

## Differences in the drought stress response of winter wheat

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### Abstract

Drought tolerance of plants is a complex character, therefore, the assessment is considerably difficult. It is most often evaluated by using physiological and morphological methods, which make the assessment of mechanisms in plants that lead to their resistance possible. The intensity of plant cell protective mechanisms can be evaluated on the molecular level. In our work, the stress response was evaluated by relative water content (RWC), osmotic pressure (OP) and the relative expression of *WDHN13* and *WRAB17* genes from the *COR/LEA* group. The evaluation included 3 varieties of winter wheat grown in the Czech Republic and one Syrian line potentially highly resistant to drought. The varieties were at the same time compared for differences in the root system size by means of specific root length (SRL). The obtained results clearly indicated a relation between the expressions of both genes and the tissue water content; RWC reduction was strongly connected with changes in the genes' activity. Particularly the expression of *WDHN13* in variety Meritto increased although the tissue hydration level was only slightly reduced, which can be related to the good adaptability of this genotype to stress.

### Keywords

*COR/LEA* genes, drought tolerance, gene expression, *Triticum aestivum*

### Introduction

Drought resistance is a polygenic complex of traits, including a range of morpho-physiological and biochemical adaptations aiming not only at the survival of plants in conditions of water deficit but also at the least yield loss under conditions of drought. The selection of tolerant genotypes becomes increasingly important with respect to the predicted effects of global warming also in Central Europe, i.e. higher temperatures, lower precipitation and an uneven distribution of precipitation during the growing season (BRÁZDIL et al. 2009). These genotypes may exhibit differences in many physiological reactions such as changes in the osmotic adaptation of plants, changes in the levels of protective proteins and other metabolites, antioxidant capacity of plants, etc. Having been exposed to stress conditions, the plants show induction of a number of biochemical and physiological changes, which lead to the development of protective mechanisms aimed at the efficient utilization of available water. The most resistant genotypes

are usually those that exhibit tolerance at multiple levels at the same time (FLEURY et al. 2010). Nevertheless, for the conditions of Central Europe it makes sense to select only traits, which do not impair yield and quality of tolerant varieties (BLÁHA 2011).

The main physiological characteristic related to tolerance to repeated drought stress, which also relates to the capacity of plant's fast regeneration is osmotic adjustment (OA). Under water deficit, the plants can control osmotic pressure by accumulating osmolyte substances, which will manifest in the maintenance of turgor and higher relative water content (RWC) in leaves (IZANLOO et al. 2008). KEYVAN (2010) reported decreased RWC in wheat plants during induced stress. Detected differences between some genotypes were likely to be connected with a better capacity to absorb water from the soil or with a better possibility to prevent water losses. According to KEYVAN (2010), genotypes that are more drought-resistant have higher RWC in leaves.

Drought is connected with a water deficit in the cell and this is why accumulation of dehydrin proteins often correlates with the plant's resistance to stress conditions. Hence, dehydrin proteins could be applicable markers for drought resistance (ATIENZA et al. 2004). In recent years, the sensitivity of plants to drought is assessed also based on the expression of protective genes from the *COR/LEA* group (RAMPINO et al. 2010).

The aim of our work was to compare intervarietal differences in some physiological traits and in the expression of protective genes from the *COR/LEA* group (*WDHN13* and *WRAB17*). These could be used as selection criteria in screening varieties suitable for further breeding in dry conditions and to study responses of different genotypes to the same stress conditions.

### Material and methods

Three winter wheat varieties ('Etelá', 'Meritto', 'Venistar') grown in the Czech Republic and one Syrian line 142670 (S1) originating from ICARDA were chosen to compare the stress response to a sudden drought spell during the vegetative growth phase. The varieties were sown into plastic containers with large-size perlite (3 containers with 20 plants per variety). The plants were subjected to 12 hours day at 18°C and 12 hours night at 10°C until the 3-4 leaves stage. The plants were then removed from the boxes and dried on filter paper. The physiological assessment of RWC and OP was made by 11 samplings (hourly intervals). RWC was determined according to BARR and WEATHERLEY (1962) on the second youngest leaf. OP was measured by vapour

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pressure osmometer model VAPRO 5600 (Wescor, Inc., South Logan, Utah) in the youngest parts of withering plants. Root system size was determined by digital image analysis using WinRHIZO software (Régent Instruments Inc., Quebec, Canada) after scanning of roots with an Epson perfection 700 photo scanner. The analyzed roots were subsequently surveyed for dry matter and specific root length (SRL). SRL values were calculated as the ratio of total root system length (m) / dry weight (g) (OSTONEN et al. 2007). The expression of *COR/LEA* genes was assessed during gradual wilting of plants under precisely defined conditions at 19 dates (K, control; 1, 10 min after pulling out the plant; 2, 15 min; 3, 20 min; 4, 25 min; 5, 30 min; 6, 60 min; 7, 90 min; 8, 2 h; 9, 3 h; 10, 4 h; 11, 5 h; 12, 6 h; 13, 7 h; 14, 8 h; 15, 9 h; 16, 10 h; 17, 12 h; 18, 24 h) in the second youngest leaf. RNA isolation (RNeasy Plant Mini Kit, (Qiagen, Hilden Germany) and purification (Turbo DNase-free<sup>TM</sup>, Applied Biosystems/Ambion, Austin, TX, USA) as well as synthesis of cDNA (QuantiTect<sup>®</sup> Reverse Transcription Kit, Qiagen, Hilden, Germany) and qPCR (quantitative polymerase chain reaction) (SybrGreen PCR Kit, Qiagen, Hilden, Germany) were conducted according to standard protocols of these commercial kits. Gene-specific primers used in the PCR were designed according known sequence AF 255053.

*Wdhn13* F: 5'GCACGGTGACCACCAGCAC3'/ R: 5'TAGCGGGTCGGGCGCGGGC3'

*Wrab17* F: 5'TCCATCAACTTCAAAAATG-3'/ R: 5'TGTGGTCTTCTTGGTGGCA-3'

Reaction conditions of qPCR were used according to HOLKOVÁ et al. (2009). The relative expression of genes was calculated according to PFAFFL (2001). The expression was assessed against ubiquitin as reference gene.

## Results and discussion

The expression of *WDHN13* and *WRAB17* was evaluated under conditions of gradual wilting of whole plants. The two genes are activated by drought and proteins coded by these genes are involved in the protective mechanisms of plant cells against drying out (KOBAYASHI et al. 2008). Tolerant genotypes should reveal a more rapid onset or a higher level of expression of corresponding genes in dependence of stress conditions. Compared with the level of *WDHN13* gene expression, the level of *WRAB17* gene expression was relatively low and considerably fluctuating during the wilting of plants. Therefore, our attention was focused on the evaluation of *WDHN13* expression (Figure 1).

The more tolerant variety 'Meritto' exhibited an earlier activation of *WDHN13* and at the same time a high relative expression (RE) level of the gene. 'Venistar' showed only a high RE level. Similar dynamics of gene expression was observed in 'Etela' and in S1, however, RE level was lower (particularly for S1) as compared to the other varieties. Com-

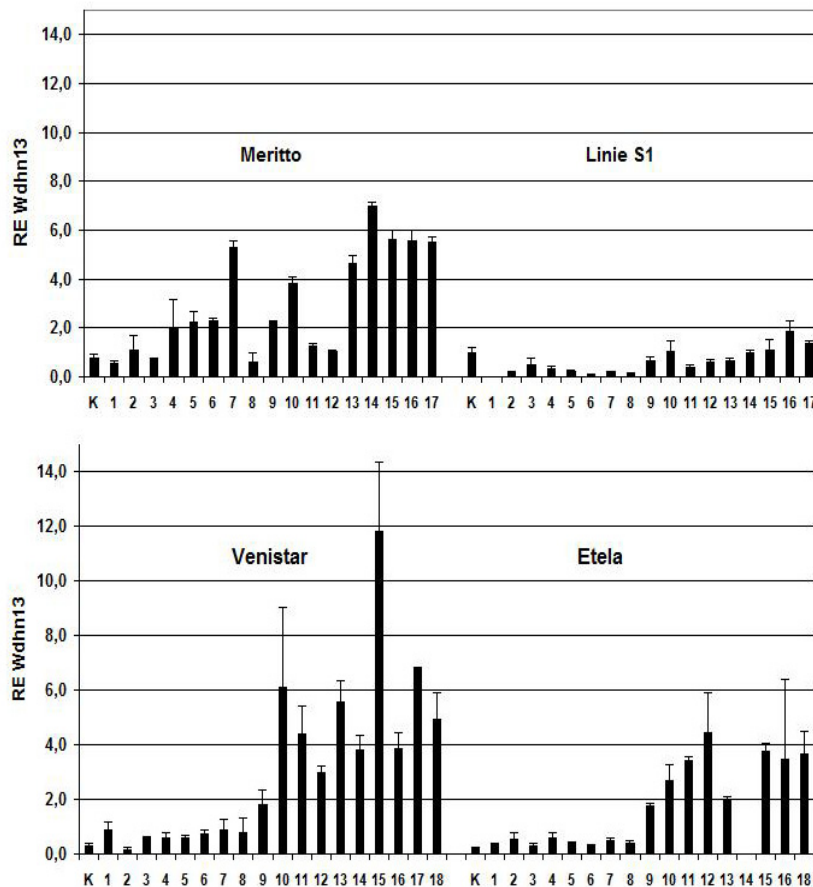


Figure 1: Evaluation of the relative expression (RE) of *WDHN13* in the course of gradual wilting of winter wheat plants. Values represent means  $\pm$  standard deviation (n=4).

**Table 1: Correlation coefficients for the relationship between relative expression (RE) of *WDHN13* and *WRAB17* and relative water content (RWC) and osmotic pressure (OP), respectively, during gradual drying out of wheat plants (\*\*  $P \leq 0.01$ ; \*  $P \leq 0.05$ ).**

	RE <i>WDHN13</i>	RE <i>WRAB17</i>	RE <i>WDHN13</i>	RE <i>WRAB17</i>
Venistar			Meritto	
RWC	-0.70**	-0.55*	-0.75**	-0.14
OP	-0.54*	-0.87**	-0.33	-0.05
Etela			S1	
RWC	-0.78**	-0.43	-0.81**	-0.58*
OP	-0.73**	-0.35	-0.64**	-0.65**

paring RE with RWC values (Figure 2), the earlier onset of increasing *WDHN13* gene expression in Meritto probably did not connect with the level of wilting.

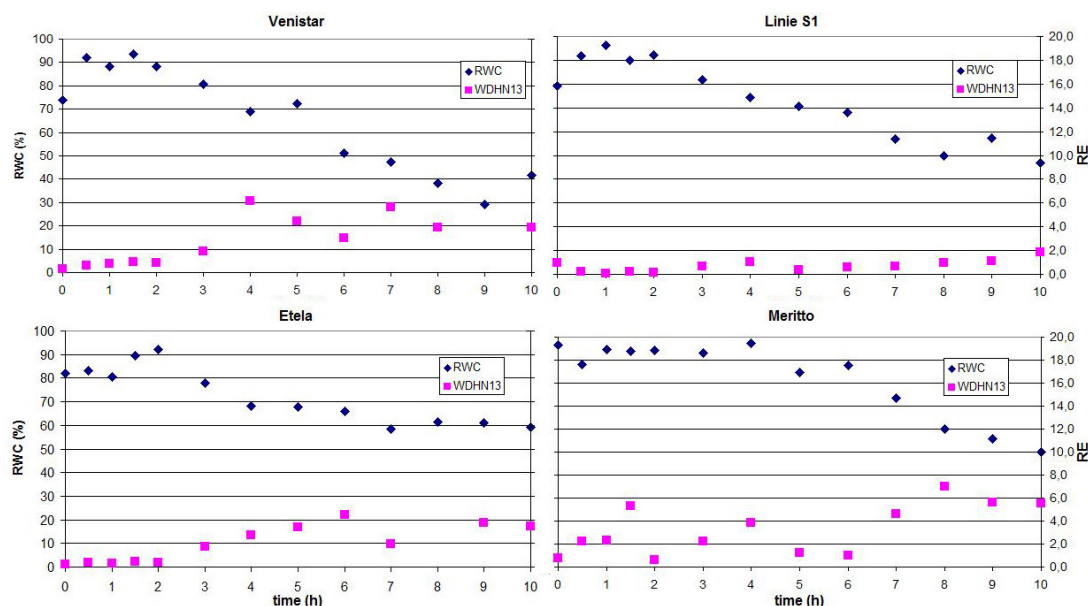
The increased activity of the gene was detected at a time when the plants were still maintaining a high water content in the leaves (RWC = 90%). RAMPINO et al. (2006) recorded a similar phenomenon in durum wheat: activation of the expression of genes from the *COR/LEA* group was recorded earlier in more drought tolerant genotypes.

Why a similar mechanism was not observed in 'Venistar' is unclear; it is probably replaced by another protective or control mechanism if grown under natural conditions. A similar hypothesis may apply to S1 in which, however, the existence of its drought tolerance is unclear. Winter damage to the stands of this line in 2011/2012 (data not shown) indicates a generally lower tolerance to abiotic stress (frost), which would be in good agreement with the result of our experiment.

The close relation between the level of desiccation and subsequent wilting of plants expressed by RWC and OP and relative gene expression of *WDHN13* and *WRAB17* is demonstrated by statistically significant correlations (Table 1). Significant correlations between the relative expression of *WDHN13* and *WRAB17* and RWC and OP were observed for

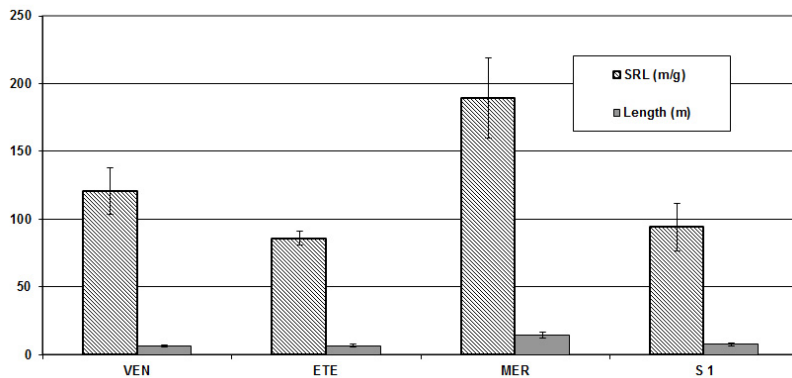
'Venistar' and S1, whereas for 'Etela' only the correlations with *WDHN13* were significant. For 'Meritto' a significant correlation was found only between RWC and *WDHN13* expression. According to KAMOSHITA et al. (2008) genotypes that can keep a higher RWC are more resistant to drought. In the present experiment the highest RWC (>70%) during desiccation was observed for 'Meritto'. SMUTNÁ and RYŠKOVÁ (2012) demonstrated that this variety shows stable yields under Czech climatic conditions. TAHARA et al. (1990) reported a positive relation between wheat grain yield and RWC measured during anthesis and mid-grain filling. Varieties with higher grain yields maintained a markedly higher RWC than low-yielding varieties. The greater decrease of RWC due to drought-induced stress is connected mainly with the capacity of more tolerant genotypes to better absorb soil water and/or with the capacity to prevent water loss through stomata (KEYVAN 2010). In our experiment the plants were pulled out from the soil. Therefore, only the second factor, i.e. transpiration control, could have been effective. Nevertheless, there could have been another factor: 'Meritto' showed faster development and had one leaf more at the prevalent conditions. During wilting, water from the lower leaves is transported to younger leaves, which have a smaller value of water potential. (KRPEŠ 2004). This is why 'Meritto' had more water available for distribution into younger parts of the plant.

According to SMUTNÁ and RYŠKOVÁ (2012) 'Venistar' showed good adaptation to drought conditions in long-term field trials. However, RWC of Venistar fell below 70% already within 25 minutes after the plant had been pulled out from the container (Figure 2). In the given growth stage, plants of 'Venistar' did not exhibit higher drought resistance compared to the other varieties. Drought-induced RWC decrease independent of drought resistance level was observed already by SCHONFELD et al. (1988). RITCHIE et al. (1990) reported that higher RWC results from either better osmotic regulation or lower elasticity of cell walls.



**Figure 2: Relationship between relative *WDHN13* gene expression and relative water content (RWC) during evaluated over the period of gradual wilting**





**Figure 3: Specific root length (SRL) and the total root length of winter wheat** (values represent means of 5 plants  $\pm$  standard deviation; VEN, Venistar; ETE, Etela; MER, Meritto; S1, Syrian line 1)

The relatively high level of *WDHN13* expression could point to a presence of other protective mechanisms that can manifest under field conditions. A part of drought adaptation mechanisms could be a higher expression of genes from the *COR/LEA* group.

The response of plants to drought stress can be affected by root system size and root architecture. *Figure 3* shows differences in the size and structure of the root systems of the tested germplasm. SRL is likely to be the most frequently assessed morphological trait expressing the architecture (fineness) of roots. SRL depends on both soil quality and genotypic effects; also genotype by environment interaction can be observed (OSTONEN et al. 2007, HAJZLER et al. 2012). Results in *Figure 3* indicate that 'Meritto' had the largest root system with the finest root hairs. Root hairs enlarge the external root surface, which results in increased absorption of water and nutrients by the plant. This property could have contributed to the observed higher biomass of 'Meritto' in the prevalent experimental conditions.

## Conclusion

A clear correlation was demonstrated between the loss of water of leaf tissues and the expression of *WDHN13* and *WRAB17* genes in winter wheat. The more tolerant varieties repeatedly showed an earlier onset and/or a higher level of the expression of genes from the *COR/LEA* group. Based on the assessed parameters the most tolerant genotype was 'Meritto'. Plants of this variety maintained water in leaf tissues >70% for the longest time and showed the most rapid onset of protective response, i.e. *WDHN13* gene activation.

## Acknowledgments

This study was supported by the Internal Grant Agency of the Faculty of Agronomy, Mendel University, Brno, project TP 10/2012.

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