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Cloning a glutathione peroxidase gene from *Nelumbo nucifera* and enhanced salt tolerance by overexpressing in rice

Ying Diao · Huaxue Xu · Guolin Li · Aiqing Yu · Xia Yu · Wanling Hu · Xingfei Zheng · Shaoqing Li · Youwei Wang · Zhongli Hu

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Abstract A full-length cDNA clone encoding an 866 bp-length glutathione peroxidase protein (NnGPX) was isolated from lotus (*Nelumbo nucifera* L.). The deduced amino acid sequence of the NnGPX gene had significant homology with ATGPX6. A 3D structural model of the NnGPX was constructed by homology modeling. The cloned NnGPX gene was expressed in *Escherichia coli*, and a fusion protein of about 40 kDa was detected after isopropyl thiogalactoside induction. Under different concentrations of Na_2SeO_3 treatments, NnGPX was found to be an enzyme that does not contain selenium. Real-time PCR analysis showed that the NnGPX gene was expressed in all organs of lotus, and its high expression mainly occurred in organs with active metabolisms. NnGPX transcript increased remarkably in response to cold, heat, mechanical damage, and salt treatment. Subsequently, the NnGPX gene was introduced in *Oryza sativa* cv. Yuetai B. PCR results verified the integration of this gene into the genome of rice and reverse transcription-PCR verified that this gene had been expressed in transgenic rice. The transgenic plants were significantly more tolerant to salt stress compared with the wild-type.

Keywords *Nelumbo nucifera* · Glutathione peroxidase (GPX) · Reactive oxygen species (ROS) · Rice · Transformation · Salt stress

Background

Reactive oxygen species (ROS) refers to some oxygen metabolites and derivatives. Plants produce a small quantity of active oxygen under normal growth conditions. However, they could be induced to produce a large quantity of active oxygen under different stress environments, such as high-concentration salt and metal or extreme temperature. Large accumulations of ROS could damage plants. The enzymatic system comprises superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione peroxidase (GPX), which can help eliminate ROS in plants [1, 2]. GPX is a big family of isoenzymes that could catalyze H_2O_2 or hydroperoxide to form water or ethanol. Studies have demonstrated higher expression of GPX under different types of environmental stress (such as invasion of pathogenic bacteria and exposure to heavy metal or high-concentration salt), indicating that it has an important function in oxidative damage induced by stress. According to the difference whether the GPXs contain selenocysteine residues, they can be divided into two categories. In animals selenium plays an important role in active site of GPX, while in plants most GPXs are non-selenium dependent which reduce the catalytic action [3, 4].

Lotus, an ancient angiosperm, is one of the earliest genera. The lotus genus has two species: *Nelumbo nucifera* L. and *Nelumbo lutea* Pers. *N. nucifera* is extensively cultivated and

Ying Diao and Huaxue Xu contributed equally to this work.

Y. Diao · H. Xu · G. Li · A. Yu · X. Yu · X. Zheng · S. Li (✉) · Z. Hu (✉)
State Key Laboratory of Hybrid Rice, College of Life Sciences, Wuhan University, Wuhan 430072, People's Republic of China
e-mail: shaoqingli@whu.edu.cn

Z. Hu
e-mail: huzhongli@whu.edu.cn

Y. Diao · Y. Wang
School of Pharmaceutical Sciences, Wuhan University, Wuhan 430072, People's Republic of China

W. Hu
Institute of WTO, Wuhan University, Wuhan 430072, People's Republic of China

utilized in China and East Asia as food and medicine; it is also an important aquatic ornamental plant. Lotus has many unique biological features. For example, the lotus seed is one of the seeds characterized by extreme longevity because it could live up to 1,000 years [5]. The lotus seed is very resistant to high temperatures, and can withstand temperatures higher than 100 °C [6]. Studies have revealed that antioxidant enzymes (POD, CAT, and SOD) have an important participation in the longevity and high-temperature resistance of the lotus seed [7–9]. Studies also show that SOD contributes remarkably to the resistance of lotus to salt stress, mechanical damage, low temperature, and other adverse situations [10, 11]. At present, only the cDNA-coded sequence (EF421198) of the GPX genes of lotus (*NnGPX*) has been published on NCBI. No further studies have been performed to date. Thus, in the present study, bioinformatics analysis and prokaryotic expression were carried out on lotus GPX genes. And their expression patterns in different tissues of lotus under different stress conditions were also examined. This study also examined the overexpression of lotus GPX genes in rice and the resistance of transgenic rice to salt to provide information for further understanding and utilization of lotus GPX genes.

Materials and methods

Plant materials and treatments

The experimental material was *N. nucifera* cv. Elian No. 3. The seed of Elian No. 3 was cut open, immersed in water, and exposed to light for 16 h and to darkness for 8 h. Lotus seedlings were allowed to grow for 10 days at 24 °C in a greenhouse and were treated in Na_2SeO_3 (0, 5, 10, 20, 30, 40, and 50 mg/L), cold stress (0, 1.5, 3.0, 4.5, and 6.0 h under 4 °C), heat stress (0, 1.5, 3.0, 4.5, and 6.0 h under 42 °C), salt stress (0, 1.5, 3.0, 4.5, and 6.0 h in 50 mM NaCl), and mechanical damage (0, 1.5, 3.0, 4.5, and 6.0 h after leaves were scratched with blades), respectively. And then fresh leaves were collected for further studies. When flower buds had appeared on the Elian No. 3 cultivated in the field for 3 weeks, the leaves, rhizomes, terminal buds, and infant flowers were collected. All the freshly collected plant materials were rapidly frozen in liquid nitrogen, and then total RNAs were extracted (RNAprep Plant Kit, Tiangen Biotech Co., Ltd.). The RNA extract was reversed to cDNA by using M-MLV reverse transcriptase kit (Promega) for genetic identification and analysis of expression models.

Bioinformatics analysis of lotus GPX genetic sequence

According to the published sequence of GPX genes in lotus (EF421198), primer pair (NnGPXF: 5′-ATGGCAAGCCAA TCGAAG-3′, NnGPXR: 5′-TCAAGAGTACCCAATAGCT-3′) was designed to amplify the full cDNA fragment of NnGPX gene. A 3D structural analysis of lotus GPX was carried out by homology modeling according to published protocols [12]. Poplar GPX 5 (PF00255) was used as the sequence template. The evolution of lotus GPX gene was analyzed by MEGA software [13].

Expression of lotus GPX gene in *Escherichia coli*

Primers for the cloned GPX gene (EF421198) were designed (forward primer: 5′-AGCCATATGGCAAGCCA ATC-3′; reverse primer: 5′-CTCGAGTCAAGAGTACCC AAT-3′) to amplify the whole encoding sequence. The 5′ end of the forward and reverse primers contained a restriction endonuclease site for NdeI and XhoI, respectively. Prokaryotic expression was performed according to published protocols [14]. The transformed *E. coli* BL21 line was induced with 0.2 mM isopropylthio-β-D-galactoside (IPTG) for 2 h to express the target proteins.

RT-PCR analysis

Specific primers NnactinF2 (5-TGCAGGAATCCATGA-GACTACC-3) and NnactinR2 (5-GGTCAGCAA-TACCAGGGAACAT-3) were designed based on the conserved sequence of lotus gene β-actin (EU131153) published on NCBI. Specific primers NnGPXqF2 (5-CGATTCAAGGCTGAGTTTCCA-3) and NnGPXqR2 (5-AATCCCACCTTTGCTTGACTT-3) were designed based on the *NnGPX* sequence. Real-time PCR was carried out in a fluorimetric thermal cycler (Rotor Gene 2000; Corbett Research, Australia) using the DNA-binding dye SYBR Green I (Toyobo Co., Ltd., Osaka, Japan) for detection of PCR products. The reaction was done with SYBR Green real-time PCR Master Mix according to specifications. The $2^{-\Delta\Delta Ct}$ methods were used to obtain relative gene expression data according to published protocols [15]. The PCR was programmed as follows: initial denaturation at 94 °C for 20 s, followed by 50 cycles at 94 °C for 5 s and at 60 °C for 10 s. All samples were run in triplicate, and the trials were repeated three times.

Construction of expressing plasmid and transformation

Gateway technology was adopted to link the sequence encoding the full-length NnGPX protein to the expression vector. The intermediate vector was pDONR201 and the overexpression vector was pH7WG2D under the control of CaMV35S promotor. The plasmid was then introduced into *Agrobacterium tumefaciens* by electroporation. NnGPX was introduced into rice (*Oryza sativa* cv. Yuetai B) mediated via Agrobacterium to obtain a T0-generation

transgenic plant, the DNA of which was extracted and amplified by PCR using primers for hygromycin (Hyg-F: GTGCCACCAAGCGTAAGG, Hyg-R: CGGATTCGGCT CCAACAA) and GPX (GPX-F: GGGGACAAGTTTGT ACAAAAAAGCAGGCTTC, GPX-R: GGGGACCACTT TGTACAAGAAAGCTGGGTC). After harvesting, the seeds were planted, and transgenic lines T1, T2, and T3 were obtained by self-fertilization. Positive plants were selected by PCR amplification using hygromycin primers. Then, T3 seedlings were used for further functional analysis.

Salt-resistant test of transgenic plants

The seeds of wild-type (non-transgenic line) and T3 transgenic line were used for the salt-resistant test. The transgenic seeds were immersed in distilled water for 2 days in a 37 °C incubator, and then underwent accelerated germination for 1 day. The seeds were first cultivated in Yoshida nutrient solution for 3 days, then transferred into a new Yoshida nutrient solution containing 150 mM NaCl and cultured in a growth chamber under a light period of 12 h and a dark period of 12 h at 28 °C for 12 days. After the height of the plant and the length of the root were measured, the plants were transferred to the routine conditions for recovery.

After accelerated germination, the seeds of the T3 transgenic line were sown on vermiculite watered with Yoshida nutrient solution and grown in a growth chamber under a light period of 12 h and a dark period of 12 h at 28 °C for 14 days. Then, the seedlings were subjected to NaCl stress (150 mM NaCl in Yoshida nutrient solution) for 12 days. The soluble sugar [16], free proline [17], and GSH and GSSG (GSH and GSSG assay kit, Beyotime Institute of Biotechnology, China) contents were tested. For the unstressed control experiment, the wild-type and transgenic rice lines maintained growth at 28 °C and were harvested at the same growth stage as the salt-treated plants. Each experiment was done with three replicates.

Results

Bioinformatics analysis of *NnGPX*

As predicted, a full cDNA fragment of approximately 866 bp length was amplified by PCR. The cDNA sequence encodes for 170 residues, including three active catalytic sites (Fig. 1). The amino acid sequence clustered with the ATGPX6 proteins in the phylogenetic tree analysis, which indicated that NnGPX had significant homology with ATGPX6 compared with the other six GPX proteins of Arabidopsis (Fig. 2).

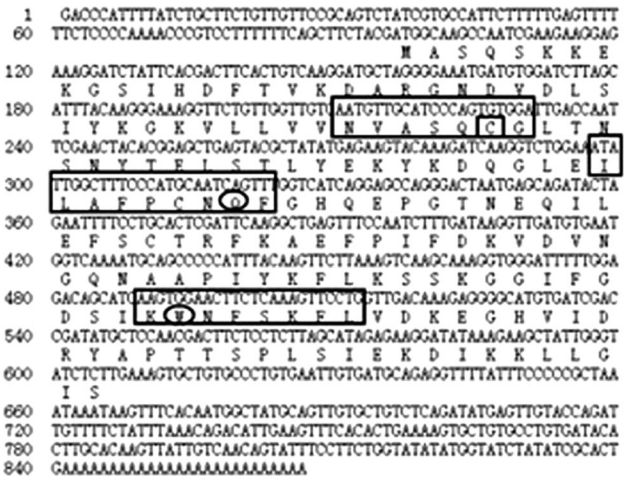


Fig. 1 The nucleotide acid sequence and deduced amino acid sequence of NnGPX cDNA. The conserved peptide sequence and active site are shown in *big boxes* and *ellipse*, respectively. The cysteine residue is *boxed*

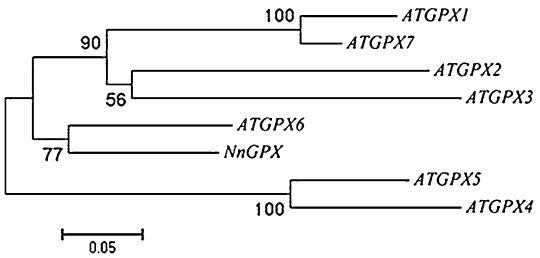


Fig. 2 Dendgrogram showing the relationships among the amino acid sequences of glutathione peroxidase from *Nelumbo nucifera* (NnGPX) and *Arabidopsis* (ATGPX1-6)

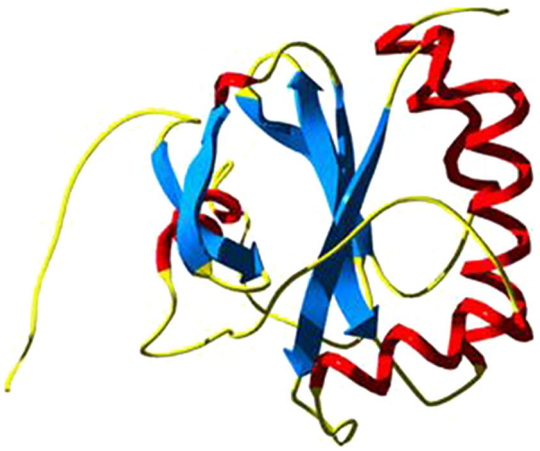


Fig. 3 The predicted molecular modeling of NnGPX. Line, helix and sheet structures are indicted in *yellow*, *red* and *blue*, respectively. (Color figure online)

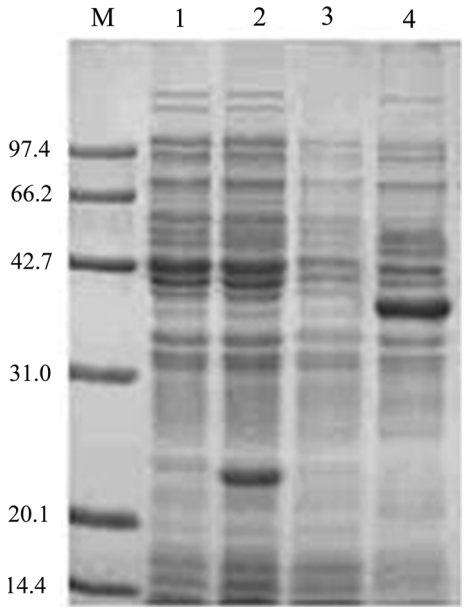


Fig. 4 The results of *gpx* expression in *Escherichia coli*. *Lanes*: *1* pET-15b without IPTG induction; *2* pET-15b with IPTG induction; *3* pET-15b-NnGPX without IPTG induction; *4* pET-15b- NnGPX with IPTG induction; *M* standard molecular-weight markers of protein

Poplar GPX 5 (PF00255) was selected as the best structural template for the query sequence of NnGPX based on the high degree of homology between the target protein and the template protein. The generated model suggested that the NnGPX protein was mixed with helix, β-sheet, and stand structures. These results indicate that the molecular model presented had good overall stereochemical qualities (Fig. 3).

Expression analysis of GPX gene in *E. coli*

About 510 bp length of encoding sequence was inserted in a pET-15b expression vector and treated with 2.0 mM IPTG. The expression of GPX protein was efficiently induced in the prokaryotic system, which was approximately 40 kDa in size (Fig. 4). Because the expressed GPX protein is a fusion protein with a His-tag at the N-terminal, the expressed fragment in *E. coli* is larger in size than 19 kDa which was predicted from the NnGPX cDNA sequence.

Expression analysis of GPX gene under Na_2SeO_3 treatment

The experimental result showed that the expression level of NnGPX did not change noticeably with the increase of Na_2SeO_3 concentration in the treatments. Significant difference between the treatment group and the control was only observed in two treatments, namely, 5 and 30 mg/L Na_2SeO_3 solution (Fig. 5). This result indicated that NnGPX is a GPX protein that is not dependent on selenium.

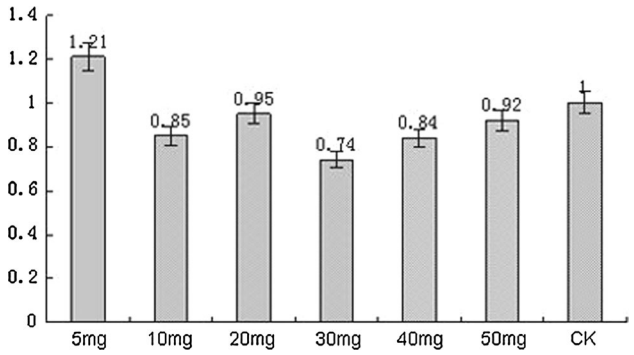


Fig. 5 Expression analysis of NnGPX with different concentrations of Na_2SeO_3 by qRT-PCR. Results are the means from three experiments with three replicates per run. The vertical bars indicate SD

Expression of the NnGPX gene in lotus

To test the expression level of *NnGPX* in different tissues of lotus during different growth periods, we collected two groups of materials: one group included the leaves, hypocotyledonary axis, stems, buds of the seedling that had grown for 7 days, and ungerminated lotus embryo; the other included the leaves, stem, terminal bud, and infant flower at the flowering stage. At the seedling stage, *NnGPX* was expressed highest in the leaves, followed by the buds and hypocotyledonary axis, and was least expressed in the stem. This result suggested that the large amount of peroxide produced from photosynthesis during the seedling stage of lotus might promote the expression level of *NnGPX* (Fig. 6a). NnGPX expression in ungerminated buds showed a relatively high level, demonstrating that NnGPX participates in physiological activities to maintain the germinating activity of seeds. At the flowering stage, the metabolism of the lotus plant was active, particularly in the terminal buds and flower organs at the meristematic zone. NnGPX expression was highest in the terminal bud, followed by the flower, leaves, and stems (Fig. 6b). Our result showed that the high expression of *NnGPX* mainly occurred in organs with active metabolisms in both the seedling and mature stages.

After 1.5 h of 4 °C cold treatment, the expression of *NnGPX* evidently increased and was maintained at a higher level in the test (Fig. 6c). After heat stress at 42 °C, the expression level of *NnGPX* gradually increased at about 140-fold higher at 6 h of treatment than that in the negative control (Fig. 6d). Our results indicated that NnGPX has an important role in the resistance of lotus to heat. The expression level of *NnGPX* also increased rapidly after the lotus was subjected to mechanical damage (Fig. 6f). After treated with 50 mM NaCl, the expression level of *NnGPX* also increased remarkably, indicating that NnGPX has the same important role in salt-stress environments (Fig. 6e). The expression level already increased by 100-fold as early

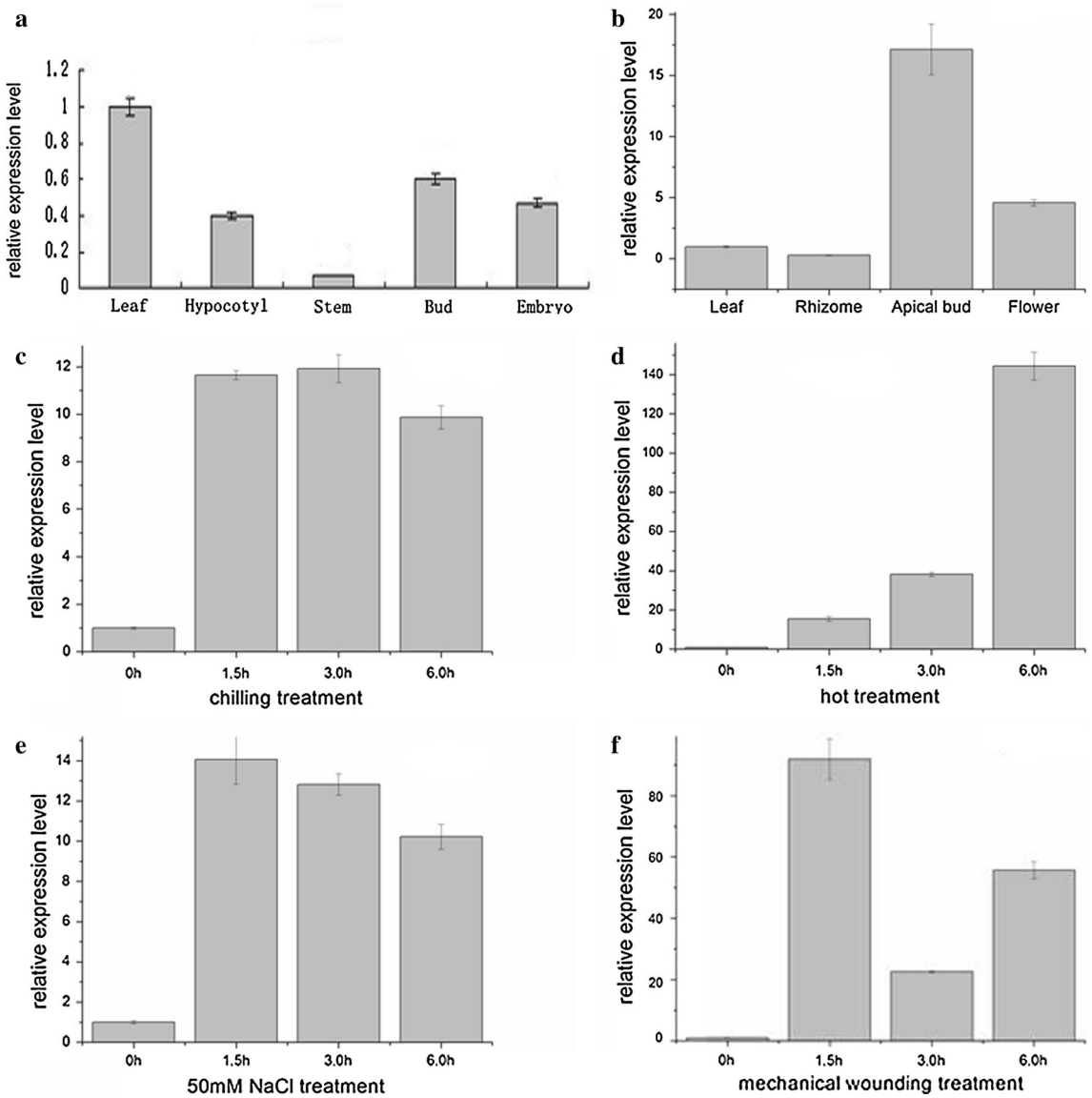


Fig. 6 Expression analysis of *NnGPX* in *Nelumbo nucifera*. **a** Expression analysis of *NnGPX* in various tissue at seedling stage; **b** Expression analysis of *NnGPX* in various tissue at blooming stage; **c** Expression analysis of *NnGPX* in leaf after chilling treatment at 4 °C; **d** Expression analysis of *NnGPX* in leaf after hot treatment at 42 °C; **e** Expression analysis of *NnGPX* in leaf after salt treatment with 50 mM NaCl; **f** Expression analysis of *NnGPX* in leaf after mechanical damage treatment

as 1.5 h after the mechanical damage, indicating that NnGPX is an important defensive factors during plant recovery after physical damage. Thus, under different stress environments, lotus can prevent oxidation-induced damage by increasing the expression of GPX.

Identification of transgenic rice

To further study the role of NnGPX in salt stress tolerance, transgenic rice "Yuetai B" was analyzed by PCR. T1 plants with segregation of 3:1 based on PCR with hygromycin specific primers (Fig. 7a) were self-fertilized and seeds were harvested for further analysis. Finally, we obtained five transgenic T2 lines. RT-PCR confirmed that the expression of NnGPX gene in different organs of the T2 plant was stable (Fig. 7b).

We simultaneously analyzed the yield of the T2 transgenic line and the wild-type, which showed an insignificant difference between the yield of transgenic and non-transgenic strains (Fig. 8).

Salt tolerance of transgenic rice

To test whether the expression of NnGPX in rice could enhance salt tolerance, wild-type and T3 generation of one transgenic line were treated with 150 mM NaCl for

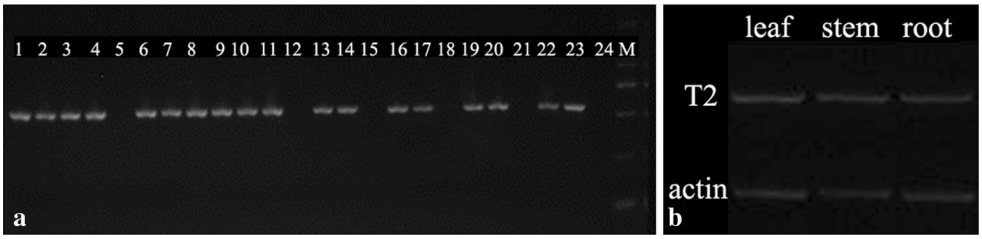


Fig. 7 PCR and RT-PCR analysis of transgenic rice plants. **a** 542 bp PCR products of hygromycin primers shows T1 plants with segregation of 3:1. **b** RT-PCR result of NnGPX gene expression in different organs of the T2 plant

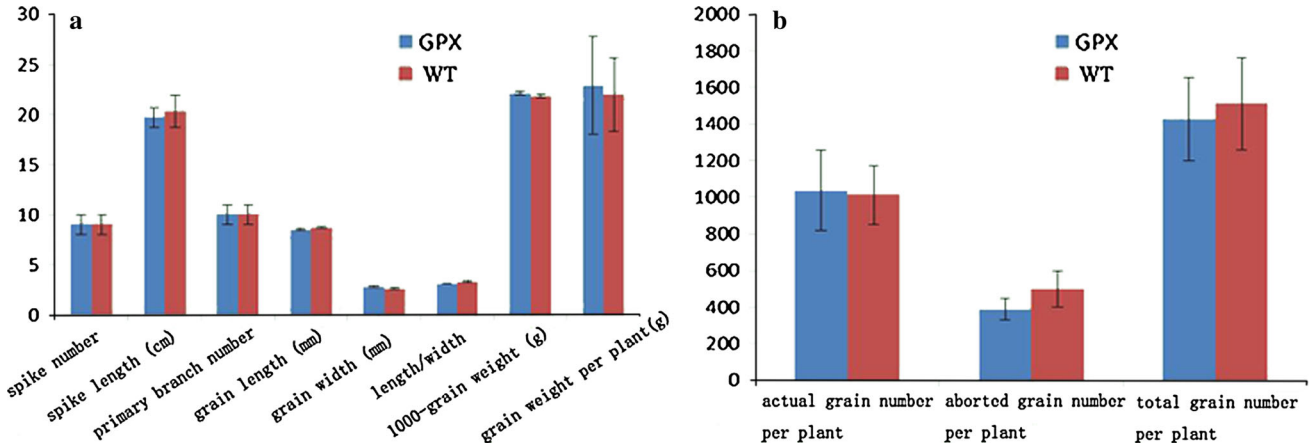


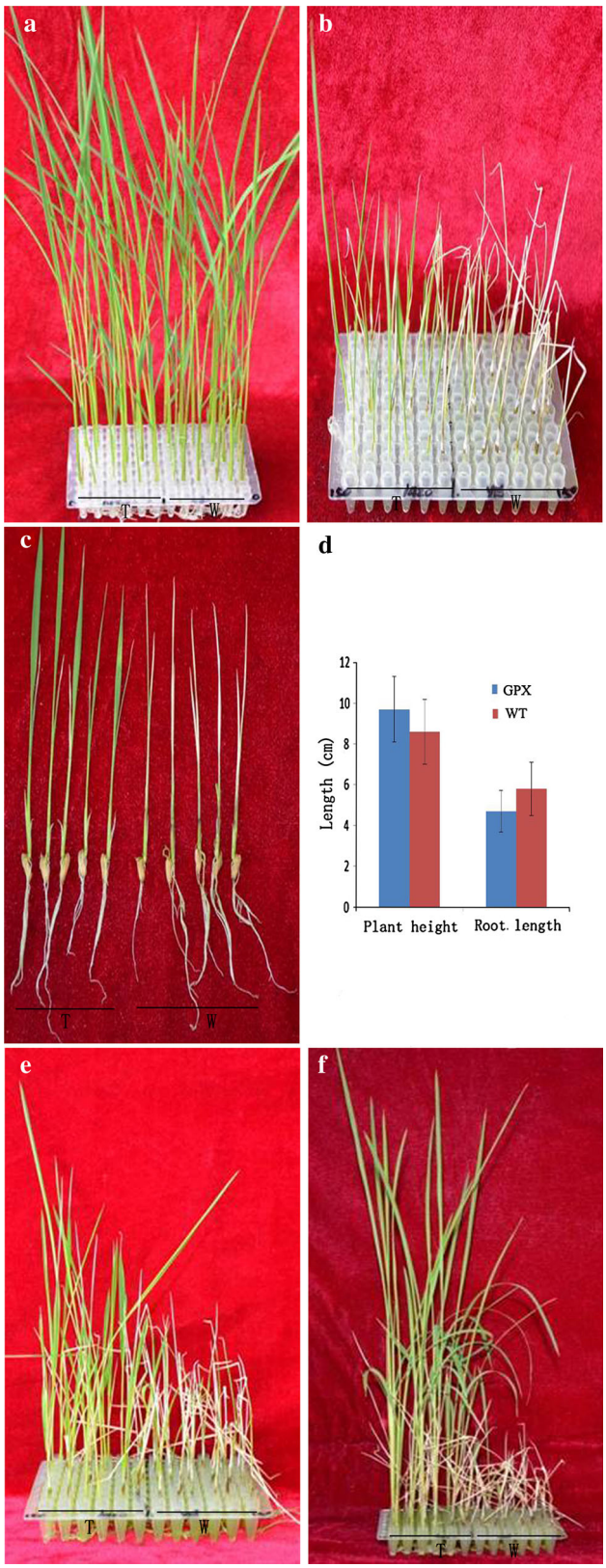
Fig. 8 Analysis of yield characters in transgenic rice. **a** Statistical analysis of yield and its components. **b** Statistical analysis of grain number

12 days. After the treatment, 83.3 % of the transgenic seedlings maintained a green color, as opposed to 16.7 % of the wild-type in the contrast group (Fig. 9a, b). The height of strain and the length of root of the GPX transgenic seedling and the wild-type showed no significant difference (Fig. 9c, d). After recovery, the green seedlings of NnGPX transgenic rice grew robustly, whereas the rest of the wild-type seedlings died (Fig. 9e, f). These results indicated the excellent salt-resistance property of the GPX transgenic rice.

To clarify the mechanism controlling the metabolic reactions representing cellular salinity damage and protection, we also determined the proline, GSSH + GSH, and soluble sugar contents (Fig. 10). The proline and GSSH + GSH contents in the transgenic and non-transgenic lines that were not treated with NaCl exhibited no remarkable difference, except for the soluble sugar content, which was much higher in the non-transgenic line than in the transgenic line. After a 12-day 150 mM NaCl stress treatment, the tested data of the transgenic and non-transgenic plants all increased remarkably compared with the untreated group. The GSH and GSSG content in the GPX transgenic line was evidently higher than that in the non-transgenic group, whereas the soluble sugar and proline contents exhibited no remarkable difference. These physiological indices demonstrated that the transgenic lines were more resistant to salt stress.

Discussion

Numerous studies have been conducted on the GPX of animals, and six types of GPX have been found [18]. GPX5, GPX6, and GPX3 have high homology, but GPX5 has no selenocysteine in its active sites [19]. A new GPX (phospholipid hydrogen peroxide) was found in animals, in which selenocysteine was replaced by cysteine in its conserved catalytic structural domain [20]. Plant GPX and animal PHGPX are highly homologous [21–26]. The plant GPX carries the cysteine (Cys) residual group, replacing the selenocysteine (Se–Cys) inserted at the terminal codon of animal GPX. GPX is a type of selenium-dependent oxidase in animals. Researchers have proven that the concentration change in selenium has a big influence on the expression level and enzyme activity of GPX genes, whereas the GPX genes in plants are not selenium dependent [27]. In this experiment, the expression level of GPX genes treated under different concentrations of selenium did not change remarkably, indicating that NnGPX is non-selenium dependent, similar to the GPX of most plants.



◀**Fig. 9** Analysis of the enhanced salt tolerance in transgenic rice. **a** Seedlings without salt treatment. **b** Seedlings after treatment with 150 mM NaCl for 12 days. **c** Plant morphology of rice seedlings after treatment with 150 mM NaCl for 12 days. **d** Statistical analysis of root length and plant height of rice seedlings after treatment with 150 mM NaCl for 12 days. **e** Recovery for 4 days after treatment with 150 mM NaCl for 12 days. **f** Recovery for 12 days after treatment with 150 mM NaCl for 12 days

The plant GPX family contains six members distributed among different cell organelles. GPX family members in plants may play a defensive role during different stages of evolution and under different stress environments. Roxas et al. [28] found that NtGPX has an important role in endurance to cold, heat, and salt stress. A study on Arabidopsis showed that the AtGPX family has an important role in stress environment response, although different stress environments induce increased expression of different AtGPXs [29]. Under drought stress and through ABA signaling in plants, a larger amount of H_2O_2 aggregates and a greater amount of ATGPX3 is expressed in plant leaves, inhibiting the phosphatase activity of ABI2. This phenomenon leads to the expression of a positive regulatory factor of the ABA signal channel, subsequent elimination of H_2O_2, and reduction of leaf pore diameter and moisture loss, ultimately helping plants in handling the stress [30]. In the present study, after cold, heat, and salt stress treatments and mechanical damage of lotus, we detected a high expression of NnGPX. In lotus plants that grow normally, NnGPX was expressed in all tissues during the seedling and flowering stages. The GPX expression patterns were different from those of Arabidopsis. Thus, NnGPX was shown to have an important role in the resistance of plants to stress environments. Its expression was not tissue specific, and it participated in the metabolic activities of anti-oxidation during the different growth stages of lotus.

Soil salinization is a major non-biological stress. Plants can produce and accumulate numerous active oxygen ingredients that are harmful to cells and directly affect the growth and yield of plants. In this study, we transferred the cloned lotus GPX gene in rice and examined the potential function of NnGPX to salt stress in rice. Under normal state, the NnGPX transgenic rice was not different from the growth features of the non-transgenic rice, particularly in terms of yield. However, under salt stress, the transgenic rice demonstrated better salt resistance than the non-transgenic rice, indicating that the overexpressed NnGPX gene can effectively protect rice from hazards caused by

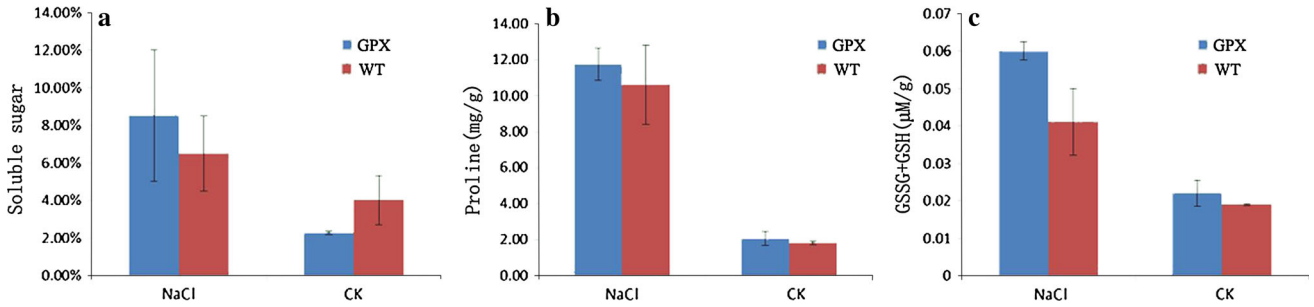


Fig. 10 Analysis of soluble sugar (**a**), proline (**b**), and GSSH + GSH (**c**) contents in *WT* and transgenic lines under normal and salt conditions. Two-week-old rice plants were treated with 50 mM NaCl for 12 days and rice leaves were collected to measure contents of soluble sugar, proline, and GSSH + GSH. Data are mean ± SD calculated from three replica

salt stress, as well as eradicate hydrogen peroxide and lipid peroxide in time. The transformed NnGPX gene did not affect the normal growth of rice but promoted its resistance to salt. This result could pave the foundation for the use of lotus NnGPX gene to cultivate salt-resistant plants.

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References

1. Bowler C, Montagu MV, Inze D (1992) Superoxide dismutase and stress tolerance. Annu Rev Plant Physiol Plant Mol Biol 4:83–116
2. Fink RC, Scandalios JG (2002) Molecular evolution and structure–function relationships of the superoxide dismutase gene families in angiosperms and their relationship to other eukaryotic and prokaryotic superoxide dismutases. Arch Biochem Biophys 399:19–36
3. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG (1973) Selenium: biochemical role as a component of glutathione peroxidases. Science 179:588–590
4. Stadman TC (1996) Selenocysteine. Annu Rev Biochem 65:83–100
5. David AP, Maarten AP (1982) Extreme longevity of lotus seeds from Pulantien. Nature 299:148–149
6. Ding YF, Cheng HY, Song SQ (2008) Changes in extreme high-temperature tolerance and activities of antioxidant enzymes of sacred lotus seeds. Sci China C Life Sci 51:842–853
7. Huang SZ, Tang XJ, Zhang L, Fu JR (2003) Thermotolerance and activity of antioxidative enzymes in lotus seeds. J Plant Physiol Mol Biol 29:421–424
8. Sujay R, Atul W, Kakali M, Bishnu PS, Pulok KM (2006) Antioxidant activity of *Nelumbo nucifera* (sacred lotus) seeds. J Ethnopharmacol 104:322–327
9. Li Y, Zhang Y, Wei S, Liu L, Chen Y (2012) Recovery of antioxidant gene expression in sacred lotus (*Nelumbo nucifera* Gaertn) embryonic axes enhances tolerance to extreme high temperature. Afr J Biotechnol 11:12011–12019
10. Dong C, Li GL, Li ZQ, Zhu HL, Zhou MQ, Hu ZL (2009) Molecular cloning and expression analysis of an Mn-SOD gene from *Nelumbo nucifera*. Appl Biochem Biotech 158:605–614
11. Dong C, Zheng XF, Li GL, Zhu HL, Zhou MQ, Hu ZL (2011) Molecular cloning and expression of two cytosolic copper-zinc superoxide dismutases genes from *Nelumbo nucifera*. Appl Biochem Biotechnol 163:679–691
12. Zheng ZL, Zuo ZY, Liu ZG, Tsai KC, Liu AF, Zou GL (2005) Construction of a 3D model of nattokinase, a novel fibrinolytic enzyme from Bacillus natto: a novel nucleophilic catalytic mechanism for nattokinase. J Mol Graph Model 23:373–380
13. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739
14. Shen G, Pang Y, Wu W, Deng Z, Liu X, Lin J, Zhao L, Sun X, Tang K (2005) Molecular cloning, characterization and expression of a novel Asr gene from *Ginkgo biloba*. Plant Physiol Biochem 43:836–843
15. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(–Delta Delta C(T)) method). Methods 25:402–408
16. Liu Y, Teng Z, Wang S, He G (2008) Effects of high temperature stress on soluble sugar and membrane protective enzyme of rice. J Southwest Uni 30:59–63
17. Zhang DZ, Wang PH, Zhao HX (1990) Determination of the content of free proline in wheat leaves. Plant Physiol Commun 94:462–465
18. Rogerio M, Christophe D, Felipe KT, Marcia MP (2008) Glutathione peroxidase family—an evolutionary overview. FEBS J 275:3959–3970
19. Lee PY, Kho CW, Lee H, Kang S, Lee SC, Park BC, Cho S, Bae KH, Park SG (2007) Glutathione peroxidase 3 of *Saccharomyces cerevisiae* suppresses non-enzymatic proteolysis of glutamine synthetase in an activity-independent manner. Biochem Biophys Res Comm 362:405–409
20. Utomo A, Jiang XZ, Furuta S, Yun J, Levin DS, Wang YCJ, Desai KV, Green JE, Chen PL, Lee WH (2004) Identification of a novel putative non-selenocysteine containing phospholipid hydroperoxide glutathione peroxidase (NPGPx) essential for alleviating oxidative stress generated from polyunsaturated fatty acids in breast cancer cells. J Biol Chem 279:43522–43529
21. Criqui MC, Jamet E, Parmentier Y, Marbach J, Durr A, Fleck J (1992) Isolation and characterization of a plant cDNA showing homology to animal glutathione peroxidases. Plant Mol Biol 18:623–627
22. Holland D, Ben-Hayyim G, Faltin Z, Camoin L, Strosberg AD, Eshdat Y (1993) Molecular characterization of salt-stress-associated protein in Citrus: protein and cDNA sequence homology to mammalian glutathione peroxidase. Plant Mol Biol 21:923–927

23. Sugimoto M, Sakamoto W (1997) Putative phospholipid hydroperoxide glutathione peroxidase gene from *Arabidopsis thaliana* induced by oxidative stress. Genes Genet Syst 72:306–311
24. Li WJ, Feng H, Fan JH, Zhang RQ, Zhao NM, Liu JY (2000) Molecular cloning and expression of a phospholipid hydroperoxide glutathione peroxidase homolog in *Oryza sativa*. Biochim Biophys Acta Gene Struct Expr 1493:225–230
25. Jung BG, Lee KO, Lee SS, Chi YH, Jang HH, Kang SS, Lee K, Lim D, Yoon SC, Yun DJ, Inoue Y, Cho MJ, Lee SY (2002) A Chinese cabbage cDNA with high sequence identity to phospholipid hydroperoxide glutathione peroxidases encodes a novel isoform of thioredoxin-dependent peroxidase. J Biol Chem 277:12572–12578
26. Nicolas N, Valérie C, José G, Eric G, Masakazu H, Pascal R, David BK, Emmanuelle Issakidis, Jean-Pierre Jacquot, Nicolas Rouhier (2006) Plant glutathione peroxidases are functional peroxiredoxins distributed in several subcellular compartments and regulated during biotic and abiotic stresses. Plant Physiol 142:1364–1379
27. Rocher C, Lalanne JL, Chaudiere J (1992) Purification and properties of a recombinant sulfur analog of murine selenium-glutathione peroxidase. Eur J Biochem 205:955–960
28. Roxas VP, Lodhi SA, Garrett DK, Mahan JR, Allen RD (2000) Stress tolerance in transgenic tobacco seedlings that overexpress glutathione S-transferase/glutathione peroxidase. Plant Cell Physiol 41:1229–1234
29. Milla M, Maurer A, Huete A, Gustafson J (2003) Glutathione peroxidase genes in *Arabidopsis* are ubiquitous and regulated by abiotic stresses through diverse signaling pathways. Plant J 36:602–615
30. Miao Y, Lv D, Wang P, Wang X, Chen J, Miao C, Song C (2006) An *Arabidopsis* glutathione peroxidase functions as both a redox transducer and a scavenger in abscisic acid and drought stress responses. Plant Cell 18:2749–2766