





Tolerance of basil cultivars to salt stress in semi-hydroponic cultivation

Sandy Thomaz dos Santos¹, Francisco de Assis de Oliveira^{1*}, Giordanio Bruno Silva Oliveira¹, Mychelle Karla Teixeira de Oliveira¹, Aline da Silva Alves¹, Carla Jamile Xavier Cordeiro¹, Mário Jonas Veras Costa¹, Carlos Eduardo Alves de Oliveira¹

¹Federal Rural University of the Semi-Arid, Mossoró, Brazil
*Corresponding author, e-mail: thikaoamigao@ufersa.edu.br

Abstract

The objective of this study was to evaluate the tolerance of basil cultivars to salt stress in semi-hydroponic cultivation. The experiment was carried out in a greenhouse, following a randomized block design, in a 5 x 3 factorial scheme, with five basil cultivars (Grecco a Palla, Alfavaca Basilicão, Alfavaca Verde, Lemoncino and Roxo) and three salinity levels of the nutrient solution (2.0, 3.5 and 5.0 dS m⁻¹), with three replications, and the experimental plot represented by three pots containing 3.0 dm³ of coconut fiber and one plant in each. At 60 days after transplanting, plants were evaluated for plant height, number of leaves, number of stems and inflorescence, length of inflorescences, stem diameter, dry mass (leaves, stems, inflorescences and total), leaf area, specific leaf area, leaf area ratio, leaf succulence and the salinity tolerance index. In general, there were different responses among the cultivars regarding salinity. Fertigation using high-salinity water promotes changes in the growth of sensitive basil cultivars. The cultivars Alfavaca Basilicão, Alfavaca Verde and Roxo were tolerant to all tested salinity levels (2.0, 3.5 and 5.0 dS m⁻¹), while Grecco a Palla and Lemoncino were tolerant up to the salinity of 3.5 dS m⁻¹.

Keywords: Hydroponics, medicinal plants, *ocimum basilicum* L., salt stress

Introduction

Basil (*Ocimum basilicum* L.) is one of the most worldwide cultivated and known medicinal plants of the Lamiaceae family, given its great importance in various market segments, such as cooking, where it can be used as a seasoning, in popular alternative medicine and pharmaceutical industry (Chiang et al., 2005; Bharti et al., 2016).

In Brazil, basil is grown mainly by small family farmers, who use conventional cultivation. Currently, many studies with basil are being carried out in protected environment in the hydroponic cultivation system (Menezes et al., 2017; Alves et al., 2019).

The cultivation of plants in inert substrate, also called semi-hydroponic, has been gaining space among researchers and rural producers because it is an alternative cultivation system to NFT hydroponic cultivation, with the advantage of providing lower consumption of electricity,

which can generate savings of up to 92% due to the shorter irrigation time (Andriolo et al. 2004). Among the substrates, coconut fiber is advantageous because it has favorable physical properties such as being an inert material in relation to the nutrients supplied, having greater durability and enabling reuse by producers (Carrizo et al., 2002).

In addition to the concentration of nutrients in the nutrient solution of hydroponic cultivation, water quality, with respect to the concentration of dissolved salts, is a primary factor for crops to reach maximum production potential, considering that the high concentration of salts in the solution can cause nutritional imbalance and/or toxicity, as well as reduction in stomatal conductance, transpiration and photosynthesis, resulting in lower growth and, consequently, reduction in biomass production (Maia et al., 2017; Silva et al., 2019; Scagel et al., 2019).

However, there are divergences regarding the

response of basil to salt stress, which may be attributed to the genotypic heterogeneity of basil crop, leading to different responses of the species to salt stress and, thus, different degrees of tolerance (Maia et al., 2017; Silva et al., 2017; Scagel et al., 2019).

In view of the above, the objective of this study was to evaluate the tolerance of basil cultivars to salt stress grown in a semi-hydroponic system.

Material and Methods

The experiment was carried out from July 4, 2018, to September 21, 2018, in a greenhouse in the Department of Agronomic and Forestry Sciences (DCAF), of the Federal Rural University of the Semi-Arid Region (UFERSA), in Mossoró, RN, Brazil (5°12'04" S Latitude; 37°19'39" W Longitude; average altitude of 18 m).

The experimental design used was randomized blocks, in a 5 x 3 factorial scheme, corresponding to five basil cultivars (Grecco a Palla, Alfavaca Basilicão, Alfavaca Verde, Lemoncino and Roxo) and three salinity levels of the nutrient solutions (S1 - standard nutrient solution (2.0 dS m⁻¹), SNS; S2 - SNS + NaCl (3.5 dS m⁻¹); S3 - SNS + NaCl (5.0 dS m⁻¹). Salinity levels of 3.5 and 5.0 dS m⁻¹ were obtained by adding NaCl in the nutrient solutions, and adjusting the salinity levels after reading with a benchtop conductivity meter. Each treatment was represented by three replicates, and the experimental unit consisted of three pots with capacity for 3 dm³, containing coconut fiber substrate and one plant per pot, totaling 135 plants.

The nutrient solutions were prepared using water from the supply system of the UFERSA campus, whose physical and chemical analyses determined the following characteristics: pH = 8.30; EC = 0.50 dS m⁻¹; Ca²⁺ = 3.10; Mg²⁺ = 1.10; K⁺ = 0.30; Na⁺ = 2.30; Cl⁻ = 1.80; HCO₃⁻ = 3.00 and CO₃²⁻ = 0.20 (mmol_c L⁻¹).

The standard nutrient solution was that recommended by Furlani et al. (1999) for leafy vegetables, containing the following quantities of fertilizers, in g per 1000 liters: 750, 500, 150 and 400 g of calcium nitrate, potassium nitrate, monoammonium phosphate and magnesium sulfate, respectively. Micronutrients were supplied using the fertilizer Rexolim®, at a concentration of 30 g per 1000 liters.

The seedlings were produced in a greenhouse using expanded polystyrene trays with 128 cells and substrate formulated with the mixture of earthworm humus and coconut fiber (1:1.5). The trays were irrigated twice a day, using a fine-mesh watering can until transplanting, which was performed when the seedlings had three to four true leaves, to plastic containers with capacity for 3 dm³, placing one plant in each pot.

The pots were arranged inside the greenhouse on wooden benches (0.5 m high, 1.0 m wide and 5.0 m long), in a pattern of 45 pots distributed in three rows with 15 pots each, at spacing of 0.45 m between rows and 0.35 m between plants.

Each nutrient solution was applied by an independent drip irrigation system, composed of a Metalcorte/Eberle EBD250076 self-venting circulation motor pump, driven by single-phase motor, with 210 V voltage and 60 Hz frequency, a plastic reservoir (60 L), lateral line with 16-mm-diameter hoses and microtube-type (Spaghetti) emitter, with internal diameter of 1.0 mm and average flow rate of 2.78 L h⁻¹.

Fertigation began to be applied one day after transplantation, using the same nutrient doses for all treatments, which differed only in the quantity of NaCl. The control was performed using a timer (Decorlux® - TE-2), adopting the frequency of 6 daily irrigations. Water consumption by the plants was not considered; however, in all irrigations, the substrate moisture content was raised to maximum water holding capacity, which was confirmed by visualizing drainage from the pots.

The plants were collected at 60 days after transplanting and evaluated for the following variables:

Plant height (PH), measured with a measuring tape graduated in cm, from the substrate surface to the stem apex;

Number of leaves (NL), obtained by counting all leaves longer than 1.0 cm;

Number of stems (NS) and number of inflorescences (NINFL), counted after the harvest of plants, considering the secondary stems emerged from the main branch;

Length of inflorescences (LINFL), measured with a ruler graduated in cm and considering the average length of all inflorescences of one plant;

Stem diameter (SD), measured with a digital caliper, at 2.0 cm distance from where the plants were cut, considering the average of two measurements taken in perpendicular positions;

Leaf dry mass (LDM), stem dry mass (SDM) and inflorescence dry mass (INFLDM), obtained after separation of the different plant parts, where each material was placed in paper bags, labeled and dried in an oven with forced air circulation at 65 °C until reaching constant weight. After drying, the materials were weighed individually on an electronic scale, and the total dry mass (TDM) was obtained by summing the dry mass of the different plant parts (TDM= LDM+SDM+INFLDM);

Leaf area (LA) was determined by the method

of collecting 25 leaf discs per plant with a hole punch, whose diameter was adjusted according to the size of the leaves of each cultivar. For its determination, Equation 1 was used.

$$LA = \frac{(LDM \times DA)}{DDM} \quad (1)$$

Where:

LA – leaf area, cm² plant⁻¹;

LDM – leaf dry mass, g plant⁻¹;

DDM – disc dry mass, g;

DA – disc area, cm².

Specific leaf area (SLA) was obtained by the quotient between leaf area (LA) and leaf dry mass (LDM), according to Equation 2.

$$SLA = \frac{LA}{LDM} \quad (2)$$

Where:

SLA – specific leaf area, cm² g⁻¹ LDM;

LA – leaf area, cm² plant⁻¹;

LDM – leaf dry mass, g plant⁻¹.

Leaf area ratio (LAR) was obtained by dividing leaf area (LA) by total dry mass (TDM), according to Equation 3.

$$LAR = \frac{LA}{TDM} \quad (3)$$

Where:

LAR – leaf area ratio, (cm² g⁻¹ TDM)

LA – leaf area, cm² plant⁻¹;

TDM – total dry mass, g plant⁻¹.

Leaf succulence (LSUC) was obtained by the ratio between the mass of water and leaf area, according to Equation 4.

$$LSUC = \frac{(LFM-LDM)}{LA} \quad (4)$$

Where:

LSUC – leaf succulence, mg⁻¹ of H₂O cm² leaf;

LFM – leaf fresh mass, g plant⁻¹;

LDM – leaf dry mass, g plant⁻¹;

LA – leaf area, cm² plant⁻¹.

The total dry mass production data were then used to calculate the percentages partitioned between the vegetative organs and the salinity tolerance index (STI), comparing the data of the salinity treatments with those of the control (ECw = 2.00 dS m⁻¹), using Equation 5.

$$STI (\%) = \frac{TDM_{\text{production in salinity treatment}} \times 100}{TDM_{\text{production in control treatment}}} \quad (5)$$

Basil genotypes were classified for salinity tolerance according to the following intervals of relative reduction in total dry mass: tolerant - zero to 20%; moderately tolerant - 21 to 40%; moderately susceptible - 41 to 60%; and susceptible - above 60% (Fageria et al., 2010).

The obtained data were subjected to analysis of variance, with follow-up analysis when there was significant response to the interaction between factors. The effect of the treatments was analyzed by a means comparison test (Tukey, 5%). Statistical analyses were performed using the statistical software Sisvar version 5.6 (Ferreira, 2011).

Results and Discussion

According to the analysis of variance, there was a significant response to the interaction between the factors cultivars and salinity for the variables number of leaves (NL), number of stems (NS), leaf area (LA), leaf area ratio (LAR), leaf succulence (LSUC) and stem dry mass (SDM) at 1% probability level. The variables length of inflorescences (LINFL), specific leaf area (SLA) and total dry mass (TDM) were significantly affected by the interaction at 5% probability level, with no significant response for the other variables ($p > 0.05$). It was also observed that all variables were significantly affected by the cultivar factor alone ($p < 0.05$). Salinity alone affected most variables ($p < 0.01$), except for NINFL ($p < 0.05$), while the variables leaf area ratio (LAR), LSUC and specific leaf area (SLA) were not affected by the salinity factor alone (**Table 1**). The cultivars differed from each other in terms of plant height, stem diameter, number of inflorescences, leaf dry mass and inflorescence dry mass (**Table 2**).

The cultivar Alfavaca Basilicão was superior to the others in terms of plant height, while Grecco a Palla showed lower PH. The cultivars Grecco a Palla and Alfavaca Basilicão showed greater stem diameter, although Alfavaca Basilicão did not differ from the others. Regarding the effect of salinity, it was observed that the use of nutrient solution with salinity of 5.0 dS m⁻¹ caused reduction in the variables PH and SD, resulting in losses of 13.62 and 19.55%, respectively, compared to the lowest salinity.

Reductions in PH and SD in the cultivation of basil subjected to salt stress have also been observed by other authors, in soil cultivation (Bione et al., 2014; Maia et al., 2017; Silva et al., 2017).

The decrease in plant height under saline conditions may be related to reductions in cell division and/or expansion and plant growth associated with low turgor pressure and reduced capacity to synthesize organic

Table 1. Summary of the analysis of variance for plant height (PH), number of leaves (NL), number of stems (NS), length of inflorescences (LINFL), number of inflorescences (NINFL), stem diameter (SD), leaf area (LA), leaf area ratio (LAR), specific leaf area (SLA), leaf succulence (LSUC), leaf dry mass (LDM), inflorescence dry mass (MSINF), stem dry mass (SDM) and total dry mass (TDM) of basil cultivars (C) subjected to different salinity levels (S) of the nutrient solution

Variables	Source of variation					
	Cultivars (C)	Salinity (S)	C x S	Block	Residual	CV (%)
PH	14422.75**	233.86**	14.83 ^{ns}	5.26 ^{ns}	15.6	11.31
SD	11.48**	11.35**	2.51 ^{ns}	1.49 ^{ns}	1.37	15.65
NINFL	13779.24**	660.68*	415.24 ^{ns}	134.96 ^{ns}	221.69	15.09
NL	940690.61**	940690.61**	940690.61**	940690.61**	940690.61**	19.09
NS	22.14**	21.49**	21.74**	2.44 ^{ns}	3.8	11.96
LINFL	306.07**	48.02**	17.52*	5.42 ^{ns}	6.07	12.47
LA	6167701**	583464.40**	146640.38**	271.22 ^{ns}	42487.12	11.55
LAR	1482.27**	36.08 ^{ns}	62.71**	15.38 ^{ns}	18.94	11.75
SLA	6463.85**	845.76 ^{ns}	942.67*	1260.96*	354.6	13.91
LSUC	610.949**	70.06 ^{ns}	522.23**	22.06 ^{ns}	23.65	9.80
LDM	258.41**	99.17**	12.94 ^{ns}	27.73*	6.93	16.37
MSINF	858.08**	17.12 ^{ns}	6.44 ^{ns}	5.21 ^{ns}	9.99	15.62
SDM	122.75**	56.50**	16.83**	1.03 ^{ns}	3.39	16.32
TDM	1329.98**	433.26**	59.99*	66.39 ^{ns}	25.65	10.64

ns: *; ** = not significant, significant at 5 and 1% probability levels, respectively.

Table 2. Mean values for plant height (PH), stem diameter (SD), number of inflorescences (NINFL), leaf dry mass (LDM) and inflorescence dry mass (MSINF) in basil cultivars fertigated with salinized nutrient solution

Cultivars	PH (cm)	SD (mm)	NINFL (unit)	LDM (g plant ⁻¹)	MSINF (g plant ⁻¹)
Grecco a Palla	35.77 d	9.58 a	30.00 b	21.54 a	5.19 c
Alfavaca Basilicão	68.66 a	8.11 ab	120.22 a	15.54 b	27.37 a
Alfavaca Verde	62.66 b	7.07 b	123.11 a	21.58 a	28.47 a
Lemoncino	49.66 c	6.75 b	103.44 a	10.43 c	24.34 a
Roxo	53.55 c	7.35 b	116.67 a	11.33 c	15.82 b
Electrical conductivity levels					
2.0 dS m ⁻¹	57.80 a	8.44 a	94.40 b	18.00 a	20.29 a
3.5 dS m ⁻¹	54.46 a	8.09 a	106.33 a	17.10 a	21.28 a
5.0 dS m ⁻¹	49.93 b	6.79 b	95.33 b	13.16 b	19.14 a

Means followed by the same letter, in the columns, do not differ from each other by Tukey test ($p < 0.05$)

compounds, due to the decrease in photosynthetic rate, thus being associated with the osmotic effects of this stress (Munns & Tester, 2008).

Regarding the number of inflorescences (NINFL), it was observed that only the cultivar Grecco a Palla differed from the others, showing the lowest values (Table 2). For salinity levels, there was a significant effect on the number of inflorescences, which increased at salinity of 3.5 dS m⁻¹ and then decreased at the highest salinity level (5.0 dS m⁻¹) (Table 2).

These results agree with those reported by Silva et al. (2017), who observed a reduction in the number of inflorescences in basil plants subjected to salt stress cultivated in soil.

The cultivars also differed from each other in terms of LDM, and Grecco a Palla and Alfavaca Verde were superior to the others, while Lemoncino and Roxo showed lower LDM (Table 2). For the effect of salinity, there was a reduction of 26.89% in LDM at the salinity level

of 5.0 dS m⁻¹ compared to the first salinity level (Table 2).

Other authors also found reduction in LDM in basil plants, such as Menezes et al. (2017). The high salinity in the nutrient solution reduces the capacity of plants to absorb water due to the osmotic effect, resulting in slower growth. In addition, excessive amounts of specific salts, such as Na⁺ and Cl⁻, negatively affect the stomata, reducing photosynthesis and, consequently, plant growth (Munns et al., 2006).

The cultivars Alfavaca Basilicão, Alfavaca Verde and Lemoncino showed higher MSINF, while the lowest values were obtained in the cultivar Grecco a Palla, despite its high production of stems and leaves (Table 2). These results confirm the information presented by Benito & Chiesa (2000), who reported that more branched basil cultivars are later in completing floral induction as they have more points of growth.

The cultivar Grecco a Palla can prioritize the distribution of photoassimilates for vegetative

development instead of reproductive development, due to the lower number of inflorescences of this cultivar as compared to the others. These characteristics are typical of each cultivar and have been observed by other authors, such as Fernandes (2014), who observed lower development in height for the cultivar Grecco a Palla.

Regarding the effect of salinity, there was no influence on MSINF, which averaged 20.23 g plant⁻¹.

The basil cultivars responded differently to the salinity of irrigation water compared to the number of leaves (NL). At salinity of 2.0 dS m⁻¹, the cultivar Grecco a Palla had higher NL compared to the others at the lowest salinity, while the cultivars Roxo and Alfavaca Basilicão showed lower values. At salinity of 3.5 dS m⁻¹, the highest values were obtained in the cultivars Grecco a Palla and Alfavaca Verde, although the latter did not differ significantly from Alfavaca Basilicão and Lemoncino, while the lowest NL was observed in the cultivar Roxo. At the highest salinity (5.0 dS m⁻¹), the highest NL was observed in the cultivars Alfavaca Verde and Grecco a Palla, which did not differ statistically from each other. On the other hand, the cultivars Alfavaca Basilicão, Lemoncino and Roxo showed a lower number of leaves (Figure 1A).

There was no effect of salinity on the number of leaves in the cultivars Alfavaca Basilicão, Alfavaca Verde, Lemoncino and Roxo, which showed average NL of 474.22, 755.88, 449.66 and 402.00 leaves per plant, respectively. On the other hand, the cultivar Grecco a Palla significantly reduced its NL with the increase in salinity, showing reductions of 43.18 and 67.94% at the salinity levels of 3.5 dS m⁻¹ and 5.0 dS m⁻¹, compared to the salinity of 2.0 dS m⁻¹ (Figure 1A).

In a study with basil cultivars, Barbieri et al. (2012) observed different responses for the number of leaves among the cultivars, with reduction in one of them and a value that remained constant in the other with increasing salinity. The divergence in the response of these cultivars, as observed in the present study, demonstrates genetic divergence in response to salinity for leaf production.

The basil cultivars differed significantly for the number of stems (NS) only at the lowest salinity (2.0 dS m⁻¹), and the cultivar Grecco a Palla was superior to the others. At the salinity levels of 3.5 and 5.0 dS m⁻¹, there was no significant difference between cultivars, with average NS of 16.73 and 14.93, respectively (Figure 1B).

Regarding the effect of salinity on NS, there was a varied response according to the cultivar analyzed. The cultivar Grecco a Palla showed a reduction in NS with the increase in NaCl concentration, with losses of

26.37 and 37.5% at the salinity levels of 3.5 and 5.0 dS m⁻¹, respectively, compared to plants subjected to the lowest salinity (2.0 dS m⁻¹). The cv. Alfavaca Basilicão initially showed an increase of 43.61% in NS at the salinity level of 3.5 dS m⁻¹; however, at the highest salinity (5.0 dS m⁻¹) there was a reduction of 5.15%, compared to the NS obtained at the lowest salinity (2.0 dS m⁻¹). In the other cultivars there was no significant effect, with means of 16.11, 15.67 and 16.11 stems per plant for Alfavaca Verde, Lemoncino and Roxo, respectively (Figure 1B).

Reduction in the number of stems in basil in response to salinity was also observed by Maia et al. (2017), who found a linear decrease in NS with increasing salinity. Thus, the results show that the cultivars have different degrees of tolerance to salt stress regarding the production of stems, confirming the results presented by Maia et al. (2017).

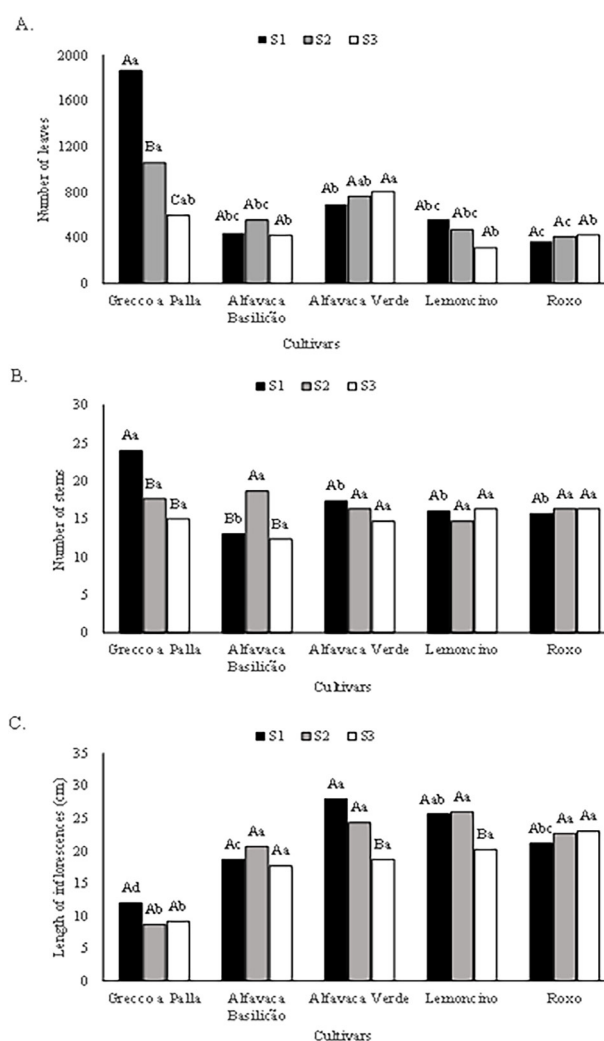


Figure 1. Number of leaves (A), number of stems (B) and length of inflorescences (C) in basil cultivars subjected to different salinity levels of the nutrient solution (Uppercase letters represent salinity for each cultivar; lowercase letters represent the cultivars for each salinity (Tukey, 0.05); S1 – 2.0 dS m⁻¹, S2 – 3.5 dS m⁻¹, S3 – 5.0 dS m⁻¹).

Salt stress can interfere with the absorption of essential nutrients, contributing to a greater inhibition of plant growth, affecting morphology or even reducing survival (Álvarez & Sánchez-Blanco, 2014).

The cultivars showed different responses regarding the length of inflorescences (LINFL) at the lowest salinity (2.0 dS m^{-1}), at which the cultivars Alfavaca Verde and Lemoncino had higher LINFL when compared to the others, despite not differing significantly from the cultivar Roxo, which in turn did not differ from Alfavaca Basilicão. The cv. Grecco a Palla had the lowest value of LINFL at this salinity. At the salinity levels of 3.5 and 5.0 dS m^{-1} , only the cv. Grecco a Palla differed significantly from the others, showing lower LINFL (Figure 1C).

It was also observed that there was no effect of salinity on LINFL in the cultivars Grecco a Palla, Alfavaca Basilicão and Roxo, which showed mean values of 9.94 , 19.00 and 22.28 cm , respectively (Figure 1C). The cultivars Alfavaca Verde and Lemoncino significantly reduced their LINFL as salinity increased, with reductions of 33.32 and 21.42% , respectively, compared to the treatment of lowest salinity (Figure 1C).

Reduction in LINFL in basil plants has also been observed by other authors, either in hydroponics (Elhindi et al., 2017a) or in soil (Elhindi et al., 2017b), which may consequently affect seed production.

Length of inflorescences is a fundamental variable from the commercial point of view, such as for ornamental purposes, as well as for seed production, because the longer the inflorescence, the greater the number of seeds per inflorescence.

For leaf area (LA), it was observed that the cultivars Alfavaca Basilicão and Alfavaca Verde had the highest values at the lowest salinity (2.0 dS m^{-1}), not differing significantly from each other. The cv. Grecco a Palla, on the other hand, had the lowest LA value. For salinity of 3.5 dS m^{-1} , the cultivars Alfavaca Basilicão and Alfavaca Verde also had the highest values of LA, not differing significantly from each other, and the cultivar Grecco a Palla had the lowest value, although it did not differ significantly from Lemoncino. At the highest salinity (5.0 dS m^{-1}), there was behavior similar to that observed at the salinity level of 3.5 dS m^{-1} , with the cultivars Alfavaca Basilicão and Alfavaca Verde showing the best performance, not differing from each other, and the cultivar Grecco a Palla had the lowest LA value, not differing significantly from Lemoncino (Figure 2A).

When evaluating the effect of salinity on LA, it was found that the cultivars Grecco a Palla and Alfavaca Basilicão were not affected by the increase in the salinity

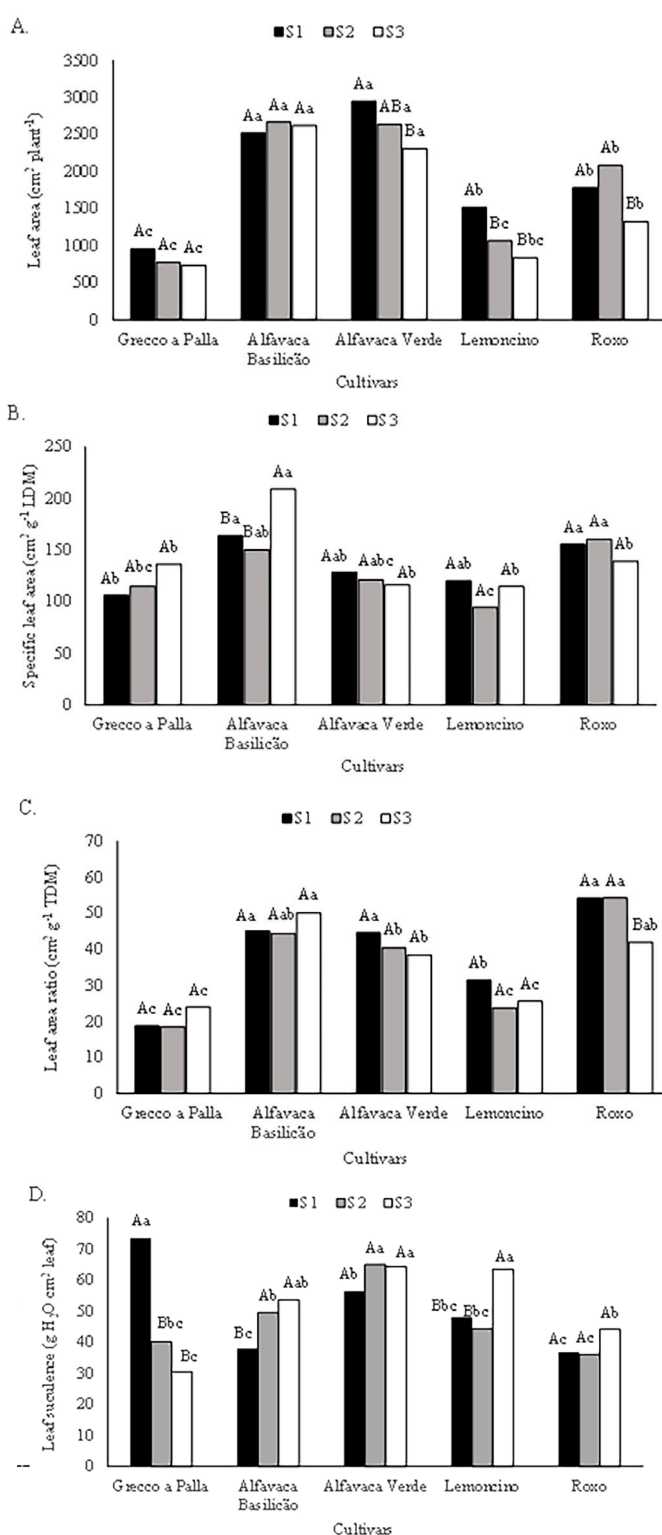


Figure 2. Leaf area (A), specific leaf area (B), leaf area ratio (C) and leaf succulence (D) in basil cultivars subjected to different salinity levels of the nutrient solution (Uppercase letters represent salinity for each cultivar; lowercase letters represent the cultivars for each salinity (Tukey, 0.05); S1 – 2.0 dS m^{-1} , S2 – 3.5 dS m^{-1} , S3 – 5.0 dS m^{-1}).

levels of the nutrient solutions, obtaining means of 819.44 and $2604.44 \text{ cm}^2 \text{ plant}^{-1}$, respectively. On the other hand, the cultivars Alfavaca Verde and Lemoncino reduced their LA as salinity increased (5.0 dS m^{-1}), with losses of

21.78 and 45.08%, respectively, compared to the lowest salinity level (2.0 dS m⁻¹). The cultivar Roxo showed first an increase of 16.7% in LA at the salinity level of 3.5 dS m⁻¹; however, it decreased at the highest salinity (5.0 dS m⁻¹), with loss of 25.83% compared to the salinity of 2.0 dS m⁻¹ (Figure 2A).

Several authors report reduction of leaf area with increased salinity, either in soil cultivation (Maia et al., 2017) or in hydroponic cultivation (Bernstein et al., 2010; Silva et al., 2019). Barbieri et al. (2012), when working with basil cultivars under saline conditions, found that this reduction is associated with the reduced number of leaves and the characteristic leaf size of each cultivar.

The reduction of leaf area is attributed to the fact that there is a lower cell division and leaf expansion in plants under conditions of high salinity, generating consequences such as lower growth, with a delay in leaf production, which consequently reduces the leaf area available for photosynthesis (Taiz et al., 2017).

Regarding the specific leaf area (SLA), the highest value at salinity of 2.0 dS m⁻¹ was observed in the cultivar Alfavaca Basilicão, although it did not differ significantly from Roxo, Alfavaca Verde and Lemoncino. The cultivar Grecco a Palla had the lowest value of SLA for this same salinity level. At salinity of 3.5 dS m⁻¹, the cultivar Roxo stood out with the highest SLA, although it did not differ from Alfavaca Basilicão and Alfavaca Verde. Alfavaca Verde, on the other hand, also did not differ significantly from the cultivars Grecco a Palla and Lemoncino, which had the lowest value of SLA for this salinity. At salinity of 5.0 dS m⁻¹, only Alfavaca Basilicão differed from the other cultivars and had higher SLA (Figure 2B).

Regarding the effect of salinity on SLA, there was a significant response only for the cultivar Alfavaca Basilicão, which showed an increase of 27.64% in SLA at the highest salinity (5.0 dS m⁻¹) when compared to the lowest salinity (2.0 dS m⁻¹). The other cultivars (Grecco a Palla, Alfavaca Verde, Lemoncino and Roxo) did not show a significant response to salinity, resulting in mean values of 119.04, 122.00, 109.86 and 151.69 cm² g⁻¹ LDM (Figure 2B).

The increase in specific leaf area observed in the cultivar Alfavaca Basilicão at the highest levels of salinity shows that the effect of salt stress was more significant on leaf biomass production than on leaf blade expansion, reducing leaf thickness. The rates of elongation and cell division depend directly on the process of extensibility of the cell wall, so the immediate response of plants to salt stress is the reduction in leaf expansion (Parida et al., 2004). On the other hand, the absence of salinity effect

on specific leaf area in the other cultivars shows that the effect of salt stress was proportional, both for leaf expansion and biomass accumulation in leaves.

For the variable leaf area ratio (LAR), at salinity of 2.0 dS m⁻¹, the cultivar Roxo showed higher LAR, although it did not differ significantly from Alfavaca Basilicão and Alfavaca Verde. On the other hand, the cv. Grecco a Palla had the lowest value of LAR. At salinity of 3.5 dS m⁻¹, the cv. Roxo also stood out, obtaining higher value than the others, but not differing from the cultivar Alfavaca Basilicão, which in turn did not differ from Alfavaca Verde. The lowest LAR was obtained in the cultivar Grecco a Palla, which did not differ significantly from Lemoncino. At the highest salinity (5.0 dS m⁻¹), the cv. Alfavaca Basilicão had the highest value of LAR, but did not differ significantly from Roxo, which did not differ from Alfavaca Verde. It is also found that, as observed at the salinity level of 3.5 dS m⁻¹, the lowest values were obtained in the cultivars Grecco a Palla and Lemoncino, which did not differ from each other (Figure 2C).

When analyzing the effect of salinity on LAR, it was found that there was a significant effect only on the basil cultivar Roxo, with a reduction of 22.53% in LAR between the salinity levels of 2.0 and 5.0 dS m⁻¹ (Figure 2C). This response indicates that there was a reduction in transpiration as a way for the plant to adjust to salt stress, increasing the photosynthetic efficiency to increase the utilization of the photosynthetic leaf area for biomass production (Freitas et al., 2014).

The cultivars Grecco a Palla, Alfavaca Basilicão, Alfavaca Verde and Lemoncino did not show a significant response to salinity for this variable, with mean values of 20.40, 46.55, 41.14 and 26.90 cm² g⁻¹ TDM, respectively (Figure 2C).

The absence of significant response to salinity for the variable LAR, observed in most cultivars, indicates that salt stress affected leaf area and dry mass production in the same proportion. The maintenance of LAR in some cultivars subjected to salt stress is an indication that salinity did not affect the photosynthetic efficiency of these plants.

For the leaf succulence variable (LSUC), the cultivar Grecco a Palla showed the highest value (73.58 mg⁻¹ H₂O cm² leaf) for salinity of 2.0 dS m⁻¹. On the other hand, the cultivars Alfavaca Basilicão and Roxo had the lowest values of LSUC at the lowest salinity, although both did not differ from Lemoncino. At salinity of 3.5 dS m⁻¹, the highest value of LSUC was obtained in the cv. Alfavaca Verde (64.95 mg⁻¹ H₂O cm² leaf) and the lowest value in the cv. Roxo (36.07 mg⁻¹ H₂O cm² leaf), which did

not differ from Grecco a Palla, which in turn did not differ from Alfavaca Basilicão and Lemoncino. For salinity of 5.0 dS m⁻¹, the highest values of LSUC were obtained in the cultivars Alfavaca Verde, Lemoncino and Alfavaca Basilicão, although this last-mentioned cultivar did not differ significantly from the cv. Roxo. The cultivar Grecco a Palla had the lowest value of LSUC for this same salinity (Figure 2D).

Regarding the effect of salinity on LSUC, no significant response was observed in the cultivars Alfavaca Verde and Roxo, which obtained means of 61.88 and 39.29 (g H₂O cm²), respectively. For the cultivar Grecco a Palla, there was a reduction of 58.51% in LSUC when comparing the values obtained at the salinity levels of 2.0 and 5.0 dS m⁻¹. On the other hand, the cultivars Alfavaca Basilicão and Lemoncino showed increments in LSUC with the increase in the salinity of the nutrient solution of 40.96 and 32.03%, respectively (Figure 2D).

Reduction in succulence due to the increase in irrigation water salinity was reported by Aquino et al. (2007) when cultivating different species (cowpea, cotton and sorghum). On the other hand, an increase in leaf succulence in plants subjected to salt stress was also observed by Leite et al. (2017) when working with cowpea.

The increase in leaf succulence is a response of plants when they are subjected to salt stress, being important to dilute the concentration of salts in leaves and avoid the toxic effect under salinity conditions, indicating capacity to adapt to salt stress (Parida et al., 2004).

For stem dry mass (SDM), it was observed that the cultivars differed from each other at all salinity levels, but the differences varied according to salinity. At the lowest salinity (2.0 dS m⁻¹), the highest values were observed in the cv. Grecco a Palla, although it did not differ significantly from Alfavaca Verde. The lowest values of SDM were obtained in the cultivars Lemoncino and Roxo, although Lemoncino did not differ significantly from Alfavaca Basilicão. At salinity of 3.5 dS m⁻¹, the cultivars Grecco a Palla, Alfavaca Basilicão and Alfavaca Verde had the highest values of SDM, not differing significantly from each other, as well as the cultivars Lemoncino and Roxo, which presented the lowest values. For the highest salinity (5.0 dS m⁻¹), the highest values of SDM were observed in the cultivars Alfavaca Basilicão and Alfavaca Verde. The cv. Alfavaca Verde did not differ significantly from Grecco a Palla at salinity of 5.0 dS m⁻¹. The cv. Grecco a Palla, in turn, did not differ from Roxo at that same salinity level. The cultivars Roxo and Lemoncino also did not differ from each other at the highest salinity level (Figure 3A).

Alfavaca Basilicão and Grecco a Palla, which did not differ significantly from each other. The two mentioned last, in turn, did not differ significantly from the cv. Lemoncino, while the lowest TDM value was observed in

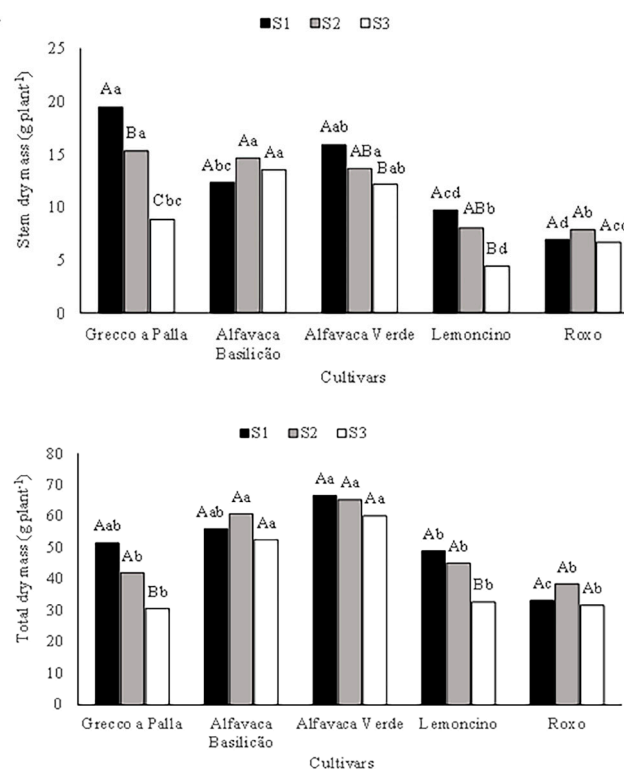


Figure 3 – Stem dry mass (A) and total dry mass (B) in basil cultivars subjected to different salinity levels of the nutrient solution (Uppercase letters represent salinity for each cultivar; lowercase letters represent the cultivars for each salinity (Tukey, 0.05); S1 – 2.0 dS m⁻¹, S2 – 3.5 dS m⁻¹, S3 – 5.0 dS m⁻¹).

Regarding the effect of salinity on SDM, there was a significant response for the cultivars Grecco a Palla, Alfavaca Verde and Lemoncino, which showed reductions of 54.64, 23.58 and 54.47%, respectively, with the increase in salinity, when comparing the salinity levels of 2.0 and 5.0 dS m⁻¹ (Figure 3A).

Reduction in stem dry mass in basil cultivars cultivated in soil and subjected to saline conditions by irrigation water was also observed by Maia et al. (2017), when these plants were subjected to salinity of 5.0 dS m⁻¹. The higher losses observed by these authors can be attributed, in part, to the cultivation system used.

It is also verified that in the cultivars Alfavaca Basilicão and Roxo there was no significant effect of salinity on SDM, obtaining means of 13.48 and 7.14 g plant⁻¹, respectively (Figure 3A), which may show that these cultivars presented higher tolerance to salinity.

For the total dry mass (TDM), it was found that the cultivars differed statistically according to the salinity used. At the lowest salinity level (2.0 dS m⁻¹), the highest value was obtained in the cultivars Alfavaca Verde,

the cultivar Roxo. At the salinity levels of 3.5 and 5.0 dS m⁻¹, the cultivars Alfavaca Verde and Alfavaca Basilicão stood out for having higher TDM, while the other cultivars did not differ from each other (Figure 3B).

When analyzing the effect of salinity levels, it was observed that there was a significant effect for the cultivars Grecco a Palla and Lemoncino, which reduced their TDM by 40.70 and 33.44%, respectively, when comparing the salinity levels of 2.0 and 5.0 dS m⁻¹. The other cultivars did not show a significant response to salinity in terms of TDM, with means of 56.40 g plant⁻¹ for Alfavaca Basilicão, 63.94 g plant⁻¹ for Alfavaca Verde and 34.30 g plant⁻¹ for Roxo (Figure 3B).

Several authors report decrease in total dry mass in basil grown under salt stress conditions (Maia et al., 2017; Silva et al., 2019).

Several factors may be associated with reduced growth in plants when subjected to salt stress, such as low osmotic potential of the solution, interaction of specific ions, nutritional imbalances or the combination of these factors (Marschner, 2012). In addition, excessive amounts of specific salts, such as Na⁺ and Cl⁻, negatively affect the stomata, reducing photosynthesis and, consequently, plant growth (Munns et al., 2006).

When analyzing the salinity tolerance index of the cultivars (Figure 4), it was found that Alfavaca Basilicão, Alfavaca Verde and Roxo showed tolerance to all salinity levels used, as they had relative loss in dry biomass production lower than 20%. The cultivar Grecco a Palla was tolerant to salinity of 3.5 dS m⁻¹ (STI < 20%) and moderately susceptible to salinity of 5.0 dS m⁻¹ (41% < STI < 60%). The cultivar Lemoncino was classified as tolerant to the salinity level of 3.5 dS m⁻¹ (STI < 20%) and moderately tolerant to the level of 5.0 dS m⁻¹ (21% < STI < 40%).

These results agree in part with those presented by Maia et al. (2017), who also observed that the cv. Roxo

was more tolerant to salinity. However, these authors found lower tolerance, probably due to the cultivation system used, because the cultivation in inert substrate promotes greater tolerance of plants to salt stress.

In summary, it was found that all cultivars were tolerant to salinity of up to 3.5 dS m⁻¹ for most of the variables analyzed. This tolerance is higher than that observed by other authors, who found tolerance up to salinity of approximately 1.5 dS m⁻¹ (Bione et al., 2014; Maia et al., 2017). This difference can be attributed to the cultivation system adopted, because the cultivation in substrate allows greater availability of water and, consequently, tolerance (Jordan et al., 2018; Rodríguez-Ortega et al., 2019).

Conclusions

The cultivars Grecco a Palla and Lemoncino reduce their growth and development at salinity of 5.0 dS m⁻¹.

The cultivars Alfavaca Basilicão, Alfavaca Verde and Roxo were tolerant to all salinity levels tested (2.0, 3.5 and 5.0 dS m⁻¹).

The cultivars Grecco a Palla and Lemoncino were tolerant up to salinity of 3.5 dS m⁻¹.

References

- Álvarez, S., Sánchez-blanco, M.J. 2014. Long-term effect of salinity on plant quality, water relations, photosynthetic parameters and ion distribution in *Callistemon citrinus*. *Plant Biology* 16: 757-764.
- Alves, L.S., Gheyi, H.R., Paz, V.P.S., Santos, N.A., Silva, M.G., Bandeira, S.S. 2019. Cultivo de manjeriço utilizando efluente doméstico tratado em sistemas hidropônicos sob diferentes espaçamentos entre plantas. *Irriga* 24: 460-472.
- Andriolo, J.L., Luz, G.L., Giraldi, C., Godoi, R.S., Barros, G.T. 2004. Cultivo hidropônico da alface empregando substratos: uma alternativa a NFT. *Horticultura Brasileira* 22: 794-798.
- Aquino, A.J.S.D., Lacerda, C.F.D., Bezerra, M.A., Gomes Filho, E., Costa, R.N.T. 2007. Growth, dry mass partitioning, and Na⁺, K⁺, and Cl⁻ retention by two sorghum genotypes irrigated with saline water. *Revista Brasileira de Ciência do Solo* 31: 961-971.
- Barbieri, G., Vallone, S., Orsini, F., Paradiso, R., Pascale, S., Negre-zakharov, F., Maggio, A. 2012. Stomatal density and metabolic determinants mediate salt stress adaptation and water use efficiency in basil (*Ocimum basilicum* L.). *Journal of Plant Physiology* 169: 1737-1746.

Benito, A. P., Chiesa, A. 2000. Physiological and productive parameters of basil (*Ocimum basilicum*) cultivars. *Revista Fave* 14: 19-28.

Bernstein, N., Kravchik, M., Dudai, N. 2010. Salinity induced

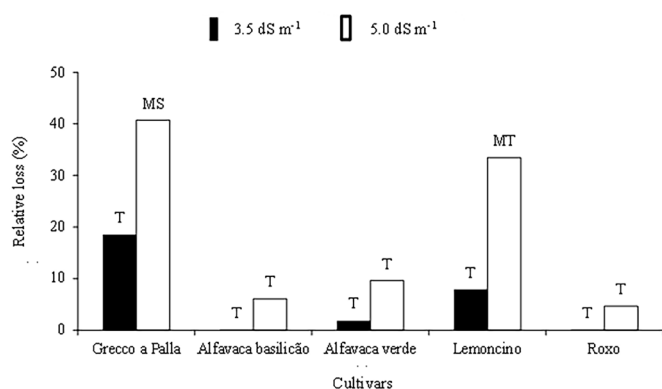


Figure 4 - Relative loss of biomass production and classification of basil cultivars in terms of salinity tolerance of nutrient solution (T – tolerant, MS – moderately susceptible, MT – moderately tolerant).

- changes in essential oil, pigments and salts accumulation in sweet basil (*Ocimum basilicum*) in relation to alterations of morphological development. *Annals of Applied Biology* 156: 167-177.
- Bione, M.A.A., Paz, V.P.S., Silva, F., Ribas, R.F., Soares, T.M. 2014. Crescimento e produção de manjeriço em sistema hidropônico NFT sob salinidade. *Revista Brasileira de Engenharia Agrícola e Ambiental* 18: 1228-1234.
- Bharti, N., Barnawal, D., Wasnik, K., Tewari, S.K., Kalra, A. 2016. Co-inoculation of *Dietzia natronolimnaea* and *Glomus intraradices* with vermicompost positively influences *Ocimum basilicum* growth and resident microbial community structure in salt affected low fertility soils. *Applied Soil Ecology* 100: 211-225.
- Carijo, A.O., Liz, R.S., Makishima, N. 2002. Fibra da casca do coco verde como substrato agrícola. *Horticultura Brasileira* 20: 533-535.
- Chiang, L.C., Ng, L.T., Cheng, P.W., Chiang, W., Lin, C.C. 2005. Antiviral activities of extracts and selected pure constituents of *Ocimum basilicum*. *Clinical and Experimental Pharmacology and Physiology* 32: 811-816.
- Elhindi, K.M., Al-Amri, S.M., Abdel-Salam, E.M., Al-Suhaibani, N.A. 2017. Effectiveness of salicylic acid in mitigating salt-induced adverse effects on different physiobiochemical attributes in sweet basil (*Ocimum basilicum* L.). *Journal of Plant Nutrition* 40: 908-919.
- Elhindi, K.M., El-Din, A.S., Elgorban, A.M. 2017. The impact of arbuscular mycorrhizal fungi in mitigating salt-induced adverse effects in sweet basil (*Ocimum basilicum* L.). *Saudi journal of biological sciences* 24: 170-179.
- Fageria, N.K., Soares Filho, W.S., Gheyi, H.R. 2010. Melhoramento genético vegetal e seleção de cultivares tolerantes à salinidade. In: Gheyi, H.R., Dias, N.S., Lacerda, C.F. *Manejo da salinidade na agricultura: estudos básicos e aplicados*. p.206-218.
- Fernandes, A.R. 2014. *Crescimento de cultivares de manjeriço (Ocimum basilicum L.) cultivadas em vasos*. 49p. (Tese de Doutorado) - Universidade Federal de Viçosa, Viçosa, Brasil.
- Ferreira, D.F. 2011. Sisvar: a computer statistical analysis system. *Ciência e Agrotecnologia* 35: 1039-1042.
- Freitas, M.A.C., Amorim, A.V., Bezerra, A.M.E., Pereira, M.S., Bessa, M.C., Nogueira Filho, F.P., Lacerda, C.F. 2014. Crescimento e tolerância à salinidade em três espécies medicinais do gênero *Plectranthus* expostas a diferentes níveis de radiação. *Revista Brasileira de Plantas Medicinais* 16: 839-849.
- Furlani, P.R., Silveira, L.C.P., Bolonhezi, D., Faquin, V. 1999. *Cultivo hidropônico de plantas*. IAC, Campinas, Brasil. 52p.
- Jordan, R.A., Ribeiro, E.F., Oliveira, F.C., Geisenhoff, L.O., Martins, E.A.S. 2018. Yield of lettuce grown in hydroponic and aquaponic systems using different substrates. *Revista Brasileira de Engenharia Agrícola e Ambiental* 22: 525-529.
- Leite, J.V.Q., Fernandes, P.D., Oliveira, W.J., Souza, E.R., Santos, D.P., Santos, C.S. 2017. Efeito do estresse salino e da composição iônica da água de irrigação sobre variáveis morfofisiológicas do feijão Caupi. *Revista Brasileira de Agricultura Irrigada* 11: 1825-1833.
- Maia, S.S., Silva, R.C., Oliveira, F.D.A.D., Silva, O.M.D.P., Silva, A.C.D., Candido, W.D.S. 2017. Responses of basil cultivars to irrigation water salinity. *Revista Brasileira de Engenharia Agrícola e Ambiental* 21: 44-49.
- Marschner, H. 2012. *Mineral nutrition of higher plants*. Elsevier, London, England. 643 p.
- Menezes, R.V., Azevedo neto, A.D., Gheyi, H.R., Cova, A.M.W., Silva, H.H.B. 2017. Tolerance of basil genotypes to salinity. *Journal of Agricultural Science* 9: 283-295.
- Munns, R., Tester, M. 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology* 59: 651-681.
- Munns, R., James, R.A., Lauchli, A. 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *Journal of Experimental Botany* 57: 1025-1043.
- Parida, A.K., Das, A.B., Mitra, B. 2004. Effects of salt on growth, ion accumulation, photosynthesis and leaf anatomy of the mangrove, *Bruguiera parviflora*. *Trees Structure and Function* 18: 167-174.
- Rodriguez-Ortega, W.M., Martínez, V., Nieves, M., Simón, I., Lidón, V., Zapata, J.C.F., Nicolas, J.J.M., Zapata, J.M.C., Sánchez, F.G. 2019. Agricultural and physiological responses of tomato plants grown in different soilless culture systems with saline water under greenhouse conditions. *Scientific Reports* 9: 6733.
- Scagel, C.F., Lee, J., Mitchell, J.N. 2019. Salinity from NaCl changes the nutrient and polyphenolic composition of basil leaves. *Industrial Crops & Products* 127: 119-128.
- Silva, T.I., Chaves, J.T.L., Bezerra, A.E., Silva, J.S., Santos, H.R., Marco, C.A. 2017. Produção de fitomassa e teor de óleo essencial de *Ocimum basilicum* L. submetido ao estresse salino. *Acta Iguazu* 6: 71-80.
- Silva, H.H.B., Azevedo, A.D., Menezes, R.V., Silva, P.C.C., Gheyi, H.R. 2019. Use of hydrogen peroxide in acclimation of basil (*Ocimum basilicum* L.) to salt stress. *Turkish Journal of Botany* 43: 208-217.
- Taiz, L., Zeiger, E., Møller, I.M., Murphy, A. 2017. *Fisiologia e desenvolvimento vegetal*. Artmed, Porto Alegre, Brasil. 888 p.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

All the contents of this journal, except where otherwise noted, is licensed under a Creative Commons Attribution License attribution-type BY.