Volume 52(2):333-356, 2008
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Engineering plant abiotic stress tolerance by the overexpression of aldo/keto reductases

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Due to the sessile life style, plants are continuously exposed to a wide range of biotic and abiotic stress factors. This stress exposure severly affects their bioproductivity by causing the rapid and excessive accumulation of reactive oxygen species (ROS). ROS production in the vicinity of biomembranes containing polyunsaturated fatty acids can lead to lipid peroxidation and generate chemically reactive cleavage products, largely represented by aldehydes. Plant aldo/keto reductases (AKRs), among other enzymes, have been shown to be effective in the detoxification of lipid peroxidation-derived reactive aldehydes (Oberschall et al. 2000; Hideg et al. 2003).

In the present work we characterize a novel rice (*Oryza sativa*) AKR protein (OsAKR1) and investigate the transcriptomic changes in the gene expression profile of additional two AKR genes (*OsAKR2, OsAKR3*) in response to different stress treatments. A wide range of stress factors (abscisic acid, hydrogen-peroxide, mannitol etc.) was shown to trigger the expression of these AKR genes in rice cell suspensions, resulting in several folds of increased transcript levels. The most effective inducers were the ABA and hydrogen-peroxide, and *OsAKR1* gene turned out to be the most stress responsive. Stimulated by these results we investigated further the properties of the encoded protein by the *OsAKR1* gene, by cloning the full-length *OsAKR1* cDNA into recombinant protein expression construct, and purifying the glutathione-S-transferase (GST)-OsAKR1 fusion protein. Results of subsequent assays revealed that the GST-OsAKR1 recombinant protein exhibited a high, NADPH-dependent catalitic activity to metabolize toxic aldehydes (methylglyoxal, phenylglyoxal, glyoxal). Since cytotoxic reactive aldehydes can produce significant damages in the plant cells, the function of OsAKR1 protein to metabolize some of these harmful products was very promising. We also showed through *in vivo* experiments, that overproduction of this enzyme in *E. coli*, increased the tolerance of bacterial cells against high concentration (2mM) of methylglyoxal. The stress induced transcription of this AKR gene, as well as the data obtained from its biochemical characterization, supported its possible involvement in the abiotic stress induced reactive carbonyl detoxification pathways.

Till now there are several approaches to increase stress tolerance by manipulating the expression of endogenous, stress-related genes. Strategies targeting transcription factor expression have been shown to be effective, but on the other hand, stress tolerance can also be achieved by changing the expression of a single gene (Zhu 2001). Following the latter approach, we overexpressed the *OsAKR1* gene in tobacco (*Nicotiana tabacum*) and verified the effects of a single gene overexpression on the stress tolerance of the transgenic plants. We found, that the transgenic lines overproducing the OsAKR1 protein, accumulated significantly lower reactive aldehydes in response to the methylviologen (MV) treatment than the wild type. MV is a strong oxidative stress inducing herbicide, linked to ROS production and consequently to the formation of toxic aldehyde degradation products. In addition, the overexpressing lines reserved their photosynthetic functions more efficiently after heat treatments then the wild type. Therefore we suggest, that overexpression of a single gene (*OsAKR1*) and the accumulation of OsAKR1 protein is mainly beneficial in the detoxification processes against the reactive aldehydes generated at increased levels under stress conditions in the transgenic plants.

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Toxicogenomics screening of small molecules using high-density nanocapillary QRT-PCR technique

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Toxicogenomics combines studies of genomics, cell and tissue-wide protein expression and metabonomics to understand the role of gene-environment interactions in healthy and diseased samples. Predictive toxicogenomics is the acquisition of advanced knowledge of the safety profile of a compound using genomic biomarkers (Fielden et al. 2006). By clustering analysis of the gene expression profiles over selected biomarkers induced by the lead molecules and relevant derivatives, the medicinal chemist can deduce the relationship between structural modifications and changes in the toxicity profile (structure-toxicity relationship). Involvement of well-characterized reference compounds can be of help in this profiling, for instance defining the specific tissue or organ toxicity.

Using in-house validated chemical reactions that are suitable for parallel synthesis and a collection of multifunctional „drug-like" scaffolds, a dedicated discovery screening library of 10,000 compounds has been enumerated by a cascading diversity building approach (www.amriglobal.com). Based on the cytotoxicity measured in MRC-5 human fibroblast assay, further on HepG2 human hepatocarcinoma assay and the interpolated IC_{50} values, 668 compounds were selected aiming for maximal diversity of scaffolds.

These selected 668 small, drug-like compounds of unknown effects and other, toxic compounds of known and of yet unknown effects and pharmaceutical active entities were screened for their gene expression profiles *in vitro*, over 56 selected biomarkers (toxicology, transporters). Our objective was to see to what extent the highly similar chemical structures induce similarities in their hepatotoxic fingerprints and to test the analytical performance of the nanocapillary QRT-PCR technique and its general applicability for the field of toxicogenomics.

Preliminary tests have been performed with our inhouse ToxicoScreen DNA-microarrays (Vass et al. 2006) and with the traditional QRT-PCR technique, following which we shifted to the OpenArray nanocapillary quantitative real-time PCR-technology (Morrison et al. 2006; Avidin Ltd.-BioTrove Inc.) that has meanwhile appeared on the market. This later technology merges the high-throughput of DNA-microarrays with the sound characteristics of QRT-PCR.

By the combination of a relatively large combinatorial chemical library and a relatively small set of selected toxicological biomarkers, we intended to avoid the two culprits of toxicogenomics: 'the curse of dimensionality' (too many genes), and 'the curse of dataset sparsity' (too few samples). The generally accepted, however rarely adapted sample-per-feature ratio for robust clustering performance is at least 5 to 10.

Based on the scaffold structure or the characteristic residues, we assigned the tested chemicals into subgroups. Different clustering methods were applied, based on results from unsupervised hiearchical clustering we performed supervised, K-means clustering. Our objective was to see wether the correlation between gene expression fingerprint and structure of the compound inducing it can be detected and to what extent can this correlation be rooted back to the scaffolds.

This work was supported by the following grants: Oszkár Asboth „XTTP" (NKTH), GVOP-3.1.1.-0280/3.0

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Tools for improving stress adaptation in cereals

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Abiotic stresses are very important factors that reduce crop productivity. Plant root is the primary organ for uptake of water and nutrients, therefore it plays important role in tolerance to stresses like drought or salinity. Plants developing stronger and deeper roots suffer less from water deficit. The aim of our work was to improve the stress-tolerance in cereals using transcriptome analysis of rice cultivars under drought stress conditions, and stress-induced and root-specific promoters.

Genes facilitating development of efficient root system can increase the survival of the plant. Fusions of drought stress-related root-specific promoters to these genes may provide environment friendly and efficient solution to improve roots of crop plants under stress conditions.

Based on published data two candidate promoters were selected: the rice *CatB* and the *RSOsPR10* promoters. The *CatB* promoter is known to be root-specific (Iwamoto et al. 2004), the expression of the *RSOsPR10* mRNA is high in salt and drought stress conditions in root (Hashimoto et al. 2004). Both promoters have stress-related transcription factor binding sites and (MYB, WRKY, DREB, LTRE) in their sequence. The 1.6 kb CatB promoter and the 2 kb of the 5` flanking region of the *RSOsPR10* were cloned, and fused to reporter genes. The constructs were transformed into rice calli and tobacco leaves.

On the regenerated T_0 rice plants, salt stress was performed that revealed the *RSOsPR10* promoter directing root-specific and stress-induced expression pattern of GFP reporter. CatB::GUS transformed T_0 and T_1 tobacco plants showed root and vascular bundle-specific GUS expression, and induction under salt stress.

The changes of the rice root transcriptome under stress conditions and its alterations during a daytime period were investigated in a greenhouse experiment including three cultivars growing in a sand-perlite soil mixture. The stressed plants were irrigated with 20% of water for one month, causing drought-stress condition. The samples were collected three times in a day from each genotype both from drought stressed and control.

To follow the transcriptional changes, root samples from the most tolerant genotype were hybridized with rice oligonucleotide DNA chip. 3200 of the genes represented on the chip gave signal in all of the hybridizations, and 11.6% were up-regulated, and 6.7% were down-regulated in the adaptive cultivar.