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ARTICLE

Monitoring the levels of *phi* and *tau* group GST genes in wheat cultivars under osmotic stress

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ABSTRACT GST isoenzymes represent a large and variable group of antioxidative enzymes, with several different activities and sequence patterns. The GST activities of the isohydric drought-tolerant *Triticum aestivum* L. cv. Kobomugi and the anisohydric cv. Öthalom were measured after one week of 400 mOsm polyethylene glycol (PEG) treatment. The GST activities were much higher in the roots than in the shoots and were induced by PEG especially in the roots. The aim of our work was to sort out the osmotic stress related wheat *GST* genes. The changes in enzyme activities and expression of several *GST*-coding sequences were in good correlation. Both cultivars responded to osmotic stress. Higher induction, especially in *phi* class *GSTs* was detectable in the isohydric Kobomugi cultivar. Elevations were measured in the transcript amounts of six different GST genes.
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KEY WORDS

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Glutathione transferases (GST, E.C.2.5.1.18) are a heterogeneous group of cell detoxifying enzymes, which catalyse the conjugation of tripeptide glutathione (GSH) to electrophilic sites on a wide range of phytotoxic substrates (Kampranis et al. 2000). GST isoenzymes have also a function in the hormone transport and homeostasis, for example in the cellular response to auxins (Bilang et al. 1993), cytokinins (Gonneau et al. 1998) and ethylene (Zhou and Goldsbrough 1993). Some GST isoforms have glutathione peroxidase (GPOX) activities, their main function can be the reduction of the toxic lipid peroxidation products and maintenance of the membrane integrity e.g. under osmotic stress (Dixon et al. 2003).

On the basis of their primary structure, the plant *GST*s may be grouped into four main classes (*phi*, *zeta*, *tau* and *theta*) and two outlying groups (Dixon et al. 2002b). *Phi* and *tau GST*s are specific to plants, and are the most abundant and variable classes (Edwards and Dixon 2005). These enzymes showed the highest conjugating activity towards 1-chloro-2-,4-dinitrobenzene (CDNB) substrate, and some members of these classes had prominently high activities against stress metabolite analogous (Cummins et al. 2003).

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* L. cv. GK Öthalom and cv. Kobomugi plants under controlled conditions as it was published earlier (Erdei et al. 2002).

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Tissue homogenization and extraction steps were carried out at 4°C. Crude protein extracts were prepared by homogenizing 0.5 g leaves and roots in 2 ml extraction buffer (0.1 M phosphate buffer pH 7.0, containing 1mmol/L phenylmethylsulfonyl fluoride and 1% polyvinyl-polypirrolidone) with mortar and pestle. The homogenate was then centrifuged at 10000 *g* for 15 min, and supernatant was decanted.

GST (EC 2.5.1.18) activity was determined spectrophotometrically by using an artificial substrate, CDNB, according to Habig et al. (1974). Reactions were initiated by the addition of CDNB, and the increase in A_{340} was determined. One U is the amount of enzyme producing 1µmol conjugated product in 1 min, ε_{340} = 9.6 mmol $L^{-1}cm^{-1}$.

RNA was extracted from root samples two day after 400 mOsm PEG treatment was applied, according to Chomczynski and Sacchi (1987). DNase digestions were applied (Fermentas, Sambrook and Russell, 2001). First strand cDNA was synthesized using MMLV reverse transcriptase (Fermentas, Gerard and D'Alessio 1993). Primers were designed using Primer express and Primer 3 softwares (Rozen and Skaletsky, 2000). Primers were synthesised in the Nucleic acid synthesis laboratory, Biological Research Center (Szeged, Hungary). Monitoring of the expression rate of *GST* genes was performed with Quantitative Real-Time PCR (BioRad, MJ Research) using SYBR green probes (Applied Biosystems; Karsai et al. 2002). Data analysis was performed using Opticon monitor software. Data were normalised using wheat 18S ribosomal RNA and elongation factor α subunit (EF-1) as high and low controls (Jukanti et al. 2006, Buchanan et al. 2005).

Results and Discussion

The *Triticum aestivum* L. cv. Kobomugi is a drought-tolerant ancient line originated from inner part of Asia, the cv. Öthalom is an anisohydric, dehydration tolerating Hungarian genotype. The GST was measured in function of time after the osmotic treatment was applied. The GST activities were much higher in the root than in the shoot and were induced by PEG in roots. In roots of Öthalom, the PEG treatment caused significant enhancement from the beginning of the experiment; the elevation of the GST activities appeared later and was more intensive in Kobomugi. Transcript amount of *phi* and *tau* genes were investigated on 13th day, two days after the 400 mOsm osmotic treatment was applied. According to the literature, the tau class GST U2 wheat enzyme showed prominently the highest conjugating activity of all investigated glutathione transferase enzymes against CDNB substrate (Thom et al. 2002). In our experiments the expression of *TaGSTU2* gene was in good correlation with the GST activity, the transcript amount of this gene was more induced in Kobomugi than in Öthalom. TaGSTU1 protein presumably plays important roles in the cell detoxification, as this enzyme has high conjugating activity towards crotonaldehyde and CDNB (Thom et al. 2002). The two alleles (*B*, *C*) of *TaGSTU1* showed overexpression due to osmotic stress in both cultivars, but some differences were detectable between the transcript amounts of the two alleles.

Two *phi* class *GST* were investigated. The *TaGSTF6* coded protein was characterized by Cummins et al. (2003) and exceeded in conjugating activity against stress metabolite analogous: crotonaldehyde and ethacrynic acid, which indicates the importance of this gene product in the stress acclimatization. *TaGSTF6* showed three times induction due to osmotic stress in Kobomugi, while less elevation was detectable in Öthalom. The other investigated *phi* group gene, *TaGST19E50* transcript level was also induced in both cultivars, but was more influenced by osmotic stress in Kobomugi.

In summary, in the isohydric Kobomugi cultivar the transcript amount of both *phi* and *tau* class GST genes were induced at least two times. In cv. Öthalom, smaller inductions appeared in the *phi* class GST gene expressions which presumably cause less effective elimination of phytotoxic stress metabolites.

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