Germination and Early Growth of
Prosopis strombulifera Seedlings in Different Saline Solutions

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Abstract: Salinity and temperature interact in their control of seed germination, with the greatest inhibition due to salinity usually found at the minimum or maximum temperature-tolerance limits. Few researchers have recently reported results on the effects of Na₂SO₄ on germination and plant growth. Besides, most salinity studies have been performed using monosaline solutions, thus limiting the possibility to extrapolate the results to field conditions. The aims of the present work were a) to evaluate the germination response of P. strombulifera seeds to monosaline iso-osmotic solutions of KCl, NaCl, Na₂SO₄, K₂SO₄, anionic bisaline iso-osmotic solutions of NaCl + Na₂SO₄ and KCl + K₂SO₄, and PEG at the maximum optimum germination °C for this species (35°C), to elucidate the possible variations in ionic interactions and osmotic effects in relation to previous results obtained at 30°C, b) to determine if cationic bisaline solutions (NaCl + KCl and Na₂SO₄ + K₂SO₄) alleviate ionic toxicity similarly to anionic bisaline solutions and c) to analyze the effect of all these treatments on radicles and hypocotyls growth. The temperature shift altered ionic interactions with no alleviation of SO₄²⁻ toxicity by the salt mixtures as previously demonstrated. Present work provides new evidence that the diversity of results of different authors trying to explain temperature-salinity interactions may be due not only to the intrinsic characteristics of each species but also to the differential effects of temperature on the osmotic and ionic components of salinity, on one side, and the chemical nature of the ions involved on the other, which interactions may change from synergistic to antagonistic or vice versa. Salt treatments inhibited hypocotyls and root growth with a similar pattern, being the main differences in their growth induced by PEG treatment which indicates that osmotic effect is the key factor regulating growth in these organs.

Key words: Prosopis strombulifera • germination • early growth • sodium chloride • sodium sulphate

INTRODUCTION

As salinity is an increasing problem affecting 20% of the world’s cultivated land and near 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, which are still lacking despite intensive research during the last decade [1].

In saline environments, adaptation of plants to salinity during germination and early seedling stages is crucial for the establishment of species [2]. Seedlings are the most vulnerable stage in the life cycle of plants and germination determines when and where seedling growth begins [3, 4].

There are conflicting reports in the literature as to the relative sensitivity of germination and seedling growth to salt stress. Nevertheless, it is well established that salt stress decreases growth in most plants, including halophytes [5]. Most seeds are located near the soil surface, where salt accumulates in salinized locations. The salt concentration at the surface of a salinized soil changes over time: continuous evaporation of ground water gradually deposits salt on the soil surface, but rainfall or melting snow can quickly leach salt from the surface and supply water to seeds. Thus, for the successful establishment of plants in saline environments, seeds must remain viable at high salinity in an imposed secondary dormancy and germinate when salinity decreases [2].

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120
Therefore, as appropriate germination responses of halophytic species to environmental parameters can determine their distribution in saline environments, it is important to investigate how typical halophytes growing in saline locations adapt to salinity in their initial life stages [6]. Examples in the literature show that their salinity tolerance at germination stage, varies in different species as *Sporobolus virginicus* [7], *Halopyrum mucronatum* [8] and *Briza maxima* [9], which germinate up to 350 mM NaCl. Some others like *Urochondra setulosa* [10] and *Aeluropus lagopoides* [11] are able to germinate in 500 mM or in yet higher NaCl concentrations as in the case of *Cressa cretica* [12], *Salicornia pacifica* [13] and *Arthrocnemum indicum* [14].

The majority of salt stress studies have used NaCl as the experimental salt. Few research works have recently reported results on the effects of Na$_2$SO$_4$ on germination and plant growth. Besides, most salinity studies have been performed using monosaline solutions, thus limiting the possibility to extrapolate the results to field conditions. Also, in few studies potassium toxicity has been reported, probably due to the fact that it is one of the main macronutrients essential for plant growth and metabolism [15, 16]. There has been increasing interest in the comparison of the NaCl effects on germination and plant growth with other salts usually present in the soil, to find out if the observed inhibitory results are due to an osmotic effect or ion toxicity [17, 18]. Salinity can affect germination of seeds either by creating osmotic potentials which prevent water uptake or by toxic effects of ions on embryo viability. Notwithstanding, the relative importance of osmotic and ionic effects on early growth of halophytes is still incomplete and depends on the species under study. In addition, many studies on seedling growth response to salt stress have used seedlings pregerminated in non saline conditions. This approach may provide a clear separation of effects, but could give an unrealistic view of the response of seeds that are sown directly into saline soils [19].

Previous results with *Prosopis strombulifera*, a halophytic spiny shrub frequently found in the salinized areas of Córdoba and San Luis (central area of Argentina) showed different responses to different compositions of iso-osmotic salt solutions of KCl, NaCl, Na$_2$SO$_4$, K$_2$SO$_4$, NaCl + Na$_2$SO$_4$ and KCl + K$_2$SO$_4$ and to the osmotic agents mannitol and polyethylene glycol (PEG) indicating specific ionic effects. The percentage of germination decreased as salinity increased. Sulphate anion in monosaline solutions with osmotic potentials -1.2 MPa and lower was more inhibitory than chloride anion at iso-osmotic concentrations. This sulphate toxicity was alleviated in salt mixtures which was more noticeable in higher concentrations. K$^+$ was more inhibitory than Na$^+$ independently of the accompanying anion. These experiments were performed under controlled conditions at 30° ± 1°C and at 80% RH [20].

Temperature (T°C) plays a key role in germination due to its known effects on biochemical processes. Salinity and temperature interact in their control of seed germination with the greatest inhibition due to salinity usually found at the minimum or maximum limits of tolerance to temperature [11].

Previous results with *P. strombulifera* seeds indicated that optimum temperature limits for germination are 25 to 35°C [21]. The aims of the present work, then, were a) to evaluate the germination response of *P. strombulifera* seeds to monosaline iso-osmotic solutions of KCl, NaCl, Na$_2$SO$_4$, K$_2$SO$_4$, anionic bisaline iso-osmotic solutions of NaCl + Na$_2$SO$_4$ and KCl + K$_2$SO$_4$ and PEG at the maximum optimum T°C (35°C), to elucidate the possible variations in ionic interactions and osmotic effects in relation to previous results obtained at 30°C, b) to determine if cationic bisaline solutions (NaCl + KCl and Na$_2$SO$_4$ + K$_2$SO$_4$) alleviate ionic toxicity similarly to anionic bisaline solutions and c) to analyze the effect of all these treatments on radicles and hypocotyls growth.

**MATERIAL AND METHODS**

Seeds of *P. strombulifera* were collected in the Province of San Luis, Argentina. The sampling location belongs to the carob tree forest located in a saline depression between the annual 300-400 mm isohyets [22] at 33° 43'S, 66° 37'W and 400-500 m a.s.l. The soil is mostly calcareous with a moderate salinity (8000 mhos/cm$^2$ electrical conductivity at the surface) [23].

Pods were collected at random from 200 plants in the first week of March (2004) and stored at 4°C until used 3 months later in the experiments, which were performed as described in Sosa et al. [20]. Treatments consisted in sowing the seeds in the following solutions: distilled water for control (osmotic potential ($\Psi_0$) = 0.0 MPa) and the corresponding solutions of NaCl, KCl, Na$_2$SO$_4$, K$_2$SO$_4$, NaCl + KCl, Na$_2$SO$_4$ + K$_2$SO$_4$, NaCl + Na$_2$SO$_4$ + NaCl, K$_2$SO$_4$ + KCl and polyethylene glycol (PEG 6000) in concentrations calculated to obtain the following osmotic potentials ($\Psi_0$): -0.4, -0.8, -1.2, -1.5 and -1.9 MPa, determined with a vapour pressure osmometer (Wescor Inc. Model 5500, Logan, UT, USA). Bisaline solutions were prepared according to Sosa et al. [20]. Seeds were allowed to germinate under
controlled conditions in a Conviron G 30 germination chamber (Winnipeg Manitoba, Canada) at 35 ± 1°C of temperature and at 80% RH.

A simple aleatory design with ten treatments was used [24]. It consisted of a distilled-water control, eight different salt treatments and PEG treatment. For statistical analysis, each osmotic potential obtained with each salt or PEG was considered a different treatment. Thirty seeds were placed in each Petri dish with six replicates per salt treatment and for PEG. The complete experiment was performed three times [20]. Final germination percentage for all treatments was recorded at 168 h (seven days) after the initiation of the experiment, according to the average germination time for most Prosopis species [20, 25, 26]. Seeds were considered germinated when the radicle was 5 mm long.

To evaluate radicle and hypocotyl growth, 20 germinated seeds were selected from each treatment after 24 h and placed in plastic trays with two filter papers moistened with the corresponding solution as described for germination treatments. They were maintained in the germination chamber under the same conditions described above. After 168 h the seedlings were harvested and the root and hypocotyl length were measured.

Data were processed using SPSS software for Windows 11.0 [27]. Results of germination percentage, root length and hypocotyl length after 168 h were first analyzed by using the Kolmogorov-Smirnov test to verify homogeneity of variance and data normality. Then, statistical differences were detected by using Kruskal-Wallis H nonparametric test and Mann-Whitney U nonparametric test. P values below 0.05 were considered statistically significant.

RESULTS

Germination percentage: Specific ionic effects were evident as different responses were obtained with different salt treatments at iso-osmotic concentrations. External Ψo of -0.4 and -0.8 MPa had no effect on germination percentage independently of the osmoticum used. From -1.2 MPa and lower, this parameter was significantly inhibited in all treatments (p<0.05) (Fig. 1).

At Ψo -1.2 MPa, NaCl and KCl had no effect on germination. Instead, Na2SO4 and K2SO4 inhibited 13% relative to the Ψo 0.0 MPa treatment (p>0.05). Bisaline solutions were more inhibitory than monosaline solutions, being the cationic mixtures (NaCl + KCl and Na2SO4 + K2SO4) the most inhibitory treatments (62% relative to the 0.0 MPa treatment (p>0.05), followed by the anionic mixtures which inhibited germination by 32% in the case of the Na-based bisaline solution and 39% in the case of the K-bisaline solution (p<0.05). PEG treatment at Ψo -1.2 MPa caused 18% inhibition of germination percentage by osmotic effect.

At Ψo -1.5 MPa there was a strong inhibition of germination percentage in all treatments except for chloride salts (24% for NaCl and 36% for KCl). SO4− in monosaline solutions caused a greater inhibition (53%) relative to Cl− anion (p<0.05). The cationic mixtures (NaCl + KCl and Na2SO4 + K2SO4) were again the most inhibitory treatments (93% inhibition in the case of the Cl− based bisaline solutions and 100% inhibition with the SO4− based bisaline solution) followed by the anionic mixtures which inhibited germination by 82% in the case of the Na-based bisaline solution and 89% in the case of the K-bisaline solution relative to the 0.0 MPa treatment (p<0.05). PEG treatment at -1.5 MPa caused 76% inhibition of germination percentage demonstrating that the osmotic effect started to be important and additive to the ionic effect already observed at Ψo -1.2 MPa in the salt treatments. The lowest germination percentages at all osmotic potentials higher than -1.9 MPa were obtained with both cationic bisaline solutions (p<0.05) suggesting a particular effect of the twice fold greater concentrations of Cl− and SO4− ions.

When Ψo was decreased to -1.9 MPa, lower germination percentages than those obtained at Ψo -1.5 MPa were registered with all treatments. Cl− monosaline solutions inhibited germination by 64 ± 1%, while SO4− monosaline solutions inhibited 82% (Na2SO4) and 86% (K2SO4). Cationic and anionic bisaline solutions inhibited germination by 97-100% and with PEG there was no germination at all.

Radical growth: Radicles showed similar final growth at osmotic potentials -0.4 and -0.8 MPa in all treatments. PEG treated radicles grew better than those in the Ψo 0.0 MPa treatment (p>0.05), while all salt solutions inhibited radical growth (p<0.05), except NaCl, KCl and Na-based anionic bisaline solution. Sulphate monosaline solutions and SO4− based cationic bisaline solutions caused the greater inhibition on radical growth. Nevertheless, at Ψo -0.8 MPa the K-based anionic bisaline solution caused an inhibition similar to them.

At Ψo -1.2 MPa osmotic effects started to be evident with 16% PEG inhibition with respect to the control treatment meanwhile Cl− monosaline solutions caused 51% inhibition. Chloride based cationic bisaline solutions inhibited radical growth by 64%, similarly to Na and K
Fig.1: Germination percentage (mean±SE) of *P. strombulifera* seeds at Ψo -0.4, -0.8, -1.2, -1.5 and -1.9 MPa with mono- and bisaline salt solutions and PEG after 168 h of treatment. Values with the same letter are not significantly different at p>0.05.
Fig. 2: Radial growth (mean ± SE) of *P. Strombulifera* seedlings at $\Psi_o$ -0.4, -0.8, -1.2, -1.5 and -1.9 MPa with monosaline and bisaline salt solutions and PEG after 168 h of treatment. Values with the same letter are not significantly different at $p>0.05$. 

124
Fig. 3: Hypocotyl growth (mean±SE) of *P. strombulifera* seedlings at Ψ₀ -0.4, -0.8, -1.2, -1.5 and -1.9 Mpa with monosaline and bisaline salt solutions and PEG after 168 h of treatment. Values with the same letter are not significantly different at p>0.05.
based anionic bisaline solutions. The greater inhibition was again obtained with SO₄²⁻-monosaline solutions and it was intensified with SO₄²⁻-based cationic bisaline solution (88% inhibition).

From $\Psi_0$ -1.5 MPa radical growth was completely inhibited in all sulphate containing treatments except in the mixtures with both anions ($SO_4^{2-} + Cl^-$). On the contrary, the best growth responses were obtained with PEG and NaCl. When concentration was increased to $\Psi_0$ -1.9 MPa the only salt treatments that allowed radical growth were chloride salts with 58% inhibition (NaCl) and 72% inhibition (KCl) relative to control.

**Hypocotyls growth:** At all osmotic potentials tested salt treatments and PEG significantly inhibited hypocotyls growth in relation to controls ($p<0.05$). At $\Psi_0$ -0.4 MPa chloride monosaline solutions, bisaline solutions NaCl + KCl, NaCl + NaSO₄ and PEG, hypocotyls length was similar, with 29% inhibition in relation to controls. Hypocotyls growth decreased under K-based anionic bisaline solution (42% inhibition) being sulphate monosaline solutions and SO₄²⁻-based cationic bisaline solution the treatments with most inhibitory tendency (52-53%) although statistics gave no significance.

With treatments at osmotic potential -0.8 MPa hypocotyls growth showed a similar tendency to that observed at $\Psi_0$ -0.4 MPa except for K-based bisaline solution and PEG, which were in this case more inhibitory (60 and 54%, respectively).

When osmotic potential was decreased to -1.2 MPa, hypocotyls growth was strongly inhibited in all treatments; from 56% inhibition with chloride monosaline solutions to 93% inhibition with SO₄²⁻-based cationic bisaline solution. All other salt treatments inhibited growth in a magnitude similar to PEG treatment (73%).

From $\Psi_0$ -1.5 MPa and lower hypocotyls growth was completely inhibited in all sulphate containing treatments except in the mixtures with both anions. On the contrary, the best growth response was obtained with NaCl. When concentration was increased to $\Psi_0$ -1.9 MPa the only salt treatments that allowed hypocotyls growth were chloride salts with 84% inhibition relative to control in both cases.

**DISCUSSION**

Germination percentages observed with monosaline solutions were higher than those previously obtained in a similar experiment performed at 30°C [20], which indicates that in the present work, the maximum optimum germination temperature for *P. strombulifera* (35°C) played an important role in the germination response by increasing the osmotic effect of salts. This was especially noticeable at $\Psi_0$ -1.5 MPa PEG where germination percentage was much lower than that with monosaline solutions. At this $\Psi_0$, only 24 and 36% of germination inhibition occurred with NaCl and KCl respectively, and 76% inhibition by the osmotic effect of PEG. Instead, at 30°C there had been 60% germination inhibition with NaCl, 75% with KCl and 40% with PEG. This increment of the osmotic effect was still more pronounced at $\Psi_0$ -1.9 (0% germination with PEG at 35°C and 10% at 30°C). These results mean that up to $\Psi_0$ -1.2 MPa the seeds could regulate their osmotic potential allowing ions to enter the seed and thus to alleviate the osmotic effect of the external medium [6], which obviously did not occur with PEG. The reason why salt had this mitigating effect in the higher temperature used in this work (35°C) may be a chemical potential increase of the salt solutions in relation to those at 30°C.

Sulphate anion in monosaline solutions was less toxic at 35°C than at 30°C from $\Psi_0$ -0.8 MPa and lower. For example, there was 53% germination inhibition at $\Psi_0$ -1.5 MPa at 35°C and 93% inhibition at the same $\Psi_0$ but at 30°C, effect which was also observed with Cl⁻-monosaline solutions. These results lead us to propose that a higher temperature alleviated the ionic effect of salts in monosaline solutions but accentuated osmotic effects. This is also true when considering bisaline solutions, Na-based anionic mixtures at $\Psi_0$ -1.2 MPa and 35°C allowed germination up to 68% and K-based anionic mixtures 61%. Instead, in our previous experiments at 30°C Na-based anionic mixtures allowed germination up to 54% and K-based anionic mixtures up to 35%.

When osmotic potential decreased to -1.5 MPa the results in the present work at 35°C gave 18% germination with Na-based anionic mixtures and 11% with K-based anionic mixtures, meanwhile the previous experiments at 30°C allowed germination up to 37% in Na-based anionic mixtures and up to 25% in K-based anionic mixtures. These results indicate that from $\Psi_0$ -1.2 MPa and lower in the present experiments carried out at 35°C, ionic interactions changed in Na and K-based anionic bisaline solutions causing a greater inhibition of germination than the corresponding monosaline solutions instead of the mitigation of anion toxicity observed at 30°C [20]. This may be due to two different reasons, one being the lower toxicity of SO₄²⁻- and Cl⁻- observed at 35°C than at 30°C when used in monosaline solutions and the other, a modification of the interactions between these two anions.
when mixed at 35°C which did not allow alleviation of SO$_4$$^{-}$ toxicity as shown in the previous work.

Then, we could say that in these experiments, from $\Psi_0$ -1.2 Mpa and lower germination inhibition increased when salt concentration increased due to the fact that the ionic effects were additive to the osmotic effects.

Cationic salinized solutions were not tested at 30°C and our results at 35°C show that the detrimental effect caused by both anions was much intensified when their proportions were duplicated in these salt mixtures, especially noticeable when SO$_4$$^{-}$ was the anion.

In the present work we corroborate the greater toxicity of SO$_4$$^{-}$ with respect to Cl$^{-}$ and the greater toxicity of K$^+$ with respect to Na$^+$ previously reported by Sosa et al. [20] suggesting that under salinity conditions P. strombulifera seeds would use preferably Na$^+$ and Cl$^{-}$ for osmotic adjustment, which would explain the reason why treatments with this chemical composition were less deleterious than PEG.

With respect to seedlings growth, radicles were more sensitive to ionic than to osmotic effects of salts. At $\Psi_0$ -0.4 and -0.8 MPa PEG radical growth was greater than that in their controls at $\Psi_0$ 0.0 MPa. From $\Psi_0$ -1.2 MPa PEG (with only 16% growth inhibition) and lower the osmotic effect of salt was additive to the ionic effect. This response of young roots to mild water stress ($\Psi_0$ -0.4, -0.8 and -1.2 MPa PEG) could be explained by the abscisic acid synthesis which stimulates root elongation through increasing the xyloglucan-endotransglycosilase activity [28], which in turn commands cell wall loosening and consequent cell elongation.

Despite KCl was more inhibitory than NaCl, both solutions allowed radical growth up to the maximum salt concentration suggesting that radicles as well as seeds, would use preferably Na$^+$ and Cl$^{-}$ for osmotic adjustment. On the contrary, this organ was very sensitive to SO$_4$$^{-}$ which toxicity was alleviated by the amionic mixture at all osmotic potentials except -1.9 MPa.

Inversely to what happened with radical growth, hypocotyl growth was more sensitive to osmotic effects than ionic effects. The PEG induced hypocotyl growth inhibition in these experiments could be explained through the diversion of seed reserves to radical elongation in detriment of hypocotyls growth, a usual response of seedlings under water stress [29]. In all salt treatments, hypocotyls growth followed a similar growth inhibition pattern to that in radicles.

In P. strombulifera as in many halophytic species [30 - 32], a number of environmental factors may be involved in the development of germination and seedling growth strategies, being temperature one of the crucial factors in determining these responses under salinity conditions. A shift in temperature may affect key processes such as membrane permeability and membrane-bound proteins, especially those involved in ion transport and cytosolic enzymes [33].

Consequently, the diversity of results obtained by different authors trying to explain temperature-salinity interactions may be due not only to the intrinsic characteristics of each species but also to the differential effects of temperature on the osmotic and ionic components of salinity. As well, ionic effects depend on the chemical nature of the ions involved which interactions may change from synergistic to antagonistic or vice versa.

To elucidate these very complex interactions in the next future it will be necessary to program multifactorial experiments including different salts, different combinations of salts and different temperatures, to assay also different species.

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