Overexpression of a specific *P. vulgaris* (*Pv*GSTU2-2) isoenzyme improves dimethenamid tolerance of transgenic tobacco plants

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Abstract

Plant glutathione transferases (GSTs) have a major role in plant herbicide detoxification system. *Pv*GSTU2-2 has been isolated from leaves of *Phaseolus vulgaris* plants. The enzyme catalyses the conjugation of glutathione with chloroacetamide herbicides alachlor and metalachlor. In order to study *in planta* the ability of *Pv*GSTU2-2 to detoxify chloroacetamide herbicides, we developed transgenic tobacco plants overexpressing *Pv*GSTU2-2. The introgression of the transgene in the plant genome was confirmed by RT-PCR and the expression of *Pv*GSTU2-2 with q-RTPCR analysis. In addition, it was verified that the transgenes codes functional proteins by measuring GST enzyme activity towards NBD-CI in transgenic lines. Three *Pv*GSTU2-2 overexpressing lines were assayed for their tolerance towards the herbicide dimethenamid (chloroacetamide). Under *in vivo* conditions, T0 lines exhibited increased tolerance at 0,5 and 1 mg/L dimethenamid, with significantly increased shoot and root elongation compared to wild type plants. These results confirm that overexpression of *Pv*GSTU2-2 in tobacco provides a way of conferring selectivity and enhancing crop safety and production.

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).PvGSTU2-2 isoenzyme catalyses *in vitro* the conjugation of glutathione with chloroacetamide herbicides alachlor and metalachlor. The aim of this work was to study the tolerance in transgenic tobacco lines overexpressing *Pv*GSTU2-2 under chloroacetamide herbicide treatment.

Materials and methods

Standard recombinant method were adopted for the construction of the plant transformation vector pART27–GSTU2-2. The construct was confirmed by restriction digest analysis and then by sequencing. *N.tabacum* leaf disks transformed by Agrobacterium-mediated co-cultivation method. The positive transgenic plants were selected by RT-PCR based on Livak and Thomas method (2001). The expression of *Pv*GSTU2-2 transgene, was studied by quantitative RT-PCR analysis in 8 transgenic lines. To assay GST activity, NBD-CI was used as substrate and GST activity was measured spectrophotometrically in the same lines. The results expressed as specific activity (SA Units/mg%). To test herbicide tolerance, *in vitro* transgenic and wt plants were transferred into vermiculite-mixed soil, after acclimation were treated with 0,5 or 1mg/L dimethenamid. Root and shoot length measurements were taken 30 days after the treatment. The experiment was performed by three replicate plants per treatment. Dunkan's test was used to reveal significant differences between wt and transgenic lines. A P-value ≤0.05 was considered significant.

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European Social Fund
OPERATIONAL PROGRAMME EDUCATION AND LIFELONG LEARNING
investing in knowledge society
MINISTRY OF EDUCATION & RELIGIOUS AFFAIRS
MANAGING AUTHORITY
NSRF 2007-2013
EUROPEAN SOCIAL FUND
Co-financed by Greece and the European Union

Results

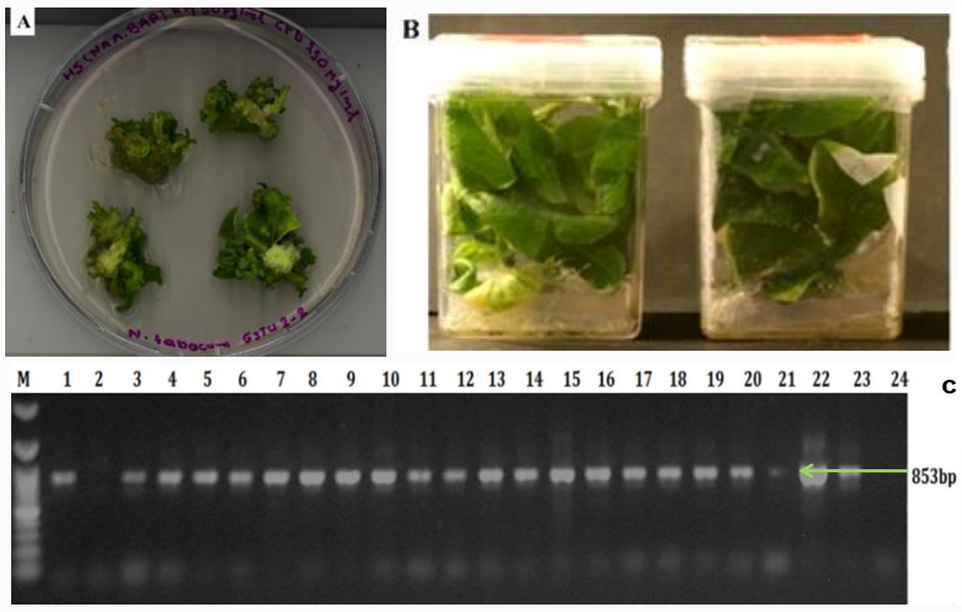
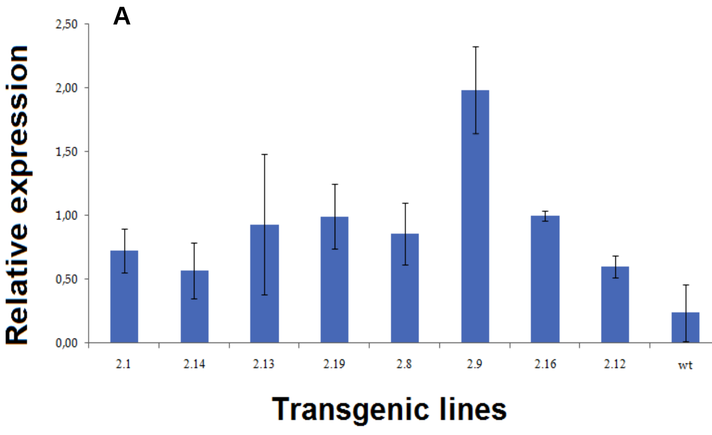


Fig.1 (A) Regeneration of putative transformed tobacco plants **(B)** Putative transgenic plants **(C)** RT-PCR analysis of independently transformed transgenic tobacco plants **(22 and 23),** positive marker) **(24)** negative marker **(M)** molecular marker 100bp. Lanes with bands at 853 bp indicate individual plants that contain the *Pv*GSTU2-*2* gene

Fig 3 The effect of dimethenamid (0,5 and 1mg/L) 30 d after treatment under *in vivo* conditions on wt tobacco plants



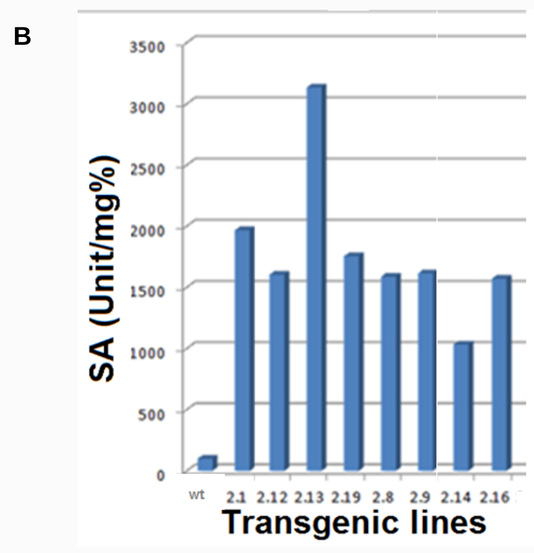


Fig 2 Expression analysis **(A)** and Enzymatic activity **(B)** of *Pv*GSTU2-2 in 8 selected transgenic lines **(wt)** Non-transformed plants **(2.1, 14, 13, 19, 8, 9,16, 12)** Independent transgenic lines

Fig.4 The effect of dimethenamid on transgenic lines 30 d after treatment **(A)** 0,5 and **(B)** 1 mg/L. **1:** wt plant s **2-4:** independent transgenic lines 2.9, 2.19, 2.13 respectively

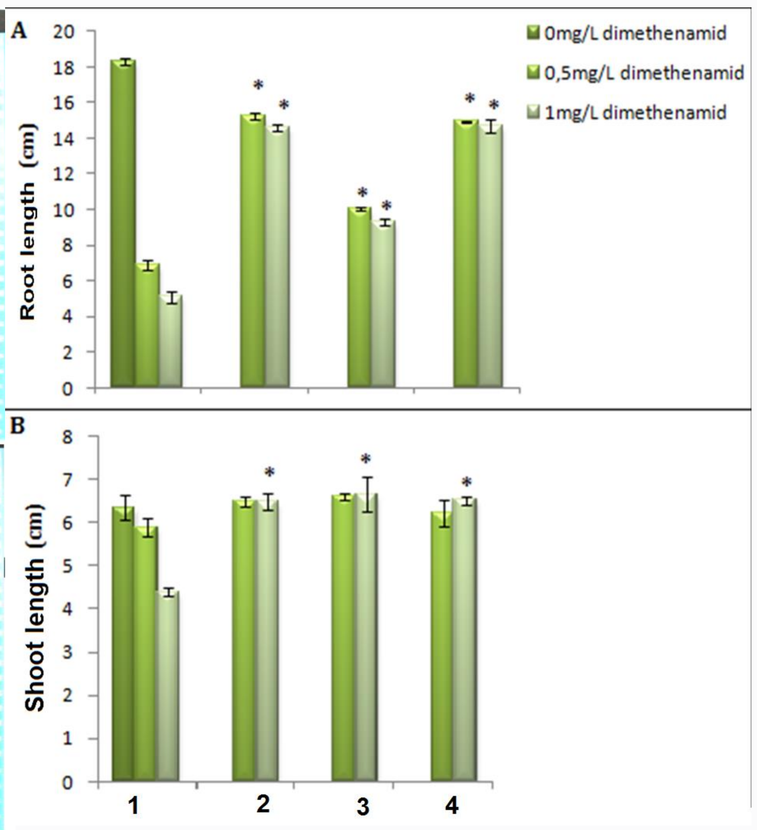


Fig 5 Tolerance of *Pv*GSTU2-2 transgenic lines and wt plants to dimethenamid (0,5 and 1 mg/L), measured by **(A)** Root and **(B)** Shoot length 30d after treatment. **1:** wt plants **2-4:** independent transgenic lines 2.9, 2.19, 2.13 respectively Data are the means (± standard error,) n=3). Lines indicated with ***** differ significantly from the wt plants P≤ 0,05

Conclusion

- **We generated 20 transgenic lines verified by RT-PCR analysis**
- **Quantitative RT-PCR revealed that expression level of *Pv*GSTU2-2 was higher in the transformants than in the wt plants. The transgenes codes functional proteins, as the specific activity of *Pv*GSTU2-2 transgenic lines was higher than in wt plants**
- **Transgenic lines exhibited statistically significant increased shoot and root elongation compared to wt plants, confirming the contribution of *Pv*GSTU2-2 in detoxification of demethenamid**
- **We postulate that transgenic plants overexpressing GST genes are good candidates for designing phytoremediation strategies for contaminated agricultural soils**