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Effects of five different salts on seed germination and seedling growth of *Haloxylon ammodendron* (*Chenopodiaceae*)

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Abstract

Saline soils contain multiple types of salt, each of which may exert a different effect on seed germination and seedling growth of plants. The effects of five types of salt on the initial growth of *Haloxylon ammodendron*, a shrub found on both saline and non-saline areas in deserts of China, were investigated. Seeds were incubated at 20°C in the dark in a solution (0 to –5.1 MPa) of a salt (NaCl, $MgCl_2$, $CaCl_2$, Na_2SO_4 or $MgSO_4$) or polyethylene glycol (PEG)-6000, or in a salt (NaCl or $MgCl_2$) or PEG solution containing a low concentration of $CaCl_2$. Seed germination, seedling growth and cation (Na^+, Mg^{2+}, Ca^{2+} and K^+) contents of seedlings were examined. Each salt had a different effect on seed germination, seedling growth and influx and outflux of cations in the seedlings. In both NaCl and $MgCl_2$ treatments, the addition of low concentrations of $CaCl_2$ favoured seed germination and seedling growth, and reduced K^+ outflux from seedlings, but caused no appreciable decrease in the influx of Na^+ or Mg^{2+} into seedlings. Marked abnormalities in seedlings were found only in treatments with Mg^{2+} salts, but these effects were completely alleviated by a low concentration of Ca^{2+} ($Ca^{2+}/Mg^{2+} = 0.012$). The different responses of the initial growth in *H. ammodendron* to different isotonic salt solutions were attributed to differences among salt components in membrane permeability, toxicity and effects on functions of the plasma membrane and/or the cell wall.

Keywords: calcium, *Haloxylon ammodendron*, magnesium, potassium, salinity, seed germination, seedling growth, sodium

Introduction

Saline soils contain multiple types of soluble salt components, each of which has a different effect on the initial growth of plants (Younis and Hatata, 1971; Redmann, 1974; Hardegree and Emmerich, 1990; Tobe *et al.*, 2002, 2003), and the compositions of soluble salts in saline soils differ greatly among locations (Tobe *et al.*, 2002). Among these salt components, Ca^{2+} is noteworthy because it significantly affects the salinity responses of plants in both initial growth (Bliss *et al.*, 1986; Marcar, 1986; Hamada, 1994; Tobe *et al.*, 2002, 2003) and later developmental stages (LaHaye and Epstein, 1969; Cramer *et al.*, 1986; Kurth *et al.*, 1986; Colmer *et al.*, 1996).

One important effect of salts on the initial growth of plants is toxicity. At least some of the effects of salt toxicity originate from the displacement of Ca^{2+} bound to the external surface of the plasma membrane by metal cations, and the subsequent impairment of membrane integrity and permeability. Ca^{2+} in the external medium alleviates this type of salt toxicity by re-displacing the cations on the membrane with Ca^{2+} (Cramer *et al.*, 1985; Kent and Läuchli, 1985; Lynch *et al.*, 1987). This type of salt toxicity and its alleviation by Ca^{2+} have been studied extensively for monovalent cations (especially Na^+) in salts (e.g. Cramer *et al.*, 1985, 1986; Kurth *et al.*, 1986; Rengel, 1992; Cramer, 2002); however, information on the toxic effects of divalent cations (e.g. Mg^{2+}) and alleviation of their effects by Ca^{2+} is limited (Marcar, 1986; Tobe *et al.*, 2002, 2003).

Also, it is known from experiments, primarily with NaCl, that salts affect the functions of the membranes and cell walls. First, NaCl affects the permeability of the plasma membrane and increases influx of external ions and efflux of cytosolic solutes (Cramer *et al.*, 1985; Kent and Läuchli, 1985; Zidan *et al.*, 1991; Allen *et al.*, 1995) in plant cells. Secondly, NaCl causes hardening of the cell wall (Neumann,

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1993; Neumann *et al.*, 1994; Nabil and Coudret, 1995) and a decrease in water conductance of the plasma membrane (Azaizeh *et al.*, 1992; Cramer, 1992). These effects of NaCl on cellular functions are alleviated by the addition of Ca^{2+} to the external medium (Cramer *et al.*, 1985; Kent and Läuchli, 1985; Kurth *et al.*, 1986; Zidan *et al.*, 1990, 1991; Azaizeh *et al.*, 1992; Cramer, 1992; Allen *et al.*, 1995). These effects of salts on the functions of the cell membranes and the cell walls may affect the water potential of the cytosol and cellular extensibility, and thus, may affect seed germination and seedling growth. Although most of these reports are based on experiments with NaCl, it is hypothesized that other salts have similar effects on cellular functions, but to different degrees, depending on the salt.

Studies to examine salinity effects on the initial growth of plants have usually been carried out with individual salts (especially NaCl); however, there remains a question as to whether the results of experiments with individual salts simulate the actual initial growth behaviour of plants in saline soils. To clarify the responses of the initial growth of plants to salinity, examination of the effects of various salts and the interactive effects of various salts with Ca^{2+} on seeds and seedlings is desirable. Furthermore, evaluation of ion movements into and out of seedlings would aid in understanding the effects of different types of salt on seedlings.

In the present study, we compared the effects of different types of salts, with or without a low concentration of Ca^{2+}, on seed germination, seedling growth and cation movement into and out of seedlings of *Haloxylon ammodendron* (C.A. Mey.) Bunge (*Chenopodiaceae*), a shrub found on both saline and non-saline soils in desert regions of north-west China (Fu and Jin, 1992). The seeds of this species have no endosperm and have only a fully differentiated spiral embryo coated by pericarp; the germination process of this type of seed seems to consist simply of water absorption by the dry spiral embryo and its subsequent elongation (Wallace *et al.*, 1968; Sharma and Sen, 1989). The seeds of *H. ammodendron* germinate even in solutions having very high NaCl concentrations, and the seedlings are highly tolerant of NaCl (Tobe *et al.*, 2000). Because the main salt components of saline soils are Na^+, Mg^{2+} and Ca^{2+} cations and Cl^- and SO_4^{2-} anions (Shainberg, 1975), we investigated the effects of individual salts (NaCl, Na_2SO_4, $MgCl_2$, $MgSO_4$ and $CaCl_2$) and combinations of salts (NaCl and $MgCl_2$) with low concentrations of $CaCl_2$ on the initial growth of *H. ammodendron*. Additionally, to evaluate the osmotic effects of the medium on seed germination and seedling growth, we examined seed and seedling responses to solutions of polyethylene glycol (PEG)-6000. Furthermore, we measured the cation (Na^+, Mg^{2+}, Ca^{2+} and K^+) contents in seedlings, to evaluate their movements into and out of the seedlings.

Materials and methods

Seeds of *H. ammodendron* [seed weight: 3.48 ± 0.48 mg (mean ± SE; *n* = 20)] were collected soon after maturation in November of 2001, from plants growing on a non-saline soil in the Tulufan Botanical Garden of the Xinjiang Institute of Pedology, Biology and Desert Research, Tulufan, China (42°56′N, 89°12′E). The collected seeds were initially stored at room temperature until transport to Japan the following January. After transport to Japan, the seeds were stored at *c.* −18°C to avoid germination loss, and germination experiments were carried out within 12 months after the maturation of the seeds.

Replicates of 25 seeds were sown on three layers of filter paper (Toyo, No. 1) in 90-mm plastic Petri dishes. About 15 ml of deionized water, a salt solution or a PEG-6000 solution was added to each Petri dish, so that about half the volume of each seed was immersed. The PEG solution of known water potential (Ψ_W) was prepared according to a calibration curve that was determined from isopiestic psychrometry (Boyer and Knipling, 1965) measurements at 20°C. The Ψ_W of each salt solution was calculated from van't Hoff's law, assuming that all salts in solution were dissociated into ions. In addition to Ψ_W, salt concentrations in solutions were expressed on a molal basis (mMolal; millimole per kilogram water). The Petri dishes were covered with lids, and the seeds were incubated at 20°C in the dark, because the seeds germinate favourably under these conditions (Tobe *et al.*, 2000). About two-thirds of the volume of the solution in each Petri dish was replaced daily with fresh treatment solution, to avoid changes in solute concentration; because no decrease in the volume of solution in each dish caused by evaporation or water uptake by the seeds was detectable, the daily solution replacement is believed to have been sufficient to maintain a constant solute concentration in the dish.

Experiment 1: Effects of individual salts and PEG on seed germination and seedling growth

Seeds were incubated in deionized water, a salt (NaCl, Na_2SO_4, $MgCl_2$, $MgSO_4$ or $CaCl_2$) solution (Ψ_W: −1.0 to −5.1 MPa) or a PEG solution (Ψ_W: −1.0 to −4.0 MPa). The seeds were observed daily for 20 d in dim light through a magnifying glass with a scale; the numbers of both germinated seeds (seeds with emerging seedlings longer than 3 mm) and seeds with emerging seedlings longer than 20 mm were counted, and seeds with emerging seedlings longer than

20 mm were discarded. After 20 d of incubation, the final germination percentage (G_F) and the final percentage of seeds with emerging seedlings longer than 20 mm (S_F) were determined. Each treatment was replicated four times.

Experiment 2: Effects of salt and PEG solutions with different $CaCl_2$ concentrations on seed germination and seedling growth

Seeds were incubated in a salt (NaCl or $MgCl_2$) or PEG solution (Ψ_W: −2.0 MPa) with different $CaCl_2$ concentrations (0, 1.0, 5.0 and 25 mMolal). Seeds were observed as described in Experiment 1, and after 20 d of incubation, G_F and S_F were determined as described in Experiment 1. Each treatment was replicated four times.

Experiment 3: Evaluation of water uptake by seedlings

Seeds were incubated in either a salt (NaCl, Na_2SO_4, $MgCl_2$, $MgSO_4$ or $CaCl_2$) solution (Ψ_W: −2.0 or −3.1 MPa) or a PEG solution (Ψ_W: −2.0 or −3.0 MPa), or in a salt (NaCl or $MgCl_2$; Ψ_W: −2.0 or −3.1 MPa) or PEG (Ψ_W: −2.0 or −3.0 MPa) solution containing 5 mMolal $CaCl_2$. After 4 d (−2.0 MPa treatments) or 6 d (−3.0 or −3.1 MPa treatments) of incubation, the percentage of seeds with an emerging seedling longer than 10 mm (S_{10}) was determined. For seeds with emerging seedlings longer than 10 mm, seedlings were detached from the pericarp and were blotted dry with tissue paper, and the fresh weight of the seedlings from each dish was measured. After drying the seedlings at 80°C for more than 3 d, the dry weight of the seedlings was determined. From fresh and dry weights of the seedlings, the water content per seedling was calculated. Each treatment was replicated four times.

Experiment 4: Measurement of cation content in seedlings

Seeds were incubated in a salt (NaCl, Na_2SO_4, $MgCl_2$, $MgSO_4$ or $CaCl_2$) solution (Ψ_W: –3.1 MPa), or in a salt (NaCl or $MgCl_2$) solution (Ψ_W: –3.1 MPa) containing 5 mMolal $CaCl_2$. After 5 d of incubation, seedlings with emerging lengths longer than 10 mm were collected, detached from the pericarp, blotted dry with tissue paper and the fresh weight of the seedlings from each dish was measured. Then, the seedlings were dipped in water and quickly blotted dry again to remove any surface salts, and the seedlings were dried at 80°C for more than 3 d. After determining the dry weights of the seedlings, they were ground, digested with HNO_3 on a hot plate, and finally dissolved in *c*. 100 ml water and the aqueous solution was analysed for cation (Na^+, Mg^{2+}, Ca^{2+} and K^+) content by ICP spectrometry (ICAP-750, Nippon Jarrell-Ash, Japan). From the results, cation content per seedling and cation concentrations in seedling water (the water contained in the seedlings) were calculated. Each treatment was replicated five times.

Statistical analysis

For comparison of multiple means, one-way ANOVA and Tukey's test were used. Percentage values were arcsine transformed prior to statistical analysis. All statistical tests were conducted at $P < 0.05$.

Results

Effects of individual salts and PEG on G_F and S_F

In all treatments, both seed germination percentage and seedling elongation progressively increased as Ψ_W in the medium increased [see Tobe *et al.* (2000) for daily changes in germination percentage of NaCl- or PEG-treated seeds], but in most treatments, the percentages of germinated seeds and of seeds with emerging seedlings longer than 20 mm substantially plateaued before the end of 20 d of incubation.

Figure 1 shows G_F and S_F for each treatment. At a Ψ_W of –3.0 MPa or less, G_F of PEG-treated seeds was lower than that of seeds treated with any salt solution of the same calculated Ψ_W. At −4.1 and −5.1 MPa, G_F was higher in treatments with SO_4^{2-} salts than in treatments with Cl^- salts. At these values of Ψ_W, G_F tended to decrease as SO_4^{2-} concentration in the solutions of the same calculated Ψ_W increased, and to decrease as Cl^- concentration increased; for example, at −4.1 MPa, G_F decreased in the order $MgSO_4$ (SO_4^{2-} concentration: 840 mMolal), Na_2SO_4 (SO_4^{2-} concentration: 560 mMolal), NaCl (Cl^- concentration: 840 mMolal), $MgCl_2$ (Cl^- concentration: 1120 mMolal) and $CaCl_2$ (Cl^- concentration: 1120 mMolal) treatments (the values of mean G_F were 97%, 76%, 65%, 49% and 46%, respectively).

The ratio S_F/G_F at a Ψ_W of −2.0 MPa or less was lowest in the $MgCl_2$ treatment (0.39 at −2.0 MPa), and highest in treatments with Na^+ salts (at −4.1 MPa, 0.83 and 0.84 for NaCl and Na_2SO_4 treatments, respectively). For $CaCl_2$ and PEG treatments, S_F/G_F was high, except for −4.1 MPa $CaCl_2$ (0.22) and −3.0 MPa PEG (0.11).

In treatments with Mg^{2+} salts, all the seedlings, including those of lengths exceeding 20 mm, were abnormal in colour (yellowish or blackish) and shape (bent and/or coiled). For treatments with other salts and PEG, although some seedlings showed slightly abnormal colour and/or shape, many seedlings appeared normal (white and straight).

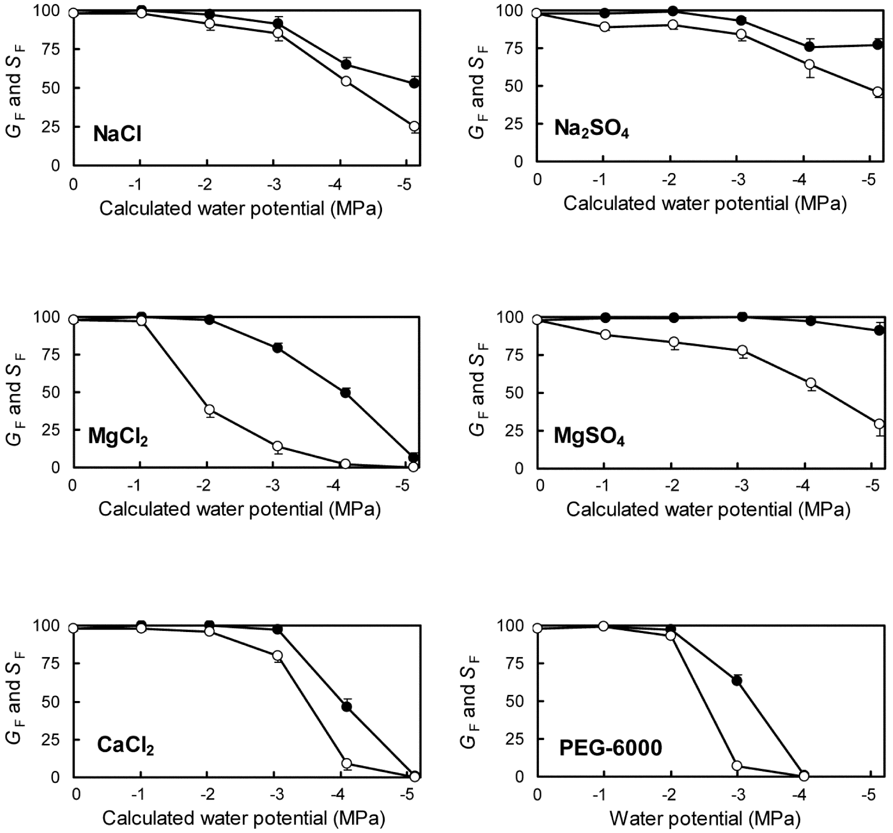

Figure 1. Effects of individual salts and polyethylene glycol (PEG)-6000 on final germination percentage (G_F) (●) and final percentage of seeds with emerging seedlings longer than 20 mm (S_F) (○). Water potentials of salts were calculated from van't Hoff's law. Each point represents the mean of four replications; bars indicating SE are shown only where SE is larger than the size of the point.

Effects of the addition of $CaCl_2$ to salt or PEG solutions on G_F and S_F

Effects of the addition of $CaCl_2$ to a salt (NaCl or $MgCl_2$) or PEG solution of −3.0 MPa on G_F and S_F were examined (Table 1). In the $MgCl_2$ treatments, both G_F and S_F were significantly increased by increasing $CaCl_2$ concentration, while in the NaCl treatments, both G_F and S_F were high irrespective of $CaCl_2$ concentration. In the PEG treatments, G_F was significantly lower at the highest $CaCl_2$ concentration.

At 0 mMolal $CaCl_2$ in the $MgCl_2$ treatment, all seedlings showed abnormalities as described above, but at 1.0 mMolal $CaCl_2$ (Ca^{2+}/Mg^{2+} = 0.0024), although many seedlings showed abnormalities, the degrees of the abnormalities were less conspicuous than those at 0 mMolal $CaCl_2$. At 5.0 and 25 mMolal $CaCl_2$ in the $MgCl_2$ treatments (Ca^{2+}/Mg^{2+} = 0.012 and 0.056, respectively), most seedlings appeared normal (white and straight). For NaCl and PEG treatments, many seedlings appeared normal irrespective of $CaCl_2$ concentration.

Effects of salts and PEG with or without 5 mMolal $CaCl_2$ on seedling water uptake

Effects of different solutions with or without 5 mMolal $CaCl_2$ on seedling water uptake were examined (Table 2a, b). For either Ψ_W, seedling water content was significantly less in the PEG treatments than in any treatment with salt. Among salt treatments without 5 mMolal $CaCl_2$, no significant difference in water content was detected. At −2.0 MPa, the addition of $CaCl_2$ caused significantly higher S_{10} and higher water content in both NaCl and $MgCl_2$ treatments, while at −3.1 MPa, the addition of $CaCl_2$ caused significantly higher water content only in the NaCl treatment. In the PEG treatments, the addition of $CaCl_2$ resulted in no significant changes in S_{10} or seedling water content at either Ψ_W. S_{10} tended

Table 1. Effects of NaCl and $MgCl_2$ solutions (water potential: −3.1 MPa) and a polyethylene glycol (PEG) solution (water potential: −3.0 MPa) with different $CaCl_2$ concentrations on final germination percentage (G_F) and final percentage of seeds with emerging seedlings longer than 20 mm (S_F). In each column, the values with the same superscript letter are not significantly different from each other ($P < 0.05$; Tukey's test)

$CaCl_2$ concentration (mMolal)	NaCl (630 mMolal)		$MgCl_2$ (420 mMolal)		PEG-6000	
	G_F	S_F	G_F	S_F	G_F	S_F
0	91^{a}	85^{a}	79^{b}	14^{c}	63^{a}	7^{a}
1.0	97^{a}	95^{a}	97^{a}	43^{b}	51^{ab}	9^{a}
5.0	97^{a}	96^{a}	99^{a}	96^{a}	47^{ab}	3^{a}
25	95^{a}	92^{a}	95^{a}	93^{a}	31^{bc}	3^{a}

Table 2. Effects of NaCl and $MgCl_2$ solutions [water potential: −2.0 (a) or −3.1 MPa (b)] and polyethylene glycol (PEG) solutions [water potential: −2.0 (a) or −3.0 MPa (b)] with (+Ca) or without (−Ca) 5 mMolal $CaCl_2$ on final percentage of seeds with emerging seedlings longer than 10 mm (S_{10}) and seedling water content. In each column, the values with the same superscript letter are not significantly different from each other ($P < 0.05$; Tukey's test)

Treatment solution		S_{10} (%)	Water content (mg seedling^{-1})
(a) PEG or salt (−2.0 MPa)			
PEG (−2.0 MPa)	−Ca	58^{cd}	3.52^{d}
	+Ca	54^{d}	3.49^{d}
NaCl (420 mMolal)	−Ca	88^{ab}	5.06^{bc}
	+Ca	96^{a}	5.91^{a}
$MgCl_2$ (280 mMolal)	−Ca	77^{bc}	4.51^{c}
	+Ca	91^{a}	5.71^{ab}
Na_2SO_4 (280 mMolal)	−Ca	64^{cd}	4.62^{c}
$MgSO_4$ (420 mMolal)	−Ca	87^{ab}	4.91^{bc}
$CaCl_2$ (280 mMolal)		90^{ab}	4.84^{c}
(b) PEG (−3.0 MPa) or salt (−3.1 MPa)			
PEG (−3.0 MPa)	−Ca	26^{c}	2.86^{d}
	+Ca	9^{c}	2.71^{d}
NaCl (630 mMolal)	−Ca	70^{b}	4.64^{bc}
	+Ca	72^{b}	6.84^{a}
$MgCl_2$ (420 mMolal)	−Ca	66^{b}	3.98^{c}
	+Ca	65^{b}	4.19^{bc}
Na_2SO_4 (420 mMolal)	−Ca	76^{b}	4.65^{bc}
$MgSO_4$ (630 mMolal)	−Ca	92^{a}	4.87^{b}
$CaCl_2$ (420 mMolal)		60^{b}	3.96^{c}

to be similar in treatments with SO_4^{2-} salts and Cl^- salts of the same Ψ_W; differences in seedling water uptake were not conspicuous between the treatments with SO_4^{2-} and Cl^- salts of the same Ψ_W.

Effects of salts with or without $CaCl_2$ on cation content in seedlings

A marked increase in Na^+, Mg^{2+} or Ca^{2+} concentration in seedling water was detected when seedlings were treated with a salt containing each cation (Table 3). Na^+ concentrations, in treatments with Na^+ salts, and Mg^{2+} concentrations, in treatments with Mg^{2+} salts, in seedling water were, respectively, 52–66% of Na^+ concentrations and 41–58% of Mg^{2+} concentrations in the treatment solutions. For 420 mMolal $CaCl_2$ treatment, the Ca^{2+} concentration in seedling water was rather low (23% of the Ca^{2+} concentration in the treatment solution).

Potassium (K^+) content per seedling was significantly higher in 420 mMolal $CaCl_2$ than in any other treatment, and was significantly lower in NaCl (−Ca) and Na_2SO_4 treatments than in any other treatment. In NaCl and $MgCl_2$ treatments, the

Table 3. Cation content per seedling and cation concentration in seedling water (in parentheses) for seedlings treated with different salt solutions (water potential: −3.1 MPa) with (+Ca) or without (−Ca) 5 mMolal $CaCl_2$. In each column, the values with the same superscript letter are not significantly different from each other ($P < 0.05$; Tukey's test)

		Cation content per seedling (nmol seedling^{-1}) and cation concentration in seedling water (mMolal) in parentheses			
Treatment solution		Na^+	Mg^{2+}	Ca^{2+}	K^+
NaCl (630 mMolal)	−Ca	1899^{b} (414^{b})	114^{c} (25^{e})	13^{bc} (3^{b})	371^{d} (81^{ef})
	+Ca	2370^{a} (418^{b})	106^{c} (19^{e})	27^{b} (5^{b})	472^{c} (83^{e})
$MgCl_2$ (420 mMolal)	−Ca	32^{c} (8^{d})	927^{b} (245^{b})	13^{bc} (3^{b})	478^{c} (127^{c})
	+Ca	47^{c} (11^{cd})	914^{b} (216^{c})	18^{bc} (4^{b})	663^{b} (157^{b})
Na_2SO_4 (420 mMolal)	–Ca	1958^{b} (439^{a})	109^{c} (25^{e})	9^{c} (3^{b})	290^{e} (65^{f})
$MgSO_4$ (630 mMolal)	−Ca	16^{c} (4^{d})	1116^{a} (255^{a})	9^{c} (2^{b})	465^{c} (106^{d})
$CaCl_2$ (420 mMolal)		108^{c} (28^{c})	138^{c} (36^{d})	377^{a} (97^{a})	832^{a} (215^{a})

addition of 5 mMolal $CaCl_2$ resulted in significant increases in K^+ content per seedling.

No significant differences were detected in Na^+, Mg^{2+} or Ca^{2+} contents per seedling among treatments in which the treatment solutions did not contain that cation (Table 3). It seemed that because the cation content in seedlings treated with a solution containing that cation was so much larger, statistical tests were not effective in determining differences in cation contents among treatments in which the treatment solutions did not contain that cation. Thus, we carried out statistical comparisons among cation contents in seedlings treated with solutions that did not contain that cation. The results (not presented) indicated that Na^+ or Mg^{2+} content was significantly ($P < 0.05$) higher in seedlings treated with 420 mMolal $CaCl_2$ than in those treated with any other solution that did not contain Na^+ or Mg^{2+}. On the other hand, the addition of 5 mMolal $CaCl_2$ to the NaCl or $MgCl_2$ solution did not have any significant effect on Mg^{2+} or Ca^{2+} content in the NaCl treatments, or on Na^+ or Ca^{2+} content in the $MgCl_2$ treatments (Table 3). Moreover, comparison of Na^+, Mg^{2+} or Ca^{2+} content among treatments in which treatment solutions contained that cation (results not presented) did not indicate significant effects ($P > 0.05$) of 5 mMolal $CaCl_2$ on any cation content.

The addition of 5 mMolal $CaCl_2$ to NaCl caused a significant increase in Na^+ content per seedling, whereas it caused no significant change in Na^+ concentration in seedling water; this was interpreted to have resulted from an increase in seedling water uptake due to Ca^{2+} and an eventual increase in Na^+ influx into the seedlings. The addition of 5 mMolal $CaCl_2$ to $MgCl_2$ caused no significant change in Mg^{2+} content per seedling, but caused a significant (but small) decrease in Mg^{2+} concentration in seedling water.

Discussion

For *H. ammodendron* seedlings treated with salts, both influx of external cations and efflux of internal cations were found, and the degree of cation movement into and out of seedlings differed among treatments with different salts (Table 3). Efflux of K^+ was prominent in treatments with Na^+ salts without 5 mMolal $CaCl_2$, suggesting that Na^+ enhances outward movement of K^+ through ion channels in the plasma membrane. Ion movements into and out of seedlings would have affected the Ψ_W of seedling water, and thus seed germination and seedling growth of *H. ammodendron*. In $CaCl_2$ treatments, although Ca^{2+} movement into the seedlings was more limited than inward movement of Na^+ or Mg^{2+} in treatments with Na^+ or Mg^{2+} salts, the G_F of seeds treated with $CaCl_2$ was similar to that of seeds treated with $MgCl_2$ of the same Ψ_W (Fig. 1). This would, at least partly, be attributable to the action of Ca^{2+} in decreasing the permeability of many ion channels (Tyerman and Skerrett, 1999), thus reducing efflux of intracellular solutes and maintaining a low Ψ_W of seedling water. The fact that seed germination was favoured more in treatments with either salt than in treatments with PEG of similar Ψ_W values may be because movement of external ions into seedlings favoured seedling water uptake. However, it should be stressed that the Ψ_W values for salt solutions presented in this study are calculated values and may deviate from the actual Ψ_W values of the solutions. For example, 1050 mMolal NaCl was calculated to have a Ψ_W of −5.1 MPa at 20°C, while its actual Ψ_W at 20°C is −4.8 MPa according to the report of Lang (1967). More strict discussion of the osmotic effects of salts on seed germination needs to be based on actual Ψ_W values. In addition to differences in the characteristics of inward and outward movements of ions, differences in the effects of different salts on cell wall extensibility and/or water conductance of membranes may have caused the different responses of G_F and S_F of *H. ammodendron* to different salts.

Low concentrations of Ca^{2+} favoured seed germination, seedling water uptake and seedling growth of *H. ammodendron* in both NaCl and $MgCl_2$ treatments. Because these effects of Ca^{2+} were not detected in the PEG treatments, they are believed to be attributable to an interaction of Ca^{2+} and salt components. For both NaCl and $MgCl_2$ treatments, the addition of low concentrations of Ca^{2+} reduced K^+ leakage from *H. ammodendron* seedlings. Similar results have been reported for crop plants (Cramer *et al.*, 1985; Kent and Läuchli, 1985; Colmer *et al.*, 1996) and charocean macroalgae (Tufariello *et al.*, 1988; Whittington and Smith, 1992), for both of which K^+ leakage from plant cells treated with NaCl was reduced by adding Ca^{2+} to the medium. On the other hand, the addition of low concentrations of Ca^{2+} did not cause any appreciable reduction in the permeation of Na^+ or Mg^{2+} into *H. ammodendron* seedlings in NaCl or $MgCl_2$ treatments. This is in contrast to the results with crop plants (Zidan *et al.*, 1991; Allen *et al.*, 1995; Roberts and Tester, 1997; Tyerman *et al.*, 1997) and charocean algae (Tufariello *et al.*, 1988; Hoffmann *et al.*, 1989; Whittington and Smith, 1992; Davenport *et al.*, 1996), for which Na^+ permeation into the plant cells treated with NaCl was reduced by adding Ca^{2+} to the medium. The promotive effects of Ca^{2+} on seed germination and seedling growth of *H. ammodendron* in salt treatments would, at least partly, have resulted from reduced efflux of K^+ (and possibly other solutes) from the seedlings, and eventual maintenance of a low Ψ_W of seedling water. Additionally, it is possible that Ca^{2+} alleviated the reduction in cell wall extensibility caused by salts (Kurth *et al.*, 1986; Zidan *et al.*, 1990) and/or water conductance of the plasma membrane (Azaizeh *et al.*, 1992; Cramer, 1992) in *H. ammodendron* seedlings, thus favouring water uptake and extension growth.

Marked abnormalities in seedlings were found only in treatments with Mg^{2+} salts, but no abnormalities were observed when low concentrations of Ca^{2+} ($Ca^{2+}/Mg^{2+} = 0.012$) were added to the medium. The most probable explanation of the interactive effects of Mg^{2+} and Ca^{2+} on *H. ammodendron* seedlings would be that the displacement of plasma membrane-bound Ca^{2+} with Mg^{2+} in the medium impaired normal growth, and that restoration by re-displacement with Ca^{2+} in the medium allowed normal seedling development (Cramer *et al.*, 1985; Kent and Läuchli, 1985; Lynch *et al.*, 1987). Because Mg^{2+} influx into *H. ammodendron* seedlings was not markedly decreased by Ca^{2+}, the abnormalities of Mg^{2+}-treated seedlings would not have resulted from cytosolic Mg^{2+} accumulation. This is in contrast to the situation in NaCl-treated charocean algae, in which Ca^{2+} alleviated salt toxicity by decreasing Na^+ influx into the cells (Tufariello *et al.*, 1988; Whittington and Smith, 1992).

Alleviatory effects of Ca^{2+} on the toxicity of Na^+ and Mg^{2+} on radicles have been found in our previous studies with a halophyte *Kalidium capsicum* (Tobe *et al.*, 2002) and three non-halophytes (Tobe *et al.*, 2003); the proportion of Ca^{2+} needed to alleviate salt toxicity differed among these species; the Ca^{2+}/Mg^{2+} ratio needed to alleviate Mg^{2+} toxicity on radicles ranged between 0.025 and 0.16, depending on the species. Compared to these species, *H. ammodendron* required a lower proportion of Ca^{2+} ($Ca^{2+}/Mg^{2+} = 0.012$) to alleviate Mg^{2+} toxicity, showing higher salt tolerance in seedlings of *H. ammodendron*. The reason for the absence of abnormalities in NaCl-treated *H. ammodendron* seedlings is believed to be because Na^+, being much less toxic than Mg^{2+} (Tobe *et al.*, 2002, 2003), did not cause appreciable injurious effects on the salt-tolerant *H. ammodendron* seedlings. The result of this study indicates that seedlings of *H. ammodendron* can survive in saline areas over a wider range of salt compositions, and reinforces the conclusions of previous studies (Tobe *et al.*, 2002, 2003) that salt composition is a factor in determining which species is dominant.

In addition to cations, anions seem to also affect seed germination of *H. ammodendron*, although they did not cause any appreciable effect on seedling growth. The promotive effect of SO_4^{2-} and suppressive effect of Cl^- on seed germination were found in *K. capsicum* in a previous study (Tobe *et al.*, 2002) as well as in *H. ammodendron* in this study. However, because the Ψ_W values presented in this study may deviate from the actual Ψ_W values of the salt solutions, the possibility cannot be ruled out that this result was caused by larger deviations of calculated values of Ψ_W in SO_4^{2-} salts than in Cl^- salts. Further research is needed to clarify the effects of anions on seed germination.

Different salts caused different responses in seed germination and seedling growth of *H. ammodendron*. This result is in agreement with the results of studies on other species (Younis and Hatata, 1971; Redmann, 1974; Myers and Morgan, 1989; Hardegree and Emmerich, 1990). The different responses of seeds and seedlings of *H. ammodendron* to different salt solutions are believed to have resulted from differences among different salt components in membrane permeability, toxicity and effects on functions of the plasma membrane and/or the cell wall. These results suggest that examination of the initial growth responses of plants to different salt components is needed to predict whether or not they will establish in saline areas.

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