Plant glutathione transferases (GSTs) have a major role in herbicide detoxification. Soybean (Glycine max L.) GmGSTs have been well studied for their correlation in herbicides selectivity towards diphenyl ether, chlorothalonil and sulfonylurea herbicides. Chloroacetanilide herbicide tolerance was assayed in vitro by measuring the growth inhibition of wild type (wt) and transgenic tobacco seedlings from cultivars (Basma, Virginia, Burley) in the presence of 7.5 and 15 mg/L of alachlor and metolachlor. Alachlor caused significant elongation, shoot and root growth of wt tobacco plants. All the transgenic Basmas lines showed significantly higher shoot and root elongation at 7.5 mg/L alachlor, with line BAGST-3 exhibiting the greatest tolerance. However, at 15 mg/L alachlor, growth was highly reduced in transgenic and wt plants. In Burley, only line BAGST-2 has statistically significant greater mean of root and shoot length compared to wt under the two doses. On the contrary, Virginia has reduced growth which was similar to the wt. Metolachlor toxicity was less severe compared to alachlor. Growth of the transgenic lines of the three cultivars was not significantly greater in either metallicor concentration tested compared to wt plants, except line BAGST-3 which showed significantly greater mean shoot elongation at 7.5 mg/L metolachlor (Fig. 2A) and also greater root elongation at both metolachlor concentrations tested (Fig 2B).

Materials and methods

Plants have developed well sophisticated detoxification systems against herbicidal and endogenous cytotoxic compounds such as xenobiotics, including herbicides. These systems incorporate a three phase detoxification procedure (Yuan et al. 2007), involving specific enzyme families in each phase: CytoP450 monooxygenases, phase I; glutathione transferases (GSTs) and glycosyltransferases (GTs) in phase II, and tonoplast localized ATP-binding cassette (ABC) transporters in phase III (Rea 2007). GSTs have a major role in the alkyl detoxification pathway, as the glutathione conjugated xenobiotics are irreversibly non toxic and can be accessible to further metabolic procedures (Schroeder 2001). Due to broad substrate specificity of different GST proteins expressed and their homo- or heterodimeric assembly, they can tolerate a broad spectrum of xenobiotics (Dixon et al. 1999). Transgenic plants overexpressing GST subunits active in herbicide detoxification confirmed GST’s role in crop’s herbicide selectivity (Dixon et al. 2003; Karavangeli et al. 2005). Soybean (Glycine max L.) glutathione transferases (GmGSTs) have been well studied for their correlation in herbicides selectivity towards diphenyl ether, chlorothalonil and sulfonylurea herbicides (Andrews et al. 2005). The aim of this work was to study the tolerance under chloroacetanilide herbicide treatment and the relative expression of GmGST4 in root and shoots of transgenic tobacco lines overexpressing GmGST4.

Results

Transgene expression analysis

The observed differences in tolerance between the transgenic lines lead us to further investigate the expression pattern of GmGST4 in root and shoot of the transgenic lines with the higher resistance (BAGST-3 and BAGST-2), in VIGST-2 which did not exhibited resistance in all treatments examined as well as in WT plants. The relative expression in shoot of BAGST-3 and BAGST-2 was 250 and 193 times higher compared to actin gene respectively without significant differences between them, while VIGST2 has only 11 times higher relative expression. The relative expression in the root was lower compared to shoot at the BAGST-3 (87 compared to 193) and BAGST-2 (177 compared to 250) without differences. On the contrary VIGST2 has higher relative expression in the root (17 compared to 11) without significant differences (Fig. 3).

Conclusions

- Transgenic lines exhibited statistically significant increased tolerance, confirming the major contribution of GmGSTs in detoxification of alachlor
- Metolachlor toxicity was less severe, probably due to the relatively high overall GST activity of tobacco towards metolachlor
- The expression analysis demonstrates that the higher GST expression in shoots compared to roots it may explain significant differences in root and shoot elongation
- We postulate that transgenic plants overexpressing GST are good candidates for designing phytoremediation strategies for contaminated agricultural soils

References

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