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Catalytic features and crystal structure of a tau class glutathione transferase from *Glycine max* specifically upregulated in response to soybean mosaic virus infections

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ABSTRACT

The plant tau class glutathione transferases (GSTs) play important roles in biotic and abiotic stress tolerance in crops and weeds. In this study, we systematically examined the catalytic and structural features of a GST isoenzyme from *Glycine max* (*Gm*GSTU10-10). *Gm*GSTU10-10 is a unique isoenzyme in soybean that is specifically expressed in response to biotic stress caused by soybean mosaic virus (SMV) infections. *Gm*GSTU10-10 was cloned, expressed in *Escherichia coli*, purified and characterized. The results showed that *Gm*GSTU10-10 catalyzes several different reactions and exhibits wide substrate specificity. Of particular importance is the finding that the enzyme shows high antioxidant catalytic function and acts as hydroperoxidase. In addition, its K_m for GSH is significantly lower, compared to other plant GSTs, suggesting that *Gm*GSTU10-10 is able to perform efficient catalysis under conditions where the concentration of reduced glutathione is low (e.g. oxidative stress). The crystal structure of *Gm*GSTU10-10 was solved by molecular replacement at 1.6 Å resolution in complex with glutathione sulfenic acid (GSOH). Structural analysis showed that *Gm*GSTU10-10 shares the same overall fold and domain organization as other plant cytosolic GSTs; however, major variations were identified in helix H9 and the upper part of helix H4 that affect the size of the active site pockets, substrate recognition and the catalytic mechanism. The results of the present study provide new information into GST diversity and give further insights into the complex regulation and enzymatic functions of this plant gene superfamily.

1. Introduction

Plant glutathione S-transferases (GSTs; EC 2.5.1.18) are enzymes that catalyze the conjugation of reduced glutathione (GSH; γ-Glu–Cys–Gly) to electrophilic centers of a wide variety of, mainly hydrophobic, compounds, both endogenous and xenobiotic [1–4]. GSTs are implicated in pesticide detoxification [5–7], in responses to abiotic and biotic stress (infection, heavy metals, UV radiation, etc) [8–11], as well as in hormonal regulation and developmental change [12–16]. GSTs can be found in plants from early embryogenesis to senescence [17].

GSTs comprise a large, complex gene family in plants. Based on a variety of criteria (e.g. sequence relatedness, immunological, kinetic and structural properties), plant soluble GSTs can be subdivided to distinct classes: phi (F), tau (U), zeta (Z), theta (T), lambda (λ), dehydroascorbate reductase (DHAR), and tetrachlorohydroquinone dehalogenase (TCHQD) [1,2,12,13]. The tau class, in particular, is the most abundant of all GST classes and its members play important roles in stress tolerance and secondary metabolism as well as catalyzing the detoxification of herbicides in crops and weeds [9,13–15,17–19].

According to numerous crystallographic studies, GSTs display significant structural conservation [8,15,16]. The soluble plant GSTs are homo- and hetero-dimeric enzymes with 23–30 kDa subunits and average length of 200–250 amino acids [1,8,12,13,16,20]. Each subunit consists of two domains, the N-terminal domain with α/β topology and the C-terminal domain with α-helical structure. Each subunit has a relatively independent active site, composed of the G-site, which is primarily responsible for binding GSH or other closely related peptides (e.g. homoglutathione), and the H-site, which is the site where hydrophobic electrophile substrates bind [8,16]. The catalytic residue of GSTs from theta, zeta, phi and tau classes is the amino acid serine [1,12,13,16]. The G- and H-sites are typically formed

Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; CuOOH, cumene hydroperoxide fluorodifen, 4-nitrophenyl 2-nitro-4-trifluoromethylphenyl ether; GSH, glutathione; GSOH, glutathione sulfenic acid; GST, glutathione transferase; *Nb*-GSH, *S*-(p-nitrobenzyl)-glutathione; SMV, soybean mosaic virus; t-BuOOH, tert-butyl peroxide

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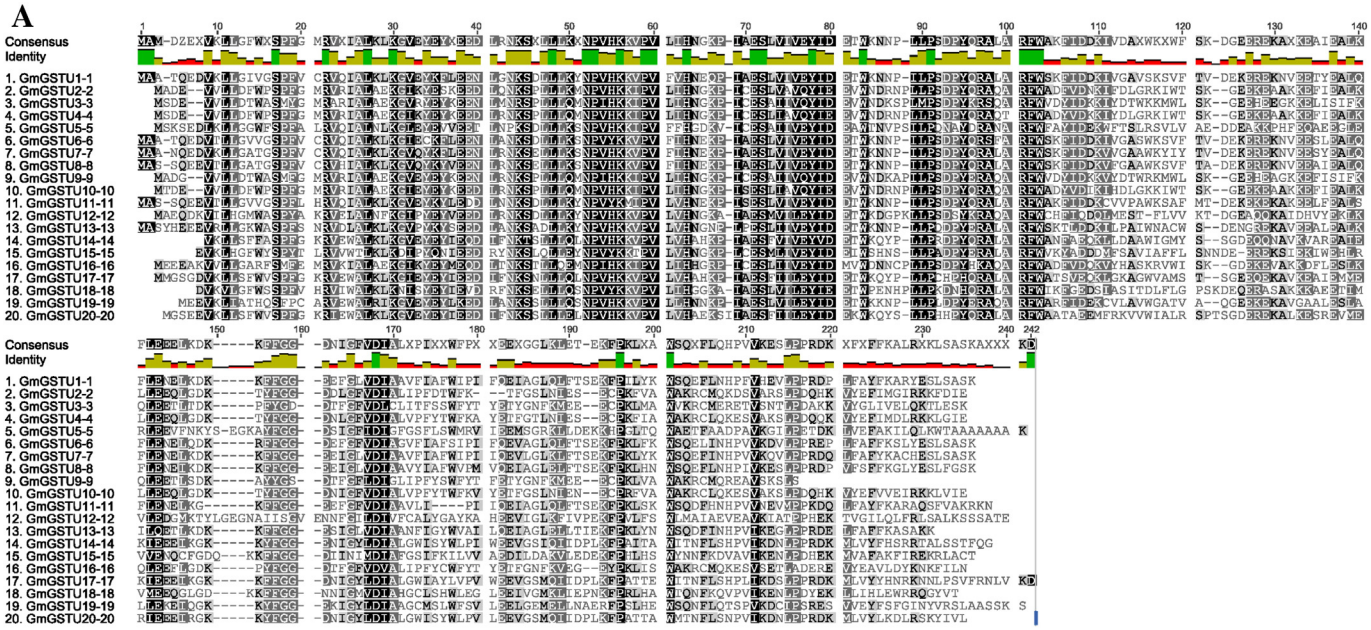
A
Consensus
Identity
1. GmGSTU1-1
2. GmGSTU2-2
3. GmGSTU3-3
4. GmGSTU4-4
5. GmGSTU5-5
6. GmGSTU6-6
7. GmGSTU7-7
8. GmGSTU8-8
9. GmGSTU9-9
10. GmGSTU10-10
11. GmGSTU11-11
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13. GmGSTU13-13
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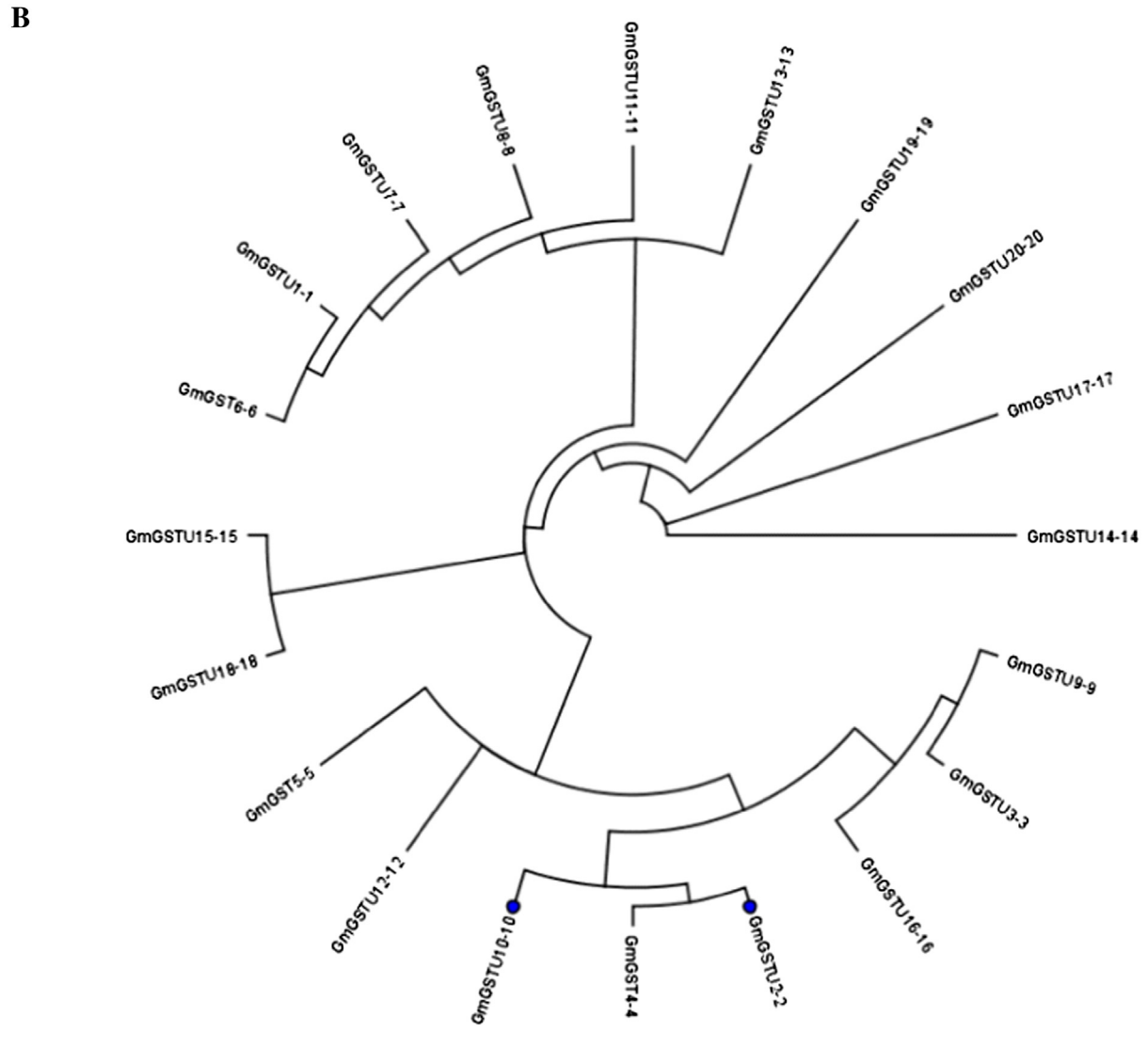
B
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GmGSTU19-19
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GmGSTU17-17
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GmGSTU9-9
GmGSTU3-3
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GmGSTU10-10
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GmGSTU8-8