Osmotic and Specific Ion Effects on the Germination of Prosopis strombulifera

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INTRODUCTION

Environmental abiotic stress conditions, especially drought and salinity, are currently the major factors that reduce crop yields worldwide. Salinity, in particular, is an increasing problem affecting 20% of the world’s cultivated land and nearly half of the area under irrigation. Breeding of salt-resistant crop varieties will require a clear understanding of the complex mechanisms of salt-stress tolerance, an understanding which is still lacking despite intensive research during the last decade (Holmberg and Bülow, 1998; Yeo, 1998; Zhu, 2001; Apse and Blumwald, 2002).

While all major crops, as well as most wild species, are glycophytes, i.e. sensitive to relatively low salt concentrations, there are also plants naturally adapted to conditions of high salinity in the soil. These plants, known as halophytes, include a large taxonomic variety and occupy diverse habitats, from extremely dry to temporarily waterlogged sites or salt marshes. Tobe et al. (2000) reported that germination responses of halophytic species to environmental parameters determine their distribution in saline environments. Nevertheless, recent investigations on the germination response of dimorphic seeds of Atriplex prostrata and Salicornia europaea to environmental conditions showed that zonation within the inland marsh is not determined at the germination stage of development (Carter and Ungar, 2003). Similar conflicting results are found in the literature related to the genus Prosopis. Catalán et al. (1994) reported that the effects of salinity on the germination of P. flexuosa seeds from habitats differing in soil salinity levels were similar. This was in agreement with results reported for P. farcta by Dafni and Negbi (1978), who found no correlation between salinity of the habitat where the seed originated and various salt tolerance criteria studied at germination. In contrast, Bazzaz (1973) found differences in the germination behaviour of seeds of the same species which originated in different habitats and suggested that they belonged to different ‘salt ecoregions’. In any case, although germination in a saline substrate does not necessarily correlate with salt tolerance at other growth stages (Noble, 1983) it is a legitimate criterion for selecting for tolerance in saline environments (Blum, 1988).

Prosopis is a genus with many widely studied species because they are an economically important arboreal component of saline zones in America (Burkart, 1976). However, within this genus there are other species which...
constitute fundamental shrub-like members of native communities such as *P. strombulifera*, a spiny shrub frequently found in the salinized areas of Córdoba and San Luis (central area of Argentina).

Previous results with *P. strombulifera* seedlings grown hydroponically under increasing NaCl concentrations showed that 80% of the treated seedlings developed stem and root growth stimulation up to 450 mM in relation to non-treated plants (Reinoso et al., 2000). This response is different to that obtained in other *Prosopis* species (Jarrell and Virginia, 1990; Catalan et al., 1994; M. A. Cony, Universidad Nacional de Mendoza, Argentina, pers. comm.) in which growth was reduced in response to increasing salinity. Anatomical and physiological studies have demonstrated that *P. strombulifera* is a salt-excluder halophytic species that constitutes an interesting model to improve knowledge on salinity tolerance (Reinoso et al., 2004).

It has been proposed that seed germination in salt-affected soils is influenced by the total concentration of dissolved salt (or the osmotic pressure) as well as by the type of salts involved (Ryan et al., 1975). Ungar (1978) reported that inorganic ions were not more inhibitory than mannitol and polyethylene glycol (PEG) in several halophytes, indicating that seeds are mainly affected by osmotic stress rather than specific ion toxicities. The same author stated that the distribution of halophytes in the United States indicates that there are species tolerant of a wide range of combinations of anions such as Cl⁻, SO₄²⁻ and CO₃²⁻ and cations such as Na⁺, K⁺ and Mg²⁺ (Ungar, 1991), suggesting that for these species specific ionic effects would have less influence on seed germination than the soil water potential. More recent results by Egan et al. (1997) on the effect of different sodium and potassium salts on the germination of *Arrípex prostrata* concluded that inhibition of seed germination and early growth was primarily due to an osmotic effect and not to a specific ion toxicity of either the chloride or sulfate salts.

The existing data on germination of *Prosopis* species under salinity have been obtained using only NaCl (López-Villagra and Galera, 1992; Villagra, 1997; Cony and Trione, 1998). The use of NaCl as the sole salinizing agent in salinity studies is due to the fact that generally it is the main component of the soluble salts mixture present in saline soils (Fan et al., 1993). Nevertheless, in the salinized areas of Córdoba and San Luis the NaCl/Na₂SO₄ proportions are similar, and in some areas Na₂SO₄ is more abundant (Sosa et al., 2002) in agreement with observations made by Cisneros et al. (1997).

Several studies compared the effects of chloride and sulfate salts on germination and seedling growth to determine if the results obtained for NaCl-affected soils could be appropriately applied to soils salinized with sulfate salts (Romo and Haferkamp, 1978; Bal and Chattopadhyay, 1985; Curtin et al., 1993). Due to conflicting evidence from different experiments, this question remains unanswered.

Few studies have recently focused on the effects of Na₂SO₄ on germination and plant growth in spite of the fact that their comparison with the NaCl effects is still of high interest (Grattan and Grieve, 1999). In addition, most studies have used monosaline solutions, which limits the extent to which one can interpret the results or relate them to field conditions.

The aims of this work, then, were (a) to obtain information about the germination response of *Prosopis strombulifera*, a native wild species common in South American salt marshes, (b) to determine the effect of increasing salinity with monosaline iso-osmotic solutions of KCl, NaCl, Na₂SO₄, K₂SO₄ and bisaline iso-osmotic solutions of NaCl + Na₂SO₄ and KCl + K₂SO₄, on germination, and (c) to differentiate the osmotic from the ion toxicity effects, through comparison of salt solutions with the osmotic agents mannitol and PEG 6000. If salts have only osmotic effects, iso-osmotic salts, PEG and mannitol solutions should affect the seeds in the same manner and to the same extent.

### MATERIALS AND METHODS

Seeds of *Prosopis strombulifera* (Lam.) Benth. were collected from an area in the south-west of the Province of San Luis, Argentina. Geographically, the location is 33°43’S, 66°37’W at 400–500 m a.s.l. with a temperate thermal regime, i.e. 15–20°C annual average temperature. This area belongs to the carob tree forest located in a saline depression between the annual 300–400 mm isohyets (Peña-Zubiate et al., 1998). The soil is calcareous, has a franc-sandy texture and moderate salinity (8000 mΩ cm⁻² electrical conductivity at the surface) (Anderson et al., 1970).

Pods were collected at random from 1000 plants in late summer (second week of March 2003) and stored at 4°C until used 2–6 months later in the experiments. Seeds were selected visually for uniform size and healthy aspect. They were chemically scarified with concentrated sulfuric acid for 10 min and then washed overnight (12 h) under flowing tap water. Before sowing, seeds were rinsed three times in distilled water and placed in sterile Petri dishes with two discs of filter paper saturated with distilled water for control treatments [osmotic potential (Ψₒ)=0·0 MPa] and the corresponding solutions of NaCl, Na₂SO₄, KCl, K₂SO₄, NaCl + Na₂SO₄, KCl + K₂SO₄, PEG 6000 and mannitol (Sigma Chemicals) in concentrations calculated to obtain the following Ψₒ: −0·4, −0·8, −1·2, −1·5, −1·9 and −2·2 MPa (Table 1). These osmotic potentials were verified with a vapour pressure osmometer (Model 5500, Wescor Inc., Logan, UT, USA). Bisaline solutions were prepared mixing equal volumes of their respective monosaline solutions at each corresponding osmotic potential. Solutions were renewed every 48 h under sterile conditions to ensure relatively constant Ψₒ in the treatments. In *Prosopis* species, germination occurs in a wide range of temperatures (25–40°C) (Passera, 2000), so optimum germination requirements were as previously determined (data not shown). Seeds were allowed to germinate under controlled conditions in a Conviron G 30 germination chamber (Winnipeg, Manitoba, Canada) at 30 ± 1°C and at 80% r.h.

A simple aleatory design (Steel and Torrie, 1995) with nine treatments was used. It consisted of a distilled-water control, six different salt treatments, a PEG treatment and a
RESULTS

Different responses with different treatments at iso-osmotic concentrations gave evidence of the existence of specific ionic effects. An external $\Psi_0$ of $-0.4$ MPa had no effect on germination percentage independently of the osmoticum used. At $\Psi_0$ of $-0.8$ MPa, germination percentage was inhibited in all salt treatments, except NaCl (Figs 1 and 2).

For the Na salts, NaCl at $-1.2$ MPa caused a significant inhibition ($P < 0.001$) of germination percentage relative to the $0.0$ MPa treatment (35% after $168\ h$), whereas $-1.2$ MPa Na$_2$SO$_4$ inhibited 60% after $168\ h$ ($P < 0.001$) (Fig. 1). In $-1.5$ MPa NaCl, germination percentage decreased by 60% relative to the $0.0$ MPa treatment after $168\ h$ ($P < 0.001$), whereas Na$_2$SO$_4$ solution inhibited it by 93% after $168\ h$ ($P < 0.001$) (Fig. 1). Thus, SO$_4^{2-}$ in monosaline Na-based solutions with osmotic potentials of $-1.2$ MPa and lower, was considerably more inhibitory than Cl$^-$ at iso-osmotic concentrations ($P < 0.05$). This SO$_4^{2-}$ inhibition was reduced in the salt mixture (NaCl + Na$_2$SO$_4$) for treatments at $-1.5$ MPa and $-1.9$ MPa ($P < 0.05$) (Figs 1 and 4).

Unexpectedly, KCl caused a greater inhibition of germination percentage than NaCl (e.g. $26\%$ vs. $65\%$ germination after $24\ h$ at $-0.8\ MPa$), which was accentuated at $-1.2$ and $-1.5$ MPa ($P < 0.001$) (Figs 2 and 4). The different

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**TABLE 1. Concentrations of salts, mannitol and PEG required to obtain the desired osmotic potentials, each of which were verified with a vapour pressure osmometer**

<table>
<thead>
<tr>
<th>$\Psi_0$ (MPa)</th>
<th>NaCl or KCl (mol L$^{-1}$)</th>
<th>Na$_2$SO$_4$ or K$_2$SO$_4$ (mol L$^{-1}$)</th>
<th>Mannitol (mol L$^{-1}$)</th>
<th>PEG 6000 (%) w/v</th>
</tr>
</thead>
<tbody>
<tr>
<td>$-0.4$</td>
<td>0.1</td>
<td>0.088</td>
<td>0.100</td>
<td>12</td>
</tr>
<tr>
<td>$-0.8$</td>
<td>0.2</td>
<td>0.178</td>
<td>0.180</td>
<td>18</td>
</tr>
<tr>
<td>$-1.2$</td>
<td>0.3</td>
<td>0.266</td>
<td>0.340</td>
<td>22</td>
</tr>
<tr>
<td>$-1.5$</td>
<td>0.4</td>
<td>0.333</td>
<td>0.425</td>
<td>26</td>
</tr>
<tr>
<td>$-1.9$</td>
<td>0.5</td>
<td>0.400</td>
<td>0.540</td>
<td>31</td>
</tr>
<tr>
<td>$-2.2$</td>
<td>0.6</td>
<td>0.480</td>
<td>0.610</td>
<td>33</td>
</tr>
</tbody>
</table>

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*Fig. 1. Percentage germination (mean $\pm$ s.e.) over time of P. strombalifera seeds exposed to different osmotic potentials of Na salts. No germination occurred at $-1.9$ MPa Na$_2$SO$_4$, so these results were not included in the graph. All treatments at $-2.2$ MPa inhibited all germination. Values with the same letter at $168\ h$ are not significantly different at $P > 0.05$, Scheffé test.*
responses with different K salts at iso-osmotic concentrations provide evidence of the existence of specific ionic effects, similar to what happened with Na salts. Thus, \( \text{SO}_4^{2-} \) in monosaline K-based solutions with \( \Psi_o \approx -1.5 \) MPa and \( -1.9 \) MPa were also more inhibitory than Cl\(^{-} \) anions, at iso-osmotic concentrations (\( P < 0.05 \)). However, the bisaline solution (KCl + K\(_2\)SO\(_4\)) showed a less pronounced reversion of SO\(_4^{2-}\) inhibition with increasing concentrations (Fig. 2), than the reversion obtained with the Na bisaline solution.

Responses of germination to treatments with PEG and mannitol were similar, showing higher germination percentages than all salt treatments at \( -0.8 \) MPa and lower. At \( -1.2 \) MPa, germination percentage was 76% in PEG and 85% in mannitol, being higher than in any of the salts. In Na salts at \( -1.2 \) MPa the germination percentages were in NaCl (63%), followed by 50% germination in the bisaline solution (NaCl + Na\(_2\)SO\(_4\)) and 40% in Na\(_2\)SO\(_4\). In K salts at \( -1.2 \) MPa, germination percentages were 30% in KCl, 25% in K\(_2\)SO\(_4\) and 37% in the bisaline solution.

When \( \Psi_o \) was decreased to \( -1.5 \) MPa, low germination percentages were obtained with all salt treatments (maximum 40% with NaCl), but 60% germination was obtained with both non-ionic treatments. At \( -1.9 \) MPa, the osmotic factor strongly inhibited germination, as demonstrated by the inhibition imposed by PEG and mannitol. There was no germination at all in both sulfate monosaline solutions. Nevertheless, the germination percentages obtained with both bisaline solutions showed again a reduction of \( \text{SO}_4^{2-} \) toxicity, giving no significant differences with their respective Cl salts (\( P < 0.05 \)).

Given that inhibition was more noticeable in \( \Psi_o \approx -1.2 \) MPa and lower in all salt treatments, the germination percentages at \( -1.2, -1.5 \) and \( -1.9 \) MPa after 168 h incubation in PEG, mannitol, monosaline and bisaline solutions were summarized in a new figure, Fig. 3. The figure also highlights the differences between the two bisaline solutions (\( P < 0.05 \)) showing that K\(^{+} \) was more inhibitory than Na\(^{+} \).

All treatments at \( -2.2 \) MPa inhibited all germination, except for mannitol, in which the germination percentage was similar to that at \( -1.9 \) MPa (Fig. 3).

**DISCUSSION**

The threshold salinity for a significant reduction in germination varies between species (Gulzar and Khan, 2001). The germination response of \( P. \) strombulifera seeds to NaCl salinity indicates that this species is within the tolerance limits cited for the majority of \( P. \) and \( P. \) species, which is between \( -1.5 \) and \( -1.9 \) MPa (Passera, 2000).

Some reports interpret the effects of salinity on the germination of seeds of different plant species in terms of \( \Psi_o \), while others explain the reduced germination as toxic effects of ionic elements (Poljakoff-Mayber et al., 1994; Al-Karaki, 2001). The present results show a general inhibition imposed by osmotic effect especially noticeable at \( \Psi_o \) \( < 1.2 \) MPa and lower in all treatments. At \( \Psi_o \approx 0.4 \) and \( -0.8 \) MPa NaCl the seeds could adjust osmotically reaching to final germination percentages equal to the controls. Nevertheless, in all the other salts used, the adjustment did not occur at \( -0.8 \) MPa, as evidenced by the statistically significant difference between K\(^{+} \) and SO\(_4^{2-}\) salt treatments and NaCl, PEG, mannitol and control treatments.

In monosaline solutions SO\(_4^{2-}\) was considerably more inhibitory than Cl\(^{-} \) at iso-osmotic concentrations. Similar results were obtained by Perez and Tambelini (1995) in \( P. \) juliflora seeds germinated in monosaline solutions of NaCl, CaCl\(_2\) and Na\(_2\)SO\(_4\) at \( \Psi_o \) from \( -0.3 \) to \( -1.5 \) MPa, in which the germination percentage was also more affected by Na\(_2\)SO\(_4\) than NaCl.
Notable toxic effects of SO$_4^{2-}$ were also observed in $P$. strombulifera plants which had been cultured hydroponically with increasing concentrations of Na$_2$SO$_4$ as the sole salinizing agent. They showed strong growth inhibition, lower leaf number, chlorosis and precocious senescence compared with control plants (Sosa et al., 2002). Notwithstanding, SO$_4^{2-}$ concentrations in root and leaf tissues were low when compared to Na$^+$: 6 g 100 g$^{-1}$ d. wt SO$_4^{2-}$ vs. 21 g 100 g$^{-1}$ d. wt Na$^+$ in leaves and 4 g 100 g$^{-1}$ d. wt SO$_4^{2-}$ vs. 9 g 100 g$^{-1}$ d. wt Na$^+$ in roots of plants growing at 531 mM Na$_2$SO$_4$ (Reginato et al., 2004).

It has been reported recently that H$^+$/SO$_4^{2-}$ co-transport in the root cells is the first step for SO$_4^{2-}$ uptake of plants from the environment, and a gene family of SO$_4^{2-}$ transporters has been identified with examples from many different plant species (Buchner et al., 2004). The availability of clones of some of these genes has enabled expression of the high-affinity SO$_4^{2-}$ transporters SHST1 and SHST2 from Stylosanthes and HVST1 from barley to be shown to be regulated at transcriptional level by the external SO$_4^{2-}$ supply. Restriction of the external SO$_4^{2-}$ supply resulted in a rapid increase in the capacity of these plants to take up SO$_4^{2-}$. Upon resupplying SO$_4^{2-}$ to such plants, SO$_4^{2-}$ uptake rates declined, thus showing that transcription of the genes encoding SO$_4^{2-}$ transporters is closely controlled by a negative feedback (Smith et al., 2000). It is considered that if such a regulatory mechanism were universal, permeability to SO$_4^{2-}$ would be blocked and SO$_4^{2-}$ inhibition of germination in salt solutions could be explained by the rapid build-up of SO$_4^{2-}$ in the cell walls of the seeds, causing deleterious effects on water uptake.

The germination percentages with bisaline solutions in the present experiment do not allow an unequivocal elucidation of the existence of a specific ionic effect of Cl$^-$ apart from those of SO$_4^{2-}$. Notwithstanding, the 60% of germination inhibition in −1.5 MPa NaCl after 168-h

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**Fig. 3.** Percentage germination (mean ± s.e.) over time of $P$. strombulifera seeds exposed to PEG and mannitol. Values with the same letter at 168 h are not significantly different at $P > 0.05$, Scheffé test.

**Fig. 4.** Percentage germination (mean ± s.e.) of $P$. strombulifera seeds at −1.2, −1.5 and −1.9 MPa with monosaline and bisaline solutions after 168 h of treatment. Data from Figs 1–3. Values with the same letter are not significantly different at $P > 0.05$, Scheffé test.
incubation may be a consequence of both, an osmotic effect plus a specific anion toxicity, taking into account that in −1.5 MPa PEG and mannitol the germination inhibition was only 40%.

Another interesting result of the present work was that K⁺, a macronutrient, was more inhibitory than Na⁺ on germination of this species independently of the accompanying anion.

Potassium is known as an important osmotic agent but is also involved in more specific metabolic roles including protein and starch syntheses and enzyme activation (Al-Karaki, 2001). Nevertheless, cases of K⁺ toxicity have been reported in glycophytes (Rathert, 1982; El-Haddad and O’Leary, 1994), and in halophytes (El-Haddad and O’Leary, 1994; Kefu et al., 1995; Egan and Ungar, 1998).

Interesting results have been reported in relation to specific K⁺ effects on the synthesis of osmoprotectants. In one report the results indicated that alfalfa cell cultures were unable to accumulate proline in response to K⁺; the results indicated that alfalfa cell cultures were unable to accumulate proline in response to K⁺. Nevertheless, cases of K⁺ toxicity have been reported in glycophytes (Rathert, 1982; El-Haddad and O’Leary, 1994), and in halophytes (El-Haddad and O’Leary, 1994; Kefu et al., 1995; Egan and Ungar, 1998).

Recent findings let us propose that future salinity studies, regardless of experimental scale or objectives, should be conducted with more realistic ion ratios, in agreement with Grattan and Grieve (1999).

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LITERATURE CITED


