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Cloning and functional analysis of a novel ascorbate peroxidase (APX) gene from *Anthurium andraeanum*[*]

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Abstract: An 888-bp full-length ascorbate peroxidase (APX) complementary DNA (cDNA) gene was cloned from *Anthurium andraeanum*, and designated as *AnAPX*. It contains a 110-bp 5′-noncoding region, a 28-bp 3′-noncoding region, and a 750-bp open reading frame (ORF). This protein is hydrophilic with an aliphatic index of 81.64 and its structure consisting of α-helixes, β-turns, and random coils. The AnAPX protein showed 93%, 87%, 87%, 87%, and 86% similarities to the APX homologs from *Zantedeschia aethiopica*, *Vitis pseudoreticulata*, *Gossypium hirsutum*, *Elaeis guineensis*, and *Zea mays*, respectively. *AnAPX* gene transcript was measured non-significantly in roots, stems, leaves, spathes, and spadices by real-time polymerase chain reaction (RT-PCR) analysis. Interestingly, this gene expression was remarkably up-regulated in response to a cold stress under 6 °C, implying that *AnAPX* might play an important role in *A. andraeanum* tolerance to cold stress. To confirm this function we overexpressed *AnAPX* in tobacco plants by transformation with an *AnAPX* expression construct driven by CaMV 35*S* promoter. The transformed tobacco seedlings under 4 °C showed less electrolyte leakage (EL) and malondialdehyde (MDA) content than the control. The content of MDA was correlated with chilling tolerance in these transgenic plants. These results show that *AnAPX* can prevent the chilling challenged plant from cell membrane damage and ultimately enhance the plant cold tolerance.

Key words: *AnAPX*, Gene expression, Cold stress, *Anthurium andraeanum*
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1 Introduction

Plants frequently encounter various unfavorable environmental conditions, such as extreme temperatures, salinity, drought, and heavy metals, which adversely affect plant growth and development. *Anthurium andraeanum*, originating from the tropic areas, is an indoor ornamental potted flower produced popularly in Zhejiang Province, China. In this area the production of this flowering plant in protected cultivation requires heating in winter as this plant can only grow well above 15 °C, and is vulnerable to low temperature below 12 °C. Therefore, the high energy cost especially in winter reduces its market profitability. A cultivar with a higher cold tolerance is thus highly desired for this flower production. Apart from traditional breeding methods, genetic engineering is now thought to be a successful approach to create new stress tolerant varieties (Bhatnagar-Mathur *et al*., 2008). However, it remains very difficult to identify the target genes for genetic manipulation or modification, resulting in such tolerant plants.

The reactive oxygen species (ROS) are ubiquitously generated in all of the aerobic organisms even under no stress conditions, but accumulated at a lower level which is fine regulated by their production and degradation (Mittova *et al*., 2004). The hydroxyl

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radical (·OH), single oxygen ($^1O_2$), superoxide anion radical ($O_2^- \cdot$), and hydrogen peroxide (H_2O_2) are four common ROS arising from many metabolic pathways such as β-oxidation, photorespiration, and purine metabolism. Among these ROS, H_2O_2 is the most stable one. Its removal by the ascorbate-glutathione cycle (AGC) constitutes one of the major ROS scavenging systems, protecting plants against oxidative stress caused by H_2O_2 surge (Asada, 1992). The AGC can be constituted with many enzymes including ascorbate peroxidase (APX), monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DAR), and glutathione reductase (GR). However, APX is the most generally employed in plants which can rapidly convert H_2O_2 to water at the expense of ascorbate (AsA) consumption as its specific electron donor (AH_2). APX has several isoenzymes which are named according to their subcellular localizations, such as cytosolic APX (cAPX) in cytosolic, membrane-bound APX (mAPX) in glyoxysome and peroxisome, thylakoid membrane-bound APX (tAPX) and stromal APX (sAPX) in chloroplasts, and mitochondrial membrane-bound APX (mitAPX) in mitochondria (Shigeoka *et al*., 2002).

APX activity was up-regulated substantially when plants were subjected to environmental stresses, for instance, heavy metals, high salinity, drought, high temperature, and wounding (Shi *et al*., 2001). *APX* has been cloned from many plant species such as pea (Mittler and Zilinskas, 1991), *Arabidopsis* (Kubo *et al*., 1992; Santos *et al*., 1996; Maruta *et al*., 2012), maize (van Breusegem *et al*., 1995), spinach (Webb and Allen, 1995; Yoshimura *et al*., 1999), tobacco (Orvar and Ellis, 1995), strawberry (Kim and Chung, 1998), barley (Shi *et al.*, 2001), potato (Kawakami *et al*., 2002), rice (Lu *et al*., 2005), *Vitis pseudoreticulata* (Lin *et al*., 2006), birch (Wang *et al*., 2009), muskmelon (Cheng *et al*., 2009), lily (Chen *et al.*, 2010), grain sorghum (Chen *et al*., 2011), and *Nelumbo nucifera* (Dong *et al*., 2011).

In this work, an *APX* gene from *A. andraeanum* was cloned and its expression patterns were analyzed. The function of this gene was also investigated and proposed to be associated with the plant tolerance to cold. These results could provide information for *APX* gene as a target for genetic engineering to improve the production of this flower plant.

2 Materials and methods

2.1 Plant and treatment

Plantlets of *A. andraeanum* 'Alabama' were grown in pots with the medium of turves:perlite (4:1, v/v) in a greenhouse. Different tissue samples of root, stem, leaf, spathe, and spadix were collected from one-year-old plants. The cold treatment was carried out by placing the pot-grown one-year-old plants in a phytotron with a 12-h light/dark photoperiod at 25 °C for 3 d pretreatment, and then at 6 °C for low temperature treatment. Samples of the first fully expanded leaves were taken at 0, 12, 24, 36, and 48 h, respectively, during treatment. All samples were immediately frozen in liquid nitrogen and then stored at −80 °C.

2.2 Cloning of *AnAPX* gene

Based on a highly conserved region of plant *APX*, the primer pairs (pAPXF: 5′-AAGGAGCAGTTCCC CATCC-3′; pAPXR: 5′-GCAAAGAAMGCRTCC TCRTC-3′) were designed and used to clone a partial complementary DNA (cDNA) fragment of *AnAPX* through polymerase chain reaction (PCR). The 5′- and 3′-rapid amplification of cDNA ends (RACE) were used to obtain a full-length gene. The total RNA from young leaves was extracted with the RNA Extraction Kit (TaKaRa) and the reverse transcription was carried out with the 5′/3′-Full RACE Kit (TaKaRa). The 5′ specific primer (5′-CAGCAACGACACCAGC CAA-3′) and 3′ primer (5′-AGCGTTCTGGCTTTG AGGGA-3′) were designed based on *AnAPX*. After purification of the expected PCR products, they were cloned with PCR instrument (BIO-RAD S1000™ Thermal Cycler) and sequenced on an ABI-PRISM3730 sequencer. The putative 5′- and 3′-RACE products and the partial *AnAPX* fragment were matched to form a putative cDNA contig which contained the initiation codon, termination codon, and open reading frame (ORF). A pair of full-length primers (APXcomF: 5′-GGGCGAGATCGAGCT GCTAAG-3′; APXcomR: 5′-GGCGATCAGCAT CACACATATTCCT-3′) was designed with the basis of this contig and then used to amplify the full-length cDNA sequence of *AnAPX*. This *AnAPX* sequence contained a 110-bp 5′-nocoding region, a 28-bp 3′-nocoding region, and a 750-bp ORF.

2.3 Sequence analysis

The full-length *AnAPX* cDNA was translated and analyzed on the website (http://www.expasy.ch/tools). The homologous sequences were searched based on the obtained amino acid sequence through BLASTP in NCBI. The secondary structure of putative AnAPX protein was predicted with DNASTAR. Multiple alignments between *A. andraeanum* and other plants were carried out using DNAMAN software (Lynnon Co., Canada). The cluster analysis was performed using MEGA5.1.

2.4 Real-time PCR (RT-PCR)

The total RNA of *A. andraeanum* was extracted using RNA Extraction Kit (TaKaRa) from different tissues and the cold-treated (6 °C) leaves as described above. The reverse transcription was performed with M-MLV (TaKaRa) and the obtained cDNA templates were applied to RT-PCR. Primer pairs (Real-FP: 5′-CTCAGCCACGGGGCCAACAA-3′; Real-RP: 5′-GGGATCTCAGGCCCTCCGG-3′) were designed using Primer Express 2.0 (ABI). This pair of primers was used for PCR amplification and generated products with the size of 100–300 bp. We used *Actin* (Actin-FP: 5′-GCTGAGCGGGAAATTGTTCG-3′; Actin-RP: 5′-CAGGCAGCTCATAGGTCTTCTC-3′) as a reference gene, which spans a 121-bp sequence. RT-PCR was performed with ABI7900 Real-Time System, carried out with Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen). Three different RNA samples were used as three biological replicates, and each biological replicate has three technical replicates. The fold changes of each gene were calculated using the $2^{-\Delta C_T}$ method.

2.5 Enzyme activity assay

The AnAPX activity was determined according to Dalton *et al*. (1987) and Kong and Yi (2008) with a modified protocol. For extraction of APX, the leaves (0.5 g) were homogenized with 50 mmol/L potassium phosphate buffer saline (PBS; pH 7.8) containing 0.3 mmol/L AsA in a cold mortar and pestle. The homogenate was quickly transferred to a test tube and centrifuged at 12000 r/min for 15 min. The supernatant was transferred to another tube and used to determine the APX activity. The mixture containing 1700 μl PBS (25 mmol/L, pH 7.0), 100 μl AsA (5 mmol/L), 100 μl H_2O_2 (20 mmol/L), and 100 μl supernatant was incubated in a quartz cuvette at 290 nm. We determined the decline in the optical density during 1 min and calculated the activity.

2.6 Transformation and transgenic tobacco analysis

The *AnAPX* gene was cloned with primer pairs (APXcomF and APXcomR), digested with *Sac*I and *pst*I, and then inserted into the expression vector pCAMBIA2300. Tobacco plants were transformed using *Agrobacterium tumefaciens* mediated transformation method according to Horsch *et al*. (1985). The transgenic tobacco plants survived in culture medium that contained 50 μg/ml kanamycin and 200 μg/ml cephalosporin were verified by PCR analysis. The controls were wild-type (WT) tobacco plants.

2.7 Tobacco cold tolerance assays

For the cold tolerance assays, the following procedures were carried out. *AnAPX* transgenic tobacco seedlings and controls were sown on holed plates at 25 °C for one month. Then the seedlings were transferred to 4 °C for 12 h in a low-temperature incubator in continuous light (2000 lux) and returned to the original growth conditions for 7 d. To study the chilling injury degree of transgenic seedlings, we used tender leaf (the first fully expanded leaf) derived from the transgenic and control seedlings subjecting to identical cold stress treatment as the whole plants. The percentage of electrolyte leakage (EL) and malondialdehyde (MDA) contents were assayed according to Hara *et al*. (2003).

2.8 Statistical analysis

Each experiment was repeated at least three times. All values were represented as mean±standard deviation (SD). The comparisons between values were evaluated within each tissue and treatment time by a single factor analysis of variance using Duncan's method. The significance of difference was set at 0.05 in all such cases.

3 Results

3.1 Cloning of full-length *AnAPX* gene

To amplify the sequences encoding APX *from A. andraeanum*, the first cDNA strands was used as the

templates for PCR. PCR products of the predicted size were sequenced and identified. Based on the 446-bp partial *A. andraeanum APX* fragment, 5′- and 3′-RACE were performed to obtain full-length sequences of *AnAPX*. The 5′-RACE products of 757 bp and 3′-RACE products of 533 bp were obtained, and the resulting sequences were consistent with the putative *APX* partial fragments. These three fragments were then aligned and a 1160-bp contig that contained 116-bp of 5′ noncoding region, 294-bp of 3′ noncoding region, and 750-bp of ORF was obtained. For the purpose of validating the sequence correctness of this contig, the full-length primers (APXcomF and APXcomR) were used for PCR, producing an 888-bp sequence, named as *AnAPX* (GenBank accession No. JQ838071) (Fig. 1a).

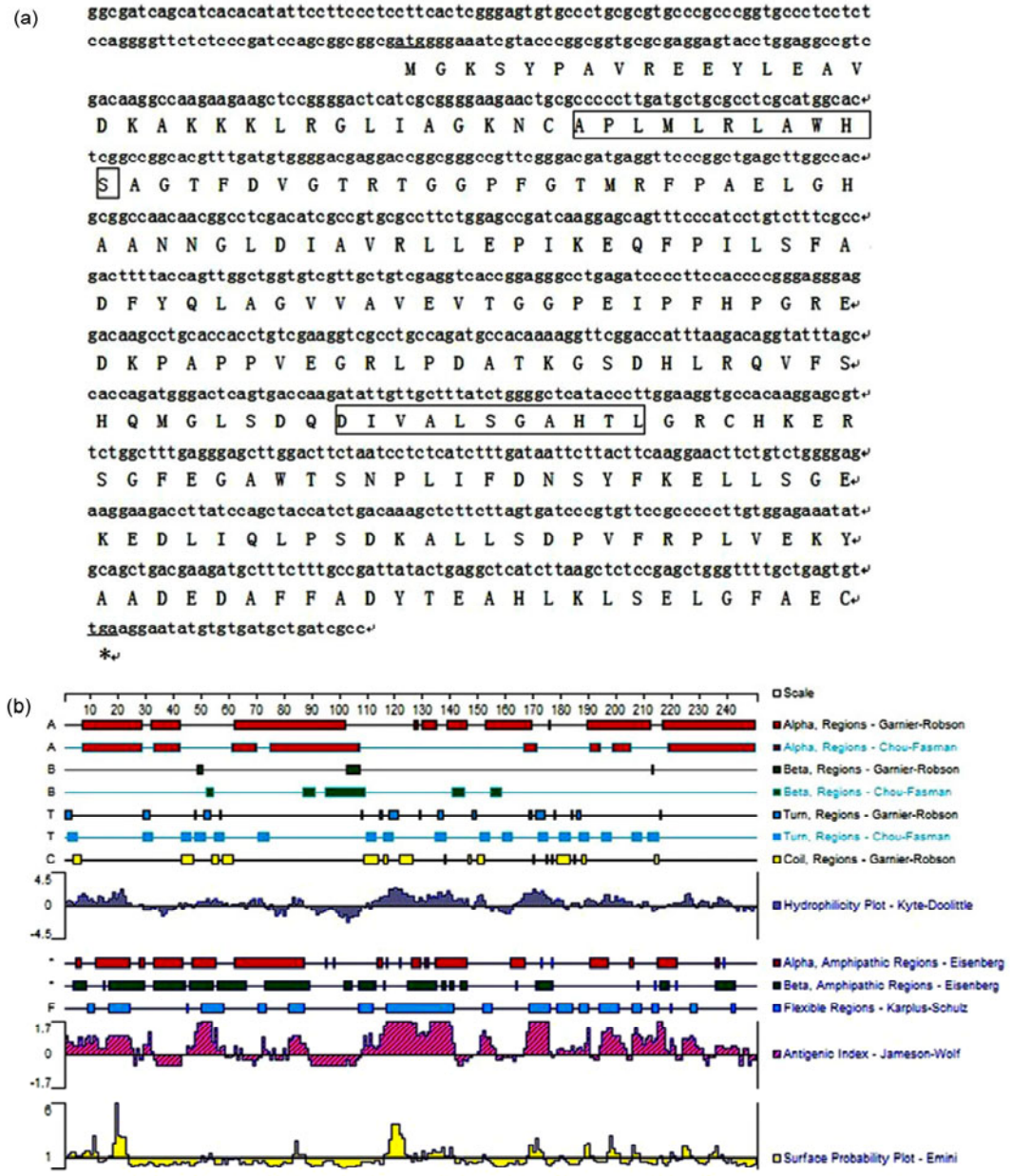

Fig. 1 Nucleotide sequences, deduced sequence of amino acid residues, and secondary structure of the predicting AnAPX protein

(a) The deduced sequence of amino acid residues was aligned based on the *A. andraeanum APX* cDNA; (b) The secondary structure of putative AnAPX protein was predicted with DNASTAR

Sequence analysis of *AnAPX* showed that the *AnAPX* sequence was perfectly associated with that of contig, indicating that the sequence is precise and accurate. The ORF of *AnAPX* encodes a 250-amino acid protein. The molecular mass of AnAPX protein is 27.4 kD and the isoelectric point (pI) is 5.40. The protein includes α-helixes, β-turns, and random coils (Fig. 1b). The protein is a hydrophilic with the aliphatic index of 81.64. Further analysis of AnAPX protein according to the GenBank of known sequences shows that the protein belongs to a typical peroxidase heme-ligand superfamily. This protein has a peroxidase active site (33–44: APLMLRLAWHSA) and a peroxidase proximal heme-ligand site (155–165: DIVALSGAHTL) (Fig. 1a).

The conserved domains of AnAPX were found with coding sequence (CDS) in NCBI, showing that APX protein belongs to a plant-peroxidase-like superfamily (Fig. 1a). Sequence analysis indicated that the deduced amino acid residues of AnAPX were highly similar to the known APX homologs from other plants, such as *Zantedeschia aethiopica* (No. AAC08576, 93%; No. AAK57005, 88%), *V. pseudoreticulata* (No. AAZ79357, 87%), *Gossypium hirsutum* (No. ABR18607, 87%; No. ACJ11731, 86%), *Elaeis guineensis* (No. ACF06591, 87%), and *Zea mays* (No. ACF87724, 86%; No. NP_001170482, 86%). Cluster analysis revealed that the deduced amino acid residues were conserved in all APX sequences. Highly conserved protein sequences appeared on the C-terminal regions, whereas non-conserved sequences existed on the N-terminal regions (Fig. 2).

A phylogenetic tree was created with the basis of AnAPX amino acids and other 16 typical plants (Fig. 3). The phylogenetic relationship between AnAPX and APXs of other 16 plants showed that AnAPX had the closest relationship with *Z. aethiopica* (No. AAC08576), while it had the most distant relationship with *A. thaliana* (No. NP_172267). Multiple alignments of those 17 sequences revealed that all amino acid sequences were highly conserved, implying their functions are evolutionarily conserved.

3.2 Expression of *AnAPX* in plant tissues

The expression of *AnAPX* in different tissues was analyzed to understand the spatial expression pattern of *AnAPX*. The abundance of the *AnAPX* gene in different tissues such as root, stem, leaf, spathe, and spadix was measured by quantitative RT-PCR. Our results revealed that *AnAPX* was expressed in all those tissues. The relative expression level varied insignificantly between root and spathe, as well as among stem, leaf, and spadix. However, the difference was significant in root vs. stem, root vs. leaf, root vs. spadix, leaf vs. spathe, and spathe vs. spadix (Fig. 4). Nevertheless, all of these showed that the expression of *AnAPX* could be detected in all those organs, indicating that *AnAPX* might have different functions in different tissues during the growth and development of *A. andraeanum*.

3.3 Expression of *AnAPX* under cold stress

To find out the possible function of *AnAPX* in response to cold stresses, we analyzed its expression level under low temperature. The results showed that *AnAPX*'s expression was remarkably up-regulated under low temperature. After 12-h treatment, the expression level of *AnAPX* was increased to 3.44 times of the control, whereas the relative expression level varied insignificantly during 12–36 h treatment, reaching the maximum after 48-h treatment (Fig. 5). These results indicated that *AnAPX* might be related to plant cold response.

3.4 Enzyme activity of AnAPX

To understand the changes of APX activity under low temperature stress, we measured the APX activity of *A. andraeanum* treated with 6 °C temperature (Fig. 6). The results showed that APX activity declined sharply during the first 12-h cold treatment in comparison with the control, then it averted to increase and reach a maximum level at 36 h; afterwards, the activity returned to normal level (Fig. 6). On the whole, the changes showed the trend of first declining, then rising and finally declining.

3.5 Analysis of *AnAPX* transgenic plants

The function of *AnAPX* has been investigated by transforming an expression construct of *AnAPX* cDNA with the CaMV 35S promoter into tobacco. The malondialdehyde (MDA) content which indicated the extent of lipid peroxidation was employed to estimate the degree of cold injury in transgenic plants (Fig. 7). The content of MDA was increased by the cold treatments, but the increased amount in

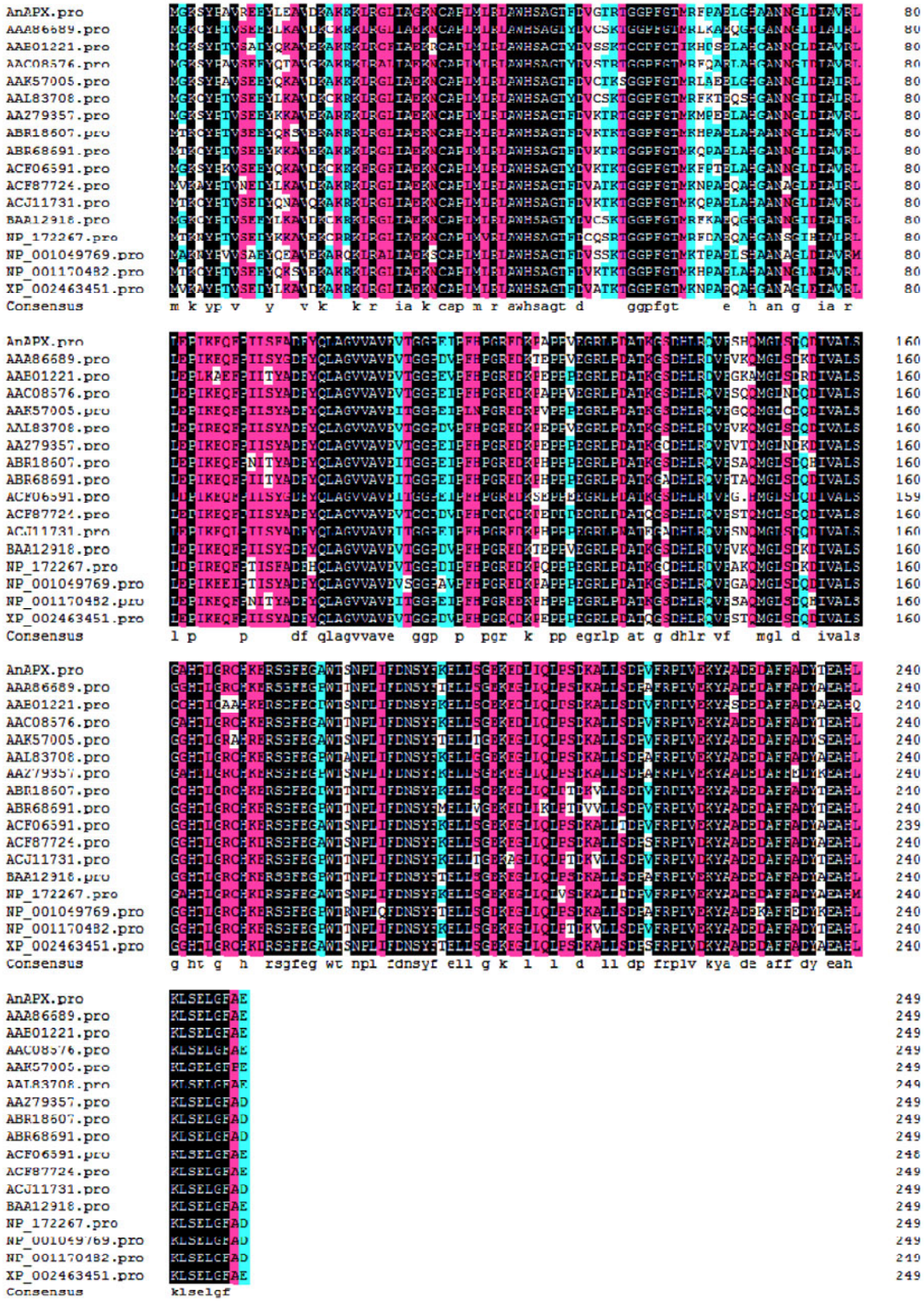

Fig. 2 CDS prognosis and multiple alignments of *AnAPX* and other plants

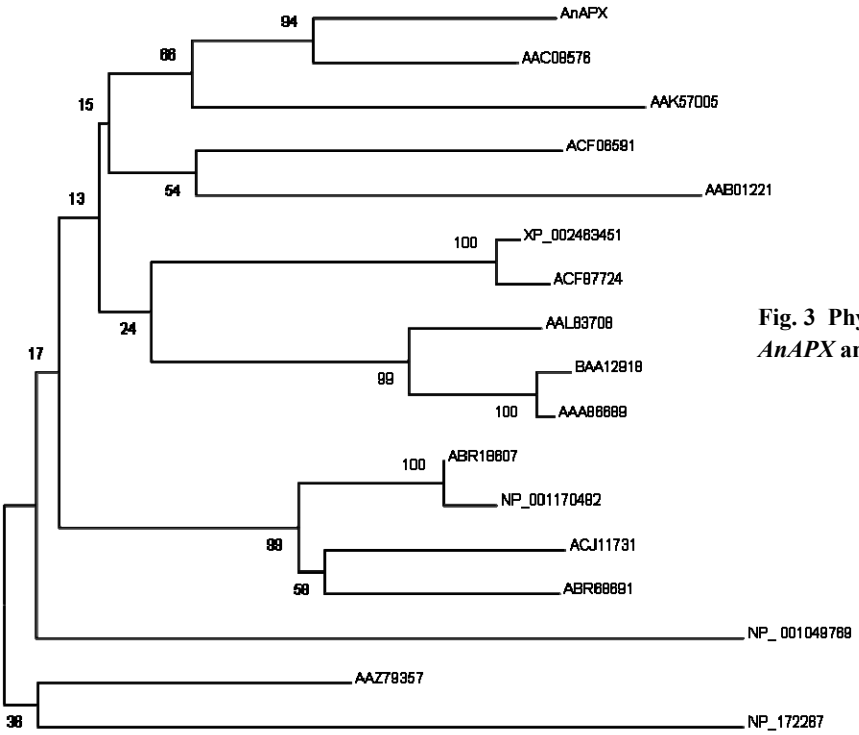

Fig. 3 Phylogenetic analysis of *AnAPX* and other plants

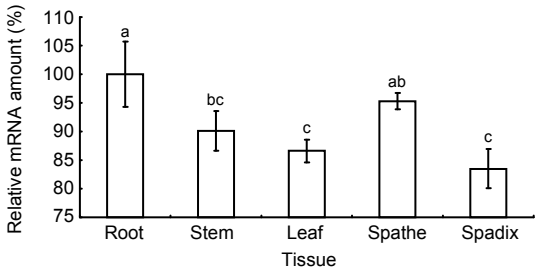

Fig. 4 Expression analysis of *AnAPX* in *A. andraeanum* tissues
Data are expressed as mean±SD, n=3. Different letters above the bar show P<0.05

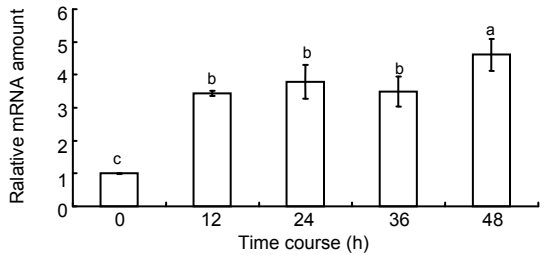

Fig. 5 Change of *AnAPX* expression in *A. andraeanum* leaves during cold treatment
Data are expressed as mean±SD, n=3. Different letters above the bar show P<0.05

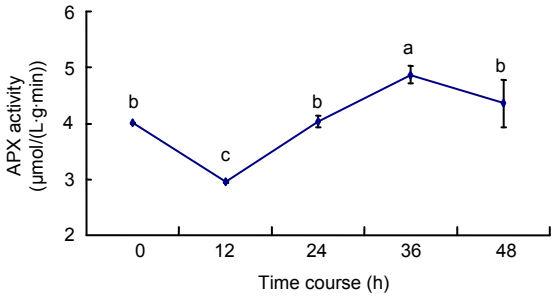

Fig. 6 Change of APX activity in *A. andraeanum* leaves during cold treatment
Data are expressed as mean±SD, n=3. Different letters show P<0.05

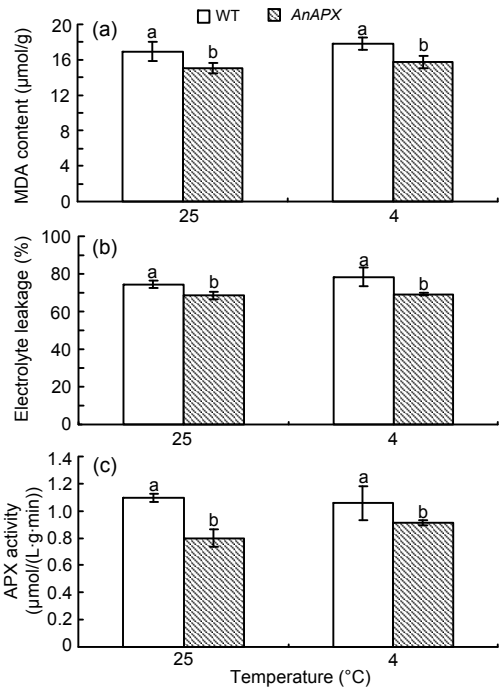

Fig. 7 MDA content (a), electrolyte leakage (b), and APX activity (c) in wild type (WT) and *AnAPX* transgenic tobacco plants after treated with low temperature

Data are expressed as mean±SD, n=3. Different letters above the bar show P<0.05

35S::*AnAPX* transgenic plants was lower than that in WT plants. In addition, less MDA content was detected in high-expression lines than in low-expression strains after cold treatment (Fig. 7a). Similar results were obtained when EL tests were applied to both transgenic plants and WT plants. When exposed to 4 °C for 12 h, the EL values were 78.4% and 69.3% for WT plants and transgenic plants, respectively, which indicated the increasing degree of WT plants was higher than that of transgenic lines (Fig. 7b). In contrast, the transgenic tobacco APX activity increased by 14.5%, while decreased by 3.3% in WT plants (Fig. 7c). In short, overexpression of *AnAPX* in tobacco plants slowed down the increase of MDA and EL due to the responses to cold stress but stimulated APX activity and consequently enhanced the cold tolerance in the transgenic plants.

4 Discussion

Under adverse conditions, a large number of ROS are often produced and accumulated in plant cells, which will bring the plants into an oxidative stress condition resulting into retarded plant growth and development. However, plants have developed its defense system against such oxidative stress both through nonenzymatic and enzymatic ROS scavenging systems. A nonenzymatic system uses antioxidants to quench ROS such as glutathione (GSH), AsA, and flavonoids, while an enzymatic system employs many enzymes including APX, superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), and catalase (CAT). Hydrogen peroxide, the natural product originated mostly from the photosynthetic electron transport chain and some enzymatic reaction in plant chloroplast, is toxic to the plant photosynthetic system. High concentration of H_2O_2 can also inhibit the enzymes of Calvin cycle. APX is a key enzyme in the enzymatic ROS scavenging system called AsA-GSH redox system that can remove the H_2O_2 especially in the chloroplast (Shen *et al*., 1997).

Cold temperature will bring plants into an oxidative stress and it was shown that APX activity was up-regulated in plants such as rice (Oidaira *et al*., 2000), *Coffea* (Fortunato *et al.*, 2010), haw (Eltelib *et al*., 2011), and pepper (Airaki *et al*., 2012) when subjected to low temperature. This is thought to be a normal defense reaction when plants are subjected to cold stress. Therefore, the increased APX activity acquired during cold acclimation can help plants tolerate or survive under even lower temperature or longer cold stress (Baek and Skinner, 2003; Kuk *et al*., 2003). In this work, the APX activity of *A. andraeanum* declined first, then gradually increased to a maximum level and finally returned to a normal level when subjected to cold stress (Fig. 6). Accordingly, we speculated that the early 12−36 h low temperature treatment on *A. andraeanum* was just like the plants acclimatizing to cold temperature conditions. When the plants got adapted to such conditions after 12 h, some unknown mechanisms triggered the adjustment of the ROS scavenging system and consequently the APX activity was reverted from a decrease into an increase trend. The rise of APX activity indicated that the capacity of *A. andraeanum* against membrane lipid peroxidation under the lower temperature was increased. Obviously, further research is required to illustrate the mechanism how *A. andraeanum* has such a transition of APX activity during the cold stress.

Previous research also indicated that the APX activity was tightly associated with the expression of *APX* gene, which could be up-regulated after the plants treated with adversities, such as salt, wounding, drought, and heat. For example, the APX activities in leaves of salt-tolerant pea cultivars increased after treated with NaCl (Hernández *et al*., 1995); the *APX* expression of *Betula platyphylla* remarkably increased after treated with NaCl (Wang *et al*., 2009). The *APX* expression of sweetpotato was remarkably up-regulated after wounding for a long time (Park *et al*., 2004). Drought combined with heat could induce the culmination of *APX1* mRNA and protein in *A. thaliana* (Koussevitzky *et al*., 2008). In this study, the expression of *A. andraeanum APX* mRNA was evidently elevated by cold stress, which is consistent to the other previous reports. Therefore, we believed that APX plays defense roles in plants under oxidative stress and its activity might be transcriptionally regulated.

Since the expression of *APX* was closely related to the degree of resistance to cold stress, it could be considered rational to increase its expression by introducing any exogenous *APX* gene to plants, then to improve their stress tolerance. As of now, *APX* genes were cloned from many kinds of higher plants, and some transgenic plants were studied and proven that the overexpression of *APX* genes in these plants can indeed provide additional protection against oxidative stress under adverse plant growth conditions. For example, overexpression of chloroplast *APX* gene in cotton has conferred higher photochemical activity of photosystem II (PSII) and stronger antioxidant capacity to transgenic plants (Kornyeyev *et al*., 2001). Overexpression of chloroplast *APX* gene in *A. thaliana* could increase its resistance to herbicide (Murgia *et al*., 2004). Not alone, overexpression of tobacco chloroplast *APX* gene improved the resistance to drought and salt stresses (Badawi *et al*., 2004). The peroxidase activity of the *CAPOA1* (a pepper ascorbate peroxidase-like 1) transgenic tobacco increased, resulting in stress resistance and pathogen tolerance (Sarowar *et al*., 2005). The peroxisomal type ascorbate peroxidase (*pAPX*) gene originated from barley was transferred to *A. thaliana*, and the transgenic plants exhibited higher resistance to salt stress (Xu *et al*., 2008). *APX* gene originated from *Hordeum vulgare* (*HvAPX*) could enhance the resistance to high temperature stress in *A. thaliana* (Shi *et al.*, 2001). As well as *tAPX* (thylakoid-bound ascorbate peroxidase gene from tomato leaf) transgenic tobacco plants, their resistance to extreme temperature has been improved (Sun *et al*., 2010). Therefore, we reckon that *APX*-overexpressed *A. andraeanum* plants should also have a higher resistance to oxidative outburst caused by cold stress and cloning this gene is certainly the first requisite for such an effort to make a transgenic plant.

In this paper, we described that a novel *APX* gene has been cloned from *A. andraeanum*. Transcript levels of *APX* could be quickly induced by the cold stress, and functional analysis also proved that the anti-cold characteristic of transgenic tobacco was enhanced by overexpression of *AnAPX*. The results implied that *AnAPX* may be a functional gene related to cold stress. Thus, it is feasible to apply *AnAPX* gene to create anti-cold plants by genetic engineering. Further investigation is obviously required to understand how many isoforms of APX in *A. andraeanum* and their respective subcellular localizations in order to maximize the utilization of the right isoform genes. Previous researches such as on tobacco (Lim *et al*., 2007), sweetpotato (Wang *et al*., 2005), and tomato (Sreenivasulu *et al*., 2007) also has reported. Nevertheless, the experiment in this study has just characterized the expression of *AnAPX* against one kind of stress. In the field, multiple stresses often occurred simultaneously (Chen *et al*., 2006), which will be undoubtedly more harmful to the growth of plants. Therefore, further studies are required to figure out whether *AnAPX* is also involved in the plant tolerance to multiple abiotic stresses.

5 Conclusions

An *APX* gene from *A. andraeanum* 'Alabama' was cloned for the first time. The full length of the *APX* cDNA was 888 bp, which consisted of a 110-bp 5′ noncoding region, a 28-bp 3′ noncoding region, and a 750-bp ORF, named *AnAPX* (GenBank accession No. JQ838071). The ORF of *AnAPX* encodes a 250-amino acid protein, which has a peroxidase active site (APLMLRLAWHSA) and a peroxidase proximal heme-ligand site (DIVALSGAHTL). Cluster analysis showed that *AnAPX* was most related to *Z. aethiopica*, which belongs to Araceae family. Biochemical

analysis of *AnAPX* showed that there was a high activity in plants at low temperature. Molecular expression and functional analysis suggested that *AnAPX* may be a cold stress responsive gene. These important data shed light on the molecular mechanisms of cold stresses in *Anthurium* and provide theoretical basis for the improvement of varieties with higher cold resistance through genetic engineering.

Compliance with ethics guidelines

Hui-chun LIU, Dan-qing TIAN, Jian-xin LIU, Guang-ying MA, Qing-cheng ZOU, and Zhu-jun ZHU declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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