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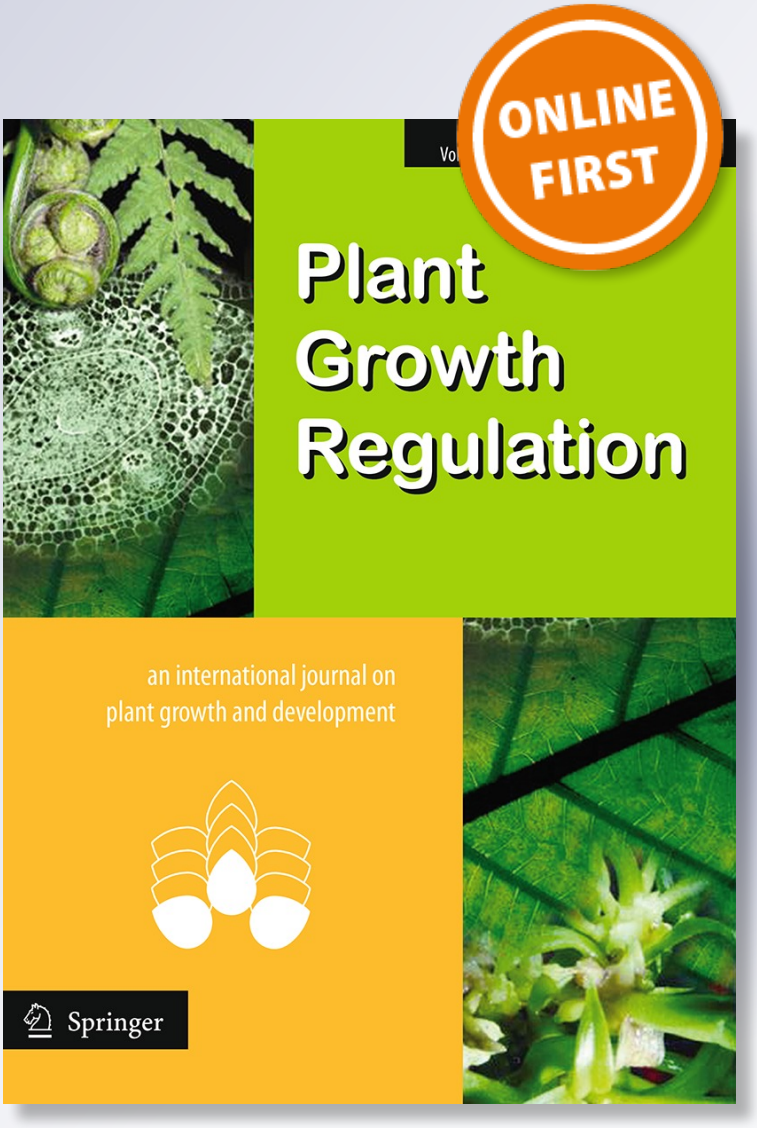

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ORIGINAL PAPER

Different relative humidity conditions combined with chloride and sulfate salinity treatments modify abscisic acid and salicylic acid levels in the halophyte *Prosopis strombulifera*

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Abstract It has been shown that abscisic acid (ABA) and salicylic acid (SA) act as endogenous signal molecules responsible for inducing abiotic stress tolerance in plants. However, our knowledge on the role of both phytohormones in response to environmental conditions in halophytic plants is still limited. In this study endogenous ABA and SA levels, growth parameters and chlorophylls content were determined in leaves and roots of the halophyte *Prosopis strombulifera* cultivated under increasing NaCl and Na_2SO_4 concentrations, at 30 and 70 % relative humidity (RH) conditions. Endogenous ABA and SA content differed depending on the salt type and concentration, RH, plant age and the organ analyzed. Under low RH conditions *P. strombulifera* growth was strongly inhibited and chlorophyll *a* and *b* content were decreased. In leaves of Na_2SO_4-treated plants at 30 % RH, high ABA levels were correlated with protection against dehydration and ion toxicity. Instead, high SA levels were correlated with the damaging effect of sulfate anion and low RH on plant growth. NaCl-treated plants growth was also inhibited at 30 % RH although levels of both hormones were not significantly increased. Taken together, the salt toxic effects on growth parameters and photosynthetic pigments were accentuated by low RH conditions and these responses were reflected on ABA and SA content.

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Keywords Abscisic acid · Humidity conditions · Plant growth · *Prosopis strombulifera* · Salicylic acid · Salinity

Abbreviations

ABA	Abscisic acid
RH	Relative humidity
SA	Salicylic acid

Introduction

The genus Prosopis includes many important arboreal and shrub like species present in high salinity areas of North and South America (Burkart 1976). Many of the species, particularly those in the Algarobia section, have economic and ecological importance, and are major components of various ecosystems, providing shade, firewood, food, and forage for wildlife and livestock (Felker 2007). Populations of some species, particularly *Prosopis pallida*, *P. juliflora*, *P. tamarugo*, and *P. alba*, include individual plants that display rapid growth at seawater salinity ($\sim$45 dS m^{-1}). This is nearly 20 times the maximal salinity concentration that can be tolerated by temperate annual legumes (Felker 2007). Several studies indicate that the NaCl tolerance of *P. strombulifera* exceeds the limits described for most halophytic plants (Almeida Viégas et al. 2004).

The spiny shrub *P. strombulifera* (Lam.) Benth (Burkart 1976) is a halophyte which grows in areas that spread from the Arizona desert (USA) to Patagonia (Argentina), and is particularly abundant in high-salinity areas of central Argentina. The major cations present in saline environments are typically Na^+, Ca^{2+}, and Mg^{2+}; the main anions are Cl^-, SO_4^{2-}, and HCO^{3-} (Grattan and Grieve 1999). In many countries, NaCl and Na_2SO_4 are the most abundant

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salts (Manivannan et al. 2008). In high-salinity soils of southern Cordoba and southwestern San Luis provinces, Argentina, proportions of these two salts are generally similar, although Sosa et al. (2005) found that Na_2SO_4 was up to three times more abundant in certain samples. It is important to compare the effects of these two salts on plant growth, in order to better understand the plants physiological responses. Our previous results showed a halophytic response of *P. strombulifera* to NaCl surviving up to 1 M NaCl in in vitro experiments, but a strong growth inhibition was found at lower Na_2SO_4 concentrations. Seedlings grown in an increasing gradient of NaCl (250–700 mmol L^{-1}) did not develop salt glands in the leaves. Some tissues displayed vacuolization, and the root system underwent precocious lignification and/or suberization of endodermal cells, with Casparian strips found much closer to the root tip than in glycophytes. These plants can therefore filter more efficiently soil solution to prevent passage of excess ions to the xylem (Reinoso et al. 2004). Na_2SO_4 treatment of *P. strombulifera* seedlings induced structural alterations in cells and tissues, with consequent changes in growth patterns at various levels of organization, and anatomical and histological differences in roots, stems, and leaflets, compared to control plants, or plants grown in high NaCl (Reinoso et al. 2005). These differential responses to the most abundant salts present in most salinized soils make this species a good model to study salt-tolerance mechanisms in halophytic plants.

Soil salinity leads to a decrease in water potential, which affects water availability; thus, the physiological mechanisms governing the plant responses to salinity and drought show high similarity, and both stresses are perceived by the plant cell as deprivation of water (Wang et al. 2001a; Flowers and Colmer 2008). In addition to the hyperosmotic shock and the generated subsequent oxidative stress (Borsani et al. 2001), the deleterious consequences of high salts concentration in the external solution of plant cells also include ion toxicity, nutrient imbalance and change the photosynthetic pigment synthesis (Hasegawa et al. 2000). It has been shown that humidity mitigates the effects of drought and salt (Asch et al. 1995). Arid atmospheric conditions enhance water and salt stresses, and therefore, crops appear to be less salt tolerant when grown under dry conditions than under humid conditions (Neue et al. 1990). Although controversially discussed in some papers (Nambara and Marion-Poll 2005), most authors agree that abscisic acid (ABA) is one of the main plant hormone which mediates adaptive responses to abiotic stress (Yamaguchi-Shinozaki and Shinozaki 2005; De Torres-Zabala et al. 2007).

ABA has multiple roles in numerous physiological processes during the plant life cycle (Zhang et al. 2006) and functions not only as a stress signal but also as a growth regulator that controls vegetative growth and determines cell fate (Benschop et al. 2005; Saika et al. 2007). The regulation of these responses is mediated by changes in endogenous ABA levels. Plants have to adjust ABA levels constantly to respond to changing physiological and environmental conditions. ABA content is also known to be affected by humidity. There are a number of reports about the regulation of ABA metabolism in response to different humidity conditions (Tan et al. 2003; Lee et al. 2006; Endo et al. 2008). In spinach (*Spinacia oleracea*) and *Tradescantia virginiana*, ABA levels were found to be lower in leaves grown in high-humidity conditions, compared with those grown in moderate humidity conditions (Rezaei Nejad and Van Meeteren 2007, 2008). In contrast to extensive progress in understanding the regulation of ABA biosynthesis in glycophytic plants, our knowledge of ABA in halophytic plant in response to environmental cues is still limited.

Salicylic acid (SA) is an endogenous growth regulator of phenolic nature, which participates in the regulation of physiological processes in plants. Recent studies point out that it also participates in the signalling of abiotic stresses (Hao et al. 2011). In the case of salt and osmotic stress, the effect of SA is rather ambiguous in consideration of different plant species, osmotic stress intensity and duration, as well as SA doses applied (Horvath et al. 2007). It has been demonstrated that pretreatment of 2- week-old maize plants with 0.5 mM SA for 1 day decreased their drought tolerance (Nemeth et al. 2002). Besides, it was shown to potentiate the generation of ROS in photosynthetic tissues of *Arabidopsis thaliana* during salt and osmotic stresses, thus participating in the development of stress symptoms (Borsani et al. 2001). However, when applied exogenously by soaking barley grains in 1 mM SA before sowing, it increased the tolerance of the plants to salt stress (Wang et al. 2001b). It has been shown that SA accumulation is a fundamental requirement of a successful acclimation to control the oxidative chain-reactions and/or maintain effective repair and detoxifying mechanisms (Tari et al. 2002). Moreover, treatment with SA leads to ABA accumulation, thus indicating a protective effect (Shakirova et al. 2003; Szepesi et al. 2009). However, the possible interplay between ABA and SA and the way of signal regulation of plant tolerance to adverse conditions is still unclear.

Because hormones are involved in plant responses to different abiotic conditions, we hypothesized that different relative humidity may lead to changes in physiological responses to different salinity conditions such as plant growth and endogenous ABA and SA levels in *P. strombulifera.*

Therefore, the aim of this study was to analyze endogenous ABA and SA content, growth parameters (shoot length, root length and photosynthetic pigment content) in leaves and roots of the halophyte *P. strombulifera* under

increasing NaCl and Na_2SO_4 concentrations, at 30 and 70 % relative humidity.

Materials and methods

Plant materials

Seeds of *P. strombulifera* were collected from an area in southwestern San Luis province, Argentina. The area is predominantly a carob tree forest in the El Monte Phytogeographic Region, 33 43′S, 66 37′W, located in a saline depression between annual 300–400 mm isohyets, altitude 400–500 m, and average annual temperature 15–20 °C. The soil has a franc sandy texture, with abundant calcareous material and moderate salinity; chemical composition of soil profile in the sampling area was determined (Sosa et al. 2005). Profile from 0 to 35 cm depth shows an increase in EC (dS/m) of 8, 4 to 11, SAR: 9 to 51, Na^+ concentration: 40 to 210, Cl^- 6 to 36, SO_4^{2-}: 28 to 33. Pods were collected at random from 100 plants within the same population. Seeds were selected visually on the basis of uniform size and healthy aspect; then they were scarified with sulfuric acid for 10 min, washed overnight under running water, rinsed in distilled water, and finally placed in Petri dishes with two layers of water-saturated filter paper for 24 h at 37 °C before sowing (Reinoso et al. 2004). Germinated seeds with roots 20 mm long were cultured under hydroponic conditions, in two black trays per treatment per experiment (200 seedlings per tray) with 10 % full-strength Hoagland's solution. Seedlings were grown for 1 week in a chamber with a cycle of 16 h light (200 µmol m^{-2} s^{-1}) (28 °C):8 h dark (20 °C), and 70 % relative humidity, then transferred to 25 % full-strength Hoagland's solution (osmotic potential −0.03 MPa). Aeration was provided by an aquarium tube system with a peristaltic pump, and pH of all media was 6.

Humidity treatments

The trays were placed in two different growth chambers (Conviron E15, Controlled Environments Limited, Manitoba, Canada) with a cycle of 16 h light (200 µmol m^{-2} s^{-1}) (28 °C): 8 h dark (20 °C). Relative humidity was 70 % in one chamber and 30 % in the other. After one week, the nutrient solution was changed to 25 % Hoagland's solution (osmotic potential (Ψo) = − 0.11 MPa). After this, the nutrient solution was changed weekly to maintain an adequate nutrient availability. Aeration was provided by an aquarium tubing system with a peristaltic pump. pH of medium was consistently 6. The experiment was performed three times (3 trays per treatment each time).

Salt treatments

Salt treatments were applied after plants had grown for 15 days, using a simple randomized design. As shown in Table 1, pulses of NaCl alone (50 mmol L^{-1}) and Na_2SO_4 alone (38 mmol L^{-1}) were applied every 48 h until reaching final osmotic potentials (Ψo) = −1.0, −1.9, or −2.6 MPa respectively (measured by a vapor pressure osmometer Model 5500, Wescor Inc., Logan, UT, USA). These Ψo values corresponded to age 29, 40, and 48 days, respectively. Iso-osmotic bisaline solutions were obtained by mixing equal volumes of the respective monosaline solutions at each osmotic potential. For each sampling, 25 treated plants were collected at random at 24 h after the medium reached a final osmotic potential as indicated above; 25 control plants (no salt added; Ψo of medium = − 0.11 MPa) were collected for each treatment. Plants were frozen with liquid nitrogen, and stored at −80 °C for a posteriori analysis. Each experiment was performed three times.

Determination of growth parameters

Root length and shoot height were measured weekly in 20 plants from each treatment, from the time that salt pulses were started (day 22, 29, 40 and 48 of culture).

Determination of photosynthetic pigments

Chlorophylls *a* and *b* were determined following the conventional method acetone extraction. Fresh leaves were ground in a mortar, extracted in 80 % acetone for 1 h at 4 °C, and centrifuged. Chlorophyll *a* and *b* concentrations were determined

Table 1 Increasing salt concentrations obtained by sequential addition of pulses every 48 h

Salt pulses	Na_2SO_4 (mM)	NaCl (mM)	Ψo (MPa)
1° pulse	37.9	50	−0.3
2° pulse	75.8	100	−0.47
3° pulse	113.7	150	−0.65
4° pulse	151.7	200	−0.82
5° pulse (sampling)	189.7	250	−1
6° pulse	227.5	300	−1.18
7° pulse	265.4	350	−1.35
8° pulse	303.3	400	−1.53
9° pulse	341.2	450	−1.71
10° pulse (sampling)	379.2	500	−1.88
11° pulse	417.1	550	−2.06
12° pulse	455	600	−2.24
13° pulse	492.9	650	−2.42
Last pulse (sampling)	530.8	700	−2.6

by absorbance of the supernatant at 650 and 665 nm, respectively, using a spectrophotometer (Helios Gamma, Thermospectronic, UK). These pigments were quantified considering the corresponding extinction coefficients.

Determination of abscisic acid and salicylic acid by LC–ESI–MS–MS

ABA and SA were extracted and purified as described by Zhou et al. (2003), with modifications. 150 mg dry weight equivalent of leaves or roots tissues were grounded in a mortar with liquid nitrogen, and extracted with 3 mL of extraction buffer (pH: 2,8); 50 ng of $^{2}H_6$-ABA and 50 ng of $^{2}H_4$-SA (OlChemIm, Czech Republic) were added as internal standards. Extracts were transferred to 50 ml tubes, centrifuged at 8,000 rpm for 15 min, and supernatants were collected and mixed with ethyl acetate. Then the organic phase was extracted and evaporated at 37 °C. Dried extracts were dissolved in 1,500 µL methanol. Extracts were resuspended in 50 µL methanol (100 %), and placed in vials.

Liquid chromatography

Analyses were performed using an Alliance 2695 (Separation Module, Waters, USA) quaternary pump equipped with auto-sampler. A Restek C18 (Restek, USA) column (2.1 9 100 mm, 5 lm) was used at 28 °C, with injected volume 10 µL. The binary solvent system used for elution gradient consisted of 0.2 % acetic acid in H2O (solvent B), and MeOH (solvent A), at a constant flow-rate of 200 µL min^{-1}. A linear gradient profile with the following proportions (v/v) of solvent A was applied [t (min), % A]: (0, 40), (25, 80), with 7 min for re-equilibration. MS/MS experiments were performed on a Micromass Quatro UltimaTM PT double quadrupole mass spectrometer (Micromass, Manchester City, UK). All analyses were performed using turbo ion spray source in negative ion mode with the following settings for ABA and SA: capillary voltage -3000 V, energy cone 35 V, RF Lens1 (20), RF Lens2 (0.3), source temp. 100 °C, de-solvation temp. 380 °C, gas cone 100 L h^{-1}, gas de-solvation 701 L h^{-1}, collision cell potential of 10 eV for ABA (Zhou et al., 2003) and 15 eV for SA (Ibanez et al. 2010).

Mass spectrometry

MS/MS parameters were optimized in infusion experiments using individual standard solutions of ABA and SA at a concentration of 50 ng μL^{-1} diluted in mobile phase A/B (40:60, v/v). MS/MS product ions were produced by collision-activated dissociation of selected precursor ions in the collision cell of the double quadrupole mass spectrometer, and mass was analyzed using the second analyzer of the instrument. In negative mode, the spectrum for ABA and SA gave deprotonated molecule [M–H]. Quantitation was performed by injection of samples in multiple reaction monitoring (MRM) mode, since many compounds could present the same nominal molecular mass. The combination of parent mass and unique fragment ions was used to selectively monitor ABA and SA in plants extracts. MRM acquisition was performed by monitoring the 263/153 and 269/159 transitions for ABA; and 137/93 and 141/97 transitions for SA and ($^{2}H_6$)-ABA and ($^{2}H_4$)-SA (internal standards) respectively, with dwell 1,000 ms for each transition.

Data were acquired and analyzed using MassLynxTM 4.1 and QuanLynxTM 4.1 (Micromass, Manchester, UK) software. For quantification, values were obtained from a calibration curve previously constructed using ABA and SA pure standard/ABA and SA deuterated internal standards (Sigma, St. Louis, MO, USA).

Statistical analysis

Data were analyzed by using INFOSTAT (2011 v.). A three factorial experiment: relative humidity (30 and 70 %), osmotic potential (Ψo) (-1.0, -1.9, or -2.6 MPa) and salt treatment (control, NaCl, Na_2SO_4) was set up in a completely randomized design. Three way ANOVA was performed and significant differences among treatments were calculated by the use of Duncan's multiple range test ($P < 0.05$).

Results

Growth parameters and photosynthetic pigments

At 30 % RH and 48 days of culture root growth of treated plants was significantly lower than NaCl and non-treated plants at 70 % RH. Interaction between humidity, salt treatments and days of culture (when treated plants arrived desired osmotic potentials) was no significant on root length (Fig. 1).

On the other hand, on shoot growth the interactive effect of humidity x type of salt x days of culture was significant. At 30 % humidity conditions both salts NaCl and Na_2SO_4 inhibited shoot growth as compared to all treatments evaluated mainly at 48 days of culture (Fig. 2).

Chlorophyll *a* and *b* were significantly affected by the triple interaction. At 30 % humidity conditions treated plants show a decrease in these photosynthetic pigments when salinity increased. However, at 70 % relative humidity chlorophyll *a* and *b* were not significantly affected by salinity (Fig. 3a, b).

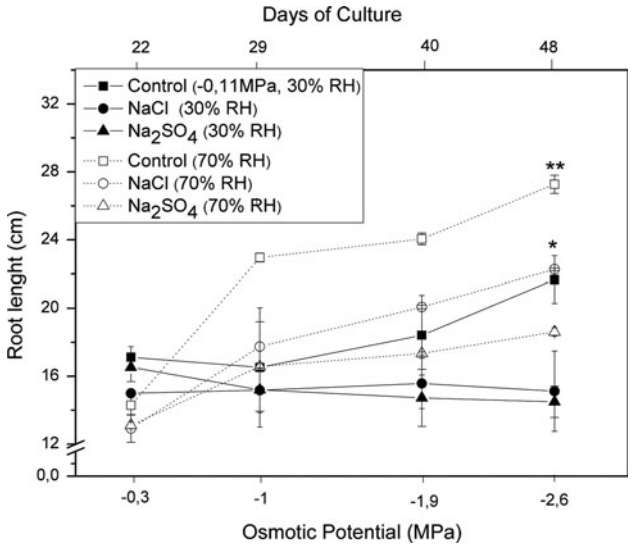

Fig. 1 Effects of NaCl and Na_2SO_4 on root length of *Prosopis strombulifera* at low (30 %) and high (70 %) humidity conditions. Data were from three replicated experiments (total n = 30 plants per treatment), and represent mean ± S.E. * and **above data indicate significant differences among treatments only at 48 days of culture ($P < 0.05$)

Abscisic acid content

At 30 % humidity conditions, roots of salt-treated plants showed no significant differences in ABA levels at low and moderate salinity (−1 and −1.9 MPa). At high salinity (−2.6 MPa) ABA levels significantly increased in Na_2SO_4-treated plants as compared to non-treated plants (Fig. 4a).

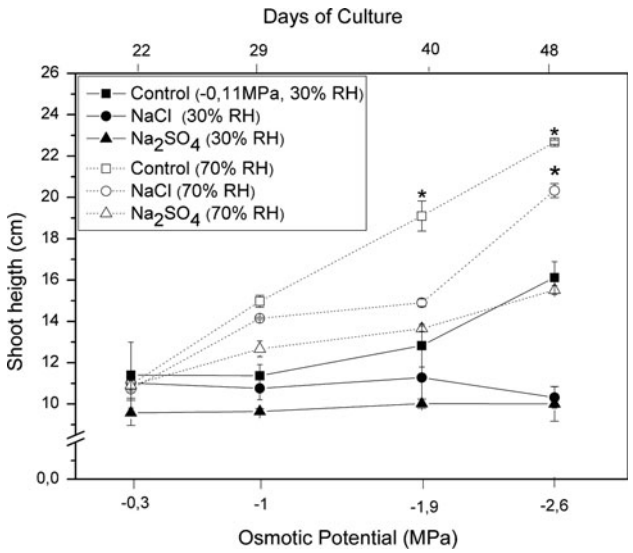

Fig. 2 Effects of NaCl and Na_2SO_4 on shoot heigth of *Prosopis strombulifera* at low (30 %) and high (70 %) humidity conditions. Data were from three replicated experiments (total n = 30 plants per treatment), and represent mean ± S.E. *above data indicate significant differences among all treatments ($P < 0.05$)

At high relative humidity the highest ABA level was observed in roots from Na_2SO_4-treated plants at −1 MPa (29 d). No significant differences were observed in ABA levels in any treatment at 40 days of culture and later.

As shown in Fig. 4b, ABA levels increased sharply in leaves of Na_2SO_4-treated plants from the beginning of the treatment (29 days), and kept constant towards the end of the assay. At high relative humidity ABA levels in leaves of Na_2SO_4-treated plants peaked later than at 30 % RH, at 40 days.

Salicylic acid content

Interaction between humidity, salinization and days of culture was significant on SA content in roots. At 30 % RH SA levels were very low through out the whole experience with no significant differences among treatments. At 70 % humidity, the highest SA level was observed in roots from

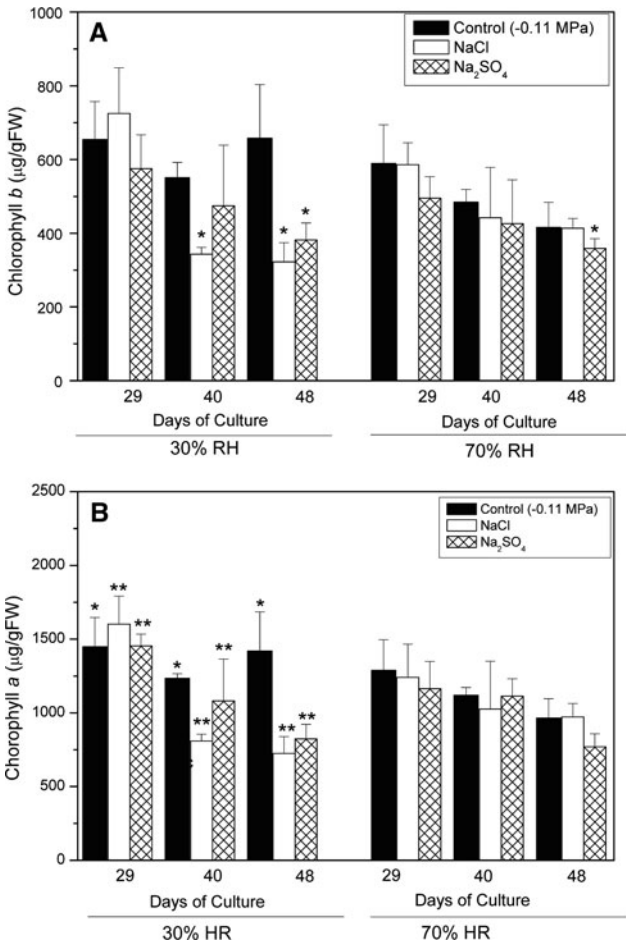

Fig. 3 Effects of NaCl and Na_2SO_4 on photosynthetic pigments of *Prosopis strombulifera* at low (30 %) and high (70 %) humidity conditions. **a** Chlorophyll *b*, **b** Chlorophyll *a*. Data were from three replicated experiments (total n = 30 plants per treatment), and represent mean ± SE * and **above data indicate significant differences among all treatments ($P < 0.05$)

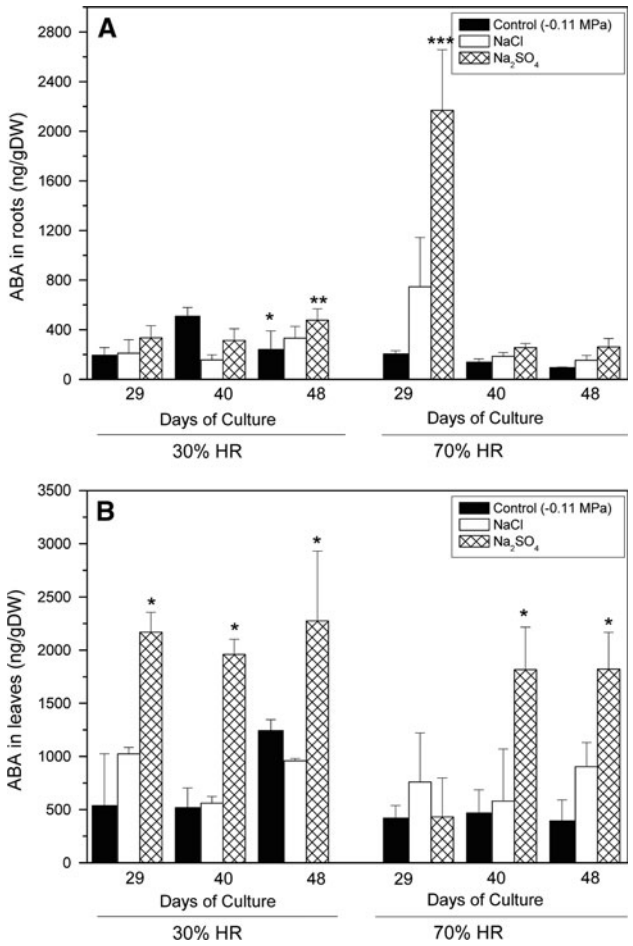

Fig. 4 Effects of NaCl and Na_2SO_4 on ABA levels of *Prosopis strombulifera* at low (30 %) and high (70 %) humidity conditions. Data were from three replicated experiments (total n = 30 plants per treatment), and represent mean ± SE. **a** Roots, ***above data indicate significant differences among all treatments ($P < 0.05$); * and **above data indicate significant differences among treatments only at 48 days of culture and 30 % RH ($P < 0.05$). **b** Leaves, *above data indicate significant differences among all treatments ($P < 0.05$)

non treated plants at the beginning of the experience (29 days of culture). A decrease in SA content was observed which continued up to the end of the experiment (Fig. 5a).

In leaves, at 30 % relative humidity SA content in Na_2SO_4-treated plants was drastically increased at 40 days of culture (Fig. 5b). Notably at 70 % RH, non treated plants showed the highest SA levels at 29 and 48 days of culture, while a significant decrease was observed at 40 days of culture. NaCl-treated plants showed a similar tendency in SA levels respect to non treated plants. SA levels in Na_2SO_4-treated plants were significantly lower than the other treatments at 29 days of culture. However, at 40 days of culture a significant increase in SA levels in Na2SO4-treated plants compared to non treated plants was observed, which was doubled at the end of the assay being significantly higher than that from NaCl-treated plants.

Discussion

Variations in the environmental conditions constitute the primary signal which brings about modifications in the plant physiology. Humidity can change rapidly (within minutes) in the natural environment, mitigating or worsening the effects of water, salt, and temperature stresses. Low atmospheric humidity conditions enhance such stresses, and therefore, plants appear to be less tolerant (Fageria 1985; Neue et al. 1990).

Our previous studies demonstrate that *P. strombulifera* does not show halophyte towards Na_2SO_4 as it is with NaCl. Plants grown in the presence of Na_2SO_4 showed immediate and sustained reduction of growth parameters, accompanied by senescence symptoms such as chlorosis, necrosis, and leaf abscission (Reinoso et al. 2005; Reginato et al. 2012). In this study, *P. strombulifera* showed a strong growth inhibition at high NaCl and Na_2SO_4 concentrations (48 days of culture) at 30 % RH. It is known that a direct effect of low RH on the leaf-to-air vapour pressure gradient is an increase in plant transpiration which is responsible of excessive ion uptake when plants are growing under salinity. This chain of events has a strong inhibitory effect on growth (Fig. 5).

On the contrary, growth parameters and content of chlorophyll *a* and *b* were less affected by salinity at 70 % RH. However, root length and shoot height of Na_2SO_4-treated plants were more affected than those in NaCl-treated plants confirming previous observations on the inhibition generated when sulfate anion is present in the medium. These plants showed a remarkably higher transpiration than those treated with NaCl, probably caused by a failure in the mechanism of closing stomata (Llanes 2010).

Reduction of chlorophyll content due to limited water availability and salinity is a commonly observed phenomenon (Chaves et al. 2003; Reynolds et al. 2005; Santos et al. 2001). In the present study, RH x salinity interaction caused a significant decrease on photosynthetic pigments at low RH conditions. Therefore, our results demonstrate that low humidity accentuated the salt toxic effects on growth parameters and chlorophyll content.

Phytohormones are essential for the ability of plants to adapt to abiotic stresses by mediating a wide range of adaptive responses. One of the most studied topics in the response of plants to abiotic stress, especially water stress and salinity, is ABA accumulation. The biosynthesis and redistribution of this hormone is one of the fastest responses of plants to abiotic stress, causing stomatal closure, thereby reducing water loss via transpiration and eventually restricting cellular growth (Blumwald and Peleg 2011).

On the other hand, there are many studies about the effect of SA exogenously applied under salt and drought stress (Senaratna et al. 2000; Borsani et al. 2001; Singh and

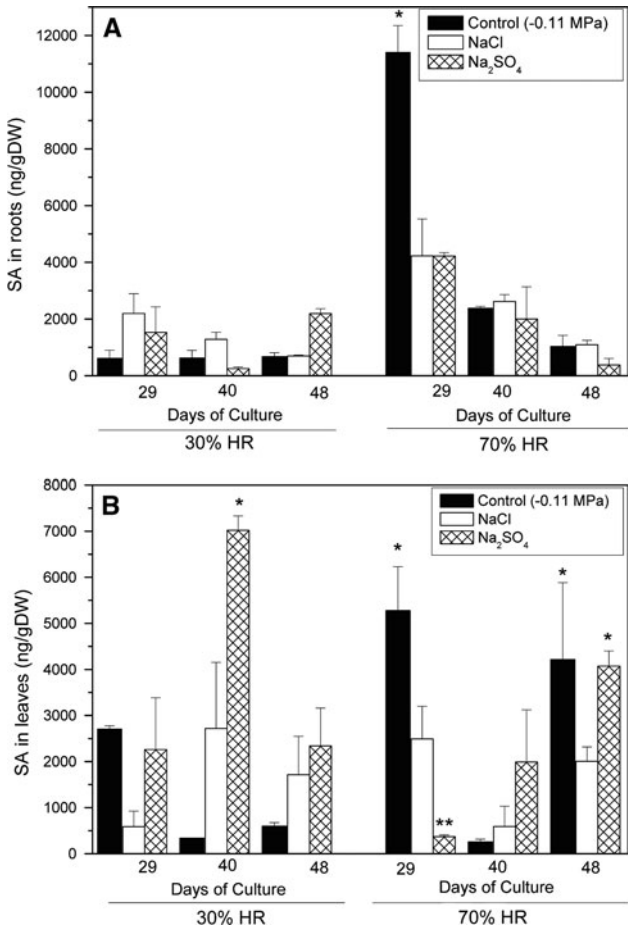

Fig. 5 Effects of NaCl and Na_2SO_4 on SA levels of *Prosopis strombulifera* at low (30 %) and high (70 %) humidity conditions. Data were from three replicated experiments (total n = 30 plants per treatment), and represent mean ± SE. **a** Roots, *above data indicate significant differences among treatments ($P < 0.05$). **b** Leaves * above data indicate significant differences among treatments ($P < 0.05$); **above data indicate significant differences among treatments only at 29 days of culture and 70 % RH ($P < 0.05$)

Usha 2003), but the synthesis of this hormone in response to salinity has been sparingly addressed (Sakhabutdinova et al. 2003).

When three stressing factors are applied simultaneously they interact bringing about a response that is different to that triggered by any of them individually. In this study, different salts at different concentrations and different RH conditions caused particular responses of phytohormones production and growth parameters. Low humidity amplified the effects of salinity by modifying the biosynthesis of ABA and SA.

Our results showed that endogenous ABA and SA levels in *P. strombulifera* varied according to the salt type and concentration, RH, days of culture and the organ analyzed. The higher ABA accumulation in leaves than in roots, independently of the environmental humidity, may be due to its rapid biosynthesis and distribution within the mesophyl, as well as to transport from roots, taking into account the relative low levels found in these organs. This was especially noticeable at low humidity conditions where ABA accumulation seemed to be triggered by the conjunction of low humidity plus salinity, especially in leaves of Na_2SO_4-treated plants from the beginning of the experience. These high ABA levels are correlated with increasing toxicity symptoms caused by this salt as reported above, and the maintenance of primary root elongation and shoot inhibition at low osmotic potentials and 30 % RH. Similar results were obtained previously in this species, where the highest levels of ABA and ABA-GE, and the highest ABA-GE glucosidase activity were observed in Na_2SO_4-treated plants. Therefore, ABA would act as a triggering signal for adaptive mechanisms for plant survival at this extremely stressing situation. However, the stress imposed by the presence of sulfate anion in the culture medium blocked activity of ABA, stomata remained open, and high transpiration values were recorded (Llanes 2010).

However, ABA content in NaCl-treated plants was slightly modified by humidity and days of culture (age of plants), compared to non treated plants but no statistical differences were found. Previous observations showed that this salt is not deleterious for this species being well tolerated up to concentrations as high as 700 Mm (−2.6 MPa).

At 70 % RH NaCl-treated plants grew well, their aspect was healthy without showing toxicity; thus, they may not need an extra ABA accumulation for adaptive mechanisms for plant survival. Possible explanations could be: (1) less accumulation of leaf ABA due to a low transpiration rate under high RH conditions; or (2) a decrease in leaf ABA levels because of increased catabolism or phloem transport out of the leaf (Llanes 2010).

On the other hand, it has been proposed that exogenously applied SA may be a potential growth regulator for improving plant growth under limited water availability (Singh and Usha 2003). Increasing evidence has shown that SA can improve plant adaptation to salinity by various mechanisms, such as lessening photosynthetic damage (Arfan et al. 2007), increasing antioxidative protection (Xu and Tian 2008), inhibiting Na^+ and Cl^- accumulation (Gunes et al. 2007) and accumulating soluble carbohydrates (Poor et al. 2010). On the contrary, a negative effect of SA in plant tolerance to salt stress has been observed in studying transgenic NahG *A. thaliana* lines, which show SA deficiency. The high accumulation of SA aggravated NaCl induced impairment in photosynthetic process, and this was effectively counteracted by the decrease of SA level due to the nahG (Hao et al. 2011). Also, endogenous SA levels have been shown to decrease with salinity in *Iris hexagona* (Wang et al. 2001a, b). Although the role of SA in plant tolerance against a variety of biotic and abiotic stresses has been

widely studied, very limited information is currently available regarding SA signaling in plants with respect to tolerance to abiotic stresses. This lack of information is reinforced by the fact that most studies have been performed by using exogenous applications of high dosis of SA which are far from being within the physiological range. This may be the cause of several contradictions found in the literature in relation to its role in stress responses.

Some studies have reported that ion sodium was shown to be essential for halophytic species like *Atriplex vesicaria* and demonstrated that small amounts of this element are specifically required for growth (Brownell and Cossland 1972; Brownell 1979). As already mentioned, optimum growth (greater than in non-treated plants) was obtained previously with 500 mM NaCl in *P. strombilufera* which is considered a true halophytic response (Reginato et al. 2012; Llanes et al. 2012). Therefore, plants growing in Hoagland solution without sodium salts may be considered as a stressing situation for this species and the highest SA levels observed in these plants at 70 % RH would imply a stress signal.

Inhibition of Na^+ and Cl^- accumulation by SA was reported by Gunes et al. (2007) and Al-Hakimi and Hamada (2001) found that exogenous applications of SA decreased Na^+ content of wheat shoot and root tissues under salinity. The high SA levels observed in Na_2SO_4-treated plants at 48 days of culture in this work are correlated with a failure in ion compartmentalization by these plants when compared with NaCl-treated plants as previously demonstrated (Reginato et al. 2012). This observation is in agreement with the report by Macri et al. (1986), that SA can greatly perturb the trans-membrane electrochemical potential of mitocondria and the ATP-dependent proton gradient of tonoplast. Since the activity of the H+/solute cotransporters depends on the proton motive force across the plasma membrane, any modification induced by SA of either transmembrane pH or Ψ gradient or both could interfere with ion uptake. Accordingly, Bourbouloux et al. (1998) demonstrated that SA decreased the acidifying activity of the tissues, without affecting either the level of ATPase transcripts or the amount of plasma membrane ATPase. The decrease in the acidifying activity of the tissues was due to the decrease of the ATP levels and of the energy charge of the cell which is considered as a good indicator of metabolic activity. A direct effect of SA on the activity of the transporters cannot be ruled out.

In fact, NaCl-treated plants accumulated similar levels of Na in their leaves than Na_2SO_4-treated plants but they succeed in ion compartmentalization and osmorregulation with direct consequences on their growth. These plants showed the lowest levels of SA. However, low humidity conditions accentuated the stressing effect of the salt and SA levels were also increased although not as much as in Na_2SO_4-treated plants.

SA levels at 70 % RH were correlated with the increasing Na_2SO_4 concentration while at 30 % RH the sharp peak of SA at −1.9 MPa (379 Mm) indicates the additive effect of low humidity and salt toxicity which was evidenced by chlorosis, necrosis and leaf abscission at the end of the experiment. These responses indicate that SA production is not a protective hormonal signal like ABA but a signal of injury in *P. strombulifera* under these adverse conditions.

SA is produced from phenylalanine by the action of the enzyme phenylanaline ammonia lyase (PAL). This enzyme is known to be induced by different types of abiotic and biotic stresses and is a key regulator of the phenylpropanoid pathway, which gives rise to various types of phenolics with multiple functions (Yalpani et al. 1993). Treatment with NaCl did not affect polyphenol content in *P. strombulifera*; instead, Na_2SO_4 sharply induced an increase in flavonoid compounds, mainly total flavan-3-ols, as well as increased levels of malondialdehyde (MDA) (Reginato et al. 2010) indicating a role for these compounds in counteracting the damage induced by severe salt stress, and providing additional evidence that PAL pathway was induced when SO_4^{2+} was present in the medium.

Finally, the importance of phytohormone balance and the cross-talk between their signal pathways is increasingly recognized as central to the outcome of plant-stress responses. For example, it has been demonstrated that ABA suppresses inducible innate immune responses by down-regulating SA biosynthesis and SA-mediated defences, thus exhibiting a complex antagonistic relationship with salicylic acid (Fan et al. 2008; de Torres-Zabala et al. 2007). On the other hand, data presented by Shakirova et al. (2003) indicate that the presowing treatment of wheat seeds with SA contributes to an increase in the resistance of these plants to salt stress; SA-treatment induces a sharp accumulation of ABA, which in turn induces a wide spectra of anti-stress reactions in the plants. Again, all these studies have been performed with exogenous SA applications.

In conclusion, the complexity of plant responses to salinity involves the complexity of the ionic interactions that take place among the various salts present in soil. To add to the complexity, each region in each country has its own specific soil salt profile. This enhances the relevance of our results regarding the need of increasing knowledge on differential plant responses to different salt composition, as well as searching for new plant models to understand the mechanisms of regulation of stress responses, which importance has been focused on recent reviews (Flowers and Colmer 2008; Mittler and Blumwald 2010).

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