Effect of Drought and Salinity Stresses on Nutrient and Essential Oil Composition in *Ocimum basilicum* L.

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Summary

Drought and salinity are serious threats to agriculture worldwide. The present study investigated the effect of drought [moderate drought stress (25–30% volumetric water content - VWC), severe drought stress (15–20% VWC)] and salinity (moderate salinity stress (100 mM NaCl), severe salinity stress (200 mM NaCl)) on essential oil chemical composition and nutrient content in *Ocimum basilicum* 'Genovese'. According to obtained results salinity stress has a stronger influence on the nutrient content as well as on essential oil composition.

Key words

basil, eugenol, linalool, minerals, radar chart

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Introduction

Adverse soil conditions and climate change increased the severity and the extent of drought conditions and soil salinity. Moreover, drought as well as soil and irrigation water salinity negatively affect plant growth and crop production and cause an enormous problem to agriculture worldwide (Forni et al., 2017). Therefore, there is a need for plants that can adjust to the new conditions without compromising yield, nutritional quality and medicinal/therapeutic potential.

Basil (*Ocimum basilicum* L.) is an annual, herbaceous plant showing high levels of morphological and chemical variability (Liber et al., 2011) and is considered to be moderately tolerant to soil salinity (Scagel et al., 2017). In addition, salinity and drought are associated with significant changes in the biosynthetic pathways of some secondary metabolites in basil, such as essential oil (Ahl and Mahmoud, 2010) and phenolic compounds as well as in mineral content in the leaf (Scagel et al., 2019).

Consequently, the aim of our study was to quantify changes in basil essential oil composition and mineral content in the herb under drought and salinity stresses.

Materials and Methods

Plant Material, Experimental Design, and Treatments

The research was carried out on *O. basilicum* 'Genovese' (MAP02282) accession from the Collection of Medicinal and Aromatic Plants held at the Department of Seed Science and Technology, University of Zagreb Faculty of Agriculture. Plant material was grown as described in Lazarević et al. (2021); fifteen days old seedlings were transplanted into 2 L plastic pots in substrate and grown in growth chamber. Stress treatments were moderate salinity stress (100 mM NaCl, 45-50% VWC; S1), severe salinity stress (200 mM NaCl, 45-50% VWC, S2), moderate drought stress (25–30% volumetric water content (VWC); D1) and severe drought stress (15–20% VWC; D2) with well-watered control (45–50% (VWC); C). The experiment was carried out in Completely Randomized Design (CRD) with the ten plants per treatment.

Isolation of Essential Oils

Basil essential oils were isolated by hydrodistillation (100 g of dried herb collected at the full flowering stage) in a Clevenger-type apparatus for 3 hours using pentane/Et2O 1:1 (v/v) for trapping. After distillation, the pentane/Et2O extracts were separated and dried with anhydrous Na_2SO_4. The extracts were concentrated by careful evaporation of the solvent mixture to a small volume, and 1 μL of these solutions was used for each gas chromatographic (GC) and GC–mass spectrometry (GC–MS) analysis.

Identification of Essential Oil Compounds

Identification of essential oil compounds was performed using a GC-MS system (Agilent Technologies (Paolo Alto, CA, USA): gas chromatograph model 8890 with a mass selective detector model 7000D GC/QT and autosampler model 7693A on HP-5MS column (Agilent J&W GC column 19091S-433), 30 m × 0.25 mm i.d., film thickness 0.25 μm).

The column temperature was programmed from 70 °C isothermal for 2 min, then increased to 200 °C at a rate of 3 °C min^{-1} and held isothermal for 18 min. Carrier gas was helium at a flow rate of 1 mL min^{-1}.

The injector temperature was 250 °C. The injected volume was 2 μL and the split ratio was 1:50. MS conditions were: ionization voltage 70 eV; ion source temperature 230 °C; mass scan range: 30–350 mass units. The transfer line temperature was held at 280 °C.

Individual peaks were identified by comparing their retention indices (relative to C9–C20 n-alkanes) with literature data (Adams, 2007) and by comparing their mass spectra against the Wiley 9N08 library (Wiley, New York) and the NIST17 (National Institute of Standards and Technology, Gaithersburg) mass spectral database. Determination of the percentage composition was based on peak area normalization (expressing the area of a given peak as a percentage of the sum of the areas of all peaks) without using correction factors.

Mineral Composition

The mineral composition of basil was determined from a homogeneous sample of the herb collected from the plants before flowering occurred. Dry weight (% DW) was determined by drying at 105 °C until constant weight, grinding and digestion in a microwave oven (Milestone, Ethos One) with nitric acid (HNO3) and perchloric acid (HClO4). After digestion, phosphorus (P) was determined using a spectrophotometer (Thermo Fisher Scientific, Evolutuion 60 S), potassium (K) using a flame photometer (Jenway, PFP-7), while calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) were determined using an atomic absorption spectrometer (AAS Solar, Thermo Scientific) (AOAC, 2015). Total nitrogen (N) content was determined using the Modified Kjeldahl method (HRN ISO 11261:2004).

Data Analysis

Two extreme data points were used to determine the range of the values for each treatment and trait. Median point was used to construct the radar plot in order to detect patterns of oil and mineral composition under different stress conditions.

Results

Effect of Drought and Salinity Stress on Essential Oil Composition

In the analyzed samples eight compounds in total were identified as the main constituents (concerning higher than 5%; calculated as % peak area) of the basil essential oil (Table 1).

As shown in Fig. 1a, a decrease in linalool content was observed under both drought and salinity stresses, while germacrene D and γ-cadinene content were higher under both severe drought and salinity stresses compared to control. Both drought and moderate salinity stresses had a positive effect on eugenol content while δ-guaiene, methyl eugenol and epi-α-cadinol content were higher under all stresses. Furthermore, moderate drought positively affected eucalyptol content (Fig. 1a).

Table 1. Value range of essential oil main compounds and mineral content in basil herb

		Treatment[1]					
		Unit	D2	D1	C	S1	S2
Oil components	LINO	(%, w/w)	17.08 - 18.77	18.46 - 23.13	20.35 - 21.51	17.55 - 19.83	16.83 - 21.01
	EUGO	(%, w/w)	17.53 - 19.03	14.73 - 18.25	14.80 - 17.23	15.98 - 17.42	14.46 - 16.42
	CADE	(%, w/w)	10.61 - 11.32	10.89 - 11.68	9.53 - 11.78	10.15 - 11.18	10.89 - 12.13
	EUGM	(%, w/w)	4.66 - 8.34	3.89 - 8.80	5.75 - 8.94	7.67 - 10.98	3.93 - 12.67
	GERM	(%, w/w)	4.21 - 6.49	3.93 - 6.24	3.99 - 5.31	4.19 - 5.09	4.06 - 6.60
	CADY	(%, w/w)	4.38 - 5.84	4.09 - 5.85	3.81 - 4.42	4.14 - 4.50	4.05 - 5.97
	GUAD	(%, w/w)	3.33 - 4.09	3.37 - 4.26	2.43 - 3.79	2.67 - 3.74	2.51 - 5.52
	EUCA	(%, w/w)	4.68 - 5.81	5.48 - 6.52	4.66 - 5.67	4.95 - 5.36	4.01 - 4.81
Nutrients	N	% DW[2]	4.19 - 4.31	4.29 - 4.36	4.92 - 5.12	4.59 - 5.05	4.69 - 4.99
	P	% DW	1.21 - 1.23	1.07 - 1.16	1.33 - 1.36	1.28 - 1.33	1.34 - 1.39
	K	% DW	4.52 - 4.71	4.69 - 4.73	4.91 - 4.96	4.95 - 4.98	4.90 - 4.99
	Ca	% DW	2.27 - 2.35	2.20 - 2.29	1.69 - 1.81	2.13 - 2.34	2.01 - 2.13
	Mg	% DW	0.57 - 0.59	0.55 - 0.60	0.46 - 0.53	0.61 - 0.71	0.55 - 0.65
	Fe	mg kg^{-1} DW	108.80 - 115.30	107.70 - 119.00	96.00 - 105.10	109.20 - 115.40	107.10 - 110.30
	Cu	mg kg^{-1} DW	2.40 - 2.60	2.50 - 2.80	3.70 - 3.90	4.20 - 4.60	4.20 - 4.50
	Mn	mg kg^{-1} DW	169.00 - 188.00	190.00 - 199.00	241.00 - 252.00	273.00 - 286.00	273.00 - 293.00
	Zn	mg kg^{-1} DW	63.50 - 65.70	51.70 - 59.20	67.70 - 74.80	93.60 - 108.10	93.70 - 99.60

Note:

[1] D2 - severe drought stress (15–20% volumetric water content); D1 - moderate drought stress (25–30% volumetric water content); C – control (45–50% volumetric water content); S1 - moderate salinity stress (100 mM NaCl, 45–50% volumetric water content); S2 - severe salinity stress (200 mM NaCl, 45–50% volumetric water content)

LINO – linalool, EUGO – eugenol, CADE - epi-α-cadinol, EUGM – methyl eugenol, GERM - germacrene D, CADY - γ-cadinene, GUAD - δ-guaiene, EUCA – eucalyptol

[2] Dry Weight

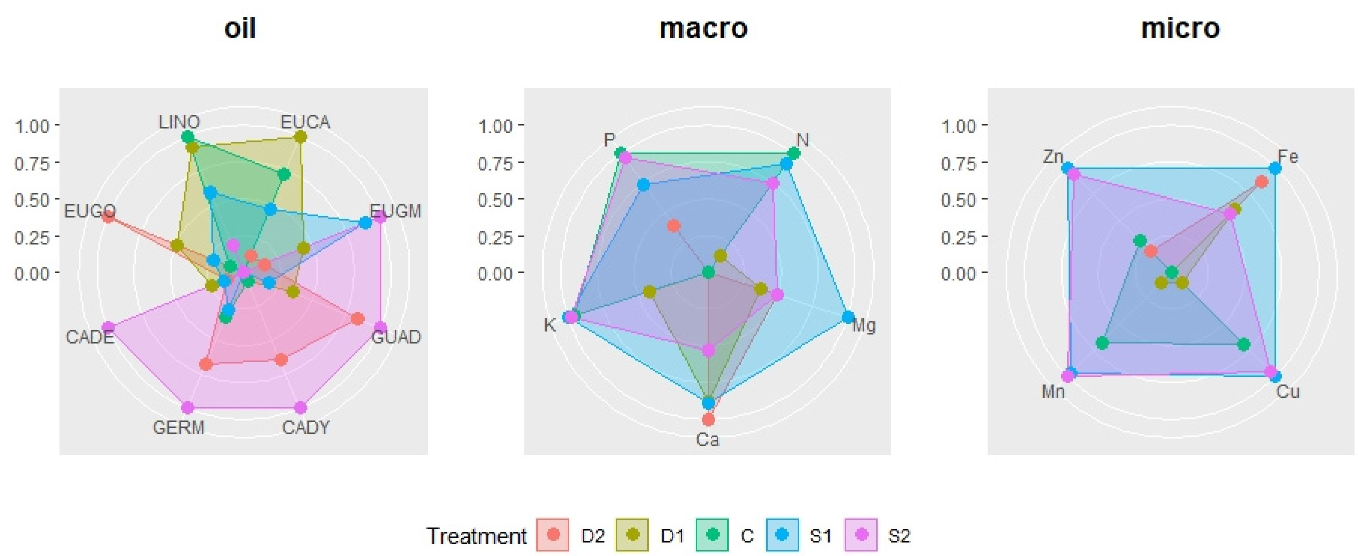

Figure 1. Radar chart of essential oil main compounds and mineral content in basil herb
Values for all traits were standardized to range 0-1

Note: D2 - severe drought stress (15–20% volumetric water content); D1 - moderate drought stress (25–30% volumetric water content); C – control (45–50% volumetric water content); S1 - moderate salinity stress (100 mM NaCl, 45–50% volumetric water content); S2 - severe salinity stress (200 mM NaCl, 45–50% volumetric water content)

Effect of Drought and Salinity Stress on Nutrient Composition

Both drought and salinity stresses positively affected Mg and Ca content (Fig. 1b), while they had a negative effect on N and P content compared to control. Higher K content was found in plants from both moderate and severe salinity treatments.

Moreover, both drought and salinity stresses positively affected Fe content (Fig. 1c) content. Higher Zn, Cu and Mn contents were found in the plants from both moderate and severe salinity treatments compared to the plants from drought treatments, indicating that salinity has a stronger influence on the plant mineral content compared to drought.

Discussion

Effect of Drought and Salinity Stress on Essential Oil Composition

Salinity stress is known to be associated with the induction of essential oil biosynthetic pathways in aromatic plants (Petropoulos et al., 2009). In the current study, both drought and salinity stresses decreased linalool while severe salinity stress decreased eugenol content. However, other stresses increased eugenol content compared to control. These results are opposite to the results of Said-Al Ahl et al. (2010), where salinity treatments increased the content of linalool and are in congruence in part where salinity treatments decreased eugenol content in *O. basilicum* var. *purpurascens*.

Effect of Drought and Salinity Stresses on Mineral Composition

Salinity stress is the second most important limiting factor for plant growth and development after drought stress (Sedaghathoor and Abbasnia Zare, 2019). Although the increase in salinity resulted in a decrease of N and P content, the other nutrients were positively affected by saline conditions. These results are in congruence with the findings of Petropoulos et al. (2019) where the mineral composition of *Cichorium spinosum* L. leaves was positively affected by medium to high and high salinity levels.

Conclusion

The preliminary results of the study highlighted some differences in basil essential oil profile as well as in nutrient composition under different stress regimes. Since it is shown that this basil cultivar tolerates drought and salinity stress, it could potentially be used for adaptation to agriculture and food security challenges in the 21st century caused by climate change.

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