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Expression Analysis of Soybean (*Glycine max*) Glyoxalase Genes Indicates their Biotic Stress Specific Alteration

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Research Article

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Abbreviations: MG: Methylglyoxal; GLYI: Glyoxalase I; GLYII: Glyoxalase II; GSH: Reduced Glutathione; DPI: Day post infection; HPI: Hour Post Infection

ABSTRACT

Detoxification of a highly cytotoxic metabolite methylglyoxal (MG) is carried out through the sequential action of two glyoxalase enzymes, glyoxalase I (GLYI) and glyoxalase II (GLYII). Physiological and metabolic role of glyoxalase proteins have been comprehensively investigated in various plant and animal species. Previously genome-wide analysis of soybean indicates the presence of forty-one GLYI and twenty-three GLYII proteins encoded by twenty-four and twelve genes, respectively. Expression of *GmGLYI* and *GmGLYII* genes was found to be modulated by different developmental stimuli and abiotic stresses. In the present study, expression of soybean glyoxalase genes has been analyzed at five distinct developmental stages and eight tissues as well as in response to various biotic stresses using publicly available microarray data. *GmGLYI*-7, *GmGLYI*-10/21, *GmGLYII*-1/8, and *GmGLYII*-6 showed high level of expression at all the developmental stages and tissues with few exceptions; while *GmGLYI*-6/9, *GmGLYI*-20, *GmGLYII*-6 and *GmGLYII*-10 showed most biotic stress specific modulation. However, the observed alteration is highly complex that depends on the duration and type of infected organisms.

INTRODUCTION

Glyoxalase system is an evolutionary highly conserved pathway, consists of two thiol-dependent enzymes; glyoxalase I (GLYI) and glyoxalase II (GLYII). Glyoxalase pathway detoxifies a cytotoxic metabolite, methylglyoxal (MG) to its non-toxic form, D-lactate with the help of reduced glutathione (GSH). Free MG and GSH forms non-enzymatic hemithioacetal adduct, which isomerized into S-D-lactoyl-glutathione (SLG) by the action of GLYI. GLYII further hydrolyzes SLG into D-lactate and regenerates GSH [1]. Glyoxalase pathway was first discovered more than hundred years back (1913) in two different organisms, rabbit and dog [2]. Later, glyoxalase genes/proteins have been reported from a wide range of species including *Escherichia coli,* yeast, fungus, plants, mammals, *Homo sapiens* and found to be highly conserved [2,3].

Chemically methylglyoxal (MG) is a reactive α, β-dicarbonyl aldehyde. It can disrupts cellular functions by forming adduct with cellular macromolecules such as DNA, RNA and proteins; and forms advance glycation end products (AGEs) [2]. Intracellular MG level was found to be increased under various adverse conditions in all living organisms and highly correlated with stress induced pathogenesis. Increased level of MG found to be connected with various diseases; diabetes, cardiovascular diseases, cancer, nephropathy, retinopathy, neuropathy [4]. Due to reduced GLYI activity, MG gets accumulated in diabetes patients, that promotes endothelial cells inflammation, dysfunction and vascular damage [5]. Enhanced accumulation of MG has been reported in various plant species under stresses that lead to stress induced senescence [6,7]. Accumulation of SLG is also toxic to living system as it could inhibit DNA synthesis [8]. Thus glyoxalase system has played a vital role to combat MG accumulation under adverse conditions. There are numerous studies in plants that have shown the ability of glyoxalase enzymes in conferring tolerance against multiple stresses by maintaining intracellular MG level [6,9,10]. Moreover, glyoxalase enzymes have been reported to be involved in different other important cellular functions, such as cell division and proliferation, microtubule assembly and protection against oxoaldehydes toxicity [11]. Thus, glyoxalase pathway has been considered as “marker for cell growth and division”. Over-expression

of *GLYI* and/or *GLYII* genes in transgenic plants enhanced tolerance against multiple abiotic stresses [6,9,12,13]. For this, both accumulation of MG and glyoxalase gene expression/enzyme activity are considered as potential plant stress biomarker [14].

Glyoxalase genes/proteins have been extensively studied in various micro-organisms and mammals, but limited information available about plant glyoxalases. The first genome wide analysis of glyoxalase genes have been reported from two model plants-*Arabidopsis thaliana* and *Oryza sativa* [10]. Authors predicted the presence of 11 potential *GLYI* and 3 *GLYII* genes in rice; and 11 putative *GLYI* and 5 *GLYII* genes in Arabidopsis [10]. Expression analysis of *AtGLYI*/II and *OsGLYI*/II genes showed their developmental and tissue specific regulation. Further, qRT-PCR analysis performed in two contrasting rice genotypes, i.e., IR64 and Pokkali identified two highly stress inducible members in rice-*OsGLYI-6* and *OsGLYI-11*. In soybean, the genome wide analysis of glyoxalase genes has been reported recently [15]. Comprehensive soybean genome analysis identified 41 putative GLYI and 23 putative GLYII proteins encoded by 24 and 12 genes, respectively. Expression profiling of these genes was carried out at different tissues and developmental stages as well as under two major abiotic stresses- salinity and drought using publicly available RNAseq data [15]. Present study revealed the biotic stress inducible expression of *GmGLYI* and *GmGLYII* genes.

MATERIALS AND METHODS

Identification of Corresponding Probeset for *GmGLYI* and *GmGLYII* Genes

To retrieve the expression data of soybean glyoxalase genes in various developmental stages, tissues and biotic stresses from Genevestigator, the corresponding probesets for all *GmGLYI* and *GmGLYII* genes are required.

Probesets for were identified using NetAffx Analysis Center (http://www.affymetrix.com/analysis/index.affx?navMode=cat 530006&aId=netaffxNav) online Probe Match tool using respective CDS sequences as query. Probe with the highest value was considered in case of genes with more than one probeset; while more than one gene showing same probeset was considered as same transcriptional profile.

Microarray-Based Expression Analysis of Soybean Glyoxalase Genes

Normalized and curated expression data at various developmental stages, tissues and biotic stresses were retrieved from publicly available Affymetrix soybean genome array using Genevestigator (https://www.genevestigator.com/gv/plant.jsp) [16,17]. Genevestigator processed a large set of manually curated and quality-controlled microarrays and RNAseq data from a large variety of samples, tissues and conditions. Various developmental stages of soybean such as germination (61 samples), seed growth (504 samples), flowering (3 samples), fruit formation (45 samples), and bean development (89 samples) are analyzed. Similarly, expression was checked at eight tissues such as root (12 samples), seedlings (2 samples), hypocotyl (14 samples), leaf (3 samples), inflorescence (2 samples), seed (39 samples), embryo (8 samples), and endosperm (8 samples). Detailed information about the corresponding experiments has been provided as **Additional File-1.** To analyze the expression in response to different microorganisms viz. *Bradyrhizobium japonicum*, *Phakopsora pachyrhizi*, *Heterodera glycines*, *Spodoptera litura*, and *Rhizophagus irregularis*, the relative signal ratio values were retrieved using Genevestigator with default parameters (using experiments with id: GM-00028, GM-00033, GM-00036, GM-00046, and GM-00048) and the log2 transformed fold change values were used to generate heap map. Detailed information of all these experiments has been included as **Additional File-2.** All these experiments were replicated atleast thrice and fold change values were calculated by comparing with the respective control with same number of replicates. Heat maps with hierarchical clustering were generated using Multi Experiment Viewer software (http://www.tm4.org/mev/) with Manhattan distance metric method [18].

RESULTS AND DISCUSSION

Analysis of *GmGLYI* and *GmGLYII* Genes at Various Developmental Stages and Tissues

Plant development is a highly complicated process that needs integrated action of several pathways and genes [19]. Different stages of development represent different sub-sets of gene population. Soybean has five distinct developmental stages-germination, shoot growth, flowering, fruit formation and bean development. Expression of all *GmGLYI* and *GmGLYII* genes were analyzed using genevestigator (https://genevestigator.com/gv/doc/intro_plant.jsp) based micro-array data and plotted in a scatter diagram with standard deviation. Some genes share same probe set which were considered as same transcript profile; while some genes did not have a respective probe, and thus no gene expression was available. Among the total of 24 *GmGLYI* and 12 *GmGLYII* genes; expression data was found for 19 *GmGLYI* and 8 *GmGLYII* genes. This analysis revealed that *GmGLYI*-7 and *GmGLYI*-10/21 maintained high transcript abundance at all the developmental stages except flowering, where *GmGLYI*-20 showed maximum expression **(Figure 1A).** Similarly, *GmGLYII*-1/8 and *GmGLYII*-6 showed higher level of expression at all the developmental stages except flowering, where *GmGLYII*-4/5 showed highest abundance **(Figure 1B).** Further, expression of all *GmGLYI* and *GmGLYII* genes were analyzed at eight distinct soybean tissues, such as- root, seedling, hypocotyl, leaf, inflorescence, seed, embryo, and endosperm. *GmGLYI*-7 and *GmGLYI*-10/21 maintained high transcript abundance at all the analyzed tissues except leaf, where *GmGLYI*-23 showed the highest peak **(Figure 1C).** In case of *GmGLYII* family members, *GmGLYII*-1/8 showed similar pattern with maximum level of expression at all the analyzed tissues except root, where *GmGLYII*-6 showed the highest expression **(Figure 1D).** This indicates the development and tissue specific alteration of *GmGLYI* and *GmGLYII* transcripts. Genes

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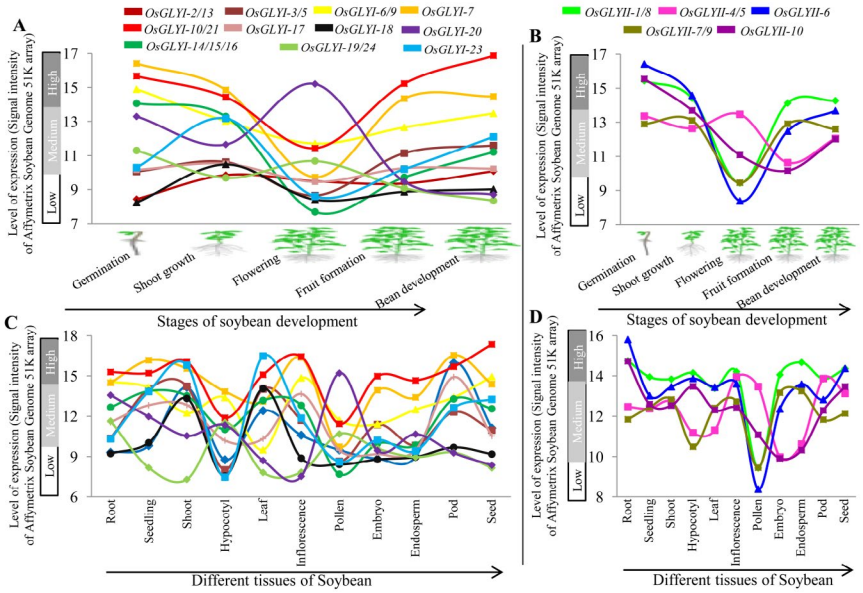
with very high level of expression at all these stages and tissues indicates the constitutive expression due to their imperative role in regular cellular/metabolic processes.



Expression of *GmGLYI* (A) and *GmGLYII* (B) genes was analyzed at five distinct developmental stages of soybean; germination, shoot growth, flowering, fruit formation and bean development as shown at the X-axis with corresponding figure. The mean signal intensity values from Soybean Affymetrix genome 51 K array were retrieved from genevestigator (https://genevestigator.com/gv/doc/intro_plant.jsp) and plotted in the scatter diagram with standard deviation. Color bar at the top of the figure represents the corresponding gene name. Expression of *GmGLYI* (C) and *GmGLYII* (D) genes was further analyzed at eight different tissues of soybean; root, seedling, hypocotyl, leaf, inflorescence, seed, embryo, and endosperm. The normalized curated expression data was collected from genevestigator and plotted in a scatter diagram. Scale at the Y-axis indicates the level of expression values and abundance of transcripts.

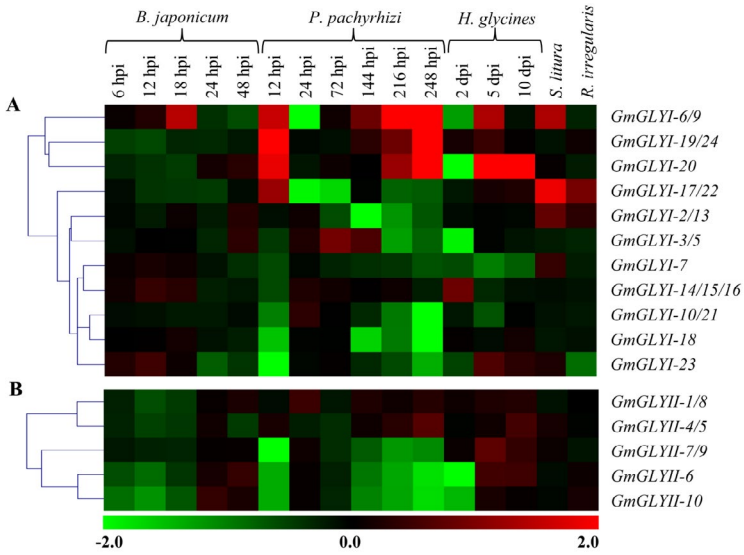
Figure 1. Expression Profiling Of *Gmglyi* and *Gmglyii* Genes Under Different Developmental Stages and Tissues Of Soybean.

Expression Analysis of *GmGLYI* and *GmGLYII* Genes in Response to Various Biotic Stresses

Abiotic stress specific response of *GmGLYI* and *GmGLYII* genes was analyzed previously [15]. Apart from plant development, tissue specificity and abiotic stress response, expression of glyoxalase genes have been reported to be modulated by biotic stress too [14]. Expression of rice *GLYI* gene was found to be down-regulated in response to *Xanthomonas oryzae* pv. oryzae or *Pyricularia grisea* infection [20]. Proteomic comparison of *Aspergillus flavus*-resistant and -susceptible maize kernel embryo showed drastic up-regulation of GLYI protein in the resistant embryos [21]. Fungal infection significantly enhances intercellular methylglyoxal (MG) level in susceptible genotypes and thus high GLYI activity is crucial. Similarly, induction of GLYI activity was observed in rice and *Brassica* in response to brown planthopper (*Nilaparvata lugens*) and *Sclerotinia sclerotiorum* infection, respectively [21,22]. All these studies convincingly indicate the role/involvement of glyoxalase genes/proteins in biotic stress modulation pathways.

To gain deep insights into the regulation of glyoxalase genes under biotic stress, the expression profiles of all *GmGLYI* and *GmGLYII* genes were analyzed in response to various types of microorganisms using publicly available microarray data. Expression of *GmGLYI* and *GmGLYII* genes were checked with *Bradyrhizobium japonicum*, *Phakopsora pachyrhizi*, *Heterodera glycines*, *Spodoptera litura*, and *Rhizophagus irregularis* infection at different time interval. *B. japonicum* is a legume-root nodulating nitrogen-fixing species which improves crop yields. *P. pachyrhizi* causes asian soybean rust disease that creates prominent dark to reddish-brown lesions. *H. glycines* are devastating soybean pest that infects mainly in the roots. It causes suppression of growth, root and stem necrosis and leaf chlorosis, and ultimately loss in the total yield. *S. litura* is a noctuid moth which is considered a major pest of many crops including soybean. *R. irregularis* is an arbuscular mycorrhizal fungus that is commonly used in scientific studies to check the effects of arbuscular mycorrhizal fungi on plant growth and development. In response to *B. japonicum* infection (6 to 48 hpi) *GmGLYI* genes showed scattered pattern up/down-regulation of expression; while all *GmGLYII* genes showed down-regulation till 18 hpi and up-regulation on/after 24 hpi **(Figure 2A and 2B).** In response to *P. pachyrhizi* infection for 12 to 248 hpi, the level of modulation was the highest **(Figure 2A and 2B).** *GmGLYI-6/9, GmGLYI-19/24* and *GmGLYI-20* showed high up-regulation at the early stage (12 hpi) as well as late stage (216 and 248 hpi) of infection; while *GmGLYI-10/21, GmGLYI-18* and *GmGLYI-23* showed strong down-regulation **(Figure 2A).** In case of *GmGLYII*, *GmGLYII-7/9, GmGLYII-6* and *GmGLYII-10* high level of down-regulation at the early stage (12 hpi) as well as late stage (216 and 248 hpi) of infection, and the rest members showed medium to low level of alterations **(Figure 2B).** Similarly, in response to *H. glycines* (2-10 dpi), most of the *GmGLYI* transcripts level were found to be down-regulated, except *GmGLYI-20* that showed strong induction at later stages (5 and 10 dpi) **(Figure 2A).** However, most of the *GmGLYII* transcripts level were found to be up-regulated, while *GmGLYII-6* and *GmGLYII-10* showed strong reduction at early stage (2 dpi) **(Figure 2B).** In response to *S. litura* and *R. irregularis*, *GmGLYI-17/22* and *GmGLYI-2/13* showed medium to high level of up-regulation, while rest of the *GmGLYI* and *GmGLYII* genes

showed minimum alterations **(Figure 2A and 2B).** This indicates the diversified effect of different pathogenic and symbiotic micro-organisms on the expression of *GmGLYI* and *GmGLYII* transcripts.



Expression of all available *GmGLYI* (A) and *GmGLYII* (B) genes were analyzed in response to *B. japonicum*, *P. pachyrhizi*, *H. glycines*, *S. litura*, and *R. irregularis* at different point of post infection (pi). Fold change expression data (log_2 scale) as compared to corresponding mock sample, was obtained from genevestigator (https://genevestigator.com/gv/doc/intro_plant.jsp). Generation of heatmap and hierarchical clustering was done using MeV software package. Colour scale below the heat map indicates the level of expression; red indicates up-regulation while green indicates down-regulation of expression.

Figure 2. Expression Analyses of Soybean Glyoxalase Genes in Response to Different Pathogenic and Symbiotic Microorganism Infection.

CONCLUSION

Taken together, this study reveals the strong correlation between biotic stress and alteration of glyoxalase transcripts. As generation of MG is a common phenomenon of stressed plant, transcript of glyoxalase genes (MG metabolizing pathway) is highly modulated by all stresses. Accumulation of MG has been reported previously in maize susceptible plants in response to *Aspergillus flavus* infection [21]. Thus, the observed information will encourage researcher to carry out further functional characterization of glyoxalase genes from various plant species in response to biotic stress.

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