (Omar et al., 2000). The mass of 10 groups of 100 seeds each was 0.577±0.3 g, and the mean (± S.E) diameter (n=100) was 2.35±0.2 mm. The seeds are flat diameter (n=100) was 2.35±0.2 mm. The seeds are flat disc like with coiled embryo. Seed bank studies indicated that although *S. imbricata* produce large number of seeds annually, most of the seeds were not found in the seed bank after a few month of dispersal indicating a transient nature of the seed bank both in the inland and coastal plant communities (Khan, 1993). Evaporation from the soil surface causes an accumulation of salts near and above the surface of desert soils during summer. The seeds only germinate when the salinity of the soil had been reduced by infiltration to a certain depth so that the soil surface has a low saline content (Gutterman, 1980, 1981a). Inhibitors or salinity also affect germination and act as “rain gauges” (Gutterman, 1993). The impact of light, temperature, salinity, seed storage temperature and water potential on germination of *S. imbricata* seeds help to understand the germination biology of seeds, the

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successful storage of this species in the seed bank and also to enhance the propagation of the seedlings in their natural habitat.

MATERIALS AND METHODS

Environmental condition of Kuwait

Kuwait has a surface area of 17,818 km^2 covering the mainland and a number of offshore islands. During summer, the desert is extremely harsh with mean maximum temperatures of over 44.7°C. The hottest months of the year are July and August. The lowest temperatures of the year occur in December and January with a daily minimum of 4°C, an average of 10°C and a daily maximum of 15 – 20°C. Rainfall is irregular in amount, frequency and distribution (Omar et al., 2000).

Seed collection

S. imbricata seeds were collected from natural rangeland population in Sabah Al Ahmad Natural Reserve (N 29 32'35.4"; E 047°42'51.6") northern region of Kuwait during 2007. The seeds were stored at 4°C prior to germination.

Germination experiment

Germination experiments were conducted in 9 cm diameter disposable Petri dishes. The Petri dishes were lined with filtered paper and moistened with distilled water. For each treatment, 4 replicates of 25 seeds were used. The germination data were recorded everyday. The seeds were considered germinated when the radical protruded to a length of 1 mm.

Seed pretreatments

Effect of light and temperature on germination

Seeds were germinated at 6 different constant temperatures (10, 15, 20, 25, 30 and 35°C) with continuous light and dark. For darkness, the Petri dishes were wrapped with aluminum foil paper.

Effect of salinity on germination and recovery

Seeds were incubated in Petri dishes moistened with 2.5 ml of distilled water or with different concentrations of NaCl solutions (0.05, 0.2, 0.4, 0.6 and 0.8 M NaCl) at room temperature. Petri dishes were sealed by parafilm to prevent evaporation. Germination was monitored every 24 h and the germinated seeds were counted. After 5 days, the ungerminated seeds were rinsed with distilled water and transferred to distilled water medium and the recovery germination rates were recorded. Final recovery germination percentages were calculated using the following formula:

$[(A-B)/(C-B)] \times 100$

Where A is the number of seed germinated in salt solution + those recovered to germinate in the distilled water. B is the number of seeds germinated in salt solution. C is the total number of seeds tested. Final germination was recorded according to Khan and Ungar (1984) as:

$(A/C) \times 100$

Effect of storage temperature

Effect of storage temperature on germination was conducted on *S. imbricata* seeds. Freshly collected seeds were stored at different temperature (ambient temperature 20 to 25, 4, -18 and 50°C) for more than 24 months and the germination percentages were determined at monthly intervals for 24 successive months using seeds per treatment and replicated four times. The germination experiments were conducted at room temperature.

Effect of water potential

Standard germination experiments were used to examine the effect of water potential using mannitol and NaCl solutions. Seeds were germinated in NaCl and mannitol solution separately over the water potential range (Ψ_w) of 0 to -2.4 MPa at room temperature.

Statistical analysis

The data were analyzed by R statistical program and the data were converted into arcsine and the levels of significance were obtained by analysis of variance.

RESULTS

Effect of temperature, light on germination

The effect of temperature, light and dark on germination of *S. imbricata* seeds are shown in Table 1. *S. imbricata* seeds are non dormant seeds. The temperature, light and dark had no effect on germination of the seeds. Germination was almost the same in all temperature ranges, with the exception of the temperature of 20°C, in which slightly lower germinability was observed. The optimum germination was observed at 10°C (Figure 1). However no significant difference in germination was found at 10, 15, 20, 25, 30 and 35°C. There were no significant differences between light and dark treatment. The germination was completed within five days in all treatments.

Effect of salinity on germination

Salinity significantly affected the germination percentage ($p<0.001$). Seed germination was higher in distilled water. As the salinity increased from 0.4 to 0.8 M NaCl, the germination decreased and no germination is observed at 0.8 M NaCl concentration (Figure 2). As the pretreatment concentration of salinity increased to 0.4, 0.6 and 0.8 M NaCl, the recovery germination percentage increased ($p<0.01$) as shown in Table 2.

Effect of storage temperature on germination

Immediately after harvest, *S. imbricata* seeds had higher germination (97%) and germinated within a day. The seeds stored at lower temperatures (-18 and 4°C) maintain

Table 1. Two way ANOVA analysis of effect of temperature, light and their interactions on germination of *Salsola imbricata* seeds.

Source of variance	df	ss	F-value	P-value
Temperature	9	40.265	1.5355	0.1841
Light	3	2.234	0.2556	0.8567
Temperature × light	7	11.396	0.5588	0.7825

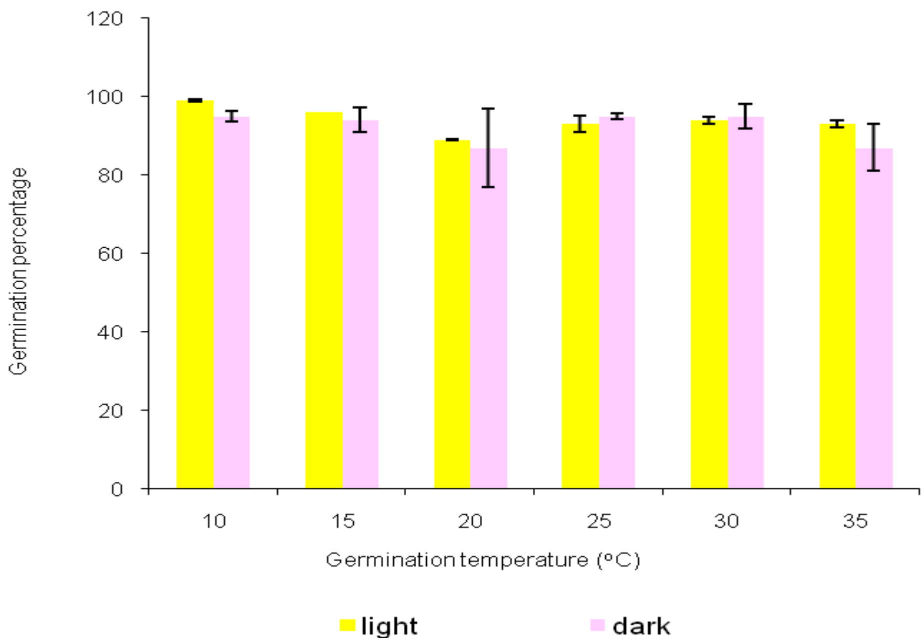

Figure 1. Effect of temperature and ligt on germinatio0n of *Salsola imbricata* seeds.

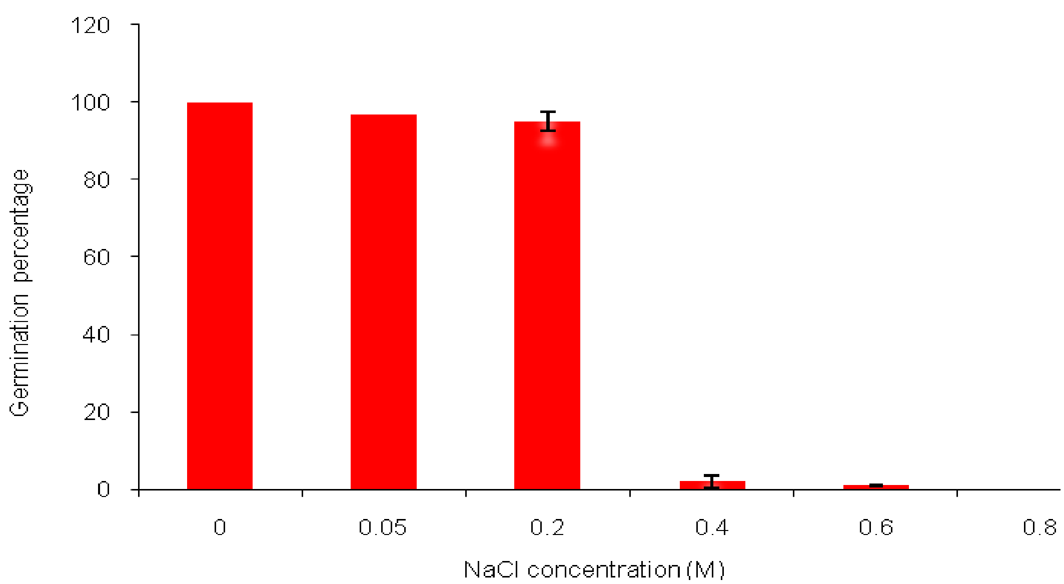

Figure 2. Effects of salinity on of *Salsola imbricata* seeds germination.

Table 2. Effect of NaCl on germination, recovery after transferred into distilled water and final germination of *Salsola imbricata* seeds.

NaCl concentration (M)	Seed germination percentage (± s.e)		
	Initial	Recovery[a]	Final
0	100±0	0±0	0±0
0.05	97±0	20±0	97±0.2
0.2	95±2.5	0±0	95±2.5
0.4	1±0.7	91±4.7	91±1.5
0.6	1±0	95±0.63	95±1.2
0.8	0±0	97±0	97±0
Significance – NaCl conc.[b]	***		
Recovery	**		
NaCl x Recovery	**		

[a] After 5 days of sowing the ungerminated seeds from NaCl treatments were washed with distilled water and transferred to Petri dishes with distilled water medium and the germination was recorded. [b] The data were analyzed by analysis of variance (ANOVA) using the R procedure. ***, ** denotes significance at p<0.001 and p<0.01.

Table 3. Two way ANOVA analysis of effect of storage temperature, duration and their interactions on germination of *Salsola imbricata* seeds.

Source of variance	df	ss	F-value	P-value
Storage temperature	3	17199.6	370.7392	<0.001
Storage duration	1	6832.3	441.8113	<0.001
Storage temperature × storage duration	3	7578.3	163.3499	<0.001

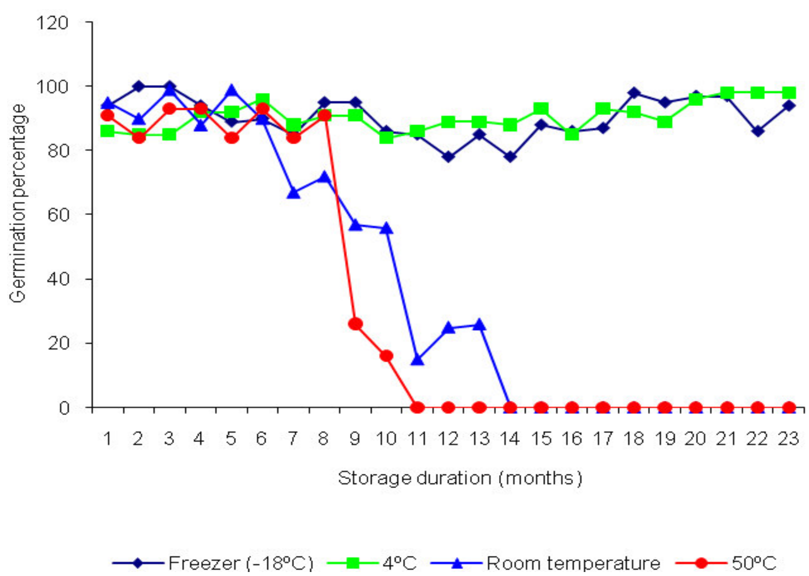

Figure 3. Effects of storage temperature on germination of *Salsola imbricata* seeds.

maintain high level of viability (p<0.001) after 24 months of storage (Table 3). The storage temperature had significant effect on the viability of the seeds during storage. The seeds deteriorated more rapidly at 50°C and room temperature. They completely lost their viability after 10 months of storage (Figure 3). Germination performance was significantly affected by higher temperature.

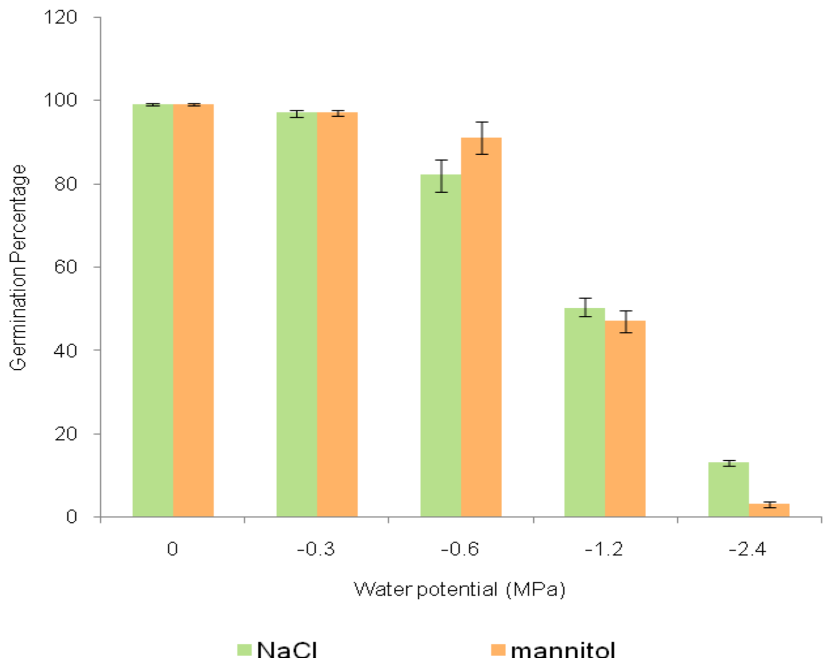

Figure 4. Seed germination of *Salsola imbricata i*n different substrate water potential.

Effect of water potential on germination

The seed germination was inhibited by the decrease in water potential of the germination medium. Highest germination was achieved in control (no NaCl or mannitol). Germination declined with decreasing water potential in both NaCl and mannitol substrate (Figure 4). It was below 15% at -2.4 MPa NaCl and mannitol. However, more than 45% of germination was achieved at -1.2 MPa mannitol and NaCl. NaCl enhancement increased germination (relative to mannitol) by 13% at -2.4 MPa. The result of germination in the 5 different water potential treatments indicated that ionic effect ($p<0.1$) and osmotic effect were significant ($p<0.05$).

DISCUSSION

S. imbricata seeds germinate very fast (within a day). This characteristic is similar to that of other desert halophytes *Haloxylon stocksii, Haloxylon salicornicum* (Sharma and Sen, 1989) and *Tamarix aucheriana* (Zaman et al., 2009). This kind of fast seed germination is the adaptive strategy of desert plants because the salt content of the soil is reduced only for short duration. In a range of temperatures from 10 to 35℃, no difference in germination was observed between light and the dark. Our results are similar with those of *Haloxylon ammodendron* species (Huang et al., 2003) indicating that the seeds may germinate if they are slightly buried in the soil. Kaul and Shankar (1988) reported that seeds of *H. salicornicum* germinated equally well in light and darkness. The optimum germination was obtained at 10℃. Non dormant seeds of many species germinate equally well in light and darkness (Baskin and Baskin, 1988). The germination was slightly lower at 20℃. However more than 90% of germination was achieved at all the other temperature tested. Some species have seeds that germinate in a wide range of temperatures (8 - 40℃). This has been found in *Blepharis* spp. (Gutterman, 1972). In nature, the seeds are dispersed during November or December and they may germinate immediately if the other environmental conditions are favorable. Germination was higher in non saline control (100%). Similar results were reported for *H. ammondendron* (Huang et al., 2003) and *Halocnemum storbilaceum* (Qu et al., 2008). Germination was fast up to 0.2 M NaCl, but at higher salinities, the germination rates were lower. The germination was higher at lower salinities, while the germination was completely inhibited at 0.8 M NaCl concentration. In contrast, seeds of *S. imbricata* from Pakistan germinated above 800 mM NaCl concentration (Mehrun-Nisa et al., 2007). In general, it is agreed that salinity affects germination by creating a potential sufficiently low to inhibit water uptake and /or by providing conditions for the entry of ions that may be toxic to the embryo (Bewley and Black, 1994). Recovery experiments showed that exposure of seeds to various salinity concentrations had little effect on viability of seeds. Similar effect was observed in *Limonium stocksii* seeds where only 5% of seeds germinated at 0.5 M NaCl concentration but 100% germination was achieved when transferred to distilled water (Zia and Khan, 2004). More investigations with halophytes (Ungar, 1991) demonstrated that seeds of several halophytic species remain dormant at higher salinities and these will germinate when returned to distilled water. Seeds of halophytes can recover the capacity to germinate after removal from salt stress that inhibits their germination (Woodell, 1985). Comparison of water potential substrate generated by NaCl or mannitol demonstrated that in *S. imbricata* seeds, the strongly negative water potential decreased the germination. Germination percentage was not adversely affected when osmotic potential was changed from 0 to -0.3 MPa. However it drastically declined as the osmotic potential approached -2.4 MPa. NaCl solution with -2.4 MPa gave 13% of germination. Mean while, a solution with -2.4 MPa mannitol resulted in 3% germination. Therefore, it is concluded that the effect of salinity on germination was not due to ionic effect, it may be due to the osmotic effect under these condition. A mannitol solution with an osmotic potential of -2.43 MPa inhibited the germination of *Z. coccineum* seeds, whereas at -2.02 MPa about 25% of them germinated (Batanouny and Ziegler, 1971a). The effect of osmotic potential on germination was more pronounced than the effect of salinity. This negative effect of osmotic potential could be due to the reduction in water absorption.

Changes in the water potential of the medium alter the properties of the tegument of seeds and low water potential induces low water content of the tegument and consequently low diffusion of water to the inside of seeds (Hadas, 1976). Reduced germination under water stress conditions may be attributed to the effect that seeds develop an osmotically enforced dormancy under water stress conditions, which may be an adaptive strategy of seeds to prevent germination under stressful environment thus ensuring proper establishment of seedlings (Singh et al., 1996; Prado et al., 2000). *S. imbricata* seeds are short lived seeds. Viability testing is crucial to the operation of seed bank because it prevents genetic erosion during storage. Storage at ambient temperature and at 50°C reduced the viability of seeds after 10 months of storage. Seeds stored at lower temperature (4 and -18°C) maintained 80 - 100% of germination after 24 months of storage. The storage temperature appeared to be the most influential factor related to the seed storability. Several studies indicated that some species of Chenopodiaceae including *Salsola* genera have short lived seeds under normal conditions (Clor et al., 1976; Sankary and Barbour, 1972; Creager, 1988; Al-Rowaily, 1999). The results obtained from this study showed that *S. imbricata* seeds are capable of establishing themselves in a wide range of environmental conditions and increase in the environmental stresses reduces the germination rate of the seeds. In addition, the seeds should be stored in air tight container at lower temperature (4 or -18°C) to maintain its viability. More research needs to be carried out on the long term influences of storage conditions on seed viability and germination of *S. imbricata* seeds.

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