1.06. COMPARATIVE INFLUENCE OF SALINITY AND TEMPERATURE ON THE GERMINATION OF SUBTROPICAL PERENNIAL HALOPHYTES

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Abstract

Seed germination responses of desert shrubs (*Haloxylon recurvum, Suaeda fruticosa, Cressa cretica, Arthrocnemum macrostachyum, Atriplex griffithii*) were studied under various salinity concentrations and thermoperiods. The halophytic shrubs differ in their response to salinity and thermoperiod. Species like *C. cretica* and *A. macrostachyum* could germinate up to 1000 mM NaCl while species like *H. recurvum* and *S. fruticosa* could tolerate up to 500 mM NaCl and *A. griffithii* about 400 mM NaCl. Species like *S. fruticosa* show little effect of temperature while germination *C. cretica* changed substantially with change in temperature. The recovery of un-germinated seeds varied significantly with variation in species and thermoperiods. *Haloxylon recurvum, S. fruticosa,* and *C. cretica* showed substantial recovery. Percentage recovery was highest in *S. fruticosa*, followed by *C. cretica* and *H. recurvum.* Effect of temperature varied with the species investigated. Recovery of *S. fruticosa* showed little variation, except in the higher salinity and at higher temperature treatments. Variation in thermoperiod appeared to play an important role in recovery of germination of halophytes from salt stress when seeds were transferred to distilled water. Ecological implications of the germination characteristics of these halophytes will be discussed.

Introduction

Seed dormancy plays a significant role in the survival of halophytic species in a harsh saline environment (Ungar, 1995). It permits seeds to remain viable in the soil during the periods when the environment is not suited for germination. Flooding, hypersaline conditions, and burial of seeds by tidal depositions or sand drift may make salt marsh and salt desert environments unsuitable for seed germination (Khan and Gul, 1998). Some seeds remain dormant because of a hard or impermeable pericarp, testa, or an immature embryo restricts germination (Ungar, 1995), while in other cases the seeds are responding to some environmental factors (e.g., light, temperature, and salinity) when they become dormant (Khan and Ungar, 1997b).

Seeds of halophytes germinate better in distilled water like non-halophytes but differ in their ability to germinate at higher salinities and also remain viable for a long period when immersed in saline water (Ungar, 1995). Species reported to have very

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high salt tolerance at germination stage include *Salicornia bigelovii* (856 mM NaCl, Rivers and Weber, 1971), *Cressa cretica* (856 mM NaCl, Khan 1991), *Tamarix* sp. (1000 mM NaCl, Waisel, 1958; Ungar, 1967), *Salicornia pacifica* var. *utahensis* (860 mM NaCl, Khan and Weber, 1986), *Suaeda torreyana, Salicornia rubra, Salsola iberica* and *Halogeton glomeratus* (1000 mM NaCl, Khan *et al.*, unpublished results).

Salinity and temperature are reported to interact in affecting the seed germination of halophyte seeds (Young *et al.*, 1980; Agami, 1986; Gutterman, 1986; Khan and Weber, 1986; Khan *et al.*, 1987; Badger and Ungar, 1989; Ismail, 1990; Khan, 1991; Naidoo and Naicker, 1992; DeVilliers *et al.*, 1994; Khan and Rizvi, 1994; Gutterman *et al.*, 1995; Noor and Khan, 1995; Khan and Ungar, 1984, 1996, 1997abcd, 1998; Khan and Gul, 1998). Seeds of *H. recurvum* showed higher germination percentages at cooler thermoperiods (Khan and Ungar, 1996) and seeds of *Zygophyllum simplex* germinated better at moderate temperature regimes (Khan and Ungar, 1997b). Germination of *Triglochin maritima* seeds was higher in distilled water but decreased significantly with an increase in salinity up to 500 mM (Khan, unpublished results). Transfer of un-germinated, salt-treated seeds to distilled water stimulated germination at 15-25°C better than other thermoperiods. Ungar (1996) reported that high salinity did not permanently injure *Atriplex patula* seeds and germination recovered fully when seeds were transferred to distilled water. Khan and Ungar (1998a) reported better germination in *Polygonum aviculare* seeds at lower thermoperiod and seeds also showed higher recovery germination at this thermoperiod (5-15°C).

Plants native to saline habitats (both coastal and inland) are exposed to various levels of moisture and salinity stress during their life cycle (Ungar, 1995). The ability of their seeds to maintain viability after an extended period of exposure to salinity has been investigated by several authors (Macke and Ungar, 1971; Woodell, 1985; Naidoo and Naicker, 1992; Keiffer and Ungar, 1995, 1997). However, few studies have focused on the effect of variation in thermoperiod on the recovery responses of halophyte seeds (Khan and Ungar, 1996, 1997abc, 1998).

The primary objective of this investigation was to compare the germination and recovery of germination from salt stress in five subtropical desert halophytes under various thermoperiods.

Materials and methods

Seeds of *Arthrocnemum macrostachyum, Atriplex griffithii, Cressa cretica, Haloxylon recurvum,* and *Suaeda fruticosa*, were collected during fall 1994 from salt flats situated on the Karachi University campus, Pakistan. Seeds were separated from inflorescence and were stored at 4°C. Seeds were surface sterilized using the fungicide Phygon. Germination was carried out in 50 x 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°C, 10°-20°C, 10°-30°C, 15°-25°C, and 25°-35°C were used. The higher temperature (15°, 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 lower temperature (5°, 10°, 15°, and 25°C) coincided with the 12-hr dark period. Seeds were germinated in distilled water, and 100, 200, 300, 400, and 500 mM NaCl solutions in the case of *S. fruticosa, H. recurvum,* and *A. griffithii* and in 0, 200, 400, 600, 800 and 1000 mM NaCl in the case of *A. macrostachyum* and *C. cretica* at the above-mentioned temperature regimes. After 20 d un-germinated seeds from the NaCl treatments were transferred to distilled water (under original temperature conditions) 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 analyzed using SPSS for windows release 7.0 (SPSS, 1996).

Results

A two-way ANOVA of percent germination and recovery of germination for *A. griffithii, A. macrostachyum, C. cretica, H. recurvum* and *S. fruticosa* indicated significant ($P < 0.0001$) main effects of salinity and temperature and their interaction (Table 1). Best germination in all halophytes occurred in non-saline control (Fig. 1-5). Seed germination of all halophytes decreased with increase in salinity. Seeds of most common halophytic species like *A. griffithii, H. recurvum* and *S. fruticosa* could germinate up to 500 mM NaCl (Fig. 1, 2, & 3). While other species like *A. macrostachyum* and *C. cretica* could germinate up to 1000 mM NaCl (Fig. 4 & 5). Change in temperature did not have any significant effect on *A. macrostachyum, H. recurvum,* and *S. fruticosa* (Fig. 2, 3, & 4). Species like *A. griffithii* and *C. cretica* showed a substantial effect of change in temperature on germination (Fig. 1 & 5). Cooler thermoperiods (10-20°C) were better for germination at all salinities. Recovery germination percentages substantially increased with increases in salinity concentrations. Almost all species studied have recovery (Fig.1-5). *Atriplex griffthii* had a relatively poor recovery (65%) response, indicating a specific ionic effect on the germination of its seeds after exposure to salinity for 20 d (Fig. 1). Change in thermoperiod has an effect on the germination of halophytes in both saline and nonsaline conditions (Fig. 1-5). *Suaeda fruticosa* at lower thermoperiods had a substantial recovery response, but higher thermoperiod (25-35°C) caused irreparable injury to the seeds (Fig. 3). *Haloxylon recurvum* seeds showed a relatively poor recovery from salt stress. Maximum recovery (40%) was obtained at 10-20°C at 400 mM NaCl. Higher thermoperiod (25-35°C) substantially inhibited the recovery of germination (Fig. 2). High salt tolerant species (*A. macrostachyum* and *C. cretica*) also recovered from salinity stress better at 10-20°C thermoperiod, however, about 65% recovery occurred at the thermoperiod (Fig. 4 & 5).

Table 1. Results of two-way analysis of variance of percent germination by Salinity (S), and Thermoperiod (T), treatments.

Name of Species	S	T	S x T
Arthrocnemum indicum	56.8^{***}	23.5^{***}	2.5^{*}
Atriplex griffithii	436.6^{***}	22.5^{***}	3.5^{***}
Cressa cretica	71.3^{***}	25.2^{***}	3.7^{**}
Haloxylon recurvum	107.4^{***}	19.5^{***}	3.4^{***}
Suaeda fruticosa	97.7^{***}	6.1^{***}	$0.5^{\text{n.s.}}$

Note: Numbers represent F values: $^{*} = P < 0.01$; $^{**} = P < 0.001$; $^{***} = P < 0.0001$; $^{\text{n.s.}}$ = non significant.

Table 2. Results of two-way analysis of variance of recovery of germination by Salinity (S), and Thermoperiod (T), treatments.

Name of species	S	T	S x T
Arthrocnemum indicum	40.2^{***}	3.8^{**}	1.9^{*}
Atriplex griffithii	55.4^{***}	20.2^{***}	3.5^{**}
Cressa cretica	71.3^{***}	25.2^{***}	3.7^{**}
Haloxylon recurvum	101.4^{***}	9.5^{**}	1.4^{*}
Suaeda fruticosa	44.3^{***}	4.5^{*}	$0.9^{\text{n.s.}}$

Note: Numbers represent F values: $^{*} = P < 0.01$; $^{**} = P < 0.001$; $^{***} = P < 0.0001$; $^{\text{n.s.}}$ = non significant.

Discussion

Tolerance of salinity by halophyte seeds may be expressed either as the ability of ungerminated seeds to tolerate high salinity without losing viability or the ability of seeds to germinate at high salinities (Khan and Ungar, 1997a). Seeds of halophytes do not only germinate at higher salinities but also remain viable for long periods of time when immersed in saline water (Keiffer and Ungar, 1997). Halophytes vary in their upper limit of salt tolerance and increase in salinity usually delays their germination (Ungar, 1995). Seeds of salt marsh species like *Atriplex triangularis, Hordeum jubatum, Polygonum aviculare,* and *Zygophyllum simplex* show little germination above 125 mM NaCl (Badger and Ungar, 1989; Khan and Ungar, 1984, 1997ab), however, species like *Atriplex griffithii, Haloxylon recurvum, Suaeda fruticosa,* and *Triglochin maritima* could germinate up to 500 mM NaCl (Khan and Rizvi, 1994; Khan and Ungar, 1996, 1998). A third group of species like *Salicornia bigelovii, S. utahensis, S. rubra, Cressa cretica, Tamarix pentandra, Salsola iberica, Suaeda torreyana, Halogeton glomeratus, Allenrolfea occidentalis*, and *Kochia scoparia* could germinate at 800 mM or higher NaCl concentrations (Khan, 1991; Rivers and Weber, 1971; Khan and Weber, 1986; Ungar, 1967; Khan *et al.*, unpublished results). Species like *A. griffithii, H. recurvum,* and *S. fruticosa* could be classified as moderately salt tolerant *A. macrostachyum* and *C. cretica* as highly salt tolerant.

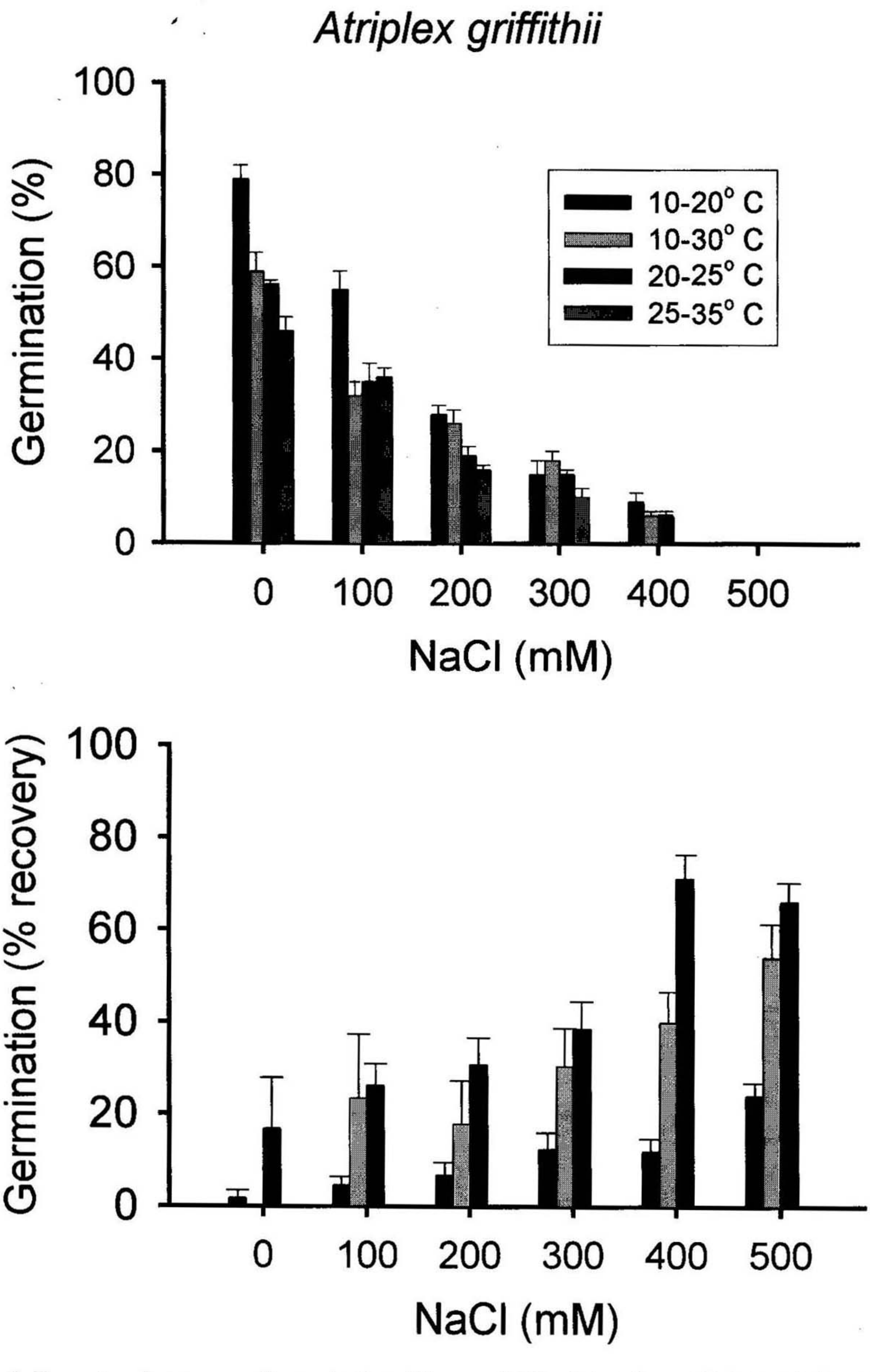

Fig. 1. Percent and recovery of germination (Mean ± S.E.) of *Atriplex griffithii* seeds in various salinities and thermoperiods.

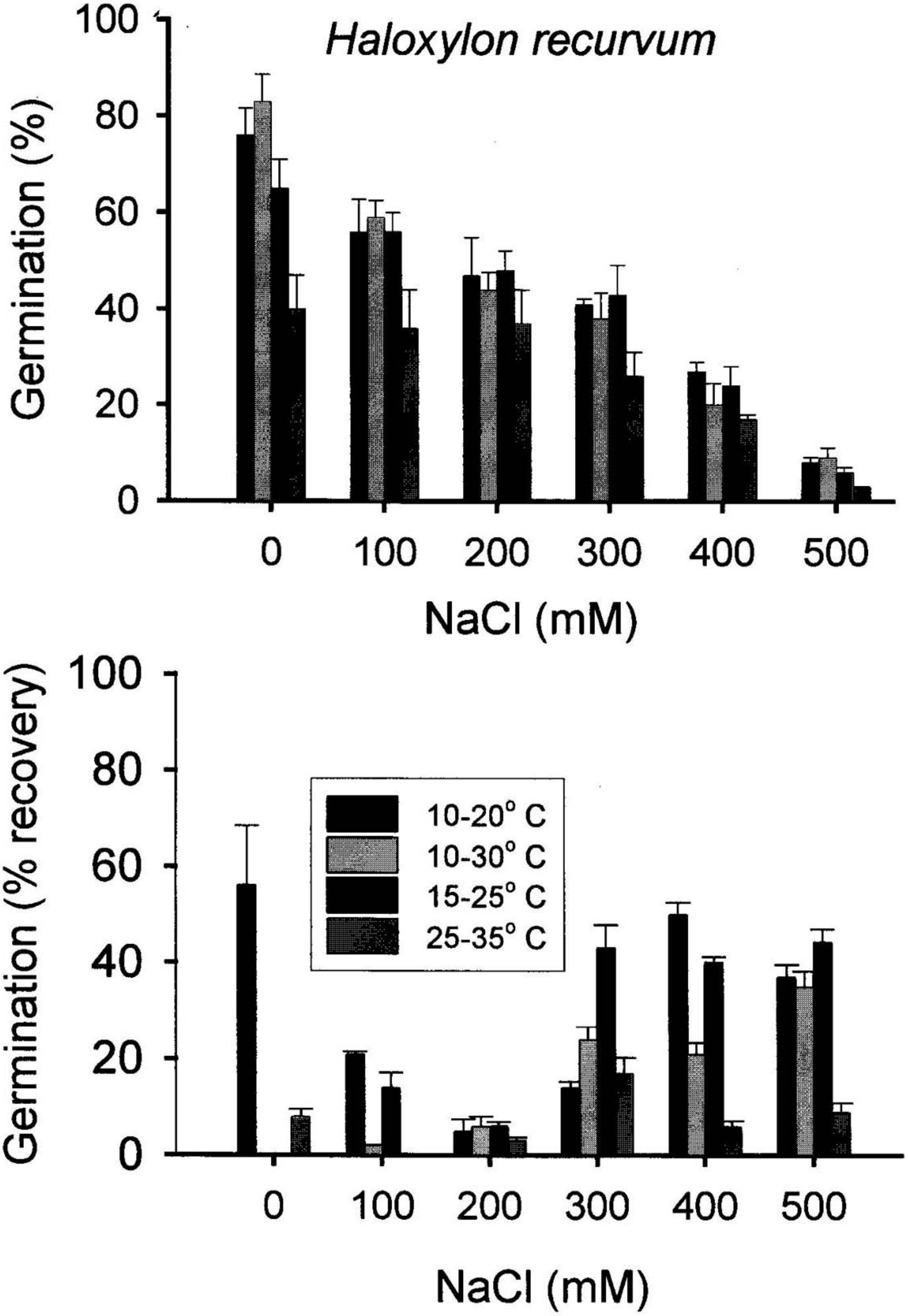

Fig. 2. Percent and recovery of germination (Mean ± S.E.) of *Haloxylon recurvum* seeds in various salinities and thermoperiods.

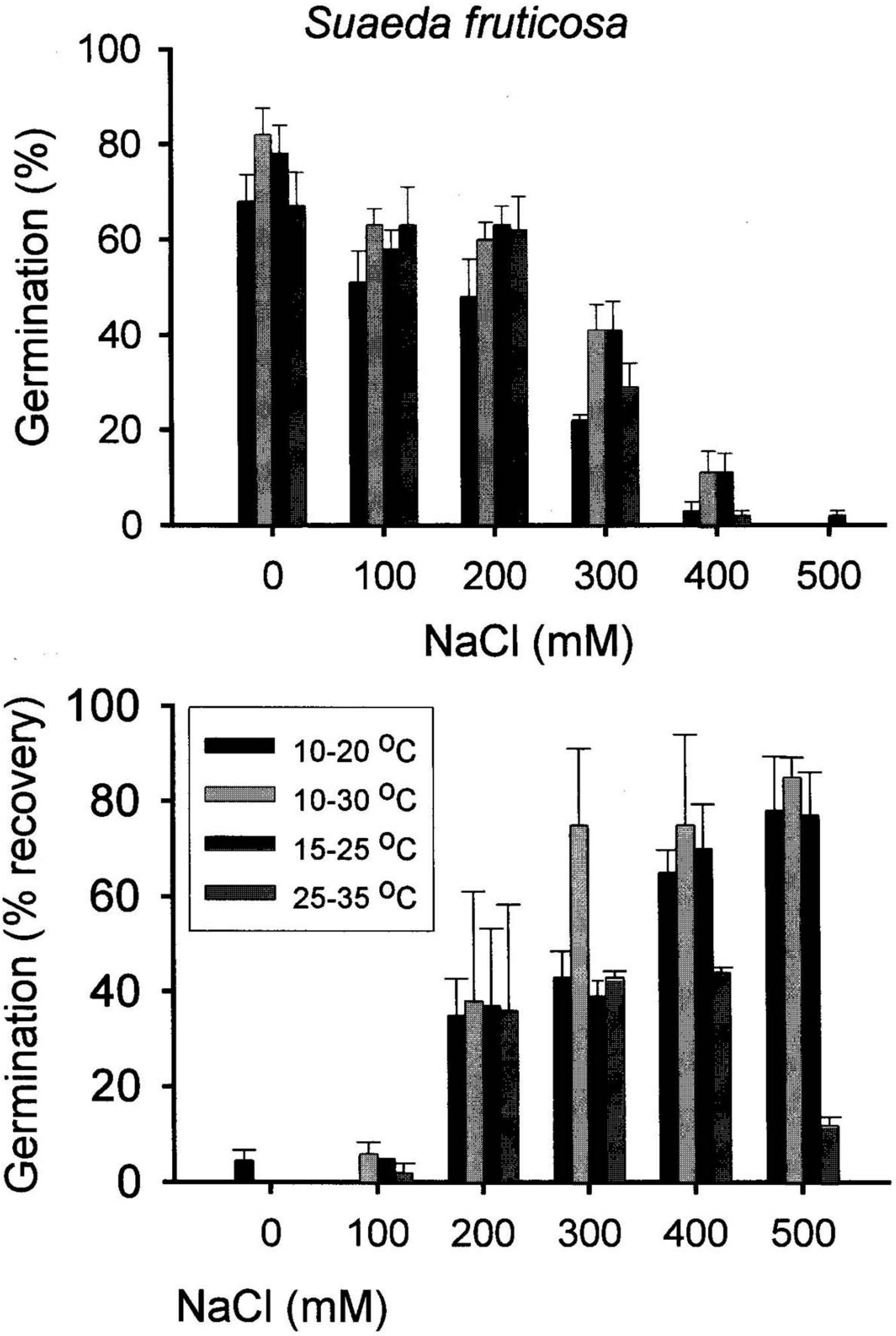

Fig. 3. Percent and recovery of germination (Mean ± S.E.) of *Suaeda fruticosa* seeds in various salinities and thermoperiods.

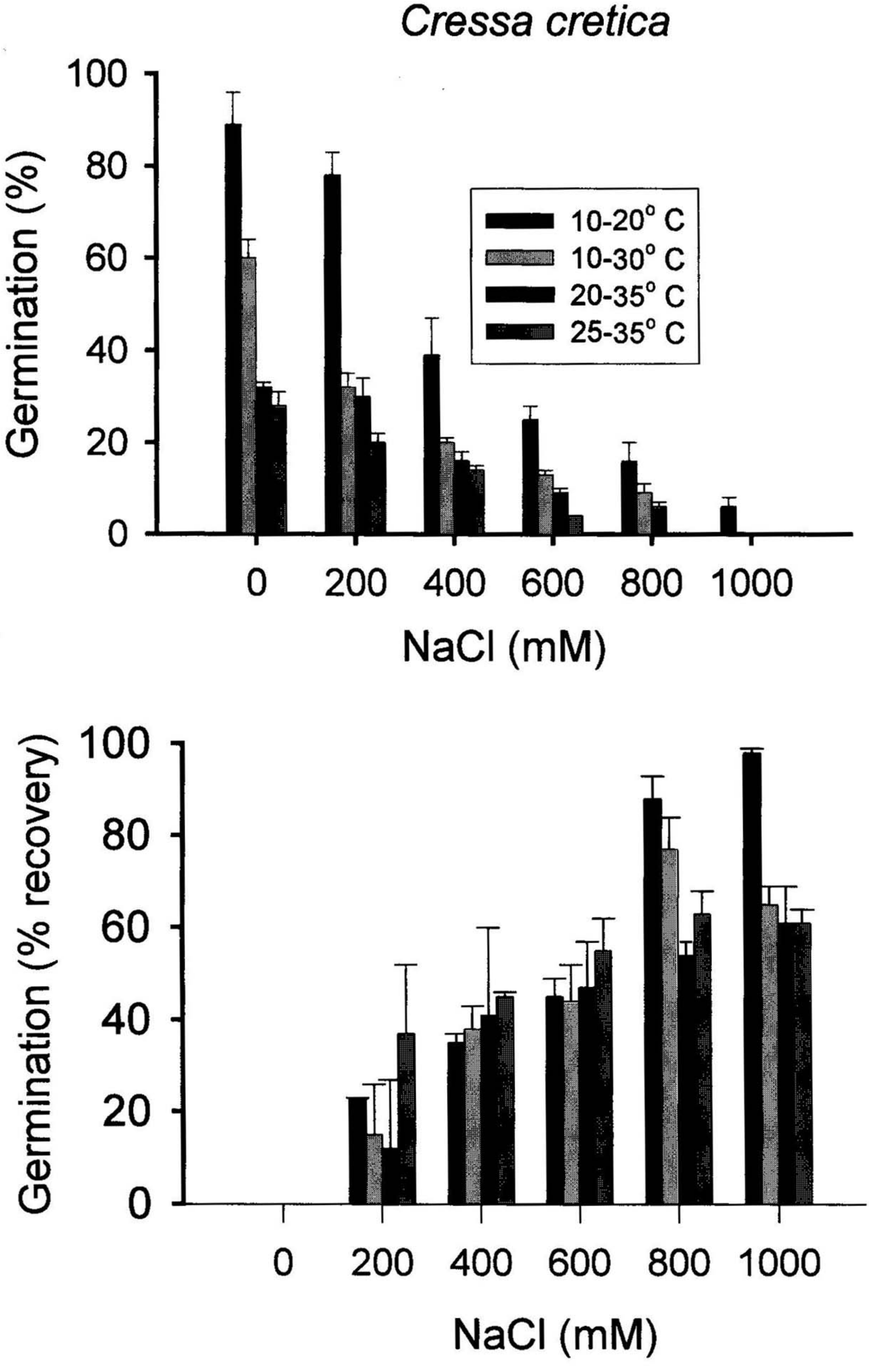

Fig. 4. Percent and recovery of germination (Mean ± S.E.) of *Cressa cretica* seeds in various salinities and thermoperiods.

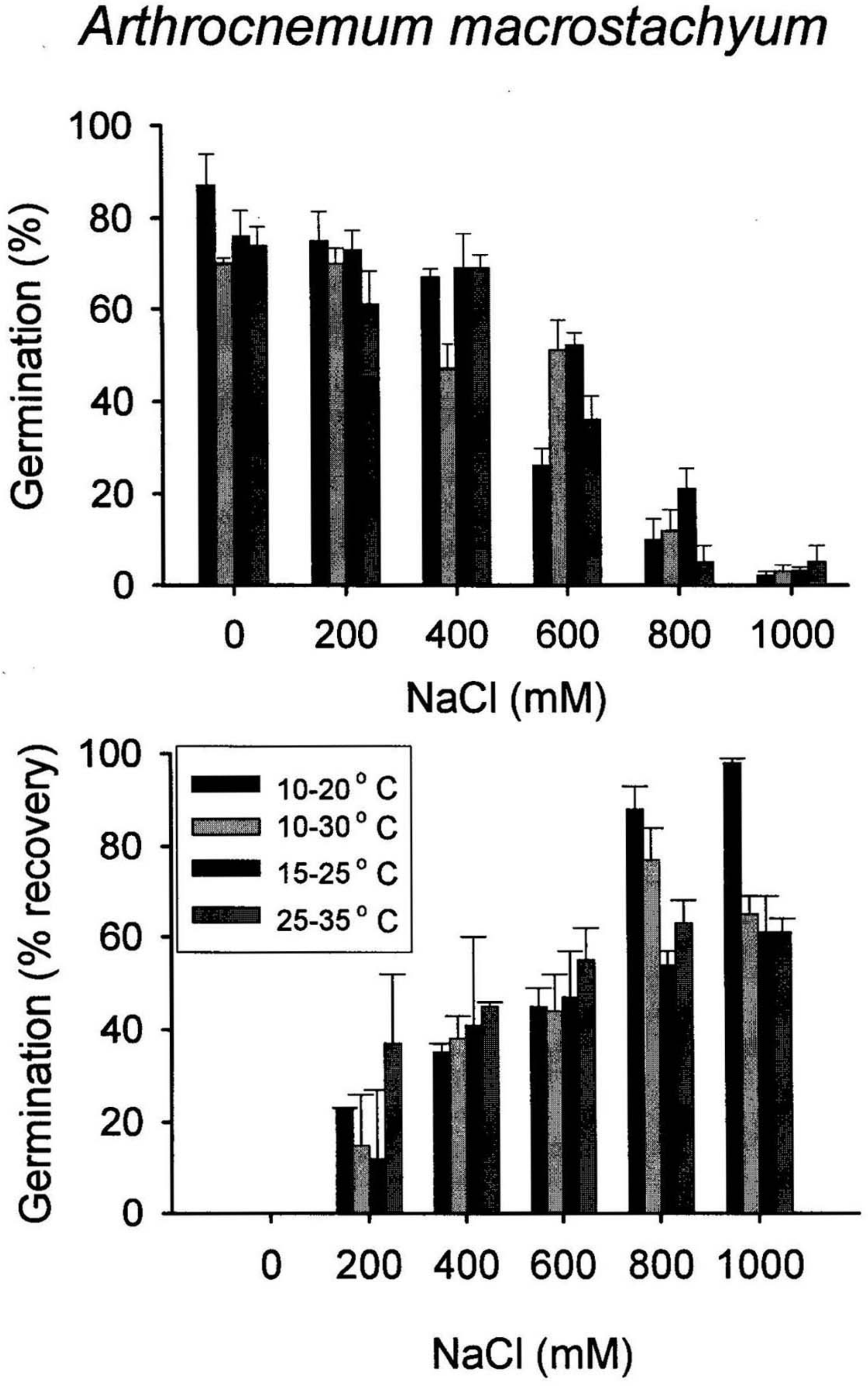

Fig. 5. Percent and recovery of germination (Mean ± S.E.) of *Arthrocnemum macrostachyum* seeds in various salinities and thermoperiods.

Salinity and temperature are reported to interact to affect seed germination of annual halophytes such as *Puccinellia nuttalliana* (Macke and Ungar, 1971), *Hordeum jubatum* (Badger and Ungar, 1989), *Suaeda depressa* (Ungar and Capilupo, 1969), *Salicornia bigellovi* (Rivers and Weber, 1971), *Atriplex triangularis* (Khan and Ungar, 1984), *Salicornia europaea* (Ungar, 1977), *Salicornia patula* (Berger, 1985) *Spergularia marina* (Okusanya and Ungar, 1983), *Polygonum aviculare* (Khan and Ungar, 1998) and tropical perennial halophytes, *Zygophyllum dumosum* (Koller, 1955; Agami, 1986), *Zygophyllum qatarensis* (Ismail, 1990), *Cressa cretica* (Khan, 1991), *Atriplex griffithii* (Khan and Rizvi, 1994), *Haloxylon recurvum* (Khan and Ungar, 1996b), *Zygophyllum simplex* (Khan and Ungar, 1997), and *Arthrocnemum macrostachyum* (Khan and Gul, 1998).

Seeds of halophytes are known to tolerate high salinity during their presence in the soil and germinate when soil salinities are reduced (Khan and Ungar, 1986; Ungar, 1995). Recovery germination responses have been demonstrated in *Salicornia europaea* (Ungar, 1962), *Spergularia marina* (Ungar, 1967), *Suaeda depressa* (Ungar and Capilupo, 1969), *Suaeda linearis* (Ungar, 1962), *Arthrocnemum australasicum, Triglochin striata, Suaeda australis, Juncus maritimus*, and *Casuarina glauca* (Clarke and Hannon, 1970). Boorman (1967, 1968) and Woodell (1985) also reported salt stimulation of seed germination following treatment with seawater for a number of salt marsh species. Woodell (1985) classified germination responses to salinity into three categories; Type 1 species, usually found in dunes or on the drift line, were all inhibited by half-strength seawater. Recovery was relatively high, but no salt stimulation was observed in this group. Seeds of Type 2 species were strongly inhibited by half-strength seawater but had recovery germination (56-98%) from seawater in distilled water that was similar to the original germination percentages in the control. Type 3 species had < 10% germination in seawater, were salt stimulated and had > 60% germination in distilled water recovery treatments. The ability of the halophyte to survive hypersaline conditions and germinate when stress is alleviated provides them with multiple opportunities for cohort establishment in these unpredictable saline environments. Salt stress induced dormancy produces a persistent seed bank of viable seeds in salt marsh and salt desert habitats that will maintain population over time (Ungar, 1995; Khan and Ungar, 1996; Khan and Gul, 1998). Keiffer and Ungar (1995) exposed seeds of five halophytes (*Atriplex prostrata, Hordeum jubatum, Salicornia europaea, Spergularia marina*, and *Suaeda calceoliformis*) to an extended period of salinity treatments and determined their recovery responses when transferred to distilled water. They used the classification system of Woodell (1985) and placed *Atriplex prostrata* seeds in the Type 1, *Hordeum jubatum* and *Spergularia marina* in the Type 2, and *Salicornia europaea* and *Suaeda calceoliformis* in the Type 3 category.

Recovery of seed germination from salt stress as affected by variation in thermoperiod is rarely reported (Khan and Ungar, 1996, 1997ab). Khan and Ungar, (1997b) studied the effect of salinity on the germination and recovery responses of *Zygophyllum simplex* under various thermoperiods. They found that *Z. simplex* showed a very poor recovery response when transferred to nonsaline medium, indicating a specific ion effect. Seeds of *H. recurvum* recovered from salt stress. Recovery in *H. recurvum* was less than in the control except for the 15°-25° C treatment where recovery from 300 mM NaCl treatment was equal to the untreated control. *Triglochin* *maritima* seeds appeared to be more sensitive to change in thermoperiod. No seed germination recovery was recorded in the 5°-15°C treatment. This would prevent germination in early spring when average temperatures in the field range from 5° to 15°C. Recovery at the lower thermoperiod (10°-20°C) was lower than in the control, but at peak thermoperiods it was similar to the control (Khan and Ungar, 1998c).

Perennial halophytic shrubs from Pakistan vary in their ability to tolerate medium salinity without loosing viability at different thermoperiods. All five halophytes studied show better recovery germination when exposed to high salinity at lower thermoperiod. Higher thermoperiod inhibit recovery in all species. Salt tolerance at germination level also varied, *A. griffithii* could germinate up to 400 mM NaCl, *Haloxylon recurvum* and *Suaeda fruticosa* up to 500 mM while *A. macrostachyum* and *C. cretica* could germinate at a concentration of 1000 mM NaCl, higher than seawater (600 mM). *Atriplex griffithii* is the only species which is annually recruited through seeds after monsoon rains when both medium salinity and temperature are reduced. Seeds of all species have the ability to germinate at high salinity and except for *C. cretica* and *H. recurvum*, they also have a persistent seed bank. It is still a puzzling question why they do not germinate under natural conditions. More studies are needed to determine the ecophysiology of halophytic seeds under natural conditions.

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