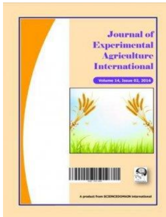

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Growth, Production and Essential Oil Content of Basil Genotypes in Hydroponic Conditions under Salt Stress

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Authors' contributions

This work was carried out in collaboration between all authors. Authors HGF and HRG participated in the conception and management of the experiment, besides writing the article. Authors PCCS, MGS, MMP and RSV were responsible for collecting, tabulating and analyzing data, besides writing the article. Authors ADAN and TMS participated in the management of the experiment and collaborated in interpreting data and writing the article. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Salinity is one of the abiotic factors that most limit the yield of crops. However, the responses may vary according to the genotypic characteristics of each species. This study aimed to evaluate the growth, production and essential oil content of three basil genotypes subjected to salinity in deep flow technique hydroponic system.
Study Design: The experimental design was in randomized blocks, in split plots, with three replicates.

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Methodology: The main plots were used to evaluate six levels of electrical conductivity of the solution (ECsol 2.45; 4.32; 6.38; 8.36; 10.37 and 12.27 dS m^{-1}), obtained by the addition of NaCl in the nutrient solution. In the subplots, three basil genotypes (‘Alfavaca Basilicão’, ‘Grecco a Palla’ and ‘Toscano Folha de Alface’) were studied.

Results: Plant height was less sensitive than stem diameter to salinity. Shoot dry matter decreased by 6.87, 6.84 and 6.47%, respectively, in the genotypes ‘Alfavaca Basilicão’, ‘Toscano Folha de Alface’ and ‘Grecco a Palla’ per unit increase in salinity (dS m^{-1}). The essential oil contents increased by 20.3 and 9.7% per unit increase in salinity (dS m^{-1}) for ‘Grecco a Palla’ and ‘Toscano Folha de Alface’, respectively.

Conclusions: The genotype ‘Alfavaca Basilicão’ was the most recommended for shoot dry matter production up to salinity level of 10.37 dS m^{-1}. At the highest salinity level tested (12.27 dS m^{-1}), there was no difference in shoot dry matter production and absolute growth rate among the genotypes evaluated. The genotype ‘Grecco a Palla’ showed higher essential oil content as salinity increased.

Keywords: Ocimum basilicum L.; Ocimum minimum L.; salinity; essential oil.

1. INTRODUCTION

Soil salinization due to anthropic action in arid and semi-arid regions has mostly been caused by inadequate management of water and soil and by the use of brackish water in irrigation. The low rainfall levels, characteristic of these regions, are not sufficient to leach the salts from the root zone to the deeper layers of the soil, further intensifying the salinization process [1].

Salinity can lead plants to stress conditions and can affect them by means of two components: (1) osmotic component, resulting from the high concentration of solutes in soil solution or irrigation water, which causes water deficit due to the reduction in the osmotic potential; (2) ionic component, resulting from the high levels of Na^+ and Cl^-, causing ionic toxicity [2]. As a consequence of the alteration in the water and ionic homeostasis, decreases in photosynthesis and growth are commonly the symptoms observed in plants under salinity [3].

In addition to the studies related to plant breeding, to increase the tolerance of species to salinity [4], the form of cultivation and management practices can bring benefits for the growth of crops under these conditions. In this context, hydroponic cultivation has been pointed as a viable technique for the use of brackish waters. [5] state that in this system the responses of plants to salinity may be better than those obtained in soil, considering the greater availability of water.

Soilless cultivation of leafy vegetables in a closed hydroponic system has gained interest and popularity among farmers due to the improvement in production and quality [6] and [7], as well as the efficient use of water and nutrients.

In semi-arid regions, brackish water sources are commonly used for agricultural production. To satisfy the local market using brackish water in agricultural cultivation, it is necessary to apply different production methods to increase crop yield. Thus, hydroponic cultivation of medicinal herbs, such as basil, is a viable alternative of cultivation to meet the consumers and increase the income of farmers in these regions.

Basil (*Ocimum basilicum* L.) has great economic importance and can be used for ‘*in natura*’ consumption and essential oil production. Some studies have shown that there may be increase [8] or even reduction [9] in the essential oil content of basil cultivated under salt stress, but these responses may also vary according to the genotype used.

Considering that different species and/or different cultivars of the same species can behave differently when subjected to the same environment, varying in growth, yield and other characteristics of agronomic interest [10], this study aimed to evaluate growth variables and content, yield and productivity of essential oil of three basil genotypes subjected to salinity under hydroponic conditions.

2. MATERIALS AND METHODS

2.1 Description of Experimental Location, Design and Structure

The experiment was carried out in a 7.0-m-wide, 24-m-long, East-West oriented greenhouse from

November to December 2016, at the Water and Soil Engineering Center/NEAS of the Federal University of Recôncavo of Bahia/UFRB, in Cruz das Almas, Bahia, Brazil (12° 40’ 19” S; 39° 06’ 23” W; altitude of 220 m).

The experimental design was in randomized blocks in split plots with three replicates and seven plants in each replicate. Treatments consisted of six levels of electrical conductivity of the nutrient solution (ECsol): 2.45 (control, without salt); 4.32; 6.38; 8.36; 10.37 and 12.27 dS m^{-1} in the main plots and three basil genotypes (‘Alfavaca Basilicão’, ‘Grecco a Palla’ and ‘Toscano Folha de Alface’) in the subplots.

Plants were grown in deep flow technique (DFT) hydroponic system adapted in PVC tubes (forming a 0.02-m-deep film of solution), according to [11]. Plants were spaced by 0.30 × 0.25 m and distributed along 6-m-long tubes. Each plot (cultivation channel) comprised a plastic tank to store the nutrient solution, composed of a ballcock to maintain constant volume (50 L), and an electric pump to pump the solution to the cultivation channel.

2.2 Crop Conduction and Nutrient Solution Management

Seeds of the basil genotypes ‘Alfavaca Basilicão’ and ‘Toscano Folha de Alface’ (*Ocimum basilicum* L.) and ‘Grecco a Palla’ (*Ocimum minimum* L.) were sown in phenolic foam (2 x 2 x 2 cm). Irrigation using public-supply water with electrical conductivity (ECw) of 0.35 dS m^{-1} was performed until seven days after sowing (DAS). Then the seedlings were transferred to a nursery (NFT system - nutrient film technique), where they received Furlani’s nutrient solution [12] at 50% for a period of nine days. Thereafter, the seedlings were transplanted to the definitive cultivation system, receiving a formulation of nutrients at 100%, and the treatments began to be applied. Each cultivation channel (main plot) had 21 plants (seven of each genotype). At transplantation, the mean shoot dry matter of the seedlings was estimated as 0.10 g and used to calculate the absolute and relative growth rates.

The water volume consumed by plants was replaced using public-supply water by an automatic supply system built with PVC tubes with a nominal diameter of 0.20 m, similar to that adopted by [13].

The solution was prepared using water with different levels of salinity (0.35, 2.22, 4.28, 6.26, 8.27 and 10.17 dS m^{-1}), obtained by adding NaCl in public-supply water. After that, the fertiliser salts were added according to the solution described by [12], to reach the respective values of electrical conductivity mentioned in the subitem 2.1. The pH of the solution in the cultivation channel was kept between 5.5 and 6.5 using KOH or HCl.

Nutrient solution recirculation was carried out using an analog timer, at intermittent intervals of 15 min (06:00 to 18:00 h) and every 2 hours (18:00 to 06:00 h), and each irrigation event lasted 15 min.

2.3 Variables Evaluated and Statistical Analysis

Harvest was carried out at 28 days after transplanting (DAT), when the following parameters were determined: plant height (PH), stem diameter (SD) and shoot fresh matter (SFM). Plant height was measured using a ruler from the collar region to the apical meristem. Stem diameter was measured in the collar region, at 2 cm height, using a digital caliper. Immediately after weighing the plants, the material was placed in paper bags and dried in an oven at 45ºC until constant weight, to quantify shoot dry matter (SDM).

Shoot dry matter values were used to calculate the absolute growth rate (AGR, in g day^{-1}) and relative growth rate (RGR, in g g^{-1} day^{-1}), using Eq. 1 and Eq. 2, respectively:

$$\mathrm{AGR} = \frac{\mathrm{SDM}_2 - \mathrm{SDM}_1}{\mathrm{T}_2 - \mathrm{T}_1} \quad (1)$$

$$\mathrm{RGR} = \frac{\mathrm{LnSDM}_2 - \mathrm{LnSDM}_1}{\mathrm{T}_2 - \mathrm{T}_1} \quad (2)$$

Where: SDM_1 (estimated as 0.1 g) and SDM_2 are the values of dry matter at times T_1 (1 DAT) and T_2 (28 DAT).

Essential oil content (OC) was determined based on the dry matter of leaves and inflorescences ($DM_{(l+i)}$) of five plants per plot, through hydrodistillation as described by [10]. Distillation time was equal to 2 h, counted from the moment the first oil drop fell in the collector. The final volume of oil extracted was determined in the Clevenger apparatus. The volumes were used to calculate oil content (OC, mL 100 g^{-1}) (Eq. 3), oil yield (OY, mL $plant^{-1}$) (Eq. 4), and oil productivity (OP, mL m^{-1} tube) (Eq. 5), as described by [10].

$$OC = \frac{Volume_{(oil)}}{DM_{(l+i)} - \left(\frac{DM_{(l+i)} \times U}{100}\right)} \times 100 \qquad (3)$$

$$OY = \frac{OC \times DM_{(l+i)}}{100} \qquad (4)$$

$$OP = OY \times \text{Number of plants} \qquad (5)$$

Where: U is the moisture content (%) of LDM, obtained after drying at 45°C; Number of plants is the number of plants in one meter of cultivation channel.

The data were subjected to analysis of variance by F test. Nutrient solution salinity levels were evaluated by polynomial regression analysis and genotypes were compared by Tukey's test (*P* = 0.05). In the linear regressions, the percentages of increase or decrease per unit of ECsol were obtained by the ratio between the angular and linear coefficients multiplied by 100.

3. RESULTS AND DISCUSSION

The variables plant height, stem diameter, shoot dry matter, absolute growth rate and essential oil content were significantly affected by the interaction between the genotypes tested and ECsol (Table 1). On the other hand, shoot fresh matter, relative growth rate, oil yield and oil productivity were affected in an isolated manner by both factors, genotypes tested and ECsol (Table 1).

Fig. 1A and 1B show that the plant height of the genotypes 'Toscano Folha de Alface' and 'Alfavaca Basilicão' had reduction of 3.34 and 3.72%, respectively. In addition, stem diameter of both genotypes decreased by 5.03 and 4.53%, respectively, per unit increase in ECsol. However, for the genotype 'Grecco a Palla', plant height decreased by 4.41% and stem diameter decreased by 3.50% per unit increase in ECsol.

When the genotypes were compared at each ECsol level (Table 2), highest values of plant height were always found in the genotype 'Alfavaca Basilicão', followed by 'Toscano Folha de Alface' and then by 'Grecco a Palla'. The genotypes 'Alfavaca Basilicão' and 'Toscano Folha de Alface' showed the highest values of stem diameter, except under ECsol of 4.32 dS m^{-1}, at which the genotype 'Toscano Folha de Alface' was superior to the others (Table 2).

The differences in plant height and stem diameter are directly associated with the intrinsic characteristics of each genotype, which have different growth habits, and may or may not be associated with the effect of salinity, as can be observed in the control treatment (Table 2). For instance, the genotype 'Alfavaca Basilicão' stands out with respect to growth in height, whereas the genotype 'Grecco a Palla' has higher lateral branching and number of leaves. Differently from the cultivation in soil, in hydroponics plant architecture is an important information for crop management practices. In hydroponic cultivation, taller plants usually need staking because the weight of the shoots may cause them to lodge.

Regardless of the genotype studied, shoot fresh matter decreased by 6.69% per unit increase in ECsol, and at the lowest salinity level (2.45 dS m^{-1}) a shoot fresh matter production of 159.24 g (Fig. 1E) was estimated. Differing from our results, some studies on salinity found differences between genotypes with respect to shoot fresh matter. [14] observed that the shoot fresh matter of the cv. 'Napoletano' was 45% higher than that of the cv. 'Genovese', under control conditions. Conversely, under salt stress (100 and 200 mM NaCl), these differences decreased to 24 and 10%, respectively, indicating that the deleterious effect of salt stress was higher on the cv. 'Napoletano' than on the cv. 'Genovese'. [15] found higher shoot fresh matter production in *Ocimum minimum* compared with *Ocimum basilicum*, using brackish waters (ECw of 3 and 6 dS m^{-1}) to prepare the nutrient solution.

Shoot dry matter decreased by 6.87, 6.84 and 6.47%, respectively, in the genotypes 'Alfavaca Basilicão', 'Toscano Folha de Alface' and 'Grecco a Palla' per unit increase in salinity (dS m^{-1}) (Fig. 1C). Based on the differences between genotypes at each salinity level (Table 2), it is possible to observe that up to ECsol of 10.37 dS m^{-1} 'Alfavaca Basilicão' had greater shoot dry matter production compared with the others and, therefore, is the most recommended for cultivation in hydroponic system with brackish water up to this limit. Nonetheless, at the highest salinity level (12.27 dS m^{-1}), there were no significant differences in shoot dry matter among the genotypes. Thus, any of the genotypes studied can be recommended for cultivation.

Table 1. Summary of analyses of variance for plant height (PH - cm), stem diameter (SD - mm), shoot fresh matter (SFM - g), shoot dry matter (SDM - g), absolute growth rate (AGR - g day^{-1}), relative growth rate (RGR - g g^{-1} day^{-1}), essential oil content (OC - mL 100g^{-1}), essential oil yield (OY - mL plant^{-1}) and essential oil productivity (OP - mL m^{-1} tube) of three basil genotypes (GEN) grown in a hydroponic system under different salinity levels of the nutrient solution (ECsol) 28 days after transplanting

SV	DF	Means squares								
		PH	SD	SFM	SDM	AGR	RGR	OC	OY	OP
Blocks	2	0.53^{ns}	0.70^{ns}	64.50^{ns}	0.15^{ns}	0.0002^{ns}	0.000006^{ns}	0.028^{ns}	0.00034^{ns}	0.0084^{ns}
ECsol	5	365.37**	34.93**	20190.43**	265.91**	0.3648**	0.00508**	1.699**	0.0072**	0.182**
Residual 1	10	12.48	0.44	203.52	4.65	0.0064	0.00006	0.056	0.00009	0.0023
Genotype (GEN)	2	4157.76**	78.62**	1285.81**	122.70**	0.1683**	0.00110**	0.303**	0.0023**	0.057**
ECsol x GEN	10	25.53**	2.92**	95.70^{ns}	8.84**	0.0121**	0.00006^{ns}	0.360**	0.00024^{ns}	0.006^{ns}
Residual 2	24	5.66	0.34	97.32	1.90	0.0026	0.00004	0.030	0.0002	0.0042
CV 1 (%)		10.90	8.65	14.61	19.78	19.97	4.75	13.08	11.19	11.19
CV 2 (%)		7.34	7.60	10.10	12.66	12.77	3.77	9.58	15.11	15.11
Genotypes		Means[1]								
A	-	-	-	105.86 a	-	-	0.176 a	-	0.0991 a	0.0504 a
G	-	-	-	98.17 a	-	-	0.162 b	-	0.0802 b	0.0218 b
T	-	-	-	88.98 b	-	-	0.163 b	-	0.0792 b	0.0436 ab

*SV – Source of variation; DF – Degrees of freedom; CV- Coefficient of variation; ** Significant at P = 0.01; ns - Not significant. A – ‘Alfavaca Basilicão’, G – ‘Grecco a Palla’, T – ‘Toscana Folha de Alface’. [1]Means followed by different letters indicate significant differences at P= 0.05 (Tukey’s test)*

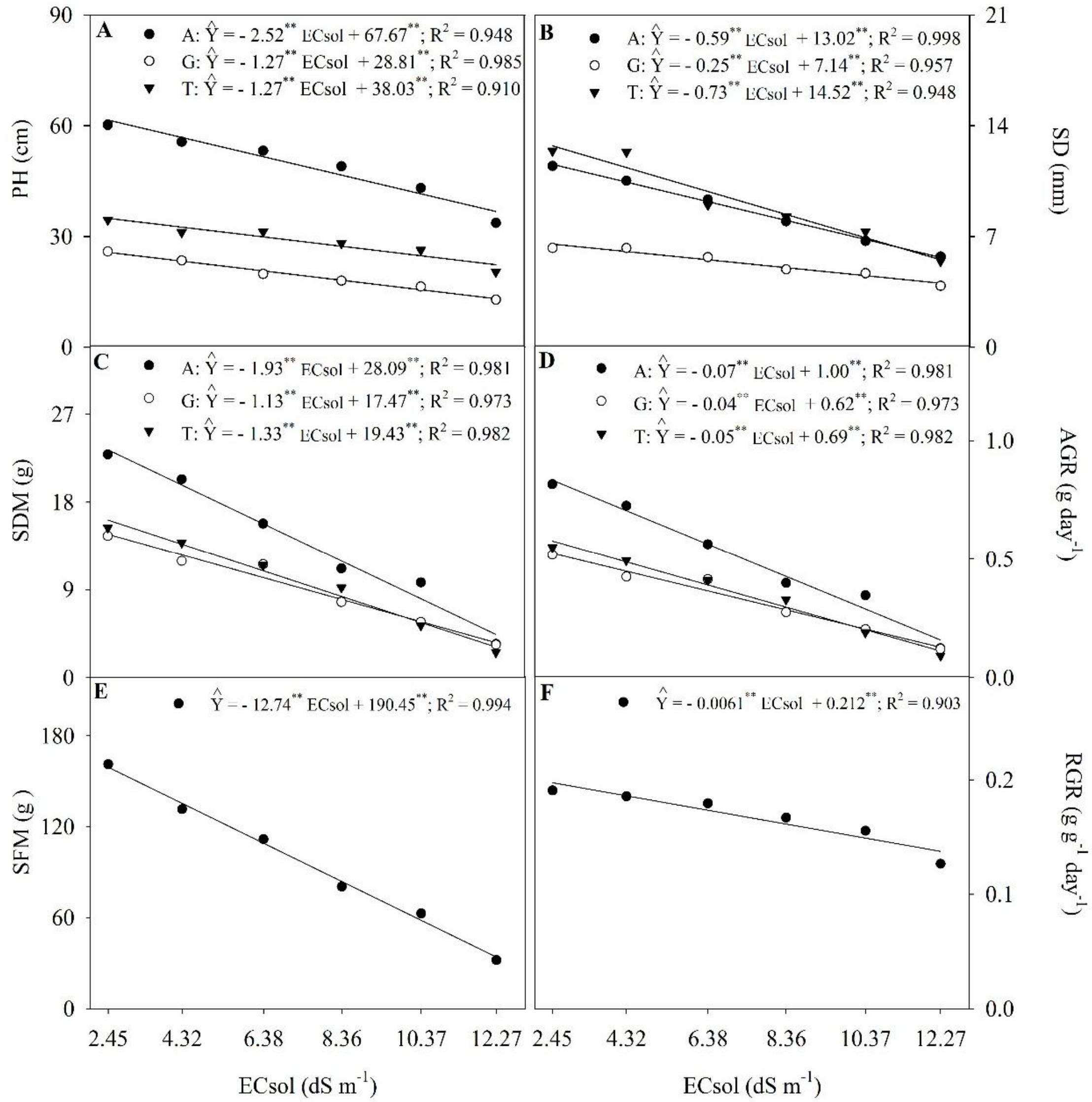

Fig. 1. Effect of salt stress - electrical conductivity of nutrient solution (ECsol) on three basil genotypes grown in a hydroponic system, 28 days after transplanting. Plant height - PH (A), stem diameter - SD (B), shoot dry matter - SDM (C), absolute growth rate - AGR (D), shoot fresh matter - SFM (E) and relative growth rate - RGR (F)

*** Significant at P = 0.01 by Student's test. A – 'Alfavaca Basilicão', G – 'Grecco a Palla' and T – 'Toscana Folha de Alface'*

The degree of severity of salt stress depends on several factors, such as species, genotype, development stage, exposure time [16] and [17] and the types of salts to which plants are subjected [18]. Evaluating the effect of salinity (80 mM NaCl) on six basil genotypes, [19] reported that the 'Toscano Folha de Alface' was more tolerant than the others evaluated. [20] associated the variations in the tolerance among basil genotypes to morphological and physiological characteristics and to metabolic adaptations to salt stress. In basil plants, [18] observed that leaf dry matter, stem dry matter, root dry matter, leaf area, shoot height and root length of basil (*Ocimum basilicum* L. cv. 'Genovese') treated with 50 mM NaCl did not differ from the values in the control treatment (0 mM), but decreased drastically when plants were treated with 25 mM Na_2SO_4.

Table 2. Effect of salt stress - electrical conductivity of nutrient solution (ECsol) on three basil genotypes grown in a hydroponic system, 28 days after transplanting. Plant height (PH), stem diameter (SD), shoot dry matter (SDM), absolute growth rate (AGR) and essential oil contents (OC)

Genotypes[#]	ECsol (dS m^{-1})					
	2.45	**4.32**	**6.38**	**8.36**	**10.37**	**12.27**
	PH (cm)					
A	60.22 a	55.61 a	53.22 a	49.00 a	43.11 a	33.61 a
G	25.94 c	23.50 c	19.83 c	18.00 c	16.44 c	12.89 c
T	34.56 b	31.17 b	31.33 b	28.17 b	26.33 b	20.39 b
	SD (mm)					
A	11.46 a	10.52 b	9.33 a	7.96 a	6.72 a	5.73 a
G	6.27 b	6.26 c	5.69 b	4.92 b	4.67 b	3.88 b
T	12.41 a	12.35 a	9.00 a	8.26 a	7.32 a	5.44 a
	SDM (g $plant^{-1}$)					
A	22.88 a	20.35 a	15.79 a	11.19 a	9.73 a	3.46 a
G	14.53 b	11.93 b	11.61 b	7.74 b	5.69 b	3.36 a
T	15.41 b	13.85 b	11.51 b	9.22 b	5.35 b	2.58 a
	AGR (g day^{-1})					
A	0.843 a	0.750 a	0.581 a	0.410 a	0.357 a	0.124 a
G	0.534 b	0.438 b	0.426 b	0.283 b	0.207 b	0.121 a
T	0.567 b	0.509 b	0.422 b	0.337 ab	0.194 b	0.092 a
	OC (mL 100 g^{-1})					
A	1.15 a	1.58 a	1.52 a	2.36 a	2.46 a	1.53 c
G	1.29 a	1.50 a	1.63 a	1.90 b	2.63 a	2.75 a
T	1.38 a	1.27 a	1.73 a	1.48 c	2.09 b	2.27 b

Means followed by different letters indicate significant differences between means of genotypes in each level of ECsol, at P= 0.05 (Tukey's test). [#]A – 'Alfavaca Basilicão', G – 'Grecco a Palla' and T – 'Toscana Folha de Alface'

Salinity decreased the absolute growth rate of all basil genotypes tested. The largest reduction per unit increase in salinity occurred in the genotype 'Toscano Folha de Alface' (7.25%), followed by 'Alfavaca Basilicão' (7.0%) and 'Grecco a Palla' (6.45%) (Fig. 1D). According to the differences among genotypes at each salinity level (Table 2), it can be noted that up to salinity level of 6.38 dS m^{-1} the genotype 'Alfavaca Basilicão' showed higher absolute growth rate than the others. However, from this level on, the differences among the genotypes decreased and, at the highest level applied (12.27 dS m^{-1}), there was no difference in the absolute growth rate values of the studied genotypes.

The deleterious effects of salinity disturb different physiological and metabolic processes of plants [21], and photosynthesis is one of the most affected [22]. Growth is a process that requires an investment of energy for biomass accumulation. However, stress tolerance mechanisms represent an additional energy cost for the plant to cope with the negative effect of salinity, diverting resources from growth to maintenance [23].

Regardless of the genotype tested, the relative growth rate decreased by 6.68% per unit increase in ECsol. (dS m^{-1}) (Fig. 1F). These results demonstrate that the boost in growth is strongly affected by salinity. This effect on rice cultivars subjected to salt stresses of 8.0 and 15.0 dS m^{-1} was observed by [24].

Essential oil content increased linearly with the ECsol in all genotypes except in 'Alfavaca Basilicão', which reached the highest content (2.15 mL $100g^{-1}$) at ECsol of 8.36 dS m^{-1}. In the 'Grecco a Palla' and 'Toscano Folha de Alface' genotypes, the essential oil content increased by 20.3% and 9.7% per unit increase in ECsol, showing in the treatment with highest ECsol estimated values of 2.62 and 2.09 mL $100g^{-1}$, respectively (Fig. 2A).

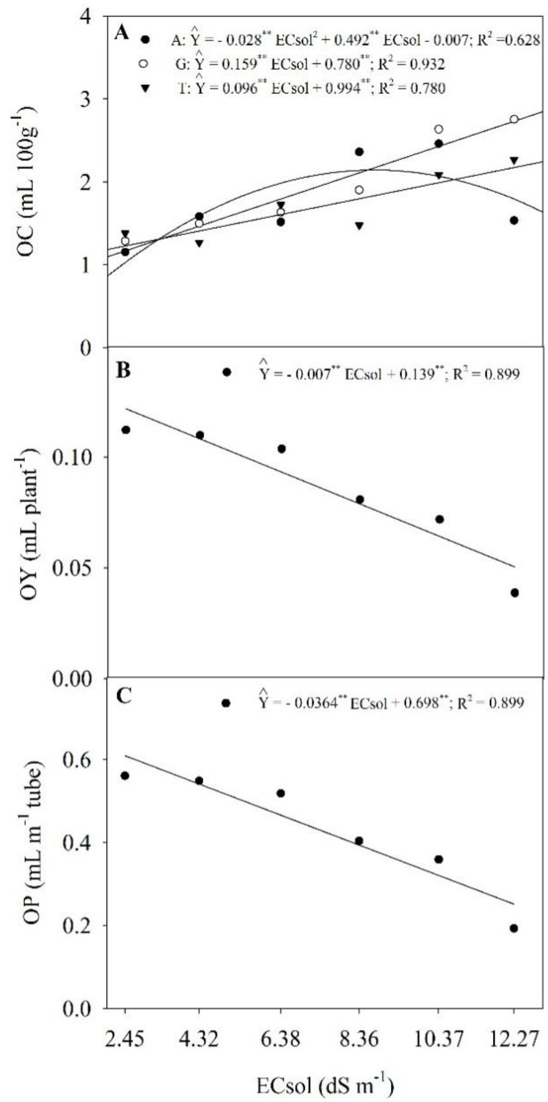

Fig. 2. Effect of salt stress - electrical conductivity of nutrient solution (ECsol) on three basil genotypes grown in a hydroponic system, 28 days after transplanting on essential oil content (A); essential oil yield (B) and essential oil productivity (C)

*** Significant to P = 0.01 by Student's test.*
A – 'Alfavaca Basilicão', G – 'Grecco a Palla' and T – 'Toscana Folha de Alface'

At the low levels of salinity (ECsol 2.45, 4.32 and 6.38 dS m^{-1}), there were no differences in the oil content among the genotypes (Table 2). However, in the higher salinity treatment (ECsol 12.27 dS m^{-1}), the oil content (1.53 mL 100 g^{-1}) of the 'Alfavaca Basilicão' genotype was 44.36% and 17.45%, respectively, lower than in 'Grecco a Palla' (2.75 mL 100g^{-1}) and 'Toscano Folha de Alface' (2.27 mL 100 g^{-1}) genotypes (Table 2).

Regardless of the genotype studied, plants showed decreases in essential oil yield (0.007 mL $plant^{-1}$) and essential oil productivity (0.0364 mL m^{-1} tube), corresponding to per unit increase in ECsol of approximately 5% (Fig. 2B and C). [25] in a study about the effect of salinity on basil, reported that the cv. 'Genovese' obtained maximum essential oil production at NaCl dose of 60 mM in comparison to the cv. 'Rubi', which showed linear increase up to the NaCl dose of 90 mM. Taken together the results of shoot dry matter and oil content, allow inferring that up to 10.37 dS m^{-1} 'Alfavaca Basilicão' is the most indicated genotype for oil production. However, at 12.27 dS m^{-1} the 'Grecco a Palla' genotype showed the same shoot dry matter and higher oil content compared with the others, suggesting that this genotype is the most recommended for oil production at this salinity level.

The salt-induced reduction in shoot dry matter production of basil plants permits the reduction of space required between plants along the profile (cultivation channel), increasing the number of plants per meter of tube and, consequently, increasing the productivity of essential oil. Some studies have shown that the optimization of plant density in hydroponic cultivation improves the production of lettuce, amaranth and basil [6,26] and [7].

According to Fig. 2.C, the mathematical simulation for oil productivity indicates values of 0.6088, 0.3205 and 0.2514 mL m^{-1} tube at ECsol of 2.45, 10.37 and 12.27 dS m^{-1}, respectively. Considering that from 10.37 dS m^{-1} the genotypes showed a reduction of shoot dry matter more than 50%, it is possible to double the oil productivity by doubling the population density. In this scenario, the oil productivity at ECsol of 10.37 and 12.27 dS m^{-1} would be respectively 0.6411 and 0.5027 mL m^{-1} tube, indicating that the basil can be grown, up to 10.37 dS m^{-1} without loss of oil productivity.

4. CONCLUSIONS

The genotype 'Alfavaca Basilicão' was the most recommended for shoot dry matter production up to salinity level of 10.37 dS m^{-1}.

At the highest salinity level tested (12.27 dS m^{-1}), there was no difference in shoot dry matter production and absolute growth rate among the genotypes evaluated.

The genotype ‘Grecco a Palla’ showed higher essential oil content as salinity increased.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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