

Glutathione Peroxidase cDNA Cloning and Expression in Soybean Roots under *Heterodera glycines* Infection

Fang Wang^{1,2}, Yuxi Duan¹, Lijie Chen¹, Yuanyuan Wang¹, Xiaofeng Zhu¹ & Wei Li³

¹ College of Plant Protection, Shenyang Agricultural University, Shenyang 110161, China

² Heilongjiang Academy of Agricultural Sciences, Qiqihar Branch Academy, Qiqihar 161006, China

³ Husbandry Research Institute of Heilongjiang Province, 2 He Yi Street, Qiqihar 161005, China

Correspondence: Yuxi Duan, College of Plant Protection, Shenyang Agricultural University, Shenyang 110161, China. Tel: 86-024-8845-4528. E-mail: duanyx64@163.com

Received: March 20, 2012 Accepted: April 1, 2012 Online Published: May 25, 2012

doi:10.5539/jas.v4n7p163

URL: <http://dx.doi.org/10.5539/jas.v4n7p163>

Abstract

A gene named GmGPX1 encoding glutathione peroxidase (GPX) was cloned and sequenced from soybean roots infested *Heterodera glycines* by RT-PCR (Reverse Transcription PCR), which is a crucial enzyme in plant cells regulating reactive oxygen species (ROX). The cDNA length of cloned gene was 693bp, flanked by a 5'-untranslated region of 7bp and a 3'-untranslated region of 185bp, containing six exons and five introns. Genomic DNA fragment was located at *Glycine max* chromosome 5. The open reading frame of cDNA which encodes a polypeptide of 166 amino acid residues and protein molecular weight was 18375.8Da, theoretical isoelectric point 6.59. The deduced amino acid sequence showed about 99% and 64% homology with *G max* putative PHGPX (XP_003532707.1) and *Arabidopsis thaliana* GPX (NP_564813.1), respectively. The expression profile of the GmGPX1 in Xiaoliheidou (*G. max*) under *H. glycines* infection, which generates oxidative stress, was analyzed. Real time PCR analysis revealed that the GmGPX1 mRNA levels were increased stabilized from 1.3 to 1.47 times after exposure to *H. glycines* from 12h to 48h, and reduced to 1.07 times at 72h comparing with non-inoculation control. These results suggest that the GmGPX1 gene is induced by *H. glycines* at early stage in soybean roots and play an important role in removing oxidative damage.

Keywords: soybean, glutathione peroxidase, expression pattern, soybean cyst nematode

1. Introduction

In plant cells, aerobic metabolisms particularly those involving in photosynthesis and respiration result in the generation of reactive oxygen species (ROS). Those ROS, such as superoxide radicals, hydroxyl radicals, or hydrogen peroxide, can damage biological molecules, including nucleic acid, lipids, and proteins. In order to protect against the oxidation stress, plants in vivo have developed several enzymatic and nonenzymatic systems (Greene, 1995). GPXs (glutathione peroxidase) play a critical role in enzymatic systems to detoxify hydrogen peroxide and protect plant cells against oxidative stress. Their principal activity is thought to catalyze the reduction of H₂O₂ and lipid hydroperoxide to water and alcohol (Bowler et al., 1992). GPX requires an ascorbate (AsA) or a glutathione (GSH) as substrate (Mittler, 2002). The first study of GPXs associated with catalytic activity and encoding gene originates from animal (Ursini et al., 1985). In 1992, the first cDNA homology to mammalian selenium-dependent GPXs is cloned from *Nicotiana sylvestris* leaves (Criqui et al., 1992). Since then, several homologous GPX cDNAs have been isolated from *Citrus sinensis*, *Arabidopsis thaliana*, *Brassica campestris*, *pinacia olerae*, *Helianthus annuus*, *Lycopersicon esculentum*, *Pisum sativum*, *Oryza sativa* and Chinese cabbage (Holland et al., 1993; Sugimoto & Sakamoto, 1997; Eshdat et al., 1997; Sugimota et al., 1997; Roeckel-Drevet et al., 1998; Depege et al., 1998; Mullineaux et al., 1998; Li et al., 2000; Jung et al., 2002). Furthermore, researches find that the activity of GPXs in plant is lower than those in mammalian because of Cys catalytic sites in plant, whereas high reactivity of catalytic sites selenium-dependent Cys in mammalian (Faltin et al., 1998; Holland et al., 1993). In addition, analyses of GPXs mRNA expression level in plants present a stable level increase under different environmental constraints, such as pathogen infections, high salt, metal stress, mechanical stimulation, aluminum toxicity (Criqui et al., 1992; Holland et al., 1993; Sugimoto & Sakamoto, 1997; Depege et al., 1998).

In the previous study, we constructed a suppression subtractive hybridization - cDNA library, which soybean cyst nematode (*Heterodera glycines*) induces *Glycine max* gene differentially expressed, and screened an EST fragment. This EST shares 98% similarity with soybean sequence (NM_001248911) by BLASTn, 86% similarity with *Arachis hypogaea* sequence (DQ889534, glutathione peroxidase 1 mRNA). So it is presumed that the EST is belonged to family member of glutathione peroxidase. In order to confirm and explicit function of the EST, we cloned the full-length ORF using reverse transcription PCR and analyzed the expression characteristic for the further study.

2. Materials and Methods

2.1 Plant Materials and Soybean Cyst Nematode Preparation

H. glycines were originated by single-cyst descent maintained on *G. max* cv. Liaodou 15 in the greenhouse of Nematology Institute of Northern China. The population of *H. glycine* genotype was identified race 3 according to Golden system (Golden et al., 1970).

The *G. max* cv. Xiaoliheidou (National code ZDD1412) used in the experiments was incompatible with race 3 determined in previous studies (Liu et al., 1994). The seeds were soaked in 10% NaClO to remove the pathogenic bacteria, and then washed with sterile water for 5min. Put seeds in the petri dishes loaded with moisture sterile filter paper, germinated at 25°C for 2d in illuminating incubator. Transplant the seeds to plastic pot until the length of radical were 1cm. Seedlings watered daily were grown in plastic pot (one plant per pot) containing 50% sterile soil and 50% sand. After 2 weeks seedling, plants were inoculated with a suspension of 4000 J2 (second-stage juveniles) by pipetting into three holes (13 cm diameter x 12 cm depth) around the roots.

The plants were challenged with nematodes were classified into one group, whereas plants without nematodes infection were used as control group. Root tips (2 cm length to tip) of five seedlings were sampled at 0, 12, 24, 48, and 72h post-inoculation, individually, for both groups.

2.2 Total RNA Isolation and cDNA Synthesis

Total RNAs were extracted following protocol of SV Total RNA Isolation Kit (Promega, USA). The quality and quantity of extracted RNA was determined, and equal amounts of root RNA per time point were pooled from the five sampled plants for each group. 1 µg of total RNA from samples was reverse transcribed according to introduction of Promega Reverse Transcription System (Promega, USA). Reaction system included MgCl₂ 4µl, reverse transcription 10X buffer 2µl, dNTP mixture 2µl, recombinant RNase in 0.5µl, AMV reverse transcriptase 0.6µl, Oligo (dT)₁₅ primer 1µl, total RNA 1µg, nuclease-free water to a final volume of 20µl. Incubate the reaction at 42°C for 60minutes, then heat samples at 95°C for 5 minutes to inactivate the AMV reverse transcriptase and prevent it from binding to the cDNA. Store the cDNA at -20°C until use.

2.3 GmGPX1 cDNA Cloning

According to reference sequence NM_001248911 in NCBI, the specific primers of gene GmGPX1 were designed as followed: sense: 5'-GGACACTATGGCCACCTCAAG-3', anti-sense primer: 5'-CACACAATAATAAGTACACAATCTC-3'. The transcribed synthesized cDNA were used as templates for PCR amplification. Reaction system included cDNA template 1.5µl, 2X Taq PCR Master Mix 12.5µl (Tiangen, China), sense primer 1µl (10mmol/L), anti-sense 1µl (10mmol/L), double distilled H₂O to total volume 25µl. Reaction program was done as followed: pre-denaturation at 95°C for 2min; denaturation at 95°C for 30s, annealing at 57°C for 30s, and extension at 72°C for 1min, followed by 30 cycles; extension at 72°C for 10min.

PCR product was separated by 1.2% (v/w) agarose gel electrophoresis, purified with DNA gel extraction kit (Tiangen, China), connected to pGEM-T Easy Vector with the size of 3105bp (Promega, USA), and transformed to competent *Escherichia coli* DH5α cells by heat strike at 42°C. White-blue selection and colonies PCR confirmation were carried out, followed by the plasmid extraction from over-night cultured lysate using plasmid extraction kit (Tiangen, China). Recombinant restriction analysis was done using EcoR I restriction enzyme, then examined on 1.2% (v/w) agarose gel electrophoresis. The plasmid containing the goal gene was sequenced.

2.4 Expression Analysis by Real-time Quantitative PCR

Real-time quantitative PCR was used to determine GmGPX1 mRNA expression level. Root tips of Xiaoliheidou were harvested at 12, 24, 36, 48 and 72h from both treatments (inoculation and non-inoculation group). First-strand cDNA was synthesized by 1 µg total RNA from each sample using the Reverse Transcription System (Promega, USA) following the manufacturer's protocol. Gene-specific primers were designed by using Primer Premier 5.0 software. Actin 11 (Accession number BW652479) was used as reference gene for normalizing the transcript profiles. Primers of actin 11 sense: 5'-CGGTGGTTCTATCTTGGCATC-3', anti-sense: 5'-

GTCTTTCGCTTCAATAACCCTA-3'; primers of GmGPX1 sense: 5'- CAAGGCTGAGTTTCCCGTT-3', anti-sense: 5'- ATACCATCCCCAAAGAGTCC-3'. The PCR reactions were prepared contained 10ul SYBR Premix Ex Taq™ II (Takara, China), 1µl forward and reverse gene-specific primers (10 µM), 1.5 µl of the five-fold diluted reverse transcribed cDNA, and 6.5 µl double distilled H₂O. The thermal cycling protocol entailed activation of Hot Start Taq DNA polymerase at 95°C for 10min, followed by 40 amplification cycles with denaturation at 94°C for 10s, primer annealing at 57°C for 30s and extension at 72°C for 30s. The real-time PCR data were calibrated following the $2^{-\Delta\Delta C_t}$ method for relative quantification of abundance (Livak & Schmittgen, 2001). Real-time quantitative PCR was performed on a Mx 3000P platform (Stratagene, Santa Clara, CA).

2.5 Sequences Analysis

Homologous comparison was carried out using BLAST at <http://blast.ncbi.nlm.nih.gov/>; ORF and amino acid sequence were determined at <http://www.ncbi.nlm.nih.gov/gorf/orfig.cgi>; number and position of exons were predicted at <http://www.ncbi.nlm.nih.gov/IEB/Research/Ostell/Spidey/>; amino acid sequence composition, molecular weight of protein, isoelectric point and other physicochemical characteristic were predicted at <http://web.expasy.org/protparam/>. Sequences alignment was done by DNAMAN6.0 software; a neighbor-joining (NJ) phylogenetic tree of GmGPX1 with other GPX from plant was constructed using MEGA4 software.

3. Results and Analysis

3.1 GmGPX1 cDNA Cloning

The cDNA from *G. max* Xiaoliheidou infected with *H. glycines* was used as PCR template. PCR amplified a sequence with the approximate size of 700bp (Figure 1). Purified this fragment, followed by blue-white screening and restriction analysis with EcoR I. Examined the fragment on 1.2% (v/w) agarose gel electrophoresis. The result showed the target gene has been successfully connected with vector (Figure 2). The vector was extracted and sequenced using T7 promoter primer and SP6 promoter primer by ABI PRISM™ 3730XL DNA sequencer. The sequenced product was BLASTn in NCBI, which the cDNA fragment has 99% similarity with soybean sequence (XP_003532707.1), so was named as GmGPX1.

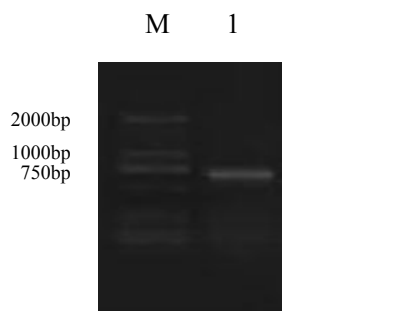


Figure 1. PCR product of GmGPX1

Note: M. DL2000 Marker; 1. PCR product from cDNA

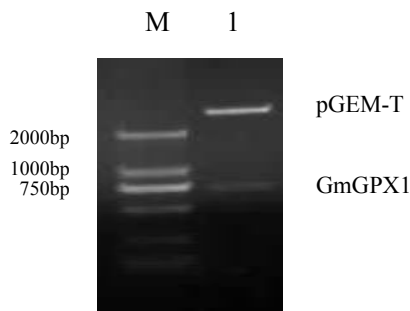


Figure 2. Analysis of recombinant plasmids digested with EcoR I

Note: M. DL2000 Marker; 1. PCR product of recombinant plasmids digested with EcoR I

3.2 GmGPX1 Bioinformatic Analysis

3.2.1 Amino Acid and Homologous Comparison

The cloned cDNA full length of GmGPX1 was 693bp, located at soybean genome 5 chromosome, flanked by a

5'-untranslated region of 7bp and a 3'-untranslated region of 185bp, including one poly A structure at 536bp, a full-length open reading frame of 501bp, encoding 166 amino acids. We attempted to obtain the genomic sequence of GmGPX1 and the result showed 6 exons using Spidey software. ExpASy software predicted the molecular weight was 18375.8Da, protein molecular formula $C_{829}H_{1293}N_{211}O_{252}S_4$, theory isoelectric point 6.59.

Homologous amino acid sequences of GmGPX1 were searched using BLASTp at <http://blast.ncbi.nlm.nih.gov/>. Use DNAMAN and MEGA4 to build amino acid alignment (Figure 3) and neighbor-joining (NJ) phylogenetic tree (Figure 4). The figure shows that amino acid sequence of GmGPX1 sharing the highest homology with *G max* (XP_003532707.1), which similarity is 99%; then followed by *Lotus japonicas* (AAP69867.1), *Medicago truncatula* (XP_003630520.1), *Nicotiana tabacum* (BAB16430.1), *Arachis hypogaea* (ACF74299.1). Lowest homology is with *Arabidopsis thaliana* (NP_564813.1), which similarity is 64%.

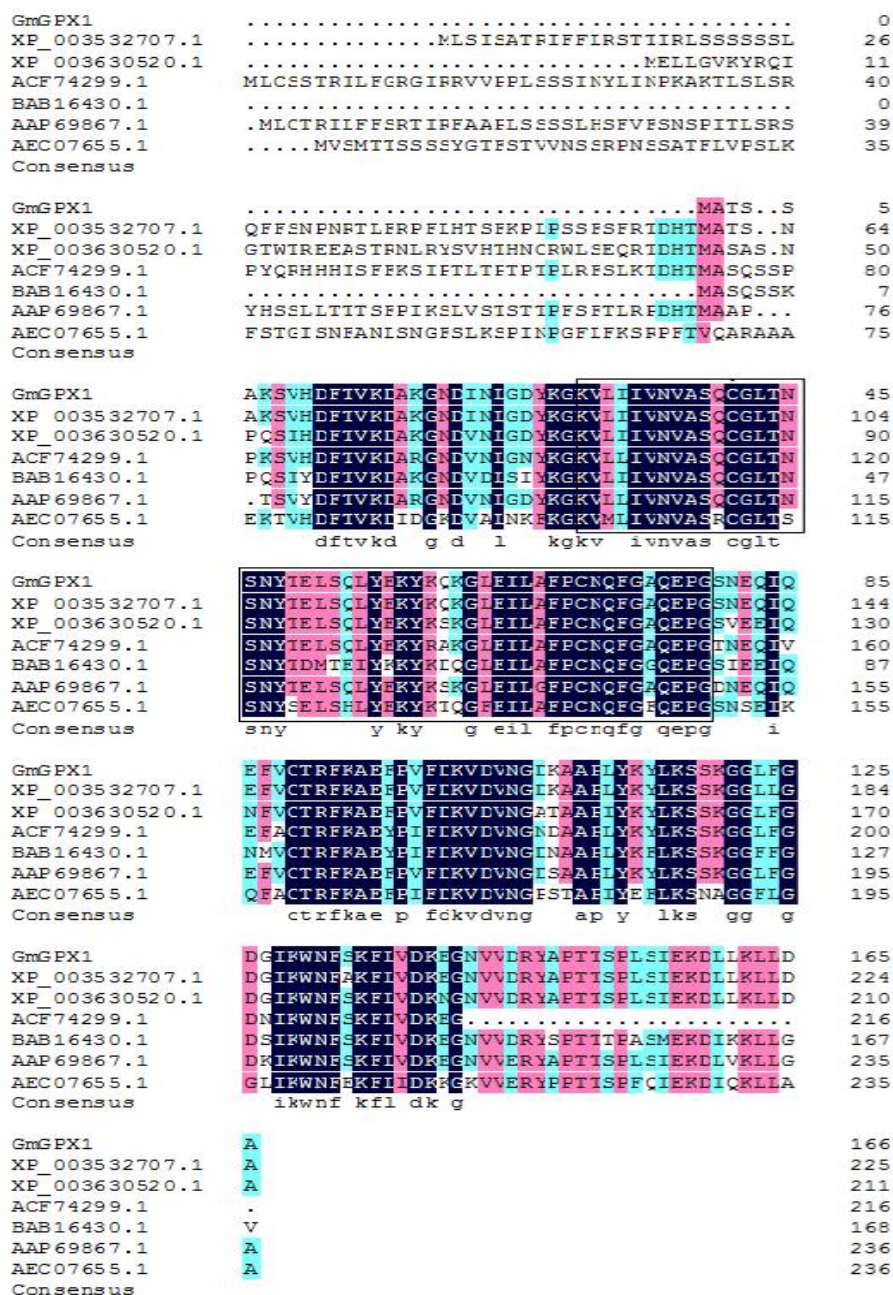


Figure 3. Amino acid alignment among GmGPX1, XP_003532707.1 (*Glycine max*), XP_003630520.1 (*Medicago truncatula*), ACF74299.1 (*Arachis hypogaea*), BAB16430.1 (*Nicotiana tabacum*), AAP69867.1 (*Lotus japonicus*), NP_564813.1 (*Arabidopsis thaliana*)

- Note: 1. Black, pink and blue boxes represent 100%, 75% and 50% similarity, respectively
 2. Catalytic triad of GmGPX1 were marked with triangles
 3. The motif of PHGPX from GmGPX1 and other plant GPX

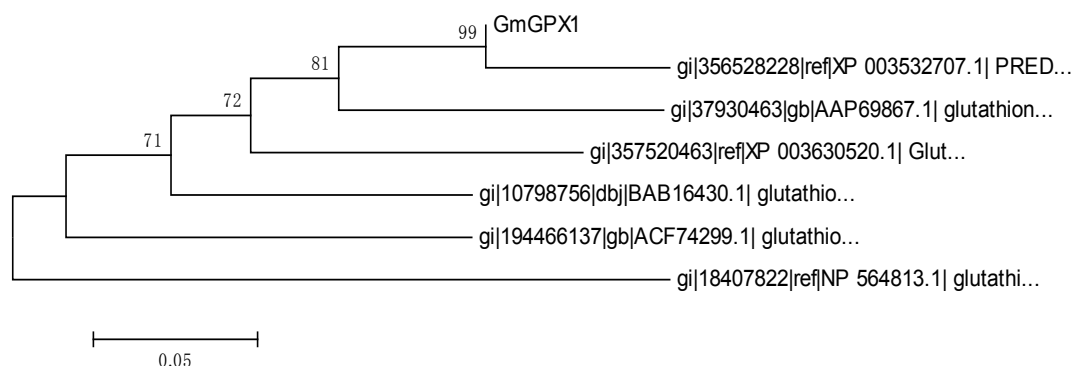


Figure 4. Phylogenetic tree of GmGPX1 with other GPXs from plant

Note: *Glycine max* (XP_003532707.1), *Lotus japonicus* (AAP69867.1), *Medicago truncatula* (XP_003630520.1), *Nicotiana tabacum* (BAB16430.1), *Arachis hypogaea* (ACF74299.1), *Arabidopsis thaliana* (NP_564813.1)

3.2.2 Structure Domain Analysis

The structure domain of gene GmGPX1 encoding protein was analyzed by CDD search in NCBI and SWISS MODEL (<http://swissmodel.expasy.org>). The result appears that GmGPX1 shares high homology with GSH peroxidase (at residues 9-117), belonged to thioredoxin _like superfamily (at residues 7-166), including three residues of catalytic triad, Cys⁴¹, Gln⁷⁶, Trp¹³⁰; six dimer interface sites (Fig.5). Multisequences comparison of GPX families proteins was done using software online <http://prosite.expasy.org/cgi-bin/prosite/>, obtained the motif of probable phospholipid hydroperoxide glutathione peroxidase (PHGPX) motif, the pattern as followed: K-x(1,2)-L-x(0,1)-I-V-N-V-A-S-x-C-G-[LM]-T-N-S-N-Y-T-[DE]-[LM]-[NST]-[EQ]-[IL]-Y-x-[KR]-Y-[KR]-[A DQS]-x-G-L-E-I-L-[AG]-F-P-C-N-Q-F-G-[ADG]-[EQ]-E-P-G.

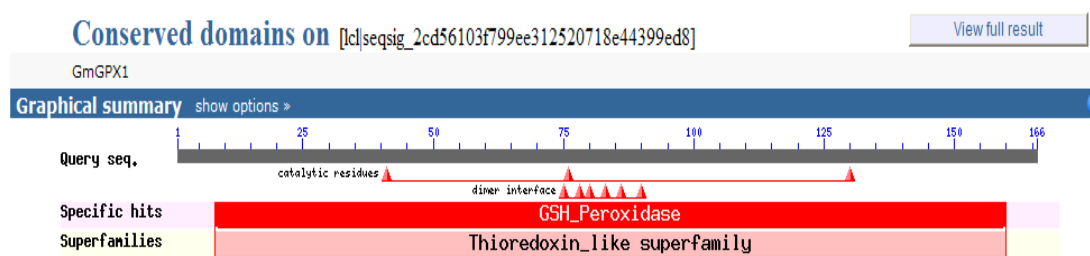


Figure 5. Conserved domain of GmGPX1 protein predicted by CDD software

3.2.3 GmGPX1 Protein Hydrophobicity Analysis

Hydrophobicity analysis of GmGPX1 protein was carried out using ProtScale program at <http://web.expasy.org/protscale/>. The analyzed content was Hphob. / Kyte & Doolittle, following Kyte & Doolittle method. The X axis is represented amino acid sequence position; Y axis shows amino acid scale, which includes positive value representing hydrophobicity; negative value representing hydrophilic; peptide with higher hydrophobicity located at protein interior, peptide with higher hydrophilic located at protein surface. As shown in Figure. 6, there is typical hydrophobic region near N-terminal of GmGPX1 protein, located at residues 30 – 40. However, residues 20, 60, 80, 105, 115, 145, 150 are belong to high hydrophilic region. It is can be inferred that GmGPX1 shares a strong hydrophobicity and is a soluble protein.

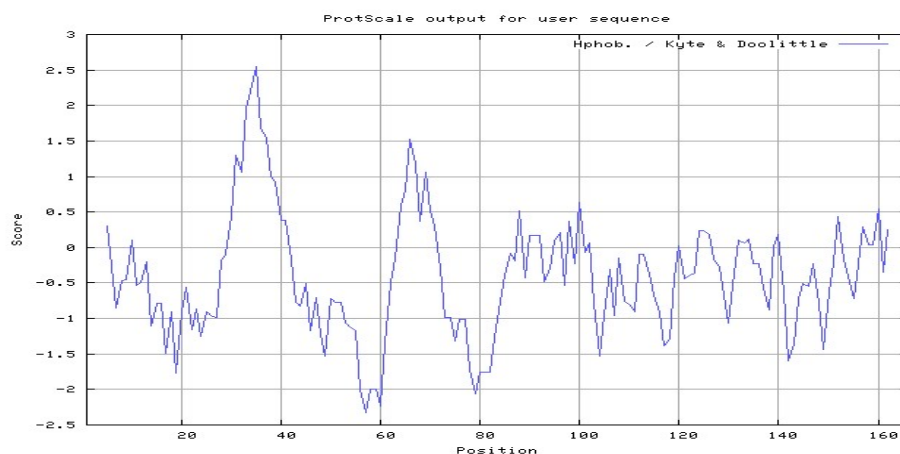


Figure 6. Hydropobicity profile of GmGPX1 protein

3.2.4 Active Sites and Subcellular Localization

The active sites of GmGPX1 amino acid sequence were predicted using software at

<https://www.predictprotein.org/>. The active sites include two N-glycosylation site, residues at 47 – 50 (NYTE) and 131 -134 (NFSK); three protein kinase C phosphorylation site, residues at 5 - 7 (SAK), 13 - 15 (TVK) and 118 -120 (SSK); two casein kinase II phosphorylation site, residues at 8 - 11 (SVHD) and 13 -16 (TVKD); four N-myristoylation site, residues at 19 - 24 (GNDINL), 42 -47 (GLTNSN), 121 -126 (GGLFGD), 127 - 132 (GIKWNF); glutathione peroxidases active site, residues at 29 - 44 (GKVLIIIVNASQCGLT); glutathione peroxidases signature 2, residues at 66 -73 (LAFPCNQF). The subcellular localization of GmGXH1 was predicted to be at chloroplast or mitochondria in plants by using LOCKey method, with 83% reliability. The protein of GmGXH1 is non-secreted and non-nuclei protein.

3.3 Expression Analysis of GmGPX1 in Root Tips

To evaluate the expression changes of GmGPX1 in soybean root tissues before and after *H. glycines* infection, we extracted RNAs from soybean root tissues with infection and those uninfected at different intervals as templates for real-time PCR. As shown in Figure 7 and 8, the main parameter analysis in real-time PCR shows that the amplification plots of the reference gene actin11 and target gene GmGPX1 represent standard S pattern; the amplification turned into index growth at 19 cycles; the peak of dissociation curve is single. Those indicate that the extracted samples with high quality, specific primers, there is no primer dimer or other non-specific products, reference gene and target gene share similar T_m value.

The mRNA expression level of GmGPX1 were up-regulated after infection from 12h to 72h, with approximately 1.30, 1.44, 1.38, 1.47 and 1.07-fold compared to uninfected controls at different intervals, individually. The up-regulated expression was more apparent from 12h to 48h, and then a slight declined (Figure 9). The entire period represented a stable up-regulated level. This result indicates that GmGPX1 is induced to be expressed after *H.glycines* infection.

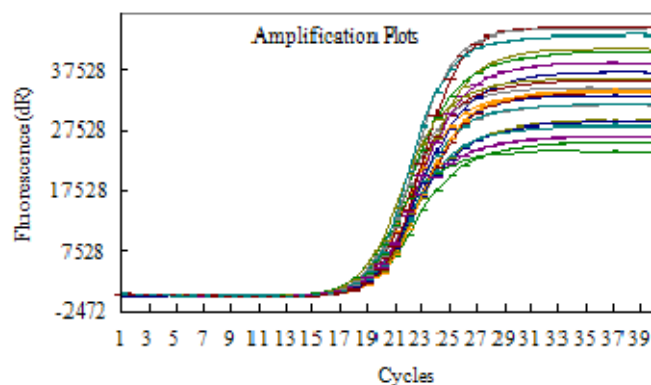


Figure 7. The amplification plots of the reference gene actin11 and target gene GmGPX1

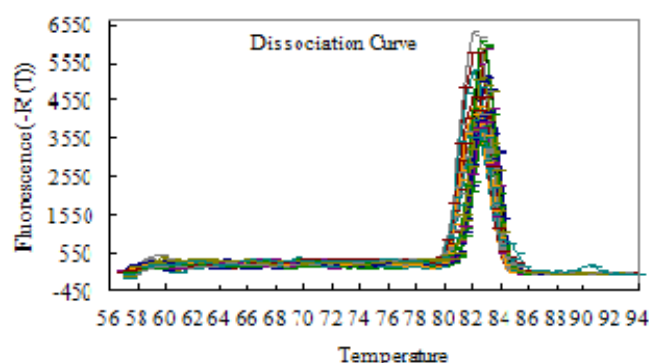


Figure 8. The dissociation curve of the reference gene actin11 and target gene GmGPX1

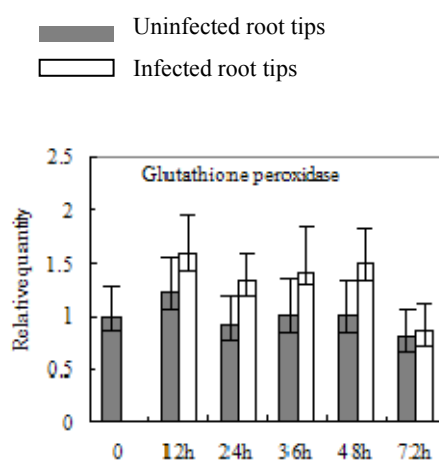


Figure 9. Relative quantity expression of GmGPX1 during 0-72h under inoculation and non-inoculation

3. Discussion

Glutathione peroxidases (GPXs) are a group of enzymes that are encoded by multiple genes. According to subcellular location, amino acid sequence, and conjuncted substrate, GPXs can be divided into several different isozymes. A family of seven genes encoding glutathione peroxidase has been identified in genome of *Arabidopsis thaliana*, named as *AtGPX1*- *AtGPX8*, respectively, which are located at the cytosol, chloroplasts, mitochondria, peroxisomes, and apoplast (Rodriguez et al., 2003). These genes are expressed ubiquitously and are regulated by abiotic stresses through diverse signaling pathways (Rodriguez et al., 2003). In mostly studies, the expression or activity of GPX is generally up-regulated in response to stress. However, there are some exceptions to this pattern. So this demonstrates that the function of these enzymes in plants is not completely the same. Two GPX isoforms increases in barley (*Hordeum vulgare*) under osmotic or methyl viologen-induced stress, a third GPX isoform (HVGPH3) is down-regulated under these conditions (Churin et al., 1999). Transcripts encoding a glutathione peroxidase (accession no. BE587404) are rapidly induced in response to low temperature stress, but other six *AtGPXs* haven't shown response to low temperature. This indicates that *AtGPXs* gene family is regulated by multiple signaling pathways at abiotic stresses (Rodriguez et al., 2002). Miao et al. (2006) finds that *AtGPX3* might play dual and distinctive roles in H_2O_2 homeostasis, acting as a general scavenger and specifically relaying the H_2O_2 signal as an oxidative signal transducer in ABA and drought stress signaling. In plant, GPXs gene family contain multiple members, some members serving as enzymes are involved in aerobic metabolism and defense response at different evolutionary period and environmental stresses, while other members play an important role in multiple signaling transduction pathways.

As predicted that gDNA of GmGPX1 included 6 exons and 5 introns, this is the same as seven members in *Arabidopsis thaliana* *AtGPX* families and the predicted soybean GPX gene (XM_003532659) in NCBI. Comparing mRNA sequences of GmGPX1 and XM_003532659 to their genome, individually, it could find that

Exon1 and Exon6 of GmGPX1 are longer than those of soybean GPX gene (XM_003532659), the length of other four exons are the same for both sequences. Analysis of structure and function of GmGPX1, it was showed that the GmGPX1 protein contains the motif of probable phospholipid hydroperoxide glutathione peroxidase (PHGPX), three conserved catalytic triads domain and three conserved Cys. GmGPX1 is belonged to non-secreted, non-nuclei protein by biological information analysis, the same as AtGPX1.

The soybean cyst nematode is an obligated sedentary endoparasite. Infective second-stage juveniles penetrate host roots and migrate intracellular where establishes feeding site. Over the course of movement and feeding site instruction, nematode causes cellular damage and stress, thereby induce the host defense response and wound-related responses. Plant cells produce superoxide (O_2^-) and its dismutation product, hydrogen peroxide (H_2O_2), both of which are toxic to nematode parasite. H_2O_2 is accumulated not only in the developing syncytium cell walls, but also in cells surrounding developing syncytia and cells which are neither in contact with the nematode nor with the syncytium, especially in cells neighboring hypersensitivity reaction. In many plants, oxidative burst is the first defense response to nematode invasion (Baker & Orland, 1995; Lamb & Dixon, 1997). Reactive oxygen species as signal molecular that control and regulate biological process, such as cell death, abiotic stress, pathogen reaction, and defense pathway (Desikan et al., 2001; Knight & Knight, 2001). In this study, the expression of GmGPX1 was increased gradually from 12h to 48h after inoculation. This indicates that after inoculation 12h, nematodes invasion causes the host cells damage or necrosis, resulting in cellular H_2O_2 increase, eventually the expression of GmGPX1 is up-regulated. Following the slow accumulation of H_2O_2 , the expression of GmGPX1 is gradually increased. This may be presumed that invasion and movement of juveniles during 12-48h inoculation could result in an increased production of H_2O_2 . There is a report that the more widespread accumulation of H_2O_2 is first apparent 12h after inoculation with *Meloidogyne incognita* and a heavy accumulation of H_2O_2 in the intercellular spaces in giant cells after 24h in resistant tomato roots (Melillo et al., 2006). At 72h after inoculation, it began to decrease to 1.07 times comparing with non-infection control. This may be that fewer plant tissues are damaged at 72h. The infective juveniles migrate in root vascular tissues and initiate the development of a feeding site after a suitable cell selected during approximately 2 days after inoculation. Another reason should be that over production of H_2O_2 is also harmful to plant cell in vivo, so a mechanism that equilibrium the H_2O_2 level is activated, resulting in the expression of GmGPX1 decreased. This study shows that GmGPX1 is playing an important role in plant resistance reaction and remove the oxidative damage induced by soybean cyst nematode infection.

References

- Baker, C. J., & Orland, E. W. (1995). Active oxygen in plant pathogenesis. *Annual Review Phytopathology*, 33, 299-321.
- Bowler, C., Montagu, M. V., & Inzé, D. (1992). Superoxide dismutases and stress tolerance. *Annual Review of Plant Physiology and Plant Molecular Biology*, 43, 83-116.
- Cho, M. J., & Lee, S. Y. (2002). A Chinese cabbage cDNA with high sequence identity to phospholipid hydroperoxide glutathione peroxidases encodes a novel isoform of thioredoxin-dependent peroxidase. *Journal of Biological Chemistry*, 277, 12572-12578.
- Churin, Y., Schilling, S., & Borner, T. (1999). A gene family encoding glutathione peroxidases homologues in *Hordeum vulgare* (barley). *FEBS Letters*, 459, 33-38.
- Criqui, M. C., Jamet, E., Parmentier, Y., Marbach, J., Durr, A., & Fleck, J. (1992). Isolation and characterization of a plant cDNA showing homology to animal glutathione peroxidases. *Plant Molecular Biology*, 18, 623-627.
- Depege, N., Drevet, J., & Boyer, N. (1998). Molecular cloning and characterization of tomato cDNAs encoding glutathione peroxidase-proteins. *European Journal of Biochemistry*, 253, 445-451.
- Desikan, R., Mackerness, S. A. H., Hancock, J. T., & Neill, S. J. (2001). Regulation of the *Arabidopsis* transcriptome by oxidative stress. *Plant Physiology*, 127, 159-172.
- Eshdat, Y., Holland, D., Faltin, Z., & Ben-Hayyim, G. (1997). Plant glutathione peroxidases. *Physiologia Plantarum*, 100, 234-240.
- Faltin, Z., Camoin, L., Ben-Hayyim, G., Perl, A., Beeor-Tzahar, T., Strosberg, A. D., Holland, D., & Eshdat, Y. (1998). Cysteine is the presumed catalytic residue of Citrus sinensis phospholipids hydroperoxide glutathione peroxidase over-expressed under salt stress. *Physiology Plant*, 104, 741-746.
- Golden, A. M., Epps, J. M., Riggs, R. D., Duclos, L. A., Fox, J. A., & Bernard, R. L. (1970). Terminology and

- identity of infraspecific forms of the soybean cyst nematode (*Heterodera glycines*). *Plant Disease Report*, 54, 544-546.
- Greene, R. (2002). Oxidative stress and acclimation mechanisms in plants. In: Someville C K, Meyerwitz E M, The *Arabidopsis* Book . *American Society of Plant Biologist*, Rockville, 1-19.
- Holland, D., Ben-Hayyim, G., Faltin, Z., Camoin, L., Strosberg, A. D., & Eshdat, Y. (1993). Molecular characterization of salt-stress-associated protein in citrus: protein and cDNA sequence homology to mammalian glutathione peroxidase. *Plant Molecular Biology*, 21, 923-927.
- Jung, B. G., Lee, K. O., Lee, S. S., Chi, Y. H., Jang, H. H., Kang, S. S., Lee, K., Lim, D., Yoon, S. C., Yun, D. J., Inoue, Y., Knight, H., & Knight, M. R. (2001). Abiotic stress signalling pathways: specificity and cross-talk. *Trends in Plant Science*, 6, 262-267.
- Lamb, C., & Dixon, R. A. (1997). The oxidative burst in plant disease resistance. *Annual Review Plant Physiology Molecular Biology*, 48, 251-75.
- Li, W. J., Feng, H., Fan, J. H., Zhang, R. Q., Zhao, N. M., & Liu, J. Y. (2000). Molecular cloning and expression of a phospholipid hydroperoxide glutathione peroxidase homolog in *Oryza sativa*. *Biochimica et Biophysica Acta*, 1493, 225-230.
- Liu, W. Z., Hong, Q. C., Liu, Y., & Duan, Y. X. (1994). Application of interorganismal genetics concept in grouping soybean genes resistant to soybean cyst nematode. *Soybean Science*, 1, 1-4. (in Chinese)
- Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_t}$ method. *Methods*, 25, 402-408.
- Melillo, M. T., Leonetti, P., Bongiovanni, M., Castagnone-Sereno, P., & Bleve-Zacheo, T. (2006). Modulation of reactive oxygen species activities and H_2O_2 accumulation during compatible and incompatible tomato-root-knot nematode interactions. *New Phytologist*, 170, 501-512.
- Miao, Y. C., Dong, L., Wang, P. C., Wang, X. C., Chen, J., Miao, C., & Song, C. P. (2006). An Arabidopsis glutathione peroxidase functions as both a redox transducer and a scavenger in abscisic acid and drought stress responses. *The Plant Cell*, 18, 2749-2766.
- Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, 7, 405-410.
- Mullineaux, P. M., Karpinski, S., Jiménez, A., Cleary, S. P., Robinson, C., & Creissen, G. P. (1998). Identification of cDNA encoding plastid-targeted glutathione peroxidase. *The Plant Journal*, 13, 375-379.
- Rodriguez Milla, M. A., Butler, E., Huete, A. R., Wilson, C. F., Anderson, O., & Gustafson, J. P. (2002). EST-based gene expression analysis under aluminum stress in rye (*Secale cereale* L.). *Plant Physiology*, 130, 1706-1716.
- Rodriguez Milla, M. A., Maurer, A., Huete, A. R., & Gustafson, J. P. (2003). Glutathione peroxidase genes in *Arabidopsis* are ubiquitous and regulated by abiotic stresses through diverse signaling pathways. *Plant Journal*, 36, 602-615.
- Roeckel-Drevet, P., Gagne, G., De Labrouhe, T. D., Dufaure, J. P., Nicolas, P., & Drevet, J. R. (1998). Molecular characterization organ distribution and stress-mediated induction of two glutathione peroxidase-encoding mRNAs in sunflower (*Helianthus annuus*). *Physiologia Plantarum*, 103, 385-394.
- Sugimota, M., Furui, S., & Suzuki, Y. (1997). Molecular cloning and characterization of a cDNA encoding putative phospholipid hydroperoxide glutathione peroxidase from spinach. *Bioscience Biotechnology Biochemistry*, 61, 1379-1381.
- Sugimoto, M., & Sakamoto, W. (1997). Putative phospholipid hydroperoxide glutathione peroxidase gene from *Arabidopsis thaliana* induced by oxidative stress. *Genes & Genetic Systems*, 72, 311-306.
- Ursini, F., Maiorino, M., & Brigelius-Flohé, R. (1995). Diversity of glutathione peroxidases. *Methods in Enzymology*, 252, 38-53.
- Ursini, F., Maiorino, M., & Gregolin, C. (1985). The selenoenzyme phospholipid hydroperoxide glutathione peroxidase. *Biochemistry Biophysics Acta*, 839, 62-70.