

The effect of drought on the composition of selected substances in barley grains

Marta Bradáčová^{1*}, Helena Pluháčková¹, Jaroslava Ehrenbergerová¹,
Ludmila Holková¹ and Eva Truhlářová¹

Abstract

Drought-induced stress affects a number of processes in plants. The protective mechanisms in plants are mainly based on reducing water losses, however, during drought stress also other enzymatic systems are activated, e.g. as a protection against oxidative stress. Vitamin E isomers (α -tocopherols and α -tocotrienols) are synthesized in plants and inhibit lipid peroxidation. The biosynthetic pathway of vitamin E isomers is very complicated. One of the important enzymes is 4-hydroxyphenylpyruvate dioxygenase (HPPD) due to its strategic location in the pathway. The content of tocotrienols is associated with HGGT enzyme. This study was aimed to assess the expression of the *Hppd* and *Hggt* genes in three barley genotypes in relation to the amount of vitamin E isomers in grains and the intensity of drought stress as an activation factor. Plants were cultivated under three different regimes (optimal conditions, early and late drought stress). The regulation of vitamin E synthesis and the composition of tocopherols were influenced both by temperature and relative air humidity, and the genotype.

Keywords

Gene expression, *Hordeum vulgare*, RT-PCR, tocopherols, tocotrienols

Introduction

A number of processes in plants which result in changes of metabolic activity is affected by drought induced stress. Plant reaction is highly influenced by the duration and intensity of exposure to stress. The protective mechanisms in plants are mainly based on reducing water losses, however, during drought stress also other enzymatic systems are activated, e.g. as a protection against oxidative stress.

To prevent oxidative damage of cellular components there is a complex network of antioxidants. Vitamin E isomers (α -tocopherols and α -tocotrienols) are synthesized in plants and inhibit lipid peroxidation. The most important source of tocopherols is plant oil and tocotrienols are abundant in seeds of monocotyledonous plants such as wheat and barley. The biosynthetic pathway of vitamin E isomers is very complicated. The content of tocotrienols in barley grains is associated with the activity of HGGT enzyme (CAHOON et al. 2003). Another important enzyme is

4-hydroxyphenylpyruvate dioxygenase (HPPD) due to its strategic location in the pathway (DÖRMANN 2007). The expression of the homologous gene in mango fruit (MiHPPD) is related to ripening and is rapidly induced by ethylene. The increase in MiHPPD transcript accumulation was followed by an increase in tocopherol levels during ripening. The ripening-related increase in MiHPPD expression was also seen in response to abscisic acid and to a lesser extent to indole-3-acetic acid (SINGH et al. 2011).

This study was aimed to assess the expression of the *Hppd* and *Hggt* genes in barley in relation to the amount of vitamin E isomers in grains and intensity of drought stress as an activation factor. Intensity of stress depends not only on the quantity of water available in the environment, but also on the stage of plant development and genotype. Therefore, plants were cultivated under three different water regimes in the years 2009 to 2011.

Material and methods

Three genotypes of spring barley (*Hordeum vulgare* L.) with different levels of tocopherols in the grain were cultivated in pots under controlled watering in the years 2009 to 2011; 'Krona' represented a malting barley, 'Wanubet' a hull-less, waxy starch barley and breeding line KM1057 a hull-less, high lysine barley developed from a cross with 'Hipoly'. Early-drought stress was induced at the end of tillering, when the water content in soil was reduced from 75% of full water capacity to 35% and kept at this level until maturity. Late-drought was applied in the same way from the stage of ear emergence to ripening. An optimal variant was continuously watered at 75% water capacity. Ear samples were taken five times (I: before pollination; II: 4 days post anthesis (dpa); III: 8 dpa; IV: 12 dpa; V: 15 dpa) for the evaluation of *Hppd* and *Hggt* genes' expression. The concentration of tocopherol isomers was measured in fully matured grains. The relative expression of *Hppd* and *Hggt* genes was assessed using RT-PCR (KOSAR et al. 2010). The content of tocopherols and tocotrienols was analysed using the HPLC with fluorescence detection (EHRENBERGEROVÁ et al. 2006). The data were analysed by two-way analysis of variance and Fisher's LSD multiple comparison test (Statistica 8.0, StatSoft, Inc.).

Results and discussion

The previous studies showed that the content of isomers of vitamin E in barley grains was significantly influenced

¹ Department of Crop Science, Breeding and Plant Medicine, Mendel University in Brno, Zemědělská 1, 613 00 BRNO, Czech Republic

* Corresponding author: Marta BRADÁČOVÁ, marta.bradacova@mendelu.cz



Table 1: Content of tocals in barley grains (Mean values with different letters within columns are statistically significant at $P \leq 0,05$; α -T, α -tocopherols; α -T3, α -tocotrienols)

Year	Water regime	cultivar/line	Content of tocals (mg·kg ⁻¹ dry matter)		
			Sum of tocals	α -T	α -T3
2009	early drought	Wanubet	29.03 ^g	4.53 ^{e-i}	15.51 ^{lm}
		Krona	28.95 ^g	6.70 ^{jk}	13.14 ^{jk}
		KM1057	38.26 ^{ij}	13.70 ^m	11.86 ^{h-j}
	late drought	Wanubet	29.42 ^g	5.61 ^{ij}	14.50 ^{kl}
		Krona	21.36 ^{d-f}	4.99 ^{fj}	9.64 ^{fg}
		KM1057	32.99 ^{gh}	11.36 ^l	10.23 ^{fh}
	optimal conditions	Wanubet	39.45 ⁱ	5.84 ^{ij}	20.98 ^o
		Krona	31.46 ^{gh}	8.12 ^k	11.73 ^{h-j}
		KM1057	37.77 ^{ij}	11.09 ^l	12.43 ^{ij}
2010	early drought	Wanubet	21.17 ^{d-f}	3.55 ^{c-h}	10.23 ^{fh}
		Krona	20.09 ^{c-f}	5.35 ^{h-j}	7.52 ^{c-e}
		KM1057	12.57 ^a	2.54 ^{a-d}	4.02 ^a
	late drought	Wanubet	22.61 ^{ef}	3.54 ^{c-g}	8.70 ^{ef}
		Krona	20.10 ^{c-f}	5.09 ^{g-j}	6.17 ^{bc}
		KM1057	15.98 ^{a-c}	3.71 ^{d-h}	4.73 ^{ab}
	optimal conditions	Wanubet	24.06 ^f	5.14 ^{g-j}	10.70 ^{g-i}
		Krona	21.03 ^{d-f}	6.16 ^{ij}	7.39 ^{c-e}
		KM1057	17.84 ^{b-d}	4.51 ^{e-i}	5.68 ^{a-c}
2011	early drought	Wanubet	12.40 ^a	1.06 ^a	6.08 ^{bc}
		Krona	14.72 ^{ab}	1.87 ^{a-c}	6.51 ^{bc}
		KM1057	19.30 ^{c-e}	3.26 ^{b-f}	8.45 ^{d-f}
	late drought	Wanubet	29.13 ^g	3.22 ^{b-f}	17.22 ^{mn}
		Krona	12.64 ^a	1.58 ^{ab}	5.79 ^{a-c}
		KM1057	13.82 ^{ab}	1.80 ^{a-c}	6.57 ^{b-d}
	optimal conditions	Wanubet	31.73 ^{gh}	3.10 ^{b-e}	18.17 ⁿ
		Krona	35.08 ^{hi}	5.33 ^{h-j}	18.39 ⁿ
		KM1057	16.41 ^{a-c}	2.97 ^{b-e}	6.52 ^{b-d}

not only by the genotype, but also by growing conditions (EHRENBERGEROVA et al. 2006). From *Table 1* it is obvious that the water regime has a significant effect on the content and composition of vitamin E isomers (tocopherols and tocotrienols). We focused on α -T and α -T3 due to low concentrations of other isomers. 'Wanubet' had the lowest content of tocals and α -tocopherols under drought conditions. The content of α -tocotrienols was lowest in the late drought variant and highest in optimal conditions. The content of α -T3 in 2009 was the highest of all years, regimes and genotypes. 'Krona' had the lowest content of tocals and α -T and α -T3 in late drought conditions and highest in optimal conditions. The content of vitamin E isomers of KM1057 was variable with respect to years and water regimes. The sunny and warm weather in 2009 and 2011 affected the content of tocals and isomers, which was highest in early drought conditions and lowest in late drought conditions. In 2010 (cold weather with high air humidity) the highest content was realized in optimal condition. The ratio α -T3/ α -T depended on water regime, year and genotype (*Table 2*). The highest α -T3/ α -T value in grains was observed for hull-less 'Wanubet'. Genotypic differences of the T3/T ratio were also shown by ZIELINSKI et al. (2007), but contrary to our results these authors observed relatively low T3/T ratios in whole grains. It is known that tocopherols and tocotrienols are distributed differently within the kernel: tocopherols have their highest concentration in the outer layers and decrease gradually to zero in the inner endosperm, while tocotrienols have their lowest concentrations in the seed coat layer (WINTER and DAVIS 2006). This justifies results of

TSOCHATZIS et al. (2012), where the tocopherol contents were higher in conventionally grown barley samples that had high ash contents, whereas organic cultivation resulted in an increase of tocotrienols and consequently in increased T3/T ratios.

The gene expressions of the two enzymes HPPD and HGGT connected with tocals biosynthetic pathway were evaluated during experiment. The results showed significant correlations between genes' expression and T3 and T contents in grains 12 dpa (*Hppd*) and 15 dpa (*Hggt*). The close correlation was found in the drought stressed variant. Hence, the influence of drought on the biosynthetic pathway was demonstrated (*Table 3*). The close relationship between *Hggt* and *Hppd* genes' expression and the content of vitamin E iso-

Table 2: Ratio of the mean content of α -tocotrienols (α -T3) and α -tocopherols (α -T) in barley grains under different water regimes

Year	Water regime	Genotype		
		Wanubet	Krona	KM1057
2009	early drought	3.42	1.96	0.87
	late drought	2.58	1.93	0.90
	optimal conditions	3.59	1.44	1.12
2010	early drought	2.88	1.41	1.58
	late drought	2.46	1.21	1.27
	optimal conditions	2.08	1.20	1.26
2011	early drought	5.74	3.48	2.59
	late drought	5.35	3.66	3.65
	optimal conditions	5.86	3.45	2.20

Table 3: Correlation between the content of α -T3 and α -T and the expression of *Hggt* gene 15 days post anthesis and *Hppd* gene 12 days post anthesis (ns, not significant; *, **, significant at $P < 0.05$ and 0.01 , respectively)

Water regime	Tocols	NRE	
		<i>HGGT (I)</i>	<i>HPPD (II)</i>
early drought	α -T3	0.44	0.52
	α -T	ns	0.87**
late drought	α -T3	0.78*	0.55
	α -T	ns	0.47
optimal conditions	α -T3	ns	ns
	α -T	ns	ns
all regimes	α -T3	0.53**	ns
	α -T	ns	0.63**

mers is determined by the position and function of vitamin E biosynthetic pathway enzymes. It remains still unclear why this relationship was not observed in optimal conditions. The plants growing under optimal water conditions could have been influenced by another type of abiotic stress, e.g. high temperature. This type of stress was not observed in the drought-stressed variants due to a masking effect of the drought stress *per se*. MUNNÉ-BOSCH (2005) described the effect of various types of abiotic stress on genes connected to the regulation of the tocols biosynthetic pathway. The regulation of vitamin E isomers synthesis and the composition of tocols isomers were influenced both by growing conditions, i.e. water availability, temperature, relative air humidity, and the genotype.

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