

## Tolerance of Basil Genotypes to Salinity

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### Abstract

Basil (*Ocimum basilicum* L.) is a medicinal species of Lamiaceae family, popularly known for its multiple benefits and high levels of volatile compounds. The species is considered to be one of the most essential oil producing plants. Also cultivated in Brazil as a condiment plant in home gardens. The objective of this study was to evaluate the effect of salinity on the growth of basil in nutrient solution of Furlani and to identify variables related to the salinity tolerance in this species. The first assay was performed with variation of five saline levels (0 - control, 20, 40, 60 and 80 mM NaCl). In the second assay six genotypes were evaluated in two salinity levels 0 and 80 mM NaCl. The height, stem diameter, number of leaves, dry mass and inorganic solutes in different organs, photosynthetic pigments, absolute membrane integrity and relative water content were evaluated. All biometric variables in basil were significantly reduced by salinity. Dry matter yield and percentage of membrane integrity were the variables that best discriminated the characteristics of salinity tolerance among the studied basil genotypes. Basil genotypes showed a differentiated tolerance among the genotypes, the 'Toscana folha de alface' being considered as the most tolerant and 'Gennaro de menta' as the most sensitive, among the species studied.

**Keywords:** biomass, saline stress, membrane integrity, *Ocimum basilicum* L.

### 1. Introduction

Basil (*Ocimum basilicum* L.) is a plant of the family Lamiaceae, a producer of essential oils of pharmaceutical importance, food, perfumery and cosmetics. It is also widely used in traditional medicine (Bharti, Barnawal, Wasnik, Tewari, & Kalra, 2016). The species is cultivated on a commercial scale in Asia, Africa, South America and the Mediterranean region, under natural conditions or in protected cultivation. The cultivation in greenhouses has the advantages of maximizing the yield and allowing a constant supply of material throughout the year (Sgherri, Cecconami, Pinzino, Navari-Izzo, & Izzo, 2010).

The use of brackish water in irrigation can result in soil salinization and compromise plant growth. The productivity of the crops in a salinized environment depends on the amount of soluble salts and the capacity of the plants to tolerate saline stress (Cova, Azevedo Neto, Ribas, Gheyi, & Menezes, 2016). The reduction of the water potential in the culture medium, due to the higher concentration of soluble salts, affects the water absorption and, consequently, the turgescence and the cellular expansion. Moreover, saline stress also leads to a reduction of photosynthesis by the closure of the stomata and, therefore, limits the absorption of carbon dioxide and, consequently, the reduction of growth and productivity occurs (Farooq, Wahid, Kobayashi, Fujita, & Basra, 2009).

In saline soils the main ions found are Na<sup>+</sup> and Cl<sup>-</sup>, which when absorbed and accumulated in excess may contribute to osmotic adjustment or become toxic (Flowers, Munns, & Colmer, 2015). Thus, the ionic

homeostasis between  $K^+$  and  $Na^+$  is fundamental for the regulation of the cellular osmotic potential (Zhu, 2003) and to avoid the deleterious effects of saline stress.

The integrity of cell membranes, enzymatic activity and photosynthesis are metabolic and physiological indicative functions of tolerance variation in the species as they are sensitive to salinity. In order to evaluate the tolerance of plants to salinity, growth is considered to be an effective measure as it integrates a set of physiological mechanisms that occur in the plant (Niknam & McComb, 2000). Tolerant plants may have ionic compartmentation mechanisms, while in sensitive plants this mechanism is not efficient. Plant responses to saline stress are complex and may vary between cultivars of the same species (Maas & Hoffman, 1977; Niknam & McComb, 2000). The genotypic variability may provide a differential tolerance to saline stress between plants from the same species, as evidenced by several authors (Azevedo Neto, Pereira, Costa, & Santos, 2011).

Knowing that basil is a very important crop, the objective of the present study was to evaluate the tolerance of six basil genotypes to saline stress and to classify them according to the degree of tolerance to stress.

## 2. Material and Methods

The study was conducted in a protected environment at the Federal Universidade do Recôncavo da Bahia, Centro de Ciências Exatas e Tecnológica, Cruz das Almas-BA (12°40'19" S 39°6'23" W), from January to March 2015. Two experiments were carried out: the first one was a completely randomized design with five saline levels and four replicates and the second one was a randomized block design with two saline levels and six genotypes with four replicates.

### 2.1 Assay I: Experimental Conditions and Treatments

Basil seeds var. *Alfavaca basilicão*, obtained at ISLA Sementes Ltda. were seeded in 150 mL plastic cups containing washed sand. At 21 days after emergence (DAE), when the seedlings presented a completely expanded pair of leaves, they were transferred to containers in a hydroponic "Floating" type system with aeration, containing 12 L of nutrient solution of Furlani (1998). After four days under these conditions, the seedlings received their respective saline treatments 0, 20, 40, 60 or 80 mM NaCl, corresponding to electrical conductivities of the nutrient solution of 2, 4, 6, 8 and 10 dS m<sup>-1</sup>. NaCl was gradually added (20 mM day<sup>-1</sup>), to avoid osmotic shock. The volume of the solutions was completed daily with water and the pH maintained between 6.0 and 6.5 by the addition of HCl or NaOH. Plants were harvested 17 days after the treatments were applied.

#### 2.1.1 Biometry and Dry Mass Production

The height of the plants, stem diameter (SD) and number of leaves (NL) were determined at the harvest. The height was measured with a graduated ruler, the main branch was measured from 0.5 cm from the insertion of the root to the apex of the main branch. The SD was measured with a digital caliper and counted the NL. Afterwards, the plants were collected and separated in leaves, stems and roots and the plant material was transferred to an oven with forced air circulation at 65 °C for 72 h. After this period, the determination of dry masses of leaf (LDM), stem (SDM) and roots (RDM) was performed on a precision scale. From the dry mass data of the plant parts, shoot dry mass (SHDM) and total dry mass (TDM) were calculated.

#### 2.1.2 Analysis of Inorganic Solutes

For the determination of  $Na^+$ ,  $K^+$  and  $Cl^-$  contents in leaves, stems and roots, the extracts were prepared as described by Jones Júnior (2001), with minor modifications. In test tubes, 0.1 g of dried (in oven) and powdered material (in Willye-type knife mill) and 10 mL of deionized water were added. The test tubes were heated at 80 °C in a water bath for 1 h, being agitated every 15 min and then centrifuged at  $5.000 \times g$ . The supernatant was collected and stored at -20 °C for further analysis. The  $Na^+$  and  $K^+$  contents were determined by flame photometry (Faithfull, 2002) and the values of  $Cl^-$  by spectrophotometry (Jones Júnior, 2001).

### 2.2 Assay II: Treatment and Execution

In the second assay, seeds of genotypes 'Gennaro de Menta', 'Alfavaca basilicão vermelho', 'Alfavaca basilicão', 'Toscano folha de alface', 'Limocino' and 'Grecco a palla', obtained from the company ISLA Sementes Ltd., were used. The seedlings production and the cultivation system were identical to Assay I. The seedlings of each genotype were submitted to 0 and 80 mM of NaCl in nutrient solution of Furlani (1998), with electrical conductivities of 2 and 10 dS m<sup>-1</sup>, respectively. Addition of NaCl and control of nutrient solutions were also identical to those of Assay I. Plants were harvested after 20 days after the treatments were applied.

### 2.2.1 Dry Mass Production and Analysis of Inorganic Solutes

At the end of the experimental period, the plants were separated into leaves, stems and roots to evaluate dry mass and determination of  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  contents according to Experiment I.

### 2.2.2 Photosynthetic Pigments

To determine the levels of chlorophyll *a*, chlorophyll *b* and carotenoids (carotenes and xanthophyll), the samples were placed in 95% ethanol. Then, the spectrophotometric readings were performed at 649, 664 and 470 nm, according to the methodology described by Lichtenthaler and Buschmann (2001) and were calculated with Equations 1, 2 and 3, respectively:

$$\text{CHL}a \text{ (}\mu\text{g ml}^{-1}\text{)} = (13.36 \times A_{664} - 5.19 \times A_{649}) \quad (1)$$

$$\text{CHL}b \text{ (}\mu\text{g ml}^{-1}\text{)} = (27.43 \times A_{649} - 8.12 \times A_{664}) \quad (2)$$

$$\text{Car (}\mu\text{g ml}^{-1}\text{)} = (1000 \times A_{470} - 2.13 \times \text{CHL}a - 97.64 \times \text{CHL}b)/209 \quad (3)$$

### 2.2.3 Absolute Integrity Percentage (AIP) of Cell Membranes and Relative Water Content (RWC)

The AIP and RWC evaluations were performed on the third fully expanded leaf pair, for all genotypes except for 'Grecco a palla', where they were determined in the main branch, adapting the methodology to the morphology of this genotype. The determination of AIP was performed according to Pimentel, Sarr, Diouf, Aboud, and Roy-Macauley (2002), where 10 leaf discs with known area were placed in threaded tubes with 10 mL of deionized water. The tubes were placed for 24 h in a dark place and after that the electrical conductivity of the water (free conductivity- FC) was measured. Afterwards, the tubes were placed in a water bath at 100 °C for one hour and after returning to the ambient temperature the electrical conductivity of the water (total conductivity TC) was again measured. It was calculated using Equation 4:

$$\text{AIP (\%)} = 100 - (\text{FC} \times 100/\text{TC}) \quad (4)$$

The RWC was determined according to Barr and Watherley (1962), in which 10 leaf discs with known area were removed and weighed to obtain the fresh mass (FM). The disks were then placed in Petri dishes, immersed in deionized water and left for 24 hours in a refrigerator. After this period, the discs were wiped with paper towel and weighed to obtain the turgid mass (TM). Afterwards, they were taken to dry in an oven until constant weight was obtained. The RWC was calculated as described in Equation 5:

$$\text{RWC (\%)} = [(\text{DM} - \text{FM})/(\text{DM} - \text{TM})] \times 100 \quad (5)$$

### 2.2.4 Statistical Analysis

For the first assay, the results were submitted to analysis of variance (F test) and regression, using the Sisvar 4.6 statistical software (Ferreira, 2011). In the second assay, the results were submitted to analysis of variance (F-test) and the means compared by the Scott-Knott test at 0.05 probability.

## 3. Results and Discussion

### 3.1 Assay I

The variables plant height, stem diameter (SD) and number of leaves (NL) presented linear decreasing behavior with the increment of sodium chloride in the nutrient solution (Figure 1). Comparing the control treatment with the one using 80 mM NaCl, reductions of 37.31 and 27.5% were observed for height, SD and NL, respectively.

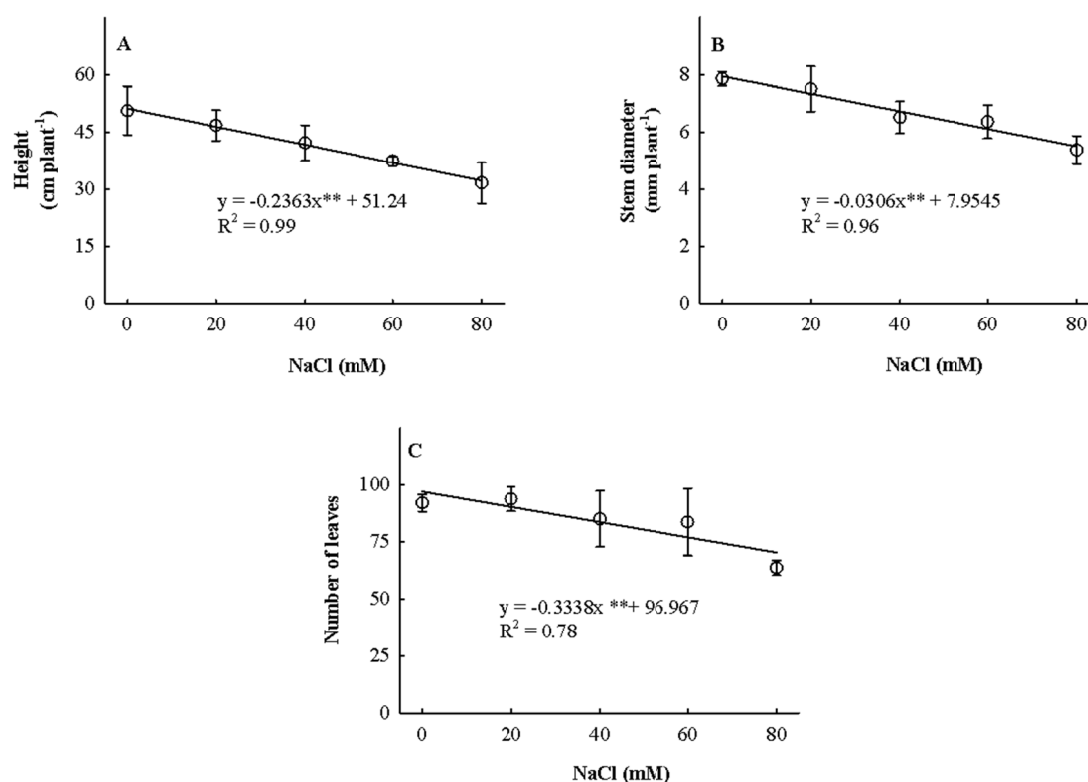


Figure 1. Height (A), stem diameter (B) and number of leaves (C) of basil plants 17 days after treatment in nutrient solution containing increasing levels of NaCl

Note. Values indicate means of four replicates and respective standard deviations.

The diameter of the stem was reduced from 7.95 to 5.51 mm. Stem diameter is an important variable in cultivation without soil, as it is responsible for the support of the aerial part thus, if too thin, it may result in plant lodging.

For NL the reduction was from 97 to 70 leaves per plant with increased salinity in the nutrient solution (Figure 1C). The leaves are reserve storage sites, sources of hormones and other plant compounds and it is the location for the photosynthetic apparatus. Therefore, a reduction in NL is related to a reduction in CO<sub>2</sub> absorption and biomass production.

Several factors may be associated with the reduction of plant growth when submitted to saline stress, such as the low osmotic potential of the solution, the interaction of specific ions, nutritional imbalances or a combination of these factors (Parida & Das, 2005; Marschner, 2012). The osmotic potential of saline environment reduces the ability of plants to absorb water, which affects the physiological processes and inhibits meristematic activity, elongation and cell division, which results in a rapid reduction in growth rate and biomass production (Ayers & Westcot, 1999; Munns, 2002; Parida & Das, 2005).

The salinity increase in the nutrient solution also linearly reduced the dry mass production in the different organs (Figure 2). The LDM and RDM showed reductions of 0.027 and 0.008 g per unit increase of mM NaCl, respectively. The SDM presented a mathematical adjustment to the quadratic model (Figure 2B), the estimated data for the minimum SDM was 0.25 g (80 mM NaCl) and the maximum was 2.08 g (0 mM NaCl). For SHDM and TDM this reduction was 0.044 and 0.051 g, respectively. Thus, the estimated reductions were 58, 88, 47, 56 and 55% for LDM, SDM, RDM, SHDM and TDM, respectively, indicating that the aerial part of the plant was more sensitive to salinity than the roots. As the reduction of dry mass production in the different parts of the plants were similar, SHDM/RDM values were not affected by salinity, presenting a mean value of approximately 4.5 (Figure 2E).

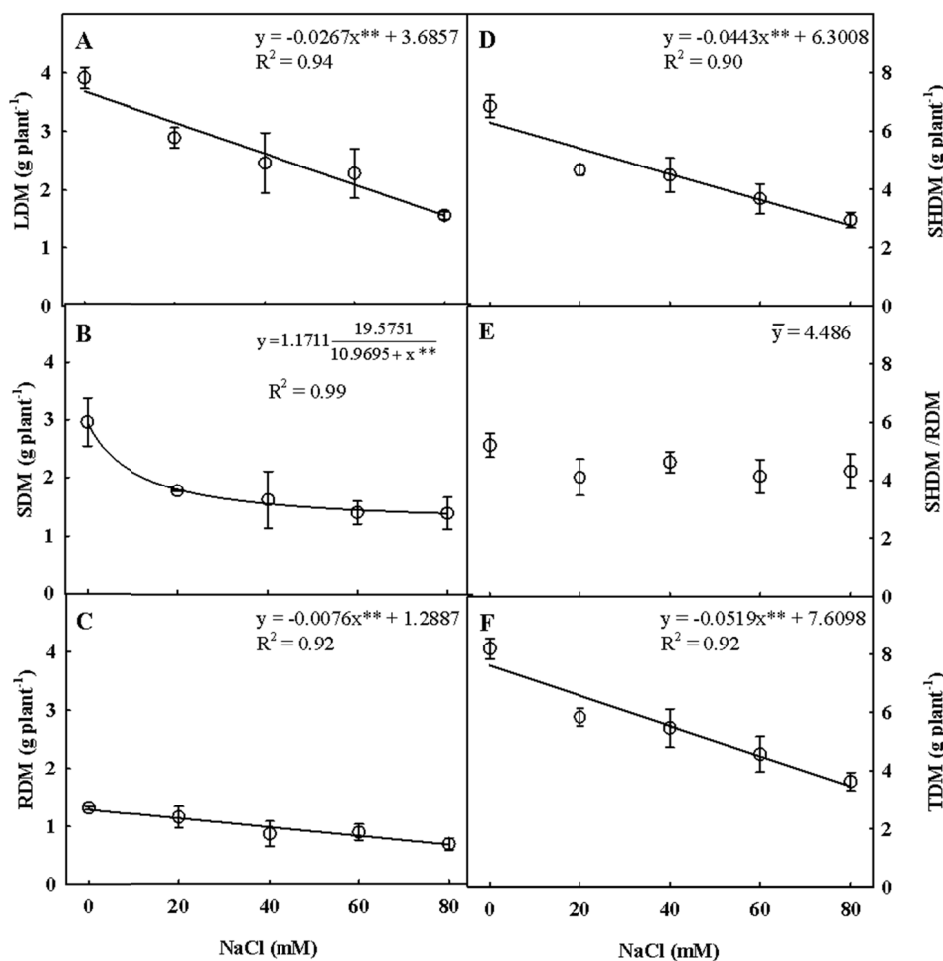


Figure 2. Leaf dry mass - LDM (A), stem dry mass - SDM (B), root dry mass - RDM (C), shoot dry mass - SHDM (F) and shoot dry mass ratio by root dry mass - SHDM/RDM (E), in basil plants 17 days after treatment in nutrient solution containing increasing levels of NaCl

Note. Values indicate means of four replicates and respective standard deviations.

Bione et al. (2014) in studies with basil, observed a reduction of 0.0786 g in SHDM for each unit increment in  $dS\ m^{-1}$ , where 8.48  $dS\ m^{-1}$  was the maximum level of electrical conductivity. Paulus, Dourado Neto, Frizzzone, and Soares (2010) and Santos, Soares, A. N. Silva, E. F. F. Silva, and Montenegro (2010), in studies with lettuce hydroponic cultivation, also reported a linear reduction in SHDM with increased saline levels. The reduction in dry mass production might be a reflection of the metabolic energy cost associated with acclimatization to saline stress and reduction in carbon gain (Atkin & Macherel, 2009). Therefore, there is a reduction in the supply of photoassimilates and plant hormones to growing tissues (Munns, 1993), which may be related to the dry mass reduction of basil different organs. As already mentioned above, another factor that affects the growth of plants under saline stress is the osmotic effect, which restricts the absorption and transport of water. Such restriction triggers a sequence of hormone-mediated reactions and consequently reducing stomatal aperture and photosynthetic assimilation of  $CO_2$  and biomass production (Odjegba & Chukwunwike, 2012; S. L. F. Silveira, Silva, E. N. Silva & Viégas, 2010).

The  $Cl^-$  and  $Na^+$  contents increased linearly in all parts of the plant with the addition of NaCl in the nutrient solution (Figure 3). The contents of  $Cl^-$  in leaves, stem and roots presented increments of 0.01; 0.008 and 0.005  $mmol\ g^{-1}\ DM$ , respectively, per mM NaCl in the nutrient solution (Figures 3A, 3B and 3C). Thus, the contents of this ion in leaves, stem and roots increased 5.15, 3.15 and 2.97 times respectively, when compared to the control treatment (0 mM NaCl) at the highest saline treatment (80 mM NaCl).

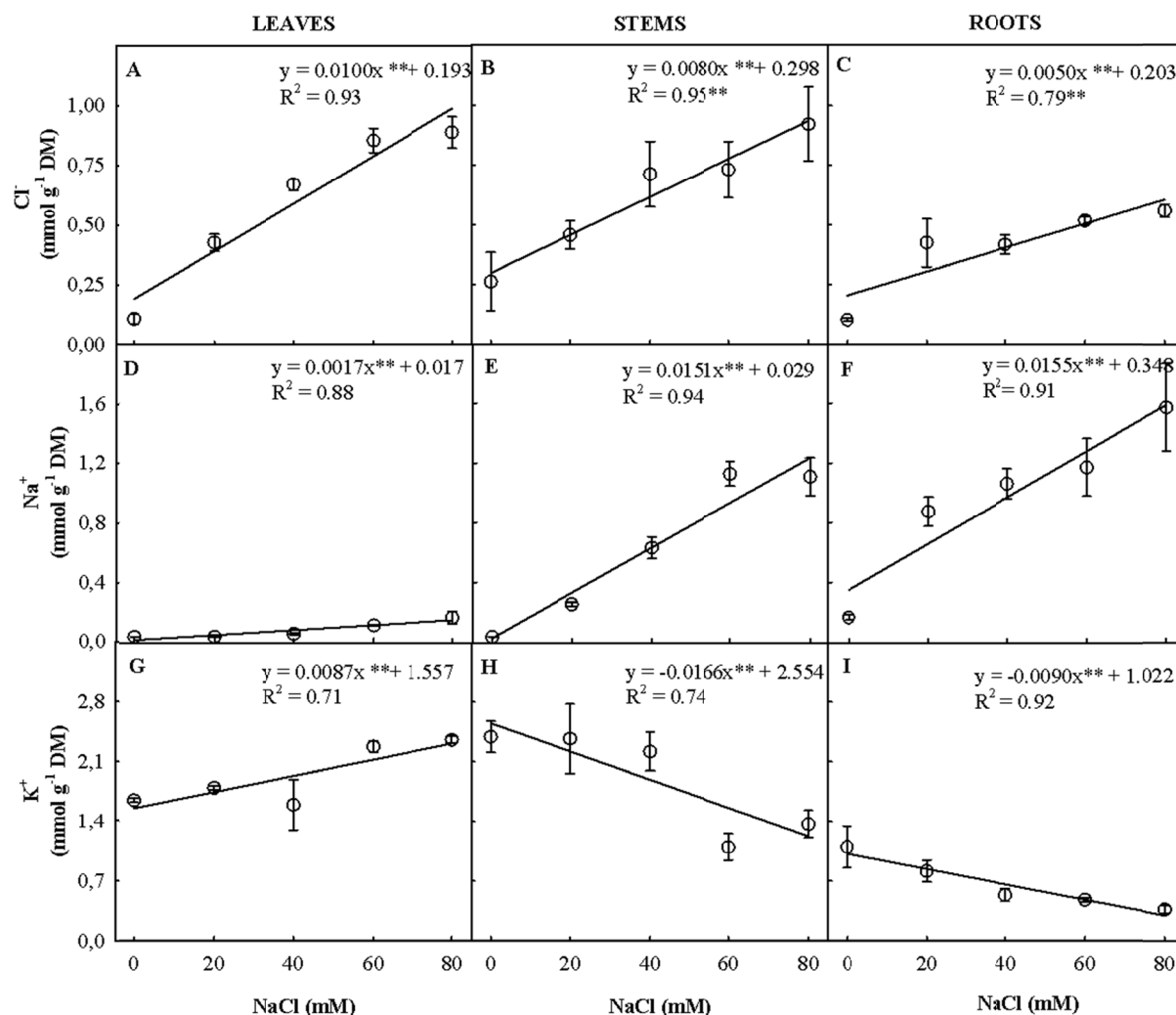


Figure 3. Chloride - Cl<sup>-</sup>, sodium - Na<sup>+</sup> and potassium - K<sup>+</sup> contents in leaves (A, D and G), stems (B, E and H) and roots (C, F and I) in basil plants 17 days after treatment in nutrient solution containing levels of NaCl

Note. Values indicate means of four replicates and respective standard deviations.

Among the growth variables and dry masses analyzed, DM was the most accurate indicator of the salinity effect, corroborating with chloride accumulation data. Therefore, a larger reduction of DM occurred in the tissues with the highest Cl<sup>-</sup> accumulation (leaves and stems), as the smallest reduction of DM was in the root, where less accumulation of this ion occurred.

The estimated concentrations of Na<sup>+</sup> at concentrations of 0 and 80 mM NaCl ranged from 0.017 to 0.153 mmol g<sup>-1</sup> for LDM, from 0.029 to 1.237 mmol g<sup>-1</sup> for SDM and from 0.348 to 1.588 mmol g<sup>-1</sup> for RDM (Figures 3D, 3E and 3F). Thus, there was a higher retention of this ion in the roots, when compared to the leaves and stem, Ning et al. (2015) found similar results. Na<sup>+</sup> retention in the root helps to maintain Na<sup>+</sup>/K<sup>+</sup> ratios required for normal cellular functions and prevents toxic accumulation of this ion in the leaves (Shabala & Mackay, 2011).

The increase of NaCl in the nutrient solution increased linearly the K<sup>+</sup> content in the leaves and decreased linearly in the stem and roots (Figures 3G, 3H and 3I). With the escalation of salinity in the nutrient solution, the K<sup>+</sup> ion increased 45% in the leaves and decreased in the stem and in the roots 52% and 70%, respectively in relation to the control treatment. Thus, we can affirm that as salinity increased, there was a translocation of K<sup>+</sup> to the leaves in detriment of the other parts.

The highest reductions in K<sup>+</sup> levels were observed in the tissues with the highest Na<sup>+</sup> accumulation. These ions compete for the same entry sites in cells, having an antagonistic relationship in plasma membrane uptake

(Marschner, 2012). These results suggest a higher selectivity of  $K^+$  ion transport in relation to  $Na^+$  for the leaves, as well as a mechanism of  $Na^+$  retention in stem and root tissues, avoiding damage due to toxicity in the leaves.

The osmotic adjustment, that is, the accumulation of solutes is an important mechanism, under conditions of saline stress, to obtain a gradient of favorable water potential and maintenance of cellular turgor. The accumulation of the inorganic solutes ( $K^+$ ,  $Na^+$  and  $Cl^-$ ) has a lower energy cost for the cells when related to the accumulation of compatible organic solutes (Flowers, Munns, & Colmer, 2015). However, there must be a balance between these ions, because  $Cl^-$  and  $Na^+$  can be toxic when in excess in plant tissues. In this way, the accumulation of  $K^+$  favors ionic homeostasis, reducing the toxic effects of  $Na^+$  (Munns & Tester, 2008).

Among all the variables studied in Assay I, the DM variable was the most accurate indicator of salinity effect, associated with the  $Cl^-$  accumulation data. In this way, a larger reduction of DM occurred in the tissues with higher  $Cl^-$  accumulation (leaves and stems) and, as the smallest DM reduction was in the root, where there was less accumulation of DM. The  $Cl^-$  is a predominant anion under saline conditions (Tavakkoli, Rengasamy, & McDonald, 2010), when absorbed by the roots is easily translocated to the tissues of the aerial part (Li, Tester, & Gilliam, 2017), justifying its large accumulation in leaves and roots.

In this study, it is considered that 80 mM NaCl significantly decreased (50 to 60%) the dry mass production of basil organs in Assay I, this concentration was used for the subsequent salt stress experiments.

### 3.2 Assay II

Salinity reduced significantly the dry matter yield of leaf (LDM), stem (SDM), roots (RDM) and total (TDM) of basil genotypes, except for the 'Toscano folha de alface' (Figure 4). The highest reductions of LDM (54%), SDM (71%), RDM (55%) and TDM (61%) were observed in the 'Gennaro de menta' genotype. The reduction percentage in biomass production has been considered an effective indicator of tolerance to salt stress in plants (Munns, 2002). These data indicate that the 'Toscano folha de alface' genotype was more tolerant and the Gennaro de menta the most sensitive to salt stress when compared to each other (Figure 4).

The results of this study corroborate with the studies of Barbieri et al. (2012) and Prasad, Lal, Chattopadhyay, V. K. Yadav and A. Yadav (2007), who reported genotypic variability of basil in tolerance to saline stress. Barbieri et al. (2012) verified that the differentiated tolerance among the genotypes of this species was related to the morphological, physiological and metabolic adaptive characteristics to stress.

The  $Na^+$  levels in the leaves (Figure 5A) of all basil genotypes were lower than in the stem (Figure 5B) and in the roots (Figure 5C). A significant variation might be observed between the mean values in the genotypes related to the rates of  $Na^+$  in leaves (0.126 to 0.337 mmol g<sup>-1</sup> DM), stem (1.814 to 3.414 mmol g<sup>-1</sup> DM) and roots (2.338 to 3.720 mmol g<sup>-1</sup> DM). It is interesting to note that the  $Na^+$  content in leaves of the 'Toscano folha de alface' genotype (0.126 mmol g<sup>-1</sup> DM) was 64% lower than the mean leaf content of the other genotypes (0.348 mmol g<sup>-1</sup> DM). In this way, it can be inferred that there was a restriction in the transport of  $Na^+$  to the leaves, with a retention of these ions in the roots of the genotype. This might explain, at least in part, the greater tolerance of this genotype to saline stress since such restriction inhibits  $Na^+$  accumulation at toxic levels in leaves (Munns & Tester, 2008).

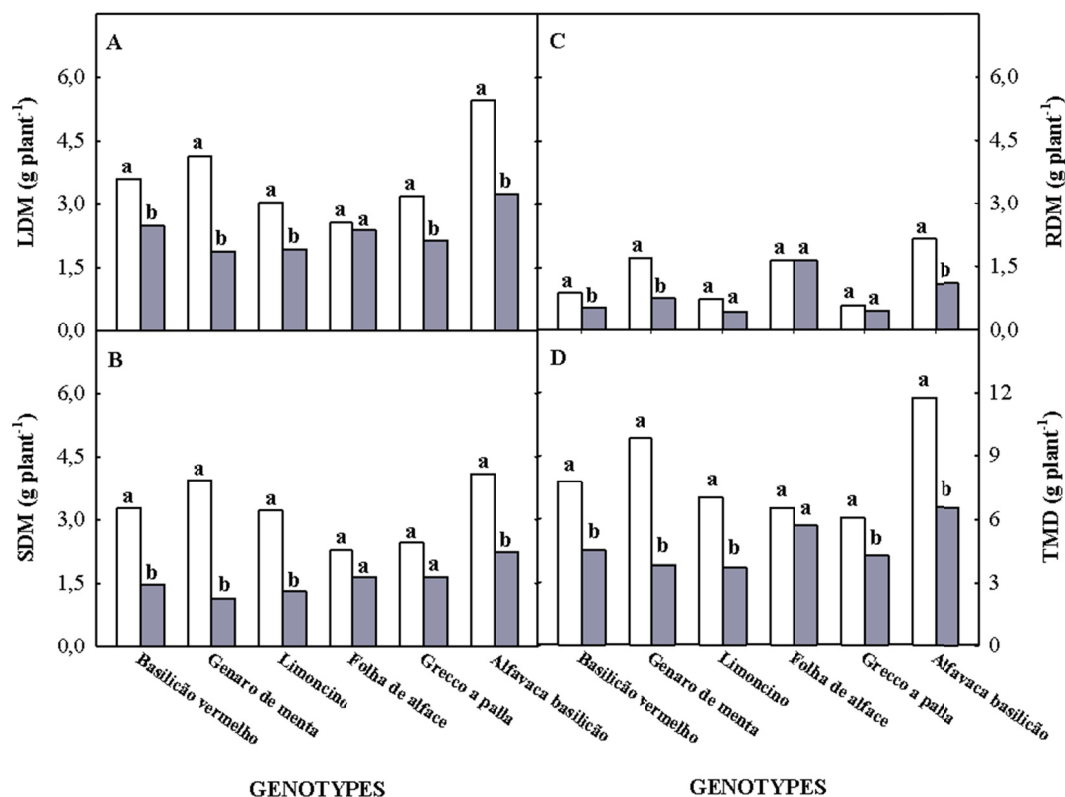


Figure 4. Dry mass of leaves - LMD (A), stem - SDM (B), roots RDM (C) and total- TDM (D) of six basil genotypes after 17 days of treatment in nutrient solution containing 0 (□) or 80 mM NaCl (■)

*Note.* For each genotype, averages followed by the same letters do not differ from each other by the Scott Knott test at 0.05 probability.

Salinity significantly increased  $\text{Na}^+$  and  $\text{Cl}^-$  contents independently of the plant organ or genotype (Figure 5). The highest increments in  $\text{Cl}^-$  per levels of NaCl in the nutrient solution were observed in the ‘Gennaro’ genotype of peppermint, both leaves (7 times) and stem (5 times) and roots (9 times). For the same ion, the smallest increments were observed in the ‘Limoncino’ genotype, which represented 2.09, 2.46 and 3.23 fold, in leaves, stem and roots, respectively (Figures 5D, 5E and 5F).

The greater increase in  $\text{Cl}^-$  levels in the organs of the ‘Gennaro’ genotype of peppermint may partly explain the sensitivity of the genotype to saline stress associated with a greater reduction of DM of this genotype. These data corroborate with the results of Assay I where there was greater reduction of DM in the tissues with greater  $\text{Cl}^-$  accumulation. Ever since accumulated concentrations of  $\text{Cl}^-$  can cause severe damage to plant tissues (Munns & Tester, 2008).

The  $\text{K}^+$  presented higher content in the leaves, in relation to the other organs of the plant. The effect of saline stress over  $\text{K}^+$  contents varied between genotypes and between parts of plants. In the leaves, salinity increased  $\text{K}^+$  levels in the ‘Alfavaca basilicão vermelho’ (16%), ‘Gennaro de menta’ (22%), ‘Toscana folha de alface’ (13%) and ‘Alfavaca basilicão’ (5%) genotypes, but, it did not affect the others (Figure 5G). In the stem, the  $\text{K}^+$  content increased in the ‘Grecco à palla’ (42%), decreased in the ‘Alfavaca basilicão vermelho’ (30%), ‘Gennaro de menta’ (13%) and ‘Alfavaca basilicão’ (14%) genotypes and unchanged in the ‘Limoncino’ and ‘Toscana folha de alface’ genotypes (Figure 5H). In the roots, saline stress did not alter  $\text{K}^+$  contents in the studied genotypes (Figure 5I).

In contrast to what was observed for the  $\text{Na}^+$  ion, it can also be observed in Figure 5G that the foliar  $\text{K}^+$  contents in the ‘Toscana folha de alface’ genotype ( $4.266 \text{ mmol g}^{-1} \text{ DM}$ ) were 26% higher than the mean foliar concentration of the other genotypes ( $3.374 \text{ mmol g}^{-1} \text{ DM}$ ). Azevedo Neto and Silva (2015) report that the mechanism for the adequate maintenance of the  $\text{K}^+$  content in the vegetal tissues, under saline condition, is in part dependent on the selectivity of  $\text{K}^+$  for the shoot, as well as the  $\text{Na}^+$  compartmentation.



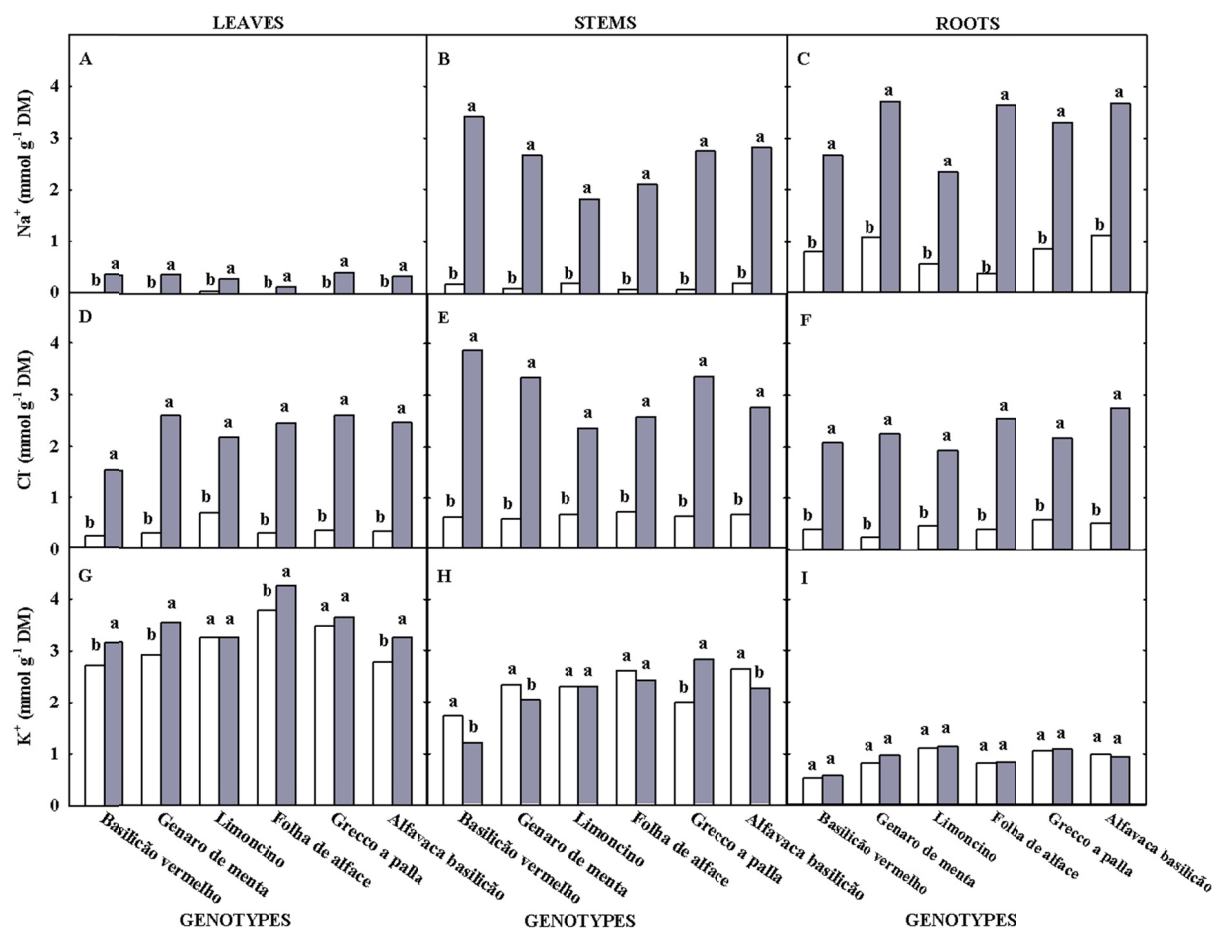


Figure 5. Contents of sodium -  $\text{Na}^+$  (A, B and C), chloride -  $\text{Cl}^-$  (D, E and F) and potassium -  $\text{K}^+$  (G, H and I) in  $\text{mmol g}^{-1}$  leaf (LDM), stems (SDM) and roots (RDM) of six basil genotypes after 17 days of treatment in nutrient solution containing 0 (□) or 80 mM NaCl (■)

*Note.* Means followed by same letters between saline levels do not differ from one another by the Scott Knott test at 0.05 probability.

$\text{K}^+$  is essential for ionic homeostasis and protection of the physiological mechanism, which favors photosynthesis and other physiological functions of plants (Munns, 2002). Ionic homeostasis is primordial for the physiology of living cells by maintaining low cellular concentrations of toxic ions and accumulating essential ions (Zhu, 2003). Such equilibrium results in maintenance of turgescence pressure, cell division, growth and biomass production, even under saline conditions. Thus, these data suggest that the increase in  $\text{K}^+$  concentration in the leaves of the 'Toscano folha de alface' genotype is directly related to its greater tolerance to saline stress. It can still be suggested that, for all genotypes, there were no salinity-induced disturbances in  $\text{K}^+$  uptake and translocation, since the contents of this ion in roots and leaves were not altered or increased with saline stress.

The levels of chlorophyll *a* (CHL*a*) and chlorophyll *b* (CHL*b*) in the studied genotypes were not altered by saline stress, except for the CHL*b* content in the 'Alfavaca basílico vermelho' genotype, which decreased by 35% (Figures 6A and 6B). Thus, the CHL*a*/CHL*b* ratio showed a significant increase of 39% only in this genotype (Figure 6C). In relation to carotenoids, the levels of these pigments decreased with salinity in 'Alfavaca basílico vermelho' (19%), 'Limoncino' (17%) and 'Toscano folha de alface' (16%), increased in 'Grecco a palla' (114%) and were not altered in 'Gennaro de menta' and 'Alfavaca basílico' (Figure 6D).

According to Heidari (2012), saline stress reduces the growth and the content of photosynthetic pigments in sensitive plants and increases in tolerant plants. However, considering that the changes in the chlorophyll content of the 'Toscano folha de alface' (tolerant) and 'Gennaro de menta' (sensitive) genotypes were similar and that of carotenoids increased in 'Gennaro de menta', these results indicate that the pigment contents showed no relation

with tolerance to salinity and should not be considered as good biochemical markers for tolerance to saline stress in these basil genotypes.

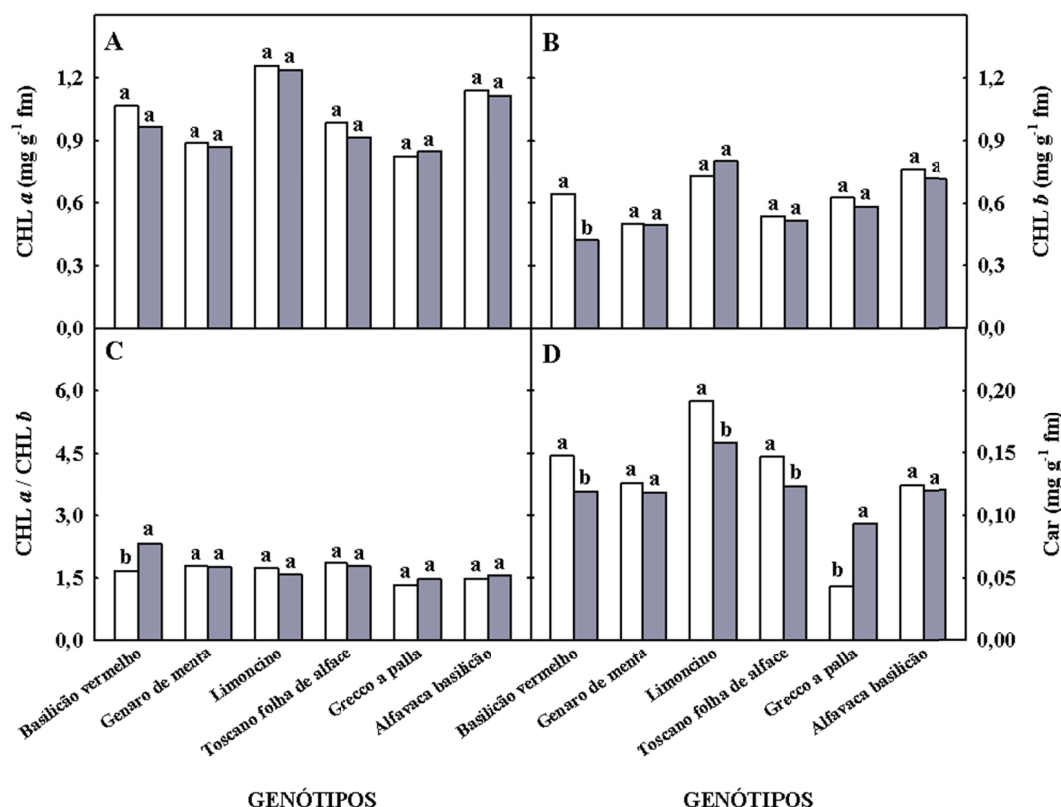


Figure 6. Concentration of Chlorophyll *a* (CHLa), Chlorophyll *b* (CHLb) and Carotenoids (Car) in leaves and CHLa and CHLb (CHLa/CHLb) ratio of six basil genotypes after 17 days of treatment in nutrient solution containing 0 (□) or 80 mM NaCl (■)

*Note.* For each genotype, averages followed by the same letters do not differ from each other by the Scott Knott test at 0.05 probability.

Changes in the relative water content (RWC) and absolute integrity percentage (AIP) are shown in Figure 7. Salinity caused a small reduction in the RWC of the genotypes 'Limocino' (9%) and 'Grecco a palla' (8%) (Figure 7A). Salinity decreased, averaging 37% of the PAI of all genotypes, with the exception of the 'Toscano folha de alface', which was not affected by stress (Figure 7B). Considering that the 'Toscano folha de alface' genotype was also the most tolerant to saline stress, when evaluated for its biomass production, the results of this study suggest that the estimation of membrane integrity expressed by AIP can be used as a physiological indicator in the identification of basil genotypes tolerant to saline stress.

According to Gupta and Huang (2014), salinity tolerance characteristics involve complex responses at molecular, cellular, metabolic, and physiological levels throughout the plant. Thus, evaluations of the morphological and physiological characteristics associated with yield provide a greater sensitivity in the identification of salinity tolerant plants (Ashraf, 2004). This author further emphasizes that the use of physiological attributes as salinity tolerance markers depends on how strong is the relationship of these markers to plant responses and it is probably more effective than selections based on individual markers.

The photosynthetic pigment contents and the relative water content were not good indicators of salinity tolerance for basil. On the other hand, the production of dry mass, the accumulation of inorganic solutes and the percentage of absolute integrity, stand out as good indicators of tolerance to saline stress among the studied genotypes. The higher accumulation of K<sup>+</sup> and a lower accumulation of Na<sup>+</sup> in the leaves of the 'Toscano folha de alface' genotype plays a key role in maintaining the integrity of the cell membranes in this genotype. The

‘Toscano folha de alface’ genotype is more tolerant to salinity and the ‘Gennaro de menta’ more sensitive when compared to each other.

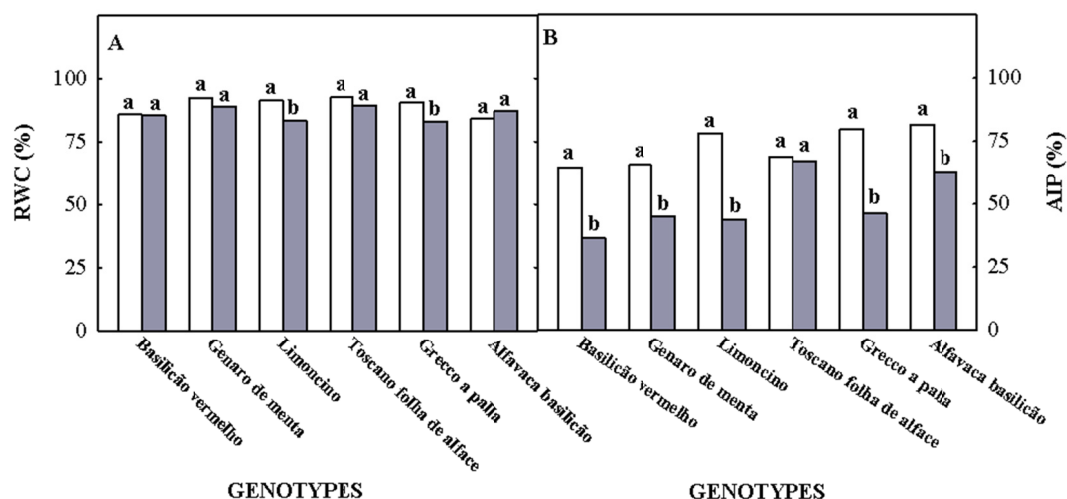


Figure 7. Relative water content in leaves (RWC) and absolute integrity percentage (AIP) off cell membranes in six six basil genotypes after 17 days of treatment in nutrient solution containing 0 (□) or 80 mM NaCl (■)

Note. For each genotype, means followed by the same letters do not differ from each other by the Scott Knott test at 0.05 probability.

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