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COMPARATIVE VOLATILOME PROFILE AND PHYSIOLOGICAL RESPONSES OF THREE BASIL GENOTYPES UNDER SALINITY

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ABSTRACT: Medicinal plants, such as basil (*Ocimum basilicum*), are widely used in folk medicine, industry and culinary. However, little is known when it is submitted to adverse environmental conditions. Thus, this work focuses on the characterization of the physiological and volatile profile of three basils cultivars challenged with 50 mM NaCl. In the absence of salinity, the cv. Limoncino exhibited higher growth followed by Alfavaca and Gennaro cultivars. Under salinity, Alfavaca presented reduced growth and increased root length and leaf K^+ content. Gennaro showed a decrease of K^+ in both leaves and roots. The cv. Limoncino exhibited a decrease in all parameters of gas exchange, while K^+ levels were increased in the leaves and decreased in the roots. Principal component analyses (PCA) displayed a separation between cultivars and treatments. Major volatile organic compounds (VOCs) were eucalyptol and linalool in cv. Alfavaca, methyl chavicol, and eucalyptol in cv. Gennaro, and citral and methyl chavicol in cv. Limoncino. Different minor VOCs were induced by salinity in Gennaro and Limoncino cultivars. In conclusion, Alfavaca and Limoncino cultivars were more sensitive and gennaro was less sensitive presenting regular growth and remarkable increase of VOCs.

KEYWORDS: *Ocimum basilicum*, salt stress, volatile organic compounds

COMPARAÇÃO DO PERFIL VOLÁTIL E RESPOSTAS FISIOLÓGICAS DE TRÊS GENEÓTIPOS DE MANJERICÃO SOB SALINIDADE

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RESUMO: Plantas medicinais como manjericão (*Ocimum basilicum*) são amplamente utilizadas na medicina, indústria e culinária. Entretanto, pouco se sabe quando submetidas às condições ambientais adversas. Assim, o objetivo desse trabalho foi a caracterização fisiológica e perfil volátil de três cultivares de manjericão sob 50 mM de NaCl. Na ausência de sal, Limoncino apresentou maior crescimento seguido por alfavaca e Gennaro. Sob salinidade, Alfavaca reduziu massa e comprimento da parte aérea, e aumentou o comprimento da raiz e teores de K^+ nas folhas. Gennaro apresentou apenas diminuição em K^+ em folhas e raízes. Limoncino apresentou diminuição nas trocas gasosas enquanto os níveis de K^+ aumentaram nas folhas e diminuíram nas raízes. Análise dos componentes principais (PCA) mostrou uma separação entre cultivares e tratamentos. Compostos majoritários foram eucaliptol e linalol na alfavaca, metil cavicol e eucaliptol no Gennaro; citral e metil cavicol no Limoncino. Compostos minoritários também foram induzidos pelo sal nas cv. Gennaro e Limoncino. Como conclusão, os cultivares Alfavaca e Limoncino foram mais sensíveis e Gennaro foi menos sensível sem prejuízo no crescimento e trocas gasosas e apresentando um aumento de compostos.
PALAVRAS-CHAVE: *Ocimum basilicum*, estresse salino, compostos orgânicos voláteis

INTRODUCTION

There are more than 25 different types of basil (*Ocimum basilicum*) in the world based on morphological, and chemical constituents (LIBER et al., 2011). Its use is linked to respiratory problems, antiseptics, anti-spasm, anti-fever, and improving digestion (BAYALA et al., 2014). Besides the healing properties, this plant can be used in many areas such as an insect repellent, food industry, and essential oils production in the cosmetic purpose (FERNANDES et al., 2019). Indeed, volatile chemical compounds (VOCs) released from leaves may improve crop production and protect plants from pathogens and environmental stresses (BRILLI; LORETO; BACCELLI, 2019). Among such environmental stresses, salinity stands as a natural phenomenon in areas with low rainfall, high evapotranspiration, high temperatures, poor quality water irrigation, and inadequate soil management practices (ZÖRB; GEILFUS; DIETZ, 2019). The behavior of different basil cultivars in saline soils is scarce, it is important to analyze the physiological responses to distinguish possible genotypes for the study of molecules and active principles, as well as tolerant and sensitive varieties. Thus, the aim of this research was to evaluate three cultivars of *O. basilicum*: Alfavaca, Gennaro, and Limoncino under 50 mM of NaCl, and its capacity to keep the growth, photosynthesis, K^+/Na^+ ions, and major VOCs production. Accordingly, this current study integrated phenotypic, biochemical and metabolic analyses concerning the importance of preserving plant diversity and productivity.

MATERIAL AND METHODS

Three basil (*Ocimum basilicum* L.) cultivars: Alfavaca, Gennaro, and Limoncino, were sown in vermiculite. Seedlings 53 days old were acclimatized in Clark solution for 14 days, then subjected to saline stress with 50 mM NaCl for seven days. Exchange parameters were performed in the third fully expanded leaf using an infrared gas analyzer (LI- 6400XT), 1200 $\mu mol\ m^{-2}\ s^{-1}$ of light. Volatiles were extracted by SPME/GCMS method using a DVB/CAR/PDMS (50/30 µm) fiber, in an Agilent model CG-7890B / EMD-5977A (quadrupole), with electron impact at 70 eV, HP-5MS methylpolysiloxane column (30 m x 0.25 mm x 0.25 µm). Compounds identification was performed by fragmentation patterns of mass spectra compared to the database provided by the equipment (NIST, 2016), and from literature data. The data were subjected analysis of variance (ANOVA F-test) and the mean treatment values were separated by a Turkey test ($p \leq 0.05$), using the SISVAR program.

RESULTS AND DISCUSSION

The variable response to salt stress displayed by Alfavaca, Genaro and Limoncino plants was interesting. Morphological, physiological and metabolic response to stress may provide insights into new approaches to growing a single genotype to obtain different compounds. Thus, in general, under control conditions, cv. Limoncino was the cultivar with high growth scores, although gas exchange measurements were not different from Alfavaca and Gennaro cultivars (Table 1). Salinity promoted the increase of root length in the cv. Alfavaca, despite, lower shoot dry and fresh weight, root dry weight, and shoot length were all decreased. Additionally, salinity did not change the growth or gas exchange parameters in cv. Gennaro, on the other hand, shoot fresh weight, and all gas exchange parameters were decreased in cv. Limoncino. Indeed, in accordance to (BARBIERI et al., 2012), basil plants under saline soil presented a decreased stomatal conductance and assimilation ratio as a response, and inhibition of growth, as observed in Alfavaca and Limoncino. Further, the cultivar Gennaro was able to keep the growth along photosynthetic maintenance in favor of secondary metabolites biosynthesis and accumulation, which may be related to antioxidative properties from VOCs.

The K^+ and Na^+ contents were also evaluated (Table 1). Under control conditions, leaf K^+ contents were similar in both Alfavaca and Limoncino cultivars, but lower than cv. Gennaro, on the other hand, ions contents in roots were similar in all three cultivars. Besides, no differences were recorded in Na^+ contents among all control basil cultivars neither leaves and roots, (BARBIERI et al., 2012). After salt treatment, Na^+ contents were considerably higher in both leaves and roots, as expected. Although in Alfavaca and Limoncino leaves the K^+ contents were increased, in cv. Gennaro, it was decreased. On the other hand, in roots of all three cultivars, the K^+ amounts were decreased. In agreement the reduced transpiration flux and lower stomatal conductance observed in Alfafaca and Limoncino may act as response to salt to decrease the movement of toxic Na^+ to the shoots.

Table 1. Physiological parameters of cultivars submitted to regular nutrition (Control) or 50 mM NaCl. Tukey test, $p \leq 0.05$, n=5. Lowercase letters represent differences among cultivars in the same salt treatment and capital letters represent differences due to different salt treatment in the same cultivar

	Alfavaca		Gennaro		Limoncino	
	Control	NaCl	Control	NaCl	Control	NaCl
Leaf Area (cm^2 $plant^{-1}$)	565.42 Aa	635.80 Aa	712.77 Aa	660.66 Aa	1040.66 Ab	816.01 Aa
SFW (g $plant^{-1}$)	38.30 Ba	26.70 Aa	35.00 Aa	26.70 Aa	56.70 Bb	31.70 Aa
RFW (g $plant^{-1}$)	13.30 Aa	13.30 Aa	10.00 Aa	15.00 Aa	21.70 Ab	25.00 Ab
SDW (g $plant^{-1}$)	3.79 Bab	2.62 Aa	3.07 Aa	2.80 Aa	4.49 Ab	3.78 Ab
RD (g $plant^{-1}$)	0.86 Bb	0.65 Aa	0.59 Aa	0.71 Aa	0.83 Ab	0.93 Ab
SL (cm)	45.23 Bb	40.71 Aa	40.62 Aa	41.69 Aa	45.18 Ab	42.44 Aa
RL (cm)	18.82 Aa	24.14 Ba	22.65 Aa	21.75 Aa	36.41 Ab	36.35 Ab
A (μmol CO_2 m^{-2} s^{-1})	24.08 Aa	20.17 Aab	24.66 Aa	25.39 Ab	26.60 Ba	14.56 Aa
gs (mmol H_2O m^{-2} s^{-1})	0.71 Aa	0.66 Aa	0.78 Aa	0.69 Aa	0.89 Ba	0.36 Aa
E (mmol H_2O m^{-2} s^{-1})	10.13 Aa	9.88 Aa	11.17 Aa	11.13 Aa	13.12 Ba	6.81 Aa
Ci/Ca	0.83 Aa	0.84 Ab	0.83 Aa	0.81 Ab	0.84 Ba	0.65 Aa
Leaf Na^+ (mmol g^{-1} DW)	165.96 Aa	3480.06 Bb	218.89 Aa	2690.83 Ba	179.06 Aa	3510.87 Bb
Leaf K^+ (mmol g^{-1} DW)	1664.91 Aa	2259.61 Bb	2736.32 Bb	1812.53 Aa	1707.56 Aa	2062.75 Bab
Root Na^+ (mmol g^{-1} DW)	265.57 Aa	2432.02 Bb	262.75 Aa	2012.82 Ba	282.12 Aa	2440.80 Bb
Root K^+ (mmol g^{-1} DW)	1407.61 Ba	511.71 Aa	1976.20 Bb	330.24 Aa	1952.95 Bb	781.55 Ab

Shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RD), shoot length (SL), root length (RL), photosynthesis assimilation (A), stomatal conductance (*gs*), transpiration (E) and ratio of internal and ambient carbon concentration (Ci/Ca).

Several basil cultivars have the genetic ability to produce distinct sets of volatile organic compounds (VOCs), building an extensive family of chemotypes inside the same species

(COUTO et al., 2019). The three basil cultivars used in this study differed in of volatile profile present in leaf extracts under non-stress conditions, including phenylpropanoids, benzenoids and flavonoids are known for their antioxidant activities (BALASUNDRAM; SUNDRAM; SAMMAN, 2006). Here the VOCs identified are listed by elution of the chromatographic column (Table 2).

Table 2. Volatile organic compounds (VOCs) of cultivars submitted to regular nutrition (Control) or 50 mM NaCl. Tukey test, p≤0.05, n=3. Lowercase letters represent differences among cultivars in the same salt treatment and capital letters represent differences due to different salt treatment in the same cultivar

Cultivars		Alfavaca				Gennaro				Limoncino			
VOCs	KI	Control		NaCl		Control		NaCl		Control		NaCl	
4-M-cyclohexanone	705	0.00	Aa	0.00	Aa	6.49	Ba	0.00	Aa	6.38	Ba	0.00	Aa
M-heptyl carbonate	738	0.00	Aa	0.00	Aa	0.00	Aa	0.00	Aa	2.82	Aa	3.64	Aa
Hydrazinecarboxylic	774	0.00	Aa	0.00	Aa	0.00	Aa	3.06	Bb	0.00	Aa	0.00	Aa
Camphene	953	0.00	Aa	0.00	Aa	0.00	Aa	15.29	Bb	0.00	Aa	0.00	Aa
β-Phellandrene	977	9.59	Aa	4.86	Aa	0.00	Aa	16.35	Bb	0.00	Aa	0.00	Aa
trans-Sabinene	977	0.00	Aa	0.00	Aa	0.00	Aa	3.84	Bb	0.00	Aa	0.00	Aa
β-Pinene	980	18.75	Bb	3.43	Aab	0.00	Aa	11.71	Bb	0.00	Aa	0.00	Aa
Hydroxycineol	989	0.00	Aa	0.00	Aa	0.00	Aa	0.00	Aa	0.00	Aa	4.46	Bb
α-Phellandrene	1005	0.00	Aa	0.00	Aa	0.00	Aa	6.73	Bb	0.00	Aa	0.00	Aa
Carene	1012	10.75	Aa	7.15	Aa	3.15	Aa	12.86	Ba	0.00	Aa	3.27	Aa
Dichlorobenzene	1015	0.00	Aa	0.00	Aa	0.00	Aa	2.89	Bb	0.00	Aa	0.00	Aa
α-Terpinene	1028	0.00	Aa	0.00	Aa	0.00	Aa	11.73	Bb	0.00	Aa	0.00	Aa
Cymene	1028	0.00	Aa	0.00	Aa	0.00	Aa	37.32	Bb	0.00	Aa	0.00	Aa
Limonene	1032	0.00	Aa	0.00	Aa	0.00	Aa	35.58	Bb	0.00	Aa	4.24	Aa
Eucalyptol/Cineole	1035	115.70	Aa	142.07	Ab	20.38	Aa	119.17	Aab	0.00	Aa	0.00	Aa
γ-Terpinene	1062	0.00	Aa	0.00	Aa	0.00	Aa	11.89	Bb	0.00	Aa	0.00	Aa
Fenchone	1091	0.00	Aa	0.00	Aa	0.00	Aa	0.00	Aa	0.00	Aa	4.86	Bb
Linalool	1100	78.25	Ab	82.31	Aa	16.71	Aab	53.60	Aa	0.00	Aa	20.74	Aa
Camphor	1150	4.03	Bb	0.00	Aa	0.00	Aa	3.03	Ba	0.00	Aa	0.00	Aa
ci-Verbenol	1185	0.00	Aa	0.00	Aa	0.00	Aa	0.00	Aa	0.00	Aa	5.14	Bb
Methyl chavicol	1203	0.00	Aa	0.00	Aa	0.00	Aa	297.53	Bb	12.20	Aa	46.06	Aa
Cyclohexanethanol	1232	0.00	Aa	0.00	Aa	0.00	Aa	0.00	Aa	0.00	Aa	4.75	Bb
Citral	1274	0.00	Aa	0.00	Aa	0.00	Aa	0.00	Aa	23.76	Aa	107.56	Bb
Anethole	1342	0.00	Aa	0.00	Aa	0.00	Aa	9.90	Ba	0.00	Aa	0.00	Aa
Eugenol	1362	14.51	Bb	0.00	Aa	0.00	Aa	0.00	Aa	0.00	Aa	0.00	Aa
α-Bergamotene	1441	11.01	Bb	2.93	Aa	0.00	Aa	2.13	Aa	0.00	Aa	0.00	Aa

Kovats index (KI), Volatile organic compounds (VOCs)

Salinity significantly alter total VOCs in the three cultivars evaluated. In the cv. Alfavaca, eight VOCs were identified in control treatments, and six were identified in salt treatment, in which eucalyptol and linalool were the two major compounds, but not statistically different. In the cv. Gennaro, only four VOCs were identified under control conditions. However, under salinity eighteen VOCs were identified, in which methyl chavicol and eucalyptol were the major compounds. In the cv. Limoncino, only four VOCs were identified under control conditions, and ten VOCs were identified under salt stress, in which citral and methyl chavicol were the major compounds. Previous works have showed methyl chavicol and linalool as the major components of several different cultivars under salt stress (HASSANPOURAGHDAM et al., 2011; TARCHOUNE et al., 2013). Increased VOCs emission exhibit by Gennaro under salinity may be possible due the maintenance of assimilation ratio, providing carbon skeletons for alternative metabolic routs (CARETTO et al., 2015). Indeed, the emissions of such compounds have been reported as an indicator of stresses (COPOLOVICI et al., 2012).

VOCs induced by abiotic stresses presented enhanced plant resistance by immediate production of reactive oxygen species (ROS) and cell membranes stabilizing (BRILLI; LORETO; BACCELLI, 2019). Moreover, many of VOC's identified are important signaling molecules, functioning as a priming stimuli, the deciphering of this chemical information will be of huge importance for the identification of plant responses (MAFFEI; GERTSCH; APPENDINO, 2011). Citral, Eugenol, α-Bergamotene were reported to be effective in inhibit germination and growth of plant pathogens, yet the mechanisms of action remain unknown (QUINTANA-RODRIGUEZ et al., 2018). In general, all VOCS also have potential medicinal applications and food industry; it could open a range of possibilities from several group of plants.

An overall representation of principal component (PC) analyses demonstrated a perfect separation between genotypes and treatments (Figure 1). PC1 represented 51.5% of total variation and PC2 represented 26.1% of the total variation. The most different response was observed by Gennaro under NaCl, which may indicate a huge modification of plant metabolism to deal with salinity. The result was an induction of major and minor organic compounds, and thus, keep other vital processes as photosynthesis and maintenance of growth.

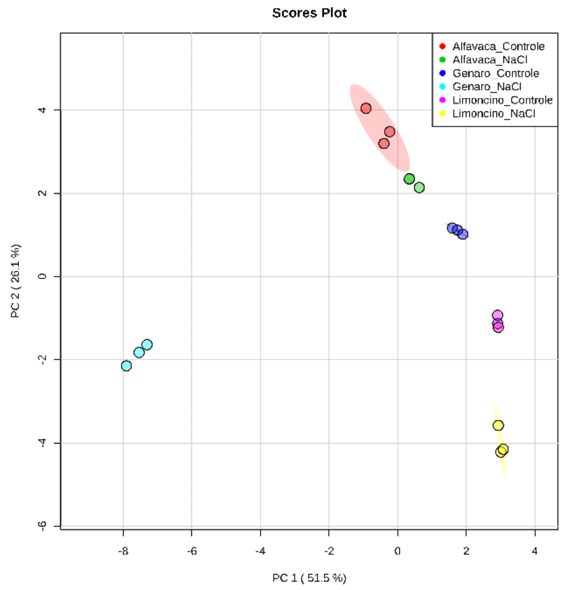

Figure 1. Two-dimensional principal component (PC) analysis.

CONCLUSIONS

The different cultivars exhibited distinct behavior under salt stress, Alfavaca and Limoncino cultivars seem to be more sensitive, since they had the growth and gas exchange impaired, respectively. Thus, moderate salinity stress along plant development changed major volatile oil components biosynthesis, and Gennaro may have a great potential to improve such compounds.

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