Pak. J. Bot. 33 (special issue) 2001

SEED GERMINATION OF *SARCOBATUS VERMICULATUS*: A HALOPHYTIC SHRUB FROM GREAT BASIN DESERT

*BILQUEES GUL, *M. AJMAL KHAN AND DARRELL J. WEBER

Department of Botany and Range Science, Brigham Young University, Provo, Utah 84602-5181, USA.

Abstract

Sarcobatus vermiculatus (Hook) Torrey (Chenopodiaceae; common name Greasewood) is a leaf succulent, sodium accumulating shrub usually found in saline substrates of Great Basin desert, Utah, USA. Laboratory experiments were conducted to determine the effect of salinity and temperature on the seed germination under various temperature (5-15°C, 10-20°C, 15-25°C, 20-30°C and 25-35°C) and salinity (0, 200, 400, 600, 800 and 1000 mM NaCl) treatments and also determine the role of growth regulators (GA_3, kinetin, thiourea and nitrate) under salinity stress. *Sarcobatus vermiculatus* showed a 100% germination in non-saline controls, at all thermoperiods. Germination decreased with increase in salinity and few seeds germinated at 1000 mM NaCl. Rate of germination decreased with an increase in salinity and variation in temperature had little effect. Exposure to high salinity caused the loss of viability in the seeds only few seeds germinated at higher temperatures when transferred to distilled water. At low salinity recovery was similar at all temperatures, but at higher salinity concentrations, it was higher at higher temperatures. Salinity-enforced germination inhibition was partially alleviated by all growth regulators at higher temperature regime (25-35 °C) and best reversal was obtained with the application of GA_3 and kinetin. Nitrate and thiourea had little effect. Rate of germination showed a similar pattern with that of percent germination. Salinity appeared to inhibit seed germination partially due to hormonal imbalance and nitrogen deficiency and this inhibition could be partially alleviated through application of germination regulating chemicals.

Introduction

Sarcobatus vermiculatus (Hook.) Torr. var. *vermiculatus* (Chenopodiaceae) is a highly salt tolerant shrub, is widely distributed on medium to heavy textured saline or saline-alkali soils in the western United States (Welsh et al., 1987). This is an important browse species for cattle and sheep, even though potentially poisonous due to oxalate salts of sodium and potassium and oxalic acid. *S. vermiculatus* is also the primary colonizer of the most recently exposed, highly saline lacustrine and shoreline substrates, where a salt crust is a visible component of the soil in some seasons (Richards et al., 1994).

Salinity alone may not be the only critical environmental factor in the germination of annual halophytes (Khan and Ungar, 1998). Interaction between salinity, temperature, and seed germination optima exist in halophytes (Rivers and Weber, 1971; Berger, 1985; Khan and Ungar, 1996, 1998b). Phillipupillai and Ungar (1984) determined that *S. europaea* had optimal germination (43%) at 5-15 °C in 860 mM NaCl in comparison to >2% in other thermoperiods. Khan and Gul (1998) showed that germination of *Arthrocnemum indicum* (Syn. *Arthrocnemum*

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

macrostachyum) was significantly higher at temperature regimes of 15-25 °C thermoperiod at 600 and 800 mM NaCl. Most salt marsh and salt desert halophytes have physiological dormancy (Baskin and Baskin, 1998).

Dormancy alleviating compounds like proline, betaine, fusicoccin, GA_3, kinetin, nitrate, thiourea and ethephon are known to alleviate the effect of salinity on the germination of halophytes (Pyler and Proseus, 1996; Khan and Ungar, 1997; Gul and Weber, 1998; 1999; Khan et al., 1998; Khan and Ungar, 1998).

The mechanism by which seeds of Greasewood are able to compensate and are thereby able to germinate in salt-affected soils is unknown. The mechanism may be external to the seed, such as a dilution effect by rainfall or flooding, it may be internal osmoregulation as postulated for adult plants, or it may be a combination of the two and other environmental factors. We examined germination response to temperature, salinity and germination regulating chemicals on the germination of *Sarcobatus vermiculatus*.

Materials and Methods

Seeds of *Sarcobatus vermiculatus* were collected during the fall 1996 from salt flats situated on 2.5 miles west of Faust, Utah, USA. 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 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 to avoid excessive water loss due to 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, 15-25 °C, 20-30 °C, and 25-35 °C, based on a 24-hr cycle were used, where the higher temperature (15, 20, 25, 30 and, 35 °C) coincided with the 12-hr light period (Sylvania cool white fluorescent lamps, 250 $\mu M.m^{-2}.s^{-1}$, 400 - 750 nM) and the lower temperature (5, 10, 15, 20, and 25 °C) coincided with the 12-hr dark period. Seeds were germinated in distilled water, 200, 400, 600, 800 and 1000 mM NaCl solutions under the earlier mentioned temperature regimes.

For growth regulators, seeds were germinated in a growth chamber at an alternating temperature regime of 25-35 °C. Nitrate concentrations of 20 mM, thiourea concentration of 10 mM, gibberellic acid concentration of 3 mM, kinetin concentration of 0.05 mM and NaCl concentrations of 0, 300, 600, and 900 mM were used. 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 assess the recovery of germination, which was also recorded at every 2-day interval for 20 days. The recovery percentages was determined by the following formula: $(a - b)/(c - b)*100$, where a is the total number of seeds germinated after being transferred to distilled water, 'b' is the total number of seed germinated in saline solution and 'c' is the total number of seeds. The rate of germination was estimated using a modified Timson index of germination velocity = $\Sigma G/t$, where G is percentage of seed germination at 2-days interval, and t is total germination period (Khan and Ungar, 1996). 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 were arcsine transformed before statistical analysis. These data were analyzed using SPSS for windows release 9.0 (SPSS Inc., 1999).

Results

Percent germination in lower salinities at different thermoperiods was not significantly different (Fig. 1). All seeds germinated in non-saline control. Different temperature regimes, salinity, and their interaction significantly ($P < 0.0001$) affected the final percent germination of *S. vermiculatus* seeds (Fig. 2).
Germination of *S. vermiculatus* was highest in distilled water and at a regime with high night (20°C) and high day (30°C) temperatures (Fig.1). Maximum germination percentages were achieved at 10d in all treatments. Germination of seeds decreased with increase in salinity since very few seeds germinated at salt concentrations higher than 800 mM NaCl (Fig. 2).

Different temperature regimes, salinity and their interaction significantly ($P < 0.0001$) affected the rate of germination of *S. vermiculatus* seeds (Fig. 3). The rate of germination, was lowest in higher salinity (Fig. 4). At all salinities the rate of germination was similar in all thermoperiods. Germination rate was similar under lower and higher thermoperiods (Fig. 4).

After 20 d of salinity treatment, seeds were transferred to distilled water to examine the recovery of germination after salt inhibition. Seeds exposed to high salinity at lower and moderate thermoperiods showed no recovery (Table 1). However, at a thermoperiod of 25-35°C, the percent recovery in all NaCl treatments was comparatively higher than the other thermoperiods. The number of seedlings germinated in all saline treatments after transfer to distilled water was significantly lower than that at the distilled water control (Table 1). Rate of recovery of seed germination decreased with increase in salinity concentration (Fig. 4).

Gibberellic acid and kinetin both were very effective in promoting germination in non-saline treatments, while both compounds significantly ($P < 0.0001$) alleviated salinity effects on germination (Fig. 4). Kinetin was more effective than gibberelic acid at 900 mM NaCl (Fig. 4). Nitrogenous compounds (thiourea and nitrate) promoted germination under low saline conditions. At 300 mM NaCl treatment, application of thiourea almost completely alleviated the salt effect on seed germination. In the presence of nitrate and thiourea some germination occurred at 900 mM NaCl (Fig. 5).

Discussion

Seed germination in Greasewood from Faust, Utah, appears to be opportunistic since most seeds germinated over a broad range on temperature and NaCl concentrations. Optimal temperature for germination appears to be 25-35°C when germination percentage, rate of germination, and stress response are considered. *Sarcobatus vermiculatus* is known accumulator of high concentrations of sodium (Rickard, 1965; Wallace et al., 1973), which is presumably used, for osmoregulation. In this study 20-30°C and 25-35°C markedly influenced germination percentage as compared to 5-15°C. We determined that seeds from the Utah population required moderate salinity and a temperature regime with high night (25°C) and a high day temperature (35°C) to promote maximum germination.

Our results indicate that seeds of this Utah population were not dormant and that high salinities and low day temperatures inhibited germination. We determined that *S. vermiculatus* seeds had their highest germination percentages in distilled water and progressively declined with increases in salinity. Similar results were reported for *Atriplex griffithii* (Khan and Rizvi, 1994), *Suaeda fruticosa* (Khan and Ungar, 1998) and a number of annual halophytes (Ungar, 1995).

The seeds tested differed in their response to salinity and in their survival after exposure to salinity. Most of the seeds were totally killed by the period of immersion that were given by a salinity of nearly 1000 mM NaCl. 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 at the germination stage. *Sarcobatus vermiculatus* had little recovery of germination when transferred to water after 20 d exposure to higher salinity concentrations at all thermoperiods studied. There was no recovery at 5-15°C temperature regime in 1000 mM NaCl, but at 25-35°C seeds incubated previously at 1000 mM NaCl had about 10 % recovery. The number of seeds germinated in all saline treatments after transfer to distilled water was significantly lower than the distilled water control. 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.

Thiourea and nitrate both stimulated the germination of *S. vermiculatus* seeds under saline conditions. The alleviating effect of thiourea on osmoinhibition gradually decreases with an increase in salinity. Some nitrogenous compounds such as nitrate, nitrite and thiourea are known to stimulate the germination of seeds (Esashi et al., 1979; Aldasaro et al., 1981; Yoshiyama et al., 1996). Thiourea counteracts the effect of ABA and reduced the level of cytokinins in plant tissues (Kabar and Baltepe, 1990). These adverse hormonal changes occur when plant tissues are subjected to water stress induced by drought, salinity or high temperatures (Kabar and Baltepe, 1990). Treatment with thiourea is highly effective in alleviating the inhibition of germination by salinity or high temperatures (Esashi et al., 1979; Gul and Weber, 1998). Thiourea is also known to break dormancy and overcome the negative effect of temperature on seed germination (Esashi et al., 1979; Aldasaro et al., 1981).

GA_3 and kinetin both alleviated the salinity effect on the germination of *S. vermiculatus* seeds. The GA_3 and kinetin are known to alleviate salinity effect in some halophytic seeds (Khan and Ungar, 1996; Khan and Ungar, 1998; Khan et al., 1998) while it was ineffective in other halophytes like *Suaeda fruticosa* and *Haloxylon recurvum* (Khan and Ungar, 1997; 1996).

Sarcobatus vermiculatus seeds had maximum germination at 25-35°C temperature regime at all NaCl concentrations tested. Inability to germinate at low day temperature in the laboratory indicates that a threshold of higher day temperatures is necessary to stimulate germination under field conditions. Because soil salinity stress usually increases during the summer months, salinity conditions early in the growing season at the germination stage determine whether halophytes will be able to successfully establish at a site (Ungar, 1995). Our investigations with seeds from this Utah population more precisely define the temperature and salinity conditions necessary for the germination and recovery of seeds of *S. vermiculatus*

from hypersalione conditions. Seeds were not dormant, but had specific temperature requirements for maximum germination. Seeds start germinating very early during spring and germination decreased with the increase in salinity. This decrease in germination appears to be mediated through reduction in germination regulating chemicals like gibberellic acid and kinetin.

Table 1. Recovery percentage (Mean ±SE) of germination of *Sarcobatus vermiculatus* seed after they transferred from 0, 200, 400, 600, 800 and 1000 mM NaCl at thermoperiods of 5-15°C, 10-20°C, 15-25°C, 20-30°C and 25-35°C.

NaCl (mM)	5-15°C	10-20°C	15-25°C	20-30°C	25-35°C
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
200	50 ± 2.9	50 ± 2.9	38 ± 2.3	15 ± 1.5	0.0 ± 0.0
400	69 ± 14	52 ± 16	46 ± 16	0.0 ± 0.0	61 ± 13
600	21 ± 6.0	37 ± 3.8	23 ± 9.0	40 ± 10	47 ± 7.2
800	12 ± 2.0	15 ± 4.9	12 ± 4.0	20 ± 5.4	22 ± 4.0
1000	0.0 ± 0.0	1.0 ± 1.0	4.0 ± 1.1	11 ± 2.2	10 ± 2.1

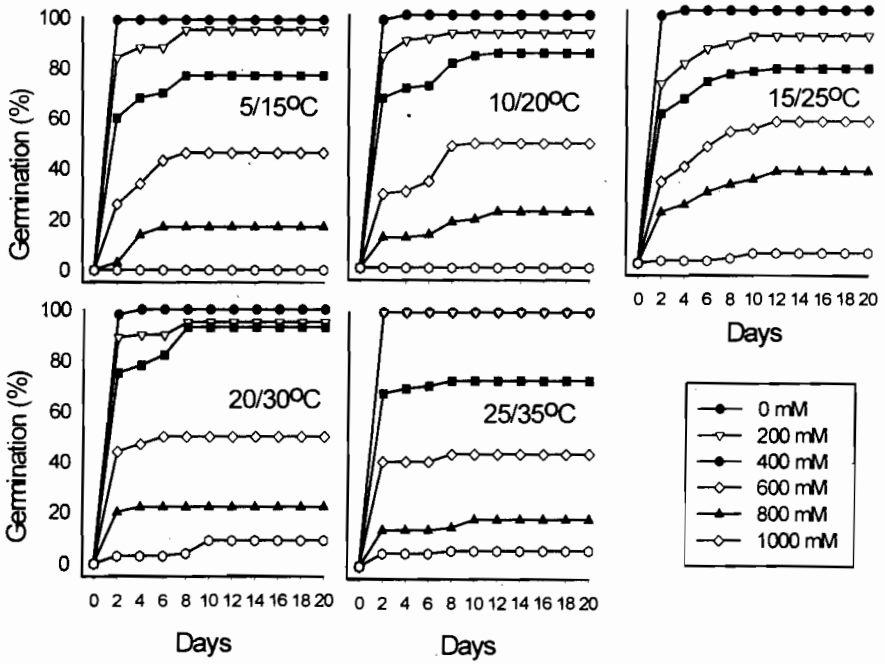

Fig. 1. Mean (±SE) final germination percentages of *Sarcobatus vermiculatus* seeds in 0, 200, 400, 600, 800, and 1000 mM NaCl at thermoperiods of 5-15 °C, 10-20 °C, 15-25 °C, 20-30 °C, and 25-35 °C.

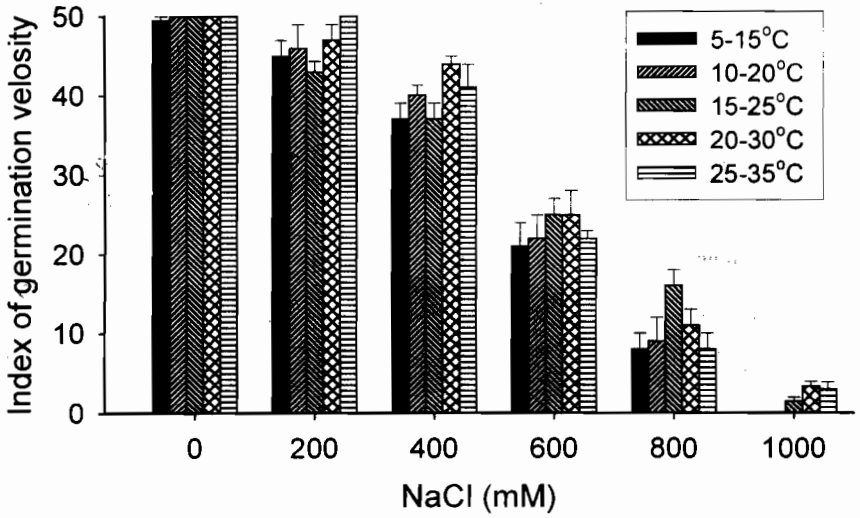

Fig. 2. Rate of germination of *Sarcobatus vermiculatus* seeds in 0, 200, 400, 600, 800 and 1000 mM NaCl at thermoperiods of 5-15 °C, 10-20 °C, 15-25 °C, 20-30 °C, and 25-35 °C.

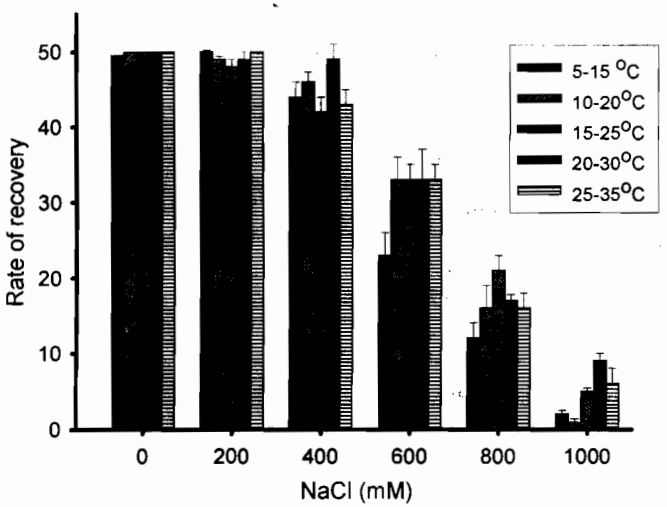

Fig. 3. Rate of germination of *Sarcobatus vermiculatus* seeds after they are transferred from 0, 25, 50, 75, 100, and 125 mM NaCl at thermoperiods of 10-20 °C, 10-30 °C, 15-25 °C, and 25-35 °C.

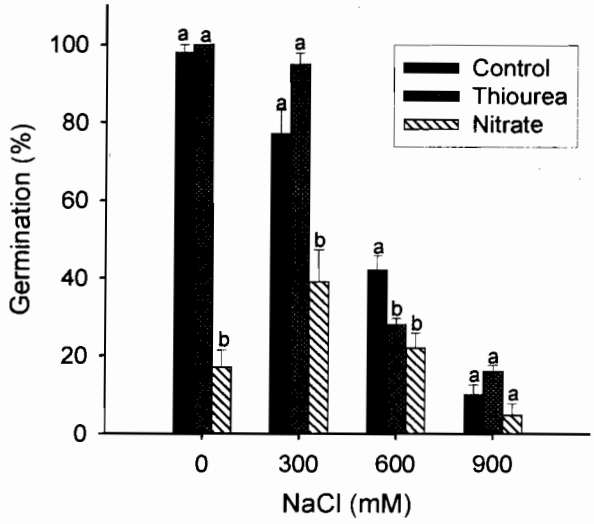

Fig. 4. Final germination percentages of *Sarcobatus vermiculatus* seeds in water, nitrate and thiourea. Values at each dormancy regulating chemicals having the same letter are not significantly different ($P > 0.05$) from control following Bonferroni test.

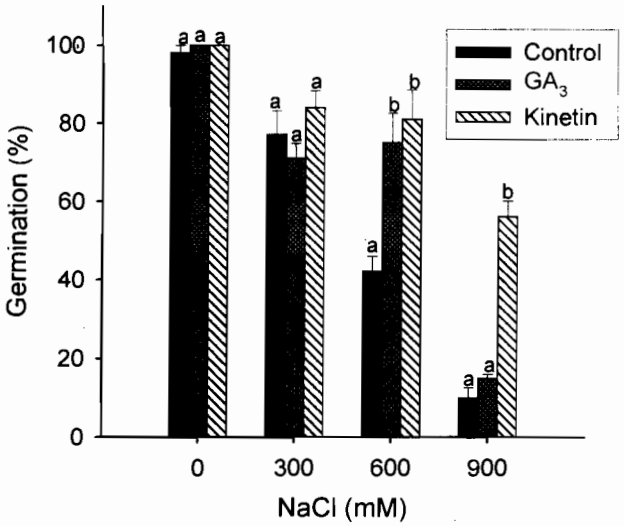

Fig. 5. Final germination percentages of *Sarcobatus vermiculatus* seeds in water, kinetin, and GA_3. Values at each dormancy regulating chemicals having the same letter are not significantly different ($P > 0.05$) from control following Bonferroni test.

References

Aldosaro, J., A. Mantilla, and G. Nicholas. 1981. Effect of ABA, fusicoccin, and thiourea on germination and K^+ and glucose uptake in chickpea seeds at different temperatures. Physiol Plantarum., 52: 353-362.

Baskin, J. M. and C. C. Baskin. 1998. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, New York.

Esashi, Y., Y. Ohara, M. Okazaki; and K. Hishinuma. 1979. Control of cocklebur seed germination by nitrogenous compounds: Nitrite, nitrate, hydroxylamine, thiourea, azide, and cyanide. Plant. Cell Physiol., 20: 349-361.

Gul, B. and D. J. Weber. 1998. Effect of dormancy compounds on the seed germination of non-dormant *Allenrolfea occidentalis* under salinity stress. Ann. Bot., 82: 555-560.

Gul, B. and D. J. Weber. 1999. Effect of salinity, light, and thermoperiod on the seed germination of *Allenrolfea occidentalis*. Can. J. Bot., 77: 1-7.

Ismail, A. M. A. 1990. Germination ecophysiology in population of *Zygophyllum qatarenses* Hadidi from contrasting habitats. J. Arid Environ., 18: 185-194.

Kabar, K. and S. Beltepe. 1990. Effect of kinetin and gibberellic acid in overcoming high temperature and salinity (NaCl) stresses on the germination of barley and lettuce seeds. Phyton., 30: 65-74.

Khan, M. A. and B. Gul. 1998. High salt tolerance in germinating dimorphic seeds of *Arthrocnemum indicum*. Intl. J. Plant. Sci., 159: 826.832.

Khan, M. A., and Y. Rizvi. 1994. Effect of salinity, temperature, and growth regulators on the germination and early seedling growth of *Atriplex griffithii* var. *stocksii*. Can. J. Bot., 72: 475-479.

Khan, M. A. and I. A. Ungar. 1996. Influence of salinity and temperature on the germination of *Haloxylon recurvum*. Ann. Bot., 78: 547-551.

Khan, M. A. and I. A. Ungar. 1997. Germination responses of the subtropical annual halophyte *Zygophyllum simplex*. Seed Sci. Technol., 25: 83-91.

Khan, M. A. and I. A. Ungar. 1998a. Seed germination and dormancy of *Polygonum aviculare* L. as influenced by salinity, temperature, and gibberellic acid. Seed Sci. Technol., 26: 107-117.

Khan, M. A. and I. A. Ungar. 1998b. Germination of salt tolerant shrub *Suaeda fruticosa* from Pakistan: Salinity and temperature responses. Seed Sci. Technol., 26: 657-667.

Khan, M. A. and I. A. Ungar. 1999. Seed germination and recovery of *Triglochin maritima* from salt stress under different thermoperiods. Great Basin Nat., 59: 144-150.

Khan, M. A., I. A. Ungar, and B. Gul. 1998. Action of osmotica and growth regulators in alleviating the effect of salinity on the germination of dimorphic seeds of Arthrocnemum indicum L. Intl. J. Plant. Sci., 159: 313-317.

Khan, M. A. and I. A. Ungar. 1999. Seed germination and recovery of *Triglochin maritima* from salt stress under different thermoperiods. Great Basin Nat., 59: 144-150.

Khan, M. A. and D. J. Weber. 1986. Factors influencing seed germination in *Salicornia pacifica* var. *utahensis*. Am .J. Bot., 73: 1163-1167.

Pyler, D.B. and T. E. Proseus. 1996. A comparison of the seed dormancy characteristics of *Spartina alterniflora* (Poaceae). Am. J. Bot., 83: 11-14.

Richards, J. H. 1965. The influence of greasewood on soil moisture penetration and soil chemistry. Northwest Sci. 39: 36-42.

Richards, J. H. 1994. Physiological limits of plants in desert playa environments. Land, air and water resources paper 100026. University of California, Davis, CA, p86.

Rivers, W. G. and D. J. Weber. 1971. The influence of salinity and temperature on seed germination in *Salicornia bigelovii*. Physiol Plantarum, 24: 73-75.

SPSS Inc. (1999). *SPSS: SPSS Base 9.0 Application Guide*. SPSS Inc. Chicago.

Ungar, I. A. 1977. Salinity, temperature and growth regulator effects on the seed germination of *Salicornia europaea* L. Aquat. Bot., 3: 329-335.

Ungar, I. A. 1982. Germination ecology of halophytes. In: Contribution to the ecology of halophytes. (Eds.) D.N. Sen and K.S. Rajpurohit, p. 143-154. Junk, The Hague.

Ungar, I. A. 1995. Seed germination and seed-bank ecology of halophytes. In: Seed Development and Germination (Eds.) J. Kigel and G. Galili, Marcel and Dekker Inc. New York.

Wallace, A., E. M. Romney and V. Q. Hale. 1973. Sodium relations in desert plants: 1. Cation contents of some plant species from the Mojave and Great Basin deserts. Soil Sci. 115:284-287.

Welsh. S. L., Atwood, N. D., Goodrich, S. and Higgings, L. C. 1987. A Utah flora. Brigham Young University, Provo, Utah-84602.

Yaniv, Z., N. Lisker, and F. Corbineau. 1995. Germination potential of *Sinapsis alba* seeds collected in Israel. J. Arid Environ., 29: 293-303.

Yoshiyama, M., A. Maruyama, T. Atsumi, and Y. Esashi. 1996. Mechanism of action of C_2H_2 in promoting the germination of cocklebur seeds. III. A further enhancement of priming effect with nitrogenous compounds and C_2H_2 responsiveness of seeds. Aust. J. Plant Physiol., 23: 519-525.