Genes for stress adaptation in cereals: from transcript profiling to functional tests

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Introduction

Traditional breeding faces many challenges due to increasing populations; higher environmental standards; climatic changes etc. One of these challenges to meet is increased yield stability under water limited conditions that plays an essential role in the reduction of economic and social consequences of global climate changes. Recent advances of molecular physiology and genetics may provide new tools and resources to support these breeding efforts.

Plants developed wide range of defense strategies to maintain the functional integrity of cells and the whole organism as well under limited water supply. The rapidly synthesized abscisic acid coordinates many of the prompt reactions while the long term adaptation can rely on extended root growth, translocation of metabolites or acceleration of the developmental program.

Transcript profiling during osmotic and drought stress

In our laboratory, experimental systems were developed for studying key molecular components of long term osmotic (ZHIPONOVA et al. 2002) and drought (SECENJI et al. 2005) adaptation of the root system in cereals using remote wheat genotypes in transcript profiling experiments. The use of barley macro-arrays (POTOKINA et al. 2002, SREENIVASULU et al. 2002) developed at IPK, Gatersleben, highlighted gene clusters with characteristic changes in their expression pattern. Unraveling genotype dependent expression profiles and allelic variations of these genes will allow development of molecular markers for breeding programs.

We found that two genotypes (Kobomugi and Plainsman) differ in root growth rate, root/shoot ratio, and adaptation to osmotic stress and low soil water content. These genotypes exhibit characteristic transcript profiles as shown by barley macroarray studies using 10500 unigenes. In our first experimental system, effect of PEG-treatment on roots was investigated and induced genes that are common between the two genotypes were searched (*Figure 1*).

In order to simulate real drought stress, a second experimental system was developed, which is based on reduced watering of plantlets for four weeks and still allows purification of RNA of proper quality. Comparison of transcript profiles of PEG-treated and water limitation adapted roots of the two cultivars revealed that osmotic stress and real drought stress induces largely different changes in transcript profile, only one single gene was found (of unknown function), which was activated in both genotypes and in both experimental systems.

In the second experimental system, reprogramming of gene expression primarily occurred during the 1-2 weeks of water stress, and 6,1% of tested genes were up-regulated in roots of the more adaptive Plainsman plants. The time course for expression of gene clusters from Kobomugi genotype revealed a prompt and transient gene activation that can help the survival of plants through function of various defense mechanisms.

In an adaptive genotype, app. 8% of the genes showed a more than 2-fold increase in their expressions due to reduced

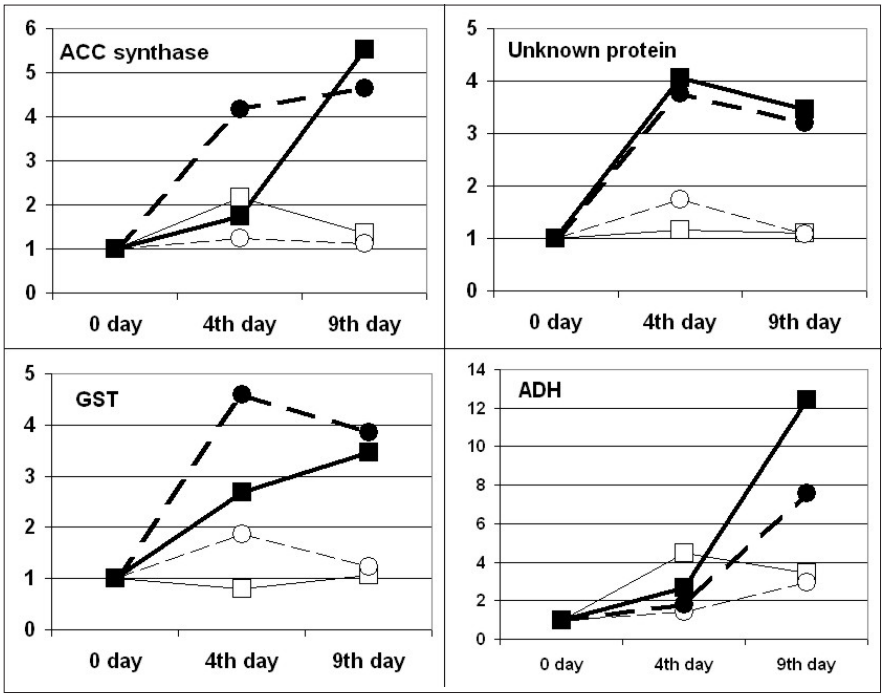

Figure 1: **The relative transcript levels of four selected genes exhibit induction during osmotic stress in wheat roots. Genes of ACC synthase, glutathione-S-transferase and alcohol dehydrogenase were found as examples which had similar transcript pattern in Plainsman (solid lines, square) and in Kobomugi (dashed lines, circle). Genes were induced during polyethylene glycol (PEG) treatment (thick lines) while remained relatively unchanged in control (thin lines).**

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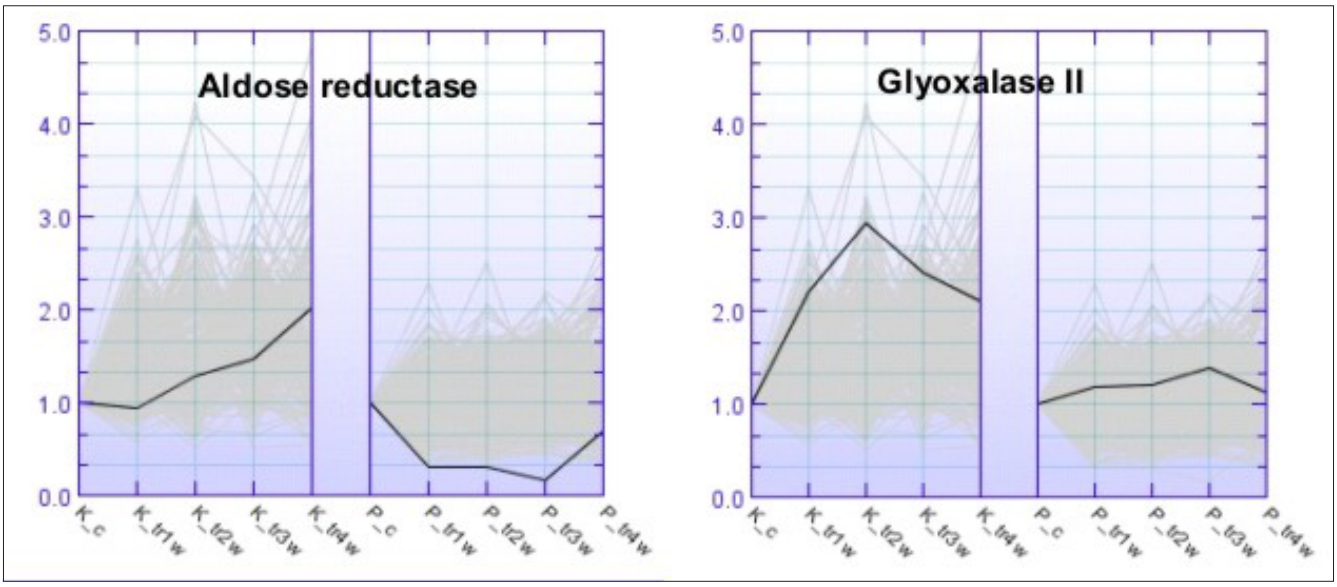

Figure 2: **Several genes encoding for detoxifying enzymes involved in oxidative stress are up regulated in Kobomugi genotype (left panels), however remain unchanged or even repressed in Plainsman (right panels). Four-weeks experiments were carried out, RNA samples were taken weekly.**

irrigation. In an escaper genotype, it was around 5%. Around 1.5% of the genes displayed increased expression level in both genotypes. Among the known genes either up- or down-regulated most of them encode metabolic enzymes. Some of them take part in detoxification [e.g. superoxide dismutase, ascorbate peroxidase, aldose reductase, glyoxalase] (*Figure 2*). Many genes were found encoding hypothetical or unknown proteins.

Genes that are up- or down-regulated during short-term desiccation stress, we were able to find only occasionally amongst our candidates. Known short-term stress-regulated genes present in this selection exhibit moderate increase/decrease of their relative transcription level in our experimental system. This indicates that long-term drought stress requires changes in gene expression and metabolism different from that of short-term [desiccation] stresses. Clustering revealed that the transcript profile of the common gene set is frequently different in the two genotypes. The observed transcription differences may provide, at least in part, the molecular background of the distinct adaptation strategies followed by either one of the two genotypes.

Conclusions of the transcript profiling experiments

Divergent drought adaptation strategies of the two genotypes are reflected in their transcript profiles. Long term adaptation is dependent on moderate changes in the expression of large set of genes in a coordinated manner.

Transient gene activation is characteristic to Kobomugi, while genes of the more adaptive Plainsman genoptype exhibit prolonged up regulation.

Based on the yield performance and photosynthetic activity, Kobomugi represents escaper strategy while Plainsman cultivar is capable to maintain physiological functions in harmony with gene expression reprogramming.

Genes found to be drought stress regulated in the two genotypes, as well as control ones (unaffected) are included in the ongoing development of a wheat test chip carrying 3200 long oligonucleotide (50-60-mers).

Functional tests of aldo-keto reductases

The aldo-keto reductases (AKRs) can detoxify lipid peroxidation products (4-

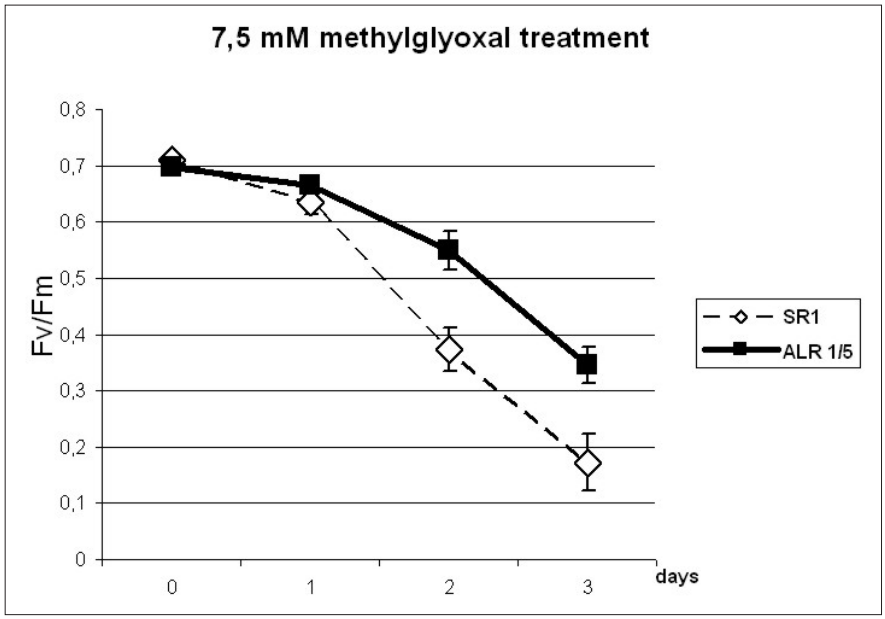

Figure 3: **Effect of methylglyoxal treatment on the photosynthetic activity of leaf discs of MsALR overproducing plant (ALR1/5) and its nontransformed control (SR1).**

hydroxynon-2-enal) and glycolysis-derived reactive aldehydes (metylglyoxal) that contribute significantly to cellular damages caused by variety of environmental stresses such as drought, high light intensity, UV-B irradiation, cold. Overproduction of AKRs in transgenic tobacco or wheat plants provides considerable stress tolerance and resistance to methylglyoxal. Molecular and physiological characterization of transgenic material will allow using AKR-based detoxification system in the improvement of stress adaptation of crop plants.

Medicago sativa derived MsALR overproduction has wide range of beneficial physiological effects on tobacco plants:

– protection against lipid peroxidation under chemical and drought stresses
– protection during drought and UV-B stresses (HIDEG et al. 2003)
– transgenic plants showed higher tolerance to low temperature and cadmium stress (HEGEDÜS et al. 2004)
– increased tolerance to the effects of high temperature and high light intensity (HORVÁTH and HIDEG, unpublished)

In collaboration with the Cereal Research Nonprofit Co. Szeged, the MsALR overproducing gene construct was introduced into wheat. Several independent transformants were regenerated and subjected to molecular and physiological characterization. Presence and expression of the transgene were proven and synthesis of the MsALR protein was also verified. Preliminary data indicate improved methylglyoxal tolerance of MsALR producing transgenic offsprings.

Based on the promising results with the alfalfa cDNA, close rice homologues of MsALR were cloned and analyzed. Expression studies revealed differential response of the members of rice AKR gene family to abscisic acid treatment in cell suspension. Enzymatic activity and substrate specificity of E. coli produced rice AKR proteins are investigated to find functional orthologue of the MsALR gene.

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References

HEGEDÜS, A., S. ERDEI, T. JANDA, E. TÓTH, G. HORVÁTH and D. DUDITS, 2004: Transgenic tobacco plants overproducing alfalfa aldose/aldehyde reductase show higher tolerance to low temperature and cadmium stress Plant Sci, 166:1329-1333.

HIDEG, É., T. NAGY, A. OBERSCHALL, D. DUDITS and I. VASS, 2003: Detoxification function of aldose/aldehyde reductase during drought and ultraviolet-B (280-320 nm) stresses. Plant Cell Env., 6:513-522.

POTOKINA, E., N. SREENIVASULU, L. ALTSCHMIED, W. MICHALEK and A. GRANER, 2002: Differential gene expression during seed germination in barley (*Hordeum vulgare* L.). Funct Integr Genomics 2:28-39.

SECENJI, M., A. LENDVAI, Z. HAJOS, D. DUDITS and J. GYÖRGYEY, 2005: Experimental system for studying long-term drought stress adaptation of wheat cultivars. Acta Biol Szeged, 49, 51-52.

SREENIVASULU, N., L. ALTSCHMIED, R. PANITZ, U. HÄHNEL, W. MICHALEK, W. WESCHKE and U. WOBUS, 2002: Identification of genes specifically expressed in maternal and filial tissues of barley cariopses: a cDNA array analysis. Mol Genet Genomics 266:758-767.

ZHIPONOVA, M., L. SZILÁK, L. ERDEI, J. GYÖRGYEY and D. DUDITS, 2002: Comparative approach for the isolation of genes involved in the osmotolerance of wheat. Acta Biol Szeged, 46, 49-51.