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Indirect evaluation of drought tolerance of barley

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Abstract

Water availability in the soil is an essential condition for growth and development of plants. The occurrence of longer and more frequent periods of drought, can significantly threaten crop production. It is not easy to evaluate drought tolerance, because sensitivity to drought is a complex trait. Abscisic acid (ABA) is a plant hormone involved in processes including defense mechanisms of plants to abiotic stress. In our work we monitored the response of selected barley genotypes to exogenous application of ABA to asses its impact on the level of tolerance to abiotic stress. Barley genotypes were selected according to their different sensitivity to abiotic stress. Physiological response of plants and plant responses at the molecular level were evaluated. Response to exogenous application of ABA was observed in all genotypes. Higher levels of defense responses to drought conditions were found in tolerant genotypes. Results of *Dhn4* gene expression show different responses of spring and winter barley genotypes.

Key words

Abiotic stress, abscisic acid, gene expression, *Hordeum vulgare*

Introduction

One of the most important factors affecting yield of agriculturally important plants is water availability in the environment. Water has a rapid cycling in the ecosystem, complementing its rainfall is irregular and random. Therefore, the possible occurrence of periods of drought can substantially affect reproduction of plants, harvest quantity and quality. Lack of water causes complex changes in metabolic processes, which precedes gene activity leading to changes in the synthesis and activity of structural proteins. Genes that code for these protective proteins are e.g. dehydrins. They belong to a group of so-called *Cor/Lea* proteins (Cold regulated/Late embryogenesis abundant). Their expression is induced both during embryo maturation, but also in response to water shortages in the cells (CLOSE et al. 1996, PARK et al. 2006, TOMMASINI et al. 2008). They can occur in small quantities in plants growing under optimal conditions, but their quantity significantly increases as a stress response excited by dehydration (KOSOVÁ et al. 2007). The *Dhn4* gene is a member of the *LEA2* group, located on chromosome 6H. Its expression is due to stress conditions, e.g. drought and abscisic acid (ABA) treatment (CHOI et al. 1999). In more tolerant genotypes the expression of these protective genes occurs earlier (ZHANG et al. 2004, PARK et al. 2006). This can be used to assess the sensitivity of genotypes to this type of stress, but also to evaluate the intensity of stress within a single genotype. ABA is a plant hormone that participates significantly in photosynthesis (transpiration, stomata conductance) and at the same time, plays a key role for water sustenance in plants (ACHARYA and ASSMANN 2009). During drought stress ABA is binded on surface of the plasmalemma resulting in activation of stomata closing. Furthermore, during hydration ABA acts as a signal molecule in the regulation of activation of the protein expression path ending with a protective function against dehydration of the cell (ZHANG et al. 2004). The aim of our experiment was to test the possibility to detect different sensitivity of barley genotypes to abiotic stress (drought tolerance, frost tolerance), after aplication of exogenous ABA.

Materials and methods

Two varieties of spring barley (*Hordeum vulgare* L.), i.e. Jersey and Malz, and the Syrian landrace Tadmor (*Hordeum vulgare* ssp. *spontaneum*) were chosen in regard to their different degrees of tolerance to drought. Moreover, two varieties of winter barley, i.e. Monaco and Okal, with different tolerance to frost, were included in the study. Plants were grown in perlite, in the nutritive solution of MS salts (MURASHIGE and SKOOG 1962) under controlled cultivation conditions. After 14 days of cultivation, ABA solution (2.10^{-5} $mol.l^{-1}$) was added to the hydroponic solution. Leaf samples for analyses were taken 3 h, 6 h, 12 h, 24 h, 2 days, 3 days, and 7 days after ABA application. Quantification of ABA was carried out radioimmunologically (RIA) according to QUARRIE et al. (1988). Monitoring of the physiological reaction of plants was conducted by scanning the thermal energy emitted from the leaves surface by TermoCam (FLIR P660, Sweden) (JONES, 1999). Simultaneously, assessment of relative expression (RE) of ABA regulated gene *Dhn4* was carried out by qRT PCR according to PFAFFL (2001). RNA was isolated according to the RNeasy Plant Mini Kit (Qiagen) protocol. cDNA was prepared using the Reverse Transcription (Qiagen) Kit and genes activity was analyzed by the QuantiTectTM SYBR Green PCR (Qiagen) Kit. Δ-Tubulin was used as reference gene (SUPRUNOVA et al. 2004). Evaluation of the *Dhn4* gene was carried out according to MIKULKOVÁ et al. (2009). Values of the relative gene expression were normalized to the values of the relative expression of the reference gene.

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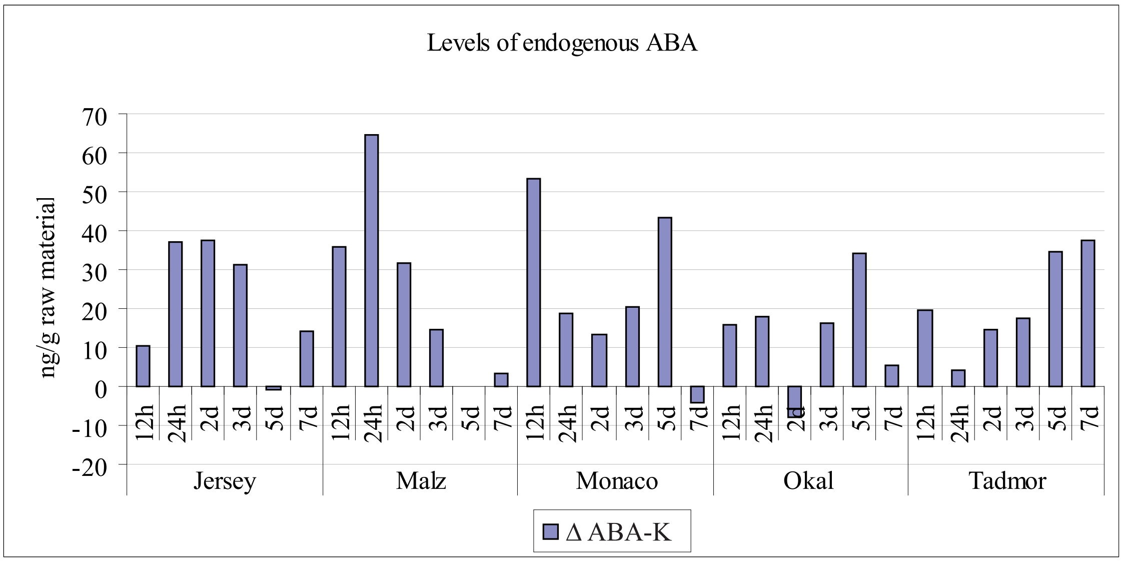

Figure 1: **Levels of endogenous ABA in barley varieties at specific times after ABA application**

Results and discussion

Measured values of ABA are presented in *Figure 1*. Mean values of leaf temperature are presented in *Figure 2*. In all tested genotypes the content of endogenous ABA in leaves was elevated after exogenous phytohormone application, which was reflected by the rise of leaf surface temperature in drought-tolerant genotypes (Tadmor, Malz). This trend prevailed during the whole experiment, while in case of the drought sensitive variety Jersey a drop in leaf surface temperature was observed on the 3rd day of measuring. A faster reaction following phytohormone application was observed in Okal, a variety which is more cold-tolerant in comparsion to Monaco. Drought results in a significant increase of ABA in the tissues (SCHWARTZ and ZEEVAART 2004).

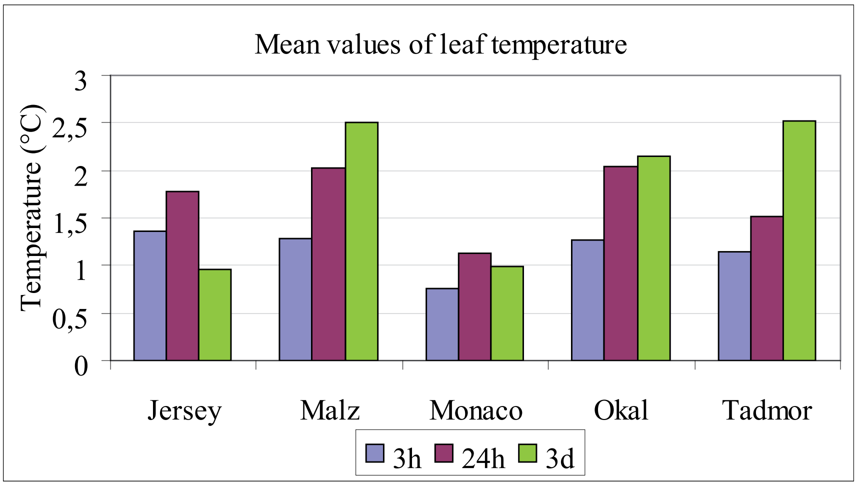

Figure 2: **Leaf temperature of barley varieties at specific times after ABA application**

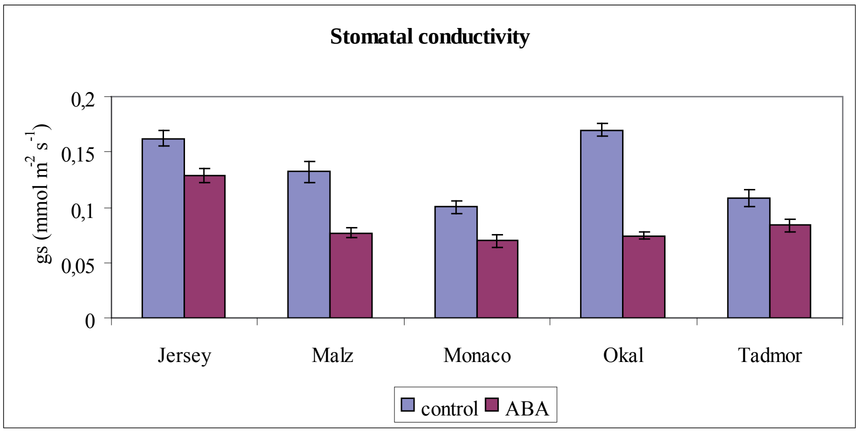

Figure 3: **Stomatal conductivity of barley varieties without and with ABA application**

Results of stomatal conductance measurements are displayed in *Figure 3*. The biggest difference between the ABA treated and the untreated control variant was observed in the winter cold-tolerant variety Okal which reacted by a very fast closure of stomata. The most closed stomata and lowest transpiration was found in Monaco and Tadmor in both the treated and untreated variant. In Tadmor the difference between the treated and untreated variant was the lowest. This variety had the most closed stomata from the very beginning. A low response to ABA in regard to the change in stomata conductance was observed for Jersey. Only a minimum drop in stomata conductance was recorded as result of exogenous phytohormone application. Under stress conditions, this variety activates its protective functions late and, therefore, loses water from its tissues.

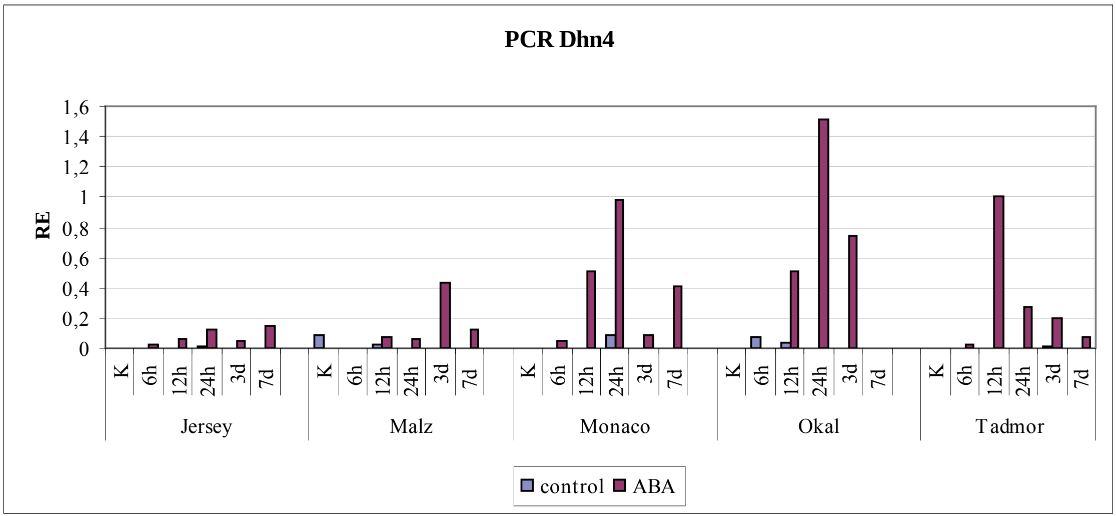

Figure 4: **Relative gene expression of Dhn4 of barley varieties at specific times after ABA application compared to non-treated (control, K) plants**

Relative expression of the *Dhn4* gene is displayed in *Figure 4*. The results show different sensitivity of genotypes to initial concentrations 2.10^{-5} mol.l^{-1} of exogenously applied ABA. Comparing varieties with different tolerance to cold, the higher value of the relative expression of was found in the cold tolerant variety Okal in contrast to the more sensitive variety Monaco. A faster reaction (i.e. more sensitive protective reaction) was found in drought tolerant Tadmor in contrast to Malz and Jersey. Jersey showed the lowest gene expression. This variety is generally sensitive to drought (MIKULKOVÁ et al. 2009) but achieves high yields under optimum climatic conditions. KOBAYASHI et al. (2008) studied wheat and also found a higher expression of *Dhn* genes in ABA sensitive mutants under identical stress conditions. Likewise, PARK et al. (2006) presented a faster and higher expression of the *Dhn3* and *Dhn4* genes, and MIKULKOVA et al. (2009) a higher expression of *Dhn4* in drought tolerant barley. The Syrian Tadmor landrace represents genotypes which are adapted to extremely dry conditions. This is caused not only by its physiological parameters such as the high osmotic potential (TEULAT et al. 1997), good water use efficiency (TEULAT 2001, MIKULKOVÁ et al. 2009), but also by its sensitive reaction to a relatively small increase of ABA as we have found in our experiments.

Differences between Tadmor and Jersey and Malz were found also on the DNA level. By evaluating the SSR polymorphism, a whole range of polymorphous areas spread on the whole genome were found (*Table 1*). The testing of their relationship to drought tolerance is ongoing.

Conclusion

Within the scope of this study, we used exogenous ABA application to signal abiotic stress and induce protective reactions to restrict water loss from tissues through transpiration by means of regulation of stomata conductance. In all tested genotypes an increase of endogenous ABA in leaves was detected, however, neither a statistically significant difference was found between genotypes nor the difference did correspond to any generally recognized tolerance. On physiological level an increase of the average leaf surface temperature was observes as result of exogenous ABA application which was manifested by lower stomata conductance. The influence of exogenous ABA was also studied by THOMAS et al. (1997) who presented that exogenous ABA application can induce drought like effects in plants. This finding was confirmed by our results. The evaluation of the relative expression of the *Dhn4* gene showed more sensitive protective reactions in more resistant genotypes. In Okal (cold-tolerant) and Tadmor (drought tolerant) a higher relative expression after ABA application was observed. Differentiation of genotypes according to their ABA sensitivity was possible on basis of relative gene expression. On physiological level (leaf temperature, stomatal conductivity) differences between treated and untreated variant were

Table 1: **Polymorphic SSR markers between Tadmor, Malz and Jersey**

Barley chromosome					
1H	2H	4H	5H	6H	7H
Bmac0213	Bmag0518	Bmag0740	Bmag0323	Bmac0316	EBmag0794
Bmag0504	Bmag0381	EBmac0775	Bmac0303b	Bmag0500	HVM04
Bmag0345	Bmag0711	Bmag0553	Bmac0113	EBmac0639	EBmac0603
GBM1451	Bmag0125	EBmac0658	Bmag0357	Bmag0613	GBM1464
Bmac032	EBmac0039	EBmac0635	Bmag0223	EBmac0602	Bmag0217
Bmac0154	EBmac0415	EBmac0679	Bmag0812		Bmag0516
			Bmag0222		Bmac0162
					GBM1419

observed after phytohormone application, however, they were not statistically significant.

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