HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

EFFECT OF DROUGHT STRESS ON PLANT PRODUCTION AND ACCUMULATION OF SECONDARY COMPOUNDS IN SELECTED *OCIMUM* SPECIES

Doctoral Thesis

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1. Introduction

1.1. Background and justification

The genus *Ocimum*, belongs to the tribe *Ocimeae*, the subfamily *Nepetoideae*, and family *Lamiaceae* (Chowdhury *et al*., 2017). The genus comprises over 60 perennial and annual herbs and shrubs, each with distinctive traits and features (Carović-Stanko *et al*., 2010, Gurav *et al*., 2022). These species are naturally found in diverse regions across the globe, including Asia, Africa, and the Americas (Paton *et al*., 1999). Well-known species within the genus include *Ocimum basilicum* (commonly known as sweet basil), *Ocimum gratissimum* (African basil), *Ocimum sanctum* (Holy basil), and *Ocimum americanum* (American basil) (Carović-Stanko *et al*., 2010, Gurav *et al*., 2022). These species exhibit noticeable variations in morphology, growth patterns, leaf shapes, and aromas, contributing to their distinct identification and classification (Nurzynska-Wierdak, 2014, Bajomo *et al*., 2022). One of the notable aspects of *Ocimum* species is their diverse chemical composition. The essential oil derived from these species is a complex mixture of volatile organic compounds that contribute to their distinct aromas. These compounds include monoterpenes, sesquiterpenes, and phenylpropanoids (Pandey *et al*., 2014, Gurav *et al*., 2022). The oil yield varies between species, sources, phenological stages of the plants, and other factors, ranging from 0.1 to 4.14% (Patel *et al.*, 2015, Mulugeta *et al*., 2022). In addition to their volatile oils, basil herbs are also rich in polyphenols (Filip *et al*., 2017, Bajomo *et al*., 2022). The aromatic essential oils and the polyphenols of basil species are used in flavor, fragrance, cosmetics, aromatherapy, and pharmaceutical industries (Pandey *et al*., 2014). They exhibit antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory activities (Pavithra *et al*., 2019, Shahrajabian *et al*., 2020, Anusmitha *et al*., 2022). In addition, the essential oils have demonstrated insecticidal and repellent properties, making them useful in pest control (Naveen *et al*., 2021).

Despite the important properties and uses mentioned above, the biomass yield, essential oil production, and essential oil compositions of *Ocimum* species are highly influenced by genetic factors (Chowdhury *et al*., 2017), ecological conditions (Chang *et al*., 2005), agro-techniques (Ciriello *et al*., 2022) and processing methods (Müller and Heindl, 2006). Among the ecological factors, drought stress is often reported to be the most limiting factor (Simon *et al*., 1992).

Studies conducted in both open field and controlled greenhouse environments have shown that water scarcity leads to reduced plant growth and biomass production in medicinal and aromatic plants (MAP) including basil. However, the impact of drought stress on the accumulation of secondary compounds in medicinal plants is inconsistent and often contradictory. An intensive review by Selmar and Kleinwächter (2015) revealed that drought stress can lead to an increase in the production of natural products. In contrast, according to Szabó *et al*. (2020) review, the available scientific evidence is not adequate to generalize due to the complex nature of the issue. Thus, the effect of drought stress on the accumulation of secondary compounds in medicinal plants in general and *Ocimum* species, in particular, is complex and can vary depending on the type of compound, different basil species or cultivars, growing conditions (greenhouse or open field), the severity and duration of drought stress.

1.2. Research objective

The main goal of the research was to investigate drought-induced physio-morphological and biochemical changes within *Ocimum* species under different growing conditions.

The specific aims of the studies are:

- → To detect intra and inter-specific morpho-chemical diversity among 15 basil genotypes preserved in the department gene bank under Hungarian (Budapest) growing conditions.
- → To investigate how the physiological parameters of specific *Ocimum* species are affected by drought stress in various growing conditions.
- → To determine the effect of drought stress on morphological characteristics of selected *Ocimum* species in various growing conditions.
- → To examine the effect of drought stress on biochemical characteristics of selected *Ocimum* species.

2. Materials and Methods

2.1. Experimental site description

Multiple experiments were conducted under three different growing conditions (open field, greenhouse, and climatic chamber) for three consecutive years (2020 - 2022). The drought experiment was carried out in an open field and greenhouse pot experiments at the Soroksár Experimental and Research Farm of the Hungarian University of Agriculture and Life Sciences (MATE). Additionally, the third pot experiment was conducted in two different plant growth chambers (Weiss-Gallenkamp Technik, Type: SGC-120 Fitotron) for two years (2021

and 2022) at the MATE Buda campus. Simultaneously, alongside the drought experiment, the intra and inter-specific variability of *Ocimum* species was assessed during 2021 and 2022 at the Soroksár Experimental and Research Farm. Tables 1 and 2 provide a summary of the daily mean air temperatures (°C), daily relative humidity (%), rainfall (mm), and soil media characteristics pertinent to each of the respective experiments.

Table 1. Metrological data of open field and greenhouse experiment (2020-2022)

Year	Months	Open field			Greenhouse	
		Temperature (°C)	RH (%)	RF (mm)	Temperature (°C)	RH (%)
2020	June	20.1	77.4	96.0	-	-
	July	21.0	74.0	67.4	26.5	-
	August	22.3	72.6	60.0	24.6	-
	September	17.0	75.3	27.2	24.6	-
2021	June	24.3	60.0	7.4	25.3	61.0
	July	23.9	66.0	71.0	24.9	65.0
	August	20.0	74.5	68.4	22.0	70.5
	September	16.0	75.3	33.2	-	-
2022	June	20.0	62.7	73.4	28.4	56.0
	July	23.5	51.6	27.0	26.7	40.0
	August	23.6	63.4	38.2	27.6	52.0
	September	15.2	79.7	123.8	-	-

RH: relative humidity, RF: Rainfall

N.B. The climatic chambers were programmed as follows: 14 hours a day/10 h night cycle, (light intensity: 14500 lux; fluorescent lamp (4200K) and incandescent lamp (2700K) temperature program: 25 °C Day/17 °C night and relative humidity 65%.

Table 2. Soil media characteristics of each consecutive experiment

Characteristics	Open field			Greenhouse			Growth chamber	
	2020	2021	2022	2020	2021	2022	2021	2022
pH (H_2O)	8.6	8.0	7.6	7.2	6.8	6.6	5.5	6.8
Humus (%)	2.0	1.7	1.7	2.8	6.9	5.3	8.2	6.2
K_A	<30.0	26.0	<25.0	-	>60.0	46.0	-	-
NO_2+NO_3-N (mg/kg)	1.6	11.9	11.4	39.2	137.9	181.8	1401.5	300.4
P_2O_5 (mg/kg)	95.5	473.9	544.4	154.0	526.6	642.8	875.6	565.5
K_2O (mg/kg)	165.0	377.3	177.4	193.0	492.2	388.5	3357.4	1610.7
Mg (mg/kg)	52.9	146.9	377.9	67.7	377.0	717.4	829.1	631.6
Na (mg/kg)	-	45.8	30.1	-	54.3	50.9	253.1	625.1
Fe (mg/kg)	8.2	-	-	43.1	-	-	-	-
Mn (mg/kg)	11.9	22.1	58.5	26.8	31.4	48.0	54.7	34.8
Zn (mg/kg)	19.7	2.1	4.9	40.6	7.8	6.1	12.4	9.3
Cu (mg/kg)	1.8	1.9	2.5	1.5	2.1	1.8	14.0	2.4
SO_4 (mg/kg)	-	24.8	71.6	-	92.5	150.5	3283.9	11179.6

2.2.Treatments and plant materials

The details of treatments of the respective treatments and experimental management are indicated in Tables 3, 4, and 5.

Table 3. Treatments of the respective experiments

Experiments	Year	Species	Treatments
I. Intra and inter-specific variability of *Ocimum*	2021 and 2022	15 *Ocimum* genotypes (as indicated in Table 4)	
II. Open field irrigation trial	2020 - 2022	1. *O. basilicum* 'Ohře' 2. *O. basilicum* 'Genovese' 3. *O.* × *africanum* 4. *O. americanum* 5. *O. sanctum* 6. *O. selloi*	a. Non-irrigated b. Irrigated ($20m^3$ m^{-2})
III. Greenhouse drought stress trial	2020 - 2022	1. *O. basilicum* 'Genovese' 2. *O.* × *africanum* 3. *O. americanum*	a. 70% SWC b. 50% SWC c. 30% SWC
IV. Plant growth chamber drought stress trial	2021 and 2022	1. *O. basilicum* 'Ohře' 2. *O. americanum*	a. 70% SWC b. 50% SWC c. 30% SWC

SWC: Soil water capacity

Table 1. List of species, cultivar name, and origin of basil accessions

Accession No.	Species	Cultivar /common name	Origin
LAMIOCI20	*O. basilicum*	Dark opal	Department gene bank
LAMIOCI38	*O. basilicum*	Thai basil	Department gene bank
LAMIOCI43	*O. basilicum*	Cinnamon	Department gene bank
LAMIOCI13	*O. basilicum*	Genovese	Department gene bank
LAMIOCI51	*O. basilicum*	Turkish basil	Department gene bank
LAMIOCI52	*O. basilicum*	Adi F1	Jelitto seed cataloge (Germany)
LAMIOCI53	*O. basilicum*	Sweet Aroma	Department gene bank
LAMIOCI19	*O. basilicum*	M. Grünes	Department gene bank
LAMIOCI41	*O. basilicum*	Ohře	Department gene bank
LAMIOCI54	*O. sanctum*	Green holy basil	Denmark
LAMIOCI56	*O. sanctum*	Purple holy basil	India
LAMIOCI57	*O. americanum*	Lime	Department gene bank
LAMIOCI58	*O. americanum*	Togo basil	Togo
LAMIOCI59	*O.* × *africanum*	Hoary basil	Department gene bank
LAMIOCI48	*O. minimum*	Törpe	Department gene bank

LAMIOCI: Lamiaceae-*Ocimum*; M. Grünes :Mittelgroβblättriger Grünes

Table 5. Experimental management of respective studies

Experimental management	Open field	Greenhouse	Plant growth chamber
Transplanting	Mid-June	Mid-June	Mid-January
Soil media mixture	Sandy loam soil	5 kg per pot of sandy loam soil and peat moss mixture with 1:1 v/v ratio	0.5 kg per pot of compost, peat moss, and perlite mixture with 3:3:1 v/v/v ratio
Plot or Pot size	2.1 m x 1.5 m	12L	1L
Plant per plot or pot	20 plants/ plot	1 plant/pot	1 plant /pot
Drought treatment (days)	30	40	47
Irrigation	Twice a week	3 times a week	3 times a week
Cultivation	Once in 2 weeks	Weekly	Once in 2 weeks
Harvesting	Full bloom	Full bloom	Full bloom

2.3. Methods of data measurements

2.3.1. Soil water capacity determination

Soil water capacity (SWC) was determined using the modified gravimetric method of Reynolds (1970) based on the water-holding capacity of the soil (Figure 1). To perform the procedure, a composite sample weighing 500 g was fully saturated by adding distilled water. The saturated soil samples were then left to drain for 24 hours to remove excess water. Afterward, the saturated soil sample (Ww) was weighed and placed in an oven set at 105 °C for 24 hours, then oven dry weight (Wd) was determined. The SWC (%) was calculated as follows:

$$SWC\ (\%) = \frac{(Ww - Wd)}{Wd} \times 100$$

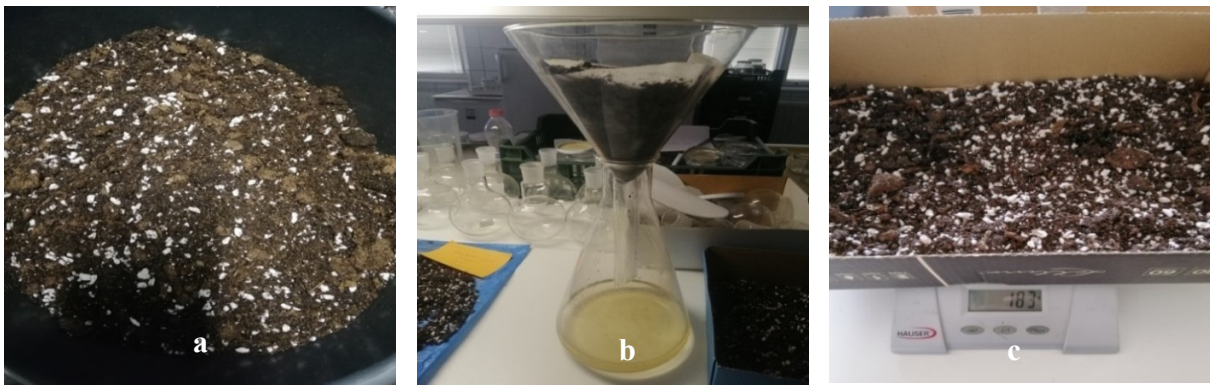

Figure 1. Soil water content determination by gravimetric method: Soil media mixture (a), saturated soil sample in volumetric flask and fennel (b), oven dry soil (c), (Photo: Mulugeta, 2022)

2.3.2. *Physiological parameter determination*

Leaf relative water content (RWC): Six to twelve fully developed leaves (depending on the species) were randomly sampled from the second and third internode of each plant. As indicated in Figure 2 below, Leaf parts were weighed to determine the fresh weight (FW) and then soaked in distilled water for 24 hrs. After that period, excess surface water of leaf parts was removed by paper towels, and turgid weight (TW) was determined. After drying at 105 °C until constant weight, the dry weight (DW) of leaf parts was determined. The RWC was then calculated according to the formula below (Weatherly, 1950, Barrs, 1968):

$$RWC\ (\%) = \frac{(FW - DW)}{(TW - DW)} \times 100$$

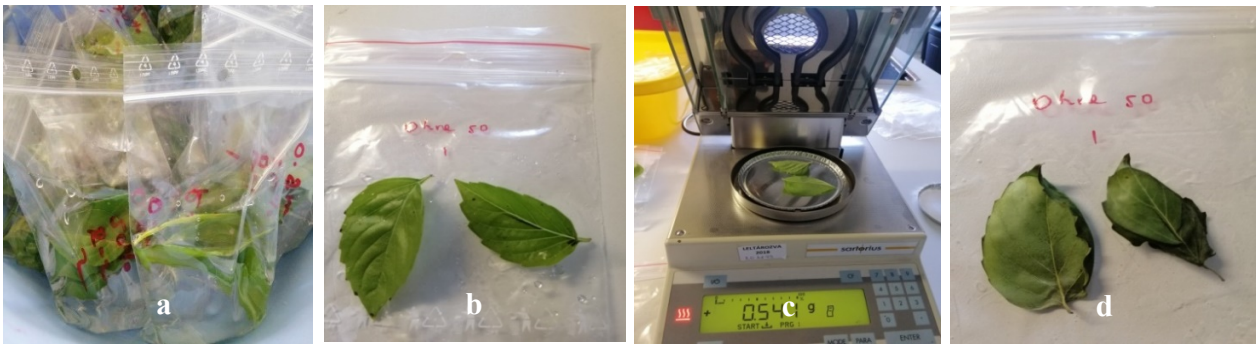

Figure 1. Relative water content determination: Soaked leaves (a), overnight soaked leaf sample (b), oven (c), oven dry leaf sample (d), (Photo: Mulugeta, 2021)

Water potential (WP): The water potential of the plants was determined in the leaves of the second and third nodes under the top of the shoots. The measurements were carried out in 3 replications (3 plants) per treatment in the full flowering phase, 2 days after watering, between noon and 2 p.m. in a pressure chamber (SKPM 1405, Skye Instruments Ltd., UK). As a gas N was used (Scholander *et al*., 1965).

Water use efficiency of the shoot (WUEs): was computed with the equation below as described by Howell (2001), dividing the dry weight of shoots by the total water (irrigation) that each treatment received. The sample pots from which the WUE measurements were recorded were wrapped with a transparent plastic film. Throughout the experiment, the amount of irrigation water applied was consistently monitored. The total irrigation water applied to each treatment was calculated as the cumulative sum of water added during the entire experiment.

$$WUEs = \frac{Dry\ shoot\ weight\ (g)}{Aplied\ irrigation\ water\ per\ pot\ (L)}$$

Chlorophyll content (SPAD value): The chlorophyll content of leaves indicated by the quantification of green color intensity was measured with a handheld SPAD meter (SPAD-502Plus Konica Minolta Inc., Japan). The values are calculated based on the amount of light transmitted by the leaf in two wavelength regions in which the absorbance of chlorophyll is different (Figure 3). The readings were taken at the third internodes from a fully developed leaf before harvesting**.** To calculate the mean, five plants per treatment (2 leaves per plant and 5 readings per leaf) were taken.

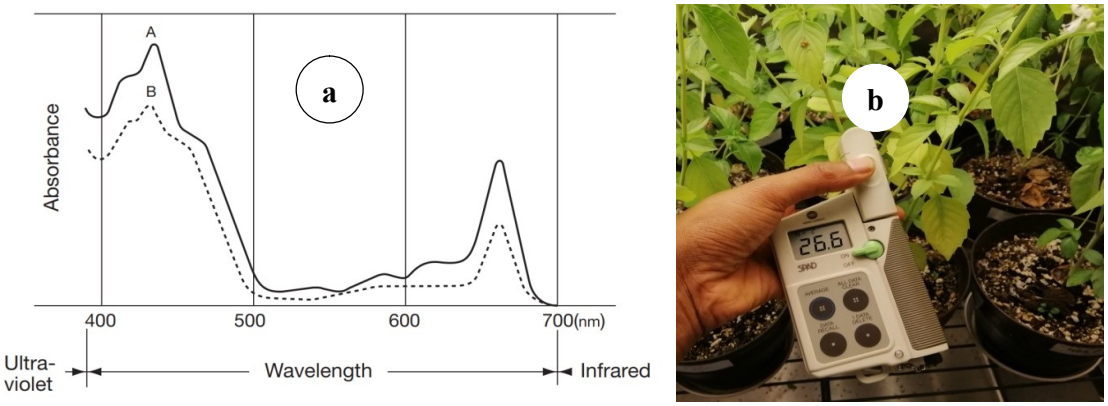

Figure 3. Spectral absorbance characteristic of chlorophyll (a) and SPAD value reading (b), (Photo: Mulugeta, 2021)

2.3.3. Morphological parameter measurement

Qualitative morphological traits: a standard basil descriptor developed by the International Union for the Protection of New Varieties of Plants (UPOV, 2003) was used.

Quantitative morphological traits: Plant height (cm), canopy diameter (cm), root fresh weight (g plant^{-1}), shoot fresh weight (g plant $^{-1}$), and shoot dry weights (g plant $^{-1}$) (after drying at room temperature in shadow) were measured from five sample plants per treatment at harvesting. Leaf area (cm^2) was measured by tracing the leaves over a square paper, and the grids covered by the leaf were counted to give the area. Randomly selected 20 leaves (2 leaves per plant and 10 leaves per treatment) were used to calculate the mean. Furthermore, additional leaf, flower, and seed parameters were taken for the morpho-chemical variability study. For leaf parameters, such as leaf length and leaf width, the mean of 20 leaves per genotype and two leaves per sample plant was calculated. The number of inflorescences was counted from 10 sample plants per genotype. We measured the inflorescence lengths (cm) of 10 plants per genotype and three inflorescences per genotype. Subsequently, after proper drying and seed extraction, 1,000 seeds of each genotype were weighed.

Glandular hair density: the glandular hair density is measured according to the Radácsi *et al*. (2020) procedure. Samples were taken from the leaf blade at the 3rd internode from the top. Circles of 4.0 mm diameter were cut out from the central part of the leaf blade excluding the main vein. Then the number of glandular peltate hairs on the abaxial surface of these blade samples was counted under a stereomicroscope (type BMS 74959). Twenty replicates per treatment were carried out.

2.3.4. Biochemical parameter measurement

Essential oil content (EOC): Ten sample plants for open field and eight sample plants for greenhouse and climatic chamber experiments were harvested per treatment and dried in well-ventilated rooms in shadow for two weeks. The essential oil content (mL $100g^{-1}$ of dry mass) was measured in six replications using a bulk sample of dried leaves and inflorescences without the stem. Using a Clevenger-type apparatus, 20 grams of dried material from each sample was hydro-distilled in 500 mL of distilled water based on the recommendation of the Hungarian Pharmacopoeia (1986). Following the collection of the oils, traces of water were removed and stored in an airtight vial in the refrigerator for a week at 4 °C before analysis.

Essential oil composition: the essential oil composition was determined by Gas Chromatography-Mass Spectrometry (GC-MS). GC analysis was carried out with an Agilent Technologies 6890 N instrument that was equipped with an HP-5MS capillary column (length: 30 m x 0.25 mm, film thickness of 0.25 μm). The initial temperature during the analysis was 60 °C, then it was raised to 240 °C at a rate of 3 °C min^{-1} and the final temperature was maintained for 5 minutes. The injector and detector were heated to 250 °C. Helium was used as a carrier gas with a constant flow rate (1 mL min^{-1}), a split ratio of 30:1, and an injection volume of 0.2 μL (1%, n-hexane). To express the proportions of individual compounds, we used total area percentages. The components were identified using an Agilent Technologies MS 5975 detector (Agilent Technologies, Inc., Waltham, MA, USA). The energy of ionization was 70 eV. Full scan mass spectra revealed total ion current chromatograms (TIC). The equation of Van Den Dool and Kratz (1963) was used to calculate linear retention indices. Homemade library mass spectra, Adams (2007) and commercial ones (NIST, Wiley) were compared with the linear retention indices (LRIs) and mass spectra. We repeated the SPME and GC samples three times.

Total polyphenol content (TPC): 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 a gram of dried and powdered plant material was extracted by 50 mL of boiling distilled water and was allowed to stand for 24 hours at room temperature. Then the extracts were filtered and stored in a freezer until the measurements were taken. Forty 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–Ciocalteau's reagent (10 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 $^{\circ}$C) for 5min, and the absorbance was measured at the wavelength of λ=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 equivalent calculated on the dry weight basis of the extract (mg GAE g^{-1} DW). The measurements were done in 6 replications.

Antioxidant capacity (AOC): 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 too. FRAP reagent was prepared that contains sodium acetate buffer (pH 3.6), TPTZ (2, 4, 6-tripiridyl-s-triazine) in HCl and $FeCl_3 \cdot 6H_2O$ solution (20 mmol L^{-1}), in proportion 10:1:1 (v/v/v), respectively. Ten µL of the test sample was added to 1.5 mL of acting FRAP reagent and 40 µL distilled water and absorbance was recorded at 593 nm after 5 minutes using the spectrophotometer above. 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^{-1} of dry extract.

2.4. Statistical analysis

Data were evaluated using a one-way analysis of variance (ANOVA). Shapiro-Wilk's test and Levene's test were used to check the normality of distribution and the homogeneity of variances, respectively. Significant mean differences were examined with t-test and Tukey HSD at $P < 5$. Hierarchical cluster analysis was performed based on the squared Euclidean distance by the Ward method, and a dendrogram was generated. Principal component analysis was also performed. All statistical analysis was performed using IBM SPSS 25 except for hierarchical cluster analysis and principal component analysis for which Originpro23b software were employed.

3. Results and discussions

The *Ocimum* genus, renowned for its medicinal properties, exhibits remarkable genetic diversity, encompassing variations in morphological traits and essential oil composition. Environmental factors further contribute to the morpho-chemical traits of this genus. This study aimed to assess the morpho-chemical variability within 15 *Ocimum* genotypes and examine their response to drought stress across different cultivation environments (open field, greenhouse, and plant growth chamber). The investigation revealed significant variations in morphology and bioactive chemical constituents among the studied genotypes. Notably, the sweet basil genotypes 'Genovese', 'Sweet Aroma', and 'Cinnamon' displayed robust morphological growth, resulting in higher fresh biomass (> 425 g plant^{-1}). *Ocimum* × *africanum* exhibited an exceptionally high essential oil yield (2.6 mL plant^{-1}) and essential oil content (3.4%), with 'Törpe' plants following closely with the second-highest essential oil content (1.9%). 'Turkish basil' stood out with the highest total polyphenol content (218.8 mg GAE g^{-1} DM) and antioxidant capacity (290.4 mg AAE g^{-1} DM). The essential oils from different basil genotypes exhibited diverse compositions. 'Thai basil' was characterized by a predominant estragole content (53.3%), while other sweet basil genotypes like 'Törpe' and 'Lime' featured linalool as a major constituent (ranging between 32% and 59%). Purple holy basil displayed *trans*-ß-caryophyllene (36.2%) and eugenol (32.2%), whereas 'Green holy basil's' essential oil comprised 1,8-cineole (22%), estragole (24%), and bisabolene (31%). Hoary basil exhibited a composition of 1,8-cineole (36%), camphor (18%), and limonene (14%), while 'Togo basil' was characterized by a significant amount of methyl cinnamate (61%). In total, fifteen genotypes were studied, and from these, three morphologically distinct and eight chemo-diversity were identified, illustrating the extensive intra- and inter-specific diversity present among basil genotypes preserved in the gene bank of the department.

Multiple experiments conducted on drought stress unveiled notable alterations in the physiological, morphological, and biochemical responses of the selected *Ocimum* species. Basil plants undergo diverse physiological changes when subjected to drought stress, and to evaluate these changes, several physiological indicators such as RWC, WP, WUEs, and SPAD values were documented. In a three-year greenhouse experiment, increasing drought intensity led to a reduction in leaf RWC. Notably, *O. americanum* exhibited the highest reduction, with 18%

under moderate drought stress and 26.8% under severe drought stress. In contrast, *O. basilicum* 'Genovese' showed reductions of 7.5% and 15.11% under moderate and severe drought stress, respectively. Similar results were observed in a plant growth chamber experiment, where *O. basilicum* 'Ohře' and *O. americanum* displayed leaf RWC reductions of over 5% and 14% under moderate and severe drought stresses, respectively. Additionally, significant changes were noted in WP and WUEs indicators with varying water supply. In a multi-year plant growth chamber experiment, the WP of *O. basilicum* 'Ohře' decreased from -0.6 MPa under control treatment to -0.8 MPa under moderate drought stress, further declining to -1.4 MPa under severe drought stress. Similar trends were observed in *O. americanum*, with WP ranging from -0.8 to -0.5 MPa under control and drought stress conditions, respectively. Soil water capacities played a crucial role in affecting the WUEs of both *O. basilicum* 'Ohře' and *O. americanum* during production years. As soil moisture decreased, WUEs increased, with severe drought stress plants showing a 1.8-fold and 3.4-fold improvement in *O. basilicum* 'Ohře' and *O. americanum*, respectively. Moreover, soil water capacities had influenced SPAD values. In the greenhouse trial, *O. basilicum* 'Genovese' and *O.* × *africanum* basil plants subjected to severe drought stress showed average SPAD values ranging from 47.5 to 52.4, while control plants had SPAD values ranging from 37.8 to 44.3. A similar trend was observed in the plant growth chamber experiment. For *O. basili*cum 'Ohře,' the average SPAD values ranged from 29.0 to 40.3 under control and severe drought stress treatments. *Ocimum americanum* plants exhibited SPAD values between 40.6 and 50.6. It should be noted that the SPAD values varied based on the production year, basil species, and growing conditions as well.

Plant growth is adversely affected by water stress, a phenomenon that has been extensively studied and well documented. Hence, multiple studies under open field, greenhouse, and plant growth chambers consistently over the years demonstrated that drought stress caused an enormous reduction in the growth of all *Ocimum* species investigated. These reductions are characterized by shorter heights and a narrower canopy. Additionally, their stems become slenderer, and the leaves exhibit reduced dimensions. Consequently, lower soil moisture levels result in a marked decrease in both fresh and dry biomass production. Taxa-specific responses were also observed under drought stress conditions. As a result, *O.* × *africanum* suffered significant losses in both non-irrigated open field plots and under greenhouse conditions when

exposed to lower soil's water capacity. On the other hand, *O. selloi*, grown in open fields without irrigation, suffered less. Consequently, *O.* × *africanum* plants grown without irrigation exhibited a decrease of 7.8 cm (16.0%) in plant height and 9.5 cm (20.7%) in canopy diameter. Similarly, *O.* × *africanum* subjected to severe drought stress in a greenhouse displayed a reduction of 29.0% in plant height and 20.8% in canopy diameter. Additionally, as a drought stress coping mechanism, both greenhouse and plant growth chamber experiments revealed that basil species (including *O.* × *africanum*) reduced their leaf area and stem diameter by over 40%. Furthermore, in terms of fresh herb yield, *O.* × *africanum* experienced a substantial loss of 39.0% (100.0 g plant^{-1}) in non-irrigated open field plots and a 73.0% reduction when cultivated with 30% SWC under greenhouse conditions. In contrast, the reduction in fresh herb yield was significantly lower at 2.3% in the case of *O. selloi* grown in non-irrigated open field plots.

The density of glandular hairs (GHD) or essential oil glands in *Ocimum* species was significantly influenced by soil water capacity and the specific species of *Ocimum*. Both moderate and severe drought stress caused an increase in GHD in *Ocimum* species during greenhouse experiments. The GHD increase due to severe drought stress ranged from 32.8% to 107.6% for *O. basilicum* 'Genovese' and *O. americanum*, respectively. Additionally, under moderate drought stress, the GHD increase for the same species varied between 23.5% and 32.0%. Over consecutive years, *O.* × *africanum* consistently exhibited the highest GHD, ranging from 869.4 to 2292.5 units 100 mm^{-2}, depending on soil water capacity and the year of production. A parallel experiment in a plant growth chamber confirmed this trend. In comparison to the control, severe drought stress increased glandular hair density by 17.0% to 74.0%, *O. basilicum* 'Ohře' and *O. americanum,* respectively.

Moreover, these various experiments consistently demonstrate that the influence of drought stress on the accumulation of secondary compounds is both heterogeneous and unpredictable. Several crucial factors, including the severity of drought, specific plant taxa, growth conditions, and the production year, play a pivotal role in determining the impact on the production of various secondary compounds such as EOC, EOY, essential oil composition, TPC, and AOC. As a result, the three-year open field results revealed that drought stress (non-irrigated) resulted in a significant increase in the EOC of *O. basilicum* 'Genovese' by 13.0%, *O.* × *africanum* plants by 22.6%, and a substantial 27% elevation in the EOC of *O. selloi*. However, no significant

differences were observed in the other species. Greenhouse experiments further indicated that severe drought stress led to an increase (>25.0%) in the EOC of *O.* × *africanum* in 2021 and 2022 but reduced *O. americanum's* EOC by over 15.0% in both 2020 and 2021. However, moderate drought stress negatively affected EOC for 'Genovese' in 2020 and *O. americanum* in 2022, with no significant changes observed in the remaining treatments. Similarly, a slight rise in EOC under the plant growth chamber conditions was also noted. Despite fluctuations in EOC due to water supply, the EOY consistently remained lower under lower soil water supply in all experiments. In the greenhouse experiment, the EOY reduction under severe drought stress conditions ranged from 74.0% (*O.* × *africanum*) to 84.0% (*O. americanum*). The moderate drought resulted in a 50.0% decline in *O.* × *africanum* and 62% in *O. basilicum* 'Genovese'. The plant growth chamber experiment indicated that severe drought stress led to a 45% reduction in EOY in both *O. basilicum* 'Ohře' and *O. americanum*. Furthermore, under moderate drought stress, EOY loss ranged from 10.0% (*O. americanum*) to 22.5% (*O. basilicum* 'Ohře'). Despite variations in the relative proportions of the primary compounds found in *Ocimum* species, the essential oil compositions of the examined basil species were influenced by factors such as the production year, cultivation environment, and the severity of drought. The overall data for each year suggested that drought stress had no significant impact on the essential oil composition of the *Ocimum* species, despite yearly fluctuations and diversity. However, subtle changes were noted, particularly in sweet basil cultivars ('Genovese' and 'Ohře'), *O. americanum*, *O.* × *africanum*, and *O. selloi*. Under lower soil water capacities, there was a minor increase in the ratios of linalool, 1,8-cineole, and tau-cadinol. Additionally, the camphor ratios of *O.* × *africanum* showed a significant increase in response to drought stress. Interestingly, non-irrigated plants of *O. selloi* exhibited notably higher ratios of methyl eugenol, with a 41% increase. While drought stress adversely affected the oxygenated monoterpene ratios in sweet basil cultivars ('Genovese' and 'Ohře'), *O. americanum*, and *O.* × *africanum*, it had a positive impact on phenylpropanes in *O. selloi* and *O. sanctum*. Phenylpropanes, in particular, displayed the highest ratios of essential oil content under drought conditions for these specific species. These findings highlight the nuanced and species-specific responses of essential oil compositions in basil species to varying drought conditions.

The impact of drought stress exhibits variability in its effects on both TPC and AOC, dependent upon factors such as the specific plant species, the severity of the drought, the year of production, and the growing environment. The three-year average of open field experiments revealed that the absence of irrigation resulted in a 17% increase in TPC for *O. sanctum* and an 18.6% increase for *O. selloi*. Conversely, positive effects were observed in the AOC for *O. basilicum* 'Genovese' (21.4%), *O.* × *africanum* (29.6%), and *O. sanctum* (20.2%). On the contrary, *O. basilicum* 'Ohře' experienced a negative impact on its TPC under drought stress (16% less). Another experiment conducted in a greenhouse indicated that moderate and severe drought stress increased the TPC of *O. americanum* by 25% and 28%, respectively, while other basil species showed no significant changes. No changes were observed on AOC. Similarly, in a plant growth chamber, severe drought stress led to a 20% increase in TPC for *O. basilicum* 'Ohře' and a 15% increase for *O. americanum*. However, their AOC capacities were adversely affected, decreasing by 15% and 11.3%, respectively. This demonstrates the complex and species-specific responses of these basil plants to different levels of drought stress across varied experimental conditions.

In conclusion, it is advisable to maintain a soil water capacity of at least 70% in protected basil cultivation to optimize the growth of biomass and the production of essential oil. For open field cultivation, especially in regions with climates similar to Hungary, it is recommended to implement a twice-weekly irrigation schedule. Specifically, the sweet basil cultivars 'Genovese' and 'M. Grünes' are recommended for herb production due to their favorable characteristics. Additionally, it is proposed that further research be conducted to delve into the mechanisms underlying the changes in secondary compounds in response to drought stress. This includes an emphasis on exploring molecular traits and phenol composition, aiming for a more comprehensive understanding of how basil plants adapt to and are affected by varying levels of water availability.

4. New research findings

- ✓ The effect of drought stress on basil's secondary compound accumulation is described as "taxa specific."

- ✓ Drought stress has been observed to enhance the density of glandular hairs on *Ocimum* species. The increase in glandular hair density can be seen as a physiological response of the plant to drought stress. Consequently, a greater density of glandular hairs may suggest a potentially increased concentration of essential oils. However, it is important to note that EOC can also be influenced by various factors.
- ✓ Severe drought stress negatively impacted the ratios of oxygenated monoterpenes while positively affecting the ratios of phenylpropanes in the studied *Ocimum* species, except *O. basilicum* 'Genovese'.
- ✓ Researchers observed significant variations in the chemical composition such as EOC, EOY, essential oil composition, TPC, and AOC of basil plants both within the same species (intraspecific) and between different species (interspecific) than the one reported in the literature.
- ✓ The year in which basil plants were grown and harvested had a more pronounced effect on the composition of their essential oils than the drought treatment.
- ✓ Various promising *Ocimum* species, including *O. selloi*, *O.* × *africanum*, as well as *O. basilicum,* and *O. americanum* of different origins, have been examined in the Hungarian environment.

5. References

1. Adams, R.P. (2007). Identification of essential oil components by gas chromatography/ mass spectrometry, 4th ed. Allured Publ., Carol Stream, IL.
2. Anusmitha, K.M., Aruna, M., Job, J.T., Narayanankutty, A., PB, B., Rajagopal, R., Alfarhan, A. and Barcelo, D. (2022). Phytochemical analysis, antioxidant, anti-inflammatory, anti-genotoxic, and anticancer activities of different *Ocimum* plant extracts prepared by ultrasound-assisted method. *Physio. Molec. Plant Path.* 117, 101746. https://doi.org/10.1016/J.PMPP.2021.101746
3. Bajomo, E.M., Aing, M.S., Ford, L.S. and Niemeyer, E.D. (2022). Chemotyping of commercially available basil (*Ocimum basilicum* L.) varieties: Cultivar and morphotype influence phenolic acid composition and antioxidant properties. *NFS J.* 26, 1-9. https://doi.org/10.1016/J.NFS.2022.01.001
4. Barrs, H.D. (1968). Determination of water deficits in plant tissues. *In*: Koslowski, T.T. (ed.): Water deficits and plant growth. Academic Press, New York, Vol. 1: 235-368.
5. Benzie, I.F. and Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* 239, 70-76. https://doi.org/10.1006/abio.1996.0292
6. Carović-Stanko, K., Liber, Z., Besendorfer, V., Javornik, B., Bohanec, B., Kolak, I. and Satovic, Z. (2010). Genetic relations among basil taxa (*Ocimum* L.) based on molecular

markers, nuclear DNA content, and chromosome number. *Plant Syst. Evol.* 285(1): 13-22. https://doi.org/10.1007/s00606-009-0251-z
7. Chang, X., Alderson, P. and Wright, C. (2005). Effect of temperature integration on the growth and volatile oil content of basil *(Ocimum basilicum L.). J. Horti. Sci. Biotech.,* 80(5): 593-598. https://doi.org/10.1080/14620316.2005.11511983
8. Chowdhury, T., Mandal, A., Roy, S.C., Sarker, D.D. (2017). Diversity of the genus *Ocimum* (Lamiaceae) through morpho-molecular (RAPD) and chemical (GC–MS) analysis. *J. genet. Eng. Biotech*. 15(1):275-286. http://doi:10.1016/j.jgeb.2016.12.004
9. Ciriello, M., Kyriacou, M. C., de Pascale, S. and Rouphael, Y. (2022). An appraisal of critical factors configuring the composition of basil in minerals, bioactive secondary metabolites, micronutrients, and volatile aromatic compounds. *J. Food Compos. Analy.*, 111: 104582. https://doi.org/10.1016/J.JFCA.2022.104582
10. Filip, S., Pavlić, B., Vidović, S., Vladić, J. and Zeković, Z. (2017). Optimization of microwave-assisted extraction of polyphenolic compounds from *Ocimum basilicum* by response surface methodology. *Food Anal. Methods*. 10, 2270-2280. https://doi.org/10.1007/s12161-017-0792-7
11. Gurav, T.P., Dholakia, B.B. and Giri, A.P. (2022). A glance at the chemodiversity of *Ocimum* species: Trends, implications, and strategies for the quality and yield improvement of essential oil. *Phytochem Rev*. 21, 879-913. https://doi.org/10.1007/s11101-021-09767-z
12. Howell, T.A. (2001). Enhancing water use efficiency in irrigated agriculture. *Agron. J*. 93, 281-289. https://doi.org/10.2134/agronj2001.932281x
13. Müller, J. and Heindl, A. (2006). Drying of Medicinal Plants. *In*: J. Bogers, L.E. Craker and D. Lange (eds.). Medicinal and aromatic plants - agricultural, commercial, ecological, legal, pharmacological and social aspects. *Springer*, 237-252. http://DOI:10.1007/1-4020-5449-1_17
14. Mulugeta, S.M., Radácsi, P. and Gosztola, B. (2022). Morphological and biochemical responses of selected *Ocimum* species under drought. *Herba Polon.* 68(4), 1-10. http://doi:10.2478/hepo-2022-0019
15. Naveen, M., Jayaraj, J., Chinniah, C., Mini, M.L., Vellaikumar, S. and Shanthi, M. (2021). Insecticidal property of methanolic leaf extract of sweet basil, *Ocimum basilicum* (L.) against cigarette beetle, *Lasioderma serricorne* (Fab.) (Coleoptera: Anobiidae). *J. Ento. Zool. Stud.* 9(1):259-262. https://doi.org/10.22271/j.ento.2021.v9.i1d.8155
16. Nurzyñska-Wierdak, R. (2014). Morphological variability and essential oil composition of four *Ocimum basilicum L. cultivars. J. Essent. Oil-Bear. Plants.* 17(1): 112-119. https://doi.org/10.1080/0972060X.2013.855362
17. Pandey, A.K., Singh, P. and Tripathi, N.N. (2014). Chemistry and bioactivities of essential oils of some *Ocimum species*: an overview. *Asian Pacific J. Trop. Biomed.*, 4(9): 682-694. https://doi.org/10.12980/APJTB.4.2014C77
18. Patel, R.P., Singh, R., Saikia, S.K., Rajeswara Rao, B.R., Sastry, K.P., Zaim, M. and Lal, R.K. (2015). Phenotypic characterization and stability analysis for biomass and essential oil yields of fifteen genotypes of five *Ocimum* species. *Ind. Crops Prod.* 77, 21-29. https://doi.org/10.1016/J.INDCROP.2015.08.043
19. Paton, A., Harley, R.M. and Harley, M.M. (1999). An Overview of classification and relationship, *In*: Hiltunen, R., Holm, Y (Eds.), The genus *Ocimum*, Harwood Academic Publishers, Amsterdam, pp. 1-7.

20. Pavithra K.H.G., Narase Gowda, P.N. and Prasad, M.P. (2019). Studies on Antimicrobial activity of *Ocimum* species of Karnataka against clinical isolates. *Ind. J. Pure App. Biosci*. 7(4), 245-254. http://dx.doi.org/10.18782/2320-7051.7732
21. Pharmacopoeia Hugarica. (1986). Pharmacopoeia Hungarica 7th ed., vol.1, Budapest, 395-398.
22. Radácsi, P., Inotai, K., Sárosi, S., Hári, K., Seidler-Łożykowska, K., Musie, S. and Zámboriné, É.N. (2020). Effect of irrigation on the production and volatile compounds of sweet basil cultivars (L.). *Herba Polon*. 66, 14-24. https://doi.org/10.2478/hepo-2020-0021
23. Reynolds, S.G. (1970). The gravimetric method of soil moisture determination Part I A study of equipment, and methodological problems. *J. Hydro.* 11(3): 258-273. https://doi.org/10.1016/0022-1694(70)90066-1
24. Scholander, P.F., Bradstreet, E.D., Hemmingsen, E.A. and Hammel, H.T. (1965). Sap pressure in vascular plants: Negative hydrostatic pressure can be measured in plants. *Sci.,* 148(3668):339-346. *http*://doi:10.1126/science.148.3668.339
25. Selmar, D. and Kleinwächter, M. (2015). New insights explain that drought stress enhances the quality of spice and medicinal plants: potential applications. *Agron. Sustain. Dev.* 35, 121-131. https://doi.org/10.1007/s13593-014-0260-3
26. Shahrajabian, M.H., Sun, W. and Cheng, Q. (2020). Chemical components and pharmacological benefits of Basil (*Ocimum basilicum*): a review. *Int. J. Food Properties*, 23(1): 1961-1970. https://DOI:10.1080/10942912.2020.1828456
27. Simon, J.E. Reiss-Bubenheim, D., Joly, R.J. and Charles, D.J. (1992). Water stress-induced alterations in essential oil content and composition of sweet basil. *J. Essen. Oil Res*. 4 (1): 71-75. https://DOI:10.1080/10412905.1992.9698013
28. Szabó, K., Zubay, P. and Zámboriné, N. (2020). What shapes our knowledge of the relationship between water deficiency stress and plant volatiles? *Acta Physiol. Plant*, 42:130. https://doi.org/10.1007/s11738-020-03120-1
29. UPOV. (2003). Basil *(Ocimum basilicum* L.*)*: Guidelines for the conduct of tests for distinctness, uniformity, and stability. International Union of New Varieties of Plant (UPOV), Geneva.
30. Van Den Dool, H. and Kratz, P. (1963). A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J. Chromatogr. A* 11, 463-471. http://doi:10.1016/s0021-9673(01)80947-x
31. Weatherley, P.E. (1950). Studies in the water relations of the cotton plant. I. The field measurement of water deficits in leaves. *New Phytologist,* 49(1):81-97.

Publication

Journal articles with IF

1. Radácsi, P., Inotai, K., Sárosi, S., Hári, K., Seidler-Łożykowska, K., **Musie, S.** and Zámboriné, É.N. (2020). Effect of irrigation on the production and volatile compounds of sweet basil cultivars (L.). *Herba Polon*., 66:14-24. https://doi.org/10.2478/hepo-2020-0021. (IF: 1.04)
2. **Mulugeta, S.M**. and Radácsi, P. (2022). Influence of drought stress on growth and essential oil yield of *Ocimum* species. *Horticulturae,* 8 (175): 1-12. https://doi.org/10.3390/horticulturae8020175 (IF:3.1)

3. **Mulugeta, S.M**., Radácsi, P. and Gosztola, B. (2022). Morphological and biochemical responses of selected *Ocimum* species under drought. *Herba polon.*, 68(4):1-10. https://doi.org/10.2478/hepo-2022-0019 (IF: 1.04)
4. **Mulugeta, S.M**., Sárosi, S., and Radácsi, P. (2023). Physio-morphological trait and bioactive constituents of *Ocimum* species under drought stress. *Ind. Crops Prod.*, *205*, 117545. https://doi.org/10.1016/J.INDCROP.2023.117545 (IF:5.9)
5. **Mulugeta, S.M.**, Pluhár, Z. and Radácsi, P. (2024). Phenotypic variations and bioactive constituents among selected *Ocimum* species. *Plants*, 13(1):64. https://doi.org/10.3390/plants13010064 (IF: 4.5)

Conference full paper

1. **Mulugeta**, S.M. and Radácsi, P. (2023). Can drought stress enhance secondary compound accumulations in *Ocimum americanum* L.? *Lippay János – Ormos Imre – Vas Károly (LOV)* Tudományos Ülésszak, November 16, 2023, Budapest. Book of Abstract, Pp..

Conference abstracts

2. **Mulugeta,** S.M. and Radácsi, P. (2021). Influence of drought stress on growth and essential oil yield of *Ocimum species.* 51st *International Symposium on Essential Oils* (ISEO), November 12-14, 2021 (Online). Book of Abstract, Pp.20.
3. **Mulugeta**, S.M. and Radácsi, P. (2021). Morpho-chemical variability among selected *Ocimum species. Lippay János – Ormos Imre – Vas Károly (LOV)* Tudományos Ülésszak, November 29, 2021, Budapest. Book of Abstract, Pp.92.
4. **Mulugeta**, S.M. and Radácsi, P. (2022). Does drought stress enhance secondary compound accumulation in *Ocimum species? EOHUB* students' mobility conference, April 27, 2022, Madrid.
5. **Mulugeta**, S.M. and Radácsi, P. (2023). Physio-morphological and biochemical changes among *Ocimum* species under drought. 6th *International Symposium on Medicinal and Aromatic Plants* (SIPAM-6), March 17-21, 2023, Djerba – Tunisia. Book of Abstract, Pp. 24.
6. **Mulugeta**, S.M., Gosztola, B. and Radácsi, P. (2023). Variation in morphology and bioactive constituents among selected *Ocimum* species. *Fiatal Gyógynövénykutatók Fóruma*, October 6, 2023, Budapest. Book of Abstract, Pp. 12
7. **Mulugeta**, S.M. and Radácsi, P. (2023). Can drought stress enhance secondary compound accumulations in *Ocimum americanum* L.? *Lippay János – Ormos Imre – Vas Károly (LOV)* Tudományos Ülésszak, November 16, 2023, Budapest. Book of Abstract, Pp..