HUNGARIAN UNIVERSITY OF
AGRICULTURE AND LIFE SCIENCES

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

Doctoral (Ph.D.) dissertation

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2024
Budapest, Hungary

DECLARATION

I declare that the thesis titled “Effect of drought stress on herb production and accumulation of secondary compounds in selected *Ocimum* species” is my work and that it has not been submitted before for any degree or examination in any other University.

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Abbreviations

AAE	Ascorbic acid equivalents
AOC	Antioxidant capacity
CD	Canopy diameter
CV	Coefficient of variation
DHW	Dry herb weight
DM	Dry matter
DNA	Deoxyribonucleic acid
DW	Dry weight
EOC	Essential oil content
EOY	Essential oil yield
FC	Field capacity
FHW	Fresh herb weight
FRAP	Ferric reducing antioxidant power assay
FW	Fresh weight
GAE	Gallic acid equivalent
GC-MS	Gas chromatography-mass spectrometry
GHD	Glandular hair density
HLW	Hundred leaf weight
I	Irrigated
IL	Inflorescence length
LA	Leaf area
LAMIOC	Lamiaceae-*Ocimum*
LL	Leaf length
LRIs	Linear retention indices
LW	Leaf width
MAE	Microwave-assisted extraction
MAP	Medicinal and aromatic plants
MATE	Magyar Agrár- És Élettudományi Egyetem
NI	Non-irrigated
NIF	Number of inflorescences
NPB	Number of primary branches
NPK	Nitrogen-phosphorus-potassium
PH	Plant height
RF	Rainfall
RH	Relative humidity
RT	Retention time
RWC	Relative water content
SPAD	Soil plant analysis development
SPME	Solid phase microextraction
SWC	Soil water capacity
TPC	Total polyphenol content
TPTZ	Tripyridyl-S-Triazine
TSW	Thousand seed weight
TW	Turgid weight
UPOV	Union for the protection of new varieties of plants
UV	Ultra-violet
Wd	Oven dry weight
WP	Water potential
WUEs	Water use efficiency of shoot
Ww	Saturated soil weight

1. INTRODUCTION

1.1. Background and justification

The genus *Ocimum* L. (basil) belongs to the family *Lamiaceae*, 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). The genus comprises over 60 perennial and annual herbs and shrubs, each with its distinctive traits and features (Paton *et al*., 1999; Carović-Stanko *et al*., 2010; Gurav *et al*., 2022). 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). The most widely grown basil species are *O. basilicum* L., *O.* × *africanum* Lour. (syn. *O.* × *citriodorum* Vis), *O. americanum* L. (syn. *O. canum* Sims.), *O. gratissimum* L., *O. minimum* L., and *O. tenuiflorum* L. (syn. *O. sanctum* L.) due to their economical and medicinal importance (Carović-Stanko *et al*., 2010; Gurav *et al*., 2022). Basil is known for its considerable genetic diversity, morphological and biochemical variabilities (Nurzynska-Wierdak, 2007; Kwee and Niemeyer, 2011; Chowdhury *et al*., 2017; Gurav *et al*., 2020; Bajomo *et al*., 2022). Genetic divergence, cytogenetics, and molecular marker studies on *Ocimum* have provided valuable insights into the diversity and evolution of this genus. These studies have revealed that there is significant genetic variation within and between *Ocimum* populations and species (Labra *et al*., 2004; Carović-Stanko *et al*., 2010; Chowdhury *et al*., 2017; Singh *et al*., 2018).

Basils are characterized morphologically by square stems, opposite leaves, brown or black seeds (also known as nutlets), and spikes of flowers. The color, shape, and texture of their flowers and leaves vary by species. The color of flowers can range from white to lavender/purple, and the color of leaves can vary from green to purple. There are also a variety of leaf textures, ranging from smooth and glossy to curled and hairy. In addition, plant height varies by species (Simon *et al*., 1990; Hiltunen and Holm, 1999; Carović-Stanko *et al*., 2011; Patel *et al*., 2015a).

The distinctive scents and flavors of the many basil species and cultivars are due to the composition of essential oils. The essential oil is bio-synthesized and stored in a structure called glandular trichomes or essential oil glands found on the leaf, stem, and flower of the *Ocimum* species (Werker *et al*., 1993; Maurya *et al*., 2019). Glandular trichomes can be classified into two types, capitate and peltate types, which can be distinguished based on their size and number of secretory cells (Werker *et al*. 1993; Martnez-Natarén *et al*., 2018). The essential oil yield (EOY) generally ranges from 0.2 to 5.22% depending on the species, source, phenological stage of the plants, and other factors

(Hiltunen and Holm, 1999; Simon *et al*., 1999; Mulugeta and Radácsi, 2022). Many species of the genus contain essential oils based primarily on monoterpene derivatives such as camphor, limonene, thymol, citral, geraniol, and linalool. Other members of the genus, including sweet 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). Apart from volatile oil basil herbs are also rich in polyphenols contents ranging from phenolic acids (rosmarinic acid, caffeic acid, caftaric acid, chicoric acid, and others), simple or complex flavonoids to colored anthocyanins (Kwee and Niemeyer, 2011; Flip, 2017; Bajomo *et al*., 2022).

The aromatic essential oils and non-volatile compounds of basil species are used in flavor, fragrance, cosmetics, aromatherapy, and pharmaceutical industries and are widely acclaimed for their biological properties that possess antimicrobial activity (Pavithra *et al*., 2019; Amor *et al*., 2021), antioxidant properties (Ahmed *et al*., 2019; Teofilović *et al*., 2021) and insecticidal effect (Bhavya *et al*., 2018; Al-Harbi *et al*., 2021; Naveen *et al*., 2021). Additionally, *Ocimum* species are known to exhibit multiple therapeutic benefits, including anti-inflammatory properties (Shahrajabian *et al*., 2020; Anusmitha *et al*., 2022), anticancer activity (Shahrajabian *et al*., 2020; Zhan *et al*., 2020; Anusmitha *et al*., 2022), cardiovascular and anti-lipidemic actions (Pandey *et al*., 2014; Shahrajabian *et al*., 2020), and immunomodulatory and CNS activity (Mediratta *et al*., 2002; Zhan *et al*., 2020). Traditionally it is also used as a treatment for cold and cough, mouthwash, diuretic, carminative, appetite stimulant, astringent, and tonic agent (Holm, 1999). Furthermore, it is used in many cultures around the world for its ritual and spiritual properties (Simoons, 1998).

1.2. Statement of the problem

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; Figueiredo *et al*., 2008), agro-techniques (Onofrei *et al*., 2018; Ciriello *et al*., 2022) and processing methods (Müller and Heindl, 2006; da Silva *et al*., 2020). Among the ecological factors, drought stress is often reported to be the most limiting factor (Simon *et al*., 1992; Bettaieb *et al*., 2011). 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. *Ocimum* species are water-intensive plants and little is known about how drought stress affects them. Except for a few sweet basil cultivars, very little is known about how drought stress affects different basil species. In order to understand drought-induced physiological, morphological, and biochemical changes in selected *Ocimum* species, a three-year experiment (2020-22) was conducted in three different growing environments (open fields, greenhouses, and growth chambers).

1.3. 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:

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

2. LITERATURE REVIEW

2.1. Taxonomy and distribution

The genus *Ocimum* L. (tribe *Ocimeae*, subfamily *Nepetoideae*, family *Lamiaceae*) is one of the largest genera in the family and includes several highly valuable medicinal and economically significant herbs and shrubs (Chowdhury *et al*., 2017). Figure 1 illustrates its global distribution in tropical, subtropical, and warm temperate regions (Kew Royal Botanical Garden, 2022; Paton *et al*., 1999). There has been taxonomic confusion among basils due to a vague understanding of genetic relationships (Grayer *et al*., 1996; Paton and Putievsky, 1996; Gupta *et al*., 2018). It is caused by widespread interspecific hybridization, polyploidy, and aneuploidy, as well as morphologically similar but distinct chemotypes in this genus (Simon *et al*., 1990). According to World Flora Online (2023), the genus comprises 67 species of perennial and annual herbs and shrubs. 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). The most widely known species with strong aromatic properties are *Ocimum basilicum* (Sweet basil), *Ocimum gratissimum* (African basil), *Ocimum sanctum* (holy basil), and *Ocimum americanum* (American basil). Scholars suggest that to get better insight into intra- and interspecific taxonomy, a combined analysis of the geographic origin, morphological traits, karyotype, molecular markers, nuclear DNA content, and chemical composition, are paramount (Grayer *et al*., 1996; Paton and Putievsky, 1996; Putievsky *et al*., 1999; Libra *et al*., 2004; Carović-Stanko *et al*., 2010).

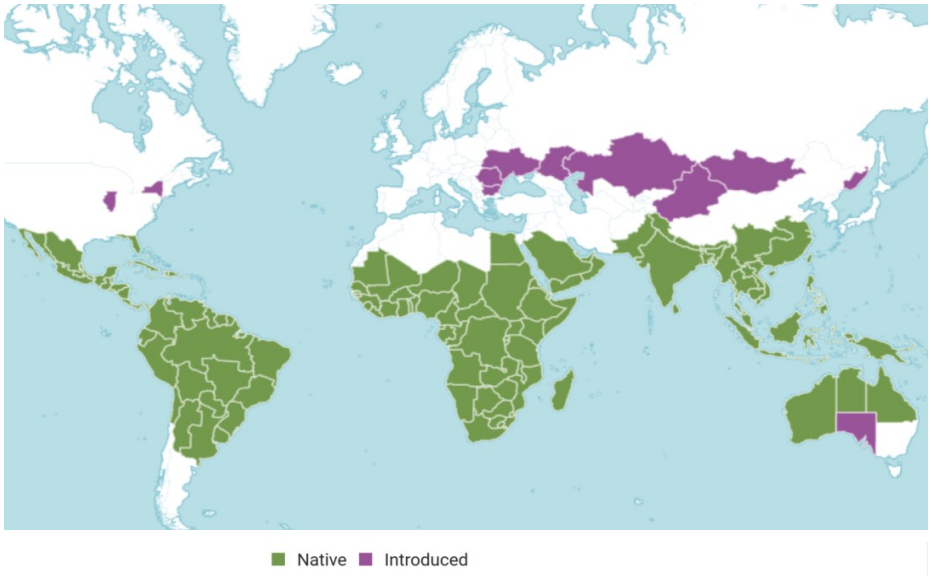

Figure 1. Worldwide distribution of *Ocimum* (Kew Royal botanical gardens, 2022)

2.2. Diversity in *Ocimum* species

The genus exists as morphotypes-differing in morphology (Marotti *et al*., 1996; Verma *et al*., 2013; Patel *et al*., 2015a), chemotypes – differing in essential oil composition (Vieira *et al*., 2006; Verma *et al*., 2013; Pyne *et al*., 2018) and genotypes-differing at the DNA level (Labra *et al*., 2004; Patel *et al*., 2015b).

2.2.1. Genetic diversity

According to genetic divergence, cytogenetics, and molecular marker studies, *Ocimum* has a high degree of genetic variability. Singh *et al*. (2018) observed a wider genetic divergence (D^2: 74 - 212.97) among 25 *Ocimum* accessions. There are numerous reports of variations in genome size and chromosome number among *Ocimum* species, suggesting that chromosomal rearrangements, sequence deletions/amplification, and polyploidization are involved (Carović-Stanko *et al*., 2010). The base chromosome number for the genus *Ocimum* is × = 8 according to Darlington and Wylie (1955). Whereas Morton (1962) suggested two base numbers × = 8, 12. However, a recent study by Carović-Stanko *et al*. (2010) suggested × = 12 (*O. basilicum* clade as tetraploid; *O. americanum* clade as hexaploids), × = 10 (*O. gratissimum*), and × = 9 (*O. tenuiflorum*) as base chromosome numbers. Based on random amplified polymorphic DNA (RAPD) analysis, Chowdhury *et al*. (2017) evaluated the level of variation in nine genotypes of *Ocimum* (Purple and green *O. tenuiflorum*, *O. basilicum* var. Babu and Marua, *O. gratissimum* var. Ram and Ajowan, *O.* × *africanum* Lour., *O. americanum* L. and *O. kilimandscharicum* Guerke). Results indicated the presence of a wide genetic variability among different genotypes. Additionally, amplified fragment length polymorphism (AFLP) analysis also showed genetic variability within nine *O. basilicum* varieties (Labra *et al*., 2004).

2.2.2. Morphological diversity

Basil plants exhibit distinctive morphological characteristics that contribute to their identification and classification. This aromatic herb typically possesses square stems, a characteristic feature of plants in the Lamiaceae family to which it belongs. The leaves are opposite, simple, and often have a serrated margin, varying shapes depending on the species or cultivar (Figure 2). *Ocimum* plants are renowned for their aromatic foliage, emitting a pleasant scent when crushed. The flowers of *Ocimum* are arranged in terminal spikes and are bilaterally symmetrical, with vibrant colors ranging from white to shades of pink, purple, or blue. Additionally, the plant often displays glandular

trichomes, which contribute to producing essential oils responsible for its distinct fragrance. These morphological traits collectively define the visual identity of *Ocimum* and play a crucial role in its taxonomy and utilization (Simon *et al*., 1990; Hiltunen and Holm, 1999; Carović-Stanko *et al*., 2011; Patel *et al*., 2015a).

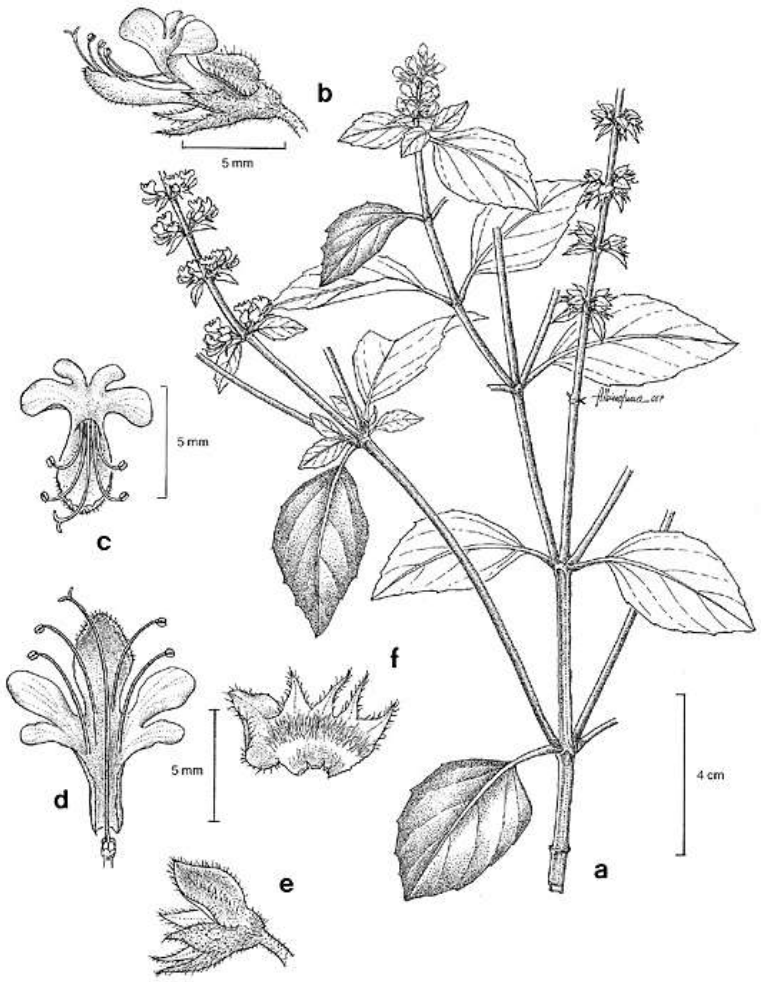

Figure 2. Morphology of *Ocimum basilicum* -a. Branch with leaves and inflorescences. -b. Flower. - c. Detail of corolla and stamens. -d. Detail of corolla, stamens, and gynoecium. -and. Chalice. -F. Detail of calyx tube and lobes (Source: Martínez-Gordillo *et al*., 2019)

2.2.3. Chemical diversity

Basil (*Ocimum* species) herbs contain a wide range of secondary compounds, including terpenoids (mono- and sesquiterpenoids), polyphenols (phenolic acids, flavonoids, anthocyanins), tannins, and steroids (Holm, 1999; Dharsono *et al*., 2022; Romano *et al*., 2022). In addition, basil seeds contain

planteose, mucilage, polysaccharides, and fixed oil that consists of linoleic acid, linolenic acid, oleic acid as well as unsaturated fatty acids (Holm, 1999).

Essential oil variability

The essential oil of the *Ocimum* species is a complex mixture of odoriferous volatile organic compounds. It is bio-synthesized and stored in a structure called glandular trichomes or essential oil glands found on the leaf, stem, and flower of the *Ocimum* species (Werker *et al*. 1993; Maurya *et al*., 2019). It comprises secretory cell(s) containing the enzymatic machinery for essential oil biosynthesis and an oil sac for storage. As illustrated in Figure 3, glandular trichomes can be classified into two types, capitate and peltate types, which can be distinguished based on their size and number of secretory cells (Werker *et al*., 1993; Martnez-Natarén *et al*., 2018).

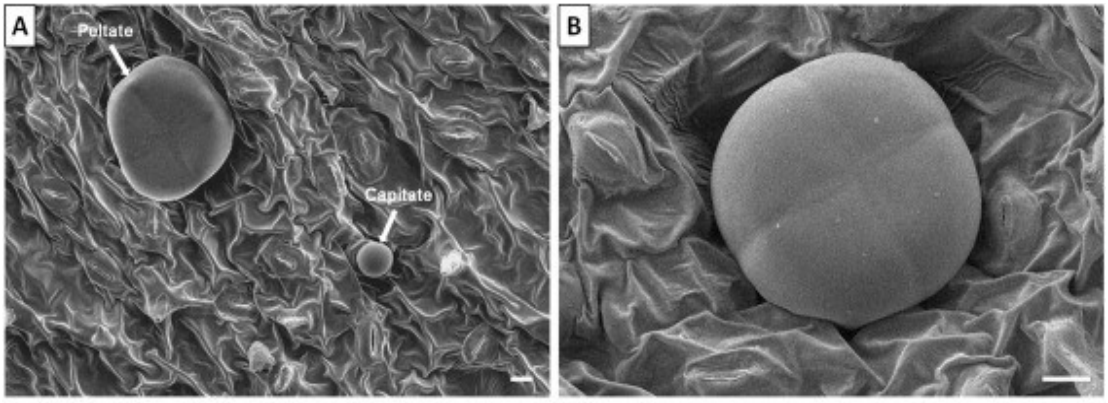

Figure 3. SEM micrographs showing the morphology of the types of glandular trichomes on the leaves of *O. campechianum*. A. Peltate and capitate glandular trichomes of *O. campechianum*. B. Fully expanded peltate trichome of *O. campechianum* on the abaxial surface. Scale bars: 10 µm in A - B (Source: Martínez-Natarén *et al*., 2018)

The essential oil can be obtained from the fresh, semi-dry, or dry aerial plant tissues at the flowering stage by steam distillation or hydro-distillation. The supercritical fluid extraction method is also used to avoid the loss of top notes from the essential oil during the distillation (Occhipinti *et al*., 2013). The EOY generally ranges from 0.2 to 5.22% depending on the species, source, phenological stage of the plants, and other factors (Hiltunen and Holm, 1999; Simon *et al*., 1999; Mulugeta and Radácsi, 2022). Essential oils are complex natural mixtures containing about 20 - 60 different components in varying concentrations. In contrast to other components present in trace amounts, they are characterized by two or three major components at relatively high concentrations (20% - 70%). Specialized metabolites like monoterpenoids, sesquiterpenoids, and phenylpropanoids majorly constitute essential oil (Pandey *et al*., 2014). Many species of the genus *Ocimum* contain essential oils based primarily on monoterpene derivatives such as camphor, limonene, thymol,

citral, geraniol, and linalool (Figure 4). Other members of the genus, including sweet 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). Their analysis and characterization of essential oil are conventionally carried out by gas-chromatography–mass spectrometry and recently with advanced liquid-chromatography–mass spectrometry (Gurav *et al*., 2022).

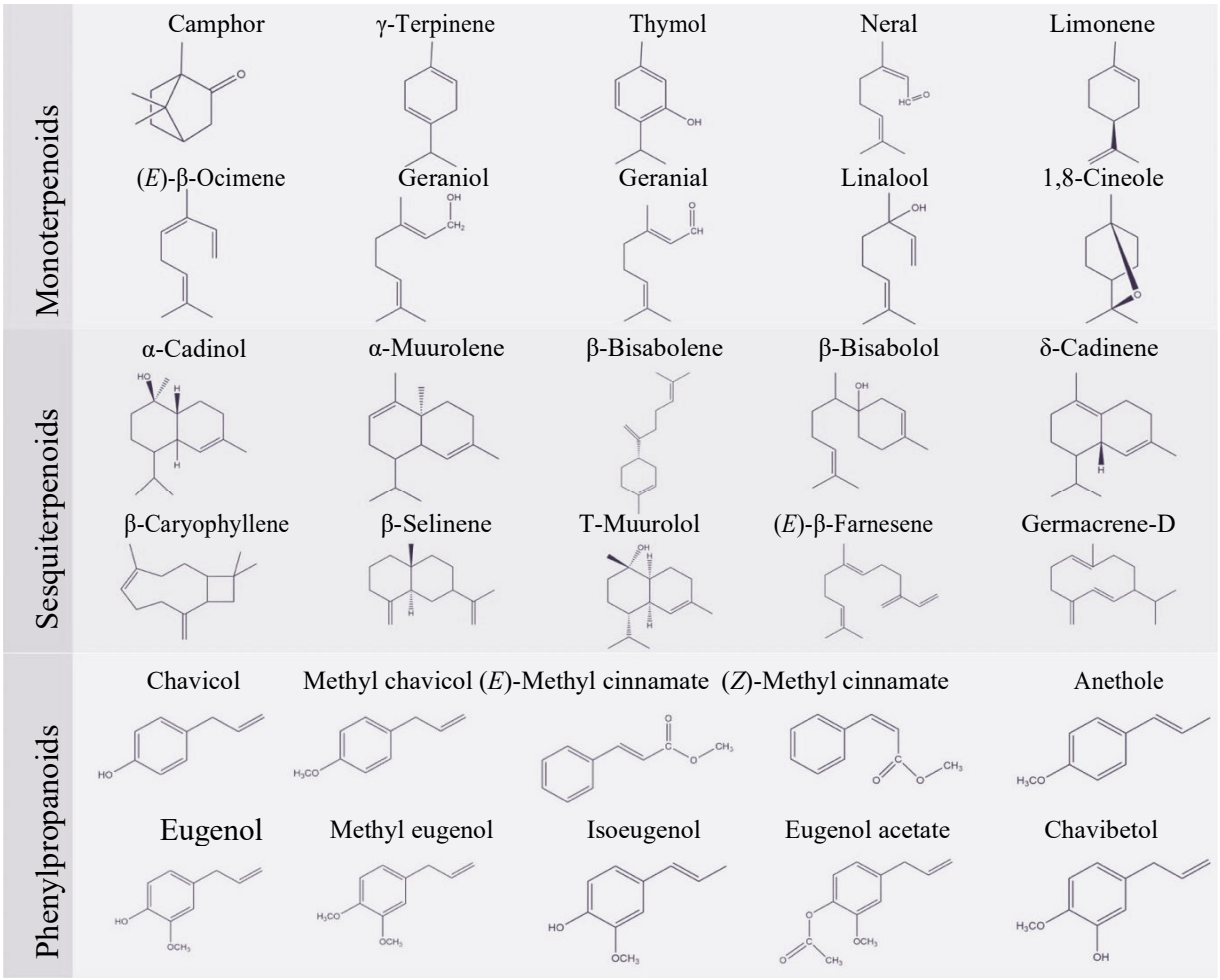

Figure 4. Major specialized metabolites of monoterpenoid, sesquiterpenoid and phenylpropanoid classes reported from the essential oil of various *Ocimum* species adopted from Gurav *et al*., 2022.

The composition of the essential oils in basil has been the subject of many studies since the 1930s (Carovic-Stanko *et al*., 2010; Kwee and Niemeyer, 2011; Labra *et al*., 2004; Gurav *et al*., 2022).

Ocimum basilicum has been the most widespread and studied species, followed by *O. gratissimum*, *O. tenuiflorum (syn. O. sanctum)*, *O. americanum*, and *O. kilimandscharicum*. According to Lawrence *et al*. (1988), *Ocimum basilicum* was classified into four main chemotypes based on their essential oil composition: (1) rich in methyl chavicol (estragole); (2) rich in linalool; (3) rich in methyl eugenol; and (4) rich in methyl cinnamate. However, these days several chemotypes have been identified from *O. basilicum*, indicating enormous chemodiversity, while others like *O. gratissimum* and *O. tenuiflorum* also have different chemotypes (Gurav *et al*., 2022). The predominately reported chemotypes of *O. basilicum* are linalool (Telci *et al*., 2006; Varga *et al*., 2017; Mulugeta *et al*., 2024), methyl chavicol (Varga *et al*., 2017; Bakhtiar *et al*., 2024; Mulugeta *et al*., 2024), methyl cinnamate (Telci *et al*., 2006), citral (Padalia *et al*., 2017; Yaldiz and Camlica, 2022), methyl eugenol (Telci *et al*., 2006; Abduche Galvão Pimentel *et al*., 2023). Additionally, various mixed ratios, such as linalool/methyl chavicol (Rawat *et al*., 2017; Varga *et al*., 2017; Raina and Gupta, 2018), linalool/methyl cinnamate (Verma *et al*., 2013; Varga *et al*., 2017; Gossa *et al*., 2023), linalool/trans-α-bergamotene (Varga *et al*., 2017), linalool/citral (Vieira and Simon, 2006; Araújo Couto *et al.*, 2019), linalool/geraniol (Gossa *et al*., 2023), linalool/eugenol (Grayer *et al*., 1996; Carović-Stanko *et al*., 2011), linalool/1,8-cineole (da Costa *et al*., 2016), methyl chavicol/methyl eugenol (Grayer *et al*., 1996) and numerous other mixed ratios, have been documented. Furthermore, uncommon chemotypes like citral/spathulenol (Vieira and Simon 2006), limonene/borneol (Ademiluyi *et al*., 2016), borneol/bocimene (Farhang *et al*., 2014), methyl chavicol/citral (Telci *et al*., 2006), 1,8-cineole /methyl chavicol (Gossa *et al*., 2023), 1,8-cineole/ß-bisabolene (Verma *et al*., 2013) have also been reported. Further, the existing literature highlighted that the main chemotypes of *O. gratissimum* include eugenol (Rao *et al*., 2011; Fuller *et al.*, 2018, Tangpao *et al.*, 2018, Saran *et al.*, 2023), thymol/p-cymene (Carović-Stanko *et al.*, 2011; de Castro *et al.*, 2019; Kumar *et al.*, 2019; Mulugeta *et al*., 2024), eugenol/caryophyllene oxide (Mulugeta *et al*., 2024), and eugenol/ *Z*-ocimene (Verma *et al*., 2013). Whereas the major essential oil chemotypes from *O. tenuiflorum* are characterized by a large amount of eugenol (Verma *et al*., 2013; Cedric *et al*., 2014; Saran *et al*., 2017; Wongpraneekul *et al*., 2022), methyl eugenol (Rao *et al*., 2011; Raina and Misra 2018), ß-bisabolene (Carović-Stanko *et al*., 2011). Moreover, eugenol/ß-caryophyllene (Mulugeta *et al*., 2022), eugenol/methyl eugenol (Rana and Blazquez 2015), methyl eugenol/ ß-caryophyllene (Piras *et al*. 2018), ß-bisabolene/1,8-cineole (Carović-Stanko *et al*., 2011) and others occur as major chemotypes.

Phenolic profile of *Ocimum*

Polyphenols are a group of small organic molecules synthesized by plants as secondary metabolites (Quideau *et al*., 2011). These molecules protect the plants from stresses, such as ultra-violet (UV) radiation, infections, cuts, etc. There are many definitions of polyphenols, but the most widely accepted is that "Compounds exclusively derived from the shikimate/phenylpropanoid and/or the polyketide pathway, featuring more than one phenolic unit and deprived of nitrogen-based functions" (Quideau *et al*., 2011). Apart from volatile oil basil herbs are also rich in polyphenols contents ranging from phenolic acids (rosmarinic acid, caffeic acid**,** caftaric acid, chicoric acid, and others), simple or complex flavonoids to colored anthocyanins (Kwee and Niemeyer, 2011; Bajomo *et al*., 2022; Dharsono *et al*., 2022). Although several factors influence it, the total polyphenol content (TPC) of *Ocimum* species ranges between 3.07 to 92.53 mg GAE g^{-1} DW (Bajomo *et al*., 2022; Mulugeta and Radácsi, 2022).

Phenolic acids

Phenolic acids are an important group of compounds found in basil that are responsible for their medicinal properties. These compounds have antioxidant and anti-inflammatory properties, making basil a useful herb. As illustrated in figure 5 below, rosmarinic acid, caffeic acid**,** caftaric acid, and chicoric acid are widely reported phenolic acids in *Ocimum* (Kwee and Niemeyer, 2011; Bajomo *et al*., 2022; Dharsono *et al*., 2022; Ullah *et al*., 2022).

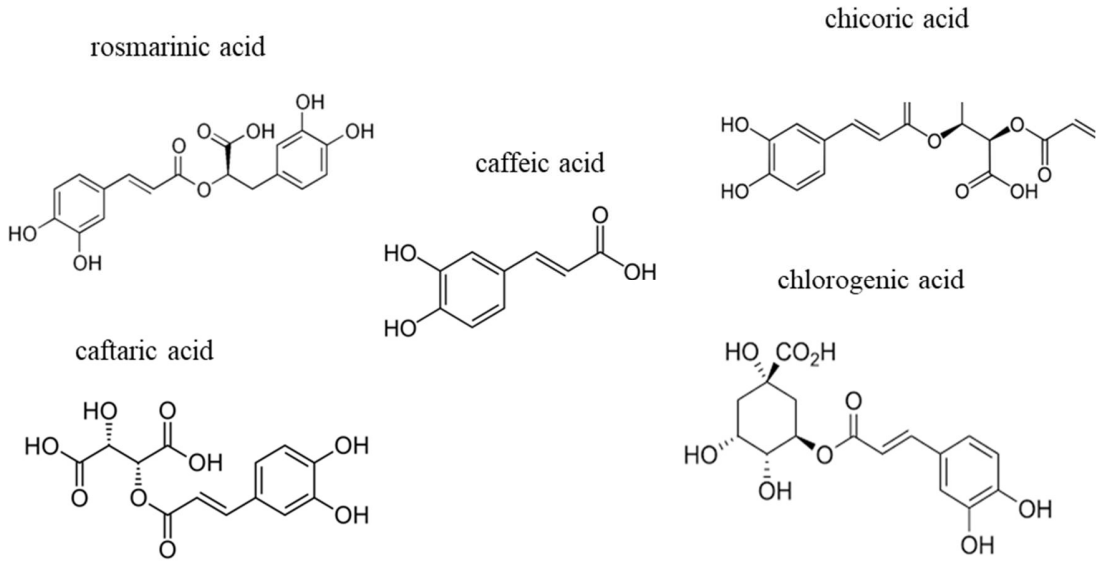

Figure 5. Major phenolic acids in *Ocimum* species

Flavonoids

The *Ocimum* genus contains a wide variety of flavonoids, including luteolin, naringenin, quercetin, rutin, kaempferol, and apigenin as indicated in figure 6. The specific compounds are present, and their concentrations can vary depending on the species and growing conditions. These compounds have been extensively studied for their potential health benefits, including antioxidant, anti-inflammatory, and anticancer properties (Mousavi *et al*., 2018; Zeljković *et al*., 2020; Dharsono *et al*., 2022; Ullah *et al*., 2022).

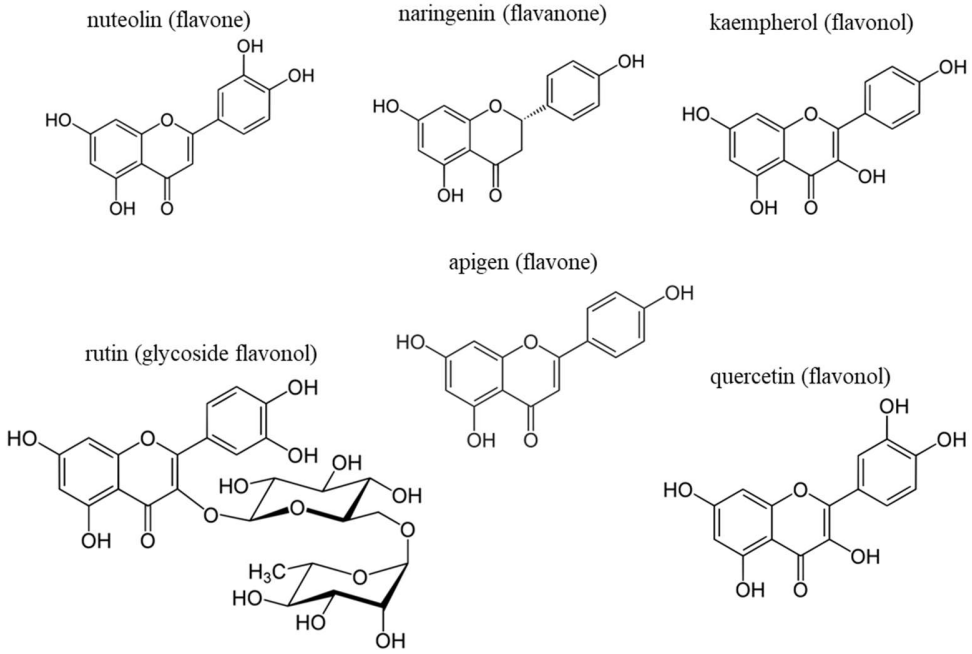

Figure 6. Common flavonoids found in *Ocimum* species.

Anthocyanins

In terms of phytochemicals, anthocyanins have high antioxidant activity and have therapeutic and prophylactic effects on human health (He and Giusti, 2010). These compounds are the sub-class of flavonoids representing the largest group of water-soluble pigments in fruit and vegetables and were correlated with the prevention of diverse human diseases (Miguel, 2011). Compared to other common red fruits and medicinal herbs, basil contains a higher level of anthocyanins (Phippen and Simon, 1998). Purple basil is a very rich natural source of anthocyanins (Phippen and Simon, 1998). Moreover, purple basil contains a peculiar kind of anthocyanins, consisting of cyanidin derivatives characterized by a high degree of acylation with coumaroyl and malonyl acids (Phippen and Simon, 1998; Luna *et al*., 2015).

2.3. Biological activity of secondary compounds of *Ocimum*

The aromatic essential oils and non-volatile compounds of basil species are used in flavor, fragrance, cosmetics, aromatherapy, and pharmaceutical industries and are widely acclaimed for their biological properties that possess antimicrobial activity, insecticidal effects, antioxidant properties, and several therapeutic benefits.

2.3.1. Antimicrobial activity

Ocimum species essential oils have been reported to be active against several bacteria (gram - and +), yeasts, and fungi due to their terpenes constituents (Pandey *et al*., 2014). There is evidence that certain essential oils and plant extracts have antimicrobial properties, some of which are discussed below. The antifungal activity of five *Ocimum* species (*O. americanum*, *O. basilicum* var. purpurascens, *O. basilicum* var. minimum, *O. miscanthi*, and *O. selloi*.) essential oils were evaluated against five *Candida* species by Vieira *et al*. (2014). Due to the presence of antifungal compounds such as eugenol and anethole, *O. micranthum* and *O. selloi* essential oils are active against *Candida* species. Vidhya *et al*. (2020) also found that ethyl acetate leaf extract of *O. americanum* has proved potentially effective in antimicrobial activity against pathogenic organisms. Furthermore, Avetisyan *et al*. (2017) also reported essential oil of *O.* × *citriodorum*, *O. basilicum* var. purpureum, and *O. basilicum* var. thyrsiflora had bactericidal properties.

2.3.2. Insecticidal and nematicidal activities

Genus *Ocimum* is known for its pesticidal properties due to the diverse group of compounds in its essential oil. Basil oil contains bioactive constituents that are insecticidal and repellent (Bhavya *et al*., 2018; Al-Harbi *et al*., 2021; Naveen *et al*., 2021). Manzoor *et al*. (2011) reported the *O. sanctum* oil as a toxicant and repellent agent against termite, *Heterotermes indicola*. According to Bhavya *et al.* (2018), *O. tenuiflorum* oil had significant fumigant activity against rice weevil (*Sitophilus oryzae* L.) by inhibiting acetyl cholinesterase activity. Additionally, *O. basilicum* oil had a repellant effect against *Sitophilus oryzae* (L.) as stated by Al-Harbi *et al*. (2021). In addition to insecticidal properties, *Ocimum* oil had nematicidal activities as indicated below. Basil oil inhibited egg hatching of rice root-knot nematode (*Meloidogyne graminicola*) with 0% egg hatch at 2000 ppm (Chavan *et al*., 2019).

2.3.3. Antioxidant properties

Antioxidants are substances that inhibit the oxidation of our cells from toxins such as free radicals. The toxins can be from the natural digestion and metabolism of foods, alcohol, nicotine from cigarette smoke, environmental factors, prescription drugs, preservatives, etc. Reactive oxygen species (ROS) including singlet oxygen ($^1O_2$), superoxide ion (O^{2-}), hydroxyl ion (OH^-), and hydrogen peroxide (H_2O_2) are highly reactive and toxic molecules generated in cells during normal metabolism (Pandey *et al*., 2014). A study is being conducted in this context to determine whether *Ocimum* species, primarily their essential oils and plant extracts, have antioxidant activity. The aqueous extract of sweet basil exhibited high antioxidant activity as well as hepatoprotective properties (Teofilović *et al*., 2021). Several authors reported that basil essential oil possesses a strong antioxidant activity (Araújo Couto *et al*., 2019; Zeljković *et al*., 2020; Zahran *et al*., 2020).

2.3.4. Therapeutic benefits

Additionally, *Ocimum* species are known to exhibit multiple therapeutic benefits, including anti-inflammatory properties (Shahrajabian *et al*., 2020; Anusmitha *et al*., 2022), anticancer activity (Shahrajabian *et al*., 2020; Zhan *et al*., 2020; Anusmitha *et al*., 2022), cardiovascular and anti-lipidemic actions (Pandey *et al*., 2014; Shahrajabian *et al*., 2020), and immunomodulatory and CNS activity (Mediratta *et al*., 2002; Zhan *et al*., 2020). The oil of *O. sanctum* was found to possess significant anti-inflammatory activity against carrageenan and another mediator-induced paw edema in rats (Singh *et al*., 1996).

2.4. Factors influencing drug quality of *Ocimum* spp.

Biosynthesis, accumulation, and distribution of secondary metabolites are strongly affected by genetic, ontogenic, morphogenetic, and environmental factors and processing methods (Paton and Putievsky, 1996; Bernhardt *et al*., 2015; Verma and Shukla, 2015; Li *et al*., 2020).

2.4.1. Genetic factors

As discussed under diversity in *Ocimum* species above (subtopic 2.2), different morphotypes correspond to different morphologies, different chemical compositions (essential oils and polyphenols), and different genotypes corresponding to different DNA sequences and chromosome numbers.

2.4.2. Plant organ and ontogenic factors

The dynamics of the accumulation of essential oil and the changes in its composition during ontogenesis are characteristics of MAP (Németh, 2005). The stage of development of the plant organ (leaf, flower, and fruit ontogeny) can influence the composition of the volatiles (Figueiredo *et al*., 2008). As stated by Werker *et al*. (1993) and Maurya *et al*. (2019), glandular trichomes or essential oil glands are found on the leaf, stem, and flower of the *Ocimum* species. Chalchat and Özcan (2008) highlighted that EOY and constituents of *O. basilicum* vary between plant organs (flowers, leaves, and stems). Accordingly, the highest EOY was obtained from the leaves (1% v w^{-1}) followed by flowers (0.5%), whereas the least was recorded from the stems (0.05%). Similarly, another experiment on *O. gratissium* by Pino *et al*. (1996) showed that the leaves (1.6% v w^{-1}) had four times more EOC than the flowers (0.4%). Furthermore, the quantity of individual components differs between the leaf and flower oils. Hence, the amount of thymol (the main constituent of the essential oil) was higher in the flower oil. For *Ocimum sanctum*, it was shown that the relative amount of eugenol and methyl eugenol decreased with the development of the leaves. The EOC of sweet basil cultivars (‘Wala’ and ‘Kasia’) increased with plant development as mentioned by Nurzyńska-Wierdak *et al*. (2012), and a change was also observed in the essential oil composition of certain compounds. Thounaojam *et al*. (2020) studied the effect of the growth stage (vegetative, flowering, and seed setting) on the EOC and composition of *Ocimum* species. They reported that harvesting plants at different growth stages affected the EOC of *O. sanctum, O. gratissimum,* and *O. basilicum* and also slightly modified the ratios of eugenol, methyl cinnamate, linalool, and methyl chavicol.

2.4.3. Environmental factors

Essential oil production and that of secondary metabolites in general is extremely dependent on the weather conditions (Chang *et al*., 2005; Figueiredo *et al*., 2008). Day length, irradiance, temperature, water supply, and nutrient availability are the major factors (Franz and Novak, 2020). Although basil is cultivated in different climatic and ecological conditions, the most favorable conditions are found in countries with warm climates. Warmth, light and moisture is the basic ecological requirements for basil cultivation. It is commonly known that basil is rather susceptible to frost. There are numerous research results reported

from countries with a temperate climate. To optimize these factors, it is important to carefully monitor and control the growing conditions of basil plants.

Light

Basil is a warm-season herb that thrives best on a long day and sunny conditions. Thus, light intensity, quality, and duration affect basil biomass production and bio-chemical constituents (Sipos *et al*., 2021). It prefers at least 6 hours of direct sunlight per day, although it can tolerate some partial shade. If grown in a greenhouse or indoor supplemental artificial light is beneficial. Basil can grow under light intensity ranging between 180 and 300 µmol $m^{-2}s^{-1}$, but the optimal radiance is 500 µmol $m^{-2}s^{-1}$ (Beaman *et al*., 2009; Bånkestad and Wik, 2016). Based on the DLI (the amount of photosynthetically active photons received in the photosynthetically active region (PAR) range in a day as a function of light intensity), it has been suggested that the optimal light for basil is 14.4 to 28.8 mol $m^{-2}d^{-1}$ (Beaman *et al*., 2009; Pennisi *et al*., 2020). Studies have shown that blue light enhances the EOC, the major oil constituents, total phenol and flavonol content of sweet basil in a controlled growing environment (Amaki *et al*., 2011; Matysiak and Kowalski, 2019; Sipos *et al*., 2021). Despite its environmental concern, UV-B is also reported to have a beneficial effect on sweet basil plant growth parameters and biochemical components in greenhouse conditions when applied at optimal doses (Sakalauskaitė *et al*., 2012).

Temperature

Temperature is one of the important physical factors, which strongly affects the ontology and developmental rate of plants (Mahajan *et al*., 2020). Basil plants are widely adapted and grow throughout the globe. However, being originated and widely cultivated in the tropics, it is most suitable for warmer temperatures (Holm, 1999; Chang *et al*., 2005; Tursun and Telci, 2020). The optimum temperature for basil growth is in the range of 25 and 30 °C, while the minimum temperature at which basil can survive is 10.9 °C (Chang *et al*., 2005; Kumar *et al*., 2014). Elevated temperature (heat stress) and low temperature (cold stress) cause changes in various physiological, biochemical, and molecular processes (Mahajan *et al*., 2020). Due to these changes, the production of secondary metabolites is also affected.

Water supply

Basil requires a continuous water supply, and it is intolerant to water stress (both drought and over watering) at all stages of development. In countries with warm climates, irrigation is an absolute precondition of basil cultivation and irrigation systems are an integrated part of the whole

production system (Putievsky and Galambosi, 1999). Extensive trials conducted in both open fields and greenhouses have illustrated the negative impact of drought stress on the production of biomass and the accumulation of secondary compounds, as emphasized in subsection 2.5.

Seasonal variation

The seasonal variation can have a significant effect on the morpho-chemical traits of MAP. Chemical variations due to season are related to a combination of factors such as precipitation, radiation, and temperature (Franz and Novak, 2020). According to a study conducted in western India on five *Ocimum* species (*O. sanctum* 'Purple' and 'Green', *O. basilicum*, *O. gratissimum* L. and Race 1 and 2, *O. viride* and *O. canum*), harvesting season affects morpho-chemical properties. Therefore, the pre-Monson harvest of respective species had higher EOC than post-Monson (Smitha and Tripathy, 2016). Additionally, Rakic and Johnson (2002) found that monoterpenes, sesquiterpenes, and phenylpropanoids are affected by the production season of sweet basil cultivars. Growth and EOC variation of *O.* × *africanum* Lour. in summer and winter were evaluated by Jnanesha *et al*. (2018). Furthermore, Jnanesha *et al*. (2018) observed growth and oil content variations in *O.* × *africanum* Lour. during summer and winter. Summer basil plants had maximum growth and EOC.

2.4.4. Cultivation practices and harvesting

Nutrient management

Fertilization, both organic and inorganic, has been identified as an agricultural practice that affects EO biosynthesis, EOC, EOY, and constituents of basil (Burducea *et al*., 2018; Onofrei *et al*., 2018; Ciriello *et al*., 2022). Considering the species requirement, soil characteristics, and growing conditions (open field or container-based cultivation), basil plants benefit from micro- and macronutrient minerals. Shalako *et al*. (2008) evaluated nitrogen (N) and sulfur (S) rates on three sweet basil (*Ocimum basilicum*) varieties in a multi-location trial. According to the results, 50-60 kg ha^{-1} of N led to the highest biomass, EOY, and essential oil ratios. Although further tuning is necessary, sweet basil varieties had higher EOC and composition with 80 kg ha^{-1} of sulfur application. Based on Sigaye *et al*. (2021) results, the maximum EOY (202.7 kg ha^{-1}), EOC (0.53%), and herb yield (47.0 tones ha^{-1}) of sweet basil were obtained with 69 kg ha^{-1} of N and 20 kg ha^{-1} of P fertilizers applied. Furthermore, 80 kg ha^{-1} of nitrogen was reported to be optimal for higher biomass and EOY of sweet basil (Alhasan *et al*., 2020). According to Onofrei *et al*. (2018),

the EOC of sweet basil was significantly enhanced by organic foliar fertilization (Fylo®, Geolino PlantsandFlowers®, Cropmax®, and Fitokondi®) at a rate of 2300 L ha^{-1} (with three split applications). Basil benefits from supplemental micronutrients in addition to the recommended macronutrient dosage. In line with that, Kanwal *et al*. (2016) demonstrated that the application of 5-6 ppm of copper along with 0.10 ppm of zinc could improve the essential oil and fresh herb yield of *Ocimum sanctum* plants. Furthermore, 95 mg L^{-1} of zinc increased vegetative growth, linalool concentration, phenolic contents, total flavonol contents, and antioxidant activity of sweet basil (Hanif *et al*., 2017).

Harvesting

Several factors influence basil harvesting time, including species, ontogenesis (as indicated in section 2.4.2 above), cultivation conditions (open or protected), the intended use (fresh herb or essential oil), geography, multiple harvesting, cultivation practices, and season as stated under 2.4.3 (Gupta, 1996; Rakic and Johnson, 2002; Tsasi *et al*., 2017). An extensive open-field study by Gupta (1996) examined the effect of harvesting basil genotypes (14 genotypes from 7 species) at various stages of maturity on biomass production, EOY, and composition. The results showed that the maximum biomass yield was obtained during the initiation of seed (180 ± 2 days) across all genotypes. During the 50% seed set stage (210 ± 2 days), the oil yield and major constituents of essential oil reach their maximum. According to Nurzyska-Wierdak *et al*. (2012), harvesting *Ocimum basilicum* (cv. 'Kasia' and cv. 'Wala') at fully developed inflorescence is optimal due to higher EOC.

2.4.5. Processing methods

Effect of drying methods

Drying is the most common and fundamental method for post-harvest preservation of MAP and for protecting their biochemical compounds (Müller and Heindl, 2006). In addition to preventing spoilage microorganism growth, drying slows enzyme activity and many moisture-mediated reactions (Garcia-Segovia *et al*., 2011). In contrast, it can cause some aroma changes, nutrient loss, color changes, and oxidation of products (Hossain *et al*., 2010; Ozdemir *et al*., 2017). The drying behavior of medicinal plants is mainly influenced by the conditions of drying air such as temperature, relative humidity, and velocity (Müller and Heindl, 2006). To reach the recommended

moisture level (10-12%), it is essential to dry the material properly. In recent decades, many studies have been conducted on herb-drying, and several new methods have been introduced to the field.

As reported by Hassanpouraghdam *et al*. (2010), different drying methods had significantly different effects on the EOC of *O. basilicum* as follows: shade drying (0.9%) > oven drying at 40°C (0.8%) > sun drying (0.5%) > oven drying at 60°C (0.4%). Essential oil constituents' number and proportional percentage are affected by drying methods as well. On the contrary, Sunita *et al*. (2018) reported that sun drying (26ºC ± 5ºC) of *O. americanum* plants had higher EOC than shade (20ºC ± 5ºC), and oven (35ºC, 45ºC, and 55ºC) drying methods. Kerekes *et al*. (2019) evaluated vacuum, hot-air, and natural air-drying methods on major volatile oil constituents of sweet basil (*O. basilicum*). They found that the major volatile oils compounds were higher under the vacuum drying method.

Extraction of essential oil

In the literature, several extraction methods have been proposed for basil essential oil, including hydro-distillation, steam distillation, microwave-assisted hydro distillation, solvent-free microwave extraction, supercritical fluid extraction, microwave-generated hydro distillation, and microwave hydro diffusion and gravity (Gurav *et al*., 2022; da Silva *et al*., 2020). Due to their economic feasibility and simplicity, steam-distillation and hydro-distillation are the most commonly used methods for extracting essential oil from basil (Shiwakoti *et al*., 2017). Several studies have shown that EOC and composition can be influenced by extraction methods. In line with that, da Silva *et al*., (2020) investigated whether extraction methods could be optimized by combining sonication time with ultrasound (0, 8, 19, 31, and 38 min) and hydro distillation (20, 30, 45, 60, and 70 min) along with herb drying temperature (20, 30, 45, 60 and 70°C) of sweet basil. Thus, the longest sonication and hydro distillation times and the lowest herb drying temperature yielded the highest EOY. Additionally, to maximize terpene extraction from *Ocimum basilicum*, Di Carro *et al*. (2013) evaluated three extraction methods: steam distillation, ultrasound-assisted extraction, and microwave-assisted extraction (MAE). With MAE, the highest results were obtained in terms of both compound identification and concentration. Furthermore, Charles and Simon (1990) evaluated essential oils extracted by solvent extraction, hydro-distillation, and steam distillation from *O. basilicum*, *O. kilimandscharicum*, and *O. micranthum.* While the yield of essential oil was consistently higher from steam-distillation than hydro-distillation, a similar number of compounds was recovered from both hydro-distillation and steam-distillation. Although the relative

concentrations of the major constituents were similar in both methods, the absolute amounts were higher with steam distillation. Compared to steam extraction, hydro distillation is faster and simpler, and it can be used in places where steam generator is not available.

Storage conditions

The composition of essential oils in basil can be affected by various factors, including storage conditions. Some studies have found that prolonged storage of basil herb or essential oil extracts can lead to changes in the concentrations of certain compounds in the essential oil due to oxidation, polymerization, and rearrangement reactions that are common among terpenoids (Turek and Stintzing, 2012; Rowshan *et al*., 2013; Turek and Stintzing, 2013). When stored at high temperatures, basil oils can degrade and lose their volatile compounds (Rowshan *et al*., 2013; Najafian, 2014). This can result in a loss of aroma and biological properties. Similarly, exposure to light can also lead to the breakdown of essential oils (Turek and Stintzing, 2013). Storage duration also affects the composition. Overall, it is important to note that the storage condition can influence the composition of the essential oil, and proper storage conditions should be considered. Thus, it is important to store basil essential oils in a cool, dark, and dry place, with minimal air flow, to maintain their composition and potency (Turek and Stintzing, 2012; Rowshan *et al*., 2013; Najafian, 2014).

Extraction of polyphenols

Extraction is the first step in the chemical isolation and utilization process. This step aims to maximize the content of target compounds in the extract for their further utilization (Filip *et al*., 2017). To obtain phenolic compounds from plant materials, several methods have been developed to promote extraction efficiency (Złotek *et al*., 2016; Filip *et al*., 2017). The extraction efficiency of phenol can be influenced by several factors such as solvent type and concentration, solvent-to-plant material ratio, temperature and pH, extraction time, agitation, particle size, and matrix composition (Złotek *et al*., 2016; Filip *et al*., 2017; Do *et al*., 2020; Alara *et al*., 2021). To extract phenol compounds from *Ocimum* species, hot water extraction is commonly used. The process involves mixing powdered material with hot water overnight. Then the filtered extract is subjected to a chemical reaction before the absorbance is measured with a spectrophotometer (Singleton and Rossi, 1965; Mulugeta and Radácsi, 2022). Additionally, a phenol extraction of sweet basil using 39% methanol concentration, 90.7 °C extraction temperature, and a 3.15-hour extraction time has been recommended by Do *et al.* (2020).

2.5. Drought stress-induced changes in *Ocimum*

Stress in plant life is defined as any unfavorable condition or substance that adversely affects plant homeostasis (Lichtenthaler, 1996). Drought stress is further defined as the lack of sufficient amount of moisture required for normal growth and development of plants to complete their life cycle (Farooq *et al.*, 2012). In addition to drought stress synonym terms including water deficiency, water stress, and water deficit are widely reported in the literature (Selmar and Kleinwächter, 2013; Szabó *et al*., 2020; Oguz *et al*., 2022). Figure 7 illustrates how drought stress induces changes in the morpho-anatomical, physiological, molecular, and biochemical composition of basil plants. Additionally, the subsequent section discusses the specific responses of *Ocimum* species under the influence of drought stress.

2.5.1. Anatomical responses

Drought stress can have a significant effect on plant anatomy, as it triggers a range of physiological and morphological adaptations in plants (Shao *et al*., 2008). The specific responses can vary among plant species or cultivars, the severity, the duration of drought, and the leaf surface (upper or lower). For instance, a study on sweet basil (*Ocimum basilicum*) conducted by Taha *et al*. (2020) demonstrated that deficit irrigation (at 50% of soil water holding capacity (SWHC)) had adverse effects on the anatomical features of stems and leaves compared to well-irrigation (at 70% of SWHC). As a result of deficit irrigation, there was a significant reduction in the thicknesses (µm) of the cortex, vascular cylinder, xylem vessels, and pith diameter (µm). The study also found that deficit irrigation led to decreased midvein length and width (µm), a lower number of xylem rows per vascular bundle, and reduced thicknesses of palisade, spongy tissue, and thinner palisade. In another study on basil (*Ocimum basilicum* L. cv. Marian) grown close to the theoretical wilting point (at 20% volumetric water content), there was a higher frequency of xylem vessels and a smaller mean vessel diameter, promoting water transport during drought conditions (Driesen *et al*., 2021). Additionally, the proportion of collenchyma area was significantly higher at the theoretical wilting point. A recent study by Driesen *et al*. (2023) revealed that leaves developed under water-deficit conditions exhibited higher stomatal densities and reduced stomatal length on both the adaxial and abaxial leaf sides of two basil cultivars, namely *O. basilicum* L. cv. Gustosa and *O. basilicum* L. cv. Marian. Furthermore, Hazzoumi *et al*. (2017) documented that *O. gratissimum* L. cultivated under drought stress (withheld water for two weeks) underwent ultrastructural changes in

glandular hair trichomes. Consequently, drought stress led to a decrease in the extracellular cavity diameter of glandular trichomes and their deflation.

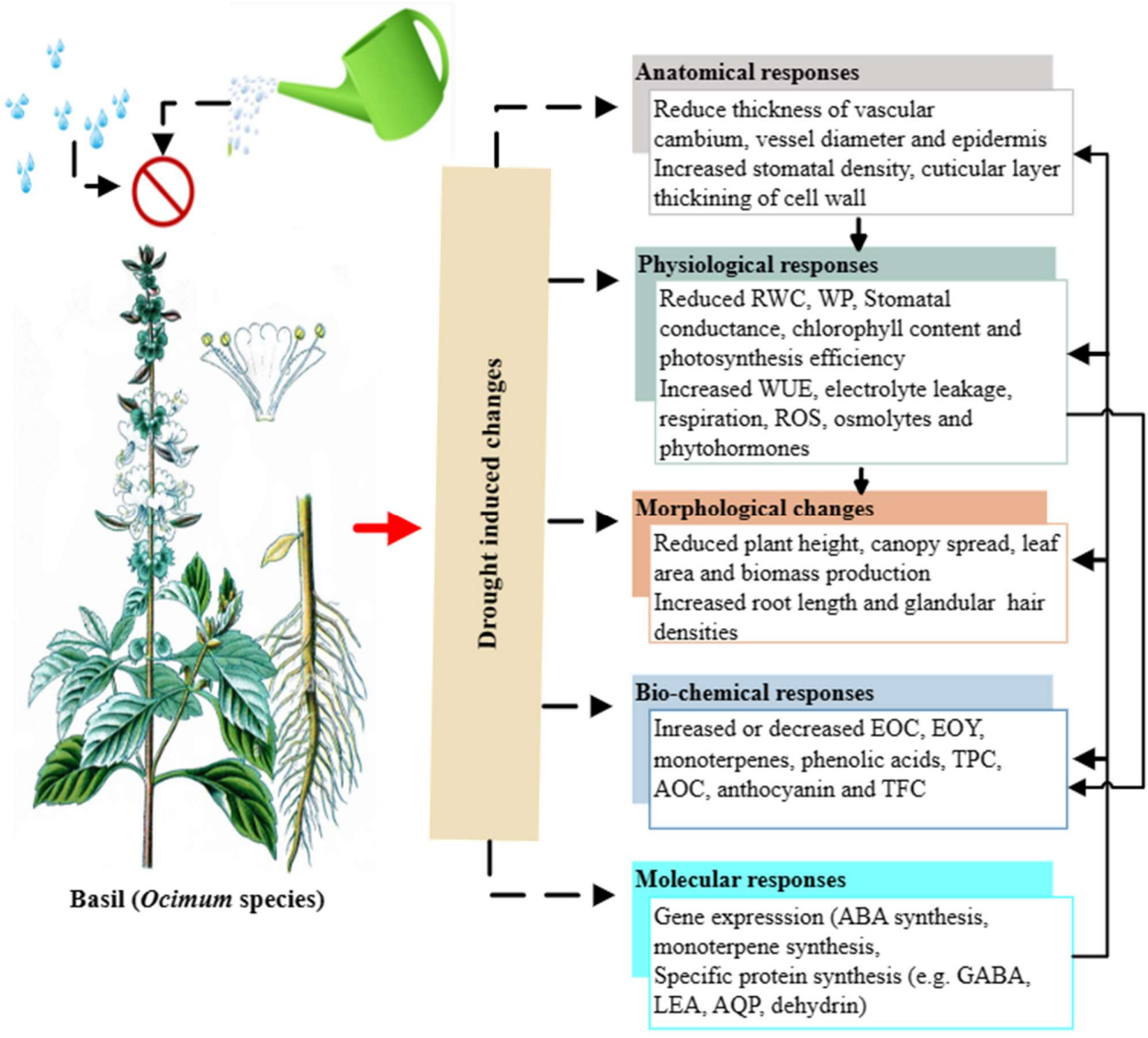

Figure 7. Illustrates the response of basil plants under drought stress. RWC: relative water content; WP: water potential: WUE: water use efficiency; ROS: reactive oxygen species, EOC: essential oil content; EOY: essential oil yield; TPC: total polyphenol content; AOC: antioxidant capacities; TFC: total flavonoid content; ABA: abscisic acid; GABA: LEA: late embryogenesis abundant; AQP: Plant aquaporin (Sources: Shao *et al*., 2008; Oguz *et al*., 2022).

2.5.2. Physiological responses

Drought stress significantly influences the physiological attributes of plants, leading to notable changes in key parameters. Under drought stress, plants in general and medicinal and aromatic plants in particular often exhibit an increase in proline accumulation, a common osmolyte associated with stress tolerance (Kordi *et al*., 2013; Damalas, 2019; Al-Huqail *et al*. 2020). Simultaneously, the elevation of malondialdehyde levels, indicative of lipid peroxidation and oxidative stress, is observed (Inotai *et al*. 2012; Weng *et al*., 2015; Al-Huqail *et al*., 2020). The relative water content, a crucial indicator of plant hydration status, tends to decrease, accompanied by reductions in water potential (Mulugeta *et al*., 2023). Stomatal conductance, responsible for water vapor exchange, generally declines as a water-saving mechanism (Clauw *et al.*, 2015; Nemeskeri *et al*., 2015; Driesen *et al*., 2023). Electrolyte leakage, a measure of cell membrane integrity, may increase under drought conditions, reflecting cellular damage (Kordi *et al*., 2013; Agami *et al*., 2016). Furthermore, photosynthesis, a vital process for plant growth, is adversely affected, with reduced rates observed due to limited water availability. These physiological responses collectively illustrate the adaptive strategies and stress-induced alterations in plants. The specific repnses of *Ocimum* taxa are elaborated below.

Proline accumulation: It serves as the initial response in plants subjected to drought stress, acting as a protective measure against cell damage. Increasing drought stress induces a substantial buildup of proline in water-stressed plants, as demonstrated by Delauney and Verma (1993). Notably, studies have shown that as the available water decreases, the proline content in the leaves of purple basil increased by 66% (Alishah *et al*., 2006). Similarly, Al-Huqail *et al*. (2020) observed a 20-fold and 36-fold increase in proline accumulation in sweet basil subjected to drought stress for 14 and 21 days, respectively. Various investigations on sweet basil, including those by Baeck and Park (2001), Khalid (2006), Khalil *et al*. (2010), Kordi *et al*. (2013), and Damalas (2019), reported substantial increases ranging from 38% to 288% in proline concentration under drought stress conditions. Beyond sweet basil, *O. americanum* exhibited a 48% higher proline level when treated with 50% field capacity (FC) (Khalid, 2006). Additionally, Rasouli and Fakheri (2016) noted a 50% increase in proline accumulation in *O. basilicum* and *O. americanum* treated with 40% FC of drought stress. Furthermore, Hazzoumi *et al*. (2015) observed heightened proline accumulation in *O. gratissimum* under drought stress. This pattern of increased proline accumulation under drought conditions extends to various medicinal and aromatic plants, including *Coriandrum sativum* L. (Aliabadi *et al*.,

2008), *Thymus vulgaris* L. (Babaee *et al*., 2010), *Mentha piperita* and *Catharanthus roseus* as reported by Alhaithloul *et al*. (2020). Consequently, the rate of proline accumulation tends to rise under drought conditions in medicinal and aromatic plants in general, with specific emphasis on *Ocimum* species.

Malondialdehyde (MDA): which is considered a reliable marker of oxidative stress and is a product of membrane lipid peroxidation by thiobarbituric acid reaction; thus, higher MDA contents should correspond to a higher degree of oxidative stress and reflect the degree of damage at adverse conditions (Weng *et al*., 2015, Al-Huqail *et al*., 2020). Sweet basil subjected to more severe drought stress (30% Soil Water Content/SWC/) exhibited elevated concentrations of MDA, measuring 1.52, 1.5, and 1.49 times higher than control plants, as reported by Baeck and Park (2001), Radácsi *et al*. (2010), and Inotai *et al*. (2012), respectively. MDA levels increased across all drought stress treatments in sweet basil plants, according to Al-Huqail *et al*. (2020). Similar studies on other medicinal plants demonstrated notable cell damage in plants exposed to severe stress conditions. For instance, *Thymus daenensis* Čelak plants subjected to drought stress treatments exhibited a 64% increase in MDA content in 2014 and a 58% increase in 2015 (Bistgani *et al*., 2017).

Electrolyte leakage (EL): EL is an index, which can quantify the damage conceived by plant cell membrane. Its relative conductivity can be used to evaluate the damage on structure and function of cell membranes under stresses (Kordi *et al*., 2013). An experiment conducted by Kordi *et al*. (2013) in Iran showed that, when sweet basils are grown under 30% Field Capacity (FC), the electrolyte leakage increased by 27% compared to the control. Agami *et al*. (2016) also stated higher percentage of EI when sweet basils are grown under 60% drought stress. On the contrary, Beack and Park (2001) reported 65% lower EI in sweet basils grown under drought treatment.

Chlorophyll content**:** It is a crucial physiological characteristic closely linked to the photosynthetic efficiency of plants, is subject to regulation by various environmental factors, with drought being one such factor (Li *et al*., 2018; Morales *et al*., 2020). Drought exerts an impact on the photosynthetic activity of leaves, altering chlorophyll a fluorescence kinetics (Strasser *et al*., 1995). As soil moisture decreases, the levels of total chlorophyll (Chl-a+b), chlorophyll a (Chl-a), and chlorophyll b (Chl-b) in leaves decrease, with maximum concentrations observed at 100% field capacity (FC) (Alshah *et al*., 2006; Kordi *et al*., 2013). Khalil *et al*. (2010) conducted a study on sweet basil, noting a significant increase in photosynthetic pigments, including Chl-a, Chl-b, and total Chl (a+b), as well as carotenoids, with an increase in soil moisture content. A 40% FC led to a

notable decrease of 23.9% in Chl-a concentration and 15.8% in Chl-b concentration compared to non-stressed plants, as reported by Damalas (2019). Hazzaumi *et al*. (2015) similarly observed a reduction in Chl-a pigments under water stress in *O. gratissimum*. Consequently, the production of chlorophyll a and b decreased, accompanied by changes in their ratios. Earlier reports documented a significant reduction in chlorophyll content in sweet basil (Hasani and Beygi, 2012), *Thymus citriodorus* (Tátrai *et al*., 2016) and chickpea (Mafakheri *et al*., 2010). This decline in chlorophyll content may be attributed to the generation of reactive oxygen species (ROS), such as O_2- and H_2O_2, leading to lipid peroxidation, protein complex imbalance, and increased activity of chlorophyll-degrading enzymes and chlorophyllase, contributing to chlorophyll breakdown under stress conditions (Foyer *et al*., 1994; Blank *et al*., 2004; Xiao *et al*., 2008).

Relative Water Content (RWC): Evaluating the water status of plants is a crucial physiological measure in response to drought stress, and one key indicator of this is RWC (Kordi *et al*., 2013). Previous findings highlighted the significant changes in the RWC of sweet basil in response to varying water availability. For instance, Damalas (2019) documented a notable 29.2% decrease in the RWC of sweet basil subjected to water deficit conditions (40% FC). Radácsi *et al*. (2010) similarly observed that the drier the soil (30% Soil Water Content (SWC)), the lower the RWC (77.4%) of sweet basil leaves, while saturated soil (70% SWC) resulted in higher RWC values (96%). Additionally, studies by Kordi *et al*. (2013) and Agami *et al*. (2016) indicated a reduction in RWC in sweet basil under drought stress compared to non-stress conditions. The decline in leaf RWC due to drought stress is associated with decreased soil humidity, leading to stomatal closure to prevent excessive water loss. Stomatal closure is prompted by the presence of Abscisic acid, produced in the roots under drought stress conditions and accumulated in stomatal cells (Chaves *et al*., 2002).

***Stomatal conductance*:** Which refers to the measurement of the rate at which carbon dioxide enters or water vapor exits through a leaf's stomata. It plays a crucial role in regulating the water balance of plants by reducing transpiration. Stomatal closure not only reduced transpiration but also hinders cell expansion and growth rates, resulting in a significant reduction in biomass and yield (Nemeskeri *et al*., 2015; Rauf *et al*., 2015). Several scholars noted that the initial response of most plants to severe drought is the closure of their stomata, a mechanism employed to prevent water loss through transpiration (Berry *et al*., 2010; Casson and Hetherington, 2010; Brodribb and McAdam, 2011; Torres-Ruiz *et al*., 2013; Clauw *et al.*, 2015; Nemeskeri *et al*., 2015). Studies conducted by

dos Santos *et al*. (2016) on *O.* × *africanum* Lour. and Driesen *et al*. (2023) *O. basilicum* demonstrated decreased stomatal conductance under water deficit conditions, accompanied by a decline in water potential.

Photosynthesis is notably sensitive to the effect of water deficiency. The plant's response to water scarcity leads to metabolic alterations and structural rearrangements within the photosynthetic apparatus. In higher plants, the photosynthesis rate decreases in correlation with reductions in RWC and leaf water potential. A reduced photosynthesis rate is a common outcome of water stress, primarily attributed to stomatal limitation and, metabolic impairments. *Ocimum* × *africanum* Lour. exhibited a reduction in net photosynthetic rate under water deficit conditions (dos Santos *et al*., 2016). This response is linked to partial stomatal closure, influencing the photosynthetic process (Akinci and Lösel, 2012; Lisar *et al*., 2012; dos Santos *et al*., 2016). Additionally, water-stressed plants experience substantial inhibition of photosynthesis due to stomatal closure, restricting CO_2 diffusion to chloroplasts, lowering internal CO_2 concentration (Cornic, 2000), and diminishing enzyme activity such as RubisCO (Li *et al*., 2018; Morales *et al*., 2020).

2.5.3. Morphological responses

Plant growth is adversely affected by water stress, a phenomenon that has been extensively studied and well documented. The growth and development of plants are dependent on the division, elongation, and differentiation of cells. Due to drought conditions, these phases are affected by the loss of turgor, disordered enzyme activities, and a decrease in photosynthesis (Bhargava and Sawant, 2013; Osakabe *et al*., 2014; dos Santos *et al*., 2016; Abid *et al*., 2018). A recent study by Mulugeta and Radácsi (2022) who reported severe drought stress (30% SWC) resulted in over 50% losses in dry and fresh biomass yield of three *Ocimum* species: *O. basilicum* 'Genovese', *O.* × *africanum*, and *O. americanum*. Although the intensities of drought stresses are different, several authors also showed the negative impact of drought stress on biomass production of *O. basilicum* cultivars (Radácsi *et al*., 2020; Mulugeta *et al*., 2023), *O.* × *africanum* Lour. (dos Santos *et al*., 2016) as well as *O. americanum* (Khalid, 2006; Mulugeta *et al*., 2023). Furthermore, Németh *et al*. (2016) reported that lower soil water content (40% SWC) resulted in significantly lower biomass production in four Lamiaceae species: lemon balm, thyme, peppermint, and marjoram.

2.5.4. Bio-chemical responses

The accumulation of secondary compounds or biochemical attributes during drought stress presents a multifaceted phenomenon marked by conflicting outcomes. On one hand, research has demonstrated that basil plants exposed to drought stress led to an increase in the concentration of essential oil percentage, major essential oil component ratio, flavonoids, and phenolic acids (Simon *et al*., 1992; Luna *et al*., 2015; dos Santos *et al*., 2016; Mandoulakani *et al*., 2017; Pirbalouti *et al*., 2017a; Mota *et al*., 2020; Radácsi *et al*., 2020). This increase in secondary compounds can vary from a minor percentage (<10%) to a substantial rise (over 2-fold). Conversely, contrasting studies indicate that drought stress might also result in a decrease in the concentration of specific secondary compounds, such as terpenoids and phenylpropanoids (Omidbaigi *et al*., 2003; Radácsi *et al*., 2010; Vilanova *et al*., 2018; Al-Huqail *et al*., 2020; Mulugeta and Radácsi, 2022). Consequently, the impact of drought stress on secondary compound accumulation in basil is intricate and contingent on factors such as the type of secondary compound, basil taxonomy, growth conditions (greenhouse or open field), and the severity and duration of drought stress, as outlined in Table 1.

2.5.5. Molecular responses

Drought stress often leads to changes in the expression of specific genes involved in stress response mechanisms. Plants may upregulate or downregulate the expression of certain genes to cope with stress. These genes play roles in various processes such as water retention, osmotic regulation, antioxidant defense, and the biosynthesis of secondary compounds (Selmar and Kleinwächter, 2015; Yahyazadeh *et al*., 2018). Mandoulakani *et al*. (2017) found that drought stress increases the content of methyl chavicol and methyl eugenol in sweet basil (*O. basilicum* cv. Keskenylevelű) by enhancing the expression levels of *chavicol O-methyl transferase* and *eugenol O-methyl transferase* genes. Additionally, drought stress induces the upregulation of monoterpene synthase expression in *Salvia officinalis* (Radwan *et al*., 2017). Furthermore, drought stress has been shown to significantly induce the expression of genes related to flavonoid biosynthesis in hybrid poplar plants, leading to an accumulation of phenolic and flavonoid compounds (Ahmed *et al*., 2021).

Table 1. Drought stress-related changes on secondary compounds accumulation of *Ocimum* species

Species /Cultivars	Country	Active compound	Effect reported	Drought stress level	References
Ocimum basilicum					
'Genovese'	Hungary	Essential oil percentage	Decreased (39%)	30% SWC	Mulugeta and Radácsi (2022)
'Keskenylevelű'	Iran		Decreased (12%)	55% FC	Omidbaigi *et al*. (2003)
	USA		Increased (200%)	-1.12 Mpa	Simon *et al.* (1992)
Purple type	Turkey		Increased (16%)	50% FC	Ekren *et al*. (2012)
'Genovese'	Hungary	Essential oil yield	Decreased (69%)	30% SWC	Mulugeta and Radácsi (2022)
	Egypt		Increased (83.7%)	50% FC	Khalid (2006)
	USA	Linalool	Decreased (37%)	-1.12 Mpa	Simon *et al.* (1992)
'Genovese'	Hungary		Decreased (26%)	30% SWC	Radácsi *et al.* (2010)
'Keskenylevelű'	Iran		Dreased (61%)	55% FC	Omidbaigi *et al*. (2003)
'Keskenylevelű'	Iran		No change	50% FC	Mandoulakani *et al*. (2017)
'Genovese Gigante'	Spain		Increased (48%)	60% FC	Mota *et al*. (2020)
'Kasia'	Hungary		Increased (44%)	Non-irrigated	Radácsi *et al.* (2020)
'Keskenylevelű'	Iran	1,8-cineole	Increased (4.5fold)	55% FC	Omidbaigi *et al*. (2003)
'Keskenylevelű'	Iran	Methyl eugenol	Increased (2.4-fold)	50% FC	Mandoulakani *et al*. (2017)
'Keskenylevelű'	Iran	Methyl chavicol	Increased (8-fold)	50% FC	Mandoulakani *et al*. (2017)
Purple basil	Iran	Anthocyanin	Increased	50% FC	Alishah *et al.* (2006)
Purple Iranian basil	Spain		No change	50% FC	Luna *et al*. (2015)
	Suadi Arabia	Flavonoid content	Decreased (41%)	21 days of drought	Al-Huqail *et al.* (2020)
Omani basil	Oman		Increased	125 mL day^{-1}	Khan *et al*. (2012)
Landrace 1	Iran	Phenolics content	Increased (62%)	30% FC	Pirbalouti *et al*. (2017a)
Landrace 2	Iran		Increased (42%)	30% FC	Pirbalouti *et al*. (2017a)
Omani basil	Oman		Increased	125 mL day^{-1}	Khan *et al*. (2012)
	Suadi Arabia		Increased (56%)	21 days of drought	Al-Huqail *et al.* (2020)
Purple Iranian basil	Spain	Phenolic acids	Increased	50% FC	Luna *et al*. (2015)
Purple Iranian basil	Spain	Chicoric acid	Increased (45%)	50% FC	Luna *et al*. (2015)
Ocimum americanum	Egypt	Essential oil percentage	Increased	50% SWC	Khalid (2006)
	Iran		Increased	40% FC	Rasouli and Fakheri (2016)
	Hungary		Decreased	30% SWC	Mulugeta and Radácsi

					(2022)
	Hungary		No change	Non-irrigated	Mulugeta *et al*. (2022)
	Egypt	Linalool	Increased	50% FC	Khalid (2006)
	Hungary	Phenolic content		Non-irrigated	Mulugeta *et al*. (2022)
Ocimum × *africanum*	Brazil	Essential oil content	Increased (26%)	-1.9 Mpa	dos Santos *et al*. (2016)
	Hungary		Increased (11%)	Non-irrigated	Mulugeta *et al*. (2022)
	Hungary		No change	30% SWC	Mulugeta and Radácsi (2022)
	Hungary	Essential oil yield	Huge loss (47%)	30% SWC	Mulugeta and Radácsi (2022)
	Brazil		Huge loss (81%)	-1.9 Mpa	dos Santos *et al*. (2016)
	Hungary	1,8-cineole	Increased (30%)	Non-irrigated	Mulugeta *et al*. (2022)
	Hungary		Increased (40%)	50% SWC	Mulugeta and Radácsi (2022)
Ocimum gratissimum	Brazil	Essential oil percentage	Decreased	60% FC	Vilanova *et al*. (2018)
	Nigeria			400 mL 5 day^{-1}	Omobolanle *et al*. (2013)
	Morocco		No change	Deprived of water for 2 weeks	Hazzoumi *et al*. (2015)
	Morocco	Phenolic content	Increased (90%)	Deprived of water for 2 weeks	Hazzoumi *et al*. (2015)
Ocimum selloi	Hungary	Essentail oil content	Increased (42%)	Non-irrigated	Mulugeta *et al*. (2022)
	Hungary	Essential oil yield	No change	Non-irrigated	Mulugeta *et al*. (2022)
	Hungary	Phenolic content	No change	Non-irrigated	Mulugeta *et al*. (2022)
	Hungary	Methyl eugenol	Increased (40%)	Non-irrigated	Mulugeta *et al*. (2022)
Ocimum sanctum					
Purple type	Hungary	Essentail oil content	No change	Non-irrigated	Mulugeta *et al*. (2022)
Purple type	Hungary	Essential oil yield	No change	Non-irrigated	Mulugeta *et al*. (2022)
Purple type		Eugenol	Increased (17%)	Non-irrigated	Mulugeta *et al*. (2022)
Purple type	Hungary	Phenolic content	No change	Non-irrigated	Mulugeta *et al*. (2022)
Ocimum ciliatum	Iranina type	Phenolic content	Increased (60%)	30% FC	Pirbalouti *et al*. (2017b)

SWC: - soil water content, FC: - Field capacity

N.B. The percentage or folds in the bracket are compared against the control treatment of respective studies.

3. MATERIALS AND METHODS

3.1. Experimental site description

For three consecutive years, the experiments were conducted under three different growing conditions (open field, greenhouse, and climatic chamber). Below is a detailed description of each experimental site.

3.1.1. Open field experiment

Over the 2020 - 2022 academic years, a drought experiment was carried out for three consecutive years. Another experiment was also conducted to determine the morpho-chemical variability of *Ocimum* species over the course of two years in parallel with the drought experiment. The experiments were conducted at the Soroksár Experimental and Research Farm of the Hungarian University of Agriculture and Life Sciences (MATE). Tables 2 and 3 summarize the monthly average of the daily air temperatures (°C), relative humidity (%), rainfall (mm), and soil media characteristics during the experiment.

3.1.2. Greenhouse experiment

The second trial, a pot experiment, was carried out under a semi-controlled greenhouse in the experimental field of MATE, Budapest-Soroksár (Hungary) from 2020 to 2022. The weather conditions and the properties of the growing soil media are summarized in Tables 2 and 3.

Table 2. Metrological data of open field and greenhouse experiments (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

Table 3. 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

3.1.3. Climatic chamber experiment

The third pot experiment was conducted in two different climatic chambers (Weiss-Gallenkamp Technik, Type: SGC-120 Fitotron) for two consecutive years (2021 and 2022) at the MATE Buda campus. Based on previous experience of the department, the climatic chambers were programed 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%.

3.2. Treatments and plant materials

3.2.1. Open field variability experiment

In the experiment, 15 basil genotypes belonging to five *Ocimum* species were tested, of which nine were *O. basilicum* cultivars (Table 4 and Figure 8). Seeds were obtained from the gene bank of the Department of Medicinal and Aromatic Plants (MATE) and other sources, as shown in Table 4. The seeds were sown in seed trays (27 × 57 × 7 cm) in a greenhouse in the second week of March. Healthy seedlings that had developed two leaves were transplanted in the open field at a spacing of 40 × 40 cm in 4 rows with 6 plants per row. These plants were replicated twice in a randomized complete block design arrangement. We irrigated the plants three times per week and cultivated them once a week, without using chemical fertilizers or protection chemicals. Harvesting took place 10 days after full bloom, and fresh herb samples were dried for two weeks in a well-ventilated room.

Table 4. 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

Figure 8. Open field experimental set of *Ocimum* genotypes (Photo: Mulugeta, 2022)

3.2.2. Open-field irrigation experiment

In this experiment, we used a two-factor randomized design with two block replications. As illustrated in Figure 9, five basil species namely *O. basilicum* 'Ohře', *O. basilicum* 'Genovese', *O.* × *africanum, O. americanum, O. sanctum* (purple type), and *O. selloi* (only in 2020) and two levels of water supply (irrigated as control and non-irrigated as a drought stress treatment) were used. All seeds of *Ocimum* species were obtained from the gene bank of the Department of Medicinal and Aromatic Plants, except *O. sanctum*, which was obtained from Assam state in India. Seeds were sown in seed trays (27 × 57 × 7 cm) in the greenhouse in March. Seedlings that had developed two leaves were transplanted into 0.1 L pots. Then in early June, seedlings were transplanted to an open field at a spacing of 40 × 40 cm. In each plot, there were 24 plants in four rows and six plants per row. Drought treatment

was initiated two weeks after transplanting and lasted for 30 days on average. Using a spraying hose connected to a water meter device, 20 mm of water was applied two times per week to the irrigated treatments (the quantity of water applied was based on the department's cultivation practices). In contrast, the drought treatment only received natural precipitation, which varies in each production year as shown in Table 2. Plant protection chemicals and fertilizer were not applied except in the first year where each plot received 8 g of complex fertilizer (Hunfert NPK 15-15-15), 10 days after transplanting. Weeding and cultivation were done twice a month. Basil plants were harvested during the full flowering stage.

Figure 9. The five *Ocimum* genotypes used in the open field experiment (a: *O. basilicum* 'Genovese', b: *O. basilicum* 'Ohře', c: *O.* × *africanum*, d: *O. americanum*, e: *O. sanctum* (purple type) (Photo: Mulugeta, 2020)

3.2.3. Greenhouse experiment

Experimental treatment consisted of three widely cultivated *Ocimum* species namely *O. basilicum* 'Genovese' *O.* × *africanum* and *O. americanum*, evaluated under three soil water capacities i.e., 70% (control), 50% (moderate drought stress), and 30% (severe drought stress). The seeds were selected from the Department of Medicinal and Aromatic Plants' gene bank of MATE. During the second week of March, seeds were sown in seed trays with dimensions of 27 × 57 × 7 cm. Two weeks after germination, seedlings were transferred to cell trays. After developing three leaves, healthy and strong seedlings were transplanted into 12 L pots (one plant per pot). A 5 kg of sandy loam soil and peat moss mixture with 1:1 v/v was used to fill the 12 L pots. Figure 10 shows a greenhouse pot experiment setup. The drought stress treatment was initiated 10 days after transplanting and lasted for 40 days afterward. Soil water capacity (SWC) was determined using the modified gravimetric method of Reynolds (1970) based on the water-holding capacity of the soil. SWC of respective treatments was maintained three times per week. Pots were kept under the greenhouse to exclude natural precipitation. A two-week cycle of weeding and cultivation was followed. Following the department's cultivation practices, the soil media mixture contains an adequate amount of nutrients. In addition, no diseases or insect

infestations were observed. Harvesting of basil plants took place two weeks after their flowering, at a height of three cm above the soil.

Figure 10. Overview of the experimental set-up in the greenhouse (Photo: Mulugeta, 2021)

3.2.4. Growth chamber experiment

The growth chamber experiment was carried out for two consecutive years using a completely randomized block design in two replicates (Figure 11). Experimental treatment consisted of two *Ocimum* species: *O. basilicum* 'Ohře' and *O. americanum*, evaluated under three soil water capacities i.e., 70%, 50%, and 30%. The SWC was determined using the modified gravimetric method as indicated in sub-section 3.2.3. Seeds were obtained from the gene bank of the department. Seeds were sown in the middle of February on a flat seed tray in the climatic chamber. After three weeks seedlings that developed two leaves were transferred to a cell tray (0.1 L). Then 10 days later seedlings were translated into bigger pots (1 L). Each pot was filled with a 500g soil media mixture of compost, peat, and perlite (3:3:1 v/v/v ratio). Drought treatments were imposed on plants after 10 days of transplanting and lasted for 47 days. The soil water capacity of respective treatments was maintained three times per week. Harvesting was done at full bloom. No fertilizer and plant protection chemicals were used.

O. basilicum 'Ohře' *O. americanum*

Figure 11. Experimental set-up under a plant growth chamber (Photo: Mulugeta, 2021)

3.3. Methods of data measurements

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 12). 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:

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

SWC of respective treatments was maintained three times per week. Pots were kept under the greenhouse to exclude natural precipitation.

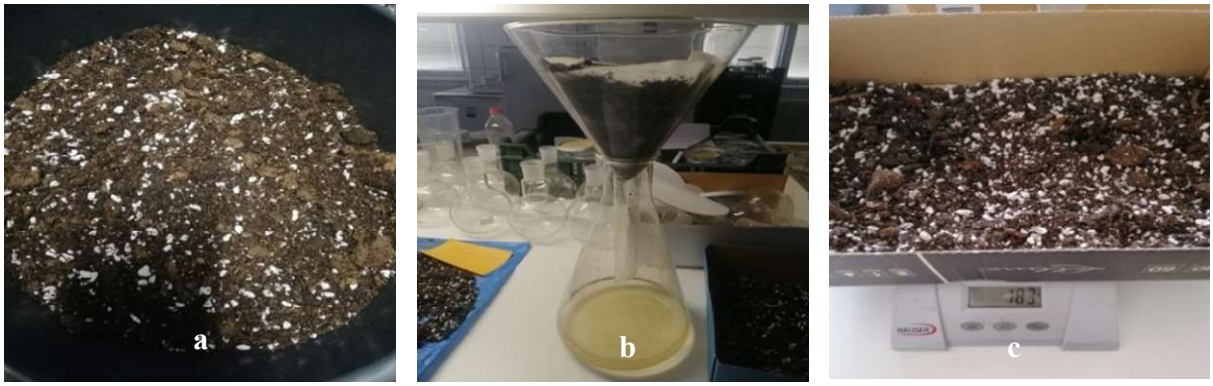

Figure 12. Soil water content determination by gravimetric method: Soil media mixture (a), saturated soil sample in volumetric flask and fennel (b), oven dry soil (c), (Photo: Mulugeta, 2022)

3.3.1. Physiological parameter determination

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 13 below, whole leaves 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):

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

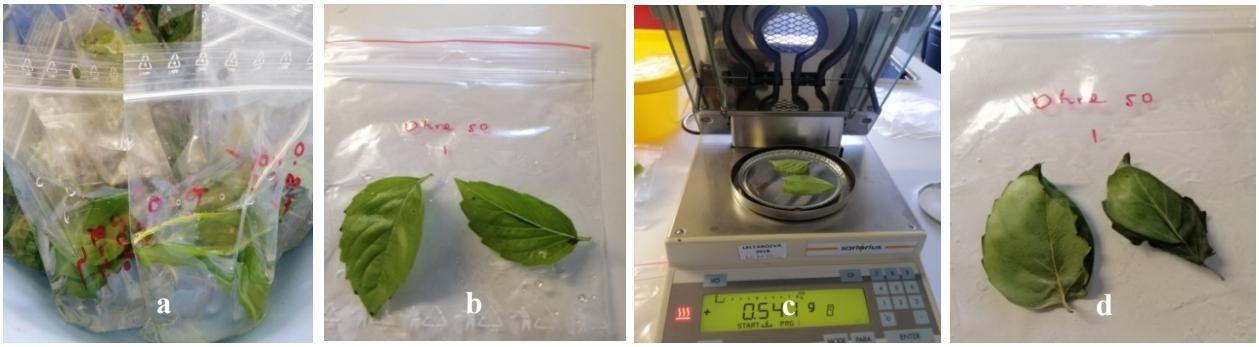

Figure 13. Relative water content determination: Soaked leaves (a), overnight soaked leaf sample (b), oven (c), oven dry leaf sample (d), (Photo: Mulugeta, 2021)

Water potential (WP): The WP 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 for that specific treatment.

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

Chlorophyll content (SPAD value): 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 14). 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.

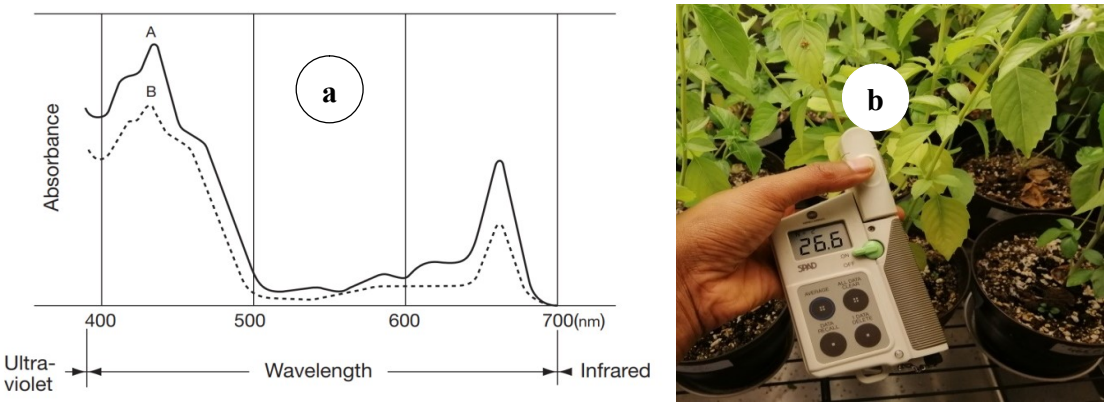

Figure 14. Spectral absorbance characteristic of chlorophyll (a) and SPAD value reading (b), (Photo: Mulugeta, 2021)

3.3.2. 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 was 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.

3.3.3. 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 EOC (mL 100 g^{-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 g 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^{-1} %). 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

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 AOC 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.

3.4. 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.

4. RESULTS AND DISCUSSIONS

4.1. Morpho-chemical diversity among *Ocimum* species

4.1.1. Qualitative morphological traits

Qualitative trait descriptions of the genotypes are shown in Table 5. Considerable variability was observed in growth habit, leaf shape, leaf margin, corolla, and stem color (see Figure 15). All sweet basil cultivars, *O.* × *africanum* (Hoary basil) and *O. americium* (Togo basil) exhibited an upright growth habit, whereas *O. sanctum* (green and purple holy basils), *O. minimum* (Törpe), and *O. americanum* (Lime basil) showed a semi-upright growth habit. The leaf blade shape varies from narrow elliptical to medium ovate, except for 'M. Grünes', which has a broad ovate blade shape. Leaf margin serration ranges from absent ('Turkish', 'Lime', and 'Törpe' basils) to strong in green holy basils and 'Togo basils'. Most genotypes have a white corolla. Holy basil has a violet (light to medium) corolla color. Although the stem color was predominantly green, a few genotypes, including 'Dark opal', 'Thai basil', 'Cinnamon', and 'Purple holy basil', had purple stems.

Table 5. Qualitative trait description of *Ocimum* species based on UPOV descriptor

Genotypes	Growth habit	Leaf-blade shape	Leaf margin	Corolla color	Stem color
Dark opal	Upright	Medium ovate	Medium	Pink	Purple
Thai basil	Upright	Medium elliptic	Weak	Light pink	Purple
Cinnamon	Upright	Narrow elliptic	Weak	Pink	Light Purple
Genovese	Upright	Medium elliptic	Weak	White	Green
Turkish basil	Upright	Medium elliptic	Absent	White	Green
Adi F1	Upright	Medium ovate	Weak	White	Green
Sweet Aroma	Upright	Narrow elliptic	Weak	White	Green
M. Grünes	Upright	Broad ovate	Weak	White	Green
Ohře	Upright	Medium elliptic	Weak	White	Green
Green holy basil	Semi-upright	Medium ovate	Strong	Light violet	Green
Purple holy basil	Semi-upright	Medium elliptic	Weak	Medium violet	Purple
Lime	Semi-upright	Narrow elliptic	Absent	White	Green
Hoary basil	Upright	Narrow elliptic	Weak	Light Purple	Green
Togo basil	Upright	Narrow elliptic	Strong	White	Green
Törpe	Semi-upright	Narrow elliptic	Absent	White	Green

M. Grünes: Mittelgroβblättriger Grünes', UPOV: The International Union for the Protection of New Varieties of Plants.

Figure 15. Morphology of 15 *Ocimum* genotypes (Photo: Mulugeta, 2022)

4.1.2. Quantitative morphological traits

Ocimum species are known to have wide morphological diversities. This study vividly demonstrated the presence of significant ($p<0.01$) morphological variations within the investigated basil genotypes (Appendix Table 4).

Plant height and canopy diameter

There was a variation in both plant height and canopy diameter among the 15 *Ocimum* genotypes as indicated in Table 6. Based on the pooled mean of the years, 'Sweet Aroma' and 'Genovese' plants had the tallest plants (62.1 cm) and the widest canopy diameters (54.0 cm), respectively, while 'Törpe' plants had short plants (30.4 cm) with a narrow canopy (35.3 cm). Due to differences in weather patterns and soil properties, basil plants showed notable variations in their height and canopy size in different years of cultivation, as depicted in Figure 15 and Table 2. In the first year of growth, most basil varieties exhibited taller plant height and a wider canopy compared to the following year.

Table 6. Growth parameters among *Ocimum* species in two production years

	Plant height (cm)		Canopy diameter (cm)	
Genotypes	2021	2022	2021	2022
Dark opal	47.3±2.7^{fg}	43.5±2.8^{de}	44.1±2.5^{d-f}	37.3±3.7^{d}
Thai basil	59.2±4.0^{c}	50.3±4.1^{a-c}	55.6±4.8^{a-c}	48.2±5.7^{ab}
Cinnamon	55.9±3.9^{c-e}	49.3±1.5^{a-c}	58.3±5.7^{ab}	48.2±1.7^{ab}
Genovese	65.2±3.2^{b}	54.0±4.9^{a}	59.5±4.8^{a}	48.5±4.6^{ab}
Turkish basil	45.8±2.0^{g}	42.0±1.4^{ef}	43.2±2.2^{ef}	40.3±2.6^{cd}
Adi F1	50.6±3.2^{e-g}	51.2±2.3^{ab}	42.3±3.8^{f}	49.3±2.7^{ab}
Sweet Aroma	71.0±2.7^{a}	53.2±2.0^{a}	47.6±2.3^{c-f}	45.0±4.6^{b-d}
M. Grünes	57.0±3.9^{cd}	50.3±1.6^{a-c}	49.9±4.8^{b-f}	44.5±3.9^{b-d}
Ohře	51.4±3.9^{d-g}	45.7±3.3^{c-e}	51.8±8.0^{a-e}	42.8±4.0^{b-d}
Green holy basil	37.5±2.9^{h}	37.0±1.1^{fg}	52.9±5.4^{a-d}	50.0±5.3^{ab}
Purple holy basil	49.3±2.5^{fg}	47.8±2.3^{b-d}	53.1±6.2^{a-c}	47.5±3.4^{a-c}
Lime	39.5±2.8^{h}	35.7±1.6^{g}	53.2±6.2^{a-c}	47.0±3.5^{bc}
Togo basil	49.5±8.2^{fg}	44.0±3.0^{de}	50.4±12.6^{b-f}	55.2±3.6^{a}
Hoary basil	52.5±3.5^{d-f}	50.2±1.9^{a-c}	48.1±4.7^{c-f}	43.3±4.0^{b-d}
Törpe	25.6±2.7^{i}	35.2±1.16^{g}	32.3±1.7^{g}	38.3±1.0^{d}

Values are presented as Mean ± SD, M. Grünes: Mittelgroβblättriger Grünes'; Different letters are for significantly different means.

Leaf related traits

Leaf growth of *Ocimum* genotypes exhibits a remarkable level of variability. Table 7 indicates a significant variation in leaf area growth among basil genotypes and the year of production. The cultivars 'M. Grünes', 'Genovese' and 'Turkish basil' had robust leaf growth (leaf length, width, area, and hundred leaf weight) in both years, whereas 'Togo basil' and 'Törpe' had the

smallest leaf growth. Based on the two-year average, ‘M. Grünes’ had the longest leaf (9.1 cm), a wider leaf (5.1 cm), a larger leaf area (29.2 cm^2 $leaf^{-1}$), and a heavier leaf weight (103.8 g 100 $leaves^{-1}$). In contrast, ‘Togo basils’ had the shortest leaf (2.4 cm), a narrow leaf (1.2 cm), a smaller leaf (2.6 cm^2 $leaf^{-1}$), and a lighter leaf weight (7.05 g 100 $leaves^{-1}$).

Reproductive parameters

The basil genotypes showed a broader and significant ($p<0.01$) variation in their reproductive traits, including the length and number of inflorescences, as well as the thousand seed weight (Appendix Table 4 and Table 8). According to the yearly average, the basil cultivars ‘Dark opal’ produced the longest inflorescence, (31.1 cm). While ‘Purple holy basil’ had the shortest inflorescence, measuring only 13 cm. Interestingly, despite having the shortest inflorescence and smaller seeds (0.2 g per thousand seeds), ‘Purple holy basil’ produced the highest number of inflorescences per plant, reaching 219.7. Even though ‘Turkish basil’ and ‘M. Grünes’ exhibited robust leaf growth, they had the lowest number of inflorescences, falling below 66.0. Additionally, sweet basil cultivars ‘Thai basil’ and ‘M. Grünes’ had larger and heavier seeds (≥1.8 g per thousand seeds).

Table 7. Leaf-related parameters among *Ocimum* species in 2021 and 2022

Genotypes	Leaf length (cm)		Leaf width (cm)		Leaf area (cm^2 $leaf^{-1}$)		Hundred leaf weight (g)	
	2021	2022	2021	2022	2021	2022	2021	2022
Dark opal	5.7±0.4^{cd}	5.7±0.5^{d}	2.9±0.3^{de}	2.9±0.4^{e-g}	9.8±2.0^{d-f}	9.9±2.0^{d-f}	40.6±1.5^{de}	41.0±1.7^{d}
Thai basil	5.7±0.7^{cd}	6.7±0.6^{c}	2.9±0.4^{de}	3.2±0.2^{d-f}	9.7±1.9^{d-f}	11.5±2.7^{de}	40.1±1.3^{de}	45.2±3.2^{d}
Cinnamon	6.2±0.8^{c}	5.7±0.4^{d}	3.7±0.5^{bc}	2.9±0.3^{d-g}	12.2±2.8^{c-e}	9.2±1.8^{d-f}	55.0±2.1^{c}	31.8±3.3^{e}
Genovese	8.7±0.9^{a}	8.7±1.0^{ab}	3.8±0.4^{b}	3.9±0.4^{bc}	18.4±4.3^{bc}	20.7±4.3^{b}	102.1±4.8^{a}	103.6±7.8^{a}
Turkish basil	7.7±0.8^{b}	7.9±0.5^{b}	3.6±0.6^{bc}	4.4±0.5^{b}	19.4±4.3^{b}	17.4±3.6^{bc}	101.2±3.7^{a}	77.2±4.5^{b}
Adi F1	5.2±0.2^{de}	5.9±0.6^{cd}	3.1±0.2^{de}	3.1±0.5^{d-g}	10.1±2.2^{d-f}	8.2±1.7^{ef}	39.3±2.9^{e}	44.9±3.2^{d}
Sweet Aroma	7.9±0.5^{ab}	6.8±0.7^{c}	3.3±0.3^{cd}	3.3±0.5^{de}	16.1±3.3^{b-d}	13.2±2.7^{cd}	69.4±4.4^{b}	57.3±3.8^{c}
M. Grünes	8.7±0.7^{a}	9.5±0.9^{a}	4.9±0.3^{a}	5.2±0.5^{a}	30.0±13.7^{a}	28.3±8.2^{a}	104.7±5.1^{a}	104.4±6.0^{a}
Ohře	5.8±0.3^{cd}	5.4±0.5^{c-e}	3.4±0.2^{b-d}	3.0±0.2^{d-g}	10.1±2.9^{d-f}	7.8±1.8^{ef}	44.5±3.0^{e}	45.4±4.3^{d}
Green holy basil	4.5±0.2^{ef}	5.4±0.3^{cd}	3.0±0.2^{de}	3.5±0.1^{cd}	7.9±1.2^{e-g}	11.3±1.7^{de}	25.3±3.6^{g}	33.8±3.8^{e}
Purple holy basil	4.7±0.5^{ef}	4.7±0.4^{d-f}	2.7±0.3^{e}	2.7±0.3^{fg}	7.9±1.1^{e-g}	7.9±1.1^{ef}	23.0±1.9^{g}	20.0±3.1^{f}
Lime	4.0±0.3^{f}	4.1±0.4^{f}	1.95±0.3^{f}	2.0±0.2^{h}	4.5±0.7^{fg}	7.6±2.2^{ef}	17.2±2.1^{j}	18.4±1.6^{f}
Togo basil	2.8±0.8^{g}	2.0±0.4^{g}	1.4±0.5^{i}	0.9±0.3^{i}	2.9±0.9^{g}	2.3±0.7^{g}	6.8±0.7^{i}	7.4±1.3^{g}
Hoary basil	6.0±0.5^{cd}	6.0±0.5^{cd}	2.6±0.2^{e}	2.6±0.2^{g}	7.7±1.9^{e-g}	7.3±1.8^{ef}	32.4±2.3^{f}	32.2±3.8^{e}
Törpe	2.7±0.3^{g}	4.4±0.4^{ef}	1.4±0.3^{hi}	2.5±0.3^{gh}	2.4±0.4^{g}	6.3±1.5^{fg}	14.0±0.9^{h}	18.4±1.5^{f}

Table 8. Variation in reproductive traits among *Ocimum* species

Genotypes	Inflorescence length (cm)		No. of inflorescence		Thousand seed weight (g)	
	2021	2022	2021	2022	2021	2022
Dark opal	36.0±4.1^{a}	26.2±1.6^{ab}	98.3±20.3^{fg}	70.0±9.5^{d-g}	1.6±0.3^{de}	1.5±0.1^{cd}
Thai basil	31.4±2.9^{ab}	26.5±3.8^{ab}	107.2±15.0^{f}	76.3±16.7^{d-f}	1.9±0.0^{a}	1.9±0.1^{a}
Cinnamon	26.8±5.1^{bc}	29.8±2.3^{a}	160.1±22.9^{cd}	84.7±15.9^{de}	1.6±0.0^{cd}	1.7±0.0^{bc}
Genovese	28.7±3.0^{b}	26.5±2.9^{ab}	175.8±27.8^{c}	167.2±31.5^{b}	1.7±0.1^{bc}	1.7±0.1^{ab}
Turkish basil	18.2±2.4^{ef}	23.5±1.4^{bc}	72.6±6.9^{g}	53.8±11.5^{e-g}	1.6±0.0^{d-f}	1.5±0.1^{cd}
Adi F1	23.6±3.3^{cd}	20.5±1.6^{cd}	114.4±26.7^{ef}	97.7±15.4^{cd}	1.4±0.1^{g}	1.4±0.0^{d}
Sweet Aroma	23.0±2.3^{cd}	21.5±2.1^{cd}	139.6±16.5^{de}	51.0±22.3^{e-g}	1.5±0.0^{f-h}	1.5±0.0^{d}
M. Grünes	30.6±3.9^{b}	17.5±1.0^{d-f}	97.0±10.1f^{g}	34.7±4.4^{g}	1.8±0.1^{b}	1.7±0.1^{ab}
Ohře	17.5±2.8^{e-g}	19.5±2.2^{cd}	257.8±39.8^{a}	53.7±8.0^{e-g}	1.4±0.0^{gh}	1.5±0.1^{d}
Green holy basil	20.3±3.3^{de}	17.8±1.5^{de}	159.9±37.6^{cd}	125.2±34.6^{c}	0.6±0.0^{h}	0.6±0.0^{e}
Purple holy basil	12.8±1.7^{g}	13.2±1.2^{f}	226.5±35.9^{b}	212.8±21.9^{a}	0.2±0.0^{j}	0.2±0.0^{f}
Lime	27.5±1.4^{bc}	22.8±2.6^{bc}	142.2±13.1^{de}	97.2±10.5^{cd}	1.5±0.0^{e-g}	1.5±0.1^{cd}
Togo basil	15.0±1.8^{g}	23.0±1.5^{bc}	29.9±7.8^{h}	191.7±27.6^{ab}	0.5±0.0^{i}	0.4±0.0^{e}
Hoary basil	19.0±2.7^{d-f}	17.3±2.7^{d-f}	207.3±13.8^{b}	183.7±14.8^{ab}	0.5±0.0^{hi}	0.5±0.0^{e}
Törpe	17.3±3.3^{e-g}	14.2±1.7^{ef}	140.8±25.8^{de}	44.3±5.8^{fg}	1.4±0.0^{gh}	1.4±0.1^{d}

Values are presented as Mean ± SD, M. Grünes: Mittelgroβblättriger Grünes'; Different letters are for significantly different means.

Fresh and dry herb yield

Ocimum species are renowned for their exceptional ability to produce a wide range of biomass. The results of the field experiments indicate that there was significant variability in both fresh and dry biomass production among the 15 basil genotypes tested, as illustrated in Appendix Table 4 and Table 9. The sweet basil cultivars were predominantly robust and vigorous in growth, with the 'Genovese' cultivar producing the maximum fresh and dry herb yields of 489.9 g plant^{-1} and 98.6 g plant^{-1}, respectively, on a yearly average basis. Additionally, 'M. Grünes', 'Cinnamon', and 'Sweet aroma' yielded over 425.0 g of fresh biomass and more than 85 g of dry mass yield per plant. In contrast, 'Purple holy basil' exhibited the lowest fresh biomass yield of 212.6 g plant^{-1} and the lowest dry biomass yield of 56.5 g plant^{-1}. Similarly, the cultivar 'Törpe' exhibited the lowest biomass yield. Furthermore, biomass production varied significantly between the years of production due to variations in soil conditions and microclimate. Hence, all genotypes produced higher fresh and dry biomass yields during the first growing year (2021) than in the second growing year (2022).

Table 9. Biomass production variability within *Ocimum* species

	Fresh herb yield (g plant $^{-1}$)		Dry herb yield (g plant $^{-1}$)	
Genotypes	2021	2022	2021	2022
Dark opal	329.8±53.4^{c-e}	151.7±43.3^{d}	47.3±6.7^{e}	32.0±4.5^{d}
Thai basil	461.0±140.7^{a-d}	328.3±153.4^{a-c}	96.9±30.1^{a-d}	55.8±22.4^{b-d}
Cinnamon	588.4±199.6^{ab}	293.7±82.2^{a-d}	110.8±34.5^{ab}	68.8±15.7^{a-c}
Genovese	635.6±81.15^{a}	344.0±33.1^{ab}	129.4±21.3^{a}	67.8±7.6^{a-c}
Turkish basil	415.1±90.1^{b-e}	371.7±43.7^{a}	74.9±17.7^{b-e}	73.7±9.8^{a-c}
Adi F1	416.3±70.3^{b-e}	356.3±80.4^{a}	74.3±12.9^{b-e}	73.3±20.6^{a-c}
Sweet Aroma	602.7±119.6^{ab}	249.7±50.6^{a-d}	112.7±27.6^{ab}	61.7±12.9^{a-d}
M. Grünes	527.3±112.9^{a-c}	381.0±52.2^{a}	97.7±21.6^{a-c}	87.2±13.2^{a}
Ohře	524.3±132.3^{a-c}	248.0±55.6^{a-d}	98.6±24.9^{a-c}	51.8±12.6^{b-d}
Green holy basil	431.0±80.8^{a-e}	330.0±81.5^{a-c}	87.0±19.7^{b-d}	86.5±13.8^{a}
Purple holy basil	234.3±47.9^{e}	190.8±23.2^{cd}	62.4±12.2^{c-e}	50.7±4.1^{cd}
Lime	446.2±155.6^{a-d}	255.3±78.8^{a-d}	101.2±35.5^{a-c}	66.7±22.8^{a-c}
Togo basil	467.8±322.1^{a-d}	326.7±130.8^{a-d}	87.9±50.3^{b-d}	82.3±25.4^{ab}
Hoary basil	513.7±68.6^{a-d}	269.0±37.4^{a-d}	94.8±17.0^{a-d}	67.7±7.5^{a-c}
Törpe	313.6±66.9^{de}	195.3±43.5^{b-d}	57.6±13.9^{de}	52.0±11.7^{b-d}

Values are presented as Mean ± SD, M. Grünes: Mittelgroβblättriger Grünes; Different letters are for significantly different means.

In addition, a morphological cluster analysis utilizing Ward's method revealed the existence of three distinct groups (as illustrated in Figure 16). The first group consisted of 'Dark opal, Tope', and 'Purple holy basil'. The second group was composed of 'Thai basil', 'Adi F1', 'Lime', 'Togo basil', 'Ohře', 'Green holy basil', and 'Hoary basil'.

On the other hand, the third group had ‘Cinnamon’, ‘Sweet aroma’, ‘Turkish basil’, ‘M. Grünes’, and ‘Genovese’. Moreover, the biplot-principal component analysis (PCA) provided insights into the relationship between the genotypes and the diverse quantitative morphological traits under investigation (as depicted in Figure 17). These two principal components collectively explained 72.0% of the variance, with PC1 accounting for 50.9% and PC2 for 21.11%.

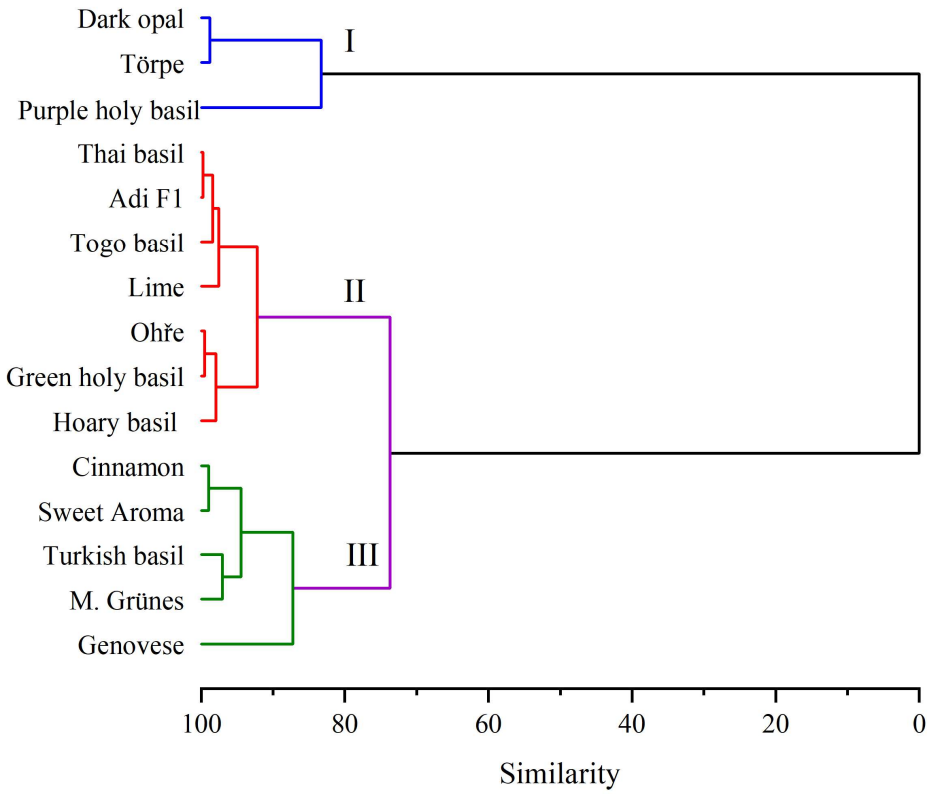

Figure 16. Similarity dendrogram showing major clusters among 15 *Ocimum* genotypes based on their morphological characteristics

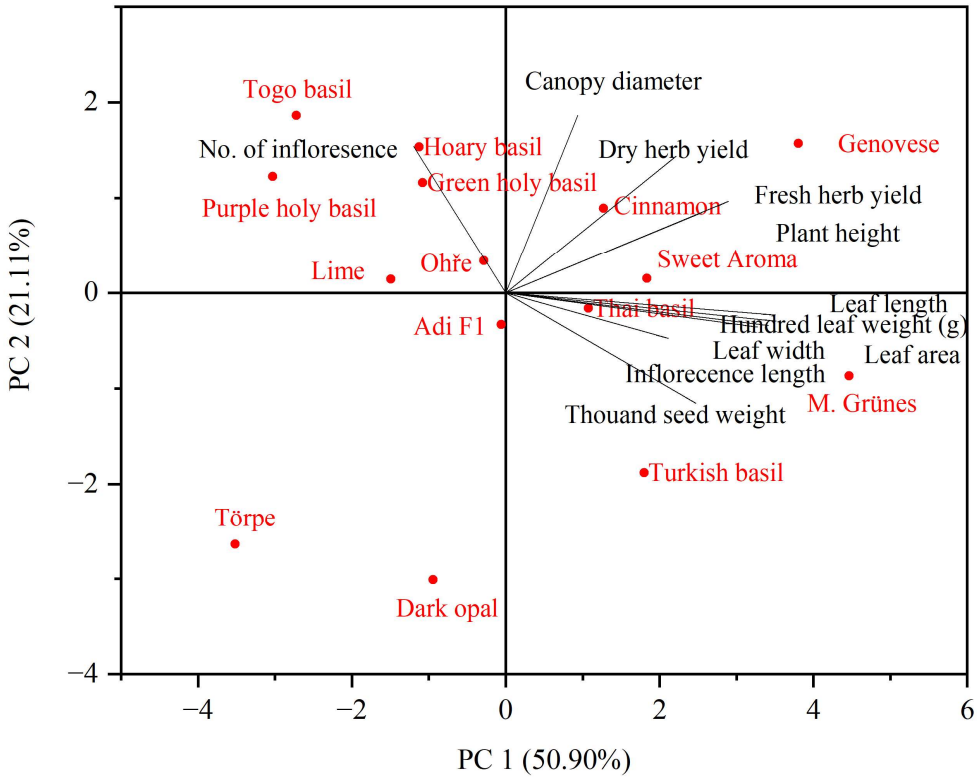

Figure 17. Principal component plot analysis of morphological traits among the 15 *Ocimum* genotypes

4.1.3. Secondary compound accumulation among Ocimum species

The presence of a wider chemical diversity was observed among the basil genotypes studied, as indicated in Figure 18 and Tables 10-12.

Essential oil content and essential oil yield

Thus, the basil genotypes and year of production had a significant ($P<0.01$) impact on both EOC and EOY (see Appendix Table 4 and Figure 18). The EOC ranged from 0.3% to 3.4% across different species, with the highest values observed in 'Hoary basil' and the lowest in 'Green holy basil'. Similarly, the EOY ranged from 0.3 to 2.6 mL plant^{-1}, with the highest yields obtained from 'Hoary basil' and the lowest from 'green holy basil'. The second highest EOC (1.3%) and EOY (1.0 mL plant^{-1}) were obtained from 'Ohře' plants.

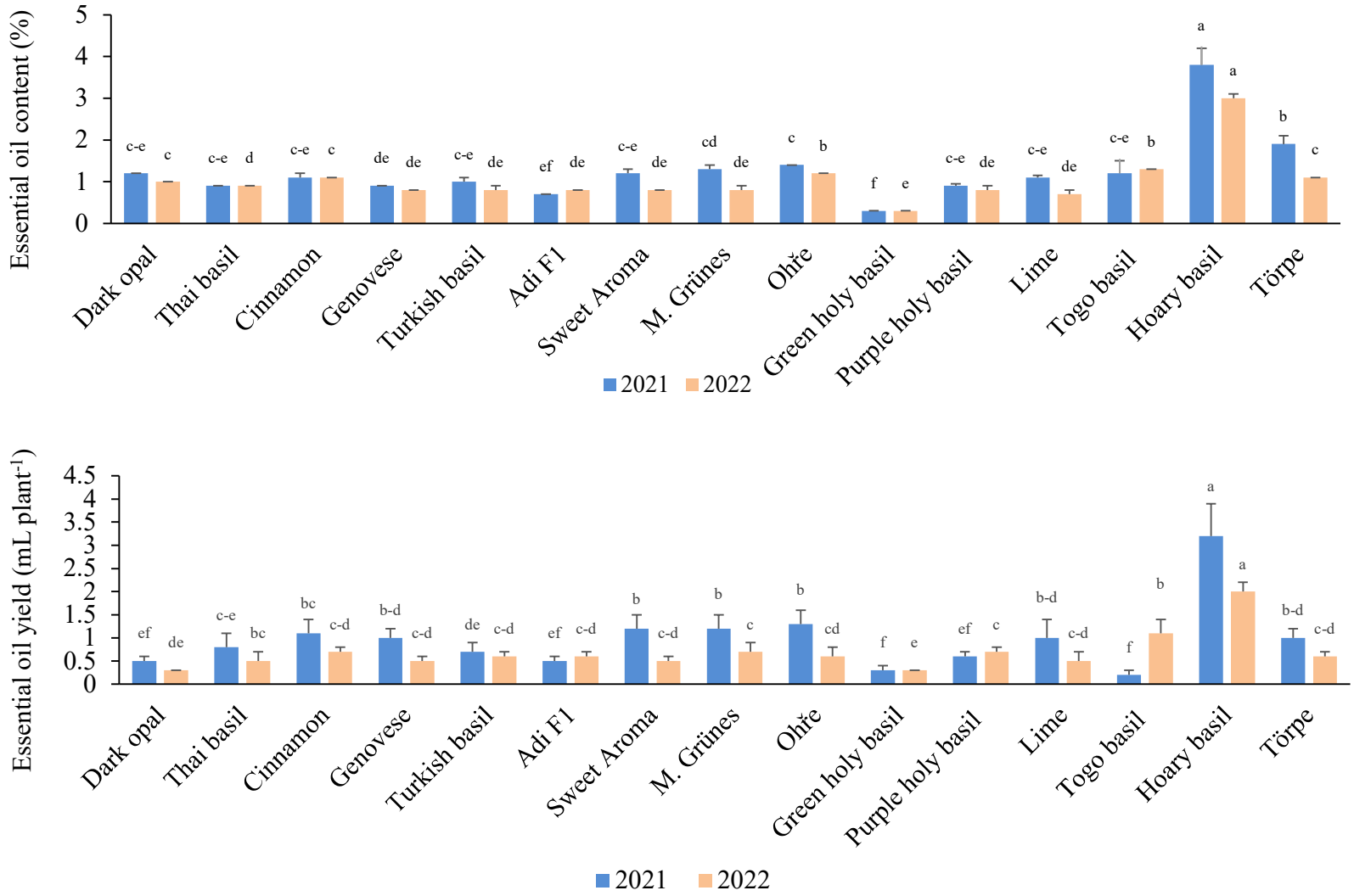

Figure 18. Variability in essential oil production of 15 *Ocimum* genotypes

Values are presented as Mean ± SD, M. Grünes: Mittelgroβblättriger Grünes; Different letters are for significantly different means.

Essential oil composition

The composition can vary significantly based on factors such as geographical location, environmental conditions, cultivation techniques, plant material (fresh or dry), and genetic variations. In addition, chemical diversification in different *Ocimum* species has been notably influenced by hybridization within and between the species through cross-pollination, as well as natural evolutionary events, polyploidy, and selective breeding, as observed in various studies. Our two-year studies also showed a wider diversity in the essential oil composition of *Ocimum* species (Tables 10 and 11). The essential oil composition was subjected to similarity cluster analysis, identifying seven distinct chemo diversity (Figure 19). The first cluster consisted of sweet basil genotypes, namely 'Dark opal', 'Sweet Aroma', 'Turkish basil', 'Genovese', and 'Ohře', which were rich in linalool ratios exceeding 54.0%. The second group comprised 'Cinnamon', 'Törpe', 'M. Grünes', and 'Lime', characterized by linalool (>32%) as a major component alongside other constituents. Specifically, 'Cinnamon' basil contained linalool/estragole/methyl eugenol mixtures. The 'Törpe' genotype displayed higher ratios of linalool (40.0%). The main compounds of 'M. Grünes' were linalool (44.0%) and estragole (19.0%). The 'Lime' basil predominantly contained oxygenated monoterpenes such as linalool (40.0%), and citral (30.0%). The third chemical group was represented by 'Thai basil', characterized by phenylpropanoid compounds, predominantly estragole (53.3%) and methyl eugenol (8.7%). Similarly, the fourth cluster also exhibited phenylpropanoids, with *trans*-ß-caryophyllene (36.2%) and eugenol (32.2%) as the primary constituents in 'purple holy basil'. The 'Green holy basil' formed the fifth cluster. It was composed of 1,8-cineole (22.0%), estragole (24.0%), and bisabolene (31.0%). Whereas hoary basil contained 1,8-cineole (36.0%), camphor (18.0%), and limonene (14.0%) as cluster six. Finally, 'Togo basil' belonged to the last chemical cluster, characterized by 61.0% methyl cinnamate. As shown in Figure 20, the biplot-PCA provided insight into the relationship between genotypes and essential oil components. The two principal components explained 56.7% of the variance, PC1 explaining 28.1% and PC2 explaining 18.6%. Thus, in our study, we observed three chemotypes of sweet basils: linalool rich, estragole rich, and mixed (linalool/estragole, linalool/citral, linalool/methyl eugenol/estragole).

Table 10. The essential oil composition of sweet basil cultivars

			Dark opal		Thai basil		Cinnamon		Turkish basil		Genovese		Adi F1		Sweet aroma		M. Grünes		Ohře	
Component	RT	LRI	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
1,8-cineole	8.40	1034	6.6	6.0	2.0	3.4	1.8	4.9	3.4	4.9	3.7	8.0	4.3	5.7	5.4	6.5	3.5	6.3	2.5	5.2
linalool	10.90	1097	68.4	48.5	3.5	25.4	29.5	34.8	74.2	37.0	63.8	44.5	58.3	34.4	66.9	43.5	45.7	42.6	60.8	57.5
α-terpineol	14.60	1189	0.5	0.9	0.3		0.3	0.8	0.5	0.9	0.5		0.7	1.3	0.5	1.1	0.5	1.1	0.2	0.6
estragole	14.80	1196	0.2	10.1	65.1	41.5	12.8	7.5		2.7			0.1	0.2	0.1	0.3	30.5	8.2		
geraniol	17.30	1252	0.5	1.1				0.7		0.1			1.8	0.4					12.4	1
eugenol	21.50	1361	0.9	4.4		0.2	0.1	3.5	1.4	4.1	2.1	3.5	1.8	5.5	2.7	5.0	0.2	1.7	0.6	0.8
ß-elemene	22.90	1391	2.6	3.0	0.6	0.7	1.8	0.6	1.2		1.7	0.5	2.8	4.1	2.0	2.6	1.0	1.9	1.8	2.9
methyl eugenol	23.50	1411	0.1	0.1	15.8	1.6	13.7	0.1	0.1	0.3	0.1		0.1	0.2	0.1	0.3	0.1	0.3	0.1	
trans-α-bergamotene	24.70	1437	0.4	1.2	2.3	2.7	0.7	1.7	1.0	1.7	3.9	4.8	0.1			0.7	1.4	1.4	0.4	2.0
germacrene D	26.50	1482	4.2	3.9	0.7	2.1	2.8	3.1	2.0	4.3	3.6	2.9	4.9	5.9	3.2	4.4	2.7	3.8	2.3	2.0
α-bulnesene	27.50	1506	3.1	2.8	0.7	0.6	2.0		1.5	3.7	2.2	2.4	3.7	4.2	2.3	2.4	1.2	1.9	2.0	1.4
cis-γ-cadinene	27.80	1515	1.1	1.2	0.8	1.6	1.2	1.9	1.8	3.0	2.3	2.6	2.6	3.3	2.0	2.7	1.4	2.4	1.6	2.1
tau-cadinol	32.60	1644	3.4	4.6	2.7	5.6	5.6	7.2	7.3	9.7	8.0	9.5	9.9	1	6.9	9.1	5.1	9.3	7.9	7.5
others (<2%)			7.4	10.8	4.7	12.3	27.2	30.9	5.2	23.9	7.4	19.6	8.0	20.3	7.5	18.9	6.2	16.8	7.0	6.8
Total			99.4	98.6	99.2	97.7	99.5	97.7	99.6	96.3	99.3	98.3	99.1	95.5	99.6	97.5	99.5	97.7	99.6	98.8
Monoterpenes			1.2	1.2	0.9	2.6	0.5	2.3	0.4	1.4	0.7	2.4	0.3	1.6	1.0	2.7	0.8	2.0	0.8	1.1
Oxygenated monoterpenes			76.9	58.1	7.7	7.8	54.7	46.4	79.5	47.5	70.1	59.2	68.7	47.8	75.1	57.1	51.5	54.5	78.1	75.0
Sesquiterpenes			15.9	17.1	6.3	8.3	10.9	13.0	1.0	23.5	16.9	18.5	17.3	24.8	12.5	18.4	1	16.1	10.8	12.9
Oxygenated sesquiterpenes			4.4	7.1	3.4	7.4	7.5	11.4	8.7	16.0	9.8	14.5	12.0	15.2	8.3	13.5	6.4	14.4	9.3	9.0
Phenylpropanes			1.1	15.0	80.9	70.4	26.6	24.1	1.5	7.6	2.2	3.9	1.9	5.8	2.9	6.0	30.8	10.3	0.7	0.8

M. Grünes: Mittelgroβblättriger Grünes, RT – retention time. LRI – linear retention index relative to C9-C23 n-alkanes on an HP-5MS capillary column.

Table 11. Major essential oil compounds of *Ocimum* species

			Purple holy basil		Green holy basil	Hoary basil		Lime		Törpe		Togo	
Component	RT	LRI	2021	2022	2021	2021	2022	2021	2022	2021	2022	2021	2022
ß-pinene	6.70	981			0.6	3.9			0.1	0.4			
limonene	8.30	1029			0.2	14.6	13.3	0.1	0.1	0.3	0.3	0.3	2.8
1,8-cineole	8.40	1034	0.2	0.3	22.3	43.8	27.9	0.3	1.7	9.1	4.3	0.1	4.6
linalool	10.90	1097	0.4	0.4	0.6	0.3	0.3	35.3	43.9	44.9	35.4	3.0	3.4
camphor	12.70	1144				16.5	18.7			1.8	2.1	0.2	6.4
estragole	14.80	1196			24.1						0.1	0.1	3.9
nerol	16.10	1227						7.8	3.7				
nerál (citrál-b)	16.70	1238						14.8	10.4				
geraniol	17.30	1252						2.9	4.7				
geranial (citral a)	18.00	1268						20.3	14.4				
(*Z*)-methyl cinnamate	19.30	1299										8.4	5.0
eugenol	21.50	1361	36.4	28.1	3.5					9.2	4.9		4.2
(*E*)-methyl cinnamate	22.60	1394										81.7	39.5
ß-elemene	22.90	1391	5.7	5.6				0.6		3.1	4.6		
methyl eugenol	23.50	1411	5.6	7.6							0.3	0.0	
trans-ß-caryophyllene	24.00	1420	38.5	34.0	0.8	0.8	2.38	2.8	2.0	0.3	1.4	1.3	2.6
trans-α-bergamotene	24.70	1437		0.1	2.2			0.7	0.5	2.3		1.5	2.4
α-humulene	25.40	1454	2.3	2.7	1.7	4.0	8.7	0.6	0.6	0.8	1.5	0.1	4.9
germacrene d	26.50	1482	0.1	0.1		0.7	2.3	1.3	1.7	3.1	4.5	0.2	0.6
α-bulnesene	27.50	1506	2.3	1.1			0.2	0.6		4.1	3.7		
β-bisabolene	27.60	1508			31.4			0.1					1.3
cis-α-Bisabolene	29.00	1544			6.4	0.1		2.3	1.5				
caryophyllene oxide	30.50	1590	4.7	9.4	1.5			1.2	0.5		0.2	0.2	1.4
tau-cadinol	32.60	1644		0.1				2.8	4.0	7.6	11.6	0.2	
others (<2%)			2.8	7.7	4.5	15	25.1	4.2	7.51	11.9	20.3	1.9	13.9
Total			99.0	97.2	99.8	99.7	98.9	98.7	97.9	98.9	95.2	99.2	96.9
Monoterpenes			0.1	0.1	1.3	23.9	19.1	0.2	1.2	1.4	1.6	0.5	6.4
Oxygenated monoterpenes			0.8	0.9	24.2	67.4	67.6	83.6	79.9	59.6	48.2	4.1	18.7
Sesquiterpenes			49.9	46.8	36.7	8.2	11.7	7.9	10.3	18.9	23.3	3.6	14.4
Oxygenated sesquiterpenes			6.2	13.7	10.0	0.2	0.6	7.0	5.3	9.5	16.1	0.8	3.6
Phenylpropanes			42.0	36.0	27.6	0.0	0.0	0.0	0.0	9.5	5.8	90.2	53.8

RT – retention time. LRI – linear retention index relative to C9-C23 n-alkanes on an HP-5MS capillary column.

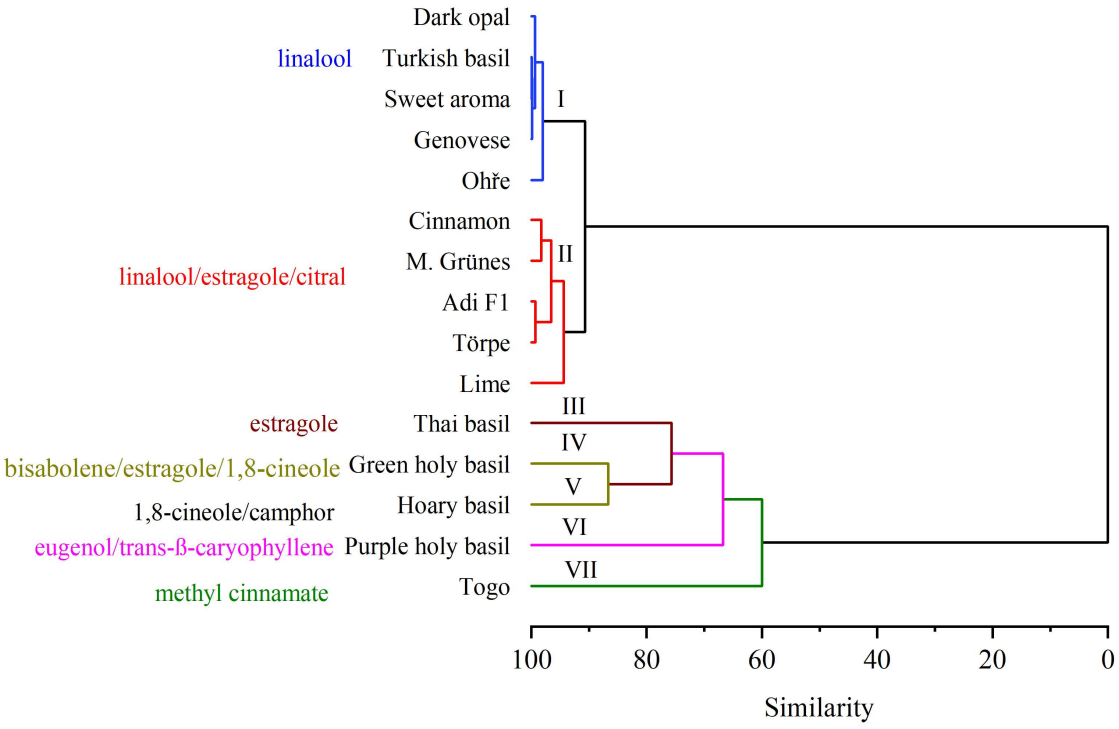

Figure 19. Similarity dendrogram showing major clusters among 15 *Ocimum* genotypes based on their essential oil composition.

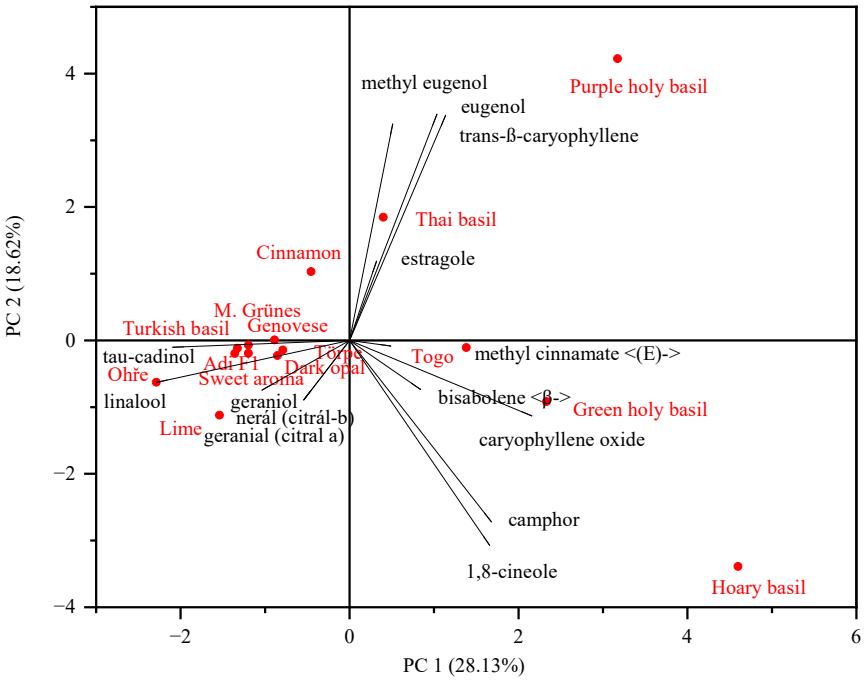

Figure 20. Principal component plot analysis on the essential oil composition of the 15 basil genotypes.

Total polyphenol content and antioxidant capacity

The TPC and its AOC were found significantly different between the genotypes as shown in Table 12. The TPC of the genotypes ranges between 127.4 and 218.8 mg GAE g^{-1} DM, in 'Adi F1' and 'Turkish basil', respectively. While 'Törpe' ranked second with 213.7 GAE g^{-1} of DM. The AOC of the genotypes varies between 67.3 and 290.4 mg AAE g^{-1} DM. Additionally, Turkish basil had the highest AOC (290.4 mg AAE g^{-1} DM) of all genotypes. The second highest AOC was obtained from 'M. Grünes' and 'Cinnamon' cultivars of sweet basils. Whereas 'Togo basil produced the lowest AOC (70.2 AAE g^{-1} DM). In general, sweet basil cultivars typically have greater AOCs than the species they were compared to except for 'Genovese' and 'Adi F1'.

Table 12. Total poly phenol content and antioxidant capacity of 15 *Ocimum* genotypes

Genotypes	Total polyphenol content (mg GAE g^{-1} DM)		Antioxidant capacity (mg AAE g^{-1} DM)	
	2021	2022	2021	2022
Dark opal	146.4±5.8^{a-d}	170.1±13.9^{d-f}	225.8±6.2^{b}	172.2±61.7^{c}
Thai basil	176.8±4.5^{a-d}	177.0±10.2^{c-e}	242.6±10.2^{b}	129.4±9.2^{c-g}
Cinnamon	186.9±8.0^{a-d}	155.8±10.3^{e-g}	246.8±11.9^{b}	232.8±7.0^{b}
Genovese	118.5±80.6^{d}	173.6±13.2^{d-f}	112.4±32.6^{cd}	154.2±8.8^{cd}
Turkish basil	224.5±9.7^{a}	234.2±15.3^{a}	410.2±38.7^{a}	170.5±18.0^{c}
Adi F1	130.7±58.8^{cd}	124.1±7.5^{h}	123.4±32.9^{cd}	129.1±12.7^{c-g}
Sweet Aroma	204.0±7.6 ^{abc}	213.0±6.1^{ab}	248.8±6.7^{b}	149.1±10.1^{c-e}
M. Grünes	190.5±8.0 ^{abc}	204.7±21.4^{bc}	224.8±7.5^{b}	296.0±31.1^{a}
Ohře	169.2±8.2 $^{a-d}$	185.2±24.3^{b-d}	217.6±32.1^{b}	119.6±16.7^{d-g}
Green holy basil	154.7±66.3^{a-d}	138.3±16.7^{gh}	145.8±69.6^{c}	115.8±12.6^{d-g}
Purple holy basil	125.3±46.6^{d}	173.5±29.6^{d-f}	143.2±54.1^{c}	98.8±18.1^{fg}
Lime	201.1±2.3 ^{abc}	172.2±5.5^{d-f}	113.3±4.4^{cd}	106.2±6.0^{e-g}
Togo basil	148.5±47.3^{b-d}	145.7±8.0^{f-h}	70.2 ±20.8^{d}	64.4±4.7^{h}
Hoary basil	151.7±5.9 $^{a-d}$	179.2±14.5^{c-e}	115.1±39.3^{cd}	93.7±5.1^{g}
Törpe	223.4±28.7^{ab}	204.1±7.3^{bc}	108.7±4.2 ^{cd}	141.1±11.7^{c-f}

Values are presented as Mean ± SD, M. Grünes: Mittelgroβblättriger Grünes' TPC: Total polyphenol content, AOC: Antioxidant capacity. GAE: Gallic acid equivalent; AAE: Ascorbic acid equivalent; Different letters are for significantly different means.

4.2. Drought-induced physiological changes among *Ocimum species*

Plants experience several physiological alterations when exposed to drought stress. To assess these changes, measurements were taken of the RWC and SPAD values (chlorophyll content expressed in green color intensity) in both greenhouse and plant growth chamber experiments. Additionally, the shoot WP and WUE were measured specifically in the plant growth chamber experiments. The findings regarding the responses of these physiological characteristics in the *Ocimum* species being studied are presented below.

4.2.1. Relative water content

As shown in the Appendix Tables 2 and 3, both greenhouse and plant growth chamber experiments show that the soil water content and the production year significantly affected the leaf RWC of various *Ocimum* species. Accordingly, to a three-year greenhouse experiment, as the drought intensity increased, the RWC of leaves decreased. Table 13 reveals that under moderate drought stress, *O. basilicum* 'Genovese', *O.× africanum*, and *O. americanum* showed a reduction of 7.5%, 13.14%, and 15.11%, respectively, based on the pooled mean of the year. Additionally, under severe drought stress, the leaf RWC reduction ranged from 18% for *O. basilicum* 'Genovese' to 26.8% for *O. americanum*. Furthermore, the plant growth chamber experiment also yielded similar results, based on the mean of the years the leaf RWC of both *O. basilicum* 'Ohře' and *O. americanum* showed a reduction of over 5% and 14% under moderate and severe drought stresses, respectively, as illustrated in table 6.

Table 13. Effect of water supply on relative water content and chlorophyll content of *Ocimum* species (greenhouse)

Species	SWC (%)	Relative water content (%)			Chlorophyll content (SPAD value)		
		2020	2021	2022	2020	2021	2022
O. basilicum 'Genovese'	70	85.4 ± 0.8^{Aa}	94.9 ± 1.8^{Aa}	85.9 ± 2.7^{Ba}	40.2 ± 3.9^{Ab}	33.0 ± 2.0^{Bb}	40.3 ± 3.0^{Ab}
	50	79.9 ± 5.5^{Aa}	89.8 ± 1.5^{Ab}	73.8 ± 3.5^{Ab}	42.6 ± 4.4^{Bb}	40.7 ± 1.7^{Ba}	42.9 ± 1.6^{Bab}
	30	71.6 ± 7.6^{Ab}	79.0 ± 2.8^{Ac}	62.5 ± 6.4^{Ac}	54.0 ± 2.8^{Aa}	42.7 ± 2.1^{Ba}	45.9 ± 3.2^{Bb}
O. × africanum	70	82.8 ± 2.7^{Aa}	92.2 ± 1.3^{Ba}	91.6 ± 4.5^{Aa}	43.3 ± 5.4^{Ab}	47.0 ± 4.9^{Ab}	42.4 ± 3.1^{Ab}
	50	75.3 ± 4.9^{Ab}	80.4 ± 4.4^{Cb}	71.5 ± 7.6^{Ab}	51.7 ± 2.5^{Aa}	47.8 ± 2.7^{Ab}	50.6 ± 1.4^{Aa}
	30	66.5 ± 5.4^{Abc}	73.6 ± 3.0^{Bc}	66.9 ± 3.9^{Ac}	53.1 ± 3.8^{Aa}	52.5 ± 1.8^{Aa}	51.6 ± 0.8^{Aa}
O. americanum	70	85.8 ± 3.5^{Aa}	95.8 ± 2.0^{Aa}	83.0 ± 2.4^{Ba}	40.5 ± 6.1^{Ab}	44.8 ± 2.0^{Ab}	42.0 ± 2.1^{Ab}
	50	73.6 ± 2.9^{Ab}	85.4 ± 1.9^{Bb}	60.3 ± 4.4^{Bb}	45.8 ± 6.0^{Ab}	51.2 ± 4.4^{Aa}	44.4 ± 2.2^{Bab}
	30	56.7 ± 6.7^{Bc}	76.0 ± 1.9^{Bc}	51.3 ± 3.4^{Bc}	51.7 ± 5.1^{Aa}	51.0 ± 2.5^{Aa}	46.7 ± 1.9^{Ba}

Values are presented as Mean ± SD, SWC: Soil Water Capacity. Different letters are for significantly different groups. Capital letters are used to differentiate between species under fixed drought stress treatment and small letters are used to differentiate drought stress under fixed species.

4.2.2. SPAD value

The soil water capacities had a significant impact ($p<0.01$) on chlorophyll content, represented by the green color intensity measured as SPAD values, in both the greenhouse and plant growth chamber experiments (See Appendix Tables 2 and 3). Across both studies, it was observed that the SPAD value increased as the intensity of drought stress increased for all tested basil species. In the greenhouse experiment, *O. basilicum* 'Genovese' and *O. × africanum* basil plants subjected to severe drought stress exhibited average SPAD values ranging from 47.5 to 52.4, whereas the control

plants had SPAD values ranging from 37.8 to 44.3 (refer to Table 13 above). Similarly, the plant growth chamber experiment yielded a comparable pattern. For *O. basilicum* 'Ohře', the average SPAD values under control and severe drought stress treatments ranged from 29.0 to 40.3. In the case of *O. americanum* plants, the range was between 40.6 and 50.6 (see Table 14). Moreover, the SPAD values varied depending on the year of production, basil species, and the growing conditions, whether it was conducted in the greenhouse or the plant growth chamber.

Table 14. Effect of water supply on relative water content and chlorophyll content of *Ocimum* species (plant growth chamber)

	SWC	Relative water content (%)		Chlorophyll content (SPAD value)	
Species	(%)	2021	2022	2021	2022
O. basilicum	70	94.6 ± 2.0^{Aa}	87.6 ± 0.8^{Ba}	24.6 ± 2.7^{Bc}	33.4 ± 2.3^{Bc}
'Ohře'	50	93.1 ± 1.3^{Aa}	78.8 ± 5.0^{Ab}	30.2 ± 2.8^{Bb}	37.2 ± 1.8^{Bb}
	30	78.6 ± 6.9^{Ab}	74.6 ± 2.9^{Ab}	38.0 ± 4.8^{Ba}	42.5 ± 2.4^{Ba}
O. americanum	70	94.6 ± 3.7^{Aa}	92.5 ± 3.7^{Aa}	39.1 ± 2.4^{Ac}	42.2 ± 4.1^{Ab}
	50	93.3 ± 3.8^{Aa}	83.2 ± 1.3^{Ab}	43.8 ± 2.1^{Ab}	47.9 ± 3.8^{Aa}
	30	82.6 ± 3.7^{Ab}	75.8 ± 0.8^{Ac}	50.1 ± 2.5^{Aa}	51.1 ± 3.4^{Aa}

Values are presented as Mean ± SD, SWC: Soil Water Capacity. Different letters are for significantly different groups. Capital letters are used to differentiate between species under fixed drought stress treatment and small letters are used to differentiate drought stress under fixed species.

4.2.3. Water potential

The variation in soil water capacities significantly ($p<0.01$) affected the WP of basil species over consecutive years (as shown in Appendix Table 3). Figure 21 below presents the average values from a multi-year experiment in plant growth chamber, revealing that the WP of *O. basilicum* 'Ohře' decreased from -0.6 MPa under the control treatment to -0.8 MPa under moderate drought stress. Furthermore, under severe drought stress, the WP further declined to -1.4 MPa. Similar trends were observed in *O. americanum* plants, with their WP ranging from -0.8 to -0.5 MPa under control and drought stress conditions, respectively.

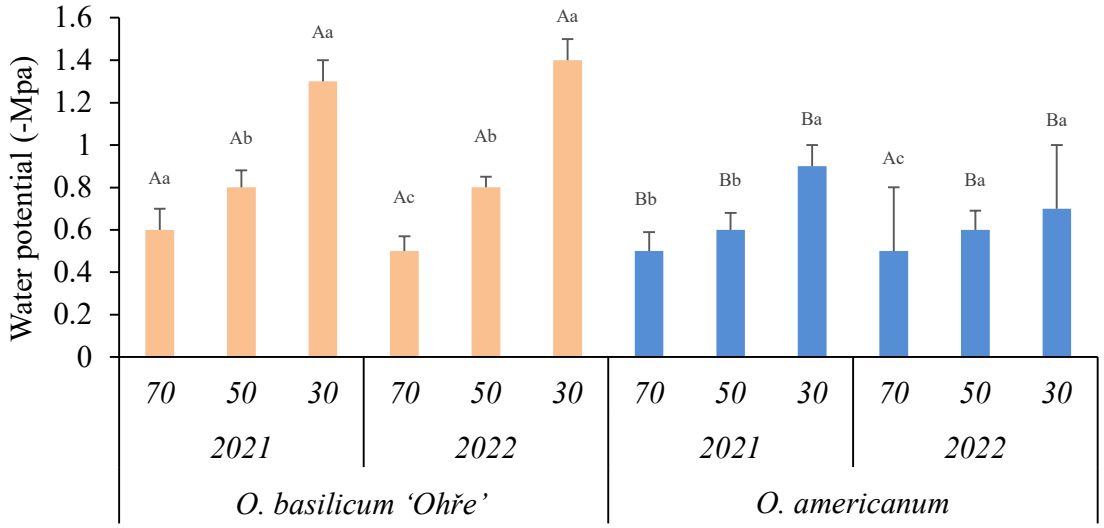

Figure 21. Effect of water supply on shoot water potential (plant growth chamber)

Values are presented as Mean ± SD, SWC: Soil Water Capacity. Different letters are for significantly different groups. Capital letters are used to differentiate between species under fixed drought stress treatment and small letters are used to differentiate drought stress under fixed species.

4.2.4. Water use efficiency

The soil water capacities significantly affected the WUE of *O. basilicum* 'Ohře' and *O. americanum* during both production years as indicated in Figure 22. Hence, as the soil moisture gets drier the WUE gets higher under both species. The WUE of severe drought stress plants improved by 1.8-fold and 3.4-fold in *O. basilicum* 'Ohře' and *O. americanum*, respectively.

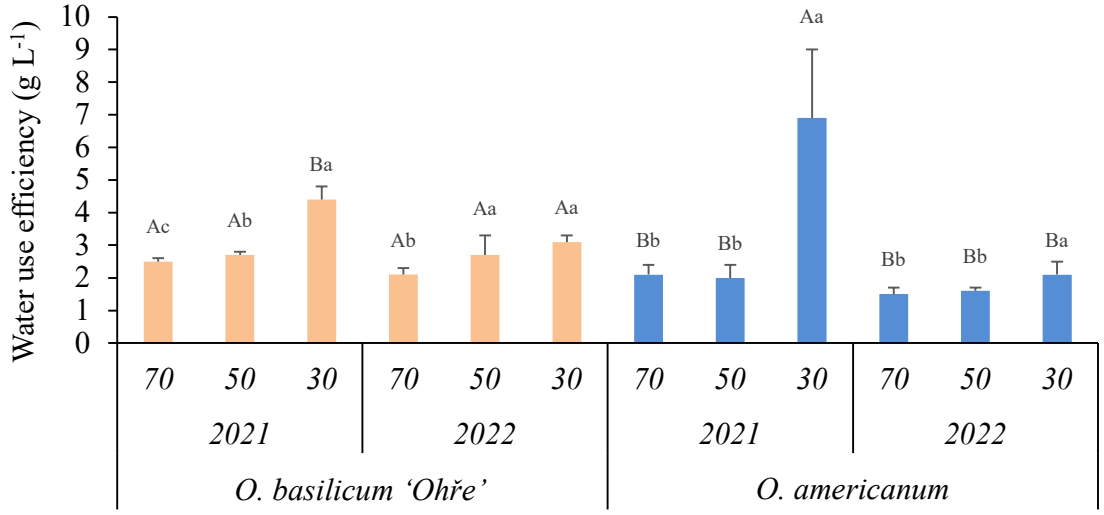

Figure 22. Effect of water supply on shoot water use efficiency (plant growth chamber)

Values are presented as Mean ± SD, SWC: Soil Water Capacity. Different letters are for significantly different groups. Capital letters are used to differentiate between species under fixed drought stress treatment and small letters are used to differentiate drought stress under fixed species.

4.3. Drought-induced morphological changes among *Ocimum* species

Plant growth is adversely affected by water stress, a phenomenon that has been extensively studied and well documented. In these experiments, it was demonstrated that basil species suffered a significant reduction in growth and productivity because of drought. This subsection provides a detailed account of drought-induced morphological changes in various *Ocimum* species grown under diverse microclimates (open field, greenhouse, and plant growth chamber).

4.3.1. Plant height and canopy diameter

The quantity of water present in the soil is a critical factor that influences basil growth, and even slight changes in the soil's moisture content had an impact on the plant's height and spread as demonstrated by open field, greenhouse, and plant growth chamber experiments. Besides soil water capacity, year of production and basil species investigated also had a significant ($p<0.01$) effect (Appendix Tables 1, 2 and 3). Hence, based on the pooled mean of the years, basil plants grown in open fields without irrigation (i.e., under drought conditions) experienced a reduction in plant height ranging from 3.4 cm (8.9%) to 7.8 cm (16.0%) in *O. americanum* and *O.* × *africanum*, respectively (Table 15). Additionally, the reduction in canopy diameter ranged from 5.6 cm (11.4% in *O. basilicum* 'Genovese') to 9.5 cm (20.7% in *O.* × *africanum*).

Table 15. Effect of irrigation on plant height and canopy diameter of *Ocimum* species in open field growing conditions from 2020 to 2022

Species	TRT	Plant height (cm)			Canopy diameter (cm)		
		2020	2021	2022	2020	2021	2022
O. basilicum 'Ohře'	I	46.9 ± 6.3^{Acd}	43.8 ± 2.5^{Aab}	50.3 ± 3.8^{Aa}	51.8 ± 7.2^{Ab}	$39.3\pm2.^{5Ab}$	50.2 ± 4.4^{a}
	NI	43.4 ± 6.7^{Ac}	40.3 ± 4.2^{Ba}	41.7 ± 4.0^{Ba}	47.6 ± 10.6^{Aab}	34.4 ± 4.4^{Ba}	42.0 ± 3.8^{a}
O. basilicum 'Genovese'	I	77.3 ± 5.5^{Aa}	47.3 ± 4.9^{Aa}	53.3 ± 4.0^{a}	62.3 ± 6.6^{Aa}	38.7 ± 4.7^{Ab}	47.7 ± 3.7^{ab}
	NI	68.5 ± 8.5^{Aa}	40.8 ± 3.6^{Ba}	43.7 ± 2.5^{a}	57.8 ± 6.0^{Aa}	34.6 ± 4.5^{Ba}	39.3 ± 5.4^{a}
O. × *africanum*	I	50.9 ± 4.2^{Ac}	44.5 ± 2.2^{Aab}	50.2 ± 1.9^{a}	49.1 ± 6.1^{Abc}	45.7 ± 4.2^{Aa}	43.3 ± 4.0^{b}
	NI	41.3 ± 4.9^{Bc}	39.7 ± 5.6^{Ba}	41.2 ± 1.8^{a}	35.3 ± 9.7^{Bc}	39.9 ± 5.6^{Ba}	34.2 ± 3.7^{b}
O. americanum	I	41.8 ± 3.6^{Ade}	35.7 ± 1.4^{Ac}	38.8 ± 2.1^{c}	42.9 ± 2.6^{Ac}	47.1 ± 4.2^{Aa}	50.1 ± 2.7^{a}
	NI	40.4 ± 4.6^{Ac}	30.2 ± 4.2^{Bb}	35.4 ± 2.9^{b}	41.4 ± 9.1^{Abc}	36.7 ± 7.2^{Ba}	41.4 ± 5.1^{a}
O. sanctum	I	64.3 ± 5.6^{Ab}	42.0 ± 1.9^{Ab}	44.5 ± 2.6^{b}	61.7 ± 6.2^{Aa}	30.9 ± 4.8^{Ac}	33.3 ± 2.6^{c}
	NI	60.2 ± 8.5^{Ab}	39.3 ± 4.9^{Aa}	35.8 ± 2.9^{b}	53.5 ± 4.6^{Ba}	25.7 ± 5.1^{A}	24.2 ± 3.2^{c}
O. selloi	I	36.7 ± 5.3^{Ae}	-	-	41.5 ± 7.7^{Ac}	-	-
	NI	38.2 ± 3.1^{Ac}	-	-	40.3 ± 8.0^{Abc}	-	-

Values are presented as Mean ± SD, TRT: Treatment; I-Irrigated; NI: non-irrigated; Different letters are for significantly different groups. Capital letters to differentiate between drought stress under fixed species and small letters are used to differentiate between species under fixed drought stress.

These studies also revealed that *O. basilicum* 'Genovese' plants under irrigation can grow up to 59.0 cm with a canopy diameter of 50.0 cm, whereas the *O. americanum* plant had a semi-upright growth habit with short height (39.0 cm) but a wider spread growth (47.0 cm). Furthermore, the year of production can also influence plant growth, with varying weather conditions and seasonal changes. Thus, the production year of 2020 had a more robust growth than the other successive years. The significant effect of drought stress on three basil species was further demonstrated under a greenhouse experiment conducted over three consecutive years as indicated in table 16. Across all basil species and years, well-watered control plants grew taller and wider. Thus, compared with the control, plant height reduction in severely stressed basil plants ranges between 24.0% (*O. basilicum* 'Genovese') to 29.0% (*O.* × *africanum*). In moderately stress treatments, plants tend to be shorter between 12.1% in *O. americanum* to 20.8% in *O.* × *africanum*. Similarly, the average of the species over the years indicated that under moderate and severe drought stress conditions, basil plants lose more than 20.0% and 45.0% of their canopy, respectively. It is also important to note that plant height and canopy diameter is influenced by the years of production in addition to drought treatment and varietal differences.

Table 16. Effect of water supply on plant height and canopy diameter of *Ocimum* species under greenhouse

Species	SWC (%)	Plant height (cm)			Canopy diameter (cm)		
		2020	2021	2022	2020	2021	2022
O. basilicum 'Genovese'	70	60.3 ± 6.3^{Aa}	55.4 ± 6.6^{Aa}	49.4 ± 6.7^{Ba}	45.9 ± 4.1^{Ba}	40.5 ± 3.2^{Ba}	35.6 ± 6.3^{Ba}
	50	60.0 ± 7.5^{Aa}	47.7 ± 6.2^{Ab}	37.6 ± 5.2^{Bb}	42.5 ± 4.9^{Aba}	34.4 ± 2.2^{Ab}	26.0 ± 5.8^{Bb}
	30	55.5 ± 4.4^{Ab}	42.4 ± 5.9^{Ab}	29.4 ± 3.5^{Bc}	35.9 ± 2.6^{Ab}	26.3 ± 3.1^{Ac}	15.5 ± 3.5^{Bc}
O. × *africanum*	70	52.5 ± 4.7^{Ba}	45.5 ± 4.1^{Ba}	63.2 ± 5.5^{Aa}	45.5 ± 3.1^{Ba}	40.1 ± 1.8^{Ba}	53.1 ± 3.7^{Aa}
	50	47.8 ± 2.9^{Bb}	37.2 ± 2.4^{Bb}	41.0 ± 5.7^{Ab}	40.3 ± 4.0^{Bb}	28.9 ± 3.3^{Bb}	23.6 ± 3.2^{Bb}
	30	45.4 ± 2.7^{Bb}	32.6 ± 4.6^{Bc}	35.3 ± 2.5^{Ac}	34.4 ± 2.3^{Ac}	18.6 ± 1.4^{Bc}	16.6 ± 2.5^{Bc}
O. americanum	70	51.1 ± 2.3^{Ba}	39.6 ± 1.6^{Ca}	39.4 ± 5.9^{Ca}	49.7 ± 2.8^{Aa}	48.6 ± 2.1^{Aa}	47.7 ± 7.5^{Aa}
	50	47.5 ± 3.7^{Bb}	33.2 ± 1.3^{Bb}	34.6 ± 3.5^{Ba}	46.8 ± 4.3^{Aa}	35.5 ± 3.4^{Ab}	38.5 ± 5.3^{Ab}
	30	41.0 ± 2.1^{Cc}	25.5 ± 2.4^{Cc}	28.7 ± 3.4^{Bb}	36.0 ± 3.5^{Ab}	23.7 ± 3.5^{Ac}	25.5 ± 4.0^{Ac}

Values are presented as Mean ± SD, SWC: Soil Water Capacity. Different letters are for significantly different groups. Capital letters are used to differentiate between species under fixed drought stress treatment and small letters are used to differentiate drought stress under fixed species.

Table 17 shows that a controlled trial under a plant growth chamber also demonstrated the negative effect of drought on the plant height and canopy diameter of two basil species. The pooled mean of the years revealed that both species exhibited a 20% reduction in plant height and canopy diameter

when the soil water capacity was low (30% SWC). Additionally, moderate drought stress resulted in a narrow canopy of 4.0% and 8.0% in *O. americanum* and *O. basilicum* 'Ohře', respectively.

Table 17. Effect of water supply on plant height and canopy diameter of *Ocimum* species under plant growth chamber conditions

Species	SWC (%)	Plant height (cm)		Canopy diameter (cm)	
		2021	2022	2021	2022
O. basilicum 'Ohře'	70	54.1±6.3^{Aa}	58.4±3.9^{Aa}	27.2±3.4^{Ba}	22.4±3.6^{Aa}
	50	50.5±3.4^{Aa}	51.0±4.0^{Ab}	24.9±6.8^{Ba}	20.6±1.4^{Bab}
	30	41.5±3.2^{Ab}	42.9±2.3^{Ac}	23.2±3.3^{Ba}	17.7±1.8^{Ac}
O. americanum	70	35.5±4.4^{Ba}	34.7±5.2^{Ba}	41.6±4.2^{Aa}	23.4±3.7^{Aa}
	50	31.1±5.3^{Bab}	32.5±2.8^{Bab}	35.9±3.3^{Aab}	24.9±2.2^{Aa}
	30	27.7±3.8^{Bb}	29.2±1.7^{Bb}	30.1±5.8^{Ab}	16.2±3.1^{Ab}

Values are presented as Mean ± SD, SWC: Soil Water Capacity. Different letters are for significantly different groups. Capital letters are used to differentiate between species under fixed drought stress treatment and small letters are used to differentiate drought stress under fixed species.

4.3.2. Leaf area and stem diameter

Changes in the soil water regime significantly influenced the leaf area and stem diameter of basil species in both greenhouse and plant growth chamber experiments (Appendix Tables 2 and 3). Accordingly, Table 18 and Figure 23 indicate that high soil water capacity had a positive effect on leaf area and stem diameter under the greenhouse. These experiments demonstrated that basil species experienced a significant decrease in leaf area and stem diameter due to drought stress. Over three years of production, the reduction in leaf area was more than 50.0%, and stem diameter decreased by 40.0% in severe drought conditions. Moderate drought stress also caused a reduction of over 20.0% in both leaf area and stem diameter, compared to well-watered plants.

Table 18. Effect of water supply on leaf area and stem diameter of *Ocimum* species under greenhouse growing condition in 2020 to 2022

Species	SWC (%)	Leaf area (cm^2 $leaf^{-1}$)			Stem diameter (cm)	
		2020	2021	2022	2021	2022
O. basilicum 'Genovese'	70	45.1±12.6^{Aa}	25.4±5.5^{Aa}	22.0±4.5^{Aa}	8.6±0.7^{Aa}	7.9±1.6^{Aa}
	50	38.3±11.4^{Aa}	23.1±5.4^{Aa}	14.1±3.1^{Ab}	6.4±0.5^{Ab}	5.4±0.5^{Ab}
	30	22.3±5.8^{Ab}	13.6±7.8^{Ab}	8.6±2.4^{Ac}	5.0±0.5^{Ac}	4.6±0.5^{Ab}
O. × *africanum*	70	10.6±2.0^{Ba}	8.6±0.9^{Ba}	15.0±7.2^{Ba}	8.6±1.1^{Aa}	7.8±1.1^{Aa}
	50	8.2±2.3^{Bb}	5.7±0.8^{Bb}	12.9±2.9^{Aa}	6.8±0.7^{Ab}	5.9±0.5^{Ab}
	30	5.5±1.5^{Bc}	3.3±0.8^{Bc}	6.1±2.0^{Bb}	5.2±0.5^{Ac}	4.4±0.9^{Ac}
O. americanum	70	11.8±3.5^{Ba}	8.1±1.3^{Ba}	7.3±2.1^{Ca}	9.2±1.0^{Aa}	5.2±0.9^{Ba}
	50	8.8±2.4^{Bb}	5.6±1.2^{Bb}	4.4±1.0^{Bb}	5.5±0.7^{Bb}	4.3±0.9^{Ba}
	30	5.5±1.3^{Bc}	3.4±1.0^{Bc}	2.6±1.0^{Cb}	4.4±0.4^{Bc}	2.6±1.0^{Bb}

Values are presented as Mean ± SD, SWC: Soil Water Capacity. Different letters are for significantly different groups. Capital letters are used to differentiate between species under fixed drought stress treatment and small letters are used to differentiate drought stress under fixed species.

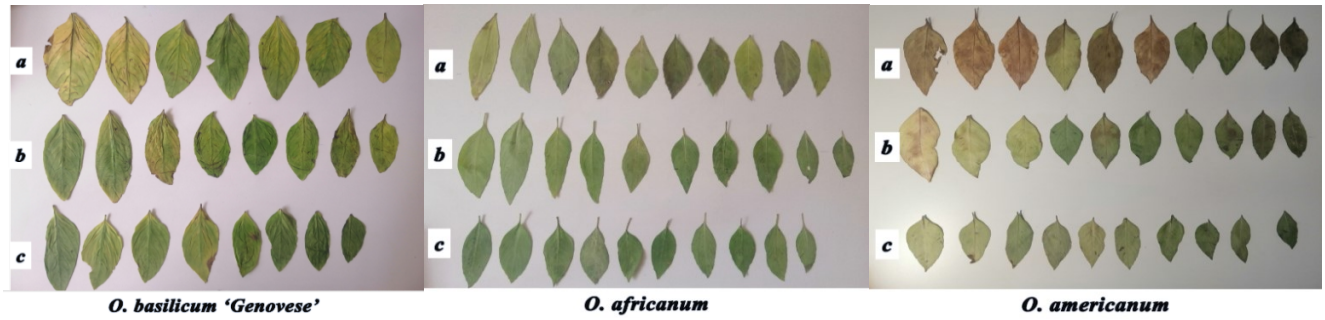

Figure 23. Effect of drought stress on leaf growth of *Ocimum* species. a: 70%; b:50%; c:30% SWC (Photo: Mulugeta, 2020)

Similar to the greenhouse experiment, the growth chamber experiment with basil plants also revealed that as the soil water content declined (as indicated in Table 19), the leaves of the plants became smaller, and the stems became thinner. In particular, *O. basilicum* 'Ohře' plants cultivated with lower moisture levels (30% SWC) experienced a significant loss of more than 40.0% of their leaf area. The reduction was even more pronounced in *O. americanum* plants, exceeding 50.0%. Additionally, both species of *Ocimum* exhibited a reduction of 36.0% in leaf area under moderate stress conditions. During severe drought conditions, both species showed a 20.0% reduction in stem diameter, according to the aggregate mean. In contrast, under moderate stress, both basil species showed a reduction of over 12.0% in stem diameter.

Table 19. Effect of water supply on plant height and canopy diameter of *Ocimum* species under plant growth chamber conditions

Species	SWC (%)	Leaf area (cm^2 $leaf^{-1}$)		Stem diameter (cm)	
		2021	2022	2021	2022
O. basilicum 'Ohře'	70	9.6 ± 2.8^{Aa}	16.4 ± 2.7^{Aa}	5.1 ± 0.1^{Aa}	4.5 ± 0.2^{Aa}
	50	7.2 ± 1.5^{Ab}	7.8 ± 1.1^{Ab}	4.4 ± 0.3^{Ab}	4.0 ± 0.2^{Ab}
	30	5.2 ± 1.3^{Ac}	7.3 ± 1.3^{Ab}	3.7 ± 0.3^{Ac}	3.1 ± 0.2^{Ac}
O. americanum	70	8.6 ± 2.3^{Ba}	11.2 ± 1.5^{Ba}	4.1 ± 0.5^{Ba}	2.9 ± 0.5^{Ba}
	50	4.9 ± 1.8^{Bb}	7.9 ± 1.1^{Ab}	3.3 ± 0.3^{Bab}	2.7 ± 0.3^{Bab}
	30	3.6 ± 1.2^{Bb}	4.3 ± 1.3^{Bc}	2.9 ± 0.2^{Bb}	2.3 ± 0.1^{Bb}

Values are presented as Mean ± SD, SWC: Soil Water Capacity. Different letters are for significantly different groups in columns. Capital letters are used to differentiate between species under fixed drought stress treatment and small letters are used to differentiate drought stress under fixed species.

4.3.3. Fresh and dry herb yield

Multiple experiments in open fields, greenhouse, and plant growth chambers showed that basil herb production (fresh and dry) was significantly influenced by soil moisture level as shown in Appendix Tables 1, 2, and 3. In addition to soil moisture levels, the type of basil species being grown and the

year of production both greatly influence herb production. Regardless of the basil species or the production year, it has been observed that lower soil water capacity leads to herb production reduction. The specific responses of each basil species to drought conditions, depending on the respective growing conditions, are thoroughly discussed below. In the open-field experiment, a notable reduction in herb yield was observed due to the absence of irrigation (Table 20).

Table 20. Effect of irrigation on herb production among *Ocimum* species in 2020 to 2022 (open field)

Species	TRT	Fresh herb yield (g plant^{-1})			Dry herb yield (g plant^{-1})		
		2020	2021	2022	2020	2021	2022
O. basilicum 'Ohře'	I	389.6±155.7^{Ab}	180.1±28.4^{A}	379.7±97.7^{Aa}	63.7±24.2^{Ab}	38.4±6.5^{A}	74.4±16.7^{Aa}
	NI	284.7±133.6^{Bb}	142.4±43.9^{B}	221.22±6^{Ba}	54.2±24.3^{Ab}	32.1±7.2^{B}	39.1±10.5^{Ba}
O. basilicum 'Genovese'	I	616.3±52.0^{Aa}	157.8±31.9^{Aa}	273.8±53.0^{Aab}	115.8±12.0^{Aa}	34.7±6.4^{A}	51.0±10.2^{Ab}
	NI	474.3±77.9^{Ba}	118.0±26.1^{B}	192.2±64.5^{Bb}	92.8±15.4^{Ba}	26.9±5.2^{B}	32.9±9.8^{Bab}
O. × *africanum*	I	268.3±118.9^{Abc}	142.7±57.5^{Aa}	269.0±37.4^{Ab}	50.6±19.0^{Abc}	42.2±19.0^{A}	67.7±7.5^{Aa}
	NI	126.1±83.4^{Bd}	108.1±79.2^{B}	164.0±47.8^{Babc}	26.6±15.4^{Bc}	32.2±17.4^{B}	38.0±10.8^{Ba}
O. americanum	I	230.2±56.8^{Acd}	146.9±30.1^{Aa}	214.8±51.7^{Ab}	38.7±13.2^{Ac}	45.9±8.5^{A}	43.3±9.9^{Abc}
	NI	165.7±49.5^{Bcd}	97.7±65.8^{B}	152.7±33.9^{Bbc}	27.7±9.3^{Bc}	30.7±16.5^{B}	35.4±5.8^{Bab}
O. sanctum	I	281.0±71.7^{Abc}	85.0±30.3^{Ab}	134.8±11.6^{Ac}	50.0±10.5^{Abc}	32.4±11.3^{A}	32.0±2.9^{Ac}
	NI	250.3±99.8^{Abc}	82.9±28.8^{A}	111.2±14.7^{Bc}	45.5±15.1^{Abc}	30.6±9.9^{A}	24.3±5.7^{Bb}
O. selloi	I	114.7±44.5^{Ad}	-	-	29.2±6.4^{Ac}	-	-
	NI	112.0±38.8^{Ad}	-	-	25.3±6.3^{Bc}	-	-

Values are presented as Mean ± SD, TRT: Treatment; I-Irrigated; NI: non-irrigated (control); Different letters are for significantly different groups. Capital letters to differentiate between drought stress under fixed species and small letters are used to differentiate between species under fixed drought stress.

The reduction in fresh herb yield ranges between 2.7 g plant^{-1} (2.3%) for *O. selloi* to 100.0 g plant $^{-1}$ (39.0%) for *O.* × *africanum.* Even though the impact of drought is dependent upon several factors, such as its intensity, duration, soil conditions, species, and others, the drier the soil is, the lower the biomass yield. The basil species showed considerable diversity in biomass, alongside the influence of drought. Irrespective of the soil moisture level, the sweet basil cultivars, specifically 'Genovese' and 'Ohře', produced the highest fresh and dried herb yield. The pooled mean of the years showed that 'Genovese' plants cultivated with irrigation produced an average fresh herb yield of 349.3 g plant^{-1}. This was followed by the cultivar 'Ohře', which yielded 316.0 g of fresh herb per plant. However, *Ocimum selloi* plants had a slow growth and their productivity was relatively low compared to the other species. These plants generated less than 115 g of fresh herb yield per plant. Furthermore, the year 2020 showed a higher biomass yield, presumably resulting from weather

fluctuations and variability in soil properties as indicated in Tables 2 and 3 under the material and method section.

Basil's sensitivity to moisture stress was also evident in the greenhouse trial, in which both moderate and severe drought stresses resulted in significant biomass losses as illustrated in Table 21 and Figure 24. When the soil moisture content was much lower (30% SWC), the fresh herb yield loss ranged from 65.0% for *O. basilicum* 'Genovese' to 73.0% for *O.* × *africanum* plants, as indicated by the pooled mean of the years. Under moderate drought stress, the fresh herb yield loss varied between 38.7% to 52.2% for *O. basilicum* 'Genovese' and *O.* × *africanum* plants, respectively. Similarly, each basil species experienced a 45.0% to 70.0% reduction in dry herb yield when exposed to moderate and severe drought stresses, respectively. Furthermore, each basil species responded differently to fluctuations in soil moisture, with *O.* × *africanum* plants being highly influenced by moderate and severe drought stress. Additionally, the production of fresh and dry biomass was influenced by the year of production due to weather fluctuations, as observed in Tables 2 and 3. The maximum biomass was obtained in the first year (2020), while the third year resulted in the lowest biomass production across all species.

Table 21. Effect of water supply on herb production of *Ocimum* species under greenhouse pot experiment

Species	SWC (%)	Fresh herb weight (g plant^{-1})			Dry herb weight (g plant^{-1})		
		2020	2021	2022	2020	2021	2022
O. basilicum 'Genovese'	70	344.7±44.3^{Aa}	211.9±24.3^{ABa}	161.0±37.4^{Ba}	75.3±4.5^{Aa}	63.5±6.4^{Aa}	74.1±8.2^{Aa}
	50	279.0±9.9^{Ab}	144.2±17.0^{Ab}	55.7±10.9^{Ab}	58.0±3.8^{Ab}	30.4±3.1^{Ab}	16.5±5.8^{Ab}
	30	169.9±19.5^{Ac}	78.1±9.5^{Ac}	29.5±2.3^{Ab}	34.9±1.7^{Ac}	13.9±1.5^{Ac}	7.5±0.8^{Ac}
O. × *africanum*	70	212.3±41.0^{Ca}	193.0±16.8^{Ba}	220.7±31.5^{Aa}	49.7±3.3^{Ca}	35.9±4.0^{Ca}	69.0±5.8^{Aa}
	50	134.8±30.8^{Cb}	101.6±8.4^{Bb}	60.2±8.5^{Ab}	38.1±2.5^{Bb}	21.1±1.7^{Bb}	20.7±4.4^{Ab}
	30	93.7±14.5^{Bc}	47.5±7.5^{Bc}	29.5±3.3^{Ac}	26.3±1.3^{Bc}	9.9±1.6^{Bc}	7.6±7.6^{Ac}
O. americanum	70	217.4±25.2^{Ba}	228.0±18.7^{Aa}	113.2±13.7^{Ca}	65.2±3.7^{Ba}	46.7±1.5^{Ba}	77.5±6.7^{Aa}
	50	191.2±24.5^{Bb}	11.0±14.7^{Bb}	53.7±5.7^{Ab}	56.7±2.0^{Ab}	29.2±4.1^{Ab}	17.5±2.1^{Ab}
	30	97.5±11.0^{Bc}	42.6±15.7^{Bc}	29.2±3.0^{Ac}	31.1±2.3^{ABc}	10.1±3.6^{Bc}	6.9±1.2^{Ac}

Values are presented as Mean ± SD, SWC: Soil Water Capacity. Different letters are for significantly different groups. Capital letters to differentiate between species under fixed drought stress treatment and small letters are used to differentiate drought stress under fixed species.

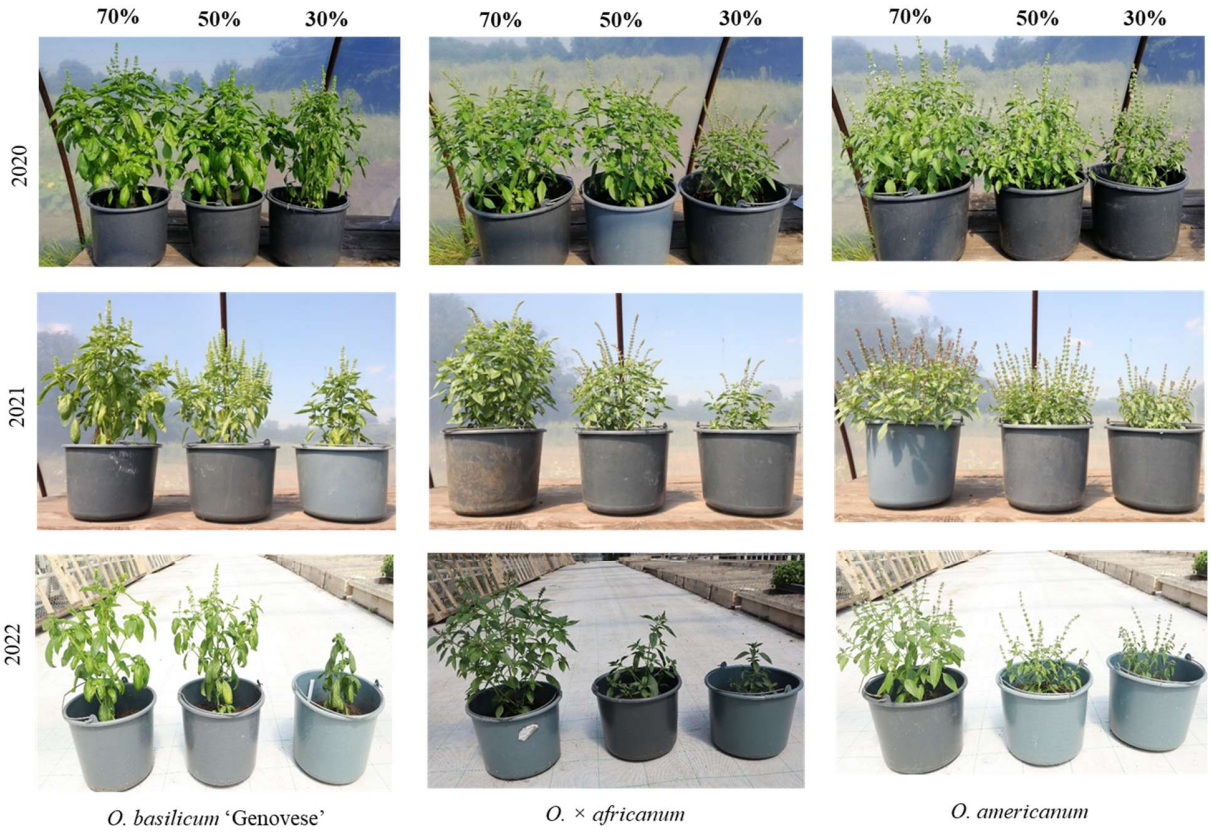

Figure 24. Effect of drought stress on the growth of *Ocimum* species in three consecutive years under greenhouse pot experiment (Photo: Mulugeta, 2020-2022)

Further investigation conducted in the plant growth chamber also revealed a significant ($p<0.01$) variation in biomass production among the different soil moisture levels and the tested basil species (see Appendix Table 3 and Table 22). Among them, *Ocimum basilicum* 'Ohře' plants displayed robust growth and higher biomass production, irrespective of soil water levels. However, when exposed to severe drought stress, *O. basilicum* 'Ohře' plants lost more than 40.0% of their fresh and dry herb weight, while *O. americanum* plants experienced a more substantial reduction of over 50.0%. Despite *O. americanum* plants having a broader canopy, their growth was less sturdy than that of other species. Additionally, biomass production differed between different years, with higher production observed in the first year. This variation in growth among species can be attributed to genetics. In contrast, differences between production years could partly be linked to fluctuations in soil chemical properties, as indicated in Table 3. The soil media mixture in the first year had a higher concentration of macro- and micronutrients.

Table 22. Effect of water supply on biomass production of *Ocimum* species under plant growth chamber pot experiment

Species	SWC (%)	Fresh herb weight (g plant^{-1})		Dry herb weight (g plant^{-1})	
		2021	2022	2021	2022
O. basilicum 'Ohře'	70	34.5±3.2^{Aa}	39.5±2.8^{Aa}	6.2±0.7^{Aa}	6.5±0.9^{Aa}
	50	26.3±2.7^{Ab}	34.0±1.6^{Ab}	4.6±0.5^{Ab}	5.4±0.3^{Ab}
	30	18.5±1.9^{Ac}	24.5±1.7^{Ac}	3.1±0.6^{Ac}	3.0±0.4^{Ac}
O. americanum	70	29.8±4.4^{Ba}	20.8±3.4^{Ba}	5.3±1.7^{Ba}	2.7±0.5^{Ba}
	50	23.3±3.6^{Bb}	11.8±1.4^{Bb}	3.7±0.9^{Bb}	2.1±0.2^{Bb}
	30	15.2±3.4^{Bc}	5.7±1.3^{Bc}	1.7±0.4^{Bc}	1.1±0.2^{Bc}

Values are presented as Mean ± SD, SWC: Soil Water Capacity. Different letters are for significantly different groups. Capital letters to differentiate between species under fixed drought stress treatment and small letters are used to differentiate drought stress under fixed species.

4.3.4. Glandular hair density

The essential oil of the *Ocimum* species is bio-synthesized and stored in a structure called glandular trichomes or essential oil glands found on the leaf, stem, and flower of the *Ocimum* species (Werker *et al*., 1993, Maurya *et al*., 2019). Through experiments conducted in a greenhouse and plant growth chamber, it was found that the density of glandular hairs was significantly influenced by factors such as the amount of water supplied, the species of *Ocimum*, and the year in which the plants were produced as indicated on Appendix Tables 2 and 3. The greenhouse experiment revealed that *Ocimum* plants experienced an increase in glandular hair density (GHD) when subjected to moderate and severe drought treatments (as shown in Figure 25). For *O. basilicum* 'Genovese' and *O. americanum*, the increase in GHD due to severe drought ranged from 32.8% to 107.6%, respectively. In moderate drought, the increase ranged from 23.5% (*O. basilicum* 'Genovese') to 32.0% (*O. americanum*). In each consecutive years, *O.* × *africanum* had the greatest densities of glandular hairs, ranging from 869.4 to 2292.5 units per 100 mm^2, which varied depending on the soil water capacity and year of production. There were substantial variations between the years. Specifically, in the third year under severe drought stress treatment, each plant had the highest GHD.

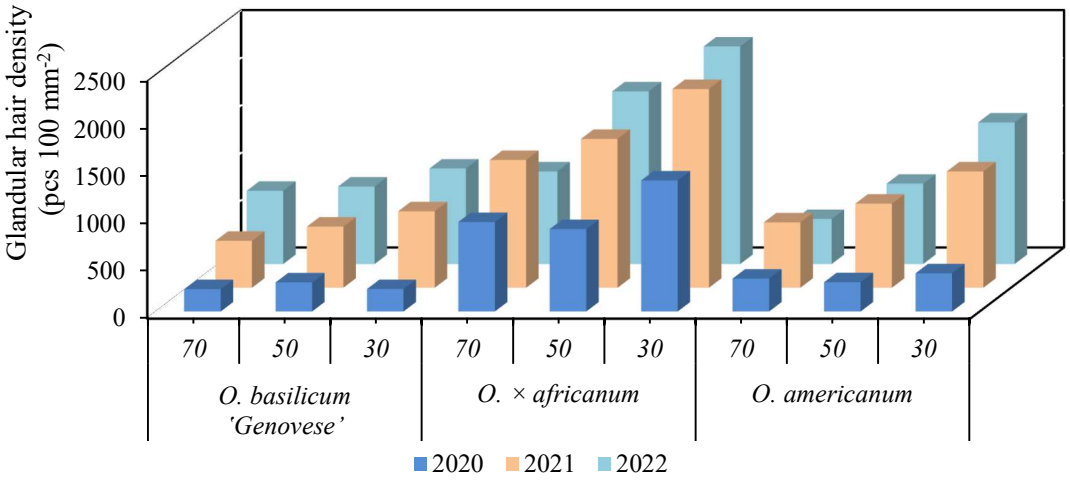

Figure 25. Effect of drought stress on glandular hair density of three *Ocimum* species under greenhouse

Similarly, the same trend was observed in the plant growth chamber experiment as well (Figures 26 and 27). The results showed that water supply significantly affected the glandular hair densities of both *Ocimum* species. It was vividly noted that the intensity of drought enhanced the density of glandular trichomes of *O. basilicum* 'Ohře' and *O. americanum* (second year). In addition to water supply, variations in glandular trichomes were observed between species and the production years. Compared to the control, severe drought stress enhanced glandular hair density in a range of 17.0 to 74.0%. Between the species, *O. basilicum* 'Ohře' had relatively higher glandular hair densities in both years.

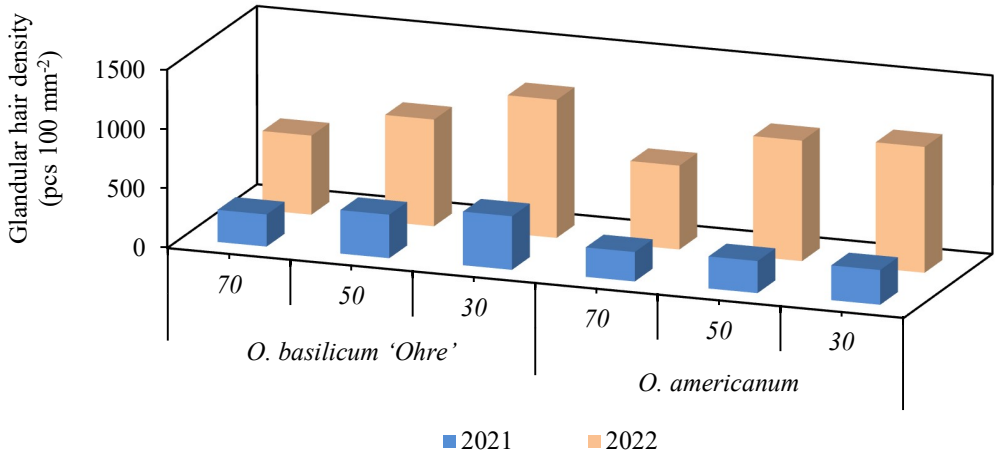

Figure 26. Effect of drought stress on glandular hair density of *O. basilicum* 'Ohře' and *O. americanum* under plant growth chamber

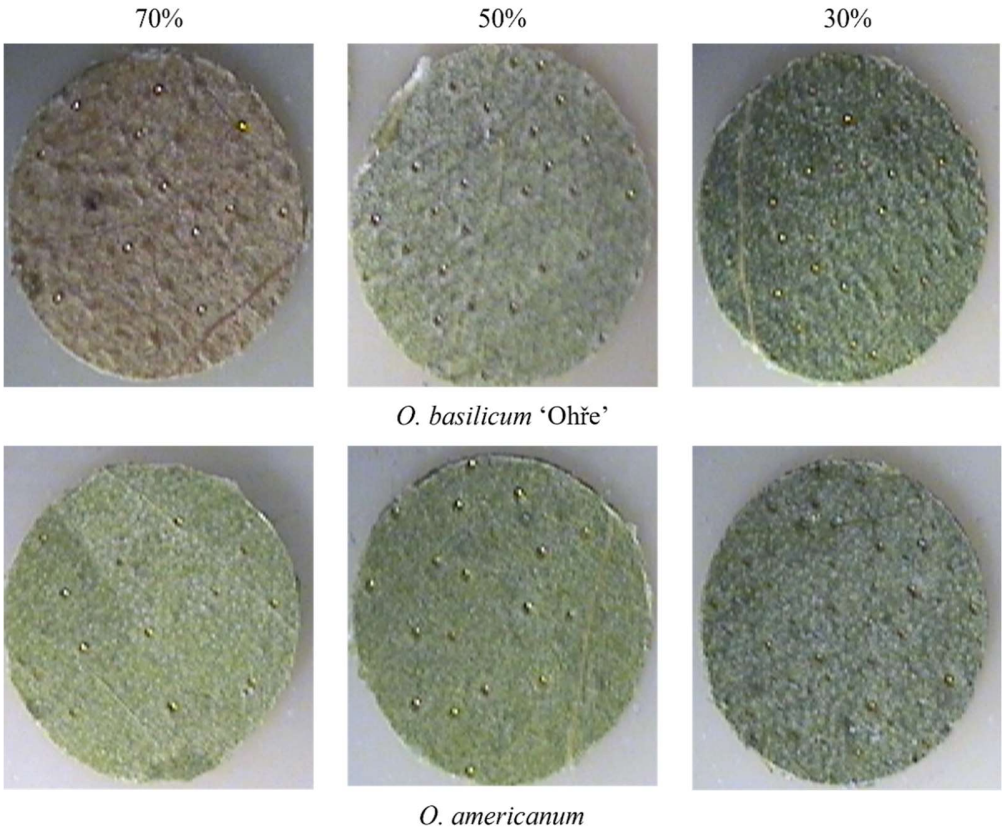

Figure 27. Effect of drought stress on glandular hair density of *Ocimum* species under plant growth chamber in 2021

4.4. Biochemical response of *Ocimum* species under drought

Secondary compounds accumulation in *Ocimum* species are influenced by an intricate interplay of several factors, including genetics, environmental conditions, and processing methods. Among these factors, drought stress has a considerable impact on the physiological and biochemical responses of medicinal plants, leading to changes in their secondary metabolism and hindering overall plant growth. To gain a deeper understanding of drought-induced biochemical changes among selected *Ocimum* species, a series of multi-year experiments were conducted across different microclimates. The following sub-section discusses the outcomes and findings of these extensive experiments.

4.4.1. Essential oil content and essential oil yield

Studies across different microclimates (open field, greenhouse, and plant growth chamber) demonstrated water supply, the type of basil species and production year significantly affect the essential oil content (EOC) and yield (EOY) as presented in Appendix Table 1 to 3.

Hence, a three-year open field experiment showed drought stress-induced changes on EOC are species-dependent (Table 23). Consequently, drought stress led to a notable 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 across each growing year. Whereas EOY declined under drought treatment depending on the years of production and the basil species. In 2021, the drought stress adversely impacted essential oil production in *O. sanctum*, while in 2022, *O. basilicum* 'Ohře' and *O.* × *africanum* were negatively affected by the dry soil, leading to reduced yields. Besides drought, the *Ocimum* genus is well-known for its extensive chemical diversity, including essential oil. A comprehensive analysis of basil species revealed a significant variation in essential oil production. Based on the average of a three-year study, *O.* × *africanum* grown under irrigation conditions demonstrated exceptional EOC (3.2%) and yield (1.8 mL plant^{-1}) compared to the other studied species. The sweet basil cultivar 'Ohře' also showed high EOC and EOY, while the remaining species produced less than 1.0% of EOC and 1.0 mL of EOY per plant. The result also showed that in the year 2020, there was a noticeable increase in EOC and EOY than the other years due to the fluctuation in weather conditions and soil properties (Tables 2 and 3).

Table 23. Effect of irrigation on essential oil production of *Ocimum* species in open field conditions in 2020 to 2022

Species	TRT	Essential oil content (%)			Essential oil yield (mL plant^{-1})		
		2020	2021	2022	2020	2021	2022
O. basilicum 'Ohře'	I	2.1±0.4^{Ab}	1.3±0.2^{Ab}	1.2±0.1^{Ab}	1.3±0.5^{Aa}	0.5±0.1^{Ab}	0.9±0.1^{Ab}
	NI	2.0±0.2^{Ac}	1.3±0.3^{Ab}	1.2±0.3^{Ab}	0.8±0.3^{Aab}	0.4±0.1^{Ab}	0.5±0.1^{Bb}
O. basilicum 'Genovese'	I	0.7±0.1^{Bd}	0.8±0.1^{Ac}	0.5±0.2^{Bc}	0.7±0.1^{Ab}	0.3±0.0^{Ab}	0.3±0.1^{Ac}
	NI	0.8±0.1^{Ae}	0.9±0.2^{Ab}	0.7±0.0^{Abc}	0.7±0.2^{Ab}	0.3±0.1^{Ab}	0.2±0.1^{Abc}
O. × *africanum*	I	3.7±0.2^{Ba}	3.2±0.5^{Ba}	2.8±0.0^{Ba}	1.3±0.4^{Aa}	1.9±0.8^{Aa}	2.0±0.11^{Aa}
	NI	4.1±0.3^{Aa}	3.9±0.1^{Aa}	2.9±0.3^{Aa}	1.5±0.9^{Aa}	1.6±0.3^{Aa}	1.1±0.2^{Ba}
O. americanum	I	1.1±0.1^{Ac}	0.7±0.1^{Ac}	0.7±0.2^{Ac}	0.4±0.2^{Ab}	0.3±0.0^{Ab}	0.3±0.1^{Ac}
	NI	1.1±0.1^{Ad}	0.9±0.3^{Ab}	0.7±0.1^{Ac}	0.4±0.1^{Ab}	0.3±0.1^{Ab}	0.2±0.0^{Abc}
O. sanctum	I	0.8±0.0^{Acd}	1.0±0.1^{Abc}	0.6±0.0^{Ac}	0.4±0.1^{Ab}	0.4±0.1^{Ab}	0.2±0.0^{Ac}
	NI	0.9±0.0^{Ae}	1.0±0.0^{Ab}	0.7±0.0^{Abc}	0.4±0.1^{Ab}	0.3±0.0^{Bb}	0.2±0.0^{Ac}
O. selloi	I	1.9±0.3^{Bb}	-	-	0.5±0.1^{Ab}	-	-
	NI	2.6±0.1^{Ab}	-	-	0.6±0.2^{Ab}	-	-

Values are presented as Mean ± SD, TRT: Treatment; I-Irrigated; NI: non-irrigated (Control); Different letters are for significantly different groups. Capital letters to differentiate between drought stress under fixed species and small letters are used to differentiate between species under fixed drought stress.

In the greenhouse experiments, the effect of drought stress on the EOC was observed to be dependent on the intensity of the drought, the tested basil species, and the year in which they were cultivated (Table 24). Consequently, compared to the control severe drought stress increased (>25.0%) the EOC of *O.* × *africanum* in 2021 and 2022. However, it reduced *O. americanum*'s EOC by more than 15.0% in both 2020 and 2021. In addition, moderate drought stress negatively affected EOC for 'Genovese' in 2020 and *O. americanum* in 2022. No significant changes were observed in the remaining treatments. However, both moderate and severe drought stresses had a negative effect on the EOY of all basil species in each consecutive year. The yield reduction of essential oils under severe drought stress conditions ranges from 74.0% (*O.* × *africanum*) to 84.0% (*O. americanum*). Whereas a moderate drought resulted in a 50.0% decline in *O.* × *africanum* and 62% in *O. basilicum* 'Genovese'. When the rate of decrease in biomass versus the rate of increase in EOC is compared, drought stress-connected genuine increase of EO production is detected contrary to the unsatisfactory result for the practical aspects of cultivation (e.g., lower drug and EO yields).

Table 24. Effect of water supply on essential oil content and essential oil yield of *Ocimum* species under greenhouse pot experiment

Species	SWC (%)	Essential oil content (%)			Essential oil yield (mL plant^{-1})		
		2020	2021	2022	2020	2021	2022
O. basilicum 'Genovese'	70	1.2±0.5^{Ba}	0.6±0.1^{Ca}	0.5±0.0^{Ba}	0.8±0.3^{Ba}	0.3±0.0^{Ca}	0.4±0.0^{Ba}
	50	0.6±0.1^{Bb}	0.6±0.1^{Ca}	0.6±0.1^{Ba}	0.3±0.1^{Bb}	0.2±0.0^{Cb}	0.1±0.0^{Bb}
	30	0.7±0.3^{Bab}	0.7±0.1^{Ba}	0.7±0.1^{Ba}	0.2±0.0^{Bb}	0.1±0.0^{Bc}	0.1±0.0^{Bb}
O. × *africanum*	70	2.8±0.6^{Aa}	2.8±0.3^{Aa}	3.0±0.1^{Ab}	1.3±0.3^{Aa}	0.9±0.2^{Aa}	2.0±0.1^{Aa}
	50	2.7±0.2^{Aa}	3.3±0.3^{Aa}	3.1±0.1^{Ab}	1.0±0.2^{Ab}	0.6±0.0^{Ab}	0.6±0.1^{Ab}
	30	2.7±0.1^{Aa}	3.7±0.8^{Aa}	3.8±0.3^{Aa}	0.7±0.1^{Ac}	0.4±0.0^{Ab}	0.3±0.0^{Ac}
O. americanum	70	0.7±0.1^{Ba}	1.5±0.1^{Ba}	0.6±0.1^{Ba}	0.4±0.1^{Ca}	0.6±0.0^{Ba}	0.4±0.0^{Ba}
	50	0.8±0.1^{Ba}	1.6±0.3^{Ba}	0.5±0.0^{Bb}	0.4±0.1^{Ba}	0.4±0.1^{Bb}	0.1±0.0^{Bb}
	30	0.5±0.1^{Cb}	1.3±0.2^{Bb}	0.6±0.0^{Ba}	0.2±0.0^{Cb}	0.1±0.0^{Bc}	0.1±0.0^{Bb}

Values are presented as Mean ± SD, SWC: Soil Water Capacity. Different letters are for significantly different groups. Capital letters to differentiate between species under fixed drought stress treatment and small letters are used to differentiate drought stress under fixed species.

Furthermore, the water supply also significantly affected the EOC and EOY of basil species tested under the plant growth chamber (Table 25). Both species had a slight rise in EOC under drought stress. However, the loss of EOY was much greater than the minor increase in EOC under moderate and severe drought stresses. Accordingly, the EOC rise ranges from 6.0% (moderate drought stress) to over 10.0% (severe drought stress). When drought conditions were severe, basil plants suffered a

45.0% EOY loss. Under moderate drought stress, EOY loss ranged from 10.0% for *O. americanum* to 22.5% for *O. basilicum* 'Ohře'.

Table 25. Effect of water supply on essential oil-related parameters of *Ocimum* species under plant growth chamber condition

Species	SWC (%)	Essential oil content (%)		Essential oil yield (mL plant^{-1})	
		2021	2022	2021	2022
O. basilicum 'Ohře'	70	1.92±0.00^{Ab}	1.37±0.12^{Aa}	0.12±0.01^{Aa}	0.10±0.01^{Aa}
	50	2.03±0.10^{Aab}	1.45±0.06^{Aa}	0.09±0.01^{Ab}	0.08±0.01^{Ab}
	30	2.10±0.10^{Aa}	1.51±0.16^{Aa}	0.07±0.01^{Ab}	0.05±0.01^{Ac}
O. americanum	70	0.89±0.11^{Ba}	0.70±0.03^{Bb}	0.05±0.00^{Ba}	0.02±0.01^{Ba}
	50	0.89±0.16^{Ba}	0.80±0.06^{Bb}	0.04±0.01^{Bb}	0.02±0.00^{Bab}
	30	1.02±0.06^{Ba}	0.93±0.04^{Ba}	0.03±0.01^{Bb}	0.01±0.01^{Bb}

Values are presented as Mean ± SD, SWC: Soil Water Capacity. Different letters are for significantly different groups in columns. Capital letters to differentiate between species under fixed drought stress treatment and small letters are used to differentiate drought stress under fixed species.

4.4.2. Essential oil composition

An extensive investigation spanning multiple years was undertaken to explore the impact of drought stress on the essential oil compounds of different *Ocimum* species. This comprehensive investigation was conducted under a range of environmental conditions, including open field, greenhouse, and plant growth chambers. Despite variations in the proportions of the main compounds observed among *Ocimum* species, the production year, the cultivation environment, and the severity of drought, the findings of this in-depth study highlighted a significant diversity in the essential oil compositions of the examined basil species. Detailed results and analyses are provided in Tables 26 to 32.

Tables 27 and 28 specifically illustrate the influence of drought stress on essential oil composition for five *Ocimum* species cultivated in an open field setting. Notably, the investigated *Ocimum* species generally had more than 40 compounds, with the exception of *O. selloi* and *O. sanctum*, which had fewer than 25 identified compounds in total. For the better transparency the components under 1 area percentage were merged under the "others <1%" category. Among *O. basilicum* cultivars, namely 'Ohře' and 'Genovese', linalool emerged as the major compound, constituting over 40.0% of the essential oil composition. Additionally, *O. basilicum* 'Ohře' plants exhibited higher ratios of geraniol (>10.0%) alongside linalool. In contrast, *O.* × *africanum* displayed limonene (13.3 – 16.2%), camphor (8.0 – 25.1%) and 1,8-cineole (27.5 – 38.0%) as its main

compounds, while *O. americanum* essential oil had higher concentrations of nerol (10.0%), linalool (8.8 - 22.6%), and citral (29.6 – 42.5%). Elemicin (28.0 – 39.4%) and methyl eugenol (32.2 – 37.0%) ratios were the major compounds in *O. selloi*. Meanwhile, *O. sanctum* had eugenol (30.7 – 50.3%) and *trans*-ß-caryophyllene (26.4 – 38.5%) as its main compounds. Although the composition ratio varies depending on the species, water supply and production year, the essential oil of *O. basilicum* cultivars (Ohře and Genovese), *O.* × *africanum* and *O. americanum* were mainly composed oxygenated monoterpenes (>50%). However, the essential oils of *O. selloi* were primarily based on phenylpropanoid derivatives (over 65%). Meanwhile, *O. sanctum* had sesquiterpene (>40%) and phenylpropanoid (>40%).

This detailed analysis provides valuable insights into the responses of different *Ocimum* species to drought stress, shedding light on the intricate interplay of environmental and genetic factors in shaping their essential oil compositions. Despite the annual fluctuations and diversity observed, the average data for each year indicated that drought stress did not exert a substantial impact on the essential oil composition of the respective *Ocimum* species. Nonetheless, a minor increase in the ratios of linalool and eugenol was observed under conditions of irrigation treatment. Furthermore, non-irrigated plants *O. selloi* had higher methyl eugenol ratios (41% more). While drought stress had an adverse impact on the oxygenated monoterpene ratios of *O. basilicum* 'Ohře', *O. americanum*, and *O.* × *africanum*, it had a positive effect on phenylpropanes in *O. selloi* and *O. sanctum*. Notably, Phenylpropanes exhibited the highest ratios of essential oil content under drought conditions for these particular species.

Table 26. Effect of irrigation of essential oil composition of sweet basil cultivars and *Ocimum selloi* under open field experiment

Components	RT	LRI	*O. basilicum* 'Ohře'						*O. basilicum* 'Genovese'						*O. selloi*	
			2020		2021		2022		2020		2021		2022		2020	
			I	NI	I	NI	I	NI	I	NI	I	NI	I	NI	I	NI
1,8-cineole	8.38	1034	1.5	1.2	2.8	3.5	5.2	4.7	6.7	8.5	6.4	8.9	8.9	10.9	8.0	6.9
trans- β-ocimene	8.85														5.6	6.1
linalool	10.76	1097	50.1	52.0	43.2	40.7	57.5	40.9	53.3	48.6	40.8	41.0	47.2	50.2		
camphor	12.68	1144	0.1	0.1			0.3	0.3	0.9	0.7			0.8	0.6	2.8	0.2
α-terpineol	14.55	1189	0.2	0.2	0.1	0.1	0.6	0.6	1.0	1.2	0.2	0.3	1.1	1.3		
estragole	14.83	1196											11.5	0.1		
geraniol	17.30	1252	15.1	18.8	16.6	10.7	1	12.2			0.4	0.2	0.1			
eugenol	21.44	1361	1.0	0.8	0.7	1.4	0.8	0.5	3.1	4.6	2.3	2.5	1.4	1.8		
β-elemene	22.55	1391	1.4	1.2	4.8	5.4	2.9	3.2	0.7	0.5	3.3	2.0	1.6	1.4		
methyl eugenol	23.31	1411													28.0	39.4
trans-ß-caryophyllene	23.68	1419	1.0	1.2			0.6	0.7	0.3	0.2			0.3	0.2	5.3	4.0
trans-α-bergamotene	24.36	1437	2.7	0.2	0.1	0.2	2.0	4.3	3.7	6.9	4.8	6.4	5.4	6.8		
germacrene D	26.18	1482	3.2	2.8	2.7	3.1	2.0	1.9	2.8	2.0	4.2	2.7	2.7	2.1	2.1	1.6
δ-guaiene,	27.18	1506	3.5	3.1					2.1	1.6						
α-bulnesene	27.48	1506					1.3	1.7					1.5	1.4		
cis-γ-cadinene	27.80	1515	2.8	2.4	2.4	2.9	2.1	2.1	2.1	2.0	3.4	2.5	2.4	2.6		
elemicin	29.30	1555													37.0	32.2
1,10-di-epi-cubenole	31.36	1621	1.0	1.1					1.2	1.1						
tau-cadinol	32.26	1644	8.1	8.8	9.7	11.2	7.5	7.0	9.2	7.8	10.0	8.8	7.9	7.9		
others (< 1%)			6.6	5.6	13.7	17.9	6.0	17.5	9.7	11.4	18.9	20.5	4.8	11.2	7.2	4.2
Total			98.3	99.5	96.8	97.1	98.8	97.6	96.8	97.1	95.7	95.8	97.6	98.5	99.3	99.5
Monoterpenes			0.7	0.7	0.9	1.3	1.1	1.1	1.9	2.6	1.4	2.4	1.9	2.1	8.1	7.9
Oxygenated monoterpenes			70.6	75.3	65.3	59.6	75.0	60.4	66.1	65.9	53.2	58.2	61.3	66.4	11.2	7.5
Sesquiterpenes			18.4	13.8	17.5	20.5	12.9	16.6	16.5	17.2	25.5	20.8	18.0	18.1	15.6	12.2
Oxygenated sesquiterpenes			9.74	10.8	12.3	13.9	1.0	8.9	11.4	9.7	13.3	11.9	1	10.4	0.9	0.6
Phenylpropanes			0.1	0.1	1.0	1.9	0.8	14.3	0.3	0.2	2.5	2.9	13.2	2.4	64.6	71.6

RT – retention time. LRI – linear retention index relative to C9-C23 n-alkanes on an HP-5MS capillary column. I- irrigated and NI- non-irrigated (control).

Table 27. Effect of irrigation of essential oil composition of *three Ocimum* species under open field experiment

Components	RT	LRI	*O. americanum*						*O. × africanum*						*O. sanctum*					
			2020		2021		2022		2020		2021		2022		2020		2021		2022	
			I	NI	I	NI	I	NI	I	NI	I	NI	I	NI	I	NI	I	NI	I	NI
α-pinene	5.56	938	0.1	0.1					2.3	2.7										
ß-pinene	6.64	980		0.1	0.1		0.1		3.0	4.0	5.4	5.4	5.7	4.0			0.1			
limonene	8.19	1028	0.2	0.1	0.6	0.1	0.1	0.1	13.3	15.5	16.2	16.1	16.1	14.1	0.2		0.2			
1,8-cineole	8.38	1034	0.2	0.2	1.9	0.7	2.0	0.1	27.5	35.7	35.4	32.1	38.0	36.2	0.3	0.3	0.3	0.2	0.2	0.2
linalool	10.76	1097	9.7	8.8	12.6	22.6	22.1	9.4	0.9	0.4	0.2	0.2	0.6	0.4	0.5	0.2	0.7	0.5	0.4	0.3
camphor	12.68	1144			1.2	0.2		0.8	25.1	15.3	14.3	9.9	8.0	24.5	0.2	0.3	0.1		0.1	
α-terpineol	14.55	1189	1.0	0.8	1.0	0.6	1.0	0.9	2.8	3.6	4.0	4.8	4.0	2.8						
nerol	16.15	1227	11.2	9.9	11.6	9.3	10.1	10.6												
neral (citral-b)	16.58	1238	17.4	17.7	15.2	10.9	12.8	16.4		0.1										
geraniol	17.20	1252	1.8	1.7	2.2	2.5	2.1	1.3	0.4											
geranial (citral a)	17.86	1268	24.3	24.8	20.3	15.3	16.8	21.7		0.1										
eugenol	21.50	1361			0.1	0.1									30.7	36.0	49.3	50.3	36.4	43.1
ß-elemene	22.90	1391			1.3	2.1	1.1	1.1			0.1	0.2	0.2	0.1	2.8	2.7	5.7	6.0	5.7	4.4
methyl eugenol	23.50	1411													9.2	7.8	7.2	3.8	5.6	6.0
trans-β-caryophyllene	23.68	1419	4.1	4.5	4.5	4.2	2.9	4.0	1.1	1.1	1.6	2.1	1.8	1.4	33.6	30.9	26.4	30.6	38.5	31.0
trans-α-bergamotene	24.36	1437	1.8	2.1	1.7	1.8	1.7	2.5	0.2	0.1		0.1						0.1		
α-humulene	25.07	1454	0.7	0.8	1.6	1.4	0.6	0.9	4.5	4.2	6.4	7.6	6.9	5.7	2.4	2.2	2.0	1.8	2.3	2.1
germacrene D	26.18	1482	1.7	1.8	1.6	2.3	0.7	1.0	1.3	1.2	1.1	1.7	1.6	1.2			0.1	0.1	0.1	0.2
(*Z*)-α-bisabolene	28.54	1544	4.6	5.1	4.1	4.0	3.3	4.6	1.8	1.30										
caryophyllene oxide	30.20	1590	4.2	4.5	3.0	2.1	5.0	7.0							9.1	11.1	3.2	2.7	4.7	7.0
Others (< 1%)			15.5	16.3	9.7	15.4	11.5	10.8	12.0	10.8	14.4	17.9	15.6	14.7	7.8	6.7	3.5	2.9	5.0	4.3
Total			98.5	99.3	94.3	95.6	93.9	93.2	96.2	96.1	99.1	98.1	98.5	98.9	96.8	98.2	98.8	99.0	99.0	98.6
Monoterpenes			0.6	0.7	1.8	1.5	1.0	0.9	22.7	25.9	26.0	25.7	24.5	27.1			0.4	0.2	0.1	0.1
Oxygenated monoterpenes			70.6	68.5	69.9	65.9	70.0	65.7	61.6	60.6	59.9	54.4	63.8	67.2	1.3	0.8	1.3	0.8	0.9	0.7
Sesquiterpenes			15.0	16.6	16.9	20.2	12.5	16.3	13.9	12.4	13.1	18.0	14.3	11.6	43.4	40.2	36.9	41.8	49.9	41.3
Oxygenated sesquiterpenes			6.4	6.5	5.7	7.7	9.2	9.4	0.2			0.1	0.3	0.1	9.6	11.1	3.7	3.2	6.2	9.0
Phenylpropanes					0.1	0.5	1.5	0.8	0.8	0.7					39.8	45.6	56.6	54.2	42.0	44

RT – retention time. LRI – linear retention index relative to C9-C23 n-alkanes on an HP-5MS capillary column. I- irrigated and NI- non-irrigated (control).

In the greenhouse experiments, over 50 compounds were identified within three distinct *Ocimum* species. Similar major compounds were identified in each species as observed in the open field experiments. However, the ratios of these compounds were significantly influenced by the specific basil species, the severity of drought stress, and the production year of the plants, as detailed in Tables 28, 29, and 30. For instance, table 28 illustrates that the main compounds identified for 'Genovese' included linalool (ranging from 30.7 to 61.2%), 1,8-cineole (in a range of 8.0 to 23.1%), *trans*-α-bergamotene (3.8 to 7.6%), and tau-cadinol (5.0 to 10.5%). In the initial two years, drought stress did not exert a significant impact on the linalool and 1,8-cineole ratios of 'Genovese'. However, in the third year, under severe drought conditions, there was a slight enhancement in the ratios of linalool and 1,8-cineole.

Table 28. Effect of water supply on essential oil composition of *O. basilicum* 'Genovese' under greenhouse pot experiment

			2020			2021			2022		
Component	RT	LRI	70%	50%	30%	70%	50%	30%	70%	50%	30%
limonene	8.31	1029	2.3	0.4	0.6	0.2	0.2	0.3	0.4	0.6	0.5
1,8-cineole	8.44	1034	9.5	8.5	9.4	9.3	8.2	10.1	8.0	9.0	23.1
linalool	10.88	1097	35.0	38.5	35.2	61.2	58.0	55.0	44.5	30.7	51.9
camphor	12.69	1144	9.5	1.2	1.1	0.8	0.5	0.5	1.0	2.4	0.5
α-terpineol	14.55	1189	1.4	1.6	1.5	0.9	1.0	1.0		1.8	1.2
isobornyl acetate	18.52	1284	1.8	2.9	3.8	1.3	2.2	1.5	3.3	4.0	5.3
eugenol	21.49	1361	3.5	2.6	2.4	2.1	1.1	0.8	3.5	3.8	0.6
ß-elemene	22.92	1391	0.8	1.2	1.0	1.0	1.2	1.5	0.5	1.3	0.1
trans-α-bergamotene	24.69	1437	3.8	5.0	6.8	5.5	7.6	5.5	4.8	7.5	4.0
α-guaiene	24.81	1439	0.7	1.1	0.9	0.3	0.4	0.5	0.5	0.5	
α-humulene	25.38	1454	1.3	1.1	1.0	0.4	0.6	0.6	0.9	1.4	0.1
germacrene D	26.49	1482	2.6	3.9	3.6	2.0	2.6	2.5	2.9	3.4	0.9
δ-guaiene	27.18	1507	2.3	3.6	3.1						
α-bulnesene	27.48	1506				1.2	1.4	1.8	2.4	2.5	0.3
cis-γ-cadinene	27.80	1515	2.4	3.7	3.3	2.1	2.4	2.5	2.6	2.8	0.9
(*Z*)-α-bisabolene	28.54	1544	1.7	0.1	0.1						
palustrol	29.77	1562							1.5	1.7	
tau-cadinol	32.62	1644	6.5	10.5	9.5	6.8	7.5	8.6	9.5	10.0	5.0
others (<1%)			11.5	11.0	13.2	3.4	3.9	6.2	12.0	12.6	3.0
Total			96.6	96.9	96.5	98.5	98.8	98.9	98.3	97.0	97.4
Monoterpenes			5.7	1.8	3.0	1.1	1.3	2.1	2.4	2.6	
Oxygenated monoterpenes			62.3	54.1	51.9	73.1	68.4	67.4	59.2	49.5	82.6
Sesquiterpenes			20.0	25.9	25.7	14.5	17.9	16.9	18.5	25.8	6.3
Oxygenated sesquiterpenes			8.4	13.1	11.8	8.6	9.0	9.9	14.5	14.9	5.1
Phenylpropanes			0.3	0.4	0.5	2.3	1.2	1.0	3.9	4.7	0.6

RT – retention time. LRI – linear retention index relative to C9-C23 n-alkanes on an HP-5MS capillary column.

The essential oil composition of *O.* × *africanum* was highly heterogenous and influenced by drought intensity and the year of production (Table 29). On average the EOC is mainly composed of monoterpenes (20%) and oxygenated monoterpenes (65%).

Table 29. The effect of water supply on essential oil composition of *Ocimum* × *africanum* under greenhouse pot experiment

			2020			2021			2022		
Component	RT	LRI	70%	50%	30%	70%	50%	30%	70%	50%	30%
α-pinene	5.58	938	1.7	2.5	2.6	2.3	2.1	2.6			
camphene	5.96	952	2.4	1.7	2.4	0.4	2.9	1.0		2.1	0.6
sabinene	6.62	976	0.5	0.8	0.7	0.9	0.4	0.9	0.5	1.2	1.7
ß-pinene	6.73	981	2.1	3.3	2.7	3.9	1.8	3.9	1.3	4.5	5.9
ß-myrcene	7.09	995	0.6	0.7	0.8	0.8	0.5	0.8	0.8	1.2	1.7
limonene	8.31	1029	10.8	12.1	11.5	11.4	11.0	11.3	4.2	13.3	14.4
1,8-cineole	8.44	1034	21.6	30.4	23.5	40.2	21.4	37.0	24.1	27.9	34.6
cis-sabinene hydrate	9.68	1068	1.7	2.1	1.9	1.6	1.0	1.6	0.5	2.0	3.0
linalool	10.88	1097	1.0	1.2	0.5	11.3	0.5	7.1	29.4	0.3	0.2
camphor	12.69	1144	35.0	21.2	27.8	7.6	41.1	15.5	0.2	18.7	7.4
δ-terpineol	13.57	1162	0.8	0.7	0.6	0.5	0.2	0.5	0.3	0.6	0.9
α-terpineol	14.55	1189	2.5	3.1	2.4	3.0	1.3	2.4	1.8	2.9	4.3
geraniol	17.29	1252							11.9		
α-cubebene	22.25	1375				1.3	1.6	1.5	0.5	2.6	2.4
α-copaene	22.03	1377	1.7	1.9	2.4						
geranyl-acetate	22.64	1381							2.7		
trans-ß-caryophyllene	24.00	1420	1.2	1.4	1.8	0.9	1.15	1.2	1.0	2.4	2.5
(*Z*)-methyl isoeugenol	24.50	1448	0.8	1.2	1.2					0.9	1.4
α-humulene	25.38	1454	4.9	5.4	6.7	4.0	5.5	5.3	1.6	8.7	8.5
germacrene D	26.49	1482	1.4	1.5	1.6	1.1	1.1	1.3	1.3	2.3	2.2
α-bulnesene	27.48	1506				0.1		0.2	1.8	0.2	0.2
cis-γ-cadinene	27.80	1515	0.1	0.1	0.2			0.2	1.1	3.0	2.7
δ-cadinene	27.80	1524	2.1	2.3	2.7						
ß-sesquiphellandrene	28.19	1527				1.4	1.8	1.6			
cis-α-bisabolene	28.95	1544	1.6	2.3	1.1						0.3
tau-cadinol	32.62	1644	0.2	0.2		0.3		0.4	4.7		
others (<1%)			4.0	2.9	3.7	4.3	3.5	2.5	9.8	4.2	4.1
Total			98.7	99.0	98.8	97.3	98.9	98.8	99.5	99.0	99.0
Monoterpenes			19.0	22.1	21.6	20.2	19.1	20.9	20.2	19.1	20.9
Oxygenated monoterpenes			65.4	60.9	59.0	65.6	67.6	65.3	65.6	67.6	65.3
Sesquiterpenes			13.6	15.6	17.2	8.3	10.1	10.5	9.6	11.6	12.0
Oxygenated sesquiterpenes			0.2	0.2		0.7	0.6	0.5	3.8	0.6	0.5
Phenylpropanes			0.8	1.2	1.2	1.5		0.3	1.5		0.3

RT – retention time. LRI – linear retention index relative to C9-C23 n-alkanes on an HP-5MS capillary column.

The heterogeneity could be attributed due to the variation in the microclimate of the greenhouse each year and the properties of the soil media mixture as indicated in Tables 2 and 3. The main compounds were 1,8-cineole (ranged between 21.4 and 40.2%), camphor (0.2 to 41.1%), linalool (0.2 to 29.4%), limonene (4.2 to 14.4%) and α-humulene (1.6 to 8.7%) as indicated on Table 29. The impact of drought stress differs across the years. In the first and third years, both moderate and severe drought stresses led to an increase in 1,8-cineole. However, in the second year, higher ratios of 1,8-cineole were obtained from the control treatment. Additionally, in the second and third years, camphor ratios exhibited a significant increase when subjected to moderate drought stress, whereas, in the first year, the ratios were relatively low. Linalool ratios were higher in a higher soil water capacity in the second and third years.

Over forty compounds were detected in the essential oil extracted from *Ocimum americanum*. Linalool, neral, geranial, and nerol were found to be the main compounds, constituting more than 55.0% of the oil's composition each year. The ratios of the essential oil compounds were influenced by both drought stress and the year of production as indicated in Table 30. When the plants were grown in soil with a higher water capacity, their linalool ratios were enhanced. On the other hand, moderate to severe drought stress treatments resulted in relatively higher proportions of citral (neral and geranial). The ratios of nerol, neral, and geranial were higher in the first production year. Whereas the ratios of linalool were much higher in the second year. In addition, the yearly average data showed, severe drought stress had a negative effect on oxygenated monoterpenes ratios of *O. americanum*.

Table 30. Effect of water supply on essential oil composition of *Ocimum americanum* under greenhouse pot experiment

			2020			2021			2022		
Component	RT	LRI	70%	50%	30%	70%	50%	30%	70%	50%	30%
1,8-cineole	8.44	1034	0.2	0.1	0.3	0.8	1.7	0.3	1.5	0.4	1.8
linalool	10.88	1097	5.7	5.2	4.8	42.4	43.9	22.8	20.9	11.9	17.9
estragole	14.83	1196	0.2	0.2	0.2	4.3	4.0	0.2	19.5	9.0	7.3
nerol	16.14	1227	9.9	9.3	8.5	3.9	3.7	4.4	3.5	5.8	5.5
neral (citral-b)	16.72	1238	21.6	21.7	24.9	8.9	10.4	19.4	9.2	14.4	12.1
geraniol	17.29	1252	0.9	1.2	1.2	5.8	4.7	0.7	0.7	0.6	0.7
geranial (citral a)	18.00	1268	30.0	30.3	32.8	12.2	14.4	26.4	12.2	19.4	15.7
neryl acetate	21.84	1366	1.6	1.1	1.0	0.2	0.5	1.0	1.0	3.2	3.9
geranyl-acetate	22.64	1381	0.4	0.3	0.3	0.8	0.6	0.4	0.8	1.7	1.8

trans-ß-caryophyllene	24.00	1420	5.7	4.5	3.6	1.5	2.0	3.0	2.7	2.5	3.2
trans-α-bergamotene	24.69	1437	2.1	1.8	1.5	0.5	0.5	1.1	1.5	1.3	2.1
α-humulene	25.38	1454	1.1	1.0	0.9	0.6	0.6	0.8	1.0	1.3	1.1
germacrene D	26.49	1482	1.7	1.9	1.5	1.5	1.7	1.0	2.0	1.0	1.3
α-bulnesene	27.48	1506	0.1	8.0	7.0	1.1	1.0	0.6	1.3	0.9	0.9
cis-γ-cadinene	27.80	1515				1.3	1.1	0.9	1.0	0.8	0.8
cis-α-bisabolene	28.95	1544	5.6	5.1	4.0	0.9	1.5	2.9	2.9	2.1	2.8
caryophyllene oxide	30.46	1590	2.5	2.8	2.8	0.4	0.5	2.1	2.7	6.2	4.4
humulene epoxide II	31.44	1615	0.5	0.4	0.4				0.3	1.1	0.6
tau-cadinol	32.62	1644	0.2	0.1	0.1	5.3	4.0	3.1	3.9	3.2	3.3
others (<1%)			7.3	2.0	2.0	5.2	1.1	4.5	8.8	8.0	8.9
Total			97.3	97.0	97.8	97.6	97.9	95.6	97.4	94.8	96.1
Monoterpenes			1.0	0.4	0.5	1.0	1.2	0.6	0.7	0.6	1.1
Oxygenated monoterpenes			72.0	71.7	76.4	79.5	79.9	76.0	71.4	64.9	64.7
Sesquiterpenes			18.6	16.1	13.0	9.3	10.3	11.9	14.9	14.1	14.1
Oxygenated sesquiterpenes			5.9	6.3	5.7	6.7	5.3	5.9	8.5	12.9	14.0
Phenylpropanes			0.1	9.0	9.0				0.2	0.2	0.2

ÎRT – retention time. LRI – linear retention index relative to C9-C23 n-alkanes on an HP-5MS capillary column.

Furthermore, the outcome of a two-year plant growth chamber study revealed that both species of *Ocimum* had 44 essential oil compounds, each with distinct main compounds and ratios. As demonstrated in Table 31, the major components of *O. basilicum* 'Ohře' essential oil, accounting for more than 5.0%, were linalool (ranged from 44.7% to 57.9%), geraniol (7.9% to 17.2%), 1,8-cineole (3.0 to 8.2%), eugenol (1.0 to 6.1%), and tau-cadinol (5.7 to 8.8%). On the other hand, Table 24 shows that the major components of *O. americanum* essential oil were linalool (14.4 to 31.8%), geranial (13.2 to 36.1%), estragole (1.3 to 33.8%), and neral (between 17.0% and 24.7%). It is important to note that the ratio of these major essential oil components is not constant and can vary depending on irrigation intensity and the year in which the oil was produced. Thus, the major components showed a slight rise or fall in their ratios, but not a significant one. Despite the proportion of tau-cadinol being less than 10%, as the severity of drought stress increased, both species showed a slight increase in the proportion of tau-cadinol during each year of production. Furthermore, there was a noteworthy increase in the proportion of estragole in the second year, which may be attributed to the fact that *Ocimum* is an outcrossing species and the seed used in the experiment was from the previous year. Additionally, SWC influenced oxygenated monoterpenes

ratios of *O. basilicum* ‘Ohře’. Consequently, moderate drought stress enhanced the oxygenated monoterpene ratios.

Table 31. Effect of drought stress on essential oil composition of *O. basilicum* ‘Ohře’ under plant growth chamber experiment

Component	RT	LRI	2021			2022		
			70%	50%	30%	70%	50%	30%
1,8-cineole	8.44	1034	8.2	3.0	4.3	4.8	7.3	4.6
linalool	10.88	1097	55.5	57.9	52.3	45.8	55.1	44.7
camphor	12.69	1144	0.1	0.3	0.6	0.1		
geraniol	17.29	1252	7.9	16.0	14.0	17.2	15.6	11.4
eugenol	21.49	1361	6.1	1.0	0.9	1.8	1.5	1.2
geranyl acetate	22.64	1381	0.7	2.5	3.7	2.8	2.5	4.3
ß-elemene	22.92	1391	1.3	1.4	1.4			
trans-α-bergamotene	24.69	1437	0.7	0.1		1.6	0.6	1.7
α-humulene	25.38	1454	0.3	0.3	0.3	1.2	0.2	0.5
germacrene D	26.49	1482	1.6	2.4	2.6	2.1	1.1	2.8
α-bulnesene	27.48	1506	1.6	1.5	1.6	1.9	1.1	2.8
cis-γ-cadinene	27.80	1515	1.6	1.8	2.1	1.9	1.0	2.7
1,10-di-epi-cubenole	31.67	1621	0.6	0.6	0.7	0.9	0.7	1.3
tau-cadinol	32.62	1644	5.8	5.7	6.5	7.0	5.9	8.8
others (<1%)			6.1	4.6	7.2	9.6	6.7	10.8
Total			98.1	99.1	98.2	98.7	99.3	97.6
Monoterpenes			1.4	1.0	1.7	1.9	2.0	2.2
Oxygenated monoterpenes			73.5	78.3	72.9	70.9	80.7	64.3
Sesquiterpenes			9.1	9.3	10.3	11.5	5.1	13.3
Oxygenated sesquiterpenes			7.0	6.5	7.5	8.7	7.1	11.2
Phenylpropanes			6.5	1.4	1.7	2.1	1.8	1.9

RT – retention time. LRI – linear retention index relative to C9-C23 n-alkanes on an HP-5MS capillary column.

The ratios of essential oils compounds were found to be significantly affected by the year of production. The analysis of *O. basilicum* ‘Ohře’ revealed that the ratio of linalool was relatively higher during the first year, whereas geraniol and tau-cadinol demonstrated a slightly elevated proportion in the second year. In addition, a higher proportion of linalool, neral, and geranial was observed in *O. americanum* during the first year. Conversely, a significant increase in the ratio of estragole was noted in the second year. Additionally, severe drought stress had a negative influence on the ratios of oxygenated monoterpenes, which constitute the primary class of compounds in the essential oil of *O. americanum* (Table 32).

Table 32. Effect of drought stress on essential oil composition of *O. americanum* under plant growth chamber experiment

			2021			2022		
Component	RT	LRI	70%	50%	30%	70%	50%	30%
1,8-cineole	8.44	1034	0.9	0.3	0.6	3.9	4.0	2.6
linalool	10.88	1097	28.0	20.7	31.8	2	21.8	14.4
estragole	14.83	1196	5.7	1.3	1.1	30.9	29.3	33.8
nerol	16.14	1227	2.4	2.6	1.8	0.9	1.3	0.5
nerál (citrál-b)	16.72	1238	19.6	24.7	18.3	12.5	10.2	10.1
geraniol	17.29	1252	2.1	0.9	0.8	0.2	0.5	0.3
geranial (citral a)	18.00	1268	26.7	36.1	25.6	16.8	13.2	13.5
methyl eugenol	23.48	1411					0.1	1.4
cis-γ-cadinene	27.80	1515	0.6	0.3	1.2	0.2	0.7	0.9
caryophyllene oxide	30.46	1590	2.6	5.0	4.2	2.1	2.2	2.4
tau-cadinol	32.62	1644	2.6	1.5	4.5	2.3	3.9	4.6
others (<1%)			6.2	5.0	7.6	8.2	11.5	13.7
Total			97.4	98.5	97.5	98.2	98.7	98.1
Monoterpenes			0.5		0.2	1.5	1.3	1.8
Oxygenated monoterpenes			87.0	88.5	82.5	88.1	83.5	78.2
Sesquiterpenes			3.3	2.1	3.7	1.6	4.0	4.9
Oxygenated sesquiterpenes			5.8	6.9	9.6	5.4	7.6	9.1
Phenylpropanes							0.1	2.1

RT – retention time. LRI – linear retention index relative to C9-C23 n-alkanes on an HP-5MS capillary column.

4.4.3. Total polyphenol and antioxidant capacity

Drought stress is known to modify the secondary compound accumulation of plants including polyphenol content and its antioxidant activities. The effects of these drought-induced changes on *Ocimum* species were demonstrated through a multiyear experiment conducted in open field, greenhouse, and plant growth chamber settings. In line with that, a three-year open field study showed significant drought-induced changes in TPC and its AOC (Table 33). Depending on the species, soil moisture level, and the year of production, the effect could be negative, positive, or no change at all. Therefore, the sweet basil cultivars ‘Ohře’ and ‘Genovese’ demonstrated a positive response to irrigation in terms of TPC in each consecutive year, except for ‘Genovese’ during the first year. Irrigation resulted in a yearly average TPC increase of 10.0% to 24.0% for the ‘Genovese’ and ‘Ohře’ cultivars, respectively. Conversely, drought conditions led to a 40.0% increase in TPC for *O.* × *africanum* in the first year and a 15% increase in the third year. Similarly, purple holy basil (*O. sanctum*) exhibited a 19.0% rise in TPC under drought stress. However, no significant changes were observed in TPC for *O. americanum* across all years. A similar pattern was

noted for AOC. Consequently, in the initial year of cultivation, drought stress increased the AOC of 'Genovese' and *O.* × *africanum*. However, in the third year, drought stress had a negative effect on the AOC of 'Genovese', *O.* × *africanum*, and *O. americanum* plants. Nonetheless, no significant changes in AOC were detected for all species in the second year, as well as for the remaining species in the first and third years. Among the *Ocimum* species, *O. selloi* had higher AOC (250 mg AAE g^{-1} DM).

Table 33. Effect of irrigation on total polyphenol content and antioxidant capacity of *Ocimum* species over the years under open field experiment

Species	TRT	Total polyphenol content (mg GAE g^{-1} DM)			Antioxidant capacity (mg AAE g^{-1} DM)		
		2020	2021	2022	2020	2021	2022
O. basilicum 'Ohře'	I	84.9 ± 16.2^{Aa}	217.8 ± 21.8^{Abc}	299.5 ± 80.6^{Aa}	129.3 ± 36.9^{Ab}	73.3 ± 10.7^{Ab}	79.9 ± 6.3^{Aa}
	NI	60.0 ± 11.5^{Bbc}	200.5 ± 43.4^{Bb}	247.8 ± 21.3^{Aa}	131.5 ± 32.4^{Ab}	70.1 ± 13.2^{Ab}	76.7 ± 5.0^{Aa}
O. basilicum 'Genovese'	I	76.4 ± 10.7^{Bab}	252.6 ± 24.7^{Ab}	241.8 ± 54.1^{Aab}	109.9 ± 33.5^{Bb}	83.9 ± 9.7^{Aab}	80.2 ± 12.6^{Aa}
	NI	92.5 ± 17.1^{Aa}	232.4 ± 21.8^{Bb}	200.0 ± 20.7^{Bbc}	149.9 ± 35.3^{Ab}	110.9 ± 66.1^{Aab}	72.1 ± 6.9^{Ba}
O. × *africanum*	I	28.5 ± 3.5^{Bd}	208.0 ± 30.9^{Ac}	189.1 ± 39.9^{Bbc}	62.9 ± 28.1^{Bc}	66.2 ± 16.7^{Ab}	83.3 ± 7.2^{Aa}
	NI	39.9 ± 8.1^{Ad}	187.9 ± 20.7^{Ab}	217.6 ± 36.5^{Aab}	134.7 ± 27.8^{Ab}	79.1 ± 12.9^{Aab}	62.6 ± 8.6^{Bb}
O. americanum	I	62.8 ± 17.3^{Abc}	211.5 ± 35.8^{Abc}	166.0 ± 5.0^{Ac}	150.2 ± 44.3^{Ab}	70.8 ± 7.9^{Ab}	53.2 ± 6.3^{Ab}
	NI	67.3 ± 13.2^{Ab}	189.6 ± 40.5^{Ab}	157.3 ± 8.7^{Ad}	137.6 ± 48.9^{Ab}	72.4 ± 23.7^{Aab}	48.4 ± 2.2^{Bc}
O. sanctum	I	41.7 ± 17.0^{Acd}	295.8 ± 45.4^{Aa}	143.3 ± 28.6^{Bc}	128.9 ± 45.4^{Ab}	100.7 ± 27.7^{Aa}	45.0 ± 9.1^{Ab}
	NI	48.9 ± 2.0^{Acd}	341.4 ± 84.1^{Aa}	170.4 ± 18.8^{Acd}	157.6 ± 4.4^{Ab}	122.9 ± 12.6^{Aa}	49.6 ± 8.3^{Ac}
O. selloi	I	62.3 ± 5.7^{Abc}	-	-	250.8 ± 17.3^{Aa}	-	-
	NI	73.9 ± 9.9^{Ab}	-	-	249.2 ± 63.7^{Aa}	-	-

Values are presented as Mean ± SD, TRT: Treatment; I-Irrigated; NI: non-irrigated (control); Different letters are for significantly different groups. Capital letters to differentiate between drought stress under fixed species and small letters are used to differentiate between species under fixed drought stress. GAE: Gallic Acid Equivalent; AAC: Ascorbic Acid Equivalent; DW: Dry matter

The influence of drought on TPC and its AOC were also demonstrated under a greenhouse experiment as illustrated in Table 34. The effect of drought stress is heterogenous on both TPC and AOC depending on the species, the drought intensity, and the production. It had no significant effect on 'Genovese' cultivated in 2022, *O.* × *africanum* in all the years and *O. americanum* in 2020. A positive effect was also noticed in 'Genovese' in 2020 and *O. americanum* in 2021 and 2022. Furthermore, a negative effect of drought on TPC was also observed among 'Genovese' plants cultivated in 2021. The same heterogenous trends were observed in AOC as well. Hence, a negative impact of drought stress was observed among all basil species in 2020 and 'Genovese' as well as *O.* × *africanum* in 2022. On the other hand, drought stress had a positive impact on all basil in 2021

and *O. americanum* in 2022. The heterogenous results could be due to the fact that beside drought stress, the variation in microclimate (see Table 2) and soil media mixture (see Table 3) between the years had influenced TPC and its AOC.

Table 34. Effect of water supply on total polyphenol content and antioxidant capacity of *Ocimum species* under greenhouse pot experiment

Species	SWC (%)	Total polyphenol content (mg GAE g^{-1} DM)			Antioxidant capacity (mg AAE g^{-1} DM)		
		2020	2021	2022	2020	2021	2022
O. basilicum 'Genovese'	70	72.7 ± 9.3^{Bb}	161.9 ± 10.7^{Aa}	111.9 ± 19.7^{Ba}	124.4 ± 12.5^{Aa}	137.2 ± 16.4^{Bb}	110.9 ± 21.8^{Aa}
	50	73.2 ± 5.2^{Ab}	128.3 ± 16.2^{Bb}	111.5 ± 7.8^{Ba}	113.9 ± 5.6^{Ab}	127.3 ± 49.0^{Bb}	126.1 ± 16.3^{Aa}
	30	81.6 ± 7.3^{Aa}	124.5 ± 15.8^{Bb}	107.8 ± 6.5^{Ba}	108.7 ± 13.8^{Ab}	180.0 ± 12.5^{Ba}	70.0 ± 11.2^{Bb}
O. × *africanum*	70	82.7 ± 6.2^{Aa}	115.3 ± 19.8^{Ba}	110.0 ± 10.3^{Ba}	126.8 ± 10.7^{Aa}	144.9 ± 22.0^{Ba}	114.3 ± 14.3^{Aa}
	50	78.0 ± 18.4^{Aa}	115.2 ± 12.8^{Ba}	114.4 ± 14.2^{Ba}	120.0 ± 27.2^{Aab}	132.4 ± 19.8^{Ba}	89.7 ± 21.5^{Bb}
	30	74.6 ± 13.1^{Aa}	114.8 ± 10.5^{Ba}	98.8 ± 19.9^{Ba}	110.0 ± 20.7^{Ab}	152.4 ± 26.5^{Ca}	106.4 ± 24.2^{Aab}
O. americanum	70	73.2 ± 8.3^{Ba}	126.5 ± 11.5^{Bb}	132.7 ± 17.3^{Ac}	96.4 ± 9.6^{Ba}	200.0 ± 15.8^{Ab}	52.8 ± 17.9^{Bb}
	50	66.7 ± 8.3^{Aa}	142.1 ± 8.8^{Ab}	207.4 ± 14.6^{Aa}	78.5 ± 14.8^{Bb}	224.6 ± 33.1^{Aa}	69.8 ± 29.2^{Bab}
	30	75.5 ± 17.5^{Aa}	167.9 ± 24.2^{Aa}	182.0 ± 21.4^{Ab}	60.7 ± 17.4^{Bc}	210.5 ± 17.9^{Ab}	85.3 ± 13.9^{Ba}

Values are presented as Mean ± SD, SWC: Soil water capacity. Different letters are for significantly different groups. Capital letters to differentiate between species under fixed drought stress treatment and small letters are used to differentiate drought stress under fixed species. GAE: Gallic acid equivalent; AAC: Ascorbic acid equivalent; DM: Dry matter

In the growth chamber experiment, the TPC and AOC of basil were found to be influenced by several factors, including the severity of drought stress, the specific cultivar of basil, and the year of production (as indicated in Table 35). For instance, in the first year, the TPC and AOC of *O. basilicum* 'Ohře' were unaffected by variations in soil water capacity. However, in the second year, the TPC increased under severe drought stress, while the AOC rose under moderate drought stress. In contrast, moderate and severe drought stress boosted the TPC of *O. americanum* in the first year, but no significant change was observed in the second year. The effect of drought stress on AOC was negative in the first year but positive in the second year. Hence, the impact of drought stress on TPC and AOC is complex and depends on multiple factors, making it difficult to draw definitive conclusions.

Table 35. Effect of water supply on total polyphenol content and antioxidant capacity of *Ocimum* species under plant growth chamber experiment

Species	SWC (%)	Total polyphenol content (mg GAE g^{-1} DM)		Antioxidant capacity (mg AAE g^{-1} DM)	
		2021	2022	2021	2022
O. basilicum 'Ohře'	70	235.9 ± 10.6^{a}	205.8 ± 9.4^{b}	234.4 ± 30.4^{a}	162.3 ± 14.3^{ab}
	50	231.2 ± 14.0^{a}	197.3 ± 16.0^{b}	215.1 ± 27.1^{a}	178.0 ± 35.1^{a}

	30	254.4±47.4[a]	284.5±32.8[a]	202.8±40.6[a]	133.8±20.5[b]
O. americanum	70	168.7±17.0[b]	154.5±11.4[a]	200.1±48.4[a]	83.2±10.5[b]
	50	170.2±35.5[ab]	173.3±9.2[a]	155.1±24.3[b]	118.0±28.7[a]
	30	199.5±20.9[a]	174.0±26.8[a]	132.6±30.7[b]	120.0±12.9[a]

Values are presented as Mean ± SD, SWC: Soil water capacity; Different letters are for significantly different groups in columns. GAE: Gallic acid equivalent; AAC: Ascorbic acid equivalent; DM: Dry matter

4.5. Discussion

Drought stress, a common environmental factor, can affect the physiological, morphological, and biochemical responses of medicinal plants and their secondary metabolism (Osakabe *et al*., 2014; Selmar and Kleinwächter; 2015; Wu *et al*., 2022). Additionally, it was also observed a presence of wider morphological and biochemical diversities among *Ocimum* species. Therefore, this sub-section discussed the specific changes induced by drought and the morpho-chemical diversity observed among *Ocimum* species.

4.5.1. Diversity in Ocimum

Morpho-chemical diversity in *Ocimum* species is quite remarkable and highlights the adaptability of these plants to different environmental conditions. Our study demonstrated the presence of intra and inter-specific variability among the 15 basil genotypes preserved in the department. Hence, variation was noted in qualitative phenotypic traits including the growth pattern, leaf appearance, corolla, and stem color. Consistent with our results, Chowdhury *et al*. (2017) similarly noted significant variation in various qualitative traits among nine *Ocimum* genotypes, including *O. basilicum, O. tenuiflorum* (both green and purple), *O. americanum*, and *O.× africanum*. These traits included stem pubescence, stem color, leaf surface, leaf margin, leaf tip, leaf shape, inflorescence type, and flower color. However, it was reported that the plant growth habit (erect) remained monomorphic across the genotypes. In addition, differences in pigmentation, leaf shape, size, and pubescence have been previously reported among basil species and cultivars (Bernhardt *et al*., 2014; Patel *et al*., 2015a; Gurav *et al*., 2020). Furthermore, quantitative morphological and chemical hierarchical cluster analysis utilizing Ward's method revealed the existence of three distinct morphological groups and six chemo-diversity. Among the genotypes, sweet basil cultivar such as 'Genovese', 'Sweet Aroma' and 'Cinnamon' had taller plants (varies between 52.6 to 62.1 cm), wider canopy (varies between 46.3 to 54.0 cm) and leaf area. As a result, the basil cultivars had maximum fresh (>425.0 g plant^{-1}) and dry (>87 g plant^{-1}) herb yield. Previous studies have also

demonstrated that the height of *O. basilicum* cultivars can vary from 29.2 to 100 cm, depending on the growing conditions, cultivation seasons, and the specific cultivars used. Similarly, canopy diameter can vary between 24.9 cm and 97.7 cm (Nurzyńska-Wierdak, 2013; Patel *et al*., 2015; Chowdhury *et al*., 2017; Saran *et al*., 2017). In addition, Patel *et al*. (2015a) also reported the fresh biomass production of 5 sweet basil cultivars varied between 140 and 295 g per plant.

Basil species are renowned for their complex essential oil compositions containing a wide range of aromatic compounds. This complexity extends across various cultivars and chemotypes, each exhibiting a unique profile of volatile compounds. Moreover, the chemical diversity observed in different *Ocimum* species has been significantly shaped by factors such as hybridization within and between species through cross-pollination, natural evolutionary events, polyploidy, and selective breeding, as evidenced in multiple studies (Carović-Stanko *et al*., 2010; Varga *et al*., 2017; Gurav *et al*., 2022). Our investigations, conducted across different cultivation practices and production years, have revealed a broadened diversity in the essential oil composition. The respective genotypes exhibited the presence of 20 to 65 compounds in their essential oils (depending on the genotypes), featuring either a predominant compound or a combination of mixed compounds. This diversity underscores the dynamic nature of essential oil composition in basil species, influenced by both genetic factors and external cultivation conditions. In line with our findings, several authors have also noted the predominant presence of the same major compound in various genotypes. Nevertheless, the proportions of these major compounds differ based on factors such as geographical location, cultivation methods, and processing techniques. Carović-Stanko *et al*. (2011) observed that linalool constituted the major compound in sweet basil genotypes like ‘Genovese’ and ‘Dark opal’. Similarly, Murarikova *et al*. (2017) and Zeljković *et al*. (2020) identified linalool, 1,8-cineole, and geraniol as the primary essential oil compounds in Ohře. Bernhardt *et al*. (2014) found estragole and linalool to be the main constituents in ‘M. Grünes’. Another study on thirty Indian accessions of *O. basilicum* revealed the presence of linalool, methyl chavicol, and various chemotypes, including methyl chavicol/linalool and *(E)*-methyl cinnamate (Raina and Kumar, 2017). In addition to linalool and estragole, Cinnamon basil was reported to have *(E)*-methyl cinnamate as its main compound (Koroch *et al*., 2017). *Ocimum minimum* essential oil was found to be rich in geranyl acetate and linalool. *Ocimum americanum* of the citral chemotype (geranial and neral) and linalool was also documented (Carović-Stanko *et al*., 2011; Bernhardt *et al*., 2015). Thai

basil predominantly contained estragole, methyl eugenol, and *(E)*-α-bergamotene (1.01%) (Łyczko *et al*., 2020). Additionally, Vieira *et al*. (2014) identified linalool and 1,8-cineole as the primary compounds in Törpe basil. In a previous study on thirty-two accessions of Indian holy basil (*Ocimum tenuiflorum*), two chemotypes abundant in eugenol and methyl eugenol were identified, along with notable quantities of β-caryophyllene and β-elemene (Raina *et al*., 2013). The *Ocimum* species, including genotypes such as 'Sweet aroma', 'Cinnamon' and 'Turkish basil' are well-suited for cultivation in Hungary. This suitability arises from their higher biomass, EOC, higher linalool ratio, and TPC. Additionally, the citral chemotype of *O. americanum* holds potential for inclusion in herbal tea blends. Moreover, *O. americanum* originating from Togo exhibits a higher EOC, featuring an increased ratio of methyl cinnamate, making it a valuable candidate for natural flavoring purposes.

4.5.2. Drought-induced physiological changes

Physiological parameters such as leaf RWC, WP, shoot WUE, and SPAD values were affected by drought stress. As the drought intensity increased, there was a consistent decline in the RWC and WP of basil leaves, observed across multiple production years. This suggests that drought stress has a negative impact on the RWC and WP of basil leaves. This is further supported by Damalas (2019) who noted a 29.2% decline in RWC of sweet basil leaves under water deficits (40% field capacity). Additionally, several other studies had also demonstrated that drought stress reduces the RWC and WP of sweet basil (Radácsi *et al*., 2010; Kalamartzis *et al*., 2020), summer savory (Radácsi *et al*., 2016) and lemon balm (Zámboriné *et al*., 2017). In drought conditions, leaf RWC and WP decreases, resulting in reduced turgor, stomatal conductance, and photosynthesis, thus reducing yield (Osakabe *et al*., 2014; dos Santos *et al*., 2016). However, drought stress had a positive effect on the WUE and SPAD values of these plants. The higher WUE observed in basil plants under drought stress could be a result of their ability to optimize water conservation, reduce transpiration, and adapt their physiological and biochemical processes to cope with limited water resources (Kalamartzis *et al*., 2020; Seleiman *et al*., 2021; Yang *et al*., 2021; Mahajan, and Pal, 2023). One mechanism is the closure of the stomata. When plants are under drought stress, stomatal closure reduces water loss through transpiration, conserving water within the plant. Even though it helps to conserve water but also limits carbon dioxide uptake for photosynthesis (Kalamartzis *et al*., 2020). Furthermore, under drought conditions, plants may undergo physiological and biochemical

adjustments to enhance their ability to capture and use water effectively. These adaptations can include changes in leaf anatomy, such as increased leaf thickness or the presence of specialized tissues that store water (Yang *et al*., 2021; Mahajan, and Pal, 2023). Additionally, basil plants may modify their metabolic pathways to prioritize the production of compounds that facilitate drought tolerance, such as osmolytes and antioxidant enzymes that help maintain cellular hydration (Seleiman *et al*., 2021; Yang *et al*., 2021; Mahajan, and Pal, 2023). On the other hand, the rise in SPAD value during moderate and severe drought stress doesn't necessarily imply a rise in chlorophyll content. The cause for this rise may be the contraction and wilting of leaves, which may result in a higher green color intensity (SPAD value) per unit area when compared to hydrated and expanded leaves of the control treatment. A previous study by Radácsi *et al*. (2016) showed higher chlorophyll levels (expressed in SPAD unit) in summer savory (*Satureja hortensis* L.) under severe drought (30% SWC). Puangbut *et al*. (2017) also found that drought treatments led to higher SPAD chlorophyll meter readings in Jerusalem artichokes than in irrigated treatments. Furthermore, Pirzad *et al*. (2011) also reported a slight enhancement of total chlorophyll content in chamomile (*Matricaria chamomilla* L.) in drought stress. Whereas no change was observed in five sweet basil cultivars by Kalamartzis *et al*. (2020). These physiological changes influence the growth, development, and drought stress tolerance of basil as detailed below.

4.5.3. Drought-induced morphological changes

Basil plants require a continuous and optimum supply of water for their normal growth. 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 level results 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. sanctum*, grown in open fields without irrigation, suffered less. The negative effects of drought stress on the growth and herb production of medicinal and aromatic plants are widely recognized and extensively studied phenomena that have been consistently documented by numerous scientific investigations.

Although the intensities of drought stresses are different, several authors also showed the negative impact of drought stress on biomass production of *O. basilicum* cultivars (Radácsi *et al*., 2020), *O.* × *africanum* (dos Santos *et al*., 2016) as well as *O. americanum* (Khalid, 2006). Additionally, Damalas (2019) demonstrated that drought stress (40% FC) reduced the shoot fresh weight, dry weight, and height of sweet basil plants by more than 40%. Furthermore, Németh *et al*. (2016) reported that lower soil water content (40% SWC) resulted in significantly lower biomass production in four Lamiaceae species: lemon balm, thyme, peppermint and marjoram. To cope with the shortage of water, basil plants undergo physiological and biochemical changes (as discussed on under section 4.5.2), which can ultimately affect their growth and biomass production.

4.5.4. Drought-induced biochemical changes

The synthesis and accumulation of secondary compounds is an intricate and dynamic process governed by various biotic and abiotic factors (Li *et al*., 2020). Our multiple experiments have demonstrated significant heterogeneity and inconsistency in this process. Consequently, factors such as drought intensity, specific taxa, the growth environment, and the year of production play a crucial role in influencing secondary compound production (EOC, EOY, essential oil composition, TPC, and AOC). As a result of drought stress, the EOC of *O.* × *africanum* increased, an effect that was additionally supported by higher densities of glandular hairs. The justification is strengthened by a strong Pearson correlation ($r= 0.87$, $P< 0.01$) observed between the GHD of *O.* × *africanum* and its EOC. Therefore, a higher density of glandular hairs can indicate a potentially higher concentration of essential oils, but it's important to note that other factors, such as basil species, the size and volume of trichomes (peltate and capitate), ages of trichomes and several others, can also influence essential oil content (Werker *et al*., 1993; Maurya *et al*., 2019; Wongpraneekul *et al*., 2022). Conversely, no significant noticeable changes were observed in *O. basilicum* 'Ohře' and *O. sanctum*. However, the impact on *O. basilicum* 'Genovese' and *O. americanum* exhibited both positive and negative outcomes, depending on the production year and the growing environment. Consistent with our findings, various authors have also noted taxa and drought intensity-specific responses in the EOC of medicinal plants. Consequently, positive impacts of drought stress on EOC were observed in *Ocimum basilicum* cultivars (Simon *et al*., 1992; Khalid *et al*., 2006; Radácsi *et al*., 2010), *Ocimum* × *africanum* (dos Santos *et al*., 2016), *Thymus citriodorus* (Tátrai *et al*., 2016), *Thymus daenensis* (Pirbalouti *et al*., 2014; Bistgani *et al*., 2017), and *Salvia officinalis* (Govahi *et*

al., 2015). Conversely, negative effects were also documented for *Salvia officinalis* (Corell *et al*., 2012; Aslani *et al*., 2023), *Matricaria chamomile* (Razmjoo *et al*., 2008), *Melissa officinalis* (Farahani *et al*., 2009), and *Mentha piperita* (Khorasaninejad *et al*., 2011). Furthermore, no discernible changes were reported in *Ocimum gratissimum* (Hazzoumi *et al*., 2015), *Hyssopus officinalis* (Khazaie *et al*., 2008), *Thymus vulgaris* (Khazaie *et al*., 2008), and *Salvia officinalis* (Corell *et al*., 2009). Our comprehensive set of experiments additionally demonstrated the negative effect of drought stress on the EOY of the species. This adverse effect can be attributed to the significant constraint imposed by drought stress on biomass production, a factor directly associated with EOY. The extent of EOY reduction varies based on the specific *Ocimum* species and the growth environment. Consequently, drought stress had a more pronounced effect in *O. × africanum* plants cultivated in a greenhouse, whereas there is a comparatively lower reduction in EOY for *O. sanctum* grown in an open field setting. Similar observations have been reported previously by Kleinwachter and Selmar (2013), Paulsen and Selmar (2016), and Yahyazadeh *et al*. (2021). Moreover, the impact of drought stress on the essential oil composition of the various *Ocimum* species was heterogeneous as well. On one hand, drought stress increased the ratios of neral and geranial in *O. americanum* across all experiments. In addition, moderate drought stress slightly enhanced the camphor ratio of *O. × africanum.* However, it reduced the ratio of linalool, a major constituent in *O. basilicum* cultivars 'Ohře' and 'Genovese', as well as in *O. americanum* plants. On the other hand, its effect on the remaining essential oil compounds varied, displaying inconsistency with both negative and positive changes or, in some cases, no detectable alteration.

A similar trend was also observed on TPC and AOC. Drought stress adversely affected the TPC of *O. basilicum* 'Ohře' plants when cultivated in an open field. Conversely, severe drought stress (30% SWC) in a plant growth chamber had a positive influence on TPC. Additionally, drought stress did not have a significant impact on the TPC of both *O. basilicum* 'Genovese' and *O. × africanum* plants when cultivated in open fields and greenhouse settings. However, under severe drought stress conditions, TPC increased in *O. americanum* plants in both greenhouse and plant growth chamber experiments, with no noticeable change observed in the open field experiment. Moreover, drought stress did not result in a noteworthy alteration or only marginally reduced AOC in all basil species across the different growing environments, with the exception of *O. basilicum*, *O.× africanum*, and *O. sanctum* cultivated in an open field, where a positive impact was observed. Previous studies also

indicated that the polyphenol content and antioxidant capacities within basil genotypes differ depending on cultivars (Kwee and Niemeyer, 2011; Bajomo *et al*., 2022) and other factors such as plant part or organ (Prinsi *et al*., 2020) and growing conditions (Chutimanukul *et al*., 2022; Brahmi *et al*., 2022). This finding aligns with the study by Pirbalouti *et al*. (2017), which found higher phenolic content in shoots of two basil varieties (*O. basilicum*) subjected to drought stress (30% FC) compared to regularly irrigated plants. Conversely, Mulugeta and Radácsi (2022) observed species-specific responses to drought, with the 'Genovese' sweet basil cultivar exhibiting higher TPC under severe drought stress, while soil water capacity had no significant effect on *O.* × *africanum* and *O. americanum*. Additionally, Mulugeta and Radácsi (2022) noted that both moderate (50% SWC) and severe drought stress (30% SWC) had a detrimental impact on the AOC of the three *Ocimum* species. Gharibi *et al.* (2016) also found that moderate drought stress treatment (50% FC) and severe drought stress (25% FC) increased TPC and AOC in three *Achillea* species.

As per Yahyazadeh *et al*. (2021), the rise in the concentration of natural products, such as isoprenoids or phenols, in response to drought stress can be ascribed to either a passive shift resulting from stomatal closure or an active increase through the up-regulation of enzymatic activity. This phenomenon, characterized by an increase in natural product biosynthesis due to over-reduction during drought, has been previously documented by Selmar and Kleinwächter (2013, 2015) and Yahyazadeh *et al*. (2018). When plants undergo water deficiency, stomatal closure occurs, leading to a decreased absorption of CO_2. Consequently, the demand for reduction equivalents ($NADPH^+H^+$) for CO_2 fixation through the Calvin cycle decreases, resulting in an excess of $NADPH^+H^+$. These surpluses leads metabolic processes toward the synthesis of highly reduced compounds, such as isoprenoids or phenols. Numerous studies have indicated that drought stress can prompt an increase in natural product production by up-regulating genes involved in their biosynthesis. Mandoulakani *et al*. (2017) discovered that drought stress enhances the content of methyl chavicol and methyl eugenol in sweet basil by elevating the expression levels of *chavicol O-methyl transferase* and *eugenol O-methyl transferase* genes. Moreover, drought stress induces the upregulation of monoterpene synthase expression in *Salvia officinalis* (Radwan *et al*., 2017). Additionally, drought stress has been demonstrated to significantly induce the expression of genes related to flavonoid biosynthesis in hybrid poplar plants, leading to an accumulation of phenolic and flavonoid compounds (Ahmed *et al*., 2021).

4.5.5. Seasonal variation

Basil is a highly adaptable plant that can thrive in a wide variety of climatic and geographical conditions. However, variations in seasons and production years can affect its growth and essential oil production. This phenomenon was demonstrated through extensive experiments involving a three-year drought study and a two-year investigation into the morpho-chemical variability of basil genotypes. Consequently, noteworthy alterations in biomass production, EOC and composition, TPC, and AOC were identified across the production years. The differences observed in factors other than the specified treatments were primarily ascribed to the annual variations in weather conditions and the variability in soil properties, as detailed in Tables 2 and 3. Several scholars have also noted the influence of these factors on morpho-chemical attributes of plants (Chang *et al*., 2005; Figueiredo *et al*., 2008; Bernhardt *et al*., 2015; Franz and Novak, 2020).

5. CONCLUSION

Biomass production, and accumulation of secondary compounds in medicinal and aromatic plants are influenced by genetic factors, environmental conditions, and processing methods. Multiple experiments conducted over several years have shown that in basil plants, drought stress, genotypes, and the year of production significantly impact biomass production and the accumulation of secondary compounds. Basil plants exhibit various physiological, morphological, and biochemical changes when subjected to drought stress.

Diversity in Ocimum

An extensive morphological and chemical diversity was observed both within and between fifteen basil genotypes. In addition to the commonly cultivated and high-yielding sweet basil varieties such as 'Genovese' and 'M. Grünes' in Hungary, other comparable species were identified. Consequently, it is recommended to introduce 'Sweet Aroma', 'Cinnamon' and 'Turkish basils' into Hungarian basil cultivation practices. Moreover, various chemotypes, including the methyl cinnamate of Togo, the linalool/citral-rich *O. americanum*, and the 1,8-cineole/camphor of *O.* × *africanum*, could be incorporated into culinary applications. Thai basils also exhibited relatively higher biomass production and essential oil content (EOC). However, caution should be exercised due to the elevated ratios of estragole, which raises safety concerns.

Drought induced physio-morphological changes

Experiments involving different basil taxa, including *O. basilicum* 'Ohře', *O. basilicum* 'Genovese', *O.* × *africanum*, *O. americanum*, and *O. sanctum*, in three distinct growth environments (open field, greenhouse, and climatic chamber) demonstrated distinct responses to drought stress. Drought-induced alterations in plant physiology were evident, with a decrease in key indicators of water status, such as leaf relative water content and water potential, under drought conditions. However, shoot water use efficiency and SPAD chlorophyll content increased. Consequently, basil plants exhibited inhibited growth, characterized by reduced plant height, a narrower canopy, smaller leaves, and a decrease in both fresh and dry biomass production.

Drought induced bio-chemical changes

Water supply also influenced secondary compound accumulations, as lower moisture levels had a positive effect on the density of glandular hairs. Despite an increase in glandular hair density, lower

moisture levels resulted in a significant reduction in the overall EOY. However, the effect of water shortage on EOC, essential oil composition, TPC, and AOC was heterogeneous. Drought stress led to a decrease in the linalool ratio but induced an increase in the ratios of citral. Additionally, it was observed that severe drought stress had a negative effect on the proportions of oxygenated monoterpenes in the examined *Ocimum* species, except in the case of *O. basilicum* 'Genovese'. Conversely, there was a positive influence on the ratios of phenylpropanes. This complexity in secondary compound accumulation shows the impact of various factors including the taxa-specific responses and the growing environment played significant roles, alongside the influence of drought stress.

In conclusion to enhance biomass and essential oil production, it is recommended to maintain a soil water capacity of not less than 70% in protected basil cultivation, with twice-weekly irrigation for open field cultivation, especially in Hungary and regions with similar climates. 'Genovese' and 'M. Grünes' are suggested for herb production along with 'Cinnamon' and Sweet Aroma'. Further research is recommended to investigate the mechanisms underlying changes in EOC and composition in response to drought stress. Future investigations should focus on exploring molecular traits and phenol composition for a deeper understanding of the subject.

6. NEW SCIENTIFIC RESULTS

i. The effect of drought stress on basil's secondary compound accumulation is described as "taxa specific." The response to drought can vary among different basil varieties or species. Some types of basil may exhibit a more pronounced change in their secondary compound content when subjected to drought, while others may be less affected.

ii. 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.

iii. Severe drought stress negatively impacted the ratios of oxygenated monoterpenes while positively affecting the ratios of phenylpropanes in the studied *Ocimum* species, with the exception of *O. basilicum* 'Genovese'.

iv. Researchers observed significant variation 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 literature.

v. The year in which basil plants were grown and harvested had a pronounced effect on the composition of their essential oils than the drought treatment. This suggests that environmental and seasonal factors strongly influenced the chemical makeup of the essential oils in different basil genotypes.

vi. 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. This marks the first reporting of their morphological characteristics, as well as their essential oil production and composition in Hungary.

SUMMARY

Basil encompasses a diverse group of aromatic herbs with over 67 recognized species, including *Ocimum basilicum*, *Ocimum* × *africanum*, *Ocimum americanum*, *Ocimum gratissimum*, and *Ocimum sanctum*. The remarkable diversity within the basil species is attributed to variations in geographical distribution, climatic preferences, and genetic makeup. Each species exhibits distinct morphological, physiological, and chemical traits, contributing to its adaptability to specific ecological niches. Basil species have been utilized for centuries in traditional medicine, culinary practices, and religious rituals across different cultures. The varied chemical profiles of basil, characterized by essential oils and polyphenols not only confer unique flavors and fragrances but also possess pharmacological properties with antimicrobial, anti-inflammatory, and antioxidant effects.

The morphological and biochemical diversities within *Ocimum* species were additionally validated through an evaluation of 15 basil genotypes (belonging to 5 species) preserved in the department's gene bank. Hierarchical cluster analysis showed three distinct morphological groups. Notably, within the sweet basil genotype category, 'Genovese', 'Sweet Aroma,' and 'Cinnamon' exhibited robust morphological growth, leading to a higher fresh biomass yield (>425 g plant^{-1}). Among the genotypes, *O.* × *africanum* demonstrated the maximum EOY (2.6 mL plant^{-1}) and EOC (3.4%), followed by 'Törpe' plants with the second-highest essential oil content (1.9%). The 'Turkish basil' had the highest TPC (218.8 mg GAE g^{-1} DM) and AOC (290.4 mg AAE g^{-1} DM). Furthermore, extensive diversity was observed in the composition of essential oils across different basil genotypes. A hierarchical cluster analysis based on essential oil composition identified seven distinct chemical profiles. The primary constituents of essential oils included linalool, estragole, methyl cinnamate, and various mixed ratios such as linalool/estragole/citral, *trans*-ß-caryophyllene/eugenol, bisabolene/1,8-cineole/estragole, and 1,8-cineole/camphor. This highlights the extensive intra- and inter-specific diversity present among the studied basil genotypes, both in terms of morphology and essential oil composition.

In addition to the genetic factors, the yield of biomass, essential oil production, and compositions of essential oils in *Ocimum* species are greatly influenced by ecological conditions, agro-techniques, and processing methods. Among the ecological factors, drought stress emerges as a predominant

limiting factor. Numerous studies conducted in both open field and controlled greenhouse environments indicate that water scarcity leads to diminished plant growth and biomass production in medicinal and aromatic plants, including basil. Nevertheless, the impact of drought stress on the accumulation of secondary compounds in medicinal plants is inconsistent and often contradictory. Consequently, to understand the intricate effects of drought stress on the physio-morphological and biochemical traits within selected *Ocimum* species, a three-year experiment (2020 to 2022), was conducted across various growing environments, including open fields, greenhouses, and growth chambers. This experiment aimed not only to unravel the intricate effects of drought stress but also to detect the intra and interspecific morpho-chemical diversity among *Ocimum* species.

In an open-field experiment setting, we utilized four basil species, namely *O. basilicum* 'Ohře', *O. basilicum* 'Genovese', *O.* × *africanum*, *O. americanum*, and *O. sanctum* (purple type). Two levels of water supply, with irrigation as the control and non-irrigation as a drought stress treatment, were implemented. The irrigated plot received 20 mm m^{-2} of water twice a week, while the non-irrigated plot solely relied on natural precipitation, ranging between 180 to 262 mm of RF during the experimental period. In controlled pot experiments, we assessed three *Ocimum* species (*O. basilicum* 'Genovese', *O.* × *africanum, O. americanum*) in a greenhouse, and two *Ocimum* species (*O. basilicum* 'Ohře' and *O. americanum*) in a plant growth chamber. The drought stress treatment included three soil water capacities: 70% (control), 50% (moderate drought stress), and 30% (severe drought stress). Furthermore, morpho-chemical diversity among 15 basil genotypes were evaluated in open field growing condition.

The results of all the experiments across the years demonstrated that drought stress significantly affected the physiological, morphological, and biochemical traits of *Ocimum* species. Physiological parameters such as leaf RWC, WP, shoot WUE, and SPAD were evaluated. As the drought intensity increased, there was a consistent decline in the RWC and WP of basil leaves, observed across multiple production years. This suggests that drought stress has a negative impact on the RWC and WP of basil leaves. However, drought stress had a positive effect on the WUE and SPAD values of these plants. The higher WUE observed in basil plants under drought stress could be a result of their ability to optimize water conservation, reduce transpiration, and adapt their physiological and biochemical processes to cope with limited water resources.

Drought stress significantly reduced the growth of all examined *Ocimum* species. This reduction is evident in shorter plant heights and a narrower canopy. Furthermore, the stems become slender, and the leaves show diminished dimensions. Consequently, the limited availability of water leads to a significant decline in both fresh and dry biomass production. Furthermore, these multiple experiments have consistently shown that the impact of drought stress on the accumulation of secondary compounds is both heterogeneous and unpredictable. Several factors, including the severity of drought, specific plant taxa, the growth conditions, and the production year, are critical in determining the influence on the production of various secondary compounds such as EOC, EOY, essential oil composition, TPC, and AOC. Generally, drought stress has been found to significantly increase the density of glandular hairs in *Ocimum* species. Moreover, among certain *Ocimum* taxa and production years, drought stress has a slight inducing effect on EOC and TPC, while no significant changes were observed in others. However, irrespective of drought intensity, *Ocimum* taxa, and production year, a consistent trend was observed: lower soil water capacity corresponded to lower EOY. Additionally, the impact of drought stress extended to the compositions of essential oils. Notably, the influence of drought stress was found to have a positive correlation with citral ratios, encompassing both neral and geranial, as well as tau-cadinol ratios. Conversely, a negative effect was noted on linalool ratios. However, it is important to highlight that the results for other compounds exhibited variations and lacked a consistent pattern under drought stress conditions. This suggests a nuanced and compound-specific response to drought, emphasizing the complexity of the relationship between drought stress and the composition of essential oils.

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APPENDICIES

Appendix tables

Appendix table 1. The mean square and significance levels of the measured plant characteristics in open field experiments

Parameters	Source of variation						
	Species	TRT	Year	Species x TRT	Species x Year	TRT x Year	Species x TRT x Year
PH	2649.57**	2692.80**	4773.83**	66.79**	862.16**	74*	27.45ns
CD	510.62**	3776.10**	4663.92**	45.85ns	1139.32**	49.98ns	69.57ns
FHY	26651.70**	389472.82**	875167.33**	14204.27*	113403.65**	32055.78**	5220.64ns
DHY	5436.04**	15023.77**	12675.94**	517.22*	5814.81**	863.75*	402.05*
EOC	23.24**	0.60**	1.9**	0.80**	1.32**	0.38*	0.51**
EOY	5.95**	0.82**	0.31*	0.21*	0.70**	1ns	0.10ns
TPC	21205.2**	1525.6ns	455073.9**	4514.7**	32059.2**	91.9ns	2573.9*
AOC	3522.5**	10525.3**	111585.0**	1605.1ns	7851.4**	8577.2**	`628.1*

TRT: Treatment; Ns: non-significant (p>0.05); *: significant (P<0.05); **: significant (P<0.01); highly significant (P<0.001); PH: plant height; CD: Canopy diameter; FHY: Fresh herb yield; DHY: Dry herb yield, EOC: Essential oil content; EOY: Essential oil yield; TPC: Total polyphenol content; AOC: Antioxidant capacity

Appendix table 2. The mean square and significance levels of the measured physio-morpho chemical characteristics of *Ocimum* species under greenhouse condition

Traits	Species	SWC	Source of variation				
			Year	Species x SWC	Species x Year	SWC x Year	Species x SWC x Year
RWC	611.8**	7498.5**	3424.7**	161.7**	369.7**	203.1**	28.6ns
SPAD	941.8**	1235.3**	130.4**	26.5ns	181.4**	42.5**	53.7**
PH	2279.9**	3484.9**	3563.9**	83.6**	686.7**	382.5**	81.5**
CD	796.3**	7206.0**	2658.4**	193.3**	228.9**	422.0**	103.1**
LA	6844.3**	1676.5**	1277.4**	236.6**	1016.3**	35.7ns	45.5*
FHY	47301.8**	398258.4**	247213.9**	3228.4**	36254.8**	6977.5**	5301.6**
DHY	2259.0**	41083.4**	8630.4**	443.3**	593.0**	3643.6**	107.8**
GHD	24272615.1**	7369310.4**	14605581.9**	984394.7**	161144.2*	1272590.4**	272288.9**
EOC	77.8**	0.1ns	1.0**	0.6**	1.6**	0.4**	0.2*
EOY	5.3**	4.4**	0.6**	0.5**	0.3**	0.3**	0.3**
TPC	23775.1**	2044.6**	109881.9**	5254.4**	14717.1**	2381.9**	2266.7**
AOC	143.5ns	226.2ns	174843.3**	1234.4*	44084.1**	5554.1**	4525.9**

Ns: non-significant (p>0.05); *: significant (P<0.05); **: significant (P<0.01); RWC: Relative water content; PH: plant height; CD: Canopy diameter; LA: Leaf area, FHY: Fresh herb yield; DHY: Dry herb yield, GHD: Glandular hair density; EOC: Essential oil content; EOY: Essential oil yield; TPC: Total polyphenol content; AOC: Antioxidant capacity

Appendix table 3. The mean square and significance levels of the measured physio-morpho chemical characteristics of *Ocimum* species under plant growth chamber

Traits	Source of variation						
	Species	SWC	Year	Species x SWC	Species x Year	SWC x Year	Species x SWC x Year
WP	1.1**	1.9**	3**		0ns	2**	0.34**
WUE	14.9**	7.7**	5.1**	0.41ns	0.48ns	0.61*	1ns
RWC	146.5**	1695.5**	131**	0.12ns	28.61ns	134.39**	36.73*
SPAD	6208.3**	1798.5**	1076.4**	15.0 ns	193.2**	47.73**	8.3ns
PH	7707.75**	863.18**	45.10ns	117.21**	10.53ns	1.31ns	20.27ns
CD	867.00**	389.22**	2228.19**	66.25*	539.13**	30.53ns	24.19ns
FHY	3317.02**	1840.20**	84.41**	4.54 ns	1585.19**	0.13 ns	14.50 ns
DHY	74.29**	41.22**	28.33**	5.10**	37.66**	2.47**	0.49ns
GHD	555253.4**	862021.1**	1444070.7**	40756.0**	71570.4**		
EOC	3.19**	1ns	20.71**	0ns	2.60**	3ns	1ns
EOY	1.19**	2*	11.24**	0ns	0.84**	4**	0ns
TPC	102392.1**	7880.2**	3751.3*	8651.7**	7.02ns	6783.9**	2707.2*
AOC	75754.5**	5360.9**	89453.4**	836.7ns	96.50ns	9246.1**	5789.1**

Ns: non-significant (p>0.05); *: significant (P<0.05); **: significant (P<0.01); SWC: Soil water capacity; WP: Water potential; WUE: Water use efficiency; RWC: Relative water content; PH: plant height; CD: Canopy diameter; LA: Leaf area, FHY: Fresh herb yield; DHY: Dry herb yield, GHD: Glandular hair density; EOC: Essential oil content; EOY: Essential oil yield; TPC: Total polyphenol content; AOC: Antioxidant capacity

Appendix table 4. The mean square and significance levels of the measured morpho-chemical variability among 15 *Ocimum* genotypes

Traits	Source of variations		
	Species	Years	Species x Years
Plant height	1113.99**	529.63**	223.98**
Canopy diameter	397.35**	184.34**	346.65**
Leaf length	65.69**	1.45*	2.71**
Leaf width	17.19**	0.67*	1.03**
Leaf length: width	0.96**	0ns	0.17**
Leaf area	832.1**	46.6ns	106.9ns
Hundred leaf weight	18939.33**	1098.14**	496.98**
Inflorenscence length	391.25**	238.93**	111.68**
No. of inflorencence	39303.29**	38970.91**	14119.53**
Thousand seed weight	1.87**	ns	ns
Fresh herb yield	118516.12**	1024070.92**	81067.23**
Dry herb yield	4386.07	14998.75**	3667.82**
Eessential oil content	6.33**	2.99**	0.32**
Essential oil yield	3.96**	3.71**	0.87**
Total polyphenol content	10566.4**	2169.7**	1954.9**
Antioxidant capacity	3905.8**	187273.1**	21971.2**

Ns: non-significant at p>0.05 *: significant at p<0.05; **: significant at p<0.01

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