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Germination of *Prosopis juliflora* (Sw.) D.C. seeds at different osmotic potentials and temperatures

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Abstract

The effects of osmolytes, osmotic potential (Ψ_s), temperature, and their interactions on the germinability, germination rate, and other germination parameters of the invasive shrub *Prosopis juliflora*, which grows in the semiarid environmental conditions of the Caatinga in northeast Brazil, were evaluated. To study the effects of polyethylene glycol (PEG) and NaCl stress and temperature on germination, two separate experiments were carried out at the Plant Ecophysiology Laboratory of the Federal University of Pernambuco in 2011. The overall germinability decreased significantly with increases in both PEG (one-way ANOVA, $F_{4,75} = 111.21$, $P \leq 0.001$) and NaCl (one-way ANOVA, $F_{4,75} = 12.82$, $P \leq 0.001$); however, the effects were more accentuated with PEG than NaCl. The PEG-treated seeds maintained their germinability, even when they were subjected to a $\Psi_s = -1$ MPa after being rinsed and allowed to germinate on deionized water. In contrast, NaCl-treated seeds usually lost their ability to germinate; this fact was possibly linked to the accumulation of Na^+ and Cl^- in the cells, which may have contributed to a loss of membrane function that led to the death of the embryos. Although numerous studies describing seed germination in the presence of osmolytes have been conducted, studies that show the interactions between osmolytes, osmotic potentials, and temperature are scarce. The present study is the first to describe these interactions for *P. juliflora* seeds.

Keywords: Caatinga, exotic species, osmotic stress, seed germination, temperature.

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Introduction

More than 900 million ha (~20% of the total agricultural land) worldwide are affected by salt (Zhang *et al*. 2010). The affected area is nearly one-fifth of the world's irrigated land, resulting in the loss of 10 million ha of otherwise arable land each year (Boyer 1982). Soil salinity is one of the most important constraints that limits crop production in arid and semiarid regions (Parida & Das 2005), resulting in reductions in crop yields by as much as 50% (Boyer 1982). Moreover, salinity impairs seed germination and reduces nodule formation, plant development, flowering, and fruit development (Khan & Gulzar 2003). Plants have diverse cellular mechanisms to protect against specific ion effects and osmotic stresses imposed by saline soils. These mechanisms include increases in proteins involved in water transport (e.g. aquaporins), ion sequestration and secretion, and increases in osmolytes or compatible solutes (Leatherwood *et al*. 2007, and references therein).

NaCl is the predominant salt that causes salinization, and it is unsurprising that plants have evolved mechanisms to regulate its accumulation. Salinity affects seed germination through osmotic effects (Almansouri *et al*. 2001), ion toxicity (Song *et al*. 2005), or a combination of the two (Meiado *et al*. 2010), and the effects of excess sodium ions (Na^+) can critically affect biochemical processes (Apse *et al*. 1999). Salt and water stress can reduce germination by limiting water absorption by the seeds (Hegarty 1977; Zeng *et al*. 2010), affecting the mobilization of stored reserves (Bouaziz & Hicks 1990), or directly affecting the structural organization or the synthesis of proteins in germinating embryos.

Seedlings are the most vulnerable stage in the life cycle of plants, and germination determines when and where seedling growth begins (Günster 1994). In saline environments, adaptation of plants to salinity during germination

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and the early seedling stages is crucial for the establishment of a species (Ungar 1978, 1982, 1995). Successful seedling establishment depends on the frequency and amount of precipitation as well as on the ability of the species' seeds to germinate and grow while soil moisture and osmotic potentials decrease. Most seeds are deposited near the surface of saline soil, where the concentration of salt is usually higher than it is below the surface (Dantas *et al*. 2006). Seed germination occurs after monsoon rains, which cause a reduction in temperature and soil salinity (Almansouri *et al*. 2001; Khan & Gulzar 2003). In this sense, seeds germinate rapidly at low NaCl concentrations but remain ungerminated at high NaCl concentrations (Song *et al*. 2005). This response may produce a persistent bank of viable seeds in saline environments that can maintain the population over time, and it may be an important strategy for seed dispersal (Ungar 1995). Therefore, rapid germination may be an adaptive strategy for the seeds of species to take advantage of transient favorable conditions during the germination stage to ensure seedling establishment.

Reductions in the germination percentage and delays in the onset of germination in saline conditions are well documented. However, salinity stress seldom occurs in isolation and its effects on seed germination may be modified by interactions with other environmental parameters, such as temperature (Pompelli *et al*. 2006; Meiado *et al*. 2010) and light (Khan & Gulzar 2003; Pompelli *et al*. 2006). Although numerous studies have described seed germination in the presence of osmolytes, studies that show the interactions between osmolytes, osmotic potentials, and temperature are scarce. The principal aim of the present study was to compare the effects of drought and salt stress on the germination of *Prosopis juliflora* seeds at different temperatures. The present study was initiated to differentiate osmotic effects from toxic effects by comparing NaCl with the metabolically inactive osmotic agent polyethylene glycol 6000 (PEG 6000). To determine the osmotic effects, we measured seed imbibition and the germination of seeds soaked in NaCl and PEG solutions at various osmotic potentials and temperatures.

Materials and methods

Study area and species studied

The Caatinga, a South American Indian name that means "white forest," is a forest that covers a 760 000 km^2 area of northeast Brazil (Sampaio 1995). Since 2003, the Caatinga has been recognized as one of "Earth's last wild places" and was classified as one of the 37 "Wilderness Areas of the World" (Russell *et al*. 2003). It is inhabited by approximately 23 million people and corresponds to the largest populated semiarid area in the world located in a single country (Prado 2003). The vegetation of the Caatinga is strongly influenced by topography, human disturbance, and, most importantly, a combination of average annual rainfall and soil attributes (Sampaio 1995; Prado 2003). The Caatinga is classified as a BSh Köppen climate with a high evapotranspiration potential (1500–2000 mm annually) throughout the year and low pluviometric precipitation (300–1000 mm annually), which is usually concentrated over 3–5 months (Sampaio 1995). The soil of the Caatinga is highly saline (Dantas *et al*. 2006), caused by the high level of water evaporation from the soil and poor soil drainage, resulting in an accumulation of salt at the soil's surface (Sampaio 1995). Moreover, it is common to drill artesian wells in the Caatinga to alleviate water shortages and facilitate agricultural and livestock activities. This action greatly increases the soil's salinity from salts that were formerly present in groundwater, which strongly contributes to the secondary salinization of the Caatinga. These events, in combination with the rapid drying of the soil due to intense sunlight, contribute to extremely limited water availability throughout most of the year and create conditions that greatly affect seed germination (Barbosa *et al*. 2003) and seedling survival in this region.

The anthropization of areas of the Caatinga has commonly led to a loss of diversity and the establishment of exotic species such as *P. juliflora*, which are introduced accidentally or intentionally, as an alternative source of protein for cattle feed during periods of water shortage.

Prosopis juliflora (Sw.) D.C. (mesquite) is a native species extending from North to South America. They occur in arid and semiarid areas of Venezuela, Colombia, and Ecuador, and extend through Panama into Mexico (Pasiecznik 2001). *Prosopis juliflora* is a species that is fast growing, highly aggressive, and able to cause substratum degradation in the arid and semiarid areas of north and northwest India (Singh 1995). *Prosopis juliflora* has a broad ecological requirement and is adapted to a very wide range of soils and site types from sand dunes to cracking clays. It is generally found in areas where water and poor soil fertility are the principal agents that limit plant growth and can survive and even thrive on some of the poorest land, which is unsuitable for any other tree species (El-Keblawy & Al-Rawai 2005). This species prefers areas subjected to high temperatures and high evapotranspiration with wide variations in rainfall (Pasiecznik 2001; El-Keblawy & Al-Rawai 2005).

Seed collection and germination

Fruits of *P. juliflora* were collected from the Experimental Station of the Agronomic Institute of Pernambuco, located in Caruaru (8°14′18″S, 35°55′20″W; 550 m a.s.l.), approximately 140 km from Recife in the semiarid Caatinga

region. The median temperature and precipitation of this region are 23°C and 671 ± 54 mm, respectively, which are irregularly distributed throughout the year (over 4–6 months). The rainy season occurs from April to August (Agritempo 2012).

The seeds were extracted from the fruits and immediately used in these experiments. Four replicates of 50 randomly selected seeds each were carried out for each treatment. The treatment factors were two osmotic salts, five osmotic potentials, and four temperatures, which were applied in a randomized complete block design. In two separate experiments, drought and salt stress were induced by different osmotic potential levels and their effects were examined. Osmotic potential of –0.25, –0.50, –0.75, and –1.0 MPa for PEG 6000 and –0.5, –1.0, –1.5, and –2.0 MPa for NaCl were studied. An osmotic-free medium was prepared to produce an osmotic potential of 0 MPa (control). The osmotic potentials of NaCl were calculated using the van't Hoff equation, $\Psi_s = -ciRT$, where Ψ_s is the osmotic potential in MPa, c is the concentration in mol/L, i is the dissociation constant of NaCl (i.e. 1.8), R is the gas constant (i.e. 0.0083 L/atm/mol/K), and T is the temperature in Kelvins. For PEG 6000, the osmotic potentials were calculated as described by Michel and Kaufmann (1973). All osmotic potentials were confirmed ($t_6 = 91.76$, $R^2 = 0.99$, $P \leq 0.001$) with a Wescor vapor pressure osmometer (model 5600, Wescor Biomedical Systems, Utah, USA).

The seeds were pretreated with H_2SO_4 for 5 min (Miranda *et al*. 2011), surface sterilized in a 1% solution of NaOCl for 10 min, and rinsed five times with sterile deionized water. Seeds were allowed to germinate in 110 mm × 110 mm × 35 mm germination boxes lined with a triple layer of filter paper and moistened with 20 mL of sterile solution plus Mycostatin solution 100 mg/L) (Bristol-Myers Squibb Pharmaceutics, New York, NY, USA) prior to incubation to control fungi. The germination boxes were covered with lids and kept in a growth chamber, where the temperature was maintained at 25, 30, 35 or 40°C with a 12 h photoperiod provided by Sylvania cool-white fluorescent lamps, which have a light intensity of 40 µmol photons/m^2/s, and the pots were randomly moved every day to minimize positional effects. Germination was recorded daily for 20 days. Seeds were considered to have germinated upon emergence of the radicle. When no seeds germinated until 20 days, we considered germination to be completed. These data were used to determine the germinability, germination rate ($\bar{t}$), germination synchrony (Z), uncertainty (U), and germination index, as described in Ranal and Santana (2006) and Zhang *et al*. (2010). To investigate the effect of darkness on germination and whether high salinities inhibited or damaged the seeds in the dark, another set of germination boxes was wrapped in aluminum foil to exclude light and placed in the same incubator. To normalize the effect of each stress on germination, the sensitivity response was calculated as the osmotic sensitivity response (OSR) and salt sensitivity response (SSR). The OSR were calculated as $[(G_w - G_{PEG})/G_w] \times 100$ and the SSR was calculated as $[(G_w - G_{NaCl})/G_w] \times 100$: where G_W is germination in water, G_{PEG} is germination on PEG solution and G_{NaCl} is germination on NaCl soluctions.

Recovery of germination

To verify the resilience of seeds exposed to low osmotic potentials, we carried out an additional experiment to determine the NaCl and PEG concentrations that completely inhibit germination and cause irreversible damage to embryos. The methodology used was the same as described above. After 20 days, nongerminated seeds were delicately rinsed with deionized sterile water, gently surface-dried with sterile filter paper and transferred to germination boxes free of osmotic agents. Seeds were allowed to germinate for 20 days at 25°C when we considered that germination had completed for that treatment. To ensure that the seeds used for the experiments were viable and maintained their viability after treatments, seed viability was determined by the ability to reduce 2,3,5-triphenyltetrazolium chloride to red colored formation (Brewer 1949).

Seed imbibition

To investigate the imbibition capacity of the seeds, four replicates of 50 seeds each were subjected to hydration at osmotic potentials of 0, –0.5 and –1 MPa with PEG and NaCl solutions. Seeds were allowed to imbibe the solutions for 120 h. During the first 12 h, the samples were weighed every hour and every 24 h thereafter. At each time point, the seeds were removed from the imbibition solution, weighed and then returned to a new solution with the same osmotic potential (Pompelli *et al*. 2010). The relative increase in the fresh weight of the germinating seeds was calculated using the formula: $[(W_f - W_i)/W_i] \times 100$, where W_i is the initial weight of seeds and W_f is the weight after n hours.

Statistical analysis

For seed germination, each treatment was composed to four replicates of 50 seeds. The germination percentages were transformed using the formula $\text{Arcsin}\sqrt{\frac{x}{100}}$, where x is the germination percentage (Ranal & Santana 2006). A Shapiro–Wilk test was used to test for normality (Shapiro

& Wilk, 1965) and a Brown–Forsyth test was used to test for equal variances (Brown & Forsyth, 1974) using the software Statistica Version 7.0 (StatSoft, Tulsa, OK, USA). All data are presented as means plus standard errors (SE). All results were analyzed with a mixed-model ANOVA, and means were compared using an SNK test with the statistical software package SigmaPlot Version 11.0 (Systat Software Inc., Chicago, USA). The parametric correlations were made with the statistical software Statistica Version 7.0 (StatSoft, Tulsa, OK, USA). The results were considered to be significant when $P \leq 0.05$.

Results

Independently of the temperature or osmotic potential, the germination of *P. juliflora* is not significantly influenced by light (*P*-value 0.642). The independency of light on the germination process is termed aphotoblastic behavior. Therefore, in this article we show only data obtained in the light because they were more reliable.

The germination percentages decreased significantly with increases in temperature and both PEG (factorial ANOVA, $F_{12,60} = 45.22$, $P \leq 0.001$) and NaCl (factorial ANOVA, $F_{12,60} = 117.72$, $P \leq 0.001$). Therefore, the effects of osmolytes (i.e. PEG and NaCl) were separated for further analysis. Independent of temperature variation, the seed germination of *P. juliflora* was verified in all osmotic potentials promoted by both NaCl and PEG. However, the PEG treatments resulted in significantly ($P \leq 0.001$) less germination than the iso-osmotic NaCl solutions (Table 1). The germinability was inversely affected by the NaCl and PEG concentrations, that is, *P. juliflora* exhibited a reduction in germination with increases in NaCl or PEG concentrations. During NaCl treatments, no germination occurred at –2 MPa at 40°C, and germination was very low in osmotic potentials below –1.50 MPa, especially at higher temperatures (Table 1). The PEG solutions inhibited the germination at osmotic potentials below –0.75 MPa at 40°C, and germination was greatly reduced at osmotic potentials below –0.5 MPa. Nevertheless, germination in the absence of NaCl/PEG or at low salinity was considerably higher. The final germination percentages of seeds soaked in PEG solutions was significantly affected by the osmotic potential ($R^2 = 0.864$, $P \leq 0.001$) and temperature ($R^2 = –0.444$, $P \leq 0.001$). Similar results were obtained for seeds soaked in NaCl as for the seeds soaked in PEG solutions, however, the correlations returned fewer negative values.

The seeds that were germinated in NaCl had the highest percentage of germination at a temperature of 30°C (Table 1) because higher temperatures, together with more negative osmotic potentials, promoted an acceleration in the metabolism of the seed and the death of the embryo; this finding was confirmed by the tetrazolium test (data not shown). However, for seeds germinated in PEG, the pattern was different because for osmotic potentials below –0.5 MPa, the optimum temperature for germination was 25°C (Tables 1, 2). The effects of temperature on the germination rate (Table 2), germination synchrony (Table 3) and uncertainty (Table 4) were not clear because the best temperature depended on the considered parameters.

For the germination rate, germination synchrony, and uncertainty, the results showed that an increase in salt concentration promoted slower or more unsynchronized germination (Tables 2–4). The $\overline{t}$ value increased with a

Table 1 Germinability (%) of *Prosopis juliflora* seeds at different concentrations of PEG 6000 and NaCl at 25, 30, 35, or 40°C

	Temperature							
Solution (MPa)	25°C		30°C		35°C		40°C	
PEG 6000								
0	100.0 ± 0.0	Aa	100.0 ± 0.0	Aa	100.0 ± 0.0	Aa	100.0 ± 0.0	Aa
–0.25	97.5 ± 2.5	Aa	99.0 ± 1.0	Aa	100.0 ± 0.0	Aa	98.0 ± 0.8	Ba
–0.50	99.0 ± 1.0	Aa	93.0 ± 3.3	Bb	95.0 ± 1.7	Bb	10.0 ± 2.2	Cc
–0.75	51.5 ± 4.3	Ba	4.0 ± 2.3	Cb	2.5 ± 0.5	Cb	0.0 ± 0.0	Dc
–1	0.0 ± 0.0	Ca	0.0 ± 0.0	Da	0.0 ± 0.0	Da	0.0 ± 0.0	Da
NaCl								
0	100.0 ± 0.0	Aa	100.0 ± 0.0	Aa	100.0 ± 0.0	Aa	100.0 ± 0.0	Aa
–0.50	100.0 ± 0.0	Aa	100.0 ± 0.0	Aa	98.0 ± 1.2	Aa	96.0 ± 0.8	Bb
–1	96.0 ± 0.8	Ba	98.5 ± 1.0	Aa	96.0 ± 1.4	Ba	98.5 ± 1.0	Aa
–1.50	95.8 ± 0.7	Bb	98.5 ± 1.5	Aa	98.5 ± 1.0	Aa	10.5 ± 1.0	Cc
–2	94.5 ± 1.3	Ba	94.0 ± 0.8	Ba	24.0 ± 2.2	Cb	0.0 ± 0.0	Dc

The values represent the media (± SE) of four replicates of 50 seeds each. Means that are followed by different uppercase letters within columns or by different lowercase letters within rows for each attribute are significantly different ($P \leq 0.05$, Newman–Keuls test)

Table 2 The germination rate ($\bar{t}$, days) of *Prosopis juliflora* seeds at different concentrations of PEG 6000 and NaCl at 25, 30, 35, or 40°C

Solution (MPa)	Temperature 25°C		30°C		35°C		40°C	
PEG 6000								
0	1.38 ± 0.03	Aa	1.27 ± 0.02	Aa	1.01 ± 0.01	Aa	1.07 ± 0.02	Aa
−0.25	2.05 ± 0.14	ABa	1.84 ± 0.10	ABa	1.78 ± 0.01	ABa	3.68 ± 0.16	Bb
−0.50	2.72 ± 0.16	Ba	2.66 ± 0.17	Ba	2.96 ± 0.07	Ca	4.23 ± 1.39	Bb
−0.75	6.03 ± 0.42	Cb	9.00 ± 0.71	Cc	3.63 ± 0.38	Ca	–	
−1	–		–		–		–	
NaCl								
0	1.24 ± 0.04	Aa	1.03 ± 0.01	Aa	1.02 ± 0.01	Aa	1.04 ± 0.01	Aa
−0.50	1.83 ± 0.05	Bb	1.10 ± 0.02	Aa	1.08 ± 0.01	Aa	2.33 ± 0.03	Bc
−1	2.70 ± 0.08	Cb	1.90 ± 0.03	Ba	1.82 ± 0.12	Ba	2.73 ± 0.09	Cb
−1.50	4.99 ± 0.02	Dd	2.99 ± 0.06	Ca	3.37 ± 0.01	Cb	4.39 ± 0.14	Dc
−2	6.52 ± 0.15	Eb	4.39 ± 0.03	Da	7.75 ± 0.34	Dc	–	

The values represent the media (± SE) of four replicates of 50 seeds each. Means that are followed by different uppercase letters within columns or by different lowercase letters within rows for each attribute are significantly different ($P \leq 0.05$, Newman–Keuls test).

Table 3 The germination synchrony (Z) of *Prosopis juliflora* seeds at different concentrations of PEG 6000 and NaCl at 25, 30, 35, or 40°C

Solution (MPa)	Temperature 25°C		30°C		35°C		40°C	
PEG 6000								
0	0.57 ± 0.04	Ab	0.63 ± 0.02	Ab	0.98 ± 0.01	Aa	0.93 ± 0.02	Aa
−0.25	0.47 ± 0.01	Bb	0.45 ± 0.01	Bb	0.65 ± 0.01	Ba	0.28 ± 0.05	Bc
−0.50	0.41 ± 0.04	Ba	0.42 ± 0.05	Ba	0.41 ± 0.05	Ca	0.15 ± 0.05	Cb
−0.75	0.17 ± 0.02	Cb	0.33 ± 0.10	Ca	0.00 ± 0.00	Dc	ND	
−1	ND		ND		ND		ND	
NaCl								
0	0.65 ± 0.04	Ab	0.94 ± 0.02	Aa	0.97 ± 0.02	Aa	0.93 ± 0.02	Aa
−0.50	0.64 ± 0.07	Ab	0.82 ± 0.03	Ba	0.89 ± 0.01	Aa	0.44 ± 0.03	Bc
−1	0.39 ± 0.01	Bb	0.72 ± 0.02	Ca	0.43 ± 0.04	Bb	0.43 ± 0.06	Bb
−1.50	0.33 ± 0.01	Bb	0.54 ± 0.03	Da	0.37 ± 0.02	Bab	0.47 ± 0.05	Ba
−2	0.34 ± 0.03	Ba	0.37 ± 0.03	Ea	0.20 ± 0.05	Cb	ND	

The values represent the media (± SE) of four replicates of 50 seeds each. Means that are followed by different uppercase letters within columns or by different lowercase letters within rows for each attribute are significantly different ($P \leq 0.05$, Newman–Keuls test). ND, not determined.

decrease in the osmotic potential for both NaCl and PEG solutions. The germination of *P. juliflora* can be classified as fast because $\bar{t}$ was less than 5 days. However, at osmotic potentials below to −0.5 MPa with PEG or −1.5 MPa with NaCl its germination was considered to be intermediate. Regardless of the temperature, germination always began on the second day, except in seeds allowed to germinate at osmotic potentials below −0.50 MPa or −1.5 MPa with PEG and NaCl, respectively, when germination began on the fourth day or later (Fig. 1).

A positive relationship between the osmotic potential and the seed water content of germinating seeds was noted, however, seeds soaked in NaCl imbibed water faster than seeds soaked in PEG (Fig. 2). Seeds allowed to imbibe a solution at an osmotic potential of −0.5 MPa exhibited increases in fresh weight of 55% and 32% in NaCl and PEG solutions, respectively. In contrast, seeds allowed to imbibe a solution at an osmotic potential of −1 MPa exhibited increases in fresh weight of 53% and 26% (Fig. 2). It is worth noting that seeds imbibing fresh water (0 MPa) exhibited increases in their fresh weight of at least 105%.

Prosopis juliflora seeds completely recovered from treatment with −1 MPa PEG because after being rinsed with

Table 4 The uncertainty (*U*) of *Prosopis juliflora* seeds at different concentrations of PEG 6000 and NaCl at 25, 30, 35, or 40°C

Solution (MPa)	Temperature 25°C		30°C		35°C		40°C	
PEG 6000								
0	1.06 ± 0.08	Ab	0.90 ± 0.05	Ab	0.07 ± 0.04	Aa	0.22 ± 0.04	Aa
–0.25	1.36 ± 0.10	Ab	1.33 ± 0.06	ABb	0.76 ± 0.01	Ba	2.08 ± 0.17	Bc
–0.50	1.41 ± 0.15	Aa	1.49 ± 0.17	Ba	1.60 ± 0.17	Cab	1.96 ± 0.33	Bb
–0.75	2.58 ± 0.12	Bc	1.00 ± 0.01	Ab	0.01 ± 0.01	Aa	ND	
–1	ND		ND		ND		ND	
NaCl								
0	0.82 ± 0.08	Ac	0.18 ± 0.07	Aa	0.10 ± 0.06	Aa	0.22 ± 0.04	Aa
–0.50	0.86 ± 0.16	Ab	0.46 ± 0.07	ABa	0.32 ± 0.01	Aa	1.32 ± 0.08	Bc
–1	1.48 ± 0.04	Bb	0.72 ± 0.07	Ba	1.29 ± 0.12	Bb	1.50 ± 0.14	Bb
–1.50	1.66 ± 0.07	Bb	1.23 ± 0.12	Ca	1.63 ± 0.05	Cb	1.94 ± 0.04	Cb
–2	1.76 ± 0.12	Bab	1.56 ± 0.16	Da	2.07 ± 0.30	Db	ND	

The values represent the media (± SE) of four replicates of 50 seeds each. Means that are followed by different uppercase letters within columns or by different lowercase letters within rows for each attribute are significantly different ($P \leq 0.05$, Newman–Keuls test). ND, not determined.

Table 5 Percentage of germinated seeds (GS), nongerminating seeds (NGS), and seeds germinated after recovery (SGR) of *Prosopis juliflora* at different osmotic potentials of NaCl and PEG

MPa, osmolyte	GS	NGS	SGR
–0.75, PEG	2.5 ± 0.5	97.5 ± 0.5	99.1 ± 0.7
–1, PEG	0 ± 0	100.0 ± 0.0	99.5 ± 0.5
–1.5, NaCl	98.5 ± 1.5	1.5 ± 1.2	0 ± 0
–2.0, NaCl	23.0 ± 1.29	77.0 ± 1.3	0 ± 0

The seeds were germinated at 35°C. The values represent the media (± SE) of four replicates of 50 seeds each.

deionized water, these embryos exhibited a similar germinability (99.5 ± 0.5%) with osmotic-agent-free treatment within 3–4 days. In contrast, seeds that did not germinate in the presence of –2 MPa NaCl completely lost their germination capacity because the germination percentage during the recovery period was 0% (Table 5).

Compared with PEG treatments, the germination indices of *P. juliflora* seeds were typically greater for NaCl treatments, and seeds incubated in NaCl germinated at more negative osmotic potentials (Fig. 3). The relationship between the external osmotic potential and the germination index was linear, and NaCl had a smaller negative effect than PEG. The amplitude increased with temperature. However, in PEG, the gradient of the lines was significantly affected by increases in temperature, with seeds at low osmotic potentials failing to germinate. No significant difference in the relationship between the osmotic potential and the germination index was observed between the NaCl and PEG treatments at 25°C and 30°C, but strong differences were observed at other temperatures.

Seeds that were allowed to imbibe PEG solutions showed the highest sensitivity responses because germination was almost completely inhibited under moderate osmotic stress. In contrast, seeds that were allowed to imbibe NaCl solutions displayed the lowest sensitivity responses because germination was only slightly affected by the same stress. The range of variation in osmotic and salt sensitivity responses was between 0 and 100% and 0 and 4% at –1 MPa of PEG and NaCl, respectively. The osmotic salt sensitivity responses were found to be highly correlated ($P \leq 0.001$) (data not shown).

Discussion

The aphotoblastic behavior of *P. juliflora* seeds described in the present study corroborates evidence from other studies of the *Prosopis* species (Peres & Moraes 1994; Villagra 1995; El-Keblawy & Al-Rawai 2005; Miranda *et al*. 2011).

The results of the present study showed that the germination parameters are inversely proportional to the NaCl or PEG concentration at all temperatures tested. Therefore, the temperature that best promotes the germination of *P. juliflora* is strongly affected by its interactions with the osmotic potential of the substrate where the germination occurs. Similar to other species (Barbosa *et al*. 2003; Meiado *et al*. 2010), *P. juliflora* is able to germinate at higher temperature ranges, such as those normally registered in the Caatinga ecosystem (Sampaio 1995). Although Meiado *et al*. (2010) did not test the combined effects of temperature and osmotic potentials, the authors found that the seeds of *Cereus jamacaru* had the highest

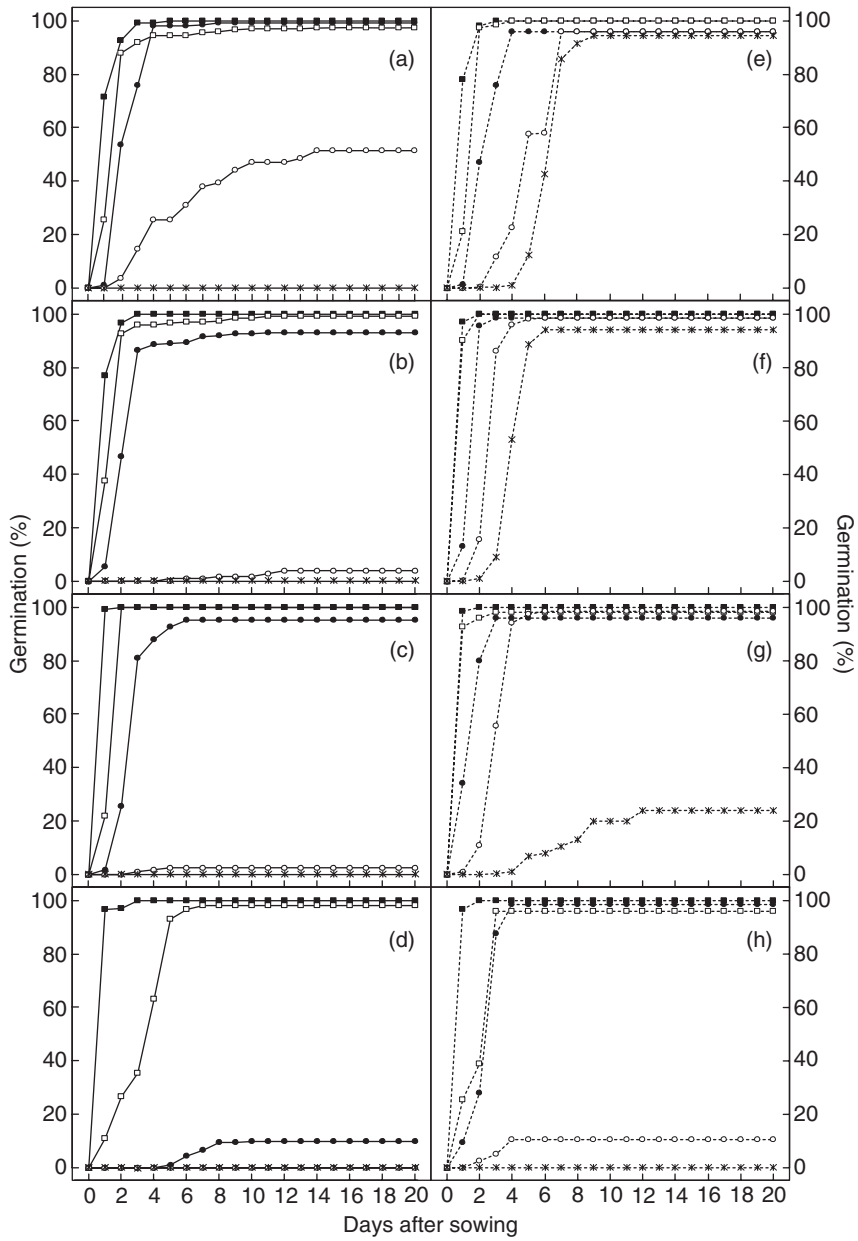

Fig. 1 Time course of germination (%) during the 20 days that seeds were in contact with distilled water (0 MPa) or solutions of PEG (a–d) or NaCl (e–h). The temperatures of incubation were 25°C (a, e), 30°C (b, f), 35°C (c, g), and 40°C (d, h). Each point represents the mean of four replicates. —■—, distilled water PEG; —▫—, –0.25 MPa PEG; —●—, –0.50 MPa PEG; —○—, –0.75 MPa PEG; —✳—, –1.00 MPa PEG. ···■···, distilled water NaCl; ···▫···, –0.50 MPa NaCl; ···●···, –1.00 MPa NaCl; ···○···, –1.50 MPa NaCl; ···✳···, –2.00 MPa NaCl.

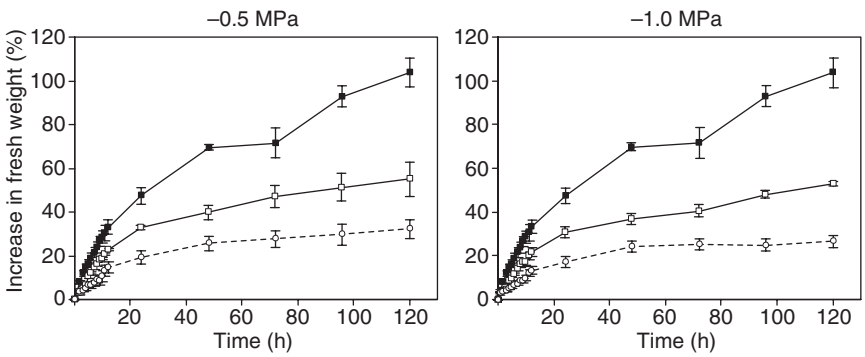

Fig. 2 Increase in fresh weight of *Prosopis juliflora* seeds that were germinated in iso-osmotic solutions of NaCl and PEG. The osmotic potentials of solutions were 0 (control), –0.5 MPa (left panel) and –1.0 MPa (right panel) after 120 h at 25°C. The vertical bars indicate the standard errors of the means. $n = 4$. —■—, control; —▫—, NaCl; - -○- -, PEG.

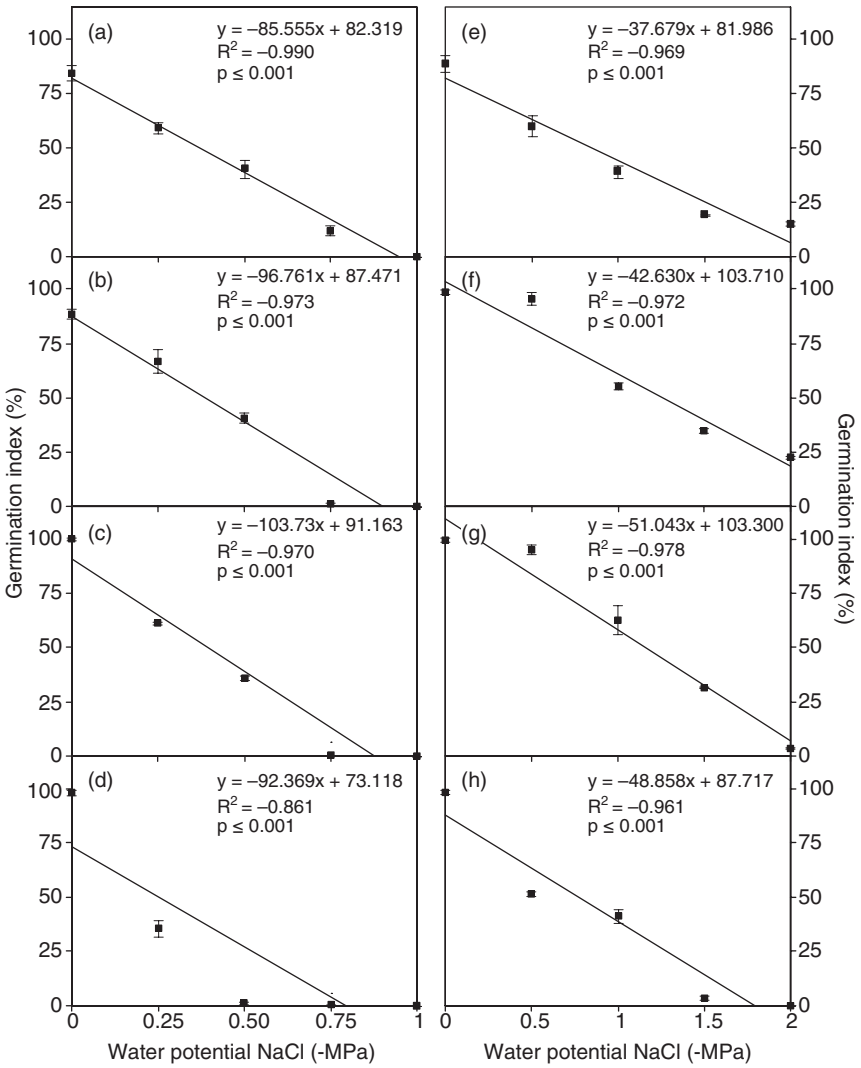

Fig. 3 The germination index plotted against the external water potential for *Prosopis juliflora* seeds in contact with PEG (a–d) or NaCl (e–h) at 25°C (a, e), 30°C (b, f), 35°C (c, g), and 40°C (d, h). Each point represents the mean of four replicates. The vertical bars indicate the standard errors of the means.

germinability and lowest $\bar{t}$ when germinated at 30°C, compared to other temperatures. Similar profiles were previously reported for *Prosopis juliflora* (El-Keblawy & Al-Rawai 2005), *Prosopis argentina* and *Prosopis alpataco* (Villagra 1995), *Dyckia encholirioides* (Pompelli *et al*. 2006) and some perennial grasses (Khan & Gulzar 2003).

Many researchers have reported that NaCl has a greater effect on germination and the early seedling stages than PEG (Mohammadkhani & Heidari 2008). However in this study PEG concentration was always more detrimental to the final germination percentage than the NaCl concentration. *Prosopis juliflora* seeds treated with PEG germinated at osmotic potentials up to –0.5 MPa, and there was an abrupt decrease of germinability at osmotic potentials lower than –0.75 MPa. In contrast, seeds treated with NaCl did not exhibit a significant decrease in their germinability until –1.5 MPa. In other non-halophyte species, the osmotic potential that completely inhibits germination is generally greater than –1.5 MPa (Hegarty 1977; Haigh & Barlow 1987), while the threshold may be less than –5 MPa in halophyte species (Ungar 1978, 1982;Debez *et al*. 2004). The results of the present study on *P. juliflora* seed germination corroborate other studies on non-halophyte species, including *P. juliflora* (Peres & Moraes 1991; Peres & Tambelini 1995; Nassif & Peres 1997; Mohammadkhani & Heidari 2008; Meiado *et al*. 2010; Zeng *et al*. 2010; Zhang *et al*. 2010).

Although Na^+ is a major cation present in the soil, it is not considered to be an essential mineral for most plants. In saline soils such as Caatinga's (Dantas *et al*. 2006), high concentrations of Na^+ disrupt the balance of other minerals such as K^+, thereby causing a reduction in cell turgor. A reduction in cell turgor leads to a drastic reduction in the rates of root and leaf elongation (Hegarty 1977; Verslues *et al*. 1998). This observation suggests that the salt acts primarily on water uptake (Hegarty 1977).

Furthermore, the high intracellular concentration of Cl^- or Na^+ ions can inhibit some metabolic pathways (Campos *et al*. 2012), slowing germination and subsequent events, which *in totum* leads to cell death. In contrast, seeds of salt-tolerant species tend to have lower osmotic potentials, allowing them to absorb water from the environment (Zhang *et al*. 2010). This decrease in osmotic potential can be achieved in one of two ways: exclusion of salt from the cytosol by vacuolar compartmentalization (Apse *et al*. 1999; Gao *et al*. 2003; Song *et al*. 2005), while maintaining an osmotic potential with organic solutes or by allowing Na^+ and Cl^- to enter the cells and using them as osmolytes while using mechanisms to mitigate the toxic effects of salt within the cell (Parida & Das 2005). These mechanisms generally involve the overexpression of the tonoplast Na^+/H^+ antiporter genes (Apse *et al*. 1999) or the proton pumps (ATPase) (Hahnenberger *et al*. 1996). Furthermore, in Arabidopsis plants, the expression of SOD2, a Na^+/H^+ antiporter on the plasma membrane, may restore the ability of the cells to export Na^+ and greatly increases their resistance to Na^+, improving seed germination and seedling growth (Gao *et al*. 2003).

Under PEG-induced water stress, a gradual decrease in osmotic potential causes a gradual decrease in germination, and this is correlated with a delay in water uptake caused by this osmolyte (Fig. 2). Moreover, the OSR2 and OSR4 quantitative trait loci (QTLs) were identified only when Arabidopsis seeds were incubated on a PEG solution (Vallejo *et al*. 2010). These results suggests that these OSR QTLs operate as a common molecular component of the signaling network that controls germination under low osmotic potentials generated by osmotic conditions, and that they are relevant in the adaptation of plant populations to different ecological environments.

The uptake of water by seeds can be considered to occur in three sequential steps: imbibition, metabolism leading to initiation of radicle growth, and radicle emergence (Bewley 1997). A threshold level of hydration is required for subsequent radicle elongation (Bewley 1997). It can be hypothesized that in the presence of PEG, that is, an inert osmoticum that cannot enter the apoplast (Carpita *et al*. 1979), water is withdrawn not only from the cell but also from the cell wall. Therefore, the inhibition of germination is attributed solely to osmotic effects and not to ionic effects (Michel & Kaufmann 1973), which may have contributed to the decrease in germinability of seeds soaked in PEG (Fig. 1). The relative increase in the fresh weight of germinating seeds was invariably highest in the control and lowest in all PEG- and NaCl-treated seeds at all temperatures (see Fig. 2 for seeds soaked at 25°C). Our results confirm that these osmolytes had some inhibitory effect on water uptake by the seeds. Khademi *et al*. (1991) reported that low osmotic potentials delay water uptake, increase the length of the water uptake plateau, and subsequently may delay or prevent germination. However, in this study, the inhibitory effect of NaCl on germination might not be solely related to water uptake because the increase in fresh weight during the first hours of germination appeared to be similar to the controls (Fig. 2). In fact, the higher rate of germination observed in NaCl than in PEG could be explained by a more rapid initial water uptake in the NaCl solution (Fig. 2) (Song *et al*. 2005) and achievement of a moisture content that allowed germination, as previously reported in maize (Mohammadkhani & Heidari 2008) or durum wheat (Almansouri *et al*. 2001) during seed imbibition and germination. In other words, NaCl did not limit the water uptake by the germinating embryo enough and, in this case, injuries appeared after ion accumulation at a later stage in the germinating process (Khademi *et al*. 1991; Almansouri *et al*. 2001).

When the osmotic potential was sufficiently low, for example –0.75 MPa (for PEG solutions) or –1.5 MPa (for NaCl solutions), the seeds could contain sufficient water to start the germination process (Phases I and II) (Fig. 2) without passing to root cell growth (Phase III) (Fig. 3). The drop in the rate of water uptake by seeds when they were soaked in NaCl and PEG solutions of increasing concentrations was most likely caused by the decrease in the osmotic potential gradient between the seeds and their surrounding media. The processes of elongation and cell wall synthesis are highly sensitive to water deficiency (Wenkert *et al*. 1978) and reductions in growth could occur due to decreases in the turgors of these cells. Very negative osmotic potentials, especially at the beginning of imbibition, influence the absorption of water, which begins the sequence of events that culminate in seed germination (Hegarty 1977). We hypothesized that the characteristics of stronger germination and higher $\bar{t}$ in drought conditions may be favorable traits in arid environments. However, in arid environments that are also saline, these traits are lethal.

Seeds of *Prosopis juliflora* present $\bar{t}$ more affected by osmotic stress than germinability in all tested osmotic solutions, temperatures, and osmolytes (Table 2); an increased $\bar{t}$ at –0.5 MPa of PEG, while for NaCl the increase was only significant at osmotic potentials below –1 MPa. Water stress can reduce both the germinability and $\bar{t}$, and there is a wide variation of responses among species (Bewley 1997). Thus, species that are more tolerant of, or better adapted to, water stress have the ecological advantage of better seedling establishment in areas subjected to water deficit. These parameters can contribute substantially to an understanding of seed germination processes and seedling recruitment in the field, which are influenced by water and/or salt stress. The decrease in germination parameters (e.g. germinability, germination rate, synchrony and uncertainty) after water or osmotic stress has been previously reported in some species

including *P. juliflora* (Peres & Tambelini 1995; Nassif & Peres 1997; Leatherwood *et al*. 2007; Zhang *et al*. 2010).

The results of the present study show that 77% of the seeds treated with NaCl at an osmotic potential of –2 MPa did not germinate even after 20 days (Table 5), and the germinability of the nongerminating seeds was not recovered even after the transfer of the seeds to deionized water. Two processes mediated this reduction: osmotic effects due to a declining osmotic potential, creating water stress on the surface of the seeds, and ionic effects due to seed ion uptake and/or accumulation. When soaked in –0.88 MPa of NaCl at 12°C, the Na^+ concentration of barley seeds was increased to 4 μg/g dry weight during the first 72 h of incubation (Zhang *et al*. 2010). This fact may have caused the damage of the seed membranes (i.e. perhaps they became more fluid), thereby preventing germination even after alleviation of the stress. The specific cause for the lack of germination in the nongerminating seeds is unclear, although the water content of the seeds treated with NaCl was generally higher, suggesting that osmotic limitation of water influx is unlikely to be the cause (Zhang *et al*. 2010). It seems plausible that the membrane integrity may have been compromised or that salt accumulation may have caused a loss of tonoplast Na^+/H^+ antiporter function, thus preventing germination (Debez *et al*. 2004). Although, in this study, we do not evaluate the membrane integrity, the lack of recovery of germination after the salt treatments provides circumstantial evidence that NaCl leads to a lethal osmotic effect in *P. juliflora* seeds. This lack of the recovery in seeds treated with NaCl was previously reported in *P. juliflora* (Peres & Moraes 1994) similar to other cultivated species (Zhang *et al*. 2010). However, several other studies showed that NaCl-treated seeds are still able to germinate after rinsing (Ungar 1978, 1982, 1995; Pujol *et al*. 2000), although most of these studies focused on halophyte species. Glycophyte seeds cannot remain viable for long periods under extremely high salinity/osmotic stress and germinate at a later time when the osmotic potential of the medium has been increased (Ungar 1978; Khan & Gulzar 2003).

When transferred to deionized water, the nongerminating seeds exhibited distinct behaviors depending on whether they been treated with PEG or NaCl. Seeds treated with PEG readily recovered their germinability (100%) (Table 5). These results indicate that *P. juliflora* seeds can remain nongerminated and viable on the soil surface; however, if the water stress is accompanied by salt stress, the viability of the seeds can be severely impaired. This may have ecological significance within highly saline environments, reflecting a physiological response that was under strong selective pressure during the evolution of these species (Ungar 1982). The suspension of germination of salt-adapted species under high salt conditions might represent an alternative strategy; it may be a method of escape or avoidance that facilitates successful completion of the life cycle in a high-stress environment without damage (Ungar 1982; Song *et al*. 2005; Leatherwood *et al*. 2007). In the Caatinga, seeds normally germinate in rainy conditions (Barbosa *et al*. 2003), instead of dry conditions to avoid the increase in the salt concentration of the soil (Dantas *et al*. 2006) caused by high levels of evaporation. This response might be favorable for germination of the species, as even during the rainy season in the Caatinga, the salt concentration at the soil interface may be rapidly alleviated (Günster 1994). This trait might be related to rainfall irregularity in the Caatinga: despite being restricted to a short period over the year (only a few days), it can reach values of up to 900 mm in some areas of the ecosystem (Sampaio 1995) and keep the soil humid for long enough to complete the germination process. Thus, for the successful establishment of plants in saline environments, seeds must remain viable at high salinity and germinate when salinity decreases (Ungar 1978, 1995). According to Flores and Briones (2001), the relationship between germination patterns and water availability highlights an important adaptation of species that germinate in arid and semiarid ecosystems, and these species would have an advantage in these environments (Pasiecznik 2001). Maybe this is one reason for the great reproductive success of *P. juliflora* in the Caatinga environment (Peres & Moraes 1994; Flores & Briones 2001; Miranda *et al*. 2011))

Prosopis species survive and grow at salinity levels equal to that of seawater (Felker *et al*. 1981) and in soils with a pH of 10.5 (Singh 1995). *Prosopis juliflora* has been found to tolerate salinity levels up to 18 g/L NaCl/L with no reduction in growth or survival and still grows at 36 g/L NaCl (Felker *et al*. 1981). However, El-Keblawy and Al-Rawai (2005) showed that *P. juliflora* has a depressive effect on the number, richness, evenness, density and frequency of associated native species because this species has the potential to alter primary productivity, decomposition, hydrology, nutrient cycling, and natural disturbance regimes. The success of exotic plants in invading some communities has been attributed to the superiority of the exotic species over the native species in some measurable traits, such as reproductive and dispersal capabilities, seedling establishment and survivorship, genome size, phenotypic plasticity, growth-related characteristics, plant height, phenology, allelopathy and plant–soil relationships (Pasiecznik 2001). The present study adds one more factor: high germinability, even in soils with high levels of salinity or soils that are affected by long periods without rain. Furthermore, the mechanical dormancy exhibited by *P. juliflora* (Miranda *et al*. 2011) allows the lifespan of the seeds to be extended, allowing the formation of a persistent seed bank in the soil and the distribution of the germination over time and

space. This strategy can increase the likelihood that the species will find conditions for the establishment of their seedlings. (El-Keblawy & Al-Rawai 2005)

Conclusion

The results of the present study showed that the seed germination of *P. juliflora* is influenced by many factors such as salinity, drought, light, and temperature. Under optimal temperature (30°C), seed germination reduced to 4% at –0.75 MPa or PEG. In this condition, germination rate was 9 days, in comparison to 1.27 days of control. *Prosopis juliflora* seeds germinated with NaCl osmotic potential until –1.5 MPa, but no seed germination was observed with PEG osmotic potential below –0.75 MPa in all temperatures or below –0.5 MPa at 40°C. The performance of the seeds was better in NaCl than in PEG at the same osmotic potential. Seed germination of *P. juliflora* was considerably reduced with increasing salt and drought stress, and this could be attributed mainly to salt stress rather than drought stress. Pretreatment of PEG may be helpful in overcoming the negative effect imposed by reduced water potential. From a practical point of view, when saline water is used for irrigation at sowing, it may be useful to sow when optimal or nearly optimal temperature may be encountered in the soil in order to avoid the combined stress of temperature and salinity or temperature and water stress. Regarding the positive effects of pretreatment with PEG on germination of *P. juliflora*, it could be used as pre-sowing treatment in field conditions. Somehow, the water stress can act positively in the establishment of this species because it promotes a delay in the germination rate and synchrony. In natural conditions, *P. juliflora* exhibited heterogeneity in germination; thus, germination is distributed over time and space, increasing the likelihood that a seedling will find conditions that are favorable for its establishment and development. Overall, the reduced seed germination due to reduced water availability is one of the special survival strategies used by *P. juliflora* in the semiarid Caatinga, because germination rate and synchrony are good indices for evaluating the occupation ability of a species in a given ecosystem. These findings could help both explain the rapid increase of the *P. juliflora* in the Caatinga and management of this species in this environment.

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