Dormancy, germination and viability of *Salsola imbricata* seeds in relation to light, temperature and salinity

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Summary

*Salsola imbricata* Forssk., a leaf succulent perennial halophyte in the family Chenopodiaceae, is distributed both in coastal and inland areas of Karachi, Pakistan. Experiments were conducted in the laboratory to determine the effect of salinity, temperature and light on the germination of seeds. Different abiotic conditions including four temperature regimes (10-20, 15-25, 20-30, 25-35°C) with five NaCl concentrations (0, 200, 400, 600, and 800 mM) were provided to *S. imbricata* seeds during germination under two light levels (12 h dark: 12 h light; and 24 h dark environment). Most seeds germinated in the non-saline control. Seed germination was progressively inhibited with an increase in salinity. About 8% seed germinated at 800 mM NaCl. Optimal germination was obtained at 15-25°C and germination inhibition was greater at cooler temperature regime (10-20°C). Light had little effect on the seed germination at all salinity and temperature treatments. When un-germinated seeds of *S. imbricata* were transferred from saline solutions to distilled water, the germination showed a temperature dependent recovery response. The highest recovery was obtained at 15-25°C. Seeds lost their viability at cooler temperature regimes (10-20°C) and under high salinity, however, poor recovery at warmer temperatures is attributed to the induction of seed dormancy.

Introduction

*Salsola imbricata* Forssk., (Chenopodiaceae) is a leaf succulent perennial halophyte shrub that is widely distributed both coastal and inland habitats. Seeds experience a wide fluctuation in both salinity and temperature. The plant is distributed from Saharan countries through Arabian peninsula, Southern Iran, Pakistan, Afghanistan and North eastern India (Freitag *et al.*, 2001). In Pakistan, it is distributed from coastal dunes and cliffs, dry river-beds, disturbed saline habitats to the mountains (up to 1500 m) Hazara district. At the Arabian sea coast near Karachi, it was found in the sand dunes and cliffs which are infrequently exposed to direct seawater inundation. It usually forms a thicket and grows in association with *Suaeda fruticosa*, *Cressa cretica*, *Cyperus arenarius*, *Heliotropium curassavicum*, *Tamarix indica*, *Atriplex stocksii*, *Sporobolus ioclados* and others. Sources of moisture are the monsoon rains and oceanic seepage. Monsoon period starts from June 15 and end on September 15. During this period, due to high tides the ocean seepage

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increases, while rainfall (220 mm/year) usually occurs during July and August. Storms are rarely reported from the Karachi coast. *Salsola imbricata* commences flowering in August and produces numerous seeds until the end of October. Seeds of other species usually germinate only after the monsoon rains. The average ambient temperature during the monsoon period ranges from 20°C at night to 30°C during the day. Seed bank studies indicated that although *Salsola imbricata* produce a large number seeds annually, most of the seeds were not found in the seed bank after a few months of dispersal indicating a transient nature of the seed bank both in coastal and inland plant communities (Khan, 1993). After considerable monsoon rains, seeds of halophytes like *Suaeda fruticosa* and *Limonium stocksii* were able to germinate but seeds of most halophytic shrubs do not. Little germination of *S. imbricata* seeds were observed under field conditions. The absence of germination could be due to the removal of seeds from the seed bank perhaps due to insect foraging, or the impositions of dormancy or loss of viability due to high salinity and temperature stress in the remaining seeds. *Salsola 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.

Abiotic environmental stresses such as drought, salinity and temperature are major limitations for seed germination of halophytes and they evolve a number of adaptations both metabolic and structural to cope with these stresses (Neo and Zedler, 2000; Al-Khateeb, 2002). A broad range of variation exists in the response of halophytes to salinity (Ungar, 1995) and some seeds of *Salicornia herbacea* germinated in up to 1700 mM NaCl (Chapman, 1960). Leaf succulent halophytes like *S. imbricata* also showed a variable response to salinity during germination (Khan, 2003a) and those with high salt tolerance at the germination stage include *Kochia americana* (1200 mM NaCl, Clark and West, 1969), *Kochia scoparia*, *Salsola iberica*, *Sarcobatus vermiculatus* and *Suaeda moquinii* (1000 mM NaCl, Khan et al., 2001ab; 2002ab), *Suaeda japonica* (900 mM NaCl, Yokoishi and Tanimoto, 1994), *Suaeda depressa* (850 mM NaCl, Ungar, 1962), *Salsola kali* (600 mM NaCl, Woodell, 1985), *Suaeda fruticosa* (500 mM NaCl, Khan and Ungar, 1998). Mohammad and Sen (1990) reported that CaCl₂ and KCl reduced the seed germination of *Salsola baryosma* more than NaCl.

Several factors (water, light and salinity) regulate seed germination and they may even co-act with the seasonal variation in temperature to determine the temporal pattern of germination (Khan, 2003a). Variation in temperature under saline conditions has differential effects on the germination of halophytes (Khan and Ungar, 1996) and this effect could be due to ecological conditions of the habitat where they belong. Pakistan has varied physiographic and climatic conditions. It stretches about 1600 km from the subtropical Arabian sea to temperate northern mountains covering an area of 800,000 km² (Khan, 2003b). Sub-tropical halophytes predominantly show optimal germination at 20-30°C and any further increase or decrease in temperature affected the germination (Khan and Rizvi, 1994; Zia and Khan, 2002; Khan, 2003a). Okusanya (1977; 1979) reported that there is an optimum temperature, which interacts with the salinity to give highest percent germination. Cooler or higher temperature from that optimum can suppress germination in the presence of salinity.
Light is another important factor in releasing seed dormancy but the role of light to regulate seed dormancy depends on the geographical origin of that plant species, time of seed production and the special conditions to which the plant is exposed to during its life cycle (Bewley and Black, 1994). Some halophytes have obligate requirement of light for seed germination while in others presence of light enhances seed germination to various degrees and still other do not require light for germination (DeVilliers et al., 1994; Khan and Rizvi, 1994). Light requiring seeds germinate at a time when drought and temperature stress are relatively low (Baskin and Baskin, 1998).

Halophyte seeds have the ability to remain viable for long periods during exposure to hypersaline conditions and then germinate when salinity is reduced (Ungar, 1995; Khan and Ungar, 1997a; 1998). However, there are halophytes which do not survive long period of hypersaline conditions (Keiffer and Ungar, 1995) and particularly seeds of sub-tropical species which loose their viability quickly under high salinity and temperature stress (Khan, unpublished data). Species differ in their recovery responses from stress (Woodell, 1985). Zygophyllum simplex showed poor recovery (Khan and Ungar, 1997b) while Haloxylon recurvum (Khan, 1999) and Suaeda fruticosa (Khan and Ungar, 1998) showed higher recovery percentages when seeds were transferred to non-saline medium. Failure of the seed to recover when transferred to distilled water could either due to death of the seeds or the induction of dormancy by high temperature and salinity stress when present in the soil. This dormancy response was perhaps mediated through metabolic changes in the imbibed seeds and could be alleviated with the application of dormancy-relieving (GA$_3$, cytokinins, nitrates, fusicoccin, ethephon etc.) chemicals (Khan and Ungar, 2002).

The present research was done to determine the effect of light, temperature and NaCl on germination, dormancy and viability of *S. imbricata* seed.

**Materials and methods**

The fruits of *Salsola imbricata* were collected during December 2000 from Clifton Karachi; Pakistan. Seeds were separated from bracts, cleaned, and dry stored at room temperature after surface sterilization with 0.85% sodium hypochlorite for 1 min. Five salinity concentrations (0, 200, 400, 600, and 800 mM NaCl) were used based on a preliminary test for salt tolerance of the species. Twenty five seeds were directly placed in 50 × 9 mm tight fitting plastic petri plates submerged in 5 ml test solution. Four replicates of 25 seeds each were used. A seed was considered to have germinated at the emergence of the radicle (Bewley and Black, 1994). Germination was tested in a programmed incubator (Percival Scientific Inc., Boone, Iowa, USA) at (dark: light) 10-20, 15-25, 20-30 and 25-35°C temperature regimes with a 12h photoperiod (25 µmol.m$^{-2}$.s$^{-1}$, 400-700 nm Sylvania cool-white fluorescent lamps). Similarly, seeds were incubated in photographic envelopes for the dark treatment under the same conditions. Germination was recorded every alternate day for 20 days. After 20 days, seeds ungerminated under photoperiodic (light: dark) were transferred to distilled water for another 20 days at their respective temperatures for the recovery and germination percentage was noted every alternate day for another 20 days. Ungerminated seeds were tested for viability using
1% of 2,3,5-triphenyl tetrazolium chloride. Rate of germination was calculated with a modified Timson’s index of germination velocity = ΣG/t, where G is percentage of seed germination after 20 days, and t is total germination period (Khan and Ungar, 1998). Rate of recovery of germination was calculated by using the equation \[(a-b)/(c-b) \times 100\] where, a is total number of seed germinated after being transferred to distilled water, b is total number of seed germinated in saline solution and c is total number of seeds. The effect of germination regulating compounds namely GA (0.3 mM), kinetin (0.05 mM), proline (0.1 mM), betaine (0.1 mM), nitrate (20 mM), thiourea (10 mM), and fusicoccin (0.05 µM) in alleviating salinity induced inhibition of seed germination of *S. imbricata* was also studied. The experiment was conducted at 15-25°C thermoperiod following the aforementioned protocol.

Germination data were analyzed using SPSS, version 9.0 (SPSS, 1999). The effects of light, salinity, and temperature on the germination and rate of germination were examined using analysis of variance (ANOVA). A Bonferroni test was carried out to determine whether significant (P < 0.05) differences occurred between individual treatments (SPSS, 1999). A linear regression analyses was used to determine the relationship between salt concentration and germination at different salinities.

**Results**

Germination of *Salsola imbricata* seeds was significantly increased at optimal temperature, decreased by salinity, little affected by light and varied with interaction of three factors (table 1). Seed germination was higher in distilled water at all temperature regimes and few seeds germinated at 800 mM NaCl (figure 1). Germination was strongly affected by temperature. Germination at 10-20°C was significantly inhibited and there was no seed germinated in 600 and 800 mM NaCl. Seeds showed the highest germination in all treatments at the moderate temperature regime (15-25°C) with 90% germination in the non-saline control (figures 1 and 2). Seeds germinated under complete darkness were significantly different from light in most tests (figure 2). However, at lower thermoperiod (10-20°C) there was significantly higher germination in light at low salinity. At 20-30°C there was a substantial reduction in germination at 400 mM NaCl and some reduction at 600 mM NaCl.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Thermoperiod (T)</th>
<th>Photoperiod (L)</th>
<th>NaCl (S)</th>
<th>T × L</th>
<th>T × S</th>
<th>L × S</th>
<th>T × L × S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination percentage</td>
<td>55.5**</td>
<td>32.1**</td>
<td>400.9**</td>
<td>0.9**</td>
<td>7.4**</td>
<td>5.1**</td>
<td>1.0**</td>
</tr>
<tr>
<td>Germination rate</td>
<td>38.6**</td>
<td>-</td>
<td>252.2**</td>
<td>-</td>
<td>5.1**</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Degrees of freedom</td>
<td>3.0</td>
<td>1.0</td>
<td>4.0</td>
<td>3.0</td>
<td>12.0</td>
<td>4.0</td>
<td>12.0</td>
</tr>
</tbody>
</table>

Note. Number represents *F*-values. ns = not significant. **P < 0.05
A linear regression indicates a higher proportion of germination response with R² values ranging from 0.71 to 0.93 in various temperature treatments (figure 3). The rate of germination showed no significant difference in non-saline control at all temperature regimes except 10-20°C where lower rate of germination was observed (figure 3). Rate of germination progressively decreased with an increase in salinity. A two way ANOVA for rate of germination indicated a significant effect of salinity and temperature (P < 0.05).

Figure 1. Percent germination of *S. imbricata* seeds in 0, 200, 400, 600 and 800mM at thermoperiods of 10-20, 15-25, 20-30 and 25-35°C. Different Bonferroni letters represent significant (P<0.05) difference between salinity treatments.

Table 2. Results of two way ANOVA of characteristics by thermoperiod (T) and NaCl (S) treatments of *Salsola imbricata*.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Thermoperiod (T)</th>
<th>NaCl (S)</th>
<th>T × S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery germination</td>
<td>59.7**</td>
<td>56.5***</td>
<td>12.3**</td>
</tr>
<tr>
<td>Recovery rate</td>
<td>3.3**</td>
<td>38.5**</td>
<td>2.5**</td>
</tr>
<tr>
<td>Degrees of freedom</td>
<td>3.0</td>
<td>4.0</td>
<td>12.0</td>
</tr>
</tbody>
</table>

Note. Number represents *F*-values. ns = not significant. **P < 0.05.
A two way ANOVA for recovery and rate of recovery indicated significant (P < 0.01) effects of salinity and thermoperiod (table 2). *Salsola imbricata* seeds responded differentially at various salinity and temperature regimes. When seeds were transferred to distilled water after 20 days of salinity treatment, the recovery germination percentages generally were low at all temperature regimes (figures 4 and 5).

The effect of light, temperature, and salinity was tested on germination, recovery, viability and death of *Salsola imbricata* seeds (figure 6). At least 5% seeds of *S. imbricata* were found dead in control at all temperature regimes. Increase in salinity progressively caused the mortality of seeds and the death of seed was relatively high at lower temperature (figure 6). At low thermoperiods, the presence of light had no effect on the mortality of seeds, however a further increase in temperature in the presence of light caused more seed death. At high salinity and higher temperature, most seeds in the dark remained dormant (figure 6).

None of germination regulating chemicals were effective in alleviating the salinity induced death or dormancy of *S. imbricata* seeds (figure 7).

![Figure 2. Effect of NaCl, light and temperature on seed germination of *S. imbricata*. Bars represent mean ± S.E. Values at each NaCl concentration having the same letter are not significant different (P < 0.05), Bonferroni test.](image-url)
Discussion

Halophytes that primarily dominate the subtropical deserts are shrubby perennials with a few annuals (Khan, 2003a). Seed germination is a critical transition between the stage that is the most tolerant to drought and extreme periods of stress (e.g. seed bank). The seedling stage is a vulnerable stage in plant development. Germination of newly produced seeds in desert species is usually prevented by the adverse climatic conditions (drought and extreme temperatures) which prevailing after seed dispersal. Perennial halophytes, including Salsola imbricata, which usually dominate the study area, often maintaining a transient rather than a persistent seed bank (Khan, 1993; Zaman and Khan, 1992). Even after a period of rainfall, few seeds germinate and the recruitment of new plants through seed germination is rare (Khan and Rizvi, 1994; Khan and Ungar, 1996) except with the exceptions of Limonium stocksii and Suaeda fruticosa (Khan, 2003a). This lack of germination could be attributed to a hard seed coat, absence of a seed bank, induced seed dormancy or death of most of the seeds.

Seeds of S. imbricata had about 95% germination in distilled water and the remaining 5% were found dead. Seeds were highly salt tolerant during germination and could germinate in the salinities higher than seawater (800 mM NaCl). Seeds of the other associated halophytes such as Cressa cretica (Khan, 1999), Arthrocnemum indicum (Khan
and Gul, 1998) could also germinate at or above 800 mM NaCl, while seeds of other halophytes like *Haloxylon stocksii* and *Suaeda fruticosa* fail to germinate at salinities beyond 500 mM NaCl (Khan and Ungar, 1996; 1998). Whereas others halophytes like *Atriplex stocksii* could germinate only in solutions up to 300 mM NaCl (Khan and Rizvi, 1994). Khan et al. (2002b) reported that seeds of *Salsola iberica* could germinate in 1000 mM NaCl and the optimal germination was obtained at 25-35°C temperature regimes, while seeds of *Salsola kali* were not dormant and the germination was reduced at 60 mM NaCl (Ignaciuk and Lee, 1980) and some seed germination occurred at 600 mM NaCl (Woodell, 1985).

Temperature and its interacts with NaCl plays a significant role in the seed germination of subtropical halophytes (Khan, 2003a). The optimal germination percentage of *S. imbricata* was observed at 15-25°C temperature regime. The germination decreases significantly, as the deviation from the optimal temperature increased. Salinity and temperature are reported to produce an interactive effect in the seed germination stage, and the limits of tolerance to salinity may be greater at one temperature than another (Ungar, 1995; Khan, 2003a). Bewley and Black (1994). Temperature changes may affect a number of processes during seed germination including the membrane permeability, activity of membrane-bound proteins, and cytosol enzymes.
Seed germination percentage of *S. imbricata* did not differ significantly between light and dark treatments. Similar results were obtained for seed germination of *Atriplex semibaccata* and *Senecio elegans* (De Villiers et al., 1994) and *Suaeda fruticosa* (Khan and Ungar, 1998). The light requirement is probably a genetic characteristic and a regulatory environmental signal for many plants (Okusanya, 1977; Khan and Ungar, 1997c) and is mediated by phytochrome (Olatoye and Hall, 1972; Jones and Hall, 1979).

Halophytes seeds are dispersed after monsoon rains (Khan and Gul, 1998) and are exposed to various environmental stresses while present in the soil (Gul and Khan, 2001). In the subtropical environment seeds are usually exposed to high salinity and temperature stress if present in salt marshes and only high temperature stress when present in the saline desert (Khan, 2003a). Halophytes can only maintain the continuity of their lineage if they can survive physicochemical stresses such as drought, salinity, extreme temperature and their interaction while in the seed bank and still maintain their viability. Many halophyte seeds have the ability to maintain seed viability for extended period of exposure to hypersaline conditions along with other factors and still germinate when conditions are favorable (Woodell, 1985; Keiffer and Ungar, 1995; Khan and Ungar, 1997a). Seeds of *S. imbricata* when exposed to high salinity and temperature stress showed a poor recovery response. Recovery percentages were higher in high salt treatments at all temperatures and vary between 20 to 30%. Low recovery responses were also reported for *Zygophyllum simplex* (Khan and Ungar, 1997b), *Arthrocnemum macrostachyum* and *Salicornia ramosissima* (Rubio-Casal et al., 2003). Our data showed that seed recovery percentage did not differ significantly with thermoperiod.
Viability tests showed varying degree of dormancy and varying loss of viability in the seeds exposed to various temperature and salinity regimes under light and dark conditions. Poor seed recovery response in *S. imbricata* appeared to be the effect of the combination of salinity, temperature, light and their interaction. It is also interesting to note that the dormancy was induced in *S. imbricata* seeds when exposed to high salinity in complete darkness at warmer thermoperiods. Seeds lose viability at cooler temperature and in light under saline conditions, while the absence of light, high salinity and warmer temperatures induced seed dormancy.

Figure 7. Effect of different germination regulating compounds on seed germination percentage of *Salsola imbricata* with NaCl at 15:25°C.
Our study indicates that all the germination regulating chemicals used were ineffective in alleviating the salinity induced dormancy of *S. imbricata* seeds. This might be due to the concentrations of the germination regulating compounds we have used in our experiment or the genetic characteristic of *S. imbricata* seeds. Results found about all of the regulating chemicals were in agreement with the earlier reports for the other associated species like *Suada fruticosa*, *Haloxylon stocksii*, *Limonium stocksii*, *Aleuropus lagopoides*, *Urochondra setulosa* and *Sporobolus ioclados* (Khan, 2003a).

*Salsola imbricata* produces a large number of ready-to-germinate seeds every year with the ability to germinate in highly saline (800 mM) conditions. Most of them, however, are lost from the seed bank in few months and those, which remained either die under cooler temperatures or have induced dormancy at warmer temperature regime in light. Failure to germinate under natural conditions could be attributed to transient seed bank, death of the seeds due to the combination of cool temperatures and high salinity or induction of dormancy at high temperature regimes. Growth regulators failed to alleviate salinity-induced inhibition of germination in *S. imbricata* seeds. It would be interesting to find out the mechanisms of decrease death and/or reduce induction of dormancy under dark, high salinity and high temperature conditions.

**References**


