

Alleviatory effects of calcium on the toxicity of sodium, potassium and magnesium chlorides to seed germination in three non-halophytes

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Abstract

Saline soils contain numerous salts with varying impact on seed germination. Seeds of three non-halophytic species found in Chinese sandy deserts (*Artemisia ordosica*, *Aristida adscensionis* and *Bassia dasyphylla*) were incubated in salt solutions (NaCl, KCl or MgCl₂, each with or without CaCl₂) at 20°C in the dark. The effects of each salt on the percentage of seeds from which visibly detectable radicles emerged, and the percentage of seeds with emerging radicles surviving to a length of at least 4 mm, were examined. NaCl, KCl and MgCl₂ were toxic to emerging radicles in all three species, but before radicle emergence these salts reduced seed germinability only in *A. ordosica*. The toxic effects of each salt on the radicles were alleviated in all three species by the addition of low concentrations of CaCl₂. MgCl₂ was more toxic than NaCl and KCl, and the concentration of CaCl₂ needed to alleviate the toxicity of MgCl₂ was several times higher than that required for alleviation of NaCl or KCl toxicity. These results suggest that Ca²⁺ present in saline soils alleviates the toxic effects of other salt components on seed germination, and that the results of germination experiments with a single salt are not always applicable to field conditions.

Keywords: calcium, non-halophyte, magnesium, potassium, salt toxicity, seed germination, sodium, *Artemisia ordosica*, *Aristida adscensionis*, *Bassia dasyphylla*

Introduction

The action of salts on seed germination differs greatly among species. The effect of salts on germination is mainly osmotic in some species (Cluff *et al.*, 1983; Myers and Morgan, 1989; Naidoo and Naicker, 1992), but salts also exert toxic effects on seeds (Ungar, 1978, 1995), although the mechanism of salt toxicity is not understood. Seeds suffer from salt toxicity before and/or after germination (protrusion of the radicle or plumule through the seed coat) begins (Ungar, 1978, 1995; Tobe *et al.*, 1999). For those species in which the seed coat is permeable to salts, moistening of the seeds with saline solutions can cause loss of germinability. However, for other species, the embryo may be protected from salt toxicity by the seed coat before the seed germinates, and is injured by salts only after it protrudes from the seed coat (Tobe *et al.*, 1999).

Saline soils contain different salt components that affect seed germination differently (Redmann, 1974; Hardegree and Emmerich, 1990; Pujol *et al.*, 2000; Tobe *et al.*, 2002). Of the salt components in saline soils, Ca²⁺ is noteworthy because it alleviates the toxicity of other salts at both germination (Bliss *et al.*, 1986; Marcar, 1986; Hamada, 1994; Hosseini *et al.*, 2002) and later developmental stages (LaHaye and Epstein, 1969; Cramer *et al.*, 1986; Kurth *et al.*, 1986; Suhayda *et al.*, 1992; Colmer *et al.*, 1996; Kinraide, 1999). Recently, in a halophyte, *Kalidium caspicum*, we found that (1) Na⁺ and Mg²⁺ have toxic effects on the radicle, (2) the toxicity of Mg²⁺ is several times stronger than that of Na⁺, and (3) the toxicity of Na⁺ and Mg²⁺ is markedly alleviated by low-concentration Ca²⁺ (Tobe *et al.*, 2002). According to some researchers (Cramer *et al.*, 1985; Kent and Läuchli, 1985; Lynch *et al.*, 1987; Marschner, 1995), the toxicity of salts to plant tissues is attributable to the displacement of Ca²⁺ bound to the external surface of the plasma

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membrane with metal cations and the subsequent impairment of membrane integrity and permeability; Ca^{2+} in the external medium may alleviate toxicity by restoring the cation-displaced Ca^{2+} on the membrane. It is not clear whether salt toxicity to seed germination in a wide range of species is attributable to the replacement of membrane-bound Ca^{2+} .

Since salinization of the soil is a serious problem in arid and semi-arid regions, it is important to gather information about salinity effects upon the germination of non-halophytes distributed in these regions. This will enable prediction of the effects of soil salinization on vegetation and the introduction of agronomically important non-halophytes to these regions. In a previous study (Tobe *et al.*, 1999), we examined the responses of seed germination to NaCl in five non-halophytes from Chinese sandy deserts, and found that the effects of NaCl differ greatly among species. The present study further investigated how different salts affected seed germination in some of these species: *Artemisia ordosica* Krasch. (*Compositae*, semi-shrub), *Aristida adscensionis* L. (*Gramineae*, annual) and *Bassia dasyphylla* (Fisch. et Mey.) O. Kuntze (*Chenopodiaceae*, annual). The effects of NaCl, KCl and MgCl_2 on germination were compared, and we investigated whether CaCl_2 ameliorated the effects of these salts on germination. We also investigated whether salts caused toxic effects before or after radicle emergence.

Materials and methods

Materials

Seeds of three test species were collected from non-saline sand dunes at Shapotou, China (latitude $37^{\circ}26'N$, longitude $104^{\circ}57'E$). Annual precipitation and annual mean temperature in Shapotou are 10.5°C and 188 mm (means from 1990–1995), respectively. Seeds were collected in October 1995 for *A. ordosica*, June 1997 for *A. adscensionis* and in November 1995 for *B. dasyphylla*. Mean seed weights of *A. ordosica*, *A. adscensionis* and *B. dasyphylla* were 0.27, 1.30 and 0.97 mg, respectively. Seeds were stored dry at $0\text{--}5^{\circ}\text{C}$. During the storage period, no indications of seed dormancy or degradation of seed germinability were observed. Experiments were carried out between January 2001 and March 2002.

Methods

Replicates of 25 seeds were sown on three layers of filter paper (Toyo, No. 1) in 90-mm plastic Petri dishes. About 10 ml of deionized water or salt solution was added to each Petri dish, so that about half the volume of each seed was immersed. Salt

solutions were prepared by dissolving reagent-grade salt(s) in deionized water, and concentrations are expressed on a millimolar basis. The Petri dishes were covered with lids, and the seeds were incubated for 10 or 15 d in the dark at 20°C (optimum conditions; K. Tobe, unpublished).

Seeds were observed daily in dim light through a magnifying glass with a scale. Seeds with radicles longer than 4 mm were counted daily, and these seeds were then discarded. About two-thirds of the solution volume in each Petri dish was replaced daily to reduce changes in solute concentration. Because there was no detectable decrease in the volume of solution in each dish due to evaporation or seed water uptake, the daily solution replacement would have sufficed to keep the solute concentration constant in the dish. On the final day of incubation, the number of radicles shorter than 4 mm was also counted, and we determined the final percentage of seedlings with visible radicle emergence (G_F) and the final percentage of seedlings with radicles longer than 4 mm (S_F). Each treatment was replicated four times.

Experiment 1: Interactive effects of CaCl_2 and other salts on G_F and S_F

Seeds were treated with deionized water; NaCl or KCl solutions (240–540 mM for *B. dasyphylla* and 60–300 mM for the other two species) with or without 5 mM CaCl_2 ; or an MgCl_2 solution (160–360 mM for *B. dasyphylla* and 40–200 mM for the other two species) with or without 25 mM CaCl_2 . Preliminary experiments showed that the concentration of CaCl_2 needed to alleviate the toxicity of MgCl_2 was about five times higher than that required for NaCl or KCl. After 15 d, G_F and S_F were determined.

Experiment 2: Effects of CaCl_2 concentration on G_F and S_F of salt-treated seeds

Seeds were treated with NaCl or KCl solutions (120, 180 and 300 mM for *A. ordosica*, *A. adscensionis* and *B. dasyphylla*, respectively) differing in the concentration of CaCl_2 (0–5 mM); MgCl_2 solution (80, 120 and 200 mM, respectively) differing in the concentration of CaCl_2 (0–25 mM); or CaCl_2 solution (80, 120 and 200 mM, respectively). After 15 d, G_F and S_F were determined.

Experiment 3: Effects of pretreatment with a salt solution on seed germinability

Seeds were treated with either 3000 mM NaCl, 3000 mM KCl, 2000 mM MgCl_2 or 2000 mM CaCl_2 for 10 d in Petri dishes. Seeds were then washed with deionized water and transferred to new Petri dishes containing deionized water and incubated for a

further 10 d, when G_F and S_F were determined. Additionally, in *A. adscensionis* the effect of pretreating seeds with either 240 mM NaCl, 240 mM KCl or 160 mM $MgCl_2$ for 10 d was examined in the same manner.

Statistical analysis

Student's *t*-test was used to test the difference between two means. One-way ANOVA was used for comparison of multiple means. Percentage values were arcsine-transformed before statistical analysis.

Results

Effects of $CaCl_2$ on the responses of G_F and S_F to salt treatments

In all salt treatments and species tested, both radicle emergence and elongation were increasingly retarded with increasing salt concentration in the media (Fig. 1: some typical treatments). Emerging radicles sometimes turned yellow or black; many ceased to elongate before their lengths reached 4 mm. Both S_F and G_F decreased with increasing salt concentration at the higher salt concentrations (Fig. 2). In all salt treatments for *B. dasyphylla*, S_F and G_F decreased at higher salinities than for the other two species. In all salt treatments and species tested, S_F was considerably higher in 5 or 25 mM $CaCl_2$ (+ $CaCl_2$) than in 0 mM $CaCl_2$ (- $CaCl_2$) (Fig. 2). In the absence of $CaCl_2$, $MgCl_2$ had more suppressive effects on S_F than NaCl or KCl in all species, especially in *A. ordosica*: for example, in - $CaCl_2$, the salt concentrations needed to suppress S_F to zero in *A. ordosica* were 40, 180 and 120 mM for $MgCl_2$, NaCl and KCl treatments, respectively.

Effects of $CaCl_2$ concentration on G_F and S_F of salt-treated seeds

To investigate quantitatively the relationship between germination, salinity and $CaCl_2$ in the medium, changes in G_F and S_F with increasing $CaCl_2$ concentration in NaCl, KCl or $MgCl_2$ solutions were

examined (Fig. 3). The effects of $CaCl_2$ alone on G_F and S_F are presented in Table 1. The concentrations of NaCl, KCl and $MgCl_2$ used were those that had led to conspicuous differences in S_F between - $CaCl_2$ and + $CaCl_2$ treatments in the previous experiment (Fig. 2), but were low enough not to cause serious osmotic effects on G_F or S_F (see + $CaCl_2$ in Fig. 2). At lower $CaCl_2$ concentrations in the NaCl, KCl and $MgCl_2$ treatments, some emerging radicles appeared yellow or black and unhealthy, and ceased to elongate before they reached 4 mm; however, in the $CaCl_2$ -alone treatment group (Table 1) and at higher $CaCl_2$ concentrations in the NaCl, KCl and $MgCl_2$ treatments, most radicles were white, and more than 90% of them continued to elongate to over 4 mm, except in the $MgCl_2$ -treated *A. ordosica* seeds (89%) (Fig. 3). No adverse effects of $CaCl_2$ alone were observed in any species (Table 1). G_F was not dependent on $CaCl_2$ concentration in the NaCl, KCl or $MgCl_2$ treatments in *A. ordosica* or *B. dasyphylla* ($P > 0.05$) (Fig. 3), but was dependent on $CaCl_2$ concentration in all of these treatments for *A. adscensionis* ($P < 0.001$). S_F was dependent on $CaCl_2$ concentration in all of these treatments for all species ($P < 0.001$).

In *A. adscensionis* and *B. dasyphylla*, the $CaCl_2$ concentration needed to give an S_F higher than 90% of G_F in $MgCl_2$ -treated seeds was 5 mM, for which the Ca^{2+}/Mg^{2+} concentration ratio (mol/mol) in the medium corresponds to 0.042 (*A. adscensionis*) and 0.025 (*B. dasyphylla*). S_F of *A. ordosica* was 89% of G_F even at 12.5 mM $CaCl_2$ ($Ca^{2+}/Mg^{2+} = 0.16$). On the other hand, the $CaCl_2$ concentrations needed to give an S_F higher than 90% of G_F in NaCl treatment were 0.5 mM ($Ca^{2+}/Na^+ = 0.004$), 1.0 mM ($Ca^{2+}/Na^+ = 0.006$) and 0.2 mM ($Ca^{2+}/Na^+ = 0.001$) for *A. ordosica*, *A. adscensionis* and *B. dasyphylla*, respectively. KCl-treated seeds required about twice the concentration of $CaCl_2$ to raise S_F than did NaCl-treated seeds in all species.

Effects of pretreatment with a salt solution on seed germinability

During pretreatment with a high-concentration salt solution, radicle emergence was not detected for any

Table 1. Final percentages of seeds with a visible radicle (G_F) or seedlings with a radicle longer than 4 mm (S_F), when incubated with 80 mM (*Artemisia ordosica*), 120 mM (*Aristida adscensionis*) or 200 mM (*Bassia dasyphylla*) $CaCl_2$ for 15 d. Data are the means of four replications; SE in parentheses

Species	G_F	S_F	S_F/G_F
<i>Artemisia ordosica</i>	87 (6.4)	84 (6.4)	0.97 (0.024)
<i>Aristida adscensionis</i>	77 (2.3)	70 (1.8)	0.91 (0.039)
<i>Bassia dasyphylla</i>	91 (1.8)	90 (1.9)	0.99 (0.011)

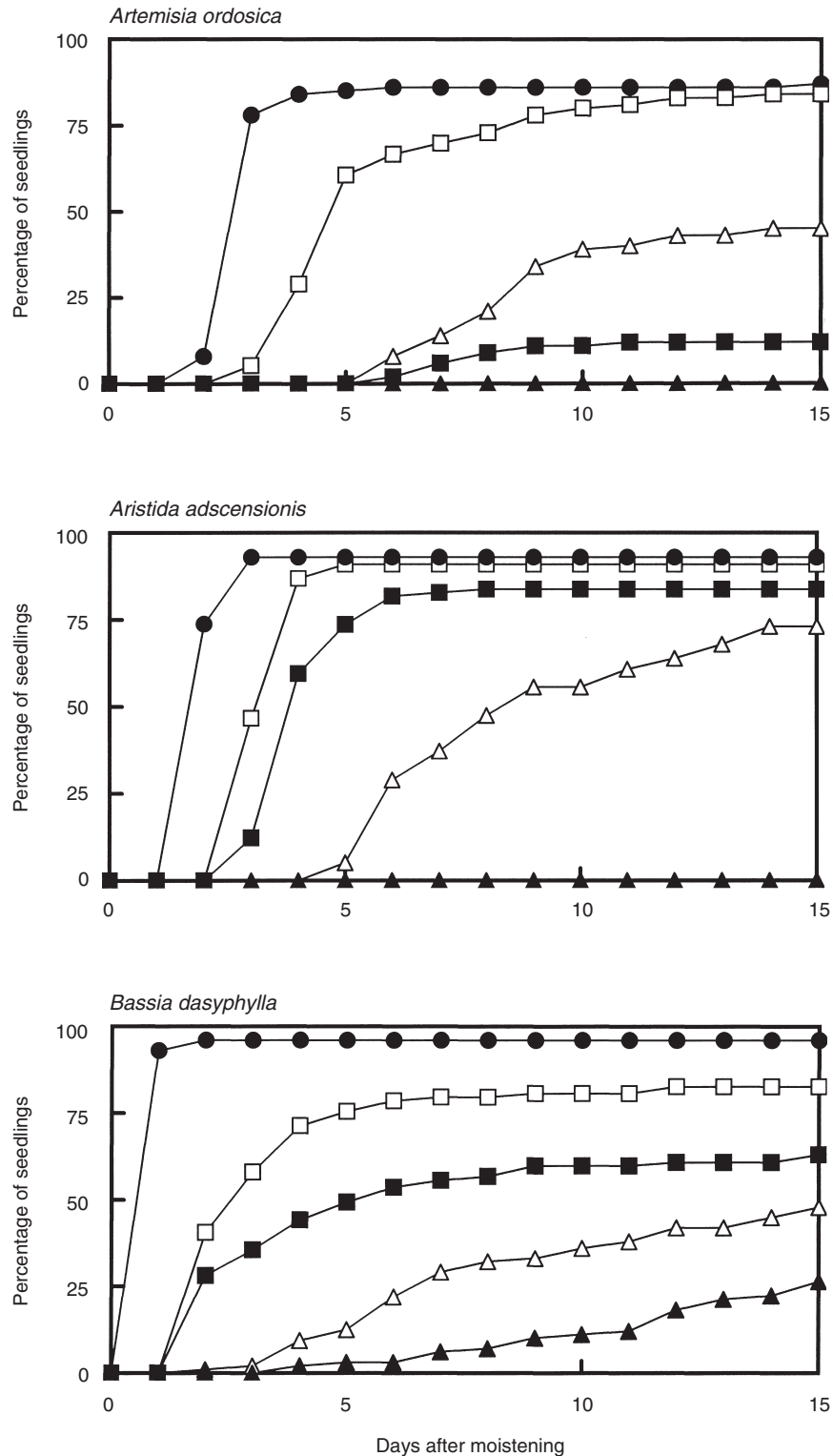


Figure 1. Changes in the percentage of seedlings with radicles longer than 4 mm (S_F). Seeds were treated with deionized water or a NaCl solution with 0 mM CaCl₂ (closed symbols) or 5 mM CaCl₂ (open symbols). ●, Deionized water; ■, □, 120 mM (*Artemisia ordosica* and *Aristida adscensionis*) or 300 mM (*Bassia dasyphylla*) NaCl; ▲, △, 240 mM (*A. ordosica* and *A. adscensionis*) or 420 mM (*B. dasyphylla*) NaCl. Each point represents the mean of four replications.

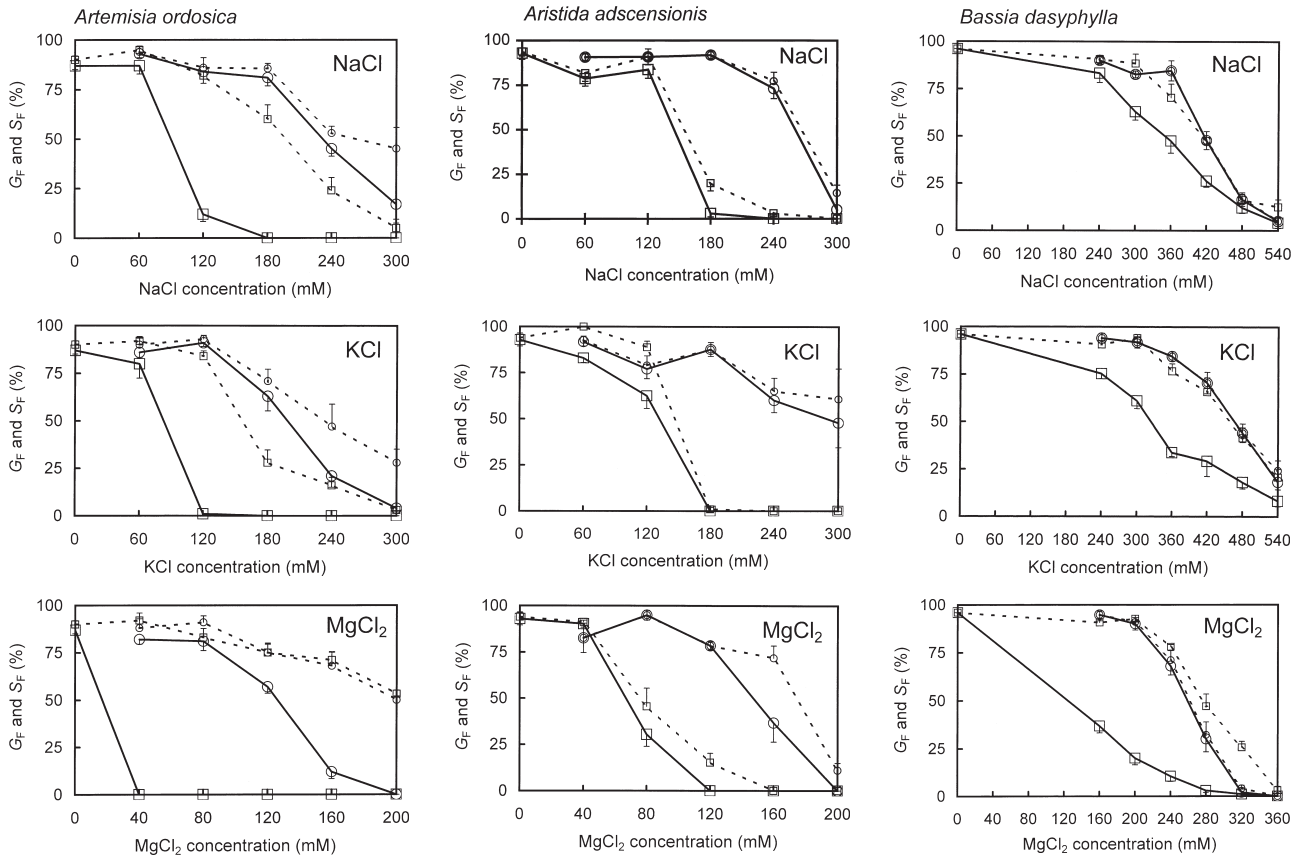


Figure 2. Effects of NaCl, KCl and MgCl₂ concentration on final percentages of seeds for which radicle emergence was visibly detectable (G_F ; small symbols connected with dotted line) and final percentage of seedlings with radicles longer than 4 mm (S_F ; large symbols connected with solid line). CaCl₂ was either added (○, 5 mM CaCl₂ for NaCl and KCl treatments or 25 mM CaCl₂ for MgCl₂ treatment) or not added (□, 0 mM CaCl₂) to the solution. Each point represents the mean of four replications; bars indicating SE are shown only where SE is larger than point size.

species. S_F and G_F were significantly and markedly decreased after pretreatment with any salt in *A. ordosica* ($P < 0.001$) (Table 2). In *A. adscensionis* and *B. dasyphylla*, pretreatment with any salt had less noticeable effects on subsequent seed germinability.

In *A. adscensionis* the effect of pretreating seeds with low-concentration salt (240 mM NaCl, 240 mM KCl or 160 mM MgCl₂) was examined. During pretreatment with any salt, radicle emergence was not visibly detected; after transfer of the seeds to

Table 2. Effect of pretreatment with a high-concentration salt solution on seed germination. After treatment with a solution for 10 d, the seeds were washed and incubated with deionized water for a further 10 d. Final germination percentages of seeds with a visible radicle (G_F) or seedlings with radicles longer than 4 mm (S_F) are presented. Data are the means of four replications; SE in parentheses

		No pretreatment	NaCl 3000 mM	MgCl ₂ 2000 mM	KCl 3000 mM	CaCl ₂ 2000 mM
<i>Artemisia ordosica</i>	G_F	90 (2.0)	10 (5.3)***	13 (2.5)***	1 (1.0)***	24 (2.8)***
	S_F	87 (1.9)	3 (1.9)***	11 (2.5)***	0 (0.0)***	8 (1.6)***
<i>Aristida adscensionis</i>	G_F	94 (2.6)	76 (4.5)*	85 (1.9)	85 (3.7)	90 (3.8)
	S_F	93 (3.4)	73 (4.4)*	85 (1.9)	83 (4.4)	90 (3.8)
<i>Bassia dasyphylla</i>	G_F	96 (0.0)	97 (1.0)	89 (1.9)**	92 (2.8)	98 (1.2)
	S_F	96 (0.0)	97 (1.0)	86 (3.8)*	92 (2.8)	97 (1.9)

*, Significantly different from 'no pretreatment' at $P < 0.05$; **, significantly different from 'no pretreatment' at $P < 0.01$; ***, significantly different from 'no pretreatment' at $P < 0.001$.

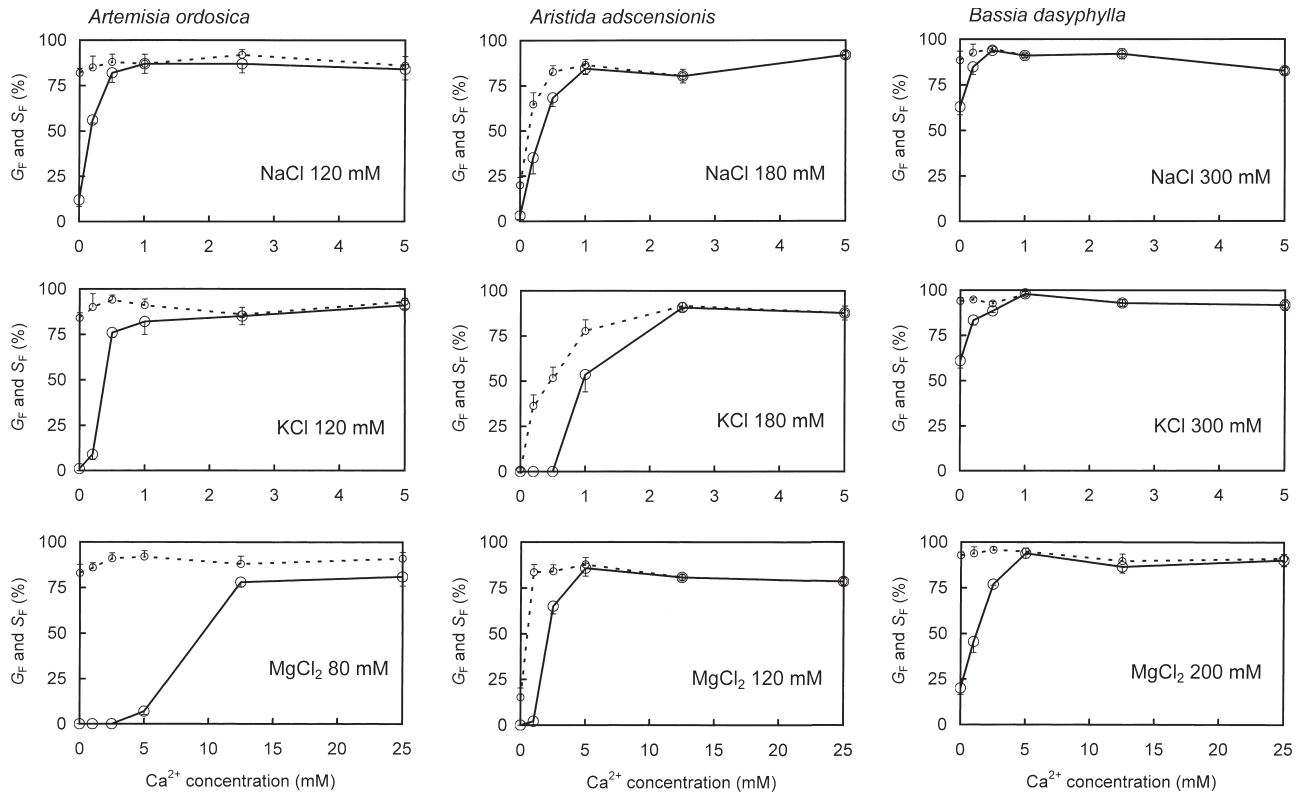


Figure 3. Effects of CaCl_2 concentration on final percentages of seeds for which radicle emergence was visibly detectable (G_F ; small open circles connected with dotted line) and final percentages of seedlings with radicles longer than 4 mm (S_F ; large open circles connected with solid line). Note that the scale of the horizontal axis for MgCl_2 differs from those for the other two salts. Each point represents the mean of four replications; bars indicating SE are shown only where SE is larger than point size.

deionized water, both S_F and G_F were very low in all salt-pretreatment groups: S_F values of seeds pretreated with NaCl, MgCl_2 and KCl were 6 ± 2.7 , 2 ± 1.1 and 4 ± 2.4 (%; mean \pm SE), respectively, and G_F was the same value as S_F in each treatment.

Discussion

NaCl, KCl and MgCl_2 have toxic effects on seeds and/or radicles of *A. ordosica*, *A. adscensionis* and *B. dasyphylla*. The considerably lower S_F in $-\text{CaCl}_2$ than in $+\text{CaCl}_2$ treatments (Fig. 2) indicates that CaCl_2 alleviates this toxicity. Different species suffer from salt toxicity at different stages of germination. The high S_F of *A. adscensionis* seeds pretreated with high-concentration salts (Table 2) suggests that *A. adscensionis* seeds are fairly insensitive to salinity exposure before radicle emergence, presumably because the embryo is protected by a salt-impermeable seed coat (Tobe *et al.*, 1999). However, the large difference in G_F of *A. adscensionis* between $+\text{CaCl}_2$ and $-\text{CaCl}_2$ treatments (Fig. 2) and marked

suppression of G_F and S_F by the pretreatment with low-concentration salts indicate that radicles emerging in salinity without CaCl_2 are killed by salt toxicity before they become visibly detectable. A similar response to NaCl was reported in barley grains (Bliss *et al.*, 1986). Thus, the G_F in *A. adscensionis* seeds treated with salts with very low added CaCl_2 concentrations does not indicate the percentage of germinated seeds (those in which radicles emerged from the seed coats). In contrast, seeds of *B. dasyphylla* seem to suffer from salt toxicity mainly after the radicles reach visibly detectable lengths, because their germinability was not conspicuously reduced by pretreatment with high-concentration salts (Table 2), and G_F in the NaCl and KCl treatments was similar between $+\text{CaCl}_2$ and $-\text{CaCl}_2$ (Fig. 2). The smaller G_F with $+\text{CaCl}_2$ than with $-\text{CaCl}_2$ in the MgCl_2 treatment group of *B. dasyphylla* (Fig. 2) could have occurred because the water potentials of the $+\text{CaCl}_2$ solutions were lower than those of the $-\text{CaCl}_2$ solutions. On the other hand, *A. ordosica* seeds appear to suffer from salt toxicity both before and after they germinate, because G_F and S_F were and considerably decreased by

pretreatment with high-concentration salts (Table 2), and G_F and S_F of salt-treated seeds were considerably increased by the addition of CaCl_2 to the solution (Fig. 2).

Besides toxicity, delayed emergence and elongation of radicles at higher salinities (Fig. 1) can be due to an osmotic effect of salt. For example, depression of both G_F and S_F at the higher salt concentrations in $+\text{CaCl}_2$ treatments (Fig. 2) would be attributable mostly to the osmotic effect of salts, because 5 or 25 mM CaCl_2 sufficed to alleviate the toxicity of NaCl , KCl and MgCl_2 salts in most situations (Fig. 3). At least some part of the inhibition of radicle elongation (resulting in a large difference between G_F and S_F) of *A. ordosica* at higher salt concentrations (Fig. 2) may have resulted from the osmotic effect of salt, because radicles of *A. ordosica* are more sensitive to osmotic inhibition than are those of the other two species tested (Tobe *et al.*, 1999). The G_F and S_F of *B. dasyphylla* were higher at high salt concentrations than were those of the other two species, because seed germination of this species is osmotically inhibited only at lower water potentials (Tobe *et al.*, 1999).

The CaCl_2 concentration needed to alleviate salt toxicity was fairly low (Fig. 3). Because the addition of a small amount of CaCl_2 to these salt solutions causes only a very small relative increase in the proportion of Cl^- in solution, marked improvement of S_F by the addition of CaCl_2 would be expected to result from the action of Ca^{2+} instead of Cl^- . The effects of Ca^{2+} may result from the action of Ca^{2+} in restoring the cation-displaced Ca^{2+} on the plasma membrane (Cramer *et al.*, 1985; Kent and Läuchli, 1985; Lynch *et al.*, 1987; Marschner, 1995; Volkmar *et al.*, 1998). However, salt toxicity to seeds does not seem to be explained solely by this mechanism, since pretreatment with CaCl_2 caused a considerable loss of germinability in *A. ordosica* seeds (Table 2), while pretreatment with -5.2 MPa polyethylene glycol-6000 had no adverse effects on germinability (Tobe *et al.*, 1999).

MgCl_2 had more adverse effects on seed germination than NaCl and KCl , and a higher concentration of Ca^{2+} was needed to alleviate its toxicity. Similar results were obtained in previous studies with a halophyte *K. caspicum* (Tobe *et al.*, 2002) and Wimmera ryegrass (Marcar, 1986). We found in the present study that KCl was more toxic than NaCl in all three species. In addition, different species showed different degrees of sensitivity to different salts; for example, both the $\text{Ca}^{2+}/\text{Na}^+$ and the $\text{Ca}^{2+}/\text{K}^+$ values needed to raise S_F to over 90% of G_F were higher for *A. adscensionis* than for *A. ordosica* in the NaCl - and KCl -treated seeds, whereas in the MgCl_2 -treated seeds *A. adscensionis* required a much smaller $\text{Ca}^{2+}/\text{Mg}^{2+}$ ratio to raise S_F than did *A. ordosica* (Fig.

3). Differences in toxicity and the Ca^{2+} concentration needed for toxicity alleviation may reflect the different abilities of these cations to displace Ca^{2+} bound to the surface of plasma membrane, as well as interspecific differences in membrane structure and/or characteristics.

In saline field soils, the most abundant cations are Na^+ , Mg^{2+} and Ca^{2+} (Shainberg, 1975), and the proportions of soluble cations differ greatly among locations (Tobe *et al.*, 2002). The results of our study suggest that the balance of soluble cations in the soil may be a crucial factor in determining seedling survival. Similar results have been obtained for a halophytic species, *K. caspicum* (Tobe *et al.*, 2002). A high proportion of soluble Mg^{2+} and/or a low proportion of soluble Ca^{2+} in the soil would inhibit seedling establishment in these species. Susceptibility to salt stress and the proportion of Ca^{2+} needed to alleviate toxicity differed greatly among species. This indicates that some species (e.g. *B. dasyphylla*) are able to establish themselves in a wider range of locations with differing cation composition, whereas one would expect others (e.g. *A. ordosica*) to become established only at limited sites, where cation composition is favourable for seedling survival. The adaptability of *B. dasyphylla* in the germination stage in the present study is consistent with observations that this non-halophyte can often be found in saline locations (Lanzhou Institute of Desert Research, 1985).

Our results indicate that a low concentration of Ca^{2+} in saline media considerably changed the responses of seed germination to salinity in the three species tested. Thus far, salinity effects on seed germination have usually been examined with single salt components (especially NaCl), and the toxic effects of salt on seed germination have been reported in many halophytic and non-halophytic species (Redmann, 1974; Bal and Chattopadhyay, 1985; Bliss *et al.*, 1986; Hardegree and Emmerich, 1990; Katembe *et al.*, 1998; Tobe *et al.*, 1999). However, saline soils contain soluble Ca^{2+} , and Ca^{2+} can alleviate the toxicity of salt to seed germination. Therefore, in some species, results of germination experiments with a single salt would not always be applicable to field conditions. For seeds that suffer from toxicity with a single salt treatment, the interactive effects of Ca^{2+} and other salts would need further examination in order to give a clearer understanding of germination behaviour in the field.

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