

Comparison of different approaches for the evaluation of response of winter wheat to drought

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Abstract

Field experiments were carried out from 2006 to 2009 in order to identify winter wheat varieties with different performance under drought stress. A set of commonly grown varieties was tested in field trials at one location on two fields with different soil water availability. Selected genotypes were further studied using physiological and molecular methods. In particular the relative expression of dehydrin genes (*Wdhn13*), the level of endogenous abscisic acid, and carbon isotope discrimination as the indirect estimation of water use efficiency were studied.

Key words

Abscisic acid, carbon isotope discrimination, dehydrin genes, drought tolerance, *Triticum aestivum*, yield

Introduction

Winter wheat (*Triticum aestivum* L.) is the most important crop in the Czech Republic. It covers more than half of the production area of cereals (2009: 793472 ha; mean yield 5.33 t.ha⁻¹). In some areas, the productivity of this crop is heavily influenced by abiotic stresses, particularly by the lack of available water. In contrast to other regions, drought periods in Central Europe fluctuate and can induce rather sudden stress in some crucial stages of plant development. HLAVINKA et al. (2009) demonstrated that at least in some areas of the Czech Republic drought is one of the key causes of interannual yield variability and has a quantifiable negative effect even within more humid regions.

Winter wheat breeding programmes are commonly aimed at better performance of new varieties under water deficient conditions (CSEUZ et al. 2009). Selection for yield under stress-alleviated conditions appears to produce superior cultivars not only for optimum environments, but also for those characterised by frequently mild or moderate stress conditions (ARAUS et al. 2002). Selection under severe stress may gain genotypes with a better survival strategy than higher yield potential; the process is also complicated by large genotype by environment interactions. It is likely that selection under both optimal and a drought conditions represents the ideal approach to maintain favourable alleles for drought resistance and at the same time, to maximise

the selection response under favourable conditions (RICHARDS et al. 2007).

Water-use efficiency from an agronomic point of view can be defined as the yield of a harvested product achieved from the water made available to the crop. Therefore, yield depends on the amount of water used by the crop, the proportion of that water actually transpired by the crop, the efficiency with which this water is converted into biomass, and the harvest index (CONDON et al. 2004). All of these processes are genetically controlled (FOULKES et al. 2002). Many selection criteria based on morphological or physiological traits have been proposed to improve yield under drought, and a few of them have been even successfully used in breeding programmes (RICHARDS 1996, REYNOLDS et al. 2009). It was proven that the measuring of carbon isotope discrimination (ratio between ¹³C and ¹²C isotopes), due to its relation to photosynthetic gas exchange, can provide an indirect estimation of water-use efficiency (CONDON et al. 1993). REBETZKE et al. (2002) suggested this trait for the selection of genotypes with higher water-use efficiency, which should be positively correlated with higher production in dry environments.

A different approach exploits defensive mechanisms in plant cells regulated by *Cor/Lea* genes. A quantitative assessment of a *Cor/Lea* genes expression was proposed for the evaluation of genotypic sensitivity of barley to abiotic stresses (SURPRUNOVA et al. 2004). For wheat, the relationship between the expression of dehydrin genes and a tolerance to frost was found by OHNO et al. (2003), KOBAYASHI et al. (2004) and HOLKOVA et al. (2009). Abscisic acid (ABA) regulates plant growth and development and plays an important role in mediating stress responses including abiotic stresses (KOBAYASHI et al. 2008b). The endogenous ABA level increases rapidly in response to drought stress, which induces stomatal closure and reduces transpiration.

However, ABA accumulation might be at least partly repressed by higher water use and the resultant improvement of plant water status (WESTGATE et al. 1996). IKEGAMI et al. (2008) demonstrated that, as response to drought stress in *Arabidopsis thaliana*, ABA is synthesised mainly in the leaves and transported to the roots and the movement of ABA from leaves to roots is activated by a water deficit in the roots.

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Materials and methods

Field experiments

The effects of limited water availability on the grain yield of winter wheat were studied in field experiments in 2005/06, 2006/07, 2007/08 and 2008/09 growing seasons. The experiments were located in Žabčice, at the Field Research Station of the Mendel University, Brno (49°01' N, 16°37' E; 179 m a.s.l.). The selection of varieties was based on the official Czech trials for varietal recommendations. Some varieties listed in the common catalogue of varieties of agricultural plant species were also added, particularly those recommended as suitable for dry conditions by seed producers. The number of varieties varied from 41 to 47 (Table 1). The trials were performed simultaneously on two fields with different soil conditions. Field A was characterized by a heavy loamy soil with good water retention (gleyic fluvisol, 51% of clayey particles). Field B was on a drought prone sandy soil of chernozem type formed on a gravel terrace (20-28% of clayey particles). The long time (1961-1990) average annual temperature in Žabčice is 9.2°C and the average sum of annual precipitation is 480 mm. The chosen fields differed mostly in the water availability during vegetation: field A with high level of sub-soil water provided the plants with sufficient moisture even in the periods without rainfall. The water availability on sandy soil (field B) was restricted mainly from the stage of stem elongation to harvest. The data provided by the continuous monitoring of soil moisture (since 2008) confirmed the occurrence of water deficiency in sandy soils (data not shown).

The trial management followed the rules of experimental practice, fertilizer application and chemical treatments were adjusted according to growing conditions and disease occurrence. An incomplete block design (alpha-design) was used for randomisation within three (field A) or four (field B) replicated blocks. The harvest area per plot was 10.5 m². The following traits were evaluated: number of ears per square meter, grain yield (kg.m⁻²), specific grain weight (g.l⁻¹) and thousand grain weight (g).

Pot experiment

Six varieties were selected (Table 2) for a pot experiment with controlled watering. Plants were grown in pots (10 litres of soil, seven plants per pot, three replications) under optimal conditions until April and since then placed under a water-proof transparent shelter to avoid rain. Two regimes of watering were applied: optimal (75% of soil saturation) and dry (30% of soil saturation). Leaf samples for analyses were collected five times at different stages of plant development (stem elongation to ripening) which corresponded to 4, 11, 18, 25 and 39 days of drought treatment. We focused on the relative expression of dehydrin genes (*Wdhn13*), the level of endogenous abscisic acid, and carbon isotope discrimination. After harvest, the yield components such as number of ears, number of grains and the weight of grains were evaluated.

Transcription activity was evaluated as a normalized relative expression calculated with real-time PCR efficiency correlation according to the method of PFAFFL (2001) and RASMUSSEN (2001). The changes in the activity of both genes normalized to ubiquitin and relative to the expression at optimal growth conditions were calculated for each sample. The sample with the highest transcription level was taken as an inner calibrator. The relative expression level reached by this sample was determined as the absolute 1. All other samples were related to this calibrator.

Carbon ¹³C isotope discrimination ($\Delta^{13}\text{C}$) in leaves was measured with the isotope ratio mass spectrometer (Delta Plus XL, Thermo Finnigan, Bremen, Germany) and the elemental analyser NC 2100 (Thermoquest, Rodano, Italy). Endogenous ABA levels in leaves were determined by ELISA (ASCH 2000).

Statistical analysis

The statistical analyses were carried out using Statistica vers. 8.0 software (StatSoft, Inc., Tulsa, USA). Relative performance of twelve varieties over eight environments (two fields in four crop seasons) was analysed by linear regression analysis. Standardised varietal means and $1 + \beta_1$ values

Table 1: Yield components and specific weight in optimal and dry conditions

Character	Year	Varieties (n)	Field A (optimal)		Field B (dry)		Difference (%)
			Mean	Std.Dev.	Mean	Std.Dev.	
Yield (kg.m ⁻²)	2006	41	8.6	0.7	4.8	0.6	-44.2
No ears m ⁻²			639	81	572	87	-10.5
1000 seed weight (g)			42.5	2.8	35.7	3.1	-16.0
Specific weight (g.l ⁻¹)	2007	41	776	25	723	31	-6.8
Yield			8.2	0.5	2.6	0.3	-68.3
No ears m ⁻²			695	113			
1000 seed weight	2008	46	42.2	3.1	35.2	2.6	-16.6
Specific weight			794	19	772	19	-2.8
Yield			9.4	0.4	3.1	0.4	-67.0
No ears m ⁻²	2009	47	605	70	386	43	-36.2
1000 seed weight			45.2	3.9	36.8	3.1	-18.6
Specific weight			791	21	763	18	-3.5
Yield	2009	47	9.5	0.7	1.8	0.5	-80.7
No ears m ⁻²			611	70	364	32	-40.4
1000 seed weight			47.5	3.8	40.3	3.4	-15.0
Specific weight			790	17	783	17	-0.9

were submitted to a biplot analysis as proposed by KEMPTON (1984).

Results and discussion

Field experiments

Mean yields on field A reached 8.6, 8.2, 9.4 and 9.5 kg.m⁻² (Table 1). Yields at the dry field B were much lower, i.e. 4.8, 2.6, 3.1 and 1.8 kg.m⁻², respectively. Hence, yield reduction on field B was from 44 to 81%. Less severe reductions were detected for the number of ears per square meter (10-40%), grain weight (15-19%) and almost none for specific weight (1-7%). The most severe drought was observed in 2007 and 2009 with similar characteristics: almost no precipitation in April (2007: 4.4 mm; 2009: 3.6 mm), a very dry May (2007: 24.8 mm; 2009: 19.7 mm until 30 May).

The biplot (Figure 1) shows that Meritto, Dromos and Biscay were the highest-yielding varieties in all environments, while Ludwig and Venistar were the lowest yielding. Varieties Hedvika, Etela and Akteur seemed to be better adapted to more humid conditions, while Alacris and Arida showed better performance in dry conditions. Six varieties were selected to represent different kinds of behaviour. Their relative yields calculated as percentage of the corresponding trial mean yield are compared in Table 2. Variety Mulan was not tested in 2005/06, but in 2007 and 2008 year showed a similar responsive pattern as Meritto. Therefore, it was included in the pot experiment.

The aim of the variety trials was the detection of genotypes able to cope better with moderate drought stress. The environmental conditions of our field B represent a common situation in South Moravia, i.e. fertile soil which cannot hold water for a longer period of time. Therefore, yield depends on the amount of rainfall and particularly its distribution. Usually no problems with drought occur during autumn and winter time. In reality, early development of winter crops is often much better here than on heavier soils due to higher temperature and soil aeration. In spring, water deficiency caused by uneven distribution of rainfall and enhanced temperatures affects plants rather suddenly and results in severe reduction of fertile tillers, ear sizes, and numbers of grains

or grain weights depending on time of stress. FOULKES et al. (2002) stated that limited water availability mainly after flowering did not affect the number of ears per area, but individual grain weight and number of grains per ear were significantly decreased. More prolonged drought affected all yield components.

Pot experiment

Results of yield traits of investigated varieties are presented in Table 3. Varieties Etela, Hedvika, Meritto and Mulan were similar in their reaction, i.e. extensive reduction of number of ears and grains followed by a substantial increase of grain size. Alacris showed similar reduction in ears and grains, but without compensation in grain size. Venistar produced the highest number of ears and grains together with a very low grain weight in both variants.

The differences in $\Delta^{13}\text{C}$ levels in leaf biomass are given in Figure 2. Controls represent average discrimination in the optimum variant for all samples of each variety. In all varieties except Venistar, Δ values distinctively decreased after 11 days of limited watering. This variety even under a normal watering regime showed lower $\Delta^{13}\text{C}$ values in comparison with the others. The correlation coefficient between grain weight and the $\Delta^{13}\text{C}$ level after 39 days of

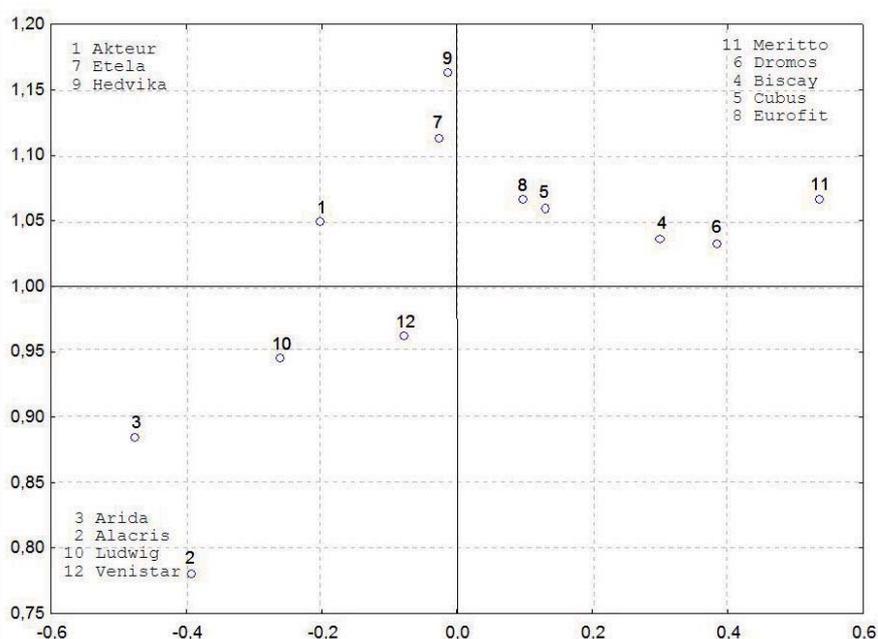


Figure 1: Biplot of wheat varieties tested in all trials (2006-2009)

Table 2: Summary of relative yield of six selected varieties

Variety (Origin)	Field A (normal conditions)					Field B (dry conditions)				
	2006	2007	2008	2009	2006-09	2006	2007	2008	2009	2006-09
Alacris (SK)	78	92	94	90	88	103	114	111	124	113
Etela (CZ)	96	103	107	108	104	91	91	91	80	88
Hedvika (NL)	104	111	105	106	107	83	66	96	85	83
Meritto (CZ)	117	105	102	113	109	103	98	112	147	115
Mulan (DE)	-	107	104	113	108	-	103	112	69	95
Venistar (SK)	92	93	106	98	97	112	117	93	92	103

drought treatment was -0.5 for the dry variant and 0.2 for the optimum variant.

The isotope discrimination method is based on higher affinity of the carbon-fixing enzyme Rubisco for the more common ^{12}C isotope over the less common ^{13}C . $\Delta^{13}\text{C}$ is reasonably negatively correlated with transpiration efficiency - the ratio of net photosynthesis to transpired water (HALL et al. 1994). According to Farquhar's model, a lower CO_2 concentration in leaves, e.g. due to stomatal closure, is associated with a lower discrimination value (FARQUHAR et al. 1982). Since the carbon incorporated in leaves is assimilated over a considerable time and under a range of environmental conditions, Δ can serve as a long-term indicator of plant metabolism (CONDON et al. 1990). Although isotope discrimination was proposed as a selection criterion for yield improvement in wheat under water deficient conditions (CONDON et al. 2002), conflicting results were also obtained, i.e. higher yielding genotypes with lower leaf water-use efficiency (higher $\Delta^{13}\text{C}$). It could be explained by a lower stomatal conductance in some genotypes connected with lower transpiration even in conditions of sufficient water supply and consequently lower photosynthesis and biomass production resulting in lower grain yield (BLUM 2009).

The levels of endogenous ABA in leaf samples were determined in the moisture-deficient variant after 25 and 39 days of drought treatment and in control (Table 4). In all varieties except Meritto, the ABA content increased with longer duration of limited watering. The highest values were found in Hedvika, the lowest in Venistar. The ABA levels in controlled plants were much lower and varied between 2 and 21 $\text{ng}\cdot\text{g}^{-1}$ of fresh weight. KURAHASHI et al. (2009) studied natural variation in drought tolerance and its relation

Table 3: Yield traits in the pot experiment with different soil water saturation

Variety	Trait	Soil saturation (%)		Difference (%)
		75	30	
Alacris	No ears pot ⁻¹	20.3	14.0	-31.0
	No grains pot ⁻¹	707.3	378.3	-46.5
	Grain weight pot ⁻¹ (g)	30.7	16.3	-46.9
	1000 seed weight (g)	43.3	43.1	-0.5
Etela	No ears pot ⁻¹	18.3	10.7	-41.5
	No grains pot ⁻¹	754.7	254.7	-66.3
	Grain weight pot ⁻¹ (g)	33.1	14.8	-55.3
	1000 seed weight (g)	43.9	58.1	32.3
Hedvika	No ears pot ⁻¹	18.7	13.7	-26.7
	No grains pot ⁻¹	791.7	360.0	-54.5
	Grain weight pot ⁻¹ (g)	33.3	17.2	-48.3
	1000 seed weight (g)	42.1	47.9	13.8
Meritto	No ears pot ⁻¹	18.7	12.0	-35.8
	No grains pot ⁻¹	778.7	363.0	-53.4
	Grain weight pot ⁻¹ (g)	35.4	18.1	-48.9
	1000 seed weight (g)	45.5	50.1	10.1
Mulan	No ears pot ⁻¹	23.3	15.3	-34.3
	No grains pot ⁻¹	839.0	351.0	-58.2
	Grain weight pot ⁻¹ (g)	33.6	18.8	-44.0
	1000 seed weight (g)	40.0	53.6	34.0
Venistar	No ears pot ⁻¹	21.0	19.7	-6.2
	No grains pot ⁻¹	969.7	657.3	-32.2
	Grain weight pot ⁻¹ (g)	33.7	21.2	-37.1
	1000 seed weight (g)	34.7	32.5	-6.3

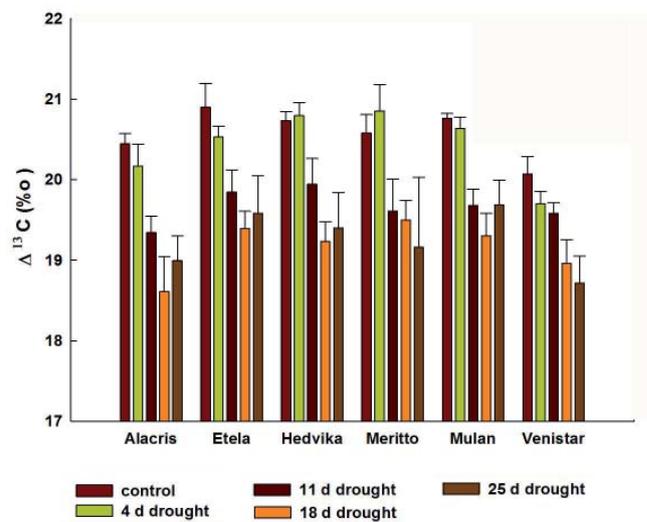


Figure 2: Comparison of $\Delta^{13}\text{C}$ in leaf biomass of wheat varieties in a pot experiment with different drought treatments

to ABA sensitivity in *Aegilops tauschii*. Drought sensitive accessions exhibited significantly lower ABA sensitivity and vice versa. However, synthetic hexaploid wheat lines showed a weaker association of ABA sensitivity with drought tolerance. Therefore, the D-genome variations in drought tolerance observed in *Ae. tauschii* were not fully transferred into hexaploid synthetic wheat.

The evaluation of the relative expression of the *Wdhn13* gene in plants grown in water deficient conditions is shown in Figure 3. An increased transcription level was already detected after 4 days of drought in Etela, Meritto and Mulan. The genotypes differed in time of reaching the maximum level of expression with the highest values observed for Etela and Meritto, while the activity of the gene was low in Hedvika and Mulan. It is interesting that the low activity in Mulan was observed also in field conditions. Leaf samples of all varieties were collected in both trials at the beginning of May 2009, after a period of more than one month without precipitation (Figure 4). It suggests that this variety has a low sensitivity of stress defence mechanisms based on protective dehydrine genes. Similar results were obtained

Table 4: Endogenous ABA levels ($\text{ng}\cdot\text{g}^{-1}$ fresh weight) of wheat varieties in a pot experiment

Variety	Days of drought	Drought treatment		Control treatment	
		Mean	Std.Dev.	Mean	Std.Dev.
Alacris	25	76	5	10	8
	39	863	15	15	68
Etela	25	61	11	3	2
	39	601	16	19	37
Hedvika	25	264	11	6	74
	39	1084	15	21	45
Meritto	25	723	11	7	41
	39	49	12	20	1
Mulan	25	48	5	11	12
	39	*		9	
Venistar	25	173	11	2	31
	39	418	16	27	85

* above calibration range

with other *Dhn* genes from the *Wcs120* family (data not shown).

An association between the accumulation of Dhn proteins and tolerance to stresses involving dehydration has been shown in several species, including wheat (LOPEZ et al. 2003). RAMPINO et al. (2006) studied *Triticum* and *Aegilops* seedlings differing in their response to drought stress and found a relation between the activation of *Dhn* genes and tissue water content. In resistant genotypes the *Dhn* gene expression was initiated even at still high hydration levels in plant tissues. It suggests that these proteins are also involved in water retention. According to SUPRUNOVA et al. (2004) and PARK et al. (2006), *Cor/Lea* genes seem to react more quickly and reach a higher level of expression in genotypes with better stress tolerance. Therefore, it is suggested to use this trait for the evaluation of genotype sensitivity to abiotic stresses (TOMMASINI et al. 2008).

KURAHASHI et al. (2009) found that synthetic hexaploid wheat lines with high ABA sensitivity and drought tolerance also showed a more rapid response in the expression of *Cor/Lea* genes (*Wrab17* and *Wdhn13*) than ABA insensitive and drought sensitive ones. It suggests that these *Cor/Lea* genes are involved in downstream genes functioning in the ABA-dependent signal pathway for the development of drought tolerance. The expression of *Wdhn13* was proven to be suitable for the determination of the frost level tolerance (HOLKOVA et al. 2009). Because the activation of this gene is induced by cold, drought, and ABA (OHNO et al. 2003, KOBAYASHI et al. 2008a) we attempted to use it as an indicator of drought tolerance. It seems that the response to particularly long term drought is much more complex than that of cold/frost and more than one system of protective mechanisms is involved. The next step will be to optimise the conditions of activation of the *Wdhn13* gene and also to study other *Cor/Lea* genes, e.g. *Wrab17*.

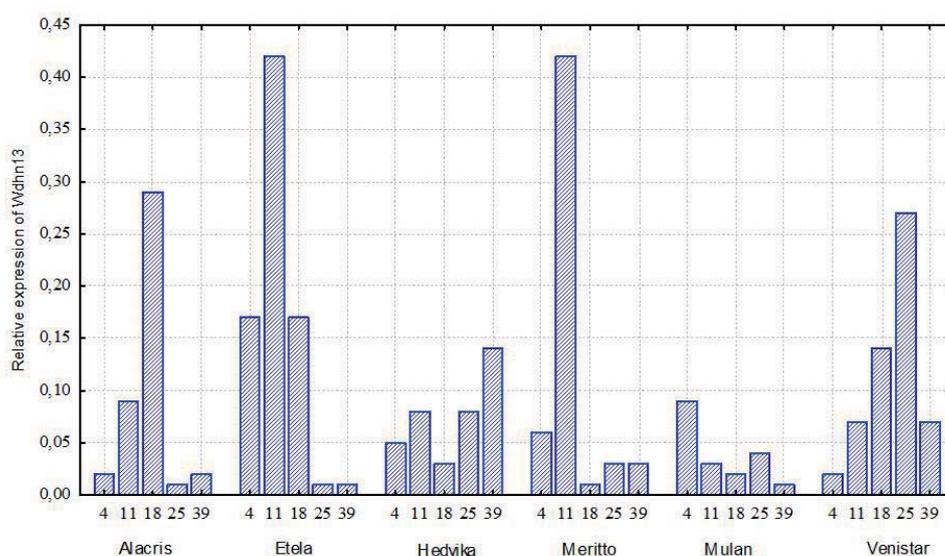


Figure 3: Relative expression of the *Wdhn13* gene of wheat varieties in a pot experiment measured after 4, 11, 18, 25 and 39 days of drought

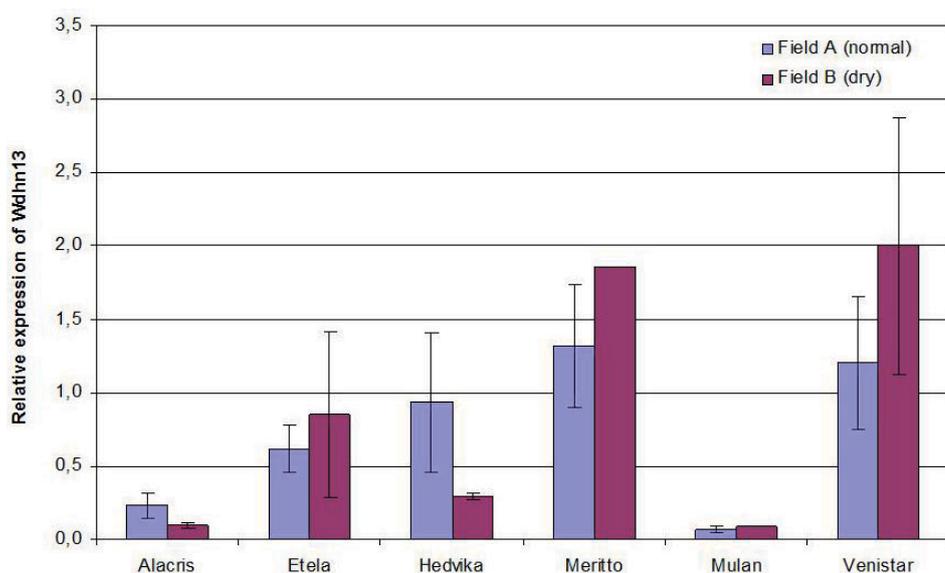


Figure 4: Relative expression of *Wdhn13* in field conditions in 2009

Conclusions

- (1) The yield of winter wheat varieties was compared on two sites, which differed mainly in water availability in the soil. Varieties with different responses to drought stress were identified.
- (2) Carbon isotope discrimination distinguished varieties Alacris and Venistar as more drought tolerant in comparison with others.
- (3) In all tested varieties, ABA content increased under drought conditions. However, ABA levels fluctuate during long-term stress and therefore, this parameter is not reliable for genotype evaluation.
- (4) Activation of the *Wdhn13* gene was already detected after four days of drought treatment. Tested varieties did not

reach a maximum level of expression at the same time. This trait might potentially be usable for field evaluation.

Acknowledgement

The authors gratefully acknowledge the financial support of the Grant Agency of the Czech Ministry of Agriculture, project QH 91192, and research plan MSM 6215648905 „Biological and technological aspects of sustainability of controlled ecosystems and their adaptability to climate change”.

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