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Induction and regulation of glutathione transferases in wheat species exposed to PEG induced osmotic stress

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ABSTRACT The large and variable family of glutathione transferases (GST) has several functions in the stress response mechanisms. Our aim was to define the roles of different GST types in defence during osmotic stress conditions in wheat seedlings and to characterise their regulation by the stress hormone abscisic acid (ABA). Two wheat cultivars with different drought tolerance ability were exposed to 400 mOsm polyethylene glycol induced osmotic stress for one week. The hyperosmolarity of the nutrient solution increased the GST activity and the transcript amount of the selected tau group GSTs in the drought tolerant Kobomugi and moderately drought tolerant GK Öthalom cultivars. The role of abscisic acid in the regulation of GST expression was examined by the inhibition of ABA biosynthesis pathway with fluridone. The tau group GST expression of the two cultivars responded differently to the ABA biosynthesis inhibition.

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KEY WORDS

glutathione transferase
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fluridone

The large and divers family of glutathione transferases (GST) has several functions in the stress response mechanisms. Besides their role in detoxification by catalyzing the conjugation of tripeptide glutathione (GSH) to electrophilic substrates, GSTs (E.C.2.5.1.18) has several other functions in cell defense reactions: e. g. some GST isoforms have glutathione peroxidase (GPOX) activities, which catalyze the reduction of the toxic lipid peroxidation products and play a role in the maintenance of the membrane integrity under stress (Dixon et al. 2002). The soluble plant GSTs are grouped into seven classes. The two largest groups are the phi and tau groups. Several members of both groups are induced by abiotic stresses (Gallé et al. 2009, Peng et al. 2009, Secenji et al. 2010, Bazargani et al. 2011). Some phi class GSTs has catalytic activity towards 4-hydroxynonenal, a toxic product of the lipidperoxidation (Gronwald and Plaisance 1998; Sappl et al. 2009). Besides the transferase activity the phi group GST plays also roles in flavonoid transport (Kitamura et al. 2004). Members of the tau group GSTs (Triticum tauschii GSTU1 and GSTU2) presumably are regulated by abscisic acid (ABA) as in the promoter of these genes ABA responsible elements were found (Xu et al. 2002).

The aim of this work was to define the roles of different GSTs in defence during osmotic stress conditions in wheat seedlings and to characterise their regulation by the stress hormone abscisic acid (ABA).

Materials and Methods

In the first experimental procedure the 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 Kobomugi plants under controlled conditions as it was published earlier (Erdei et al. 2002).

In the second experimental system the fluridone (15 µM) was added to the nutrient solution of the one week old wheat seedlings. In this experiment the osmotic stress was induced by increasing the osmolarity of the nutrient solution of the 7 days old seedlings to 200 mOsm with PEG. The sampling for the relative GST expression was 24 hours after the fluridone and PEG treatment.

GST activity was determined spectrophotometrically by using an artificial substrate, 1-chloro-2,4-dinitrobenzene (CDNB), according to Gallé et al. (2009). Reactions were initiated by the addition of CDNB, and the increase in A_{340} was determined. One U is the amount of enzyme producing 1 µmol conjugated product in 1 min, $\epsilon_{340} = 9.6 \text{ mmol L}^{-1}\text{cm}^{-1}$.

The detection of the GST relative transcript amounts was performed with Quantitative Real-Time PCR (BioRad, MJ Research) using SYBR green probes (Applied Biosystems). For data analysis Opticon monitor software was used. Data were normalised using wheat 18S ribosomal RNA and elongation factor α subunit (EF-1) as high and low controls.

Results and Discussion

Two wheat cultivars, with different drought tolerance sus-

ceptibility, were exposed to 400 mOsm polyethylene glycol induced osmotic stress for one week in the first experiment. The GST activity was induced by stress in case of moderately drought tolerant GK Öthalom and in drought resistant Kobomugi. The 100 mOsm PEG treatment (the first step of treatment) increased the GST activity and expression level of the selected tau group GSTs in the drought tolerant Kobomugi and in the moderately drought tolerant GK Öthalom cultivars.

The role of abscisic acid in the regulation of GST expression was examined by the inhibition of ABA biosynthesis pathway with fluridone. The tau group GST expression (namely TaGSTU1C and TaGSTU2 genes) of the two cultivars responded differently to the ABA biosynthesis inhibition. The fluridone treatment decreased the TaGSTU1C transcript amount both in control and PEG-treated conditions in GK Öthalom. In case of TaGSTU2 similar inhibition was detectable, which suppose the ABA regulation of these genes GK Öthalom. The inhibition of the ABA biosynthesis had less effect in Kobomugi, which suggest a less ABA dependent tau group GST expression in this cultivar, than in GK Öthalom.

In summary, in the isohydric Kobomugi and moderately drought tolerant GK Öthalom cultivars the osmotic stress induced the transcript amount of both phi and tau class GST genes, but mostly in case of TaGSTU1C and TaGSTU2. ABA biosynthesis inhibition decreased the expression of TaGSTU1C and TaGSTU2 in GK Öthalom, which refer to the different ABA regulation of the GST isoenzymes in the two wheat lines.

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