Influence of Salinity and Temperature on the Germination of *Haloxylon recurvum* Bunge ex. Boiss.

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The stem succulent perennial halophyte *Haloxylon recurvum* Bunge ex. Boiss. grows and produces seed under highly saline conditions and seeds are deposited in saline soils. Experiments were conducted to determine the effect of salinity and temperature on the germination of seeds. Results indicate that seeds can germinate at very high salt concentrations (500 mM). However, highest germination percentages were obtained in distilled water. Cooler thermoperiods promoted germination, while high temperatures significantly inhibited the germination of seeds at all NaCl concentrations tested. Rate of germination decreased with increases in salinity. At higher thermoperiods the rate of germination was significantly lower in comparison to lower thermoperiods. Seeds recover after being transferred to distilled water and recovery was higher from higher salinity concentrations and lower thermoperiods. Final recovery germination percentages in high salt treatments were significantly lower than non-saline controls, indicating that exposure to high concentration of NaCl permanently inhibited germination.

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INTRODUCTION

Haloxylon recurvum Bunge ex. Boiss. (Chenopodiaceae) is a leaf succulent perennial that is distributed from southern Sindh and Balochistan up to the Northern Himalyan mountain valley of Chitral, Pakistan (Stewart, 1972). *Haloxylon recurvum* is commonly found in highly saline patches in association with *Suaeda fruticosa*, *Salsola baryosma*, and *Sporobolus arabicus*.

Seed germination under field conditions occurs after monsoon rains, which not only provide much needed moisture but also help in the substantial reduction in soil salinity due to leaching. Recruitment of seedlings depends on the duration of rainfall. If rainfall persists for 7–10 weeks, the chance of recruitment increases. However, in the case of brief periods of rain the soil dries out quickly, resulting in a substantial rise in soil salinity concentrations that ultimately increase seedling mortality.

There is little information available on the seed germination responses of *H. recurvum*. Sharma and Sen (1989) reported that seeds of *H. recurvum* germinate rapidly only 75–120 min after the imbibition. They attributed rapid seed germination in *H. recurvum* as an adaptive strategy to the availability of water when reduced levels of NaCl content is reached in soil for short durations during the rainy season. This is because evaporation of moisture under full sunlight and higher temperatures causes an increase in the salt content by capillary movement. If a seed can take advantage of a short period of reduction in soil salinity and germinate rapidly, then seedling establishment is ensured (Sharma and Sen, 1989). Rajpurohit and Sen (1977) reported the effect of

salinity on the germination of some shrubby perennial halophytes from Jodhpur, India. They demonstrated that *H. recurvum* germinated well under non-saline conditions. However, increase in salinity substantially inhibited germination. No seeds were able to germinate in greater than the 83 mM NaCl treatment.

Thermoperiod and salinity interact in determining germination of *Atriplex triangularis* (Khan and Ungar, 1984). Temperature regimes have been shown to effect the germination of halophyte seeds at various salinities (Khan and Ungar, 1985; Khan and Weber, 1986; Khan *et al.*, 1987; Badger and Ungar, 1989, DeVilliers *et al.*, 1994; Noor and Khan, 1995). The ability of halophyte seeds to recover from exposure to salt stress has been reported (Woodell, 1985), but little data is available on the recovery rate of seeds from osmotically induced salt stress at different thermoperiods. We address this question in our studies with *Haloxylon recurvum*.

Haloxylon recurvum is one of the dominant halophytic species of salt deserts present around Karachi, Pakistan. Its germination responses to environmental variables are poorly understood. The present study describes the germination response of *H. recurvum* seeds under various thermoperiod and salinity regimes.

METHODS

Seeds of *Haloxylon recurvum* were collected during Autumn 1994 from salt flats situated on the Karachi University campus, Pakistan. Seeds were separated from inflorescences and stored at 4 °C. These seeds were brought to Ohio University, USA and germination studies were started in Feb. 1995. Seeds were surface sterilized using the

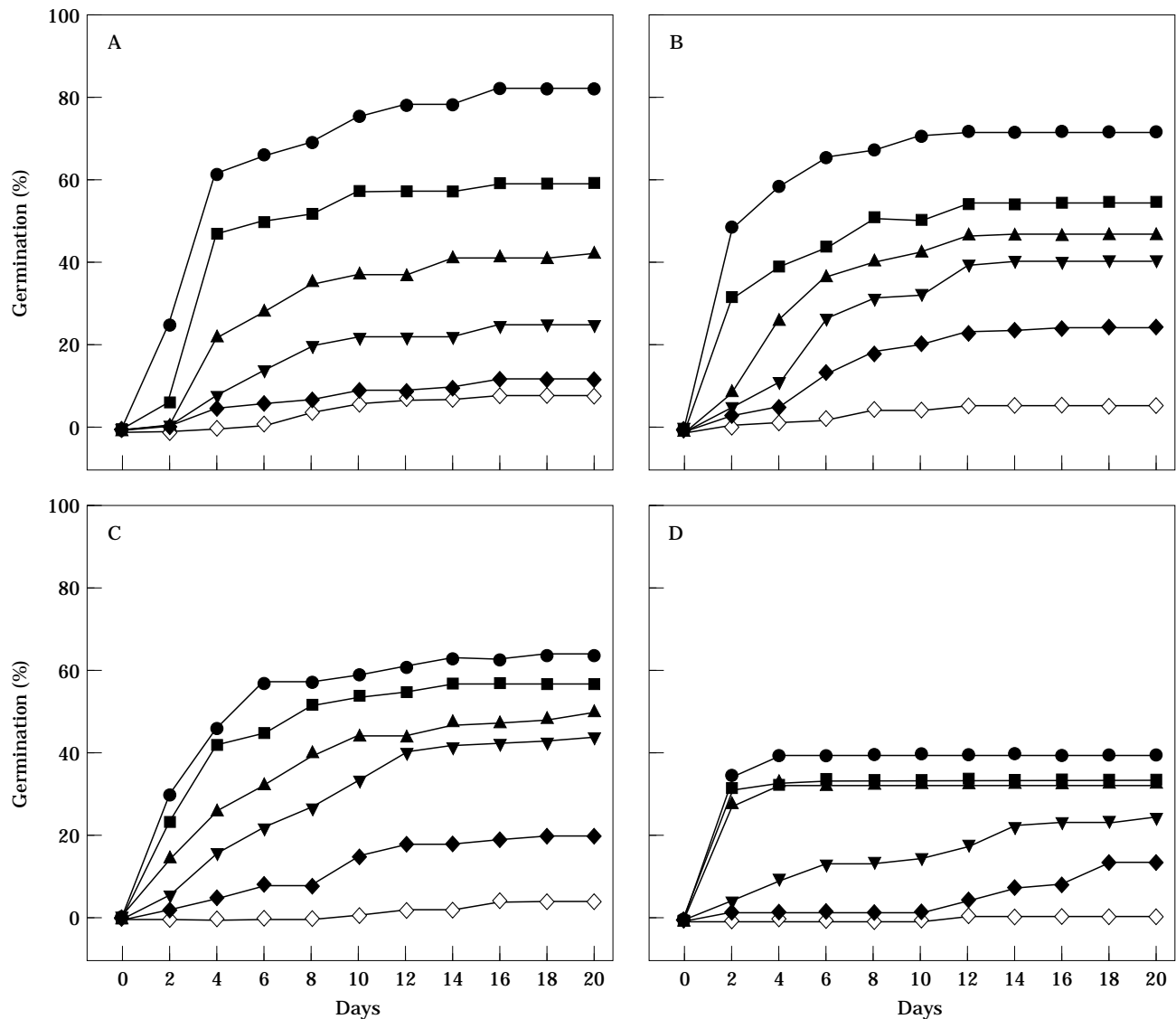* Permanent address: Department of Botany, University of Karachi, Karachi-75270, Pakistan.

FIG. 1. Mean germination percentage as a function of time of *Haloxylon recurvum* seeds in 0(●), 100(■), 200(▲), 300(▼), 400(◆), and 500(◇) mM NaCl at thermoperiods of 10–20 (A), 10–30 (B), 15–25 (C), and 25–35 °C (D).

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 10–20, 10–30, 15–25, and 25–35 °C based on a 24-h cycle were used, where the higher temperature (20, 25, 30 and 35 °C) coincided with the 12-h light period (Sylvania cool white fluorescent lamps, 25 μmol m^{-2} s^{-1}, 400–750 nm) and the low temperature (10, 15 and 25 °C) coincided with the 12-h dark period. Seeds were germinated in distilled water, 100, 200, 300, 400, and 500 mM NaCl solutions under the above mentioned temperature regimes. Percent germination was recorded every alternate day for 20 d. After 20 d ungerminated seeds from

the NaCl treatments were transferred to distilled water to study the recovery of germination, which was also recorded at 2 d intervals for 20 d. The rate of germination was estimated by using a modified Timson index of germination velocity, $\Sigma G/t$, where G is percentage of seed germination at 2-d intervals, and t is total germination period (Khan and Ungar, 1984). The maximum value possible using this index with our data was 50 (i.e. 1000/20). The higher the value, the more rapid the rate of germination.

Germination data was transformed (arcsine) before statistical analysis. These data were analysed using SPSS for Windows release 6.1 (SPSS Inc., 1994).

RESULTS

Seed of *H. recurvum* germinated rapidly in non-saline controls at all temperature regimes tested and reached final germination percentages in less than 10 d (Fig. 1). Change in
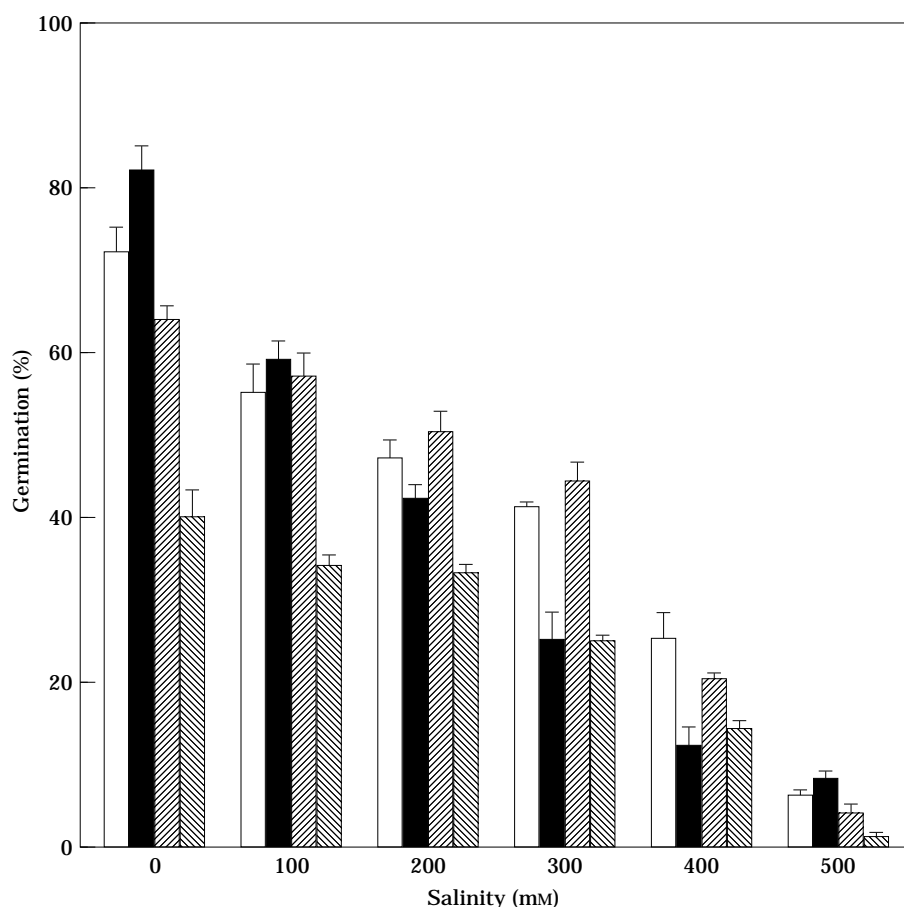
FIG. 2. Mean (±s.e.) final germination percentages of *Haloxylon recurvum* seeds in 0, 100, 200, 300, 400, and 500 mM NaCl at thermoperiods of 10–20 (□), 10–30 (■), 15–25 (▨), and 25–35 °C(▧).

TABLE 1. *Index of rate of germination (Mean±s.e.: n = 4) using modified Timson index (Khan and Ungar, 1984)*

NaCl (mM)	Thermoperiod (°C)			
	10–20	10–30	15–25	25–35
0	34·9±3·6	32·9±2·9	32·9±2·9	19·8±3·1
100	25·2±1·2	24·7±2·4	24·7±3·4	16·9±1·1
200	16·2±1·1	19·6±1·7	19·6±1·7	16·3±1·1
300	9·2±2·5	15·7±0·7	15·7±0·6	8·6±1·0
400	4·1±1·4	9·4±2·2	9·4±2·2	3·0±0·7
500	2·5±0·4	2·4±0·5	2·4±0·9	0·2±0·2

temperature regimes significantly affected the germination of *H. recurvum* seeds (Fig. 2). At lower thermoperiods (10–20 °C) seeds in non-saline controls had about 80% germination as compared to less than 50% germination at the higher thermoperiod (25–35 °C). Increases in temperature and salinity progressively inhibited the seed germination of *H. recurvum*. Few seeds germinated in the 500 mM NaCl treatment. Seeds in the distilled water control and low salinity at the lower thermoperiod (10–20 °C) had highest germination percentages, while at higher salinity moderate thermoperiod treatments had better germination percentages. A two way ANOVA indicated that germination

of *H. recurvum* seed was significantly affected by thermoperiod ($F = 19.57$, $P < 0.0001$), salinity ($F = 107.2$, $P < 0.0001$) and the interaction of two factors ($F = 3.416$, $P < 0.0001$).

The index of rate of germination calculated using a modified Timson index showed that the rate decreased with an increase in salinity (Table 1). Germination rate was similar under lower and moderate thermoperiods but was significantly reduced at the highest thermoperiod.

After 20 d of salinity treatment, seeds were transferred to distilled water to study the recovery of germination after salt inhibition. The results presented in Table 2 show that at lower NaCl concentrations there was no recovery of the seed germination. Seeds exposed to high salinity at lower and moderate thermoperiods showed a significant increase (40%) in germination. However, at a thermoperiod of 25–35 °C there was no change in germination percentages. The recovery germination percentages after transfer from salinity to water are reported in Table 2. At the highest thermoperiod (25–35 °C) the percent recovery in all NaCl treatments was very low, whereas, in all other thermoperiods recovery percentages of ungerminated seeds increased with increases in NaCl concentration. The number of seeds germinated in all saline treatments after transfer to distilled water was significantly lower than the distilled water control (Table 2).

TABLE 2. *Mean (±s.e.: n = 4) germination (%) indicating the initial salinity effect (S), recovery (R) after 20 d of transfer to distilled water, and total germination (T)*

NaCl (mM)	10–20 °C			10–30 °C			15–25 °C			25–35 °C		
	S	R	T	S	R	T	S	R	T	S	R	T
0	82±3·0	56±12·5	92±2·7	72±2·9	0±0·0	72±2·8	64±1·4	0±0·0	64±1·4	40±3·2	8±1·5	45±3·2
100	52±2·2	21±0·5	62±2·3	55±3·9	2±0·1	56±4·3	57±2·6	14±3·2	63±2·6	34±1·3	0±0·0	34±1·2
200	42±1·7	5±2·5	45±2·1	47±2·2	6±2·1	50±2·8	50±2·6	6±1·0	53±2·7	33±1·0	3±0·7	35±0·9
300	25±3·3	14±1·3	36±2·8	41±0·5	24±2·6	55±0·9	44±2·3	43±4·8	68±4·2	25±0·5	17±3·3	38±1·4
400	12±2·2	50±2·5	56±2·1	25±3·0	21±2·4	41±2·6	20±0·8	40±1·2	52±1·6	14±1·0	6±1·2	19±2·1
500	8±0·8	37±2·5	42±2·5	6±0·6	35±3·2	39±4·0	4±0·8	44±2·7	46±1·8	1±0·5	9±1·8	10±2·3

DISCUSSION

Deserts are regions of low and irregular rainfall in which potential evapotranspiration exceeds precipitation. In certain areas the water table is high and a high rate of evaporation causes an accumulation of salts on the surface of the soil. These harsh conditions have led to differential life history strategies in desert plants in order to maximize their fitness (Kigel, 1995). Desert species may differ in their life cycle (annual *vs.* perennial), life form (shrubs *vs.* herbaceous), response to drought (tolerance *vs.* avoidance), time of flowering, reproductive effort, seed dispersal, and germination behaviour.

Some aspects of the seed ecology of perennial halophytes differ from those of annuals. Annual halophytes which are commonly found in temperate saline marshes have a large persistent seed bank with germination and seedling recruitment occurring in early spring (Khan and Ungar, 1986). Seed dormancy that occurs in these annual halophytes is regulated by salinity and thermoperiod to fit the climatic pattern of the area (Ungar, 1991). Halophytes that primarily dominate the subtropical deserts are shrubby perennials. Germination is a critical transition between the stage most tolerant to drought and extreme periods of stress (e.g. seed) and the seedling stage that is the most 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) prevailing after seed dispersal. Perennial halophytes, including *H. recurvum,* which usually dominate in tropical areas, often maintain a transient rather than a persistent seed bank (Khan, 1991, 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, unpubl. res.). Most of the seeds of perennial halophytes have hard seed coats that help them to survive the harsh stressful conditions of high temperatures and drought. They are ready to germinate when temperatures are reduced (about 25 °C), and moisture in the form of annual monsoon rains is plentiful (Khan, 1991; Khan and Rizvi, 1994). Ungar (1978) showed that with an increase in salinity and temperature there is a corresponding decrease in germination. Khan (1991) reported that in *Cressa cretica,* a perennial shrub from subtropical maritime desert habitats, acid scarification alleviated the seed dormancy. Scarified seeds were able to germinate at 850 mM NaCl concentrations. However, maximum germination was achieved in non-saline controls. Seeds of *C. cretica* germinated better at cooler thermoperiods (10–20 °C) at all salinities tested (Khan, 1991). Similar results were also reported for other shrubby halophytic species like *Zygophyllum qatarensis* (Ismail, 1990), *Chrysothamnus nauseosus* (Khan *et al.,* 1987), *Atriplex griffithii* (Khan and Rizvi, 1994) and *Suaeda fruticosa* (Khan and Ungar, unpubl. res.).

Germination of *H. recurvum* decreased with an increase in salinity, and was substantially inhibited at 500 mM NaCl. Maximum germination was obtained in the non-saline control. Similar results were reported for *Atriplex griffithii* (Khan and Rizvi, 1994), *Cressa cretica* (Khan, 1991), *Suaeda fruticosa* (Khan and Ungar, unpubl. res.) and a number of annual halophytes (Ungar, 1995). It is very well established from the survey of previous literature that most halophytes can not only germinate, but germinate better, under non-saline conditions (Ungar, 1995). However, halophytes have the ability to germinate at higher salinities than the glycophytes and halophyte seeds remain viable after long periods of exposure to salinity (Keiffer and Ungar, 1995; Ungar, 1995). Seeds of halophytes vary in their ability to tolerate salinity at the germination stage of development, e.g. *Atriplex patula,* 170 mM NaCl (Ungar, 1996) and *Tamarix pentandra,* 850 mM NaCl (Ungar, 1967).

Haloxylon recurvum had little recovery of germination when transferred to water after 20 d exposure to lower salinity concentrations at all thermoperiods studied. Seeds exposed to high salinity concentrations (400 and 500 mM) had about 40–50 % recovery germination in distilled water at low and moderate thermoperiods. These data indicate that seeds could remain in the soil under field conditions when salinity levels are beyond their tolerance limits, and germinate during the monsoon rainy period when salinity levels are reduced. The combination of high Apr. to Jun. temperatures (30 °C) and high soil salinity (> 500 mM total salts) would inhibit seed germination of *H. recurvum.* Recovery germination experiments, after soaking of seeds of annual halophytes (*Salicornia europaea, Spergularia marina, Suaeda depressa,* and *Suaeda linearis*) for 30 d in 850 mM NaCl, indicated that there was no specific ion toxicity and that an osmotic effect limited germination (Ungar, 1995). Seeds of *Suaeda fruticosa* recovered rapidly

from hypersaline conditions when transferred to distilled water (Khan and Ungar, unpubl. res.). Seeds of *S. fruticosa* exposed to higher salinity levels demonstrated the priming effect of salinity on germination. Final germination percentages in these recovery experiments were 20 % higher than the control. No such priming effects were noted in the case of *H. recurvum*. In addition, final germination percentages after the 20 d recovery period were much lower than for the non-treated control, indicating that exposure to high concentration of NaCl permanently inhibited germination.

At lower thermoperiods seeds of *H. recurvum* germinated better in non-saline controls. Increase in salinity progressively inhibited seed germination and few seeds germinated in the 500 mM NaCl treatment. Seeds germinated rapidly and no significant change in germination was noticed after 10 d. When seeds were transferred to non-saline medium after 20 d exposure to salinity there was a substantial recovery of germination. However, all recovery treatments had lower germination than the germination percentages determined in the non-saline control, indicating that even at lower temperatures exposure to high concentration of NaCl permanently inhibited germination.

ACKNOWLEDGEMENTS

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