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EFFECTS OF THERMOPERIOD ON RECOVERY OF SEED GERMINATION OF HALOPHYTES FROM SALINE CONDITIONS[1]

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Recovery of seed germination from NaCl salinity of desert shrubs (*Haloxylon recurvum* and *Suaeda fruticosa,* and the herbs *Zygophyllum simplex* and *Triglochin maritima* was studied under various thermoperiods. The percentage of ungerminated seeds that recovered when they were transferred to distilled water varied significantly with variation in species and thermoperiods. *Zygophyllum simplex* had little recovery from all NaCl concentrations in all thermoperiods. *Haloxylon recurvum, S. fruticosa,* and *T. maritima* showed substantial recovery. Percentage recovery was highest in *S. fruticosa,* followed by *T. maritima,* and *H. recurvum.* Thermoperiodic effects varied with the species investigated. There was little thermoperiodic effect on the percentage recovery of *S. fruticosa,* except in the higher salinity treatment at higher thermoperiods. Variation in thermoperiod appears to play an important role in recovery of germination of halophytes from salt stress when seeds are transferred to distilled water.

Key words: halophyte; *Haloxylon recurvum*; recovery of seed germination; *Suaeda fruticosa*; thermoperiod; *Triglochin maritima*; *Zygophyllum simplex*

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 ((Ungar, 1962, 1978; Parham, 1970; Seneca and Cooper, 1971; Barbour, 1970; Macke and Ungar, 1971; Woodell, 1985; Naidoo and Naicker, 1992; Keiffer and Ungar, 1995). However, few studies have focused on the effect of variation in thermoperiod on the recovery responses of halophytic (Khan and Ungar 1996a,b). Salinity and temperature are known to interact to affect the germination of halophytes ((Khan and Ungar, 1984; Gutterman, 1986; Khan and Weber, 1986; Khan et al., 1987; Badger and Ungar, 1989; Khan, 1991; Khan and Rizvi, 1994; Ungar, 1996; Khan and Ungar, 1996a,b). Seeds of *Haloxylon recurvum* showed higher germination percentages at cooler thermoperiods (Khan and Ungar, 1996a) and seeds of *Zygophyllum simplex* germinated better at moderate temperature regimes (Khan and Ungar, 1996b). Naidoo and Naicker (1992) studied the effect of light, temperature, and salinity on the germination of *Triglochin maritima* and *T. striata.* Germination was best in distilled water and at salinities below 250 mmol/L NaCl but decreased significantly with an increase in salinity up to 500 mmol/L. Transfer of ungerminated, salt-treated seeds to distilled water stimulated germination more in *T. striata* than in *T. bulbosa.* Ungar (1996) reported that high salinity did not permanently injure *Atriplex patula* seeds and germination recovered fully when seeds were transferred to distilled water.

Thermoperiod seems to play an important role in the seed germination of inland and coastal salt marsh species. Little work has been done to ascertain the effect of thermoperiod on the recovery response of halophytic seeds when salinity stress is removed. The primary objective of this investigation was to compare the recovery of germination from salt stress in four halophytes under various thermoperiods.

MATERIALS AND METHODS

Seeds of *Haloxylon recurvum, Suaeda fruticosa,* and *Zygophyllum simplex* were collected during fall 1994 from salt flats situated on the Karachi University campus, Pakistan. Seeds of *Triglochin maritima* L. were collected during summer 1995 from a salt marsh located ≈50 km south of the Great Salt Lake, at Faust, Utah. Seeds were separated from inflorescence and then brought to Ohio University where they were stored at 4°C. Germination studies were started in September 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. 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, 5°−25°C, 10°–20°C, and 15°–25°C in *Triglochin maritima* and 10°–20°C, 10°–30°C, 15°–25°C, and 25°–35°C in *Suaeda fruticosa, Haloxylon recurvum,* and *Zygophyllum simplex.* The higher temperature (15°, 20°, and 25°C) coincided with the 12-h light period (Sylvania cool white fluorescent lamps, 25 μmol photons$\cdot m^{-2} \cdot s^{-1}$, 400–750 nm) and the lower temperature (5°, 10°, and 15°C) coincided with the 12-h dark period. Seeds were germinated in distilled water, and 100, 200, 300, 400, and 500 mmol/L NaCl solutions in the case of *Suaeda fruticosa, Haloxylon recurvum,* and *Triglochin maritima* and in 0, 25, 50, 75, 100, and 125 mmol/L NaCl in the case of *Zygophyllum simplex* at the abovementioned temperature regimes. After 20 d ungerminated seeds from the NaCl treatments were transferred to distilled water (under original temperature conditions) to study

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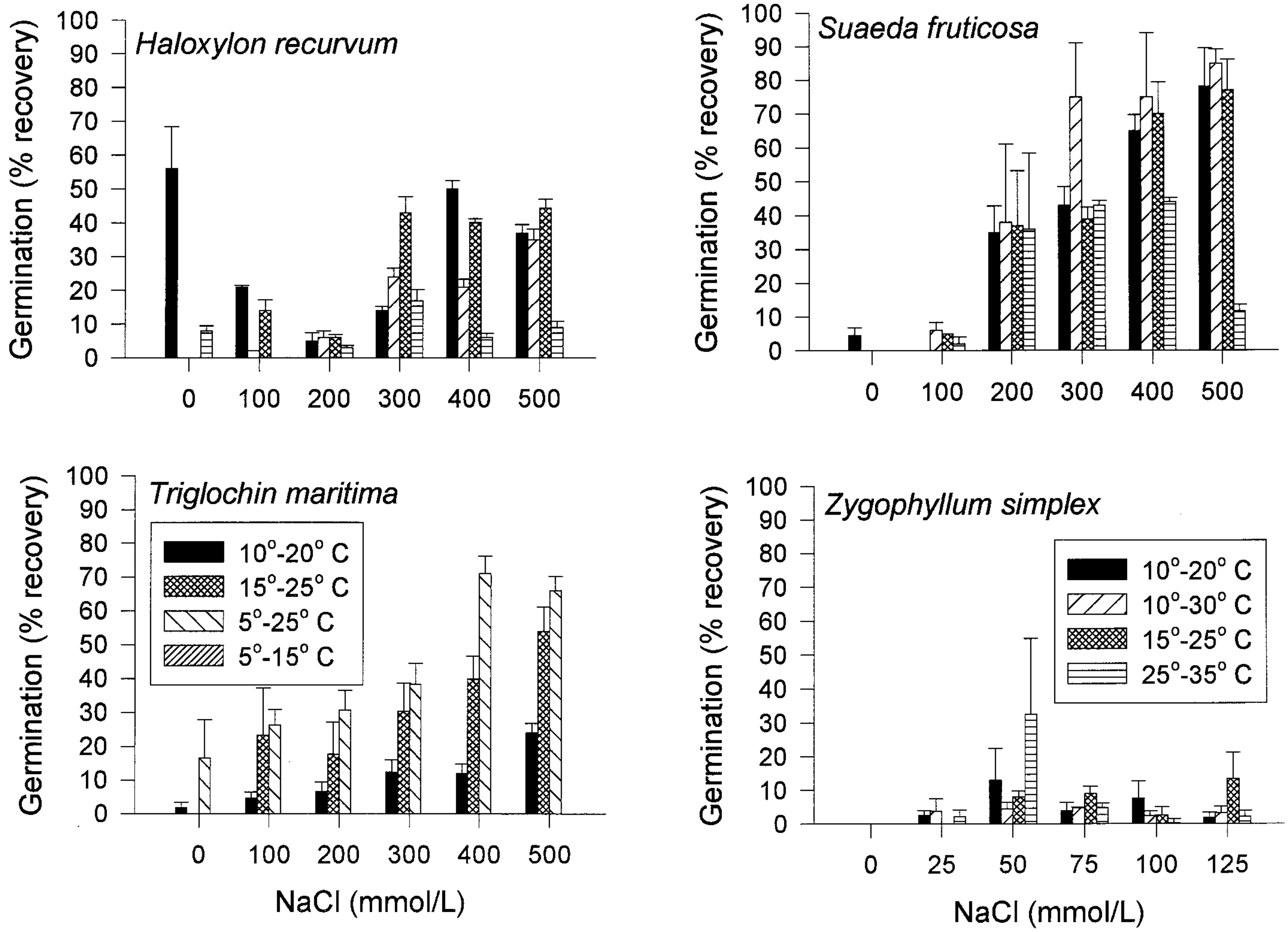

Fig. 1. Percentage recovery of germination of *Suaeda fruticosa, Haloxylon recurvum, Zygophyllum simplex,* and *Triglochin maritima* seeds in various salinities and thermoperiods.

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 6.1 (SPSS, 1994).

RESULTS

Effect of thermoperiod and salinity on percentage germination recovery—Recovery germination percentages substantially increased with increases in salinity concentrations except in *Z. simplex* (Fig. 1). *Zygophyllum simplex* had a very poor recovery response, indicating a specific ionic effect on the germination of its seeds after exposure to salinity for 20 d. Change in thermoperiod had an effect on the germination of halophytes in both saline and nonsaline conditions (Fig. 1). This effect varies with the species. *Triglochin maritima* seeds are most severely affected by change in thermoperiod. Low night and high day temperatures significantly increased the recovery percentages. However, at low night and low day temperature there was no recovery. *Suaeda fruticosa* at lower thermoperiods had a substantial recovery response, but high thermoperiods caused irreparable injury to the seeds. *Haloxylon recurvum* seeds had a better recovery response at the moderate thermoperiod but any increase or decrease in temperature substantially prevented recovery (Fig. 1).

Effect of thermoperiod and salinity on rate of germination recovery—Rate of recovery of germination of *Suaeda fruticosa* seems to be unaffected by the pretreatment concentration of NaCl. Recovery rate was similar in all treatments. At higher salt concentrations, the warmer thermoperiod significantly decreased the germination rate (Fig. 2). In the case of *Haloxylon recurvum,* at lower salinity concentrations cooler and moderate thermoperiods had the same germination rate but warmer thermoperiods significantly reduced the rate of germination. However, the rate of recovery from the high salt treatment was optimal in moderate (15°–25°C) and minimal in warmest (25°–35°C) thermoperiods. Rate of recovery

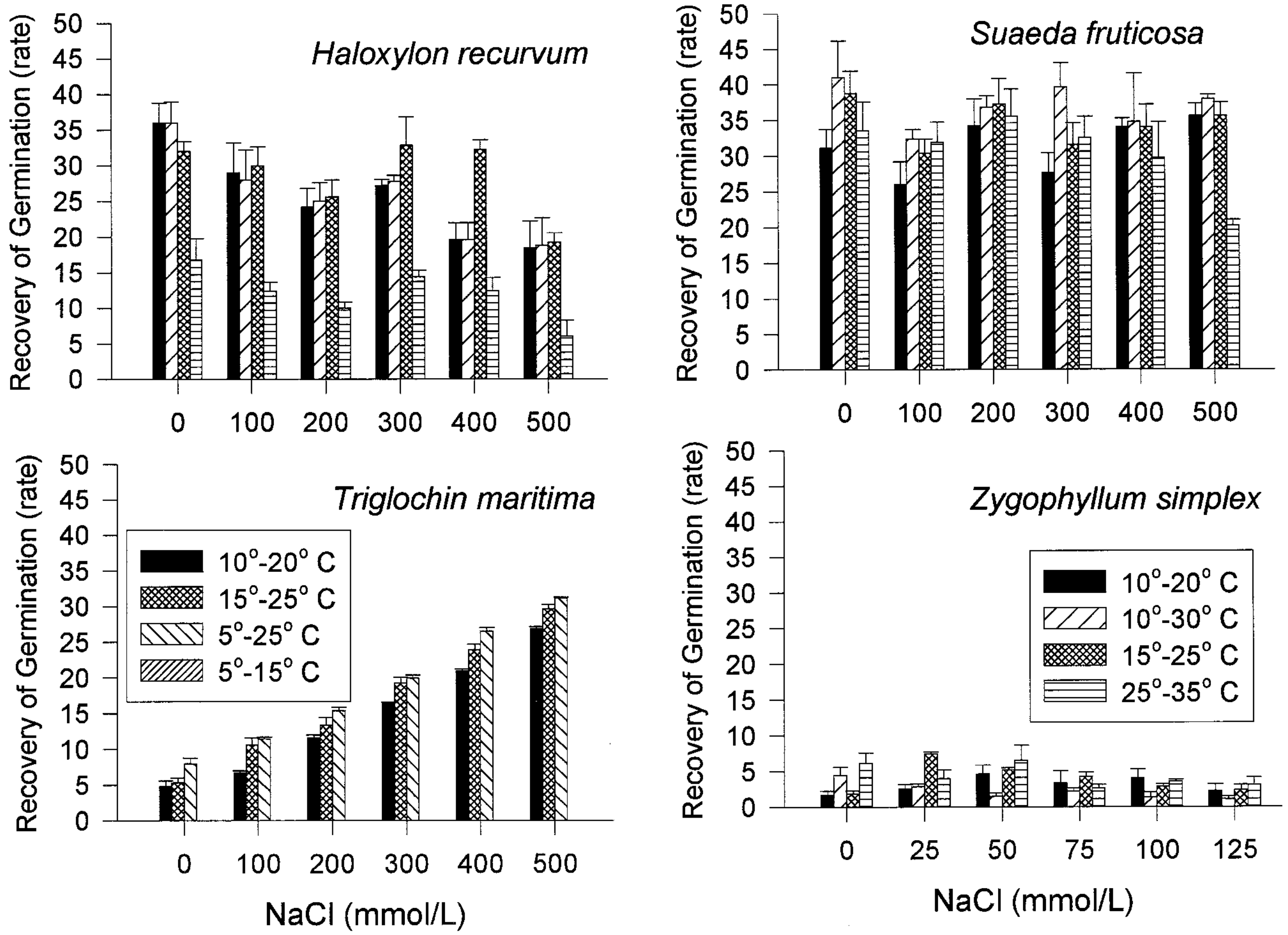

Fig. 2. Rate of recovery germination of *Suaeda fruticosa, Haloxylon recurvum, Zygophyllum simplex,* and *Triglochin maritima* seeds in various salinities and thermoperiods.

of germination in *Triglochin maritima* progressively increased with increases in the NaCl pretreatment concentration. There is little difference between thermoperiods except at the 5°–15°C thermoperiod where there was no recovery. *Zygophyllum simplex* had a low rate of recovery of germination in all treatments (Fig. 2).

Effect of thermoperiod and salinity on days required for 50% germination recovery—*Suaeda fruticosa* and *Haloxylon recurvum* seeds showed 50% recovery after just 2 d of transfer from saline solutions (Fig. 3a), irrespective of change in salinity and thermoperiods. In *Triglochin maritima* it took 6 d to reach 50 % recovery at all salinity concentrations. At low and high salinity 50% recovery was faster at 10°–20°C and 10°–30°C. However, with increase from low to moderate salinity the recovery germination was delayed at the 10° −30° C thermoperiod. Days required to reach 50 % recovery of germination increased with increases in thermoperiod and decreased again at the highest thermoperiod.

DISCUSSION

Seed germination of halophytes under natural conditions is regulated by variation in soil salinity and ambient thermoperiod (Khan and Ungar, 1984; Badger and Ungar, 1989; Ungar, 1995). The salt tolerance of seeds appears to be affected by thermoperiod (Morgan and Myers, 1989; Khan and Ungar, 1996a, b). 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 australsicum, 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 >

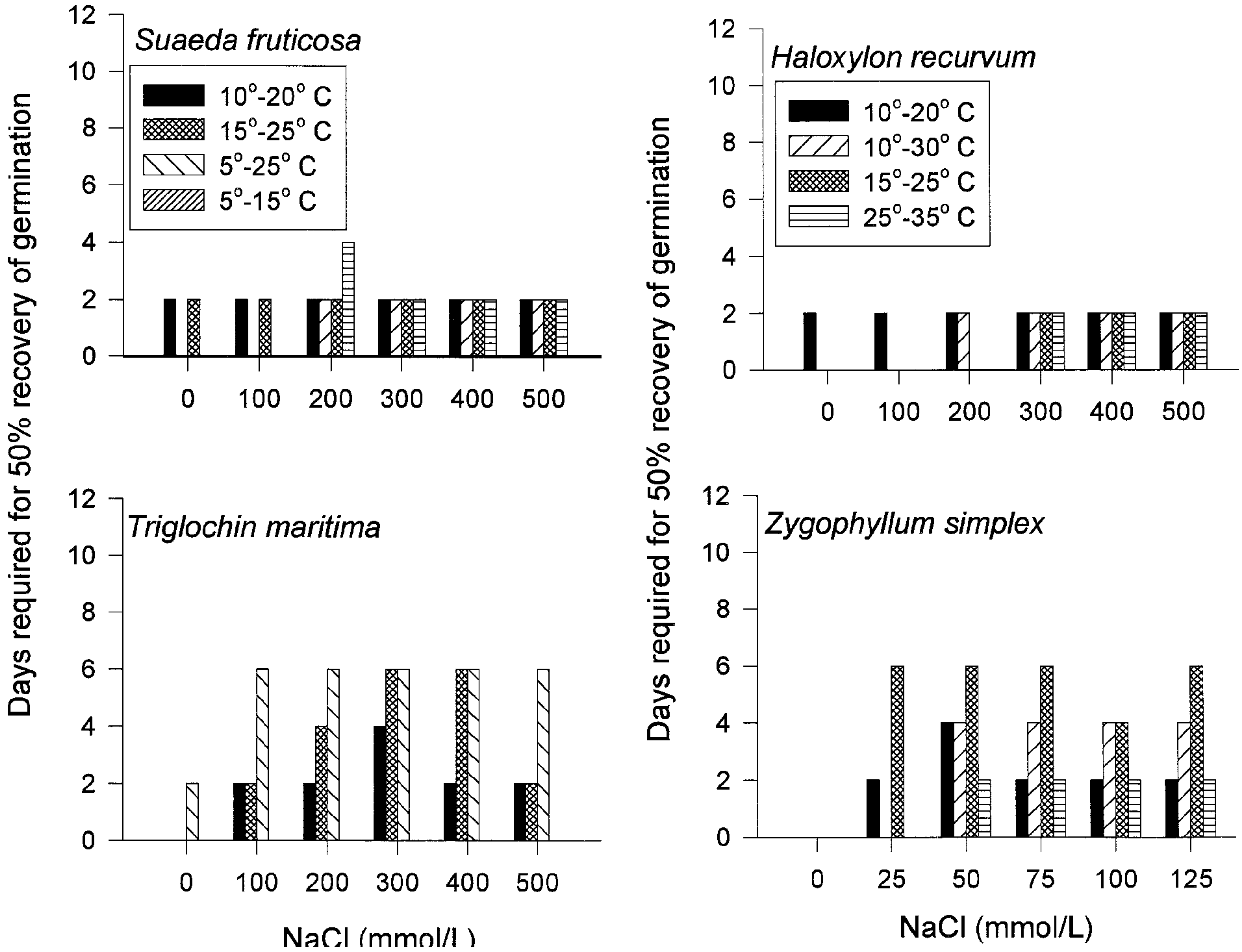

Fig. 3. Time required to attain 50% recovery of germination of *Suaeda fruticosa, Haloxylon recurvum, Zygophyllum simplex,* and *Triglochin maritima* seeds in various salinities and thermoperiods.

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 marshes and salt desert habitats that will maintain the population over time (Ungar, 1995; Khan and Ungar, 1996a). 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 Woodell (1985) classification system 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, 1996a, b). Khan and Ungar (1996a, b) studied the effect of salinity on the germination and recovery responses of *Haloxylon recurvum,* and *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 mmol/L NaCl treatment was equal to the untreated control (Khan and Ungar, 1996a). *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 temperature 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, unpublished data). The results presented here indicate that species vary greatly in their germination recovery responses when exposed to various salinities and thermoperiods. Annuals like *Z. simplex* had little recovery after exposure to salt stress. However, other species studied such as *Suaeda fruticosa, Haloxylon recurvum,* and *Triglochin maritima* did demonstrate recovery of germination (Khan and Ungar 1996a, and unpublished data). Recovery percentages of the previously ungerminated seeds were higher in the case of *S. fruticosa* and lower in *H. recurvum.* The two species usually grow in a similar association. Thermoperiodic effects are also varied. Change in thermoperiod had little effect on the recovery responses of *S. fruticosa* in low salinity treatments. However, at higher thermoperiods there was an interaction with the higher salt concentration to inhibit recovery. Germination responses of *T. maritima* indicated a high sensitivity to variation in thermoperiod. No recovery occurred at the 5°–15°C thermoperiod, but in the 5°–25°C treatment recovery from high salinity approached 72%. Recovery was low at 10°–20°C and moderate at 15°–25°C. Responses of *H. recurvum* were similar to *S. fruticosa*, but recovery percentages were low. Rate of germination was similar at all thermoperiods, but the low thermoperiod in *Triglochin maritima* and high thermoperiod in the case of *Haloxylon recurvum* and *S. fruticosa* significantly reduced the germination rate.

The present study clearly indicates the significance of thermoperiod in determining the recovery of germination responses of halophyte seeds. Seeds of the same species could show from 0 to 72% recovery with a change in thermoperiod. Further studies on the effect of thermoperiod on the recovery of germination in coastal and inland halophytes are suggested.

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