Salinity reduces nutrients absorption and efficiency of their utilization in cassava plants

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ABSTRACT: The objective of this research was to evaluate the influence of salinity on the absorption and utilization of nutrients by cassava. For the study, cassava was submitted to four saline concentrations: 0, 20, 40, and 60mM NaCl. Results showed that the absorption of all nutrients, except nitrogen (N), was reduced by salinity, with highest reduction for potassium (K). However, all nutrients were maintained at concentrations which did not indicate mineral deficiency problem. The abnormal concentration of calcium in the tuberous roots may have been one of the factors that contributed to the lower growth of this organ and of the plant as a whole. Transports of nitrogen, potassium, phosphorus and sulfur from root to the aerial part was higher under salinity treatment. Efficiency in the use of all the nutrients, mainly N, was reduced due to salinity. Given that: (i) the absorption of K was the most impaired, (ii) there was abnormal accumulation of Ca in tuberous roots, and (iii) the efficiency in the use of N was the most affected, it is suggested to prioritize studies on these three issues, as a way to better understand the aspects related to the tolerance/sensitivity of cassava plants to salinity.

Key words: Manihot esculenta Crantz, sodium, Na:K ratio, nutrients absorption, nutrient transport.

INTRODUCTION

Many areas destined for agricultural production are affected by several abiotic stresses, such as salinity that, for some authors, is the problem that more have contributed to the reduction of crop productivity around the world (GUPTA & HUANG 2014). The salinity problem includes saline soils (relatively high concentrations of salts), sodium soils (those with high sodium concentrations), and alkaline soils (those with a high pH, mainly due to the high concentration of carbonates) (van BEEK & TÓTH 2012; DALIKAPOULOS et al. 2016). The increase of production areas with salinity and sodicity issues has grown in the last decades due to global climate change (KUMAR et al. 2017; PARIHAR et al. 2015), the pressure for food production that leads to intensive and disorganized soil use, and the inadequate management of irrigated areas (RIBEIRO et al. 2003).

In Brazil, saline and sodic soils occur (i) in the Pantanal, in the region of Mato Grosso, (ii) in the state of Rio Grande do Sul, and mainly (iii) in the Northeast region, in places where pluviometric precipitation is low and water evaporation from soil is high (RIBEIRO et al. 2003). Worldwide, salinity affects more than 800 million of suitable lands for commercial production (RENAGASAMY 2010).

Saline stress induces changes in various physiological and metabolic processes of the plants, whose magnitude depends on the genotype, mineral composition of the nutrient solution, environmental...
conditions (TEDESCHI et al., 2017), and the severity and duration of stress (GUPTA & HUANG, 2014). In cassava, some negative effects due to salinity are related to the reductions in leaf area, photosynthetic rate and biomass accumulation (GLEADOW et al., 2016). Several mechanisms can be developed by different species and varieties aiming to face the inhospitable conditions generated by the presence of high concentrations of Na+ and Cl– in the soil solution. One of the main consequences of high concentrations of Na+ and Cl– is the negative effect on the absorption and/or use of several essential nutrients. The logic is that the mechanisms of absorption, transport, and use of the nutrients could not operate efficiently when they are grown under salt stress.

Recently, CRUZ et al. (2017) published a report on the effect of salinity on cassava growth. They observed that the use of only 20mM of NaCl in the soil solution reduced photosynthesis and accumulation of dry mass, mainly in tuberous roots. Due to the drastic effect of NaCl on biomass accumulation, that authors concluded that cassava behaved as susceptible to such abiotic stress. However, these authors did not discuss the effect of salinity on the absorption and use of any macronutrients. Several authors have suggested that maintenance of the absorption and increase in nutrient use efficiency are two of the main mechanisms of plant adaptation to salt stress (PETTIGREW, 2008; NIU & CABRERA, 2010; GUPTA & HUANG, 2014; BORZOUEI et al., 2014). As an example, KAFKATI et al. (1992) reported that more tolerant tomato and melon varieties absorb greater amount of NO3 from saline substrate than the sensitive ones.

Each ion has specific and important functions on plant physiology; therefore, reductions in its absorption, under saline conditions, can induce deficiency and potentiates the negative effect of such stress on plant growth and productivity. In addition, one of the main objectives of studies that relate salinity to plant nutrition is to evaluate if the addition of certain nutrients to the soil solution, especially K+ and Ca2+, can increase the tolerance of the cultivated plants to salinity (MORGAN et al., 2014; CHAKRABORTY et al., 2016), since that the established ionic competition can reduce the absorption of Na+. However, prior knowledge about which nutrients and how they are impaired are essential to the development of those studies.

The literature clearly indicates that salinity inhibits the absorption and use of various nutrients, causing nutritional imbalances in the plants. Thus, the objective of this research was to evaluate the effect of salinity on the absorption, transport, and efficiency in the use of macronutrients by cassava.

MATERIALS AND METHODS

The methodology was similar to the one used by CRUZ et al. (2017). The experiment was conducted in a greenhouse of Embrapa Mandioca e Fruticultura, located in Cruz das Almas/BA. The study area present geo-coordinates of 12°40’12”S and 39°06’07” and altitude of 220m. The variety used in the experiment was Verdinha (BGM 116), which has shown good productive potential in field conditions. Cuttings with 15cm long were selected and placed to sprout in plastic pots with capacity for 14 liters. The substrate used was formed by a mixture of washed coarse sand, perlite and vegetal substrate in equal proportions. The substrate was composed of 60% pine bark + 30% coconut fiber + 10% vermiculite. Before planting the pots with the final substrate were washed several times with tap water, aiming to leach chemical elements. Two cuttings were placed in each pot. During the initial 15 days, the substrate was irrigated twice a day with only tap water, always to reach the field capacity. After this phase, the less developed plant was removed from the pot and the experiment was conducted with only one plant per pot. After thinning, the substrate was fertilized with growth solution, containing the following concentration in mM: N (12), P (1.5), K (6.5), Ca (3.5); Mg (1.5), and S (1.5). The micronutrient solution was similar to the one used by HOAGLAND & ARNON (1950). Saline stress was also imposed fifteen days after sowing by the addition of NaCl to the growth solution. Saline treatments were equivalent to concentrations of 0, 20, 40, and 60mM of NaCl, which presented, respectively, electrical conductivities of 1.1, 3.6, 5.2, and 6.8dS m⁻¹. It is noteworthy that the solutions were modified to vary the NaCl concentrations, but to maintain the same concentration of all nutrients. Aiming to reduce the possibility of osmotic shock to the plants at the beginning of the experiment, the induction of saline stress was gradually applied (set to 20mM every 48 hours). The initial pH of the nutrient solutions was always adjusted to maintain a value between 6.3 and 6.5 and replacement of water lost by evapotranspiration was performed daily. Every 10 days, the substrate was washed vigorously with tap water to prevent its salination and on the same day a new growth solution, along with NaCl, was added to the substrate. Within the 10-day period, each treatment received four liters of their respective nutrient solutions. Ninety days after the induction of treatments, the experiment was terminated. Initially, the leaves and stem + petiole were removed from the...
Salinity reduces nutrients absorption and efficiency of their utilization in cassava plants. Chemical analyses of N, P, K, Ca, Mg, S, and Na were performed in the plant tissues of 0 and 60mM NaCl treatments following the analytical procedures described by MALAVOLTA et al. (1989). Other researchers have also used the same strategy of determining nutrients in only two concentrations of NaCl, without prejudice to the understanding the research (WU et al., 2015; SUZUKI et al., 2016). At the final, our interpretation was that the evaluation of nutrients in only two concentrations allowed to achieve the objectives, as can be seen by the final conclusions presented.

With the data of dry mass and the concentration of the nutrients, the following parameters were calculated: (a) Total nutrient content: the nutrient content for each plant organ was calculated by the product between the organ dry mass and their respective nutrient concentrations. Subsequently, the contents present in the leaves, stems + petioles, absorption roots, and tuberous roots were added together to form the total content of each nutrient per plant; (b) Translocation Index (TI) obtained by dividing the content of each nutrient present in the aerial part by the total plant content of each nutrient, multiplied by 100; and (c) Nutrients Use Efficiency (NUE) obtained by the division between total plant dry mass and the total content of each nutrient.

Statistical analysis
The plants were distributed in an experimental randomized block design with six replicates. Due to the combination of two plants to form the composite samples, statistical analysis was performed taking into account only the three replicates. The analysis of variance was performed and means were compared on the basis of the F test. Level of significance for all variables analyzed was p≤0.05.

RESULTS
Total dry mass of cassava grown under 0mM and 60mM of NaCl were, respectively, 365.3g and 132.5g (Table 1), setting a reduction of 63%; for the tuberous roots, the dry mass values were 261.1g and 50.3g, respectively, for 0 and 60mM NaCl treatments, corresponding to a reduction of 81%.

For all the plant organs, the nitrogen concentration (henceforth referred to as [N]) was higher for plants grown under salinity. The value of [N] in the tuberous roots was 125% higher for plants grown with 60mM of NaCl (Figure 1A). In the leaves, the [N] were 35.7g kg⁻¹ and 28.6g kg⁻¹, respectively, for plants grown with and without salinity, establishing an increase of only 25%.

With very few exceptions, the concentrations of phosphorus ([P]) and potassium ([K]) were also higher for the treatments submitted to salinity (Figures 1B e 1C). The exceptions occurred in the absorption roots (for P) and stem + petiole (for K) that presented similar values between the two saline treatments. In the leaves, the [P] and [K] of the plants grown without salinity were, respectively, 3.4g kg⁻¹ and 1.5g kg⁻¹ of dry mass. Calcium ([Ca]) concentrations for stem + petiole were similar for both treatments (Figure 1D), while for the leaves the lowest value of 13.0g kg⁻¹ of dry mass was obtained by the plants cultivated under salinity. For the tuberous roots

<table>
<thead>
<tr>
<th>NaCl (mM)</th>
<th>Tuberous roots</th>
<th>Stem + petiole</th>
<th>Leaves</th>
<th>Absorbing roots</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>261.1a¹</td>
<td>51.8a</td>
<td>37.6ab</td>
<td>14.4a</td>
<td>365.3a</td>
</tr>
<tr>
<td>20</td>
<td>198.3b</td>
<td>50.5a</td>
<td>42.5a</td>
<td>14.8a</td>
<td>306.1b</td>
</tr>
<tr>
<td>40</td>
<td>126.3c</td>
<td>47.9a</td>
<td>33.6bc</td>
<td>15.5a</td>
<td>223.3c</td>
</tr>
<tr>
<td>60</td>
<td>50.3d</td>
<td>37.4b</td>
<td>28.1c</td>
<td>16.7a</td>
<td>132.5d</td>
</tr>
</tbody>
</table>

*The values followed by the different lower case letters within column are significantly different (p<0.05).
The [Ca] was higher for 60mM NaCl treatment, with a surprising increase of 355% in relation to the control plants. That value can be considered much higher than the one required by the tuberous roots to perform their metabolic activities. The leaves of the two treatments had the same concentrations of magnesium ([Mg]) and sulfur ([S]) (Figures 1E and 1F). The absorption roots and stem + petiole showed higher [Mg] in the treatments without salinity, whereas for the tuberous roots the saline treatment had a higher [Mg]. The [S] of the plants grown without salinity was higher only for the absorption roots.

The ratio between Na:K of the different organs was higher for plants grown under 60mM of...
Salinity reduces nutrients absorption and efficiency of their utilization in cassava plants. NaCl, mainly for the stem (0.74) and absorption roots (0.47) (Figure 2A). Tuberous roots and leaves of plants cultivated under stress showed similar values of Na:K ratio (close to 0.18). The values of Na:Ca ratio of leaves, stem + petiole, and absorption roots were higher for plants cultivated under salt stress (Figure 2B). However, for the tuberous roots the value of this ratio was higher for plants grown without salinity.

The total content of a given nutrient is directly related to the plant’s ability to absorb it from the soil solution. Some authors even replace the term “content” with the term “absorption” (ALV A et al., 2002; MIN et al., 2014). Following this concept, nitrogen was the only nutrient whose absorption capacity was not reduced by salinity (Figure 3A). For all the others, there was a reduction in the absorption, whose percentage varied as a function of the nutrient. For example, K (-50%) and Mg (-43%) were the nutrients whose absorption was most impaired. Sulfur (-26%) and Ca (-29%) were the ones that presented the lowest reductions in their respective absorptions. For P, the reduction was 36%.

The translocation rates of N, P, K, and S to aerial part were stimulated by salinity (Figure 4). Of these nutrients, the highest proportional increase was for K, whose translocation to the aerial part was almost 80% in favor of the salinized plants, while the smallest stimulus was for the nitrogen with only 13%. Salinity did not affect Ca and Mg translocations. The efficiency in N use was the most affected by salinity (-59%), followed by S (-50%) and Ca (-48%) (Figure 5). The efficiencies that presented the lowest

Figure 2 - Na:K (A) and Na:Ca (B) ratios of leaves (L), stem + petiole (S+P), absorption roots (AR) and tuberous roots (TR) of cassava plants grown under salt conditions.
reductions due to salt stress were K (-27%) and Mg (-35%). Phosphorus was in an intermediate range, with a 43% reduction in the efficiency of its use.

DISCUSSION

Salinity did not reduce nutrient concentration for most of the evaluated organs. The exceptions were [Ca] in the leaves and absorption roots and the [Mg] in stem + petiole and absorption roots. However, even in these cases, the concentrations of Ca and Mg were not characterized as values that would indicate deficiency, as reported by ASHER et al. (1980). Salinity increased the concentration of N in all organs; of P in the leaves, stem, and tuberous roots; and of K in the leaves, absorption roots and tuberous roots. Unlike our results, GLEADOW et al. (2016), in another research with cassava, observed that the salinity negatively affected the leaf concentration of N, P, and Mg, but did not affect leaf K concentration. Regarding the tuberous roots, GLEADOW et al. (2016) observed that salinity increased K concentration by approximately 40%, while in our research the increase was only 19%.

Absorption of all nutrients was higher for plants grown without salinity, except for N which was similar for both treatments, while the total dry mass of the salinized plants was 61% lower. These two results, taken together, indicated that the high concentration of nutrients observed in plants cultivated under 60mM of NaCl was a consequence of the lower growth of the plants; that is, the lower growth of the salinized plants avoided the dilution of the mineral elements, a phenomenon that normally occurs with plants cultivated without salt stress. In fact, plants growing under no stress conditions increase the total nutrient content; however, under these circumstances, the nutrient concentration will tend to be lower due to a dilution effect (HÖGY & FANGMEIER, 2008; WANG et al., 2016).

The leaf N concentrations were of 28.6g kg⁻¹ and 35.5g kg⁻¹ of dry mass, respectively, for the plants cultivated with and without salinity. Considering that the value described as sufficient for upper leaves of cassava plants is between 50g kg⁻¹ and 60g kg⁻¹ of dry mass (ASHER et al., 1980), the value observed in our experiment could be considered indicative of N deficiency. However, the visual evaluation did not indicate symptoms of deficiency in the treatment conducted without salinity. It is also noteworthy that this value of [N] is an average of all the leaves that were present in the plant at the time of harvest and not only the last fully expanded leaf, which is normally used as reference for detection of possible deficiencies in cultivated plants. Thus, basal leaves with lower [N] may have contributed
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Figure 4 - Translocation index of macronutrients in cassava plants grown under saline conditions.

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et al., 2003a), the possibility is that this likely reduced availability of P in the tuberous roots may have, directly or indirectly, impaired the assimilation of sucrose from the “source”, and seriously inhibited the accumulation of dry mass in the tuberous roots and in the plants as a whole. In addition to this possibility, HE et al. (2014) reported that Ca in excess can be toxic to plants, as it interferes negatively with several metabolic processes, including signaling and energy-related metabolism.

In summary, this accumulation of Ca may help to explain the high sensitivity of tuberous roots to salinity (CRUZ et al., 2017) and reinforces the suggestion that tuberous roots may be one of the target organs in studies aiming to improve the tolerance of cassava to salinity. However, calcium toxicity is uncommon in plants since, normally, calcium in excess is precipitated and stored in the vacuole (NAKATA, 2003). Thus, the advantage or disadvantage of the high Ca accumulation and the higher Ca:Na ratio (or lower Na:Ca ratio) of the tuberous roots of salinized cassava plants needs to be better investigated. The higher Na:Ca ratio of the absorption roots of plants grown under salinity may also have been another factor that contributed to the lower growth of cassava plants this is because high ratio of Na:Ca reduces cell membrane integrity and selectivity, increasing Na passive absorption and decreasing nutrient absorption, especially K (KRAMER et al., 1977; CRAMER 1992, 2002), as seen in the present research.

Cassava plants grown under saline stress have their transpiration reduced (CRUZ et al., 2017), which explains, at least partially, the lower nutrient absorption (except N) observed for the treatment corresponding to 60mM of NaCl. Another factor that also contributes to the lower absorption of nutrients is the antagonism between the Cl⁻ and the anions and between Na⁺² and the cations (GRATTAN & GRIEVE, 1998). The competition that Na⁺² established with K⁺ (reduction of 50% in absorption) was stronger than that which was established with Ca⁺² (-29%), confirming that low K absorption, along with the low K:Na ratio for all organs, was one of the main factors limiting the growth of cassava grown under saline conditions. In fact, for some authors, the ability to absorb and retain K⁺ in the cytoplasm is one of the most important characteristics associated with salinity tolerance (CUIN et al., 2008; WU et al., 2013; ADEM et al., 2014; PERCEY et al., 2016). Cassava, at least for the cultivar evaluated in the present study, failed to maintain or minimize the reduction in K⁺ absorption.

Thus, due to the great reduction in K⁺ absorption, it is possible that the increase of this
element in the soil solution and/or studies aiming to increase its absorption efficiency may contribute to reduce the effect of NaCl on growth of cassava plants, similar to what has occurred for barley (MAHMOOD, 2011), bean (DAWOOD et al., 2014), and wheat (AHANGER & AGARWAL, 2017).

According to FAYEZ & BAZAID (2014), increases in K⁺ absorption from soil solution may attenuate saline stress because in the plant it can contribute to reducing the production of malonic dialdehyde and to increasing the antioxidant capacity.

The salinity did not affect N absorption, evidencing that the absorption of this nutrient was not a limiting factor of the growth reduction of the salinized cassava plants and that the use of cultivars with greater capacity to absorb N, as suggested by HU & SCHMIDHALTER (2005), could contribute in a limited way to the reduction of the negative effect of salinity on the physiology and growth of cassava. Similar to our research, NaCl salinization also had little effect on N uptake in winter barley (HELAL & MENGEL, 1979). According CEREZO et al. (1997), two factors can explain the maintenance of NO₃⁻ uptake observed in our research (i) for some species, high concentrations of NaCl in the uptake medium do not inhibit the net absorption of NO₃⁻ in the low affinity transport system (LATS) and (ii) the affinity of the nitrate HATS (high affinity transport system) for Cl⁻ is so low that very high concentrations of Cl⁻ must be used to bring about inhibitory effects on NO₃⁻ uptake.

The efficiency in the use of all the nutrients was reduced by the salinity. Of the nutrients, K was the one that had the lowest reduction in use efficiency, again indicating that improving the absorption and, to a lesser degree, the efficiency in the use of K also may contribute to increasing the tolerance of cassava to salinity. However, the efficiency in N use was the one that suffered the greatest reduction (-60%). In fact, salinity has been shown to reduce several parameters related to N metabolism in plants, such as NO₃⁻ absorption, free aminoacid, leaf protein, and activity of the enzymes nitrate reductase, glutamine synthase, and glutamate synthetase (SOUZA et al., 2016; KAUSAR et al., 2014; MENG et al. et al., 2016; IQBAL et al., 2015; WANG et al., 2012; DEBOUBA et al., 2006; SILVEIRA et al., 2001), and these reductions have been higher in the varieties more sensitive to salinity (KAUSAR et al., 2014). Thus, given the importance of this nutrient for plant physiology and growth, studies related to N metabolism could also be prioritized in studies of cassava tolerance to salinity.

CONCLUSION

In short, salinity impaired absorption (except N) and utilization of all nutrients. However, lower K absorption, high Ca accumulation in tuberous roots, and lowest efficiency in N use seem to be more associated with lower cassava growth under salinity. Thus, studies on these three items should be prioritized as a way to better understand the aspects related to the effects of salinity on cassava.

DECLARATION OF CONFLICTING INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study, in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS’S CONTRIBUTIONS

The authors contributed equally to the manuscript.

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