THE EFFECT OF WATER DEFICIT ON SOME STRESS PARAMETERS OF *OCIMUM BASILICUM* L. AND *SATUREJA HORTENSIS* L.

DOCTORAL THESIS

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1. SCIENTIFIC BACKGROUND AND AIMS OF THE STUDY

As a result of the indicated environmental changes as well as the strict quality requirements the former cultivation techniques of medicinal plants has to be optimized in order to make them more economical. The basic condition of this optimization process is the ability to influence or to change the formerly elaborated cultivation techniques according to the requirements in as many aspects as possible. In the case of the more frequently occurring climate induced stress adaptation an influencing point can be a good choice of an eco-type or cultivar, the reasonable selection of the field, elimination of the competitor plant species, optimization of the nutrient and water supply. Nowadays the acquisition of the scientific data basic for the intensive cultivation techniques' elaboration and the operation of the modern agricultural methods cannot exist without each other.

Because of the insufficient water supply (smaller than the optimal) the plant-growth and the drug yields can decrease. As a result of the stress the plant metabolic reactions change, the plant suffers from oxidative stress too, therefore its antioxidant defence system is activated. Because of this reason the determination of the optimal water supply is extremely important in those annual, herbaceous spices, where the aim is the maximization of the biomass production and the drug is coming from the areal flowering shoots; and at the same time the drug yield and quality also need to be optimized.

Summer savory (*Satureja hortensis* L.) belongs to the *Lamiaceae* family, and it is an annual, herbaceous plant species. It is native to the Mediterranean region and West-Asia (Halászné, 2000). It prefers warm weather conditions and tolerates the drought quite well; therefore in Hungary the cultivation of this plant species do not need irrigation according to the practice. The flowering, areal parts contain essential oil in 0.3-2 %, as well as tannins, mucilage, resin and saccharides (Halmai and Novák, 1963).

Basil (*Ocimum basilicum* L.) also belongs to the *Lamiaceae* family; it is a popular, wide-spread annual, herbaceous plant species used for flavouring and medicinal purposes as well. Basil, native to South-Asia, is characterised by great morphological and chemical diversity.

Its essential oil content varies between 0.2-5.2% (Simon et al., 1999). The essential oil composition is rather variable, up until nowadays all together 140 volatile compounds have been identified in the essential oil of basil (Hiltunen and Holm, 2006). Three main chemotypes can be differentiated according to the main essential oil compounds (linalool, methylchavicol and eugenol) (Telci et al., 2006).

Aims of the study

Our main target was to evaluate the extreme weather conditions – basically the effect of the drought periods becoming more frequent also in Hungary – in medicinal plant cultures, since this field of science is less examined. According to the literature data the effect of the different water supply was to be characterised in the first step by some physiological parameters playing role in the stress response reactions. For our investigations two, frequently cultivated model species were chosen, basil (*Ocimum basilicum* L.) and summer savory (*Satureja hortensis* L.), both species have been less evaluated from this scientific point of view until our study.

During our investigations we wanted to know, how the presumed water deficit influence the following parameters:

- *membrane lipid peroxidation processes*; that was characterised by the main product of the lipid peroxidation – the malondialdehyde (MDA) concentration;
- the *antioxidant enzyme system*, especially the activity changes of superoxide dismutase (SOD);
- the *defence actions* induced by the osmotic stress reactions, that was mainly determined by the osmoprotectant macromolecules concentrations' changing. Besides, the water soluble sugar-content, and the accumulation dynamism of prolin – used as an important marker molecule having cell protective and regulator properties as well – was also evaluated.

Further aim was to clarify whether the above mentioned stress response reactions and the amount of those biochemical markers can be used for their characterisation are in correlation with

- the plant growth dynamics, and the different phenological stages;
- their characteristic active compound - the essential oil - accumulation.

Finally, we wanted to compare the investigated parameters and the direction, measure and main characteristics of the response reactions given by the two model species of different origins to define the species-specific attributes.

Important aspect of our research work was to get not only theoretical but practical knowledge as well on the anticipated effect of the irrigation in drought periods.

2. MATERIAL AND METHOD

Our research work was carried out in 2008-2010 in the Experimental and Research Farm of the Corvinus University of Budapest, Unit of the Department of Medicinal and Aromatic Plants.

2.1. Experiments carried out in growth chambers, plant growing conditions

In each research year from 2008 until 2010 the plant cultures were maintained in a Conviron E-15 type growth chamber. As an experimental treatment 3 different soil water capacities (SWC) were used: rich water supply meant 70 % SWC (signed by „K"), slight drought was induced by 50% SWC (signed by „S1"), and significant drought was induced by 30 % SWC (signed by „S2"). In all treatments 13 individual pots were used as replications. The soil water capacity was determined by using gravimetric method. In the growing chamber 14 hour-daylight-period was applied at 25°C, night-temperature was 17°C. The relative air humidity was 65%.

In 2008 the following cultivars were investigated: *Ocimum basilicum* L. 'Keskenylevelű' and *Satureja hortensis* 'Budakalászi'. In both cases 4-4 individuals were taken into one pot. In 2009 and 2010 the cultivar 'Keskenylevelű' was substituted by the more frequently cultivated 'Genovese', because it showed great heterogeneity in 2008.

2.2. Open-field experiments

In a small plot field trial the *Ocimum basilicum* 'Keskenylevelű' and *Satureja hortensis* 'Budakalászi' cultivars have been investigated.

In both experimental years seedling bearing 4-6 leaves were taken into open field with a spacing of 50×30 cm, in the Experimental and Research Farm of the Corvinus University of Budapest. Irrigated and non-irrigated (control) treatments were applied in two replications. In the irrigated plot fields 20 mm additional water amount was taken out two times a week.

2.3. Sampling

In the growing chambers as well as in the open field sampling was done three times in a leafy stage, before flowering, in full flowering, and after flowering. In all cases average samples were collected, in 3-5 replications.

2.4. Analytical methods

- Relative water content (RWC) determination: according to the modified method of Schonfeld and his co-workers (1988).
- Malondialdehyde (MDA) quantity determination: in a spectrophotometric measurement by using thiobarbituric acid reagent.

- Superoxide-dismutase (SOD) activity and the total-protein content determination: protein content was measured according to the method of Bradford (1976). SOD activity was characterised by the photochemic reduction inhibition of nitro blue tetrazolum (NBT) in the presence of riboflavin.
- Analysis of the sugar components was determined by HPLC method, by using authentic standards.
- Prolin content determination: spectrophotometrically, by using ninhydrin reagent.
- Essential oil content determination: by Clevenger-type distillation, according to the descriptions of the 7th Hungarian Pharmacopoeia (expressed as ml/100 g dry weight).

Statistical analysis was done by ANOVA, using PASW software (http://www.spss.com).

3. RESULTS AND CONCLUSIONS

According to our results the stress response reactions of basil and summer savory can be easily observed; while the activity of the superoxide dismutase (SOD) was almost the same in both species, the MDA content was 1.5 times higher in summer savory than in the basil samples. The prolin accumulation was similar in both plant species. On the contrary, the water soluble sugar content was 2 times lower in the case of basil, than in the summer savory samples.

In the investigated plant species the malondialdehyde (MAD) content is increasing during the ontogenesis, the highest concentration can be measured in the most intensive metabolic stage – in the full flowering period. Based on our results, watering plays an important role in the preservation of the membrane integrity. In most cases the applied treatments did not have any negative influence on the lipid peroxidation, especially in the case of the induced slight drought stress (50% SWC). This is possibly due to the activation of the antioxidant enzymes; therefore the lipid peroxidation does not increase significantly as a result of the slight drought stress.

In both plant species the highest level of the hydrogen peroxide producing SOD enzyme activity was observed in full flowering period, and the elevated values, in most of the cases, were still detected after flowering as well. Referring to basil, the SOD activity was less connected to the phenophases than in the case of summer savory, therefore, this characteristic can be regarded species specific. The similar reaction given by the two test species to the drought stress, is not in accordance with the expectations. Knowing that summer savory is

less sensitive to the drought, than basil, a more significant reaction was expected in the case of these samples.

In our investigations the total protein content of both plant species was increasing during the ontogenesis: in certain cases until flowering, or after flowering as well. Significant difference between the two species has not been detected.

In our research work the effect of watering did not influence significantly the amount of the water soluble sugar (glucose, fructose, sucrose) content. Similar results were given by the two investigated plant species: among the applied treatments only in certain cases can be seen significant difference – as a result of less watering (30% and 50% SWC, and non irrigated open field trial) the sugar content increased. With relevance to the more drought tolerant summer savory the sugar content was 5.5 and 13.5 times higher in the growing chamber and in the open field trial, than in the leaves of basil. The ratio of the monosaccharides – especially the glucose proportion – increased.

However, in our investigations, the sugar accumulation was not clearly correlated to the phenophases. The concentration of the analysed mono-and disaccharides slightly increased at the end of the vegetation cycle in the case of summer savory. Even less significant correlations could be seen referring to basil.

On the contrary, the prolin content showed a more characteristic dynamic pattern in both plant species: the accumulation level increased during the ontogenesis, highest values were detected in the last phenophase, after flowering. According to our observations we assume that the continuous water deficit has an increasing effect on the prolin level; therefore the cumulated stress effect possibly induce an increasing synthesis, or – on the contrary, inhibit the decomposition of prolin.

Higher essential oil content was connected to the less watering, in most of the cases. However, it is probably due to an indirect reaction: as a result of less watering the plant growing slows down, the leaf/stem ratio increases, therefore the essential oil amount calculated on a certain dry drug unit can increase as well.

Summarizing our results it is obvious that the stress response reactions given by the analyzed plant species are complex. Strong activity of the SOD antioxidant enzyme system was detected in both plant species. Although during the ontogenesis this reaction is increasingly expressed in both species, more characteristic dynamics can be seen in the case of summer savory. Because of this fact we came to the conclusion that the plant tissues of summer savory are more adaptable, having stronger defence potential. We assume, that due to the strong antioxidant enzyme activity the lipid peroxidation, characterised by the MDA

concentration, do not change significantly. Of course presence and activation of other defence systems cannot be excluded; however, these unknown mechanisms are seemed to be equally expressed in both plant species.

According to our results the non-enzymatic defence systems also play a significant role in the drought-tolerance of the model species. Significant prolin accumulation was observed in both plant species as a result of the drought stress. Prolin is probably an important osmolite, however, it can also take part in the signalling, that cannot be definitely determined during the analysis. Therefore, besides SOD this molecule seem to be the other important compound in the defence system against drought stress during the vegetation cycle. From this point of view the analyzed plant species showed quite similar results. The presence of other protectant proteins cannot be excluded; however, in our research we could not have the possibility to analyze them.

Another important part of the non-enzymatic defence system is the accumulation of the low molecular weight, water soluble sugars as osmolites; however its importance is less than the prolin accumulation in both plant species. Since the sugar content was 3-5 times higher in summer savory than in the fresh leaves of basil, we assume that sugar accumulation can play a more significant role in the case of summer savory. The better drought tolerant characteristic of summer savory can also be proven by the fact that the sugar content continuously increased during the ontogenesis; while in the case of the other plant species this correlation to the phenophases was less strong. More complex sugar composition was observed in the summer savory samples, sucrose could only be detected in these samples.

From a practical point of view both of the applied treatments (30 and 50% SWC) induced stress reactions in our model species, therefore none of them could be regarded advantageous to the production.

In the future drought tolerance will be able to be increased by the enforcement of the protectant mechanism by using several plant breeding techniques. However, it can be worked out only the far future, because a more complex knowledge of the biosynthetic pathways and molecular background is necessary.

From a practical point of view, in the optimization of the species specific water supply conditions the analyzed characteristics, especially the SOD, MDA and the prolin content, can be regarded as perfect biochemical markers.

4. NEW SCIENTIFIC RESULTS

1. We came to the conclusion, that the water deficit caused similar reactions in the two analyzed, annual, frequently cultivated plant species from the *Lamiaceae* family - basil and summer savory – although, according to the practice, they have quite different need on the water supply. At the same time there are some elements of the drought tolerance which can be activated only in the case of summer savory assuring a better adaptation to the arid climatic conditions, and better yields even without irrigation. Especially the long, dry summer periods can induce additional reactions in the protectant mechanisms.

2. As a result of the different water supply the SOD activity changed in both plant species. 1.3-2.3 and 1.2-2 times higher activity was measured in the case of summer savory and basil in the growing chamber by applying 30 % SWC compared to the control plants. Referring to summer savory the most significant increase of the SOD activity was observed in full flowering period. In the case of basil clear correlation between the SOD activity and the phenophases could not be detected, therefore this characteristic seems to be species specific. Presumably, owing to the above mentioned details, the negative effect on the lipid-peroxidation caused by the water deficit could not be detected in most of the cases.

3. In summer savory the osmoprotectant like molecules' accumulation was influenced by the water supply, therefore these molecules play an important role in the stress protectant mechanism. By applying 30% SWC the water soluble sugar content (glucose, fructose, sucrose) can be 4 times higher in summer savory and even 8 times higher in basil compared to the control plants (70% SWC).

4. Among the non-enzyme type protectant mechanisms prolin seems to be determinant in the drought tolerance in both plant species. Prolin content showed a characteristic dynamism in both cases: the accumulation increased during the ontogenesis, highest results were measured after flowering.

5. Water supply can also influence the essential oil content of both plant species; essential oil content can increase by 28% and 10% in summer savory and basil as a result of the water deficit compared to the control plants.

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