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Identification and expression analysis of nuclear factor Y families in *Prunus mume* under different abiotic stresses

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Abstract

The nuclear factor Y (NF-Y) is one of the largest transcription factor families in plants consisting of NF-YA, NF-YB, and NF-YC subunits. It could play important roles in various processes such as flowering time, seed development, and response to drought. In this study, 6 NF-YA, 13 NF-YB, and 8 NF-YC proteins were identified and characterized in *Prunus mume*. Analyses of a conserved domain indicated that the PmNF-Y subunits shared an elevated degree of homology with the corresponding *Arabidopsis* NF-Y ones. Phylogenetic analysis showed that each NF-Y subunit family from *Prunus mume* and *Arabidopsis* could be divided into 4 or 2 clades based on their full-length proteins. The gene expression patterns of all 27 *PmNF-Y* genes were examined under abscisic acid (ABA), osmotic, salt, and H_2O_2 treatments using real-time quantitative PCR analyses. *PmNF-YA1/2/4/5/6*, *PmNF-YB3/4/8/10/11/13*, and *PmNF-YC1/2/4/5/6/8* were found to be up-regulated under the ABA and osmotic treatments. *PmNF-YA1/2/3/4/5/6*, *PmNF-YB1/3/8/10/11/13*, and *PmNF-YC1/2/5/6/8* were obviously induced by the H_2O_2. In addition, only *PmNF-YA2* and *PmNF-YB3* expressions were enhanced under the salt stress. These findings could provide an entry point to investigating the roles of *PmNF-Y* genes during abiotic stress responses.

Additional key words: abscisic acid, *Arabidopsis thaliana*, gene expression, H_2O_2, osmotic stress, salinity.

Introduction

A CCAAT box is *cis*-element and is widely present in the eukaryotic promoter region (Bucher 1990). A nuclear factor Y (NF-Y), which is also called the CCAAT-binding factor (CBF) or heme activator protein (HAP), can bind to the *cis*-element. The NF-Y is trimeric transcription factor complex composed of NF-YA (CBF-B/HAP2), NF-YB (CBF-A/HAP3), and NF-YC (CBF-C/HAP5) subunits in most species (Mantovani 1999). In animals and yeast, each subunit of NF-Y is encoded by a single gene, whereas there are some members of each subunit gene family in plants. For example, 10 *NF-YA*, 13 *NF-YB*, and 13 *NF-YC* genes encode different subunits of NF-Y in *Arabidopsis* (Siefers *et al.* 2009), and 10 NF-YA, 11 NF-YB, and 7 NF-YC proteins are identified in rice (Thirumurugan *et al.* 2008). These members play important parts in mediation of diverse genes involved in regulating various processes such as embryogenesis (Lotan *et al.* 1998, Kwong *et al.* 2003), abiotic stress tolerance (Nelson *et al.* 2007, Leyva-González *et al.* 2012, Li *et al.* 2013a,b, Yan *et al.* 2013), flowering time control (Cai *et al.* 2007, Kumimoto *et al.* 2008, Siefers *et al.* 2009), nitrogen nutrition (Zhao *et al.* 2011), abscisic acid (ABA) response, seed germination (Warpeha *et al.* 2007), chloroplast biogenesis (Miyoshi *et al.* 2003), and photosynthesis (Stephenson *et al.* 2011).

In recent years, more evidence demonstrates the functions of the NF-Y family in abiotic stress responses (Petroni *et al.* 2012). An altered expression of *TaNF-YA10* could greatly affect a response to salinity in *Arabidopsis* (Ma *et al.* 2015), and expression of *NF-YA1* in *Arabidopsis* is shown to result in hypersensitivity to salt and ABA stress during early growth (Li *et al.* 2013b). Moreover, *NF-Ys* have been identified as regulators of

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Abbreviations: ABA - abscisic acid; CBF - CCAAT-binding factor; CDS - coding sequence; HAP - heme activator protein; NF-Y - nuclear factor Y; ORF - open reading frame; qPCR - quantitative PCR.

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drought tolerance in different plant species. Several drought-related *NF-YB* genes are identified in *Triticum aestivum*, *Populus euphratica*, and *Hordeum vulgare* (Stephenson *et al.* 2007, Liang *et al.* 2012, Yan *et al.* 2013). The over-expressions of *NFYA5*, *NF-YB1*, *PdNF-YB7*, and *GmNF-YA3* in *Arabidopsis*, as well as of *ZmNF-YB2* in maize improve plant performance and survival under drought (Nelson *et al.* 2007, Li *et al.* 2008, Han *et al.* 2013, Ni *et al.* 2013). Over-expression of *CdtNF-YC1* in rice confers tolerance to drought and salinity (Chen *et al.* 2015). Until now, biological roles of most of the *NF-Y* family members in the abiotic stress responses remain to be explored.

Prunus mume has outstanding ornamental features and is one of the longest-living species of the ornamental trees in China. It is one of the first genomes which have been sequenced within *Prunus* genus of *Rosaceae* (Zhang *et al.* 2012). In this study, we identified *PmNF-Y* genes from the *Prunus mume* genome and analysed their conservation domains, phylogenetic relationships, and gene structures and compared them with *Arabidopsis* homologues. We also detected the expression patterns of the *PmNF-Y* genes under ABA, drought, high salinity, and H_2O_2 conditions. The results of this study could provide a basis for investigation of the roles of *PmNF-Y* genes in response to abiotic stress.

Materials and methods

Detection of PmNF-Y members in *Prunus mume:* The *Prunus mume* genome database was downloaded from the *Prunus mume* genome project (http://prunusmumegenome.bjfu.edu.cn/). *Arabidopsis* NF-Y protein sequences retrieved from the *Arabidopsis* information resource (http://www.arabidopsis.org) were used as *BLASTP* tool to search *PmNF-Y* genes in the *Prunus mume* genome database. Additionally, the candidate sequences were queried against *InterPro* (*v. 51.0*, http://www.ebi.ac.uk/interpro/) to validate their identity as *NF-Y* subunits. Some of the initially identified candidate sequences that had incomplete domains or open-reading frames (ORFs) were excluded from further analysis.

Multiple alignments and phylogenetic analyses: Multiple sequence alignments were done by *Clustal X* (*v. 2.1*) and crested with the *BoxShade* server (http://www.ch.embnet.org/software/BOX_form.html) using the amino acid sequences of the full-length proteins. Phylogenetic trees of PmNF-Y and *Arabidopsis* NF-Y subunit families were constructed using the neighbor-joining method with the following parameters: the Jones-Taylor-Thornton model and a bootstrap of 1 000 replicates in molecular evolutionary genetic analysis (*MEGA*, *v. 5.0*). The exon-intron structure of PmNF-Y subunits and *Arabidopsis* NF-Y was drawn using the Gene Structure Display Serve tool (http://gsds.cbi.pku. edu.cn/) by comparing the coding sequences (CDS) with genomic DNA (without the untranslated region) sequences. *Mus musculus* NF-YA (CAA39023.1), NF-YB (CAA39024.1), and NF-YC (AAC52892.1) protein sequences were used for the out-groups to conduct the multiple alignments and root phylogenetic trees.

Expression analysis of *PmNF-Y* genes under abiotic stresses: Dormant cuttings with five winter buds were collected from five-year-old *Prunus mume* Sieb. & Zucc. cv. Xue Mei trees at the Huazhong Agriculture University (Wuhan, China) and incubated with their lower ends dipped in water at a temperature of 25 °C, a 16-h photoperiod, an irradiance of 80 μmol m^{-2} s^{-1}, and an air humidity of 85 % until young leaves emerged from the buds. The two-week-old young leaves (1 - 2 cm long) were floated in a MES medium (50 mM KCl and 10 mM MES, pH 5.7, Toumi *et al.* 2010) with 100 μM ABA, 300 mM mannitol, or 250 mM NaCl for 3, 6, 12, and 24 h, or soaked with 3 % (v/v) H_2O_2 for 3, 6, 12, 24, 36, or 48 h. Control leaves were floated in the MES medium without other reagents at each stage. Twelve to 15 leaves were used in each experiment and each experiment was repeated three times. All samples were frozen in liquid nitrogen, and stored at -80 °C until use.

Real-time quantitative PCR (qPCR) was applied to evaluate transcription of *PmNF-Y* genes expressed under the different abiotic stresses. The total RNA of every sample was extracted using an RNA *Easyspin* isolation system (*AidlabBiotech*, Beijing, China). A cDNA was synthesised using a *PrimeScript* RT reagent kit (*RR047A*, *TaKaRa*, Dalian, China). The real time qPCR was performed using an *ABI 7500* fast real-time PCR system (*Applied Biosystems*, Foster City, CA, USA) with *SYBR®Premix Ex Taq™ II* (*RR820A*, *TaKaRa*) using the following cycling regime: 95 °C for 30 s followed by 40 cycles at 95 °C for 3 s and 60 °C for 30 s. A volume of 10 mm^3 of real time qPCR solutions contained 5 mm^3 of *SYBR®Premix Ex Taq II*, 0.4 μM forward and 0.4 μM reverse primers, 2 mm^3 of a real time reaction solution (*TaKaRa*), and 1 mm^3 of a cDNA template. The *elongation factor 1 alpha* from *Prunus mume* was used as internal control gene to calculate a relative gene expression using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001, Guo *et al.* 2014). The trascription of each gene in the control was set to 1.0. The experimental data are means of at least three independent replicates. Significance of differences between the treated samples and controls were evaluated by Dunnett's method of one-way *ANOVA* in the *SAS* software (*v. 8.1*). Primers used in real time qPCR are listed in Table 1 Suppl.

Results

The *Arabidopsis* NF-Y protein sequences were used to search *PmNF-Y* genes in the *Prunus mume* genome database. The obtained sequences were queried against the *InterPro* domain database to confirm their identity as NF-Y subunits. A total of 27 proteins were identified in the *Prunus mume* genome based on their conserved domains corresponding to the NF-Y *Interpro* IDs of IPR001289, IPR003956, IPR003958, and IPR027170, including 6 PmNF-YAs, 13 PmNF-YBs, and 8 PmNF-YCs proteins (Table 2 Suppl.). These protein sequences shared an elevated degree of homology (> 60 %, Fig. 1 Suppl.) with the corresponding conserved domains of the *Arabidopsis* NF-Y ones.

The multiple sequence alignments showed that the conserved core region of PmNF-YAs was ~53 amino acids long (Fig. 2*A* Suppl) and was characterized by the presence of Gln (Q)- and Ser/Thr (S/T)-rich NH_2 termini, a subunit interaction domain (NF-YB/NF-YC interaction), a DNA-binding domain for specific DNA binding to CCAAT boxes (Olesen and Guarente 1990, Maity and De Crombrugghe 1992, Xing *et al.* 1993, 1994), and well conserved amino acids between plant and other eukaryote lineages located in protein interaction and DNA binding domains. The conserved regions of NF-YB and NF-YC had a structural and amino acid homology to histone fold motifs. Specifically, NF-YB was related to the histone fold motifs of H2B histones, whereas NF-YC subunits were related to H2A histones (Mantovani 1999).

The NF-YA subunit members from *Prunus mume* and *Arabidopsis* were divided into four groups, each of which contained three to six members (Fig. 1*A*). *PmNF-YA3* and *PmNF-YA6* formed a subgroup and constituted one group with the couples *NF-YA3/8* and *NF-YA5/6*. *PmNF-YA1* with the couple *NFYA4/7* and *NFYA1* with the couple *PmNF-YA2/NF-YA9* formed one group. *NF-YA2/10* clustered in a group with *PmNF-YA4/5*. With respect to gene structures, most of the *PmNF-YA* family members were in accordance with their putative orthologues of *Arabidopsis* (Fig. 1*A*) where most *PmNF-YA* or *Arabidopsis NF-YA* subunits included four or five exons except *NF-YA5/9* with three and six exons, respectively. Nevertheless, the intron lengths of the *PmNF-YA* subunits were different from those of the *Arabidopsis NF-YA* ones. Compared to the range of the *Arabidopsis NF-YAs* intron lengths from 76 to 385 bp, the *PmNF-YAs* had some introns longer than 1 000 bp.

The genes could be divided into two major groups in the *NF-YB* tree (Fig. 1*B*). The first group comprised phylogenetically distant *Arabidopsis* family members, such as *NF-YB11/12/13* and *PmNF-YB1/13*, which much more likely underwent to changes in required amino acids (Fig. 2*B* Suppl.). In the second group, another bifurcation generated two secondary subgroups. *Arabidopsis NF-YB4/5/7/6/9* and *PmNF-YB4/ 12/2/5/9/6/7* were on one side, comprising *Arabidopsis NF-YB6 (L1L)* and *NF-YB9 (LEC1)*, whose expression was highly organ specific and developmentally regulated, and *Arabidopsis NF-YB1/2/3/8/10* and *PmNF-YB10/8/3/11* were on the other side.

The phylogenetic analyses and amino acid alignments of PmNF-YC suggest that there were two distinct clades (Fig. 1*C*). One clade consisted of *Arabidopsis NF-YC1/4* and *PmNF-YC8* with a short last exon, and *Arabidopsis NF-YC2/3/9* as well as *PmNF-YC2/4/5* with a single exon. And the amino acid sequences of *PmNF-YC* members in the clade were still very similar to distant mammalian *NF-YC* lineages (Fig. 2*C* Suppl). The members of the second clade consisting of eight members of *Arabidopsis NF-YC* (*NF-YC5/6/7/8/10/11/12/13*) and four members of *PmNF-YC* (*PmNF-YC1/3/6/7*), were increasingly divergent from the ancestral *NF-YC*. Suggesting that they might have evolved functions inconsistent with mammalian NF-Y functions, the members of this clade had numerous nonconservative changes from required amino acids.

To investigate the function of *PmNF-Y* genes, transcription of *PmNF-Y* genes under multiple abiotic stresses was quantified by real-time qPCR. The expression patterns of all 27 *PmNF-Y* genes were examined in the ABA, osmotic, salt, and H_2O_2 stresses at different treatment time points. But only 20 *PmNF-Y* genes, whose transcription was detectable, were selected for further analysis, and the other 7 *PmNF-Y* genes (*PmNF-YB2/5/6/7/9/12* and *PmNF-YC7*), whose transcripts were undetectable by real-time qPCR (data not shown), were not used for subsequent comparison.

The highest expressions of all *PmNF-YA*s were reached after 24 h of the ABA treatment where expressions of *PmNF-YA1/2* were modestly up-regulated (~7.1-fold and 5.5-fold at 24 h, respectively), and other *PmNF-YA* genes were similarly enhanced after 24 h of the ABA treatment but at a relatively lower level than *PmNF-YA1/2*. Some differences existed between the expression patterns of *PmNF-YA* genes at ABA treatment: expression of *PmNF-YA3/5* decreased after 3 h followed by up-regulation after 6 h and reached a peak after 24 h; expression of *PmNF-YA4* was constant after 3 h, followed a fall after 6 h and 12 h, and an increase to the control level after 24 h (Fig. 2*A* and Fig. 3*A* Suppl.).

The transcript abundance of four members of the *PmNF-YB* subfamily (*PmNF-YB3/8/10/13*) increased due to the ABA treatment, but time to reach peaks was different (after 24, 6, 12, and 24 h, respectively). *PmNF-YB4/11* were down-regulated after 3 h following a rise after 6 h of the ABA treatment and reaching a peak after 24 h, but *PmNF-YB1* basically kept a steady expression (Fig 3*B* Suppl.).

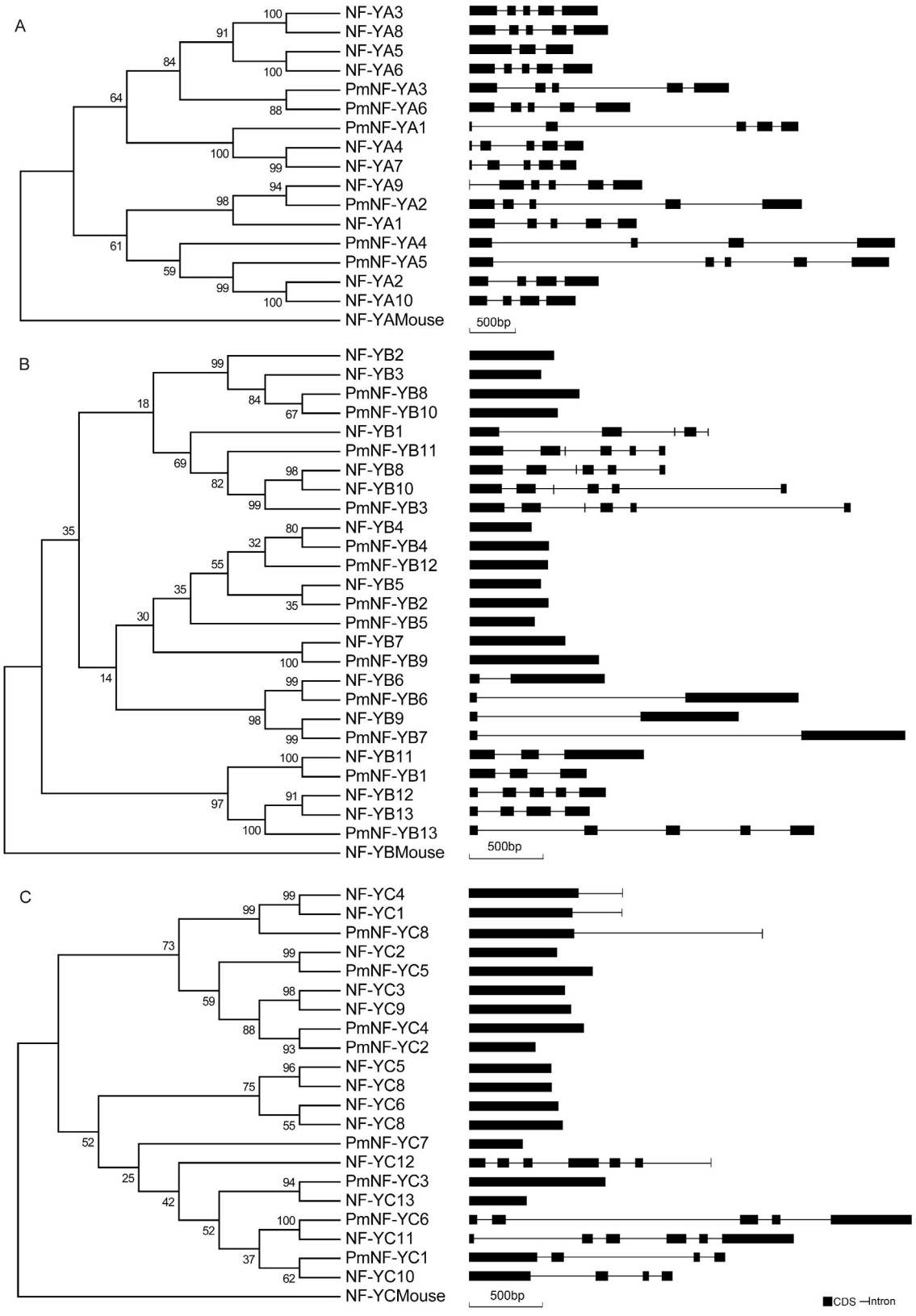

Fig. 1. Phylogenetic trees and gene structures for *PmNF-Y* and *Arabidopsis NF-Y* families. *A* - *PmNF-YA* and *Arabidopsis NF-YA* phylogenetic tree. *B* - *PmNF*-YB and *Arabidopsis NF-YB* phylogenetic tree. *C* - *PmNF-YC* and *Arabidopsis NF-YC* phylogenetic tree.

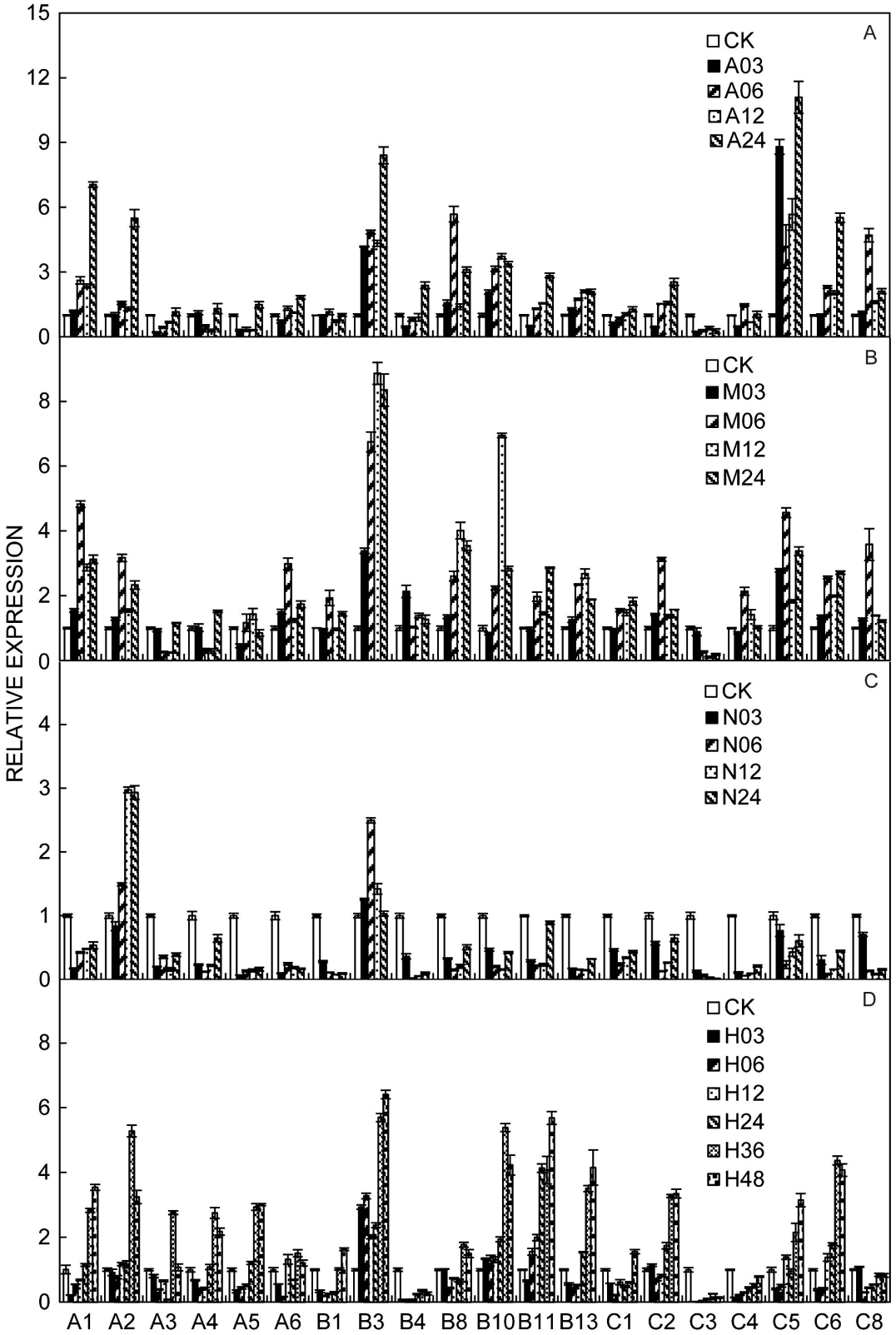

Fig. 2**.** Expression patterns of *PmNF-YA* (A1 - A6), *PmNF-YB* (B1 - B13), and *PmNF-YC* (C1 - C8) in response to abiotic stresses at different time points [h]: *A* - 100 μM ABA (A). *B* - 300 mM mannitol (M). *C* - 250 mM NaCl (N), *D* - 3 % H_2O_2 (H). CK - controls. The expressions were normalized to that of *PmEF1α*, and the expression in the control was set to 1.0. The *error bars* indicate SDs of means of three independent experiments.

The mRNA content of all *PmNF-YC* genes increased with the exception of *PmNF-YC3*. Expressions of *PmNF-YC5/6/8* were obviously up-regulated in the ABA stress, and especially *PmNF-YC5* expression was ~11.1-fold higher than in the control 24 h after the application of 100 μM ABA (Fig 3*C* Suppl.).

PmNF-YA1/2/6 were obviously up-regulated in response to the osmotic stress (4.8-,3.2-, and 3.0-fold 6 h after the application of 300 mM mannitol, respectively) and showed a similar expression pattern: the expression slightly increased after 3 h, reached a peak after 6 h, and gradually decreased after 12 and 24 h. *PmNF-YA3*/*4* were down-regulated after 6 h and up-regulated after 12 h of the mannitol treatment and reached a peak after 24 h (Fig. 2*B* and Fig 4*A* Suppl.).

All seven *PmNF-YB* genes were up-regulated under the osmotic stress; the *PmNF-YB3* gene had the highest expression level (8.9-fold higher than in the control after 12 h of the mannitol treatment). Transcriptions of most *PmNF-YC* genes increased by the osmotic stress, but the *PmNF-YC3* gene was an exception as its expression kept stable after 3 h, however, it steadily decreased 6, 12, and 24 h after the treatment (Fig. 4*B* and Fig 4*C* Suppl).

Transcriptions of most *PmNF-Y* genes were obviously down-regulated under the salt stress (Fig. 2*C* and Fig 5 Suppl) except *PmNF-YA2* and *PmNF-YB3*, whose expressions increased ~3.0-fold after 12 h and ~2.5-fold after 6 h under 250 mM NaCl, respectively.

Expressions of all *PmNF-YA* genes reached peaks 36 or 48 h after the H_2O_2 treatment; *PmNF-YA2* had the highest mRNA abundance. However, transcriptions of all *PmNF-YA* genes were lower than in the control before 24 h of the treatment with the exception of *PmNF-YA6* (Fig. 2*D*, Fig 6*A* Suppl.).

Two main expression patterns existed for the *PmNF-YB* genes in response to the H_2O_2 treatment (Fig 6*B* Suppl). One shows that the mRNA abundance was down-regulated to the level of the control before 36 h and enhanced sharply after 36 h and 48 h such as *PmNF-YB1/8/13*. On the contrary, another displays that transcription was higher than in the control and significantly increased after 36 or 48 h like *PmNF-YB3/10/11*. *PmNF-YB4* is exception, its expression was lower than in the control at any treatment point. Transcriptions of four genes (*PmNF-YC1/3/4*/8) were lower than in the control at any H_2O_2 treatment point with the exception of *PmNF-YC1* after 48 h and *PmNF-YC8* after 3 h, whereas the mRNA levels of *PmNF-YC5*/*6* were down-regulated after 6 h following an increase after 12 h of the H_2O_2 treatment and reaching peaks after 48 and 36 h, respectively (Fig 6*C* Suppl). *PmNF-YC2* expression was down-regulated after 6 h, kept steady and then rised after 12 and 24 h, and its highest expression was reached after 48 h of the treatment.

Discussion

Unlike mammals and yeasts, each NF-Y subunit in a plant genome is encoded by multiple genes. In *Arabidopsis*, there are 10 *NF-YA* genes, 13 *NF-YB* genes, and 13 *NF-YC* genes (Siefers *et al.* 2009). Rice harbors 10 *NF-YA* genes, 11 *NF-YB* genes, and 7 *NF-YC* genes (Thirumurugan *et al.* 2008). In *Triticum aestivum*, 10 *NF-YA*, 11 *NFYB*, and 14 *NF-YC* are characterized (Stephenson *et al.* 2007). Compared to these species, a less number of *PmNF-Y* family genes (6 *PmNF-YAs*, 13 *PmNF-YBs*, and 8 *PmNF-YCs*) were identified in this study. This might be partly due to a contraction of these gene families in *Prunus mume*, as well as by the incompleteness of the *Prunus mune* genome sequence (Zhang *et al.* 2012).

In *Arabidopsis*, *NF-YA1* is obviously induced by NaCl, mannitol, PEG, and ABA treatments, and *NF-YA1* was involved in regulation of post-germination growth arrest under salt stress (Li *et al.* 2013b). Moreover, overexpression of *NF-YA1/5/6/9* genes causes a hypersensitivity to ABA during seed germination. Growth of these transgenic seedlings is retarded and their flowering time is delayed to a different degree (Mu *et al.* 2013). In this study, *PmNF-YA2* grouped with *Arabidopsis NF-YA1*/*9*. Expression analysis shows that the *PmNF-YA2* gene was the only one whose expression was up-regulated in response to the ABA, salt, osmotic, and H_2O_2 stresses. Overexpression of *NF-YA2*/*3*/*7*/*10* in *Arabidopsis* results in dwarf growth, late senescence, and enhances tolerance to drought, flooding, cold, and heat stresses (Leyva-González *et al.* 2012). Expression of *PmNF-YA1*, homologous with *Arabidopsis NF-YA4*/*7*, and one of the *PmNF-YA4/5* clusters with *NF-YA2*/*10* is regulated by the ABA, osmotic, salt, and H_2O_2 stresses. The *Arabidopsis NF-YA5* transcript is strongly induced by the drought stress in an ABA-dependent manner and its overexpression could be connected with a reduced leaf water loss and higher resistance to drought stress (Li *et al.* 2008). *PmNF-YA3/6* class with *Arabidopsis NF-YA3/5/6/8*, and their expressions are induced by the osmotic stress.

Overexpression of *NF-YB1* in *Arabidopsis* and *ZmNF-YB2* in *Zea mays*, increases tolerance to drought stress and grain yield under water-limited conditions (Nelson *et al.* 2007), and *TaNF-YB2* and *PeNF-YB8/11* homologues of *Arabidopsis NF-YB1* are up-regulated in wheat and poplar under drought treatment, respectively (Stephenson *et al.* 2007, Yan *et al.* 2013). *PmNF-YB3* and *PmNF-YB11* are clustered with *Arabidopsis NF-YB1*/*8*/*10*. *PmNF-YB3* is induced by the ABA, osmotic, salt, and H_2O_2 stresses, whereas *PmNF-YB11* expression

is up-regulated under the ABA, osmotic, and H_2O_2 treatments. So, it can be suggested that *PmNF-YB3/11* may be important for drought resistance. *PdNF-YB7*, the homologue of *Arabidopsis NF-YB3*, is induced by the PEG and ABA treatments in poplar, and its ectopic overexpression in *Arabidopsis* confers drought tolerance and improves water-use efficiency, which indicates that *PdNF-YB7* regulates ABA-dependent dehydration response (Han *et al.* 2013). *PmNF-YB8/10* group with *Arabidopsis NF-YB2/3* are found to be induced and have similar expression profiles after the ABA, osmotic, salt, and H_2O_2 treatments. *TaDr1A* and *TaDr1B* are orthologous to *NF-YB12/13* of *Arabidopsis*, and their mRNA abundance is up-regulated under drought (Stephenson *et al.* 2007). *PmNF-YB1* and *PmNF-YB13* are classed with *NF-YB11/12/13* in *Arabidopsis*, but expression of only *PmNF-YB13* is induced by the ABA, osmotic, and H_2O_2 treatments, which suggests that it may be involved in drought stress response.

There were two distinct clades in the *PmNF-YC* family and *Arabidopsis NF-YC* family. One clade consists of *PmNF-YC2/4/5/8* and *NF-YC1/2/3/4/9*. In *Arabidopsis*, *NF-YC1/2/3/4/9* are found to control flowering time (Siefers *et al.* 2009, Kumimoto *et al.* 2010, Hackenberg *et al.* 2012), and *NF-YC2* also shows the strongest inducibility towards oxidative stress (Hackenberg *et al.* 2012). *Arabidopsis* transformed with *PwHAP5*, an orthologue of *Arabidopsis NF-YC2*, improves tolerance to salinity and decreases sensitivity to ABA (Li *et al.* 2013b). In this study, expression of *PmNF-YC8*, the homologue of *Arabidopsis NF-YC1/4*, is up-regulated under the ABA, mannitol, and H_2O_2 treatments. *PmNF-YC5*, the orthologue of *Arabidopsis NF-YC2*, is also induced by the ABA, osmotic, and H_2O_2 stresses. Expressions of *PmNF-YC2/4*, the homologues of *Arabidopsis* NF-YC3/9, are both up-regulated under the osmotic stress, and *PmNF-YC4* expression is also enhanced under the ABA and H_2O_2 treatments. Therefore, *PmNF-YC2/4/5/8* may be involved in multiple abiotic stress responses and flowering time control. The other clade contains *PmNF-YC1/3/6/7* and *Arabidopsis NF-YC10/11/12/13*. In our study, *PmNF-YC1/3/6* expression is regulated by the ABA, osmotic, and H_2O_2 stresses, so we infer that *PmNF-YC1/3/6* may be related to drought tolerance.

Some *NF-Ys* were reported to promote drought resistance, and also to be involved in oxidative stress responses (Li *et al.* 2008, Hackenberg *et al.* 2012). In this study, gene expression results exhibit that the expression levels of most *PmNF-Y* genes are up-regulated in the osmotic and H_2O_2 stresses, which is speculated that *PmNF-Ys* may be involved in responses to drought and reactive oxygen species stresses.

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