



Research Paper

Morphological and biochemical responses of selected *Ocimum* species under drought

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ABSTRACT

Drought stress is known to induce biochemical activity, altering plant growth and development. Examining the relationship between drought stress and morphological changes as well as essential oil accumulation is important for basil species due to shortages of research evidence. Hence, an open field experiment was conducted in 2020 to determine the effect of drought stress conditions on the morphological and biochemical responses of selected *Ocimum* species. Five basil species, namely, *O. basilicum* 'Genovese', *O. basilicum* 'Ohre', *O. x africanum*, *O. americanum*, *O. selloi*, and *O. sanctum* were grown under irrigated (control) and non-irrigated (drought stress treatment) plots with two block replications. The results showed that irrigation had a positive effect on production. On average, irrigated plots produced 39% and 33% higher fresh and dry herb yield, respectively. No significant change was detected between the treatments in essential oils (EO), yield, and composition of the major compounds. However, a slight increase in camphor (*O. x africanum*), nerol (*O. americanum*), and trans- β -Caryophyllene (*O. sanctum*) ratios were observed under irrigated treatment. On contrary, the EO content, total polyphenol content, and antioxidant capacity were higher under drought stress condition. In addition, drought stress conditions had a positive effect on 1, 8-cineole (*O. africanum*) and eugenol (*O. sanctum*) ratios. The observed slight enhancement of EO content under drought stress is not comparable to the significant yield reduction recorded. Morphological and biochemical variation was also detected among basil species. Accordingly, higher biomass (616.33 g plant⁻¹) and EO yield (3.72 ml/100 g) among the species were obtained from *O. basilicum* 'Genovese' and *O. x africanum*, respectively. In conclusion, for maximum biomass and higher EO yield, irrigation is paramount.

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INTRODUCTION

The genus *Ocimum* L. belongs to the family Lamiaceae, it comprises around 35–150 species of annual and perennial herbs and shrubs, which are native to the tropical and warm temperate regions of the world, but extensively disseminated worldwide (Hiltunen and Holm, 1999; Shasany and Kole, 2018; Maddi et al., 2019). Because of their economical and medical importance, the most widely grown species throughout the world are *O. basilicum* L., *O. africanum* Lour. (syn. *O. x citriodorum* Vis), *O. americanum*

L. (syn. *O. canum* Sims.), *O. gratissimum* L., *O. minimum* L., and *O. tenuiflorum* L. (syn. *O. sanctum* L.) (Carović-Stanko et al., 2010). Along with the above species, there are also many varieties, as well as several related species or hybrids (Shasany and Kole, 2018; Maddi et al., 2019). *Ocimum* species occur as several morphotypes, chemotypes, and cultivars that differ in morphology, oil composition, and yield (Simon et al., 1990; Hasegawa et al., 1997). The essential oil yield generally ranges from 0.2 to 2.34 but can

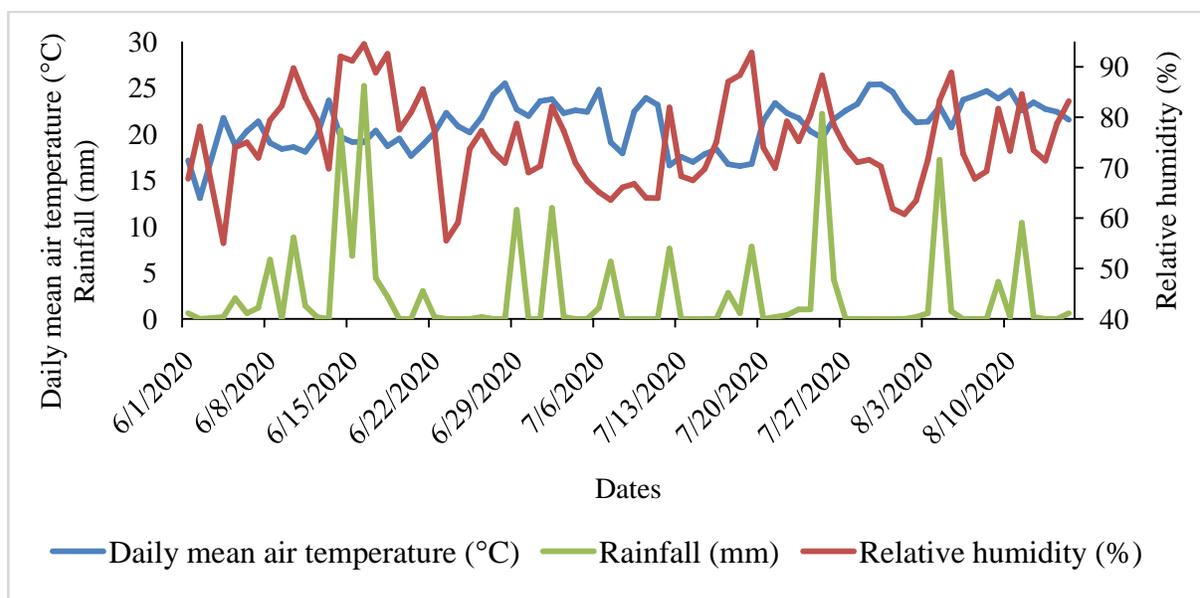


Figure 1: Air temperature (°C), rainfall (mm), and relative humidity (%) of the experiment field

Table 1: Soil characteristics of the experimental plot (Budapest, 2020)

pH	Humus	K _A	NO ₃ -N	P ₂ O ₅	K ₂ O	Ca	Mg	Fe	Mn	Zn	Cu
H ₂ O	%		mg/kg	mg/kg	mg/kg	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
8.61	2.02	<30	1.62	95.5	165	1.6	52.9	8.19	11.9	19.7	1.77

be as high as 5.22%, depending on the species, source, and phenological stage of the plant (Hiltunen and Holm, 1999; Simon et al., 1999). Many species of the genus *Ocimum* contain essential oils based primarily on monoterpene derivatives such as camphor, limonene, thymol, citral, geraniol, and linalool. Other members of the genus, including common basil (*O. basilicum*), contain an essential oil based primarily on high proportions of phenolic derivatives, such as eugenol, methyl chavicol (estragole), and methyl cinnamate, often combined with various proportions of linalool (Hiltunen and Holm, 1999, Labra et al., 2004).

The levels of essential oil and other compounds vary between different basil species and cultivars and are also influenced by growing conditions and genetic factors (Hiltunen and Holm, 1999; Bowes and Zheljzakov, 2004). Drought stress is a known factor that induces biochemical, physiological, and developmental alterations in plants. Several scholars examined the relationship between water stress and essential oil accumulation in certain *Ocimum* species, thus their results indicated restricted plant growth and slightly enhanced essential oil content (Simon et al., 1992; Omobolanle et al., 2013; Martielly et al., 2016; Rasouli and Fakheri, 2016; Vilanova et al., 2018). On the contrary, others observed that drought stress has no significant change in the essential oil content of *Thymus*

vulgaris and *Hyssopus officinalis* (Khazaie et al., 2008). García-Caparrós et al. (2019) also observed no change on *Menthapiperita*, *Salvia sclarea*, *Salvia lavandulifolia*, and *Thymus mastichina*. The effect of drought stress on different basil species is not well known except for sweet basil. Studying the effect of water supply on drug quantity and quality is important for producers. Therefore, the objective of this article is to evaluate the morphological and biochemical responses of selected *Ocimum* species to drought conditions.

MATERIALS AND METHODS

Experimental site description

The experiment was carried out at the Experimental and Research Farm of Hungarian University of Agriculture and Life Sciences in Soroksár during the year 2020. The mean max and min temperatures during the experimental period were 29.81°C and 13.92°C, respectively; and 198 mm rainfall was observed during the experiment. The daily mean temperature (°C), daily rainfall (mm), and daily relative humidity (%) during the experimental period are indicated in Figure 1. The characteristics of the soil media are summarized in Table 1.

Plant materials and treatments

The experiment was laid out as a two-factor randomized design with two block replications. Five basil species, namely, *O. basilicum* 'Ohre', *O. basilicum* 'Genovese', *O. x africanum*, *O. americanum*, *O. selloi*, and *O. sanctum*, and two trays (27×57 cm) in the greenhouse in the middle of March. Seedlings that developed two leaves were transplanted into 0.1 L pots. In the first and second week of June, 50 seedlings were allocated for each treatment and planted in open field plots at a spacing of 40×40 cm except for *O. basilicum* 'Genovese' which was transplanted at a spacing of 50×50 cm. Drought treatment was initiated two weeks after transplanting and lasted for 31 days. In the irrigated treatments, 20 mm of water was applied two times per week using a spraying hose attached to the water meter device. While the drought treatment received only natural precipitation which was 198 mm of rainfall well distributed throughout the growing season. Eighty grams of complex fertilizer (Hunfert NPK 15-15-15) per plot was applied 10 days after transplanting but no plant protection chemical was used. Weeding and cultivation were done twice a month. Harvesting was done 10 days after full bloom.

Plant growth measurement

Plant height (cm), plant canopy diameter (cm), fresh herb yield (g plant⁻¹), and dry herb yield (g plant⁻¹) (after drying at room temperature in shadow) were measured from nine sample plants per treatment from the middle row at harvesting.

Essential oil content determination

Nine sample plants were harvested per plot separately and after drying at room temperature, a bulk sample from the dried leaves and inflorescence (excluding the stem) were used to measure essential oil content in six replications per treatment with a Clevenger-type apparatus according to VII Hungarian Pharmacopoeia.

Total polyphenol content

The total amount of phenolic compounds in each extract was determined using the Folin-Ciocalteu method following the procedure of Singleton and Rossi (1965) with slight modifications. Half gram of dried and powdered plant material was extracted by 50 mL of boiling distilled water and was allowed to stand for 24 h at room temperature. Then the extracts were filtered and stored in a freezer until the measurements were taken. 40 µL of the test sample and 460 µL of distilled water were placed into a test tube and then mixed with 2.5 mL Folin-Ciocalteu's reagent (10

levels of water supply (irrigated as control and non-irrigated as drought stress treatment) were used with two block replications. Seeds of *Ocimum* species were selected from the gene bank of the Department of Medicinal and Aromatic Plants except for *O. sanctum* which was obtained from the Assam state of India. Seeds were sown into seed v/v%). After 1 min of incubation, 2 ml of sodium carbonate (0.7 M) was added. Then the mixture was kept in hot water (50°C) for 5 min and the absorbance was measured at the wave length of $\lambda=760$ nm with a Thermo Evolution 201 spectrophotometer. Gallic acid (0.3 M) was used as a chemical standard for calibration. The total phenolic content of the samples was expressed in gallic acid equivalents calculated on the dry weight basis of the extract (mg GAE/g DW). The measurements were done in 6 replications.

Antioxidant capacity

The FRAP assay was performed according to the Benzie and Strain (1996) procedure with slight modifications. The same extract mentioned above was used for antioxidant capacity. FRAP reagent was prepared that contains sodium acetate buffer (pH 3.6), TPTZ (2,4,6-tripiridil-striazin) in HCl and FeCl₃ · 6H₂O solution (20 mmol/L), in proportion 10:1:1 (v/v/v), respectively. 10 µL of the test sample was added to 1.5 mL of acting FRAP reagent and 40 µL distilled water and absorbance were recorded at 593 nm after 5 min using the spectrophotometer above. The blank contained distilled water instead of extract. FRAP values of samples were calculated from the standard curve equation and expressed as mg ascorbic acid equivalent (AAE)/g of dry extract.

Essential oil composition

GC-MS method was used to determine the EO composition. GC analysis was carried out using an Agilent Technologies 6890 N instrument equipped with an HP-5MS capillary column (30 m x 0.25 mm, 0.25 µm film thickness), with the following temperature program: initial temperature 60°C, heating at a rate of 3°C/min up to 240°C; the final temperature was maintained for 5 min; injector and detector temperatures: 250°C; carrier gas: helium (constant flow rate: 1 ml min⁻¹); split ratio: 30:1, injection volume 0.2 µl (10%, n-hexane). The proportions of individual compounds were expressed as total area percentages. The same equipment was used to identify the components with an Agilent Technologies MS 5975 detector. Ionization energy was 70 eV. The mass spectra were recorded in full scan mode, which revealed the total ion current (TIC) chromatograms. A mixture of aliphatic hydrocarbons (C9-C23) in n-hexane was injected to calculate the linear retention indices using the generalized equation of (Van

Table 2: Effect of drought stress on plant height and plant canopy diameter of *Ocimum* species (Budapest, 2020)

Species	Plant height (cm)		Plant canopy diameter (cm)	
	Irrigated	Non-irrigated	Irrigated	Non-irrigated
<i>O. basilicum</i> 'Ohre'	46.94Acd	43.39Ac	51.83Ab	47.56Aab
<i>O. basilicum</i> 'Genovese'	77.33Aa	68.50Aa	62.33Aa	57.83Aa
<i>O. africanum</i>	50.94Ac	41.28Bc	49.06Abc	35.28Bc
<i>O. americanum</i>	41.78Ade	40.44Ac	42.89Ac	41.44bAc
<i>O. selloi</i>	36.72Ae	38.22Ac	41.50Ac	40.28Abc
<i>O. sanctum</i>	64.33Ab	60.17Ab	61.67Aa	53.50Ba

Different letters are for significantly different groups. Capital letters to differentiate between drought stress under fixed species and small letters are used to differentiate species under fixed drought stress.

Den and Kratz, 1963). The linear retention indices (LRI) and mass spectra were compared with commercial ones (NIST, Wiley) and homemade library mass spectra built up from data obtained from standard (Sigma/Aldrich), pure compounds. SPME and GC samples were repeated three times.

Data analysis

Data were evaluated using a one-way analysis of variance (ANOVA) and t-test. Shapiro Wilk's test and Levene's test were used for checking the normality of distribution and the homogeneity of variances, respectively. Significant mean differences were examined with Turkey HSD at $P < 0.05$. All statistical analysis was performed using IBM SPSS 25.

RESULTS AND DISCUSSION

Plant height and canopy diameter

In drought stress conditions, plants tend to reduce their growth as a stress tolerance strategy. Although the non-irrigated plants received higher rainfall during the experimental period, a slight reduction in plant height and plant canopy diameter was observed (Table 2). Among the species *O. africanum* shows a 19% and 28% reduction in plant height and canopy diameter respectively. Former studies also indicated that water shortage significantly decreases the growth parameters of sweet basil cultivars across different growing areas (Sirousmehr et al., 2014; Damalas, 2019 and Al-Huqail et al., 2020). Besides, reduced growth as a result of drought was also reported in *O. africanum* (Martielly et al., 2016) and *O. gratissimum* (Omobolanle et al., 2013; Vilanova et al., 2018). Almost all evidence revealed that drought restricted the growth of *Ocimum* species and other medicinal and aromatic plants. These observed reductions in growth parameters in drought stress conditions were due to the disturbance in

the metabolic process of the plant, including photosynthesis and transpiration, chlorophyll destruction, and cell division. Statistically significant variability in plant height and plant canopy diameter were also detected between the tested species. Of the species, *O. basilicum* 'Genovese' were found higher in production followed by *O. sanctum*, whereas *O. selloi* plants were shorter in height and narrow in diameter.

Fresh herb yield and dry herb yield

Significantly notable fresh and dry herb yield reduction was measured in drought stress treatment (Table 3). As a result, the fresh herb yield reduction ranges from 2.78 g/plant in *O. selloi* to 142 g/plant in *O. basilicum* 'Genovese'. Similarly, drought stress reduced dry herb yield in a range of 3.84 g plant⁻¹ in *O. selloi* to 24 g plant⁻¹ in *O. africanum*. In an experiment conducted by Al-Huqail et al. (2020), where sweet basil plants were exposed for 15 and 21 days of drought stress treatment, the fresh weight dropped by 25% and 89%, respectively. Radácsi et al. (2010) also observed a 34% dry mass reduction due to drought stress. Additionally, Forouzandeh et al. (2012) mentioned that dry yield of sweet basil was reduced by 12% in 60% field capacity (FC) compared to the control (100% FC). Moreover, water shortage conditions resulted in a 48.3% and 50.6% reduction in shoot fresh and dry weight of *Ocimum basilicum* L. 'Genovese gigante', respectively (Damalas, 2019). Furthermore, Khalid (2006) also reported a total herb fresh and dry weight of *O. americanum* reduced under 50% FC of drought stress in two seasons, unlike the control which gave the highest. Besides, drought, variability among basil species in fresh and dry herb yield was also observed. Higher biomass yield was obtained from sweet basil cultivar 'Genovese' followed by cultivar 'Ohre' whereas significantly lower biomass yield was recorded from *O. selloi* plants. The observed yield variation among species is mainly genetic. Previous studies showed the presence of abundant morphological variability in the *Ocimum* genus due to polyploidy, aneuploidy, and inter- and intra-specific hybridization in addition to targeted

Table 3: Effect of irrigation on fresh and dry herb weight of *Ocimum* species (Budapest, 2020)

Species	Fresh herb yield (g plant ⁻¹)		Dry herb yield (g plant ⁻¹)	
	Irrigated	Non-irrigated	Irrigated	Non-irrigated
<i>O. basilicum</i> 'Ohre'	389.56Ab	284.72Bb	63.72Ab	54.22Ab
<i>O. basilicum</i> 'Genovese'	616.33Aa	474.33Ba	115.83Aa	92.83Ba
<i>O. africanum</i>	268.33Abc	126.06Bd	50.61Abc	26.61Bc
<i>O. americanum</i>	230.22Acd	165.72Bcd	38.67Ac	27.72Bc
<i>O. selloi</i>	114.78Ad	112.00Ad	29.17Ac	25.33Bc
<i>O. sanctum</i>	281.00Abc	250.33Abc	50.00Abc	45.50Abc

Different letters are for significantly different groups. Capital letters to differentiate between drought stress under fixed species and small letters are used to differentiate species under fixed drought stress.

Table 4: Effect of irrigation on essential oil content and yield of *Ocimum* species (Budapest, 2020)

Species	Essential oil content (ml/100g)		Essential oil yield (ml/plant)	
	Irrigated	Non-irrigated	Irrigated	Non-irrigated
<i>O. basilicum</i> 'Ohre'	2.05Ab	2.04Ac	1.25Aa	0.83Aab
<i>O. basilicum</i> 'Genovese'	0.66Bd	0.78Ae	0.71Ab	0.69Ab
<i>O. africanum</i>	3.72Ba	4.14Aa	1.32Aa	1.46Aa
<i>O. americanum</i>	1.12Ac	1.1Ad	0.40Ab	0.36Ab
<i>O. selloi</i>	1.86Bb	2.64Ab	0.46Ab	0.62Ab
<i>O. sanctum</i>	0.85Acd	0.91Ae	0.40Ab	0.38Ab

Different letters are for significantly different groups. Capital letters to differentiate between drought stress under fixed species and small letters are used to differentiate species under fixed drought stress.

cultivation and breeding practices for desired morpho-chemotypes (Gupta et al., 2018).

Essential oil content and essential oil yield

The effect of drought on essential oil (EO) content varies among the species as indicated in Table 4. Drought conditions lead to a higher EO percentage in *O. basilicum* 'Genovese', *O. africanum*, and *O. selloi* while no change was detected on *O. basilicum* 'Ohre' and *O. americanum*. Non-irrigated plants showed slightly lower EO yield per plant, but statistically, no significant differences were seen. Previous researchers also reported different results on the effect of water deficit on the EO content and yield, which could be related to stress level, species investigated, and other environmental conditions. On one hand, an increased EO content as a result of drought stress was reported in *O. basilicum* (Simon et al., 1992; Forouzandeh et al., 2012; Sirousmehr et al., 2014), *O. americanum* (Khalid, 2006; Rasouli and Fakheri, 2016), *O. africanum* (Martielly et al., 2016), and *O. gratissimum* (Omobolanle et al., 2013; Vilanova et al., 2018). In addition, no significant change was also reported on *Menthapiperita*, *Salvia sclarea*, *Salvia lavandulifolia*, and *Thymus mastichina* (García-Caparrós et al., 2019). Despite all above effects, drought significantly reduced biomass yield, which in turn affected EO yield.

Hence, the biomass loss is much higher than a slight increase in EO content as indicated by many scholars. Of all species, *O. africanum* has produced higher EO content and yield per plant followed by *O. basilicum* 'Ohre'. Singh et al. (2018) also observed the presence of high genetic diversity in essential oil content between species and varieties of basil.

Total polyphenol content and antioxidant capacity

Drought stress is known to modify the secondary compound accumulation of plants, including polyphenol content and antioxidant activities. In line with that, a significant change in total polyphenol content (TPC) and antioxidant capacity (AOC) was detected among *Ocimum* species grown under irrigated and non-irrigated plots (Table 5). Drought stress had a positive effect on the TPC and AOC of basil species investigated except *O. basilicum* 'Ohre' where a negative or no effect was observed. In general, a significant positive correlation ($r=0.5$) was observed between total polyphenol content and antioxidant capacity. The increment in TPC and AOC in the drought stress plot could be a response induced to cope with oxidative stress. Previous studies also reported that drought increased TPC and antioxidant activity of green and purple sweet basil cultivars (Pirbalouti et al., 2017).

Table 5: Effect of irrigation on total polyphenol content and antioxidant capacity of *Ocimum* species (Budapest, 2020)

Species	Total polyphenol content (mg GAE /g DM)		Antioxidant capacity (mg AAE /g DM)	
	Irrigated	Non-irrigated	Irrigated	Non-irrigated
<i>O. basilicum</i> 'Ohre'	84.88Aa	60.04Bbc	129.30Ab	131.48Ab
<i>O. basilicum</i> 'Genovese'	76.40Bab	92.53Aa	109.95Bb	149.93Ab
<i>O. africanum</i>	28.53Bd	39.92Ad	62.95Bc	134.72Ab
<i>O. americanum</i>	62.78Abc	67.33Ab	150.2Ab	137.59Ab
<i>O. selloi</i>	62.32Abc	73.94Ab	250.86Ba	249.2Aa
<i>O. sanctum</i>	41.74Acd	48.94Acd	128.92Ab	157.60Ab

Different letters are for significantly different groups. Capital letters to differentiate between drought stress under fixed species and small letters are used to differentiate species under fixed drought stress.

Table 6: Effect of the water supply on essential oil composition of *Ocimum* species (Budapest, 2020)

Components	RT	LRI	<i>O. basilicum</i> 'Ohre'		<i>O. basilicum</i> 'Genovese'		<i>O. americanum</i>		<i>O. africanum</i>		<i>O. selloi</i>		<i>O. sanctum</i>	
			W	D	W	D	W	D	W	D	W	D	W	D
α -pinene	5.56	938	0.03	0.04	0.21	0.22	0.07	0.06	2.32	2.69	0.2		-	-
β -pinene	6.64	980	0.07	0.11	0.41	0.53	0.05	0.06	3.00	4.01	0.7	0.56	-	-
limonen	8.19	1028	0.11	0.14	0.47	0.43	0.16	0.14	13.3a	15.5a	0.4	0.24	-	-
1,8-cineole	8.38	1034	1.53	1.22	6.65b	8.49a	0.15	0.16	27.5b	35.7a	8a	6.9a	0.33	0.29
(E)-ocimene (trans- β -ocimene)	8.85	1046	0.23	0.19	0.18	0.35	-	-	0.29	0.21	5.56	6.12	-	-
Sabinene hydrate <cis->	9.66	1068	-	-	0.22	0.28	1.97	2.40	-	-	-	-	-	-
linalool	10.76	1097	50.1a	52.0a	53.3a	48.6a	9.69a	8.82a	0.91	0.42	0.3	0.4	0.54	0.21
camphor	12.68	1144	0.08	0.11	0.94	0.70	-	-	25.1a	15.3b	2.8	0.2	0.2	0.29
α -terpineol	14.55	1189	0.23	0.23	0.97	1.37	0.98	0.77	2.80	3.59	-	-	-	-
Nerol	16.15	1227	0.12	0.17	-	-	11.2a	9.9b	-	-	-	-	-	-
neral(citral-b)	16.58	1238	0.13	0.13	-	-	17.4a	17.7a	-	0.06	-	-	-	-
geraniol	17.2	1252	15.13a	18.84a	-	-	1.85	1.70	0.45	-	-	-	-	-
geranial (citral a)	17.86	1268	0.22	0.21	-	-	24.26a	24.84a	0.05	0.08	-	-	-	-
eugenol	21.44	1361	0.99	0.78	3.06	4.58	0.10	0.10	0.06	0.06	-	-	30.7b	36a
β -elemene	22.55	1391	1.36	1.24	0.71	0.51	0.12	0.12	0.07	0.06	0.1	0.1	2.82	2.66
metil-eugenol	23.31	1411					-	-	-	-	28b	39.4a	9.26	7.83
trans- β -caryophyllene	23.68	1419	1.02	1.23	0.27	0.16	4.10	4.51	1.07	1.11	5.3	4.01	33.6a	30.9b
trans- α -bergamotene	24.36	1437	2.73	0.18	3.73b	6.93a	1.82	2.12	0.20	0.14	-	-	-	-

Table 6: Continued

α -humulene	25.07	1454	0.47	0.47	0.88	0.42	0.71	0.77	4.47	4.24	0.5	0.32	2.4	2.25
germacrene d	26.18	1482	3.23	2.84	2.82	2.00	1.70	1.77	1.28	1.18	2.1	1.6	-	-
α -bulnesene	27.23	1508	-	-	-	-	0.21	0.22	-	-	-	-	3.8	3.9
Bisabolene <(Z)- α ->	28.54	1544	-	-	-	-	4.65	5.07	1.81	1.30	-	-	-	-
elemicin	29.3	1555	-	-	-	-	-	-	-	-	37a	32.2a	0.5	0.93
caryophyllene oxide	30.2	1590	-	0.10	-	-	4.21	4.50	-	-	0.9	0.6	9.14a	11.1b
tau-cadinol	32.26	1644	8.12a	8.82a	9.24	7.78	0.56	0.39	0.24	-	-	-	-	-
Others (< 2%)			12.37	10.43	12.7	13.8	12.57	13.17	11.3	10.4	7.22	4.15	3.51	1.86
Total identified			98.27	99.48	96.80	97.14	98.53	99.29	96.17	96.07	99.31	99.52	96.80	98.22
Monoterpenes			0.72	0.75	1.95	2.60	0.59	0.68	22.74	25.94	8.13	7.94	0.00	0.00
Oxygenated Monoterpenes			70.59	75.31	66.10	65.92	70.57	68.48	61.58	60.58	11.17	7.53	31.99	36.79
Sesquiterpenes			18.40	13.80	16.47	17.16	15.01	16.59	13.88	12.40	15.57	12.12	43.42	40.22
Oxygenated Sesquiterpenes			9.74	10.82	11.39	9.67	6.38	6.50	0.24	0.00	0.86	0.57	9.65	11.14
Phenylpropanes			0.08	0.13	0.32	0.20	0.00	0.00	0.79	0.73	64.63	71.65	9.72	8.76

RT – retention time, LRI – linear retention index relative to C9-C23 n-alkanes on an HP-5MS capillary column, W- watered (irrigated) and D- drought (non-irrigated), Different letters are for significantly different groups.

Higher variability in TPC and AOC was detected between basil species as well. The maximum TPC and AOC were measured from sweet basil cultivars (Genovese and Ohre) and *O. selloi* plants, respectively whereas the minimum was from *O. africanum* plants. A former study by Kwee et al. (2011) also reported a significant variation in TPC and antioxidant capacity between 15 sweet basil cultivars. In addition, variation in TPC and antioxidant activity among eight basil species (including *O. americanum*, *O. africanum*, and *O. selloi*) was reported by Hakkim et al. (2008).

Essential oil composition

Basil species have a higher level of chemical variability due to inter-specific and intra-specific hybridization. As a result, the investigated basil species showed different essential oil compositions

(Table 6). On average, more than 40 compounds were identified except for *O. selloi* and *O. sanctum* where 20 compounds were identified in total. Linalool was the major compound in *O. basilicum* cultivars, while camphor and 1,8-cineole were the main compounds in *O. africanum*. Whereas for *O. americanum* nerol, neral (Citral-b) and geranial (Citral-a) were measured to be higher in concentration. *O. selloi* accumulated a higher concentration of elemicin and methyl-eugenol. In the case of *O. sanctum*, the main compounds were eugenol and trans- β -Caryophyllene. Similarly, Hasegawa et al. (1997) reported linalool as the main compound in Genovese and observed EO chemical diversity in 9 basal species due to polymorphism caused by inter-specific hybridization. The study also revealed that drought slightly increased 1,8-cineole in *O. basilicum* 'Genovese' and *O. africanum*; eugenol and caryophyllene oxide in *O. sanctum*, and methyl-eugenol in *O. selloi*. On the contrary, irrigation

increased the camphor ratio of *O. africanum*, nerol ratio of *O. americanum*, and trans- β -Caryophyllene in *O. sanctum*. Despite that, no change was detected between the treatments in the accumulation of linalool (*O. basilicum* 'Ohre' and 'Genovese' and *O. americanum*), geraniol (*O. basilicum* 'Ohre'), neral, geranial (*O. americanum*), and elemicin (*O. selloi*). Radácsi et al. (2020) also reported no significant difference in linalool concentration and a slight increment in 1,8-cineole among the investigated sweet basil cultivars.

CONCLUSION

Basil plants possess multiple morphological and biochemical responses under drought stress conditions. This study observed that drought stress conditions significantly lowered the biomass yield of all tested basil species although the impact level

was species dependent. Of the species, *O. africanum* was highly affected by drought stress conditions whereas the essential oil yield and the major essential oil components remain mostly unchanged. On the contrary, drought stress conditions enhanced EO content, total polyphenol content, and antioxidant capacity. The slight essential oil enhancement under drought treatment cannot be compensated due to significant biomass reduction. Morphological and biochemical variability were also detected among the species investigated. Sweet cultivars 'Genovese' and 'Ohre' were higher in biomass production. Regarding EO yield, *O. africanum* and *O. basilicum* 'Ohre' produced the highest. Irrigating basil plants is beneficial for higher biomass and essential oil accumulation. Besides, during production, taking the specific requirements of the species into account is important.

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