

Ecophysiological and biochemical responses of saltbush subjected to salinity¹

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ABSTRACT

Saltbush, a halophyte species tolerant to salt stress, is an excellent material for the identification of physiological and biochemical mechanisms involved in the resistance to salinity. This study aimed at evaluating the ecophysiological and biochemical responses of saltbush plants at the concentrations of 0 mM, 150 mM, 300 mM, 450 mM and 600 mM of NaCl. Biometry, gas exchange and relative content of chlorophyll and proline in the leaves were measured. Plants presented growth stimulation mainly at the levels of 0 mM, 150 mM and 300 mM of NaCl. Regarding gas exchange, plants showed variation in the photosynthetic rate and stomatal regulation, with a small increase in leaf transpiration. Salinity did not significantly alter the chlorophyll contents, but induced an increase in proline contents in all NaCl levels, demonstrating a growth maintenance capacity and indicating the occurrence of osmotic adjustment.

KEY-WORDS: *Atriplex numulária*; gas exchange; proline.

RESUMO

Respostas ecofisiológicas e bioquímicas de erva-sal submetida a salinidade

A erva-sal, uma halófito tolerante ao estresse salino, constitui excelente material para a identificação de mecanismos fisiológicos e bioquímicos envolvidos na resistência a salinidade. Objetivou-se avaliar as respostas ecofisiológicas e bioquímicas de plantas de erva-sal, nas concentrações de 0 mM, 150 mM, 300 mM, 450 mM e 600 mM de NaCl. A biometria, trocas gasosas e teor relativo de clorofilas e de prolina nas folhas foram mensurados. As plantas apresentaram estímulo de crescimento principalmente nos níveis de 0 mM, 150 mM e 300 mM de NaCl. Quanto às trocas gasosas, houve variação na taxa fotossintética e regulação estomática, gerando um pequeno aumento na transpiração foliar. A salinidade não alterou significativamente os teores de clorofilas, mas induziu incremento nos teores de prolina em todos os níveis de NaCl, demonstrando capacidade de manutenção do crescimento e evidenciando ocorrência de ajustamento osmótico.

PALAVRAS-CHAVE: *Atriplex numulária*; trocas gasosas; prolina.

INTRODUCTION

Soil salinization is a serious problem in the Brazilian semiarid region, due to a climate characterized by high evapotranspiration and low rainfall rates. Salinity can interfere with the development of crops, reducing plant production and yield to uneconomical levels (Souza et al. 2000, Leal et al. 2008).

Halophytes are the only plants that can live in soil with high salt concentration (Taiz & Zeiger 2013). Species of the *Atriplex* genus are considered authentic halophytes, since they are characterized by high tolerance to drought and soil salinity, and

are able to complete their life cycle under these environmental constraints (Porto et al. 2000).

Atriplex nummularia Lindl., commonly known as saltbush, is a halophyte known for its tolerance to salt and water stress. Additionally, saltbush produces large amounts of biomass, and it is able to extract considerable amounts of salt from contaminated soils (Leal et al. 2008, Souza 2010).

Saltbush is a species with C₄ metabolism that naturally occurs in saline soils in the semiarid region of Australia. The species has been established in cattle grazing soils affected by salts (Barrett-Lennard et al. 2003). The plant was introduced in the Northeast region of Brazil in the 1930s, through research, due

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to its tolerance to saline and brackish soil, as well as to drought (Obras... 1938). Thus, it constitutes a useful plant for studying physiological mechanisms involved in salt stress resistance (Cabello-Hurtado & Ramos 2004).

Salinity can inhibit plant growth by several factors in the short and long term. The short-term effects include growth reduction, due to the osmotic effect of salt, which reduces cell expansion. Long-term effects include ionic stress, due to excessive salt absorption, leading to a reduction in the photosynthetic leaf area available for growth maintenance (Munns 2002). In both cases, growth reduction is often accompanied by low photosynthetic CO₂ assimilation, due to stomatal and non-stomatal limitations, such as stomata closure and metabolism reduction caused by stress (Silva et al. 2008). However, regardless of the limitation, salinity can still alter stomatal conductance, transpiration, CO₂ availability in the absorption sites in the chloroplast, and photosynthetic pigments (Parida & Das 2005).

An important factor in the salt tolerance mechanism is the ability of plant cells to osmotically adjust themselves by accumulating organic solutes, such as proteins, sugars, free amino acids, proline, etc. (Munns 2002). Among these solutes, proline is an amino acid that protects cells from denaturation processes under conditions of salt stress, assisting in cellular ionic homeostasis, photosynthesis preservation, proteins and membranes stabilization, and free radicals removal (Azevedo Neto et al. 2009).

Given its halophyte nature and importance for soil remediation, and as a potential fodder crop for semiarid regions, this study aimed at evaluating the ecophysiological responses of saltbush plants under increasing NaCl levels over time.

MATERIAL AND METHODS

The experiment was carried out in a climatized greenhouse, with average temperature of 29 °C and relative humidity of 80 %, at the Universidade Federal de Sergipe, in São Cristóvão, Sergipe State, Brazil, in August 2013.

Forty days old saltbush plants were placed in 4 L polypropylene vessels (one plant per pot and five pots for each solution) filled with about 3.80 kg of soil, which was collected at 0.0-0.20 m depth, from a dystrophic Red Ultisol. Its chemical characterization was carried out at the Soil Analysis Laboratory of

the Universidade Federal de Lavras, as it follows: phosphorus: 5.4 mg dm⁻³; potassium: 76.00 mg dm⁻³; exchangeable calcium: 14.18 cmol_c dm⁻³; exchangeable magnesium: 10.85 cmol_c dm⁻³; exchangeable aluminum: 0.10 cmol_c dm⁻³; hydrogen + aluminum: 7.87 cmol_c dm⁻³; pH: 4.9; organic matter: 3.14 dag kg⁻¹; sum of exchangeable bases: 25.22 cmol_c dm⁻³; cation exchange capacity: 33.09 cmol_c dm⁻³; base saturation: 76.23 cmol_c dm⁻³. Fertilization was carried out at the time of plants transplantation with 1.39 g of urea, 3.13 g of superphosphate and 1.08 g of potassium chloride per pot.

During 45 days, the same volume of saline concentration specific for each treatment (0 mM, 150 mM, 300 mM, 450 mM and 600 mM of NaCl) was added to keep the soil at field capacity. Mean values for electric conductivity (at 25 °C) of the irrigation solutions were 0.0075 mS cm⁻¹, 12.11 mS cm⁻¹, 16.36 mS cm⁻¹, 22.1 mS cm⁻¹ and 28.5 mS cm⁻¹, respectively.

Treatments were arranged in a completely randomized design, in a 5 x 4 factorial scheme, with five salinity levels (0 mM, 150 mM, 300 mM, 450 mM and 600 mM of NaCl) and four periods of evaluation over time (0, 15, 30 and 45 days after the application of treatments), with five replications.

Every two weeks, plant height and stem diameter were measured, using a graduated ruler and a digital caliper. Leaves from the upper third of the plants were also sampled for the determination of free proline (Bates et al. 1973).

Gas exchange was measured using the infrared gas analyzer IRGA (LI-6400xt model, LI-COR, Nebraska, USA), with 300 mL min⁻¹ air flow and 1,500 μmol m⁻² s⁻¹ coupled light source, on fully expanded leaves of the upper third part of the seedlings. Measurements were carried out every two weeks, between 8 am and 9 am, under room relative humidity and air temperature. The obtained gas exchange data were: photosynthetic CO₂ assimilation, stomatal conductance, instantaneous transpiration, vapor pressure deficit between leaf and air and carbon internal concentration.

For the determination of leaf chlorophyll *a*, *b* and total, a non-destructive method was used, with a Clorofilog chlorophyll meter (CFL 1030 model, Falker, Porto Alegre, BR). Measurements were carried out at the end of the gas exchange analysis, in the middle portion of the leaf, located in the upper third of the seedlings. Data were subjected to analysis of

variance by the F test and to regression analysis. Data analyses were carried out with Sisvar (Ferreira 2000).

RESULTS AND DISCUSSION

Plant height growth was affected by increasing NaCl levels during the irrigation (Figure 1a). The 0 mM, 150 mM and 300 mM doses of NaCl resulted in higher regression coefficients (slope), when compared to the higher doses (450 mM and 600 mM), with relative height increases of 38.41 %, 29.69 % and 24.63 %, respectively, if compared to the beginning of the experiment.

Stem diameter also increased over time for all NaCl levels (Figure 1b), with increments of 72.64 %, 69.39 % and 70.96 %, respectively for levels of 0 mM, 150 mM and 300 mM of NaCl. Plants subjected to 450 mM and 600 mM of NaCl also followed the same trend, showing that salt stress did not affect stem diameter as much as height. However, under higher salt concentrations (450 mM and 600 mM of NaCl), stem diameters presented the lowest average values in the whole experimental interval. In general, this behavior proves that the species has adjustment or resistance mechanism to the salinity condition imposed, which may have influenced plants to keep plant cell expansion. Thus, the limited growth of plants subjected to high levels of electric conductivity may be predominantly due to osmotic stress (Munns et al. 2006).

Araújo et al. (2006), studying *A. nummularia* plants grown under increasing NaCl levels, reported growth stimulation as the concentration of Na⁺ and Cl⁻ ions to 300 mmol L⁻¹ in the irrigation solution increased. These authors observed that above this

concentration level, growth is negatively affected due to disruption of plant metabolism, with a subsequent reduction in growth. Bazihizina et al. (2012) evaluated the growth of *A. nummularia* under 10 mM, 120 mM, 230 mM, 450 mM and 670 mM of NaCl and observed, 21 days later, an increase in growth at the interval of 120-230 mm of NaCl. However, at 450 mM, growth was similar to that of plants exposed to 10 mM of NaCl. At 600 mM, there was a 71 % reduction in the stem growth rate, in relation to the NaCl dose of 10 mM.

Photosynthetic CO₂ assimilation (Figure 2a) for all the salinity levels decreased immediately after the treatment, and then increased with time under salt stress imposition, reaching minimum values of 12.84 μmol m⁻²s⁻¹, 6.07 μmol m⁻²s⁻¹, 3.28 μmol m⁻²s⁻¹, 6.30 μmol m⁻²s⁻¹ and 2.49 μmol m⁻²s⁻¹, respectively for the levels of 0 mM, 150 mM, 300 mM, 450 mM and 600 mM of NaCl. There was little variation in the time of maximum inflection of photosynthetic CO₂ assimilation curve for salinity, reaching a minimum at 22-24 days for all the salt treatments. After this period, the photosynthesis rate increases, reaching average values close to those obtained in the stress-free period at 45 days under stress, demonstrating high capacity of adaptation to the conditions of this study.

Stomatal conductance showed greater variation among the different salinity levels. However, similarly to photosynthetic CO₂ assimilation, it also showed a slight upward trend for all NaCl levels (Figure 2b). For the levels of 450 mM and 600 mM, this increase was linear (p < 0.01), whereas, for the levels of 150 mM and 300 mM, effects of the time under salt stress imposition were quadratic (p < 0.01). Mean values obtained at 45 days for the

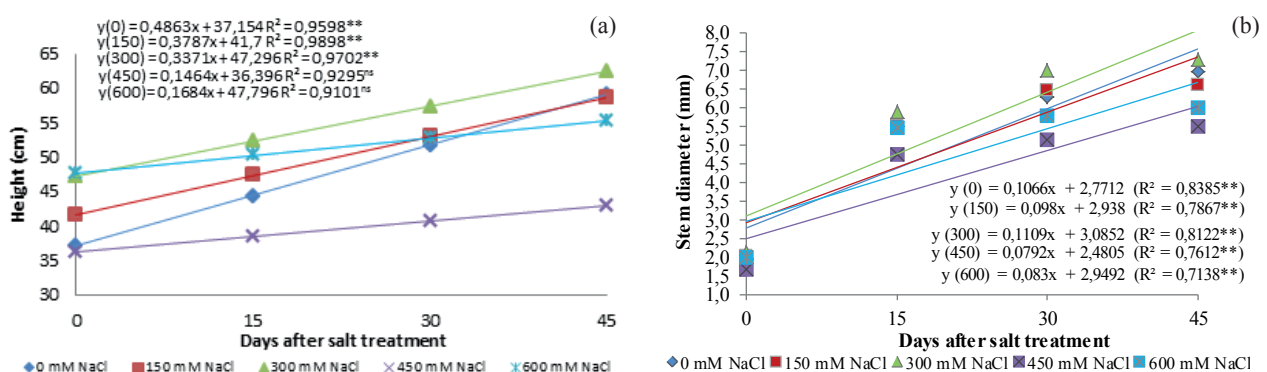


Figure 1. Plant height (a) and stem diameter (b) of saltbush plants grown under different NaCl levels, as a function of the time under salt stress imposition (days). *, ** and ns: significant at 5 % and 1 % and not significant, respectively.

levels of 300 mM, 450 mM and 600 mM of NaCl were respectively $0.41 \text{ mol m}^{-2} \text{ s}^{-1}$, $0.53 \text{ mol m}^{-2} \text{ s}^{-1}$ and $0.83 \text{ mol m}^{-2} \text{ s}^{-1}$.

Responses of these two variables show that saltbush plants did not keep their stomata predominantly closed. This behavior suggests a possible adaptation of the plants to maintain favorable photosynthetic assimilation, even under higher salinity levels, when there are limitations in water absorption. Studies have shown that plants of the *Atriplex* genus subjected to salinity decreased photosynthetic CO_2 assimilation due to reduced stomatal conductance (Hassine et al. 2008, Bazihizina et al. 2009).

Stomatal response usually occurs before photosynthesis inhibition, and limits CO_2 availability in the chloroplast. This defense mechanism is probably the most important factor limiting carbon fixation. However, non-stomatal photosynthesis limitations have been attributed to the reduction in carboxylation efficiency, which may be caused by the accumulation of salts in the mesophyll, resulting in changes in the intracellular CO_2 concentration (Larcher 2004).

CO_2 internal concentration (C_i) presented quadratic response ($p < 0.01$) to all NaCl levels over time (Figure 2c), except at the concentration 0 (linear), with upward trend until the 45th, 45th, 29th, 30th and 31st day, respectively for concentrations of 0 mM, 150 mM, 300 mM, 450 mM and 600 mM of NaCl. The maximum CO_2 internal concentration values were $350.38 \text{ mmol m}^{-2}$, $375.06 \text{ mmol m}^{-2}$, $368.35 \text{ mmol m}^{-2}$, $374.76 \text{ mmol m}^{-2}$ and $394.63 \text{ mmol m}^{-2}$. After this increase, mean values did not change much until the last evaluation period. The decrease in CO_2 assimilation coincided with the increase in C_i , demonstrating that it may

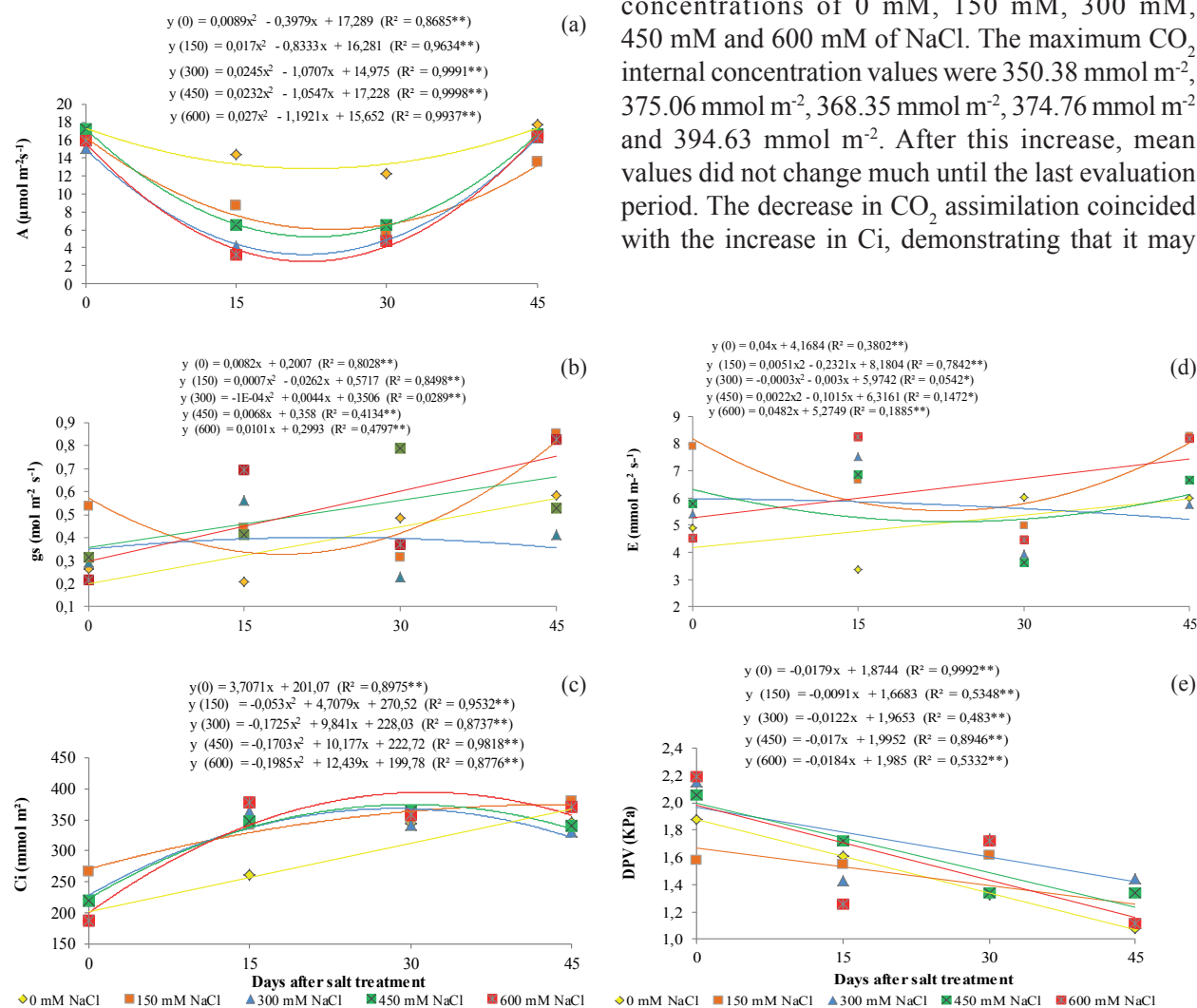


Figure 2. Photosynthetic CO_2 assimilation (A) (a), stomatal conductance (gs) (b), internal carbon concentration (C_i) (c), transpiration (E) (d) and vapor pressure deficit between leaf and air (VPDleaf-air) (e) of saltbush plants under different NaCl levels, as a function of the time under salt stress imposition (days). *, ** and ns: significant at 5 % and 1 % and not significant, respectively.

have occurred inhibition of photosynthetic carbon fixation. This may be due to a non-stomatal response, such as ionic damages on the photosynthetic apparatus. However, the small variation of C_i values in the last periods is consistent with the maintenance of photosynthetic CO_2 assimilation until the last day of evaluation, which may be caused by the period of adaptation to the imposed stress.

According to Machado et al. (2009), C_i reflects the substrate available for photosynthesis, and may indicate whether the stomatal closure is restricting the photosynthetic activity or not. Therefore, decreases in C_i could reduce photosynthetic assimilation due to the reduction of CO_2 concentration for rubisco activity. However, lower C_i values also stimulate the opening of the stomata, allowing increased inflow of CO_2 for the substomatal cavity, which tends to a balance between CO_2 intake and exhaust, maintaining C_i approximately constant.

Transpiration, as well as stomatal conductance, was significantly influenced by salinity levels, showing a slight trend to increase (Figure 2d). Transpiration followed quadratic response models for levels of 150 mM and 450 mM of NaCl, with a slight increase from the 30th day ($p < 0.01$). Moreover, levels of 300 mM and 600 mM of NaCl showed linear and quadratic response ($p < 0.01$), respectively. Therefore, it can be inferred that saline treatments did not cause severe stress in plants. Salts might have stimulated the stomata opening, increasing transpiration in some of the treatments. Thus, salt excess caused by the application of saline solutions may have promoted an increase in plant transpiration, due to decreases in the osmotic potential of the soil. According to Chen & Jiang (2010), this fact can be explained by the need for more energy from the plant, such as in the osmotic adjustment, in order to generate more negative potential, than that found in the soil, for water absorption. Such energy expenditure involves more transpiration by the plant, as soil salinity increases up to a certain point. From this point, the plant may dehydrate.

The vapor pressure deficit between leaf and air ($VPD_{leaf-air}$) showed a linear decrease trend ($p < 0.01$) for all salinity levels over time (Figure 2e). At 45 days, mean values of 1.44 KPa, 1.34 KPa and 1.12 KPa were observed respectively for levels of 300 mM, 450 mM and 600 mM of NaCl. It has been found that the increase in $VPD_{leaf-air}$ when the plant is under water/salt stress, reduces the water vapor

flow in the substomatal chamber to the atmosphere, causing reduction in the stomatal conductance and transpiration (Thomas & Gausling 2000). However, in the present study, the reduction in $VPD_{leaf-air}$ along the time under salt stress imposition seems to have favored saltbush plants to maintain small stomatal opening and transpiration rates at levels consistent with the salinity conditions to which they were exposed. Chlorophyll *a* and *b* contents, as well as their relative contents (Chlor *a*/Chlor *b* ratio), at all NaCl levels, did not differ ($p > 0.05$) over time, indicating that chlorophyll is not affected by salt concentration.

The salinity effect on proline content over time was significant ($p < 0.01$), resulting in a linear increase. The highest contents were found at 45 days, for the levels of 450 mM and 600 mM of NaCl, respectively with $3.44 \mu\text{mol g}^{-1}$ and $3.01 \mu\text{mol g}^{-1}$ of fresh matter (Figure 3). Therefore, saltbush plants responded to salinity by maintaining larger proline concentrations. This accumulation can be considered a physiological indicator of response to salt stress.

Plants subjected to increasing NaCl levels in the irrigation solution had a progressive proline accumulation (Hussin et al. 2012). The highest salt treatment (750 mM of NaCl) resulted in a significant increase (six and eight times) of proline in the saltbush leaves. The increase in proline content with the increase in salinity and in salt exposure time suggests a protective role of proline against water loss and/or reactive oxygen species. Proline generates a solute concentration gradient to lower the osmotic potential of cells, helping them to avoid water loss by osmosis. Proline also aids free radicals removal, helping plants to cope with ionic stress (Azevedo Neto et al. 2009).

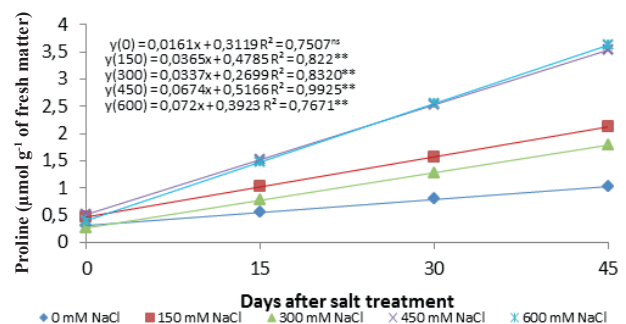


Figure 3. Proline content in saltbush plants under different NaCl levels, as a function of salt stress imposition time (days). *, ** and ns: significant at 5% and 1% and not significant, respectively.

CONCLUSIONS

1. *Atriplex nummularia* can grow under salt stress, with its growth being affected only at higher concentrations (> 600 mM of NaCl).
2. Ecophysiological parameters are little affected by salt concentration. Both photosynthetic CO₂ assimilation and stomatal conductance showed adaptive capacity to salinity, avoiding stomatal closure and reduction in the photosynthetic rate at 45 days.
3. Chlorophyll *a* and *b* contents remained virtually stable in function of time, under salt stress imposition.
4. The proline content increases with the time after stress imposition and with higher salinity levels.

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