ROLE OF GLUTATHIONE TRANSFERASES IN THE HARDENING EFFECT OF SALICYLIC ACID IN TOMATO

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Introduction

Plant glutathione transferases (GSTs) are a diverse group of multifunctional enzymes that catalyze the conjugation of the reduced form of glutathione (GSH) to diverse electrophilic centres of lipophilic molecules and play important roles in detoxification of cytotoxic endogenous and xenobiotic compounds. Some GST isoforms have glutathione peroxidase (GPOX) activity, suggesting that their main function could be the reduction of toxic lipid peroxidation products and the maintenance of membrane integrity. GSTs catalyze alternative GSH-dependent reactions e.g. reduction of dehydroascorbate and also have a role in the metabolism of secondary products such as anthocyanins and cinnamic acid. Arabidopsis contains 55 GST genes which can be divided into 8 classes: phi, tau, theta, zeta, dehydroascorbate reductase (DHAR), lambda, tetrachlorohydroquinone dehalogenase (TCHQD) and microsomal GST (Dixon and Edwards 2010). Some GSTs have important role in hormone metabolism and they can be induced by auxin, ethylene and salicylic acid (SA). SA is a signal molecule and it was shown that SA-generated pre-adaptation responses led to salinity tolerance in tomato. In the present work the role of GSTs were investigated in the improved acclimation to salt stress of SA-treated tomatoes.

Materials and methods

Solanum lycopersicum L. cvar. Rio Fuego plants were used in our experiments. After 3 weeks control condition we started to pre-adapt the tomato plants with 10-4M or 10-7M SA and 100 mM NaCl was added to the nutrient solution from the 6th week. The level of total ascorbic acid (by the method of Gillespie and Ainsworth 2007) and glutathione (Rahman et al. 2007), GST, GPOX enzyme activities (Csiszár et al. 2004) and DHAR activities (Edwards and Dixon 2005) were analyzed spectrophotometrically after one week of salt treatment. Phylogenetic analysis of tomato GSTs was performed in silico using the genetic sequence database at the National Center for Biotechnical Information (NCBI). Additional GST coding sequences were identified using the known Arabidopsis GST sequences found in The Arabidopsis Information Resource (TAIR) database. A family tree was constructed using the ClustalW (Thompson et al, 1994) and Dendroscope (Huson et al. 2007) programs. Primers were designed using Primer express and Primer 3 softwares and were synthesised in the Nucleic Acid Synthesis Laboratory, Biological Research Center (Szeged, Hungary). Monitoring the expression rate of selected GST coding sequences was performed by Real-Time PCR after purification of RNA (Gallé et al. 2009). Gene-specific primers were designed with Primer3 software (frodo.wi.mit.edu/primer3). Primer pairs are shown in Table 1. Data were normalized using the tomato elongation factor α subunit (EF-1) gene as internal control.

Investigated coding sequences	Direct and reverse primer pairs sequences (5' to 3')
TC243130 (BI-GST)	F: ttgcgagtctattattggagttga R: ggatcagaagggaggaaaagg
TC218553 (LeGSTU5)	F: tgctaggttttgggctgatt R: ttggctgttttgtgctcttc
TC218296 (GSTU25)	F: ggtgatttttgttggatttttgg R: agcagagggctttttgttcg
TC229067 (GSTF8)	F: cgtgtgagtgtatggtgtgct R: catcttctccaaccccttca
TC225442 (GSTF9)	F: ccgatctctctcaccttcca R: tgctctgtctgtcccgttc
TC227422 (GSTZ1)	F: cacattggtggatggagatg R: atgctcgctggggatactt
TC217416 (DHAR1)	F: ttcctaccttcgtctatttctg R: aacaaacattctgcccattga
TC226588 (DHAR2)	F: ggtgccagattcagatgtca R: cggagtctttgcttttcagg
TC218155 (GSTL1)	F: gtgcccctctctggaacaca R: aacggaccctcaagttgc
EF1-α	F: ggaacttgagaaggagcctaag R: caacaccaacagcaacagtct

Table 1. Specific primers used in QRT-PCR. Nine primer pairs amplify glutathione transferase sequences; the last primer pair were utilised to amplify expression standard.

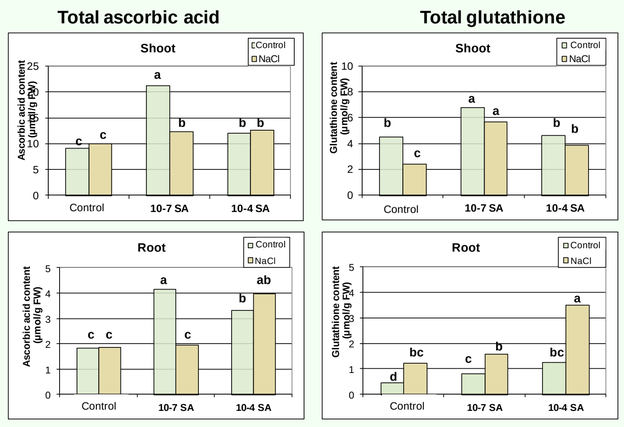

Figure 1. Effect of 100 mM NaCl after one week on total ascorbic acid and glutathione contents of 7-week-old tomato plants applied after 3 weeks pre-treatment with 10^{-7} M or 10^{-4} M SA. .

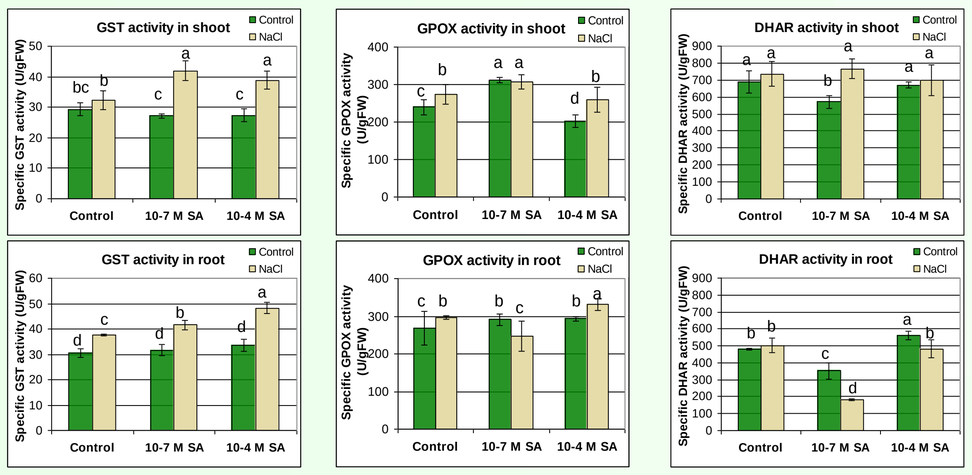

Figure 2. Glutathione transferase, glutathione peroxidase and dehydroascorbate reductase activities in 10^{-7} M or 10^{-4} M SA pre-treated tomato plants after applying salt stress for one week. Means denoted by different letters indicate a significant difference between the treatments ($P<0.05$, Duncan test).

Results and discussion

10^{-4} M or 10^{-7} M SA treatment of tomato plants after 3 weeks resulted in enhanced ascorbic acid and glutathione levels, indicating the role of these redox molecules in the enhanced stress tolerance of SA-pre-treated plants. (Figure 1).

The GST activities were enhanced by SA, suggesting the role of these enzymes in the elevated stress tolerance of SA pre-treated plants under the subsequent salt stress (Figure 2).

For further analysis a family tree was constructed from GST-coding TCs found in databases. In tomato only 6 GST genes are known, therefore other GST-coding sequences were identified from databases using the Arabidopsis GST homolog genes. Nine GST-coding sequences belonging to different GST classes were chosen and their expression levels were determined after SA and NaCl treatments by qRT-PCR (Figure 3). SA differently enhanced their expression during the hardening period and several GSTs were induced by concentration-dependent and tissues specific manner (Figures 4, 5). The expression level of the *BI-GST* (Bax-Inhibitor GST, which can protect the tomato cells from oxidative stress and programmed cell death) was only slightly increased in the presence of 10^{-7} M SA in leaves and at 10^{-4} M SA in roots. The expression level of *DHAR1* was induced by SA in leaves while that of *DHAR2* in roots, indicating tissue specific involvement in maintaining the reduced ascorbate pool. Higher inductions were detected in the case of *LeGSTU5* gene and TCs coding GSTs similar to Arabidopsis GSTU25, GSTF8 and GSTL1 proteins. Some of the investigated sequences were elevated both by NaCl and SA (e.g. *LeGSTU5, GSTU25, GSTF8, GSTL1*) suggesting that the products of these genes have a general role in stress responses (Figures 4 and 5). Elevated GST expression levels by the end of the SA pre-treatment indicate that GSTs may participate in the maintenance of the redox state of cells and/or in elimination of toxic metabolites during oxidative stress. These mechanisms may contribute in the improved salt stress tolerance of tomato plants.

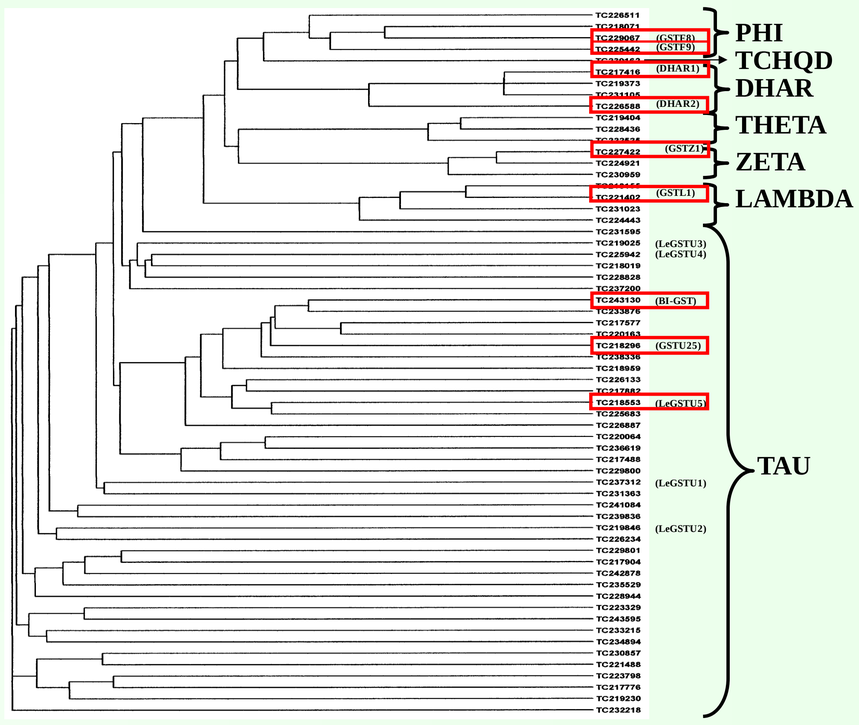

Figure 3. Phylogenetic tree and classes of GSTs in tomato. TCs and the corresponding GST genes with high similarity are shown. Red boxes are for the GST coding sequences selected for further analysis.

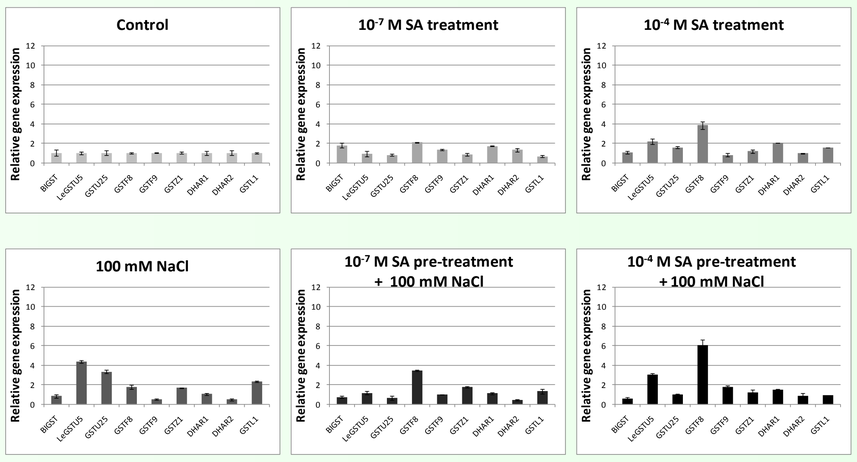

Figure 4. Transcript amount of selected tomato GSTs in 10^{-7} M or 10^{-4} M SA pre-treated tomato leaves after applying salt stress for one week.

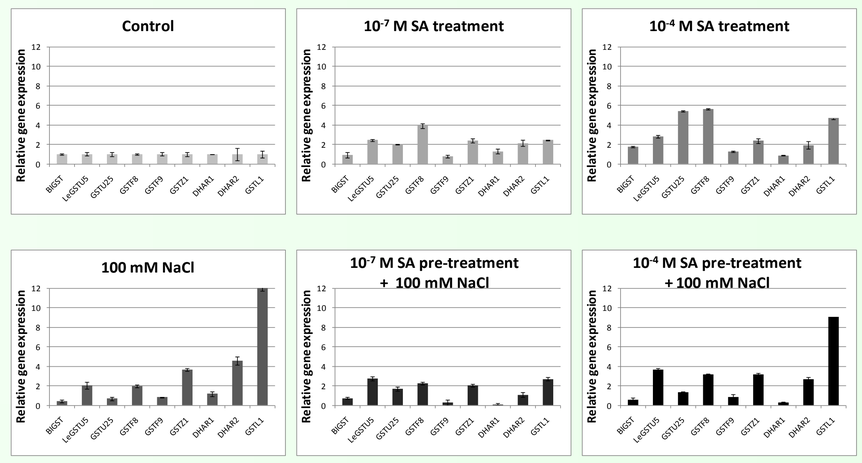

Figure 5. Transcript amounts of selected tomato GSTs in 10^{-7} M or 10^{-4} M SA pre-treated tomato roots after applying salt stress for one week.

Conclusion

Our results indicate that GSTs can participate in the maintenance of the redox state of cells and improving the salt stress tolerance of tomato plants. The increased antioxidant capacity and the decrease in H_2O_2 confirm that this phenomenon is the result of the hardening effect of SA. The process may be part of the SA effect on enhancing the antioxidant capacity.

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