PLANT GLUTATHIONE S-TRANSFERASES FUNCTION DURING ENVIRONMENTAL STRESSES: A REVIEW ARTICLE

MARYAM SHAHRTASH[1]

Glutathione S-transferases are a superfamily of multifunctional enzymes that present at every stage of plant development and in every tissue types. Plant soluble (cytoplasmic) GSTs are presented as seven groups of Phi, Tau, Zeta, Theta, Lambda, Dehydroascorbate reductase, and Tetrachlorohydroquinone dehalogenase. In plants the amino acid sequence identity between classes is usually less than 30%. They are most known for their function to protect the cell from oxidative damage by quenching reactive molecules with the addition of glutathione (GSH). This review points out some recent findings about GSTs function in stressful situations such as plant-pathogen and pest interactions, herbicide detoxification and heavy metal stress.

Key words: GST, Herbicide detoxification, Stress, Phi classe, Tau class.

PLANT-PATHOGEN AND PEST INTERACTIONS

Many plant GSTs such as Theta, Phi and Tau classes have glutathione peroxidase activity that detoxify cytotoxic alkenals and lipid hydroperoxides (Mauch and Dudler, 1993), and reduce organic hydroperoxides of fatty acids and nucleic acids to the corresponding monohydroxyalcohols. This reduction plays a pivotal role in preventing the degradation of organic hydroperoxides to cytotoxic aldehyde derivatives (Dixon *et al*., 2002). The most likely role for GSTs in pathogen-infected plants was to suppress necrosis by detoxifying lipid hydroperoxides produced by peroxidation of membranes (Dean *et al*., 2005). It has postulated that antioxidative activity of GSTs plays a role in the reduction of damage caused by pathogens or in limiting the extent of cell death during the hypersensitive response (HR) (Lieberherr *et al*., 2003). This induction is correlated with increasing concentrations of H_2O_2 (Venisse *et al*., 2001). GSTs may detoxify organic peroxides, which are highly reactive molecules that can produce during pathogen attack (Mauch and Dudler, 1993). GSTs might induce in plants because of increased levels of auxins produced by fungal pathogens (Hahn and Strittmatter, 1994).

[1] Islamic Azad University, Kazerun Branch, Kazerun, Iran
E-mail: maryam.shahrtash@gmail.com

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So far, GSTs have been found to be induced following infection of potato by *Phytophthora infestans*, wheat by *Erysiphe graminis* f. sp. *hordei* and *Arabidopsis* by *Peronospora parasitica* (Dean *et al*., 2003). Multiple GST sequences have been identified in soybean infected by *Sclerotinia sclerotiorum*, *Brassica napus* infected by *Leptosphaeri maculans,* and wheat spikes infected by *Fusarium graminearum* (McGonigle *et al*., 2000; Kruger *et al*., 2002; Fodor *et al*., 1997).

Changes in glutathione levels and in activities of DHAR, glutathione reductase (GR), and GST were investigated in tobacco mosaic virus (TMV)-inoculated lower leaves and in non-inoculated upper leaves of *Nicotiana tabacum* L. cv *Xanthi-nc* (Hamid and Strange, 2000). GSTs also have non-catalytic roles and are called ligandins, because of their ability to bind structurally diverse compounds, such as steroids, carcinogens and some drugs (Scalla and Roulet, 2002).

Prp1-1, a Tau GST from potato was induced during disease because of auxin produced by *P. infestans*; auxin competitively binds PRP1-1, thereby inhibiting GST function. A potato GST, prp1-1, was competitively bound by *P. infestans* auxin produced by and maintenance of auxin homeostasis has been proposed to be the role for the GST genes. The inhibition of GST activity by indole-3-acetic acid is reminiscent of the inhibitory effect of salicylate, another endogenous signal molecule, on the activity of tobacco catalase, inducing an increase of the H_2O_2 concentration *in vivo*. Prp1-1 is an auxin binding protein but no detection of IAA-GSH conjugates formation. It is known that the binding of nonsubstrate ligands inhibits GST activity toward xenobiotics, but the precise functions of GST binding to non-substrate ligands remain unclear (Hahn and Strittmatter, 1994).

However, this non-enzymatic binding capacity may allow the suggestion that GSTs are involved in the storage and rapid transport of these nonsubstrate ligands in the cell to specific receptors or cellular compartments. In doing so, they prevent cellular damage from cytotoxic and genotoxic compounds which can oxidize protein and insert into DNA (Scalla and Roulet, 2002). Among 10 GST genes of *A. thaliana* tested, one Zeta, one Tau and two Phi GST genes showed increased expression by 3 day after inoculation in interaction with *P. parasitica.* These genes are involved in the detoxification of oxidative stress products (Booth *et al*., 1961).

GSTs also have glutathione peroxidase activity, thereby protecting cells from oxidative injury by organic peroxides are created in plants during processes such as photosynthesis, pathogen attack (Mauch and Dudler, 1993), detoxification of microbial toxins (Edwards *et al*., 2000), and detoxification of phytoalexins produced during the hypersensitive response. If not reduced, these peroxides will convert to cytotoxic derivatives that can damage plant cells (Dean *et al*., 2005). By functioning as GPOXs, they protect cells from the effects of active oxygen species (AOS), which produced during oxidative stress (Scalla and Roulet, 2002).

Colletotrichum destructivum infection induced ethylene production in *N. tabacum*. The rapid production of ethylene may explain the rapid induction of NbPR2, NbGSTU1, and NbGSTU3 expression. Ethylene treatment induced the

expression of a GST gene, AtGSTF2 in *A. thaliana*. The signaling pathways that lead to the rapid induction of GSTs by pathogens are not well understood. Individual GSTs from *Arabidopsis* and other plants have shown to be induced by salicylic acid (SA), ethylene, jasmonic acid (JA), auxin and hydrogen peroxide (Lieberherr *et al*., 2003).

In wheat, a Phi GST, GSTA1, was induced with *E. graminis* f. sp. *Tritici*. The proposed function of GSTA1 involved the detoxification organic proxides to prevent continuing cell death caused by free radicals produced during the hypersensitive response in the incompatible interaction. After inoculation of *A. thaliana* with a compatible strain of *P. parasitica*, higher expression of Phi, Tau and Zeta GST genes was observed and these may have a role in restricting cellular damage by functioning in antioxidative reactions. Treatment of poppy cell suspension cultures with a fungal elicitor extracted from *Botrytis spp*. resulted in the induction of a class Phi GST one hour after exposure to elicitor, and the GST was believed to be involved in the translocation or metabolism of phenylpropanoids both as part of the normal developmental physiology and the defense response (Dean *et al*., 2005). GSTs are active in the process of binding of xenobiotics to produce less toxic metabolites, and chickpea GSTs may be involved in detoxifying two toxins produced by the chickpea blight fungus, *Ascochyta rabei* (Hamid and Strange, 2000). In *Nicotiana benthamiana* infected by *colletotrichum orbiculare*, NbGSTU1 might act by conjugating and detoxifying toxins produced by *C. orbiculare*. NbGSTU1 could also possibly reduce infection by *C. orbiculare* by maintaining auxin homeostasis. Auxin production by plant pathogenic fungi may be involved in pathogenesis (Dean *et al*., 2005).

Tissue damage caused by insect feeding activates an octadecanoid signaling cascade that culminates in JA biosynthesis and production of antifeedant proteinase inhibitors and other putative defense molecules. The phytohormone ethylene is another wound-response regulator. In addition, SA interferes with wound-related gene expression by inhibiting the octadecanoid pathway. The balance between different signaling pathways adjusts defense characteristics against particular insects. GST6 mRNA increased approximately 4-fold after herbivory and about 3-fold after wounding in *Arabidopsis*. The rapid induction of GST6 by insect feeding and wounding in *Arabidopsis* may relate to H_2O_2 signaling, because the effects of an oxidative burst caused by mechanical damage are faster than regulation by phytohormones (Stotz *et al*., 2000).

HERBICIDE DETOXIFICATION

GST isoenzymes have a well-defined role in plant detoxification reactions. They are capable of catalyzing the binding of various xenobiotics and their

electropholic metabolites with GSH to produce less-toxic conjugates (Rochalska and Grabowska, 2007). GSTs in plants were identified first and were intensively studied because of their ability to detoxify herbicides, and individual GSTs conferring herbicide tolerance were characterized from most major crop species (Xu *et al*., 2002). Molecules that were conjugated with GSH are efficiently imported into vacuoles via ATP-binding cassette transporters (McGonigle *et al*., 2000). GSTs are the predominant detoxification enzymes in maize and cereal crops that are responsible for triazine herbicides, acetamid herbicides, and certain graminicides, such as fenoxaprop-ethyl in wheat. Herbicide detoxifying GSTs have been well characterized in maize and soybean, and have also been identified and partially characterized in wheat. Herbicide safeners protect the crop plant by increasing herbicide metabolism and detoxification pathways. The increase in metabolism results from an increase in the activity of herbicide detoxification enzymes such as GSTs (Xu *et al*., 2002).

Plant GSTs are multifunctional enzymes that catalyze the conjugation of glutathione (g-glutamyl-cysteinyl-glycine) or homoglutathione (in legumes) to various substrates (R-X) to form a polar S-glutothionylated (R-SG) product. GSTs are considered important detoxification components involved in Phase II detoxification, even though GSTs might directly conjugate herbicides. The conjugation reaction leads to R-SG products are often transported into the vacuole by Phase III proteins such as ABC transporters. GSTs are involved in non-target-site herbicide resistance comes from GST activity assays in herbicide-resistant weeds. GST activity is normally studied by using a model substrate, such as1-chloro-2, 4-dinitrobenzene (CDNB), whereby the conjugation of GST with artificial substrates is detectable by light absorbance. An increase in GST activity is accompanied by increased GST gene expression (Yuan *et al.*, 2006).

Herbicide resistance in a weed and increased GST activity were established in velvetleaf (*Abutilon theophrasti*), and GSH conjugation of atrazine was observed in the resistant biotype (Anderson and Gronwald, 1991). In plants, the most commonly observed GSH conjugation reaction is the nucleophilic displacement of a halogen from an electrophilic site on an aromatic ring, a heterocyclic ring, or an alkyl group. Conjugations of the herbicides atrazine, fluorodifen, pentachloronitrobenzene (PCNB), propachlor, chlorimuron ethyl or insecticide, methidathion are examples for this type of reaction (Scalla and Roulet, 2002). Safener-induced protection in cereals is associated with increased expression of herbicide detoxifying enzymes, including GSTs. Treatment of *Arabidopsis* seedlings growing in liquid medium with various safeners similarly resulted in enhanced GST activities toward a range of xenobiotics with benoxacor, fenclorim, and fluxofenim being the most effective (DeRidder *et al*., 2003). In maize GST IV appears to be the principal detoxifying enzyme for alachlor, although GST I and II are involved in the process. GST I, II and II are also reactive against CDNB (1-Cl-

2, 4-dinitrobenzene), a chromogenic artificial substrate commonly used for GST activity assays (Rossini *et al*., 1998).

HEAVY METAL STRESS

Chemical detoxification of heavy metals within the plant cell may be achieved by binding or sequestration by metal-complexing agents, and transport of the heavy metals to cellular compartments (*i.e*., vacuole), or a combination of both mechanisms. Heavy metals interact with a number of cellular constituents in plant cells and inhibit important life processes. On the other hand, plants have developed different defense mechanisms (reduced uptake, active efflux, and sequestration processes) (Xiang and Olive, 1998).

In maize (Marrs *et al*., 1996), the bronze two gene (Bz2) encodes for the GST enzyme, as does the soybean counterpart gene GmGST26-A (Ulmasov *et al*., 1995; Marrs *et al*., 1996). This enzyme, located in the cytosol, performs the last genetically defined step in anthocyanin biosynthesis, namely tagging an anthocyanin precursor, cyandin-3-glycoside with glutathione, allowing for recognition and targeting of anthocyanin into the vacuole via and Mg/ATP-dependent, ABC-type GSH pump located in the tonoplast. This mechanism was confirmed by the use of vanadate, which inhibits transport into the vacuole by inhibiting the tonoplast GSH pump (Marrs *et al*., 1996). Both Bz2 and GmGST26-A belong to a group of type III GSTs, which are induced by a variety of environmental stresses, including heavy metals such as Cadmium (Cd). Cd induces a higher transcription rate of Bz2, and apparently two, not one, species of mRNA are produced. In addition to one mRNA species coding for GST, a second mRNA species codes for a new truncated GST protein which is missing the enzyme activity domain, but retains the dimerization and GSH-binding domains. Therefore, the result is two proteins, one of which is the normal GST, which targets the anthocyanin precursor to the vacuole, while the other appears to be involved in the heavy metal transport into and accumulation within the vacuole (Marrs and Walbot, 1997). Expression of the GST gene remained at elevated levels for at least 48 hr (Richards *et al*., 1998).

Heavy metals were found to rapidly and markedly induce osgstu4 and osgstu3 in *Oryza sativa*. Stress responses of osgstu4 and osgstu3 suggest involvement in the detoxification of heavy metals or stress metabolites in rice roots. Osgstu4 and osgstu3 were induced by heavy metals and hypoxic stress and were differentially responsive to salt stress in rice roots, and suggested that reactive oxygen species and redox changes are involved in the complex stress response regulation of osgstu4 and osgstu3 expression (Moons, 1993).

Heavy metal stress leads to considerable GST induction in various plants. GST proteins may be involved in heavy metal detoxification also as ligandins (xenobiotic-binding proteins) (Marrs *et al*., 1996). In *Arabidopsis* a gene encoding a GST has recently been isolated. This gene which is called TT19 is required for the accumulation of anthocyanin in the vacuole of vegetative tissues and proanthocyanidin in the seed coat (Kitamura *et al*., 2004).

CONCLUSION

As it was mentioned, GST plays an important role in plant resistance against biotic and abiotic stresses. There are still many unanswered questions about plant GSTs. Why plant species are different in having GST classes? Do these differences result in strong or weak points in dealing with stressful situations? Why do Tau- and Phi classes outnumber other GST classes? How do plant GSTs regulate? Are there more than eight GST classes in plants?

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