

Growth, production and essential oil content of basil genotypes in hydroponic conditions under salt stress

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 DFT hydroponic system.

Study design: The experimental design was in randomized blocks, in split plots, with three replicates.

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. Plant height was less sensitive than stem diameter to salinity.

Results: SDM decreased by 6.87, 6.84 and 6.47%, respectively, in the genotypes 'Alfavaca Basilicão', 'Toscano Folha de Alface' and 'Grecco a Palla' with 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 between the genotypes evaluated. The genotype 'Grecco a Palla' showed higher essential oil content as salinity increased.

Keywords: *Ocimum basilicum* L.; *Ocimum minimum* L.; salinity.

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 the 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

28 toxicity [2]. As consequence of the alteration in the water and ionic homeostasis, decreases
29 in photosynthesis and growth are notable symptoms observed in plants under salinity [3].

30 Different species and/or different cultivars of the same species can behave differently when
31 subjected to the same environment, varying in growth, yield and other characteristics of
32 agronomic interest [4].

33 In addition to the studies related to plant breeding, to increase the tolerance of species to
34 salinity [5], the form of cultivation and management practices can bring benefits for the
35 growth of crops under these conditions. In this context, hydroponic cultivation has been
36 pointed as a viable technique for the use of brackish waters. Soares et al. [6] state that in
37 this system the responses of plants to salinity may be better than those obtained in soil,
38 considering the greater availability of water.

39 Soilless cultivation of leafy vegetables in a closed hydroponic system has gained interest
40 and popularity among farmers due to the improvement in production and quality [7] and [8],
41 as well as the efficient use of water and nutrients.

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

47 Basil (*Ocimum basilicum* L.) has great economic importance and can be used for 'in natura'
48 consumption and essential oil production. Some studies have shown that there may be
49 increase [9] or even reduction [10] in the essential oil content of basil cultivated under salt
50 stress, but these responses may also vary according to the genotype used.

51 In this context, this study aimed to evaluate growth variables and production, content and
52 yield of essential oil of three basil genotypes subjected to salinity under hydroponic
53 conditions.

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55 2. MATERIAL AND METHODS

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57 2.1 Description of Experimental Location, Design and Structure

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59 The experiment was carried out in a 7.0-m-wide, 24-m-long, East-West oriented greenhouse
60 from November to December 2016, at the Water and Soil Engineering Center/NEAS of the
61 Federal University of Recôncavo of Bahia/UFRB, in Cruz das Almas, Bahia, Brazil (12° 40'
62 19" S; 39° 06' 23" W; altitude of 220 m).

63 The experimental design was in randomized blocks in split plots with three replicates and
64 seven plants in each replicate. Treatments consisted of six levels of electrical conductivity of
65 the nutrient solution (ECsol): 2.45 (control); 4.32; 6.38; 8.36; 10.37 and 12.27 dS m⁻¹ in the
66 main plots and three basil genotypes ('Alfavaca Basilicão', 'Grecco a Palla' and 'Toscano
67 Folha de Alface') in the subplots.

68 Plants were grown in DFT (deep flow technique) hydronic system adapted in PVC pipes
69 (forming a 0.02-m-deep film of solution), according to [11]. Plants were spaced by 0.30 ×
70 0.25 m and distributed along 6-m-long pipes. Each plot (cultivation channel) comprised a

plastic tank to store the nutrient solution, composed of a ballcock to maintain the 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), when 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. Then, 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 growth rate.

The water volume consumed by plants was replaced using public-supply water (ECw 0.35 dS m⁻¹) by an automatic supply system built with PVC pipes with 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), by adding NaCl in public-supply water. After that, the fertilizer 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). PH was measured using a ruler from the collar region to the apical meristem. SD was measured in the collar region using a digital caliper, at 2 cm height. Immediately after weighing the plants, the material was placed in paper bags and dried in a forced-air oven at 45°C until constant weight, to quantify shoot dry matter (SDM).

SDM 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:

$$AGR = \frac{SDM_2 - SDM_1}{T_2 - T_1} \quad (1)$$

$$RGR = \frac{\ln SDM_2 - \ln SDM_1}{T_2 - T_1} \quad (2)$$

Where: SDM₁ (estimated as 0.1 g) and SDM₂ are the values of dry matter at times T₁ (1 DAT) and T₂ (28 DAT).

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

$$119 \quad OC = \frac{Volume_{(oil)}}{DM_{(l+i)} - \left(\frac{DM_{(l+i)} \times U}{100} \right)} \times 100 \quad (3)$$

$$120 \quad OY = \frac{OC \times DM_{(l+i)}}{100} \quad (4)$$

$$121 \quad OP = OY \times \text{Number of plants} \quad (5)$$

122 Where: U is the moisture of LDM, obtained after drying at 45°C; N° plants is the number of
 123 plants in one meter of cultivation channel.

124

125 The data were subjected to analysis of variance by F test. Nutrient solution salinity levels
 126 were evaluated by polynomial regression analysis and genotypes were compared by
 127 Tukey's test ($P = 0.05$).

128 **3. RESULTS AND DISCUSSION**

129

130 The variables PH, SD, SDM, AGR and OC were significantly affected by the interaction
 131 between the genotypes tested and ECsol. On the other hand, SFM, RGR, OY and OP were
 132 affected by both factors, genotypes tested and ECsol (Table 1).

133

134

135 **Table 1. Summary of analyses of variance for plant height (PH), stem diameter (SD), shoot fresh matter (SFM), shoot dry matter**
136 **(SDM), absolute growth rate (AGR), relative growth rate (RGR), essential oil content (OC), essential oil yield (OY) and essential oil**
137 **production (OP) of three basil genotypes (GEN) under different salinity levels of the nutrient solution (ECsol) in hydroponic**
138 **system, at 28 days after transplantation.**

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
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

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

According to the follow-up test, the genotypes 'Toscano Folha de Alface' and 'Alfavaca Basilicão' had lower reductions in PH (3.34 and 3.72%) than in SD (5.03 and 4.53%) with per unit increase in ECsol (Fig. 1A and B). However, for the genotype 'Grecco a Palla', PH decreased by 4.41% and SD decreased by 3.50% per unit increase in ECsol. When the genotypes were compared at each ECsol level (Table 2), highest values of PH 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 SD, except under ECsol of 4.32 dS m⁻¹, at which the genotype 'Toscano Folha de Alface' was superior to the others (Table 2).

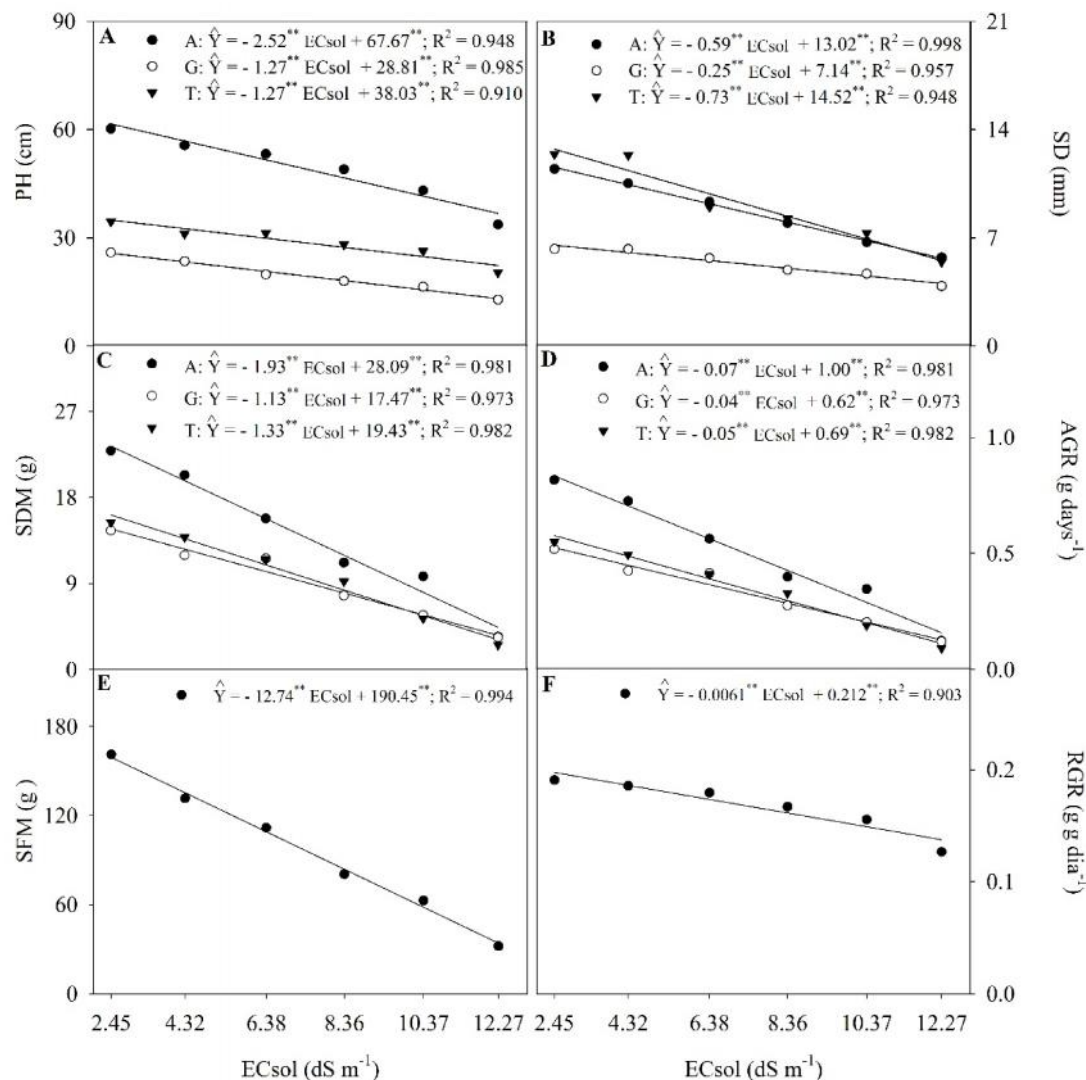


Fig. 1. Follow-up tests of the interaction between nutrient solution electrical conductivity levels (ECsol) and basil genotypes for plant height (A), stem diameter (B), shoot dry matter (C), absolute growth rate (D); individual effect of ECsol on shoot fresh matter (E) and relative growth rate (F) in hydroponic system, at 28 days after transplantation.

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

The differences in PH and SD 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, SFM decreased by 6.69% per unit increase in ECsol, and was estimated a SFM production of 159.24 g plant⁻¹ at the lowest salinity level (2.45 dS m⁻¹) (Fig.1E). Differing from our results, some studies on salinity found differences between genotypes with respect to SFM. [14] observed that the SFM of the cv. 'Napoletano' was 45% higher than that of the cv. 'Genovese', under control conditions. Conversely, under salt stress (100 and 200 mM of NaCl), the difference 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 SFM production in *Ocimum minimum* compared with *Ocimum basilicum*, using brackish waters (ECw of 3 and 6 dS m⁻¹) to prepare the nutrient solution.

SDM decreased by 6.87, 6.84 and 6.47%, respectively, in the genotypes 'Alfavaca Basilicão', 'Toscano Folha de Alface' and 'Grecco a Palla' with 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 10.37 dS m⁻¹ the genotype 'Alfavaca Basilicão' had greater SDM production compared with the others and, therefore, is the most recommended for cultivation in hydroponic system. Nonetheless, at the highest salinity level (12.27 dS m⁻¹), there were no significant difference in SDM among the genotypes. Thus, any of the genotypes studied can be recommended for cultivation.

Table 2. Follow-up tests of the interaction between nutrient solution electrical conductivity levels and basil genotypes for plant height (PH), stem diameter (SD), shoot dry matter (SDM), absolute growth rate (AGR) and essential oil contents (OC) in hydroponic system, at 28 days after transplantation.

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 dia ⁻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 100g ⁻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 – 'Toscano 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 of NaCl) on various basil genotypes, [19] reported that the genotype 'Toscano Folha de Alface' was more tolerant than the others evaluated. [20] associated these variations in the tolerance of each genotype 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 of NaCl did not differ from the values in the control treatment (0 mM), but decreased drastically when plants were treated with 25 mM of Na₂SO₄.

Salinity negatively affected the AGR 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 between 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 growth rate than the others. However, from this level on, the differences in the growth rate between genotypes gradually decreased and, at the highest level applied (12.27 dS m⁻¹), there was no difference in AGR between the studied genotypes.

The deleterious effects of salinity affect different physiological and metabolic processes of plants [21], and photosynthesis is one of the most affected [22]. Plants depend on a set of factors to survive stress, which occurs when the amount of energy acquired by plants is reduced (reduction in photosynthetic rate or leaf area), being redistributed from growth to its maintenance under stress [23].

Regardless of the genotype tested, the RGR decreased by 6.68% with per unit increase in salinity (dS m⁻¹) (Fig. 1F). These results demonstrate that the boost in growth is heavily affected by salinity. It has been demonstrated that the parameter RGR is strongly influenced by the concentrations of Na⁺ ions in plant tissue. 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 cultivars except in the genotype 'Alfavaca Basilicão', which reached the highest content (2.15 mL 100g⁻¹) at ECsol of 8.36 dS m⁻¹. In the other genotypes, 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. In the treatment with highest ECsol (12.27 dS m⁻¹), oil contents of 2.62 and 2.09 mL 100g⁻¹ were estimated for the genotypes 'Grecco a Palla' and 'Toscano Folha de Alface', respectively (Fig. 2A).

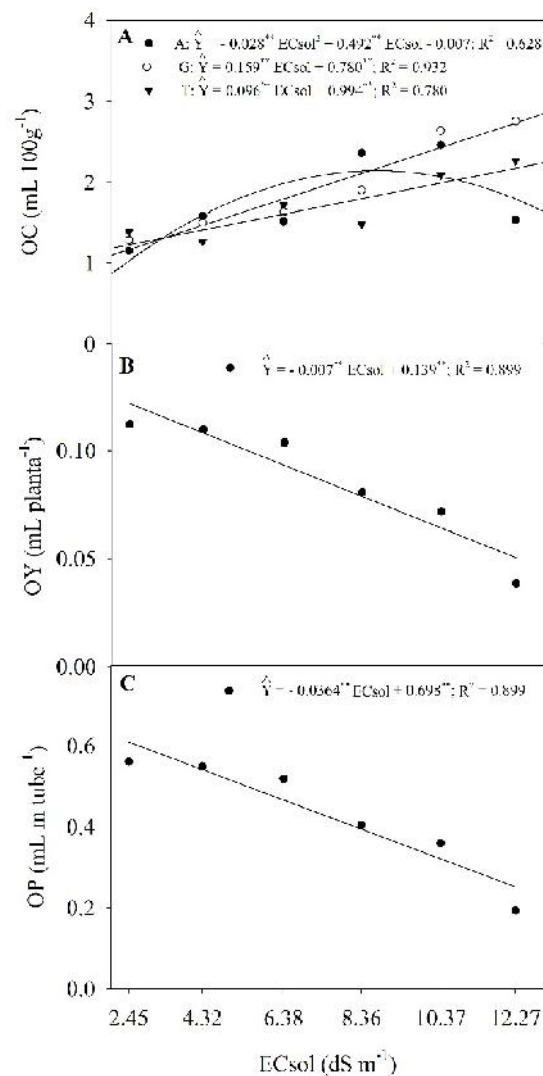


Fig. 2. Follow-up tests of the interaction between nutrient solution electrical conductivity levels (ECsol) and basil genotypes for essential oil content (A); effect of ECsol on essential oil yield (B) and essential oil production (C) in hydroponic system, at 28 days after transplantation.

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

At the low levels of salinity (2.45, 4.32 and 6.38 dS m⁻¹), there were no differences in the oil contents between the genotypes (Table 2). However, in the treatment of highest salinity (12.27 dS m⁻¹) there was a strong reduction (44.36%) in the oil content of the genotype 'Alfavaca Basilição', in comparison to 'Grecco a Palla' (44.36%), whereas the genotype 'Toscano Folha de Alface' showed a reduction of 17.45% (Table 2).

Regardless of the genotype studied, essential oil yield and production decreased by approximately 5% per unit increase in salinity (dS m⁻¹) (Fig. 2B and C). Based on a joint analysis of the results for SDM and essential oil content, it can be inferred that at the low levels of salinity (2.45, 4.32 and 6.38 dS m⁻¹) the genotype 'Alfavaca Basilição' had higher SDM and the same OC compared with the others. However, at the highest salinity level tested (12.27 dS m⁻¹) this genotype showed the same SDM and lower OC compared with the others, which suggests that, under high salinity, this genotype may not be recommended for oil production. Talebi et al.[25], in a study about the effect of salinity on basil, reported that the cv. 'Genovese'

249 obtained maximum essential oil production at NaCl dose of 60 mM in comparison to the cv.
250 'Rubi', which showed linear increase up to the NaCl dose of 90 mM.

251 Reduction in SDM production of basil plants with increasing salinity permits the reduction of
252 space between plants along the profile (cultivation channel) and, consequently, the increase in
253 the number of plants and in essential oil yield and production per linear meter. Some authors
254 claim that optimization of plant density in hydroponic cultivation improves the production of
255 lettuce, amaranth and basil [7]. [26] and [8].

256 The analysis of the results of this study indicates that, at the highest salinity level (12.27 dS m⁻¹
257 ¹), even with a reduction in SDM, the increase in essential oil content in the genotype 'Grecco a
258 Palla' would allow higher production of essential oil with an increase in plant density. Simulating
259 if half of the plant spacing (12.25 cm) was adopted in the treatment of highest salinity (12.27 dS
260 m⁻¹), it would allow to double the essential oil production in the genotype 'Grecco a Palla'.

261

262 4. CONCLUSIONS

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264 In general, basil growth was negatively affected by salinity, and plant height is the least
265 sensitive variable to the increase in nutrient solution salinity.

266 The genotype 'Alfavaca Basilicão' is the most recommended for shoot dry matter production up
267 to salinity of 10.37 dS m⁻¹ in the solution.

268 The genotype 'Grecco a Palla' has higher essential oil content under salt stress conditions.

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