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Research Article

Identification of salt and drought inducible glutathione *S*-transferase genes of hybrid poplar

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Abstract Recent genome annotation revealed that *Populus trichocarpa* contains 81 glutathione *S*-transferase (GST) genes. *GST* genes play important and varying roles in plants, including conferring tolerance to various abiotic stresses. Little information is available on the relationship − if any − between drought/salt stresses and *GST*s in woody plants. In this study, we screened the PatgGST genes in hybrid poplar (*Populus alba* × *Populus tremula* var. *glandulosa*) that were predicted to confer drought tolerance based on our expression analysis of all members of the poplar *GST* superfamily following exposure to salt (NaCl) and drought (PEG) stresses, respectively. Exposure to the salt stress resulted in the induction of eight *PatgGST* genes and down-regulation of one *PatgGST* gene, and the level of induction/repression was different in leaf and stem tissues. In contrast, 16 *PatgGST* genes were induced following exposure to the drought (PEG) stress, and two were down-regulated. Taken together, we identified seven *PatgGST*s (*PatgGSTU15*, *PatgGSTU18*, *PatgGSTU22*, *PatgGSTU27*, *PatgGSTU46*, *PatgGSTU51* and *PatgGSTU52*) as putative drought tolerance genes based on their induction by both salt and drought stresses.

Keywords Glutathione *S*-transferases, salt stress, drought stress, *Populus alba* × *Populus tremula* var. *glandulosa*

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Introduction

Gene/genomic duplication events have been very common occurrences during the evolution of eukaryotes. Some of these duplicated genes/genomic sequences would have been prone to mutation and may have acquired novel functions in additional to their original function(s), or they may have changed their original function(s) completely. In extreme cases, they might have become pseudogenes (Hughes 1994; Force et al. 1999; Moore and Purugganan 2005). Thus, it is not at all uncommon to identify duplicated genes in eukaryotes, especially in plants (Tuskan et al. 2006). These duplicated copies of the genes form a unique gene family and it is a commonly accepted assumption that all of those genes have evolved and diversified after repeated duplication events, as exemplified by the glutathione *S*-transferase (GST) gene family in plants. GSTs (EC 2.5.1.18) are multi-functional proteins that can be found in all organisms (Smith et al. 2004). They protect cells from both biotic and abiotic stresses, including xenobiotics, heavy metals, pathogens, and oxidative bursts (Kampranis et al. 2000; Mueller et al. 2000; Agrawal et al. 2002).

Plant GSTs are subdivided into eight distinct classes. These are Phi, Tau, Theta, Zeta, Lambda, glutathione-dependent dehydroascorbate reductases (DHARs), tetrachlorohydroquinone dehalogenase (TCHQD) (Basantani and Srivastava 2007) and γ-subunit of the eukaryotic translation elongation factor 1B (EF1Bγ) with last subfamily only being included as a member of the GST family based on structural similarities to GSTs (Jeppesen et al. 2003; Oakley 2005; Lan et al. 2009). Among these, Phi and Tau are plant specific whereas the Theta and Zeta classes are found in mammals, fungi, and insects (Sheehan et al. 2001; Smith et al. 2004). In plants, each GST family has a large number of members. For example, the GST gene family in *Arabidopsis thaliana* has 53 members (Dixon et al. 2002; Wagner et al. 2002), 79 members in *Oryza sativa* (Soranzo et al. 2004) and 81

members in *Populus trichocarpa* (Lan et al. 2009). In *Populus trichocarpa,* the Tau and Phi GSTs were the most numerous, being represented by 58 and 9 members, respectively (Lan et al. 2009). The Lambda, DHAR, and EF1Bγ GST classes were each represented by three members, both the Zeta and Theta classes by two members, and the TCHQD class by just one member (Lan et al. 2009).

GST genes play very important and varying roles in plants. Some of the roles reported to date include conferring tolerance to oxidative stress and UV-radiation, protecting cells from biotic and abiotic stress, and providing defense against cadmium toxicity (Kampranis et al. 2000; Loyall et al. 2000; Agrawal et al. 2002; Bianchia et al. 2002). Tau- and Phi class GTSs, the two largest classes and the most abundant GSTs in plants, participate in endogenous cellular metabolism by functioning as glutathione peroxidases that counteract oxidative stress, as flavonoid-binding proteins and as stress signaling proteins (Dixon et al. 2002; Mueller et al. 2000; Loyall et al. 2000; Basantani and Srivastava 2007). Although detailed knowledge of the physiological and molecular mechanisms of tolerance to stress in plants is not yet available, considerable evidence has been accumulated to indicate that GSTs play a protective role in mitigating the effects of drought and salinity stresses in plants (George et al. 2010; Ji et al. 2010; Wei et al. 2010; Jha et al. 2011; Chen et al. 2012). For example, Ji et al. (2010) observed that the overexpression of *Glycine soja* GST gene in tobacco enhanced the drought and salt stress tolerance of the transgenic tobacco plants. In another study, transgenic tobacco plants overexpressing the *Salicornia brachiata* GST gene were more tolerant of the salt stress condition than wild-type tobacco plants (Jha et al. 2011).

Poplar species are fast-growing temperate woody plants with high potential for biomass production. The recent sequencing of the entire genome of P. trichocarpa has led to an abundance of gene expression data becoming available. Based on poplar genome annotation, Lan et al. (2009) showed that *Populus* spp. have the largest GST family known to date. Thus, this plant would appear to be an ideal model species to study the function of GSTs in plants. GSTs are a particularly interesting research topic since the functions of some GST genes might be fully or partially overlapping in terms of salt and drought stress tolerance.

In this study, our aim was to screen the members of the poplar GST family that respond to salt and drought stresses by semi-quantitative reverse transcriptase (RT)-PCR analysis. Seven members of the Tau class of the GST family were screened based on transcript levels in the hybrid poplar (*Populus alba* × *P. tremula* var. *glandulosa*) under conditions of salt (NaCl) and drought [polyethylene glycol (PEG)] stresses.

Materials and methods

Plant culture and stress treatments

A hybrid aspen clone BH1 [*Populus alba* × *P. tremula* var. *glandulosa* (*Patg*)] was vegetatively propagated *via* shoot-tip culture on solid rooting medium [half-strength MS medium (Murashige and Skoog 1962) supplemented with 3% sucrose, 0.2 mg/L indole-3-butyric acid, and 0.8% agar; pH 5.8]. The cultures were maintained at 22℃ and 40% humidity under white-fluorescent lighting provided at an intensity of 200 μmol/m^{2}s for 16 h per day. The plants were subcultured at 4-week intervals until being transferred into liquid medium prior to the stress treatments. For the stress treatments, plants were removed from the solid medium, washed with sterile distilled water, and placed in test tubes containing liquid medium with the same composition as the rooting medium (except for a higher concentration of agar) supplemented with 150 mM NaCl or 10% PEG 6000. Plants were harvested after 0 (Control), 30 m, or 2 h exposure to the stress (Fig. 1).

RNA extraction and cDNA library construction

Fresh tissues (leaves and stems) were ground into a fine powder in liquid nitrogen using a mortar and pestle. After homogenization, total RNA was purified using the RNeasy Plant Mini kit (Qiagen, Venlo, The Netherlands). The quality and quantity of purified total RNA were confirmed by spectrophotometric analysis and gel electrophoresis. Total RNA (5 μg) was used to synthesize first-strand cDNAs by the PrimeScript™ RT Reagent kit (TaKaRa, Otsu, Japan).

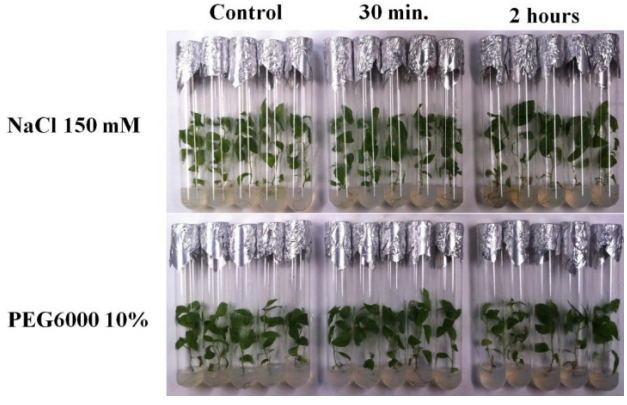

Fig. 1 Shoot-tip cultures of the hybrid poplar [*Populus alba* × *P. tremula* var. *glandulosa* (*Patg*)] for stress treatments

Transcript analysis by semi-quantitative RT-PCR

The expression of GST genes under our drought or salt treatment conditions was analyzed with five replicates. The poplar β-actin gene was used as a control gene. The actin forward primer sequence was 5'-GCCATCTCTCATCGGA ATGGAA-3', and the reverse primer sequence was 5'-AGG GCAGTGATTTCCTTGCTCA-3'. RT-PCR assays were performed with the PCR PreMix 20 Reaction kit (Bioneer, Daejeon, Korea) on a thermocycler (model T3000; Biometra GmbH, Göttingen Germany). Some of the GST primers for the semi-quantitative RT-PCR assays were designed according to the previously reported primer sequences for *P. trichocarpa* GST genes (Lan et al. 2009), and the remaining ten pairs were designed in our study (Table 1). The cycling conditions of the semi-quantitative RT-PCR assays consisted of 30 cycles of an initial denaturation at 95℃ for 5 m, denaturation at 95°C for 30 s, and annealing at 54 ~ 63°C, and extension at 72°C for 40 s. The amplified products were run on a 1 % agarose gel and visualized by UV-light.

Table 1. Primers for reverse transcription (RT)-PCR that were newly designed based on the sequence information of *Populus trichocarpa* in the Phytozyme database (http://www.phytozome.net)

Gene	Primer name	Sequence (5′ to 3′)
PatgGSTU6	GSTU6-F	TTGGAAGGAGAGCTTGGAGA
	GSTU6-R	CTTCCTCAGCATCACAACGA
PatgGSTU41	GSTU41-F	GAAGGGACATGGAAGCAAAA
	GSTU41-R	TCCACAATTCCGATCCTCTC
PatgGSTU43	GSTU43-F	CCAGTGCTTGTTCATGATGG
	GSTU43-R	TTGCCTTTTCCTGTTGCTCT
PatgGSTU44	GSTU44-F	AGGGACAAGAGAAGGCAACA
	GSTU44-R	GATGCAAACGAGGGAAGCTA
PatgGSTU45	GSTU45-F	CGAGAAGTGTATGCCTGCAA
	GSTU45-R	CCCAACCAGAAGCCTATGAA
PatgGSTU55	GSTU55-F	TTTTGGGGATGAGAGCTTTG
	GSTU55-R	AAACCTCCTTCTGGCCACTT
PatgDHAR3	DHAR3-F	GAAAGGTCCCAGTGGTGAAA
	DHAR3-R	TCCAATGCCTTCAATTCCTC
PatgGSTT2	GSTT2-F	TTTGGTCGACCTCTGAATCC
	GSTT2-R	TGCGATCCGTTTCATCTGTA
PatgGSTZ1	GSTZ1-F	AACCTTGCAAAAGGAGAGCA
	GSTZ1-R	CACCAAAGCCAGCATATGAA
PatgEF1Bγ3	EF1Bγ3-F	CTGAACAAGGTCGGTGGATT
	EF1Bγ3-R	TGTCAACCTTGGTCCATTCA

Results

Design of RT-PCR primers for hybrid poplar transcript analysis

Prior to the semi-quantitative RT-PCR assays, primers designed on the basis of the genome of *P. trichocarpa* (Lan et al. 2009) were tested using cDNA synthesized from our hybrid poplar grown in the normal growth media. We detected 43 putative *PatgGST* transcripts among the 81 *PatgGSTs* identified in our hybrid poplar (data not shown), of which only ten *PatgGST* transcripts were detected using the newly designed primers listed in Table 1. The transcripts of the remaining 38 *PatgGST* genes were not detected by RT-PCR. Possible explanations for this failure to obtain amplification products include sequence differences at the priming sites between the two poplar species, development- and/or or tissue type-specific expression, and/or inducible expression under specific environmental conditions.

Isolation of salt stress response genes in hybrid poplar

Semi-quantitative analysis of the expression of the 81 *PatgGST* genes of hybrid poplar (*P. alba* × *P. tremula* var. *glandulosa*) was performed following exposure of the plants to 150 mM NaCl treatment for 30 m and 2 h (Fig. 1). Eight of the 43 *PatgGST* genes amplified under the salt stress condition were up-regulated. Of these, five (*PatgGSTU15*, *PatgGSTU18*, *PatgGSTU27*, *PatgGSTU45* and *PatgGSTU46*) were up-regulated in either the leaves (*PatgGSTU27 and Patg GSTU46*) or the stem (*PatgGSTU15*, *PatgGSTU18 and Patg GSTU45*), and three (*PatgGSTU22, Patg GSTU51 and Patg GSTU52*) were induced in both the leaves and stems (Fig. 2). In contrast, *PatgGSTU9* expression was down- regulated by the NaCl treatment (Fig. 2). Almost all of the other *Patg GST* genes amplified under this specific stress condition did not show any visible change in the expression level when compared with untreated control tissues (data not shown).

Isolation of PEG response genes in hybrid poplar

Drought-associated oxidative stress was induced in our study by adding 10% PEG 6000 to the growth media and sampling the plants at 30 m and 2 h. Of the 43 *PatgGST* genes amplified, 16 were up-regulated at the transcription level following the 10% PEG 6000 treatment (Fig. 3). Most of them were up-regulated in both the leaf and stem tissues, but *PatgGSTF8* was induced only in the stem (Fig. 3). Among the 16 *PatgGST* genes that were up-regulated in the presence of 10% PEG were seven *PatgGST* genes that were induced

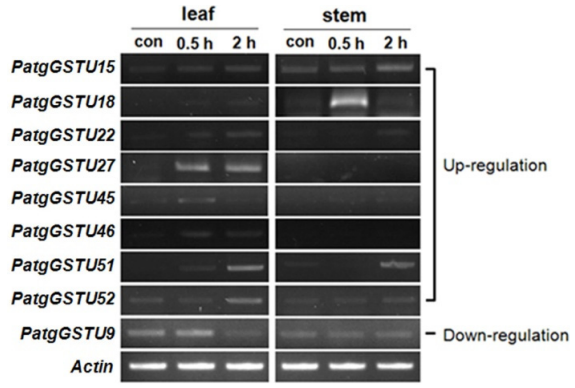

Fig. 2 Transcript analysis of *Patg* glutathione S-transferases (*PatgGSTs*) with NaCl treatment by semi-quantitative reverse transcriptase (RT)-PCR. *con* ; Control (baseline)

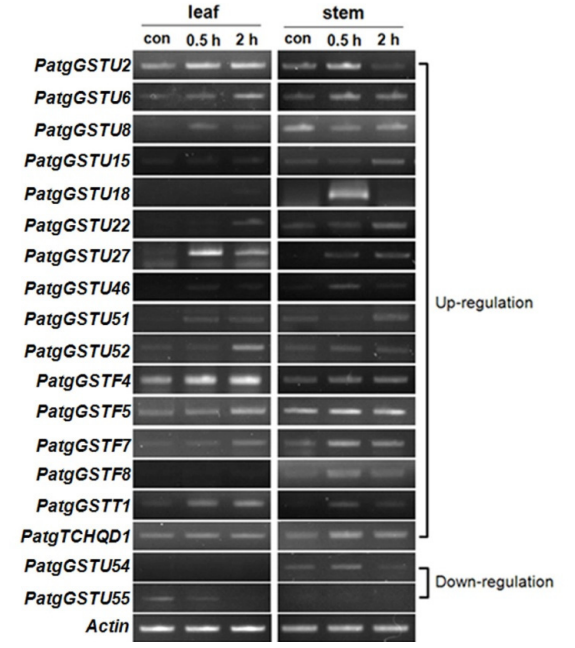

Fig. 3 Transcript analysis of *PatgGSTs* with 10% PEG 6000 treatment by semi-quantitative RT-PCR. *TCHQD* ; Tetrachloro-hydroquinone dehalogenase, *con* ; Control (baseline)

in the NaCl treatment: *PatgGSTU15*, *PatgGSTU18*, *PatgGSTU22*, *PatgGSTU27*, *PatgGSTU46*, *PatgGSTU51*, and *PatgGSTU52* (Figs. 2 and 3). In contrast, *PatgGSTU54* and *PatgGSTU55* were down-regulated following exposure to PEG 6000 (Fig. 3).

Discussion

Glutathione *S*-transferases of many plant species, including *Arabidopsis* (Bianchi et al. 2002), wild soybean (Wei et al. 2010), *Amaranthaceae* (Jha et al. 2011), and wheat (Gallé et al. 2009), have been implicated in salt stress and drought tolerance. However, poplar GSTs have not been characterized in terms of tolerance against abiotic stress, including salt and drought. Consequently, our aim was to isolate putative candidate genes as first step toward understanding the mechanism of salt and drought tolerance in woody plants.

Based on our semi-quantitative RT-PCR results, we were able to identify 17 poplar *PatgGST* genes that responded to either salt- or oxidative stress-mediated drought: *PatgGSTU2*, *PatgGSTU6*, *PatgGSTU8*, *PatgGSTU15*, *PatgGSTU18*, *PatgGSTU22*, *PatgGSTU27*, *PatgGSTU45*, *PatgGSTU46*, *PatgGSTU51*, *PatgGSTU52*, *PatgGSTU58*, *PatgGSTF4*, *PatgGSTF5*, *PatgGSTF7*, *PatgGSTF8*, *PatgGSTT1*, and *PatgTCHQD1*. These genes were subjected to real-time RT-PCR to confirm their induction at the transcription level under the specific stress condition tested (data not shown). Among the eight subclasses of the poplar GST gene family, several GST genes belonging to the Tau subclass were identified as inducible under both the salt and PEG stress (Figs. 2 and 3). Members of the Tau class of GST (GSTU) genes are plant specific and well-known for their role in herbicide detoxification (Axarli et al. 2009). Recently, extensive analysis of 81 GST family members in *Populus* were conducted by Lan et al. (2009), which includes phylogenetic relationships among them, their chromosomal locations, gene expression patterns in the different tissue types, and protein structural features. However, their physiological roles regarding to the stress tolerance have not been reported yet. In our study, analysis of the transcriptional levels of *Populus* GSTs by semi-quantitative RT-PCR led to the identification of candidate genes for salt and drought tolerance. These include *PatgGSTU15*, *PatgGSTU18*, *PatgGSTU22*, *PatgGSTU27*, *PatgGSTU46*, *PatgGSTU51* and *PatgGSTU52*. These genes can be used in future investigations to study the mechanism(s) of abiotic stress tolerance as well as in genetic engineering attempts to improve the tolerance of woody plants to various stresses.

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Supplementary Table 1. Primers designed by Lan et al. (2009) were initially used for RT-PCR in this experiment

Gene	Primer name	Sequence (5'-3')	Gene	Primer name	Sequence (5'-3')
GSTU1	PtGSTU1RT1	TGTTGACAACAAGGTGTATTTTCCT	*GSTU42*	PtGSTU42RT1	ACTTGAGCAACGGAAAGAGTGAT
	PtGSTU1RT2	GCCTCTATGCTAAAGTTACCAAATG		PtGSTU42RT2	TCCATATCTTCCTAGAAAGGTTATTACC
GSTU2	PtGSTU2RT1	ATTTTGTTGACAAAAAGATGTATGACT	*GSTU43*	PtGSTU43EX1	AGATATCATGGTAGGTGTGAAGCTCTTCG
	PtGSTU2RT2	ACATCTTCCTCAGCATCACAACA		PtGSTU43EX2	TGAATTCTTACTAATGGTGATGGTGGCAAG
GSTU3	PtGSTU3RT3	CTGGGCAGATTTTGTTGATAAGA	*GSTU44*	PtGSTU44EX1	AGATATCATGGAAGAAGTGAAGCTACTAGG
	PtGSTU3RT4	ACATCAACGTAGCCGAGAGTCTT		PtGSTU44EX2	TAAGCTTTTATCATCTTGGTGGAAAAATAC
GSTU4	PtGSTU4RT3	GTCCATGATGGGAAACCTATTTT	*GSTU45*	PtGSTU45-EX1	AGATATCATGGCTGAAGTGAAGTTGCT
	PtGSTU4RT4	CCTCCCAAGGTCATATACCTTCTTA		PtGSTU45-EX2	TGAATTCTTACTAATACTTCCAAGAGGAAGC
GSTU5	PtGSTU5RT1	ATTTGTGGAACAAAGGTGCTTTATA	*GSTU46*	PtGSTU46RT3	AATTTACTGAGGAGAAGTGTCTGATG
	PtGSTU5RT2	CATATCTTCTTCCCAAAATAATTTACC		PtGSTU46RT4	CAGCCCATTGCTAGATCTAAATAA
GSTU6	PtGSTU6RT1	GATTTGTGGAACAAAGGTGATTTACT	*GSTU47*	PtGSTU47RT1	AACTTCAAGGACGAGCCCATAA
	PtGSTU6EX2	TCTCGAGTTACTACTCAAGCCCAAACT		PtGSTU47RT2	ACGGTATGCCCTTCAGTTTCAG
GSTU7	PtGSTU7RT3	GAATCCTGTTCACTCGAAAATCCT	*GSTU48*	PtGSTU48RT1	AACTTCAAGGACGAGCCCATAA
	PtGSTU7RT4	CGAGGTCATATATCTTTTTGTCAACA		PtGSTU48RT2	ACGGTATGCCCTTCAGTTTCAG
GSTU8	PtGSTU8RT3	TGTCGACAAAAAGATATATGACTTTG	*GSTU49*	PtGSTU49RT1	TGACAAGAAGGCGTACCGTTC
	PtGSTU8RT4	GAAAGGAAGTAGTGCTACATCAACAT		PtGSTU49RT2	GCAATCAACTTGGGGCACTC
GSTU9	PtGSTU9RT3	GTCCATGATGGAAAACCTATTTG	*GSTU50*	PtGSTU50RT1	CATGGCCTAGTCCTTTCAGTTATA
	PtGSTU9RT4	CCAAGGTCATATAACTTTTTGTCAA		PtGSTU50RT2	GAGGAAATTTCTGGGGTTCA
GSTU10	PtGSTU10RT1	TGTTGACAAAAAGATATATGCCCTC	*GSTU51*	PtGSTU51RT2	CACTCCATTCATCTTCTCTATGGC
	PtGSTU10RT2	TCATAGGCATAAAACCAGCAAAG		PtGSTU51EX3	AGAATTGCCCTTAATATTAAATCTGC
GSTU11	PtGSTU11RT1	TGTTAAAGAGGAAGAGCTTGAGATG	*GSTU52*	PtGSTU52RT1	TTGTTATTCTTGAGTTTATCGATGAG
	PtGSTU11RT2	AAGGCTATATCTGCCATCCCTAC		PtGSTU52RT2	GCTTCAACGGCCAGTTTTAT
GSTU12	PtGSTU12RT3	GGTGGAGACAGTATTGGGATAGC	*GSTU53*	PtGSTU53RT1	TTAAGCTGCTAGGAACATGGC
	PtGSTU12RT4	CTTAAAGAAGGTAACCGACCAGC		PtGSTU53RT2	CGAGGAAATTTCTGACGTTCC
GSTU13	PtGSTU13RT1	TTGCGAGTCCATGATCATTGTG	*GSTU54*	PtGSTU54RT3	CTGGGCAGATTTTGTTGATAAGA
	PtGSTU13RT2	CCTTTAGCATGAAACATTCTCCG		PtGSTU54RT4	CATCAACGTAACCGAGGGTGTC
GSTU14	PtGSTU14RT1	CATAAGAAGGTTCCGGTACTTGTTC	*GSTU55*	PtGSTU55RT1	CATCGACAAGAAGATGTATCCTACTG
	PtGSTU14RT2	TTCTCTTCTTCAGTCGCTCGG		PtGSTU55RT2	AACTCGGCAACCATGCTCC
GSTU15	PtGSTU15RT3	TTGACAAGAAGATATATAACAATGCC	*GSTU56*	PtGSTU56RT1	TCAGATGTTCAGGAGAGAACTCGA
	PtGSTU15RT4	TCCATGCAGTGATCTTTGGAC		PtGSTU56RT2	GTATCGGTCAGGTAGGTTCTTGG
GSTU16	PtGSTU16RT1	ATTGAGGCTGAGTGTCCAAGGT	*GSTU57*	PtGSTU57RT1	GCCTCTTGCCTGATGATCCTT
	PtGSTU16RT2	TTCTTCTTGAGCAGCAACACGT		PtGSTU57RT2	CTGAGACTCCATTGGGCAAAAG
GSTU17	PtGSTU17RT3	CATTGACAAGAAGATATATAACAATGC	*GSTU58*	PtGSTU58RT1	TGAAACGTCGGAAGCAGTCTCC
	PtGSTU17RT4	CATGCAGCGATCATTGGAA		PtGSTU58RT2	GCAATAAAAAGGGACTAACGCAAC
GSTU18	PtGSTU18RT1	TGATGACAAGGTCCTCCAGTCA	*GSTF1*	PtGSTF1RT1	TAGTGTCCCAGTAGACGAAGCCT
	PtGSTU18RT2	CAAGGTCTGCTAGTCCAATTGC		PtGSTF1RT2	AGTCCACCACCTGTTTACATTCTT
GSTU19	PtGSTU19RT1	TGACATCTCATATGGACTATTCGTTTA	*GSTF2*	PtGSTF2RT1	TTCGCACCAAGAATGAATATCC
	PtGSTU19RT2	GCAACTTATCATTGTCGCGATG		PtGSTF2RT2	AAAGATGTCCCTTGTCCGTAGC
GSTU21	PtGSTU21RT1	GCTCGGTTTTGGATTCAATATG	*GSTF3*	PtGSTF3RT1	ACCTCTCGTGTTCTGACTTGTCTG
	PtGSTU21RT2	AAGCCAACAAGTGAAGAGTCCAA		PtGSTF3RT2	CGTAGCCAGTTTCCTTGAATCTT
GSTU22	PtGSTU22RT3	TTGGGGTGGTTGAGAACCAC	*GSTF4*	PtGSTF4RT1	TAAAGTCCACGGAAGCACCC
	PtGSTU22RT4	TAGGAGTTCGAGCTGGCTGC		PtGSTF4RT2	CCGTCGGCAATGTATTGAGTG
GSTU23	PtGSTU23RT1	GAGCCATGATACGCTTTTGGA	*GSTF5*	PtGSTF5RT1	TGAAACTCCATGGAAGCGTTT
	PtGSTU23RT2	TATCTTTTCTATGACGTCAACGGTT		PtGSTF5RT2	CCAGTTGAGTCCCCTTGCTG
GSTU24	PtGSTU24RT1	ACTCTTGCAATACAATCCTATCCAC	*GSTF6*	PtGSTF6RT1	TTGAAACTCCATGGAACCCCTA
	PtGSTU24RT2	TTGATTGCAAAACCTTATCATCAC		PtGSTF6RT2	CCCAGTTGAGTCCCCTTGCTA
GSTU25	PtGSTU25EX1	AGATATCATGGCAGAAGTGAAGCTACATG	*GSTF7*	PtGSTF7RT1	CTCTCGCGTGCTGACATGC
	PtGSTU25EX2	TGAATTCTTACTAGTTCTTGCCTAACTTCTCC		PtGSTF7RT2	GTAGCCAGTCCCCTTGAACTTC

Supplementary Table 1. Primers designed by Lan et al. (2009) were initially used for RT-PCR in this experiment (continued)

Gene	Primer name	Sequence (5'-3')	Gene	Primer name	Sequence (5'-3')
GSTU26	PtGSTU26RT1	TTTGCTGAAGAGAAGTGTTTGATG	*GSTF8*	PtGSTF8RT1	TGAAGACCGTGGACCGAATC
	PtGSTU26RT2	CAGCTTCTTCCATAACATTCAGC		PtGSTF8RT2	TTTTCCTCGTAAGTCCTGACCAG
GSTU27	PtGSTU27RT1	CTACTGGATTTTGAGATGAGTCCC	*GSTF9*	PtGSTF9RT1	CCCGTGTATTGCTTTGCTTATCT
	PtGSTU27RT2	CAATAGGATAAATCTTCTTGTCGATG		PtGSTF9RT2	TTGCTCGTGATTCTGACAATAGTG
GSTU28	PtGSTU28RT1	TGGATAAACATATTTTCCCAAACG	*DHAR1*	PtGSTH1RT1	AAGGGTATTGCTTACTTTGGAGG
	PtGSTU28RT2	TAGAAAAAGTTGAAGAAGGGAATAAGT		PtGSTH1RT2	AGGTGGAATTGCCAATGGTG
GSTU29	PtGSTU29RT1	TGGATAAACATATTTTCCCAAACG	*DHAR2*	PtGSTH2RT1	TCATCTTAAAGCACACGGTCCA
	PtGSTU29RT2	AATCGGCTTTCCTTCATGGA		PtGSTH2RT2	ATGATATGTTCCTTAGCAGCCTTG
GSTU30	PtGSTU30RT1	TTGATGAGGTGTGGAAGGATAGC	*DHAR3*	PtGSTH3RT1	GGCATCTCAAGGGTCCTTTCAT
	PtGSTU30RT2	AGTGCTATATCAACGTAGCCGATG		PtGSTH3RT2	ACATGTTCCTCGGCAGCTCTT
GSTU31	PtGSTU31RT1	GGACTTGAGGGACAAGAGTGCA	*GSTL1*	PtGSTL1RT1	CACAATAACGAAGTGAAAGGAGAGTC
	PtGSTU31RT2	GAAGGTAGCAAAGGAGTCTTGCC		PtGSTL1RT2	TTTGAATGTGGAATTATTGGCTTT
GSTU32	PtGSTU32RT1	CGAGGGACGAGATCACAAAAT	*GSTL2*	PtGSTL2RT1	CTCCTACACTGATACATTCAACCGT
	PtGSTU32RT3	ATCTCAAATACCTTCTTGTCAATAAAA		PtGSTL2RT2	AACACTTCTGACAGGAAGATGCAG
GSTU33	PtGSTU33RT1	GAACTTGAGGGACAAGAGTGCAT	*GSTL3*	PtGSTL3RT1	AATTCAACGGCACAGTGTATACTG
	PtGSTU33RT3	TCTCGAATACCTTCTTGTCAATAAAA		PtGSTL3RT2	GCAAGAAGATATTGAGTCTTTCAACA
GSTU34	PtGSTU34RT1	TGACAAGAAGGTTTTTGATGCAAC	*GSTT1*	PtGSTT1RT1	CATGGTGCAGCTGAATATGTCA
	PtGSTU34RT2	CCACTATATCGACAAACCCAAAGG		PtGSTT1RT2	GCTGCAATCCTTTTCATCCAA
GSTU35	PtGSTU35RT1	TGACAAGAAGGTTTTTGATGCAAC	*GSTT2*	PtGSTT2RT1	TCGCGGCTCAGTTGAATTTAT
	PtGSTU35RT2	CCACTATATCGACAAACCCAAAGG		PtGSTT2RT2	GCTATGGATGGTTGGCTACTGC
GSTU36	PtGSTU36RT3	GAGTTATCAAGGGTGCTTCCTTT	*GSTZ1*	PtGSTZ1RT1	AAATCTCCAAGTTGCCAGTATCG
	PtGSTU36RT4	CTTTCACCGCCGAAAAACTT		PtGSTZ1RT2	GTAGCAATCTGTGGGGCCAA
GSTU37	PtGSTU37RT1	AAGGGTGCTGCCTTTAGTGC	*GSTZ2*	PtGSTZ2RT1	TGGTCCAGATGAGGTGATTCC
	PtGSTU37RT2	AGTCTCTTCATGGAGCCAGTCAC		PtGSTZ2RT2	CCTTGATAGAAGAGGGAACTGAGTC
GSTU38	PtGSTU38RT1	CAAGGGTGCTTCCTTTAGGGA	*EF1Bγ1*	PtGSTG1RT1	ATTTTGCGCTGGTTCATACCA
	PtGSTU38RT2	CGAGGCAAAGTGGCTGGTTC		PtGSTG1RT2	AAATCCCAAAGTCAGGTTACAGG
GSTU39	PtGSTU39RT1	GACTTGATGAACGGAAAGAGTGAT	*EF1Bγ2*	PtGSTG2RT1	CGTATTGTATGCCGGTAAGACG
	PtGSTU39RT3	CCTAGAAAGATCATTTATCTTTTTATCAA		PtGSTG2RT2	CAATTGCATTGCTCTCGAAGATA
GSTU40	PtGSTU40RT3	ACTACATCGACAAGAAGATTTATCCTAT	*EF1Bγ3*	PtGSTG3RT1	ACTCCTGAAGGTCCCGTGTTT
	PtGSTU40RT4	GGTGTAAAAGAAGCTGTAGAACGG		PtGSTG3RT2	CTGGAGGAAGGTACGGTTGAT
GSTU41	PtGSTU41EX1	AGATATCATGGAAAAGGGAGTGAAACTTTTTAA	*TCHQD1*	PtTCHQ1RT1	TGGCTCGCTTAGTCCTCTTGAA
	PtGSTU41EX2	TGAATTCTTATCAGGCATGAGGTGCTGC		PtTCHQ1RT2	ACTTCTGAAACGAACAAAGCACC