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Effect of Water Supply on the Physiological Characteristic and Production of Basil (*Ocimum basilicum* L.)

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Summary

Different levels of soil water capacity (SWC 30-50-70 %) resulted in significant changes in physiological parameters of *Ocimum basilicum* L. 'Genovese' in a pot experiment. Compared to the control plants (water supply to 70 % SWC) the driest condition (30 % SWC) caused a decrease of the relative water content (RWC) in the plants by 20 %. Additionally, their water potential was reduced to 45 % of the control plants. The same treatment increased the concentration of malondialdehyde (MDA) indicating the level of oxidative stress, by 52 % compared to the leaves of the control plants. However, under a moderate water deficit (50 % SWC), the plants exhibited a less severe decrease in RWC and water potential, as well as less oxidative stress. Although the reduction of the SWC to 50 % did not significantly affect the dry shoot mass, a more severe water stress (30 % SWC) reduced it by 34 %, compared to the control plants. The essential oil concentration per plant dry mass showed only a slight increase in consequence of drought stress (significant at 90 % confidence level). The essential oil production calculated to a single individual is determined basically by the changes in the biomass, thus, the lowest results were found in the driest soil. Water supply modified the quantitative composition of the oil: the proportion of linalool decreased from 59.68 to 44.39 %. The samples of the stressed plants contained some minor components, (ß-myrcene, 2-octanone) not present in the control samples.

Key words. 'Genovese' – essential oil – malondialdehyde – stress – RWC – water deficit – water potential

Introduction

Basil (*Ocimum basilicum* L.) is a well-known aromatic plant from the Lamiaceae plant family. Its wide range of applications; such as phytotherapy and as a spice, include new areas too (e.g. pest control). This plant species is morphologically variable regarding size, shape and colour of the leaves, for example. The essential oil content varies between 0.2–5.2 ml 100g^{-1} dry weight (DW) (Szabó et al. 1996; Simon et al. 1999; Telci et al. 2006) and until now almost 140 components have been identified in the essential oil of *O. basilicum* (Hiltunen and Holm 2006). Based on the main components – linalool, estragole, eugenol, methyl cinnamate – different chemotypes have been described (Grayer et al. 1996; Telci et al. 2006).

Basil, native to the subtropical areas, prefers humus rich, well drained loamy or sandy-loamy soils. Although in Central and South Europe the biomass production is mainly limited by the amount of precipitation, there is no adequate data for the optimal parameters. The results regarding the optimal water-supply of essential oil bearing plants are often contradictory, indicating that this scientific question calls for a complicated answer.

A mesophyte species, *Tanacetum vulgare* L., needs regular irrigation with high amounts of water in order to produce both optimal drug yield (dried shoot mass) and high essential oil content (Zámboriné and Héthelyiné 1985). In the case of chamomile (*Matricaria chamomilla* L.) a plant species also adapted to arid conditions, irrigation does not influence the essential oil content, while in the xerophyte *Hyssopus* species its level may be decreased significantly as a result of abundant precipitation (Németh et al. 2001). According to Penka (1978), the essential oil content of angelica (*Archangelica officinalis* L.), caraway (*Carum carvi* L.), fennel (*Foeniculum vulgare* Mill.) and anise (*Pimpinella anisum* L.) has been increased due to regular irrigation, while in the case of coriander (*Coriandrum sativum* L.) and lavender (*Lavandula spica* L.) the opposite effect occurred. Also water supply may significantly influence the essential oil composition (e.g. Pank 1991; Gosztola et al. 2010). However, the effect seems to be highly dependent on the target species and influenced by other factors, too.

According to our previous results a water supply of 140–480 mm year^{-1} can positively influence the drug yields of basil; however, it has no effect on the essential oil content (Zámboriné et al. 2005). The drought stress indicated decrease in the drug yield and a possible increase of the essential oil level was confirmed by other authors, too (Simon et al. 1992; Khalid 2006). Concerning the results of Simon et al. (1992), the excessive lack of water

can positively influence the ratio of linalool and methyl chavicol in the essential oil, while the ratio of total sesquiterpenes decreases. In contrast to the previous results KHALID (2006) did not find any obvious relation between the water supply and the essential oil composition of basil.

Consequently, the optimal water supply needs to be determined for each species specifically. The range of optimal water supply for essential oil accumulation and biomass production for basil is still unknown. Therefore our aim was to detect the physiological modifications indicating water stress conditions for *O. basilicum* L. Further we intended to discover changes in biomass, drug production, essential oil content and composition as a consequence of different levels of water supply.

Material and Methods

Plant growing parameters

O. basilicum L. 'Genovese' cultivar was used as plant material. This cultivar is absolutely free of methyl-chavicol (COPETTA et al. 2006; CAROVIĆ-STANKO et al. 2010). Plants were grown in a growth chamber (type Conviron E-15) in pots (1.6 L). The medium above a gravel drainage (140 g, diameter: 5–8 mm) consisted of a combination of Rekyva Remix D soil mixture, black peat and perlite in a ratio of 7:2:1. Three plants were planted in each pot in a two-leaf stadium. The stress treatment was initiated 1 week after planting and the experiment spanned 95 d from sowing until harvesting.

The climatic program of the chamber was the following: 14 h day/10 h night cycle, (light intensity: 14500 lux; fluorescent lamp (4200 K) and incandescent lamp (2700 K)) temperature program: 25 °C day/17 °C night, relative humidity 65 %. Plants were fertilized weekly with Wuxal Super complex fertilizer (Aglukon Spezialdünger GmbH & Co. KG; Düsseldorf, Germany), 100 ml 0.4 % solvent was added weekly to each pot.

Treatments

In every treatment two levels of water supply were applied: for modelling a slight drought stress effect (S1) we used a 50 % saturation of SWC, while to induce a severe stress effect (S2), the saturation level of SWC was 30 %. The control (K) plants were grown in soil with a 70 % saturation level of SWC. SWC was determined using the gravimetric method. Both SWC and the water supplement were checked out 3 times per week.

Water potential

The water potential (ψ) 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 9 replications (9 plants) per treatment in full flowering phase, 2 d after watering, between noon and 2 p.m. in a pressure chamber (Model 610, PMS Instrument Company, Albany, USA). As a gas CO_2 was used (SCHOLANDER et al. 1965).

Relative water content (RWC)

The relative water content (RWC) was analysed in full flowering period from leaf samples from the third nodes under the top of the shoots (in 6 replications per treatment). Disks with a diameter of 12.7 mm were cut from the leaves. After determining fresh weight (FW), they were immersed in distilled water for 6 hours (to estimate turgid weight – TW), then the disks were dried at 60 °C for 24 h (to measure dry weight – DW). The RWC was determined with the following formula:

RWC (%) = ((FW–DW)/(TW–DW))×100 (WEATHERLY 1950; BARRS 1968).

Malondialdehyde (MDA) content

The determination of MDA content was carried out in the full-sized leaves collected in full flowering period. Lipid peroxidation was measured in terms of MDA content described by DHINDSA et al. (1981). A fresh sample of 0.150 g of leaves was extracted in 450 μL 0.1 % (v/v) trichloroacetic acid. The homogenate was then centrifuged at 10000 g for 10 min. The supernatant (250 μL) was mixed with 1.0 ml of 20 % (v/v) trichloroacetic acid containing 0.5 % (v/v) thiobarbituric acid, then incubated in boiling water bath for 30 min, and cooled immediately. Absorbance was recorded at 532 nm and corrected for non-specific absorbance at 600 nm. MDA content was calculated using the extinction coefficient of 156 $mM^{-1}cm^{-1}$. All measurements were made in three replications.

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Determination of dry plant mass, essential oil concentration and composition

The essential oil content and the biomass production were determined in the shoots collected in full flowering period. The plant material was dried at room temperature (22–24 °C). All the dried shoots existing above-ground were measured by analytical scale in 9 replications (9 plants). The essential oil content was measured from this material, in three replication per treatment with a Clevenger-type apparatus according to the VII. Hungarian Pharmacopoeia.

GC analysis was carried out using an Agilent Technologies 6890 N instrument equipped with HP-5 and HP-5MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness), working with the following temperature program: initial temperature 60 °C, heating by a rate of 3 °C min^{-1} up to 240 °C; the final temperature was maintained for 5 min; injector and detector temperatures: 250 °C; carrier gas: helium (constant flow rate: 1 ml min^{-1}); split ratio: 30:1, injection volume 0.2 ml (10 %, n-hexane).

The GC-MS analysis was carried out using an Agilent Technologies 6890 N GC equipped with an Agilent Technologies MS 5975 detector according to the parameters given above. Ionization energy was 70 eV. The mass spectra were recorded in full scan mode, which revealed the total ion current (TIC) chromatograms. A mixture of aliphatic hydrocarbons (C_9–C_{23}) in n-hexane was injected under the above mentioned temperature program to calculate the linear retention indices using the generalized equation of VAN DEN DOOL and KRATZ (1963). The mass spectra and linear retention indices (LRI) were compared with those of commercial (NIST, Wiley) and home-made library mass spectra built up from data obtained from standards (Sigma/Aldrich). The proportion

of the individual compounds are expressed as total area percentages. The GC-MS analysis was carried out in 2 replications per treatment.

Statistical analysis

The results were analysed with a SPSS.17.0 statistical program. One-way ANOVA was applied, and for the pair wise comparisons of the variances, the Duncan test was used.

Results

Relative water content (RWC)

The RWC values of the plants changed significantly as a result of the varying water supply (Table 1). The highest RWC values (an average of 96 %) were measured in the control plants, in a soil saturated to 70 % water capacity (SWC). In the treatments of lower soil saturation levels the RWC values decreased; in the 50 % (S1) treatment we measured 84.5 %, while under the driest circumstances only 77.4 % water content was observed. This shows that in consequence of the increasing lack of water in the soil, the RWC of the basil leaves can decrease by almost 20 %.

Water potential

The varying water supply significantly influenced the water potential of the plants, too (Table 1). The lowest leaf water potential was measured under the highest drought stress conditions (S2). The increasing water saturation level in the soil positively influenced the water potential of the plants. The highest value (−0.48 MPa) was detected in the control (K) plants, while, according to the expectations, the results measured under the medium stress parameters (S1) were between the values of K and S2.

Malondialdehyde (MDA) content

According to our results (Table 1) higher concentrations of MDA could be measured in the plants grown in drier soils. The highest concentration was found in the case of the more severe drought stress (S2); the values were 1.5 times higher than that of the control plants.

Dry mass

The water supply influenced the production of basil, too. The dry mass of the control (K) plants and those originating from the moderate stress conditions (S1) were statistically equal, however, the S2 treatment resulted in significantly lower values (Table 2).

Essential oil content

The highest essential oil concentration was measured under the strongest drought stress (S2) conditions (0.52 ml $100g^{-1}$ DW), while the control (K) plants were characterised by the lowest accumulation level (0.438 ml $100g^{-1}$ DW). Although the detected difference in the essential oil concentration was more than 15 % between the treatments K and S2, it could be proved with only 90 % confidence (Table 2).

Essential oil production

Based on the dried shoot (drug) mass and the detected essential oil concentration we calculated the production

Table 1. Main physiological parameters indicating the drought-stress in basil.

Parameter	Treatment			Sign. (α)
	K (mean ± S.D.)	S1 (mean ± S.D.)	S2 (mean ± S.D.)	
RWC (%)	96.70 a ± 2.21	84.76 b ± 4.56	77.47 c ± 2.37	0.05
Water potential (MPa)	−0.48 a ± 0.04	−0.73 b ± 0.07	−1.05 c ± 0.06	0.05
MDA (nmol g^{-1})	7.05 b ± 0.64	8.58ab ± 2.99	10.71 a ± 1.24	0.10

Water saturation in the soil: K = 70 %, S1 = 50 %, S2 = 30 % of SWC; S.D.: Standard Deviation
The different letters represent significant differences in the means in rows

Table 2. The effects of variations in water supply on the dry shoot mass, essential oil content and essential oil production of basil.

Parameter	Treatment			Sign. (α)
	K (mean ± S.D.)	S1 (mean ± S.D.)	S2 (mean ± S.D.)	
Dry mass(g)	15.220 a ± 2.860	13.300 a ± 1.890	10.000 b ± 1.500	0.05
Ess. oil content (% DW)	0.440 b ± 0.030	0.470 ab ± 0.050	0.520 a ± 0.060	0.10
Ess. oil production (ml $plant^{-1}$)	0.066 a ± 0.004	0.062 b ± 0.004	0.052 b ± 0.006	0.05

Water saturation in the soil: K = 70 %, S1 = 50 %, S2 = 30 % of SWC, S.D.: Standard Deviation; DW: dry weight
The different letters represent significant differences in the means in rows

Table 3. The effects variations in water supply on the essential oil composition (relative composition %) of basil.

	RT	LRI	Treatment		
			K	S1	S2
ß-pinene	6.64	981	n.d.	0.18	n.d.
ß-myrcene	6.99	995	n.d.	n.d.	0.10
2-octanone	7.04	997	n.d.	0.11	n.d.
1,8-cineol	8.38	1034	11.72	12.91	15.45
linalool	10.76	1097	59.68	47.17	44.39
isobornyl-acetate	18.64	1286	3.11	3.48	3.99
eugenol	21.67	1367	6.86	11.85	9.17
metyl-eugenol	23.67	1420	n.d.	0.71	1.02
trans-α-bergamotene	24.36	1437	4.11	5.22	6.08
α-guajene	24.45	1439	0.40	0.37	0.57
α-humulene	25.07	1454	0.21	0.56	0.27
germacrene-D	26.18	1482	1.90	1.87	2.29
bicyclogermacrene	26.81	1497	n.d.	0.33	0.23
δ-guajene	27.16	1506	1.49	2.76	2.97
cis-γ-cadinene	27.49	1515	2.60	2.94	3.63
tau-cadinol	32.26	1644	7.32	7.56	8.91
Total			99.38	97.98	99.04

RT = retention time; LRI = linear retention index relative to C9–C23 n-alkanes on a HP-5MS capillary column; K = control (SWC 70 %); S1 = stress 1 (SWC 50 %); S2 = stress 2 (SWC 30 %); n.d. = not detected

of essential oil per plant realized under the different water regimes. According to the results the relative increase of the accumulation of essential oil under the strongest stress effect (S2) cannot compensate the loss of the dry mass. Therefore, the essential oil production, calculated for an individual plant, is significantly lower compared to both the control plants (K) and the plants under medium stress conditions (S1), (Table 2).

Essential oil composition

16 compounds could be identified in the essential oil (Table 3). In each treatment linalool was the main component of the essential oils, however, its ratio decreased in accordance with the low water-level in the soil (K=59.68 % S1=47.17 % S2=44.39 %). Parallel with the decrease of the linalool content, the ratio of the minor compounds increased slightly (1,8-cineol, eugenol, trans-α-bergamotene and tau-cadinol). In the essential oil of the control (K) plants the number of detected compounds is less than in the ones receiving a restricted water supply (S1, S2).

Discussion

The different water saturation levels in the soil (SWC 30-50-70 %) caused significant differences in the physiological characteristics of *O. basilicum* L. 'Genovese' in a pot experiment carried out in a growth chamber.

The RWC of the plants was influenced by the level of water supply. Compared to the control (K) plants grown in a soil with 70 % saturation level of SWC, the strongest drought stress (S2) caused a 20 % decrease in the RWC values. Our results were comparable to results from wheat and tobacco (Broin et al. 2000; Bai et al. 2006). Considering all the applied treatments 70 % SWC seems to be optimal for basil, since under this value the plant needs to decrease its osmotic potential significantly. The decrease of the water potential of basil can reach more than 50 % if it is grown in a soil containing only 30 % SWC.

Even the medium water supply (S1) seems to induce an oxidative stress condition in the plant's tissues, which becomes even more emphasized under higher water shortage (S2). The increasing level of the free radical scavenger MDA might be a consequence of the increased level of free radicals in the organism (Ge et al. 2006). The MDA content changes according to the intensity of the stress effect, as a result of the stronger drought stress (S2), the MDA content increased by 52 % compared to the control (K) plants. Similar results were reported in the case of turfgrass species (Jiang and Huang 2001) and maize (Ge et al. 2006).

It can be concluded that a 50 % saturation level of SWC represent a condition which may induce significant changes in the water balance of the plant tissues and detectable changes in the physiological characteristics of basil. The differences in shoot mass can be proven statistically only in the S2 treatment which shows that the slight drought stress did not influence the shoot mass severely, although at metabolism level we already find measureable changes such as limited RWC and slight oxidative stress. This may have great importance for agricultural practice.

In contrast to the above mentioned characteristics in the case of the essential oil accumulation, a slightly in-

creasing tendency can be observed as a consequence of the drought stress. Our statement is in accordance with the results of SIMON et al. (1992) and KHALID (2006). However, this is a characteristic which has hardly been influenced by the water supply. The increase of the essential oil concentration in plants treated with the lowest water supply (S2) was confirmed by the statistical analysis at only of 90 % confidence level. Therefore, the essential oil production of a plant individual is mainly affected by the biomass production, which is much lower in the drier soils.

Changes in the composition of essential oil caused by drought stress were also measured. The ratio of the main compound, linalool, decreased, beside which the spectrum of components (number of the detected essential oil compounds) changed. Although the main compounds remained the same, some minor compounds occurred in the essential oils of the S1 and S2 treatments (ß-myrcene, 2-octanone). These compounds are known in basil (HILTUNEN and HOLM 2006) however, there has not been any reference to their connection with water supply till now.

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References

BAI, L., F. SUI, T. GE, Z. SUN, Y. LU and G. ZHOU 2006: Effect of soil drought stress on leaf water status membrane permeability and enzymatic antioxidant system of maize. Pedosphere **16**, 326–332.

BARRS, H.D. 1968: Determination of water deficits in plant tissues. In: KOSOLWSKI, T.T. (ed.): Water deficits and plant growth. Vol. **1**. Academic Press, New York, 235–368.

BROIN, M., S. CUINÉ, G. PELTIER and P. REY 2000: Involvement of CDSP 32, a drought-induced thioredoxin, in the response to oxidative stress in potato plants. FEBS Letters **467**, 245–248.

CAROVIĆ-STANKO, K., S. ORLIĆ, O. POLITEO, F. STRIKIĆ, I. KOLAK, M. MILOS and Z. SATOVIC 2010: Composition and antibacterial activities of essential oils of seven *Ocimum* taxa. Food chem. **119**, 196–201.

COPETTA, A., G. LINGUA and G. BERTA 2006: Effect of three fungi on growth, distribution of glandular hairs, and essential oil production in *Ocimum basilicum* L. var. 'Genovese'. Mycorrhiza **16**, 485–494.

DHINDSA, R.S., P. PLUMB-DHINDSA and T.A. THORPE 1981: Leaf senescence: correlated with increased leaves of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J. Exp. Bot **32**, 93–101.

GE, T., F. SUI, L. BAI, Y. LU and G. ZHOU 2006: Effects of water stress on the protective enzyme activities and lipid peroxidation in roots and leaves of summer maize. Agr. Sci. in China **5**, 291–298.

GOSZTOLA, B., Sz. SÁROSI and É. NÉMETH 2010: Variability of the essential oil content and composition of chamomile (*Matricaria recutita* L.) affected by weather conditions. Nat. Prod. Com. **5**, 465–470.

GRAYER, R.J., G.C. KITE, F.J. GOLDSTONE, S.E. BRYAN, A. PATON and E. PUTIEVSKY 1996: Infraspecific taxonomy and essential oil chemotypes in sweet basil, *Ocimum basilicum*. Phytochemistry **43**, 1033–1039.

HILTUNEN, R. and Y. HOLM 2006: Essential oil of *Ocimum*. In: HILTUNEN, R. and Y. HOLM (ed.): Basil. The Genus *Ocimum*. Harwood Academic Publisher, Amsterdam, 77–111.

JIANG, Y. and B. HUANG 2001: Drought and heat stress injury to two cool-season turfgrasses in relation to antioxidant metabolism and lipid peroxidation. Crop. Sci. **41**, 436–442.

KHALID, Kh.A. 2006: Influence of water stress on growth, essential oil, and chemical composition of herbs (*Ocimum* sp.). Int. Agrophy. **20**, 289–296.

NÉMETH, É., É. VARGA and R. FRANKE 2001: Variabilität des ätherischen Ölgehaltes und deren Ursachen in *Hyssopus officinalis* L. Z. Arzn. Gew. Pfl. **6**, 29–34 (In German).

PÁNK, F. 1991: The influence of sprinkler irrigation on the quality of medicinal and aromatic plants, Planta Med. **57**, 32–33.

PENKA, M. 1978: Influence of irrigation on the contents of effective substances in officinal plants, Act. Hort. **73**, 181–197.

SCHOLANDER, P.F, H.T. HAMMEL, E.A. BRADSTREET and E.A. HEMMINGSEN 1965: Sap pressure in vascular plants. Science **148**, 339–346.

SIMON, J.E., D. REISS-BUBENHEIM, R.J. JOLY and D.J. CHARLES 1992: Water stress-induced alterations in essential oil content and composition of sweet basil. J. Ess. Oil Res. **4**, 71–75.

SIMON, J.E., M.R. MORALES, W.B. PHIPPEN, R.F. VIEIRA and Z. HAO 1999: Basil: A source of aroma compounds and a popular culinary and ornamental herb. In: JANICK, J. (ed.): Perspectives on new crops and new uses. ASHS Press, Alexandria, 499–505.

SZABÓ, K., É. NÉMETH, L. PRASZNA and J. BERNÁTH 1996: Morphological variability of basil genotypes. Proc. International Symposium Breeding Research on Medicinal and Aromatic Plants (June 30–July 4, 1996), Quedlinburg, Germany, 76–79.

TELCI, I., E. BAYRAM, G. YILMAZ and B. AVCI 2006: Variability in essential oil composition of Turkish basils (*Ocimum basilicum* L.). Biochem. Syst. and Ecol. **34**, 489–497.

VAN DEN DOOL, H. and P. KRATZ 1963: A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatogr. A **11**, 463–471.

WHEATHERLEY, P.E. 1950: Studies in the water relations of the cotton plant. 1. The field measurements of water deficits in the leaves. New Phytol. **49**, 81–97.

ZÁMBORINÉ, N.É. and I. HÉTHELYI 1985: Data on the cultivation of a new medicinal plant, *Tanacetum vulgare*. Herba Hung. **24**, 77–96.

ZÁMBORINÉ, N.É., G. TANÍTÓ, I. NOVÁK and P. RAJHÁRT 2005: Optimalisation of production of some medicinal species in consequence of the climatic change. Agro 21 Series, Hungarian Academie of Sciences, Budapest, 158–168.

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