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‘Doce do Havaí’ Maize Cultivar Presents Tolerance to Saline Stress in Hydroponic Cultivation

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Abstract

This study evaluated the effects of salinity on the development, growth and biomass production of two sweet maize genotypes (‘Tropical Plus®’ and ‘Doce do Havaí’) and compared the oxidative stress marker responses of plant tissues from roots and leaves of different seedlings submitted to different amounts of NaCl in a nutrient solution. The experiment was carried out in a complete random design, in a 2x4 factorial arrangement (two sweet maize genotypes and four salt concentrations: 0, 50, 100 and 150 mM NaCl). Previously, the seeds were distributed in Styrofoam trays containing commercial substrate and, 10 days after sowing, the seedlings were transferred to 2-litter plastic pots, containing nutrient solution without NaCl addition, where they were kept for 8 days. NaCl was added to the nutrient solution, according to the treatments. Each treatment consisted of four pots containing six plants each. The plants were kept in B.O.D. at 25 ℃ with 18/6 light for 14 days. The nutrient solution with NaCl addition was changed every 7 days until the end of the experiment. Saline stress reduced root (13% and 29% for Hawaiian and ‘Tropical Plus®’, respectively) and shoot length (36% for ‘Doce do Havaí’ and 48% for ‘Tropical Plus®’), fresh shoot (29% for ‘Doce do Havaí’ genotype, and 70% for ‘Tropical Plus®’) and root mass (18% and 38% for ‘Doce do Havaí’ and ‘Tropical Plus®’, respectively), shoot diameter (18% and 20% for ‘Doce do Havaí’ and ‘Tropical Plus®’, respectively) and chlorophyll content in both genotypes, with results more significative in ‘Tropical Plus®’ hybrid seedlings. However, the concentrations of proline and malondialdehyde in roots and leaves, as well as conductivity, increased in response to the addition of NaCl, mainly in ‘Doce do Havaí’. These results suggest that the ‘Doce do Havaí’ genotype is more tolerant to salinity compared to ‘Tropical Plus®’ hybrid, and may be indicated for breeding programs aiming to develop saline tolerant plants.

Keywords: *Zea mays* L., NaCl, salinity, nutrient solution

1. Introduction

Sweet maize has been developed from a common maize natural mutation caused by mutant recessive genes that are responsible for alterations in the endosperm composition, making it highly nutritious and sweet (Tracy, 2001). Botanically classified as *Zea mays* L. *saccharata* Sturt, this product is exclusively utilized “*in natura*” or processed for human consumption (Oliveira Junior et al., 2006). Its total production is nearly all directed to the canning industry (Abreu de Jesus et al., 2016).

In Brazil, sweet maize is not a very popular crop. Its cultivation occurs in approximately 36 thousand acres and 90% of it is concentrated in the state of Goiás (Barbieri, 2010). Moreover, its productivity is 28% lower than the one reached by countries with temperate weather such as the USA and Canada. However, there is a potential to improve these rates since it is a product with great aggregated value and that has been introduced to big consuming markets (Bordallo et al., 2005).

One of the reasons of the lack of sweet maize popularity is farmers’ unfamiliarity with the crop, high production cost especially for the acquisition and treatment of seeds, great expenses with manual operations, and lack of available cultivar options in the market (Abreu de Jesus et al., 2016). However, the interest in sweet maize has been growing, making seed companies keep plant breeding programs, and consequently generating commercial hybrids with high productivity and grain yield (Luz et al, 2014).

Despite the advances observed in sweet maize cultivation, more studies are needed to evaluate other essential characteristics for the industry and farmers such as corncob shape, its behavior after industrial processing, seed quality, tolerance to plagues and diseases, and aspects that interest the consumer like color, taste and texture (Luz et al, 2014). Moreover, factors such as saline stress should also be studied because they influence the growth and productivity of crops in general worldwide (Islã and Araguês, 2010).

Soil salinization is a typical process of arid and semiarid regions due to low rainfall, high evaporation rate, and low natural draining capacity, making non-leaching salts accumulate at harmful levels to plant growth (Soares Filho et al., 2016). The exploitation of natural resources utilizing inappropriate techniques contributes to worsen this problem. An increase in salt concentration can cause farming land abandonment in few years; the great amounts of salt in the soil cause alterations in the soil chemical characteristics, retarding or preventing plant growth, due to the increase of its osmotic potential and indirect toxicity by specific elements (Pedrotti et at., 2015).

Maize is classified as moderately sensitive to salinity, with a threshold salinity of 1.1 dS m^{-1} and 1.7 dS m^{-1} (Ayers and Westcot, 1999), however, salinity tolerance varies between in species and genotypes, the effect of saline stress is dependent on factors such as developmental stages, environmental factors, cultivar, salt type, intensity and duration of salt stress, crop and irrigation management and edaphoclimatic conditions. Therefor it is recommended that all essential nutrients be applied in appropriate amounts in order to evaluate genotypes regarding their tolerance to salinity. Therefore, the utilization of nutrient solutions, applied through hydroponic systems, allows a control of slat balance in the solution as well as the available nutrient contents to plants (Soares Filho et al., 2016). Thus, this study aimed to evaluate saline stress in the growth parameters and oxidative stress markers of leaves and roots of ‘Doce do Havaí’ and ‘Tropical Plus®’ genotypes cultivated in nutrient solutions.

2. Materials and Methods

2.1 Plant Material and Salinization of the Cultivation Medium

The experiment was carried out in Campus III and the Main Campus of Paranaense University – UNIPAR, in the city of Umuarama, PR. The plant material consisted of two sweet maize (*Zea mays* L. *saccharata* Sturt) genotypes: ‘Doce do Havaí’ and ‘Tropical Plus®’, a commercial hybrid. None of the evaluated materials have been reported regarding their tolerance to abiotic stresses. The experiment had a 2 x 4 (genotypes x NaCl concentrations) complete random design with 3 replications per treatment: 0, 50, 100, and 150 mM of NaCl.

Previously, seeds of two genotypes were distributed in Styrofoam trays containing commercial substrate. Ten days after seeding, the seedlings were transferred to plastic pots (12 x 15.50 x 11cm), each one containing 2 L of whole Hoagland nutrient solution (1950). After 7 days, the solution was substituted for half Hoagland solution with or without addition of NaCl according to the treatments, and the seedlings were kept under these conditions for 14 days. Each one of the pots had six seedlings of each genotype, totaling 24 plants per treatment.

Each pot consisted of an experimental unit that was kept in a B.O.D. incubator, regulated at a temperature of 25 ℃, a photoperiod of 18/6h light, daily ventilation for 30 min and luminosity of 126.97 µmol m^{-2} s^{-1}.

2.2 Growth Parameters

The following parameters were evaluated 31 days after sowing in three plants per treatments: shoot (cm) and root (cm) lengths, fresh shoot mass (g), fresh root mass (g) and shoot diameter (cm), indirect measurement of chlorophyll rates, malondialdehyde concentration (nmol MDA g^{-1}FM), and proline concentration (µg proline g^{-1} FM).

The plant height and root length were measured with a 150-mm universal digital pachymeter. The shoot base until the last visible leaf was measured to estimate the shoot length. The fresh shoot mass was weighed in a semi-analytical scale. The shoot diameter was also determined with a digital pachymeter, measuring the medial part of the stem.

2.3 Chlorophyll Index

The chlorophyll index was determined in the area units through a portable chlorophyll meter SPAD-502 (Soil and Plant Analysis Development - Minolta Co), through two readings in each of the evaluated plants. The index (µg cm^{-2}) was obtained from the average of both values according to the recommendations by Amarante et al. (2010).

2.4 Oxidative Stress Markers

2.4.1 Malondialdehyde

The lipid peroxidation level was determined according to Cakmak and Hosrt (1991) by estimating malondialdehyde (MDA) content. The lipid peroxidation was estimated as the total content of reactive (Thiobarbituric Acid) TBA substances and expressed as MDA equivalents. The MDA molar extinction coefficient (155 mmol cm^{-1}) was utilized for calculations.

2.4.2 Membrane Damage

The membrane damage percentage (%MD), estimated from electrolyte leakage, was determined from five 3-cm diameter leaf disks immersed in distilled water. The disks were kept in assay tubes under agitation during 24h at 28 ℃. Next, the electric conductivity reading of the solution (L_1) was done. Afterwards, the tubes were incubated in water bath for 1h at 100 ℃, and after cooling at ambient temperature, another electric conductivity reading of the solution (L_2) was done. The % MD was estimated by the equation MD = $(L_1/L_2)*100$ (Blum and Ebercon, 1981; Silveira et al., 2001).

2.4.3 Proline Content

The proline concentration was determined according to the methodology by Bates et al. (1973). The proline concentration was determined using a standard L-proline curve (Synth).

2.5 Data Analysis

After treatment, the data were submitted to analysis of normality using Shapiro-Wilk's test. After confirming parametricity, they were evaluated by ANOVA at 5% of significance. When significant by F test, they were submitted to regression analysis for the concentration deployment in each genotype, and to Tukey's for the genotypes in each concentration at 5% probability. The analyses were done using the statistical program Sisvar 5.6 (Ferreira, 2011).

3. Results

3.1 Growth Parameters and Biomass Production

Salinity reduced shoot length (SL). At the concentration of 150 mM, there was a reduction of 36% and 48% in 'Doce do Havaí' and 'Tropical Plus®' seedlings, respectively. The effect of salt addition on SL was significant to 'Doce do Havaí' as well as 'Tropical Plus®', with an average difference of 18 and 35% ($p \leq 0.05$), respectively at the concentrations of 50 and 100 mM (Table 1). The relation between the salt concentration in the cultivation medium and SL in both varieties was shown by a decreasing linear equation (Table 1). Thus, it was evident that the addition of NaCl in the cultivation medium reduced the height of 'Doce do Havaí' and, mainly, of 'Tropical Plus®'.

Table 1. Shoot length and Root length (cm), Fresh shoot mass and Freshh root mass (g) and Shoot diameter (cm) of *Zea mays* L. *saccharata* genotypes, 'Doce do Havaí' and 'Tropical Plus®', cultivated in nutrient solution at different NaCl concentrations

Treatments	NaCl concentration (mM)					
	[0]	[50]	[100]	[150]	Regression Equation	R^2 (%)
	Shoot length - SL (cm)					
'Doce do Havaí'	52.5*a	50.16a	41.00a	33.50a	$y = -0.132333x + 54.2167$	95.51
'Tropical Plus®'	45.66a	37.33b	24.33b	23.66a	$y = -0.1580x + 44.600$	91.46
CV (%)	16.02	General average	38.52	± 11.64		
	Root length - RL (cm)					
'Doce do Havaí'	26.46a	30.46a	24.00a	23.00a	$y = -0.0005x^2 + 0.41267x + 27.263$	61.73
'Tropical Plus®'	22.33a	26.16a	16.33a	15.83a	$y = -0.00043x^2 + 0.0063x + 23.483$	64.34
CV (%)	20.53	General average	23.08	± 6.199		
	Fresh shoot mass - FSM (g)					
'Doce do Havaí'	4.00a	4.09a	2.83a	3.32a	$y = -0.006593x + 4.05533$	51.26
'Tropical Plus®'	3.44a	2.69a	1.19a	1.04b	$y = -0.017395x + 3.398167$	92.49
CV (%)	37.37	General average	2.83	± 1.417		
	Fresh root mass - FRM (g)					
'Doce do Havaí'	1.95a	1.7a	1.59a	3.40a	$y = 0.000205x^2 - 0.022307x + 2.0347$	92.66
'Tropical Plus®'	1.23a	0.73b	0.76b	0.81b	$y = 0.000055x^2 - 0.0108 + 1.20795$	92.33
CV (%)	31.66	General average	1.52	± 0.94		
	Shoot diameter - SD (cm)					
'Doce do Havaí'	4.54a	4.09a	3.67a	6.09a	$y = 0.000285x^2 - 0.034237x + 4.67483$	88.49
'Tropical Plus®'	3.63a	3.39a	2.90a	3.34b	$y = 0.000068x^2 - 0.01288x + 3.686$	74.47
CV (%)	20.16	General average	3.96	± 1.159		

*Averages followed by the same letters in the column did not differ statistically among themselves by Tukey's test at 5% of probability. CV: Coefficient of Variation.

Salinity also reduced root length (RL). The decrease in seedlings cultivated at 150 mM of NaCl was 13% for 'Doce do Havaí', and 29% for 'Tropical Plus®'. In the genotype comparison (Table 1), the effect of the saline stress on the averages was not significant ($p>0.05$). The regression model for both genotypes was quadratic (Table 1). Therefore, the quadratic equation confirmed the analysis of variance and showed that a greater effect of saline stress on RL occurred for 'Tropical Plus®' hybrid.

The saline stress reduced fresh shoot mass (FSM) of seedlings up to 29% for 'Doce do Havaí' genotype, and 70% for 'Tropical Plus®'. Between the genotypes, the effect of NaCl addition on FSM was significant ($p \leq 0.05$) at the concentration of 150 mM of NaCl (Table 1). The relation between the salt concentration in the cultivation medium and FSM inof both varieties was demonstrated by a decreasing linear equation (Table 1), making evident that the addition of NaCl in the cultivation medium reduced the fresh shoot mass of 'Doce do Havaí' and, mainly, of 'Tropical Plus®'.

Regarding fresh root mass (FRM) and shoot diameter (SD), the responses to saline stress in general were similar and statistically significant ($p \leq 0.05$) for both parameters. However, they were different for each genotype. In 'Doce do Havaí' seedlings cultivated at the concentration of 100 mM of NaCl, there was a reduction of 18% in FRM in well as SD. In 'Tropical Plus®' seedlings, the decrease was 38% in FRM and 20% in SD. SD presented statistically significant difference ($p \leq 0.05$) between the seedlings genotypes cultivated at 150 mM of NaCl. FRM significantly differed ($p \leq 0.05$) from one genotype to another in all treatments, except for the control (Table 1).

3.2 Determination of Chlorophyll Index

The chlorophyll content was reduced up to 26% in Hawaiian seedlings and up to 18% in Tropical Plus when cultivated at 150 mM of NaCl, but the effect of NaCl addition on the chlorophyll content was not significant ($p > 0.05$) for both genotypes (Table 2). There was statistical difference ($p \leq 0.05$) of the salinity content effect on the chlorophyll index alone at all concentrations. The quadratic regression model better adjusted to the salt effects on chlorophyll concentration for both genotypes (Figure 1A). Thus, it was evident that NaCl addition to the cultivation medium reduced chlorophyll content in both genotypes, mainly in 'Tropical Plus®'.

Table 2. Chlorophyll content, malondialdehyde concentration in roots and leaves, proline concentration and conductivity of *Z. mays saccharata* seedlings cv. 'Doce do Havaí' and *Z. mays saccharata* cv. 'Tropical Plus®', cultivated at different NaCl concentrations

Treatments	NaCl concentrations (mM)					
	[0]	[50]	[100]	[150]	Regression Equation	R^2 (%)
	Chlorophyll content					
'Doce do Havaí'	24.40a	25.75a	23.82a	18.00a	$y = -0.725x^2 + 3.005x + 22.517$	56.12
'Tropical Plus®'	28.56a	26.82a	24.52a	23.33a	$y = -0.925x^2 + 1.3317x + 27.817$	96.07
CV (%)	14.39	General average	24.4	±4.08		
	Malondialdehyde concentration in root (nmol MDA g^{-1} FM)					
'Doce do Havaí'	2.36a	3.97a	5.82a	3.44a	$y = -0.000399x^2 + 0.070003x + 2.140167$	84.1
'Tropical Plus®'	1.60a	4.02a	5.23a	1.78a	$y = -0.000586x^2 + 0.09135x + 1.430833$	93.62
CV (%)	33.45	General average	3.96	± 1.159		
	Malondialdehyde concentration in leaf (nmol MDA g^{-1} FM)					
'Doce do Havaí'	5.47a	7.08a	4.43a	5.78a	$y = -0.000026x^2 + 0.000377x + 5.887167$	4.66
'Tropical Plus®'	8.60a	8.26a	8.94b	10.96b	$y = 0.000237x^2 - 0.0199127x + 8.613667$	99.87
CV (%)	34.02	General average	7.44	± 2.949		
	Proline concentration (μg proline g^{-1} FM)					
'Doce do Havaí'	6.09a	6.10a	6.13a	6.15a	$y = 0.000425x + 6.0843$	97.88
'Tropical Plus®'	6.08b	6.09b	6.11b	6.14a	$y = 0.000425x + 6.072667$	96.92
CV (%)	0.08	General average	6.11	± 0.025		
	Membrane damage (%)					
'Doce do Havaí'	25.59a	17.02a	29.21a	92.48a	$y = 0.0065x^2 – 0.5646x + 28.1135$	96,15
'Tropical Plus®'	16.26a	23.74a	46.45a	99.27a	$y = 0.0052x^2 – 0.2240x + 15.9935$	99,97
CV (%)	26.58	General average	43.75	± 33.48		

* Averages followed by the same letters in the column do not differ statistically by Tukey's test at 5% level of significance. CV: coefficient of variation. Source: Research data.

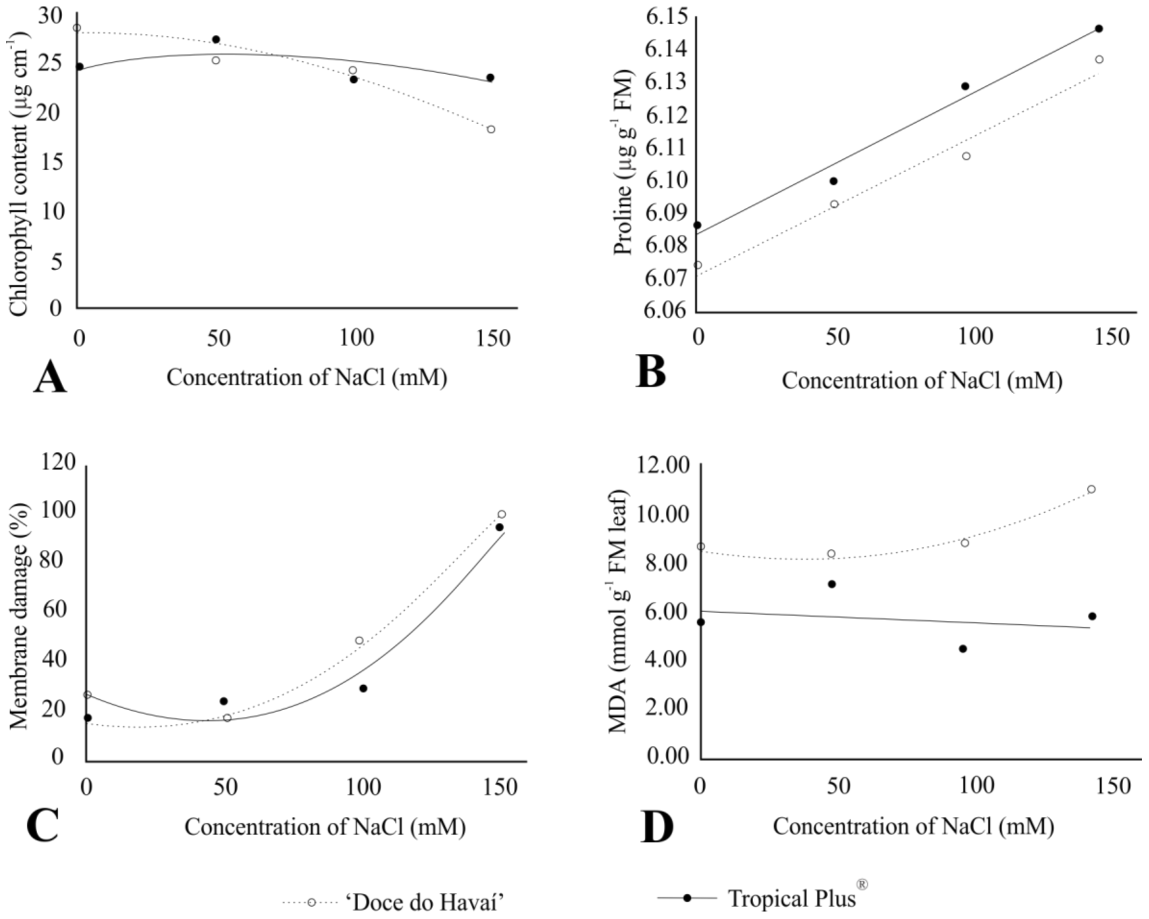

Figure 1. Regression analysis for chlorophyll content (A), proline concentration (B), membrane damage (C) and malondialdehyde concentration in leaves (D) in *Z. mayssaccharata* seedling cv. 'Doce do Havaí' and *Z. mays saccharata* cv. 'Tropical Plus®', cultivated under different NaCl concentrations

3.3 Oxidative Stress

3.3.1 Malondialdehyde (MDA) Concentration

Salinity caused an increase in malondialdehyde (MDA) concentration in roots and leaves of 'Doce do Havaí' seedlings as well as of 'Tropical Plus®' ones. The effect was greater in the leaves of the former when compared to the latter and, the opposite occurred for the roots. However, statistically, the effect of salt addition on MDA/root parameter did not present any significant difference ($p>0.05$) in both genotypes (Table 2), that is, they responded in the same way to salinization. Regarding the treatment effect on the cultivars, the treatment with 100 mM of NaCl triplicated MDA amount in 'Tropical Plus®' seedlings and increased 1.5 fold in 'Doce do Havaí'. The regression model was quadratic for both genotypes (Table 2). Therefore, NaCl addition to the cultivation medium increased malondialdehyde content in roots of both genotypes, mainly in 'Tropical Plus®' at the concentration of 100 mM.

Salinity increased MDA leaf content in 29% for 'Doce do Havaí' genotype at the concentration of 50 mM of NaCl, and in 27% for 'Tropical Plus®' seedlings at the concentration of 150 mM. Regarding the salt addition effect on MDA amount in leaves, the result was significant ($p \leq 0.05$) for the concentrations of 100 and 150 mM of NaCl (Table 2). The regression model was quadratic for both genotypes (Figure 1D). Comparing both genotypes, both responded to salinity similarly, that is, without significant difference ($p \leq 0.05$). However, the negative values of the quadratic regression coefficient for 'Doce do Havaí' indicate that this plant is more tolerant to the effects of saline stress on the lipid peroxidation of leaf tissues compared with 'Tropical Plus®' hybrid.

3.3.2 Proline Concentration

The increase in salt concentration resulted in significant proline content increment ($p \leq 0.05$) in the seedlings of 'Doce do Havaí' variety as well as for 'Tropical Plus®' hybrid. However, the analysis of variance of the averages showed that there was a statistical difference ($p \leq 0.05$) between the genotypes, except at the concentration of 150 mM of NaCl (Table 2). The relation between salt concentration in the cultivation medium and proline concentration in both varieties was demonstrated by a linear equation whose positive coefficient equals to 0.00042 for 'Doce do Havaí' as well as for 'Tropical Plus®' (Figure 1B). Therefore, salinity increased proline concentration in both cultivars.

3.3.3 Membrane Damage

NaCl addition increased conductivity of both sweet maize genotypes seedlings without statistical difference between them ($p \leq 0.05$) for all concentrations (Table 2). However, at the concentration of 150 mM of NaCl, salinity increased conductivity 3.15 fold in 'Doce do Havaí' and 4.18 fold in 'Tropical Plus®', when compared to the concentration of 100 mM. The regression model for both genotypes was quadratic (Figure 1C). Therefore, it was verified that for both genotypes and for the highest salt concentration (150 mM), the membrane damage leakage had exponential increase compared to lower concentrations.

4. Discussion

Munns and Tester (2008) stated that salinity reduces plant growth and productivity. This occurs because the presence of salt affects water availability and reduces water potential in a plant, causing turgor loss and stoma closing. Plants exposed to saline stress decrease their energy expenditure destined to osmoregulation as an adaptive response to the salt presence, aiming to save energy to keep their vital processes (Mansour et al., 2005). These physiological alterations reduce the development and productivity of plants as verified in this study.

The shoot length reduction of 36% for 'Doce do Havaí' and 48% for 'Tropical Plus®' is similar to the results obtained by Shtereva et al. (2015), who observed a reduction of 32% and 31% in the height of pure strains of *Line 6-13 and Line C-6* sweet maize cultivated with addition of 150 mM of NaCl for 14 days.

The height reduction of plants exposed to saline stress can be explained by the decrease of growth-promoting hormone synthesis (such as auxins and gibberellins) and by the increase in the production of growth-inhibiting substances (Costa et al., 2007; Rady and Hemida, 2016).

This occurs because salt alters rates and metabolic pathways of plants, affecting their development (Rahimi et al., 2012; Farooq et al., 2015). Regarding that, Taiz and Zeiger (2009) stated that height is one of the most affected parameters by salinity because the osmotic pressure of the medium decreases water availability to the plant, harming cell division and elongation and, therefore, limiting plant growth.

The shoot length reduction of 29% for ‘Doce do Havaí’, observed in this study, is in accordance to the results by Shtereva et al. (2015) who verified a decrease of 25 and 24% in FSM of *Line 6-13* sweet maize pure strain and *Zaharina* hybrid, cultivated in nutrient solution with addition of 150 mM of NaCl for 14 days. However, the decrease of 70% in FSM of ‘Tropical Plus®’ makes evident that this hybrid is more sensitive to salinity. Nevertheless, similar results under similar cultivation conditions were not found in the literature.

Regarding the effect of salinity on roots, it was observed that the reduction of 13% and 29% for root length of Hawaiian and ‘Tropical Plus®’, respectively. This is similar to the results obtained by Shtereva et al. (2015), who verified a decrease of 27%, 23% and 19%, respectively, in Line *6-13*, *Line C-6* and *Zaharina* RL, cultivated with the addition of 150 mM of NaCl for 14 days. In that same experiment, a reduction of 39% and 43% occurred in *Line C-6* and *Zaharina* FRM, respectively. This result is similar to the reduction of 38% in ‘Tropical Plus®’ FRM, verified in this study. The reduction of only 18% for ‘Doce do Havaí’ FSM was smaller when compared to the studies on saline stress in sweet maize (like it had occurred in RL), suggesting that this genotype is tolerant to saline stress considering that the decrease was smaller than the values verified by Shtereva et al. (2015) in *Zaharina*, a commercial sweet maize hybrid considered tolerant to salt.

The smallest percentage reduction of RL and FRM when compared to SL and FSM, verified in our study (Table 1), corroborated the results by Munns and Tester (2008), Silva et al., (2011) and Richter et al., (2015), who observed greater reduction shoot growth and productivity rates of plants exposed to saline stress when compared to roots. This explains the fact that roots react more quickly to salinity effects (Azevedo-Neto and Tabosa, 2000; Hsiao e Xu, 2000), balancing water relations in cells and gradually increasing growth rate that, after the osmotic adjustment, becomes stable at lower levels due to stress (Munns, 2002).

Regarding the shoot diameter reduction of 18% and 20% for ‘Doce do Havaí’ and ‘Tropical Plus®’ seedlings, respectively (Table 1), Souza et al. (2014) verified a decrease of 6.4% in plant shoot diameter. This was observed when those authors studied the saline effects on Super sweet Aruba (Feltrim®) sweet maize cultivated in soil irrigated with water with low and high salinity (0.5 and 4.5 dS m^{-1} of NaCl). Morales et al. (2001) state that salinity affects each plant part differently, and that the adaptation to the saline medium is different for each species or even to the same variety when at different phenological stages.

There are studies that demonstrate the growth and productivity reduction of plants exposed to saline stress (Mansour et al., 2005). However, as it can be observed in this study, when submitted to this type of stress, different genotypes respond distinctly because the response is a plant reaction to an adverse situation and varies according to the genetic factors that regulate the cellular metabolism (Shtereva et al., 2015).

Decreases in nutrient accumulation in several plant parts have been justified by the effect of NaCl excess on the medium that competes for absorption sites in roots like it occurs with phosphorus, potassium, calcium and magnesium in maize (Sousa et al., 2010). These reductions, however, are not homogeneous, not even in plants of the same species. Plants that synthesize and moderately accumulate a variety of solutes with osmoprotective action of macromolecules show a marginal increase in stress tolerance (Willadino and Camara, 2010).

Proline can attenuate saline stress in young plants. Studies by Hajlaoui et al. (2010) showed that free proline content was significantly increased in plants submitted to stress when compared to control plants of Arper and Aristo maize in all treatments. Free proline concentration showed significant differences between both genotypes in young, mature and senescent leaves. At the concentration of 102 mmol L^{-1} of NaCl, the average leaf proline content for young, mature and senescent leaves in Arper was 38, 25 and 14 µmol g^{-1} FM, respectively. In Artiso, a slight reduction of leaf proline was observed with values of 32, 22, and 13 µmol g^{-1} FM. At the concentration of 100 mmol L^{-1}, very close to 102 mmol L^{-1} mentioned before, proline content in ‘Doce do Havaí’ and ‘Tropical Plus®’ seedlings were, respectively, 6.13 and 6.11 µmol g^{-1} FM.

In studies by Hajlaoui et al. (2010) as well as in our study with ‘Doce do Havaí’ and ‘Tropical Plus®’, there was an increase in proline content according to the increase in the evaluated saline concentration. However, studies by Silva et al. (2009), utilizing species with different capacities to accumulate proline in tissues under stress, showed that the tested concentrations were not sufficiently high to cause a significant contribution to the cellular osmotic potential or even to the cellular protection.

Because of divergent results, which seemed contradictory, a better explanation is still needed for the fact that proline contributes to the resistance to saline stress or for the fact that its accumulation is merely related to a metabolic disorder symptom (Silveira et al., 2010).

A plant photosynthetic system is strongly influenced by the availability of factors such as mineral nutrients which can be observed when submitted to stress that affects its osmoregulation (Kaya et al., 2013) such as saline stress. Its deleterious effect results from the increase of chlorophilase enzyme that degrades chlorophyll molecules. The consequence of this is that plants that grow under salinity conditions have their photosynthetic activity damaged/compromised and as a result there is a growth reduction of a smaller leaf area and lower content of this pigment (Mendonça et al., 2010), as observed in ‘Tropical Plus®’ when compared to ‘Doce do Havaí’.

Results that corroborate the ones observed in this study were found by Lima et al. (2004) when studying rice cultivars submitted to saline stress. When evaluating three genotypes, two were possibly not affected by salinity. On the other hand, the third cultivar presented a decrease in chlorophyll content compared to the control, showing that salinity affected significantly ($p \leq 0.05$) the formation of this pigment at concentrations over 25 mM of NaCl, as it occurred with ‘Tropical Plus®’ genotype, indicating that it is more sensitive to saline stress than ‘Doce do Havaí’.

Lipid peroxidation of root and leaf tissue membrane, caused by free radicals, produces Malondialdehyde (MDA). These radicals are formed in stress situations such as the saline one, and affect many biological functions due to structural damages that they cause in the nucleic acid (Bhattacharjee, 2005) and proteins (Zhu et al., 2010). Results that make these effects evident were found by Hajlaoui et al. (2010) in an experimental study aiming to compare the phenolic content of both maize varieties submitted to different NaCl concentrations and determine more trustworthy leaf indicators to distinguish varieties that are sensitive and tolerant to salt.

Hajlaoui et al. (2010) showed that lipid peroxidation was influenced by saline stress and, increased leaf MDA content as observed in ‘Doce do Havaí’ and Troplical Plus®. The authors verified that the amount of MDA significantly and progressively increased with saline stress and that the highest values were recorded in seedlings treated with 102 mmol.L^{-1} of NaCl, which accumulated 45 and 55 nmol MDA.g^{-1} FM for both evaluated varieties (Arper and Aristo), respectively.

The capacity to tolerate saline stress was evident in the study by Santos et al. (2016), with AL Bandeirantes maize cultivar. In that study, the water content did not differ statistically, showing that there was not a variation due to the accumulation of soluble compounds responsible for osmotic adjustment. This response possibly indicates an adaptive mechanism in which the stability of water content under saline stress reflects the soluble compound accumulation in leaves, allowing osmoregulation and hydration maintenance as verified by Ragagnin et al. (2014).

Osmoregulation and hydration maintenance are possible because a plant can have several tolerance mechanism (high metabolic activity) and leakage (reduced metabolic activity) to stresses, or a combination of both when compared to another. The individual adjustment in response to these environmental factors consists of resistance mechanisms known as acclimatization (Bray et al., 2000) which is a combination of behavioral, morphological, anatomical, physiological and biochemical processes dependent of molecular processes (Gaspar et al., 2002). Therefore, new studies should be carried out with ‘Doce do Havaí’ cultivar to confirm its tolerance to NaCl stress.

5. Conclusion

Salinity reduced growth and development of sweet maize plants with different responses for ‘Doce do Havaí’ and ‘Tropical Plus®’ genotypes. The salt addition to the cultivation medium reduced the growth and biomass production as well as the chlorophyll content of seedlings, with smaller effects on ‘Doce do Havaí’; it also increased the conductivity and concentrations of malondialdehyde and proline with greater effects on ‘Tropical Plus®’ hybrid. These results pointed out that ‘Doce do Havaí’ is more tolerant to saline stress. However, it is recommended that further studies be carried out to investigate the responses of different sweet maize genotypes in salinized soil and/or irrigated with saline water.

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