Hardening effect of salicylic acid – role of glutathione transferases in the improved stress tolerance

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Introduction

Glutathione transferases (GSTs) are a diverse group of multifunctional enzymes that catalyze the conjugation of glutathione and play important roles in detoxification. Some GST isoforms have glutathione peroxidase (GPOX) activity and they are important in hormone metabolism. GSTs can be induced by auxin, ethylene and salicylic acid (SA). SA is a signal molecule, which can be involved in the activation of defence mechanisms in plants, so generate the acclimation responses in tomato plants under salt stress. We investigated the role of GSTs in the improved tolerance to salt stress of SA pre-treated plants.

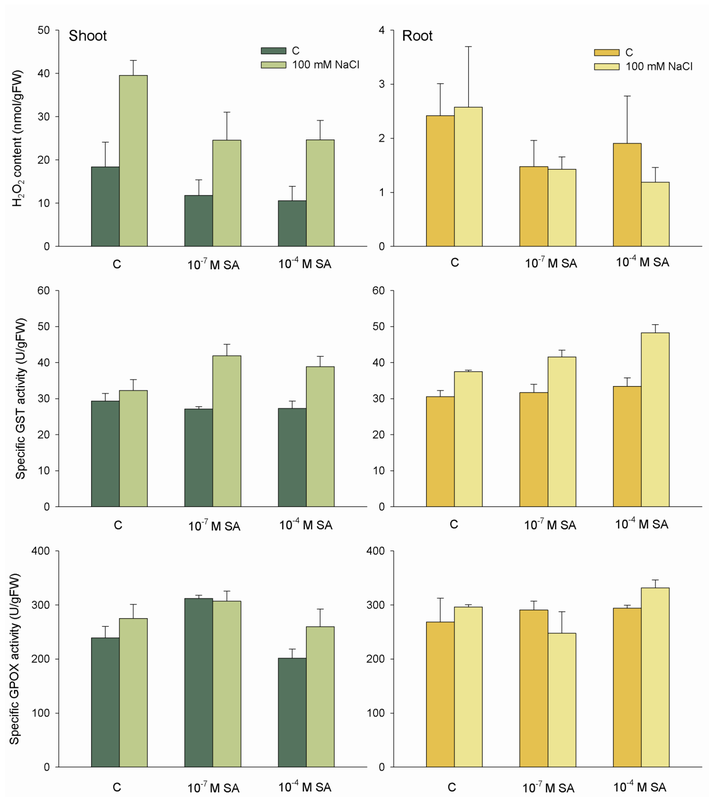

Figure 1.: Hydrogene peroxide content, glutathione transferase (GST) and glutathione peroxidase (GPOX) activities in 10^{-7} M or 10^{-4} M SA pre-treated tomato plants after applying salt stress for one week. (Mean ±SD)

Materials and Methods

Solanum lycopersicum L. cvar. Rio Fuego, tomato plants growing in hydroponic culture were pre-treated with 10^{-4} M and 10^{-7} M SA from the 3rd until the 6th week, on which 100 mM NaCl were added to induce salt stress. This experimental system was transferred also on *Arabidopsis thaliana* L. (Col-0) plants, which were grown in Hoagland solution for 5 weeks and after that 10^{-5} M and 10^{-7} M SA were applied as pre-treatments. Salt stress was applied after 2 weeks of SA pre-treatment. The measurements were done after one week of 100 mM NaCl treatment in both experiments. The fluorescent dye 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA) was used for investigation of reactive oxygen species (ROS). Hydrogene peroxide content (Velikova et al. 2000), glutathione transferase and glutathione peroxidase activities (Csiszár et al,. 2004) were measured spectrophotometrically. For molecular analysis, GST protein sequences were aligned using the ClustalW program to determine their homology (Larkin et al. 2007) and a phylogenetic tree was drawn using Dendroscope 3 program (Huson et al. 2012). Monitoring the expression rate of selected GST coding sequences was performed by Real-Time PCR (Gallé et al. 2009).

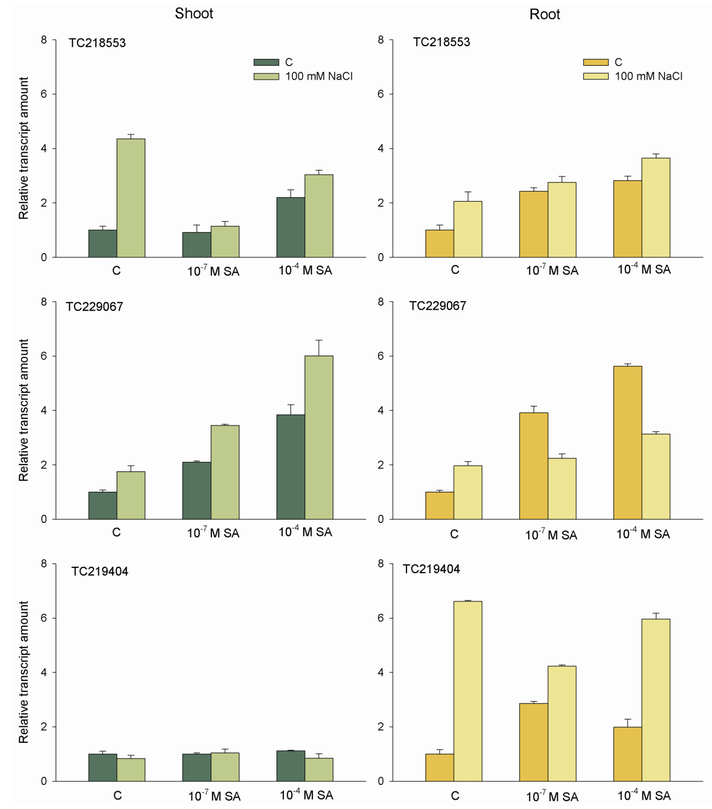

Figure 2,: The expression rate of tomato GST genes. Data were normalized using the tomato elongation factor α subunit (EF-1) gene as internal controls. Relative transcript level in the control samples was equalled to one for each gene. (Mean ±SD)

Results and Discussion

Our previous results indicated that treatment of seedlings with sub-lethal concentrations of SA may improve the stress tolerance of plants to the subsequent salt stress. (Gémes et al. 2011)

The temporary enhancement of H_2O_2 production in Arabidopsis and tomato plants may play an important role in this effect, but by the end of the hardening process the ROS levels were lower in the pre-treated plants than in controls either in non-saline conditions or under salt stress (Figs. 1. and 4.). The changes in total ROS level of the Arabidopsis root tips differ from those of the H_2O_2 content, suggesting that not only H_2O_2 is involved in this process, but also e.g. $ONOO^-$ (Fig. 4.).

The GST and GPOX activities were enhanced by SA in tomato (Fig. 1.), however, in Arabidopsis the GST activity was elevated only in roots of 10^{-5} M SA pre-treated plants and after addition of 100 mM NaCl to the hydroponic solution (Fig. 4.). These results indicate the role of GSTs in the successful acclimation and elevated stress tolerance.

Several tomato GST tentative consensus (TC) sequences were chosen for further investigations: TC218553, belonging to the tau class GSTs, TC229067, a phi class GST and TC219404, which encodes a theta class GST (Fig. 3). The expression of different GST-coding sequences exhibited tissue specific pattern. The transcript amounts of TC218553, TC229067 and TC219404 were elevated by NaCl and/or SA in roots, suggesting that the products of these genes are involved in elimination of toxic metabolites during oxidative stress (Fig. 2.).

As a conclusion, we can assume that GSTs belonging to different GST classes are involved in the hardening effect of SA.

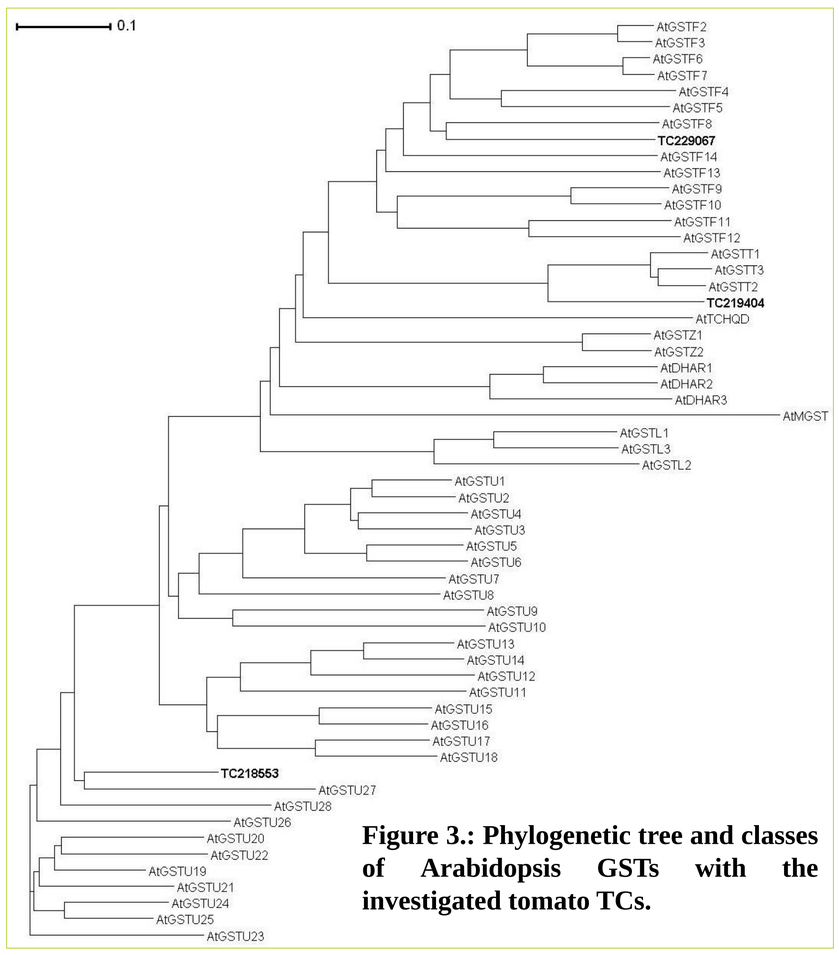

Figure 3.: Phylogenetic tree and classes of Arabidopsis GSTs with the investigated tomato TCs.

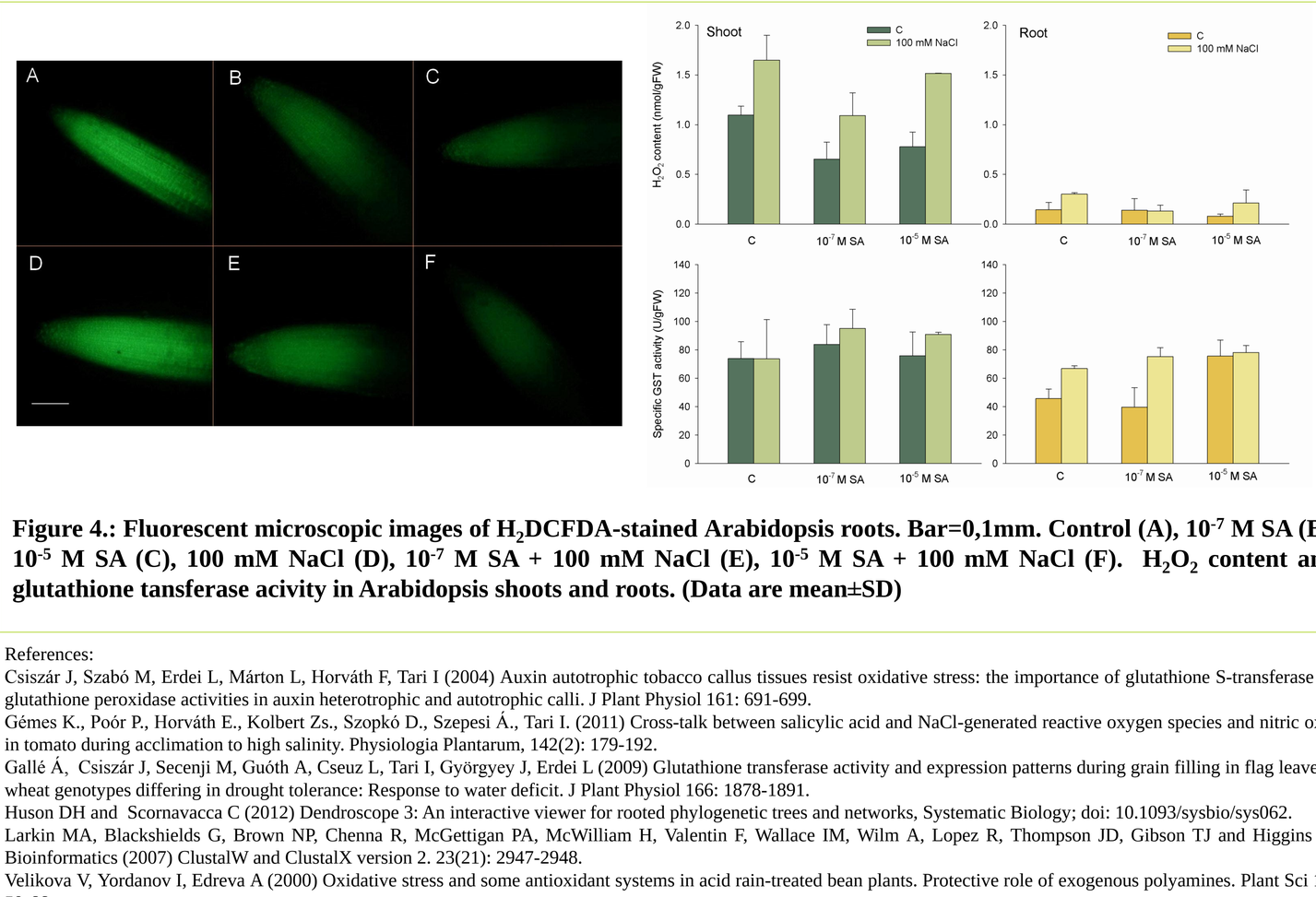

Figure 4.: Fluorescent microscopic images of H_2DCFDA-stained Arabidopsis roots. Bar=0,1mm. Control (A), 10^{-7} M SA (B), 10^{-5} M SA (C), 100 mM NaCl (D), 10^{-7} M SA + 100 mM NaCl (E), 10^{-5} M SA + 100 mM NaCl (F). H_2O_2 content and glutathione tansferase acivity in Arabidopsis shoots and roots. (Data are mean±SD)

Acknowledgement

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