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EFFECTS OF SALT AND WATER STRESS ON THE GERMINATION OF *CHENOPODIUM GLAUCUM* L., SEED

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Abstract

Effect of salts (Na_2SO_4, Na_2CO_3, $MgSO_4$, NaCl, $MgCl_2$), soil extract and polyethylene glycol (PEG-6000) on the germination of *Chenopodium glaucum* L., seed was studied. Maximum germination was obtained in distilled water. Germination decreased with increase in salinity. The inhibition of germination by salt solutions was in the order of $MgCl_2 > Na_2SO_4 > Na_2CO_3 > NaCl >$ Soil extract > $MgSO_4$. Germination also decreased with decrease in osmotic potential caused by PEG treatment. Seed germination percentage was lower in NaCl than in iso-osmotic PEG solutions at osmotic potential less than -0.5 MPa. Non-germinated seeds under various salt treatments when transferred to distilled water recovered completely, indicating little ionic effect of salinity on seed germination and viability. Germination inhibition, therefore, appears to be osmotic. Similar recovery response was noted when seeds from PEG solution were transferred to water.

Introduction

Seed of halophytes under natural conditions are subjected to saline stress and usually it is dominated by NaCl. However, other chloride, sulfate and carbonate salts and their interaction plays a significant role in affecting seed germination (Khan, 2002). Salts can affect seed germination either by restricting the supply of water (osmotic effect) or causing specific injury through ions to the metabolic machinery (ionic effect). Studies have been carried out on the effect of various chloride and sulfate salts on the germination of halophytes where all the salts exhibited some osmotic effects but no specific ion effect (Mohammed & Sen, 1990; Egan *et al.,* 1997; Agboola, 1998; Pujol *et al.*, 2000) while others reported both osmotic and ionic effects (Mohammed & Sen, 1990). Seed germination under isotonic solutions of PEG 6000 and NaCl had similar effect on the seed germination of halophytes (Myers & Couper, 1989; Naidoo & Naicker, 1992; Ungar, 1995; Bajji *et al.*, 2002). High salinity could be injurious to most glycophytes, however, seeds of halophytes usually recover completely when saline stress is removed indicating an osmotic effect (Hardegree & Emmerich, 1990).

Many halophyte seeds have the ability to maintain seed viability for extended period of exposure to hypersaline conditions with other physicochemical factors and germinate when conditions are favorable (Woodell, 1985; Keiffer & Ungar, 1995; Khan & Ungar, 1997). Seeds of some halophytes when pretreated with salinity show the priming effect of salinity on germination, while others showed no effect of salinity and recover immediately after salinity stress is removed and still other halophytes failed to germinate when exposed to high salinity (Ungar, 1995; Keiffer & Ungar, 1995; Khan & Ungar, 1997). *Suaeda fruticosa* showed a high recovery percentage when transferred to distilled water. Potassium chloride, potassium sulfate, sodium chloride and sodium sulfate had no effect on the germinability of the seeds and all of them germinated to control level when

transferred to distilled water except for potassium chloride where recovery percentages were about 20% higher than non-treated control (Mohammed & Sen, 1990). Exposure to potassium chloride had a priming effect and germination was significantly increased in comparison to control. There was no specific ion toxicity and that an osmotic effect limited germination as in other halophytes (Mohammed & Sen, 1990; Egan *et al.,* 1997; Pujol *et al.,* 2000).

Chenopodium glaucum L. of the family *Chenopodiaceae* is an annual halophyte that grows in temperate saline areas of China in association with other chenopods and salt tolerant grasses (Gu *et al.,* 2003). It can be used for improving the soil texture, reducing soil salinity and increasing soil organic matter. In addition, it is a good source of food and forage additive due to the high protein content in the leaves (Zhao & Li, 1999). The present report describes the effect of different salts, soil solution and various concentrations of PEG on seed germination and recovery of *Chenopodium glaucum* L.

Materials and Methods

Seeds of *Chenopodium glaucum* L., were collected from the coastal salt marshes in Haixing county, Hebei province (China) in October 2002. Seeds were separated from inflorescence in the laboratory and surface sterilized by treating with ozone gas for 30 minutes. Seeds were germinated on 3 sheets of 9 cm diameter filter paper in10 cm diam., Petri dishes with 10 ml of solutions. The Petri dishes were sealed with transparent tape. Forty seeds were used for each treatment with four replicates each.

To determine the effect of different salts (NaCl, Na_2SO_4, Na_2CO_3, $MgSO_4$, $MgCl_2$ and soil extract) and PEG-6000 iso-osmotic of -0.2 MPa, -0.5 MPa, -0.9 MPa, -1.4 MPa, -1.8 MPa, and -2.7 MPa) were used (Michel & Kaufmann, 1973). The major ions in the soil extract collected from the area where *Chenopodium glaucum* L., was present were Cl^- (53.4%) and $Na^+ + K^+$ (30.5%), SO_4^{2-} (12.5%), Mg^{2+} (2.3%), Ca^{2+} (1.2%) and HCO_3^- (0.1%).

The experiment was carried out at 15-25 °C and 12 h dark/12 h light. Germination was recorded at every alternate day and after 9 days un-germinated seeds were transferred to distilled water to determine the recovery of germination. The percent recovery was calculated using the following index:

$$\text{Percent recovery} = \frac{a-b}{c-b} \times 100$$

where, a = total number of seed germinated after being transferred to distilled water, b = total number of seed germinated in saline solution and c = total number of seeds. High recovery germination percentages would indicate that previous seed germination was inhibited by an osmotic effect, whereas, low germination would indicate specific ion toxicity (Khan, 2002).

Statistical analyses were carried out using SPSS 9.0 (2000). A one-way ANOVA was carried out to determine the differences among treatment group means for percent germination, rate of germination and recovery percent germination. A Bonferroni test was also done to determine if significant ($p < 0.05$) differences occurred between individual treatments.

Results and Discussion

Seed germination in temperate salt marshes is the most critical phase for the survival and continuity of halophytic lineage (Khan, 2002) because most plants are annuals whereas, in sub-tropical environment, perennials use vegetative methods to induce new ramets and maintain continuity at proximate scale. Salinity is a major environmental stress factor that affect seed germination in coastal salt marshes (Khan, 2002) where salinity ranges from 0.8% to 2.4%. Saline sodic and non-sodic soils have high salt content of sodium, calcium and magnesium that could reach 8% (Waisel, 1972) in these soils while the more important anions are chloride, sulfate and bicarbonates (Bewley & Black, 1994).

Seeds of *C. glaucum* showed 73% germination in non-saline control (Table 1). Seed germination decreased with increase in NaCl, Na_2CO_3, Na_2SO_4, $MgSO_4$, $MgCl_2$ salts and salt solutions extracted from soil. $MgCl_2$ inhibited germination more than other salts and only 11% seeds germinated at 0.2 M, whereas 21% seeds germinated at 0.4 M $MgSO_4$ solution. Little seed germination was observed in 0.6 M salt treatments (Table 1). The data on the salt tolerance of only 7% of coastal species are reported (Baskin & Baskin, 1998) and the available data indicate that the level of salinity required to reduce the seed germination of coastal species to about 10% ranges from 0.06 - 0.6 M with an average of 0.36 M (Baskin & Baskin, 1998; Woodell, 1987; Ungar, 1995; Khan, 2002). Optimal germination of halophytes is reported in non-saline control (Khan & Weber, 1986; Katembe *et al.*, 1998; Gulzar & Khan, 2001; Li *et al.*, 2002; Khan, 2002) and their germination decreased with increases in salinity. Annual halophytes vary in their level of salt tolerance at the germination stage like *Salicornia bigelovii* (856 mM NaCl, Rivers & Weber, 1971); *Kochia americana* (1712 mM NaCl, Clarke & West, 1969); *Triglochin maritima* (400 mM NaCl, Khan & Ungar, 1999); *Salicornia rubra, Ceratoides lanata* and *Halogeton glomeratus* (1000 mM NaCl, Khan *et al.* 2001, 2002, 2004). Our data clearly indicate that the inhibition of *C. glaucum* seed germination at high salt concentration was mostly due to osmotic effects while some ionic effects were noted at lower salt concentrations. The inhibition of different salt solutions to halophyte seed germination varied greatly. Ungar (1996) reported germination of *Puccinellia festucaeformis* seeds by various salts was in the order of $CaCl_2$, $MgCl_2$ > NaCl > KCl > $NaNO_3$ > KCl > $MgSO_4$. Our study showed that the inhibition to seed germination of *C. glaucum* is in the order of $MgCl_2$ > $NaSO_4$ > $NaCO_3$ > NaCl > SES > $MgSO_4$. The single salt solutions inhibited germination more in comparison to multiple salt solutions extracted from soil (Yuying, 1991). Similar results were reported for the two turf grasses viz., *Lolium perenne* and *Festuca elata* Keng (Li *et al.*, 2002).

Ungerminated seeds from all salt treatments when transferred to distilled water germinated to levels similar to non-saline control (Table 2). Iso-osmotic solutions of NaCl and PEG have similar effect on seed germination at similar concentrations (Fig. 1) and no seed germinated at or above -1.8 MPa PEG. When recovery of germination from various salts and PEG were compared at -1.8 MPa and -2.7 MPa PEG, 88% seeds germinated at -1.8 MPa in all salts, however, a few seeds germinated when pretreated with -2.7 MPa Na_2CO_3 and PEG (Fig. 2). Comparison of recovery of germination when treated with various concentrations of NaCl and PEG had a similar pattern showing about 60% germination at the highest osmotic potential (Fig. 3). It was also reported that after the stress was removed, the seed germination and early seedling growth were higher than those of the untreated seeds (Yuying *et al.*, 1999). *Chenopodium glaucum* seeds showed a priming effect when treated with high salt concentrations.

Table 1. Seed germination (Mean ± S.E.) of *Chenopodium glaucum* when treated with different salt solutions. Different letters indicate significant difference among the data in same column at P= 0.05. Bonferroni test.

Salt c oncentration	Germination (%)					
	NaCl	SES	Na_2CO_3	Na_2SO_4	$MgSO_4$	$MgCl_2$
0.0	72.5^{de}	72.5^{d}	72.5^{c}	72.5^{d}	72.5^{e}	72.5^{d}
	±0.2	±0.2	±0.2	±0.2	±0.2	±0.2
0.1	65.0^{d}	68.8^{d}	62.5^{c}	56.9^{c}	73.8^{e}	48.8^{c}
	±2.2	±2.0	±2.3	±1.5	±1.2	±2.7
0.2	44.4^{c}	46.3^{c}	41.3^{b}	16.9^{b}	53.8^{d}	11.3^{b}
	±2.7	±1.5	±2.5	±1.0	±2.3	±0.3
0.3	12.5^{b}	16.3^{b}	4.4^{a}	0.6^{a}	36.3^{c}	0^{a}
	±0.9	±0.6	±1.1	±0.3	±2.5	±0
0.4	2.5^{b}	4.4b^{a}	0^{a}	0^{a}	21.3^{b}	0^{a}
	±0.4	±0.9	±0	±0	±2.6	±0
0.6	0^{a}	0^{a}	0^{a}	2.5^{a}	0^{a}	0^{a}
	±0	±0^{a}	±0	±0.4	±0	±0

Table 2. Recovery of germination (Mean ± S.E.) of *Chenopodium glaucum* when treated with different salt solutions. Different letters indicate significant difference among the data in same column at P= 0.05. Bonferroni test/

Salt concentration	Germination recovery (%)					
(M)	$MgSO_4$	SES	NaCl	Na_2CO_3	Na_2SO_4	$MgCl_2$
0	18.2^{d}	18.2^{f}	18.2^{g}	18.2^{f}	18.2^{f}	18.2^{f}
	±2.1	±2.1	±2.1	±2.1	±2.1	±2.1
0.1	19.1^{d}	38.2^{d}	26.4^{e}	64.0^{d}	50.6^{d}	40.2^{d}
	±3.1	±3.3	±2.4	±4.7	±4.8	±4.1
0.2	44.3^{c}	55.0^{c}	62.6^{d}	79.4^{c}	74.3^{bc}	78.2^{c}
	±3.7	±4.2	±3.6	±5.7	±5.0	±4.9
0.3	68.8^{b}	74.1^{a}	74.5^{c}	79.8^{c}	75.3^{c}	74.4^{b}
	±4.6	±5.1	±4.9	±5.5	±5.1	±4.7
0.4	73.5^{a}	80.0^{b}	82.7^{b}	68.1^{b}	73.8^{b}	74.4^{b}
	±5.2	±6.0	±6.1	±4.7	±5.1	±5.2
0.6	73.6^{a}	73.1^{a}	71.3^{a}	56.9^{a}	70.6^{a}	66.3^{a}
	±5.1	±5.3	±5.5	±4.1	±4.9	±4.3

Seeds of many halophytes have the ability to maintain viability for extended period of exposure to hyper-saline conditions with other physicochemical factors and germinate when conditions are favorable (Woodell, 1985; Keiffer & Ungar, 1995, 1996; Khan & Ungar, 1997). Seed of some halophytes when pretreated with salinity showed a priming effect of salinity on germination, while others showed no effect of salinity and recovered immediately after salinity stress was removed. Other halophytes failed to germinate when exposed to high salinity (Ungar, 1995; Keiffer & Ungar, 1995; Khan & Ungar, 1997). *Suaeda fruticosa* showed high recovery percentages when transferred to distilled water. Potassium chloride, potassium sulfate, sodium chloride, and sodium sulfate had no effect and all seeds germinated to control level when transferred to distilled water except for potassium chloride where recovery percentages were about 20% higher than non-treated

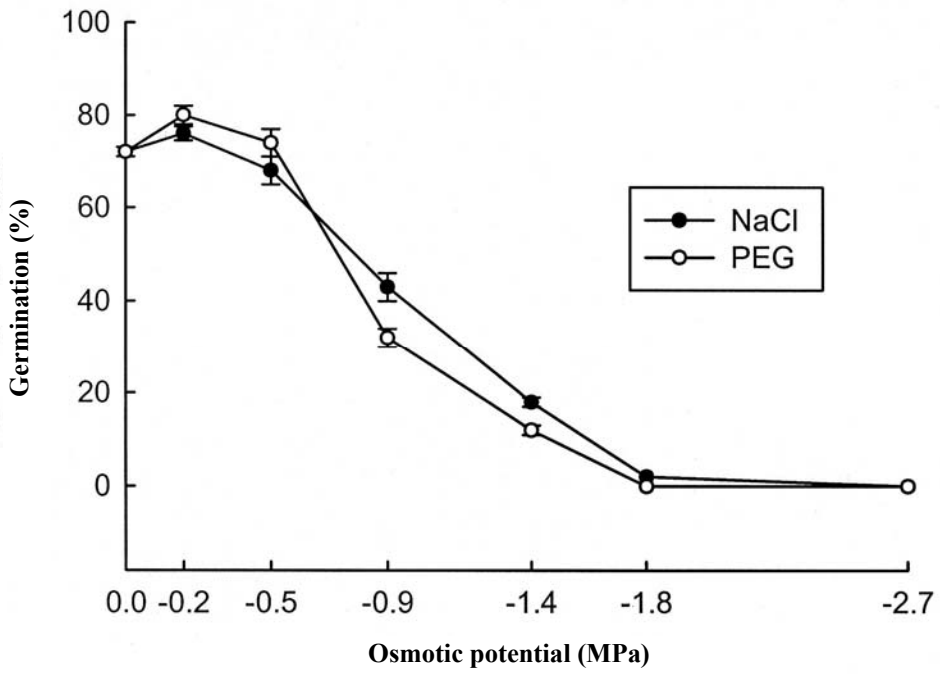

Fig. 1. Seed germination of *Chenopodium album* in different concentrations of NaCl and PEG solutions.

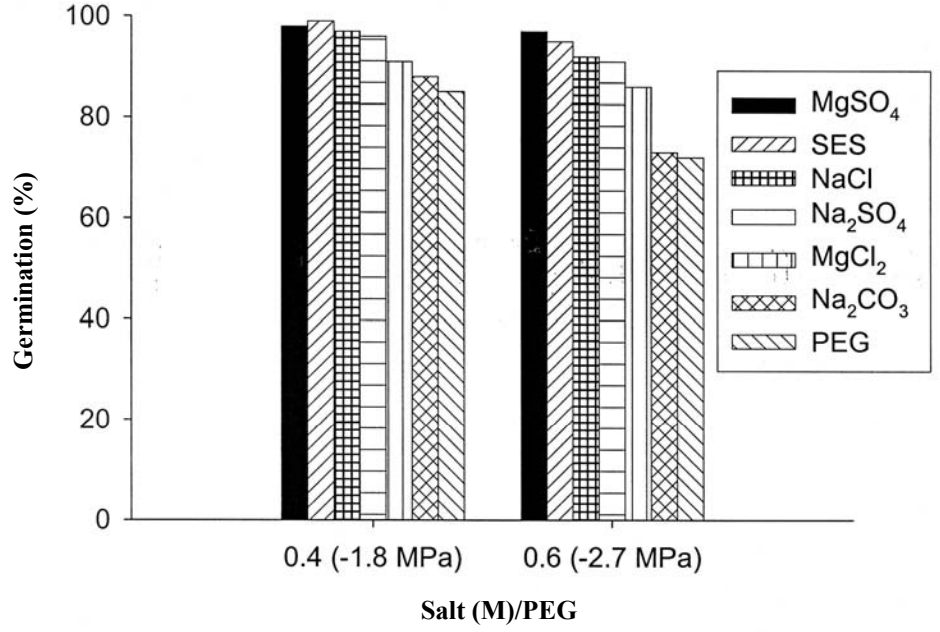

Fig. 2. Relative germination percentages of seed treated with 0.4 / 0.6 M salt or -1.8 / -2.7 Mpa PEG concentrations.

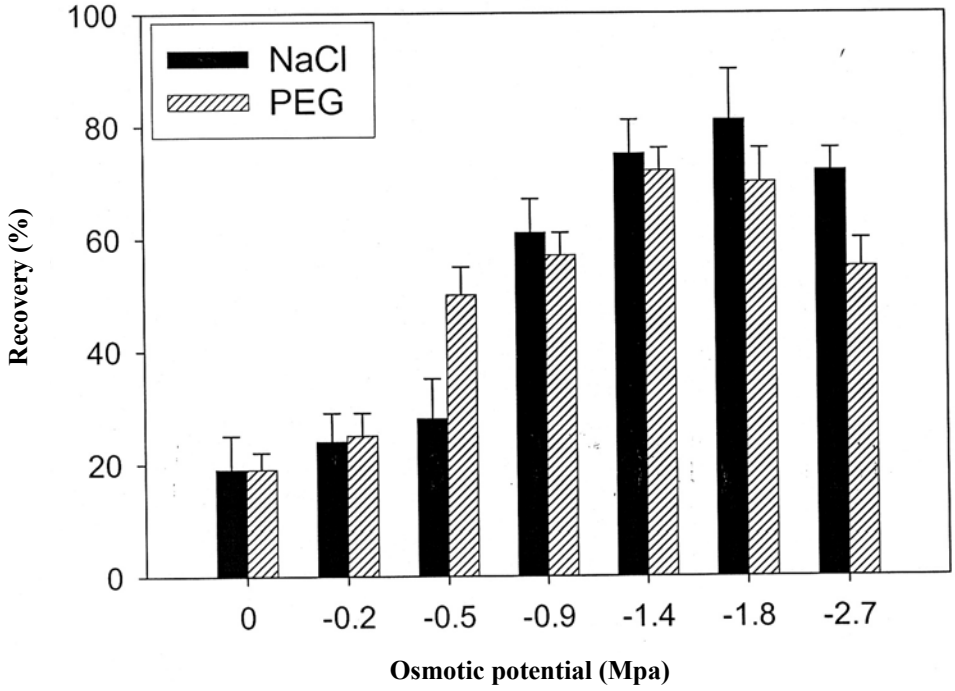

Fig. 3. Recovery of seed germination in *Chenopodium album* with different concentrations of NaCl and PEG solutions.

controls (Khan, unpublished data). It shows that exposure to potassium chloride had a priming effect and germination was significantly increased in comparison to control. There was no specific ion toxicity and an osmotic effect limited germination as in other halophytes (Mohammed & Sen, 1990; Egan *et al.*, 1997; Pujol *et al.,* 2000). Other species are also reported to show osmotic effects on germination rather than specific ion toxicity and saline pretreatment stimulated germination (Macke & Ungar, 1971; Williams & Ungar, 1972; Khan & Ungar, 1998).

Seeds of *Chenopodium glaucum* germinated better in non-saline controls and any increase in salinity progressively inhibited seed germination and few seed germinated beyond the 0.4 M salt treatment. $MgCl_2$ was more toxic and $MgSO_4$, the least effective in inhibiting seed germination. When seed were transferred to non-saline medium after 9 days exposure to salinity, there was a substantial recovery of germination. However, seed germination under natural conditions is more complicated and is influenced by many factors such as salinity, drought, light and temperature. Future studies would focus on the interactive effects of these factors. *Chenopodium glaucum* is an important cash crop halophyte which could be used to improve the quality of degraded saline land as well as a high protein diet for animals.

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References

Agboola, D.A. 1998. Effect of saline solutions and salt stress on seed germination of some tropical forest tree species. *Trop. Biol*., 45: 324-331.

Bajji, M., J.M. Kinet and S. Lutts. 2002. Osmotic and ionic effects of NaCl on germination, early seedling growth and ion content of *Atriplex halimus* (Chenopodiaceae). *Can. J. Bot*., 80: 297-304.

Baskin, C.C. and J.M. Baskin. 1998. *Seeds: ecology, biogeography, and evolution of dormancy and germination*. San Diego: Academic Press.

Bewley, J.D. and M. Black. 1994. *Seeds: physiology of development and germi*nation. Plenum Press, London, UK.

Clarke, L.D. and N.E. West. 1969. Germination of *Kochia americana* in relation to salinity. *Agron. J*., 20: 286-287.

Egan, T.P., I.A. Ungar and J.F. Meekins. 1997.The effects of different salts of sodium and potassium on the germination of *Atriplex prostrata* (Chenopodiaceae). *J. Plant Nut*., 20: 1723-1730.

Gu, F.T., Zh.Y. Liu and Zh.G. Yao. 2003. *Wild Cashcrop Resources in Yellow River delta*. Shandong Atlas Press. p. 68-70.

Gulzar, S. and M.A. Khan. 2001. Seed germination of a halophytic grass *Aeluropus lagopoides*. *Ann. Bot*., 87: 319-324.

Hardegree, S.P. and W.E. Emmerich. 1990. Partitioning of water potential and specific salt effects on seed germination of four grasses. *Ann. Bot*., 66: 587-595.

Katembe, J., I.A. Ungar and P. Mitchell. 1998. Effect of salinity on germination and seedlings growth of two *Atriplex species* (Chenopodiaceae). *Ann. Bot*., 82: 167-175.

Keiffer, C.W. and I.A. Ungar. 1995. Germination responses of halophyte seeds exposed to prolonged hyper-saline conditions. In: *Biology of Salt Tolerant Plants.* (Eds): M.A. Khan and I.A. Ungar. Karachi: Department of Botany, University of Karachi, Pakistan. pp. 43-50.

Khan, M.A. 2002 Halophyte seed germination: Success and Pitfalls. In: *International symposium on optimum resource utilization in salt affected ecosystems in arid and semi arid regions* (Eds.): A.M. Hegazi, H.M. El-Shaer, S. El-Demerdashe, R.A. Guirgis, A. Abdel Salam Metwally, F.A. Hasan & H.E. Khashaba. Desert Research Centre, Cairo, Egypt. pp. 346-358.

Khan, M.A., B. Gul and D.J. Weber. 2001. Effect of salinity and temperature on the germination of *Kochia scoparia*. *Wetland Ecol. & Manag*., 9: 483-489.

Khan, M.A., B. Gul and D.J. Weber. 2002. Effect of temperature, and salinity on the germination of *Sarcobatus vermiculatus. Biol. Plant*., 45: 133-135.

Khan, M.A., B. Gul and D.J. Weber. 2004. Temperature and high salinity effect in germinating dimorphic seeds of *Atriplex rosea. West. North Amer. Nat*., 64: 193-201.

Khan, M.A. and I.A. Ungar. 1997. Effect of thermoperiod on recovery of seed germination of halophytes from saline conditions. *Amer. J. Bot*., 84: 279-283.

Khan, M.A. and I.A. Ungar 1998. Germination of the 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. and D.J. Weber. 1986. Factors influencing seed germination in *Salicornia pacifica var. utahensis. Amer. J. Bot*., 73: 1163-1167.

Li, H., Z. Kefu and W. Xiufeng. 2002. The inhibition of salinity on the germination of halophyte seeds. *J. Shandong Agric. Uni.* (*natural science*). 33: 170-173.

Macke, A. and I.A. Ungar. 1971. The effect of salinity on germination and early growth of *Puccinellia nuttalliana. Can. J. Bot*., 49: 515-520.

Michel, E. and R. Kaufmann. 1973. The osmotic potential of Polyethylene Glycol 6000. *Plant Physiol*., 51: 914-916.

Mohammad, S. and D. N. Sen. 1990. Germination behavior of some halophytes in Indian desert. *Int. J. Exp. Biol*., 28: 545-549.

Myers, B.A. and D.I. Couper. 1989. Effects of temperature and salinity on the germination of *Puccinellia ciliata* (Bor.) cv. Menemen. *Aust. J. Agric. Res*., 40: 561-571.

Naidoo, G. and K. Naicker. 1992. Seed germination in the coastal halophytes *Triglochin bulbosa and Triglochin striata*. *Aquat. Bot*., 42: 217-229.

Pujol, J.A., J.F. Calvo and L.R. Díaz. 2000. Recovery of germination from different osmotic conditions by four halophytes from southeastern Spain. *Ann Bot.*, 85: 279-286.

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

Ungar, I.A. 1996. Effect of salinity on seed germination, growth and ion accumulation of *Atriplex patula* (Chenopodiaceae). *Amer. J. Bot*., 83: 604-607.

Ungar, I.A. 1995. Seed germination and seed-bank ecology in halophytes. In: *Seed Development and Seed Germination*, (Eds.) J. Kigel and G. Galili. pp. 599-628, Marcel Dekker, New York.

Waisel, Y. 1972. Biology of Halophytes. Academic Press. New York and London, pp: 437.

Williams, M.D. and I.A. Ungar. 1972. The effect of environmental parameters on the germination, growth and development of *Suaeda depressa* (Pursh) Wats. *Am. J. Bot*., 59: 912-918.

Woodell S.R.J. 1985. Salinity and seed germination patterns in coastal plants. *Vegetatio,* 61: 223-229.

Yuying S. 1991. Effect of salt concentration on seed germination of *Puccinellia tenuiflora*. *Pratacultural Sci*., 8:68-71.

Yuying S., W. Suomin and C. Yaming. 1999. The effect of saline condition on the germination and its recovery of pasture species. *Acta Prataculturae Sinic*., 8:54-60.

Zhao, K. and F. Lee. 1999. *Halophytes in China*. Science Press, Beijing, pp: 157.

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