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Responses of basil cultivars to irrigation water salinity

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ABSTRACT
The objective of this work was to verify the response of basil cultivars to the salinity of irrigation water during the period from January 15 to March 20, 2013, in a greenhouse, at the Federal Rural University of the Semi-Arid, Mossoró, RN, Brazil. The experimental design was completely randomized, with treatments arranged in 2 x 4 factorial scheme, constituted of two cultivars of basil ('Verde' and 'Roxo') and four levels of irrigation water salinity (0.5; 2.0; 3.5 and 5.0 dS m^{-1}), with four replicates. The plants were collected at 65 days after transplanting and the following variables were evaluated: stem diameter; plant height; number of stems and leaves; leaf area; dry matter of leaves, stems, roots, and total dry matter. The increase in salinity was detrimental to all variables evaluated in both cultivars, but the cultivar 'Roxo' proved to be more tolerant than the cultivar 'Verde'. Both cultivars are tolerant to irrigation water salinity of up to 1.5 dS m^{-1}.

Palavras-chave:
Ocimum basilicum L.
estresse salino
plantas medicinais

Respostas de cultivares de manjericão à salinidade da água de irrigação

RESUMO
O objetivo do trabalho foi verificar as respostas de cultivares de manjericão à salinidade da água de irrigação, no período de 15 de janeiro a 20 de março de 2013, em casa de vegetação, na Universidade Federal Rural do Semiárido, Mossoró, RN. O delineamento experimental foi inteiramente casualizado e os tratamentos arranjados em esquema fatorial 2 x 4, relativo a duas cultivares de manjericão (Verde e Roxo) e quatro níveis de salinidade (0,5; 2,0; 3,5 e 5,0 dS m^{-1}), com quatro repetições. As variáveis avaliadas foram diâmetro do caule e altura das plantas, número de hastes, número de folhas e área foliar, massa seca de folhas, hastes, raiz e total. O aumento da salinidade prejudicou todas as variáveis avaliadas em ambas as cultivares, mas a cultivar Roxo mostrou-se mais tolerante que a cultivar Verde; as duas cultivares são tolerantes à salinidade da água de irrigação até 1,5 dS m^{-1}.

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Introduction

Basil (*Ocimum basilicum* L.) has gained high commercial value due to its use in various market segments in Brazil, such as in food industry, cosmetics and perfumery, pharmaceutical industry and other uses, such as repellent, natural insecticide and ornamental plant, besides being used in popular medicine (Martins et al., 2010; Pereira & Moreira, 2011; Pitaro et al., 2012).

There is a great interest in the commercial production of basil in the Northeast region, because of its multiple uses, especially fresh. However, before starting cultivation on a commercial scale, it is necessary to know the behavior of the species regarding the climatic effects of the planting region, cultivation practices and abiotic factors, since these factors influence the production of basil essential oil (Blank et al., 2010).

In the Northeast region of Brazil, one of the main abiotic factors that can affect the development of the plants is related to the quality of the water used in irrigation, particularly regarding the concentration of dissolved salts (Santos et al., 2016; Vasconcelos et al., 2013).

In the literature, there are few studies on the response of basil to salinity (Fatemi & Aboutalebi, 2012; Heidari, 2012; Bione et al., 2014), reporting that, under salt stress, basil plants suffer reduction in all evaluated growth variables. On the other hand, Delavari et al. (2014) report that basil proved to be tolerant to up to 100 mmol of NaCl (≈ 10 dS m^{-1}) and point out that this species maintains the osmotic adjustment with accumulation of Na^+ and increases the regulation of the enzymatic antioxidant systems. However, these authors evaluated the effect of salinity only during the stage of seedlings.

As presented above, there is not yet a consensus on the tolerance of basil plants to salinity and it is scarce the classification of the basil crop with respect to tolerance to salinity. Therefore, this study aimed to evaluate the difference in the behavior of two basil cultivars in response to irrigation water salinity.

Material and Methods

The study was carried out from January 15 to March 20, 2013, in a greenhouse at the Department of Environmental and Technological Sciences of the Federal Rural University of the Semi-Arid (UFERSA) in Mossoró, RN, Brazil (5° 11’ 31” S; 37° 20’ 40” W; 18 m). The greenhouse had an arch-shaped, transparent cover made of low-density polyethylene, with thickness of 0.10 mm, treated against the action of ultraviolet rays, with width of 7.0 m and length of 21 m. The side and front walls were made of anti-aphid screen, with 0.30-m-high wall made of reinforced concrete.

According to Köppen’s classification, the climate in Mossoró is BSwh', i.e., semi-arid tropical very hot and with rainy season in the summer-winter, with mean temperature of 27.4 °C, very irregular rainfall, with annual mean of 673.9 mm and relative air humidity of 68.9% (Carmo Filho & Oliveira, 1995).

The experimental design was completely randomized with treatments arranged in a 2 x 4 factorial scheme, with four replicates represented by one pot containing 10 dm^3 of substrate, with two plants. The treatments consisted of the combination of two basil cultivars (‘Verde’ and ‘Roxo’) and four levels of irrigation water salinity (0.5, 2.0, 3.5 and 5.0 dS m^{-1}). The saline solutions were obtained by the dissolution of NaCl in water from the supply system of the UFERSA campus, extracted from a deep well, whose physico-chemical analysis showed the following characteristics: EC = 0.50 dS m^{-1}, pH = 8.30; Ca^{2+} = 2.10; Mg^{2+} = 1.10; K^+ = 0.30; Na^+ = 2.30; Cl^- = 1.80; HCO_3^- = 3.00 and CO_3 = 0.20 ($cmol_c$ L^{-1}), obtained using the methodology of EMBRAPA (1997). A benchtop conductivity meter with automatic adjustment of temperature was used to adjust the electrical conductivity of each salinity level.

The seeds were planted on expanded polystyrene trays using coconut fiber as substrate and placing three seeds per cell, with later thinning to maintain the most vigorous plant in each cell. When the seedlings showed five true leaves, they were transplanted to plastic pots with capacity for 12 dm^3, containing 10 dm^3 of soil material classified as eutrophic Red Yellow Argisol (EMBRAPA, 2013), with the following chemical characteristics: pH = 6.5; OM = 10.2; N = 0.6 (g kg^{-1}); P = 10.7; K = 176.7; Na = 35.4 (mg dm^{-3}); Ca = 2.9; Mg = 1.44; H = 1.2 $cmol_c$ dm^{-3} (Donagema et al., 2011).

A drip irrigation system with microtube-type emitters was used and the water was supplied by a tank (100-L fiber box) suspended on sawhorses, maintaining a water column of 1.0 m. The water distribution system was composed of four lateral lines with 16 mm and microtube-type emitters, with length of 0.5 m and mean flow rate of 1.7 L h^{-1}.

Harvest was performed at 65 days after transplantation, when the following variables were evaluated: stem diameter, plant height, number of stems and leaves, leaf area and dry matter of leaves, stems and roots.

Plant height was measured with a graduated ruler. Stem diameter was measured using a digital caliper. Leaf area was obtained using an area integrator (model LI-3100, Licor).

Plants were packed in previously identified paper bags and dried in a forced-air oven, at temperature of 65 °C (±1) until constant weight. Then, plants were weighed on an analytical scale (0.01 g) for the determination of dry matter of leaves, stems, roots and total dry matter.

The obtained data were subjected to analysis of variance. The means of the cultivars were compared by the F test, while the means of the salinity levels were subjected to regression analysis using the software Sisvar (Ferreira, 2011).

The total dry matter production was used to determine the reference of the classification of the genotypes regarding their tolerance to salt stress, considering the percent calculations of relative performance (increase or decrease) and taking as 100% the absolute value of the non-salinized treatment (Fageria et al., 2010), Eq. 1.

$$RP = \left[\frac{(PWoST - PWST)}{PWoST} \right] \times 100 \quad (1)$$

where:

RP - reduction of production, %;

PWoST - production without salinity treatment, g; and, PWST - production with salinity treatment, g.

The basil genotypes were classified regarding their tolerance to salinity according to the following intervals of relative reduction of total dry matter: tolerant, zero to 20%; moderately tolerant, 21 to 40%; moderately susceptible, 41 to 60%, and susceptible, above 60% (Fageria et al., 2010).

Results and Discussion

As indicated in Table 1, except stem growth in height, the interaction between water salinity and cultivars had significant effects on all variables of biometric growth and dry biomass production. These results confirm those obtained by Bione et al. (2014) and Attia et al. (2011), who also observed that basil growth was significantly affected by salinity.

Figure 1 shows the results referring to the effect of salinity on the variables stem diameter, plant height, number of stems, number of leaves and leaf area. Except for the variable plant height, there were different responses of the cultivars to the increase in irrigation water salinity.

There was no significant difference between the cultivars with respect to stem diameter, regardless of the salinity level (Figure 1A). For both cultivars, stem diameter was linearly affected by the increase in irrigation water salinity, exhibiting reductions of 7.3 and 10.7% per unit increase in salinity and total reductions of 32.7 and 48.2% for the cultivars 'Verde' and 'Roxo', respectively (Figure 1A).

Figure 1B shows the results of plant height, which indicates that the highest values were obtained in the cultivar 'Verde', at all salinity levels, which was superior to the cultivar 'Roxo' by approximately 65.5%. For both cultivars, the data fitted to a decreasing linear model, with reduction of 5.77 cm (7.7%) per unit increase in the electrical conductivity of the irrigation water, resulting in total loss of 34.6% at the highest salinity level.

These results agree with those of Bione et al. (2014), who observed linear reduction of 2.09% per unit increase of salinity in basil plants under hydroponic cultivation and, therefore, a reduction much lower than that obtained in the present study. This difference in the response of the crop to salinity for the variable plant height can be attributed to the adopted cultivation system, since in the hydroponic cultivation the plants tend to increase their tolerance to salinity.

The cultivar 'Roxo' was superior to the cultivar 'Verde' for all salinity levels regarding the number of stems. However, the cultivar 'Roxo' showed negative linear response to irrigation water salinity, with reduction of 6.8% in the production of stems per unit increase in salinity, resulting in total loss of 30.6%. On the other hand, the cultivar 'Verde' showed quadratic response to the increase in salinity so that the highest number of stems occurred at the salinity level of 1.9 dS m^{-1} (12.3 stems). From this level on, there was a reduction in this variable and, at the lowest salinity level, there were approximately 11.5 stems per plant (Figure 1C).

These results evidence that the tolerance to salinity is variable among species and, even within the same species, the effect of salt stress is dependent on factors such as the studied cultivar, confirming the results found by Heidari (2012), also working with basil cultivars under saline conditions.

Based on the variables plant height (Figure 1B) and number of stems (Figure 1C), the studied cultivars differ with respect to the growth habit, since the lower height observed in the cultivar 'Roxo' is compensated by the greater production of stems.

For the number of leaves, as observed in the number of stems, the cultivar 'Roxo' surpassed the cultivar 'Verde' at all levels of irrigation water salinity (Figure 1D). Both cultivars had the same number of leaves, decreasing with the increase in irrigation water salinity. However, the cultivar 'Roxo' was more damaged, reducing by 36.15 leaves per plant (12.7%) per unit increase in the electrical conductivity of the irrigation water, while the cultivar 'Verde' showed reduction of 13.34 leaves per plant (13.5%). Comparing the values between plants irrigated with water of highest and lowest salinity levels (0.5 and 5.0 dS m^{-1}), there were reductions of 57.1 and 60.8% in the cultivars 'Verde' and 'Roxo', respectively (Figure 1D).

The results demonstrate that the leaves are sensitive organs, reducing in size and number in the presence of high concentrations of salts. Besides the reduction in the production of new leaves, the reduction in leaf area occurs because of the acceleration of leaf senescence, which may cause the death of the leaves (Mahmoud & Mohamed, 2008). Under salt stress conditions, there are morphological and anatomic alterations in the plants, resulting in the loss of transpiration as an alternative to maintain the absorption of water (Munns & Tester, 2008).

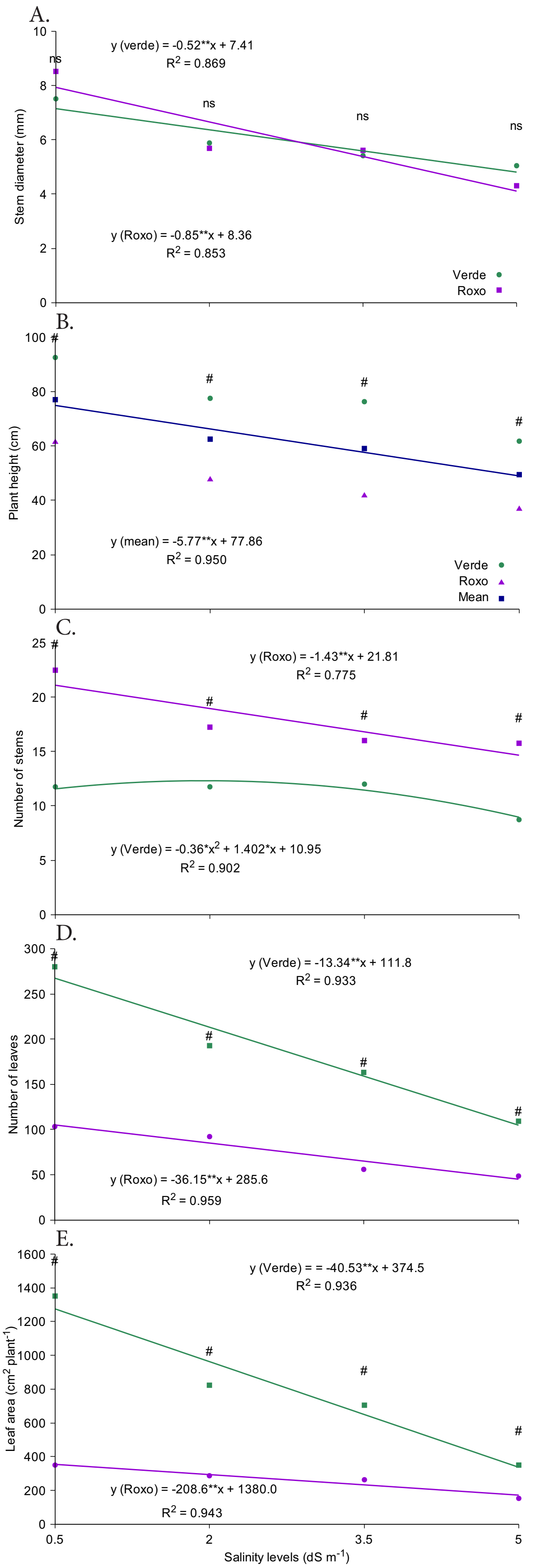
The cultivar 'Roxo', for having higher number of leaves, also showed larger leaf area in comparison to the cultivar 'Verde' at the first three salinity levels (0.5, 2.0 and 3.5 dS m^{-1}). However, the cultivars did not differ significantly at the salinity level of 5.0 dS m^{-1} (Figure 1E). The study conducted by Fernandes (2014), evaluating the development of four basil cultivars ('Anão', 'Folha Larga', 'Grecco' and 'Roxo'), reported that the cultivar 'Roxo' has, as a genetic characteristic, a greater leaf expansion, resulting in larger photosynthetic area.

For both cultivars, the increase in the electrical conductivity of the irrigation water linearly inhibited leaf area, but the

Table 1. Summary of the analysis of variance and test of comparison of means for the variables stem diameter (SD), plant height (PH), number of stems (NS), number of leaves (NL), leaf area (LA), dry matter of stems (SDM), leaves (LDM), roots (RDM) and total dry matter (TDM) of two basil cultivars subjected to irrigation water salinity

Source of variation	DF	Mean squares								
		SD	PH	NS	NL	LA	SDM	LDM	RDM	TDM
Cultivars	1	0.03 ns	7564.50**	371.28**	98679.03**	2257562.82**	32.26**	0.68 ns	1.31 ns	28.75**
Salinity	3	16.28**	1044.58**	32.53**	18627.28**	434792.71**	101.86**	57.86**	11.03**	428.81**
C x S	3	3.27**	41.08 ns	16.78*	4661.36*	267284.08**	4.54*	6.16**	7.01**	38.65**
Residual	24	4.75	39.00	5.36	2200.76	22226.68	0.95	0.55	0.68	2.40
CV (%)		7.41	10.13	16.05	33.22	27.54	19.05	17.92	32.97	23.12

CV - coefficient of variation, ns - not significant, * - significant at 0.05 probability level, ** - significant at 0.01 probability level by F test



**, * - significant at 0.01 and 0.05 probability levels, respectively; #, ns - significant and non-significant differences between the cultivars for each salinity level by the F test (0.05)

Figure 1. Stem diameter (A), plant height (B), number of stems (C), number of leaves (D) and leaf area (E) in two cultivars of basil (*Ocimum basilicum* L.) subjected to different levels of irrigation water salinity

cultivar 'Roxo' showed higher sensitivity, with loss of 16.4% per unit increase in salinity, resulting in total loss of 73.6%, while the cultivar 'Verde' showed linear reduction of 11.4% and total loss of 51.5% at the highest salinity level (Figure 1E).

Negative effect of the increase in salinity on leaf area was also reported by Bernstein et al. (2010) and Tounekti et al. (2012), working with basil and common sage (*Salvia officinalis* L.), respectively. The reduction in leaf area is a response of the plant to salt stress, which has been attributed to the decrease in cell division and to the expansion of leaf surface. As a consequence, it may reduce the capacity of production of photoassimilates, limiting plant growth and yield (Taiz & Zeiger, 2013).

The smaller leaf area of the plants under salt stress reflects the effect of the osmotic potential of the soil solution, inhibiting water absorption by the plant, and the decrease in leaf area is possibly related to one of the adaptive mechanisms of the plant to the salt stress and to the reduction of the transpiring surface (Munns & Tester, 2008).

Regarding the accumulation of stem dry matter (Figure 2), the cultivar 'Verde' was superior at all salinity levels. This variable of biometric growth was compromised in both cultivars, with reductions of 19.8 and 16.8% per unit increase in salinity and losses of 89.3 and 75.6%, in the cultivars 'Roxo' and 'Verde', respectively, when the plants were irrigated with water of highest salinity level (5.0 dS m^{-1}) (Figure 2A).

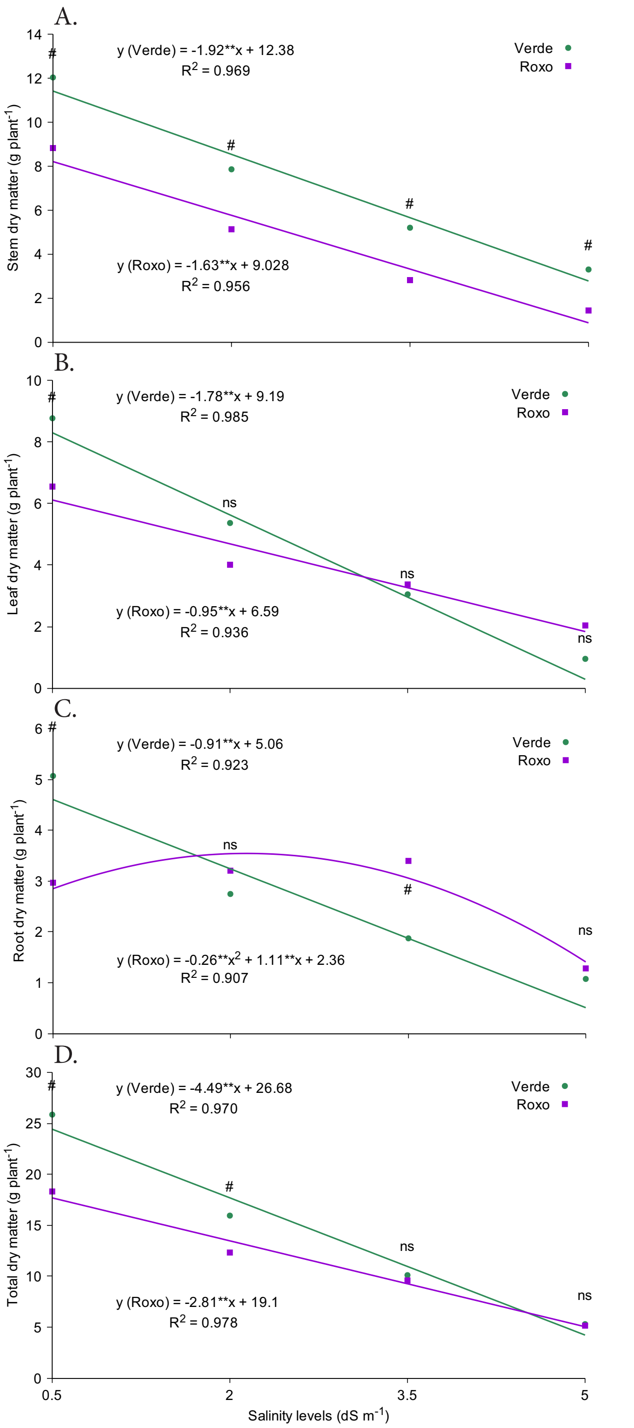
The cultivars were statistically different regarding leaf dry matter when plants were irrigated with water of lowest salinity level (0.5 dS m^{-1}), but there was no difference between the cultivars at the other salinity levels (Figure 2B).

As observed for the variables number of leaves (Figure 1D) and leaf area (Figure 1E), leaf dry matter was also reduced by the increase in salinity. However, different from the number of leaves and leaf area, the cultivar 'Verde' showed lower values, evidencing lower sensitivity to salinity for the accumulation of dry matter in the leaf tissue, with linear reduction of the order of 21.4% per unit increase in salinity and total reduction 96.5% at the highest salinity level (5.0 dS m^{-1}). On the other hand, the cultivar 'Roxo' showed total reduction of 69.9% at the salinity level of 5.0 dS m^{-1} and loss of 15.5% per unit increase in irrigation water salinity (Figure 2B).

Variation in the response of basil genotypes to salinity for biomass production was also reported by Heidari (2012), 17.8 to 28.2%. In study with hydroponic cultivation of basil, Bione et al. (2014) observed linear decrease of 6.76% in the accumulation of shoot dry matter per unit increase in salinity.

As to the accumulation of root dry matter, there were differences between the cultivars at the salinity levels of 0.5 and 3.5 dS m^{-1}, and the cultivar 'Verde' was superior at the first salinity level, but inferior at the second one (Figure 2C).

Still according to Figure 2C, there were different responses to the increase in salinity in both evaluated cultivars. For the cultivar 'Verde', there was a linear reduction in root dry matter with the increase in salinity so that the highest salinity level (5.0 dS m^{-1}) led to loss of 88.9%, which is equivalent to a reduction of 19.7% per unit increase in salinity. On the other hand, the cultivar 'Roxo' showed a quadratic response, with an initial positive response in relation to the increase in salinity up to



** - significant at 0.01 probability level; #, ns - significant and non-significant differences between the cultivars for each salinity level by the F test (0.05)

Figure 2. Dry matter of stems (A), leaves (B), roots (C) and total (D) in two cultivars of basil (*Ocimum basilicum* L.) subjected to different levels of irrigation water salinity

2.1 dS m^{-1} and maximum accumulation of 3.5 g per plant. From this salinity level on, there was a reduction in root dry matter, leading to a decrease of 50.5% at the salinity level of 5.0 dS m^{-1} in comparison to the values obtained at the lowest salinity level (Figure 2C). In study conducted by Bione et al. (2014), using the NFT hydroponic system, the authors also observed reduction in root dry matter in response to salinity; however, in lower intensity.

Regarding the total dry matter (Figure 2D), the cultivar 'Verde' was superior to the cultivar 'Roxo' at the first two salinity levels (0.5 and 2.0 dS m^{-1}), with no significant difference at the other levels. In addition, as observed in most variables, both cultivars showed linear reduction in TDM with the increase in salinity, and the cultivar 'Verde' was more affected by the salt stress. There were total reductions of 82.7 and 71.5%, corresponding to reductions of 18.4 and 15.9% per unit increase in irrigation water salinity (Figure 2D).

Other authors also observed significant reduction in basil dry matter in response to salt stress (Zahedi et al., 2011; Fatemi & Aboutalebi, 2012; Heidari, 2012; Bione et al., 2014; Elhindi et al., 2016). In a study conducted by Bione et al. (2014) in hydroponic system (NFT), the authors observed reduction in TDM of 6.02% per unit increase in salinity, a value lower than that obtained in the present study. This difference may have occurred because, in hydroponic system, plants tend to show greater tolerance to salinity in comparison to cultivation in soil, because the lack of matric potential over the water potential reduces the difficulty of water absorption by plants.

In an overall evaluation of the obtained results, there was a reduction in the growth of both cultivars due to the increase in the salinity level of the irrigation water.

The methodology proposed by Fageria et al. (2010) and the regression equations fitted to the total dry matter production were used to determine the estimate of dry matter production and the classification of the cultivars regarding their tolerance to salinity levels ranging from 0.5 to 5.0 dS m^{-1}, with intervals of 0.5 dS m^{-1} (Table 2).

Both cultivars were classified as tolerant and moderately tolerant to salinity of up to 1.5 and 2.5 dS m^{-1}, respectively. From this salinity level on, there were differences between the cultivars and the cultivar 'Verde' was classified as moderately sensitive to the salinity levels of 3.0 and 3.5 dS m^{-1} and sensitive to salinity levels from 4.0 dS m^{-1} on. On the other hand, the cultivar 'Roxo' was classified as moderately tolerant to the salinity level of 3.0 dS m^{-1}, moderately sensitive to the levels of 3.5 and 4.0 dS m^{-1} and sensitive to salinity above 4.5 dS m^{-1} (Table 2).

In a recent study conducted by Bione et al. (2014) using the cultivation in NFT hydroponic system, basil was classified as tolerant to the salinity up to 1.45 dS m^{-1}, moderately sensitive to salinity levels of 3.80 and 6.08 dS m^{-1} and sensitive to the salinity level of 8.48 dS m^{-1}.

Although there was a reduction of growth in both cultivars due to the increase in salinity, no visual damages with consequences, such as plant death, were observed. Additionally,

Table 2. Relative reduction of the production in % total dry matter of two basil cultivars subjected to different levels of irrigation water salinity

Salinity (dS m^{-1})	Cultivars	
	'Verde'	'Roxo'
1.0	9.2 ^{T}	7.9 ^{T}
1.5	18.4 ^{T}	15.9 ^{T}
2.0	27.6 ^{MT}	23.8 ^{MT}
2.5	36.8 ^{MT}	31.8 ^{MT}
3.0	45.9 ^{MS}	39.7 ^{MT}
3.5	55.1 ^{MS}	47.6 ^{MS}
4.0	64.3 ^{S}	55.6 ^{MS}
4.5	73.5 ^{S}	63.5 ^{S}
5.0	82.7 ^{S}	71.5 ^{S}

T - tolerant; MT - moderately tolerant; MS - moderately susceptible; S - susceptible as observed by Bione et al. (2014), even in plants subjected to the highest salinity levels, there were no deleterious symptoms that could compromise the marketing of the basil plants.

Conclusions

1. The cultivars have different growth habits, with superiority of the cv. 'Verde' for growth in height and cv. 'Roxo' for lateral branching and leaf development.

2. Both cultivars are classified as tolerant to irrigation water salinity of up to 1.5 dS m^{-1}.

3. The cultivar 'Roxo' proved to be more tolerant to irrigation water salinity than the cultivar 'Verde'.

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