Germination of the salt tolerant shrub *Suaeda fruticosa* from Pakistan: salinity and temperature responses

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**Summary**

*Suaeda fruticosa*, a leaf succulent perennial in the family Chenopodiaceae, is widely distributed in the salt marshes and salt deserts of Karachi, Pakistan. We are reporting here the effect of salinity and thermoperiod on the percentage germination and recovery of germination of *Suaeda fruticosa* after exposure to salinity under laboratory conditions. Cumulative germination percentages decreased with an increase in salinity. Seed germination occurred up to the 400 mM NaCl treatment. A 12h 15°C/25°C thermoperiod was more suitable for germination than the higher (12h 25°C/35°C) and lower (12h 10°C/20°C) thermoperiod. When ungerminated seeds from all thermoperiods were transferred to distilled water after 20 days of exposure to salinity, they initiated germinated in 48h. There was up to 80% recovery of germination for seeds that initially did not germinate in 500 mM NaCl. Seeds exposed to the low thermoperiod (12h 10°C/20°C) demonstrated a higher priming effect from salinity exposure than did those in the high thermoperiod (12h 25°C/35°C).

**Introduction**

Halophytes are distributed in coastal and inland saline habitats throughout the world (Ungar, 1991a; Ungar, 1991b) and their populations are subjected to high mortality risks because of the direct action of high salinity stress or other associated abiotic factors (Ungar, 1987). Germination is a crucial stage in the life cycle of plants and salt tolerance during the germination stage is critical for the establishment of plants that grow in saline soil (Khan and Ungar, 1996; Ungar, 1996b). Halophytes vary in their upper limits of salt tolerance, and increases in salinity usually delay seed germination (Ungar, 1995). Seeds of salt marsh species *Cressa cretica* L. (Khan, 1991), *Salicornia bigelovii* Torr. (Rivers and Weber, 1971), *Salicornia pacifica* (Tidestrom) Munz (Khan and Weber, 1986) and *Tamarix pentandra* Pall. (Ungar, 1967) have been shown to germinate in up to 860 mM or higher NaCl. However, species like *Atriplex triangularis* Willd. (Khan and Ungar, 1984) and *Zygophyllum simplex* L. (Khan and Ungar, 1996b) show little germination above 125 mM NaCl. Seeds of halophytes are known to maintain viability for an extended period of time during exposure to high salinity and they initiate germination when the salinity is reduced (Williams and Ungar, 1972; Ungar, 1991a; Ungar, 1991b; Ungar, 1995; Keiffer and Ungar, 1995).

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Suaeda fruticosa (L.) Forssk. maintains a large seed bank in inland saline communities (Khan, 1991) and their seeds germinate soon after monsoon rains when temperature as well as salinity concentrations are lowered due to leaching (Mohammad and Sen, 1991). Salinity plays an important role in determining the germination and survival of Suaeda sp. (Sheikh and Mahmood, 1986) and it can tolerate high salinity during germination (Rajpurohit and Sen, 1977; Sheikh and Mahmood, 1986; Mohammad and Sen, 1991). The limit of salt tolerance of different species of Suaeda varies from 400 mM to 800 mM NaCl (Ungar, 1962; Ungar and Capilupo, 1969; Clarke and Hannon, 1970; Okusanya, 1979; Yokoishi and Tanimoto, 1994).

Temperature interacts with the salinity in determining the rate and total germination percentages of seeds of halophytes (Ismail, 1990; Khan, 1991; Khan and Rizvi, 1994; Noor and Khan, 1995; Khan and Ungar, 1996a; 1996b). Binet and Boucaud (1968) studied the effect of salinity and temperature on the germination of four Suaeda species viz. Suaeda macrocarpa Moq., S. splendens Gren. and G., S. flexilis Focke, and S. fruticosa Forsk. They found that lower constant temperature regimes and a 15-day cold pretreatment significantly promoted germination. However, alternating temperature regimes there was no stimulation by a stratification pretreatment except in the case of S. fruticosa, where it significantly promoted seed germination. The ability of halophyte seeds to maintain viability after an extended period of exposure to salinity has been reported (Macke and Ungar, 1971; Woodell, 1985; Keiffer and Ungar, 1995; Nolasco, Vega-Villasante, Romero-Schmidt, and Diaz-Rondero, 1996). However, few studies have focused on the effect of variation in thermoperiod on the recovery of halophytes (Khan and Ungar, 1996a; 1996b). Based on these latter data, it is important to determine the effect of thermoperiod and salinity on the germination and recovery of S. fruticosa seeds to understand its germination behavior under field conditions.

Suaeda fruticosa (Chenopodiaceae) is a leaf succulent perennial, with a woody stem distributed widely in the coastal and inland salt marshes and deserts of Karachi, Pakistan (Stewart, 1976). Rainfall is seasonal, averaging 22 cm per year during the monsoon rains that occur from June to August. Soil salinity increases after these rains and after a few months salt crystals precipitate on the soil surface. The average low temperature during winter is 15°C and the average high temperature during summer is 35°C (Maximum reported in 47°C). Suaeda fruticosa seeds present in the seed bank are exposed to various thermoperiods and salinity concentrations. Little information is available on the effect of salinity on the germination and recovery of germination of Suaeda fruticosa under different thermoperiods. The purpose of this investigation is to determine how thermoperiod and salinity interact to affect the rate and total germination percentages of Suaeda fruticosa seeds. We also wish to ascertain the effect of salinity and thermoperiod on the viability of seeds.

Materials and methods

Seeds of Suaeda fruticosa (L.) Forssk. were bulk collected during the fall 1994 from salt flats situated on the Karachi University campus, Pakistan. Seeds were separated
from the inflorescence and stored at 4°C. They were brought to Ohio University, USA and germination studies were started in February 1995. Seeds were surface-sterilized using the fungicide (Phygon). Germination was carried out in 50 × 9-mm, Gelman No. 7232, tight-fitting plastic petri dishes with 5 ml of test solution. The dishes were placed in 9-cm-diameter plastic petri dishes as an added precaution to prevent loss of water by evaporation. Four replicates of 25 seeds each were used for each treatment. Seeds were considered to be germinated with the emergence of the radicle.

To determine the effect of temperature on germination alternating temperature regimes of 10–20°C, 10–30°C, 15–25°C and 25–35°C. The higher temperature coincided with a 12-hr light period (Sylvania cool white fluorescent lamps, 25 µM m⁻² s⁻¹, 400–700 nm) and the low temperature with a 12-hr dark period. Seeds were germinated in distilled water, 100, 200, 300, 400 and 500 mM NaCl solutions. Percentage germination was recorded on alternate days for 20 days. After 20 days ungerminated seeds from the NaCl treatments were transferred to distilled water to study the recovery of germination, which was also recorded at 2 day intervals for 20 days. The recovery percentages was determined by the following formula: (a−b)/(c−b) X 100, where a is the total number of seeds germinated after being transferred to distilled water, b is the total number of seed germinated in saline solution and c is the total number of seeds. The rate of germination was estimated by using a modified Timson index of germination velocity = \( \Sigma G/t \), where G is the percentage of seed germination at 2-days intervals, and t is the total germination period (Khan and Ungar, 1984). The maximum value possible using this index with our data was 50. The higher the value, the more rapid the rate of germination.

Germination data were transformed (arcsine) before statistical analysis. A two way ANOVA analysis was used to determine if significant differences were present among means. If significant differences occurred, a Bonferroni analysis (Multiple range test = Modified LSD, P < 0.05) was carried out to determine if significant differences occurred between individual treatments (SPSS, 1994).

Results

A two-way ANOVA of percent germination, rate of germination and recovery of germination indicated significant main effect of salinity and temperature (Table 2). Interaction between salinity and temperature was not significant.

In the non-saline control the seeds of *Suaeda fruticosa* germinated rapidly at 15–25°C, 10–30°C, and 25–35°C, while at the cooler thermoperiod (10–20°C) their germination reached its peak on the 10th day in comparison to days 3 to 8 in other treatments (Figure 1). Maximal germination was obtained at the thermoperiod 15–25°C in the non-saline control. Increase in salinity caused a progressive decrease in germination (Figure 1). Extreme temperature conditions (25–35°C and 10–20°C) inhibited germination more than the moderate thermoperiods (Figure 2). Final germination percentages at 10–20°C in all salinity treatments was low in comparison to other thermoperiods (Figure 2). One-way ANOVA of germination for each temperature regime revealed that salinity signifi-
Table 1. Index of germination velocity (mean ± SD at different salinities and day/night temperature using a modified Timson index (Khan und Ungar 1984).

<table>
<thead>
<tr>
<th>NaCl (mM)</th>
<th>Thermoperiod °C</th>
<th>10–20</th>
<th>10–30</th>
<th>15–25</th>
<th>35–25</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>20.0 ± 2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.6 ± 5.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.3 ± 2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.7 ± 3.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>15.5 ± 2.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>28.3 ± 1.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>22.1 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.8 ± 3.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>200</td>
<td></td>
<td>11.9 ± 1.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.5 ± 3.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>21.2 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.6 ± 4.0&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>300</td>
<td></td>
<td>4.0 ± 0.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.0 ± 1.9&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>12.1 ± 1.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.3 ± 4.6&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>400</td>
<td></td>
<td>0.2 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.5 ± 0.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.6 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.8 ± 0.4&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>500</td>
<td></td>
<td>0.0 ± 0.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.1 ± 0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.7 ± 0.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.0 ± 0.0&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values in each column having the same letter are not significantly different P<0.05, Bonferroni test).

Significantly affected germination of seeds at each temperature regimes (10–20°C, F = 27.8, P < 0.00001; 10–30°C, F = 20.7, P < 0.00001; 15–25°C, F = 43.5, P < 0.00001; 25–35°C, F = 19.2, P < 0.00001).

Rate of germination under non-saline control conditions was highest at a 15–25°C.

![Figure 1. Rate of germination of *Suaeda fruticosa* seeds in 0, 100, 200, 300, 400, and 500 mM NaCl at thermoperiods of 10–20°C, 10–30°C, 15–25°C, and 25–35°C.](image-url)
thermoperiod (Table 1). At all salinity treatments, the 10–20°C thermoperiod showed the lowest germination rate in comparison to other thermoperiods. One-way ANOVA of rate of germination for each temperature regime revealed that salinity significantly affected rate of germination of seeds (10–20°C, $F = 29.9$, $P < 0.00001$; 10–30°C, $F = 19.0$, $P < 0.00001$; 15–25°C, $F = 68.5$, $P < 0.00001$; 25–35°C, $F = 18.4$, $P < 0.00001$).

Table 2. Results of two way analysis of variance of characteristics by salinity (S) and temperature (T) treatments.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variable</th>
<th>Salinity (S)</th>
<th>Temperature (T)</th>
<th>S x T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent germination</td>
<td></td>
<td>97.7***</td>
<td>6.19**</td>
<td>0.553n.s.</td>
</tr>
<tr>
<td>Rate of germination</td>
<td></td>
<td>95.1***</td>
<td>12.7***</td>
<td>1.479n.s.</td>
</tr>
<tr>
<td>Recovery of germination</td>
<td></td>
<td>44.3***</td>
<td>4.5*</td>
<td>0.947n.s.</td>
</tr>
</tbody>
</table>

Note: Numbers represent F value: • = $P < 0.05$, *** = $P < 0.001$, n.s. = not significant.
NaCl at all thermoperiods recovered very quickly when transferred to distilled water (Figure 3). Seeds exposed to 400 and 500 mM salinity at the three lower thermoperiods (10–20, 10–30, 15–25°C) showed a priming effect of salinity. The percentage germination of seeds exposed to 500 mM NaCl had 20% higher germination than the controls at 10–20 and 10–30°C. At 15–25C recovery germination from the highest salinity was similar to the non-saline control, while at the higher thermoperiod (25–35°C) recovery was about 50% less than in the non-saline control. However, at lower salinities recovery germination was similar to the control.

Percentage recovery germination varied with thermoperiod (Figure 4). At lower thermoperiods (10–20, 10–30 and 15–25°C), seeds initially exposed to 400 and 500 mM NaCl treatments had a recovery of 70 to 80%, while at 25–35°C germination was reduced to 40–50%. Seeds exposed to moderate salinity treatments (200 and 300 mM NaCl) had about 40 to 50% recovery except in the 10–30 C thermoperiod where recovery was 70% at 300 mM NaCl (Fig. 4). Seeds in the 100 mM NaCl treatments showed no significant recovery when compared to the distilled water control. One-way ANOVA of recovery of germination for each temperature regime revealed that salinity sig-
Figure 4. Percent recovery of *Suaeda fruticosa* seeds in 0, 100, 200, 300, 400, and 500 mM NaCl at thermoperiods of 10–20°C, 10–30°C, 15–25°C, and 25–35°C. Same symbols as in figure1.

Significantly affected recovery germination of seeds (10–20°C, F = 35.5, P < 0.00001; 10–30°C, F = 5.8, P < 0.003; 15–25°C, F = 11.8, P < 0.00001; 25–35°C, F = 18.3, P < 0.00001). Time required for 50% of the seeds to germinate (relative value per treatment) in a treatment usually increased with an increase in salinity and with a decrease in temperature regime (Figure 5). However, at 400 mM and 500 mM NaCl this trend did not occur with a decrease in temperature.

**Discussion**

Germination of *Suaeda fruticosa* seeds decreased with an increase in salinity, and few seeds germinated at 400 mM NaCl. A cooler thermoperiod (10–20°C) inhibited germination whereas, a moderate thermoperiod was better suited for germination. Contrary to previous reports (Chapman, 1947; Binet and Boucaud, 1968) indicating a very low viability of seeds (8 to 50%), our *Suaeda fruticosa* seeds displayed from 79–94% germination in distilled water at various thermoperiods. Chapman (1947) reported about 66% reduction in germination of *Suaeda fruticosa* at 100 mM NaCl, and similar data was reported by Rajpurohit and Sen (1977) and Sheikh and Mehmood (1986). However, Binet
Fig. 5. Days required to attain 50% of total germination (mean +SE) in a treatment of *Suaeda fruticosa* seeds in 0, 100, 200, 300, 400, and 500 mM NaCl at thermoperiods of 10–20°C, 10–30°C, 15–25°C, and 25–35°C.

and Boucaud (1968) found a substantial reduction in germination at about 400 mM NaCl. Our results are in agreement with the findings of Binet and Boucaud (1968). Mohammad and Sen (1991) determined that there was a difference in salt tolerance in *S. fruticosa* species collected from different habitats. At 1% NaCl, seeds from highly saline areas had 40% germination as compared to 67% in non-saline controls, while seeds from low salinity areas did not germinate.

*Suaeda fruticosa* seeds recovered quickly from hypersaline conditions (>400 mM NaCl) when transferred to distilled water at all thermoperiods. After transfer to distilled water, seeds initially exposed to 500 mM salinity at 10–20°C had 20% more germination than the control. Recovery experiments, after soaking seeds of *S. calceolaria* and *S. linearis* (Elliot) Moq. for 30 days in 5% NaCl, indicated no specific ion toxicity and that an osmotic effect limited germination (Ungar, 1962; Ungar and Capilupo, 1969; Williams and Ungar, 1972). *Suaeda calceolaria* (Hook.) Moq. had 63% germination in the recovery treatment compared to 42% in distilled water controls, indicat-
ing that a NaCl pretreatment might stimulate germination (Ungar and Capilupo, 1969). *Suaeda calceoliformis* seeds exposed to 3, 5 and 10% NaCl solutions for 2 years were significantly stimulated when compared to the distilled water control (Keiffer and Ungar, 1995). A similar promotion of germination was also reported for *Suaeda australis* R. Br., *Arthrocnemum australasicum* (Moq.) Moss., *Triglochin striata* Ruiz and Pav., *Juncus maritimus* Lam., and *Casuarina glauca* Sieb. Ex Spreng (Clarke and Hannon, 1970) and *Limonium* sp (Boorman, 1968). Woodell (1985) classified germination responses to salinity into three categories; Type 1 species, were all inhibited by half strength seawater. Recovery in distilled water was relatively high, but no salt stimulation of germination was observed in this group. Seeds of Type 2 species were strongly inhibited by half-strength seawater but had recovery germination (56% to 98%) from seawater that was similar to the control. Type 3 species, which had less than 10% seed germination in seawater, were salt stimulated and had greater than 60% recovery germination. Keiffer and Ungar (1995) exposed seeds of five halophytes to an extended period of salinity treatments and determined their recovery responses when transferred to distilled water. They used Woodell (1985) classification system and placed *Atriplex prostrata* Boucher ex DC seeds in Type 1, *Hordeum jubatum* L. and *Spergularia marina* (L.) GRÎSEB. in the Type 2 and *Salicornia europaea* L. and *Suaeda calceoliformis* in Type 3 category. Germination and recovery responses of *S. fruticosa* in the present study can be categorized as Type 3.

Percentage recovery in *S. fruticosa* varied with temperature. At lower thermoperiods the seeds initially exposed to high salinities had 70 to 80% recovery. However, at high thermoperiods (15–25 and 25–35°C) the recovery response was reduced to 40–50%. Seeds exposed to the low salinity treatment had a recovery rate that was similar to controls. Our data indicate that the ambient temperature plays a significant role in determining the recovery responses of halophytic seeds.

*Suaeda fruticosa* usually grows in warm, dry and highly saline habitat. After dispersal seeds are exposed to high salinity and remain dormant in the soil. Monsoon rains substantially from June through August, decrease the salinity level of the soil due to leaching and moisture in the atmosphere causes high cloud cover, which results in a decrease in the mean ambient temperature from a high of 31°C in June to 27°C in August. This provides conditions, which are apparently ideal for the germination of *S. fruticosa* seeds. Exposure to high salinity instead of having any deleterious effect on seeds has a priming effect and promotes germination significantly when compared to those seeds that are not exposed to saline conditions. *Suaeda fruticosa* is adapted to germinate at low thermoperiods and reduced salinity conditions after being exposed to high salinity for nine months.

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GERMINATION OF SUAEDA FRUTICOSA


