Over-expression of a specific soybean *Gm*GSTU4 isoenzyme improves chloroacetanilide herbicide tolerance of transgenic tobacco plants

Christos Kissoudis[1], Kostantinos Benekos[1] , Georgia Voulgari[1], Nikolaos E. Labrou[2], AthanasiosTsaftaris[1,3], Panagiotis Madesis[3], Irini Nianiou-Obeidat [1]*

[1]Department of Genetics and Plant Breeding, School of Agriculture, Aristotle University of Thessaloniki, P.O. Box 261, Thessaloniki GR-54124, Greece
[2]School of Food, Biotechnology and Development, Agricultural University of Athens, 75 Iera Odos Street, 11855 Athens, Greece
[3] Institute of Applied Biosciences, CERTH, 6th km Charilaou-Thermis Road, P.O. Box361, Thermi GR-57001,Thessaloniki, Greece

Abstract

Plant glutathione transferases (GSTs) have a major role in herbicide detoxification. Soybean (*Glycine max* L.) *Gm*GSTs have been well studied for their correlation in herbicides selectivity towards diphenyl ether, chloroacetanilide 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 (Basmas, Virginia, Burley) in the presence of 7.5 and 15 mg/L of alachlor and metolachlor. Alachlor caused strong inhibition of 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 BUGST-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 metolachlor concentrations tested, compared to wt plants, except line BAGST-3 which exhibited significantly greater mean of shoot and root elongation at 7.5 mg/L. Transgene expression was determined quantitatively using Real Time qPCR, lines BAGST-3 and BUGST-2 showed greater expression of *Gmgstu4* in shoot compared to root. These results confirm that overexpression of GmGSTU4 in tobacco provides higher catalytic activities towards xenobiotics, resulting for future use in environmental cleanup of alachlor.

Introduction

Plants have developed sophisticated detoxification systems against endogenous and exogenous 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: CytP450 monooxygonases in 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 above detoxification procedure, 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 heterodimerization, plants are able to 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 (*Gm*GSTs) have been well studied for their correlation in herbicides selectivity towards diphenyl ether, chloroacetanilide 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 *Gmgstu4* in root and shoots of transgenic tobacco lines overexpressing *Gmgstu4.*

Materials and methods

Plant material and Chloroacetanilide treatments

T1 seeds from three cultivars of transgenic tobacco (*Nicotiana tabacum)* lines, Basmas (BAGST-1,2,3 and 4) Virginia(VIGST-2 and 3) and Burley (BUGST-1 and 2) overexpressing the *Gm*GST4 and wild type (wt) plants, were surface sterilized and placed for germination in MS medium (Murashige and Skoog 1962) supplemented with 100 mg/L kanamycin. The plants were grown at 25^{o}C under a 16 h photoperiod using artificial light. Three-week old transgenic and wt tobacco seedlings were transferred in MS medium supplemented with the herbicides alachlor and S-metolachlor (7.5 and 15 mg/L), both as commercial formulations (ALANEX® 48 EC, Makhteshim-Agan and DUAL® 96 EC, Syngenta respectively. The tobacco plants tolerance was assessed after 40 days by measuring growth parameters such as shoot and root elongation.

Transgene expression analysis with RT-PCR

We further investigate the relative expression of *Gm*GST4 in roots and shoots of the lines (BAGST-3, BUGST-2 and VIGST-2). Total RNA was isolated from six week old plants grown *in vitro* using the RNeasy plant RNA isolation kit (Qiagen).The RNA was subsequently treated with DNase I First-strand cDNA was synthesized using the SuperScript II Reverse Transcriptase Kit (Invitrogen).

Primers used for the *actin* gene were Forward 5'-GGTGACGAAGCTCAGTCCAAAAGGGGT-3' and reverse 5 ACGGCCACTGGCGTATAGGGACAACA3'.

Primers used for the *gst4* gene were Forward 5' TGGCCAAGTCCATTTGGGATGAGGG-3' and reverse 5'-TGGGTTTGCCATTGTGGATGAGAACCG-3'.

Results

Chloroacetanilide herbicide tolerance

Alachlor caused strong inhibition of shoot and root growth of WT tobacco plants (Fig.1 A and B). The transgenic lines showed different responce in the presence of this herbicide. Lines BAGST-1, BAGST-2, BAGST-3 and BAGST-4 has significant higher shoot and root elongation at 7,5 mg/L alachlor compared to WT plants. However at 15 mg/L alachlor, shoot and root growth was highly reduced, resulting in non significant differences (Fig.1A and B). Metolachlor toxicity was less severe compared to alachlor. Growth of the transgenic lines was not significantly greater in either metolachlor concentrations tested, compared to WT plants, except from line BAGST-3 which exhibited 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)

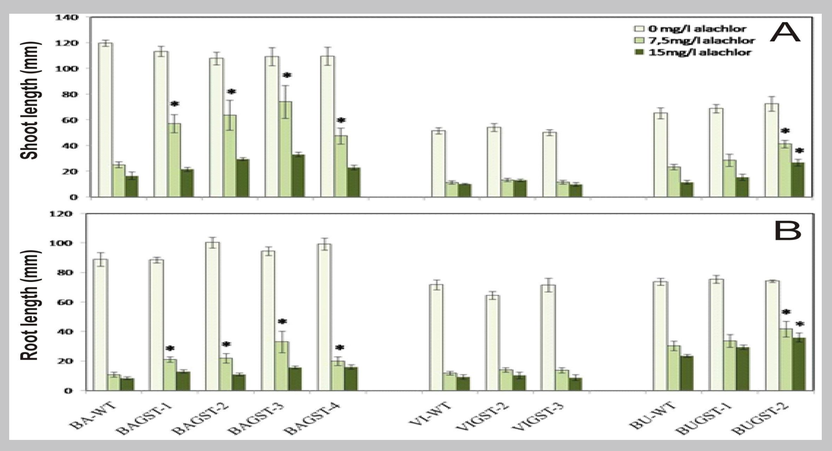

Fig.1 The effect of alachlor treatment (7.5 and 15 mg/L) on shoot (A) and root length (B) on transgenic lines and wt tobacco varieties after 40 days of growth under *in vitro* conditions. Data are the means (± standard deviation, n=4). Lines indicated with * differ significantly from the wt plants P≤ 0,05

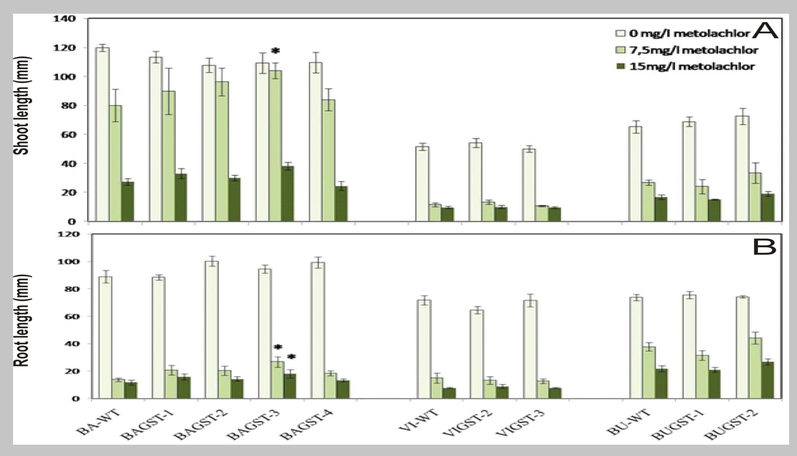

Fig.2 The effect of metalachlor treatment (7.5 and 15 mg/L) on shoot (A) and root length (B) on transgenic lines and wt tobacco varieties after 40 days of growth under *in vitro* conditions. Data are the means (± standard deviation, n=4). Lines indicated with * differ significantly from the wt plants P≤ 0,05

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 BUGST-2), in VIGST-2 which did not exhibited resistance in all treatments examined as well as in WT plants. The relative expression in the shoot of BUGST-2 and BAGST-3 was 250 and 193 times higher compared to actin gene respectively without significant differences between them, whilst 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 BUGST-2 (177 compared to 250) without differences. On the contrary VIGST-2 has higher relative expression in the root (17 compared to 11) without significant differences (*Fig. 3*).

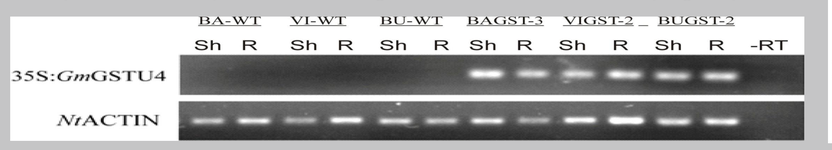

Fig. 3 Expression of 35S:GmGST4 and endogenus actin gene in shoot (Sh) and root (R) of transgenic lines and wt plants of the three cultivars

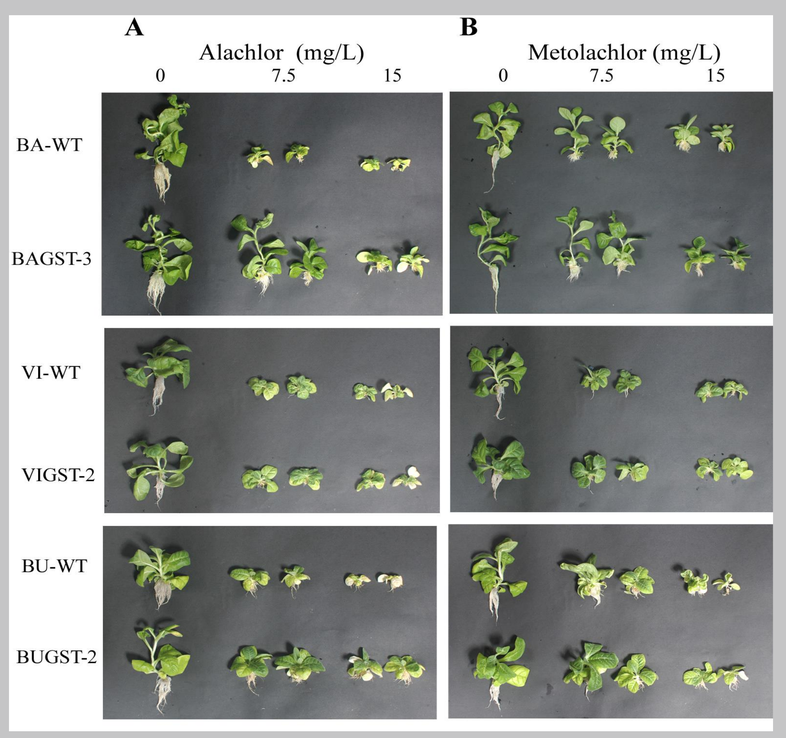

Fig.4 Transgenic and WT tobacco plants after 40d in MS medium with 7.5 and 15mg/L (A) alachlor και (B) metolachlor

Conclusion

- **Transgenic lines exhibited statistically significant increased tolerance, confirming the major contribution of *Gm*GST4 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 play a significant role in chloroacetanilide resistant plants**
- **We postulate that transgenic plants overexpressing *GST* are good candidates for designing phytoremediation strategies for contaminated agricultural soils**

References

Andrews CJ, Skipsey M, Townson JK, Morris C, Jepson I, Edwards R (1997) Glutathione transferase activities toward herbicides used selectively in soybean. Pesticide Science 51: 213-222

Dixon DP, McEwen AG, Lapthorn AJ, Edwards R (2003) Forced evolution of a herbicide detoxifying glutathione transferase. Journal of Biological Chemistry 278: 23930-23935

Dixon DP, Cole DJ, Edwards R (1999) Dimerisation of maize glutathione transferases in recombinant bacteria. Plant Molecular Biology 40: 997-1008

Karavangeli M, Labrou NE, Clonis YD, Tsaftaris A (2005) Development of transgenic tobacco plants overexpressing maize glutathione S-transferase I for chloroacetanilide herbicides phytoremediation. Biomolecular Engineering 22: 121-128

Schroeder P (2001) The role of glutathione and glutathione S-transferases in plant reaction and adaptation to xenobiotics. Significance of Glutathione to Plant Adaptation to the Environment: 155-183

ACKNOWLEDGEMENTS.

The present was supported by the action "THALIS: Glutathione transferases, multifunctional molecular tools in red and green biotechnology" falling under the Operational Programme "Education and Lifelong Learning (EdLL)" and is co-financed by the European Social Fund (ESF) and National Resources.