Biosaline Agriculture and Salinity Tolerance in Plants
Edited by M. Öztürk, Y. Waisel, M.A. Khan and G. Görk

Comparative effect of NaCl and seawater on seed germination of *Suaeda salsa* and *Atriplex centralasiatica*

Xiaojing Liu[1], Hailong Qiao[1], Weiqiang Li[1], Toshiaki Tadano[2] and M. Ajmal Khan[3]

[1] *Center for Agricultural Resources Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, 286 Huaizhong Road, Shijiazhuang, Hebei 050021, P.R.China*
[2] *Department of Applied Biology and Chemistry, Faculty of Applied Bio-Science, Tokyo University of Agriculture, Tokyo, Japan*
[3] *Department of Botany, University of Karachi, Karachi, Pakistan*

Introduction

Seed germination in annual halophytes usually occurs when soil salinity levels are low and soil moisture is relatively high [1]. Optimum germination of halophyte seeds is often obtained under freshwater and inhibited by increasing salinity concentrations [2–4], but the ability to germinate at higher salinities is varied with species, for example *Salicornia herbacea* germinated at 1,700 mM NaCl [5], *Arthrocnemum macrostachyum* can germinate at 1,000 mM NaCl solution with 10 % germination percentage [6]. Some secreting halophytes could also germinate above seawater salinity [7–11]. Most secreting halophytes show germination at NaCl concentrations ranging from 0.34–0.52 M NaCl. Few of them have low salt tolerance during germination [12–14].

Seed of halophytes under natural conditions are subjected to saline stress dominated by NaCl. However, other chloride, sulfate and carbonate salts and their interaction play a significant role in affecting seed germination [1, 4]. There has been much research on halophyte seed germination in NaCl solutions. Such tests may not be relevant to the field conditions because in the field the soil solutions contains different cations (Na^+, Mg^{2+}, Ca^{2+}, K^+) and anions (Cl^-, ${SO_4}^{2-}$, HCO_3^- etc.), which compositions are similar to seawater. Different salt sources have different effects on seed germination [15]. Zia and Khan [16] reported that seawater inhibited seed germination of *Limonium stocksii* more than NaCl solutions, similar to Joshi et al. [17] reported for *Salvadora persica*. However, Duan et al. found that *Suaeda salsa* [18] and *Chenopodium glaucum* [19] germinated better in soil extracted solutions than in NaCl solutions, so did some combinations of salts have the similar effects on *Securigera securidaca* [20] and *Rhus chinensis* [21]. Those compared studies are still limited on the relative tolerance of seawater and NaCl solution on seed germination of halophytes.

Recovery of germination responses in temperate halophytes has been demonstrated in *Salicornia europaea, Suaeda linearis* [22], *Spergularia marina* [9], *Suaeda depressa* [23], *Arthrocnemum australsicum, Triglochin striata, Suaeda australis, Juncus maritimus,* and *Casuarina glauca* [24]. Boorman [25, 26] and Woodell [7] also reported salt stimulation of seed germination following treatment with seawater for a number of saltmarsh species. Keiffer and Ungar [27] 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 Woodell [7] 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.

Suaeda salsa, a leaf succulent annual plant, and *Atriplex centralasiatica*, a secreting annual plant from the family Chenopodiaceae, are widely distributed in China and are quite common in coastal areas. Both species have the potential to be used as cash crops [28–30]. The present study investigates the effects of seawater and NaCl on the seeds germination of *S. salsa* and *A. centralasiatica* and compared the differential response of these two species.

Materials and methods

Seeds of *S. salsa* and *A. centralasiatica* were collected in October 2004 in the coastal saline soils of Haixing County, Hebei Province of China. Before storage they were surface sterilized using clorox (0.5 %) for 1 minute followed by thorough rinsing with distilled water and air-drying for a few days. After cleaning, seeds were stored in paper bags at room temperature with relative humidity 30–40 % in the laboratory and germination experiments were initiated in January 2005. Germination experiments were carried out in 10 cm diameter tight fitting plastic Petri dishes with two-layer wet filter paper. 10 mL of test solution were added in the Petri dishes to investigate the effect of salinity on seeds germination of *S. salsa* and *A. centralasiatica*. Germination was carried out in different dilutions (0, 5, 10, 20, 30, 40, 50 dS m^{-1}) of NaCl and seawater separately. The electrical conductivity for both salt solutions was maintained at the desired level with the help of a conductivity meter. Four replicates of 40 seeds each were used for all treatments. Seeds with visible radicle were considered to have germinated.

A 24 h cycle was used where day temperature (25° C) coincided with 12 h light period (cool white fluorescent lamps, 25 uM $m^{-2}.s^{-1}$ 400–750 nm) and night temperature (15° C) coincided with the 12 h dark period. Percent germination was recorded every day for 15 days for all experiments. Un-germinated seeds from the salinity treatments were transferred to distilled water to study the recovery of germination, which was recorded each day for 7 days. The recovery percentage was determined by the following formula: (a–b)/(c–b) $\times$ 100; where a = the total number of seeds germinated after being transferred to distilled water, b = the total number of seeds germinated in saline solution and c = the total number of seeds. The rate of germination was estimated by using modified Timson's index of germination velocity: $\sum G/t$ – where G is the percentage of seed germination at one day interval, and t is the total germination period [2, 31]. The maximum number in our system could be 100. The higher value the more rapid the germination.

Data were analyzed using SPSS for window release 11.0. LSD test was used ($p < 0.05$) to determine significant differences among salinity treatments and species in seawater and NaCl separately.

Results

Seed germination in both *S. salsa* and *A. centralasiatica* remained unaffected up to 20 $dS.m^{-1}$ NaCl and seawater treatments (Fig. 1). A further increase in salinity inhibited germination in both species; however, only 8 % of seeds germinated at highest salinity treatments in the case of *A. centralasiatica* in comparison to 56 % germination in *S. salsa* in NaCl solution. NaCl inhibited germination more in comparison to seawater solutions (Fig. 1).

In non-saline control, highest germination was recorded at 2 days in *S. salsa* and 6 days in *A. centralasiatica* (Fig. 2). Increase of salinity delayed the germination and at EC 50 $dS.m^{-1}$ salinity level first seed germinated after 9 days in NaCl solution and only 11 % seeds germinated in seawater solution in *A. centralasiatica*. While in *S. salsa* 46 % and 58 % seeds germinated in NaCl and seawater solutions, respectively.

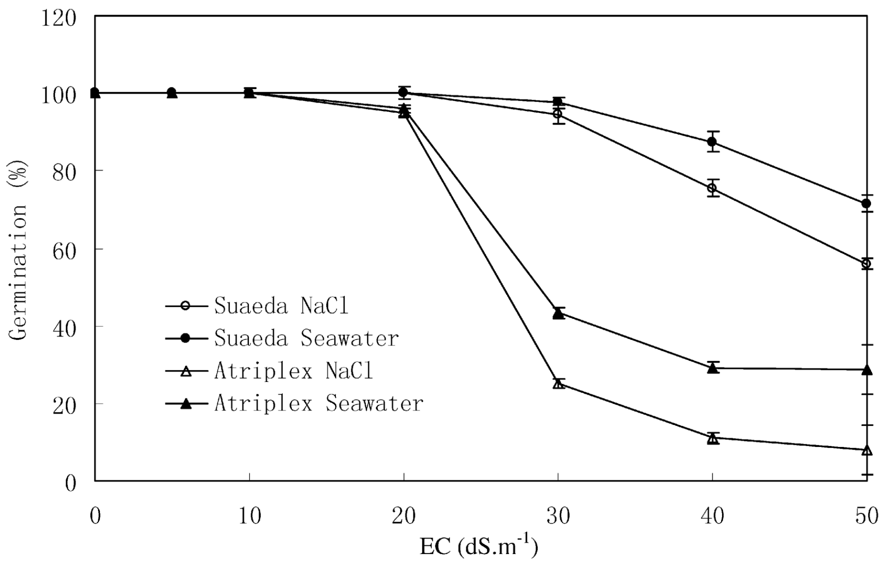

Figure 1. Mean final germination percentage of *Suaeda salsa* and *Atriplex centralasiatica* in various NaCl and seawater solutions. Bars represent means (± s.e., n=4).

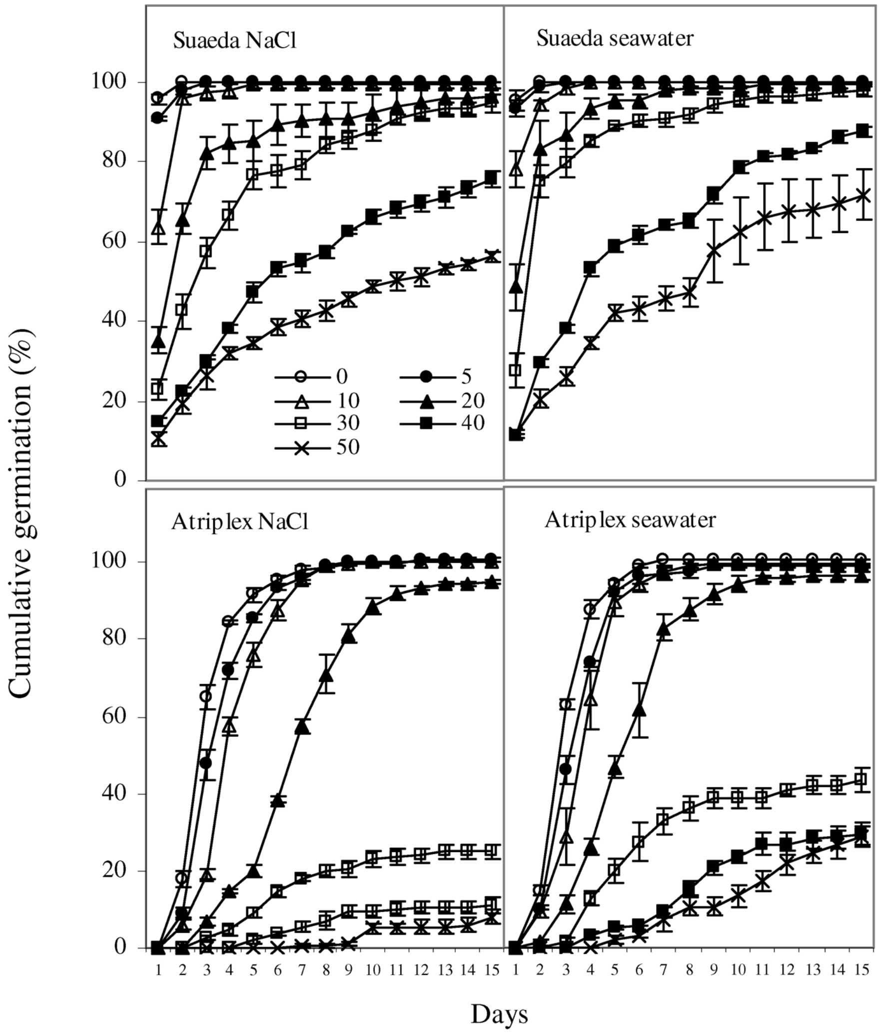

Figure 2. Cumulative mean germination percentage of *Suaeda salsa* and *Atriplex centralasiatica* over time in various NaCl and seawater concentrations. Bars indicate s.e. of means (n=4).

Rate of germination was highest in non-saline controls, and higher in *S. salsa* seeds than in *A. centralasiatica* seeds in all treatments and decreased with the increase of salinity (Fig. 3). Seeds of both species germinated slowly in NaCl solutions than in seawater solutions.

Un-germinated seeds from both NaCl and seawater solutions when transferred to distilled water recovered completely, but higher salinities affected the recovery of *A. centralasiatica*, and greater in seawater solutions than in NaCl solutions, for

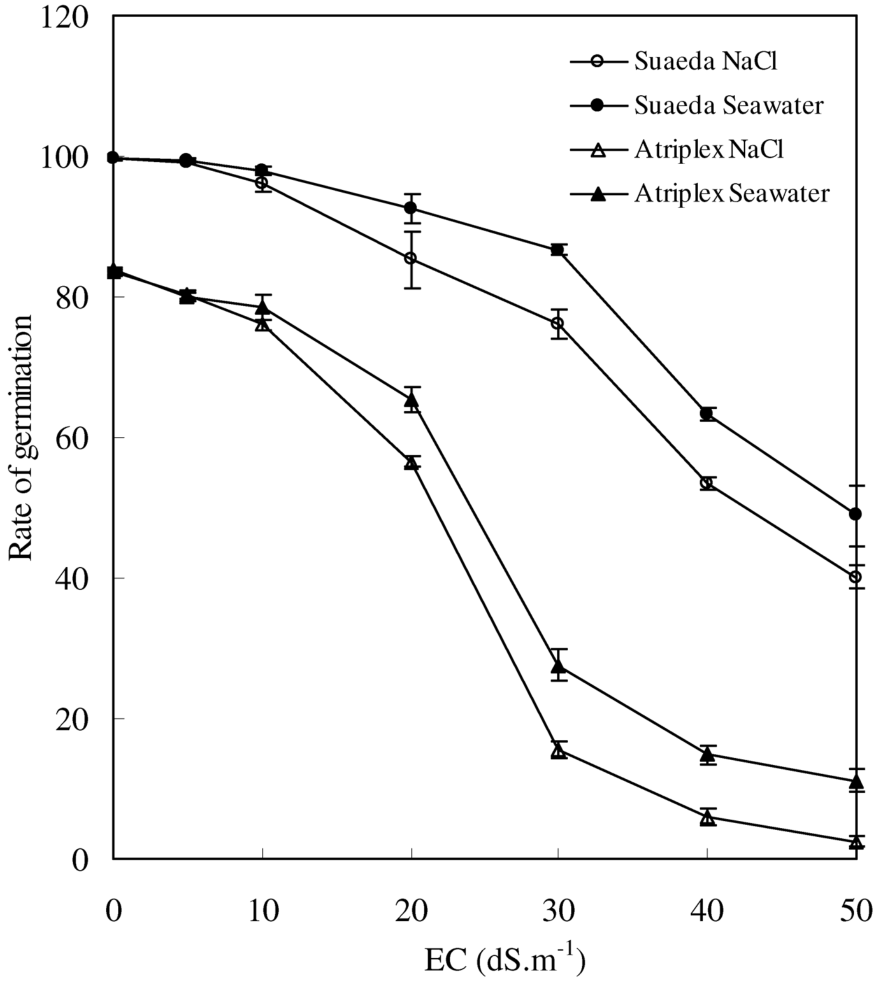

Figure 3. Rate of germination of *Suaeda salsa* and *Atriplex centralasiatica* seeds under various NaCl and seawater concentrations. Bars represent means (±s.e., n=4).

example, un-germinated seeds from 50 dS.m^{-1}, *S. salsa* can fully recovered at 1 day and 97 % in NaCl and 82 % in seawater of *A. centralasiatica* recovered at 7 days (Fig. 4).

Discussion

The vegetation along the coast of Northern China is dominated by a stem succulent halophyte *Suaeda salsa* and a secreting halophyte *Atriplex centralasiatica*. Coastal communities are usually mono-specific and their distribution is controlled by inundation gradient and their frequency. *S. salsa* occupy low marsh habitat with high

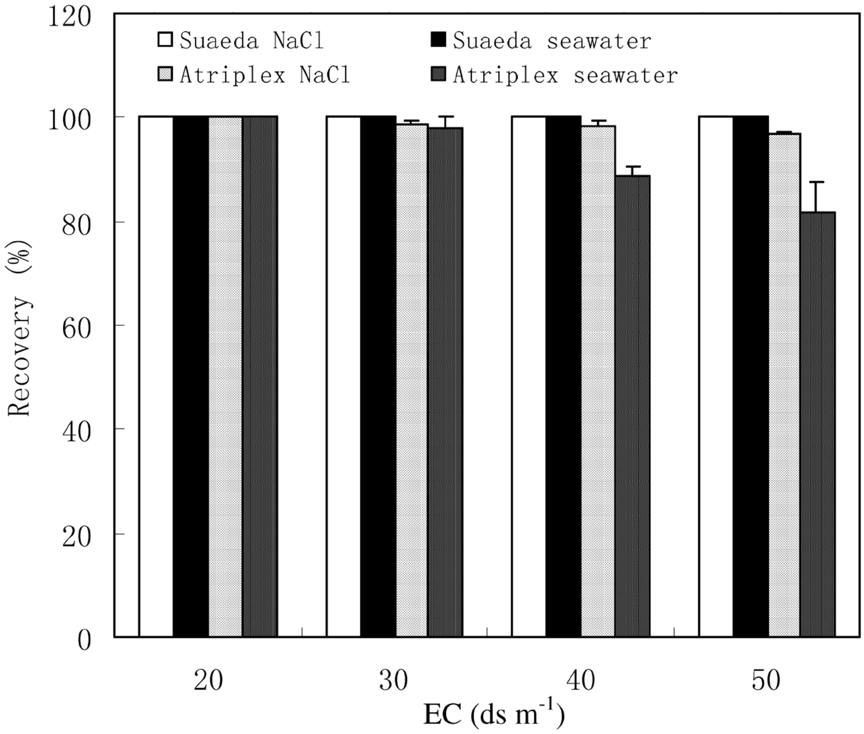

Figure 4. Mean recovery percent germination for *Suaeda salsa* and *Atriplex centrlasiatica* in distilled water under the treatments of various NaCl and seawater concentrations. Bars represent means (±s.e., n=4).

salinity and high moisture, while *A. centralasiatica* occupy a higher ground with infrequent inundation and low salinity and moisture conditions. This difference in ecological habitat has conferred varied ecological strategies to both species particularly at germination level. Seeds of *S. salsa* are highly salt tolerant and could germinate at seawater salinity, while few seeds of *A. centralasiatica* could germinate at that level. Halophytes vary in their ability to tolerate salinity at different stages of the life cycle. They are usually very highly salt tolerant while stored in the soil; however, their tolerance decreases at the germination and in most cases it increases again at the growth stage [4]. Seeds of leaf succulent species are highly tolerant to salinity [4]. Salinity plays an important role in determining the germination and survival of *Suaeda* spp [32] and it can tolerate high salinity during germination [32, 33]. The limit of salt tolerance of different species of *Suaeda* varies from 400–800 mM NaCl [22, 23, 34–36]. Secreting halophytes that could germinate above seawater salinity are few [7–11]. Most secreting halophytes show germination at NaCl concentrations ranging from 0.34–0.52 M NaCl [4, 37] while few of them have low salt tolerance during germination [12–14].

NaCl inhibited more seed germination in comparison to seawater solution both in *S. salsa* and *A. centralasiatica*. There is little information available on the effect of seawater on the germination of halophytes [7, 16, 17, 38, 39] and on the relative tolerance of seawater and NaCl solutions during seed germination [16, 17, 40]. Some results of combined salts effects on seed germination showed that the inhibitory effect of single salts can be considerably alleviated in natural soil systems by synergistic interactions between salts [18–21]. The increased seed germination in seawater may follow the same rule as combined salt effect because in seawater a lot of ions were included. However, seeds of *Limonium stocksii* [16] and *Salvadora persica* [17] were inhibited more by seawater; they also attributed this effect to seawater composition. This needs the further investigation.

Seeds of halophytes have the unique property of surviving extremely high salinity during the storage in the seed bank [41] and they germinate readily when soil salinity is reduced. However, recovery responses vary from one species to the other and against the level of salinity they are exposed to [27]. Seeds of *S. salsa* recovered completely when exposed to higher concentrations of NaCl and sea salt. Recovery of *A. centralasiatica* was also very high, except at 50 $dS.m^{-1}$ where 12–20 % of seeds failed to recover. A substantial recovery from germination occurred at the NaCl concentrations up to 600 mM NaCl in *Halogeton glomeratus* [42], *Sarcobatus vermiculatus* [43], *Suaeda moquinii* [36] and *Triglochin maritima* [44].

Suaeda salsa and *A. centralasiatica* are highly salt tolerant halophyte species where *S. salsa* is more salt tolerant than *A. centralasiatica*. Seeds of both species could germinate at seawater level but more germination was recorded in the seeds of *S. salsa*. Seed germination of *S. salsa* was more rapid in comparison to *A. centralasiatica*. Both species have a high ability to survive under extreme conditions; the un-germinated seeds can recover completely when the salinity stress was removed. Seed germination was inhibited more by NaCl than seawater. The literature suggests that the germination of halophyte seeds have differential response to seawater and NaCl. Some reports indicate that seawater inhibits more seed germination, while others believe that it is NaCl. Further studies will be carried out to understand the difference between NaCl and seawater effects on germination.

References

1 Ungar IA (1991) *Ecophysiology of Vascular Halophytes*. CRC Press, Boca Raton, Florida
2 Khan MA, Ungar IA (1984) The effect of salinity and temperature on germination of polymorphic seeds and growth of *Atriplex triangularis* Willd. *Am J Botany* 71: 481–489
3 Ungar IA (1995) Seed germination and seed bank ecology in halophytes. In: J Kigel, G Galili (eds): *Seed Development and Seed Germination*. Marcel Dekker, New York 599–628
4 Khan MA, Gul B (2002) Some ecophysiological aspects of seed germination in halophytes. In: X Liu, M Liu (eds): *Halophytes Utilization and Regional Sustainable Development of Agriculture*. Meteorological Press. Beijing 56–68
5 Chapman VJ (1960) *Salt marshes and salt deserts of the world*. Interscience Publishers, New York
6 Khan MA, Gul B (1998) High salt tolerance in germinating dimorphic seeds of *Arthrocnemum indicum*. *Int J Plant Sci* 159: 826–832

7 Woodell SRJ (1985) Salinity and seed germination patterns in coastal plants. *Vegetation* 61: 223–229
8 Khan MA (1991) Studies on germination of *Cressa cretica* L. seeds. *Pakistan J Weed Sci Res* 4: 89–98
9 Ungar IA (1967) Influence of salinity and temperature on seed germination. *Ohio J Sci* 67: 120–123
10 Binet P (1965) Action de divers rythmes thermiques journaliers sur la germination de semences de *Triglochin maritimum* L. *Bulletin Societe. Normandie (Caen), 10e series*. 6: 99–102
11 Ignaciuk R, Lee JA (1980) The germination of four annual strand-line species. *New Phytologist* 84: 581–587
12 Mahmoud A, El Sheikh AM, Abdul Baset S (1983) Germination of two halophytes: *Halopaplis perfoliata* and *Limonium axilare* from Saudi Arabia. *J Arid Env* 6: 87–98
13 Ladiges PY, Foord PC, Willis RJ (1981) Salinity and water logging tolerance of some populations of *Melaleuca ericifolia* Smith. *Australian J Ecol* 6: 203–215
14 Fernandez G, Johnston M, Olivares PA (1985) Rol del pericarpio de *Atriplex repanda* en la germinacion. III. Estudio histological y quimico del pericarpio. *Phyton* 45: 165–169
15 Ungar IA (1978) Halophyte seed germination. *Botanical Review* 44: 233–264
16 Zia S, Khan MA (2002) Comparative effect of NaCl and seawater on seed germination of *Limonium stocksii*. *Pakistan J Botany* 34: 345–350
17 Joshi AJ, Mali BS, Hingalajia H (1995) Halophytes – A good source of forage production under salt stress conditions l. *Salvadora persica*. In: MA Khan and IA Unger (eds): *Biology of Salt Tolerant Plants.* Department of Botany, University of Karachi 353–360
18 Duan D, Liu X, Feng F, Li C (2003) Effects of salinities on seed germination of halophytes *Suaeda salsa*. *Chinese Agricultural Science Bulletin* 19(6): 168–172
19 Duan D, Liu X, Khan MA, Gul B (2004) Effects of salt and water stress on the germination of *Chenopodium glaucum* L. seed. *Pakistan J Botany* 36: 793–800
20 Al-Jibury LK, Clor MA, Talabany D (1986) Effects of certain salts and their combinations on germination and seedling development of *Securigera securidaca* L. *Arabic Gulf J Sci Res* 4: 5–16
21 Zhang J, Li J, Xing S, Song Y, Chi J (2003) Experiment of seed germination of *Rhus chinensis* under salt stress. *J Northeast Forestry Uni* 31: 79–80
22 Ungar IA (1962) Influence of salinity on seed germination in succulent halophytes. *Ecology* 3: 329–335
23 Ungar IA, Capilupo F (1969) An ecological life history study of *Suaeda depressa* (Pursh.) Wats. *Advancing Frontiers of Plant Sciences* 23: 137–158
24 Clarke LD, Hannon NJ (1970) The mangrove swamp and saltmarsh communities of the Sydney District. III: plant growth in relation to salinity and water logging. *J Ecol* 58: 351–369
25 Boorman LA (1967) Experimental studies in the genus *Limonium.* Ph.D. dissertation, University of Oxford, UK
26 Boorman LA (1968) Some aspects of the reproductive biology of *Limonium vulgare* Mill. and *L. humile* mill. *Annals of Botany* 32: 803–824
27 Keiffer CW, Ungar IA (1995). Germination responses of halophyte seeds exposed to prolonged hypersaline conditions. In: MA Khan and IA Unger (eds): *Biology of Salt Tolerant Plants*. Department of Botany, University of Karachi 43–50
28 Zhao K, Li F (eds) (1999) *Halophytes in China.* Science Press. Beijing
29 Zhao K, Fan H, Ungar IA (2002) Survey of halophyte species in China. *Plant Sci* 163: 491–498

30 Bai Yajun, Liu Xiaojing, Li Weiqiang (2003) Primary analysis of four salt tolerant plants growing in Hai-He Plain, China. In: H Lieth, M Mochtchenko (eds): *Tasks for vegetation science-38, Cash crop halophytes recent studies*. Kluwer Academic Publishers, Dordrecht, the Netherlands 135–138
31 Timson J (1965) New methods of recording germination data. *Nature* 207: 216–217
32 Sheikh KH, Mahmood K (1986) Some studies on field distribution and seed germination of *Suaeda fruticosa* and *Sporobolus arabicus* with reference to salinity and sodicity of the medium. *Plant and Soil* 94: 333–340
33 Rajpurohit KS, Sen DN (1977) Soil salinity and seed germination under water stress. *Transaction of Indian Society of Desert Technology and University Center of Desert Studies* 2: 106–110
34 Okusanya OT (1979) An experimental investigation into the ecology of some maritime cliff species. II. Germination studies. *J Ecol* 67: 293–304
35 Yokoishi T, Tanimoto S (1994) Seed germination of the halophyte *Suaeda japonica* under salt stress. *J Plant Res* 107: 385–388
36 Khan MA, Gul B, Weber DJ (2001) Germination of dimorphic seeds of *Suaeda moquinii* under high salinity stress. *Australian J Botany* 49: 185–192
37 Khan MA, Rizvi Y (1994) The effect of salinity, temperature and growth regulators on the germination and early seedling growth of *Atriplex griffithii* Moq. var. *stocksii* Boiss. *Canadian J Botany* 72: 475–479
38 Rivers WG, Weber DJ (1971) The influence of salinity and temperature on seed germination in *Salicornia bigelovii*. *Physiologia Plantrum* 24: 73–75
39 McMillan C (1988) Seed germination and seedling development of *Halophphila decipiens* Ostenfeld from Panama. *Aquatic Botany* 31: 169–176
40 Tirmizi SAS, Khan KM, Qadir SA (1993) Study on salt tolerance of *Hippophae rhamnoides* L., during germination. *Pakistan J Sci Ind Res* 36: 252–257
41 Khan, MA, Ungar IA (1997) Effect of thermoperiod on recovery of seed germination of halophytes from saline conditions. *American J Botany* 84: 279–283
42 Khan MA, Gul B, Weber DJ (2001) Seed germination characteristics of *Halogeton glomeratus*. *Canadian J Botany* 79: 1189–1194
43 Khan MA, Gul B, Weber DJ (2002) Effect of temperature, and salinity on the germination of *Sarcobatus vermicullatus*. *Biologia Plantarum* 45: 133–135
44 Khan MA, Ungar IA (1999) Seed germination and recovery of *Triglochin maritima* from salt stress under different thermoperiods. *Great Basin Naturalist* 59: 144–150