Khan M. A. and Ungar, I. A. (1996), *Seed Sci. & Technol.*, **25**, 83–91

Germination responses of the subtropical annual halophyte *Zygophyllum simplex*

M. A. KHAN[1] and I. A. UNGAR

Department of Environmental and Plant Biology, Ohio University, Athens, Ohio 45701-2979. USA

(Accepted August 1996)

Summary

Seeds of the succulent annual halophyte *Zygophyllum simplex* germinate after rainfall during July or August. *Zygophyllum simplex* L. is one of the few annual halophytes found in subtropical salt marshes and deserts. It has a life span of about 80 days. Experiments were conducted to determine the effects of NaCl and thermoperiods on the germination of seeds and their recovery responses after being transferred to distilled water. Cooler temperatures significantly inhibited germination at all treatments and the highest temperature also caused some inhibition. *Zygophyllum simplex* seeds are moderately tolerant to NaCl concentrations at the germination stage of development. Highest germination percentages in all salinity treatments were obtained at a moderate (15–25 °C) thermoperiod. Few seed germinated at concentrations higher than 100 mM NaCl treatment. When seeds were transferred to distilled water, after 20 days of salinity treatment and at various thermoperiods, there was some recovery. Recovery ranged from 0 to about 20% germination at extreme thermoperiods for seeds that were germinated at high salinity concentrations.

Introduction

Salt tolerance at the germination stage is critical for plant survival and growth in saline soils (Khan and Ungar, 1986; Ungar, 1991). Seedlings are more vulnerable to physical changes in the environment than at later life-cycle stages. Seed dormancy is a mechanism that prevents germination in an unsuitable environment (Fenner, 1985). Halophytes often have some type of dormancy mechanism that distributes seed germination within and between growing seasons (Ungar, 1987). Germination of halophytes in saline environments occurs when seasonally low salinities are reached (Khan and Ungar, 1986; Khan and Rizvi, 1994; Aziz and Khan, 1996). This enables plants to complete critical stages of their life cycle during a time of reduced salinity stress.

Several factors (water, temperature, light, salinity) interact in the soil interface which regulate seed germination. They may even co-act with the seasonal variation in temperature to determine the temporal pattern of germination. Osmotic and matric potential narrow the range of temperature that is effective for the germination of seeds (Hegarty, 1978). Salinity and temperature is reported to interact to affect seed germination of temperate annual halophytes such as *Puccinellia nuttalliana* (Mack and Ungar, 1971), *Hordeum jubatum* (Badger and Ungar, 1989), *Suaeda depressa* (Ungar and Capilupo,

[1] Author for correspondence and reprints.

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) and tropical perennial halophytes, *Zygophyllum dumosum* (Koller, 1955; Agami, 1986), *Zygophyllum qatarensis* (Ismail, 1990), *Cressa cretica* (Khan, 1991), *Atriplex griffithii* (Khan and Rizvi, 1994), *Suaeda fruticosa* (Khan and Ungar, 1996a) and *Haloxylon recurvum* (Khan and Ungar, 1996b). 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 and Ungar, 1995). However, halophytes differ in their capacity to recover from salinity stress (Woodell, 1985). This variation in recovery responses could be due to difference in the thermoperiod to which they are exposed (Khan and Ungar, 1996ab). *Haloxylon recurvum* and *Suaeda fruticosa* clearly demonstrate that recovery responses are significantly affected by the change in thermoperiods (Khan and Ungar, 1996ab).

Zygophyllum simplex L. (Zygophyllaceae) is a succulent ephemeral that is widely distributed in the dry subtropical deserts of Africa and Asia (Schmida, 1985). In Pakistan, it is distributed from coastal areas of Sindh and Balochistan to the plains of Punjab (Stewart, 1972). It is also found in moderately saline areas on the flat plains of Karachi, Pakistan. It germinates when salinity is reduced after monsoon rains, which usually occurs during July or August, and completes its life cycle in 75 to 80 days. During the later stage of its life cycle, the shoots of *Z. simplex* become very succulent and turn red or almost black in color. At maturity plants produce many seeds and die. The species has economic value since leaves and seeds are used to cure some eye diseases and it has antibacterial and anthelmintic properties, and shoots are grazed by animals (Dagar, 1995).

There are few reports on the responses of annual halophytes from dry subtropical habitats to various salinity and temperature regimes. The present study describes the effect of salinity and thermoperiod on the germination and recovery responses of seeds of *Zygophyllum simplex*.

Materials and methods

Seeds of *Zygophyllum simplex* were collected during the fall of 1994 from salt flats situated on the Karachi University campus, Pakistan. Seeds were separated from the inflorescence and stored at 4 °C. These seeds 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. 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 °C, 10–30 °C, 15–25 °C, and 25–35 °C, based on a 24-hr cycle were used, where the higher temperature (20, 25, 30 and, 35 °C) coincided with the 12-hr light period (Sylvania cool white fluorescent lamps, 25 μmol.m^{-2}.s^{-1}, 400–700 nm) and the lower temperature (10, 15, and 25 °C) coincided with the 12-hr dark period. Seeds were germinated in distilled water, 25, 50, 75, 100, and 125 mM NaCl solutions under the above mentioned 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 rate of germination was estimated by using a modified Timson index of germination velocity = $\sum G/t$, where G is percentage of seed germination at 2-days 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 (Statistical Package for Social Sciences) for Windows release 6.1 (SPSS Inc., Chicago, USA, 1994).

Results

Seeds of *Z. simplex* can tolerate low NaCl concentrations at the time of germination. Seed germination was progressively inhibited with increases in salinity and only a few seeds germinated at 125 mM NaCl treatment (Figure 1). Maximum germination was ob-

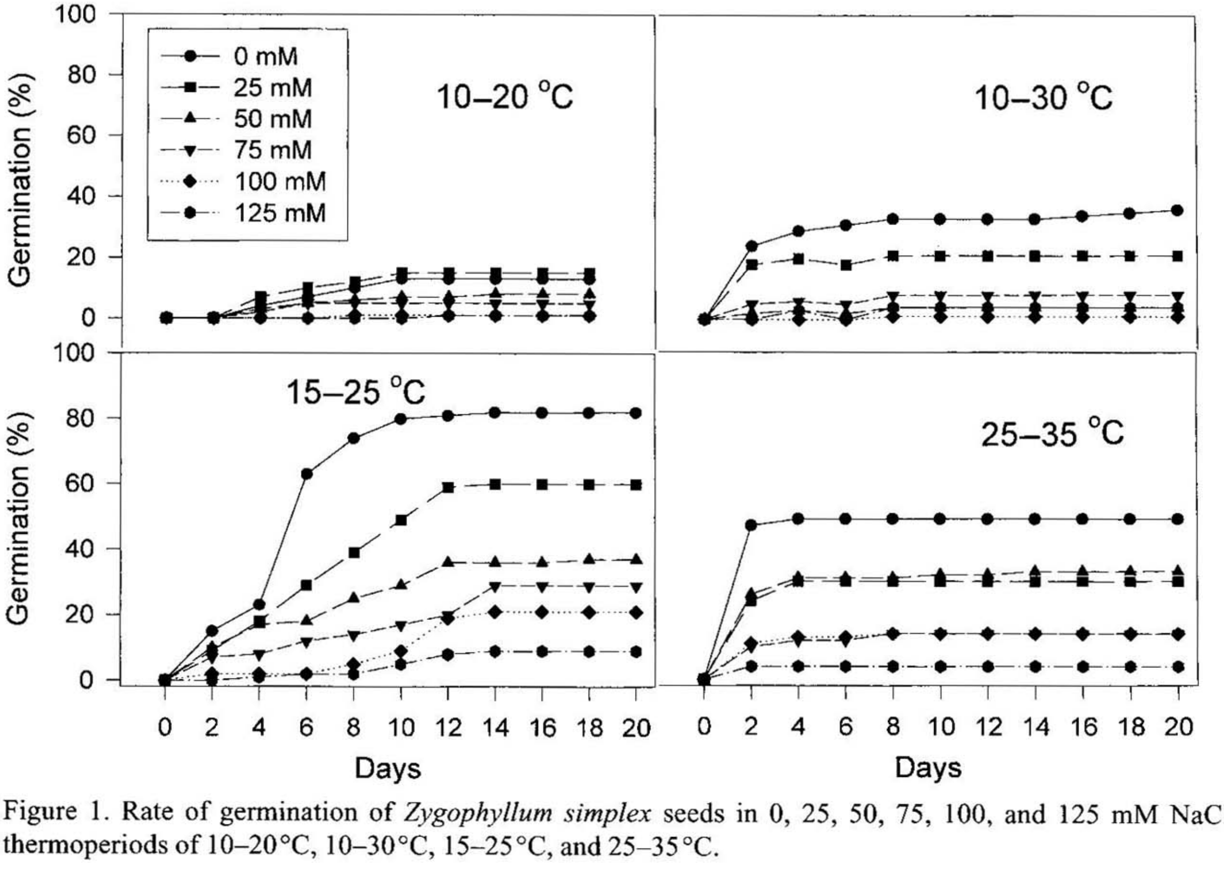

Figure 1. Rate of germination of *Zygophyllum simplex* seeds in 0, 25, 50, 75, 100, and 125 mM NaCl at thermoperiods of 10–20 °C, 10–30 °C, 15–25 °C, and 25–35 °C.

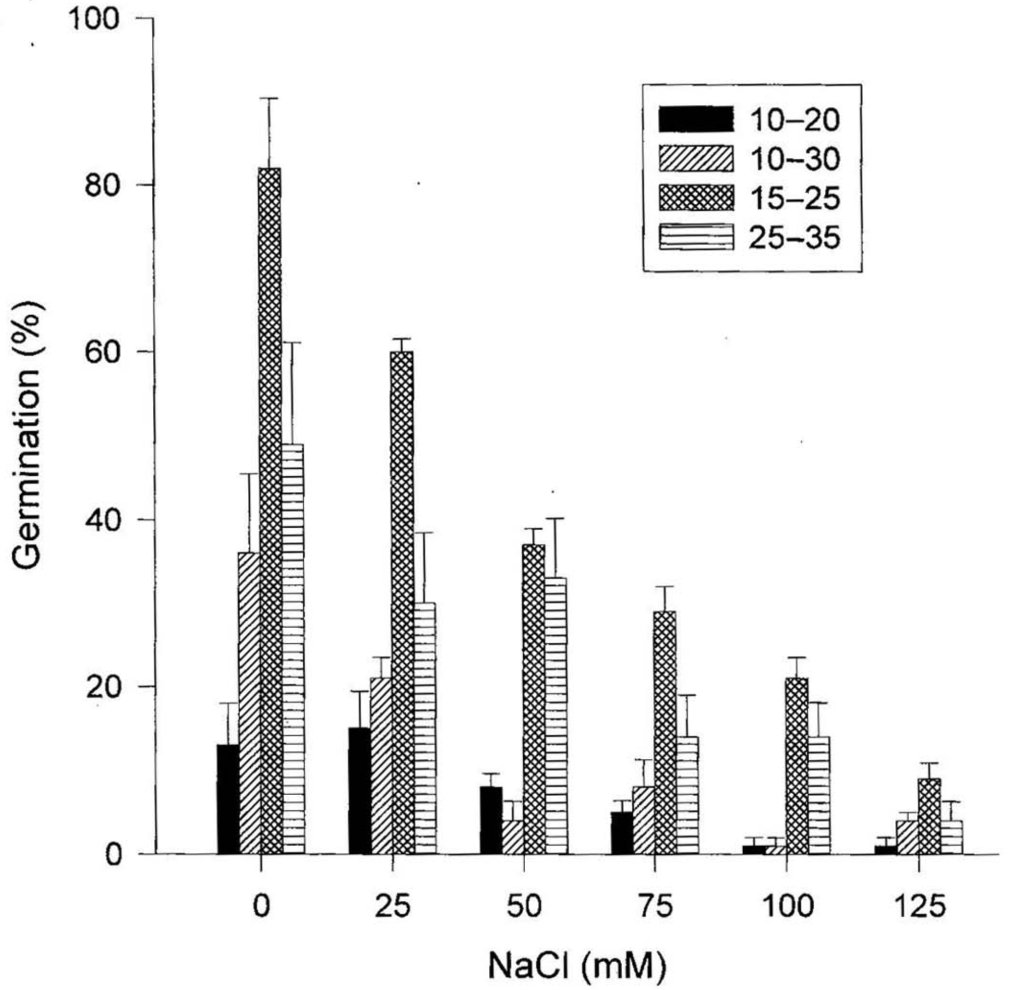

Figure 2. Mean (±SE) final germination percentages of *Zygophyllum simplex* seeds in 0, 25, 50, 75, 100, and 125 mM NaCl at thermoperiods of 10–20 °C, 10–30 °C, 15–25 °C, and 25–35 °C.

tained in distilled water. *Zygophyllum simplex* had considerable variation in germination in the different thermoperiods. Germination at the cooler (10–20 °C) thermoperiod was reduced to only 15% in non saline controls and seed germination was further inhibited with an increase in NaCl concentrations. Higher thermoperiods (25–35 °C) were also inhibitory. However, at the moderate thermoperiod (15–25 °C) there was more than 80% germination in distilled water treatments (Figure 1, 2), and germination of seeds at all other salinity treatments were also higher than at the other thermoperiod treatments (Figure 2). Seeds germinated rapidly and reached their maximum germination generally in less than 10 days. At 10–30 °C and 25–35 °C maximum germination percentages were achieved in 2 days after the initiation of germination. Different thermoperiods and various concentration of salinity individually, and their interaction were significant ($P < 0.0001$) in affecting the germination of *Z. simplex* seeds (Table 1).

Highest rate of germination was obtained in the 15–25 °C thermoperiod and lowest in 10–20 °C (Figure 1 and 4). Rate of germination decreased with an increase in salinity and the thermoperiod effect remained the same at all NaCl concentrations (Figure 1).

Table 1. Result of two-way analysis of variance of percent germination by salinity and temperature treatments.

Source of variation	Sum of squares	DF	Mean square	F	Significance of P
Temperature	14969.8	3	4989.9	51.5	0.0001
Salinity	18328.8	5	3665.8	37.9	0.0001
Temperature × Salinity	5893.2	15	392.9	4.1	0.0001

Different thermoperiods and various concentration of salinity individually, and their interaction were significant ($P < 0.0001$) in affecting the rate of germination of *Z. simplex* seeds (Table 2).

Generally, at all thermoperiods little recovery was observed (Figure 3). However, in the highest and lowest temperature regimes the high salinity treatment had some recovery (20%) (Figure 3).

Discussion

The principal adaptation desert plants have to make is to the scarcity of water. Although water is generally the limiting factor for growth in the desert, there are short variable

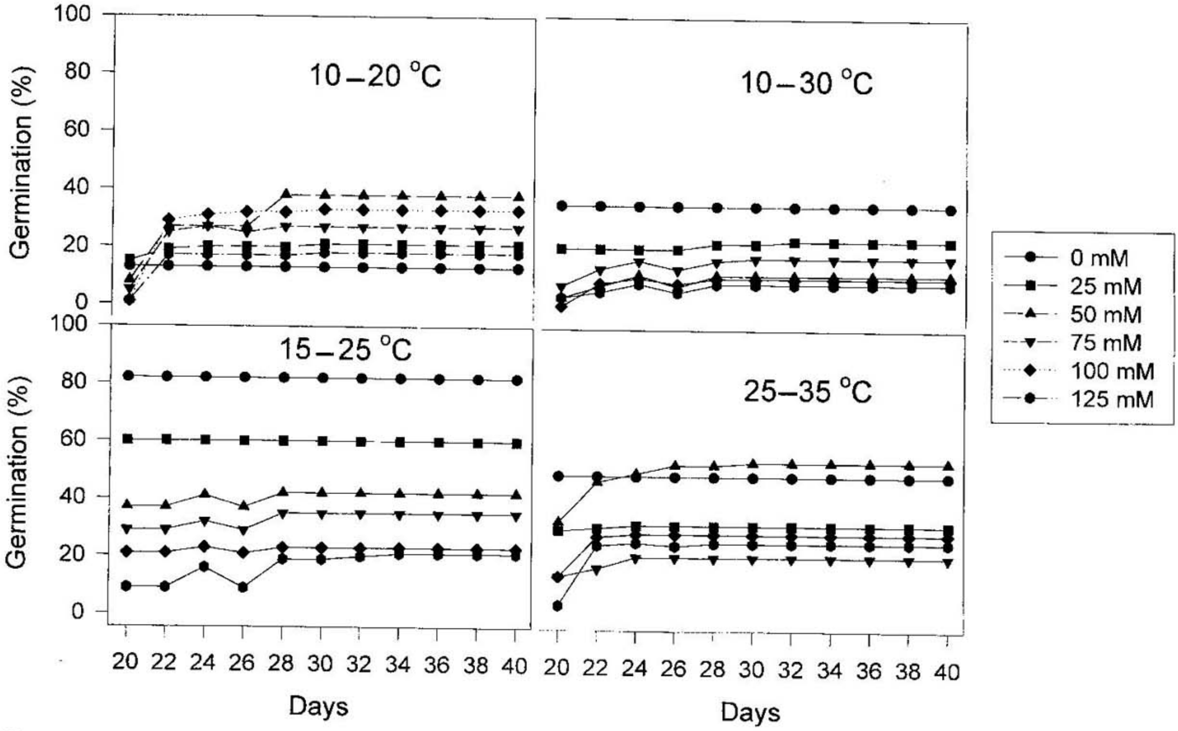

Figure 3. Recovery percentage of germination of *Zygophyllum simplex* seeds after they are transferred from 0, 25, 50, 75, 100, and 125 mM NaCl at thermoperiods of 10–20°C, 10–30°C, 15–25°C, and 25–35°C.

Table 2. Result of two-way analysis of variance of germination rate by salinity and temperature treatments.

Source of variation	Sum of squares	DF	Mean squares	F	Significance of P
Temperature	2144.4	3	714.8	34.1	0.0001
Salinity	3643.5	5	728.7	34.8	0.0001
Temperature × Salinity	1103.9	15	73.6	3.5	0.0001

periods where water may be found in abundance (Evenari et al., 1982). Some desert plants have adapted themselves to the short duration of these periods. Since the variability of rainfall from year to year is very great, plants must be able to survive drought years with little or no rain. High soil salinity and high environmental temperatures also play a role in the life of desert plants and so make their adaptation to moisture stress even more difficult. *Zygophyllum simplex* is a subtropical desert annual halophyte.

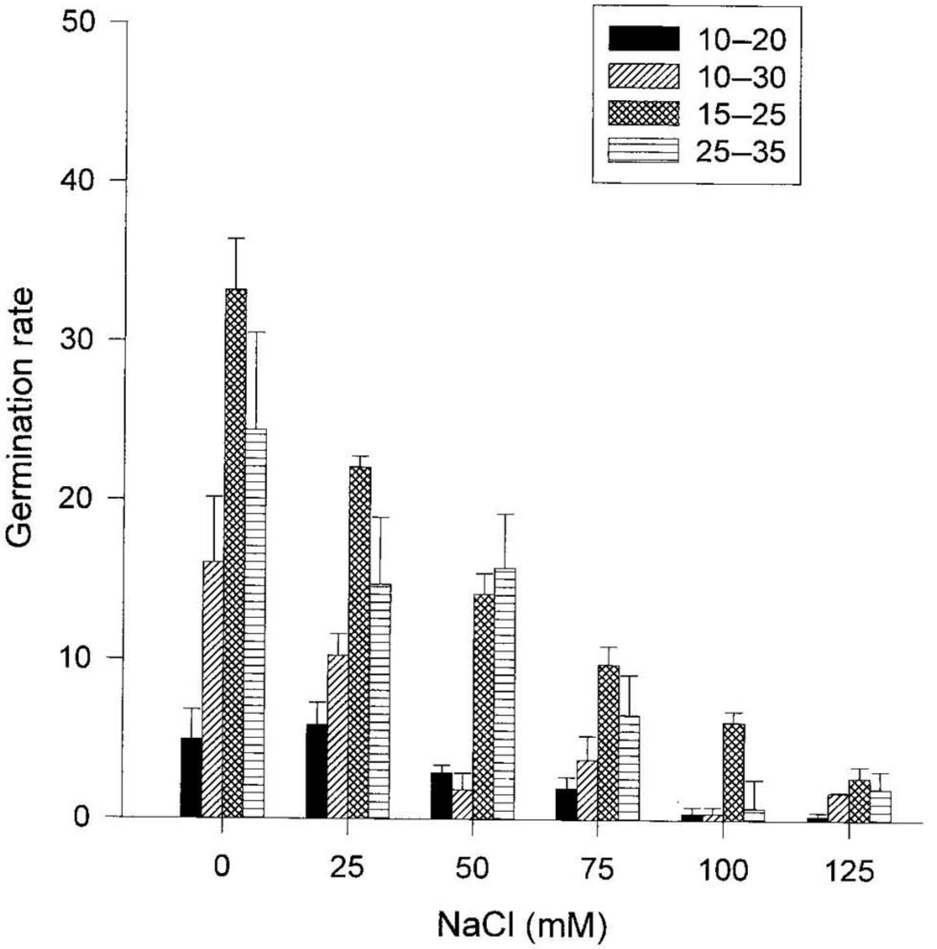

Figure 4. Rate of germination of *Zygophyllum simplex* seeds after they are transferred from 0, 25, 50, 75, 100, and 125 mM NaCl at thermoperiods of 10–20 °C, 10–30 °C, 15–25 °C, and 25–35 °C.

Seeds of *Z. simplex* germinate soon after substantial monsoon rains, which results in a decrease in the soil salinity by leaching and considerable reduction in the ambient and soil temperatures. *Zygophyllum simplex* has been described as a true halophyte (Sen et al., 1982). It is distributed in the dry tropical and subtropical regions of Asia and Africa. Our study showed that it is a moderately salt tolerant annual, whose germination was substantially inhibited at 100 mM NaCl treatment. Few seeds germinated at NaCl concentrations higher than 100 mM. The cooler thermoperiod (10–20 °C and 10–30 °C) considerably inhibited the germination and the warmer thermoperiod was also inhibitory. Best germination percentages were obtained at the 15–25 °C thermoperiod. This correlates with the thermoperiod that occurs at the time of monsoon rains in Karachi, Pakistan. Salinity and temperature have a differential effect on the germination of halophytes and the limits of tolerance to salinity may be greater at one thermoperiod than at others (Ungar, 1991; Ungar, 1995). Ismail (1990) showed that germination of *Zygophyllum qatarense* decreased with an increase in salinity and temperature. Agami (1986) studied the effect of temperature on the germination of *Zygophyllum dumosum* and reported that germination of seeds was independent of temperature in the range of 10–25 °C, but strongly inhibited at 30 and 35 °C. At 20 °C germination was inhibited by salinity of the medium but still occurred (0.5%) even at a concentration of 500 mM NaCl. Cruz et al. (1995) reported that seeds of three coastal tropical dune species had their optimal germination in all of the salinity concentrations studied at 28 °C. The optimal salinity and temperature for germination was found to vary for the halophyte *Salicornia bigelovii* (Rivers and Weber, 1971). At a constant temperature of 26.6 °C the greatest germination occurred in the distilled water controls, whereas at 4.4 and 15.5 °C optimal germination was at 4.0% NaCl. The annual halophyte *Atriplex triangularis* varied in its capacity to germinate at higher salinity levels at different thermoperiods (Khan and Ungar, 1984). Optimal germination at all salinities for large seeds of *A. triangularis* occurred at 5–25 °C. A combination of low night temperatures and high day temperatures was most stimulatory to germination at all salinities tested, yielding both a higher rate of seed germination and higher total germination. A very significant ($P < 0.0001$) interaction (temperature x salinity) was determined for their effects on germination of *A. triangularis*. Similar effects were also noted for *Hordeum jubatum* (Badger and Ungar, 1989), *Chrysothamnus nauseosus* (Khan et al., 1987), *Cressa cretica* (Khan, 1991), *Atriplex griffithii* (Khan and Rizvi, 1994), *Suaeda fruticosa* and *Haloxylon recurvum* (Khan and Ungar, 1996ab), *Salicornia pacifica* var. *utahensis* (Khan and Weber, 1986).

Seeds of *Z. simplex* failed to recover in most of the salinity and thermoperiods treatments when transferred to distilled water. There was 20% recovery in high salinity concentrations at the extreme thermoperiods. Woodell (1985) studied the recovery responses of a number of coastal species and reported that after immersion in half or full and one and a half strength seawater all seeds recover at least partly. Dune species were more adversely affected by salinity than those from shingle, driftline or salt marsh. He also reported salt stimulation in several salt marsh species. Khan and Ungar (1996ab) studied the recovery responses of salt desert species under various thermoperiods.

Suaeda fruticosa recovered quickly from hypersaline conditions when transferred to distilled water at all thermoperiods. Seeds exposed to the highest salinity at the lower thermoperiod showed stimulation by salinity (Khan and Ungar, 1996a). *Haloxylon recurvum* seeds when transferred to distilled water had about 50% recovery germination at low and moderate thermoperiods (Khan and Ungar, 1996b). The present study indicates that temperature plays an important role in recovery responses. Seeds of halophytes including *Z. simplex* when exposed to salinity at cooler temperatures recover better and in some cases show a salt stimulation response (Khan and Ungar, 1996ab).

Zygophyllum simplex germinated well under a moderate thermoperiod and in distilled water. Seed can germinate in up to a 100 mM NaCl treatment. This species showed some recovery responses at high salinity and low temperature treatments. It appears that thermoperiod during the exposure of seeds to salinity plays a crucial role in determining the recovery of germination responses of halophytic species. More research is necessary to quantify the interaction of temperature and salinity on the recovery of germination.

Acknowledgements

M. A. Khan would like to thank CIES, Washington for a Fulbright Scholar Research Grant, Department of Environmental and Plant Biology, Ohio University for provision of facilities, and the University of Karachi for granting a foreign service leave.

References

Agami, M. (1986). The effects of different soil water potentials, temperature and salinity on germination of seeds of the desert shrub *Zygophyllum dumosum*. *Physiologia Plantarum*, **67**, 305–309.

Aziz, S. and Khan, M. A. (1996). Seed bank dynamics of a semi-arid coastal shrub community. *Journal of Arid Environment*, **32**, (in press).

Badger, K. S. and Ungar, I. A. (1989). The effects of salinity and temperature on the germination of the inland halophyte *Hordeum jubatum*. *Canadian Journal of Botany*, **67**, 1420–1425.

Berger, A. (1985). Seed dimorphism and germination behaviour in *Salicornia patula Vegetatio*, **61**, 137–143.

Cruz, M. S. D., Perez-urria, E., Martin, L. Avalos, A. and Vicente, C. (1995). Factors affecting germination of *Canavalis brasiliensis*, *Leucaena leucophyhala*, *Clitoria ternatea* and *Calopogonium mucunoides* seeds. *Seed Science and Technology*, **23**, 447–454.

Dagar, J. C. (1995). Characteristics of halophytic vegetation in India. In *Biology of Salt Tolerant Plants*, (eds. M. A. Khan and I. A. Ungar), pp. 255–276, Department of Botany, University of Karachi, Pakistan.

Evenari, M., Shanan, L. and Tadmor, N. (1982). The Negev. The challenge of a desert. 2nd ed. Harvard University Press, Cambridge.

Fenner, M. (1985). Seed Ecology. Chapman and Hall, New York, p. 151.

Hegarty, T. W. (1978). The physiology of seed hydration and dehydration, and the relation between water stress and the control of germination: a review. *Plant Cell and Environment*, **1**, 101–119.

Ismail, A. M. A. (1990). Germination ecophysiology in populations of *Zygophyllum qatarenses* Hadidi from contrasting habitats. *Journal of Arid Environment*, **18**, 185–194.

Keiffer, C. H. and Ungar, I. A. (1995). Germination responses of halophyte seeds exposed to prolonged hypersaline conditions. In *Biology of Salt Tolerant Plants*, (eds. M. A. Khan and I. A. Ungar), pp. 43–50, Department of Botany, University of Karachi, Pakistan.

Khan, M. A. (1991). Studies on germination of *Cressa cretica*. *Pakistan Journal of Weed Science Research*, **4**, 89–98.