

# Evaluation of HPPD gene expression in relation to vitamin E content in barley

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The term vitamin E encompasses the range of components with a similar chemical structure, which are also called tocopherols. A group of four tocopherols and four tocotrienols, marked with Greek letters  $\alpha$  -  $\delta$ , is included among these components with a key antioxidant effect. Vitamin E has a very important function in plants. It protects photosynthetic apparatus from damage caused by free radical elements and helps plants to survive stress conditions such as cold and drought. Moreover, vitamin E has positive effect on human health. One of the important enzymes in a biosynthetic pathway of vitamin E is 4-hydroxyphenylpyruvate dioxygenase (HPPD). HPPD is considered as one of the essential factors, which control activity of the whole biosynthetic pathway due to its strategic location. The highest vitamin E content among cereals was detected in spring barley (HOLASOVÁ et al. 1995) and the barley cultivars with higher level of vitamin E have been already developed (WANG et al. 1993).

This study was focused on the determination of regulation of the gene expression for HPPD in cultivars with contrastive level of vitamin E during the growth and development of plants and grains. Cultivars (see Table 1) were grown in a phytotron under optimal growing conditions. Evaluation of gene expression for HPPD was carried out in the different growth and development stages of leaves and grains.

Evaluation of relative gene expression in leaves confirmed published information (Kleber-Janke and Krupinska, 1997). In the majority of varieties the highest gene expression was detected during grain

	Tocopherols			Tocotrienols			Total
	$\alpha$ -T	$\beta$ + $\gamma$ -T	$\delta$ -T	$\alpha$ -T3	$\beta$ + $\gamma$ -T3	$\delta$ -T3	
Krona (Kr)	6,96	3,64	0,84	27,59	10,38	0,55	49,96
Kompakt (Kom)	7,05	5,75	1,10	24,84	12,25	0,71	51,70
KM 1771 (KM)	5,87	2,72	0,69	22,79	13,76	0,93	46,76
Carina (Car)	9,79	4,64	0,97	23,54	8,02	0,56	47,52
Wabet (Wb)	7,35	4,08	1,17	34,80	12,45	0,60	60,45
Wanubet (Wnb)	6,17	3,78	1,16	36,32	12,4	0,53	60,36
Washonubet (Wsnb)	6,56	3,13	0,93	42,09	13,86	0,62	67,19

Table 1: Content of tocopherols in grain [mg/kg], locality Žabčice, Czech Republic, 2005

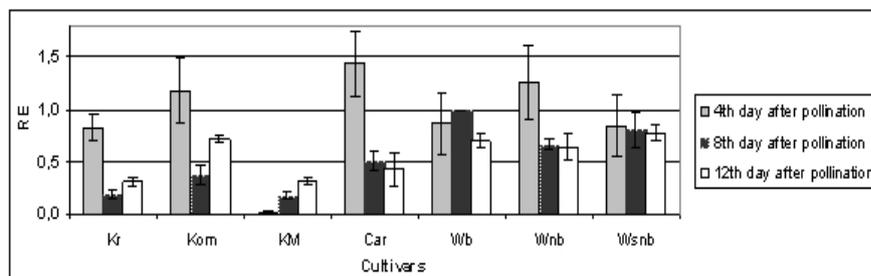


Figure 1: Normalized relative expression (RE) of HPPD gene in barley grain was evaluated according to PFAFFL (2001). Quantification is based on Ct (threshold cycle) values that were normalized using the Ct value corresponding to a barley housekeeping gene for  $\alpha$  tubulin.

filling in fourth day after pollination (see Figure 1).

The expression of HPPD gene in cultivars with low content of vitamin E was descending during sampling whereas the expression of this gene in cultivars with high level of vitamin E was identical or descending very slowly. Results have confirmed the relationship between the activity of the gene on the eighth and the twelfth day after pollination and content of vitamin E, which is typical for the cultivars in field condition. Higher gene expression was found in the cultivars with higher level of vitamin E than in the cultivars with lower vitamin E content. This relationship was statistically significant and the correlation coefficient of this relation was 0,85.

## References

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