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Enhanced rosmarinic acid accumulation and rosmarinic acid synthase gene expression under drought stress in thyme (*Thymus vulgaris*)

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Rosmarinic acid (RA) is the ester of 3,4-dihydroxyphenyllactic acid and caffeic acid and widely occurs in the Lamiaceae. It has numerous biological effects such as antiviral, antibacterial, anti-inflammatory and antioxidant activity. In an earlier study RA accumulation correlated with the expression of rosmarinic acid synthase (RAS) gene in in vitro lemon balm (*Melissa officinalis* L.) cell cultures [1].

Our goal was to study the effect of irrigation and water deficiency in thyme (*Thymus vulgaris* L.) on RA accumulation and RAS gene expression.

The plants were grown in pots in 2014. The soil water capacity was 70% in the controls (irrigated) and it was 40% in the drought stress (water deficiency). Four genotypes were involved in the experiment; 'Varico' – thymol chemotype, TV17 candidate cultivar - thymol chemotype, TV115 - geraniol chemotype, TV143 - α-terpineol chemotype. The samples were collected 3 times at 3 weeks intervals. RA content was determined by HPLC. RNA was extracted with a CTAB-based method. RNA samples were DNase treated and cDNA was transcribed. Primers were designed based on the lemon balm RAS sequence and the amplified region was sequenced. The gene expression study was done by real-time PCR with actin and EF1 α reference genes.

According to the HPLC results the RA accumulation was 50-60% higher under drought stress. However each chemotypes reacted differently. The sequenced region of thyme showed 89% similarity to the corresponding region of the RAS sequence of lemon balm. The gene expression study showed twice as high relative RAS gene expression in the non-irrigated samples during the summer.

Based on these results RAS expression correlated with the RA accumulation in thyme plants. According to our results drought is favourable for higher RA content in thyme, while watering has an adverse effect on the RAS expression and hence on the RA accumulation.

[1] Weitzel, C. & Petersen, M. Phytochemistry 2011; 72: 572–578.