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 hyperosmolality 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.

**Key words**

glutathione transferase  
wheat  
osmotic stress  
abscisic acid  
fluridone

**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 1µmol conjugated product in 1 min, \( r_{340} = 9.6 \text{ mmol L}^{-1}\text{cm}^{-1} \).

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 \( \alpha \) 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 Óthalom and in drought resistant Kobomugi. The 100 mOsm PEG treatment (the first step of treatment) induced the GST activity and expression level of the selected tau group GSTs in the drought tolerant Kobomugi and in the moderately drought tolerant GK Óthalom 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 Óthalom. In case of TaGSTU2 similar inhibition was detectable, which suppose the ABA regulation of these genes GK Óthalom. 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 Óthalom.

In summary, in the isohydric Kobomugi and moderately drought tolerant GK Óthalom 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 Óthalom, which refer to the different ABA regulation of the GST isoenzymes in the two wheat lines.

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