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Germination responses of *Salicornia rubra* to temperature and salinity

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Salicornia rubra Nels. is one of the most salt tolerant species in the western half of the United States and Canada. *Salicornia rubra* seeds were collected from Goshen, Utah. Seeds were germinated at five temperature regimes and six salinities to determine optimal conditions for germination and recovery from saline conditions. A temperature regime of 25°C night and 35°C day yielded maximum germination. The lower temperature regime 5–15°C significantly inhibited seed germination. Rate of germination decreased with increase in salinity. Final recovery germination percentages in high salt treatments were significantly higher indicating that exposure to high concentration of NaCl did not permanently inhibit germination.

Keywords: *Salicornia rubra*; halophyte; seed germination; recovery from salinity; temperature regime; salt tolerance; NaCl

Introduction

Several factors (water, temperature, light, and salinity) that regulate seed germination interact in the soil interface (Ungar, 1995). Other variables may co-act with the seasonal variation in temperature to determine the temporal pattern of germination (Khan & Gul, 1998). Osmotic and matric potential of soils narrow the range of temperature that is effective for the germination of seeds (Hegarty, 1978).

Annual *Salicornia* sp. are highly salt tolerant but vary in their response to salinity (Langlois, 1966; Ungar, 1977; Philipupillai & Ungar, 1984). *Salicornia europaea* showed a 10% germination at 5% (860 mM) NaCl (Ungar, 1967*a*), *Salicornia bigelovii* had 63% germination at 8% (1376 mM) NaCl (Rivers & Weber, 1971), and *Salicornia stricta* was reported to have 10% germination at 10% (1720 mM) NaCl (Chapman, 1974). Salinity alone may not be the only critical environmental factor in the germination of annual halophytes (Khan & Ungar, 1998*a*). Interactions between salinity and temperature occur and they determine optimal conditions for seed germination in halophytes (Hogan, 1968; Rivers & Weber, 1971; Philipupillai & Ungar, 1984; Badger & Ungar, 1989; Khan & Ungar, 1996, 1998*b*). Philipupillai & Ungar (1984) determined that

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S. europaea had optimal germination (43%) at 5–15°C in 860 mM NaCl in comparison to <2% in other temperatures. Khan and Gul (1998) observed that germination of *Arthrocnemum indicum* (= *Arthrocnemum macrostachyum*) was significantly higher at 15–25°C at 600 and 800 mM NaCl.

Halophyte seeds have the ability to maintain seed viability for extended periods of time during exposure to hypersaline conditions and then to commence germination when salinity stress is reduced (Ungar, 1982; Woodell, 1985; Keiffer & Ungar, 1995; Khan & Ungar 1996, 1997; Gul & Weber, 1999). However, halophytes differ in their capacity to recover from salinity stress (Woodell, 1985). This variation in recovery responses could be due to the difference in the temperature regime to which seeds are exposed (Khan & Ungar, 1996, 1998*a, b*). Germination studies with *Arthrocnemum indicum, Haloxylon recurvum*, *Suaeda fruticosa, Zygophyllum simplex*, and *Triglochin maritima* clearly demonstrate that recovery responses are significantly affected by changes in temperature regimes (Khan & Ungar, 1996, 1997, 1998*a, b*, 1999; Khan & Gul, 1998). Recovery of germination responses have been demonstrated in *Salicornia europaea* (Ungar, 1962; Keiffer & Ungar, 1995) and *Salicornia rubra* (Hogan, 1968).

Salicornia rubra Nels. (Chenopodiaceae) is a highly salt tolerant annual species occurring in salt playas of the Great Basin desert (Ungar, 1965, 1974). It forms a pioneer community, with plants being shorter and more dense at the higher salinity (Chapman, 1974). It was found in pure stands on the most saline location of an inland salt playa at Goshen, Utah. It was also associated with *Salicornia utahensis, Allenrolfea occidentalis*, and *Distichlis spicata* along a gradient of reduced salinity. The present study describes the effect of salinity and alternating temperature regimes on the seed germination and recovery responses of *Salicornia rubra*.

Materials and methods

Seeds of *Salicornia rubra* were collected during the fall 1994 from salt flats located at Goshen, Utah, U.S.A. Seeds were separated from the inflorescence and stored at 4°C. 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. Each dish was placed in a 10 cm diameter plastic petri dish as an added precaution against 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 5–15, 10–20, 15–25, 20–30, and 25–35°C were used based on a 24-hr cycle of 12 hr (Growth chambers with Sylvania cool white fluorescent lamps, 25 $\mu mol\ m^{-2}\ s^{-1}$, 400–750 nM). These temperatures regimes were averages for minimum and maximum temperatures if the growing season is divided into five periods. Seeds were germinated in distilled water and 200, 400, 600, 800 and 1000 mM NaCl under the aforementioned temperature regimes. Percent germination was recorded every alternate day 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 percentage was determined by the following formula:

$$(a - b)/(c - b)^{\star}100,$$

where a is the total number of seeds germinated after being transferred to distilled water, b is the total number of seeds 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 & Ungar, 1996). The maximum value possible using this index with our data was 50 (i.e. 1000/20).

Germination data were transformed (arcsine) before statistical analysis. These data were analysed with a two-way ANOVA using SPSS for windows release 9.0 (SPSS Inc., 1999). *Post hoc* tests were used and significance is indicated in the form of letters.

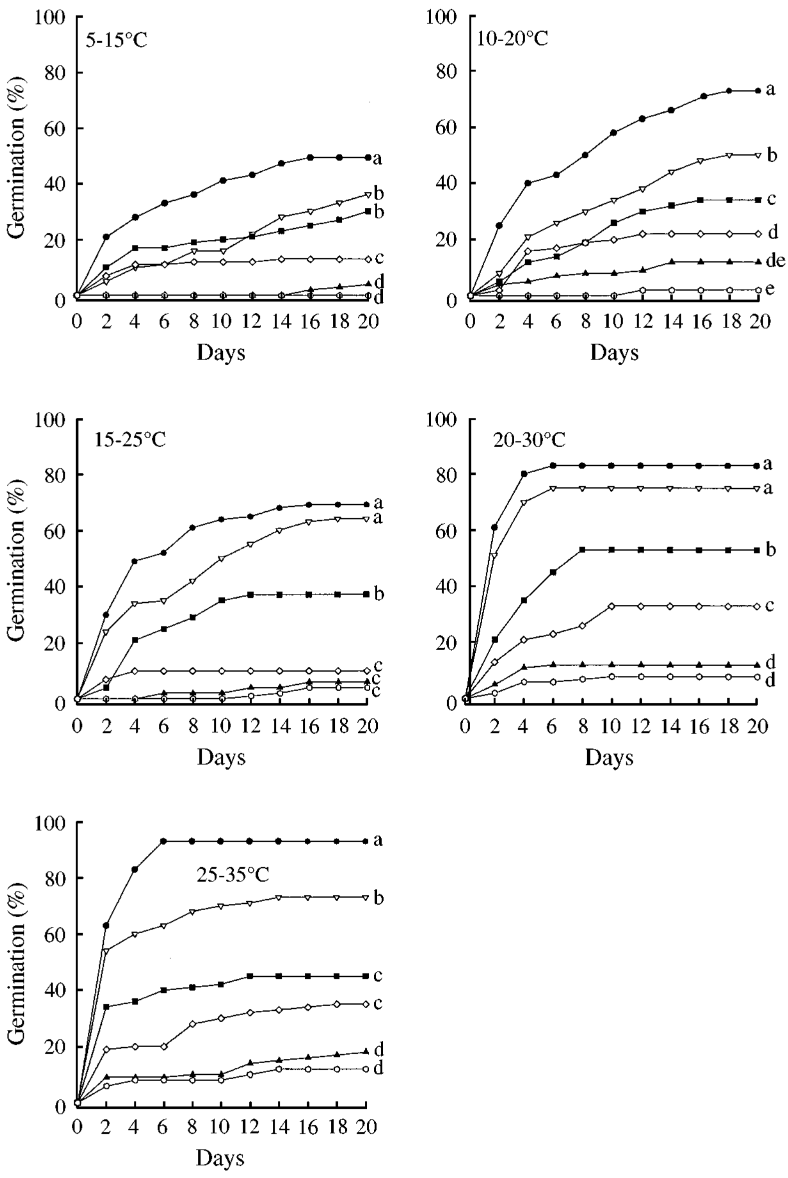

Figure 1. Rate of germination of *Salicornia rubra* seeds in 0 (—●—), 200 (—▽—), 400 (—■—), 600 (—◇—), 800 (—▲—) and 1000 (—○—) mM NaCl at 12 hr night-12 hr day at temperature of 10–20, 10–30, 15–25 and 25–35°C. Values of the final germination percentage (Mean ± S.E.) having the same letter are not significantly different at $p < 0.05$, Bonferroni test.

Results

Optimal germination of *Salicornia rubra* in 12/12 hr light/dark conditions occurred in distilled water at temperatures of 20–30°C and 25–35°C (Fig. 1). Seed germination decreased with an increase in NaCl concentrations at all temperatures. A delay in germination was more obvious at 5–15°C than with other temperatures regimes (Fig. 1). Change in temperature regimes significantly affected the germination of *S. rubra* seeds. At the higher temperature regime, 25–35°C, seeds in non-saline controls had about 98% germination compared to less than 50% germination at the lower temperature regime 5–15°C (Fig. 2). Few seeds germinated in the 1000 mM NaCl treatment. A two-way ANOVA indicated that germination of *S. rubra* seed was significantly affected by the temperatures ($F = 21{\cdot}81$, $p < 0{\cdot}0001$), salinity ($F = 141{\cdot}20$, $p < 0{\cdot}0001$), and the interactions of two factors ($F = 4{\cdot}03$, $p < 0{\cdot}0001$).

The rate of germination calculated using a modified Timson index showed that the rate decreased with an increase in salinity (Fig. 3). Highest rate of germination was obtained in the 25–35°C temperature regime and lowest in 5–15°C (Fig. 3). Different temperatures and various concentrations of salinity individually, and their interaction significantly ($p < 0{\cdot}0001$) affected the rate of germination of *S. rubra* seeds (Table 1). After 20 days of salinity treatment, seeds were transferred to distilled water to study the recovery of germination. Seeds showed recovery from salinity stress at all thermoperiods (Table 2). A 80% recovery in control treatment at 5–15°C indicated a temperature-mediated delay in germination (Table 2). There was relatively lower

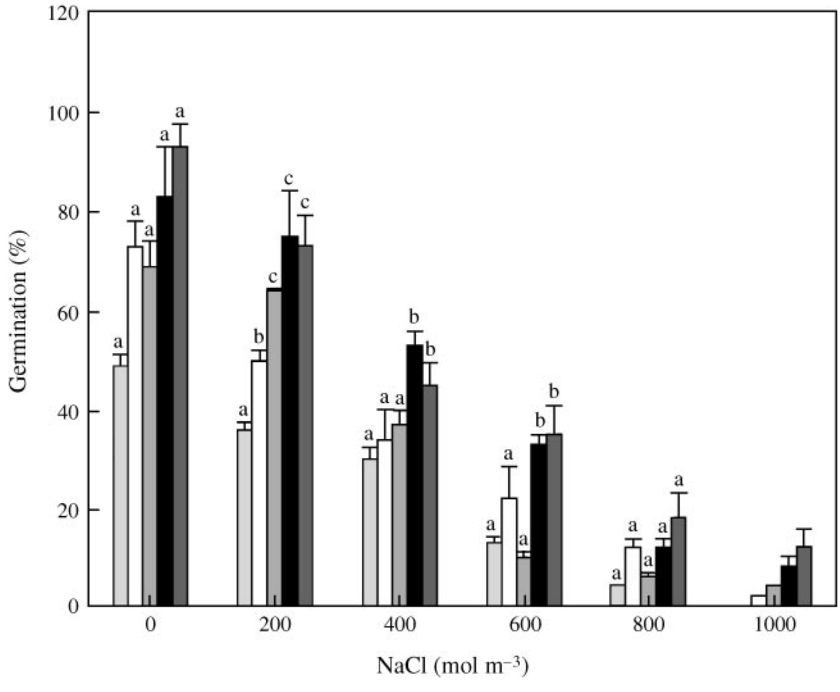

Figure 2. Mean (±S.E.) final germination of *Salicornia rubra* seeds in 0, 200, 400, 600, 800 and 1000 mM NaCl at 12 hr night-12 hr day at temperatures of 5–15 (□), 10–20 (□); 15–25 (■), 20–30 (■), 25–35 (■)°C. Values of the final germination percentages (Mean ± S.E.) having the same letter are not significantly different $p < 0{\cdot}05$, Bonferroni test.

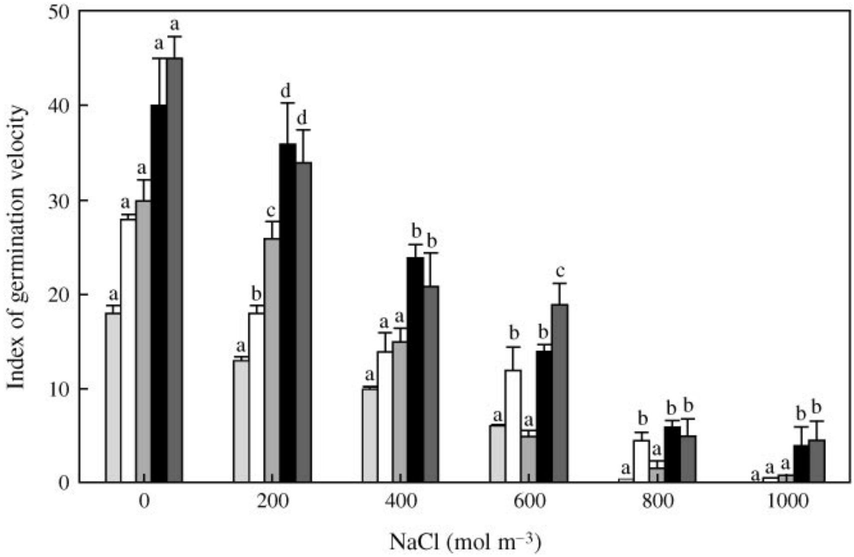

Figure 3. Rate of germination of *Salicornia rubra* seeds after they are transferred from 0, 25, 50, 75, 100 and 125 mM NaCl at 12 hr night-12 hr day at temperatures of 5–15 (▨), 10–20 (□); 15–25 (▨), 20–30 (■), 25–35 (▨) °C. Values of the final germination percentages (Mean ± S.E.) having the same letter are not significantly different $p < 0{\cdot}05$, Bonferroni test.

recovery at lower temperatures in high salinity treatments. However, at higher temperatures there was substantial recovery at higher salinities (Table 2).

Discussion

Salicornia rubra plants grew in the most saline portion of an inland salt playa at Goshen, Utah, and then produced seeds during September and October. Seeds germinated during April when the snowmelt and the water from the surrounding mountains accumulated in the playa decreasing the soil salinity concentration. Field observations also showed that seeds continued to germinate until early summer when the evaporation caused by high temperature caused a corresponding increase in soil salinity. Our laboratory studies indicated that *Salicornia rubra* germinated well under non-saline conditions. Lower temperatures delayed germination but with increased temperature there was a substantial increase in germination. In addition the salinity tolerance of *S. rubra* seeds also increased with higher day temperatures. *Salicornia rubra* is capable of

Table 1. *Results of two-way ANOVA of characteristics by salinity, thermoperiods and their interaction*

Independent variable	Salinity	Thermoperiod	Salinity × Thermoperiod
Percent germination	141·20***	21·81***	4·03***
Rate of germination	130·70***	32·91***	5·10***
Percent recovery	3·90**	4·22**	6·02***

Note. Number represents *F*-values. $^{**}p < 0{\cdot}01$; $^{***}p < 0{\cdot}001$.

Table 2. *Recovery percentage (Mean ± S.E.) of germination of* Salicornia rubra *after seeds are transferred from 0, 200, 400, 600, 800 and 1000 mM NaCl at thermoperiods of 5–15, 10–20, 15–25, 20–30 and 25–35°C.*

NaCl (mM)	5–15°C	10–20°C	15–25°C	20–30°C	25–35°C
0	81 ± 15·7	54 ± 21·3	45 ± 7·3	0·0 ± 0·0	2 ± 2·0
200	35 ± 3·5	43 ± 7·1	17 ± 9·6	0·0 ± 0·0	2·5 ± 2·5
400	58 ± 7·5	33 ± 6·9	15 ± 5·2	54 ± 12·3	22 ± 9·0
600	48 ± 7·0	48 ± 4·2	36 ± 3·4	48 ± 8·4	34 ± 5·6
800	48 ± 7·7	46 ± 3·1	45 ± 2·7	32 ± 7·4	59 ± 12·1
1000	40 ± 10·8	45 ± 5·8	28 ± 5·7	69 ± 11·7	78 ± 11·3

germinating at 860 mM NaCl (5% NaCl) at 15°C constant temperature. An increase in temperature inhibited germination at 860 mM NaCl (Hogan, 1968). Our *S. rubra* is more tolerant to salinity and the seeds germinated better at higher alternating temperatures regimes. Hogan (1968) on the contrary reported better germination at cooler and constant temperatures. Seeds of *S. rubra* had 15% germination at 1000 mM NaCl. Halophyte seed germination has been reported to occur optimally under reduced salinity stress (Ungar, 1962, 1977; Khan & Ungar, 1997, 1998*b*; Khan & Gul, 1998; Gul & Weber, 1999). Seeds of halophytes vary greatly in their ability to germinate under hypersaline conditions but seeds could also germinate at high salinities (Khan & Gul, 1998). Maximum salinity tolerance ranges from 200 to 1720 mM NaCl in halophytes such as *Salicornia bigelovii, S. europaea, S. stricta, Cressa cretica, Suaeda moquinii* and *Arthrocnemum indicum* (Ungar, 1967*b*; Rivers & Weber, 1971; Chapman, 1974; Khan, 1991, Khan & Gul, 1998, Khan *et al.*, unpublished data). Khan & Weber (1986) reported that the germination of *Salicornia pacifica* var. *utahensis* was reduced from 55% in distilled water control to 3% germination at 860 mM NaCl. These reports indicate that *S. rubra* seeds could be classified as one of the most salt tolerant during germination.

Seed germination of *S. rubra* was affected by change in temperature. The lower temperatures either delayed germination in the control and low salinity treatments or completely inhibited germination at higher salinity concentrations. Seeds germinated faster at higher temperatures where maximum germination occurred under both non-saline and saline conditions. Similar increases in germination under higher temperatures were reported for other Great Basin desert species like *Salicornia pacifica* var. *utahensis*, (Khan & Weber, 1986), *Allenrolfea occidentalis* (Gul & Weber, 1999), *Triglochin maritima* (Khan & Ungar, 1999) and *Suaeda moquinii* (Khan *et al.* unpublished data). However, halophytes from subtropical maritime deserts of Pakistan e.g. *Haloxylon recurvum* (Khan & Ungar, 1996), *Zygophyllum simplex* (Khan & Ungar, 1997), *Suaeda fruticosa* (Khan & Ungar, 1998*b*), and *Arthrocnemum macrostachyum* (Khan & Gul, 1998) had better germination at lower temperatures.

Halophyte seeds have the ability to maintain their viability for extended periods when exposed to hypersaline conditions and then to commence germination when salinity stress is reduced (Hogan, 1968; Ungar, 1982; Woodell, 1985; Keiffer & Ungar, 1995; Khan & Ungar, 1996, 1997; Khan & Gul, 1998; Gul & Weber, 1999). Seeds of *S. rubra* from the Goshen, Utah population, when transferred to distilled water after 20 days of treatment at various salinity concentrations responded differentially under different temperature regimes. Recovery at lower temperatures under highly saline conditions was lower in comparison to seeds exposed to higher temperatures. Keiffer & Ungar (1995) exposed seeds of five halophytes (*Atriplex prostrata, Hordeum jubatum, Salicornia europaea, Spergularia marina*, and *Suaeda calceoliformis*) to an extended period of salinity and determined their recovery responses after the seeds were transferred to distilled water. *Hordeum jubatum* seeds failed to germinate after 2 years of exposure; seeds of *S. marina* were stimulated after long exposures. *Atriplex prostrata* decreased over time, and *S. europaea* and *S. calceoliformis* were stimulated by the exposure of salinity and had higher germination in comparison to the control. Seeds of *S. fruticosa* recovered quickly from hypersaline conditions when transferred to distilled water at all temperatures (Khan & Ungar, 1998*b*), but only those exposed to high salinity at a lower temperatures showed salt stimulation of germination. *Haloxylon recurvum* and *Triglochin maritime* seed had about 50% recovery at low to moderate temperatures (Khan & Ungar, 1996; 1998*a*).

Salicornia rubra seeds were able to germinate in high salinity (1000 mM NaCl) under laboratory conditions and may be included among those halophytes which have the ability to withstand high salinity stress during germination. *Salicornia rubra* seeds had maximum germination at the 25–35°C temperature regime for all NaCl concentrations tested. This is consistent with the results of other Great Basin desert species indicating a higher temperature is better suited for their germination. In early spring, lower temperatures would delay germination. Goshen can receive heavy snow and frost during spring and this would cause high mortality. However, during late spring when it is warmer with little chance of frost. The ability of *S. rubra* to germinate quickly at the higher temperatures confers an ecological advantage in maintaining the fitness of the population. Seeds are highly tolerant to salinity when stored in the soil, however, when salinity of the playa is reduced due to snowmelt, they do not germinate quickly but can remain dormant until higher temperatures occur.

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