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Salinity reduces carbon assimilation and the harvest index of cassava plants (*Manihot esculenta* Crantz)

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ABSTRACT. This study was developed to evaluate the effects of salinity on the growth and gas exchange of cassava plants, cultivar Verdinha. The four concentrations of NaCl (mM) were as follows: 0, 20, 40, and 60. Under salinity, the lowest concentration of Na^+ ions was observed in the tuberous roots; however, the dry matter of tuberous roots was reduced with an application of just 20 mM NaCl. The harvest index was reduced 50% with the highest salt concentration. Salinity reduced carbon assimilation (*A*), stomatal conductance (g_s), transpiration, and the instantaneous water use efficiency. The correlation between g_s and *A* was high and positive, showing that stomatal movement was one of the responsible for the lower *A*. Under salt stress, there was an increase in intercellular CO_2 concentration, indicating the impairment of carbon metabolism. Based on the reduction of dry matter of the tuberous roots (reduction of 81% under 60 mM NaCl), it was concluded that cassava is sensitive to salinity. The growth of shoots and the absorbing roots were minimally affected by salinity, even in the situation where *A* was reduced; therefore, the sensitivity of cassava was related to the high sensitivity of the tuberous roots to the ionic and/or osmotic effects of salinity. Thus, tuberous roots can be the target organ in studies that aim to improve the tolerance of cassava to salinity.

Keywords: photosynthesis, dry matter, water use efficiency, harvest index, root:shoot ratio, salt stress.

Salinidade reduz a fotossíntese e o índice de colheita da mandioca (*Manihot esculenta* Crantz)

RESUMO. Esse estudo foi desenvolvido para avaliar os efeitos da salinidade sobre as trocas gasosas e crescimento da mandioca, cultivar Verdinha. Quatro concentrações de NaCl, em mM, foram utilizadas: 0, 20, 40 e 60. A menor concentração de Na^+ foi observada nas raízes tuberosas; entretanto, a matéria seca dessa parte da planta foi reduzida pela aplicação de apenas 20 mM de NaCl. O índice de colheita foi reduzido em até 50% pela salinidade. A aplicação de NaCl reduziu a assimilação de carbono (*A*), condutância estomática (g_s), transpiração (*E*) e eficiência no uso de água (EUA). A correlação entre g_s e *A* foi alta e positiva, evidenciando que o movimento estomático foi um dos responsáveis pela menor *A*. Sob salinidade também existiu aumento na concentração interna de CO_2, indicando que o NaCl pode ter inibido o metabolismo de carbono das plantas. Com base na redução do acúmulo de matéria seca das raízes tuberosas (redução de 81% sob 60 mM de NaCl) é concluído que essa cultivar de mandioca é bastante sensível à presença de NaCl na solução do solo. A sensibilidade da mandioca esteve relacionada à alta sensibilidade das raízes tuberosas aos efeitos iônicos e/ou osmótico do NaCl. Assim, as raízes tuberosas podem ser o órgão alvo em estudos que visem melhorar a tolerância da mandioca à salinidade.

Palavras-chave: fotossíntese, matéria seca, uso eficiente de água, índice de colheita, relação raiz:parte aérea, estresse salino.

Introduction

The inadequate management of irrigation water, coupled with the intensive use of soluble fertilizers with a high salt index, has contributed to the increase of agricultural areas with salinity problems. Indeed, in addition to natural salinization, a significant proportion of areas incorporated into the production process have become salty due to: (i) the replacement of evergreen vegetation with annual crops, which causes top soil erosion; and (ii) inadequate irrigation, which increases salt concentrations in the root zone (Munns & Tester, 2008). This is particularly important in arid and semi-arid regions due to the lack of rainfall and high evaporative demand, which hinders the leaching of salts found in the soil arable layer. The salinity problem is increasing considerably, and there are estimates that worldwide more than one billion hectares are affected by salinity (Wicke et al., 2011).

Some authors consider salinity the main environmental factor limiting plant growth and productivity worldwide (Dasgupta, Nandy, & Das, 2013; Gupta & Huang, 2014). Thus, by studying the negative effects of salinity on the physiology and growth of crops, it is possible to generate information that helps improve crop yields when cultivated in this adverse condition. Crop species show a spectrum of responses to NaCl, though the majority has their growth reduced by salinity (Manchanda & Garg, 2008). Salt stress involves changes in various physiological and metabolic processes depending on the species or genotype, salt and nutrient composition in the solution, environmental conditions (Tedeschi et al., 2017), and stress severity and duration (Gupta & Huang, 2014). According Munns and Tester (2008), soil salinity inhibits plant growth for two reasons: (i) a reduction in a plant's ability to absorb water, which is the osmotic effect or water-deficit induced by salinity; and because (ii) NaCl ions can enter the transpiration stream and eventually harm a plant's cell metabolism. In fact, the growth reduction induced by NaCl has also been correlated with low photosynthate supply to the sink organs as a consequence of the reduction in the photosynthetic capacity under these conditions (Eisa, Hussin, Geissler, & Koyro, 2012). Decreases in photosynthesis can occur by two main mechanisms: (i) restricted diffusion (CO_2 flux to carboxylation sites) caused by decreases in stomatal and internal conductance; and (ii) the inhibition of the metabolic potential for photosynthesis (Pérez-López, Robredo, Lacuesta, Mena-Petite, & Muñoz-Rueda, 2012). Plants develop several physiological and biochemical mechanisms in order to survive in soil with high salt concentrations. In a comprehensive review, Manchanda and Garg (2008) indicated that the main mechanisms include ion absorption, transport, homeostasis and compartmentalization, the biosynthesis of compatible solutes and osmoprotectants, the synthesis of antioxidant compounds, the activation of antioxidant enzyme, polyamine synthesis, the generation of nitric oxide (NO) and hormonal modulation. For different species, it is important to know these mechanisms in order to develop technologies that reduce the negative effects of salinity.

Cassava is considered a robust species with tolerance to acidic and low fertility soils. It also shows a high level of tolerance to high temperatures and drought. Good production capacity, even in unfavorable conditions, has led researchers to suggest cassava as a strategic crop option to mitigate hunger in many poor regions of the world (Burns, Gleadow, Cliff, Zacarias, & Cavagnaro, 2010). However, few studies have been developed to evaluate the response of cassava to salinity. Anon (1976), cited by Hawker and Smith (1982), described a field study in which cassava had an approximately 50% decrease in yield at a salinity level as low as 6 to 8 mM NaCl (0.7 mS cm^{-1}). Carretero, Cantos, García, and Troncoso (2007) observed that salinity adversely affected survival, development, and mineral composition of three cassava clones. In research conducted by Hawker and Smith (1982), salinity seriously affected cassava growth; because of this, they considered it a moderately salt sensitive species. Recently, studies have been exploring salinity effects with a focus on the proteome and genome analysis of cassava (Costa et al., 2011; An et al., 2014; Santa Brígida et al., 2014).

Although the salinity problem is growing, the literature regarding cassava's response to salinity is scarce (Setter and Fregene, 2007, Santa Brígida et al., 2014). However, cassava has shown to have potential to be improved for salt stress through screening and selection (Carretero et al., 2007).The objective of this study was to evaluate the effects of NaCl concentrations on cassava growth, leaf gas exchange, and dry matter accumulation.

Material and methods

Experimental conditions and plant culture

The study was carried out in a greenhouse at *Embrapa Mandioca e Fruticultura* located in Cruz das Almas, Bahia State, Brazil; the greenhouse was equipped with the automatic control of temperature (30°C ± 2°C) and relative humidity (64% ± 2%). The cultivar Verdinha (*Manihot esculenta* Crantz, BGM 116) was chosen for the experiment. This variety has good yield potential, which was determined by the National Center for Research on Cassava and Tropical Fruits (CNPMF/EMBRAPA), for commercial plantations in some areas of the Brazilian Northeast region. Two stem cuttings (15 cm long) were planted in pots with a 14 L capacity. Before planting, the pots were filled with a mixture of sand, perlite, and vegetable substrate (composed of 60% pinus bark + 30% granulated coconut fiber + 10% vermiculite) in equal proportions. During the first 15 days, the pots were watered twice a day with tap water until field capacity. After this period, the less vigorous plant was discarded, and the experiment proceeded with only one plant per pot. After thinning, the substrate was fertilized with a growth nutrient solution, modified from the solution used by Cruz, Alves, LeCain, Ellis, and Morgan (2014). The solution was modified to have varying NaCl concentrations but maintained the same concentration of the other nutrients. The saline treatments were applied 15 days after sowing by the addition of NaCl

to the growth solution to obtain the following final concentrations: 0, 20, 40, and 60 mM NaCl, which had electrical conductivities of 1.1, 3.6, 5.2, and 6.8 dS m^{-1}, respectively. To avoid the possibility of osmotic shock to the plants, the salt stress treatments were added at 20 mM increments every 48 hours until the desired concentration for each treatment was reached. The pH of the nutrient solutions was adjusted to maintain values between 6.3 and 6.5. For every 10-day period, the plants received four liters of growth nutrient solution, and irrigations were performed daily to replace the water lost by evapotranspiration. Every 10 days, the substrate was washed thoroughly with tap water to avoid salinization and, on the same day, new growth solutions together with NaCl were added to the substrate. During the experiment, some leaves (central lobe shorter than 4.0 cm) were labeled, with the aim of measuring gas exchange in leaves at similar ages.

Measurements

One hundred and ten days after planting, the following variables were evaluated: CO_2 assimilation (*A*), intercellular CO_2 concentration (*Ci*), stomatal conductance (g_s), and transpiration (*E*) in the central lobes of the youngest fully expanded leaves (YL) and the old leaves (OL) located in the lower part of the stem. For the determination of leaf gas exchange in the older leaves, the leaves with only a little yellowing in the highest saline treatment were chosen. For all saline treatments, precaution was taken to select leaves of similar age. Evaluations were performed using a portable photosynthesis system (LCpro, ADC Bioscientific LTD., UK) with an air flow rate of 200 µmol s^{-1}. The LCpro system provided temperature and humidity control for the measurements, which were adjusted on the measurement day to replicate growth conditions. The LCpro leaf chamber was connected to an artificial source of light, which at the time of measurement projected a photosynthetic flux density of 1,200 µmol photons m^{-2} s^{-1} on leaf surfaces. Measurements were performed between 8:45 and 10:45 am, and the values were recorded only when the CO_2 exchange rates remained stable. The instantaneous water use efficiency (iWUE) was calculated as the ratio between *A* and *E*.

The leaves still attached to the stem were recorded as living leaves, regardless of their yellowness level. The nodes of the stem that had no leaves were computed as the number of abscised leaves. The sum of leaves attached to the stem and abscised leaves allowed the estimate of the total number of leaves produced during the experimental period. The total leaf area was estimated using the same procedure described for cassava by Alves and Setter (2004). Posteriorly, the plants were separated into leaves, stems + petiole, absorption roots, and tuberous roots (diameter greater than 0.5 cm). These parts were placed to dry at 70°C for 96 hours, and subsequently, the dry matters were determined. The root:shoot ratio was calculated by dividing the dry matter of the absorption roots by the dry matter of the shoot. The harvest index (HI) was defined as the ratio of economic yield (tuberous roots yield) to biological yield (total plant dry matter) multiplied by 100 and expressed as a percentage. Dry matter were ground in a Willy type mill using a 20 mesh sieve, and chemical analyses of sodium (Malavolta, Vitti, & Oliveira, 1997) were conducted using plant tissues from the treatments 0 and 60 mM NaCl.

Statistical analysis

The plants were distributed in a randomized block experimental design with six replications. Each plant was considered an experimental unit. An analysis of variance was performed with SISVAR software (Ferreira, 2011). When significant effects were observed, means were compared using the Student-Newman-Keuls (SNK) method. Correlation analyses were performed between *A* and g_s. The significance level for all variables was $p \leq 0.05$.

Results

For plants grown without salt stress (control plants), the highest sodium (Na^+) concentration was observed in the absorption roots (AR) and the lowest in the stem + petiole (Figure 1). The increase of NaCl from 0 to 60 mM considerably increased Na^+ concentration in all parts of the plant. For plants grown under salt stress, the following Na^+ concentrations were observed (g $kg^{-1}$of dry matter): stem + petioles (15.6), absorbing roots (10.9), leaves (3.67) and tuberous roots (2.56).

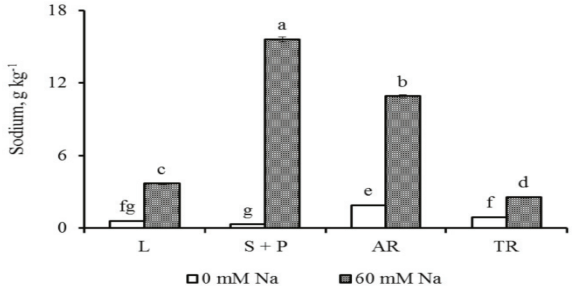

Figure 1. Sodium concentrations in the organs of cassava plants grown under different NaCl concentrations. L = leaf; S + P = stem + petiole; AR = absorption roots; and TR = tuberous roots. Values given are means ± standard error, and means followed by the same letter are not significantly different at $p < 0.05$.

The total number of leaves produced by plants during the growth period was not changed by the salt present in the solution (Figure 2A); on average, the plants produced 55 leaves. That is, even 60 mM NaCl did not affect the meristematic activity that leads to the development of new leaves. However, the number of living leaves (attached to the stem) at the end of the experiment was 45.6 for the control plants and 35.0 for the plants grown under 40 and 60 mM NaCl (Figure 2B), which was an average reduction of 23%; 20 mM NaCl did not negatively affect this characteristic. The reduction in the number of living leaves was due to the occurrence of larger leaf abscission (Figure 2C) in the plants grown under the highest salinity concentrations evaluated (40 and 60 mM NaCl). For the control plants, the final leaf area was 94.6 dm^2, while for the plants grown under 60 mM NaCl, it was 73.59 dm^2, which was a reduction of 22% (Figure 2D). The average size of each leaf was not affected by the solution salt levels (Figure 2E), indicating that salinity did not affect the leaf expansion rate. The number of tuberous roots, an important determinant of cassava production, was also not affected by NaCl concentrations (Figure 2F). Of the above parameters, leaf abscission was the most affected by salinity, with the concentrations of 40 and 60 mM NaCl resulting in an increase of approximately 100% in the number of abscised leaves when compared to the control.

The dry matter of the stem + petioles and leaves were similar in plants grown in 0, 20, and 40 mM NaCl (Figure 3A and B). However, the highest NaCl concentration reduced the dry matter of the stems + petioles and leaves by 28% and 26%, respectively, compared to the control.

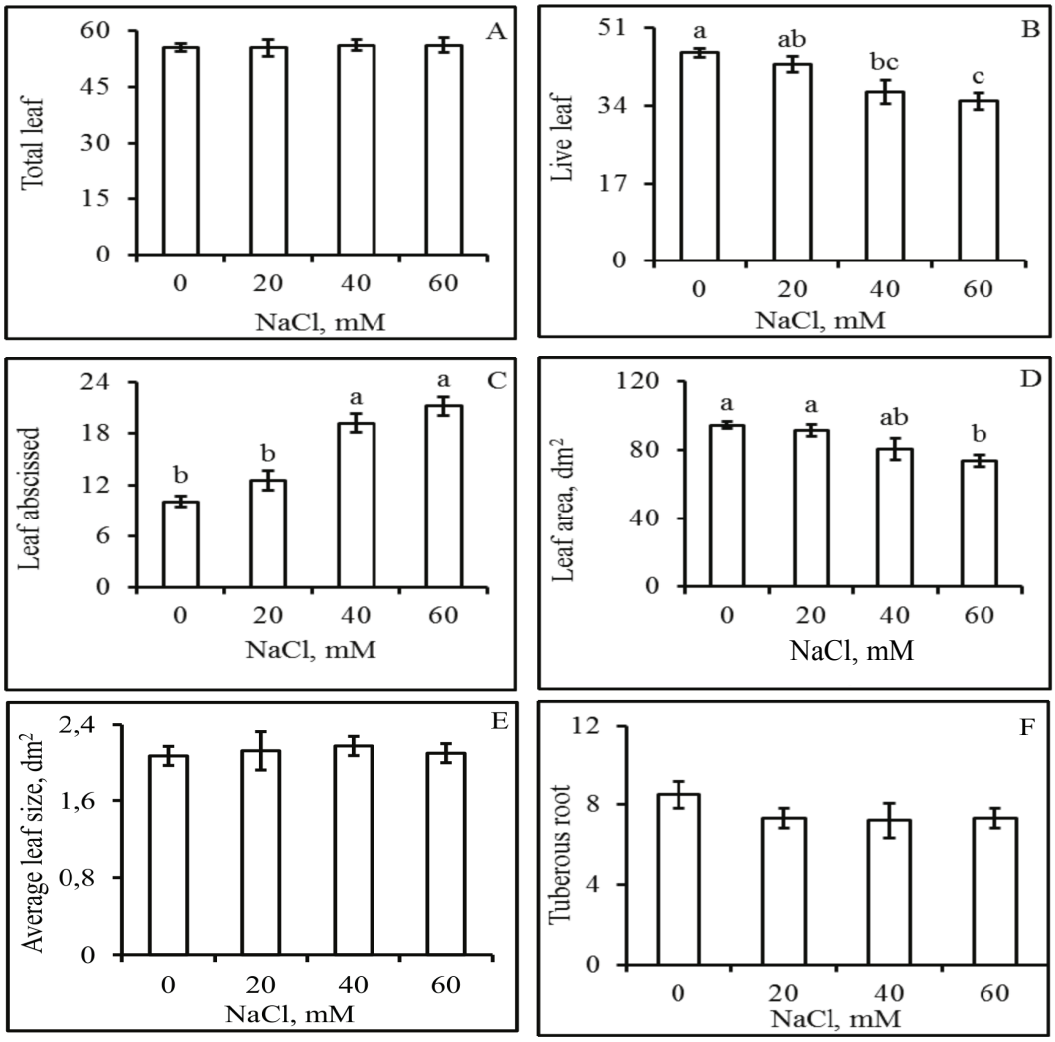

Figure 2. Total number of leaves produced during the experimental period (A), the number of leaves attached to the stems or living leaves (B), the number of abscised leaves (C), leaf area (D), average leaf size (E), and the number of tuberous roots (F) of cassava plants grown under different concentrations of NaCl. Values given are the means ± standard error, and the means followed by the same letter are not significantly different at $p < 0.05$.

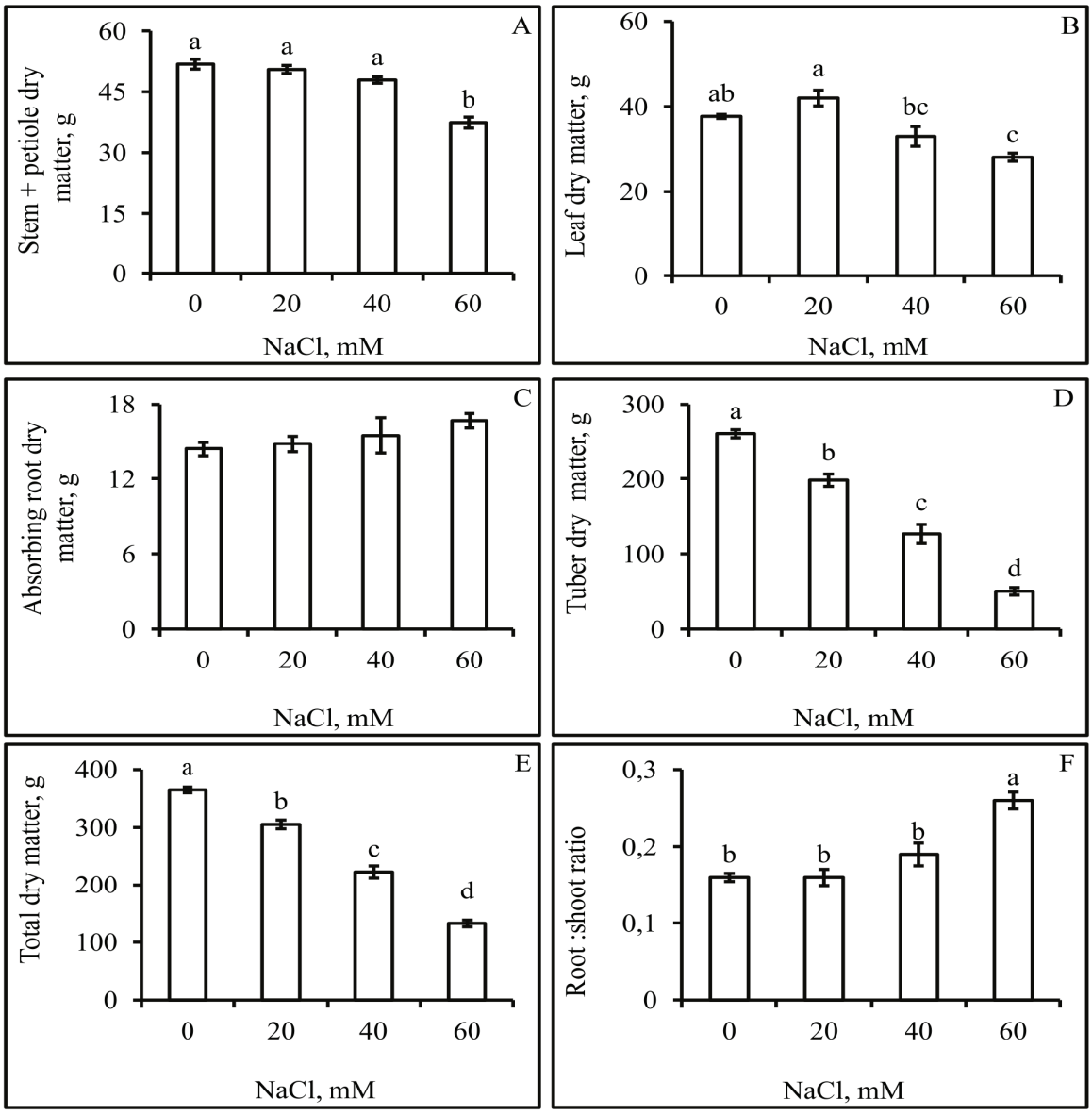

Figure 3. Dry matter of the stem + petiole (A), leaves (B), absorption roots (C), tuberous roots (D), and total (E) and the root:shoot ratio(F) of cassava plants grown under different concentrations of NaCl. Values given are means ± standard error, and means followed by the same letter are not significantly different at $p < 0.05$.

For the absorption roots, there was not negative effect of salt stress (Figure 3C). The concentrations of 20, 40, and 60 mM NaCl reduced the tuberous roots dry matter by 24%, 52%, and 81%, respectively (Figure 3D). These results show that among the evaluated organs, the dry matter accumulation in tuberous roots was the most affected. Total dry matter was reduced by 16%, 39%, and 64% with the applications of 20, 40, and 60 mM NaCl, respectively (Figure 3E). The root:shoot ratio was not affected by the 0 to 40 mM NaCl concentrations; however, it significantly increased (62%) when plants were grown under 60 mM NaCl (Figure 3F). This difference was due exclusively to lower shoot growth since there were no differences between the dry matter of the absorption roots. The harvest index proved to be a very sensitive parameter to salinity, because the use of only 20 mM NaCl caused a reduction compared to the control (Figure 4). From 40 to 60 mM NaCl concentrations, the reduction in the harvest index was 22 and 48%, respectively. However, the harvest index results should be used as a baseline because they were taken just 110 days after planting.

The application of 60 mM reduced stomatal conductance (g_s) of the newly expanded leaves by 37% (Figure 5A); for older leaves, this reduction was approximately 80%. The newly expanded leaves of the plants grown under 60 mM NaCl had the lowest transpiration rates (Figure 5B). Compared to the

control, the concentration of 60 mM NaCl reduced the transpiration of older leaves by 67%. The highest carbon assimilation (*A*) was observed in the newly expanded leaves of the control plants (Figure 5C.); however, the plants grown in 40 and 60 mM NaCl had a reduction in *A* by 13% and 37%, respectively, compared to control plants. For older leaves, the negative effects of salinity on *A* were more evident since the values of the stressed plants at 40 and 60 mM were 63 and 82% lower, respectively, compared to the control. As a result of having the lowest *A*, plants grown under salinity also showed the lowest values of iWUE; this effect was stronger in the older leaves (Figure 5D). The correlation between g_s and *A* was positive and high (Figure 5E), but *Ci* was higher in older leaves and plants grown under highest saline conditions (Figure 5F).

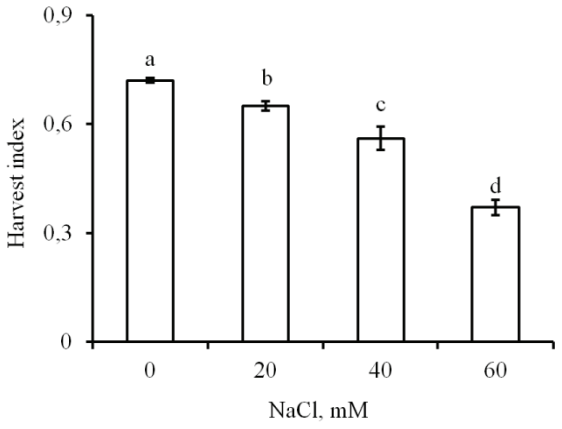

Figure 4. Harvest index of cassava plants grown under different concentrations of NaCl. Values given are means ± standard error, and means followed by the same letter are not significantly different at $p < 0.05$.

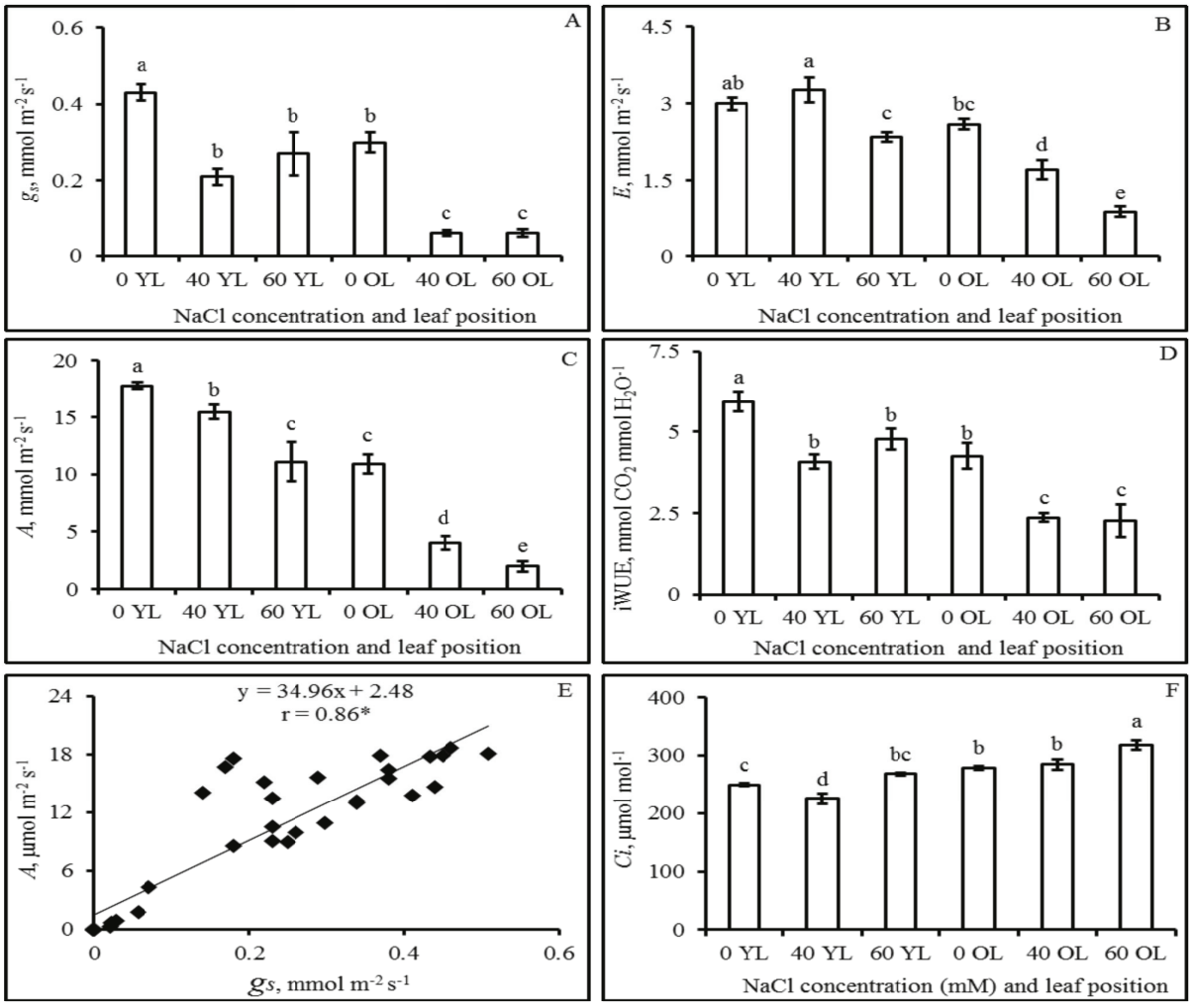

Figure 5. Stomatal conductance (A), transpiration (B), carbon assimilation (C), instantaneous water use efficiency (D), correlation between g_s and carbon assimilation (E), and intercellular CO_2 concentration (F) of cassava plants grown under different NaCl concentrations. The 0 YL, 40 YL, and 60 YL treatments correspond to NaCl concentrations and the newly expanded leaves. The 0 OL, 40 OL and 60 OL treatments correspond to NaCl concentrations and the older leaves. Values given are the means ± standard error, and the means followed by the same letter are not significantly different at $p < 0.05$. * indicates significance at $p < 0.05$.

Discussion

Na^+ concentrations in plants

A considerable part of absorbed Na^+ was retained in the absorption roots; however, large amounts of Na^+ were translocated to the shoot. The stem + petiole retained most of the translocated Na^+, preventing it from being directed to the leaves, which is the place where important physiological processes and biochemical reactions occur. The high concentration of Na^+ in the absorption roots also prevented the tuberous roots from accumulating high concentrations of Na^+. The yellowing and abscission of the older leaves suggest that there was a large accumulation of Na^+ and Cl^- ions in this part of the plant, displaying an important strategy that protects the shoot apexes and the younger leaves. The high rate of leaf senescence in plants grown under 40 and 60 mM NaCl supports the hypothesis that there were higher Na^+ and Cl^- concentrations in the older leaves. Munns, James, and Läuchli (2006) and Cruz et al. (2006) also confirmed that, in general, the concentrations of these two ions are much greater in older leaves than in younger leaves, which is called the exclusion mechanism; in certain circumstances, this concentration is so high that it can determine the senescence and death of older leaves. Thus, our data, when taken together, indicates that Na^+ was distributed mainly to the stem + petiole, absorption roots, and old leaves, which may have contributed to the reduction of the negative effects of salinity on cassava growth and dry matter accumulation.

It is worth noting that the amount of Na^+ in the leaves is an average, which includes the older leaves that are known to accumulate more Na^+; therefore, the presented amounts overestimate the concentrations of Na^+ in the younger leaves and underestimate the ones in older leaves. For some species, a Na^+ concentration of 10 mg kg^{-1} dry matter is not considered a toxic value (Silva, Ribeiro, Ferreira-Silva, Viégas, & Silveira, 2010). Thus, our data allows us to hypothesize that the Na^+ concentration found in the newly expanded cassava leaves was not at a toxic concentration. Hawker and Smith (1982) also observed that the leaf Na^+ concentration remained low in cassava leaf even with an application of 75 mM NaCl.

Growth and dry matter accumulation

The average size of each leaf, an indirect measure of leaf expansion, and the total number of leaves produced during the experimental period were not affected by salinity, even at 60 mM NaCl. This aspect may be related to the low concentration of Na^+ observed in this part of the plant. The highest NaCl concentration reduced leaf area, and this result was due solely to leaf abscission, which was one of the most affected parameters. This means that under salinity, the leaf production rate of cassava was lower than the abscission rate, which determined the smaller carbon assimilation areas; this is a negative aspect for plants growing under salt stress. The effects of salinity on the increase of abscission has also been described for other crops, such as strawberry (Orsini, Alnayef, Bona, Maggio, & Gianquinto, 2012) and tomato (Maggio, Raimond, Martino, & De Pascale, 2007). The drastic effects of salinity on leaf abscission have been explained by the increases in abscisic acid and ethylene concentrations that usually occur in organs that store high concentrations of Na^+ and/or Cl^- (Arango, Wüst, Beyer, & Welsch, 2010; Amjad et al., 2014; Siddikee, Chauhan, & Sa, 2012). Salinity did not affect the differentiation process that leads to the formation of tuberous roots, demonstrating that this process, as well as the total number of leaves produced, was less sensitive to NaCl stress.

The total dry matter was linearly reduced with the increase of NaCl concentration in the growth medium. This decrease was primarily due to the reduction in tuberous roots dry matter since the absorbing roots dry matter was not reduced with salt stress, and the stem + petioles and leaves dry matter were reduced only at the highest NaCl concentration. That is, except for the tuberous roots, the other organs proved to be less sensitive to salt stress, even after accumulating large amounts of Na^+ in its tissues, such as that seen in the stem + petiole and absorption roots. This aspect indicates that cassava has the ability to isolate the ions Na^+ and Cl^- from compartments associated with important metabolic processes.

The absorbing roots were less sensitive to salinity than the shoot components because NaCl did not reduce dry matter accumulation, even at 60 mM NaCl. Other authors have also shown that the shoot is more sensitive to salinity than the roots (Astolfi & Zuchi, 2013; Maggio et al., 2007; Carretero et al., 2007). The root:shoot ratio was affected only by the highest NaCl concentration in the growth medium. Thus, the reduction in the total dry matter of plants cultivated under the highest NaCl concentration may have been a consequence of the higher carbon allocation to the absorption roots than to the photosynthetic organs. The absorption roots growth maintenance under saline conditions is beneficial because they can accumulate large amounts of Na^+, thus preventing a portion of this element from being

transported to the leaves (Abideen et al., 2014), which was in accordance to what occurred in the present study.

Under the highest NaCl concentration, the tuberous roots accumulated the lowest concentrations of Na^+ at 2.56 g kg^{-1} dry matter, a value 84% lower than that in the stem + petiole; however, it was the organs that had the highest growth reduction. Even the 20 mM NaCl concentration reduced dry matter accumulation of the tuberous roots. In addition to the lower accumulation of Na^+, cassava also not accumulate high concentrations of Cl^- in their tuberous roots, as reported by Hawker and Smith (1982), who cultivated cassava under 50 mM NaCl. It can be inferred that root tuberization process is sensitive to the presence of those ions, even in low concentrations.

The lower photosynthetic rates of plants grown under saline conditions may have been one reason for the strong growth inhibition of tuberous roots. However, other factors may also be associated with this problem. According to Indira (1978), when cassava is grown under saline conditions, a degeneration of the cortical parenchyma and the formation of tyloses in the vessels of the tuberous roots occurs, inhibiting dry matter accumulation in these parts of the plant; according to the author, salinity delays the onset of tuberous roots formation, which may have caused less time for dry matter accumulation in these roots. Another problem is associated with the lower harvest index (HI), that provides a good indication of the balance between total carbon fixation and its distribution to the tuberous roots. The lower HI and the drastic reduction in dry matter accumulation in this organ seem to indicate that the flow and/or the use of carbon by tuberous roots were reduced by salt stress. In fact, the negative effects of salinity on the sink organs have been partly explained by damage to the transport and metabolism of sucrose (Balibrea, Cuartero, Bolarín, & Pérez-Alfocea, 2003) and starch formation and breakdown (Gimmler & Möller, 1981). Thus, the adverse effects of NaCl on the anatomy, the use of carbon, and the shorter time for filling, in addition to lower photosynthesis, may have been crucial to the lower growth of the tuberous roots.

Based on the shoot dry matter accumulation, cassava could be considered moderately tolerant to salinity. However, the drastic effects of NaCl on the tuberous roots dry matter indicates that, at this growth stage and for these experimental conditions, cassava behaves as a sensitive plant to salinity. Other studies have also classified cassava as moderately sensitive to salinity (Indira, 1978; Hawker & Smith, 1982).

Gas exchanges

Stomatal closure, as observed in this study, is a typical plant response to salt stress (Chaves, Flexas, & Pinheiro, 2009). The stomatal closure of salinized plants is attributed to lower leaf water potential and a reduction in relative water content, which results in a loss of cell turgor (Cabot, Sibole, Barceló, & Poschenrieder, 2014). However, the present study demonstrated that the leaf expansion rate, which may be indicative of turgor potential, was not affected by salinity, and the leaves of plants grown under 60 mM showed low Na^+ concentrations. Moreover, even when grown in severe water deficit conditions, cassava closed the stomata, but it did not significantly change its leaf water potential (El-Sharkawy, 2007). In addition, according to Alves and Setter (2004), signals derived from the roots and/or leaves could better explain stomatal movement of cassava grown under drought conditions than leaf water potential. All of these results seem to indicate that water relations are not the main determinants of stomatal closure in salinized cassava plants. The maintenance of good water status in cassava plants grown under moderate to high salinity has been observed by Carretero et al. (2007).

The highest NaCl concentration reduced *E* and this is a positive characteristic of plants grown under salinity because there is a positive relation between salt accumulation in the different organs and transpiration rate (Parihar, Singh, Singh, Singh, & Prasad, 2015). Thus, lower transpiration rates contributed to the lower accumulation of Na^+, and possibly of Cl^-, in the plant as a whole. The lower iWUE that occurred in plants grown under 40 mM NaCl was only due to a lower *A*, while for plants grown in 60 mM NaCl the lower iWUE was due to lower *A* and lower *E*. Lower iWUE have been associated with lower plant tolerance to salinity (Omamt, Hammes, & Robbertse, 2006).

The carbon assimilation rate (*A*) was reduced even under 40 mM NaCl, which was considered an intermediate level of stress. This result was more drastic in the older leaves than in the newly expanded leaves. It is worth noting that this concentration of NaCl did not reduce the growth of the shoots and absorption roots or reduce leaf expansion, showing that, except for tuberous roots, the photosynthetic process was more sensitive to NaCl concentration than the growth parameters. The lower stomatal conductance of the plants grown under salinity was essential to a lower *A*, as can be deduced from the correlation between g_s and *A* that

was high and positive. This result imply that the restriction of CO_2 diffusion from the outside air into the chloroplast was one of the factors responsible for lower *A*, as was the case of the newly expanded leaves of the plants grown with 40 mM NaCl, which showed lower intercellularCO_2 concentrations. All other saline treatments showed an increase in intercellular CO_2 concentration, clearly indicating that in addition to lower stomatal conductance, salinity may have increased mesophilic resistance to the ingress of atmospheric CO_2 into carboxylation sites and/or decreased the enzymatic activities associated with photosynthetic carbon metabolism, a common effect in plants grown under saline conditions (Chaves et al., 2009; Pérez-López et al., 2012). Another important aspect, as deduced from a lower HI, is that the tuberous roots' ability to metabolize sucrose unloaded from phloem could have been impaired, leading sugar accumulation in the source leaves, which can determine photosynthesis inhibition, a process known as "photosynthetic repression feedback". This has already been observed in cassava by Cruz, Mosquim, Pelacani, Araujo, and DaMatta (2003). Albacete et al. (2014) also indicated that the reduction in sink activity might be an indirect effect of salinity on *A*. Thus, the lower photosynthesis in the leaves of cassava plants grown under salinity was due to stomatal and, most likely, to biochemical factors. The probable accumulation of sugars in the leaves may have also been one of the components that led to the stomatal closure of plants grown under salinity. In fact, the importance of sucrose and other compounds derived from cellular metabolism in the regulation of stomatal movement has been well-established (Lawson, Simkin, Kelly, & Granot, 2014).These assumptions, however, need to be further investigated with salinized cassava plants.

It is most likely that the drastic reduction of photosynthesis in older leaves, compared to the newly expanded leaves, is related to Na^+ and Cl^- ion accumulation at toxic levels, a situation that normally occurs when plants are grown under high salt conditions (Cruz et al., 2006; Munns & Tester, 2008). Indeed, it has been shown that *A* is inversely proportional to the concentration of Na^+ and Cl^- in leaf tissue (Wang et al., 2015). These older leaves were also more yellow, indicating photosynthetic pigment loss and probably protein reduction (Qiao et al., 2010), which likely contributed to the reduction of *A* in these leaves.

Conclusion

Our results indicate that salt stress affects carbon assimilation in cassava plants via its effects on stomatal resistance and its negative interference on the biochemical reactions that occur in chloroplast stroma. The detrimental effect of salt stress on the dry matter of cassava plants is associated with an alteration of the sink-source relationships, lower carbon assimilation, and the high sensitivity of tuberous roots to NaCl. In this respect, the tuberous roots could be the target organ in studies that aim to improve the tolerance of cassava to salinity.

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