

Full Length Research Paper

Effect of salt stress on growth and contents of organic and inorganic compounds in noni (*Morinda citrifolia* L.)

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Salinity is one of the abiotic stresses that most affect agricultural production, especially in arid and semi-arid regions; however, among species there are large differences in salt tolerance. In this study, the effects of salinity on the growth and accumulation of organic and inorganic solutes were evaluated in 'noni' seedlings at 1, 10, 20, 30 and 40 days of salt stress in a 5 x 2 completely randomized experimental design. Seedlings of 'noni' were grown in the nutrient solution at two salinity levels (0 and 100 mM sodium chloride). Plant height, number of leaves, stem diameter and dry matter of leaves, stems and roots, the allocation of biomass and the contents of organic and inorganic solutes were determined in the different plant organs. Salinity reduced all growth variables, being less expressive in stem diameter. The biomass allocation in leaves was higher than in roots, regardless of treatment or time considered. The contents of organic and inorganic solutes varied according to the plant part and the time of exposure to salinity. In general, salinity increased the contents of sodium ion (Na⁺), chloride ion (Cl⁻) and reduced potassium ion (K⁺), nitrogen (N) and phosphorus (P). Soluble carbohydrates and free amino acids were the main organic solutes contributing to osmotic potential of noni to salt stress. The salinity increased proline content in roots more than in leaves, but the proline content in the leaves was, on average 17 and 6 times higher than that of roots of plants under control and stressed conditions, respectively. Quantitatively, proline does not contribute substantially to the osmotic potential of noni, however its increase suggests that it plays a role in the salt stress acclimation or is an indicator of salt-induced metabolic disorders.

Key words: Amino acids, carbohydrates, toxic ions, proline, proteins.

INTRODUCTION

Medicinal plants are used since the antiquity by the population for combating various diseases. Among the

different species, noni (*Morinda citrifolia* L.), which belongs to the *Rubiaceae* family, has stood out for its

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phytotherapeutic properties. It is a fruit crop with medicinal and nutritional value that has been used for more than 2000 years by the Polynesians (Chan-Blanco et al., 2006). In noni seeds, peel and pulp, significant amounts of carbohydrates, proteins, vitamin C, total carotenoids and total phenolic compounds have been found (Costa et al., 2013). In addition, the plant acclimates to the most diverse climatic situations; soils and environmental stresses including high levels of salts in the soil (Mian-Ying et al., 2002). Salinity is one of the abiotic stresses that most affect agricultural production especially in arid and semi-arid regions. In these regions, the edaphoclimatic conditions and the inadequate management of soil and water favor the process of salinization. However, these areas can be explored with the cultivation of plants tolerant to salts, and noni can be an alternative of extra income for farmers. Sodium chloride is one of the main salts in the areas affected by salinity. The ions Na^+ and Cl^- are toxic to most glycophytes, but the plants may have mechanisms to survive to certain concentrations of salts in the soil (Munns and Tester, 2008). However, among the species there are large differences in the tolerance to salt stress (Severiano et al., 2014). Plants when cultivated in saline environment may compartmentalize ions like; potassium ion (K^+), chloride ion (Cl^-) and sodium ion (Na^+) in the vacuole and accumulate compatible organic solutes in the cytoplasm to maintain the osmotic homeostasis of the cell (Quéro et al., 2014). The most studied compatible solutes contributing to the osmotic potential in plants under salt stress are soluble carbohydrates, free amino acids, soluble proteins and free proline (Azevedo Neto et al., 2004, Silva et al., 2008, Sacramento et al., 2014). Among the compatible solutes, the accumulation of proline has been considered as one of the adaptive mechanisms to minimize the adverse effects of salinity (Iqbal et al., 2014). In noni, proline accumulation has proved to be an indicator of damages caused by salt stress (Souza et al., 2014).

The accumulation of organic solutes is extremely important to guarantee the survival of plant, under saline conditions. Hence, in the selection of plants tolerant to salinity, the accumulation of these compounds has been proposed as a physiological marker (Azevedo Neto et al., 2004, 2009). The knowledge on the accumulation and compartmentalization of organic and inorganic solutes, in the different organs in noni plants under salt stress may help to understand physio-biochemical mechanisms of tolerance to salinity. In this context, this study aimed to evaluate the growth and accumulation of organic and inorganic solutes in noni seedlings under salt stress.

MATERIALS AND METHODS

Experimental conditions and treatments

The experiment was carried out in a greenhouse, in completely randomized design using a factorial scheme with five time intervals

(1, 10, 20, 30 and 40 days after transplanting) x two salinity levels in the nutrient solution (0 and 100 mM sodium chloride), with four replicates. Noni seedlings at recommended age for transplanting that is, three months with four pairs of leaves (de Sousa et al., 2010) were transferred to containers with 12 L of nutrient solution of Furlani (1998), in a floating hydroponic system. Noni seedlings remained in nutrient solution for four days for acclimation. After this period, the seedlings received the respective treatments [nutrient solution without NaCl (control) or nutrient solution containing 100 mM of NaCl (salt stress)]. NaCl was gradually added (25 mM day^{-1}), in order to avoid the osmotic shock. The volume of the nutrient solutions was completed daily with water and the renewal was performed weekly. The pH was evaluated every two days and adjusted to 6.0 by using sodium hydroxide (NaOH) or hydrochloric acid (HCl). The system was maintained under intermittent aeration of 15 min every hour, using an air compressor coupled to a timer.

Growth

At the end of each time period, the plants of all treatments were carefully removed from the nutrient solution. The roots were washed with distilled water and plants were divided into different organs. After drying at 65°C in an oven for 72 h, the dry masses of stem (SDM), leaf (LDM) and root (RDM) were determined. Based on these data, the total dry mass of the plant (TDM) was calculated. These data were used to calculate the biomass allocation of leaves (ABL), stem (ABS) and roots (ABR) according to the equations proposed by Benincasa (2003):

$$\text{AB organ (\%)} = (\text{DM organ/DM total}) \times 100$$

Extract preparation and inorganic solute analysis

Plants were separated into leaves, stems and roots, which after drying were ground for the determination of the inorganic solute contents. For the determination of the Na^+ , K^+ and Cl^- contents, the extracts were prepared as described by Jones (2001), with minor modifications. 100 mg of the ground material of leaves, stems or roots were mixed with 10 mL of deionized water in test tubes. The tubes were maintained for 1 h at 80°C in water bath, shaking every 15 min. After this period, the tubes were centrifuged at $5,000 \times g$ for 15 min, at room temperature. The Na^+ and K^+ contents were determined by flame photometry (Faithfull, 2002) and Cl^- contents by spectrophotometry (Jones, 2001).

The N and P contents were determined by acid digestion of 0.5 g of plant material in a mixture of concentrated H_2SO_4 and H_2O_2 (30%), as described by Jones (2001). The N and P contents were determined by phenol-hypochlorite and molybdovanadate spectrophotometric methods, respectively (Faithfull, 2002).

Extract preparation and organic solute analysis

The organic solutes contents were analyzed in the first pair of fully expanded leaves and in the samples of the lower third of the roots. The leaves were collected, immediately frozen, lyophilized, ground and stored in ultra-freezer (-80°C). For organic solute determinations, the extracts were prepared by maceration of lyophilized leaf and root tissues (200 mg) in a mortar and pestle with 6 mL of 0.1 M potassium phosphate buffer, pH 7.0, containing 0.1 mM ethylenediaminetetraacetic acid (EDTA). The extract was filtered through muslin cloth, centrifuged at $12,000 \times g$ for 15 min, and the supernatant was stored in ultra-freezer and used for soluble carbohydrates, free proline, free amino acids and soluble proteins determinations (Azevedo Neto, 2009).

The soluble carbohydrates were determined at 490 nm by

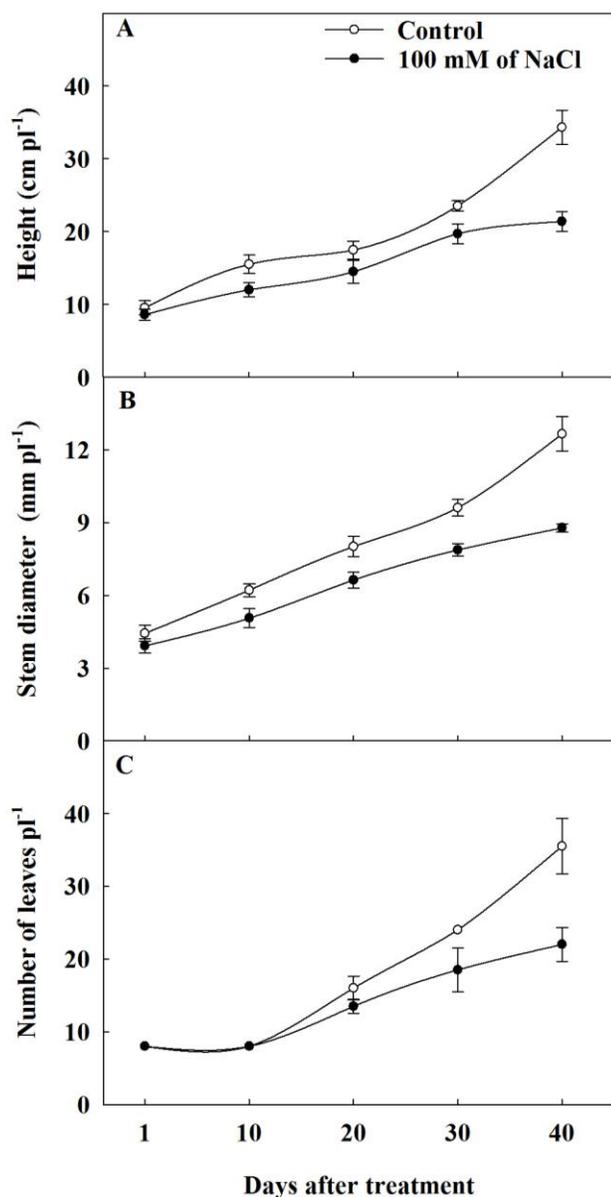


Figure 1. Plant height (A), stem diameter (B) and number of leaves (C) of noni plants cultivated for 40 days in hydroponic system under conditions of control (○) or presence of 100 mM of NaCl (●) in the nutrient solution. The values indicate the mean of four replicates and respective standard deviations.

sulfuric acid-phenol method, using D-(+)-glucose as the standard (Dubois et al., 1956). Free proline was determined at 520 nm by acid-ninhydrin method, using L-proline as standard (Bates et al., 1973). Free amino acids were determined at 570 nm by ninhydrin method, using L-leucine as standard (Yemm and Cocking, 1955). Soluble proteins were determined at 595 nm by protein-dye binding method, using bovine serum albumin as standard (Bradford, 1976).

Statistical analysis

The obtained data were subjected to analysis of variance (F test)

and the means were compared through their respective standard deviations, according to Snedecor (1956).

RESULTS

Plant height (Figure 1A), stem diameter (Figure 1B) and number of leaves (Figure 1C) increased in the plants of both treatments, during the experimental period. However, it can be observed that, in all variables, this increment was more evident in control plants (without NaCl) than in salt stressed ones. In the comparison between treatments, salinity reduced plant height and stem diameter from 10 days onwards and the number of leaves from 30 days onwards. It can also be observed in this figure that these reductions were more pronounced at 40 days of stress, of the order of 31% for stem diameter and 38% for plant height and number of leaves. Thus, stem diameter was less affected by salinity than plant height and number of leaves.

The dry mass of leaves (Figure 2A), stem (Figure 2B) and roots (Figure 2C) of noni plants increased during the experimental period in both treatments. However, comparing the dry mass production at the end of the experimental period, it is observed that the salinity decreased LDM, SDM and RDM by 26, 62 and 71%, respectively. It can also be observed that the salt stress decreased RDM and SDM after 10 days while LDM was reduced only at 40 days.

The data of biomass allocation in the different plant organs are also shown in Figure 2. In stressed plants, ABL increased from 10 days onwards in relation to the respective controls (Figure 2D). In the stem, biomass allocation decreased only at 40 days (Figure 2E). In contrast to ABL, the ABR decreased from 10 days onwards, compared with the control plants (Figure 2F). It can also be observed in this figure that ABL was higher than ABS and ABR, regardless of treatment or time considered.

The Na⁺ and Cl⁻ contents in all parts of the stressed plants abruptly increased at 10 days, remaining relatively stable up to the end of the experimental period (Figures 3A, 3B, 3C, 3D, 3E and 3F). The contents of Na⁺ in the leaves of stressed plants were about 1.4 and 1.7 times, respectively, higher than in stem and roots. However, there were no substantial differences between Cl⁻ contents in the different plant organs. Unlike the result observed for Na⁺ and Cl⁻ contents, from 10 days onwards, salinity decreased K⁺ contents by approximately 31% in leaves, 45% in stem and 25% in roots (Figures 3G, 3H and 3I). In the roots of stressed plants, the K⁺ contents were about 1.5 and 2.0 times higher than those in leaves and stems, respectively. The K⁺/Na⁺ ratios were reduced by salinity in all organs (Figures 3J, 3K and 3L). It is important to point out that, from 10 days of salt stress, the values of this ratio were lower than 0.5 in leaves and stems. In contrast, in the roots, the values of K⁺/Na⁺ in all times varied between 1.0 and 2.5.

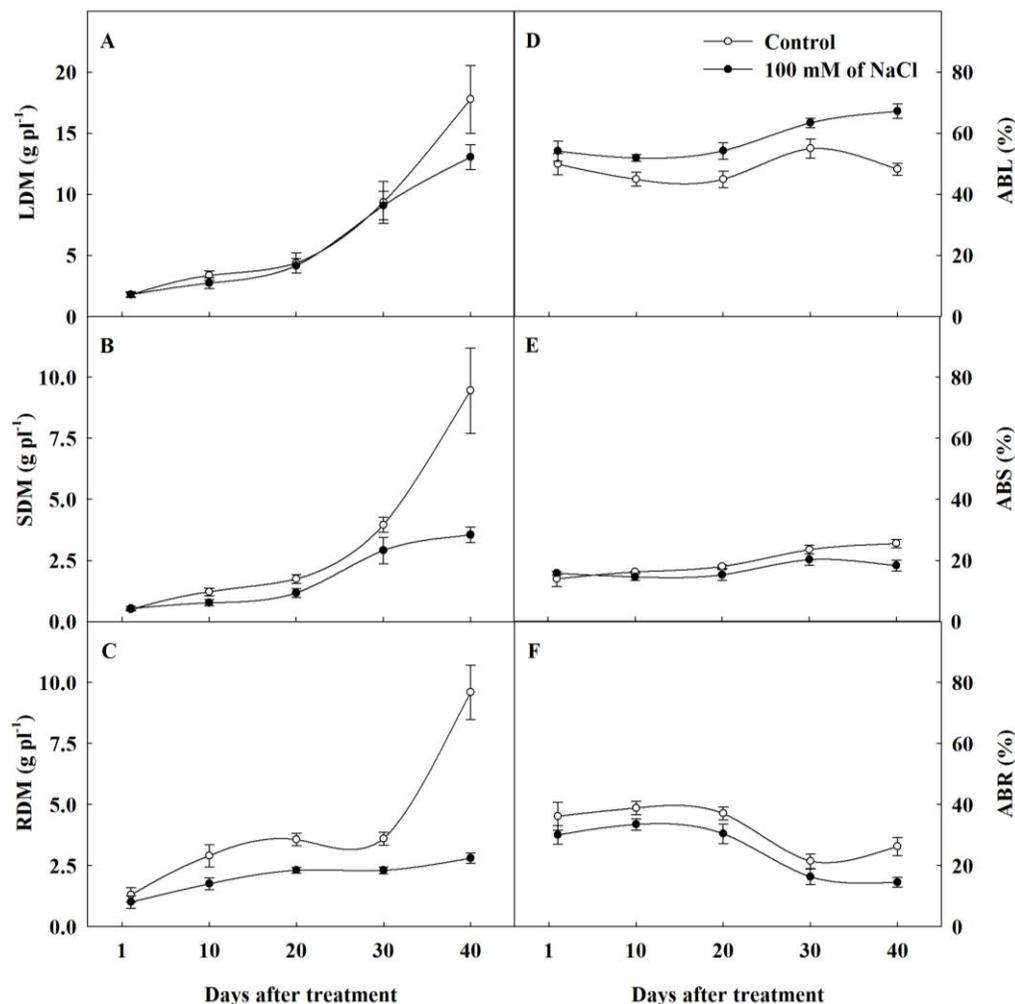


Figure 2. Dry mass of leaves - LDM (A), stems - SDM (B) and roots - RDM (C) and allocation of biomass in leaves - ABL (D), stem - ABS (E) and roots - ABR (F) of noni plants cultivated for 40 days in hydroponic system under conditions of control (○) or presence of 100 mM of NaCl (●) in the nutrient solution. The values indicate the mean of four replicates and respective standard deviations.

In the leaves of stressed plants, N contents decreased during the experimental period and, in the stem, until 20 days (Figures 4A and 4B). The leaf P contents decreased until 20 days and, in the stem, until 10 days (Figures 4D and 4E). In the roots, the salinity did not affect the N and P contents during the studied period (Figures 4C and 4F).

The variations in the soluble carbohydrates, free amino acids, soluble proteins and free proline contents in leaves and roots are presented in Figure 5. By comparing the treatments, the NaCl in the nutrient solution decreased by 14% the contents of soluble carbohydrates in the leaves after 40 days of stress (Figure 5A). In the roots, there were reductions of 30 and 36% in the contents of these solutes at 30 and 40 days of stress, respectively (Figure 5B).

Salinity decreased the leaf free amino acids contents in day one (50%) and day ten (28%) (Figure 5C). In the roots, the amino acids decreased by about 30% after 20

days of stress (Figure 5D). The soluble proteins in leaves and roots increased along the experimental period in both treatments. However, there were no differences between the protein contents in control and stressed plants (Figure 5E and 5F). Salinity increased the content of free proline in leaves (Figure 5H) and in roots (Figure 5G), from day 10 of stress onwards. However, by comparing the proline contents in both plant parts, it is observed that they were, on average, 17 and 6 times higher in the leaves than in the roots of control and stressed plants, respectively.

DISCUSSION

The accumulation of salts in the rhizosphere can limit water absorption, cause ionic imbalance and affect plant growth (Iqbal et al., 2014). One of the most harmful effects of salt stress is the metabolic disorders caused by

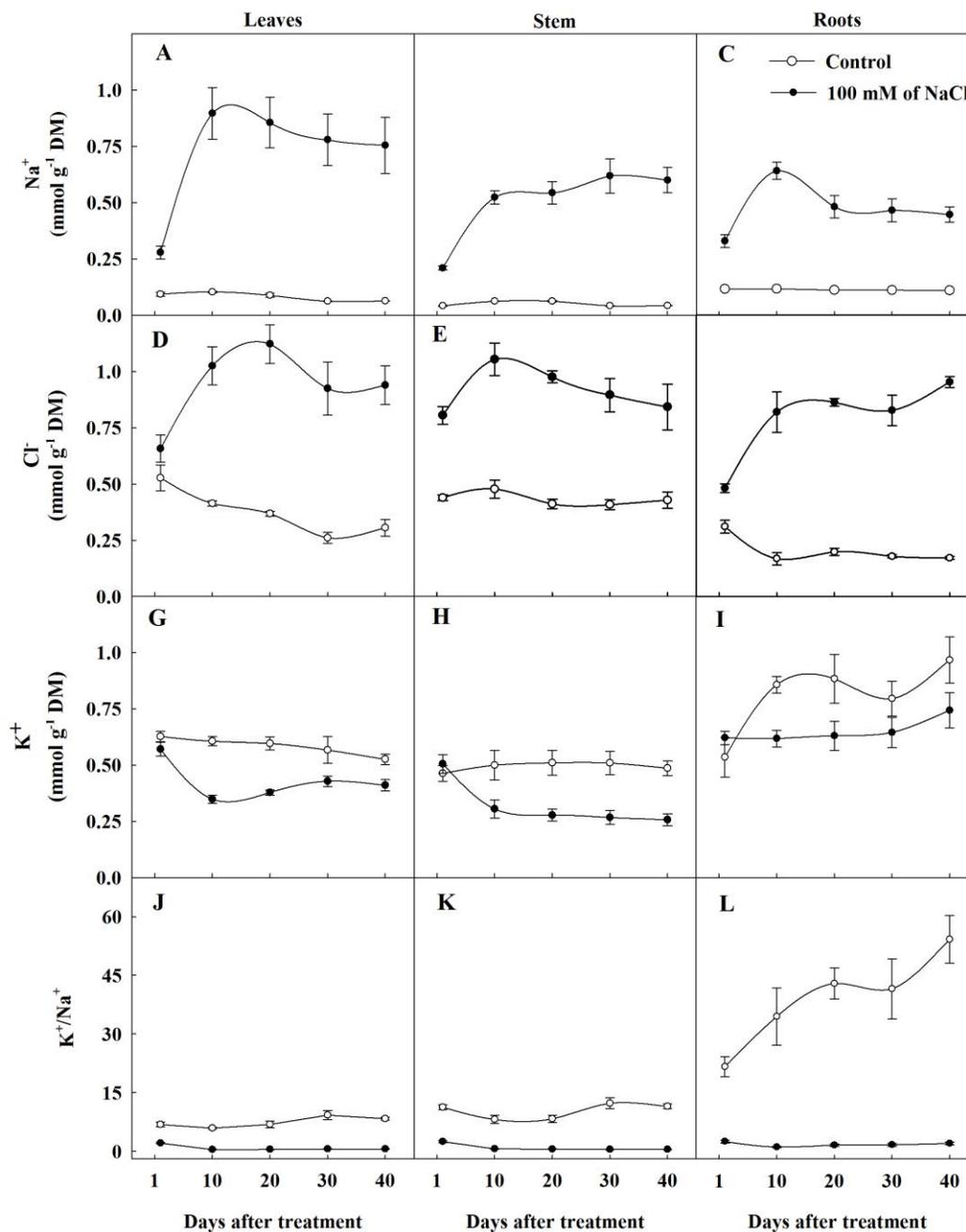


Figure 3. Contents of sodium - Na⁺ (A, B, C), chloride - Cl⁻ (D, E, F), potassium - K⁺ (G, H, I) and K⁺/Na⁺ ratio (J, K, L) in the different organs of noni plants cultivated for 40 days in hydroponic system under conditions of control (○) or presence of 100 mM of NaCl (●).

Na⁺ and Cl⁻ accumulation in plant cells (Geilfus et al., 2015). Consequently, the reduction in height, stem diameter, number of leaves and dry mass production are the main effects of the salts observed in the whole plant level (Silva et al., 2008). The salinity reduced all the analyzed growth variables, but the stem diameter and LDM were the least affected. Souto et al. (2013), working with the noni crop, also reported reductions in plant height, stem diameter, number of leaves, leaf area and

shoot dry mass with the increase of salinity in irrigation water. The plant stem diameter shows a positive correlation with the survival rate after transplanting (Tatagiba et al., 2010), the vigor and strength (Marçal, 2011), and the initial yield (Carvalho et al., 2010). It is also important to point out that noni plants can produce up to 70,000 kg ha⁻¹ year⁻¹ of fruits (Chan-Blanco et al., 2006), requiring stems resistant to breaking and damping-off. Considering that stem diameter was sensitive variable

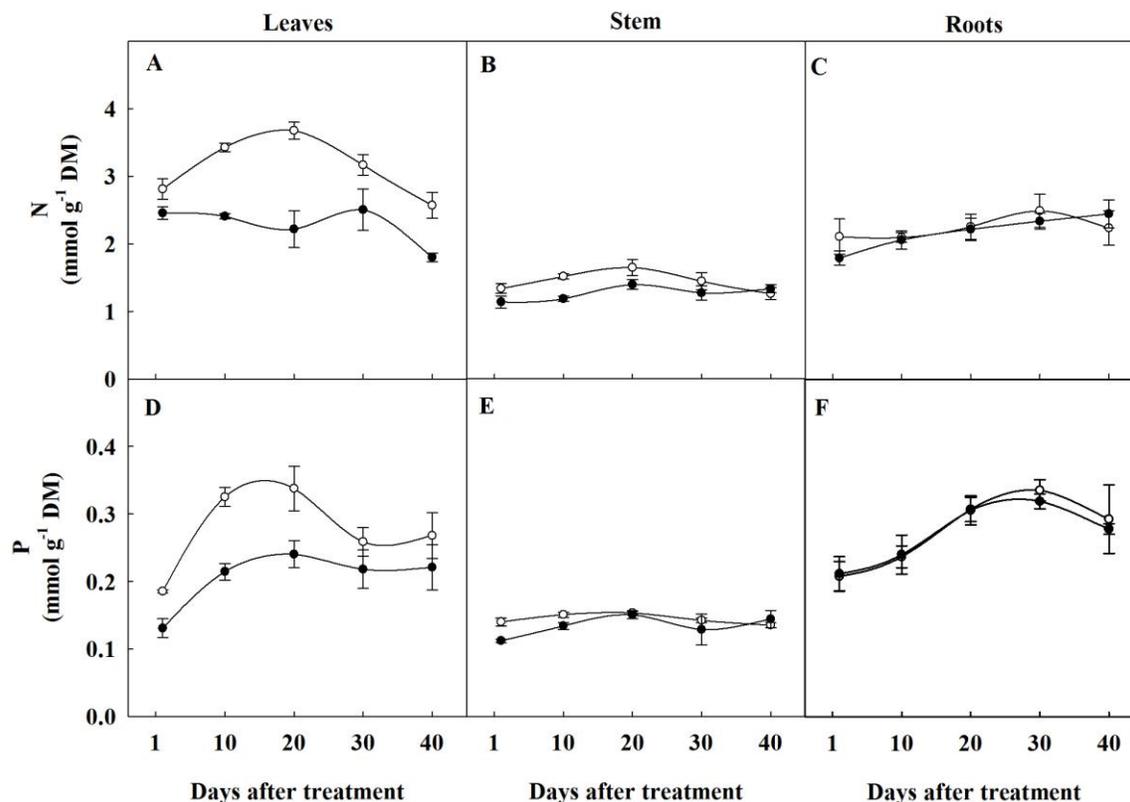


Figure 4. Contents of nitrogen - N (A, B, C) and phosphorus - P (D, E, F) in different organs of noni plants cultivated for 40 days in hydroponic system under conditions of control (○) or presence of 100 mM of NaCl (●) in the nutrient solution. The values indicate the mean of four replicates and respective standard deviations.

to salt stress, these data suggest that noni plants are less subjected to the damages resulting from the breaking of stems due to the weight of the fruits.

Silva et al. (2008) and Souza et al. (2014) also reported that, plant height and number of leaves of noni also decreased in the presence of salts. According to Mazher et al. (2007), the reduction in plant height may be due to the negative effects of salts on photosynthetic rate, enzyme activity and on the contents of carbohydrates and growth hormones. Additionally, the salt accumulation in cell walls and cytoplasm limit the production of leaves. In contrast, the reduction in number of leaves and leaf area has a negative effect on photosynthesis, water and nutrient uptake and, consequently, on growth and production (Souza et al., 2014).

Regarding the dry mass production, roots and stems were the most sensitive organs to salt effect when compared with the leaves. Abreu et al. (2008) reported that roots have higher capacity of osmotic adjustment and better protection against oxidative stress under salt stress conditions. However, the higher reduction in RDM observed in noni may be a consequence of the roots which are directly exposed to salt stress. Souza et al. (2014) also observed that RDM of noni was more reduced by salinity than shoot dry mass. In plants of

Ricinus communis, the roots were more affected by salinity than stems and leaves when subjected to 150 mM NaCl (Rodrigues et al., 2014).

Analyzing the effect of duration of stress on the growth variables, it can be inferred that plant height, stem diameter, SDM and RDM were precocious indicators of salinity effect on the noni crop.

The increase in ABL occurred simultaneously to the reduction in ABR. These results corroborate with those of dry mass production with respect to the higher sensitivity of noni roots to salt stress and are similar to those observed in *Ricinus communis* seedlings (Rodrigues et al., 2014). This response suggests a sensitivity of noni to salt stress, due to the higher growth of the parts with greater transpiration capacity in relation to the roots (Rodrigues et al., 2014). This hypothesis is supported by the significant reduction in growth, as well as the presence of toxicity symptoms, such as curving of older leaves, coriaceous texture and greenish-blue color, observed at 40 days (data not shown), which are considered symptoms of sensitivity to salinity (Munns and Tester, 2008).

In the present study, the increase of salinity in the nutrient solution caused a sharp increase in the Na⁺ and Cl⁻ contents in all plant organs. However, these

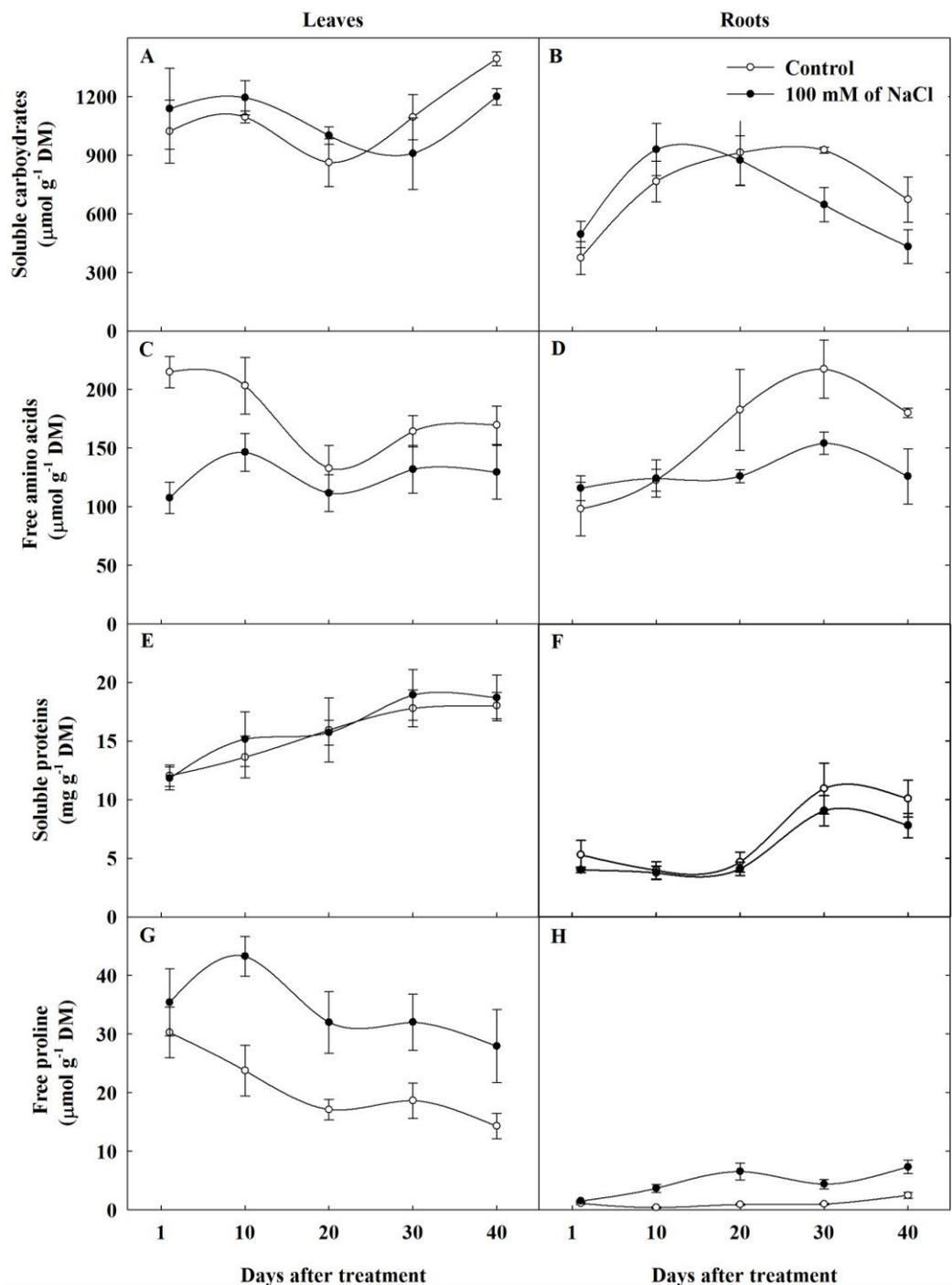


Figure 5. Contents of soluble carbohydrates (A and B), free amino acids (C and D), soluble proteins (E and F) and free proline (G and H) in leaves and roots of noni plants cultivated for 40 days in hydroponic system under conditions of control (○) or presence of 100 mM of NaCl (●) in the nutrient solution. The values indicate the mean of four replicates and respective standard deviations.

increments were more pronounced in the leaves than in the roots. Similar results were also observed in *Spondias tuberosa* (Silva et al., 2008). The Na^+ accumulation in the leaves may be due to the transpiration flow rate and/or

the reduction in cell volume induced by the lower water absorption caused by salinity (Munns and Tester, 2008). Na^+ and Cl^- are the most abundant ions in saline soils and the excessive absorption of these salts may affect

the electrochemical stability and the plant growth. Na^+ is a cytotoxic ion that destabilizes membranes and proteins, disturbs fundamental physiological processes such as cell division and expansion, and alters the primary and secondary metabolisms and nutrient homeostasis (Munns and Tester, 2008). Cl^- at high concentrations induces chlorophyll degradation, which may result in structural impact in photosystem II, reducing the photosynthetic capacity and yield (Marschner, 2012) thus, salt-induced growth inhibition in noni plants can at least partially be related to the toxic effects of Na^+ and Cl^- ion accumulation.

In contrast with Na^+ , salinity reduced the K^+ contents in the different plant organs. It is well established that K^+ uptake can be affected by the antagonism between this ion and the Na^+ in the absorption sites resulting from physico-chemical similarities between them (Abreu et al., 2008; Rodrigues et al., 2013). This antagonism frequently results in decrease of K^+ contents thereby leading to metabolic disorders in the plants (Mekawy et al., 2015).

Salinity decreased the contents of K^+ and increased those of Na^+ in all plant organs thereby altering the K^+/Na^+ ratios. Similar results were reported by Azevedo et al. (2004), Rodrigues et al. (2014) and Sacramento et al. (2014). According to Greenway and Munns (1980), the K^+/Na^+ ratio in glycophytes must be higher than 1.0 for the maintenance of ion homeostasis and an optimal metabolic efficiency. Recently, Rodrigues et al. (2013) reported that K^+/Na^+ ratios between 1.0 and 2.0 were the ones that promoted maximum photosynthesis and growth in *Jatropha curcas* plants under salt stress. In the present study, from day 10 onwards, the values of K^+/Na^+ ratio in stressed plants were below 1.0 which suggests that the plant metabolism was affected by salinity and can partially explain the salt-induced growth reduction.

The contents of N and P also decreased with salinity especially in the leaves. This behavior corroborates the growth reduction observed in the present study, because biomass production is directly related to the nutritional balance of the plants (Lucena et al., 2012; Marschner, 2012). A few physiological and biochemical reasons have been proposed for the reduction of N and P uptake in salt-stressed plants. NO_3^- and H_2PO_4^- are the main sources of N and P in agricultural soils, and high salt concentrations may affect their absorption, resulting from the competitive mechanism established with Cl^- (Feijão et al., 2013; Lucena et al., 2012).

In the present study, the reductions in N and P contents in conjunction with the salt-induced growth decrease indicate an antagonism (Imo, 2012) between these nutrients and Cl^- ions of the nutrient solution. In addition, the observation that salinity decreased N and P contents in shoot organs, but did not affect those in the roots, suggests the occurrence of disorders in the translocation of these nutrients from roots to shoots (Marschner, 2012). In the present study, the salinity reduced the soluble carbohydrate contents in leaves and roots. This reduction

was probably due to the decrease in net photosynthesis observed after 30 and 40 days of stress (data not shown). Consequently, the transport of carbohydrates from leaves to roots was also reduced (Silva et al., 2008). The reduction in the leaf carbohydrates is frequently associated with disorders in their biosynthesis or in the translocation to the other plant parts (Azevedo Neto et al., 2004, 2009). Various studies have reported changes in the soluble carbohydrates in different organs and plant species under salt stress conditions. In *Zea mays* and *Anacardium occidentale*, the carbohydrates were not affected in the different organs (Abreu et al., 2008, Azevedo Neto et al., 2004). In *Spondias tuberosa* there was an increment in the leaves and reduction in the roots (Silva et al., 2008) and in *Spartina alterniflora* an increment in the shoots (Li et al., 2010). Thus, the content of carbohydrates may vary with the species, plant organ and time of exposure to salts.

The NaCl addition in the nutrient solution reduced the free amino acid contents, in both leaves and roots (Figures 5C and D), but did not affect the soluble protein contents (Figures 5E and F). The decrease in the amino acids is often associated with increased degradation or inhibition of their biosynthesis, along with the reduction in protein degradation or increase in protein synthesis (Silva et al., 2008). N availability is another factor that can limit the amino acids synthesis (Feijão et al., 2013). Thus, the data of this study suggest that the reduction in the amino acid contents in stressed plants is resulted from the biosynthesis inhibition. The observation that salinity did not affect the soluble proteins (Figures 5E and F) and decreased the N content (Figures 4A and B) supports this hypothesis.

Unlike soluble carbohydrates, free amino acids and soluble proteins, the contents of proline significantly increased in salt-stressed noni plants, along the entire experimental period. Similar results were obtained by Souza et al. (2014), which indicate that the proline accumulation was primarily due to "de novo" synthesis coupled with the reduction of proline degradation and utilization (Carillo et al., 2011).

Proline is an osmolyte that accumulates in various plant species in response to biotic and abiotic stresses, however its role in the osmotolerance still remains controversial. Thus, while some authors consider proline as an important amino acid for the osmotic adjustment in plants under salt stress (Iqbal et al., 2014, Li et al., 2010), others consider that the proline contents are not sufficient for a significant contribution to the osmotic adjustment (Oliveira et al., 2013). In this study, salinity increased proline contents in noni leaves and roots however, the contents of soluble carbohydrates and free amino acids were quantitatively much higher than those of proline. This indicates that in noni, soluble carbohydrates and free amino acids were the main organic solutes involved in the osmotic potential.

Furthermore, since proline was the only organic solute

whose concentration increased with the salt stress despite its importance in osmoregulation, this result suggests that proline accumulation plays an important role in the acclimation of noni to salinity. In this scenario, other important functions have been attributed to the proline accumulation, such as stabilization of proteins, protein complexes and membranes, removal of free radicals, maintenance of cell redox homeostasis, increase in the enzyme activities, reserve of carbon and nitrogen, cytosolic pH control, and detoxification of NH_4^+ excess (Azevedo Neto and Silva, 2015). Alternatively, the increase in proline concentration in conjunction with the growth reduction suggests that the sensitivity to stress is conditioned to the salt-induced metabolic disorders, as proposed by Oliveira et al. (2013).

Conclusions

The increase in the contents of sodium and chloride ions (Na^+ and Cl^-) leads to nutritional imbalance of N, P and K. This accumulation of toxic ions associated with the nutritional imbalance can at least explain the growth reduction and the change in the pattern of biomass allocation in noni seedlings under salt stress. Among the evaluated growth variables, plant height, stem diameter and root dry mass are the earliest indicators of the salt effects on noni seedlings. The results also show that soluble carbohydrates and free amino acids are the main organic solutes contributing to the osmotic potential in leaves and roots of noni and that proline, although not contributing substantially to the osmotic potential, it either play a role in noni acclimation to salt stress or is an indicator of the salt-induced metabolic disorders.

Conflict of Interests

The authors have not declared any conflict of interests.

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