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# Plant Glutathione Transferases: Structure, Antioxidant Catalytic Function and *in planta* Protective Role in Biotic and Abiotic Stress

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**Abstract:** Plant cytosolic glutathione transferases (GSTs) belong to an ancient enzyme superfamily with multiple and diverse functions which are important in counteracting biotic and abiotic stress. GSTs catalyze the conjugation of xenobiotics and endogenous electrophilic compounds with glutathione (GSH), leading to their detoxification. GSTs not only catalyze detoxification reactions but they are also involved in GSH-dependent isomerization reactions, in GSH-dependent reduction of organic hydroperoxides, biosynthesis of secondary metabolites, and exhibit thioltransferase and dehydroascorbate reductase activity. The applications of 'omics' technologies have allowed the classification of GSTs and the study of their evolution and sequence diversity, while enzymology has provided powerful insights into their catalytic role. This review focuses on plant GSTs, and attempts to give an overview of the new insights into their catalytic function and biological role in biotic and abiotic stress tolerance mechanisms in plants.

**Keywords:** Glutathione transferase, herbicide detoxification, biotic stress, abiotic stress.

## 1. INTRODUCTION

GSTs are ubiquitous enzymes in aerobic organisms and are encoded by large gene families of cytosolic, mitochondrial, and microsomal proteins. GSTs mainly catalyse the conjugation of reduced glutathione (γ-L-Glu-L-Cys-Gly; GSH) via the sulfhydryl group, to electrophilic centres on a wide variety of compounds, both endogenous and xenobiotic [1-4].

The tripeptide GSH is mostly present in reduced form (GSH), while the oxidized form (GSSG) is a marker of oxidative stress [5-8]. Under physiological conditions, free GSH is present in concentrations ranging from 1 to 10 mM [6, 7]. The distribution of GSH is significantly different between gametophyte and sporophyte. Gametophyte is equally distributed among mitochondria, plastids, nuclei and the cytosol, while in sporophyte the highest concentration was found in mitochondria followed by nuclei, the cytosol, peroxisomes and plastids. High levels of GSH in mitochondria are essential for the proper plant development [9].

The conjugation of GSH to xenobiotics serves several important roles: (a) limit and restrict the reactivity of the chemicals; (b) increases their solubility and facilitates their membrane transport

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from the cell and organism; and (c) in some cases, it leads to the formation of secondary metabolites or essential biological mediators [1, 10].

The soluble GSTs have an ancient monophyletic origin shared with the respective enzymes from nearly all eukaryotic and prokaryotic species [10]. The Cytosolic GSTs from mammalians, insects, plants, and bacteria comprise a complex enzyme superfamily that has been subdivided into a number of classes based on a variety of criteria (e.g. amino acid/nucleotide sequence, and immunological, kinetic and structural properties) [11]. GST genes and proteins from mammalian sources have been well characterized, but studies of GSTs from non-mammalian sources such as plants and microorganisms have revealed the existence of several different classes (for more details see Sheehan *et al.*, 2001 [11]). For example, the soluble GSTs of vascular plants according to their can be subdivided into the following distinct classes: phi (F), tau (U), zeta (Z), theta (T), lambda (λ), dehydroascorbate reductase (DHAR), EF1Bγ and tetrachlorohydroquinone dehalogenase (TCHQD) [4, 10-19]. Two new classes, *ι (iota)* and *hemerythrin* have been found in the moss *Physcomitrella patens* [16]. The majority of the plant GSTs are classified as tau (GSTU) and phi (GSTF). The DHAR class is essentially present in terrestrial plants, while is absent in cyanobacteria and a single gene, that likely represents the ancestor DHAR gene, is found in a few algae of the *Chlorophyceae* and *Trebouxiophyceae* classes [20].

GSTs are promiscuous enzymes capable of catalyzing the conjugation of GSH with a broad range of electrophilic substrates [21-25]. This functional promiscuity of GSTs correlates with structural flexibility, which allows for recognition of diverse structures at minimal energetic cost [26]. GSTs exhibit wide substrate specificity toward electrophile molecules including organic halides, organic hydroperoxides, epoxides, arene oxides, α- and β-unsaturated carbonyls, organic nitrate esters, and organic thiocyanates [24]. GSTs not only catalyze the conjugation of GSH to electrophilic compounds but they also have more functions, including double-bond *cis-trans* isomerization, dehydroascorbate reduction and binding "ligandin" activity [25]. For example, some members (zeta class) are involved in GSH-dependent isomerization reactions (e.g. in GSH-dependent isomerization of maleylacetoacetate to fumarylacetoacetate), in the synthesis of sulfur-containing secondary metabolites such as volatiles and glucosinolates, and the conjugation, transport and storage of reactive oxylipins, phenolics and flavonoids [10]. In addition, Lo Piero *et al.*, (2006) have reported the involvement of GST from *Citrus sinensis L.* in anthocyanin glutathionylation [27]. Typical GST-catalyzed reactions are schematized in (Fig. **1**).

GSTs can be found in plants from early embryogenesis to senescence [17]. They play a crucial role in the protection of cells from a wide range of biotic and abiotic stresses, including pathogen attack, xenobiotic and heavy metal toxins, oxidative stress and UV radiation [28-31]. The diversity of potential xenobiotics and stressors, causes functional divergence of this enzyme family which has major adaptive significance. Therefore, the supergene GST family shows extensive functional diversity in gene expression, enzymatic activities, and substrate specificities [17]. Their role in stress tolerance in plants is less characterized than their detoxification function [32], however, GSTs are thought to be evolved as part of the cell protection system against oxygen toxicity [33, 34]. The antioxidant catalytic function of GSTs [14] is displayed through peroxidase [35], thioltransferase and dehydroascorbate reductase activity [32, 36] (Fig. **2**). Recently, *in silico* analysis revealed that GSTs might be subjected in post translational regulation [37].

Proteins able to participate in unrelated biological processes have been grouped under the generic name of moonlighting proteins [38, 39]. Work with different organisms has uncovered a great number of GST isoenzymes that are able to participate in unrelated biological processes. In addition to their role in catalyzing the conjugation of electrophilic substrates to GSH, these enzymes also carry out a range of other functions. Different activities of GST isoenzymes include their role as modulators of signal transduction pathways that control cell proliferation and cell death, regulation of the metabolic pathways, bind non-catalytically and transfer a wide range of endogenous and exogenous ligands [10, 11, 40-42].

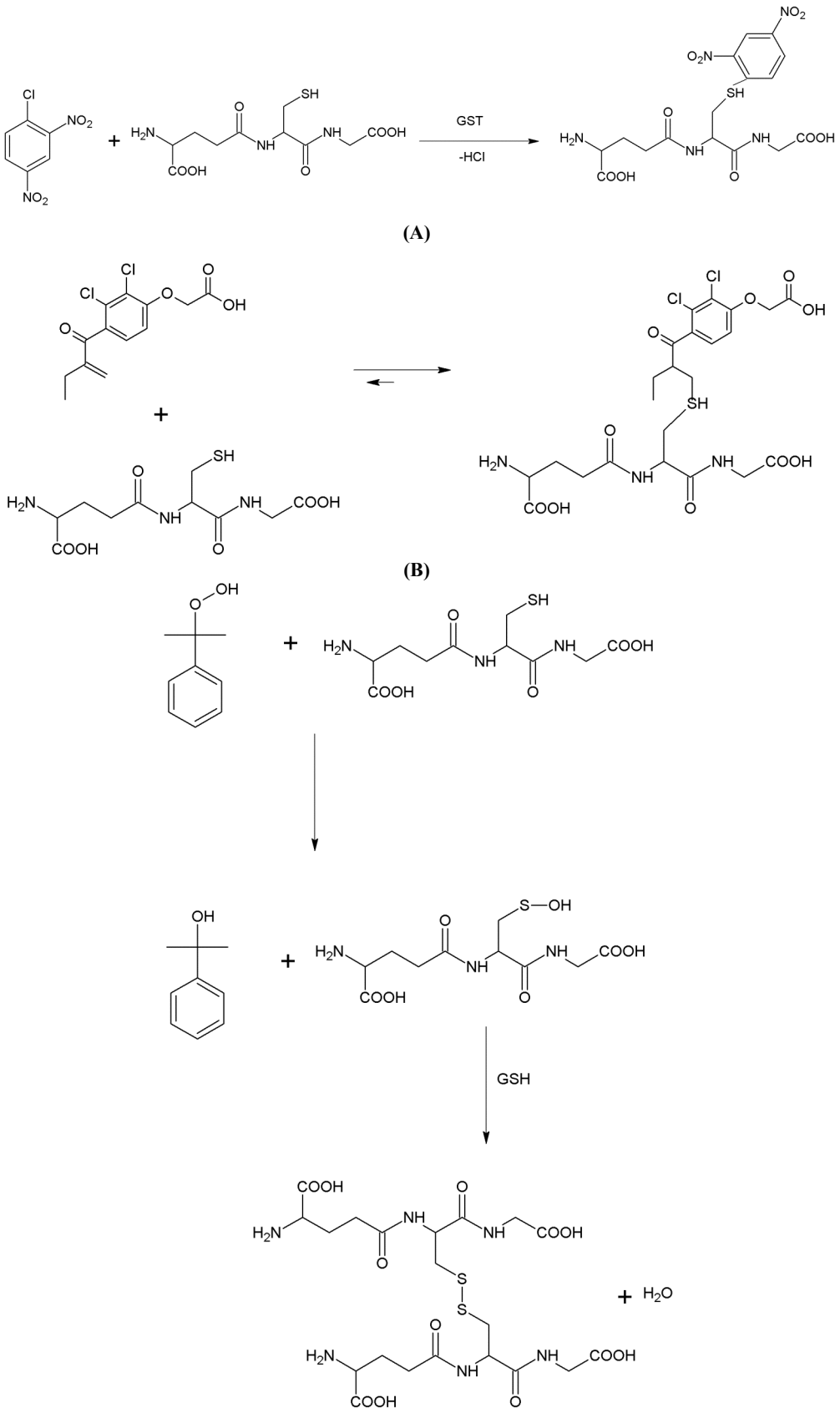



**Fig. (1).** Typical GST-catalyzed reactions. (**A**): nucleophilic aromatic substitution with 1-chloro-2,4-dinitrobenzene, (**B**): Michael-type addition reaction with ethacrynic acid, (**C**): hydroperoxide reduction with cumene hydroperoxide.

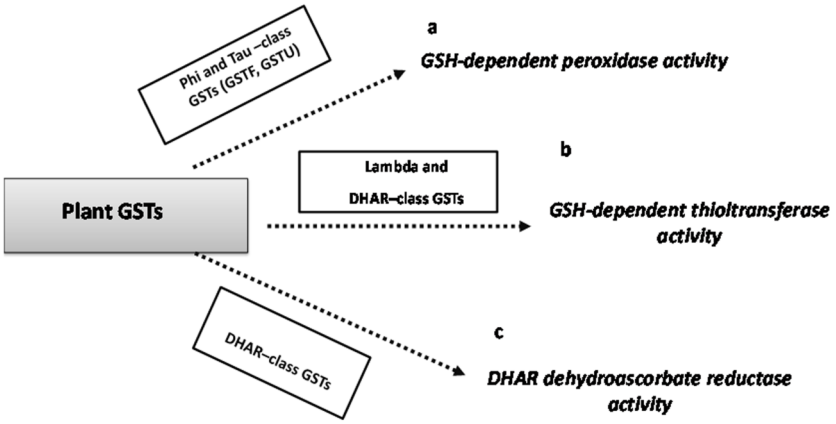



**Fig. (2).** Catalytic activity in relation to the antioxidant function of GSTs: **a**) peroxidase activity, **b**) GSH-dependent thioltransferase activity, and **c**) dehydroascorbate reductase activity.

For example, the isoenzyme GSTP1-1 from human is an ubiquitously expressed protein that plays an important role in the detoxification and xenobiotics metabolism. This isoenzyme, has been associated with the development of tumor resistance to anticancer drugs, acts as a repressor of JNK and other protein kinases involved in stress response, cell proliferation, and apoptosis, and plays an important regulatory role in TNF-α-induced signaling by forming ligand-binding interactions with TRAF2 [43, 44]. Another example of moonlight activity comes from the protein Ure2 [45]. Ure2 is an important regulator of nitrogen catabolite repression, the process that controls the utilization of available nitrogen sources by *S. cerevisiae*. Ure2 does not have a typical GST substrate specificity but belongs to a subset of GST proteins that exhibits glutathione peroxidase activity and are active against different oxidants [46].

## 2. ANTIOXIDANT CATALYTIC FUNCTION OF GSTs

GSH can function as an antioxidant and as a substrate or cofactor of GSTs [12, 47-52]. GSH is mainly known for its antioxidant function against Reactive Oxygen Species (ROS) and hydrogen peroxide ($H_2O_2$) [53, 54]. The high concentration of ROS can lead to a non-controlled oxidation of DNA, proteins and membrane lipids which can cause disruption of metabolism and cellular structure destruction [52, 55]. Plant GSTs of tau and theta classes exhibit GSH-dependent peroxidase activity (GPx, EC 1.11.1.9) [35, 56] and act protectively against cytotoxicity by reducing organic hydroperoxides of fatty acids and nucleic acids to monohydroxyalcohols which are less toxic [1, 15, 36]. This reaction is important as prevents the formation of cytotoxic aldehyde derivatives from organic hydroperoxides degradation [15]. Plant GSTs with GPx activity contribute to defence against oxidative injury during various stresses, including oxidative stress, pathogen attack, herbicide treatment, and to abiotic stresses [57]. It was suggested that in addition to the direct protective effect of the GPx activity, the enhanced tolerance may be due to the GPx-mediated increase in GSSG concentration in the cells, which then function as a signal to activate further protective stress responses [58-60].

The GPxs in plants can be divided into three types. These are the selenium-dependent GPxs [61], the non-selenium dependent phospholipids hydroperoxide glutathione peroxidases (PHGPxs), and glutathione transferases showing glutathione peroxidase activity [62]. The selenium-dependent GPxs composed of four 16 kDa subunits, contain selenocysteine at the catalytic site and appears to be similar to mammalian cytosolic GPx. PHGPx contain cysteine at the catalytic site and appears to be different to the mammalian type PHGPxs. These enzymes can be widely found in plant cells including chloroplasts, mitochondria, cytoplasm, peroxisome and apoplast [62-64].

Plant theta and tau class GSTs exhibit high GPx activities toward organic hydroperoxides [65]. For example, the isoenzymes from wheat [28], peas [13], soybean [66], monocot weeds such as *Alopecurus myosuroides* (blackgrass), and dicot weeds such as *Arabidopsis thaliana* [62, 67] display wide substrate specificity towards organic

hydroperoxides. In particular, the phi and tau class GSTs from *Arabidopsis thaliana* have shown high peroxidase activity with linoleic acid hydroperoxides (13-hydroperoxy-9,11,15-octadecatrienoic acid and 13-hydroperoxy-9,11-octadecadienoic acid) [67].

The isoenzymes of the GST-like class with dehydroascorbate reductase (DHAR) activity catalyze the reduction of dehydroascorbate (DHA) to ascorbic acid using GSH. Members of this class have already been found in *Arabidopsis* [14], rice and soybean [32]. The DHARs do not exhibit GSH conjugating activity. Unlike most other GSTs, DHARs are monomeric and form mixed disulfides with GSH [14].

Members of the lambda and DHARs classes of GSTs, exhibit thioltransferase activity using the 2-hydroxyethyl disulfide (HED) as a substrate [14]. In cases of oxidative stress, when there is a lack of GSH, some protein thiols are S-thiolated making protein-thiol disulfides (Fig. **3**). This modification affects the activity of the proteins or enzymes. Whereas many proteins are active when the key sulfhydryls are in the thiol form, others require them to be in the oxidized, disulfide form [68, 69]. For example, glutathione disulfide (GSSG) can activate enzymes such as glucose-6-phosphatase, acid phosphatase, γ-aminolaevulinate synthetase, creatine kinase, etc. On the other hand, GSSG inhibits glycogen synthetase, pyruvate kinase, adenylate cyclase, phosphorylase/phosphatase, ribonucleotide reductase, phosphofructokinase, etc [15, 68, 70-72].

The involvement of elevated GST expression as a marker for plant response to herbicide stress is continuously gaining ground. GST enzymes can play both a direct role (detoxification of herbicides by GSH conjugation) and an indirect role (involvement in stress response) in the mechanism of herbicide resistance [4, 73-76].

## 3. STRUCTURE OF GSTs

GSTs belong to the thioredoxin superfamily (also including thioredoxin, glutaredoxin, and disulfide-bond formation facilitator) classified by the common GSH binding domain-adopted thioredoxin fold (Fig. **4**) [77, 78]. So far, the available three-dimensional (3D) that have been solved can be summarized as follows: (i) one phi class GSTs from *Arabodopsis thaliana* [79], two from maize (*Zm*GSTF1 and *Zm*GSTF3) [80, 81], (ii) a zeta class GST from *Arabodopsis thaliana* [82], (iii) and five tau class GSTs, one from wheat (*Ta*GSTU4) active in herbicide detoxification [30], one from rice (*Os*GSTU1), and three from *Glycine max* (*Gm*GSTU4-4) [83-85]. Recently the structures of two isoenzymes from *Populus trichocarpa* that belong to lambda class have been reported [86]. Because of the important role of the tau class GSTs, the structure of the *Gm*GSTU4-4 [83, 84] will be presented and discussed with regards to the other plant classes.

### 3.1. Overall Structure

Each soluble GST is, in general, active as dimer of approximately 23–30 kDa subunits of and an average length of 200–250 aminoacids [79-86] (Fig. **4**). Sequence identity within class is typically >40%. For example, sequence identity within tau class GSTs is shown in (Fig. **5A**). Interclass identities are significantly lower, usually <20% in plants (Fig. **5B**). Although there is little sequence similarity between enzymes of different classes, there is significant conservation in overall structure (Fig. **6**).

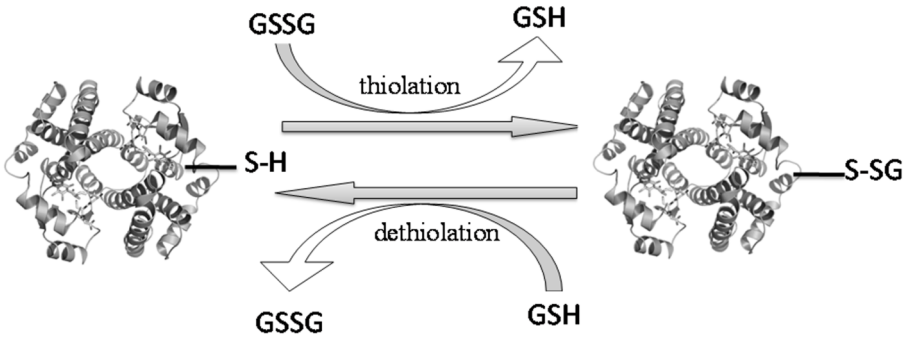



**Fig. (3).** Thioltransferase activity plays regulatory and protective role through reversible thiolation and dethiolation reactions.

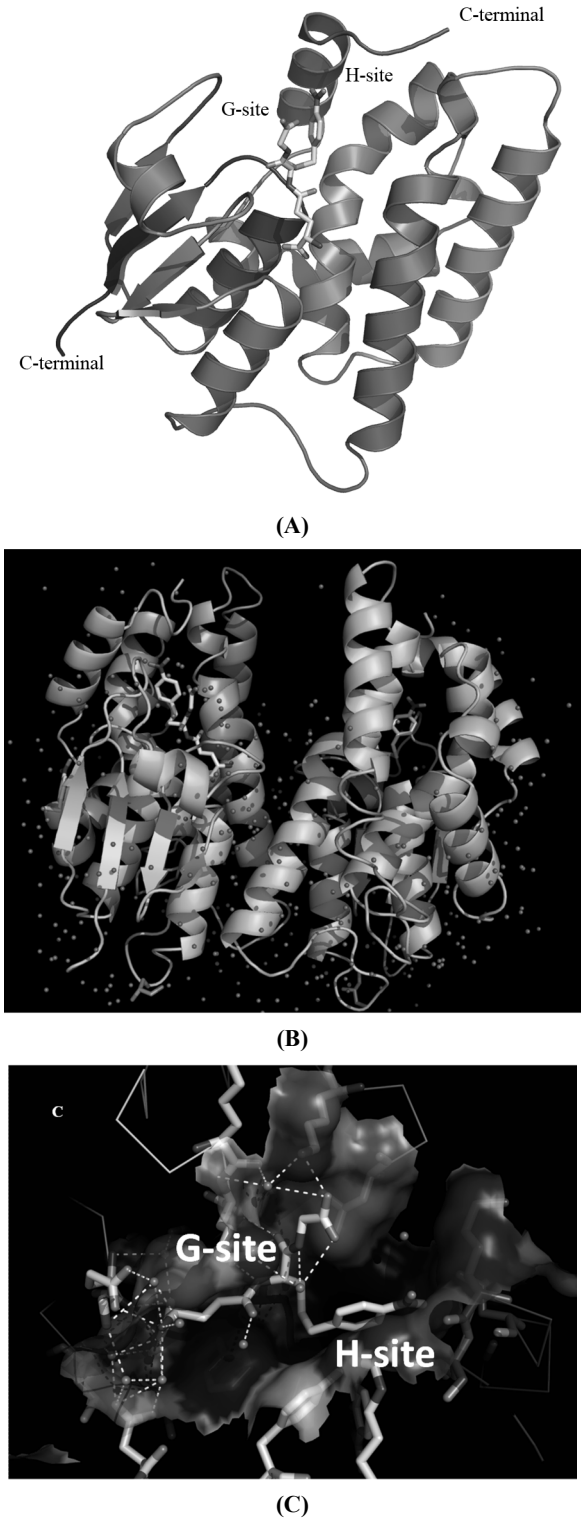



**Fig. (4).** A cartoon representation of the tau class *Gm*GSTU4-4 monomer (**A**), dimer (**B**) and the substrate binding site (**C**). Secondary structure elements and the location of G- and H-site are labelled. The water molecules are represented by spheres. The bound inhibitor S-(p-nitrobenzyl)-glutathione (Nb-GSH) is shown in a stick representation. The figures were produced using PyMol.

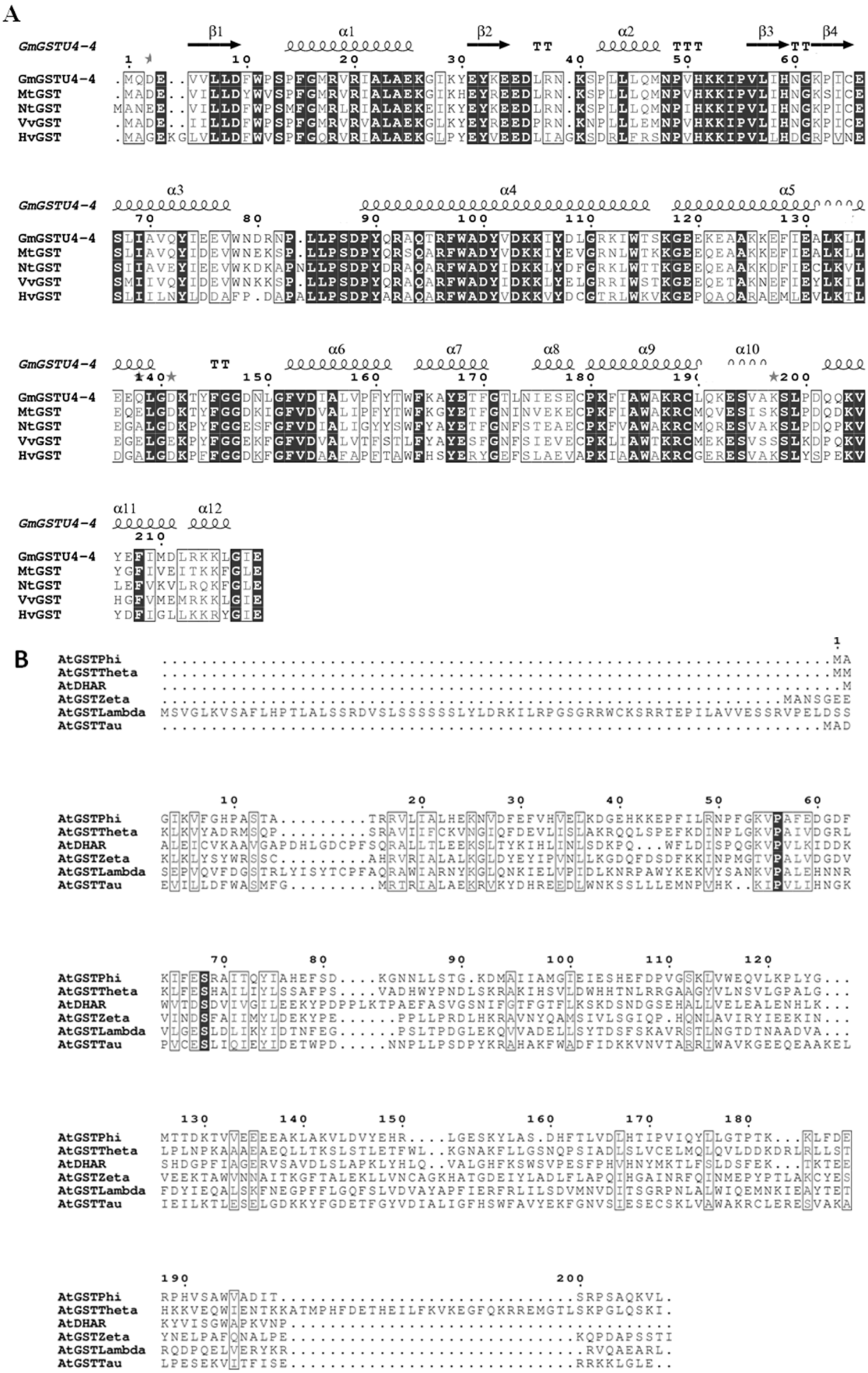
Each subunit adopts the same folding pattern, which is called 'GST fold', and consists of two distinct domains: a highly conserved N-terminal GSH binding domain and structurally diverse C-terminal hydrophobic domain [76]. The N-terminal domain (approximately one third of the protein sequence), consisting of β-strands and α-helices as secondary structure elements, usually βαβαββα, similar to the thioredoxin fold [78, 79, 86] and the all helical C-terminal domain composed of α-helices arranged in a right-handed spiral (Fig. **4**) [34, 87, 88]. Each subunit has an independent active site, consisting of two regions: a GSH binding site (G-site) in the N-terminal domain and a xenobiotic (hydrophobic) substrate binding site (H-site) in the C-terminal domain [30, 80, 81, 83, 84, 89] (Fig. **4A,C**).

### 3.2. Interactions between Subunits

The interactions that are involved in assembling the quaternary structure of GSTs include salt bridges, hydrogen bonds, hydrophilic and hydrophobic interactions, including a lock-and-key motif that physically anchors the two subunits together [90-92]. The lock-and-key motif is a common feature of GSTs of the tau, phi, alpha, mu and pi classes [82, 90, 91]. Only subunits with the same interfacing type appear to be compatible for dimerization. Subunits from different classes of GST are not able to dimerize because of the incompatibility of the interfacial residues [93, 94].

### 3.3. GSH Binding Site (G-site)

In each monomer the G-site is located in a polar region, formed by the beginning of helices H1, H2, and H3 in the N-terminal domain, (Fig. **4A**,**C** Fig. **7**) [83]. The G-site contains specific residues critical for GSH binding and catalytic activity. In particular, a highly conserved, catalytically essential Ser of the tau (Ser13 in *Gm*GSTU4-4) [83, 95], phi, zeta, and theta classes plant and of insect delta class GSTs and Tyr of the mammalian alpha, mu, pi classes GSTs have a crucial role in the mechanism of GSH activation [11]. The Ser/Tyr hydroxyl group acts as hydrogen bond donor to the thiol group of GSH, contributing to stabilization of reactive thiolate anion which is the nucleophile group for the electrophilic substrate [87, 96]. GSTs that belong to the, omega, beta, lambda and DHAR classes contain instead of Ser/Tyr, a catalytically essential Cys, that changes enzyme properties,


A
GmGSTU4-4
GmGSTU4-4
MtGST
NtGST
VvGST
HvGST
B
AtGSTPhi
AtGSTTheta
AtDHAR
AtGSTZeta
AtGSTLambda
AtGSTTau

**(Fig. 5) Contd….**

**(Fig. 5) Contd….**

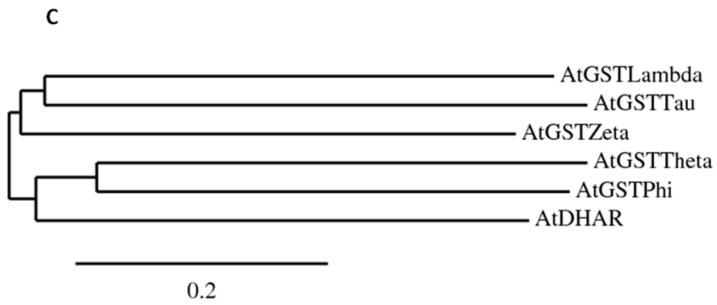



**Fig. (5). A**: Sequence alignment of members of the tau family of GSTs compared with the secondary structure of *Gm*GSTU4-4 (PDB code 2VO4) produced using ESPript (http://espript.ibcp.fr/ESPript/ESPript/). *Gm*GSTU4-4 numbering is shown above the alignment. Alpha helices and beta strands are represented as helices and arrows, respectively, and beta turns are marked with TT. Conserved areas are shown shaded. A column is framed, if more than 70 % of its residues are similar according to physico-chemical properties. This sequence alignment was created using the following sequences (NCBI accession numbers are in parentheses): *Gm*GSTU4-4: *Glycine max* (AAC18566), *Nt*GST: *Nicotiana tabacum* (CAA39707), VvGST: *Vitis vinifera* (XP_002263395), *Mt*GST: *Medicago truncatula* (ACJ85907), *Hv*GST: *Hordeum vulgare* (ABI18247). **B**: Sequence alignment of representative members of the *Arabidopsis thaliana* GST family (phi, theta, DHAR, lambda and tau). Conserved areas are shown shaded. A column is framed, if more than 70 % of its residues are similar according to physico-chemical properties. This sequence alignment was created using the following sequences (NCBI accession numbers are in parentheses): *At*GST Phi (NP_171792); *At*GST theta (NP_198937); *At*DHAR (Q9FWR4); *At*GST zeta (Q9ZVQ3); *At*GST tau (AAS76278); *At*GST lambda (NP_191064). C: Phylogenetic analysis of representative members of the *Arabidopsis thaliana* GST family (phi, theta, DHAR, lambda and tau) (TreeDyn program run at http://www.phylogeny.fr/).

which is involved in forming a mixed disulfide with GSH [14, 20, 97]. Cys residue is highly conserved in all plant DHARs' and is thought to be responsible for binding to DHA [98].

The analysis of crystal structures of soluble GSTs clearly demonstrates that, several active-site residues and a functionally conserved electron-sharing network contributes to the formation and stabilization of the thiolate anion. Amino acids mainly with positive charges for instance Arg18 (α-helix H1) located at the bottom of the G-site, which is conserved among all tau GST sequences, although not involved directly in the formation of the G-site, seems to have an indirect role in GSH binding, and in stabilization of G-site architecture through a network of hydrogen bonds and electrostatic interactions [83].

### 3.4. Electrophilic Binding Site (H-site)

Unlike the conserved N-terminal domain, the sequence of C-terminal domain is variable [83, 84]. The H-site is composed of non-conserved residues from the C-terminal domain (Fig. **4**), showing diversity in substrate specificity (Fig. **5A**) [99]. For example, the H-site of *Gm*GSTU4-4 is typically hydrophobic, and is built predominantly by hydrophobic residues from the C-terminal domain: helix H4a, (Tyr107, Arg111), helix H6 (Trp163) helix H9 (Phe208, Leu212, Lys215 and Leu216), and Phe10 and Leu37 from the N-terminal domain [83, 84].

Regarding variability, the most variable regions include the C-terminal residues and the upper part of the two long helices in the C-terminal domain. Moreover, plant GSTs possess a larger H-site for hydrophobic substrate binding, compared to mammalian GSTs, and therefore are able to accept a larger and much more diverse substrates [83, 84].

### 3.5. Ligand Binding Site (L-site)

In addition to their catalytic function GSTs act as ligand-binding proteins and bind hydrophobic molecules (azo-dyes, bilirubin, heme, polycyclic aromatic hydrocarbons, steroids, thyroid hormones, plant hormones and flavonoids) in a non-substrate manner into a distinct site. This site is termed L-site [12, 83, 100-105] and seems to play a role in storage and transport of these compounds in the cell [106].

Little information is available about the exact localization and the nature of the L-site in GSTs.

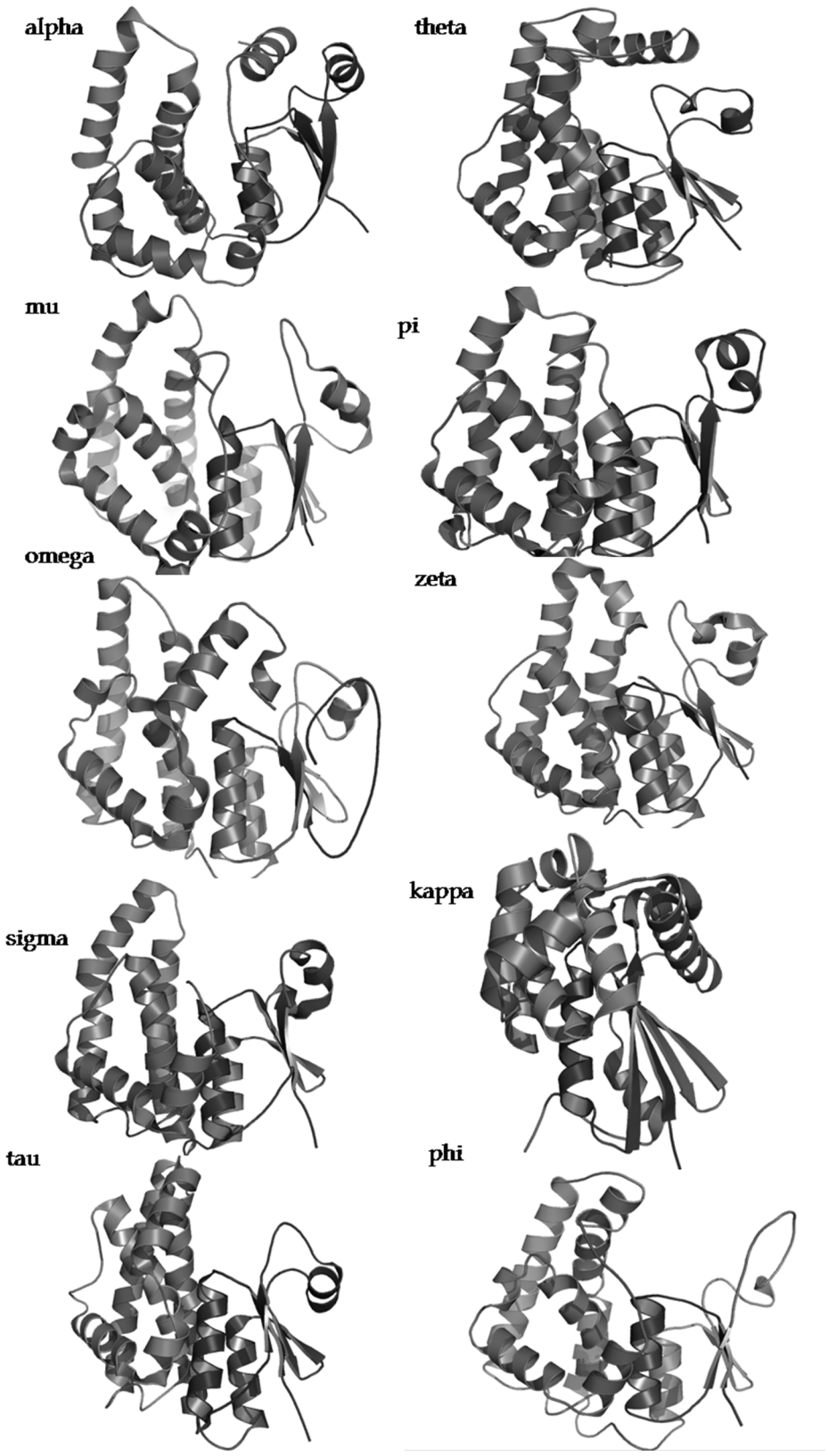
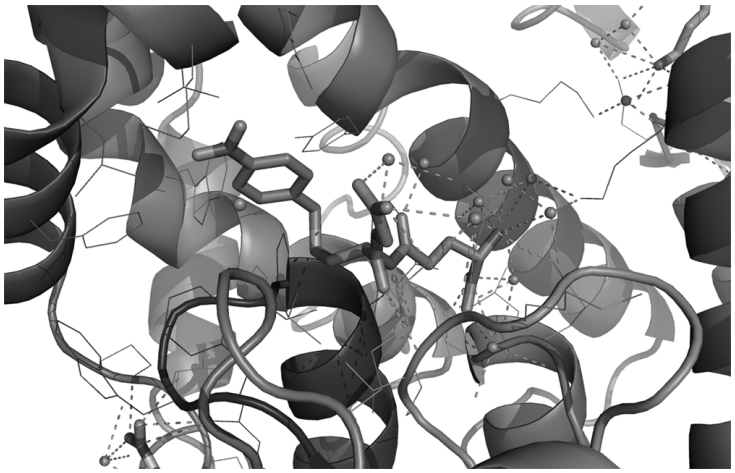
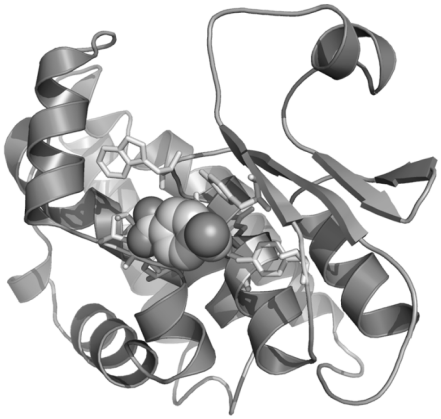



**Fig. (6).** Ribbon representations of the structures of the GST classes: alpha (PDB code: 1gse), mu (PDB code: 1hna), pi (PDB code: 1glp), theta (PDB code: 1ljr), zeta (PDB code: 1fw1), omega (PDB code: 1eem), sigma (PDB code: 1mou), kappa (PDB code: 1yzx), phi (PDB code: 1aw9), tau (PDB code: 1gwc). The figure was produced using PyMoL.



**(A)**



**(B)**

**Fig. (7). A:** Cartoon representation of the G- and H-site of *Gm*GSTU4-4 with the inhibitor S-(p-nitrobenzyl)-glutathione. Amino acid side chains that contribute directly to G and H-site formation are shown in a stick representation. **B:** A representation of the putative L-site of GmGSTU4-4 with the ligand (4-nitrophenyl)-methanethiol. The ligand (4-nitrophenyl)-methanethiol is represented as a ball-and-stick. Amino acid side chains that contribute to L-site formation are shown in a stick representation. The figure was produced using PyMol.

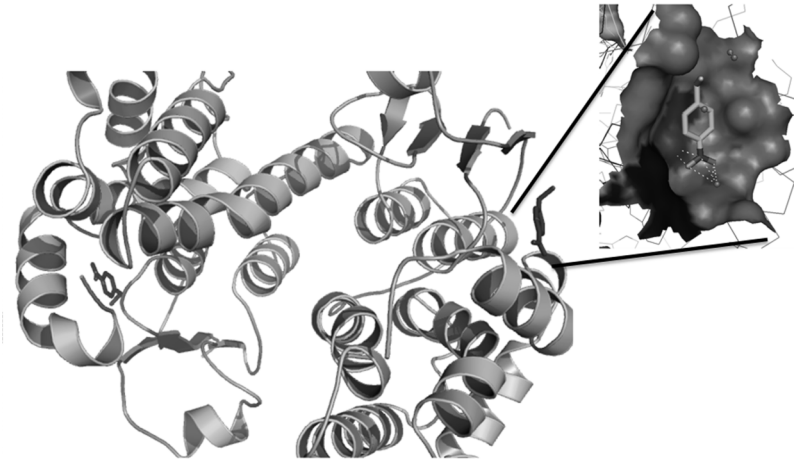


**Fig. (8).** A representation of the putative L-site of *Gm*GSTU4-4 with the ligand (4-nitrophenyl)-methanethiol. The ligand (4-nitrophenyl)-methanethiol is represented as a stick. The figure was produced using PyMol.

Variation in the location of L-site between different GST isoenzymes is a well-known feature of GSTs. For example, the L-site in *Gm*GSTU4-4 was found to bind the molecule (4-nitrophenyl) methanethiol [83] and is located in a hydrophobic surface pocket formed by Trp11, Arg20, Tyr30, Tyr32, Leu199 and Pro200 (Fig. **7B**, **8**). The main binding residues (Trp11, Arg20, Tyr30 and Tyr32) are, in general, conserved within the tau GST family (Fig. **5A**). On the other hand, the L-site of GST from *Schistosoma japonica* [100] is located at the dimer interface. In the case of the *Arabidopsis* enzyme [107], the L-site is located next to the G-site between the side chains of helices α3''/α3''' and α5'', whereas the L-site of the human pi class GST and the maize GST I is located into the H-site [101, 104]. Dixon *et al.*, (2011) [108] demonstrated that the *Arabidopsis At*GSTF2 binds camalexin and flavonol quercetin-3-O-rhamnoside with high affinity (typically $K_d$<1 μM). The binding is enhanced in the presence of GSH and by the other heterocyclic ligands. With GSTF2, these secondary ligand associations resulted in an allosteric enhancement in GSH-conjugating activity. The authors concluded that *At*GSTF2 play important role in regulating the binding and transport of defence-related compounds *in planta*.

The precise role of L-site is unclear. However, it has been proposed that binding of non-substrate ligands to GST prevents modification (e.g. degradation, oxidation) of the molecules *in vivo*. Another possibility is that GST prevents cellular damage that may be caused by cytotoxic and genotoxic compounds. The other possibility is that binding to L-site may help to the delivery of the ligands to specific cellular protein receptors or compartments [83, 101, 102, 104]. Lu and Atkins (2004) have demonstrated the possible antioxidant role for the ligandin activity of GSTs [40]. More recently, Dixon and Edwards have shown that GSTUs from *Arabidopsis thaliana* are able to bind tightly thioester of fatty acids with varied chain length ($C_{(6)}$ to $C_{(18)}$), oxygen content, and desaturation, with $K_{(d)}$ approximately 1 μM [109]. The strong binding of various fatty acids by GSTUs and the conservation in binding observed in the different hosts suggest that GSTUs have selective roles in binding and conjugating these unstable metabolites *in vivo*. In addition, the same group of researchers has demonstrated the ability of GSTs to act as ligand binding proteins of porphyrins *in vitro* [110]. This ability results in highly specific interactions with porphyrinogen intermediates, which can be demonstrated in both plants and bacteria *in vivo* [111].

## 4. ROLE OF GSTs IN ABIOTIC STRESS TOLERANCE

Significant progress has been achieved in the previous years regarding the ability of GSTs to confer resistance to abiotic stresses like herbicides, drought salinity and heavy metals.

Plants in order to overcome stresses have evolved sophisticated and coordinated defense responses [112, 113] against endogenous and exogenous cytotoxic compounds, such as xenobiotics, including herbicides. These systems incorporate a three phase detoxification mechanism [112-116]. GSTs are phase II detoxyfing enzymes, catalyzing the conjugation of the xenobiotic with GSH.

Salinity, drought and temperature stresses, are the primary causes of crop loss worldwide. These abiotic stresses affect plant metabolism and cause important changes in growth, development and gene expression of plants [117]. The recent advances on GSTs have increased our understanding of their role mediated stress tolerance.

*Glycin max L*. under salt stress (200 mM NaCl) showed significant increase in GST activity, but when plants were sprayed with, sodium nitroprusside (SNP), a widely used NO donor, presented lower activity in soybean leaves at 0 h and 12 h, while it increased at 6 h, supported by GST isoenzyme activities. This action could be attributed to the exogenous NO application which induced GST activity in an ABA-dependent manner. Moreover, *G. max* plants showed increase, *GST1* and *GST4* transcript levels in both salt-stressed and SNP pretreated and subsequently stressed samples at 6 h and 12 h, while a more variable regulation pattern was observed in plants treated only with SNP [118].

*Solanum lycopersicum* salt treatment resulted in the overexpression of selected GSTs (*SlGSTU23*, *SlGSTU26*) in the leaves while other like GSTs from lambda, theta, dehydroascorbate reductase and from the zeta classes (*SlGSTL3, SlGSTT2, SlDHAR5, SlGSTZ2)* in the roots [119].

It is interesting that a GST from *Tamarix hispida* found to be downregulated by drought and salinity stress [120]. Transgenic *Arabidopis* plants overexpressing this GST showed enhanced tolerance to drought and salinity stress while found to have increased levels of GST, GSH peroxidase, superoxide dismutase and peroxidase activity, along with decreased malondialdehyde content, electrolyte leakage rates and reactive oxygen species (ROS) levels under salt and drought stress conditions [120]. These results suggest that the enzyme per se has the ability to confer tolerance to abiotic stress caused by drought and salinity but in *Tamarix hispida* there must be an alternative mechanism operating regulating the expression of GSTs [120]. In barley, five GST genes were investigated all were up-regulated significantly under drought stress and/or showed a higher level of transcripts in the tolerant cultivar. In addition, it showed increased GST enzyme activity while it did not changed in the sensitive genotype under drought conditions. The sensitive genotype showed also higher levels of lipid peroxidation, suggesting that GSTs might be an important factor in the drought tolerance of barley genotypes [121].

Another role of GSTs is their involvement in tolerance to heavy metals. This role of GSTs could also be used for the detoxification of polluted soils contaminated by the extensive use of hexavalent chromium [Cr(VI)] in the industry. Tripathi *et al*. reported that yeast cells overexpressing two rice (*Oryza sativa*) GSTs *OsGSTU30* and *OsGSTU41* had normal growth, but had much higher levels of GST activities and showed enhanced resistance to Cr(VI) as compared to control cells. Moreover, yeast cells showed increased accumulation of chromium compared to the control cells [122].

GSTs have also been found to be able to detoxify the explosive 2,4,6-trinitrotoluene (TNT) is a major worldwide military pollutant. More specifically two GSTs, GST-U24 and GST-U25, from *Arabidopsis thaliana* have been found to be upregulated when exposed to TNT and to react with it forming three TNT glutathionyl products [123].

Although we have gained a better understanding on the resistance the GSTs and how they confer tolerance to plants against various stresses [124-126], very little is known on the regulatory mechanism and promoter analysis of specific GST genes. Yet, it has been found that certain GSTs, are induced by a wide range of xenobiotics or other biotic/abiotic stresses, suggesting that there is a specific mechanism. So far the analysis of the GST promoters failed to identify elements related to biotic or abiotic stresses like those found in in animal GST promoters [32]. However, an *ocs* element which is an plant enhancer sequence has been characterized in some of the GST promoters [128]. *Ocs* elements have been found to be induced by auxin or auxin analogs, the plant defense signal, and salicylic acid [129]. In addition, other elements like auxin-responsive elements [130] and ethylene-responsive elements [131] have been identified in GST promoters that might be responsible for auxin- and ethylene-induced GST expression, respectively.

Csiszar *et al* [132] have found significant changes in the expression of specific GSTs when *Solanum lycopersicum* was treated by salycilic acid at doses as low as $10^{-4}$ M. The differential expression of GSTs might be a mechanism of maintaining redox homeostasis during adverse conditions [132].

However, the molecular mechanisms regulating plant GST expression have yet to be identified. The completion of genome sequence from many different plants is expected to facilitate the identification of the *cis*-acting regulatory elements [133, 134]. Furthermore the trans-acting DNA binding factors are also expected to be identified thus allowing plant GST transcriptional regulation to be clarified.

## 5. ROLE OF GSTs IN BIOTIC STRESS TOLERANCE

Pathogen infection elicits the expression of disease related genes resulting in the production of several toxic plant products as well as reactive oxygen species [135]. In a number of studies, it has been reported the biological relevance of GSTs to pathogen attack. However, little is known about their regulatory or catalytic role during pathogen infection. In general it has been proposed that GSTs play a role in the reduction of damage caused by pathogens or diminishing the extent of cell death caused by the hypersensitive response (HR) [136].

A GST gene (*Pv*GST3-3) from *P. vulgaris* is induced after the infection with the fungus *Uromyces appendiculatus*. A number of findings further

support that *Pv*GSTU3-3 plays a crucial role under biotic stress conditions. Expression of *Pv*GSTU3-3 in *E. coli* revealed that it exhibits hydroperoxidase, thioltransferase, and dehydroascorbate reductase catalytic function. In addition, due to its low $K_m$ for GSH relative to other plant GSTs, is possibly able for efficient catalysis under low reduced GSH concentration (e.g., oxidative stress). Finally, a regulatory role in the release of isothiocyanates has been proposed due to its ability to conjugate GSH with isothiocyanates [124].

Global transcriptome analysis of poplar plants infected by *B. dothidea* revealed that GSTs transcripts were accumulated to high levels. Ten of the most highly expressed transcripts had high sequence homology to GSTs from other plant species. Based on present annotation, in addition to transferase activity these GSTs implicated in defense responses and auxin-mediated signaling pathways, involved in aromatic amino acid metabolism, induced by $H_2O_2$ and by the pathogens *Botrytis cinerea* or *Pseudomonas syringae*. According to these results, it seems that GSTs are closely associated with responses to *B. dothidea* in infected poplar plants and, each member of GST subfamilies performed a slightly different functional role to defend against these pathogen [137].

Four tau GST genes (*Nb*GSTU1, *Nb*GSTU2 and *Nb*GSTU3) and one phi GST (*Nb*GSTF1) from *N. benthamiana* plants were examined for their roles in fungal infection. Expression levels of *Nb*GSTU1 and *Nb*GSTU3 increased remarkably post infection while those of *Nb*GSTU2 and *Nb*GSTF1 were not changed. Furthermore, it was examined the performance of inoculated *N. benthamiana* plants following gene silencing. A significant increase in susceptibility was recorded only for *Nb*GSTU1-knockdown plants. These findings suggest that distinct GST genes involved in disease development and that the responsiveness to fungal infection differs between the GST genes [136].

A negative role of GST genes has also been proposed in plant responses against pathogen infection. *N. tabacum* plants infected with *P. parasitica* var. nicotianae showed a major increase in a specific GST gene. In order to verify its involvement in the host response to fungal infection, GST silenced plants was developed. A significant increase in resistance was recorded in GST silenced plants. Possibly, the combined action of several gene products are under the direct or indirect control of this GST gene which appears to act as a negative regulator in the defense response of tobacco to *P. parasitica* [138].

A tau GST gene for *N. benthamiana Nb*GSTU4 with 80% identity to *Nb*GSTU2 [136] upregulated in *N. benthamiana* post Bamboo mosaic virus (BaMV) infection. With the view to functionally characterize the role of *Nb*GSTU4 in BaMV infection, the *Nb*GSTU4 was knocked down or was transiently expressed in *N. benthamiana*. When the expression level of *Nb*GSTU4 is reduced, a significant decrease in BaMV RNA accumulation was recorded. In contrast, the accumulation of viral RNA increases when *Nb*GSTU4 is transiently expressed. The results suggest that *Nb*GSTU4 involved in the infection cycle of BaMV. The *Nb*GSTU4 enables the unhindered synthesis of minus-strand RNA by providing either an antioxidative moiety or by changing the redox state of replicase complex [139].

A growing body of evidence suggests that GST enzymes play a role in plants disease susceptibility. However, no clear picture has manifested yet. A number of important questions remain to be addressed. Which are the exact roles of GSTs in infected plants? Is there specific GSTs that respond to different pathogens? What is the molecular mechanism that governs plant GST expression under biotic stress conditions? Further research is needed to answer the above questions.

In conclusion, the plant GST family of enzymes belongs to the thioredoxin superfamily classified by the common GSH binding domain-adopted thioredoxin fold. The GST family represents a group of catalysts with multiple roles many of which are important in counteracting biotic and abiotic stress. These roles can be relevant to maintaining cellular homeostasis as well as in the direct detoxification of toxic compounds. The detoxification roles of GSTs arise for their ability to catalyze the conjugation of GSH to a large number of electrophilic molecules. The antioxidant catalytic function of GSTs is exhibited through peroxidase, thioltransferase and dehydroascorbate reductase activity. Further analysis and study of this protein family will inevitable reveal many examples of functional and catalytic diversification and will highlight the importance of these enzymes in the

protection against the oxidative stress and in other cellular processes.

## LIST OF ABBREVIATIONS

CDNB = 1-chloro-2,4-dinitrobenzene

GSH = Glutathione

GST = Glutathione transferase

G-site = GSH binding site

GPx = Glutathione peroxidase

H-site = Hydrophobic binding site

Nb-GSH = S-(p-nitrobenzyl)-glutathione

ROS = Reactive Oxygen Species.

## CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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## DISCLOSURE

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