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Overexpression of *Pp14-3-3* from *Pyrus pyrifolia* fruit increases drought and salt tolerance in transgenic tobacco plant

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Abstract: Drought and salinity are the major abiotic stresses, which reduce agricultural productivity. In plants, 14-3-3s function as regulators of many target proteins through direct protein-protein interactions and play an important role during response to abiotic stresses. Here we report that CaMV 35S promoter driven overexpression of a *Pyrus pyrifolia* 14-3-3 gene, *Pp14-3-3*, improves drought and NaCl tolerance in T1 generation plants of transgenic tobacco (*Nicotiana tabacum* L. cv Xanthi). Under drought and NaCl stresses, the *Pp14-3-3* was largely expressed in T1 transgenic tobacco lines, and compared with the wild-type (WT), transgenic tobacco plants showed relatively normal growth condition. In addition, the levels of membrane lipid peroxidation in T1 transgenic lines were definitely lower than that in WT according to the significantly decreased content of malondialdehyde. Meanwhile, the T1 transgenic tobacco lines showed significantly slower superoxide anion production rate than the WT under abiotic stress. Moreover, both the glutathione S-transferase (GST) and ascorbate peroxidase (APX) activities in T1 transgenic lines were markedly higher than those in WT. GSTs and APXs are important components of plant antioxidant system, and the results of present study suggested that *Pp14-3-3* should play a crucial role in reducing oxidative damage caused by drought and salt stresses.

Key words: 14-3-3; drought tolerance; oxidative stress; *Pyrus pyrifolia*; salt tolerance; transgenic tobacco.

Abbreviations: ABA, abscisic acid; APX, ascorbate peroxidase; GST, glutathione S-transferase; MDA, malondialdehyde; ORF, open reading frame; QRT-PCR, quantitative reverse transcription – polymerase chain reaction; ROS, reactive oxygen species; WT, wild-type.

Introduction

With rapid growth in global population, it is urgently need to increase the yields of crop plants in order to meet the expanded demand of food production. However, crop plants often encounter a wide range of environment stresses, such as drought, salinity, extreme temperatures, heavy metals, and ultraviolet radiation, which negatively affect the growth and productivity of plant. It is noteworthy that a huge part of crop losses were caused by salinity, drought, and other abiotic stresses. Plants have evolved a series of complex mechanisms to cope with the abiotic stresses including molecular networks involved in stress perception and signal transduction as well as the induction of specific stress-related genes (Vinocur & Altman 2005). The expression regulation of stress-related genes can lead to various kinds of biochemical and physiological changes in plant during response to stresses, such as activation of detoxifying enzymes, clearance of reactive oxygen species (ROS), compartmentalization of toxic ions in the vacuoles, and accumulation of compatible solutes (Foolad 2007).

Previous studies indicated that 14-3-3s were involved in the molecular networks protecting plant cells from damages of biotic and abiotic stresses (Chelysheva et al. 1999; Chen et al. 2006; Denison et al. 2011). The 14-3-3s are encoded by multigene family, and it has been reported that 13 14-3-3 isoforms were found in *Arabidopsis thaliana* (Sun et al. 2011) and 16 isoforms in soybean (*Glycine max*) (Li & Dhaubhadel 2011). Phylogenetic analysis of the core region of 14-3-3s from different species showed that plant 14-3-3s fall into two groups, the epsilon and the non-epsilon group (Ferl et al. 2002). Many members of plant 14-3-3 gene family responded to abiotic stresses. When sugar beet (*Beta vulgaris*) cells were exposed to cold or osmotic stresses, the 14-3-3 proteins were increased in plasma membrane accompanied by the increased activity of H^+-ATPase (Chelysheva et al. 1999). Under each stressful condition of salinity, potassium, and iron deficiency, the expression level of a tomato (*Solanum lycopersicum*) 14-3-3 gene *TFT7* was strongly up-regulated (Xu & Shi 2006). In addition, the levels of 4 rice (*Oryza sativa*) *14-3-3* genes, *GF14b*, *GF14c*, *GF14e*, and *GF14f*, were differentially regulated by drought, salt, and abscisic acid

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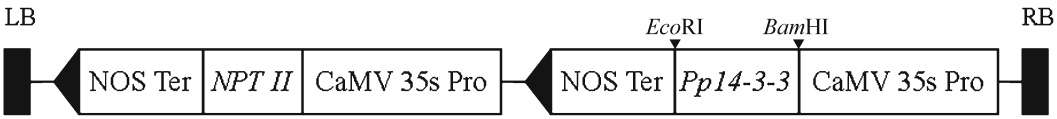

Fig. 1. Schematic diagram of *Pp14-3-3* expression construct. *Pp14-3-3* gene was under control of the CaMV 35S promoter and linked to the kanamycin resistance gene *NPT II*.

(ABA) (Chen et al. 2006). Under salinity or drought condition, the transcript levels of a few cotton (*Gossypium hirsutum*) 14-3-3 genes, such as *Gh14-3-3b*, *Gh14-3-3c*, *CGF14_4*, and *CGF14_10* were significantly induced in roots (Wei et al. 2009; Sun et al. 2011). Moreover, gene expression analysis indicated that a maize (*Zea mays*) 14-3-3 gene *ZmGF14-6* was up-regulated by salt, but its expression was down-regulated by drought stress (Campo et al. 2012).

In plants, 14-3-3s are known to function in regulating many biological processes including metabolism, hormone signalling, growth and cell division, light signalling, transcription, cell-cycle control, protein trafficking, and stress responses through binding to their phosphorylated client proteins and modulating their functions (Denison et al. 2011). Under abiotic stresses, the 14-3-3 proteins were involved in various signal transduction processes and interact with many targets including plasma membrane H^+-ATPase, ion channels, ABA, and ascorbate peroxidase (APX). The 14-3-3 proteins are regulatory partners of plant K^+ channel through which protect plants against the salt stress (Roberts et al. 2002). To mitigate the damages caused by stresses, one response of the ABA signalling pathway is to increase the expression of necessary genes, and 14-3-3s are the genes which interact with some transcription factors involved in ABA signal transduction (Schoonheim et al. 2007). APX is an important defence enzyme that protects plant cells from oxidative damage, and the interaction of 14-3-3 isoforms with APX hinted the potential role of 14-3-3 in regulation of antioxidant status of plant cells (Lukaszewicz et al. 2002). Overexpression of *TFT7*, a gene encoding a 14-3-3 protein in tomato, improved salt tolerance in *Arabidopsis* (Xu & Shi 2007). Compared with wild-type (WT), the *TFT7* transgenic plants showed increased germination rate, dry mass, total chlorophyll concentration, and root length under salt stress.

Pyrus pyrifolia Nakai cv Huobali is a kind of local sand pear in Yunnan province of China. It possesses a strong adaptability to soil and showed high level of resistance to biotic and abiotic stresses. 'Huobali' has been widely used to develop novel pear cultivars, however, there are few studies on resistance-related genes isolated from 'Huobali'. In previous study, we isolated a novel *14-3-3* gene, *Pp14-3-3* (GenBank accession No. JF810599) from the fruit of 'Huobali' (Wang et al. 2012). The full-length cDNA of *Pp14-3-3* was 1,107 bp with an open reading frame (ORF) of 786 bp which encoded a predicted polypeptide of 261 amino acids. The phylogenetic analysis of *Pp14-3-3* with some known plant 14-3-3s grouped *Pp14-3-3* into the class of non-epsilon. Moreover, *Pp14-3-3* was abundantly expressed in pericarps of 'Huobali' fruits, hinted that the *Pp14-3-3* may be involved in response to stress during the pear fruit development. In the present study, the plant expression vector of *Pp14-3-3* was constructed and introduced into tobacco through *Agrobacterium*-mediated transformation. After the positive transgenic tobacco lines were screened out, the growth condition and tolerance of T1 transgenic lines and non-transgenic plant were analyzed under drought and NaCl stresses.

Material and methods

Plant materials

The sterile seedlings of *Nicotiana tabacum* L. cv Xanthi were cultivated in our laboratory and used for genetic transformation.

Construction of Pp14-3-3 binary vector for tobacco transformation

The ORF of *Pp14-3-3* from pMD-18T-*Pp14-3-3* was inserted into pCAMBIA2300s vector via *Bam*HI and *Eco*RI restriction sites to generate a plant expression construct pCAMBIA2300s-*Pp14-3-3* in the present study (Fig. 1). The primers with recognition sites of *Bam*HI and *Eco*RI were respectively designed to amplify the full-length ORF of *Pp14-3-3* (forward primer 5'GGATCCGACCAGAGAAG GTTTCAGATTTAGG3' and reverse primer 5'GAATTCT GCCATCTATTACAAAGGTCCCAAG3'), then the specific PCR product was cloned into the pMD-18T vector (Takara, Japan). The full-length ORF of*Pp14-3-3* was obtained through double digestion of pMD-18T-*Pp14-3-3* with *Bam*HI and *Eco*RI, and ligated into the digested pCAMBIA2300s with the same two restriction endonucleases. Then the ligation product was transferred into competent cells of *Escherichia coli* DH5α, and the recombinant plasmids of pCAMBIA2300s-*Pp14-3-3* were screened out through PCR.

Tobacco transformation and PCR screening of transgenic tobacco

The*Agrobacterium tumefaciens* strain LBA4404 containing the binary plasmid pCAMBIA2300s-*Pp14-3-3* was obtained with electro-transformation and the clones on the Luria-Bertani plate with kanamycin were confirmed by PCR. Then the *A. tumefaciens* positive clones were used to transform leaf discs of tobacco (*N. tabacum* L. cv Xanthi) (Horsch et al. 2007). The infected tobacco leaf discs were cultured on the inductive differentiation medium containing kanamycin (0.05 mg/mL) and cefotaxime (0.2 mg/mL) to regenerate plantlet. The regenerated plants were selected in the Murashige and Skoog medium plates supplemented with 0.05 mg/mL kanamycin. In order to confirm the stable integration of transgene in the kanamycin resistant plants, subsequently, the tobacco genomic DNA was extracted with CTAB (hexadecyltrimethylammonium bromide) method,

then the positive transgenic tobacco plants were screened through PCR for presence of the *Pp14-3-3* gene.

Southern blot analysis
To detect the insertion copy numbers of transgenic plants, genomic DNA (10 μg each) of WT and 5 randomly selected positive transgenic tobacco plants were analyzed with the specific probe of *NPTII* in southern blotting, and the detailed experimental procedure was as presented in Liu et al. (2012).

Abiotic treatments
A total of three T1 transgenic lines (each contained 9 plants) together with WT seedlings were transplanted to pots filled with humus and soil, and cultured in the Phytotron for 2 weeks under a 16:8 h light/dark photoperiod, room temperature, and relative humidity of 50%. Then the cultures were placed in green house for 2 weeks. During this period, all the tobacco plants were supplemented with Hoagland nutrient solution at 2-day intervals. The T1 transgenic and WT plants were assessed for tolerance to drought stress by withholding irrigation. After the soil water content decreased to 9%, the drought stress treatment was last for 7 days. To evaluate salt tolerance, the transgenic and WT plants were carefully watered by 500 mM NaCl solution for 7 days. After drought and NaCl treatments, the growth status of all the transgenic and WT plants was recorded, and young leaves of transgenic and WT plants were collected and used in the following assays.

Quantitative reverse transcription-polymerase chain reaction (QRT-PCR)
The specific primers of *Pp14-3-3* designed with the software Primer Premier 5 were as follows: forward primer: 5'CGAGACAATCTGACACTCTGGACTTC3', and reverse primer: 5'ACCCACATGCCATCTATTACAAAGG3'. The expression level of tobacco *actin* gene (GenBank accession No. AB158612.1) was used as internal control to standardize the RNA samples. The protocol of QRT-PCR was same as that in Liu et al. (2012).

Determination of superoxide anion production rate and malondialdehyde (MDA) content
As for determination of superoxide anion production rate, a modified method of Wang & Luo (1990) was used. Approximately, 0.5 g leaves were grounded into powder with liquid nitrogen, then 1.5 mL 50 mM potassium phosphate buffer (pH 7.0) was added and homogenized. The supernatant was obtained after centrifugation. The incubation mixture contained the 0.5 mL supernatant, 0.5 mL 50 mM phosphate buffer (pH7.0), 1 mL 1 mM hydroxy lamine hydrochloride; then incubation at 25 ℃ for 1 h. Subsequently, 17 mM sulfanilic acid (1 mL) and 7 mM α-naphthylamine (1 mL) were added. After incubated at 25 ℃ and coloured for 20 min, the absorbance at 530 nm was measured. A standard curve was established according to the method of Wang & Luo (1990).

The MDA content was determined with method described in Le Martret et al. (2011). After the leaves were grounded into powder with liquid nitrogen and homogenized with 0.2 mL distilled water, an equal volume of 0.5% (w/v) thiobarbituric acid in 20% (v/v) trichloroacetic acid was added. After incubated at 95 ℃ for 30 min, the mixture was cooled on ice for 15 min to stop the reaction. After centrifugation, the supernatant of reaction product was taken to measure the absorbance at wavelengths of 532 and 600 nm. The total value for non-specific absorption at 600 nm was subtracted from the 532 nm value. MDA contents were calculated by its absorption coefficient of 155 nmol/cm.

Determination of glutathione S-transferase (GST) and APX activity
About 0.2 g leaves were grounded into powder with liquid nitrogen and homogenized in 0.2 mL protein extraction buffer [50 mM phosphate buffer (pH 7.0), 1 mM EDTA, 0.05 (v/v) of Triton-100, 2% polyvinylpyrrolidone, and 0.5 mM phenylmethanesulfonylfluoride]. The supernatant was taken after centrifugation and used as crude enzymes preparation for determination of GST and APX activities.

GST was determined with the method of Veal et al. (2002). The GST assay buffer (1 mL) contained the crude enzyme extract 50 μL, 50 mM phosphate buffer (pH 7.0), 1 mM 1-chloro-2, 4-dinitrobenzene, and 1 mM reduced glutathione, then the solution was incubated at 25 ℃, and the absorbance at 340 nm was measured at timed intervals. APX activity was determined by monitoring the decline in absorbance at 240 nm in accordance with Nakano & Asada (1981). Each 3 mL reaction mixture included 20 μL enzyme extract, 50 mM potassium phosphate buffer (pH 7.0), and 0.1 mM H_2O_2. The extinction coefficient of 2.8 mM^{-1} cm^{-1} was used for calculating the amount of oxidized ascorbate.

Statistical analysis
The relative expression level of *Pp14-3-3*, MDA content, superoxide anion production rate, GST and APX activities are presented as means and standard deviations of the means, and the statistical analyses were performed with the SPSS software. The statistical differences between WT and T1 transgenic plants were analyzed by Student's *t* test.

Results

Selection of Pp14-3-3 transgenic tobacco plants
In the present study, nearly 210 leaf discs of *N. tabacum* L. cv Xanthi were transformed by *A. tumefaciens* LBA4404 with *Pp14-3-3* expression cassette, and a total of 41 independent transgenic tobacco lines which resisted to kanamycin were generated. The kanamycin-tolerant plants were analyzed by PCR to produce an amplicon of 894 bp with the *Pp14-3-3* specific primers. Then the positive transformants were screened out. Finally, 24 lines contained the *Pp14-3-3* gene were confirmed as positive transgenic lines among the 41 lines. Moreover, the morphology and growth of positive transgenic plants were observed, and there were no visible differences between the transgenic plants and WT.

Southern blot analysis of Pp14-3-3 transgenic tobacco and development of T1 generation transgenic plants
To determine the insertion copy number of integrated foreign *Pp14-3-3* gene in transgenic plants, genomic DNA of 5 randomly selected transgenic lines (3-2, 3-5, 3-7, 3-9, and 3-10) and WT was digested by *Eco*RI and hybridized with the gene-specific probe of *NPTII*. The result is shown in Figure 2. It is easy to see that the transgenic tobacco lines were integrated 1-3 copies of *Pp14-3-3* expression cassette. The 3 lines, 3-5, 3-7, and 3-10, were single-copy insertion, the line 3-2 was double-copied, and the line 3-9 had 3 copy numbers of T-DNA. Obviously, different profiles of transgene were observed in 5 randomly chosen T0 tobacco plants. The 3 lines, 3-5, 3-7, and 3-10, which had one copy of T-DNA, were

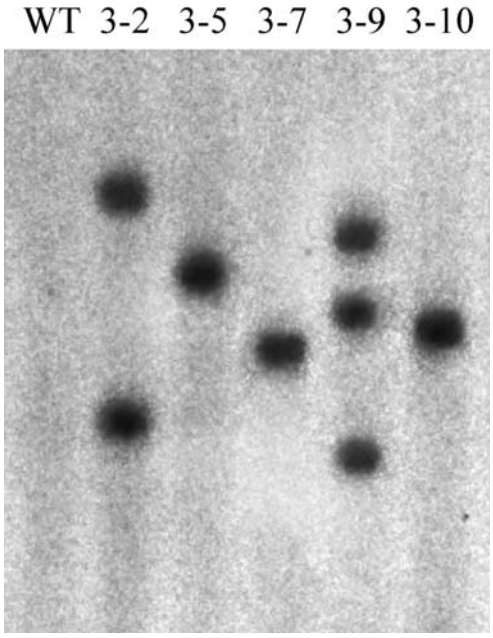

Fig. 2. Copy number analysis of *Pp14-3-3* transgenic tobacco lines. The DNA analyzed in southern blotting was isolated from WT and 5 randomly selected transformants, 3-2, 3-5, 3-7, 3-9, and 3-10, then digested by *Eco*RI and hybridized with the*NPT II* probe.

chosen to generate T1 generations. Co-segregation analysis was performed by kanamycin selection and PCR confirmation of inbred progenies. The T1 generations of all the 3 lines showed normal phenotype (data not shown), and they were further analyzed in the following experiments.

Pp14-3-3 was highly expressed in T1 transgenic tobacco
In order to detect whether the *Pp14-3-3* gene was expressed under normal and stressful conditions, the total RNA was extracted from young leaves of 3 T1 tobacco lines (3-5, 3-7, and 3-10) for QRT-PCR. As shown in Figure 3, the transcripts of*Pp14-3-3* largely accumulated in all the 3 transgenic lines under normal and stressful conditions. Besides, *Pp14-3-3* was slightly upregulated by drought and NaCl stresses compared with the expression level during normal development. Additionally, transcription levels of *Pp14-3-3* in the transgenic lines 3-5 and 3-10 were visibly higher than that in line 3-7 under normal condition or abiotic stresses. The data of present assay indicated that the *Pp14-3-3* was highly expressed in T1 transgenic tobacco plants under driving of the CaMV 35S promoter.

Phenotype of T1 transgenic plants under drought and salt stresses
After drought treatment for 7 days, the WT plant was markedly dehydrated and withered, while the T1 transgenic plants showed a little of dehydration but no obvious wilting (Fig. 4). As for the salt stress, after 500 mM NaCl treatment for 7 days, the WT was notably etiolated and the growth of plant was severely inhibited; by contraries, *Pp14-3-3* transgenic plants did not show evident chlorotic symptom (Fig. 4). In conclusion, the *Pp14-3-3* transgenic tobacco showed relatively normal growth condition compared with WT under stresses, which suggested that overexpression of *Pp14-3-3* enhanced resistance of transgenic tobacco lines to drought and NaCl stresses.

Physiology of T1 transgenic plants under drought and salt stresses
In the present study, both the superoxide anion production rate and MDA content were stable and maintained low levels during normal development, and no significant difference was observed between WT and T1 transgenic lines (Fig. 5). Under stressful conditions, the above physiological parameters in WT and all the 3 transgenic lines were greatly increased. But the *Pp14-3-3* transgenic tobacco plants showed significantly slower

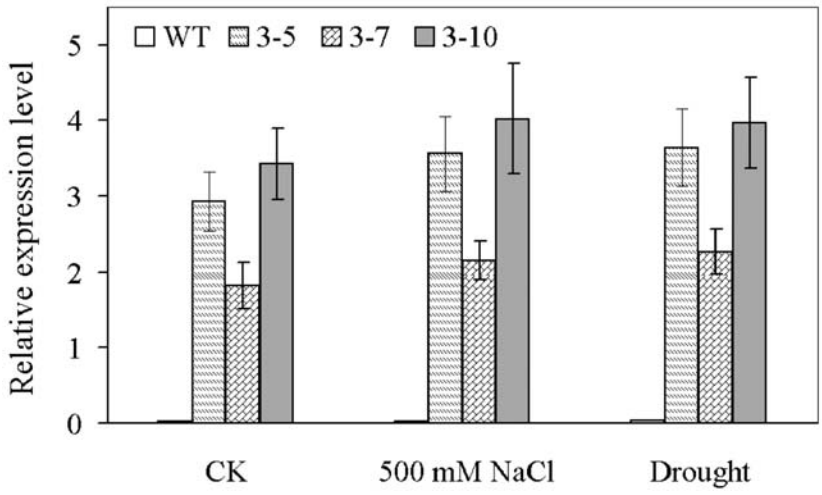

Fig. 3. Expression analysis of *Pp14-3-3* in T1 transgenic tobacco lines and WT by QRT-PCR. Relative values of *Pp14-3-3* expression in young tobacco leaves under normal and stressful conditions are shown. Data represent the means ± SD of three independent experiments. The *Pp14-3-3* was expressed at the transcriptional level in all the 3 transgenic tobacco lines.

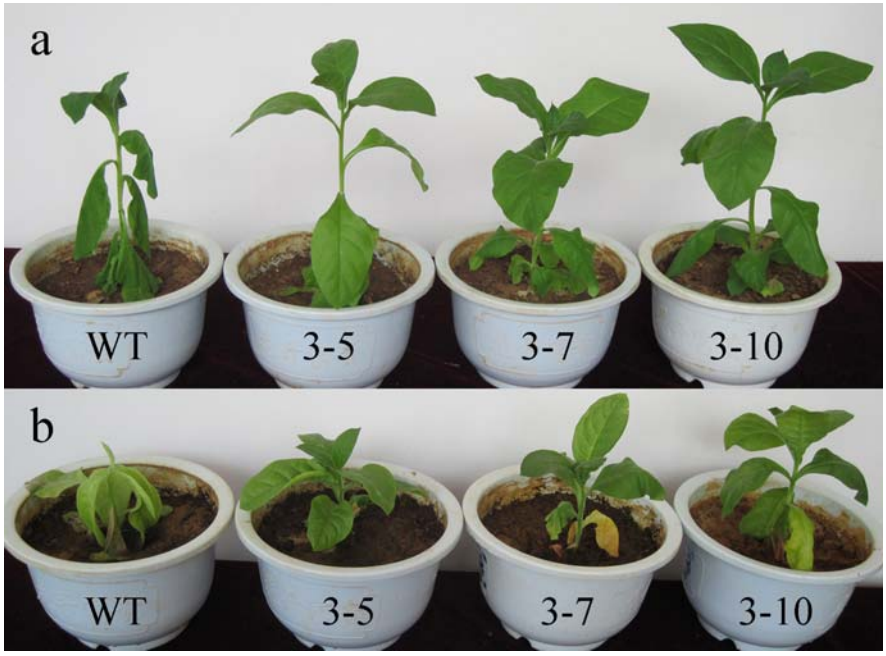

Fig. 4. Growth of *Pp14-3-3* transgenic tobacco plants and WT under drought (a) and NaCl (b) stresses. The T1 generations of *Pp14-3-3* overexpressing tobacco showed relatively normal growth condition under stresses.

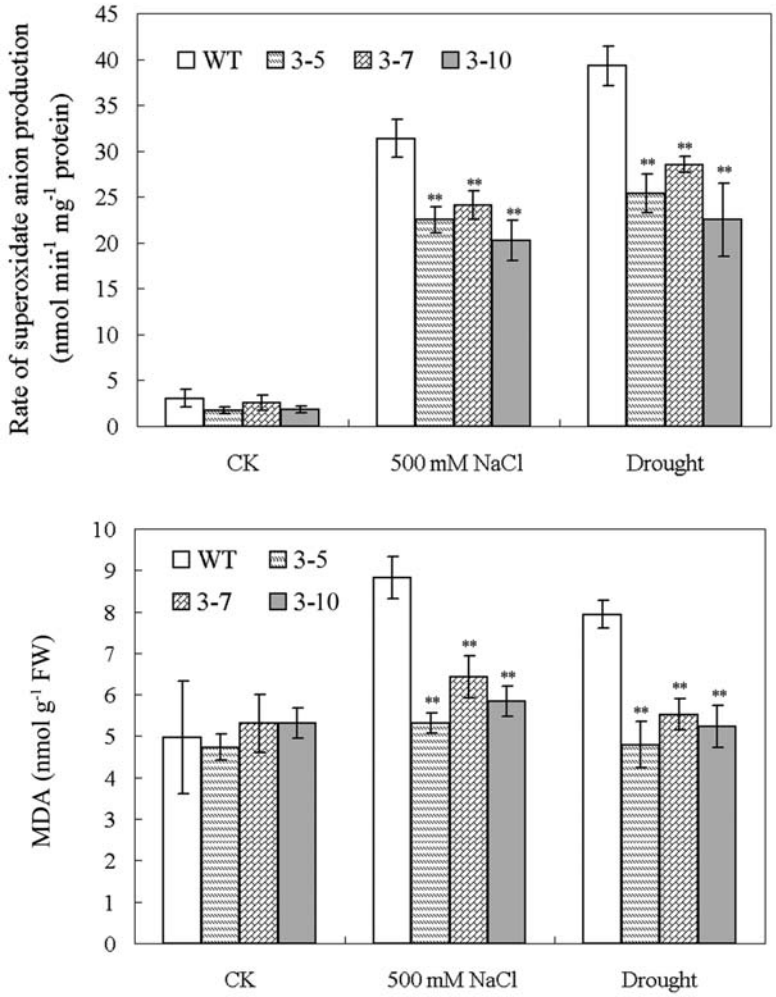

Fig. 5. Changes in the rate of superoxide anion production and MDA content in leaves of *Pp14-3-3* transgenic tobacco lines and WT under drought and NaCl stresses. Data represent the means ± SD of three independent experiments. A single asterisk and double asterisks mean significant values at $p < 0.05$ and $p < 0.01$, respectively.

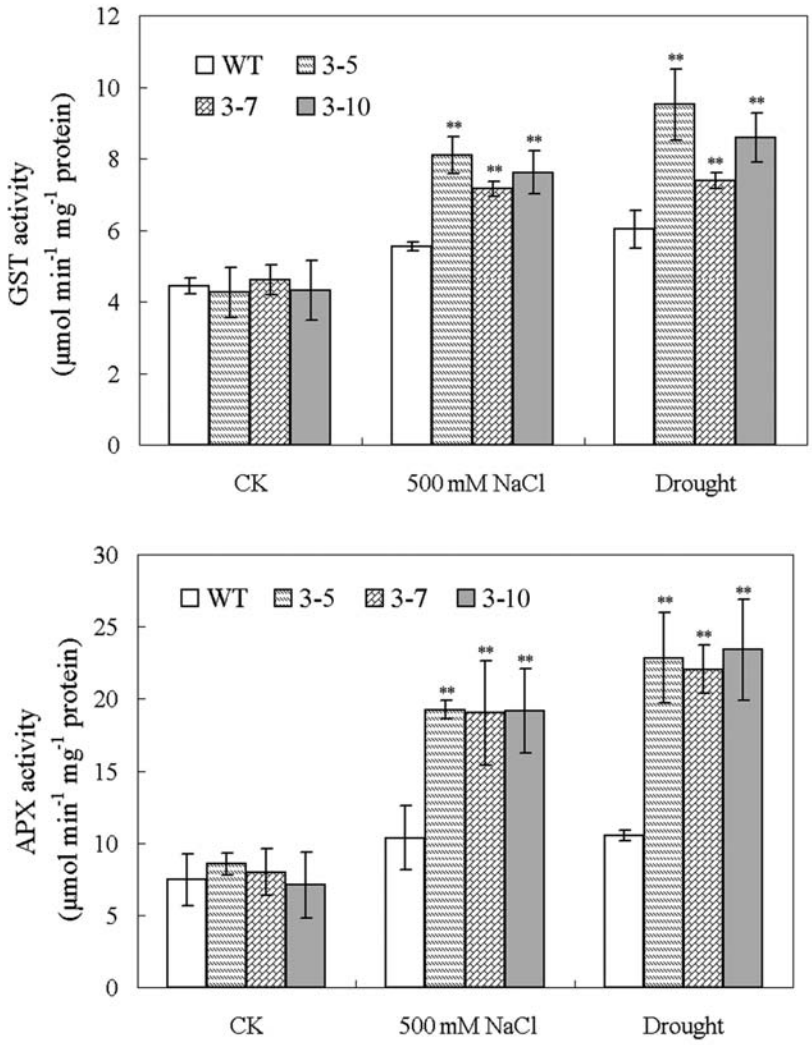

Fig. 6. Enzymatic assay for GST and APX in *Pp14-3-3* transgenic tobacco plants and WT under normal and stressful conditions. Data represent the means ± SD of three independent experiments. A single asterisk and double asterisks mean significant values at $p < 0.05$ and $p < 0.01$, respectively.

superoxide anion production rate and MDA content than WT during stressed by drought and NaCl, respectively (Fig. 5). Apparently, the oxidative damages caused by drought and NaCl stresses were greatly reduced in *Pp14-3-3* overexpressing tobacco plants, and protection on membranes was evidenced through the reduction of MDA level in *Pp14-3-3* transgenic tobacco lines under abiotic stresses.

The assay of GST and APX activities in this study indicated that the 2 enzymes in WT and *Pp14-3-3* transgenic tobacco lines did not show significant difference during normal development (Fig. 6). After stressed by drought and NaCl, both the GST and APX activities increased in WT and transgenic lines, but the increase amplitude of APX and GST activities in 3 T1 transgenic lines was greatly higher than that in WT (Fig. 6). Besides, the GST and APX activities showed similar behaviour under stressful conditions in all the transgenic lines. Based on these results, it is reasonable to conclude that the overexpression of *Pp14-3-3* in tobacco up-regulated GST and APX activities through this mechanism to scavenge ROS and enhance tolerance of transgenic tobacco to oxidative stresses.

Discussion

The 14-3-3s are a large family of regulatory molecules existing in virtually all eukaryotes. In higher plants, the 14-3-3s are involved in regulating complex environmental signalling pathways and networks. In addition, they allow crosstalk among different pathways (Denison et al. 2011). Previous studies indicated that 14-3-3s play important roles in response to drought and salt stresses (Yan et al. 2004; Xu & Shi 2007; Wei et al. 2009). In the preceding study, we isolated a 14-3-3 gene *Pp14-3-3* from *P. pyrifolia* Nakai cv. Huobali, and *Pp14-3-3* was largely expressed in the pericarp of 'Huobali' fruit. In order to reveal whether *Pp14-3-3* is associated with tolerance to oxidative damage under drought and NaCl stresses, the plant overexpression vector of *Pp14-3-3* was constructed and transferred into tobacco. According to the results of Figures 2 and 3, *Pp14-3-3* was successfully integrated into the genome of transgenic

tobacco lines and expressed as expected in the transformants under normal and stressful conditions.

It is well known that abiotic stresses cause redundant accumulation of ROS, which results in oxidative damages in cells (Sairam et al. 2004; Sairam & Tyagi 2004). As one of the strongest ROS among free radicals, superoxide anion can damage biomolecules, such as lipids, proteins, and nucleic acids. MDA is formed as an end-product of lipid peroxidation caused by ROS and other free radicals in membranes (Smirnoff 2005). The present study indicated that *Pp14-3-3*-overexpressing T1 transgenic plants were more tolerant to drought and NaCl stresses than WT (Fig. 5). Under normal condition, the superoxide anion production rate and MDA level were not significantly different among all the tobacco plants, nevertheless, the 2 physiological parameters of T1 transgenic plants were much lower than WT under drought and salt stresses. Similarly, it had been reported that overexpression of a tomato 14-3-3 gene *TFT7* reduced H_2O_2 generation and the degree of MDA accumulation in the transgenic plants (Xu & Shi 2007). Therefore, the drought and salt tolerance of *Pp14-3-3*-overexpressing tobacco plants may be explained by reduction of oxidative stress damage.

Under drought and high salt conditions, plant growth is severely inhibited due to ionic, osmotic, and oxidative stresses. Plants have developed antioxidant defence systems to cope with the damages resulting from different stresses, such as expression of a series of antioxidant enzymes including APX, GST, superoxide dismutase, catalase, and glutathione peroxidase (Smirnoff 2005; Xu & Shi 2007). GSTs are ubiquitous enzymes that provide physiological flexibility to deal with numerous stresses. The transgenic tobacco plants overexpressing a GST cDNA (*Nt107*) exhibited a substantial improvement in seed germination and seedling growth under chilling and salt stresses, moreover, the transgenic tobacco seedlings showed lower level of lipid peroxidation, but maintained higher level of metabolic activity compared to WT (Roxas et al. 1997, 2000). Previous studies suggested that GSTs may participate in rapid clearance of superoxide radicals to protect plant cells from oxidative damage. In this study, overexpression of *Pp14-3-3* in tobacco improved the GST activity under stressful conditions, which hinted that the *Pp14-3-3* might interact with GST and regulates its activity. In addition, the 14-3-3 proteins also interact with APX, which plays an important role in abiotic stresses (Zhang et al. 1997; Lukaszewicz et al. 2002). APXs are found in almost every compartment of plant cell and involved in removal of ROS as a part of ascorbate-glutathione or Asada-Halliwell-Foyer pathway (Smirnoff 2005). It was reported that the signal(s) generated at the very early stage of oxidative stress can be perceived by an *Arabidopsis* 14-3-3 protein, AFT1, and then the effect of AFT1 on APX3 regulates the subsequent down-stream events including a rapid detoxification in cytosol or peroxisome where large amounts of ROS are produced (Zhang et al. 1997). In this paper, under drought and NaCl stresses, the APX activity in transgenic plants were higher than that in WT, proved that *Pp14-3-3* up-regulated the APX activity. All the results of this paper suggest that overexpression of *Pp14-3-3* in tobacco enhanced the activities of GST and APX, and the stronger tolerance of transgenic plants to drought and NaCl were associated with the higher activities of GST and APX.

In conclusion, our study demonstrated that overexpression of *Pp14-3-3* from *P. pyrifolia* fruit in tobacco plants significantly enhanced the drought and salt tolerance. Therefore, it is reasonable to point out that *Pp14-3-3* plays an important role in response to drought and salt stresses and could be a candidate gene for developing drought- and salt-tolerant crops. Further understanding the actual roles and the complex interactions of 14-3-3 proteins are the challenging goals for future research. The study on determination of cellular and subcellular localizations and target molecules of *Pp14-3-3* will be performed to completely understand the roles and molecular mechanism of *Pp14-3-3* in response to abiotic stresses.

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