Isolation of GST isoenzymes from *Phaseolus vulgaris* L. and characterization of detoxifying mechanism under biotic and abiotic stress

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Abstract

Three Glutathione transferase (GSTs) isoenzymes have been isolated from *P. vulgaris* leaves after *in vivo* treatment with 1/250 fluazifop-p-butyl herbicide. The inducible GST isoenzymes were identified and separated by affinity chromatography. They found to belong to phi and tau classes. Moreover, the fluazifop-inducible glutathione transferases from *P. vulgaris* (PvGSTs, termed PvGSTU2-2, PvGSTF1-1 and PvGSTU3-3) were found to catalyze a broad range of reactions and exhibit quite varied substrate specificity. Another GST isoenzyme was isolated from *P. vulgaris* (PvGST, termed PvGSTU3-3), after its induction with biotic stress treatment (*Uromyces appendiculatus* infection). PvGSTU3-3 shares high homology the tau class plant and catalyzes several different reactions and exhibits wide substrate specificity. Of particular importance are the high antioxidant catalytic function and hydroperoxidase, thioltransferase, and dehydroascorbate reductase action of Pv-GSTU3-3. Transgenic tobacco plants over-expressing PvGSTU2-2 isoenzyme have been developed via *Agrobacteriun tumefaciens* in order to study their *in planta* potential to confer biotic and abiotic resistance as a means of plant breeding. Our results provide new insights into catalytic and structural diversity of GSTs and the detoxifying mechanism used by *P. vulgaris*. Moreover, highlight the functional and catalytic diversity of plant GSTs and demonstrate their pivotal role for addressing biotic stresses in *P. vulgaris.*

Introduction

The glutathione transferases (GSTs) are a family of enzymes that catalyze the conjugation of glutathione (GSH) to reactive xenobiotic chemicals. Metabolic detoxification is probably the major mechanism involved in plant tolerance to herbicides or other abiotic and biotic stresses which act through oxidative stress. In the present work we have studied the functional and catalytic diversity of selected members of the GST family from *P vulgaris*. We identified three isoenzymes (PvGSTF1-1, PvGSTU1-1 and PvGSTU2-2) that are putatively involved in the herbicide fluazifop-pbutyl stress response mechanism. The interest in *P. vulgaris* stems from the fact that is one of five cultivated species from the genus *Phaseolu*s and is a major grain legume crop, third in importance after soybean and peanut, but first in direct human consumption. Fluazifop-p-butyl is a selective aryloxyphenoxy propionic herbicide used for post-emergence control of annual and perennial grass weeds, absorbed rapidly through leaf surfaces and disrupts the synthesis of lipids in susceptible species by inhibiting acetyl-CoA carboxylase. The study of herbicide stress mechanism and detoxification systems in plants provide new insights into catalytic and structural diversity of GSTs and the detoxifying mechanism used by *P. vulgaris*. The lack of a basic understanding of the molecular mechanisms underlying herbicide detoxification remains the greatest obstacle to the use of eco-friendly approaches to deal with this problem. In addition we report the function and catalytic diversity of another isoenzyme from *P. vulgaris,* PvGSTU3-3 which is expressed during infection of *P. vulgaris* with the rust fungus *U. appendiculatus*. Furthermore, plants over-expressing PvGST isoenzyme can be used in plant breeding programs in order to develop resistant cultivars to biotic and abiotic stress conditions. Transgenic tobacco plants over-expressing PvGSTU2-2 isoenzyme have been developed via *Agrobacteriun tumefaciens* in order to study their *in planta* potential to confer biotic and abiotic resistance as a means of plant breeding.

Results

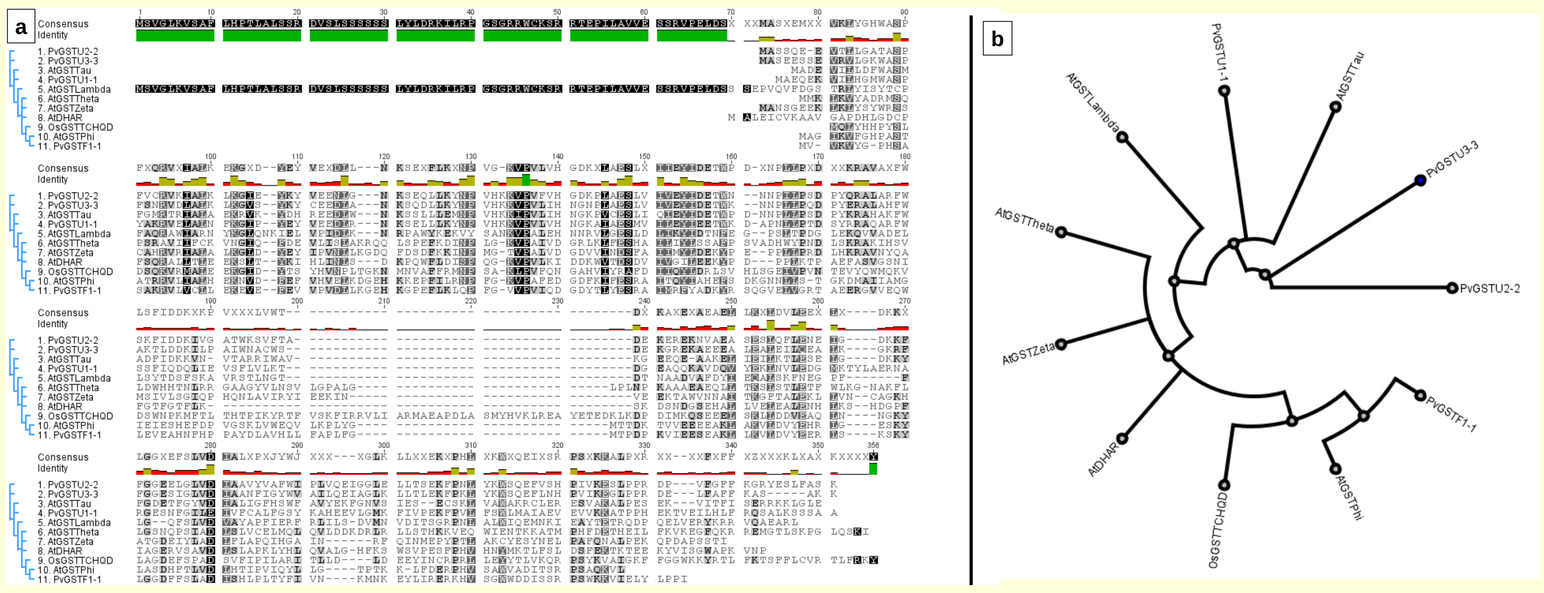

Fig. 1 (a) Sequence alignments (ClustalW, Thompson et al.) of PvGSTs and representative members from all known GST classes: phi, tau, theta, zeta, lambda, dehydroascorbate reductase (DHAR), and tetrachlorohydroquinone dehalogenase (TCHQD). Conserved areas are shown shaded, with gray gradient: 100 % identity, 80–100 % identity, 60–80 % identity, and <60 % identity. (b) Neighbor joining phylogenetic analysis of PvGSTs. The tree was formed after alignment of the protein sequences using ClustalW.

Table 1. Substrate specificity for purified recombinant *Pv*GSTF1-1, *Pv*GSTU1-1 and *Pv*GSTU2-2. Enzyme assays were carried out under standard conditions as described in Methods section. Results represent the means of triplicate determinations, with variation less than 5% in all cases.

Substrate	*Pv*GSTF1-1	*Pv*GSTU1-1	*Pv*GSTU2-2
	Specific activity (U/mg)		
1-Chloro-2,4-dinitrobenzene	3.5	0.28	16.6
1-Bromo-2,4-dinitrobenzene	8.4	0.64	27.9
4-Chloro-7-nitrobenzofurazan	3.9	0.073	148.2
Fluorodifen	ND	0.002	ND
Alachlor	0.08	0.04	0.09
Atrazine	0.01	0.005	0.02
trans-2-Nonenal	ND	0.009	0.13
Allyl isothiocyanate	2.7	7.6	66.1
2-Hydroxyethyl disulfide (2,2-dithiodiethanol)	9.5	0.058	4.1
Bromosulfophthalein	3.6	ND	ND

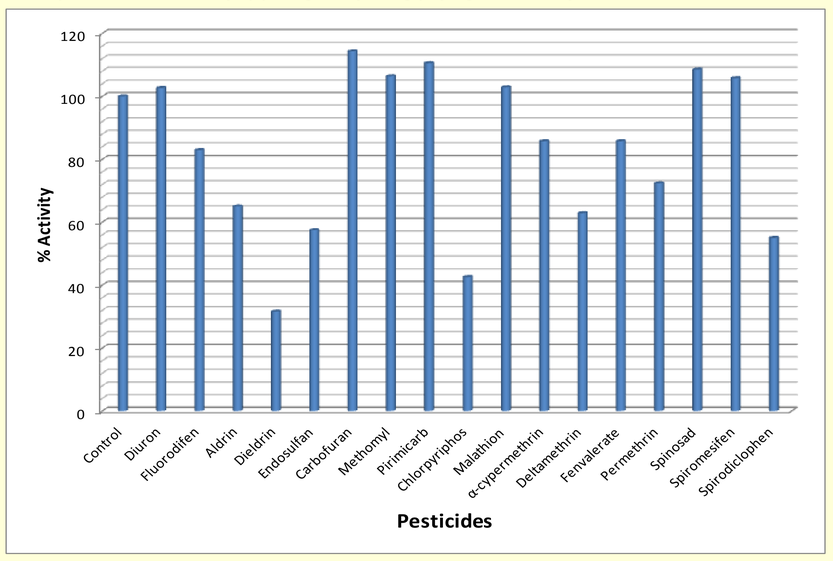

Fig. 2 Probing the PvGSTU3-3L-site. Screening of the inhibition potency of different pesticides towards PvGSTU3-3. GST activity was assayed using the CDNB/GSH assay system

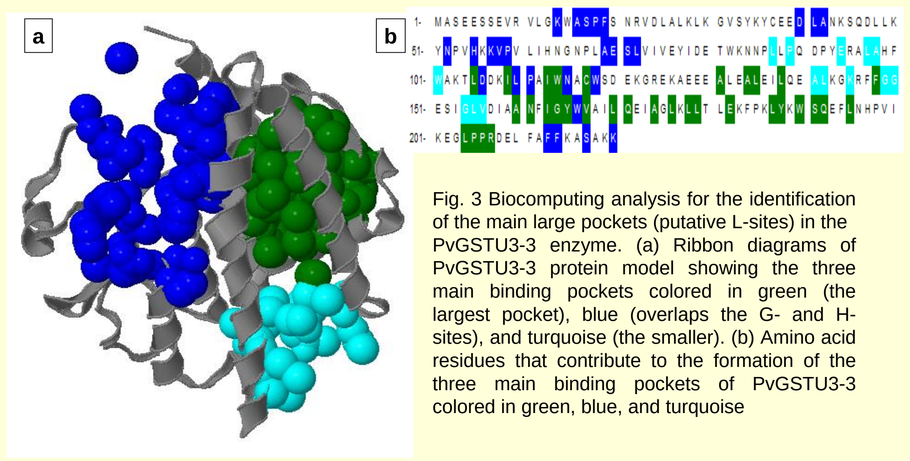

Fig. 3 Biocomputing analysis for the identification of the main large pockets (putative L-sites) in the PvGSTU3-3 enzyme. (a) Ribbon diagrams of PvGSTU3-3 protein model showing the three main binding pockets colored in green (the largest pocket), blue (overlaps the G- and H-sites), and turquoise (the smaller). (b) Amino acid residues that contribute to the formation of the three main binding pockets of PvGSTU3-3 colored in green, blue, and turquoise

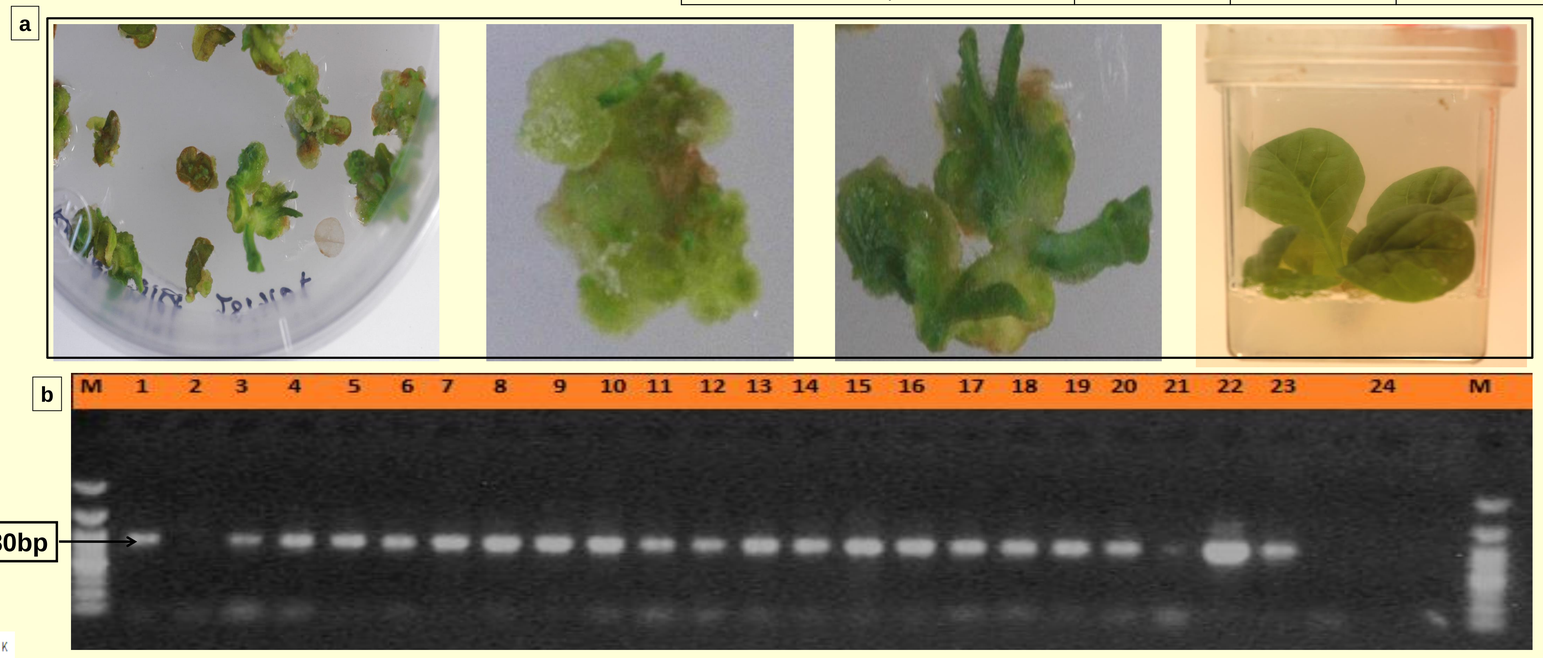

Fig. 4 (a)Regeneration of putative transformed tobacco plants (b)RT-PCR analysis of independently transformed transgenic tobacco plants (1-21), positive marker (22,23), negative marker (25) ,molecular marker (M). Lanes with bands at 680bp indicate individual plants that contain the *Ph. vulgaris GSTU2-2 gene*

Material and Methods

P. vulgaris seeds were pre-germinated on plates, on Whatman filter paperlants were treated 3–4 weeks after germination having 3-4 pairs of leaves. Plants were sprayed with fluazifop-pbutyl (Syngenta) diluted 1/250 with deionised water (used field dose), covered with cling film and leaf samples were collected after 24 h. Control plants were sprayed with fluazifop-p-butyl-free solvent. Standard molecular techniques were used in order to isolate and clone the 4 GST genes. Enzymatic activity was meaured against a wide range of substances. Moreover bioinformatics analysis and molecular modeling were used in order to reveal their structure. *Agrobacterium tumefaciens* harboring pART27::GST plasmid was used in order to develop transgenic plants

Conclusion

- **Structural analysis showed that PvGSTs (PvGSTU2-2, PvGSTF1-1 and PvGSTU3-3) share the same overall fold and domain organization of other plant cytosolic GSTs, with major differences at their active site and some differences at the level of C-terminal domain and the linker between the C- and N-terminal domains. The structural heterogeneity within the C-terminal domain seems to be responsible for the substrate variability and specificity across PvGSTs.**
- **PvGSTs are capable of catalyzing several different reactions and substrates, including herbicides, and exhibit wide substrate specificity.**
- **An induced PvGST following *U. appendiculatus* infection has been isolated.**
- **PvGSTU3-3 catalyzes several different reactions and exhibits wide substrate specificity.**
- **Structural analysis showed that PvGSTU3-3 shares the same overall fold and domain organization of other tau class plant cytosolic GSTs, with major differences at the H-site.**
- **Transgenic plants have been developed in order to achieve the production of stress tolerant plants**
- **Our findings shed light on better understanding the PvGSTU3-3 functional and catalytic diversity and highlight the pivotal role of GSTs used by plants to cope with biotic stress.**

References

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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.

European Union
European Social Fund
OPERATIONAL PROGRAMME
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MANAGING AUTHORITY
NSRF 2007-2013
EUROPEAN SOCIAL FUND
Co-financed by Greece and the European Union