

## Effects of NaCl on seed germination of five nonhalophytic species from a Chinese desert environment

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### Summary

The effects of NaCl on seed germination were investigated among five nonhalophytes distributed in Chinese desert. To evaluate the specific effects of NaCl on the seeds, germination, viability and water uptake of the seeds in NaCl solutions were compared with those in isotonic solutions of polyethylene glycol-6000. The seeds of the five species in the same habitat showed wide ranges of differences in susceptibility to NaCl both before and after radicle emergence. It is suggested that some species distributed naturally in nonsaline locations are potentially salt-tolerant in the germination stage and can be candidates to be utilized for the rehabilitation of vegetation in salinized locations.

### Introduction

Salinization of the soil is a serious problem in arid and semiarid regions. For species to become established in saline environments, adaptation of the species to salinity in the germination stage is crucial (Ungar, 1991, 1995). Therefore, to predict the effects of salinization of the soil on vegetation in these regions, or to undertake to introduce agronomically important nonhalophytes to salinized locations, information is important on how salinity affects the seed germination of nonhalophytes distributed in these regions.

In this study, we investigated the effects of NaCl on five species distributed in non-saline sand dunes in Chinese desert (Table 1). In China, all these five species serve not only as livestock feed but also as vegetative cover that stabilizes sand dunes (Lanzhou Institute of Desert Research, 1985).

The effect of salt on seed germination is mainly osmotic for some species (Cluff, Evans and Young, 1983; Myers and Morgan, 1989; Naidoo and Naicker, 1992), but for others salt can also exert salt-specific effects on the germinating seeds. At least some of the salt-specificity is attributable to permeation of salt into the seeds during the imbibition stage. Indeed, solutes can significantly permeate through the testa of seeds (Manohar and Heydecker, 1964; Manohar, 1966), and moistening of seeds with NaCl solution increases the Na content of the seeds (Bliss, Platt-Aloia and Thomson, 1986a;

Torres-Schumann *et al.*, 1989; Saleki, Young and Lefebvre, 1993). When salt enters the seed tissues, it decreases the water potential ( $\Psi_w$ ) in the seeds and promotes water uptake by them (Sharma, 1973; Romo and Eddleman, 1985; Saleki *et al.*, 1993; Ungar, 1995). However, salt absorbed into seeds can also have toxic effects on the tissue and reduce the germinability of the seeds (Redmann, 1974; Bal and Chattopadhyay, 1985; Hardegree and Emmerich, 1990). If imbibed salt can act toxically on the seed germinability, hypersalinity that completely inhibits seed germination can also be lethal to the seeds. However, Bliss *et al.* (1986a,b) have reported that NaCl inhibits the germination of barley seeds only osmotically during the initial imbibition stage, and acts toxically only by injuring the emerging radicles. In this case, salt can act toxically only at concentrations that allow the initiation of radicle elongation. It seems that effects of salt on the seeds differ widely among different species.

In this study, to evaluate the specific effects of salt on the seeds, germination in NaCl solutions was compared with that in isotonic solutions of polyethylene glycol (PEG)-6000, which cannot permeate plant cell walls and acts solely as an osmoticum on seeds (Sharma, 1973; Redmann, 1974; Hardegree and Emmerich, 1990; Bradford, 1995). Aspects investigated in this study included germination in moderate  $\Psi_w$ , effects of pretreatment with solutions of low  $\Psi_w$  on seed germinability, and water uptake by the seeds in solutions of different  $\Psi_w$ .

## Materials and methods

Seeds of the five tested species (Table 1) were gathered in nonsaline sand dunes in Shapotou, China (37°26'N, 104°57'E). At this site the ground is covered with nonsaline fine sand, and the annual mean temperature and annual precipitation in 1995 were 9.9°C and 220 mm, respectively. Seeds were collected in October, 1996 for *H. scoparium*, October, 1995 and January, 1996 for *A. ordosica*, and October, 1995 for the other three species. Before the experiments, the seeds were stored dry at about 5°C. The germination experiments were carried out during the period March 1996–August 1997.

In all the experiments, the seeds were sown in petri dishes on three layers of filter paper (Toyo, No.1) moistened with deionized water or a solution of NaCl or PEG-6000, so that about half the volume of each seed was immersed in the solution. The NaCl and PEG solutions of known  $\Psi_w$  were prepared according to the respective equations

Table 1. Characteristics of the five tested species.

Species	Family	Life form	Seed weight (mg)*
<i>Hedysarum scoparium</i> Fisch. et Mey.	Leguminosae	shrub	23.9 ± 4.8
<i>Artemisia ordosica</i> Krasch.	Compositae	semi-shrub	0.27 ± 0.11
<i>Bassia dasyphylla</i> (Fisch. et Mey.) O. Kuntze	Chenopodiaceae	annual	0.97 ± 0.22
<i>Agriophyllum squarrosum</i> (L.) Moq.	Chenopodiaceae	annual	1.78 ± 0.63
<i>Aristida adscensionis</i> L.	Gramineae	annual	1.30 ± 0.25

\*: mean ± SD (n=20)

of Lang (1967) and Michel and Kaufmann (1973), but the  $\Psi_w$  of the prepared PEG solutions was finally determined with an isopiestic psychrometer (Boyer and Knipling, 1965) at 20°C. The petri dishes were covered with lids and maintained at 20°C under continuous darkness in an incubator. About two thirds the volume of the water or solution in each petri dish was replaced daily to avoid a change in the  $\Psi_w$  of the solution. The seeds were considered to have germinated when the radicle length exceeded 5 mm for *H. scoparium* and 3 mm for the other four species and the radicles showed no abnormalities.

### Experiment 1: Examination of effects of solutions of moderate $\Psi_w$ on seed germination

The number of seeds sown in each petri dish (inner diameter: 90 mm) was 20 for *H. scoparium* or 25 for the other four species. After moistening, the number of seeds that had germinated in each dish was counted every day for 15–25 days until the germination percentage became almost steady; the germination percentage at this time is designated as the final germination percentage ( $G_F$ ). Time needed for the germination percentage to reach 50% of  $G_F$  ( $\tau_{1/2}$ ) was interpolated from the germination percentages at the consecutive two days.

### Experiment 2: Examination of effects of pretreatments with solutions of low $\Psi_w$ on seed germination

The seeds of each species were moistened for either 5 days (*H. scoparium*) or 10 days (other four species) in petri dishes (inner diameter: 90 mm: the number of the seeds in each dish being the same as that in experiment 1) with –5.0 MPa NaCl or –5.2 MPa PEG solution. Thereafter the seeds were lightly washed with deionized water and transferred to other petri dishes in which they were moistened with deionized water. The number of germinated seeds was counted for 10 days after transfer, and  $G_F$  and  $\tau_{1/2}$  after transfer were calculated.

### Experiment 3: Examination of effects of solutions differing in $\Psi_w$ on water uptake by seeds

One seed of *H. scoparium* sown in each dish (inner diameter: 25 mm) or ten seeds of each of the other four species in each dish (inner diameter: 50 mm) were moistened with NaCl or PEG solution of known  $\Psi_w$ . Because it had proved from a preliminary experiment that the seed weight of all these species becomes almost steady within 7 days after moistening, changes in the weight of the seed(s) in each dish were measured from just before to 7 days after moistening with various types of solutions. In addition, changes in the weight of the same single seed of *H. scoparium* were measured at different times after moistening with various types of solutions for 7 days, and subsequently transferred to filter papers moistened with deionized water. Water uptake by the seeds was evaluated in terms of relative weight gain ( $W_r$ ) which was calculated as  $(w-w_0)/w_0$ , where  $w$  and  $w_0$  are the seed weights at a given time and just before moistening, respectively.

## Results

### Effects of NaCl and PEG solutions of moderate $\Psi_w$ on seed germination

For *A. squarrosom*, germination was abnormal in about 3.5% of seeds in that a plumule emerged from the ruptured testa distant from the micropyle, while normal germination of this species was characterized by radicle protrusion from the micropyle. Most of the abnormally germinated seeds did not develop into normal seedlings. The abnormal germination was mostly detected 1–4 days after seed moistening, whereas normal germination usually occurred more than 3 days after moistening. There was no correlation between the percentage of abnormal germination and the type of the solution with which the seeds had been treated. Abnormal germination seemed to result from protrusion of the embryo from an abraded or cracked part of the testa under the turgor pressure of the embryo before the turgor pressure pushed the radicle through the micropyle. In this study, abnormally germinated seeds were excluded from calculation of the  $G_F$  and  $\tau_{1/2}$  of *A. squarrosom*.

For *A. ordosica*, when the seeds were treated with either NaCl solution of  $\Psi_w$  lower than  $-0.4$  MPa or PEG solution of  $\Psi_w$  lower than  $-1.0$  MPa, some or most of the radicles emerging from the seeds were necrotised and ceased to elongate when the radicle was less than 3 mm long; however, the radicles emerging from the other seeds elongated to more than 3 mm and showed no abnormalities. In this study,  $G_F$  of *A. ordosica* was calculated for both seeds in which radicle emergence was visibly evident and those in which the radicle elongated to more than 3 mm without abnormalities. For all the seeds of *H. scoparium*, *B. dasyphylla* and *A. adscensionis*, and normally germinated *A. squarrosom* seeds, the emerging radicles that were visibly detectable elongated to 3 mm or more and showed no abnormalities.

Figure 1 shows changes in  $G_F$  with changing  $\Psi_w$  of PEG and NaCl solutions with which the seeds were treated. Mean  $G_F$  of the seeds treated with deionized water (0 MPa) exceeded 89% for all the tested species (Figure 1 and Table 2). With decreasing  $\Psi_w$  of the media,  $G_F$  of all the tested species was initially unchanged, and then gradually decreased to near 0% (Figure 1). Both the  $\Psi_w$  at which  $G_F$  began to decrease and the  $\Psi_w$  at which  $G_F$  approached 0% varied among the species for both treatments (Figure 1). For PEG treatment, the  $\Psi_w$  at which  $G_F$  decreased by 50% was the lowest (approximately  $-1.6$  MPa) for *B. dasyphylla* and highest (approximately  $-0.6$  MPa) for *A. squarrosom*, and approximately  $-1.2$  MPa for the other three species.

There was not substantial difference in  $G_F$  of *A. squarrosom* between treatments with isotonic NaCl and PEG solutions. However, for the other four species, NaCl and PEG solutions of the same  $\Psi_w$  had different effects on  $G_F$ . Germination of *B. dasyphylla* was favoured more in NaCl than in isotonic PEG solution, while the other three species showed higher  $G_F$  in PEG than in isotonic NaCl (Figure 1). In *A. ordosica*, treatment with NaCl solutions of  $\Psi_w$  lower than  $-0.4$  MPa caused the death of most of the emerging radicles. *B. dasyphylla* was distinct from the other four species in that the seeds were able to germinate at higher salinity (Figure 1).

$\tau_{1/2}$  of the five species is shown in Figure 2 as a function of  $\Psi_w$  of the NaCl and PEG

solutions.  $\tau_{1/2}$  of the seeds treated with deionized water (0 MPa) was within the range 0.55 day (*B. dasyphylla*) to 3.3 days (*A. ordosica*) (Figure 2 and Table 2). As  $\Psi_w$  decreased,  $\tau_{1/2}$  increased in both media in all the tested species. The relationship between  $\tau_{1/2}$  and  $\Psi_w$  is steeper at lower values of  $\Psi_w$ , but in *A. squarrosom* it becomes less steep at very low values of  $\Psi_w$ . The increases in  $\tau_{1/2}$  with decreasing  $\Psi_w$  were most pronounced in *B. dasyphylla*:  $\tau_{1/2}$  of *B. dasyphylla* seeds treated with the NaCl solution at  $-2.4$  MPa was 16.9 days, which was about 30 times longer than that in deionized water.  $\tau_{1/2}$  of *B. dasyphylla* was longer at low  $\Psi_w$  in PEG than in the isotonic NaCl solutions, but *H. scoparium* seeds showed longer  $\tau_{1/2}$  in NaCl than in isotonic PEG solutions. For the other three species, no substantial difference in  $\tau_{1/2}$  was detected between NaCl and PEG treatments.

### Effects of pretreatment with NaCl or PEG solution of low $\Psi_w$ on seed germination

During pretreatment with  $-5.0$  MPa NaCl and  $-5.2$  MPa PEG solutions, seed germination was completely suppressed in all the five tested species, but transfer of these seeds to filter paper moistened with deionized water resulted in seed germination in most situations (Table 2). In Table 2,  $G_F$  and  $\tau_{1/2}$  of non-pretreated seeds in deionized water from the data in Figure 1 and Figure 2 are also shown. For *H. scoparium*,  $G_F$  was significantly reduced by pretreatment with PEG solution, and germination was completely inhibited by pretreatment with NaCl. For all the other four species, the  $G_F$  of PEG-pretreated seeds was not significantly ( $P = 0.05$ ) lower than those of non-pretreated seeds. NaCl pretreatment, however, significantly ( $P = 0.01$ ) lowered the  $G_F$  in *H. scoparium*, *A. ordosica* and *A. adscensionis*. On the other hand, *B. dasyphylla* and *A. squarrosom* did not show a significant difference ( $P = 0.05$ ) in  $G_F$  between NaCl pretreatment and no pretreatment.

For *B. dasyphylla* and *A. squarrosom*,  $\tau_{1/2}$  was significantly shortened by pretreatment with both the NaCl and PEG solutions. However,  $\tau_{1/2}$  of *A. ordosica* seeds was increased by the NaCl pretreatment.

Table 2. Effects of pretreatment with a NaCl or PEG solution on seed germination. After moistening with a solution of  $-5.0$  MPa NaCl or  $-5.2$  MPa PEG for 5 days (*Hedysarum scoparium*) or 10 days (other species), the seeds were transferred to filter paper moistened with deionized water, and thereafter the numbers of germinated seeds were counted. Final germination percentages ( $G_F$ ) and times needed for the germination percentages to reach 50% of final germination percentage ( $\tau_{1/2}$ ) are shown as mean  $\pm$  SE ( $n=4$ ).

Species	No pretreatment		PEG-pretreated		NaCl-pretreated	
	$G_F$	$\tau_{1/2}$	$G_F$	$\tau_{1/2}$	$G_F$	$\tau_{1/2}$
<i>Hedysarum scoparium</i>	95.0 $\pm$ 2.9	1.35 $\pm$ 0.05	78.8 $\pm$ 3.1**	1.43 $\pm$ 0.04	0.0 $\pm$ 0.0**	–
<i>Artemisia ordosica</i>	100.0 $\pm$ 0.0	3.28 $\pm$ 0.08	97.0 $\pm$ 1.0	3.08 $\pm$ 0.13	30.0 $\pm$ 6.6**	5.92 $\pm$ 0.80*
<i>Bassia dasyphylla</i>	89.0 $\pm$ 3.4	0.55 $\pm$ 0.01	96.0 $\pm$ 1.6	0.5 $\pm$ 0.0*	90.0 $\pm$ 3.8	0.50 $\pm$ 0.0*
<i>Agriophyllum squarrosom</i>	99.0 $\pm$ 1.0	3.12 $\pm$ 0.06	93.9 $\pm$ 2.1	2.10 $\pm$ 0.16**	96.8 $\pm$ 2.1	1.68 $\pm$ 0.11**
<i>Aristida adscensionis</i>	90.0 $\pm$ 2.6	1.68 $\pm$ 0.08	82.1 $\pm$ 5.0	1.58 $\pm$ 0.03	54.5 $\pm$ 4.7**	1.42 $\pm$ 0.02*

\*: significantly different from 'no pretreatment' at  $P=0.05$ , \*\*: significantly different from 'no pretreatment' at  $P=0.01$

### Effects of NaCl and PEG on water uptake by seeds

Figure 3 shows change over time in  $W_r$  of *H. scoparium* seeds which were treated with different types of solutions for 7 days and subsequently moistened with deionized water. Moistening the dry seeds caused initial rapid increases in  $W_r$  in all treatments. *H. scoparium* seeds treated with deionized water and  $-0.9$  MPa PEG solution began to germinate 1 day and 4 days after moistening, respectively, when the  $W_r$  was approximately

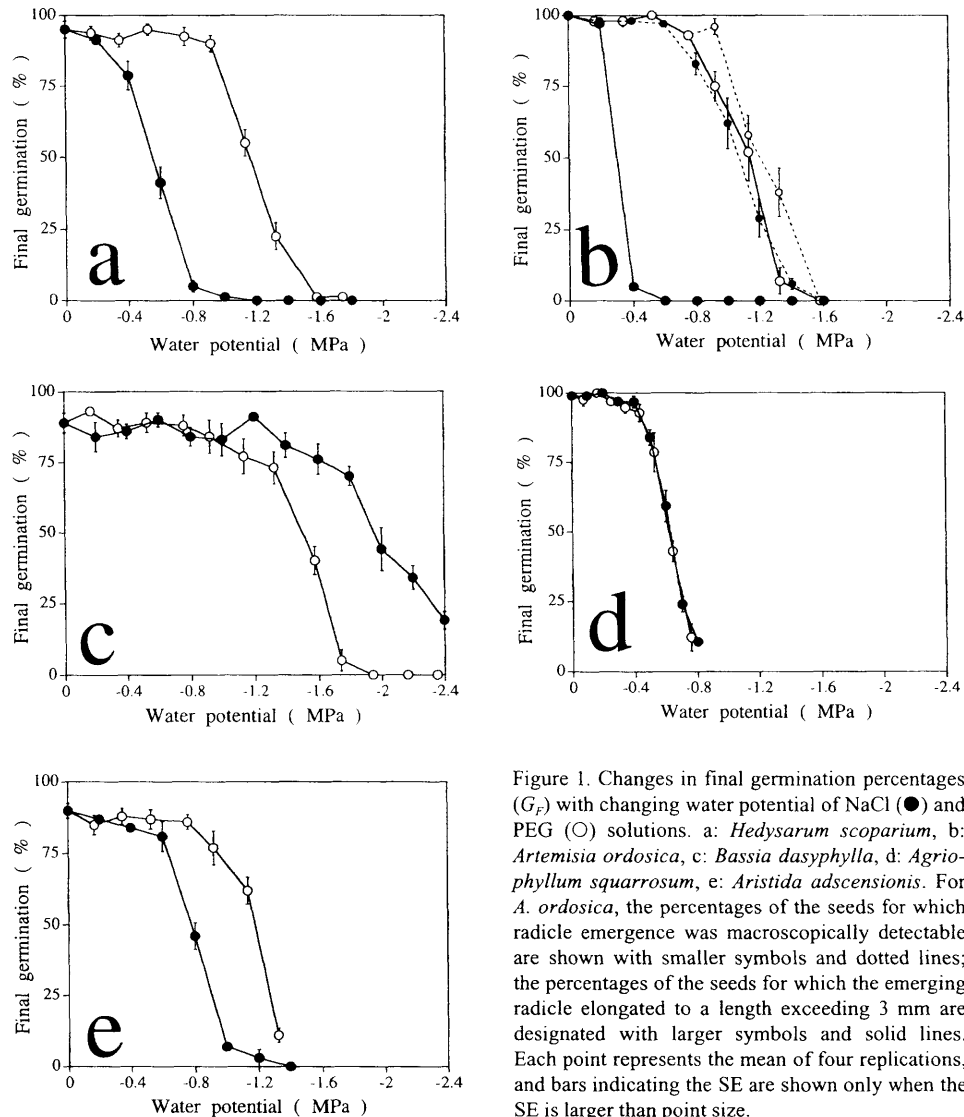


Figure 1. Changes in final germination percentages ( $G_r$ ) with changing water potential of NaCl (●) and PEG (○) solutions. a: *Hedysarum scoparium*, b: *Artemisia ordosica*, c: *Bassia dasyphylla*, d: *Agriophyllum squarrosum*, e: *Aristida adscensionis*. For *A. ordosica*, the percentages of the seeds for which radicle emergence was macroscopically detectable are shown with smaller symbols and dotted lines; the percentages of the seeds for which the emerging radicle elongated to a length exceeding 3 mm are designated with larger symbols and solid lines. Each point represents the mean of four replications, and bars indicating the SE are shown only when the SE is larger than point size.

1.1; the germinated seeds gained  $W_r$  quickly in deionized water, but slowly in  $-0.9$  MPa PEG solution. However, in all the other treatments, seed germination was completely suppressed for 7 days, and the  $W_r$  became steady after the initial rapid rises. The steady-state  $W_r$  of the PEG-treated seeds decreased significantly with decreasing  $\Psi_w$  (Figure 3a and 4a). For the NaCl treatments, there were not significant differences in steady-state  $W_r$  among the seeds treated with solutions of different  $\Psi_w$  (Figure 3b and 4a). When the

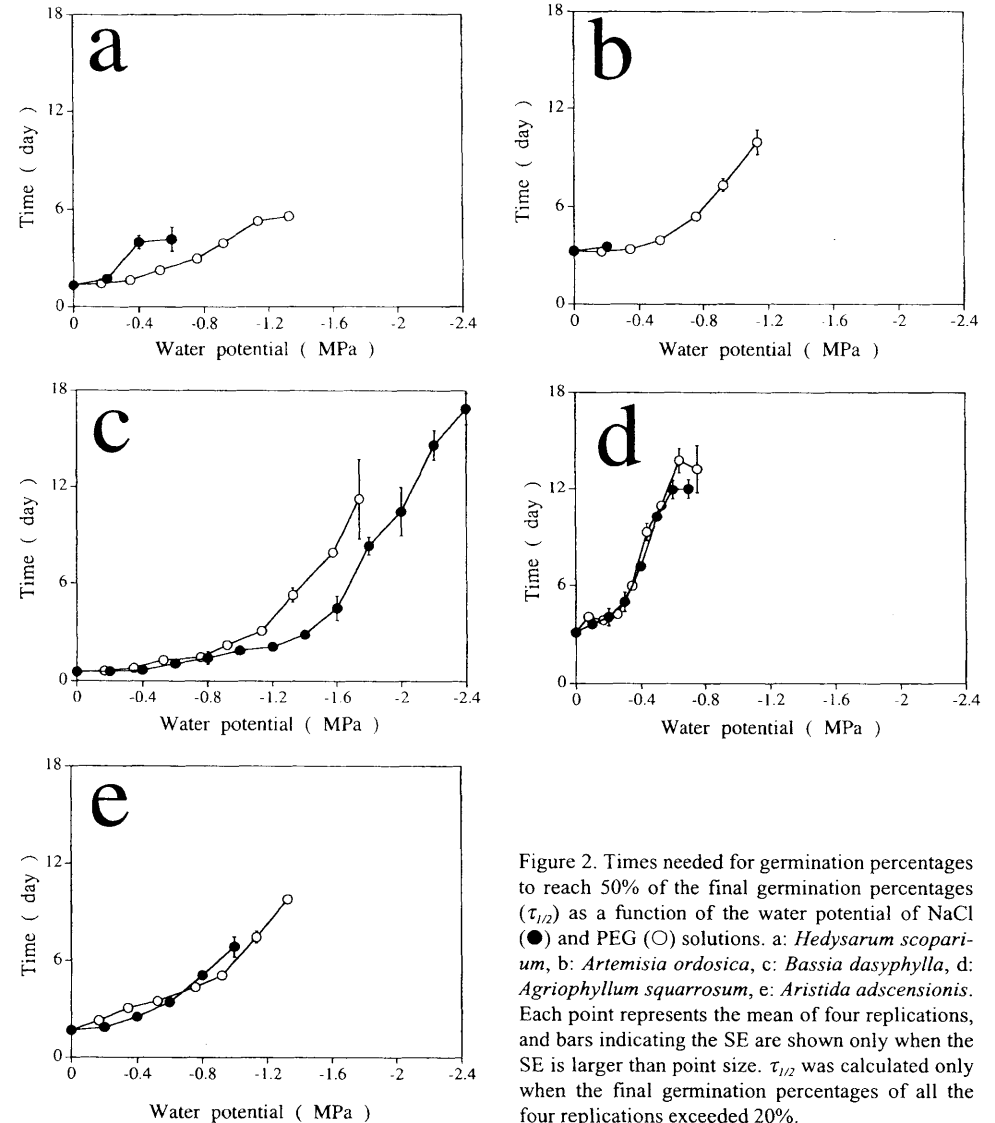


Figure 2. Times needed for germination percentages to reach 50% of the final germination percentages ( $\tau_{1/2}$ ) as a function of the water potential of NaCl (●) and PEG (○) solutions. a: *Hedysarum scoparium*, b: *Artemisia ordosica*, c: *Bassia dasyphylla*, d: *Agriophyllum squarrosum*, e: *Aristida adscensionis*. Each point represents the mean of four replications, and bars indicating the SE are shown only when the SE is larger than point size.  $\tau_{1/2}$  was calculated only when the final germination percentages of all the four replications exceeded 20%.

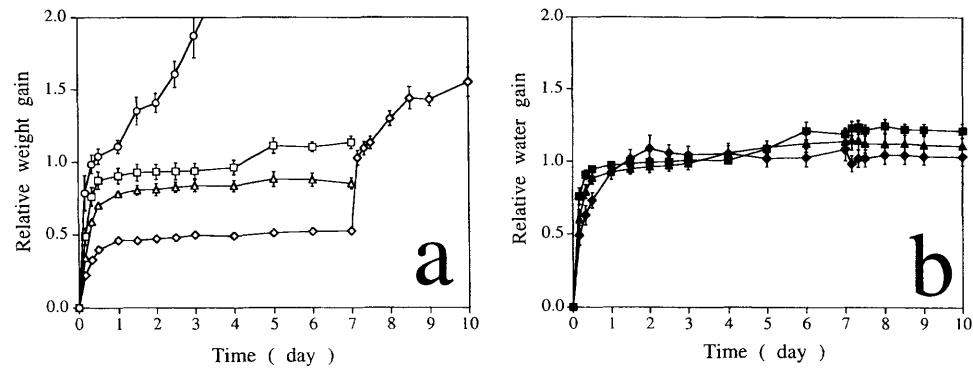


Figure 3. Relative weight gain ( $W_r$ ) over time of *Hedysarum scoparium* seeds on filter papers moistened with deionized water (O), a PEG solution of  $-0.9$  MPa (□),  $-1.9$  MPa (Δ) or  $-5.2$  MPa (◇) (a), or a NaCl solution of  $-1.0$  MPa (■),  $-2.0$  MPa (▲) or  $-5.0$  MPa (◆) (b). On the seventh day, seeds moistened with NaCl and  $-5.2$  MPa PEG solutions were transferred to filter papers moistened with deionized water. Each point represents the mean of six replications, and bars indicating the SE are shown only when the SE is larger than point size.

seeds treated with  $-5.2$  MPa PEG solution for 7 days were transferred to filter papers moistened with deionized water,  $W_r$  increased quickly and the seeds began to germinate, although the increase in  $W_r$  was slower than that of non-pretreated seeds in deionized water (Figure 3a). However, transfer of either of the NaCl-treated seeds to deionized water caused neither a rise in  $W_r$  nor seed germination (Figure 3b).

Figure 4 shows the  $W_r$  of the seeds of five species after moistening with NaCl or PEG solutions of different  $\Psi_w$  for 7 days. During the treatments, seed germination of all the five species was completely suppressed. For all the species, mean  $W_r$  was higher for the NaCl than for the isotonic PEG treatments. The difference in  $W_r$  between the treatments with isotonic NaCl and PEG solutions was conspicuously smaller for *A. squarrosus* than for the other four species.  $W_r$  of the PEG-treated seeds of all the species generally decreased with decreasing  $\Psi_w$ . Regression analysis to test the significance (Mendenhall and Sincich, 1995) of the relationship between  $W_r$  and  $\Psi_w$  revealed that  $W_r$  of PEG-treated seeds was significantly ( $P = 0.01$ ) affected by  $\Psi_w$  in all the five species. For NaCl-treated seeds, the effect of  $\Psi_w$  was significant ( $P = 0.01$ ) in *B. dasyphylla*, *A. squarrosus* and *A. adscensionis*, and  $W_r$  was not significantly ( $P = 0.05$ ) affected by  $\Psi_w$  in *H. scoparium* and *A. ordosica*.

## Discussion

NaCl and PEG solutions of the same  $\Psi_w$  caused different effects on the seed of all five tested species, indicating that the effect of NaCl on the seeds of these species was not solely osmotic. First, water uptake by the seeds of all species was more favoured in NaCl than in the isotonic PEG solutions (Figure 3 and 4), suggesting that NaCl entered the seeds and alleviated the osmotic inhibitory effects of the media on water uptake (Shar-

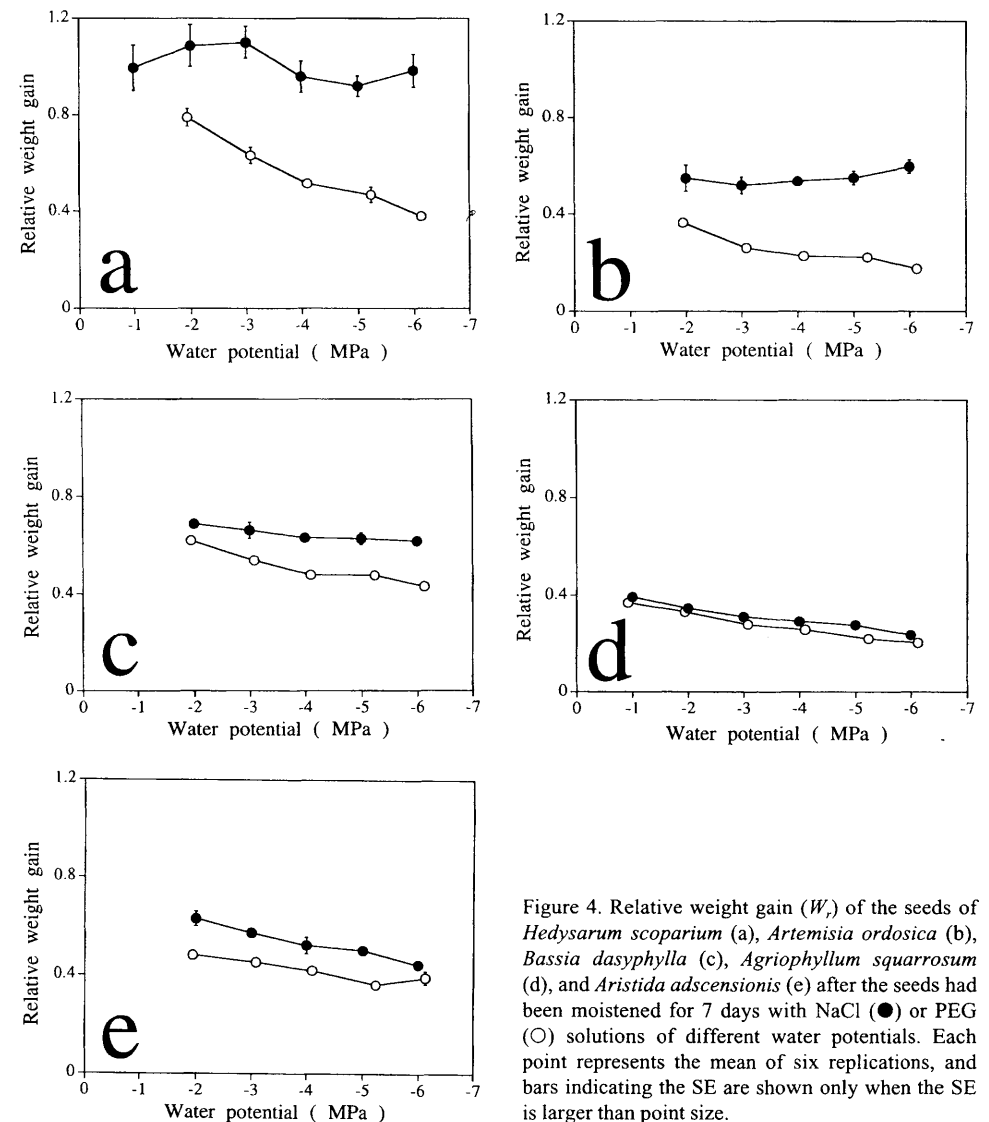


Figure 4. Relative weight gain ( $W_r$ ) of the seeds of *Hedysarum scoparium* (a), *Artemisia ordosica* (b), *Bassia dasyphylla* (c), *Agriophyllum squarrosus* (d), and *Aristida adscensionis* (e) after the seeds had been moistened for 7 days with NaCl (●) or PEG (○) solutions of different water potentials. Each point represents the mean of six replications, and bars indicating the SE are shown only when the SE is larger than point size.

ma, 1973; Romo and Eddleman, 1985).

For *H. scoparium*,  $W_r$  of approximately 1.1 would correspond to a sufficiently imbibed state at which radicle elongation was able to be initiated (Hegarty, 1978; Bliss *et al.*, 1986a,b; Bradford, 1995). Though  $-1.9$  MPa PEG solution was insufficient for attaining this level (Figure 3a and 4a), imbibition of the seeds in  $-5.0$  MPa NaCl solution resulted in this state (Figure 3b and 4a). This would indicate that a considerable amount

of NaCl entered the seeds of this species and lowered the internal  $\Psi_w$ , thus facilitating water uptake.

Among the seeds of the five species, the seeds of *H. scoparium* and *A. ordosica* seem to be highly permeable to NaCl because the  $W_r$  of the NaCl-treated seeds was not significantly changed with changing  $\Psi_w$ , and the difference in  $W_r$  between the isotonic NaCl and PEG treatments was larger. For the other species, the  $W_r$  of the NaCl-treated seeds decreased with decreasing  $\Psi_w$ , suggesting that NaCl permeation into these seeds was more limited. Especially for *A. squarrosom*, the difference in  $W_r$  between the NaCl and PEG treatments was small over the entire range of  $\Psi_w$  (Figure 4d). This suggests that NaCl permeation into the seeds of *A. squarrosom* is considerably lower than in the other species.

For *A. squarrosom* seeds, the effect of NaCl on  $G_F$  was substantially the same as that of isotonic PEG. Shorter  $\tau_{1/2}$  for NaCl than for PEG pretreatment (Table 2) and larger mean  $W_r$  of the NaCl-treated than of PEG-treated seeds (Figure 4d) suggest that water uptake by the seeds was favoured more in NaCl than in isotonic PEG solution. It seems that the slightly higher water uptake was not effective to increase  $G_F$  of this species. For *H. scoparium*, *A. ordosica* and *A. adscensionis*, despite greater water uptake by the seeds in NaCl than in the isotonic PEG solution,  $G_F$  was lower in NaCl than in isotonic PEG (Figure 1). This indicates that NaCl had a toxic effect on the seeds at the stage of either initial imbibition (Sharma, 1973; Hardegree and Emmerich, 1990), final radicle emergence (Bliss *et al.*, 1986b), or both.

The pretreatments with NaCl and PEG solutions of low  $\Psi_w$  produced different responses in the seeds of the five species (Table 2). PEG pretreatment brought about a significant decrease of  $G_F$  only in *H. scoparium* seeds. The reason for this response is unclear, but one possibility is that solute leakage from the seeds into the medium (Simon, 1974; Hendricks and Taylorson, 1976) reduced the germinability of the seeds. NaCl pretreatment caused a more marked decrease in  $G_F$  in *H. scoparium*, and significant  $G_F$  decreases in *A. ordosica* and *A. adscensionis*, for both of which PEG pretreatment did not affect  $G_F$ . The most probable explanation for this NaCl effect is that NaCl that has entered the seeds acts toxically on the embryos before the initiation of radicle emergence. However, considering that treatment with hypersaline solutions resulted in a highly imbibed state in the seeds of these species (Figure 4a, b and e), the possibility cannot be completely ruled out that radicle emergence did begin, but that the emerging radicles suffered NaCl toxicity in this solution and died before the radicle had become macroscopically detectable. In any event, hypersalinity irreversibly reduced the seed germinability of these three species. For *A. squarrosom*, lower permeation of NaCl through the seed coat would have protected the seeds from salt toxicity during the pretreatment with NaCl (Table 2). The maintenance of germinability of *B. dasyphylla* seeds in hypersaline solution would have resulted from their NaCl tolerance.

In *A. ordosica*, most of the radicles died in NaCl solutions of  $\Psi_w$  lower than  $-0.4$  MPa, showing that the seedling radicles of this species are exceptionally sensitive to NaCl. Furthermore, it is interesting that some radicles emerging from the seeds of this species treated with PEG solution died in the same media. Hegarty and Ross (1978) and

Ross and Hegarty (1979) have reported that for many species, germination is initiated only at a  $\Psi_w$  that is sufficient to allow the elongation of emerging radicles. This seems to hold true in the present study for the other four tested species, where all the emerging radicles macroscopically detectable continued to elongate in PEG solutions until they exceeded 3 or 5 mm in length, *A. ordosica* being the only exception. However, the deleterious effect of NaCl solution on the radicles of *A. ordosica* was much more marked than that of the isotonic PEG solution (Figure 1b). Indeed,  $-0.4$  MPa NaCl solution killed most of the radicles, while PEG solution of  $\Psi_w$  higher than  $-0.8$  MPa did not affect them (Figure 1b). The effect of PEG solution on the *A. ordosica* radicles would be osmotic; that is, the solution would have caused dehydration of the radicles. Then, the more marked deleterious effect of NaCl on the radicles suggests salt-specific toxicity. When seeds of which the emerging radicles died without elongation were counted as having germinated (dotted lines in Figure 1b), the difference in  $G_F$  between the isotonic NaCl and PEG treatments was smaller. Moreover, it is possible that some radicles having emerged from the seeds died before reaching a visibly detectable size. Similarly, for *A. adscensionis*, NaCl solutions of  $-0.8$  to  $-1.2$  MPa seemed to act more toxically than that of  $-5.0$  MPa. For example, treatment with  $-1.0$  MPa NaCl solution, where osmotic  $G_F$  depression was not conspicuous as seen in the  $G_F$  of PEG-treated seeds at this  $\Psi_w$  (Figure 2e), decreased the mean  $G_F$  to 7%, whereas pretreatment with  $-5.0$  MPa NaCl solution for 10 days decreased the mean  $G_F$  only to 54.5% (Table 2). These results suggest that NaCl has injurious effects on radicles emerging from the seeds of *A. ordosica* and *A. adscensionis*, and decreases the apparent  $G_F$  of these species. According to Bliss *et al.* (1986a,b), NaCl inhibits the germination of barley seeds only osmotically during their initial imbibition stage, and acts toxically only by injuring the emerging radicles. In this study, *A. ordosica* and *A. adscensionis* seemed to show marked inhibition of germination at the stage of radicle emergence, although their seeds seemed to suffer from salt toxicity also at the imbibition stage.

In this study, there was no evidence that NaCl exerted any toxic effects on the seeds of *B. dasyphylla* and *A. squarrosom*. For *A. squarrosom*, the seeds seem to be protected from salt toxicity by lower salt permeability through the seed coat, and germination of the seeds only at higher  $\Psi_w$  would protect the emerging radicles from suffering from salt toxicity. On the other hand, the seed coat of *B. dasyphylla* seem to be considerably permeable to NaCl (Figure 4c). However, seed germinability was not affected after moistening with hypersaline solution (Table 2). Moreover, the seeds were able to germinate in NaCl solutions with  $\Psi_w$  higher than  $-2.0$  MPa (Figure 1c), at which the germination of seeds of the other four species was completely inhibited, and the radicles survived in these solutions. Thus, the tissues of this species seem to have a conspicuously higher tolerance to salinity than those of the other four species.

One important factor that determines whether seeds suffer from salt toxicity would be salt permeation through the seed coat. For seeds with seed coats showing low permeability to salt, the embryos would be protected from salt toxicity before the radicles emerged from the seed coats (e.g. *A. squarrosom* seeds), while the embryos in the seeds with seed coats that are highly permeable to salt would tend to suffer from salt toxicity

before radicle emergence (e.g. *H. scoparium* seeds). After radicle elongation has been initiated, radicles emerging from seed coats are directly exposed to salt in the external medium, but this situation is brought about only when the salinity in the external medium is decreased. In any event, the embryos would suffer from salt toxicity inside and/or outside the seed coat. Therefore, the salt tolerance of embryo tissues would be another important factor determining the susceptibility of seeds to salinity. The seeds of different species would show differing degrees of susceptibility to salt toxicity both before and after radicle emergence, depending on the salt permeability of the seed coat, and accordingly would be inhibited from establishing themselves in saline environments either before or after the start of germination.

The seeds of the five species collected from the same nonsaline habitat showed a wide variety of adaptability to NaCl in the germination stage. Although this study examined only the initial stage of development, a wide variety in salt susceptibility of the seedling radicles (e.g. *B. dasyphylla* vs. *A. ordosica*) suggests a wide variety in salt susceptibility of the tissue among the different species. If a habitat of these species is salinized, salt-tolerant species (especially, *B. dasyphylla*) will dominate there over the other salt-susceptible species (especially, *A. ordosica*). It seems that some species distributed naturally in nonsaline locations are potentially salt-tolerant and can be candidates to be utilized for the rehabilitation of vegetation in salinized locations.

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#### References

- Bal, A. R. and Chattopadhyay, N. C. (1985). Effect of NaCl and PEG 6000 on germination and seedling growth of rice (*Oryza sativa* L.), *Biologia Plantarum*, **27**, 65–69.
- Bliss, R. D., Platt-Aloia, K. A. and Thomson, W. W. (1986a). Osmotic sensitivity in relation to salt sensitivity in germinating barley seeds, *Plant, Cell and Environment*, **9**, 721–725.
- Bliss, R. D., Platt-Aloia, K. A. and Thomson, W. W. (1986b). The inhibitory effect of NaCl on barley germination, *Plant, Cell and Environment*, **9**, 727–733.
- Boyer, J. S. and Knipling, E. B. (1965). Isopiestic technique for measuring leaf water potentials with a thermocouple psychrometer, *Proceedings of the National Academy of Science of the United States of America*, **54**, 1044–1051.
- Bradford, K. J. (1995). Water relations in seed germination. In *Seed Development and Germination*, (eds. J. Kigel and G. Galili), pp. 351–396, Marcel Dekker, New York.
- Cluff, G. J., Evans, R. A. and Young, J. A. (1983). Desert saltgrass seed germination and seedbed ecology, *Journal of Range Management*, **36**, 419–422.
- Hardegreve, S. P. and Emmerich, W. E. (1990). Partitioning water potential and specific salt effect on seed germination of four grasses, *Annals of Botany*, **66**, 587–585.
- 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.
- Hegarty, T. W. and Ross, H. A. (1978). Differential sensitivity to moisture stress of seed germination and

- seedling radicle growth in calabrese (*Brassica oleracea* var. *italica*) and cress (*Lepidium sativum*), *Annals of Botany*, **42**, 1003–1005.
- Hendricks, S. B. and Taylorson, R. B. (1976). Variation in germination and amino acid leakage of seeds with temperature related to membrane phase change, *Plant Physiology*, **58**, 7–11.
- Lang, A. G. R. (1967). Osmotic coefficients and water potentials of sodium chloride solutions from 0 to 40°C, *Australian Journal of Chemistry*, **20**, 2017–2023.
- Lanzhou Institute of Desert Research (1985). *Zhongguo shamo zhiwuzhi*, vol. 1–3. [Chinese desert flora, vol. 1–3]. Science Press, Beijing.
- Manohar, M. S. (1966). Effects of “osmotic” systems on germination of pea (*Pisum sativum* L.), *Planta*, **71**, 81–86.
- Manohar, M. S. and Heydecker, W. (1964). Effects of water potential on germination of pea seeds, *Nature*, **202**, 22–24.
- Mendenhall, W. and Sincich, T. (1995). *Statistics for engineering and the sciences*, 4th ed., pp. 553–557, Prentice-Hall, Inc., New Jersey.
- Michel, B. E. and Kauffman, M. R. (1973). The osmotic potential of polyethylene glycol 6000, *Plant Physiology*, **51**, 914–916.
- Myers, B. A. and Morgan, W. C. (1989). Germination of the salt-tolerant grass *Diplachne fusca*. II. Salinity responses, *Australian Journal of Botany*, **37**, 239–251.
- Naidoo, G. and Naiker, K. (1992). Seed germination in the coastal halophytes *Triglochin bulbosa* and *Triglochin striata*, *Aquatic Botany*, **42**, 217–229.
- Redmann, R. E. (1974). Osmotic and specific ion effects on the germination of alfalfa, *Canadian Journal of Botany*, **52**, 803–808.
- Romo, J. T. and Eddleman, L. E. (1985). Germination response of greasewood (*Sarcobatus vermiculatus*) to temperature, water potential and specific ions, *Journal of Range Management*, **38**, 117–120.
- Ross, H. A. and Hegarty, T. W. (1979). Sensitivity of seed germination and seedling radicle growth to moisture stress in some vegetative crop species, *Annals of Botany*, **43**, 241–243.
- Saleki, R., Young, P. G. and Lefebvre, D. D. (1993). Mutants of *Arabidopsis thaliana* capable of germination under saline conditions, *Plant Physiology*, **101**, 839–845.
- Sharma, M. L. (1973). Simulation of drought and its effect on germination of five pasture species, *Agronomy Journal*, **65**, 982–987.
- Simon, E. W. (1974). Phospholipids and plant membrane permeability, *New Phytologist*, **73**, 377–420.
- Torres-Schumann, S., Godoy, J. A., Pintor-Taro, J. A., Moreno, F. J., Rodrigo, R. M., Garcia-Herdugo, G. (1989). NaCl effects on tomato seed germination, cell activity and ion allocation, *Journal of Plant Physiology*, **135**, 228–232.
- Ungar, I. A. (1991). *Ecophysiology of vascular halophytes*, pp. 9–48, CRC Press, Boca Raton.
- Ungar, I. A. (1995). Seed germination and seed-bank ecology in halophytes. In *Seed Development and Germination*, (eds. J. Kigel and G. Galili), pp. 599–628, Marcel Dekker, New York.