Transgenic Arabidopsis Plants Expressing Tomato Glutathione S-Transferase Showed Enhanced Resistance to Salt and Drought Stress

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Abstract

Although glutathione S-transferases (GST, EC 2.5.1.18) are involved in response to abiotic stress, limited information is available regarding gene function in tomato. In this study, a GST gene from tomato, designated LeGSTU2, was cloned and functionally characterized. Expression profile analysis results showed that it was expressed in roots and flowers, and the transcription was induced by salt, osmotic, and heat stress. The gene was then introduced to Arabidopsis by Agrobacterium tumefaciens-mediated transformation. Transgenic Arabidopsis plants were normal in terms of growth and maturity compared with wild-type plants. Transgenic plants also showed an enhanced resistance to salt and osmotic stress induced by NaCl and mannitol. The increased tolerance of transgenic plants was correlated with the changes in proline, malondialdehyde and antioxidative enzymes activities. Our results indicated that the gene from tomato plays a positive role in improving tolerance to salinity and drought stresses in Arabidopsis.

Introduction

Environmental stress factors, such as drought and salinity, affect plant growth and development by causing osmotic stress and water deficit [1]. Plants develop various biochemical and physiological mechanisms to respond and adapt to drought and salt stresses. One of these mechanisms is the expression of some stress-inducible genes (e.g. enzymes involved in the biosynthesis of various osmoprotectants) to counteract the detrimental conditions [2-3].

Glutathione S-transferases (GSTs; EC 2.5.1.18) are a well-characterized detoxification enzyme family involved in stress tolerance, which catalyze the conjugation of reduced tripeptide glutathione (GSH) to electrophilic substrates. GSTs were first discovered because these enzymes can metabolize various toxic exogenous compounds (xenobiotics) by GSH conjugation [4]. Plant GSTs are commonly known for their role in herbicide detoxification. Besides,
Plants GSTs are also considered as glutathione peroxidases that directly detoxify electrophiles. Furthermore, GSTs function as non-enzymatic carriers (ligandins) in intracellular transport and catalyze anthocyanin-GSH conjugates, thereby allowing transport into vacuoles via a glutathione pump [5]. Moreover, several plant GSTs exhibit peroxidase activity and may play roles in enhancing tolerance to chilling, osmotic dehydration, and herbicide-induced damage [6–8].

Plants GSTs are classified into eight classes: phi, tau, zeta, lambda, glutathione-dependent dehydroascorbate reductases (DHARs), tetrachlorohydroquinone dehalogenase (TCHQD) and membrane associated proteins in eicosanoid and glutathione metabolism (MAPEG). Among these eight classes, phi and tau are the largest plant-specific and often highly stress-inducible GSTs [9]. The role of GSTs in stress has been demonstrated in several transgenic studies, and over-expression of GSTs in tobacco [8] and rice [10] increased transgenic plant capability to endure harsh treatments such as high and low temperatures and salt concentration. Kumar et al. showed that over-expressing of OsGSTL2 enhanced the tolerance to heavy metals and other abiotic stresses like cold, osmotic and salt in GST transgenic plants [11]. Moreover, expression of ThGSTZ1 from Tamarix hispida in Arabidopsis can improve drought and salinity tolerance in transgenic plants [12]. Transgenic tobacco over-expressing cotton GST showed enhanced resistance to methyl viologen [13]. A recent study on AtGSTU17 showed that it plays a negative role in drought and salt stress tolerance. When AtGSTU17 was mutated, plants were more tolerant to drought and salt stresses compared with wild-type plants [14].

Although many GST genes have been cloned from Arabidopsis [14–15], rice [16], tobacco [17], soybean [18], maize [19], poplar [20], sorghum [21] and other plants, the role of a GST gene from tomato has been rarely investigated. In this study, a GST coding sequence named LeGSTU2 from tomato (Lycopersicon esculentum) was cloned by PCR method and was functionally characterized by heterologous expression in Arabidopsis. Detailed analysis carried out on transgenic lines developed in this study suggests the role of LeGSTU2 in abiotic stresses tolerance, particularly in osmotic and salt stress conditions.

Materials and Methods

Plant materials, growth conditions, and treatments

Plants (Arabidopsis thaliana ecotype Columbia L.) were grown in a growth chamber at 22°C on MS medium or in pots filled with vermiculite/peat moss/perlite (9:3:1) mixture. The plants were kept in a 16/8h day/night cycle at a light intensity of ~120μmol photons m⁻² s⁻¹.

Tomato seeds (Lycopersicon esculentum) were sterilized with 75% (v/v) ethanol for 5 min, followed by commercial bleach (0.5% sodium hypochlorite) for 20 min, then rinsed thrice with sterile deionized water and sown onto plates containing MS with 1% agar and 4% (w/v) sucrose. The plates were placed in the dark for 3–4 d. After the seeds germinated, the plates were transferred to a controlled environment chamber at 22°C and subjected to a 16/8 h day/night cycle. After 7 d, the seedlings were transplanted to pots (vermiculite/peat moss/perlite mixture, 9:3:1). Three-week-old tomato seedlings were watered with 200 mM NaCl, 50 mM mannitol, 4°C (cold) or 40°C (heat) for 0, 1, 3, 6, 12, and 24 h, respectively. The whole plants at each treatment or the different organs (roots, stems, leaves, flowers or fruits) from untreated plants were pooled, frozen in liquid N₂, and stored at -70°C.

Total RNA extraction and reverse transcription

Total RNA was isolated using a Multisource Total RNA Miniprep kit (Axygen Scientific, CA, USA) according to the manufacturer’s instructions. First-strand cDNA synthesis was
conducted with 5 μg of total RNA by using a TransScript Fly First-strand cDNA Synthesis SuperMix (Transgen Biotech, Shanghai, China).

Cloning and sequence analysis of LeGSTU2

The LeGSTU2 (GenBank accession number: AY082341) coding sequence was cloned from tomato (L. esculentum) cDNA using PCR method with specific primers for LeGSTU2 genes, forward: LeGSTF2: 5’-AAGGATCCATGGCTAATGATCAGGTG-3’ and reverse: LeGSTZ2: 5’-AAGAGCTCTTATTCAAGTGTTCCAG3’). Clones containing the LeGSTU2 coding sequence were further sequenced from both sides to confirm their sequences. Molecular weight (MW) and isoelectric point (pI) predictions for the deduced protein were performed using the Compute pI/Mw tool (http://web.expasy.org/protparam/). Phylogenetic tree construction was performed using the Clustal W2 program.

Real-time PCR

Tomato seedlings from different treatments (as described in the previous section) were harvested to analyze LeGSTU2 expression profile. Total RNA was isolation and reverse-transcribed as described in a previous section. PCR amplification was performed with specific primers for LeGSTU2 genes, forward: LeGSTF1: 5’-GGGAGACGAACAAGAGGC-3’ and reverse: LeGSTZ1: 5’-CCACAAGGTTTGGGCACT-3’. Amplification of tomato Actin gene (GenBank accession number: BT012695) was used as an internal control [22]. Primers as following: forward: LeAcF: 5’-TGAAATGTGACGTGGATATTAGG-3’ and reverse: LeAcZ: 5’-TGAGGGGAGCCAAGGATAGGC-3’.

Arabidopsis plants of three-week-old were harvested to analyze the positive transformants. Specific primers information for LeGSTU2 was mentioned in the previous part. A. thaliana actin gene (AtActin2, GenBank Accession number: U41998) was used as an internal control, primers as following: forward: AtAc2F: 5’-AGTAAGGTCACGTCCAGCAAGG-3’ and reverse: AtAc2Z: 5’-GCACCTGTGTTCTTACCGAG-3’.

The cDNA was amplified using SYBR Premix Ex Taq (TaKaRa, Shanghai, China) as described [23]. The PCR program was 94°C, 1min, 35 cycles of 94°C, 20s; 54°C, 20s; 72°C, 20s, 81°C for 1s for plate reading. Each sample reaction was carried out in triplicate to ensure the reproducibility of the results.

Vector construction and Arabidopsis transformation

The complete ORF of LeGSTU2 was amplified by PCR using gene-specific primers as described, cloned in TA clone vector Simple pMD-18 (Takara) and sequenced. The fragment was then cloned in the plant expression vector pYK4102 [23] at BamHI and SacI sites under the control of CaMV35S promoter. The construct was mobilized into A. tumefaciens GV3101 and transformed in plants to generate GST transgenic lines of Arabidopsis by using a floral dip method [24]. Positive transformants in hygromycin (50 μg/ml) plates were selected and confirmed by PCR using the same primers. T3 generation was selected for further experiments.

GST activity measurement

GST activity was determined according to Ji et al. [25] with minor modification. Fresh leaf tissues (~ 0.1 g) were obtained from three-week-old plants grown under normal conditions. The leaves were harvested and homogenized in 1.5 ml of chilled 50 mM phosphate buffer by using a chilled pestle and mortar. The homogenate was centrifuged at 20,000g for 10 min in a refrigerated centrifuge at 4°C. The supernatant was stored at 4°C and used for enzyme assays within
Enzyme activity was evaluated in the presence of 1mM glutathione in 100mM sodium phosphate buffer (pH 6.5). The reaction was initiated by adding 1-chloro-2, 4-dinitrobenzene (CDNB) to a final concentration of 1mM. Changes in A340 were measured and background levels of spontaneous CDNB decay were subtracted.

### Analysis of stress tolerance in transgenic lines

Stress tolerance was analyzed using three homozygous transgenic lines (OE3, OE7 and OE10) expressing *LeGSTU2* in T3 Arabidopsis generation. To evaluate the effect of abiotic stresses on WT and transgenic plants, seeds were germinated on MS media containing NaCl (150 mM and 200 mM) and mannitol (150 mM and 300 mM). Seed germination was subsequently monitored and recorded at an interval of 12h during growth. Root length was recorded after 10 d of germination.

To evaluate salt/drought stress tolerance, three-week-old plants were watered with NaCl (250 mM) as salt stress, or withheld watering for two weeks as water deficit stress. Then the plants were irrigated again and the performances of WT and transgenic plants were compared.

To measure the physiological parameters involved in stress tolerance, three-week-old plants were treated with 250mM NaCl (as salt stress) or 300 mM Mannitol (as osmotic stress) for 5d, and the proline content, MDA content, chlorophyll content, SOD and POD activity were measured according to the study of Diao et al. [26]. Proline was assayed on water-extracted seedlings using the ninhydrin assay. The malondialdehyde (MDA) content was determined by the reaction of thiobarbituric acid (TBA). Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined by monitoring the inhibition of photochemical reduction of nitro blue tetrazolium.

Aerial parts from three-week-old plants were excised to analyze the standardized water content. The loss in fresh weight was monitored at indicated times. Detached aerial parts were then dried at 80°C for 5h to determine dry weight. It was calculated as \((\text{FW} - \text{DW}) / (\text{FW}_0 - \text{DW})\) × 100, where FW₀ and FWᵢ are fresh weight for original and any given internal fresh weight, respectively, and DW is dry weight. These tests were conducted in the controlled environment chamber at 22°C.

### Statistical analysis

ANOVA was performed using Duncan’s multiple comparison tests. Statistically significant differences \((P < 0.05)\) are reported in the text and shown in the figures.

### Results

#### Cloning and characterization of *LeGSTU2*

The coding region of *LeGSTU2* was 663 bp in length and was predicted to encode a 220 amino acid protein with a calculated MW of 25.4 kDa and a pl of 5.51. Multiple sequence alignments showed that *LeGSTU2* share the highest similarity with *AtGSTU19* (At1g78380), *AtGSTU6* (At2g29440), *AtGSTU2* (At2g29480) and *AtGSTU4* (At2g29460), which suggests that *LeGSTU2* may be a lambda-type GST (Fig 1).

#### Expression of *LeGSTU2* transcripts

Different organs of tomato seedlings were harvested, and the expression of *LeGSTU2* was analyzed by real-time PCR to examine the expression profile. The results showed that *LeGSTU2* was highly expressed in roots and flowers, suggesting that *LeGSTU2* could participate in root growth and flower development (Fig 2A).
The expression of LeGSTU2 under stress conditions was determined by Real-Time PCR. The results showed similar patterns in response to salt, osmotic and heat treatment. LeGSTU2 transcripts were detected in 3h and the highest transcript level was reached at 6h after salt or osmotic treatment was initially administered (Fig 2B). Under heat stress, LeGSTU2 transcripts were analyzed and the highest level was reached at 3h. These observations suggested that LeGSTU2 was induced by salt, osmotic and heat stress. However, LeGSTU2 expression was repressed by low temperature.

Expression of LeGSTU2 in Arabidopsis

LeGSTU2 cDNA was cloned into plant expression vector pYK4102 (Fig 3A) and introduced to Arabidopsis cells by using A. tumefaciens-mediated floral dip method. Ten independent lines of transgenic plants (T1 generation) were generated. Among these lines, three homozygous
Fig 2. Expression profile of LeGSTU2 in tomato. a. The expression of LeGSTU2 in different organs by real-time PCR. Total RNA was isolated from different organization (roots, stems, leaves, flowers or fruits) of tomato seedlings. The experiment was replicated three times and the mean value is shown. b,
transgenic lines (named OE3, OE7 and OE10) expressing LeGSTU2 of T3 generation were selected for further experiments.

RT-PCR analysis results confirmed that LeGSTU2 was detected in the three transgenic lines in the T3 generation, and no amplification was observed in WT (Fig 3B). GST activity levels were analyzed in both transgenic plants and WT. Compared with WT, different transgenic lines showed a significantly enhanced GST activity when CDNB was used as substrate (Fig 3C). No obvious effects on growth and development were observed in LeGSTU2 transgenic plants under normal growth conditions.

Response of transgenic lines to salt stress and osmotic stress

Transgenic Arabidopsis lines over-expressing LeGSTU2 gene were germinated in medium containing different concentrations of NaCl (150 and 200mM) as salt stress or Mannitol (150 and 300mM) as osmotic stress to analyze the role of LeGSTU2 under stress. There was no significant difference in the germination rates and root length between WT and transgenic plants under normal growth conditions. However, the germination rate and root length were increased in the transgenic lines compared with WT under salt and osmotic stress conditions (Fig 4). Under salt stress, the average germination rates of transgenic seedlings were 93.5%, 63.4%, compared with 68.9%, 29.4% of the WT in the medium (96h) containing 100 and 150 mM NaCl, respectively (Fig 4A). Under 300 mM mannitol stress conditions, the germination rate of WT was 68.6%, while the average germination rate of transgenic plants was 96.5% (Fig 4B).

The growth of both transgenic plants and WT was significantly repressed by both salt and osmotic stress, but the growth of WT was obviously decreased compared to that of the transgenic lines. The average root lengths of the transgenic lines were 2.2-, 1.7-fold more than the WT under 150mM, 200mM NaCl treatments, respectively (Fig 4C). Likewise, the average root lengths of the transgenic lines were 1.3-, 1.8-fold longer than WT under 150mM, 300mM mannitol, respectively (Fig 4D). These results suggested that transgenic plants enhanced tolerance to salt and osmotic stress.

Stress tolerance of transgenic plants

Three-week-old seedlings were watered with NaCl (250 mM) for salt stress, or with withheld watering for water deficit stress to further analyze salt/drought tolerance. After two weeks, some plants exhibited lethal effects, then the plants were irrigated again. The plants were photographed at 15 d after these plants were exposed to stress, the survival rate was then measured (Fig 5). Under salt stress condition, >80% of the transgenic plants survived, whereas only 20% of WT survived. Under water deficit stress for two weeks, >80% of the transgenic plants survived, whereas approximately 20% of WT plants survived.

The water content was analyzed further. When the leaves of WT and transgenic plants were exposed to air for 2h, the average water content rate of the transgenic plants was 82.3%, whereas the rate was 79.3% in WT. At 8h, the average water content rate of transgenic plants was 45.7%, which was 1.4-fold higher than that of WT. These results indicated that transgenic plants presented a higher capacity to conserve water than those of WT plants (Fig 6).

Proline, MDA and chlorophyll contents were analyzed (Fig 7A–7C) and the results showed that there were no difference between transgenic plants and WT. Under stress condition,
proline and total chlorophyll contents increased in both transgenic plants and WT. However, the contents of proline and chlorophyll were significantly higher in transgenic plants than in
WT plants during NaCl and mannitol stresses. The MDA content was also increased in both genotypes under stress condition, and WT showed significantly higher MDA content than that of transgenic plants, which means WT plants exhibited higher rates of cell damage than transgenic lines under stress conditions.
The activities of SOD and POD were also measured (Fig 7D and 7E) and the results showed no significant difference between transgenic and WT plants under normal conditions. Under...
stress conditions, the SOD and POD activity increased in both the transgenic and WT plants. However, SOD and POD activities in transgenic lines were significantly higher than in the WT plants during NaCl and mannitol stresses. Specifically, after 5d of NaCl stress treatment, the SOD activity levels of transgenic lines were 33% higher than that of WT, and the POD activity levels were 62% higher than in the WT. After 5d of mannitol treatment, the SOD and POD activity levels of transgenic plants were 34%, 73% higher than WT levels, respectively. The transgenic plants exhibited higher levels of GST activity during NaCl and mannitol stresses.

**Fig 6. Standardized water content of different lines.** Each data point is the mean ± SD of three replicates, each from ≥10 plants.

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Taken together, these results suggest that \textit{LeGSTU2} maintains cellular ROS homeostasis by scavenging ROS and preventing cell membrane damage. \textit{LeGSTU2} appears to play an important role in plant resistance to salt and drought stress.

**Discussion**

Plants often encounter various environmental stresses, thereby generating reactive oxygen species (ROS), which likely cause membrane lipid peroxidation and yield highly cytotoxic products of oxidative DNA damage [5]. GST can be induced by diverse environmental stimuli. For instance, increased GST levels are used to maintain cell redox homeostasis and protect
organisms against oxidative stress [14]. Although many GST genes have been cloned and analyzed, the role of a GST gene from tomato has been rarely investigated. In this study, LeGSTU2, a GST gene from tomato, was cloned and introduced to Arabidopsis to evaluate gene function during abiotic stress. Our results revealed that transgenic plant lines over-expressing the LeGSTU2 gene improved drought and salinity tolerance of Arabidopsis.

Expression pattern analysis results revealed that LeGSTU2 was highly expressed in roots and flowers; the transcript expression levels of LeGSTU2 were induced by salt, osmotic and heat stress. A similar pattern was previously observed in AtGSTU19, (also named AtGST8, [27]), OsGSTU3 and OsGSTU4 [28]. The accumulation of AtGSTU19 was induced by drought-associated oxidative stress [27], and its role in drought/oxidative tolerance was demonstrated in our recent findings. OsGSTU3 and OsGSTU4 are polyethylene glycol (PEG)-induced tau class GSTs, which were identified in rice roots. Salt stress, phytohormones, antioxidants and hydrogen peroxide (a strong oxidant) can rapidly induce OsGSTU3 and OsGSTU4 expression in rice roots. However, the roles of these two genes in stress tolerance have not been reported. LeGSTU2 in stress tolerance was functionally analyzed using the transgenic lines of Arabidopsis. Under normal conditions, transgenic plants showed no superiority in terms of germination and root growth to WT. However, when treated with NaCl or mannitol, transgenic plants showed higher germination rate and longer root growth than the WT, which suggests that LeGSTU2 may play a role in the tolerance phenotype. Previous studies have shown that transgenic parB (GST) lines showed higher levels of root growth and relative biomass than Ler-0 when exposed to 100mM Na stress, which indicates that such phenotypes are derived from the expression of parB [29]. A recent study on the characterization of ThGSTZ1 from Tamarix hispida showed similar results to those described above [12]. Furthermore, transgenic plants displayed the tolerance to salt/drought stress and conserved water to a higher extent than WT plants (Figs 5 and 6); these results suggest that LeGSTU2 may play a positive role in the tolerance to salt/drought stress.

ROS homeostasis is important in protecting the normal metabolism. Plants can regulate ROS levels through ROS scavenging enzymes, such as SOD, POD and GST [30]. Moreover, over-expression of some GST genes has been shown to improve oxidative stress tolerance levels in transgenic plants [31]. In this study, over-expression of LeGSTU2 showed increased activities of SOD and POD under stress conditions. It has been reported that over-expression of a specific antioxidant gene could influence the expression of other antioxidant genes; indeed transgenic GST expressing seedlings were shown to have a higher APX activity and MDHAR activity compared to nontransformed seedlings [8]. Tobacco plants expressing the GST gene showed an increase in GR activity [32]. Our results suggested that LeGSTU2 may act as a stress regulator through increasing the activity of antioxidant enzymes to strengthen the ROS scavenging ability or maintain ROS homeostasis.

Proline, as a compatible solute, functions in defense to maintain turgor pressure against osmotic challenge caused by water deprivation or extreme salinity. Proline also protects plant cells against oxidative damage by quenching \textsuperscript{1}O\textsubscript{2} and directly scavenging HO. [33]. Proline accumulation is also positively correlated with drought/salinity tolerance [34–35]. Our experiments showed that transgenic plants accumulated more proline contents under salt and drought stress than WT (Fig 7); thus, stress tolerance of transgenic plants was enhanced. Water deficit and salinity often cause rapid and excessive accumulation of ROS in plant cells, subsequently resulting in lipid peroxidation and accumulation of MDA [36–37]. In our study, MDA accumulation occurred in transgenic plants under salt stress to a less extent than in WT plants, this result suggested that lipid peroxidation was decreased in LeGSTU2 transgenic plants. Relatively stable chlorophyll content was also observed in transgenic plants, this result indicated that these plants can maintain photosynthesis better than WT under stress conditions.
Photosynthesis, photorespiration and light signaling in defense responses are correlated with ROS regulation [38]. Far-red insensitive 219 (FIN219)-interacting protein 1 (FIP1), a tau class GST gene from *Arabidopsis*, can interact with FIN219 to regulate cell elongation and flowering in response to light [39]. *AtGSTU17*, another tau class GST gene, is also regulated by multiple photoreceptors, particularly phytochrome A under all light conditions, *AtGSTU17* also participates in various aspects of seedling development [15]. In conclusion, *LeGSTU2* possibly plays a positive role in the tolerance to salt/drought stress in *Arabidopsis*. Further analyses, particularly on the role of phytohormones, are awaited to elucidate the function and the regulatory mechanism of *LeGSTU2* in plant stress responses.

**Author Contributions**

Conceived and designed the experiments: JX QHY. Performed the experiments: JX XJX. Analyzed the data: YST YX. Contributed reagents/materials/analysis tools: RHP WZ. Wrote the paper: JX XJX.

**References**

1. Jakab G, Ton J, Flors V, Zimmerli L, Metraux JP, Mauch-Mani B. Enhancing Arabidopsis salt and drought stress tolerance by chemical priming for its abscisic acid responses. *Plant Physiol.* 2005; 139 (1):267–74. Epub 2005/08/23. doi: 10.1104/pp.105.056598 PMID: 16113213; PubMed Central PMCID: PMC1203376.

2. Saijo Y, Hata S, Koyozuka J, Shimamoto K, Izui K. Over-expression of a single Ca2+-dependent protein kinase confers both cold and salt/drought tolerance on rice plants. *Plant J.* 2000; 23(3):319–27. Epub 2000/08/06. doi: tpj787 [pii]. PMID:10929125.

3. Zhu JK. Salt and drought stress signal transduction in plants. *Annu Rev Plant Biol.* 2002; 53:247–73. Epub 2002/09/12. doi: 10.1146/annurev.arplant.53.091401.143329 PMID: 12221975; PubMed Central PMCID: PMC3128348.

4. Cummins I, Dixon DP, Freitag-Pohl S, Skipsey M, Edwards R. Multiple roles for plant glutathione transferases in xenobiotic detoxification. *Drug Metab Rev.* 2011; 43(2):266–80. Epub 2011/03/24. doi: 10.3109/03602532.2011.552910 PMID: 21425939.

5. Marrs KA. The Functions and Regulation of Glutathione S-Transferases in Plants. *Annu Rev Plant Physiol Plant Mol Biol.* 1996; 47:127–58. Epub 1996/06/28. doi: 10.1146/annurev.arplant.47.1.127 PMID: 15012285.

6. Bartling D, Radzio R, Steiner U, Weiler EW. A glutathione S-transferase with glutathione-peroxidase activity from Arabidopsis thaliana. Molecular cloning and functional characterization. *Eur J Biochem.* 1993; 216(2):579–86. Epub 1993/09/01. PMID: 8375395.

7. Cummins I, Cole DJ, Edwards R. A role for glutathione transferases functioning as glutathione peroxidases in resistance to multiple herbicides in black-grass. *Plant J.* 1999; 18(3):285–92. Epub 1999/06/23. PMID: 10377994.

8. Roxas VP, Lodhi SA, Garrett DK, Mahan JR, Allen RD. Stress tolerance in transgenic tobacco seedlings that overexpress glutathione S-transferase/glutathione peroxidase. *Plant Cell Physiol.* 2000; 41(11):1229–34. Epub 2000/11/28. PMID: 11092907.

9. Dixon DP, Edwards R. Glutathione transferases. *Arabidopsis Book.* 2010; 8:e0131. Epub 2010/01/01. doi: 10.1199/tab.0131 PMID: 22303257; PubMed Central PMCID: PMC3244946.

10. Takesawa T, Ito M, Kanzaki H, Kameya N, Nakamura I. Over-expression of a glutathion S-transferase in transgenic rice enhances germination and growth at low temperature. *Mol Breed.* 2002; 9:93–101.

11. Kumar S, Asif MH, Chakrabarty D, Tripathi RD, Dubey RS, Trivedi PK. Expression of a rice Lambda class of glutathione S-transferase, OsGSTL2, in Arabidopsis provides tolerance to heavy metal and other abiotic stresses. *J Hazard Mater.* 2013; 248–249:228–37. Epub 2013/02/06. doi: S0304-3894(13)00009-5 [pii] doi: 10.1016/j.jhazmat.2013.01.004 PMID: 23380448.

12. Yang G, Wang Y, Xia D, Gao C, Wang C, Yang C. Overexpression of a GST gene (ThGSTZ1) from *Tamarix hispida* improves drought and salinity tolerance by enhancing the ability to scavenge reactive oxygen species. *Plant Cell Tiss Organ Cult.* 2014; 117:99–112. doi: 10.1007/s12410-014-0424-5

13. Yu T, Li YS, Chen XF, Hu J, Chang X, Zhu YG. Transgenic tobacco plants overexpressing cotton glutathione S-transferase (GST) show enhanced resistance to methyl viologen. *J Plant Physiol.* 2003; 160(11):1305–11. Epub 2003/12/09. PMID: 14658362.
14. Chen JH, Jiang HW, Hsieh EJ, Chen HY, Chien CT, Hsieh HL, et al. Drought and salt stress tolerance of an Arabidopsis glutathione S-transferase U17 knockout mutant are attributed to the combined effect of glutathione and abscisic acid. Plant Physiol. 2012; 158(1):340–51. Epub 2011/11/19. doi: 10.1104/pp.111.181875 pp.111.181875 [pii]. PMID: 22095046; PubMed Central PMCID: PMC3252094.

15. Jiang HW, Liu MJ, Chen IC, Huang CH, Chao LY, Hsieh HL. A glutathione S-transferase regulated by light and hormones participates in the modulation of Arabidopsis seedling development. Plant Physiol. 2010; 154(4):1646–58. Epub 2010/10/12. doi: 110.15915 [pii] doi:10.1104/pp.110.159152 PMID: 20935176; PubMed Central PMCID: PMC2996023.

16. Jain M, Ghanashyam C, Bhattacharjee A. Comprehensive expression analysis suggests overlapping and specific roles of rice glutathione S-transferase genes during development and stress responses. BMC Genomics. 2010; 11:73. Epub 2010/01/30. doi: 10.1186/1471-2164-11-73 1471-2164-11-73 [pii]. PMID: 20109239; PubMed Central PMCID: PMC2825235.

17. Hoque MA, Uraji M, Baru MN, Mori IC, Nakamura Y, Murata Y. The effects of methylglyoxal on glutathione S-transferase from Nicotiana tabacum. Biosci Biotechnol Biochem. 2010; 74(10):2124–6. Epub 2010/10/15. doi: JST.JSTAGE/bbb/100393 [pii]. PMID: 20944411.

18. Dalton DA, Boniface C, Turner Z, Lindahl A, Kim HJ, Jelinek L, et al. Physiological roles of glutathione s-transferases in soybean root nodules. Plant Physiol. 2009; 150(1):521–30. Epub 2009/03/13. doi: pp.109.136630 [pii] doi:10.1104/pp.109.136630 PMID: 19279195; PubMed Central PMCID: PMC2675717.

19. Sytynska-Hinz H. Expression patterns of Glutathione Transferase Gene (Gt) in maize seedlings under juglone-induced oxidative stress. Int J Mol Sci. 2011; 12(11):7982–95. Epub 2011/12/17. doi: 10.3390/ijms121117982.ijms-12-07982 [pii]. PMID: 22174645; PubMed Central PMCID: PMC3234541.

20. Lan T, Yang ZL, Yang X, Liu YJ, Wang XR, Zeng QY. Extensive functional diversification of the Populus glutathione S-transferase supergene family. Plant Cell. 2009; 21(12):3749–66. Epub 2009/12/10. doi: tpc.109.070219 [pii] doi:10.1105/tpc.109.070219 PMID: 19999377; PubMed Central PMCID: PMC2814494.

21. Chi Y, Cheng Y, Vanitha J, Kumar N, Ramamoorthy R, Ramachandran S, et al. Expansion mechanisms and functional divergence of the glutathione s-transferase family in sorghum and other higher plants. DNA Res. 2011; 18(1):1–16. Epub 2010/12/21. doi: dsq031 [pii] doi:10.1093/dnares/dsq031 PMID: 21169340; PubMed Central PMCID: PMC3041506.

22. Hu ZL, Deng L, Yan B, Pan Y, Luo M, Chen XQ, et al. Silencing of the LeSGR1 gene in tomato inhibits chlorophyll degradation and exhibits a stay-green phenotype. Biologia Plantarum. 2011; 55(1):27–34. PubMed Central PMCID: PMC3268380.

23. Xu J, Tian YS, Peng RH, Xiong AS, Zhu B, Jin XF, et al. AtCPK6, a functionally redundant and positive regulator involved in salt/drought stress tolerance in Arabidopsis. Planta. 2010; 231(6):1251–60. Epub 2010/03/11. doi: 10.1007/s00425-010-1122-0 PMID: 20217124.

24. Zhang X, Henrques R, Lin SS, Niu QW, Chua NH. Agrobacterium-mediated transformation of Arabidopsis thaliana using the floral dip method. Nat Protoc. 2006; 1(2):641–6. Epub 2007/04/05. doi: nprot.2006.97 [pii] doi:10.1038/nprot.2006.97 PMID: 17406292.

25. Ji W, Zhu Y, Li Y, Yang L, Zhao X, Cai H, et al. Over-expression of a glutathione S-transferase gene, GsGST, from wild soybean (Glycine soja) enhances drought and salt tolerance in transgenic tobacco. DNA Res. 2011; 18(1):1–16. Epub 2010/12/21. doi: dsq031 [pii] doi:10.1093/dnares/dsq031 PMID: 21169340; PubMed Central PMCID: PMC3041506.

26. Zhao X, Chen IC, Huang CH, Chao LY, Hsieh HL. A glutathione S-transferase regulated by light and hormones participates in the modulation of Arabidopsis seedling development. Plant Physiol. 2010; 154(4):1646–58. Epub 2010/10/12. doi: 110.15915 [pii] doi:10.1104/pp.110.159152 PMID: 20935176; PubMed Central PMCID: PMC2996023.

27. Moons A. Osgstu3 and osgtu4, encoding tau class glutathione S-transferases, are heavy metal- and specific roles of rice glutathione S-transferase genes during development and stress responses. BMC Genomics. 2010; 11:73. Epub 2010/01/30. doi: 10.1186/1471-2164-11-73 1471-2164-11-73 [pii]. PMID: 20109239; PubMed Central PMCID: PMC2825235.

28. Moons A. Osgstu3 and osgtu4, encoding tau class glutathione S-transferases, are heavy metal- and specific roles of rice glutathione S-transferase genes during development and stress responses. BMC Genomics. 2010; 11:73. Epub 2010/01/30. doi: 10.1186/1471-2164-11-73 1471-2164-11-73 [pii]. PMID: 20109239; PubMed Central PMCID: PMC2825235.

29. Eziuki B, Gardner RC, Ezaki Y, Matsumoto H. Expression of aluminum-induced genes in transgenic Arabidopsis plants. Plant Physiol. 2000; 122(3):657–65. Epub 2000/03/11. PMID: 10712528; PubMed Central PMCID: PMC58900.

30. Jiang Y, Yang B, Harris NS, Deyholos MK. Comparative proteomic analysis of NaCl stress-responsive proteins in Arabidopsis roots. J Exp Bot. 2007; 58(13):3591–607. Epub 2007/10/06. doi:erm027 [pii] doi:10.1093/jxb/erm027 PMID: 17916636.

31. Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 2002; 7(9):405–10. Epub 2002/09/18. doi: S1360-1385(02)02312-9 [pii]. PMID: 12234732.
32. Le Martret B, Poage M, Shiel K, Nugent GD, Dix PJ. Tobacco chloroplast transformants expressing genes encoding dehydroascorbate reductase, glutathione reductase, and glutathione-S-transferase, exhibit altered anti-oxidant metabolism and improved abiotic stress tolerance. Plant Biotechnol J. 2011; 9(6):661–73. Epub 2011/04/01. doi: 10.1111/j.1467-7652.2011.00611.x PMID: 21450042.

33. Matysik J, Bhalu B, Mohanty P. Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. Current Science. 2002; 82:525–32.

34. Kishor PBK, Hong Z, Miao GH, Hu CAA, Verma DPS. Over-expression of [delta]-pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. Plant Physiol. 1995; 108:1387–94. PMID: 12228549.

35. O’Regan BP, Cress WA, Van Staden J. Root growth, water relations, abscisic acid and proline levels of drought-resistant and drought-sensitive maize cultivars in response to water stress. S Afr J Bot. 1993; 59:98–104.

36. Bartels D. Targeting detoxification pathways: an efficient approach to obtain plants with multiple stress tolerance. Trends Plant Sci 2001; 7:284–6.

37. Zhu JK. Plant salt tolerance. Trends Plant Sci. 2001; 6(2):66–71. Epub 2001/02/15. doi: S1360-1385 (00)01838-0 [pii]. PMID: 11173290.

38. Kangasjarvi S, Neukermans J, Li S, Aro EM, Noctor G. Photosynthesis, photorespiration, and light signalling in defence responses. J Exp Bot. 2012; 63(4):1619–36. Epub 2012/01/28. doi: err402 [pii] doi: 10.1093/jxb/err402 PMID: 22282535.

39. Chen C, Gao M, Liu J, Zhu H. Fungal symbiosis in rice requires an ortholog of a legume common symbiosis gene encoding a Ca2+/calmodulin-dependent protein kinase. Plant Physiol. 2007; 145(4):1619–28. Epub 2007/10/30. doi: pp.107.109876 [pii] doi: 10.1104/pp.107.109876 PMID: 17965173; PubMed Central PMCID: PMC2151686.