EFFECT OF WATER SUPPLY ON THE PHYSIOLOGICAL CHARACTERISTICS, PRODUCTION AND ACTIVE SUBSTANCES OF SWEET BASIL (*OCIMUM BASILICUM* L.) AND SUMMER SAVORY (*SATUREJA HORTENSIS* L.)

DOCTORAL THESIS

PÉTER RADÁCSI

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1. SCIENTIFIC BACKGROUND AND AIMS OF THE STUDY

For a long time it was the typical characteristic of the Hungarian medicinal- and aromatic (MAP) plants sector that MAPs were cultivated on poor quality fields where other crops could not be grown in a profitable way. MAPs could be cultivated effectively with a small financial investment. However, for a long time medicinal plants were not irrigated at all because the expenditure of watering could make the cultivation unprofitable. Intensively cultivated and labour work required crops (e.g. sweet basil, marigold) were only irrigated in case of extremely arid conditions. The changes of the market make these tendencies questionable: nowadays in many countries of the world an intensive MAP cultivation takes place, although formerly they had only been importing medicinal plants. This tendency motivated us to improve the quality of cultivation of Hungarian MAPs. Based on the publication of Lange (1998) Hungary is an important exporter of the medicinal plants. To solidify and strengthen our current position on the world market, Hungarian MAP cultivation and processing must be improved and optimized. Previous studies proved that the production of secondary metabolites, the quality and quantity of biologically active compounds strongly depend on the environmental factors (Bernáth, 1986). The predicted changes in our climate and weather conditions may significantly modify the cultivation system of our medicinal plants.

Basic condition of the effective and profitable MAP cultivation is the successful modification of the cultivation technologies as it is required by the predicted climate changes. The volume of water supply and irrigation technology are getting more important as well as the well-chosen cultivars and agro-technology. The volume and way of watering need a well-established decision before they would be used in the cultivation.

Being able to decide the need of supplementary watering we definitely have to know the responses of the crops on the increased air temperature and dryer weather conditions.

Based on previous studies, the predicted warming-up may not have the same effect on each of the plant species. Most likely it modifies the quality and quantity of the the level of biologically active compounds of species or chemotaxa (Bernáth és Németh, 2004).

Our experiments were carried out with two model species which (based on our previous experiences and scientific literature available) have different water needs: sweet basil (*Ocimum basilicum* L.) and summer savory (*Satureja hortensis* L.).

Aims of our studies:

- Investigating the production biology of the two plant species with different water needs. Detecting the effect of drought stress on the production biology of the two species.
- Investigation of the physiological parameters which can describe the water balance of the model species. Measurements of relative water content, chlorophyll content, stomatal conductance and water potential, and proving their connection with the biomass and production of secondary metabolites.
- Effect of the modification of water supply on the essential oil and its main active components of basil and savory. Detection of changes in the essential oil concentration and the components of the oil.
- It was also the aim to clarify if the response on drought stress is universal for both species or a characteristics of the genus, species or cultivars.

To strengthen the reliability of our experiments, they were carried out in open field and under controlled conditions (climatic chamber and pot experiment with exclusion of natural precipitation) as well.

2. MATERIALS AND METHODS

In our experiments two model species (sweet basil – *Ocimum basilicum* L. and summer savory – *Satureja hortensis* L.) were investigated. The different species and cultivars used in the experiments are listed in Table 1.

Table 1. Investigated species and cultivars

Year	Type of experiment	Species	Cultivar
2008	Climatic chamber	*Ocimum basilicum*	’Keskenylevelű’
		Satureja hortensis	’Budakalászi’
	Field experiment	*Satureja hortensis*	’Budakalászi’
2009	Climatic chamber	*Ocimum basilicum*	’Genovese’
		Satureja hortensis	’Budakalászi’
	Field experiment	*Ocimum basilicum*	’Genovese’
			’Kasia’
			’Keskenylevelű’
			’Wala’
		Satureja hortensis	’Budakalászi’
2010	Climatic chamber	*Ocimum basilicum*	’Genovese’
		Satureja hortensis	’Budakalászi’
	Field experiment	*Ocimum basilicum*	’Genovese’
			’Kasia’
			’Keskenylevelű’
			’Wala’
		Satureja hortensis	’Budakalászi’
	Semi-controlled field experiment	*Ocimum basilicum*	’Genovese’
		Satureja hortensis	’Budakalászi’

2.1. Growing conditions in the climatic chambers

In our experiments two Conviron E-15 type climatic chambers were used.

Based on our previous experiences the climatic program of the chambers was the following: 14 hours day/10 hours night cycle, (light intensity: 14500 lux; fluorescent lamp (4200K) and incandescent lamp (2700K)) temperature program: 25°C day/ 17°C night, relative humidity 65%.

Plants were grown in a growth chamber in pots (1.6 l). In each pot a gravel drainage (140 g, diameter: 5-8 mm) was set. The pots were irrigated through a pipe which drove the water to the drainage level.

Three levels of water supply were applied: for modelling a slight drought stress effect (S1) we used a 50 % saturation of soil water capacity (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 3 times per week.

2.2 Growing conditions in the open field experiments

Open field small plot experiments were carried out in the Experimental and Research Farm of the Corvinus University of Budapest in Soroksár.

The area of the experimental field is one of the driest parts of Hungary. The soil is mainly sandy with low mould content and the water holding capacity is low.

Propagation was carried out under greenhouse conditions. In the 1-2 leaf stadium the seedlings were transplanted to 0.1 litre pots. After a short incubation period the plants were planted to small plots in a spacing of 50×30 cm. In each parcel 36 plants were planted.

To identify the effect of water supply, irrigated and non-irrigated control treatments were applied. The irrigated plots were watered with 20 mm water twice a week while the non-irrigated ones got only the natural precipitation. A spraying system was used. The amount of water was checked by a water meter. 1 m isolation distance was kept between the irrigated and non-irrigated plots.

2.3. Growing conditions in the semi-controlled pot experiments

Pots (n = 5 per treatment) without drainage were filled with 10 L sandy soil of the Experimental Field. Three seedlings of 2 normal leaves were planted into each pot (19.05.2010). The soil volumetric water content (SVWC) was determined by HH2 moisture meter and ML2x Theta probe (Delta-T, Cambridge, UK). Two irrigation regimes were set while natural precipitation was locked out. In the first treatment (T1) plants were irrigated with 1L water per pot when the SVWC decreased less than 20%. In the second treatment (T2) the plants were irrigated with 0,5 L water when the SVWC decreased under 10%.

A second factor of the experiment was the harvesting time. Each plant was harvested two times: in full flowering phenophase and after re-shooting again, in the flowering phase.

2.4. Methods of measurements

2.4.1. Measurement of water potential

The water potential (ψ) of the basil plants was determined in the leaves of the second and third nodes under the top of the shoots. In case of savory the top of the shoots was used. The measurements were carried out in 9 replications (9 plants) in full flowering phase. The water potential was measured 2 days 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.

In the semi-controlled experiment SKYE SKPM 1405 type pressure chamber was used while the used gas was nitrogen. 10 replications per treatment were carried out.

2.4.2. Measurement of chlorophyll content

Chlorophyll content of the leaves was determined by SPAD-502 chlorophyll meter. Measurements were carried out in the day before harvesting. Leaves of 3-4th internode from the top of the shoots were measured. 8 measurements were carried out in each leaf and 10 leaves were measured in each treatment.

2.4.3. Determination of relative water content

Relative water content was determined by the modified method of Turner and Thomas (1998).

In 2009 disks with a diameter of 12.7 mm were cut from the basil leaves. After determining fresh weight (FW), they were immersed in 0,5 mM $CaSO_4$ for 24 hours (to estimate turgid weight – TW), then the disks were dried at 105 °C for 24 hours (to measure dry weight – DW). The relative water content (RWC) was analysed in full flowering period from leaf samples from the third nodes from the top of the shoots (in 6 replications/treatment).

The leaf disks of savory were so small it was not possible to determine the DW that with an analytical scale. From 2009 on, whole leaves of basil and savory plants were collected in a full flowering stage in 8 replications for each treatment.
The RWC was determined according to the following formula:

$$\mathbf{RWC\ (\%) = [(FW-DW)/(TW-DW)] \times 100}$$

2.4.4. Determination of stomatal conductance

Stomatal conductance of both species was determined by AP4 porometer (Delta-T Devices, UK). Samples of basil were taken from the 3-4th internode from the top of the shoots. In case of the savory, samples could only be taken from the lower – wide – leaves because the size of the upper leaves did not cover the sampling chamber.

Measurements were implemented on full flowering plants two days after the irrigation treatment in 18 replications.

2.2.5. Measurement of plant height and parameters of production biology

The plants in the growth chamber were harvested once in a full flowering phenophase.

Before harvesting, the plant height was measured in 10 replications in each treatment. The fresh weight of plants was measured immediately after cutting, with a digital scale.

Plant material was dried in a shadow in room temperature until the stems could be easily broken. The mass of dry plant material was measured. Leaves and flowers were separated from the stems and the leaf-stem ratio was determined. Leaf ratio includes not only the leaves but also the generative organs.

Plants in open field were harvested also in full flowering phenophase. Plants were cut above the first internodes from the ground level. Height (tallest shoot) was measured in 10 replications. After harvesting, the fresh weight was measured and the plant material was dried in a drying frame in shadow. After drying the dry weight and leaf-stem ratio was determined.

2.2.6. Measurement of glandular hair density

Identification of "peltate-type" glandular hair density was carried out in 2009 in the growth chambers and also in open field. Plants were in full flowering phenophase.

Basil leaf samples were collected from the 3rd internodes from the top. 4 mm diameter samples were cut from the middle of the leaf avoiding cutting the main vain of the leaf. Samples were investigated under a stereo microscope.. Gland density was investigated on the abaxial surface.

Savory samples were collected from the leaves 5-6 cm from the top. Because of the narrow leaves of savory, 3.2 mm diameter disks – with the main vain in the centre - were cut. Gland density was investigated also on the abaxial surface.

From the plants which were grown in the growth chambers 15 replication were carried out. In the open field experiments only the basils gland density was measured in 10 replications per treatments. In the field experiments savory's gland density was not measured.

2.2.7. Measurement of essential oil concentration

Essential oil concentration was measured with Clevenger-type apparatus according to the VII. Hungarian Pharmacopoeia. Dry plant material was used for the distillation. In 2008 the above-ground plant parts were distilled, including the stems. From 2009 the stems were removed from the distilled plant materials in this way only the leaves and flowers were used. From the beginning of boiling the distillation was continued for two hours. Distillation was repeated 2-4 times depending on the amount of available plant material. Until the identification of essential oil compounds the essential oil was stored in a fridge.

2.2.8. Identification of essential oil composition

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). Ionization energy was 70 eV.

The mass spectra were recorded in full scan mode, which revealed the total ion current (TIC) chromatograms. The mass spectra and linear retention indices (LRI) were compared with mass spectra of those of commercial (NIST, Wiley) and home-made libraries built up from data obtained from standards (Sigma/Aldrich).

2.2.9. Analysis of flavour compounds with SPME technique

For analysing the flavour compounds of the two species 20 ml glass vials with screw cap were used. The vials can be closed hermetically; the samples were taken through a septum. Into each vials 2.5 g fresh plant material collected from the flowering shoots was filled. After closing the vials for 30 minutes the vials were incubated in a room where the temperature was 19 °C. Sampling was carried out due to septum for 10 minutes. Injection was done manually. The absorbent fibre (Supleco, 100 µ polydimethyle-siloxane) was injected for 33 seconds. The temperature and flow programme was the same as it was used for identification of essential oil compounds.

2.3. Methods of statistical analysis

Data was analysed by the Microsoft Office 2003 and PASW 20.0 software.
If the criteria of parametric probes were met, one way or multiple factor analysis of variance was carried out depending on the number of factors. If the normal distribution of data was not met and the data allowed it, they were transformed. For the pairwise comparisons of the variances – if the homogeneity of deviation was accepted - the Tukey HSD post hoc test was used. If the homogeneity of deviation did not meet for the pairwise comparison Games-Howel test was used.

The correlation between measured parameters was tested by Pearson type correlation test.

3. RESULTS AND DISCUSSION

During our experiments we determined that in both species the decreasing water saturation level in the soil negatively influenced the water potential of the plants. Under controlled conditions, the drought stress caused 25-170 % decrease in the water potential of basil while the decrease in savory was 44-380 %. Based on our experiences the water potential of savory is usually lower than the water potential of basil. The changes of chlorophyll content (SPAD unit) were more expressed in the climatic chambers. In some experiments the SPAD unit increased by 50 % due to the applied drought stress. In the open field experiments this tendency was less characteristic: the chlorophyll content of savory has not been modified by the irrigation level. Positive correlation was found between the RWC of the plants and the soil water level but no significant differences were found between the relative water content of the two studied species. Highest RWC of basil was 97.5 % while the lowest was 65.4 %. In savory, the corresponding values were 93.1 % and 75,2 % respectively.

Stomatal conductance of the basil and savory plants decreased intensively if the soil water level was lower. The basil's stomatal conductance was higher (131 $mmol \cdot m^{-2} \cdot s^{-1}$) than that of the savory's (77 $mmol \cdot m^{-2} \cdot s^{-1}$) if the soil was well saturated.

Lack of water influenced negatively the production parameters and plant height in case of both model plants. 19-26 % decrease in the plant height of basil was measured as the result of lower water supply. In climatic chamber the height of savory also decreased (22-23 %) compared to the control but in open field we did not find significant difference. Fresh- and dry weight of the plants decreased if the soil water capacity was reduced from 70 % to 30 %. The difference in basil was 28-30 % while in savory 32-47 % was measured. In 2010, when the natural precipitation was more than usual, only the basil's fresh weight was modified by the irrigation. Fresh weight of savory has not changed significantly. The leaf ratio of basil slightly increased (1-7 %) under drought stress while that of savory did not alter (1-3 % changes).

The density of glandular hairs on the leaf surfaces of basil and savory plants increased under lower water capacity. Increase of glands under low soil water capacity was more than 40 % in savory and more than 78 % in basil compared to the control treatment. In open field experiments the irrigation did not influence the density of glandular hairs; however the no differenc among basil cultivars was detected. The highest gland density was measured on the leaves of 'Genovese'.

In contrary to the high number of experiments it is not possible to formulate a clear statement on the effect of the water supply on the essential oil concentration. Under controlled conditions the different water capacity influenced only the savory's essential oil concentration (EOC): highest value was measured in 50 % of the soil water capacity. Compared to this, 8 % decrease was measured in the control pots and 10% under 30 % of soil water capacity. In 2009 (this year was extremely dry) the EOC of both basil and savory was higher in the irrigated control plants, however the essential oil yield of the plants was significantly higher on the irrigated plots. In 2010 (wet, rainy year) we could not detect any differences in the EOC between the different water supply treatments. In the semi-controlled condition the savory's EOC increased under the lower water supply while in case of basil the irrigation treatment did not influence it. The harvesting time did not have an effect on the EOC of basil.

We did not find any tendency in changes of the essential oil composition of savory due to the treatments. Main components were the *carvacrol* and *γ-terpinene* in each case. In the contrary different water supply influenced the essential oil composition of basil: ratio of *linalool* decreased (10-15 %) while the *1,8-cineole* and *tau-cadinole* increased due to the lack of water.

Headspace volatile composition of the two investigated species has been identified. The major components of the plants were the same as in the distillate oil. We did not find significant modifications in the essential oil composition due to the different water supply.

During our research work we described the physiological parameters, production biology and active substances of two annual plants: the sweet basil and summer savory. We established that the investigated physiological parameters (water potential, chlorophyll content, relative water content and stomatal conductance) represent well the ratio of drought stress. The tendencies of changes in the physiological parameter were the same in the two model plants however their scale were different. These differences may explain the different water demand of the two species but also could reflect to the drought tolerance.

The production of secondary materials was negatively influenced by the lack of water. In some cases the drought stress increased the accumulation level of the essential oil however, due to biomass reduction, the yield of it was always lower compared to the control. Based on this information we may declare that during the cultivation of both species, additional irrigation seems to be necessary. However, to identify the optimal irrigation regimes and methods of the two examined species (and other medicinal plants) further investigations would be highly recommended.

4. NEW SCIENTIFIC RESULTS

In our research the following new scientific results have been achieved:

The response of two plant species on the different water supply was examined. Detailed information was given about the physiological parameters, production biology, essential oil concentration and composition of sweet basil and summer savory.

1. We determined that the differences in the water potential and stomatal conductance might play an important role in the different drought tolerance of the two investigated species. Both species's water potential decreased intensively as the consequence of lower soil water content. In basil we measured 25-170 % higher water potential in a soil with 30 % of soil water capacity (SWC) while the measurements in savory show even higher (by 44-380 %) values compared to the control treatments (70 % of SWC). Savory's water potential was always more negative than that of basils. The stomatal conductance of the two species was published by us firstly in the scientific literature. If the water supply decreased the stomatal conductance of basil decreased to one sixth. The reaction of savory was less intensive: stomatal conductance decreased until 30 %.

2. Chlorophyll content (SPAD units) and density of essential oil glands on the leaf surface do not seem to be proper features to characterize the effect of drought stress. Although we observed the raise of SPAD units due to decreasing SWC but here a strong correlation with the water content of the leaves and the turgor of the cells might be anticipated. The gland density increased in both species if the water supply decreased. The gland density does not have a definite effect on the essential oil content. It seems to have connection with the turgor of cells. We observed that the SPAD value and the gland density have intraspecific characteristic in the case of sweet basil.

3. The lower water capacity of soil has strong effect on the biomass and dry mass of both species however the changes in the dry mass are less intensive. In basil – if the SWC was 30 % - we measured 28-35 % lower dry mass production compared to the control treatments. In savory the decrease was between 32-47 %. The drought stress has more visible effect on the height of basil than on that of savory. In the case of lower water supply we measured a 1-7 % increase in the leaf ratio of sweet basil and a 1-3 % one in savory, however these increases cannot compensate the reduction of biomass due to lack of water.

4. We proved that the essential oil yield is mainly influenced by the drug yield. It was observed that the essential oil concentration was not strongly influenced by the water supply. I

although in some cases a small increase could be observed in soil with lower SWC. The different water supply influenced the essential oil composition only in basil. We measured an intensive decrease of the ratio of *linalool* as the response of decreased water supply while the ratios of *1,8-cineole* and *tau-cadinole* were increased. These features have not been published previously. The mentioned changes in the composition are relevant only for the distilled essential oil. The flavour compounds (determined by SPME technique) did not change significantly in savory, we did not recognize important changes in the essential oil composition in neither of the extraction methods.

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