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Changes of glutathione S-transferase activities and gene expression in *Triticum aestivum* during polyethylene-glycol induced osmotic stress

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ABSTRACT Glutathione S-transferase (GST) isoenzymes represent a large and variable group of antioxidative enzymes, with several different activities and sequence patterns. The GST activities of drought-tolerant *Triticum aestivum* L. cv. Kobomugi and cv. Plainsman were measured after one week 400 mOsm polyethylene glycol (PEG) treatment. The GST activities were much higher in the root than in the shoot and were induced by PEG especially in root. The aim of our work was to sort out the drought stress related wheat GST genes. Phylogenetic analysis of wheat *GSTs* was performed *in silico* and using the tentative consensus sequences a dendogram was composed. According to the conserved sequences used for classification of GST proteins, we could identify six groups of wheat GSTs. The phi GSTs are the most heterologous group, containing 25 sequences. The zeta, theta and tau GSTs are represented by 10, 9 and 8 TCs respectively. There are two other unidentified groups containing 8 and 6 sequences. Homology found between the osmotic stress upregulated sequences and the GST coding TCs were identified.
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KEY WORDS

Glutathione-S-transferase
wheat
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upregulated sequences

Plants have evolved very effective defence mechanisms against stress-induced oxidative damages. One of them relies on glutathione S-transferases (GSTs), which are ubiquitous enzymes performing a range of functional roles using the tripeptid glutathione (GSH) as a cosubstrate or coenzyme. The GSH-dependent catalytic functions include the conjugation and resulting detoxification of cytotoxic products, e.g. organic hydroperoxides formed during oxidative stress and the isomerization of maleylacetoacetate to fumarylacetoacetate, a key step in the catabolism of tyrosine. GSTs also have non-catalytic roles, binding plant hormones (and thus perhaps controlling their level) or flavonoid natural products in the cytosol prior to their deposition in the vacuole. Recently, it was reported that some isoenzymes have glutathione-dependent dehidroascorbate reductase activity and GSTs are components of UV-inducible cell signaling pathways and potential regulators of apoptosis (Edwards et al. 2000; Kampranis et al. 2000; Dixon et al. 2002a). These different functions coincide with high diversity in the protein and nucleotide sequence. On the basis of their primary structure, the plant GSTs may be grouped into four main classes (phi, zeta, tau and theta) and two outlying groups (Dixon et al. 2002b). The long term goal of the present work is to sort out the drought stress related members of this gene family and to reveal their particular role in drought adaptation.

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Materials and Methods

Osmotic stress treatment was applied gradually reaching 400 mOsm polyethylene glycol (PEG 6000) treatment (-0.976 MPa) on one-week-old *Triticum aestivum* plants under controlled conditions as it was published earlier (Erdei et al. 2002). Enzyme activities were measured both in roots and shoots of the treated and control plants after one week PEG treatment. GST activity was determined spectrophotometrically by using the artificial substrate CDNB according to Habig et al. (1974). Phylogenetic analysis of wheat *GSTs* was performed *in silico* using e.g. ClustalX and Bioedit softwers on the TIGR and GenBank databases. The family tree was composed from approximately 300 amino acid long sequences (Phylodendron, for drawing phylogenetic trees, by D.G. Gilbert version 0.8d).

Results and Discussion

The *Triticum aestivum* cv. Kobomugi is a drought-tolerant ancient line originated from inner part of Asia, the cv. Plainsman is a drought- and frost-tolerant canadian cultivar. The enzyme activity was higher in the cv. Kobomugi than in cv. Plainsman both in control and in the osmotic stress treated plants. The GST activities were much higher in the root than in the shoot and were induced by PEG especially in root. The increase of enzyme activities was not-significantly higher in the cv. Kobomugi than in cv. Plainsman after one week osmotic stress treatment.

The expression of GST genes raised due to osmotic stress in different wheat cultivars (Zhiponova et al. 2002). The molecular analysis of treated Kobomugi and Plainsman plants revealed that expression of several *GST* genes becomes elevated under osmotic stress in both lines (Györgyey et al., unpublished results). To identify the upregulating GST coding sequences 700-1500 bp cDNAs coding wheat GSTs were taken from GenBank database and 8 wheat *GST* genes were selected for homology searching of tentative consensus sequences (TCs) in TIGR database with high homology to known *GSTs.* The minimum cut-off E value was more than e-20 using as standard criteria. The search resulted 105 sequences using these genes and an alignment was created. The independent sequences were translated by Bioedit, the contradicting TCs eliminated and finally an alignment with 66 sequence was used for further estimation. The homology based tree was created after selecting ca. 300 amino acid long sequence region exhibiting the highest similarity.

According to the conserved sequences used for classification of GST proteins, we could identify six groups of wheat GSTs. The phi GSTs are the most heterologous group, containing 25 sequences. The zeta, theta and tau GSTs are represented by 10, 9 and 8 TCs respectively. There are two other unidentified groups containing 8 and 6 sequences. Homology found between the osmotic stress upregulated sequences and the GST coding TCs were identified. For further investigation different *GST* genes from 5 GST classes were chosen.

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