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Overexpression of three orthologous glutathione S-transferases from *Populus* increased salt and drought resistance in *Arabidopsis*

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ABSTRACT

Glutathione S-transferases (GSTs) are multifunctional proteins and play a role in detoxification of xenobiotics as well as prevention of oxidative damage. This study exogenously overexpressed *PtGSTF4* from *Populus trichocarpa* and its two orthologs from *Populus yatungensis* and *Populus euphratica* in *Arabidopsis thaliana*, respectively. To elucidate the function of three GSTF4 proteins in stress response, we compared germination and seedling growth in transgenic *Arabidopsis* with salt and drought treatments. All three *Populus* GSTF4 genes overexpressed *Arabidopsis* showed enhanced resistance to salt stress and drought. GSTF4 transgenic plants accumulated less hydrogen peroxide and more chlorophylls and decreased levels of lipid peroxidation under salt stress and drought comparing to the mock control plants. The difference observed by GSH and GSSG measurements indicated GSTF4 proteins may involve in glutathione-dependent peroxide scavenging which lead to reduced oxidative damage. The *Arabidopsis* transformed with the GSTF4 gene form *P. euphratica* showed higher germination rate and different performance of affecting GSSG contents comparing with the other two orthologous GST genes under NaCl treatment. These results suggested three *Populus* GSTF4 orthologs may have functional divergence in stress responding. This study provides insights into molecular mechanisms that underlie salt and drought stress tolerance of Phi GSTs and gives evidence for the functional divergence among orthologs in vivo.

1. Introduction

Plants are constantly subjected to a combination of different abiotic stresses (Mittler, 2006). In northwest of China, for example, many plants suffered salt stress in arid and semiarid regions (Zou et al., 2005). The lack of rainfall and high temperature aggravated the salinity problem. Like other abiotic stresses, salt stress and drought often lead to oxidative stress by generating superfluous reactive oxygen species (ROS) (Dat et al., 2000; Mittler, 2002; Apel and Hirt, 2004). Excess of ROS, such as hydrogen peroxide (H_2O_2), superoxide and hydroxyl radicals, could cause programmed cell death by damaging the lipids, proteins, carbohydrates and DNA (Inzé and Montagu, 1995; Mittler et al., 2004; Das and Roychoudhury, 2014). Plants have evolved a complex defense anti-oxidative system to scavenge ROS. Various low-molecular mass antioxidants are involved in the equilibrium between the production and the scavenging of ROS.

As a major component of endogenous antioxidants, γ-glutamyl-cysteinylglycine (GSH) play a crucial role in plant defense mechanisms against various stresses by scavenging free radicals and reducing peroxides (Noctor and Foyer, 1998; Foyer and Noctor, 2005). In the ROS-scavenging systems, GSH is oxidized to its disulfide (GSSG) by participating the ascorbate-glutathione cycle and the glutathione peroxidase (GPX) cycle (Foyer and Halliwell, 1976). The balance between GSH and GSSG is necessary in maintaining cellular redox state and a high ratio of GSH/GSSG is important for ROS scavenging in plants. Glutathione S-transferase (GST) is an important enzyme that regulating the cellular GSH/GSSG pool in plant defense mechanisms (Droog, 1997). Knowing for its key role in enzymic detoxification, GSTs catalyze the conjunction of GSH with various xenobiotic and endobiotic compounds (Edwards et al., 2000; Dixon et al., 2003; Basantani and Srivastava, 2007). GST can also function as GPX which directly involved in the ROS-scavenging system by catalyzing the reaction of GSH with hydrogen peroxide as showed by studies in moss, soybean and conifer (Roxas et al., 2000; Lan et al., 2013; Liu et al., 2013; Liu et al., 2015; Yang et al., 2014).

Plant GSTs constitute a large, ubiquitous gene family with multifunction. In *Arabidopsis*, poplar (*Populus trichocarpa*) and rice (*Oryza sativa*), there are more than 54 GST genes in each genome (Dixon et al., 2009; Lan et al., 2009; Jain et al., 2010). Based on protein sequence identity, structure and substrate specificity, plant GSTs are divided into eight classes: tau, phi, lambda, theta, zeta, dehydroascorbate reductase

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(DHAR), tetrachlorohydroquinone dehalogenase (TCHQD), and γ-subunit of the eukaryotic translation elongation factor 1B (EF1Bγ) (Oakley, 2005; Basantani and Srivastava, 2007). Tau and Phi GSTs are the most abundant classes in plant GST family. GSTs protect plants cells when exposing to various biotic and abiotic stressors such as pathogens, salt, heavy metal toxins and UV radiation (Frova, 2003; Jiang et al., 2010). Plenty of previous studies have shown that GST gene family displays extensive functional divergence in gene expression and enzymatic activities (Lan et al., 2009; Dixon et al., 2009; Jain et al., 2010; Yang et al., 2014; Liu et al., 2015). Unfortunately, the cellular and molecular biology of GST proteins defense mechanisms in response to stress is still poorly understood.

Populus trichocarpa are native to northwest of America and grow up to an altitude of 2600 m, *Populus yatungensis* are native to southwest of China (mainly distributed in Tibetan Plateau) and grow on mountain slopes with altitude 2400–3600 m, and *Populus euphratica* are native to desert regions ranging from western China to north Africa and adapted to drought and salt stress (Fang et al., 1999; Ma et al., 2013). The three *Populus* species grow in significant different habitats which give us the opportunity to study the function of the orthologous GST proteins from different species. Our previous study had identified 21 orthologous GST groups from *P. trichocarpa*, *P. yatungensis*, and *P. euphratica*, and gene expression together with enzymatic analysis showed that functional divergences were prevalent within ortholog GSTs (Yang et al., 2018). To study the biological function of ortholog GSTs in vivo, a Phi class GST (PtGSTF4) from *P. trichocarpa* and its two orthologs from *P. yatungensis* and *P. euphratica* were exogenously overexpressed in *Arabidopsis* plants, respectively. To elucidate the role of three GSTF4 proteins in stress response, we compared germination and seedling growth in transgenic *Arabidopsis* under normal, salt and drought treatments. A set of physiological and biochemical indexes related to stress tolerance were investigated in these overexpressed lines. Our data here clearly elucidated three *Populus* GSTF4 proteins playing a role in tolerance of drought and salt stress.

2. Materials and methods

2.1. Plant materials and growth conditions

The ecotype Columbia-0 plants of *Arabidopsis thaliana* was selected as the wild-type to be transformed with the *Populus* genes or empty plasmid. The Arabidopsis seeds were sterilized with 70% ethanol for 1 min, and followed by 2% sodium hypochlorite for 10 min. Then the seed were rinsed five times with sterile deionized water and sown onto half-strength Murashige and Skoog (1/2 MS) medium with 2% sucrose and 0.8% agar. After sowing on a plate, the seeds were stored at 4 °C for 2 days before they were transferred to growth cabinet under 22 °C and 16-h-light/8-h-dark cycle. Seven days after germination, the seedlings were transplanted into pots.

2.2. Vector construction and A. thaliana transformation

To construct binary vectors for gene overexpression in *A. thaliana*, the coding sequence of GSTF4 genes were amplified from three corresponding pET30/GST vectors which were constructed previously (Yang et al., 2018). The primers were listed in Supplemental Table S1. The PCR fragments were cloned into pEASY-T3 Vector by using a pEASY-T3 Cloning Kit (TransGen) and then confirmed by sequencing. Subsequently, these genes were subcloned into a modified pCAMBIA1302 vector (http://www.cambia.org.au/, Supplemental Fig. S1) and then introduced into *Agrobacterium* strain *EHA105*, respectively. *A. thaliana* Col-0 wild-type plants were transformed with empty vector pCAMBIA1302 or with different pCAMBIA1302-GSTF4 constructs using floral dip method (Zhang et al., 2006).

2.3. Identification and isolation of transformed Arabidopsis plants

To identify the positive transformed *Arabidopsis* plants, the seeds of each T0 transgenic plant were sown onto 1/2 MS medium with 50 mg^{-1} L hygromycin. All hygromycin-resistance seedlings were transplanted into pots. Total DNA were extracted from seedlings and three pairs of primers were designed to detect the insertion of subject fragments (Supplemental Table S2). Total RNA of T3 homozygotes were extracted with an Aurum Total RNA kit (Bio-Rad) and reverse transcribed into cDNA with RNA PCR kit (TaKaRa). T3 homozygous lines were used for further analysis.

2.4. Salt and drought treatments

To determinate seed germination rate under salt stress, 90–120 seeds per transgenic line were sterilized and pointed to 1/2 MS plates supplemented with 150 mM NaCl. Plates were stored at 4 °C in the dark for 2 days and then incubated in growth cabinet under 22 °C to count germinated seeds. The other salt-stress experiment was accomplished by irrigating the 3-week old seedlings with 200 mM NaCl solution and the phenotype was observed 7 days later. The effect of drought on seedling growth was examined by withholding water from 3-week old plants grown under greenhouse conditions for 14 days. Each experiment included 12 seedlings per transgenic line. At the end of experiments, the leaves were harvested for further analysis. The samples form seedlings without any treatment were used as control.

2.5. Measurements of chlorophylls contents, hydrogen peroxide and malondialdehyde contents

Chlorophylls were extracted and measured as described by Arnon (1949). For each sample, 0.2 g of the leaf tissue was cut into small pieces (0.5–1 mm width) and soaked in 10 ml aqueous acetone (80%) for 72 h in −20 °C fridge. The contents of chlorophyll *a* and *b* were measured at 663 and 645 nm. Malondialdehyde (MDA) content was determined as described by Hagege et al. (1990). 0.2 g leaf tissue were homogenized in 10 ml of 10% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 4000 rpm for 10 min. The supernatant was mixed with equal volume of 0.6% thiobarbiturice acid, then heated at 100 °C for 30 min and cooled on ice for 5min. The absorbance of supernatant was determined at 450 nm, 532 nm and 600 nm. Hydrogen peroxide (H_2O_2) was measured according to the method of Alexieva et al., (2001). For each sample, 0.2 g of the leaf tissue was homogenized in 2 ml 3% (w/v) precooled TCA, following a centrifugation at 4000 rpm for 10 min. For every 1 ml of supernatant, 1 ml of 50 mM phosphate buffer (pH 7.0) and 2 ml of 1 M potassium iodide (KI) were added. The reaction was carried at room temperature in the dark and then absorbance was determined at 390 nm. Data were calculated against a standard curve of H_2O_2. The microplate reader (Multiskan GO, Thermo Scientific) were used for all measurements. All experiments were repeated three times.

2.6. Measurements of GSH and GSSG contents

The GSH content and GSSG content were assayed using a GSH/GSSG Assay Kit (Beyotime). The leave tissues were homogenized in the buffer M of this commercial kit and then stewed at 4 °C for 10 min. After a centrifugation at 10000*g*, 4 °C for 10 min, the supernatant was used for GSH/GSSG assay according to the manufacturer's instructions. All measurements were repeated three times.

3. Results and discussion

3.1. Orthologs of GSTF4 from three Populus species shared similar sequence

Our previous study had identified orthologs of GSTF4 from *P. trichocarpa*, *P. yatungensis* and *P. euphratica* (Lan et al., 2009; Yang et al.,

2018). Phylogenetic tree showed the three GSTF4 orthologs grouped into one clade with high bootstrap support and protein sequence alignment demonstrated the PyGSTF4 were more conserved to PtGSTF4 than to PeGSTF4 (Supplemental Fig. S2). The pairwise protein sequence identities among the three *Populus* GSTF4 proteins were 93.8–99.5% while sequence identities among three *Populus* GSTF4 against other Phi class GSTs from *P. trichocarpa* and *A. thaliana* were much lower (Supplemental Fig. S3).

3.2. Overexpression of three Populus GSTF4 genes in transgenic Arabidopsis confer salt and drought tolerance

Although the three GSTF4 orthologs had conserved sequence, the diverged enzymatic profile indicated they may have diverged functions in plants (Yang et al., 2018). To study the function of the three GSTF4 orthologs from three *Populus* species in vivo, these GSTF4 genes were constitutively overexpressed in *Arabidopsis thaliana* Col-0 under the control of the 35S promoter, respectively. Col-0 transformed with empty vector were used as mock control. Three positive transformed homozygous lines for each gene were selected to perform the experiments (Fig. 1A). GSTF4 genes in every transgenic line were highly expressed (Fig. 1B). Germination and growth of the transgenic and mock control plants was evaluated on 1/2 MS medium and in soil, and no phenotype was observed among those transgenic plants compare to wild-type.

The Phi class GSTs in plants were involved in various abiotic stresses response, especially the oxidative stress (Edwards et al., 2000). To determine whether overexpression GSTF4 in transgenic *Arabidopsis* plants enhance the resistance to oxidative stress, salt and drought treatment were performed, respectively. The 3-week old transgenic and mock control plants were treated with 200 mM NaCl and subsequently monitored for 7 d. All three transgenic lines of each GSTF4 gene showed

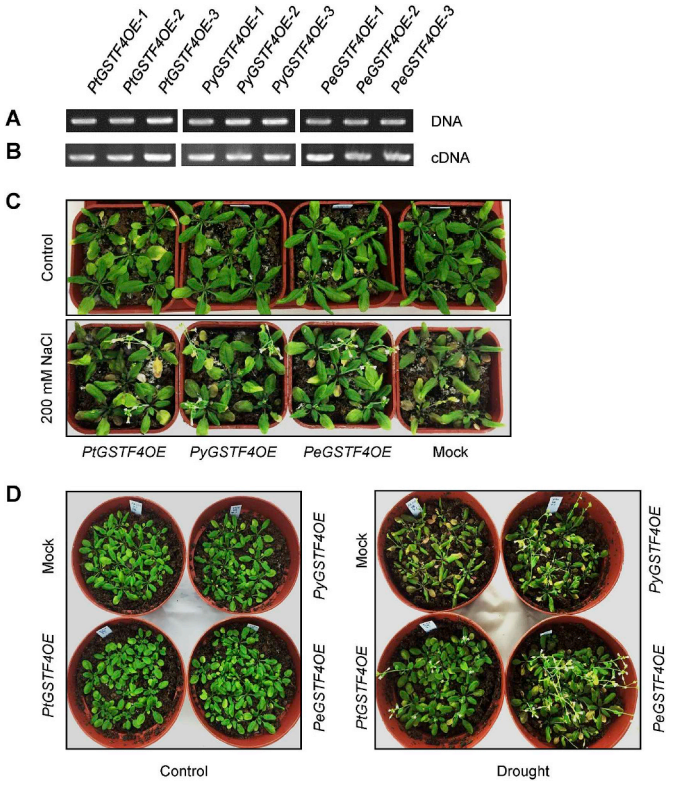

Fig. 1. Identification of transgenic plants and their tolerance to drought and salt stresses. A, detection of the transgenic plants by PCR. B, detection of the transgenic plants by RT-PCR. C, 4-week old seedlings with or without NaCl treatment. D, 5-week old seedlings with or without drought treatment.

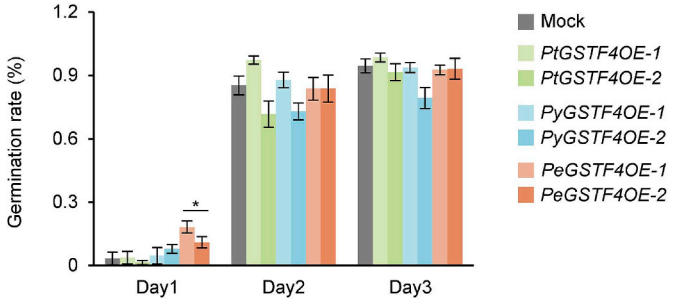

Fig. 2. The germination rate of transgenic *Arabidopsis* under 150 mM NaCl treatment. Each column represents an independent line of transgenic *Arabidopsis*. Error bar show standard deviation from three replicates. Asterisks indicate significant differences between GSTF4 over-expression lines and mock plants with $P < 0.05$.

improved resistance to salt stress than mock control plants (Fig. 1C). We also investigated the response of 3-week old plants to drought by withholding water for 12 days. The result showed that transgenic plants had stronger resistance to water deficits than the mock control plants (Fig. 1D). Whereas among all the *Arabidopsis* plants transformed with different GSTF4 genes, no visible difference was found in the resistance to salt and drought stress.

Germination rate of transgenic plants under NaCl stress were examined. Seeds were sown on the 1/2 MS plates containing 150 mM NaCl. Over ninety percent seeds from all examined lines were germinated within three days except PyGSTF4-OE2 line (Fig. 2). There was no significant difference in germination rate among transgenic lines with control plants by third day. However, the PeGSTF4-OE lines showed faster germination rate than the lines of the other two GST genes or the mock control in the beginning. This result may indicate the PeGSTF4 had different functions in plants comparing with PyGSTF4 and PtGSTF4.

3.3. Overexpression GSTF4 plants exhibit reduced oxidative damage under salt and drought stress

Salt stress and drought have been assumed to cause oxidative damage to the plants by generating ROS (Dat et al., 2000; Mittler, 2002; Apel and Hirt, 2004). Previous study had shown Phi class GST genes had displayed the GPX activity which may involve in the ROS scavenging in various species (Lan et al., 2013; Liu et al., 2013; Liu et al., 2015; Yang et al., 2014). To test if the GSTF4 contributed to the reduced levels of oxidative damage, several indicators of cellular oxidative damage including H_2O_2 accumulation, chlorophyll content and lipid peroxidation were determined.

The hydrogen peroxide is one of the reactive oxygen species which will accumulate when plants exposed to oxidative stresses. In mock control plants, the contents of H_2O_2 showed significant increase under salt stress comparing to the normal growth condition ($P < 0.05$, Fig. 3A). Whereas, there's no significant accumulation in all *Populus* GSTF4 lines under salt ($P > 0.05$). For the drought treatment, the result show that all plants increased their H_2O_2 accumulation compare to the control condition. But the contents of H_2O_2 in *Populus* GSTF4 overexpression lines were significantly lower than in mock control plants ($P < 0.05$). These results indicated all the three *Populus* GSTF4 proteins played a role in scavenging the ROS in transgenic plants under stresses.

The oxidative stresses imposed on plants could cause the decrease of the chlorophyll content (Lichtenthaler and Rinderle, 1988). We extracted and measured the chlorophylls form the rosette leaves which were applied with salt and drought treatments. The contents of chlorophyll *a* and *b* in mock control plants were both decreased under salt and drought conditions while that in GSTF4 overexpressed lines didn't

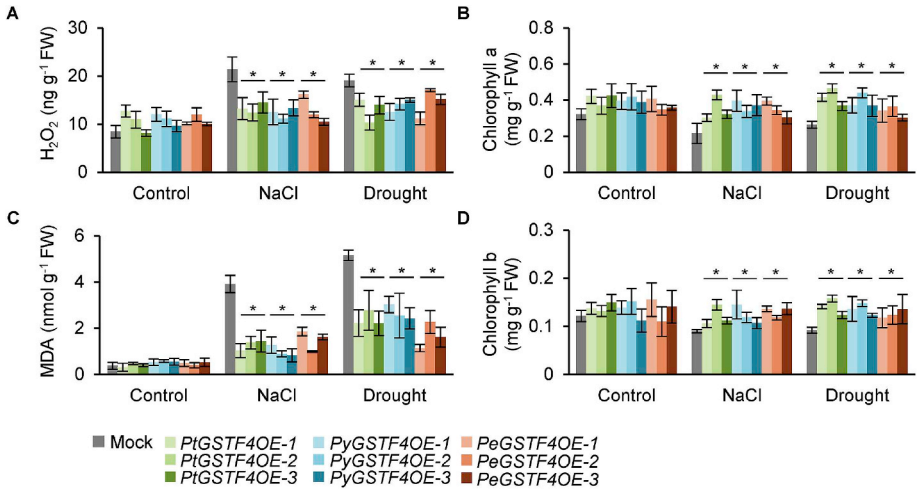

Fig. 3. The effects on the contents of H_2O_2, chlorophylls and lipid peroxidation in transgenic *Arabidopsis* under abiotic stresses. A, the content of H_2O_2. B, the content of chlorophyll *a*. C, the content of malondialdehyde (MDA) indicating lipid peroxidation. D, the content of chlorophyll *b*. Each column represents an independent line of transgenic *Arabidopsis*. Error bar show standard deviation from three replicates. Asterisks indicate significant differences between GSTF4 over-expression lines and mock plants with $P < 0.05$.

display significant changes (Fig. 3B and D). Oxidative stresses could also cause the free radical-mediated lipid peroxidation which can damage the membranes of plant tissues (Mittler, 2002; Willekens et al., 1997). The content of malondialdehyde (MDA) is an index of lipid peroxidation in plants (Draper and Hadley, 1990). The MDA contents in mock control plants were significantly increased under salt stress or drought comparing to the normal growth condition ($P < 0.05$, Fig. 3C). However, the MDA contents in GSTF4 overexpressed lines were much lower under stress conditions comparing to the mock control. Taken together, all three GSTF4 proteins contributed to decrease the accumulation of oxidative stress product and protecting the plants from oxidative damage.

3.4. Overexpression GSTF4 plants affect the glutathione contents

The GSH and GSSG equilibrium is critical to maintain cellular redox state (Noctor and Foyer, 1998). The high GSH/GSSG ratio is essential for the ROS scavenging in cells which protecting plants from oxidative damages (Mittler, 2002). Phi class GST genes were involved in the regulating of cellular GSH/GSSG pool in plant ROS defense mechanisms (Droog, 1997). To verify whether GSTF4 proteins protect plants from oxidative damage via adjusting GSH/GSSG ratio, the contents of GSH and GSSG were analyzed.

Transgenic and control plants contained similar amount of oxidized (GSH) and reduced (GSSG) glutathione under normal growth condition (Fig. 4). The GSH contents were increased while the GSSG contents were decreased when mock control plants were stressed (Fig. 4). Whereas the GSH content were significantly lower and the contents of GSSG were significantly higher in GSTF4 overexpressed lines than mock control plants under the salt stress ($P < 0.05$). As a result, the GSH/GSSG ratio in GSTF4 overexpressed lines showed similar values with or without stress and were much lower than mock control plants (Fig. 4C). These results demonstrated Populus GSTF4 proteins would contribute to maintain the GSH/GSSG balance under salt stress. Interestingly, the PeGSTF4OE lines didn't show significant changes of both GSH and GSSG contents between salt stress and control condition ($P > 0.05$). While the PtGSTF4OE and PyGSTF4OE lines indeed show significant decrease of GSSG contents compare salt stress to control condition ($P < 0.05$). This result indicated the function of PeGSTF4 could be diverged with its orthologs PtGSTF4 and PyGSTF4. The GSH and GSSG contents in the GSTF4 overexpressed plants were higher than that in mock control plants under drought treatment. Whereas the ratio of GSH/GSSG in GSTF4 overexpressed plants didn't show significant difference with mock control plants (Fig. 4C). In summary, our results suggested *Populus* GSTF4 proteins protected plants from salt stress through adjusting GSH/GSSG pool while may have different mechanisms for drought tolerance.

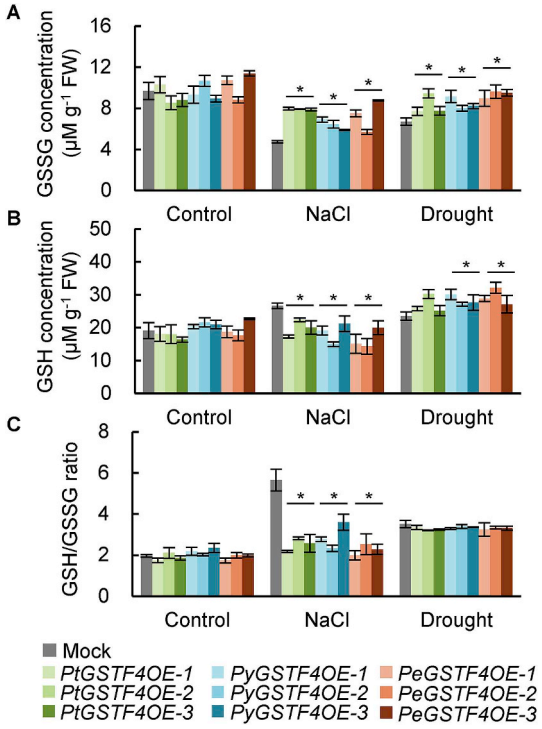

Fig. 4. The changes in the quantifications of GSH and GSSG in transgenic *Arabidopsis* under abiotic stresses. A, the content of GSSG. B, the content of GSH.C, the GSH/GSSG ratio. Each column represents an independent line of transgenic *Arabidopsis*. Error bar show standard deviation from three replicates. Asterisks indicate significant differences between GSTF4 over-expression lines and mock plants with $P < 0.05$.

4. Conclusion

In this study, three GST orthologs from *P. trichocarpa*, *P. yatungensis* and *P. euphratica* were transformed into *Arabidopsis*, respectively. We found all three GSTF4 genes were contributed to increase the tolerance of salt stress and drought and the adjustment of GSH/GSSG pool. The plants transformed with the PeGSTF4 form *P. euphratica* showed higher germination rate and different performance of affecting GSSG contents comparing with the other two GST genes under NaCl treatment. Our result indicated all three GSTF4 genes play a role in protecting seedlings from various stress-induced oxidative damages, and the function of PeGSTF4 form *P. euphratica* may diverged with its orthologs from *P. trichocarpa*. and *P. yatungensis*. This work provides insights into

molecular mechanisms that underlie salt and drought stress tolerance of Phi GSTs and gives evidence for the functional divergence of orthologous genes in vivo.

Author contributions

QY conducted the research. QY and Y-JL. performed the data analysis. QY and Q-YZ. conceived the research, designed the experiments and drafted the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bse.2019.01.001.

References

Alexieva, V., Sergiev, I., Mapelli, S., Karanov, E., 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ. 24 (12), 1337–1344. https://doi.org/10.1046/j.1365-3040.2001.00778.x.

Apel, K., Hirt, H., 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 55, 373–399. https://doi.org/10.1146/annurev.arplant.55.031903.141701.

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in beta vulgaris. Plant Physiol. 24 (1), 1–15.

Basantani, M., Srivastava, A., 2007. Plant glutathione transferases — a decade falls short. Can. J. Bot. 85 (5), 443–456. https://doi.org/10.1139/B07-033.

Das, K., Roychoudhury, A., 2014. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Front. Environ. Sci. 2, 53. https://doi.org/10.3389/fenvs.2014.00053.

Dat, J., Vandenabeele, S., Vranova, E., Van Montagu, M., Inze, D., Van Breusegem, F., 2000. Dual action of the active oxygen species during plant stress responses. Cell. Mol. Life Sci. CMLS 57 (5), 779–795. https://doi.org/10.1007/s000180050041.

Dixon, D.P., Hawkins, T., Hussey, P.J., Edwards, R., 2009. Enzyme activities and subcellular localization of members of the *Arabidopsis* glutathione transferase superfamily. J. Exp. Bot. 60 (4), 1207–1218. https://doi.org/10.1093/jxb/ern365.

Dixon, D.P., McEwen, A.G., Lapthorn, A.J., Edwards, R., 2003. Forced evolution of a herbicide detoxifying glutathione transferase. J. Biol. Chem. 278 (26), 23930–23935. https://doi.org/10.1074/jbc.M303620200.

Draper, H., Hadley, M., 1990. Malondialdehyde determination as index of lipid peroxidation. In: Methods in Enzymology, vol. 186. Elsevier, pp. 421–431. https://doi.org/10.1016/0076-6879(90)86135-I.

Droog, F., 1997. Plant glutathione S-transferases, a tale of theta and tau. J. Plant Growth Regul. 16 (2), 95–107. https://doi.org/10.1007/pl00006984.

Edwards, R., Dixon, D.P., Walbot, V., 2000. Plant glutathione S-transferases: enzymes with multiple functions in sickness and in health. Trends Plant Sci. 5 (5), 193–198. https://doi.org/10.1016/S1360-1385(00)01601-0.

Fang, C., Zhao, S., Skvortsov, A.K., 1999. Salicaceae. In: In: Wu, Z.Y., Raven, P., Hong, D.Y. (Eds.), Flora of China, vol. 4. Science Press/Missouri Botanical Garden Press, Beijing, China/St. Louis, MO, USA, pp. 158.

Foyer, C.H., Halliwell, B., 1976. The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta 133 (1), 21–25. https://doi.org/10.1007/BF00386001.

Foyer, C.H., Noctor, G., 2005. Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. Plant Cell 17 (7), 1866–1875. https://doi.org/10.1105/tpc.105.033589.

Frova, C., 2003. The plant glutathione transferase gene family: genomic structure, functions, expression and evolution. Physiol. Plantarum 119 (4), 469–479. https://doi.org/10.1046/j.1399-3054.2003.00183.x.

Hagege, D., Nouvelot, A., Boucaud, J., Gaspar, T., 1990. Malondialdehyde titration with thiobarbiturate in plant extracts: avoidance of pigment interference. Phytochem. Anal. 1 (2), 86–89. https://doi.org/10.1002/pca.2800010208.

Inzé, D., Montagu, M.V., 1995. Oxidative stress in plants. Curr. Opin. Biotechnol. 6 (2), 153–158. https://doi.org/10.1016/0958-1669(95)80024-7.

Jain, M., Ghanashyam, C., Bhattacharjee, A., 2010. Comprehensive expression analysis suggests overlapping and specific roles of rice glutathione S-transferase genes during development and stress responses. BMC Genomics 11 (1), 73. https://doi.org/10.1186/1471-2164-11-73.

Jiang, H.W., Liu, M.J., Chen, I.C., Huang, C.H., Chao, L.Y., Hsieh, H.L., 2010. A glutathione S-transferase regulated by light and hormones participates in the modulation of *Arabidopsis* seedling development. Plant Physiol. 154 (4), 1646–1658. https://doi.org/10.1104/pp.110.159152.

Lan, T., Wang, X.R., Zeng, Q.Y., 2013. Structural and functional evolution of positively selected sites in pine glutathione S-transferase enzyme family. J. Biol. Chem. 288 (34), 24441–24451. https://doi.org/10.1074/jbc.M113.456863.

Lan, T., Yang, Z.L., Yang, X., Liu, Y.J., Wang, X.R., Zeng, Q.Y., 2009. Extensive functional diversification of the Populus glutathione S-transferase supergene family. Plant Cell 21 (12), 3749–3766. https://doi.org/10.1105/tpc.109.070219.

Lichtenthaler, H.K., Rinderle, U., 1988. The role of chlorophyll fluorescence in the detection of stress conditions in plants. CRC Crit. Rev. Anal. Chem. 19 (Suppl. 1), S29–S85. https://doi.org/10.1080/15476510.1988.10401466.

Liu, H.J., Tang, Z.X., Han, X.M., Yang, Z.L., Zhang, F.M., Yang, H.L., Liu, Y.J., Zeng, Q.Y., 2015. Divergence in enzymatic activities in the soybean GST supergene family provides new insight into the evolutionary dynamics of whole-genome duplicates. Mol. Biol. Evol. 32 (11), 2844–2859. https://doi.org/10.1093/molbev/msv156.

Liu, Y.J., Han, X.M., Ren, L.L., Yang, H.L., Zeng, Q.Y., 2013. Functional divergence of the glutathione S-transferase supergene family in *Physcomitrella patens* reveals complex patterns of large gene family evolution in land plants. Plant Physiol. 161 (2), 773–786. https://doi.org/10.1104/pp.112.205815.

Ma, T., Wang, J., Zhou, G., Yue, Z., Hu, Q., Chen, Y., Liu, B., Qiu, Q., Wang, Z., Zhang, J., Wang, K., Jiang, D., Gou, C., Yu, L., Zhan, D., Zhou, R., Luo, W., Ma, H., Yang, Y., Pan, S., Fang, D., Luo, Y., Wang, X., Wang, G., Wang, J., Wang, Q., Lu, X., Chen, Z., Liu, J., Lu, Y., Yin, Y., Yang, H., Abbott, R.J., Wu, Y., Wan, D., Li, J., Yin, T., Lascoux, M., Difazio, S.P., Tuskan, G.A., Wang, J., Liu, J., 2013. Genomic insights into salt adaptation in a desert poplar. Nat. Commun. 4, 2797. https://doi.org/10.1038/ncomms3797.

Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7 (9), 405–410. https://doi.org/10.1016/S1360-1385(02)02312-9.

Mittler, R., 2006. Abiotic stress, the field environment and stress combination. Trends Plant Sci. 11 (1), 15–19. https://doi.org/10.1016/j.tplants.2005.11.002.

Mittler, R., Vanderauwera, S., Gollery, M., Van Breusegem, F., 2004. Reactive oxygen gene network of plants. Trends Plant Sci. 9 (10), 490–498. https://doi.org/10.1016/j.tplants.2004.08.009.

Noctor, G., Foyer, C.H., 1998. ASCORBATE and glutathione: keeping active oxygen under control. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49, 249–279. https://doi.org/10.1146/annurev.arplant.49.1.249.

Oakley, A.J., 2005. Glutathione transferases: new functions. Curr. Opin. Struct. Biol. 15 (6), 716–723. https://doi.org/10.1016/j.sbi.2005.10.005.

Roxas, V.P., Lodhi, S.A., Garrett, D.K., Mahan, J.R., Allen, R.D., 2000. Stress tolerance in transgenic tobacco seedlings that overexpress glutathione S-transferase/glutathione peroxidase. Plant Cell Physiol. 41 (11), 1229–1234. https://doi.org/10.1093/pcp/pcd051.

Willekens, H., Chamnongpol, S., Davey, M., Schraudner, M., Langebartels, C., Van Montagu, M., Inze, D., Van Camp, W., 1997. Catalase is a sink for H_2O_2 and is indispensable for stress defence in C3 plants. EMBO J. 16 (16), 4806–4816. https://doi.org/10.1093/emboj/16.16.4806.

Yang, Q., Han, X.M., Gu, J.K., Liu, Y.J., Yang, M.J., Zeng, Q.Y., 2018. Functional and structural profiles of GST gene family from three *Populus* species reveal the sequence–function decoupling of orthologous genes. New Phytol. https://doi.org/10.1111/nph.15430.

Yang, Q., Liu, Y.J., Zeng, Q.Y., 2014. Biochemical functions of the glutathione transferase supergene family of *Larix kaempferi*. Plant Physiol. Biochem. 77, 99–107. https://doi.org/10.1016/j.plaphy.2014.02.003.

Zhang, X., Henriques, R., Lin, S.S., Niu, Q.W., Chua, N.H., 2006. Agrobacterium-mediated transformation of *Arabidopsis thaliana* using the floral dip method. Nat. Protoc. 1 (2), 641–646. https://doi.org/10.1038/nprot.2006.97.

Zou, X., Zhai, P., Zhang, Q., 2005. Variations in droughts over China: 1951–2003. Geophys. Res. Lett. 32 (4), L04707. https://doi.org/10.1029/2004GL021853.