Strategies for Adaptation of *Suaeda physophora*, *Haloxylon ammodendron* and *Haloxylon persicum* to a Saline Environment During Seed-Germination Stage

JIE SONG¹,³, GU FENG¹,* , CHANGYAN TIAN² and FUSUO ZHANG¹

¹College of Resource and Environmental Science, China Agricultural University, Beijing 100094, PR China, ²Institute of Ecology and Geography, Xinjiang, Academia Sinica, Urumqi 830000, PR China and ³College of Life Science, Shandong Normal University, Jinan 250014, PR China

Received: 18 March 2005 Returned for revision: 15 April 2005 Accepted: 11 May 2005 Published electronically: 7 July 2005

- *Background and Aims* Germination is very important for plant establishment in arid regions. The strategies taken by halophytes during the seed germination stage to adapt to saline environments in an arid zone were investigated in *Suaeda physophora* (euthalophyte), *Haloxylon ammodendron* (xero-halophyte) and *Haloxylon persicum* (xerophyte).
- *Methods* Seeds of *S. physophora*, *H. ammodendron* and *H. persicum* were exposed to a range of iso-osmotic NaCl and PEG solutions. Seed germination in, and recovery germination from, high NaCl were recorded. The effects of iso-osmotic NaCl and PEG on seed water uptake and changes in ion content were measured. In addition, the structure of seeds and Na⁺ distribution in the seed coat and embryos of dry seeds were investigated.
- *Key Results* The relative increase in fresh weight of germinating seeds was markedly reduced in −2.24 MPa PEG compared with that in −2.24 MPa NaCl, while the opposite trend was found in concentration of K⁺ during the initial 9 h for all species. *Haloxylon ammodendron* and *S. physophora* had a higher recovery germination from −3.13 MPa NaCl compared with *H. persicum*. Seeds of all species had no endosperm. More Na⁺ was compartmentalized in the seed coats of the two halophytic species compared with that in the xerophyte *H. persicum*.
- *Conclusions* The effect of NaCl on seed germination was due to both osmotic stress and ion toxicity for the three species. High soil salinity and a high content of Na⁺ in seeds may induce more seeds to remain ungerminated in *S. physophora* and *H. ammodendron*. Morphological structure and adaptation to salinity during seed germination may determine the geographical distribution of *H. ammodendron* and *S. physophora* in certain saline regions.

Key words: Adaptation, embryo, germination, halophyte, *Haloxylon ammodendron*, *Haloxylon persicum*, ion compartmentalization, ion toxicity, osmotic stress, seed coat, *Suaeda physophora*, xerophyte.

INTRODUCTION

Germination is a key stage in the life cycle of plants in saline environments as it determines whether or not the plants can establish successfully in certain areas. Most seeds are deposited near the surface of saline soil where the concentration of salt is usually much higher than it is below the surface (Esechie, 1995). In general, non-halophytes and halophytes respond to salinity in a similar way during the germination stage; the initial germination process is delayed under salt stress (Keiffer and Ungar, 1997; Khan and Ungar, 1997). The mechanism for how salinity inhibits seed germination of halophytes in a highly saline environment has received a lot of attention in recent years. The inhibitory effect of NaCl stress on seed germination is mainly due to an osmotic effect, rather than specific ion toxicity, in some halophytes, e.g. *Atriplex prostrata* (Egan et al., 1997) and *A. halimus* (Baji et al., 2002). A similar conclusion has been reported for other halophytes (Pujol et al., 2000) and non-halophytes (Almansouri et al., 2001). However, some studies have shown that the inhibitory effect of NaCl on seed germination was more severe than that of iso-osmotic PEG, as in *Atriplex prostrata* and *A. patula* (Katembe et al., 1998), *Aristida adscensionis* and *Artemisia ordosica* (Tobe et al., 1999). Therefore, the effect of salinity on seed germination can be attributed to an osmotic effect and/or a specific ion toxicity, depending on the plant species (Petruzzelli et al., 1992; Poljakoff-Mayber et al., 1994).

The establishment of populations of halophytes mainly depends on the responses of seed germination to soil salinity. Germinating seeds cannot exclude Na⁺ or Cl⁻ by roots (Tester and Davenport, 2003) or accumulate them in vacuoles like older seedlings (Greenway and Munns, 1980). Therefore, it is believed that seeds or young seedlings may be less stress tolerant than adults (Dodd and Donovan, 1999). Seeds of halophytes can remain viable or germinate in salt solution, which indicates that seeds can resist salt stress to some extent, and sometimes they may be even more salt-tolerant than actively growing plants (Ungar, 1996). Germination at higher tissue Na⁺ concentrations has been associated with higher K⁺ and Ca²⁺ concentrations in the embryo axis of *Glycine max*, compared with growing seedlings, suggesting that these ions may protect the seeds in the pre-germination stage against salinity stress (Hosseini et al., 2002). However, the mechanisms taken by the seeds of halophytes to adapt to saline stress are still poorly understood.

*Suaeda physophora*, *Haloxylon ammodendron* and *Haloxylon persicum* (Chenopodiaceae) are common plant species in the Zhunger Basin, which is located in Xinjiang, the north-west province of China. *Haloxylon persicum* is a
Stem-succulent xerophytic shrub, which only occurs in non-saline sandy deserts. *Haloxylon ammodendron* is a stem-succulent shrub, which occurs in both saline lands and non-saline sandy deserts. *Stauea physophora* is a leaf-succulent euhalophytic shrub, occurring on highly saline soil in which the total salt content reaches a maximum of 60 g kg$^{-1}$ dry soil in the 0- to 20-cm soil layer (Kong and Ma, 1996). *Haloxylon ammodendron* and *H. persicum* are traditionally used as livestock feed and firewood, and are also crucially important in stabilizing sand dunes (Tobe et al., 2000). Great attention has been paid to the salt tolerance of halophytes at different seedling stages (Glenn and Brown, 1999; Tester and Davenport, 2003). Tobe et al. (2000) reported that *H. ammodendron* shows greater adaptability to salinity at an older seedling stage than does *H. persicum*. They further suggested that adaptations to salinity at the later developmental stage of these species determine their distribution with respect to salinity. However, little is known about how the tolerance to salinity of seeds of halophytes and xerophytes is related to the edaphic conditions in which these plants are growing (Tobe et al., 2000; Huang et al., 2003). It has been hypothesized that the distribution of plants in certain saline or arid edaphic conditions might be directly related to adaptations to salinity during seed germination. Therefore, the aim of the present study was to reveal what strategies are taken by *H. ammodendron*, *S. physophora* and *H. persicum* to adapt to saline or arid environments during their seed-germination stage.

**MATERIALS AND METHODS**

**Seeds**

Seeds of *S. physophora* Pall. and *H. ammodendron* (C.A.Mey.) Bunge were collected from a saline habitat, while seeds of *H. persicum* Bunge ex Boss. et Buhse were collected from a non-saline habitat (44°13′−22′N; 87°40′−54′E) in Xinjiang, the north-west province of China in October 2003. Dry seeds were stored in a refrigerator at <4°C for 2 months before being used.

**Dynamic observation of seed germination during the initial 10 d**

Solutions of NaCl and iso-osmotic PEG-6000 were prepared at $\Psi_s$ of −0.45, −1.34, −2.24 and −3.13 MPa with a freezing-point osmometer (Fiske 210; Advanced Instruments Inc., MA, USA). In all the germination experiments, 25 seeds of each species were sown in Petri dishes (9 cm in diameter) on two layers of filter paper moistened with 10 mL distilled water (control) or test solution. Four replicates of each treatment were used in all experiments. All Petri dishes were placed in an incubator (LRH-250-G Illuminating Incubator; Guangdong Medicine Apparatus Manufactory, Guangdong, China) with constant temperature (20°C) and darkness. Every 2 d, the solution in each Petri dish was removed, a further 10 mL test solution added and removed again as completely as possible, before 5 mL of the test solution was added. Germinated seeds were recorded daily. Seeds were considered to have germinated when the emerging radicle was at least 2 mm.

**Determination of final germination percentage**

To measure the final percentage germination of the three species in different solutions, another germination test was carried out under the same conditions. The treatments included distilled water (control), −1.34, −2.24, −3.13 MPa NaCl and iso-osmotic PEG solutions. Every 2 d the solution in each Petri dish was renewed as described above. Germinated seeds were removed daily, and the final percentage germination was recorded 20 d after incubation. Seeds were considered to have germinated when the emerging radicle was at least 2 mm.

**Recovery test**

To understand whether seeds exposed to a high concentration of NaCl lost the ability to germinate, 140 seeds of each species were submerged in 20 mL of −3.13 MPa NaCl solution in a plastic bottle with a tight cap at a constant temperature (20°C) and in darkness. The NaCl solutions were changed completely every day. Seed germination in the NaCl solution was recorded for 10 d. The ungerminated seeds were rinsed three times with distilled water, and four replicates of 25 ungerminated seeds each were sown in each Petri dish, as described above, using water to moisten the filter paper. Seed was considered to have germinated when the emerging radicle was at least 2 mm. Seedlings elongated to a length of at least 10 mm and almost completely uncoiled were regarded as normal seedlings; otherwise they were recorded as abnormal seedlings.

**Determination of water uptake**

Seeds sown in Petri dishes (9 cm in diameter) on two layers of filter paper moistened with 10 mL of distilled water (control), −2.24 MPa NaCl or iso-osmotic PEG solution were weighed after incubation for 9 h at constant temperature (20°C) in the dark. The relative increase in fresh weight ($W_f$) was calculated as $W_f = (W_i - W_f)/W_i \times 100$, where $W_i$ is the initial weight of seeds, and $W_f$ is the weight after 9 h (Almansouri et al., 2001).

**Determination of inorganic ions in seeds**

Seeds were imibed for 9 h on two layers of filter papers moistened with 10 mL distilled water (control), −2.24 MPa NaCl or an iso-osmotic PEG solution in Petri dishes (9 cm in diameter). The seed coats of seeds that had not germinated were removed. Embryos were quickly washed with distilled water, surface-dried with filter paper, and dried in an oven for 2 d at 80°C. Meanwhile, the seed coats of dry seeds were removed, and dry seeds, seed coats and embryos were also oven-dried for 2 d at 80°C. Dry samples were placed in a muffle furnace to be ashed at 550°C. The ash was dissolved with concentrated nitric acid, and then distilled water was added to obtain a final volume of 20 mL. Concentration of Na$^+$ and K$^+$ were determined by flame emission (2655-00 Digital Flame Analyzer; Cole-Parmer Instrument Company, Chicago, IL, USA); four replicates of 30 seeds each were used for all species.
Microscopy

Seed coats of dry seeds were removed carefully, and the embryos were observed and photographed under a light microscope (XTL30; Beijing Taike Instrument Co. Ltd, Beijing, China).

Statistical analysis

Germination data were transformed (arcsine) before statistical analysis to ensure homogeneity of variance. Data were subjected to a one-way ANOVA using the SAS™ software (SAS Institute, 1989). Treatment means were compared by LSD at $P = 0.05$.

RESULTS

Effects of NaCl and iso-osmotic PEG on seed germination during the initial 10 d

Seeds of the three species germinated rapidly in distilled water during the initial 2 d. Percentage germination was 17% on the first day, and 50% on the second day in *H. persicum* (Fig. 1A), 7% and 45%, respectively, in *H. ammodendron* (Fig. 1B), and 26% and 70%, respectively, in *S. physophora* (Fig. 1C).

When seeds were germinated on filter papers moistened with solutions of NaCl or iso-osmotic PEG, percentage germination declined with decreasing $C_s$ in all species, except for *S. physophora* at $-0.45$ MPa NaCl. The inhibitory effect of PEG on germination was more severe than that of iso-osmotic NaCl treatments in *H. persicum* (Fig. 1A). There were no significant changes in the percentage germination at $-0.45$ and $-1.34$ MPa NaCl compared with at iso-osmotic PEG treatments in both *H. ammodendron* (Fig. 1B) and *S. physophora* (Fig. 1C).

Percentage germination declined severely at $-2.24$ and $-3.13$ MPa NaCl started with that at iso-osmotic PEG solutions in *H. ammodendron* during the initial 5 d, but it increased rapidly, and was higher than that at iso-osmotic PEG solutions during the following days (Fig. 1B). The inhibitory effect of $-2.24$ MPa NaCl on *S. physophora* was invariably greater than that in the iso-osmotic PEG treatment. Percentage germination was inhibited more severely under $-3.13$ MPa NaCl compared with that in the iso-osmotic PEG treatment during the initial 6 d; after that, the opposite was found (Fig. 1C).

Effects of NaCl and iso-osmotic PEG on final germination percentage

Final germination percentages were over 96% in distilled water for all species, but decreased with decreasing $C_s$ in all species. The final germination percentages under $-1.34$, $-2.24$ and $-3.13$ MPa PEG were 43%, 48% and 76% lower, respectively, than that under iso-osmotic NaCl treatments in *H. persicum*, and 42% and 72% lower under $-2.24$ and $-3.13$ MPa PEG, respectively, than that under iso-osmotic NaCl treatments in *H. ammodendron*. However, there were no significant differences in final percentage germination between various NaCl and iso-osmotic PEG treatments in *S. physophora* (Fig. 2).

Inorganic ions in embryos and relative increase in fresh weight of germinating seeds treated at $-2.24$ MPa NaCl and iso-osmotic PEG for 9 h

$Na^+$ concentration of embryos at $-2.24$ MPa NaCl increased to 147%, 143% and 154% of control seeds, while the $K^+$ concentration decreased to 84%, 90% and 67% of the control seeds in *H. persicum*, *H. ammodendron* and *S. physophora*, respectively. There were no significant
changes in the concentration of Na\(^+\) and K\(^+\) in embryos at -2-24 MPa PEG compared with the control seeds in all species (Table 1).

The relative increase in fresh weight of germinating seeds (\(W_r\)) was invariably the highest in the control, and the lowest in the -2-24 MPa PEG treatments in all species (Table 1).

**Na\(^+\) content in whole seeds, seed coats, embryos and Na\(^+\) in the seed coat : Na\(^+\) in embryo ratio**

The Na\(^+\) content was 1-17 mmol g\(^{-1}\) d. wt in whole seed of *S. physophora*, which was 1-5 and 9-8 times that in seeds of *H. ammodendron* and *H. persicum*, respectively (Table 2). The highest Na\(^+\) content in seed coats and embryos was also seen in *S. physophora*, followed by *H. ammodendron*, with lowest concentrations in *H. persicum* (Table 2). The ratio of Na\(^+\) in seed coat : Na\(^+\) in embryo in *S. physophora* was 9-8, compared with 6-1 and 2-5 in *H. ammodendron* and *H. persicum*, respectively (Table 2).

**Recovery of pretreated seeds in NaCl solution**

When seeds were pretreated with -3-13 MPa NaCl for 10 d, seed germination was 7%, 9% and 10% in *H. persicum, H. ammodendron* and *S. physophora*, respectively. Following transfer of the ungerminated seeds, pretreated with -3-13 MPa NaCl for 10 d, to distilled water for another 16 d, the recovery percentage germination was 25% in *H. persicum, 73% in H. ammodendron* and 51% in *S. physophora*. All seedlings were normal under control conditions after 16 d treatment in all plant species. For the recovery germination, the percentage of normal seedlings in *S. physophora* was 98%, and it was 81% in *H. ammodendron*, and 68% in *H. persicum* (Table 3).

**DISCUSSION**

The effect of salinity on seed germination is due to an osmotic effect and/or ion toxicity (Rehman *et al.*, 1996). NaCl had a specific ion toxicity effect on the seed.
germination for nonhalophytic species, e.g. *Hedysarum scoparium*, *Artemisia ordosica* and *Aristida adscensionis* (Tobe et al., 1999). However, the effects of different salts of sodium and potassium on seed germination were due to osmotic effect rather than specific ion toxicity for the halophyte, *Atriplex prostrata* (Egan et al., 1997). Therefore, salinity-induced reductions in germination are usually attributed to an osmotic effect only for halophytes, but non-halophytes are more likely to exhibit an additional ion toxicity (Bajji et al., 2002). In the present study, the relative increase in fresh weight of germinating seeds at −2.24 MPa NaCl was lower than in control for all species (Table 1), which indicated that NaCl had an inhibitory effect on water uptake of seeds. Moreover, external Na⁺ may damage the plasma membranes or negatively influence intracellular K⁺ influx (Hasegawa et al., 2000), because K⁺ concentration in the embryos was lower at −2.24 MPa NaCl than in controls when seeds were moistened for 9 h (Table 1). Therefore, the adverse effects of NaCl on seed germination of the three species might result from both osmotic and ion toxicity effects. More than 50 enzymes are activated by K⁺, and Na⁺ cannot substitute in this role. In addition, protein synthesis requires high concentrations of K⁺, and the disruption of protein synthesis by high concentrations of Na⁺ appears to be an important cause of damage (Tester and Davenport, 2003). Seeds under PEG treatments did not suffer ion toxicity (Table 1). However, the final germination percentages were higher at −2.24 and −3.13 MPa NaCl compared with iso-osmotic PEG treatments for *H. persicum* and *H. ammodendron* (Fig. 2), suggesting that Na⁺ or Cl⁻ entered the seeds and alleviated the osmotic inhibitory effects of the media on water uptake for all species (Table 1). Therefore, higher germination percentages were found at high NaCl concentrations compared with iso-osmotic PEG solutions.

Normally, halophytes germinate in spring, rather than in summer, in order to avoid the increase of salt concentration in soil solution caused by high evaporation (Keiffer and Ungar, 1997). High levels of salt can induce seeds to remain dormant in a saline environment when they meet unfavourable edaphic condition (Khan and Ungar, 1997). When seeds of two high salt-tolerant species, *Salicornia europaea* and *Suaeda calceolaris*, were removed to fresh water after being exposed to a high salinity solution for 30–730 d, germination was stimulated (Keiffer and Ungar, 1997). Therefore, the ability to maintain seed viability for extended periods of time during exposure to hypersaline conditions and then to initiate germination when the salinity stress is reduced is a characteristic of halophytes, distinguishing them from non-halophytes (Keiffer and Ungar, 1997). The results in the present experiment (Table 3) were consistent with the previous report (Keiffer and Ungar, 1997). More interesting results in the present experiment were that high NaCl markedly inhibited germination of the two halophytic species during the initial 4 d (Fig. 1B and C), then seed germination increased rapidly in the following days, especially for *H. ammodendron* (Fig. 1B). Favourable conditions are always transient in natural arid environments during spring. Soil salt content may increase soon after a rain event because evaporation of moisture under full sunlight and higher temperatures causes an increase in the salt content by capillary movement (Khan and Ungar, 1996a). Thus, the survival of seedlings may not be ensured. Seeds germinated rapidly at low NaCl but remained ungerminated at high NaCl during the initial germination stage for *H. ammodendron* and *S. physophora* in the present study. This response may produce a persistent seed bank of viable seeds in salt desert habitats that will maintain the population over time, and it may be an important strategy taken by the two halophytic species in the present experiment to adapt to the saline conditions in the Zhunger Basin, one of the driest regions in the world.

It has been suggested that desert halophytic shrubs may have evolved a mechanism to accumulate high ion content to induce dormancy, and seeds only germinate when temperature and edaphic conditions are favourable (Khan and Ungar, 1996b). In the present study, the Na⁺ content of whole seeds of the euhalophyte *S. physophora* was much higher than that in the xero-halophyte *H. ammodendron*, while being lowest in the xerophyte *H. persicum* (Table 2), and this may be related to their environmental factors. High Na⁺ or other ions in seeds may alleviate the osmotic inhibitory effects of the media on water uptake for the two halophytic species, especially for seeds of *S. physophora*. As a result, there was no significant difference in the final germination percentage between NaCl and PEG at iso-osmotic levels in *S. physophora* (Fig. 2). The seed coat may have an important role, greater than simply as a physical protective barrier and may play a role in ion exchange, and it has been suggested that the high uptake of Na⁺ by the seed coat of *Acacia tortilis* and *A. coriacea* may reduce uptake of Na⁺ by the embryo (Rehman et al., 1998). Interestingly, more Na⁺ was compartmentalized in the seed coat of the two halophytic species, especially in *S. physophora*, compared with the xerophyte *H. persicum*. Seeds of *S. physophora* and *H. ammodendron* accumulated less Na⁺ in embryos but more Na⁺ was compartmentalized in the seed coats, which might protect embryos from ion toxicity to ensure seed viability during seed development, and may be an adaptive mechanism for *S. physophora* and *H. ammodendron* to a saline environment.

### Table 3. Seed germination when seeds were submerged in −3.13 MPa NaCl for 10 d, recovery germination after ungerminated seeds pretreated with −3.13 MPa NaCl for 10 d were transferred to distilled water in continuous darkness at 20°C for another 16 d and the percentage of normal seedlings in the recovery test

<table>
<thead>
<tr>
<th>Plant</th>
<th>NaCl</th>
<th>Control</th>
<th>Recovery</th>
<th>Normal seedlings (%)</th>
<th>Control</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. persicum</em></td>
<td>7</td>
<td>93 a</td>
<td>25 b</td>
<td>100 a</td>
<td>68 b</td>
<td></td>
</tr>
<tr>
<td><em>H. ammodendron</em></td>
<td>9</td>
<td>98 a</td>
<td>73 b</td>
<td>100 a</td>
<td>81 b</td>
<td></td>
</tr>
<tr>
<td><em>S. physophora</em></td>
<td>10</td>
<td>95 a</td>
<td>51 b</td>
<td>100 a</td>
<td>98 a</td>
<td></td>
</tr>
</tbody>
</table>

Means within a species that have the different letter are significantly different at *P < 0.05*; percentage of normal seedlings in the recovery test was calculated as: normal seedlings/germinated seeds × 100.
The morphological structure of seed has hardly been related to salt tolerance strategy in previous studies. Seeds of *H. persicum* and *H. ammodendron* have no endosperm and contain only a fully spiral seedling coated by a pericarp (Tobe et al., 2000). Seed of *S. physophora* had a similar structure in the present investigation (Fig. 3). Such a morphological structure may help these plants to germinate as rapidly as possible. Seeds of the three plant species germinated rapidly in the initial 2 d in distilled water (Fig. 1A–C). Therefore, rapid germination may be an adaptive strategy for seeds of the three species to take advantage of transient favourable conditions during the germination stage, to ensure seedling establishment.

In conclusion, the effect NaCl has on the seed germination of the three species is due to both an osmotic effect and ion toxicity. High soil salinity and a high content of Na⁺ in seeds may induce more seeds to remain ungerminated for the two halophytic species *S. physophora* and *H. ammodendron* when environmental conditions are unfavourable, and seeds will germinate only when salinity stress is alleviated. Morphological structure and adaptations to salinity during seed germination may determine the geographical distribution of *H. ammodendron* and *S. physophora* in certain saline regions.

**ACKNOWLEDGEMENTS**

This study was supported financially by Nature Science Foundation of China (30470341) and ‘863’ program of Chinese Ministry of Sciences and Technology (2004AA227110-2). We thank Professor Zhang Liyun for his valuable help in identification of plant species when we collected seeds in the field. We thank Professor Hans Lammers for critically reading and revising the manuscript, Dr Hu Yuncai for suggestions on the manuscript and Dr Fan Hai for analysing the osmotic potential of NaCl and PEG solution. The financial support from the Institute of Ecology and Geography, Xinjiang, Academia Sinica is also greatly appreciated.

**LITERATURE CITED**


