

## Isolation and Characterization of a Theta Glutathione S-transferase Gene from *Panax ginseng* Meyer

Yu-Jin Kim<sup>1</sup>, Ok Ran Lee<sup>1</sup>, Sungyoung Lee<sup>2</sup>, Kyung-Tack Kim<sup>3</sup>, and Deok-Chun Yang<sup>1\*</sup>

<sup>1</sup>Department of Oriental Medicinal Materials and Processing, College of Life Science, Kyung Hee University, Suwon 449-701, Korea

<sup>2</sup>Department of Computer Engineering, Kyung Hee University, Yongin 449-701, Korea

<sup>3</sup>Processing Technology Research Group, Korea Food Research Institute, Seongnam 463-746, Korea

Plants have versatile detoxification systems to encounter the phytotoxicity of the wide range of natural and synthetic compounds present in the environment. Glutathione S-transferase (GST) is an enzyme that detoxifies natural and exogenous toxic compounds by conjugation with glutathione (GSH). Recently, several roles of GST giving stress tolerance in plants have demonstrated, but little is known about the role of ginseng GSTs. Therefore, this work aimed to provide further information on the *GST* gene present in *Panax ginseng* genome as well as its expression and function. A GST cDNA (*PgGST*) was isolated from *P. ginseng* cDNA library, and it showed the amino acid sequence similarity with theta type of GSTs. *PgGST* in ginseng plant was induced by exposure to metals, plant hormone, heavy metals, and high light irradiance. To improve the resistance against environmental stresses, full-length cDNA of *PgGST* was introduced into *Nicotiana tabacum*. Overexpression of *PgGST* led to twofold increase in GST-specific activity compared to the non-transgenic plants, and the GST overexpressed plant showed resistance against herbicide phosphinothricin. The results suggested that the *PgGST* isolated from ginseng might have a role in the protection mechanism against toxic materials such as heavy metals and herbicides.

**Keywords:** *Panax ginseng*, Environmental stress, Glutathione-S-transferase, Phosphinothricin

### INTRODUCTION

Glutathione S-transferases (EC 2.5.1.18) are ubiquitous family of multifunctional proteins that catalyze the conjugation of the tripeptide glutathione (GSH) to large variety of hydrophobic compounds containing electrophilic centers. This reaction yields a GSH conjugate, which is often inactive, water-soluble, and is usually less toxic than the parent compound [1]. In plants, these conjugates are sequestered into the vacuole where they are further processed and detoxified [2]. Most studies are done using mammalian glutathione S-transferases

(GSTs), which are categorized into five classes, alpha, mu, pi, theta, and sigma, based on the amino acid identity, immuno-cross reactivity, and substrate specificity [3]. The theta class is the largest class of GST isozymes with more than 50 members and finds in the widest range of species including bacteria, insects, plants, fish, and mammals. Many GSTs have been purified from animals over the last 30 yr and classified [4], and several plant GSTs have been characterized [2].

GSTs appear to play an important role in protecting

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\*Corresponding author

E-mail: dcyang@khu.ac.kr

Tel: +82-31-201-2100, Fax: +82-31-205-2688

plants as well as animals against oxidative damage [5]. Plants often encounter various kinds of environmental stress such as pathogen attack, wounding, heavy metals, and extreme high or low temperatures. In all these processes, a common result is the production of reactive oxygen species (ROS), which would then generate membrane lipid peroxidation and the formation of highly cytotoxic products. GSTs can remove membrane lipid peroxides as well as products of oxidative DNA degradation by conjugating them with GSH [6]. Expression of GSTs in plants is highly responsive to biotic and abiotic stresses and to a wide variety of stress-associated chemicals [7].

Despite the presence of GST genes in various plants, up to now there was no report of any type of GST in ginseng plant. As most of crop plants have to endure various stresses during their life cycle, perennial ginseng plant also faced these kinds of difficulties. Because ginseng is a shade-loving perennial herb, which is cultivated 4 to 5 years, oxidative stress may seriously affect its growth. Ginseng (*Panax ginseng* Meyer) is one of the most widely used herbal medicines in the Orient. Despite the medicinal importance of ginseng, few antioxidant genes in ginseng plant have studied in molecular levels [8]. The present study aims to provide further information about a cloning of *GST* gene present in *P. ginseng* genome using available ginseng expressed sequence tag (EST) library [9], and as well as its expression and functional characteristics against various environmental stresses.

## MATERIALS AND METHODS

### Plant materials and growth conditions

Ginseng (*P. ginseng*) was collected from the Ginseng Genetic Resource Bank in Korea. Three-week-old ginseng plants were used for gene expression analysis using reverse transcriptase polymerase chain reaction (RT-PCR). Ginseng seeds were *in vitro* germinated in Murashige and Skoog (MS) media (Duchefa Biochemie, Haarlem, the Netherlands) with 3% sugar at 25°C under a 16-h photoperiod.

### Glutathione S-transferase gene sequence and its sequence analysis

The cDNA library [9] constructed previously was utilized for searching of GST gene in ginseng. Homologous sequences of GST EST were searched against the GenBank databases using a BLASTX algorithm. A pTriplEx phagemid for full-length of GST cDNA was excised from the  $\lambda$ pTriplEx2 and used as templates for

sequence analysis. Nucleotide and amino acid sequence analyses were performed using DNASIS program (Hitachi, Tokyo, Japan). These deduced amino acid sequences were searched for homologous proteins in the databases using BLAST network services at the National Center for Biotechnology Information. We used ClustalX with default gap penalties to perform multiple alignment of GST isolated in ginseng and previously registered in other species. The protein property was estimated using ProtParam [10].

### Environmental stresses treatment

To investigate the response of the *PgGST* against various stresses, ginseng plantlets were treated with abiotic stimuli in about 3 wk after zygotic embryo germination as described previously [11]. For chemical stress or plant hormone treatments, the plantlets were placed for various periods in MS medium containing indicated concentrations of chemicals; 100 mM NaCl, 10 mM H<sub>2</sub>O<sub>2</sub>, 20% sucrose, 11% mannitol, 500  $\mu$ M CuSO<sub>4</sub>, 500  $\mu$ M CdSO<sub>4</sub> or 1 mM salicylic acid (SA), 0.2 mM jasmonic acid (JA), 0.1 mM abscisic acid (ABA). Chilling stress was applied by exposing the plantlets at 4°C. For the UV treatment, the plantlets were irradiated under UVC lamps at 1.35  $\mu$ E m<sup>-2</sup>s<sup>-1</sup> (below 280 nm). To monitor the effect of light stress, plants were kept in the white light with intensity of about 500  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>. In all cases, stress treatments were carried out on the MS media and 10 plantlets were treated with each stress for 1, 4, 8, 24, 48, or 72 h. The stressed plant materials from all completed treatments were immediately frozen in liquid nitrogen and stored at -70°C until required.

### Semi-quantitative reverse transcriptase polymerase chain reaction analysis

Total RNA was extracted from different parts of whole plant tissues (leaves, stems, and roots) and seedlings of *P. ginseng* using RNeasy mini kit (Qiagen, Valencia, CA, USA). For RT-PCR, 200 ng of total RNA was used as a template for reverse transcription using oligo (dT)<sub>15</sub> primer (0.2 mM) and AMV Reverse Transcriptase (10 U/ $\mu$ L; Intron Biotechnology Inc., Seongnam, Korea) according to the manufacturer's instructions. The PCR reaction was then performed using a 1  $\mu$ L aliquot of the first strand cDNA in a final volume of 20  $\mu$ L containing 5 pmol of specific primers for coding region of *PgGST* (forward, 5'-CGT TCT CAT CTT CTG CAA GGT C-3'; reverse, 5'-GCT GAG ATC TGC TAT GGA TGG T-3'). As a control, the primers specific to *P. ginseng* actin gene were used (forward, 5'-CGT GAT CTT ACA GAT AGC

TTG ATG A-3'; reverse, 5'-AGA GAA GCT AAG ATT GAT CCT CC-3'). PCR was carried out using 2X *Taq* Premix (SolGent Co., Daejeon, Korea) in a thermal cycle programmed as follows: an initial denaturation for 5 min at 96°C, 30 amplification cycles, (20 s at 95°C [denaturation], 45 s at 60°C [annealing], and 1 min at 72°C [polymerization]), followed by a final elongation for 10 min at 72°C. The actin gene was PCR-amplified in the same PCR conditions as *PgGST* with the same amplification cycles (30 cycles), and was used as an internal control to normalize each samples. Seven microliters of the reaction mixture was analyzed on a 1% (W/V) agarose gel containing ethidium bromide in 1X TAE buffer and then photographed for the expression analysis.

### Vector construction and plant transformation

The entire coding region of GST gene was amplified from *P. ginseng* cDNA using PCR with specific primers embedding *Nco*I and *Bam*HI restriction sites. The constructed PCR product was ligated into the same restriction sites between double 35S cauliflower mosaic virus (CaMV) promoter followed by alfalfa mosaic virus enhancer and the nopaline synthase (NOS) terminator in 524Xba cassette vector (NRC Plant Biotechnology Institute, Saskatoon, Canada). The 524Xba recombinant vector carrying the *PgGST* was digested with *Xba*I and then the expression cassette was ligated into the same restriction sites within pRD400 (Canada). The construct was verified by automated DNA sequencing and then transformed into *Agrobacterium tumefaciens* strain LBA4404 according to the tri-parental mating method [12]. Leaf disk explants of *Nicotiana tabacum* cv. Xanthi were cocultured with *A. tumefaciens* LBA4404 containing pRD400 binary vector. Transgenic plants were selected on the basis of their ability to regenerate on the MS medium containing 2 mg/L benzylaminopurine, 0.2 mg/L 1-naphthaleneacetic acid (NAA), 100 mg/L kanamycin sulfate, and 500 mg/L cefotaxime. Upon transfer to MS medium containing 0.2 mg/L NAA, transgenic seedlings developed into plantlets.

### Analysis of transgenic plants

The presence of neomycin phosphotransferase II (*NPTII*) (forward, 5'-GAG GCT ATT CGG CTA TGC TG-3'; reverse, 5'-ATG CCA TAG CGG CGA GGG CTA-3'), *PgGST*, CaMV promoter (forward, 5'-ATT GTG CGT CCC TTA CGT CAG TGG-3'; reverse, 5'-ATC GTT GAA GAT GCC TCT GCC G-3'), and NOS terminator (forward, 5'-GCG GAG AAT TAA GGG AGT CAC G-3'; reverse, 5'-GGA TAC CGA GGG GAA

TTT ATG G-3') transgenes were analyzed by PCR from regenerated leaf tissue samples. For control, internal transcribed spacer primers (forward, 5'-TAC CGA TTG AAT GGT CCG-3'; reverse, 5'-ATA TGC TTA AAC TCA GCG GGT-3') were used for PCR analysis. In addition, Southern blot analysis was carried out to confirm the successful insertion of *PgGST* into genome of transgenic *N. tabacum* plants. Southern blotting involved digesting 20 µg of leaf genomic DNA with *Eco*RI and *Hind*III, then electrophoresing the digested products on a 1.3% agarose gel (50 V, overnight). DNA was transferred to a nylon membrane using a Turbo blotter apparatus (Schleicher and Schuell, Maidstone, UK). Labeling of the probes, hybridization, and detection was carried out using DIG High Prime DNA Labeling and Detection Starter Kit following the instruction given by the manufacturer (Roche, Mannheim, Germany).

### Analysis of transcripts of transgenic plant against chemical stresses

Differential transcriptional level against chemical stresses was carried out by RT-PCR using gene-specific primers. As a loading control, actin gene was amplified using actin specific primers (forward, 5'-GAA CCG GAA ATT GTT CGA GA-3'; reverse, 5'-GCA GAT TCC ATT CCG ATC AT-3'). For stress treatment, we sprayed on tobacco seedlings with distilled water containing each of 100 µM CdSO<sub>4</sub>, CuSO<sub>4</sub>, ZnSO<sub>4</sub> or phosphinothricin (PPT), and 1 mM 2,4-D or SA. After 4, 12, 24, and 48 h, respectively, treated leaves were immediately frozen in liquid nitrogen and stored at -70°C. Total RNA was isolated using RNeasy mini kit (Qiagen, Valencia, CA, USA), and RT-PCR was conducted by the method described above.

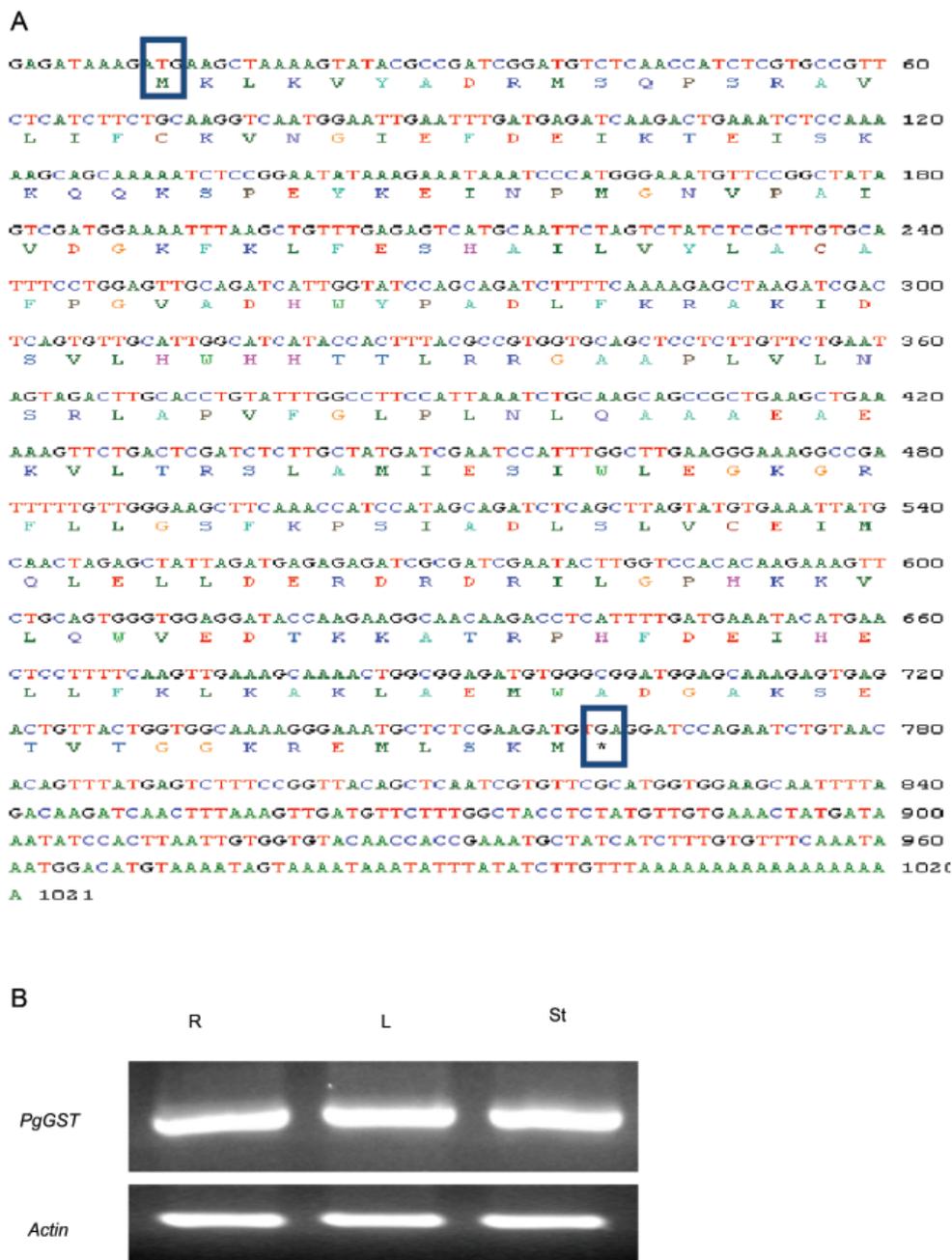
### Glutathione S-transferase activity assay

GST activity was determined according to the procedure described by Dean et al. [13]. The substrates 1-chloro-2,4 dinitrobenzene (CDNB) and reduced GSH were used to measure GST activity. Leaf samples were homogenized by grinding with sodium phosphate buffer and then centrifuged at 12,000 rpm for 10 min at 4°C. The supernatant was used for enzyme activity assay. The reaction mixture contains final concentrations of 20 mM reduced GSH and 20 mM CDNB which are dissolved in 2.5% ethanol and potassium phosphate buffer (0.1 M, pH 6.5). GST activity was determined by absorbance readings at 340 nm using an extinction coefficient of 9.6 mM<sup>-1</sup> cm<sup>1</sup>.

### Treatment of herbicide phosphinothricin and analysis of chlorophyll contents

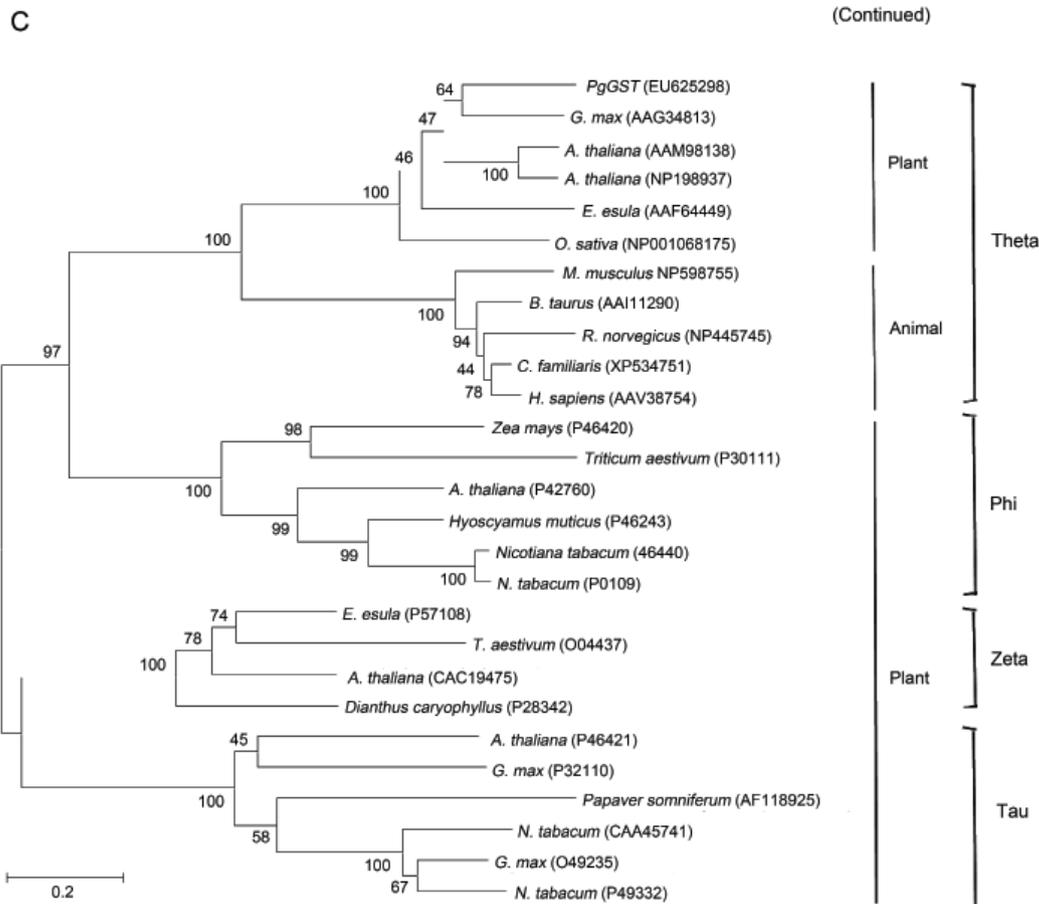
To treat PPT, leaves of 3-week-old tobacco plantlets cultivated in a controlled growth chamber were analyzed. Leaf discs (6 mm diameter), prepared from the leaves of control and transgenic plants, were floated on petri dishes containing 17 mL of 0.1, 1, 10, 100, or 1,000

mM PPT solutions. Each treatment was replicated three times, and each replicate consisted of five leaf discs (6 mm diameter) explants. After incubating in the light for 4 and 24h at 25°C, they were ground in liquid nitrogen using a mortar and pestle, and samples were extracted with 700 µL of 85% (v/v) acetone. Extracts were centrifuged for 15 min at 3,000 rpm. Chlorophyll contents in



**Fig. 1.** *PgGST* sequence analysis. (A) nucleotide and deduced amino acid sequence of a *PgGST* cDNA isolated from *Panax ginseng*. The deduced amino acid sequence is shown in single-letter code below the nucleotide sequence with the open reading frame from 10 to 763 bp. The position of nucleotides is shown on the right. Black box shows the transcription start codon and termination codon. The sequence has been deposited in GenBank as accession no. EU625298. (B) Expressions of *PgGST* in various organs of *P. ginseng*. Total RNAs were extracted from leaves (L), root (R), and stems (St). The actin gene of *P. ginseng* was used as a control.



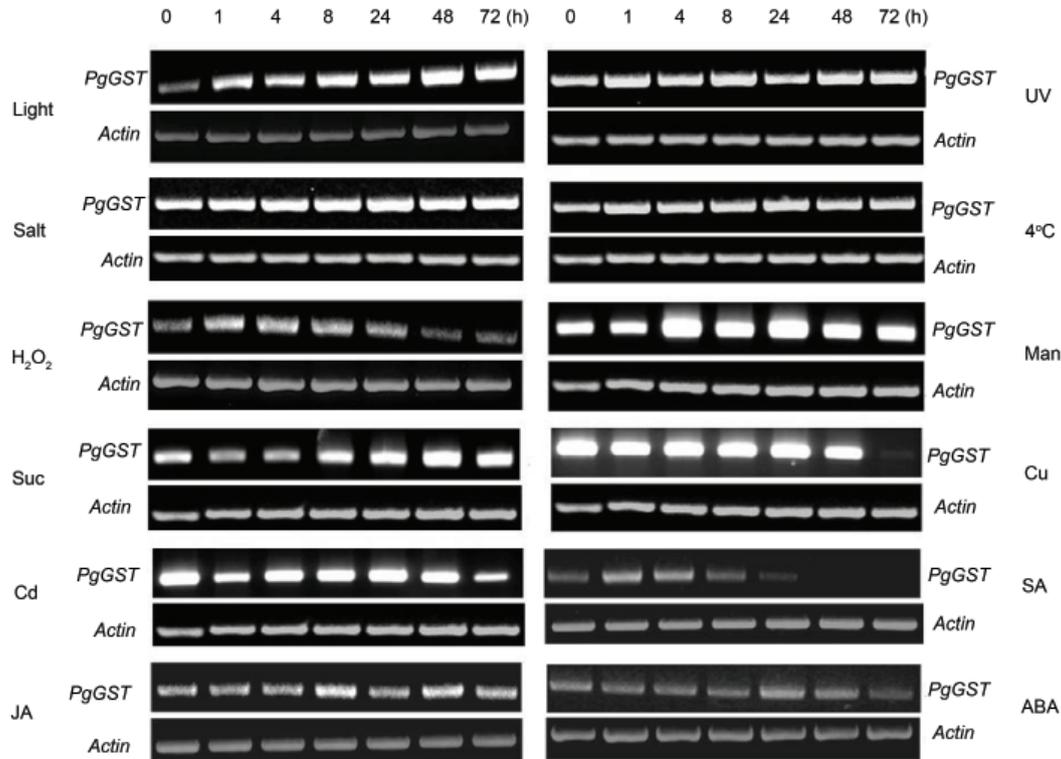


**Fig. 2.** Sequence homology and phylogenetic analysis of *PgGST* with other glutathione S-transferase (GST) proteins. (A) comparison of the putative amino acids sequence of *PgGST* with those of GST genes of theta class from other species with GenBank accession no. in parenthesis; *Glycine max* (AAG34813), *Arabidopsis thaliana* (AAM98138), *Euphorbia esula* (AAF64449), *Oryza sativa* (AAK98534), *Canis familiaris* (XP534751), *Homo sapiens* (AAV38754), *Bos taurus* (AAI11290), *Mus musculus* (NP598755), *Rattus norvegicus* (NP445745), *Danio rerio* (AAH56725), *Anopheles gambiae* (AAM61892), and *Aedes aegypti* (AAV68399). Hyphen was inserted within amino acid sequence to denote gap. Shadow box means well conserved residues, \* represents conserved amino acid and, : represents very similar amino acid. Glutathione-binding residues are indicated by red boxes. (B) Phylogeny of the theta class GST protein family from *Panax ginseng* and other species. Phylogenetic analysis is based on the deduced amino acid sequences of GST genes from various species. Neighbor-joining method was used and the branch lengths are proportional to divergence, with the scale of 0.1 representing 10% changes.

open reading frame of 753 bp, and this ORF encodes a GST protein of 251 amino acids (Fig. 1A). Aliphatic index, which may be regarded as a positive factor for the increase of thermostability, was calculated as 96.80 and instability index is computed to be 41.40 by using Prot-Param, and this result means *PgGST* classifies as unstable [10]. The calculated molecular mass of the matured protein is approximately 28.3 kDa with a predicted isoelectric point of 9.11. By calculating the hydropathy value by the method of Kyte and Doolittle [15], *PgGST* was estimated to be hydrophobic. The ubiquitous expression patterns of *PgGST* in different organs, leaf, stem and root, were examined using RT-PCR analysis (Fig. 1B).

### Phylogenetic analysis

An amino acid sequence comparison with other known GSTs led to the conclusion that *PgGST* belongs to subgroup theta (Fig. 2). The putative protein encoded by *PgGST* shares high degrees of homologies with *Glycine max* (AAG34813, 74.2% identity), *Arabidopsis thaliana* (AAM98138, 71.4% identity), *Oryza sativa* (AAK98534, 66.3% identity), and *Euphorbia esula* (AAF64449, 65.1% identity). Alignment of the GST theta family indicated that amino-acid residues in the N-terminal region are highly conserved, while the C-terminal region shares lower degrees of homology (Fig. 2A). Putative conserved glutathione-binding residues are found (Fig. 2A, red-boxed) [3]. Phylogenetic tree of *PgGST* with other ho-



**Fig. 3.** Reverse transcriptase polymerase chain reaction (RT-PCR) analysis for the expression of *PgGST* genes in *Panax ginseng* at various time points (h) post-treatment with various stresses including high light, UV, 100 mM NaCl, chilling (4°C), 10 mM H<sub>2</sub>O<sub>2</sub>, 11% mannitol (Man), 20% sucrose (Suc), 500 μM CuSO<sub>4</sub> (Cu), 500 μM CdSO<sub>4</sub> (Cd), 5 mM salicylic acid (SA), 200 mM jasmonic acid (JA), or 0.1 mM abscisic acid (ABA). A RT-PCR employing actin specific primer was carried out to confirm equal RNA loading.

mologous proteins reveals the closest amino acid identity (Fig. 2B).

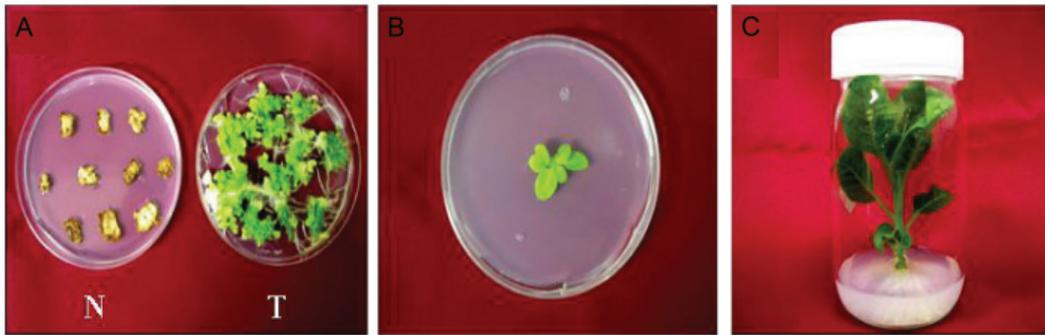
### Differential expression of *PgGST* in response to various stresses

A high light stress caused a rapid induction of *PgGST* within 1 h (Fig. 3), whereas *PgGST* was not responsive against UV. In response to salinity and chilling treatment, *PgGST* transcript level maintained at a steady state until 72 h. Exogenous application of H<sub>2</sub>O<sub>2</sub> caused a strong induction immediately (1 h), but transcription was gradually reduced from 4 h treatment. Under mannitol stress, *PgGST* transcription level was increased at 4 h and was the highest at 24 h post-treatment. At a sucrose treatment, *PgGST* expression was weak at 4 h treatment, then increased and strongest at 48 h post-treatment. After copper stress treatment, the *PgGST* expression continued to be strong from 4 to 24 h. However, a 3-day copper exposure caused a clear decrease in the expression of *PgGST*. In the case of cadmium stress, its expression was reduced at 1 h, and remained at a slightly increased level until 2 d, then decreased after 3 d. SA caused induction in the

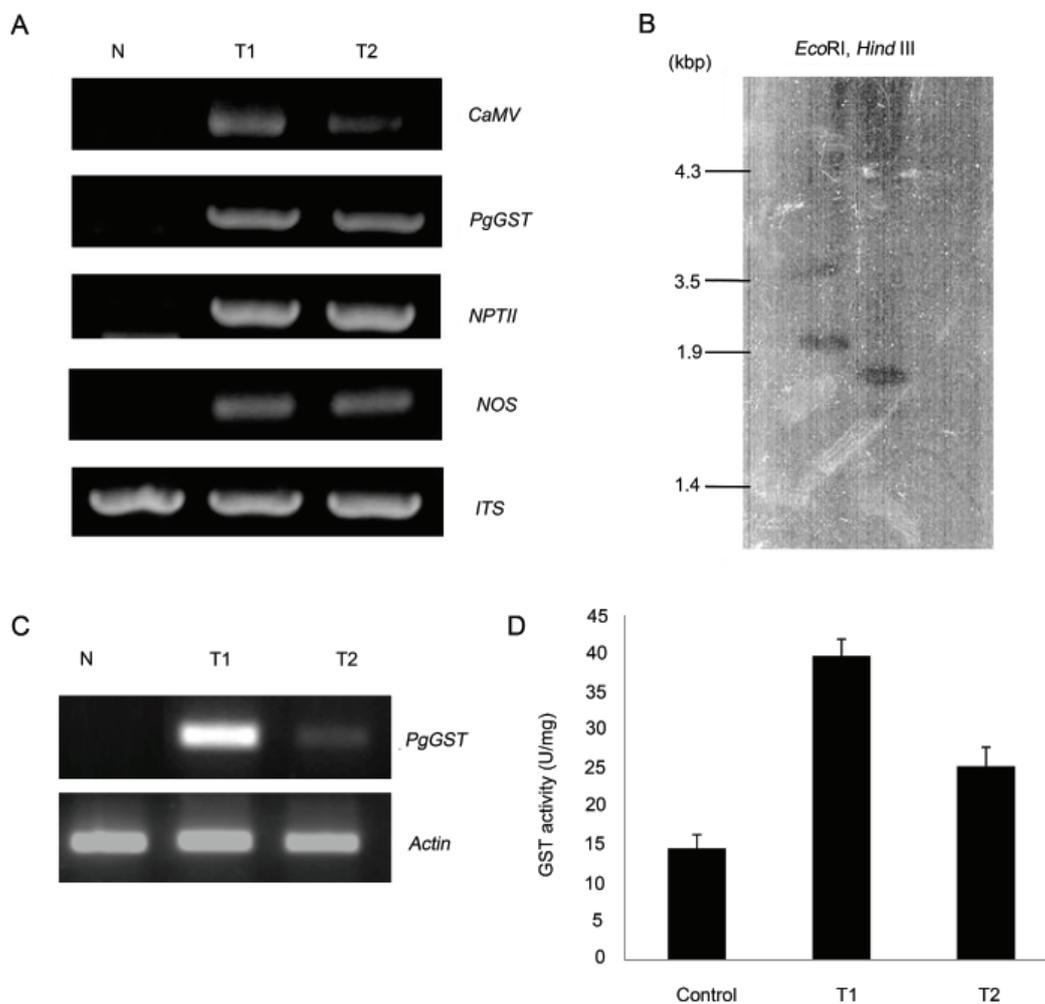
expression of *PgGST* mRNA at an immediate time (1 h), and then, its expression was continuously decreased after 8 h treatment. When continuous SA treatment was maintained, *PgGST* expression was decreased and no transcripts were observed at 2 to 3 days treatment. JA caused its upregulation at 8 and 48 h treatment. Under ABA treatment, it seems that *PgGST* transcription was strongly expressed at 24 h post-treatment and was kept decreased in other time zones.

### Overexpression *PgGST* showed enhanced glutathione S-transferase activity and resistance against phosphinothricin

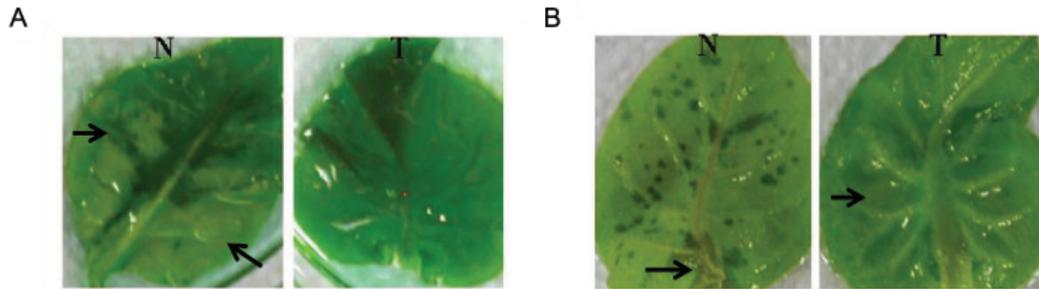
Tobacco was transformed using *A. tumefaciens* with the *PgGST*, under the control of CaMV promoter and NOS terminator (*PgGSTox*). Several independent transgenic lines were established by selection on kanamycin (Fig. 4). Kanamycin resistant callus was developed on the surfaces of explants during the subculture on selection medium. Upon transfer to MS medium containing 0.1 mg/L NAA, transgenic seedlings developed plantlets. The integration of the *PgGST*, *NPTII*, CaMV promoter,



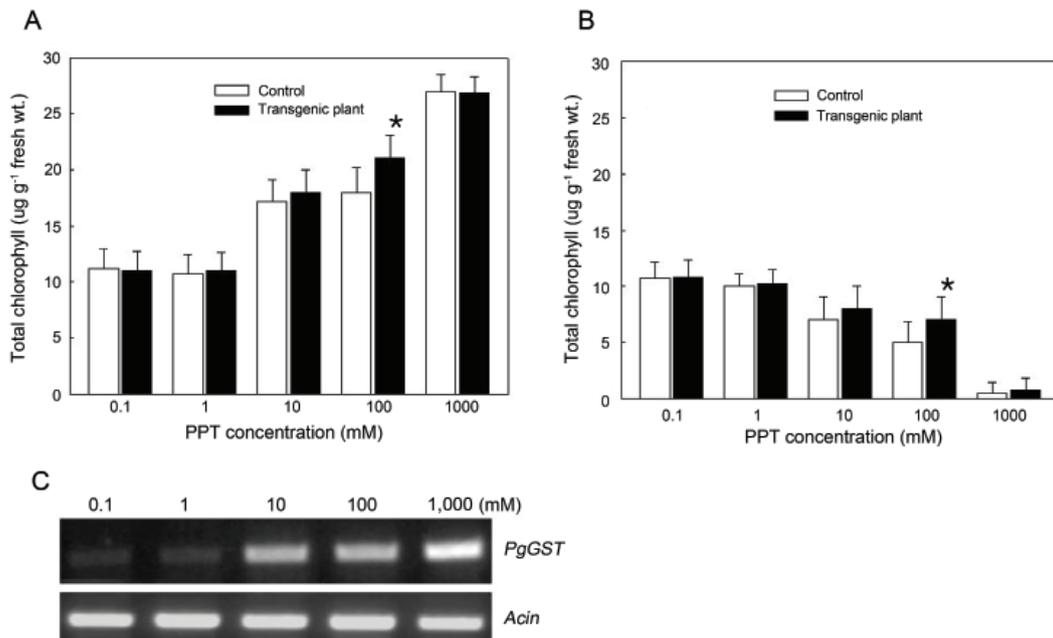
**Fig. 4.** Genetic transformation of *Nicotiana tabacum* with ginseng glutathione S-transferase gene. Survived transgenic (T) calli compared with non-transgenic line (N) (A) on selection medium containing 100 mg/L kanamycin, and 500 mg/L cefotaxime produced resistant shoot (B), and transgenic tobacco explants acclimated *in vitro* (C).



**Fig. 5.** Confirmation of transgenes in tobacco by polymerase chain reaction (PCR) and southern blot analysis. (A) PCR analysis of 35S cauliflower mosaic virus promoter (*CaMV*), glutathione S-transferase of *Panax ginseng* (*PgGST*), neomycin phosphotransferase II (*NPTII*), nopaline synthase terminator (*NOS*), and internal transcribed spacer (*ITS*) gene as control from non-transgenic (N) and two transgenic tobacco lines (T1 and T2) was conducted. (B) Southern blot hybridization of DNA prepared from transgenic tobacco plants. DNA (20 µg) was cut by restriction enzymes *EcoRI* or *HindIII*, separated on 1.3% agarose gel, transferred to a membrane and hybridized to a DIG-labeled *PgGST*-specific probe. (C) reverse transcriptase-PCR and (D) glutathione S-transferase (GST) activity of non-transgenic (N) and two transgenic lines (T1 and T2).



**Fig. 6.** Leaves of non-transgenic (N) and transgenic (T and T2 line) tobacco plants treated with 0.1 M (A) or 1 M (B) phosphinothricin for 4 h.



**Fig. 7.** Effect of phosphinothricin (PPT) on the chlorophyll content and *PgGST* expression in transgenic tobacco leaves (T2). Total chlorophyll content from leaf discs of wild-type and transgenic plants treated with 0.1, 1, 10, 100, or 1,000 mM of PPT for 4 h (A) and 24 h (B) was analyzed. Means ( $\pm$ SE) are for 10 replicates per treatment. Averages for treated samples were significantly different compared to the control at  $*p < 0.01$ . *PgGST* expression by PPT (0.1, 1, 10, 100, or 1,000 mM) for 4 h was performed by reverse transcriptase polymerase chain reaction (C).

and NOS terminator genes were confirmed by PCR analysis (Fig. 5A). To determine the number transgene in the tobacco genome, we performed a Southern blot analysis. When probed under high-stringency conditions, a single band was detected from genomic DNA after digestion with *EcoRI* or *HindIII* (Fig. 5B). RT-PCR analysis and GST activity were assayed in transgenic and wild-type plants. Transgenic *PgGST* gene was expressed highly in transgenic tobacco lines (Fig. 5C) and GST-expressing transgenic lines had an approximately twofold higher activity in leaf extracts compared to the control (Fig. 5D). These results confirmed that *P. ginseng* GST transgene encode an active GST in transgenic *N. tabacum* plants.

Generally reported GST-mediated herbicide detoxification suggested us to test its functional resistance against

PPT. Fig. 6 shows that the leaf damage of transgenic (T, T2 line) and non-transgenic (N) plants when they were treated with 0.1 M (Fig. 6A) and 1 M (Fig. 6B) of PPT for 4 h. The necrotic area in the control leaf is much larger than in the transgenic plants.

Since chlorophyll content is one of well-known indication to access plant damage after stress treatment, its content was analyzed. Chlorophyll contents of tobacco treated with high concentration of PPT at 4 h treatment were higher than those at low concentration (Fig. 7A). Increased chlorophyll content was correlated with increased transcript level of *PgGST* (Fig. 7C). Chlorosis developed throughout the leaves according to the treatment time prolonged. Chlorophyll content at 24 h after PPT treatment was decreased as PPT concentra-

tion increased (Fig. 7B). At 100 mM PPT, control plant showed significantly lowered total chlorophyll level than transgenic plant (T2). However, higher concentrations of PPT (1 M) application resulted in chlorosis and extensive necrosis in both control and transgenic plants within 24 h after treatment.

## DISCUSSION

We describe for the first time the isolation and characterization of *GST* gene in *P. ginseng*. A cDNA encoding a GST, designated *PgGST* was isolated and sequenced. Genbank BlastX search, phylogenetic study, and analysis of protein confirmed that the *PgGST* should be theta group of GST. The calculated size of *PgGST* is 28.3 kDa, which is approximately the same size with other reported theta class GSTs [16]. Hydrophathy value can explain that *PgGST* easily interact with hydrophobic molecules. Each GST has known to contain an independent catalytic site composed of two components. One is a binding site for GSH (G-site) and the other is a site that binds the hydrophobic substrate (H-site) [2]. Alignment of the GST theta family indicated that amino-acid residues in the amino-terminal region are highly conserved, while the C-terminal region shares lower degrees of homology (Fig. 2). One explanation is that the G-site is highly specific for glutathione, which formed a conserved group, and the H-site interacts with electrophilic and hydrophobic xenobiotic substrates, which is much more structurally variable [17]. Although the theta class is the largest class of GST isozymes and its sequence is diverse, several strict sequence conservations are present to distinguish it from the other classes. In the region considered crucial for glutathione binding, a conserved glutamine residue (Q) is present in the other GSTs, which is shown to interact directly with GSH [4]. On the other hand, at this same position of the theta class, a conserved glutamic acid (E) is present instead [3]. A second essential difference between the theta class and the other four classes is a conserved tyrosine residue (Y) near the amino-terminal end that is part of the active site and is located within hydrogen bonding distance of the thiol group of bound GSH. *PgGST* also has the same residues in the G-site domain as other theta class (Fig. 2).

Plant GST genes exhibit a diverse expression patterns during biotic and abiotic stresses. It becomes obvious that unique combinations of multiple, often interactive signaling pathways from various phytohormones and ROS render the distinct transcriptional activation patterns of individual GSTs during stress [18]. Droog et al. [3]

reported that the activity of GST is needed under many different conditions. In case of ginseng *GST*, it was not significantly responsive against UV, which is distinctly different comparing to the case of GST from *Petroselinum crispum* where the transcript was induced within 2 h by UV irradiation [19]. However, *PgGST* showed significant upregulation by H<sub>2</sub>O<sub>2</sub> (Fig. 3). *Arabidopsis* GST gene was also reported to be induced by H<sub>2</sub>O<sub>2</sub> [20]. GSTs participate in the detoxification of products formed by oxidative damage and thereby protect cells against such stresses [2]. ROS can affect cell viability by causing enzyme inactivation, therefore extensive treatment of H<sub>2</sub>O<sub>2</sub> could reduce *PgGST* expression. In this study, expression of *PgGST*, which belongs theta class, was not significantly changed against chilling and salinity stresses, whereas tobacco overexpressing tau class of GST was tolerant to low temperature and salt stress exposure [21]. Compatible solutes, such as sucrose and mannitol, are demonstrated to more or less closely relate to antioxidant or osmoprotectant mechanisms [22]. Following similar mechanism, *PgGST* expression under these compatible solutes was also induced. Expression pattern of *PgGST* against heavy metals were corresponding with that of theta class *GST* (*GST-theta*) from *Neanthes succinea* where the upregulation of *GST-theta* was concentration and time dependent [16]. Stress-induced plant growth regulators like ABA, JA, or SA differentially activate *GST* gene expression [18]. When there was a continuous SA treatment, *PgGST* expression was decreased, which can be explained by the role of SA. SA induces cell death via the hypersensitive response, and recent studies have suggested that GSTs act as potential regulators of apoptosis [2].

Previous studies indicated that many theta class members are related with herbicide detoxification. Herbicide detoxification activity was shown for most of theta class subunits [3]. A wheat theta *GST* gene was found to be closely related to the herbicide-detoxifying GSTs [6], and transgenic tobacco plants overexpressing GST were tolerant to herbicide exposure [21]. Therefore, transgenic tobacco plants were continuously studied to find the evidence of the increasing tolerance against herbicide. PPT, an herbicide commonly used for weed control in agriculture, which is a structural analog of glutamate that binds to glutamine synthetase (GS), a key enzyme of nitrogen metabolism in plants. It leads to the accumulation of ammonia, leaf chlorosis, and an inhibition of photosynthesis [23]. When chlorophyll content was determined from leaves of transgenic and control tobacco plants treated with PPT, interestingly, chlorophyll contents was

increased by 4 h treatment of PPT. (Fig. 7A). This result can be explained with previous study that large subunit of Rubisco (LSU) increased after 4 h PPT treatment to overcome PPT-mediated inhibition of GS [24]. It was suggested that LSU accumulation could be considered as a mechanism to recover from amino acid depletion and ammonia accumulation, when photosynthesis is inhibited by PPT.

The increased *PgGST* expression according to the PPT concentration suggests that enhanced GST expression could offer resistance against PPT exposure (Fig. 7). Overall, our results show that the *PgGST* may have a role in the protection against toxic material such as several heavy metals and herbicides. Further studies are still needed to understand the physiological function of GST in *P. ginseng*. Ginseng transformant might have possibility to endure under the sun light and against heavy metal and herbicide.

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## REFERENCES

1. Droog F, Hooykaas P, Van Der Zaal BJ. 2,4-Dichlorophenoxyacetic acid and related chlorinated compounds inhibit two auxin-regulated type-III tobacco glutathione S-transferases. *Plant Physiol* 1995;107:1139-1146.
2. Dixon DP, Laphorn A, Edwards R. Plant glutathione transferases. *Genome Biol* 2002;3:REVIEWS3004.
3. Droog F. Plant glutathione S-transferases, a tale of theta and tau. *J Plant Growth Regul* 1997;16:95-107.
4. Wilce MC, Parker MW. Structure and function of glutathione S-transferases. *Biochim Biophys Acta* 1994;1205:1-18.
5. Park MS, Cho EJ, Lee SK, Lee EJ, Lee DS, Lee KH, Jeon BH. Korean red ginseng protects oxidative injury caused by lead poisoning. *J Ginseng Res* 2010;34:132-137.
6. Dudler R, Hertig C, Rebmann G, Bull J, Mauch F. A pathogen-induced wheat gene encodes a protein homologous to glutathione-S-transferases. *Mol Plant Microbe Interact* 1991;4:14-18.
7. Marrs KA. The functions and regulation of glutathione S-transferases in plants. *Annu Rev Plant Physiol Plant Mol Biol* 1996;47:127-158.
8. Lee OR, Sathiyaraj G, Kim YJ, In JG, Kwon WS, Kim JH, Yang DC. Defense genes induced by pathogens and abiotic stresses in *Panax ginseng* C.A. Meyer. *J Ginseng Res* 2011;35:1-11.
9. Kim MK, Lee BS, In JG, Sun H, Yoon JH, Yang DC. Comparative analysis of expressed sequence tags (ESTs) of ginseng leaf. *Plant Cell Rep* 2006;25:599-606.
10. Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins MR, Appel RD, Bairoch A. Protein identification and analysis tools on the ExpASY server. In: Walker JM, ed. *The proteomics protocols handbook*. Totowa: Humana Press, 2005. p.571-607.
11. Kim YJ, Shim JS, Krishna PR, Kim SY, In JG, Kim MK, Yang DC. Isolation and characterization of a glutaredoxin gene from *Panax ginseng* C. A. Meyer. *Plant Mol Biol Report* 2008;26:335-349.
12. Ditta G, Stanfield S, Corbin D, Helinski DR. Broad host range DNA cloning system for gram-negative bacteria: construction of a gene bank of *Rhizobium meliloti*. *Proc Natl Acad Sci U S A* 1980;77:7347-7351.
13. Dean JV, Gronwald JW, Eberlein CV. Induction of glutathione s-transferase isozymes in sorghum by herbicide antidotes. *Plant Physiol* 1990;92:467-473.
14. Lichtenthaler HK. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods Enzymol* 1987;34:350-382.
15. Kyte J, Doolittle RF. A simple method for displaying the hydropathic character of a protein. *J Mol Biol* 1982;157:105-132.
16. Rhee JS, Lee YM, Hwang DS, Won EJ, Raisuddin S, Shin KH, Lee JS. Molecular cloning, expression, biochemical characteristics, and biomarker potential of theta class glutathione S-transferase (GST-T) from the polychaete *Neanthes succinea*. *Aquat Toxicol* 2007;83:104-115.
17. Choi CY, An KW, An MI. Molecular characterization and mRNA expression of glutathione peroxidase and glutathione S-transferase during osmotic stress in olive flounder (*Paralichthys olivaceus*). *Comp Biochem Physiol A Mol Integr Physiol* 2008;149:330-337.
18. Moons A. Regulatory and functional interactions of plant growth regulators and plant glutathione S-transferases (GSTs). *Vitam Horm* 2005;72:155-202.
19. Loyall L, Uchida K, Braun S, Furuya M, Frohnmeyer H. Glutathione and a UV light-induced glutathione S-transferase are involved in signaling to chalcone synthase in cell cultures. *Plant Cell* 2000;12:1939-1950.
20. Chen W, Singh KB. The auxin, hydrogen peroxide and salicylic acid induced expression of the Arabidopsis GST6 promoter is mediated in part by an ocs element. *Plant J* 1999;19:667-677.

21. Roxas VP, Smith RK Jr, Allen ER, Allen RD. Overexpression of glutathione S-transferase/glutathione peroxidase enhances the growth of transgenic tobacco seedlings during stress. *Nat Biotechnol* 1997;15:988-991.
22. Jouve L, Hoffmann L, Hausman JF. Polyamine, carbohydrate, and proline content changes during salt stress exposure of aspen (*Populus tremula* L.): involvement of oxidation and osmoregulation metabolism. *Plant Biol (Stuttg)* 2004;6:74-80.
23. Wendler C, Barniske M, Wild A. Effect of phosphinothricin (glufosinate) on photosynthesis and photorespiration of C3 and C4 plants. *Photosynth Res* 1990;24:55-61.
24. Pascual MB. Expresión de glutamina sintetasa e isocitrato deshidrogenasa en árboles transgénicos: papel en el desarrollo [dissertation]. Malaga: Universidad de Malaga, 2007.