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Effects of water deficit on morphophysiology, productivity and chemical composition of *Ocimum africanum* Lour (Lamiaceae)

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Mechanisms by which plants acclimatize to water deficit conditions can affect development and, especially in aromatic plants, the essential oil synthesis. To evaluate the effects of water deficit on *Ocimum africanum*, seedlings were exposed to water levels corresponding to 100, 80, 70 and 60% of the substrate field capacity. The different watering regimes corresponded to predawn water potentials of –0.5, –0.9, –1.6 and –1.9 MPa, respectively. Decreased stomatal conductance and increased glandular and tector trichome density were observed under water deficit. In addition, reductions in leaf gas exchange and water consumption under water deficit led to decreased plant height, numbers of leaves and inflorescences, and total biomass production. As a result, lower essential oil yield and water use efficiency for biomass production occurred in the –1.9 MPa treatment. The content and production efficiency of essential oil (quantity of oil produced per unit water consumed) were higher in the –0.9 and –1.6 MPa treatments. There were no significant differences among treatments in the chemical composition of essential oil, and the major component of essential oil in leaves and inflorescences was isoeugenol in all treatments. The 80% field capacity watering regime (branch water potential of –0.9 MPa) was best for the growth and production of essential oil in *O. africanum*, for the cultivation of aromatic plant under drought economically viable in the semi-arid climatic regions.

Key words: Drought adaptation, essential oil, irrigation, water use efficiency.

INTRODUCTION

The production and composition of secondary metabolites in plants is affected by environmental conditions, physiological characteristics, genetics and evolutionary factors (Selmar and Kleinwächter, 2013).

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For essential oil-producing aromatic species, the quantity and chemical composition of secondary metabolites varies according to the cultivation conditions (Marchese et al., 2010; Saeidnejad et al., 2013), among which water availability is a major factor influencing oil yield and quality (Baghalian et al., 2011; Bahreininejad et al., 2013).

Water is a critical factor for the maintenance of primary and secondary metabolism in plants. Drought conditions are normally associated with low precipitation, high evaporative demand, and high temperatures and irradiation, which makes the water deficit a multidimensional stress (Larcher, 2000). Under water deficit conditions, plant growth and development are affected by changes in turgor pressure, cell wall elasticity, carbon assimilation and opening and closing of stomata (Akinci and Lösel, 2012; Wang et al., 2012). In addition, metabolic changes can occur, including increased photorespiration (Lambers et al., 2008), antioxidant enzyme activity (Reddy et al., 2004) and production of secondary metabolites (Saeidnejad et al., 2013; Selmar and Kleinwächter, 2013). In many species, stomatal and biochemical adjustments occur to maintain photosynthesis under moderate water deficits. Partial stomatal closure early after the onset of drought is essential for the control of transpiration rate and may be an important biological strategy for increasing water use efficiency (WUE) by enabling more CO₂ to be absorbed per unit of transpired water. Irrigation techniques designed to examine plant responses to water stress and to improve WUE have been developed; these techniques include partial root zone drying and regulated water deficit (Kriedemann and Goodwin, 2003) and cutback (Valipour, 2013). The water deficit approach has been used with aromatic species including *Matricaria recutita* L. (Baghalian et al., 2011), *Thymus daenensis* (Bahreininejad et al., 2013), *Catharanthus roseus* (Jaleel et al., 2008), *Ocimum basilicum* and *Ocimum americanum* L. (Khalid, 2006), with the goal of increasing essential oil production.

Species of the genus *Ocimum* (Lamiaceae) stand out for the quality, quantity and chemical diversity of the essential oils they produce. These oils have been used in applications in the pharmaceutical, cosmetic and food industries (Vani et al., 2009). The main components of essential oils in *Ocimum* species are estragol, eugenol, methyl eugenol, citral, linalool, geraniol and thymol (Blank et al., 2004; Khalid, 2006; Vani et al., 2009). Some of these compounds have antibacterial (Stefan et al., 2013; Sneha and Sastry, 2011), insecticidal (De Paula et al., 2004) and antioxidant properties (Kaurinovic et al., 2011). According to Blank et al. (2004), the price of *Ocimum basilicum* essential oil reached US\$110,000/L in the international market because of demand from the fine perfumery industry for its main component, linalool, which is used as a fixative. However, despite the economic importance of *Ocimum* species, its botanical and

agricultural studies are scarce.

An understanding of plant drought tolerance is necessary to determine cultivation conditions that optimize harvest time and essential oil yield and will contribute to improved oil production and quality to meet market standards. This study aimed to investigate the effects of water deficit on gas exchange and biomass partitioning and accumulation. The authors examined plant water use efficiency based on biomass and essential oil production, content, yield and chemical composition in leaves and inflorescences of *O. africanum* under controlled experimental conditions.

MATERIALS AND METHODS

Plant and growing conditions

The experiment was conducted in a greenhouse in the Campus of Universidade Estadual de Santa Cruz (UESC), Ilhéus, Bahia, Brazil (14°47'00"S, 39°02'00"W), from January 07 to April 17, 2012. Voucher materials are deposited at the herbarium of the UESC (number VIC 16.344). Seedlings of *Ocimum africanum* Lour. were grown from seed and transferred to 10-L pots containing a mixture of soil and sand (3:1) as substrate. After potting, the seedlings were irrigated to field capacity, and the weight of the pots was measured gravimetrically until constant weight was obtained. After acclimatization for 20 days, irrigation was stopped and the pots were covered to control evaporative losses. The evaluations began 5 days after irrigation was stopped. Irrigation was controlled by weighing the pots on alternate days, and the amount of water to be replaced was determined by the difference between the current weight and the corresponding water holding capacity. Four treatments were applied: T1, 100% of field capacity (the control); T2, 80%; T3, 70%; and T4, 60% of field capacity.

During the experiment, photosynthetically active radiation (PAR) in the greenhouse was monitored using S-LIA-M003 light radiation sensors coupled to a Hobo Micro Station data logger (Onset Computer Corp., Bourne, MA). The mean daily PAR measured in the greenhouse was between 8.6 and 23.2 mol photons m⁻² d⁻¹; temperature ranged from 24 to 30.7°C; and relative humidity (RH) was between 64 and 94%.

Water potential (Ψ_w)

Water potential (Ψ_w) was measured in branches at predawn using a pressure chamber 1000 (PMS Instrument Company, Corvallis, OR) according to Scholander et al. (1965). The measurements were performed every 8 days in 60 plants for the entire experimental period.

Leaf gas exchange

Measurements of leaf gas exchange were performed in mature and completely expanded leaves localized in the third or fourth node from the apex at 20, 40, and 60 days from the start of treatment. The evaluations were performed between 08:30 and 12:00 h in 10 plants per treatment using a portable Li-6400 photosynthesis system (Li-Cor, Lincoln, NB). Net photosynthetic rate (A), stomatal conductance to water vapor (g_s), transpiration (E) and the ratio between internal and external CO₂ concentrations (C_i/C_a) were evaluated under an artificial saturating light of 1000 μmol photons m⁻² s⁻¹ and an atmospheric CO₂ concentration (C_i/C_a) of 380 μmol

mol⁻¹.

Growth measurements

The number of leaves and inflorescences per plant was manually counted on day 60. The dry biomass of roots (DBR), stems (DBS), leaves (DBL), inflorescences (DBI) and total biomass (TDB) were obtained by drying at 75°C in a circulating air oven until constant biomass was reached. Leaf area (LA) was estimated using an LI-3100 Area Meter (Li-Cor), and 30 millimeter ruler was used to determine the height of the plants (HP). The following variables were calculated from the dry biomass and leaf area: specific leaf mass (SLM) = DBL/LA; relative growth rate (RGR) = $\ln TDB2 - \ln TDB1 / (T2 - T1)$; and net assimilation rate (NAR) = $[(\ln TDB2 - \ln TDB1) / (T2 - T1) \times (\ln LA2 - \ln LA1) / (LA2 - LA1)]$, according to Hunt (1990).

Micromorphology

For scanning electron microscopy (SEM), leaf fragments from each treatment were fixed at room temperature in 2.5% glutaraldehyde (v/v) prepared in 0.1 mol/L sodium cacodylate buffer (pH 6.9) for 48 h. The fragments were then dehydrated twice in an increasing ethanol series and brought to critical drying point using a CPD-030 (Bal-Tec, Balzers, Liechtenstein) with liquid carbon dioxide. The dried fragments were mounted on aluminum stubs, coated with gold, and examined under a JSM-6390 low-vacuum (LV) SEM (Jeol, Peabody, MA). Determination of stomata and glandular and tector trichome density was performed using electron micrographs of the same magnification.

Water-use efficiency (WUE), production and chemical composition of essential oil

Water use efficiency (WUE) was determined for biomass (total dry weight/water transpired) and essential oil production in leaves and inflorescences (total essential oil produced/water transpired) (g).

Production and chemical composition of essential oil

Leaves and inflorescences were dried in a circulating air oven at 75°C until constant dry biomass was reached. The essential oil was extracted from 100 g samples of dry biomass through hydrodistillation in 1500 mL distilled water for 90 min using a Clevenger apparatus; the extractions were performed in quadruplicate. The essential oil content was determined based on the volume extracted per 100 g dry biomass (% w/v); essential oil yield was determined by multiplying the content by the average value of leaf or inflorescence dry biomass (g plant⁻¹).

The essential oil was analyzed by gas chromatography using a Varian Saturn 3800 (Varian, Palo Alto, CA) equipped with a flame ionization detector (GC-FID) and VF-5ms capillary column (30 mm × 0.25 mm × 0.25 µm film thickness), using helium as the carrier gas at a flow rate of 1.2 mL min⁻¹. The injector and detector temperatures were 250 and 280°C, respectively. The column temperature program began at 70°C, increased by 8°C min⁻¹ to 200°C and by 10°C min⁻¹ to 260°C, and was held at 260°C for 5 min. One milliliter of a 10% solution of oil in chloroform was injected in the 1:10 split mode. The concentration of volatile constituents was calculated based on the full area of each peak in relation to the total area of all constituent peaks. Qualitative analysis of the essential oil was carried out using a Chromopack 2000 MS/MS mass spectrometer (Varian) with the same VF-5ms column and temperature program. The transfer line temperature was 250°C and

the trap temperature was 220°C. The chemical constituents were identified by computer comparison with the apparatus library and literature, and linear retention indices were calculated by injecting a series of *n*-alkanes (C₈–C₂₆) under the same chromatographic conditions as used for the samples (Adams, 1995).

Experimental design

The experiment was conducted following a completely randomized design, in a split-plot array with four water conditions: 100% (T1), 80% (T2), 70% (T3) and 60% (T4) of field capacity; four evaluation times (0, 20, 40 and 60 days); and 10 replications per treatment, (n = 160 plants). The data were examined by analysis of variance (ANOVA, *F*-test). For the treatment averages, polynomial regression models were adjusted using Sisvar 5.3 (Ferreira and Sisvar, 2010). Tukey tests were used to assess the average values within treatments for each evaluation time. Significance was defined as *P* ≤ 0.05.

RESULTS

Water relations and leaf gas exchange

Water deficit treatments in *O. africanum* resulted in a significant decrease (*P* ≤ 0.001) in predawn branch water potential (Ψ_w) throughout the experimental period. Values ranging from -0.3 to -0.5 MPa and from -0.4 to -2.2 MPa were measured in the well watered and water deficit (more severe) treatments, respectively (Figure 1).

A significant (*P* < 0.01) interaction was observed between water deficit treatment and time of evaluation for leaf gas exchange variables: *A*, *g_s* and *E* decreased linearly with increasing treatment time. Photosynthetic parameters decreased with increasing duration of drought stress (Figure 2A–C). *A* and *g_s* were positively correlated in all treatments (*R*² ≥ 0.92) (Figure 2D); the reduced transpiration rate indicated that stomatal limitation was an efficient mechanism for reducing water loss (Figure 2C).

Morphological aspects of biomass partitioning

O. africanum plants cultivated under the highest water deficit conditions showed reduced growth. Dry biomass of all plant parts (roots, stems, leaves and inflorescences) decreased linearly with increasing water deficit (Table 1). At Ψ_w of -1.9 MPa (severe deficit), biomass was reduced most in stems and inflorescences, with values that were 60 and 69% lower, respectively, than those of the control treatment (Ψ_w ~0.5 MPa) (Table 1). The allometric relationship observed in the ratio of roots to aerial parts (R/AP) showed a quadratic response with a maximum value of 0.106 g at Ψ_w = -1.04 MPa.

Plant height, leaf area, number of leaves and inflorescences, relative growth rate and net assimilation rate decreased linearly, with the lowest values in the plants subjected to the highest water stress (Table 1).

Figure 1. Predawn water potential (Ψ_w) measured in branches of *O. africanum* under different watering regimes. Points are means ($n = 5$) and bars represent standard error.

Figure 2. Net photosynthetic rate, A (A); stomatal conductance, g_s (B); transpiration rate, E (C); and relationship between g_s and A in leaves of *O. africanum* subjected to soil water deficit as indicated by the predawn water potential measured in branches (Ψ_w) (D). Points are means ($n = 5$); bars represent standard error.

Table 1. Plant growth variables in *O. africanum* after 60 days of water deficit treatments at different branch water potentials. Values are means ($\pm$ s.e.) of five replicates.

Variable	Predawn water potential (–MPa)				Equation	R^2
	0.5 MPa	0.9 MPa	1.6 MPa	1.9 MPa		
RDB	3.1 $\pm$ 0.5	1.9 $\pm$ 0.1	1.5 $\pm$ 0.2	1.2 $\pm$ 0.1	$\hat{y} = -1.278^{**}x + 3.513^{**}$	0.90
SDB	11.7 $\pm$ 0.2	7.9 $\pm$ 0.9	5.3 $\pm$ 0.2	4.7 $\pm$ 0.3	$\hat{y} = -4.864^{**}x + 13.423^{**}$	0.92
LDB	6.3 $\pm$ 0.6	4.8 $\pm$ 0.5	3.7 $\pm$ 0.	3.5 $\pm$ 0.3	$\hat{y} = -1.988^{**}x + 7.003^{**}$	0.93
IDB	15.7 $\pm$ 0.6	9.5 $\pm$ 0.9	5.3 $\pm$ 0.5	4.8 $\pm$ 0.5	$\hat{y} = -7.522^{**}x + 18.001^{**}$	0.91
TDB	36.9 $\pm$ 0.9	24.2 $\pm$ 2.3	15.7 $\pm$ 0.5	14.2 $\pm$ 1.0	$\hat{y} = -15.652^{**}x + 41.941^{**}$	0.92
R:PA	0.09 $\pm$ 0.02	0.1 $\pm$ 0.02	0.1 $\pm$ 0.01	0.09 $\pm$ 0.01	$\hat{y} = -0.069x^2 + 0.142x^{**} + 0.032^*$	0.63
HEI	63.2 $\pm$ 2.7	59.2 $\pm$ 1.1	52.6 $\pm$ 0.7	53.2 $\pm$ 0.9	$\hat{y} = -7.678^{**}x + 66.412^{**}$	0.94
SLM	42.8 $\pm$ 1.9	45.0 $\pm$ 2.0	50.1 $\pm$ 3.3	55.2 $\pm$ 3.3	$\hat{y} = 8.478^{**}x + 37.909^{**}$	0.95
NI	129.4 $\pm$ 6.8	88.0 $\pm$ 9.1	62.2 $\pm$ 5.8	53.6 $\pm$ 6.8	$\hat{y} = -51.251^{**}x + 146.08^{**}$	0.93
NL	1149 $\pm$ 96	853 $\pm$ 80	620 $\pm$ 30	558 $\pm$ 50	$\hat{y} = -407.87^{**}x + 1294.4^{**}$	0.94
LA	1478 $\pm$ 110	1066 $\pm$ 90.3	734 $\pm$ 23.3	644 $\pm$ 86	$\hat{y} = -576.63^{**}x + 1686.6^{**}$	0.95
RGR	33.7 $\pm$ 0.3	29.1 $\pm$ 1.1	24.7 $\pm$ 0.3	23.5 $\pm$ 0.8	$\hat{y} = -7.125^{**}x + 36.488^*$	0.97
NAR	0.6 $\pm$ 0.02	0.5 $\pm$ 0.02	0.4 $\pm$ 0.01	0.4 $\pm$ 0.01	$\hat{y} = -0.165^{**}x + 0.659^{**}$	0.90

RDB (root dry biomass, g); SDB (stem dry biomass, g); LDB (leaf dry biomass, g); IDB (inflorescence dry biomass, g); TDB (total dry biomass, g); HEI (height, cm); SLB (specific leaf biomass, g m⁻²); NI (number of inflorescences); NL (number of leaves); LA (leaf area, cm²); RGR (relative growth rate, mg g⁻¹ d⁻¹); NAR (net assimilation rate, mg cm⁻² d⁻¹).

The most evident morphological differences between treatments were reduced height (16%), number of leaves (51%) and inflorescences (59%) at low Ψ_w (–1.9 MPa) as compared to Ψ_w = –0.5 MPa (Table 1). However, drought stress corresponded to a significant (29%) increase in specific leaf mass at Ψ_w = –1.9 MPa, which was related to the 56% reduction in foliage in this treatment.

Leaf micromorphology

O. africanum had stomata and glandular and tector trichomes on both epidermal faces. The trichomes were classified as peltate glandular, with four secretory cells in the head and protected by a cuticle; multicellular tector trichomes had cuticular ornaments (Figure 3A–C).

The density of capitate, peltate glandular and tector trichomes was influenced by water deficit. The density of glandular trichomes adjusted linearly on the adaxial surface of the epidermis; these trichomes showed a 9-mm² quadratic adjustment on the abaxial surface, with maximum mean values of 10 trichomes mm⁻² for Ψ_w of –1.5 MPa. At Ψ_w = –1.9 MPa, tector trichomes showed a quadratic adjustment with maximum mean value of 23 trichomes mm⁻² for the abaxial surface, and a linear adjustment for the abaxial face with 31 trichomes mm⁻² (Figure 4A and B).

Stomatal density showed quadratic adjustments with significant increases ($P < 0.01$) in the number of stomata in the adaxial and abaxial surfaces at Ψ_w = –1.6 and –1.9 MPa. The maximum stomatal density occurred at Ψ_w = –1.6 MPa, with 10 and 12 stomata mm⁻² in the adaxial and

abaxial surfaces, respectively (Figure 4C).

Essential oil production and water use efficiency

The essential oil content in leaves and inflorescences was significantly affected by water deficit and was highest at the lowest water potentials in branches (Figure 5A). The essential oil yield of leaves and inflorescences decreased significantly with increasing severity of the water deficit ($R^2 = 0.95$) (Figure 5B).

The WUE based on the productivity of total biomass (WUE_{bm}) and essential oil (WUE_{oil}) showed quadratic responses, reaching a maximum of 5.54 g/L at Ψ_w = –1.1 MPa. In leaves and inflorescences, the maximum WUE was 0.75 and 2.7 g/L at Ψ_w = –1.24 and –1.15 MPa, respectively (Figure 6).

Water deficit had little effect on the chemical composition of the essential oil extracted from leaves and inflorescences of *O. africanum* (Tables 2 and 3). Nine chemical compounds were identified in the essential oil; the major component was isoeugenol. There were difference in the composition of the essential oil between leaves and inflorescences: linalool was present only in the leaves; and γ -curcumen, caryophyllene oxide, fenchol and (*endo*)-fenchol only occurred in the inflorescences (Tables 2 and 3). Changes in the content, yield and WUE of essential oil production after 60 days of the treatments caused only small variations in the chemical composition of the oil (Tables 2 and 3). In leaves, the concentration of isoeugenol remained stable at the different water potentials, whereas in inflorescences, the relative

Figure 3. Scanning electron micrograph of leaf surface of *O. africanum*; (A) Overview of epidermis, abaxial surface; (B) Tector trichome; (C) Peltate glandular trichome. Scale bars = 100 μm (A and C), 20 μm (B).

concentration of isoeugenol varied among the treatments (Table 3).

DISCUSSION

Plants subjected to water deficit have mechanisms for acclimating to these environmental conditions, including high level of expression of many genes and transcription factors (Rehem et al., 2012; Osakabe et al., 2014), increase in abscisic acid biosynthesis, stomatal closure and intracellular somatically active solutes accumulation (Lisar et al., 2012; Osakabe et al., 2014). These biochemical and physiological adjustment helps maintain cellular turgor and metabolic homeostasis and can be indicator of capacity of plant respond to drought stress (Akinci and Losel, 2012). All changes can interfere with growth patterns and, in aromatic plants, with essential oil biosynthesis.

Water deficit caused reductions in net photosynthetic rate and stomatal conductance in *O. africanum*. This response pattern was attributed to partial stomatal closure, which affects photosynthesis (Akinci and Lösel, 2012; Lisar et al., 2012), showing that stomata in this species are strongly sensitive to drought. The decreases in stomatal conductance were accompanied by a drop in

water potential throughout the experimental period. The reduced conductance is thus related to a decrease in water lost to transpiration (Guha et al., 2010) and an increase in water use efficiency, which is an important protection mechanism against leaf dehydration and loss of enzyme activity, both of which affect carbon fixation (Parry et al., 2002; Saeidnejad et al., 2013).

Reduced growth in response to drought is common in plants and is one of the first responses to water limitation. Growth is affected by drought through decreased cell turgor pressure, which leads to inhibition of cell expansion (Cominelli et al., 2008; Harb et al., 2010). In the aromatic plants, *Matricaria recutita* L. (Baghalian et al., 2011), *Thymus daenensis* subsp. *daenensis* (Bahreininejad et al., 2013), *Artemisia annua* L. (Marchese et al., 2010) and *Salvia officinalis* L. (Bettaieb et al., 2009), growth changes under water deficit included reductions in leaf area, pigment contents, and leaf, flower and stem biomass. In *O. africanum*, the reduction in biomass production under the most severe water deficit conditions was mainly related to the decreased stomatal conductance and photosynthetic rate. Water availability is essential for cell expansion and division, which are also important during the flowering stage. Water deficit reduced flower size and nectar volume in *Epilobium angustifolium*, although nectar concentration was

Figure 4. Density of peltate glandular trichomes (A) and tector trichomes (B); and stomatal density on the adaxial (circles) and abaxial (squares) surfaces of *Ocimum africanum* at different water potentials (C). Values are means of five replicates; bars indicate standard error.

unchanged (Carroll et al., 2001).

The reduction in relative growth rate of *O. africanum* at lower water potential ($\Psi_w = -1.6$ and -1.9 MPa) suggested physiological changes, which were reflected in the reduced net assimilation rate. Relative growth rate is indicative of adaptations of plants to environmental conditions and is determined by changes in biomass (Hunt, 1990). The reduction in leaf number and area in *O. africanum* with increased water deficit (Table 1) showed strong plasticity to adapt to water availability. A decrease in photosynthetic area is an important mechanism under drought conditions, resulting in less light absorption and water loss by transpiration, and thus in higher water use efficiency (Bahreininejad et al., 2013; Shao et al., 2008).

The allometric relationship observed in the increased

ratio of roots to aerial parts in *O. africanum* reflected a complex system that involves nutrient uptake and vegetative growth to maximize water absorption and assimilate flow in roots, consistent with the functional balance theory (Poorter et al., 2012).

The increased density of stomata and glandular and tector trichomes in *O. africanum* was associated with decreased leaf area in treatments with lower water potential. The presence of tector trichomes on leaf surfaces may contribute to the formation of a humid microclimate that reduces transpiration and heating by helping to reflect solar radiation (Guerfel et al., 2009). This an important morphological characteristic for tolerance to water stress in leaves of amphistomatic species such as *O. africanum*.

Figure 5. Essential oil contents in leaves (○) and inflorescences (Δ) (A); Essential oil yield in leaves (○) and inflorescences (Δ) of *Ocimum africanum* 60 days after water deficit was imposed (B). Values are means of five replicates; bars indicate standard error.

Figure 6. Water use efficiency of biomass production (A) and of essential oil production (B) by *O. africanum* leaves (○) and inflorescences (Δ), 60 days after the start of the treatments. Values are means of five replicates; bars indicate standard error.

In some species in the Lamiaceae family, synthesis and storage of essential oils occur in secretory structures in leaves and inflorescences (Bahreininejad et al., 2013; Bettaieb et al., 2009; Khalid, 2006; Singh-Sangwan et al., 1994). The effects of water deficit on biomass production and glandular trichome density may influence the content, yield (Baghalian et al., 2011) and chemical composition of essential oils (Saeidnejad et al., 2013). In qualitative and quantitative aspects, the essential oils are chemically unstable and may undergo changes

depending on the severity of stress (Sangwan et al., 2001). *O. africanum* under water deficit increased the glandular trichomes density and essential oil content. There were no significant changes in the relative percentage of the main constituents of the essential oil from the leaves and inflorescence.

An effect of drought on water use efficiency per liter of essential oil produced in aerial parts was observed in *Thymus daenensis* (Bahreininejad et al., 2013), and here, flowers of *O. africanum* had higher essential oil yield and

Table 2. Relative percentages of components in essential oil extracted from leaves of *Ocimum africanum* under water deficit treatments. KI: (Kovats indices).

Compound	KI literature	KI experiment	Predawn water potential (–MPa)			
			0.5	0.9	1.6	1.9
			Relative percentage			
Linalool	1098	1095	0.76	0.28	0.70	0.38
Methyl(Z)-cinnamate	1301	1302	4.95	4.51	5.09	5.19
(Z)-isoeugenol	1402	1396	92.00	94.24	92.89	93.54
(E)-caryophyllene	1418	1425	0.63	0.49	0.71	0.47
Cis-thujopsene	1429	1433	0.54	0.43	0.62	0.43
Oxygenated monoterpenes			5.71	4.79	5.79	5.57
Phenylpropanoids			92.00	94.24	92.89	93.54
Sesquiterpenes			1.17	0.92	1.33	0.90
Total identified (%)			99.88	99.94	100	100

Table 3. Relative percentages of components in essential oil extracted from inflorescences of *Ocimum africanum* under water deficit treatments.

Component	KI literature	KI experiment	Predawn water potential (–MPa)			
			0.5	0.9	1.6	1.9
			Relative percentage			
Fenchol	1087	1096	0.91	0.84	0.58	0.58
(Endo)-fenchyl acetate	1220	1218	0.11	0.20	-	-
(Z)-methyl cinnamate	1301	1302	2.25	2.41	1.56	2.36
(Z) isoeugenol	1402	1400	89.27	88.70	92.59	90.50
(E)-caryophyllene	1418	1427	2.92	3.09	1.97	2.26
Cis-thujopsene	1429	1434	230	2.73	1.76	2.18
γ-Curcumene	1480	1484	1.56	-	1.54	1.32
Caryophyllene oxide	1581	1580	0.31	0.21	-	0.53
Oxygenated monoterpenes			3.27	3.45	2.14	2.93
Phenylpropanoids			89.27	88.70	92.59	90.50
Sesquiterpenes			6.77	5.83	5.27	5.77
Oxygenated Sesquiterpenes			0.31	0.21	-	0.53
Total identified (%)			99.62	98.19	100	99.2

KI: Kovats indices.

WUE at the lowest water potential.

These results suggest that the plants invested proportionally more photoassimilate in the production of essential oil per water unit consumed at Ψ_w of -0.9 MPa, primarily in flowers. Understanding the effects of drought on essential oil production in these organs will help to maximize the raw material obtained before and during harvest. In *O. africanum*, essential oil content was higher in leaves than in inflorescences, and the largest density of glandular trichomes was found in the leaves. In addition, the highest essential oil content occurred under the most severe water deficit conditions. Simon et al. (1992) observed an increase in essential oil content in *O. basilicum* from mild (-0.68 MPa) to moderate (-1.12 MPa) water deficit, from 3.1 to $6.2 \mu\text{L g}^{-1}$.

Increased essential oil content under water stress has been attributed to the production of high concentrations of terpenes, isoprenoids and phenylpropanoids by plants (Delfine et al., 2005). In *O. africanum*, the essential oil yield of leaves and inflorescences was inversely proportional to the essential oil contents in those organs. Thus, this study verified that water deficiency promoted increased essential oil content but decreased essential oil yield in this species. This finding is attributed to reduced numbers of leaves and inflorescences under water deficit, which led to lower biomass.

Understanding the relationship between water use and essential oil production and quality is fundamental in order for the cultivation of aromatic plants under drought to be economically viable. Furthermore, the presence of

essential oil in leaves and inflorescences, and differential responses of essential oil content and yield as observed in this study, may reduce the cost of processing by the harvest of the organs and drying period. Here, for *O. africanum*, increased essential oil content and water use efficiency in the production of essential oil were demonstrated for both organs under moderate water deficit. Methyl (Z)-cinnamaldehyde, caryophyllene and tujupesno were observed in the essential oil of leaves and inflorescences. However, isoeugenol was the major component in both organs, while derivatives of fenchol were observed in the oil of inflorescences. Isoeugenol is a chemical isomer of eugenol, which is known for its antifungal, antibacterial and antioxidant properties (Dubey et al., 2000; Lemos et al., 2005; Pereira and Maia, 2007), and which is broadly used in the perfume, cosmetic and pharmaceutical industries (Oliveira et al., 2008; Costa et al., 2010). Furthermore, eugenol and isoeugenol obtained from essential oils are viable economical sources for the production of valine by bioconversion (Daugusch and Pastore, 2005; Zhao et al., 2006; Ashengroph et al., 2012). Thus, considering the elevated concentration of isoeugenol in leaves and inflorescences even under water deficit, *O. africanum* may hold potential as a source of eugenol and vanillin for industry. In addition, the cost of managing the harvest and post-harvest may be reduced because leaves and inflorescences do not need to be processed.

Conclusion

The results presented here showed that irrigation at 80% of the field capacity (water potential of -0.9 MPa) provides the best condition for the growth and production of essential oil in *O. africanum*. Under these conditions, the yield and quality of essential oil make cultivation of *O. africanum* economically viable. No change were observed in the chemical composition of the essential oil in leaves and inflorescences, and the essential oil content and the quantity of oil produced per unit water consumed increased under moderate water deficit. More detailed studies are necessary to support the development of appropriate field management conditions to achieve maximum essential oil yield while conserving water resources.

Conflict of Interests

The authors have not declared any conflict of interests.

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