

Performance on tolerance of the AL Bandeirantes maize cultivar under salt stress

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Abstract: The maize (*Zea mays* L., Poaceae) is a high productivity plant, but its agricultural yield is compromised in areas with soil salinity problems. This study aimed to evaluate the performance of maize plants (AL Bandeirantes cultivar) submitted to different levels of salinity. The experiment was conducted in greenhouse conditions under completely randomized design, with 4 treatments (0, 50, 100 and 150 mM of NaCl). An additional group of plants was not exposed to NaCl (control). The plants were evaluated for height, root volume, shoot and root dry weight, shoot/root ratio, relative water content and proline concentration in leaves. The NaCl reduced the dry weight and the root volume in comparison with that observed for the control. The plants height was affected only after 21 days of exposure to salt stress, while no significant difference in relative water content was observed among treatments. The proline accumulation was expressive in the plants treated with 150 mM of NaCl, indicating a mechanism for regulating osmotic. Salt stress did not affect the survival of the AL Bandeirantes cultivar, as was observed by plants development at all concentrations applied.

Key words: *Zea mays* L., Salinity, Sodium chloride.

Desempenho na tolerância da cultivar de milho AL Bandeirantes sob estresse salino

Resumo: O milho (*Zea mays* L., Poaceae) é uma planta de alta produtividade, mas o seu rendimento agrícola é comprometido em áreas com problemas de salinidade do solo. Este estudo teve como objetivo avaliar o desempenho de plantas de milho (cultivar AL Bandeirantes) submetidas a diferentes níveis de salinidade. O experimento foi conduzido em condições de casa de vegetação sob delineamento inteiramente casualizado, com 4 tratamentos (0, 50, 100 e 150 mM de NaCl). Um grupo adicional de plantas não foi exposto ao NaCl (controle). As plantas foram avaliadas quanto à altura, volume radicular, peso seco dos brotos e raízes, razão parte aérea/raiz, teor relativo de água e concentração de prolina nas folhas. O NaCl reduziu o peso seco e o volume radicular em comparação com o observado no controle. A altura das plantas foi afetada somente após 21 dias de exposição ao estresse salino, enquanto que no teor relativo de água não foi observada diferença significativa entre os tratamentos. O acúmulo de prolina foi expressivo nas plantas tratadas com 150 mM de NaCl, indicando um mecanismo de regulação osmótica. O estresse salino não afetou a sobrevivência da cultivar AL Bandeirantes, como foi observado pelo desenvolvimento das plantas em todas as concentrações aplicadas.

Palavras chave: *Zea mays* L., Salinidade, Cloreto de sódio.

Introduction

Maize (*Zea mays* L.) belongs to the Poaceae family and isn't just a food product. Maize-derived products have been used in many aspects in our lives, and maize also have been considered potential bioenergy crop in recent years (Niu et al., 2012). Furthermore, maize plants have high production potential and great physiological capacity evidenced in the conversion of mineral carbon in organic compounds, which are translocated to the photosynthetically active tissues, where they are stored and metabolized. Maize crop exhibits low CO₂ compensation point, high photosynthetic rate and reduced water consumption for biomass production (Farooq et al., 2015).

Brazil is a major producer of maize; however, due to soil salinity many areas are not explored, once the maize was considered moderately salt sensitive (Salinity threshold: water salinity of 1.1 dS m⁻¹ and soil salinity of 1,7 dS m⁻¹), undergoing physiological changes and progressive reduction in growth in salt-affected soils (Gondim et al., 2011, Hoque et al., 2015 & Cunha et al., 2016).

Salt stress is one of the most serious limiting factors for agricultural productivity in countries of arid and semi-arid regions, where soil salt content is naturally high and precipitation can be insufficient for leaching, along with the use of poor quality water (Oliveira et al., 2007 & Farooq et al., 2015). In northeastern Brazil, irrigated lands have been intensively affected by salinity and thus subject to abandonment with the permanence of the problem (Oliveira et al., 2007 & Lima et al., 2017).

The direct effect of salt stress on plant growth is result of disturbance in the biochemical and physiological functions and may involve (1) reduction in the osmotic potential of the soil solution that reduces plant-available water, and (2) toxicity of excessive Na⁺ or Cl⁻ on the plasma membrane (Shirmardi et al., 2009). The high concentrations of Na⁺ and/or Cl⁻ in the soil causes disorder in the nutrient availability, transport or partitioning within the plant, effects associated with a nutritional imbalance (Ali et al., 2012). Accordingly, there is inhibition of the growth and crop yield, besides changes in morphology and metabolism of plants (Graciano et al., 2011).

Numerous studies reported variations in salt tolerance among genotypes and negative

implications of salinity on the maize growth (Gomes et al. 2011, Godim et al. 2011, Lacerda et al. 2011, Carvalho et al. 2012, Niu et al., 2012, Richter et al., 2015 & Hoque et al., 2015). However, there is no information available on the effect of salinity in the AL Bandeirantes cultivar. Thus, this study aimed to evaluate the growth, relative water content and changes in the proline concentration from maize plants (AL Bandeirantes cultivar) at different levels of salinity with NaCl, in order to verify resistance traits of this cultivar under salt stress.

Material and methods

The experiment was conducted in greenhouse conditions in the city of Mossoró-RN (06°12'43"S; 37°20'39"W), during the period May-June 2011. The city is located in northeastern Brazil, in the semi-arid region of the country, and has an annual average temperature of 27.5°C, an annual average relative humidity of 68.9%, an annual average cloud cover of 4.4 tenths, and an annual average rainfall of 673.9 mm (Carmo-Filho & Oliveira, 1995).

Maize seeds were planted in 2-kg pots containing a substrate composed of washed sand, clay and earthworm humus, at 1: 1: 1. The water retention capacity of the substrate was evaluated before the seeds were planted. In each pot were placed 3 seeds on the substrate, which remained being moistened two times a day with system of automatic microaspersión. At 15° day after emergence of the plants was done thinning, leaving only one plant per pot. Then, selected plants were exposed to no salt stress (controls) or to one of four levels of salt stress resulting from the addition of 0, 50, 100, and 150 mM of sodium chloride (NaCl) to the irrigation water. The experiment was maintained at 27 °C with shading that allowed 70.0% luminosity reduction. Maize plants cultivation was conducted until 28 days after the onset of stress, time period in which we determine growth, relative water content and proline concentration.

We evaluated growth by measuring the plant height every week (0, 7, 14, 21 and 28 days) after exposure to salt. At 28 days of exposure was determined the root volume (mL) and dry weight (g plant) of shoots and roots. The volume of roots was obtained by measuring the water displacement in a beaker, while the plant material

was dried in a forced-air kiln at 70 °C from the leaves and roots collected.

The relative water content (RWC) was determined in leaf discs of approximately 8 mm diameter, using the fourth leaf from the apex of the plant. The RWC (expressed as percentage) was calculated by the following equation:

$$RWC = \left(\frac{W_f - W_d}{W_t - W_d} \right) \cdot 100$$

where W_f , W_d , and W_t are the fresh, dry, and turgid weights, respectively. The RWC was analyzed at 14, 21 and 28 days of exposure. The discs were immediately weighed to determine the fresh weight, after which they were placed in Petri dishes over water-soaked filter paper in a biological oxygen demand incubator (at 25 °C and 80.0% relative humidity) for 10 h in the dark. Subsequently, the discs were weighed to determine the turgid weight and dried in a forced-air kiln at 70 °C by three days (Ragagnin et al., 2014).

Proline concentration was quantified at 28 days of exposure in leaves harvested between 5:00 a.m. and 6:00 a.m. Fresh leaves (750 mg) were crushed and placed in a test tube containing 15 ml of 5-sulfosalicylic acid and centrifuged at 2000 rpm for 3 min. A mixture of 3 ml of supernatant, 3 ml of acetic acid, and 3 ml of ninhydrin was heated in a water bath at 100 °C for 1 h. After color development, samples were cooled in an ice bath and 6 ml of toluene was added for phase separation. The colorless fraction was discarded, and the color fraction was read at 520 nm (Ragagnin et al., 2014). Absorbance values were logged in the proline standard curve equation; results were expressed in μmol of proline/g of fresh weight.

The experimental design was completely randomized with four treatments. The experimental plot consisted of five pots, each pot containing an established plant. Data were subjected to analysis of variance and means were compared statistically by Tukey test at a 5% probability. The results of dry weight, root volume and shoot/root ratio were transformed into $\sqrt{x + 0,5}$, however, in the tables are presented the original values. Data analysis was performed using the ESTAT Software 2.0 (Unesp, 1994).

Results and discussion

All plants of AL Bandeirantes cultivar used in this study survived the salt treatment, as was observed by plants development at all concentrations applied. However, salt stress affects the dry weight, so that the total dry weight (shoots and roots) decreased as the concentration of sodium chloride was increased (Table 1). Among the evaluated vegetal parts, dry weight of roots was more impaired, with 66.51% reduction compared to control, in the plants exposed to 150 mM NaCl. The dry weight of shoot and total showed reductions of 48.18% and 57.27%, respectively, at the highest salinity level (150 mM). Thus, the AL Bandeirantes cultivar showed root system more sensitive to salt stress than shoots.

Conversely, Azevedo-Neto e Tabosa (2000) reported that two maize varieties BR-5011 and P-3051 grown in saline solution (100 mol.m⁻³ NaCl) reduced the dry weight of shoots by 74.0% and 56.0%, respectively, as well as dry weight of roots at 66.0% and 42.0%, when

Table 1 - Effect of treatment with different concentrations of NaCl on the dry weight and root volume of AL Bandeirantes maize cultivar.

NaCl concentration	Dry weight (g plant ⁻¹)				Root Volume (mL)
	Shoot	Root	Total	Shoot/root	Root
0 mM	6.51 a	5.67 a	12.19 a	1.20 a	54.16 a
50 mM	5.96 ab	3.74 ab	9.70 ab	1.59 a	41.25 ab
100 mM	4.18 bc	3.05 ab	7.27 bc	1.35 a	32.50 bc
150 mM	3.31 c	1.90 b	5.20 c	1.74 a	20.16 c
CV (%)	6.97	10.69	8.31	6.13	6.51

Means followed by the same letter in each column are not statistically different ($p \leq 0.05$), according to Tukey's test.

compared to their controls. Therefore, shoot systems from cultivars BR-5011 and P-3051 are more affected by salinity than are roots.

The salt sensitivity of shoots can reflect differences in the conditions under which the studies were conducted, since in the work cited above the plants were grown in nutrient solution, whereas in this study, the cultivation of the AL Bandeirantes variety was performed on the substrate.

According to Borzouei et al. (2012), more pronounced effect on root weight with respect to shoot weight could be due to direct exposure of the roots to salt solution. Additionally, limited space for root development might have contributed to this result, considering that ability of the pots (2 kg) may not have been sufficient for the full growth of roots up to 28 days of exposure.

The dry weight from plants under salinity condition is affected due the salt stress cause a diversion of growth energy to maintain the metabolism. In stress adaptation mechanism, the plant loses turgor, causing stomatal closure and consequently, leading to reduction in carbon fixation (Monreal et al., 2007, Graciano et al., 2011 & Farooq et al., 2015).

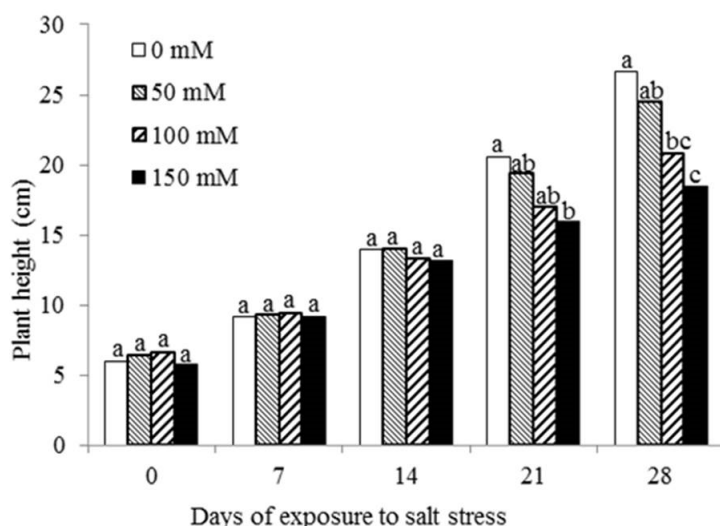
Evaluating the shoot/root ratio we found a

slight increase associated with the intensity of the salt stress (Table 1), although not statistically significant. The increase of the shoot/root ratio shows that salinity has an adverse effect on the reported that root systems are less affected by salinity than are shoots (Munns, 2002, Silva et al., 2011 & Richter et al., 2015).

In order to monitor the influence of the salts accumulation in the root volume, this variable was too measured and a decrease of the root volume in response to higher levels of salinity was observed (Table 1), as reported by Borzouei et al. (2012). The roots from maize plants treated with 150 mM of NaCl displaced approximately 20.16 mL of water, water roots; however, other authors have content 37.22% lower than the control plants (54.16 mL).

Salt stress also inhibits plant growth by reducing the height (Figure 1). Until 14 days of exposure, there was no significant difference between treatments. However, after 21 days of exposure, the plants exposed to 150 mM of NaCl showed lowest shoot length. Concentrations \geq 100 mM of NaCl were sufficient to cause a 30.62% reduction in plant height salt treated at 28 days of exposure.

Figure 1 - Effect of treatment with different concentrations of NaCl on the height (cm) of AL Bandeirantes maize cultivar. Significant differences ($p \leq 0.05$) between treatments are indicated by different letters, according to Tukey's test.



Conus et al. (2009) obtained similar results, reporting a decrease in shoot length from maize

plants exposed to increasing concentration saline using different salts. Gomes et al. (2011) studied

the negative effects of salt stress on maize hybrid (AG 1051) and found that, the height of the plants irrigated with saline water was lower than plants irrigated without salt. It was hypothesized that the suppression of development in response to salt stress was related to the restriction of the synthesis of plants growth promoters such as cytokinins and the increase in the production of the inhibitors such as abscisic acid (De Costa et al., 2007 & Rady & Hemida, 2016). Thus, the salt affects the plant growth directly through its interaction with metabolic rates and pathways within the plants (Rahimi et al., 2012 & Farooq et al., 2015).

The RWC did not differ statistically among the treatments evaluated here (Table 2), indicating that the accumulation of soluble compounds responsible for osmotic adjustment has prevented the significant variation of the RWC. This response of the AL Bandeirantes cultivar may possibly indicate an adaptive mechanism in which the stability of the RWC under salt stress reflects the accumulation of proline in the leaves, which allowed osmoregulation and the maintenance of hydration, as observed by Ragagnin et al. (2014).

Table 2 - Effect of treatment with different concentrations of NaCl on the relative water content (RWC) of AL Bandeirantes maize cultivar.

NaCl concentration	Days of exposure to salt stress		
	14	21	28
	RWC (%)		
0 mM	86.11 a	81.32 a	80.14 a
50 mM	85.83 a	78.93 a	88.57 a
100 mM	80.51 a	80.19 a	89.52 a
150 mM	86.76 a	82.83 a	75.01 a
CV (%)	5.34	3.00	8.74

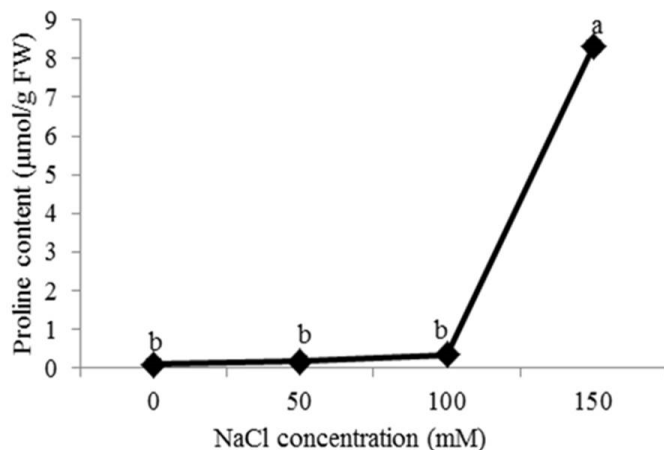
Means followed by the same letter in each column are not statistically different ($p \leq 0.05$), according to Tukey's test.

This is consistent with our results indicating that proline content was greatest in the leaves of plants exposed to 150 mM of NaCl (Figure 2). Under salt stress, plants accumulate several compatible solutes, such as polyols, betaine, trehalose, ectoine, proline and others (Farooq et al., 2015 & Rady & Hemida, 2016) in order to balance the water potential of the cytosol with the apoplast and vacuolar lumen (Szabados & Savoure, 2010). Besides regulation of osmotic pressure, proline has been shown to stabilize proteins and membranes, protecting plant cells against free radical-induced damage (Hayat et al., 2012).

Tissue accumulation of proline with 150 mM

of NaCl may result mainly from: (1) de-novo biosynthesis or/and activation of enzymes involved with proline synthesis (Dobrá et al., 2011 & Huang et al., 2013); (2) depression of proline degradation (Huang et al., 2013); (3) mobilization of stored carbohydrates to supply carbon skeletons used in the proline production (Monreal et al., 2007). Independent of the reported mechanisms, the relationship between proline accumulation and salt treatment in the AL Bandeirantes cultivar could indicate a trait of tolerance to osmotic stress, thought to be important for plant cellular homeostasis (Kazama et al., 2014 & Richter et al., 2015).

Figure 2 - Effect of treatment with different concentrations of NaCl on the proline accumulation of AL Bandeirantes maize cultivar, after 28 days of exposure to salt stress. Significant differences ($p \leq 0.05$) between treatments are indicated by different letters, according to Tukey's test.



Conclusion

The results demonstrate that, under the conditions evaluated in this studied, salinity does not affect the survival of AL Bandeirantes cultivar, since the maize seedlings remained viable throughout the experiment. Traits of salt tolerance can be attributed to the stability of the RWC and plant height, which was reduced only after 21 days of exposure. Addition, free proline is accumulated in leaves as a possible protective metabolic adaptation to prevent leaf tissue from damage under salinity. Thus, our experiments indicate that the limit salinity tolerance of AL Bandeirantes cultivar is high, keeping their development, even when subjected to the 150 mM of NaCl concentration for a time period of 28 days of exposure.

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