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ORIGINAL ARTICLE

Increased glutathione S-transferase activity in 35S(CaMV)-*Zmgstf4* transgenic *Arabidopsis thaliana*

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Abstract *- Clones of 35S-Zmgstf4 transgenic Arabidopsis thaliana expressing the glutathione S-transferase F4 gene of Zea mays, were tested for stress-inductive GST (glutathione S-transferase) activity following treatments with the heavy metals Zn (150 and 1500 µM), Cd (20 and 30 µM) and chloroacetanilide herbicide metolachlor (2000 µM). The overexpression of Zmgstf4 gene in Arabidopsis resulted in an extreme resistance to all treatments. The GST activity of the transgenic plants was almost the double compared to the wild type plant in the untreated samples. After Cd (20 and 30 µM), and Zn (150 and 1500 µM) exposure the stress response activity of GSTs increased in both wild type and transgenic plants, however with significantly higher levels in transgenic plants with extreme level at 20 µM $CdSO_4$ treatment (0.24 in transgenic and 0.13 in wild-type). To compare GST responsivity, Zn treatments was less inductive compared to Cd. Metolachlor (200 µM) was totally tolerated by transgenic plants, compared to wild type plants, which died in 11 days.*

Keywords - transgenic Arabidopsis, zinc, cadmium, metolachlor, glutathione S-transferase

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Introduction

Plant GSTs (glutathione S-transferases) (EC 2.5.1.18) are a large and diverse stress-protective enzyme family, which catalyze the conjugation of the tripeptide glutathione (gamma-L-glutamyl-L-cysteinylglycine, GSH) with a wide variety of harmful electrophilic xenobiotics. E.g. plant resistance to chloroacetanilide herbicides is mainly caused by the functions of GSTs in maize and soybean, which, in case of atrazine, results in GSH-atrazine a complex (Cummins et al. 2011; Dixon, Skipsey, and Edwards 2010; Labrou et al. 2015). Recent results revealed that GSTs are also involved in heavy metal stress defence mechanisms in plants (Lyubenova and Schröder 2011; Schröder 2001). Genes encoding for GSTs are grouped in diverse gene families of A, B, D, K, M, O, F, T, U, Z, L, M, S. In *Arabidopsis*, 51 *At*GST isoenzyme genes were cloned belonging to F (Phi), T (Theta), U (Tau) and Z (Zeta) families (Fig. 1). In maize 42 *Zm*GSTs (12 F, 28 T, and 2 Z), and in soybean 25 *Gm*GSTs (20 T, 4 F, and 1 Z) are known.

Here we report a study of GST enzyme activity of wild type and 35S(CaMV)-*Zmgst*F4 transgenic *Arabidopsis* following exposure to Zn, Cd and metolachlor.

Materials and methods

Plant material

Arabidopsis thaliana (ecotype: Col-5) were transformed with cDNA clone overexpressing Zm*gstf4* gene (*Zea mays*, NCBI: U12679 / X79515; Uniprot: P46420) driven by cauliflower mosaic virus 35S promoter. cDNA was introduced with floral dip transformation method (Clough and Bent 1998) using the hygromycin phosphotransferase (*hpt*) gene as selectable marker. The pCAMBIA1301 binary vector was used for transformation.

Seeds of transgenic 35S(CaMV)-*Zmgst*F4 and wild type *Arabidopsis thaliana* (ecotype: Col-5) were germinated *in vitro* on aseptic hormone free agar media. First, seeds were stratified in dark for 72 hour at 4°C to break seed dormancy. Seeds were surface sterilized with 70 % ethanol (2 min), followed by 0.5% NaOCl (3 min) and rinsing with ddH_2O, sown on ½ MS media, solidified with 7% agar, and supplemented with different chemicals. After germination, seedlings were illuminated with Osram Fluora fluorescent lamps for 16/8 photoperiod (16-27 µmol m^{-2} s^{-1}). Metolachlor was applied to plants as irrigation at rosette stage grown on Jiffy peat.

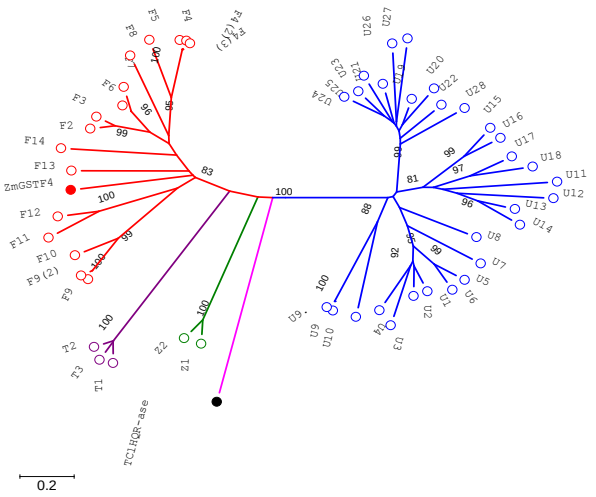

Figure 1. Minimum Evolution (ME) cladogram of the amino acid (aa) sequences of *At*GST isoenzyme families (1 - 51) compared to *Zm*GSTf4 (223 aa, NCBI #NP_001105366). ME cladogram was edited by MEGA4, with x1000 bootstrap based on the sequences of NCBI data bank. GST gene families (F - Phi, T - Theta, U - Tau, Z - Zeta) are indicated with different colours. Genetic distance (scale) indicates amino acid substitution rate per loci.

Biochemical analysis

The aerial parts of plants were harvested, frozen in liquid nitrogen, and stored at -80ºC prior to enzyme extractions (Schröder et al. 2003). The GST enzyme activities were measured according to Lyubenova and Schröder (2009) with CDNB model substrate at 22°C by using a Shimadzu UV-1601 Spectrophotometer at 22°C.

Statistics

At least three independent parallel experiments were carried out in each case. The significant differences between mean values were evaluated by Student's *t*-test at P=0.05.

Results

Symptoms

Wild type plants treated with Zn and Cd showed typical heavy metal toxicity symptoms: loss of chlorophyll and leaf turgor. The symptoms of metolachlor (200 µM) were also significant (Fig. 2); apparently, transgenic plants were unaffected by metolachlor compared with wild type plants, which died after 11 days (Fig. 2). The root development of wild type plants was also seriously inhibited in wild type.

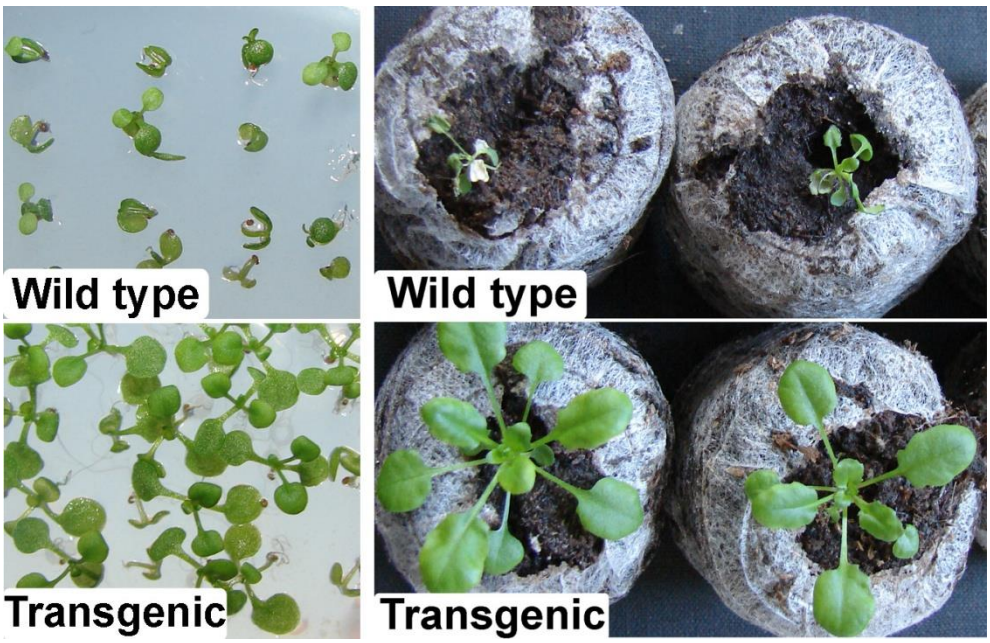

Figure 2. Phytotoxic symptoms of wild-type and 35S(CaMV)-*Zmgst*f4 transgenic *Arabidopsis thaliana* plants following a 10-day exposure to metolachlor (2000 µM) in agarose media, (left) and in pots (in Jiffy peat, right)

Treatments

Zinc ($ZnSO_4$) (150 and 1500 µM), and cadmium ($CdSO_4$) (20 to 200 µM) were applied to the agar media *in vitro* (Fig. 2). Metolachlor (2000 µM) was applied (in agar media, or with irrigation) at concentrations lethal to wild type, which was determined in preliminary measurements.

Enzyme activities

The GST activity of the transgenic plants is 75% higher than in the wild type plant (Fig. 3). After Cd (20 and 30 µM), but not Zn (150 and 1500 µM) exposure, increased GST activities were measured in both wild-type and transgenic plants, but the induction in transgenic plants was significantly higher (Fig. 3).

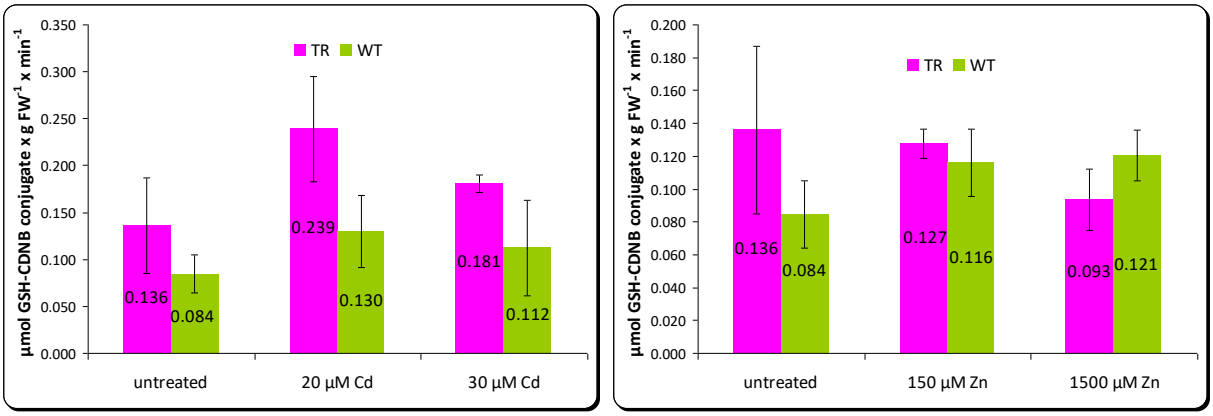

Figure 3. Example The activity of GST (glutathione S-transferase) enzymes in wild-type (WT; green columns) and 35S(CaMV)-*Zmgstf*4 transgenic (TR; pink column) *Arabidopsis thaliana* under stresses of Cd ($CdSO_4$) *(left)* and Zn ($ZnSO_4$) *(right)*. Mean values ± SEM are indicated in percent of untreated wild type plant.

Discussion

Genetic engineering is a powerful tool to study plant metabolic pathways. Overexpression of specific genes helps to clarify their physiological roles in the metabolism grown under different stress conditions (Bittsánszky et al. 2015). Deeper understanding of the biochemical pathways contributing to the processes of uptake, translocation and accumulation of heavy metals, and tolerance of phytotoxic chemicals will greatly help the improvement of phytoremediation potential of plants. Glutathione S-transferases seem to be valuable targets for these purposes.

Glutathione S-transferases are considered to play an important role in heavy metal stress (Mendoza-Cozatl et al. 2011; Schröder et al. 2003; Saraswat and Rai 2011), especially through detoxification (Lyubenova et al. 2009).

Our results has indicated that overexpression of GST enzymes can play important roles in the detoxification of heavy metals, and tolerance to herbicides. The overexpression of *Zmgstf*4 gene was also found to increase resistance against chloroacetanilide herbicides (Milligan et al. 2001).

In conclusion, *Zmgstf*4 transgenic *Arabidopsis* plant investigated in this study provided new data on the understanding of plant GST functions with indications in their use in phytoremediation. Results also show the applicability of the *Zmgstf*4 gene in molecular plant breeding for phytoremediation purposes.

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