Overexpression of the poplar NF-YB7 transcription factor confers drought tolerance and improves water-use efficiency in Arabidopsis

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Abstract

Water deficit is a serious environmental factor limiting the growth and productivity of plants worldwide. Improvement of drought tolerance and efficient water use are significant strategies to overcome this dilemma. In this study, a drought-responsive transcription factor, NUCLEAR FACTOR Y subunit B 7 (PdNF-YB7), induced by osmotic stress (PEG6000) and abscisic acid, was isolated from fast-growing poplar clone NE-19 [Populus nigra × (Populus deltoides × Populus nigra)]. Ectopic overexpression of PdNF-YB7 (oxPdB7) in Arabidopsis enhanced drought tolerance and whole-plant and instantaneous leaf water-use efficiency (WUE, the ratio of biomass produced to water consumed). Overexpressing lines had an increase in germination rate and root length and decrease in water loss and displayed higher photosynthetic rate, instantaneous leaf WUE, and leaf water potential to exhibit enhanced drought tolerance under water scarcity. Additionally, overexpression of PdNF-YB7 in Arabidopsis improved whole-plant WUE by increasing carbon assimilation and reducing transpiration with water abundance. These drought-tolerant, higher WUE transgenic Arabidopsis had earlier seedling establishment and higher biomass than controls under normal and drought conditions. In contrast, Arabidopsis mutant nf-yb3 was more sensitive to drought stress with lower WUE. However, complementation analysis indicated that complementary lines (nf-yb3/PdB7) had almost the same drought response and WUE as wild-type Col-0. Taken together, these results suggest that PdNF-YB7 positively confers drought tolerance and improves WUE in Arabidopsis; thus it could potentially be used in breeding drought-tolerant plants with increased production even under water deficiency.

Key words: Arabidopsis, drought tolerance, NF-YB, poplar, transcription factor, water-use efficiency.

Introduction

Poplar is an important tree species of great economic and ecological importance worldwide. It is also one of the fastest growing trees and its high productivity requires a high consumption of water (Ridge et al., 1986; Tschaplinski and Blake, 1989; Zsuffa et al., 1996; Bradshaw et al., 2000; Monclus et al., 2005). Environmental abiotic stress, such as drought, salinity, and cold, could have harmful effects on the development and growth of poplars (Tschaplinski et al., 1994; Renaut et al., 2005; Escalante-Pérez et al., 2009). North China is a mostly dry or semi-dry area. Water resources are key factors in plant yields (Sun et al., 2006). Water-use efficiency (WUE), measured as the biomass produced per unit transpiration, describes the relationship between water use and plant productivity. The basic physiological definition of leaf WUE is equal to the ratio of photosynthesis to transpiration, also referred to as transpiration efficiency (Karaba et al., 2007). High WUE could increase the total production of plants under variable soil water content in the soil as an adaptation to water scarcity.
Water-deficit-inducible genes can be classified into two major groups: the first group encodes function proteins, such as aquaporins, LEA proteins, and chaperones; the second group includes genes encoding regulatory proteins, such as transcription factors, protein kinases, hormones, and other signal molecules (Shinozaki et al., 2003; Valliyodan and Nguyen, 2006). Transcription factors play critical roles in controlling intrinsic developmental processes and responses to external stimuli by influencing the expression of downstream targets and have been confirmed to improve drought resistance in transgenic plants (Shinozaki et al., 2003). The transcription factors involved in drought-responsive pathways are distributed mainly in the AP2/ERF, bZIP, NAC, MYB, C2H2 zinc finger, and WRKY families (Abe et al., 1997; Kizis et al., 2001; Sakamoto et al., 2004; Lu et al., 2007; Xiang et al., 2008; Wang et al., 2009). AtMYB61, a member of the R2R3-MYB family of transcription factors in Arabidopsis thaliana, closes stomata to limit water loss while directing the establishment of water-conducting xylem vessels with larger vessel diameter and root system proliferation to better seek, acquire, and transport water (Romano et al., 2012). Overexpression of TaWRKY2 and TaWRKY19 wheat WRKY transcription factors could regulate drought stress tolerance in Arabidopsis by activating downstream target genes related to drought resistance, such as DREB, RD29, and COR6.6 (Niu et al., 2012). In addition, another transcription factor family, GTL1, could control stomatal density by transrepression of SDD1 and negatively regulate WUE and drought tolerance in Arabidopsis (Yoo et al., 2010).

Recently, the NUCLEAR FACTOR Y family (NF-Y) binding specifically to the CCAAT box, was identified in drought-responsive pathways and also named for the CCAAT-box binding factor (CBF) (Nelson et al., 2007; Stephenson et al., 2007; Li et al., 2008; Thirumurugan et al., 2008). NF-Y is a ubiquitous nuclear transcription factor that consists of three subunits: NF-YA (CBF-B, HAP2); NF-YB (CBF-A, HAP3); and NF-YC (CBF-C, HAP5). In yeast, the subunits are called HEME ACTIVATED PROTEIN (HAP) 2, 3, and 5, respectively, and are encoded by single genes (Mantovani, 1999). The CCAAT box is a cis-acting element widely found in the eukaryotic promoter region, which is bound specifically with proteins encoded by the NF-Y family and regulates a series of related gene expressions (Testa et al., 2005). Recent reports elucidate some functions of the NF-Y family. Several NF-Y and NF-YC members in Arabidopsis, tobacco, and wheat play roles in light regulation and flowering time (Kumimoto et al., 2008, 2010; Stephenson et al., 2010, 2011; Hackenberg et al., 2012). AtNF-YB6 (LIL) and AtNF-YB9 (LEC1) are involved in embryo development in seeds (Kwong et al., 2003; Yamamoto et al., 2009). In recent years, NF-Y family members in plants have been found to function in drought stress. Li et al. (2008) found that the plants overexpressing AtNF-YA5 display reduced stomatal aperture and leaf water loss and significantly promote drought resistance and AtNF-YA5 is regulated transcriptionally by abscisic acid (ABA) and posttranscriptionally by miR169. The overexpression of AtNF-YB1 confers improved performance in Arabidopsis with higher water potential and photosynthesis rates under drought treatments. Transgenic maize plants with ZmNF-YB2, the homologue of AtNF-YB1, show drought tolerance based on increased chlorophyll content, stomatal conductance, and photosynthesis rates and decreased leaf temperature under drought conditions and exhibit a grain yield advantage (Nelson et al., 2007). However, little has been reported about the relationship between NF-Y transcription factors and WUE.

This study identified a poplar drought-responsive NF-Y family member from the fast-growing black cottonwood with high WUE, which conferred drought tolerance and improved plant WUE under water deficit.

Materials and methods

Plant materials and growth conditions

The poplar genotype NE-19 [Populus nigra × (Populus deltoides × Populus nigra)] was used in this study. NE-19 cuttings with 15-cm-long stems were planted in April 2010, in the nursery of Beijing Forestry University, Beijing, China (40° 0’ N 116° 15’ 1.60’ E) for further gene analysis.

Arabidopsis Col-0 was selected as the wild-type control. Arabidopsis mutant nf-y3 (stock name SALK_074951) was ordered from the Arabidopsis Biological Resource Center and the homozygous mutant for T-DNA insertion within AtNF-YB3 (AT4G14540) was verified by PCR. Arabidopsis seeds were sterilized by a 60-s 70% ethanol treatment followed by 1% NaClO within 10 min and four washes in distilled water. Seeds were sown on half-strength Murashige and Skoog (MS) plates with 3% sucrose and 0.6% agar and stratified for 2 d at 4 °C before being transferred to the culture room at 22 °C under a 16/8 light/dark cycle. After germination, 10-d-old Arabidopsis seedlings were transplanted and grown at a density of four plants per 7 × 7 × 6.5 cm pot containing a mixture of soil and vermiculite (2:1) at 22 °C under a 16/8 light/dark cycle (150 μmol m−2 s−1 and 70% relative humidity).

Poplar gene cloning, transformation, and expression analysis

Total RNA was extracted from the leaves of poplar NE-19 seedlings using the CTAB reagent method described by Chang et al. (1993). First-strand cDNA synthesis was performed using M-MLV Reverse Transcriptase and an oligo (dT) primer (Promega, Madison, WI, USA) according to the manufacturer’s instructions (Xing et al., 2011). The PdNF-YB7 cDNA sequence was amplified by PCR using the primers PdNFYB7f and PdNFYB7r (Supplementary Table S1, available at JXB online).

To obtain 3SS:PdNF-YB7 and nf-y3/PdB7 transgenic plants, the PdNF-YB7 cDNA was cloned into the pCAMBIA-1304 binary vector under the control of the cauliflower mosaic virus (CaMV) 3SS promoter and transformed into Arabidopsis Col-0 and mutant lines respectively by the floral dip method (Bechtold et al., 2003) using Agrobacterium tumefaciens GV3101. The transgenic lines were identified using half-strength MS plates containing 100 mg l−1 hygromycin.

For promoter expression analysis, the PdNF-YB7pro:GUS construct, including a 2.3-kb fragment upstream from the initiation codon extracted from poplar NE-19 genomic DNA, was cloned into the pBI121 vector and transformed into Arabidopsis Col-0.

For subcellular localization of PdNF-YB7 in plant cells, GFP fusion proteins were observed using a confocal laser scanning microscope (DMI6000 CS; Leica, Wetzlar, Germany).

To analyse the expression levels of related genes, total RNA was extracted from transgenic, wild type, mutant, and complementation plants by the CTAB method. Real-time PCR analysis was performed using primers PdB7 and PdActin (Supplementary Table S1).
Quantitative real-time PCR (qPCR) analysis followed the procedure described by Chen et al. (2009). SYBR Green was used to monitor the kinetics of PCR product formation in qPCR. The 18S rRNA transcript, as an internal control, was used to quantify the relative expression levels of genes in samples. The primer sequences are shown in Supplementary Table S2.

Histochemical staining analysis

To test the induction of GUS expression by osmotic stress, 10-d-old Arabidopsis seedlings were transferred from half-strength MS plates to half-strength MS liquid medium containing 25 mM PEG6000 or 200 mM mannitol for osmotic treatment. The controls were treated with half-strength MS liquid medium. GUS staining was performed by incubating the plants in GUS solution containing 100 mM Na2HPO4 buffer, 1 mM K3(Fe(CN)6), 1 mM K4(Fe(CN)6), 10 mM EDTA, 1% (v/v) Triton X-100 and 0.5 mg ml−1 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid overnight at 37 °C in the dark, followed by clearing with 75% ethanol for another hour.

Physiological experiments

Three independent batches of seeds were used to confirm the germination rate. Twenty seeds for a line in one batch were used for germination comparison between oxPB7s, Col-0, nf-yb3 and nf-yb3/PdB7 plants. Based on the diversity of germination time, the seeds would be separately sown on the plates to unify germination time. The Arabidopsis lines after germination were grown vertically to maintain the saturated field capacity. Additionally, filled pots were used to calculate water loss by evaporation. The soil collected at the three stages (well-watered, drought, rewatered) were oven-dried to a constant weight for 16 h at 90 °C and weighed for measurement of soil water content. The photosynthetic rates, instantaneous leaf WUE, and total biomass were described as previously. The leaf water potential was measured in situ nondestructively at the leaf surface of Arabidopsis seedlings using psychrometers (L-51A; WESCOR, Utah, USA) connected to the PSYPRO Water Potential System (WESCOR).

Water loss measurements

Rosette leaves of oxPB7s, Col-0, nf-yb3, and nf-yb3/PdB7 plants, which were grown under normal conditions for 25 d after germination, were excised, weighed immediately (leaves weighing approximately 1 g were harvested and used immediately for experiments), and incubated on a bench at room temperature and at 70% humidity and 150 μmol m−2 s−1. Losses in fresh weight were monitored at the times indicated (Ma et al., 2010). Water loss is expressed as the percentage of initial fresh weight.

Results

Identification and molecular characterization of differentially expressed genes

According to the microarray profile analysis of Populus euphratica response to drought stress (Yan et al., 2012a), several NF-YB family genes are induced by drought. Further qPCR analysis validated that PeNF-YB7 was especially upregulated in the leaves of drought-stressed poplars (Gray et al., 2009; Cao et al., 2011; Yan et al., 2012b). To study the role of the drought-related gene NF-YB in P. nigra × (P. deltoides × P. nigra), the current study characterized the poplar NUCLEAR FACTOR Y subunit B7 (PdB7) (GenBank accession KC460319), the homologue of P. euphratica PeNF-YB7, for future research. The PdB7 cDNA is 672 bp in length and encodes 223 amino acid residues with a predicted molecular mass of 24,644 kDa and an isoelectric point of 7.56.

The protein structure alignment using InterPro (http://www.ebi.ac.uk/interpro/) showed that the PdB7 sequence domain includes a NF-YB transcription factor conserved site (IPR003956), NF-YB binding site (IPR003957), and NF-YB archaeal histone (IPR003958) (Supplementary Fig. S1). The results indicate that PdB7 is a member of the NF-YB transcription factor family. Multiple sequence alignment revealed that the PdB7 secondary structure has a basic helix–loop–helix motif composed of four helices and three loops as in NF-YB family conserved domains (Fig. 1A). This highly conserved domain plays a central role in the junction between the NF-Y transcription factor and DNA and interaction between NF-Y and proteins (Mantovani, 1999). Additionally, the Arg in loop 2 and Asp in helix 3, which function in combinations of NF-YB and NF-YC, are highly conserved in the PdB7 protein. The two Glu in helix 2 are important for NF-YA binding (Romier et al., 2003). The phylogenetic relationship between the poplar and Arabidopsis
NF-YB family members was further analysed by amino acid sequence alignment (Dereeper et al., 2008, 2010). As shown in Fig. 1B, PdNF-YB7 did not cluster with known-function NF-YB proteins from Arabidopsis, such as drought-resistance protein, AtNF-YB1 (Nelson et al., 2007), and seed development-related proteins AtNF-YB9 and AtNF-YB6 (Kwong et al., 2003; Yamamoto et al., 2009). The results suggest that PdNF-YB7 differs from known-function NF-YB genes in Arabidopsis and has a specific function in poplars. The closest homologue to PdNF-YB7 was poplar protein PtNF-YB7 (NF-YB for Populus trichocarpa), which exhibits 99% identity. Moreover, this study found that, although not entirely orthologous to PdNF-YB7 (PtNF-YB6, PtNF-YB19, and PtNF-YB16 are even closer), AtNF-YB3 has the closest relationship to PdNF-YB7 among the Arabidopsis NF-YB family members using PdNF-YB7 as a phylogenetic control. For this reason, nf-yb3 was chosen for complementation experiments.

**Expression pattern of poplar PdNF-YB7**

To investigate the involvement of PdNF-YB7 in poplar responses to osmotic stress, the expression level of PdNF-YB7 under 30% PEG6000 was tested by PCR (Yan et al., 2012b). The results indicate that the expression level of PdNF-YB7 rose gradually with increased stress intensity and peaked at 15 d of PEG treatment (Fig. 2A). ABA is an important secondary signalling molecule and its exogenous application can cause similar effects to osmotic stress and mediate some drought-responsive genes (Zhu, 2002). Thus, 200 μM ABA treatment was used to test the response of PdNF-YB7 in poplar (Chen et al., 2009). The expression of PdNF-YB7 increased ~2.7-fold by 6 h, and then decreased slightly after 6 h (Fig. 2B). The results indicate that ABA mainly functions in the early stage of osmotic stress. ABA-responsive stress signalling first modifies the constitutively expressed transcription factors, resulting in the expression of early response genes and then activates downstream stress tolerance effector genes. The early response genes typically encode transcription factors (Zhu, 2002).

To study the tissue-specific presentation of expression of PdNF-YB7 in poplar, the expression of PdNF-YB7 was detected in roots, stems, young leaves, mature leaves, and senescent leaves of NE-19 under normal growth conditions. The results showed that PdNF-YB7 was expressed more highly in mature leaves, young leaves, and roots than in stems and senescent leaves (Fig. 3A).

To assess the expression pattern of PdNF-YB7, this study cloned the promoter of PdNF-YB7 from poplar genomic DNA. A predicted analysis of the PdNF-YB7 promoter
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using the PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) revealed a series of water-related and abiotic stresses responsive elements, including ABRELATERD1, ACGTATERD1, CBFHV, DPBFCOREDCC3, MYB, and MYC (Supplementary Table S3). The results suggest that PdNF-YB7 plays roles in the response to environmental stresses and in plant growth and development.

To analyse the regulatory activity of PdNF-YB7 during the stress response, this study constructed the PdNF-YB7pro:GUS expression vector and transformed Col-0 wild-type Arabidopsis. Then 25mM PEG6000 and 200mM mannitol were used to determine the osmotic stress response in transgenic plants. Histochemical staining analysis revealed that GUS was expressed in transgenic seedlings and that expression was enhanced in response to drought and osmotic treatment and observed throughout the entire plant (Fig. 3B–E).

To test the subcellular localization of PdNF-YB7 in plant cells, the 35S:PdNF-YB7-GFP fusion was constructed and transformed into Arabidopsis Col-0. Expression of the PdNF-YB7-GFP fusion in Arabidopsis predominantly accumulated in the nucleus (Fig. 4), consistent with its function as a transcriptional regulator.
Phenotype of overexpressing lines under well-watered conditions

To evaluate the performance of overexpressing *PdNF-YB7* (*oxPdB7*) lines grown in well-watered conditions, the growth phenotype of overexpressing *Arabidopsis* was observed during different developmental stages. The germination time for *oxPdB7* was 1 or 2 d earlier than the wild-type control Col-0 and mutant *nf-yb3*. Eight days after germination, the primary root length of *oxPdB7* also was longer than Col-0 (1.3-fold) and *nf-yb3* (2.1-fold) (Fig. 5A, D). In 18-d-old seedlings grown in soil, transgenic plants had larger leaves than Col-0 and the mutant (Fig. 5E). The leaf area of *oxPdB7* was about 1.00–1.37-fold larger than Col-0 and 3.07–3.82-fold larger than the mutant (Fig. 5B). The *oxPdB7* lines bolted 22–23 d after germination, whereas the Col-0 and mutant plants bolted at approximately 23–24 d and 24–25 d, respectively. Additionally, the transgenic plants showed higher average stem elongation rate at the earlier shooting stage (25–34 d) compared to Col-0 and mutant (Fig. 5C, F). At 34 d, the inflorescence length of *OxPdB7* varied from 28.2 cm to 32.6 cm, that of Col-0 varied from 21.3 cm to 24.9 cm and that of the mutant varied from 15.5 cm to 18.3 cm. However, at the late stage, Col-0 and *nf-yb3/PdB7* (34–37 d), and *nf-yb3* (37–40 d) displayed higher average elongation rate, respectively (Fig. 5C). The results indicate that the transgenic lines had faster stem elongation at the early shooting stage and earlier seeding establishment.

Overexpressing *PdNF-YB7* improves WUE in *Arabidopsis*

The *oxPdB7* lines had higher net photosynthetic rates than Col-0 and the mutant under the same conditions and the mutant, with the slowest net photosynthetic rate, had a rate equivalent to Col-0 after complementation with *PdNF-YB7* (Fig. 6A). For the transpiration rate, the *oxPdB7* lines showed slower rates than Col-0 and the mutant (Fig. 6B). Based on the higher photosynthetic capability and lower transpiration level, the *oxPdB7* lines had higher leaf WUE than Col-0 and the mutant (Fig. 6C). In addition, whole-plant WUE of *oxPdB7* lines was higher than Col-0 and the *nf-yb3* mutant (Fig. 6D).

Overexpressing *PdNF-YB7* increases drought tolerance under water deficit

To decipher the mechanism by which water deficit affects plant development and growth, various experimental setups were developed. The seeds of transgenic lines, Col-0, *nf-yb3*, and the complemented line *nf-yb3/PdB7* were sown on half-strength MS culture with 200 mM mannitol for osmotic stress. After 4 d, *oxPdB7* lines had more vigorous germination (75.3%) than that of Col-0 (40.4%) and the mutant (16.3%) (Fig. 7A). However, by comparison, all seeds of transgenic lines, Col-0, *nf-yb3*, and the complemented line *nf-yb3/PdB7* had sprouted after 4 d under normal conditions (data not shown). Additionally, the primary root lengths of 8-d-old seedlings were also different. The *oxPdB7* plants had considerably longer (1.4- and 2.4-fold, respectively) primary roots than that of Col-0 and *nf-yb3* (Fig. 7B). Compared to the primary roots of *Arabidopsis* under well-watered conditions, primary root lengths of *oxPdB7* lines decreased by 21.7% to 27.3% while that of Col-0 decreased by 35.3% and that of *nf-yb3* reduced by 44.8% under drought conditions. Additionally, the detached leaves of transgenic plants lost water more slowly than Col-0 and the mutant (Fig. 7C).
After the seedlings were transplanted to soil, water deficit was imposed for 10 d, followed by a rewatering period of 8 d (Fig. 7D). Analysis of soil water status for the genotypes explained that soil water contents of three phases in drought experiment were 53.20 ± 1.19% (well-watered), 6.45 ± 1.00% (drought), and 46.01 ± 0.95% (rewatered), respectively. During water deprivation, Col-0 and the \textit{nf-yb3} mutant withered and showed more severe wilting than overexpressing plants, but the \textit{oxPdB7} lines exhibited continued development and growth resulting in more biomass (Fig. 8A). Photosynthesis analysis showed that transgenic lines maintained a significantly higher photosynthetic rate than Col-0, and the mutant under stress treatment (Fig. 8B), resulting in an increase in instantaneous leaf WUE (Fig. 8C). The leaf water potential of \textit{Arabidopsis} seedlings showed significant differences among the transgenic, Col-0, \textit{nf-yb3}, and \textit{nf-yb3/PdB7} seedlings under drought. The transgenic lines had higher leaf water potential compared to Col-0 and the mutant under drought stress (Fig. 8D). Thus, the expression of \textit{PdNF-YB7} was demonstrated to be sufficient to improve tolerance to water scarcity in \textit{Arabidopsis}.

### Expression analysis of stress-responsive genes regulated by the PdNF-YB7 transcription factor

To determine the improved drought tolerance by altered expression of \textit{PdNF-YB7}, the expression levels of some drought-related genes in the leaves were analysed by qPCR in an independent experiment using the \textit{oxPdB7} lines, Col-0, mutant, and complementation under well-watered, drought, and rewatered conditions. The results showed that ABA pathway markers (\textit{RD29B}, \textit{RAB18}, and \textit{CBF4}), CBF pathway markers (\textit{COR15B}, \textit{KIN1}, and \textit{LEA76})(Nelson \textit{et al.}, 2007), and several predicted candidate target genes of \textit{AtNF-YA5} (\textit{BAM5}, \textit{LTP}, \textit{GST}, and \textit{COR15A}) (Li \textit{et al.}, 2008) were differentially expressed in the \textit{oxPdB7} lines compared to the 35S:\textit{NF-YB1} and 35S:\textit{NF-YA5} lines (Fig. 9). \textit{CBF4}, \textit{COR15B}, \textit{LEA76}, \textit{BAM5}, and \textit{GST} were more highly expressed in the \textit{oxPdB7} lines under well-watered conditions. Under water deficit, these stress-responsive genes were strongly induced in transgenic plants. However, the majority of these genes did not significantly change in \textit{nf-yb3}, suggesting that for many of these genes, \textit{PdNF-YB7} was required for induction by dehydration.

### Discussion

Plants have evolved regulatory mechanisms to adapt to environmental water deficit. Transcription factors regulate expression of the stress-responsive genes by binding specifically to the motif of the promoters to modulate resistance to drought and lower productivity loss (Zhu, 2002;...
Nuclear factor Y is one of the largest transcription factor gene families in plants. A number of NF-Y proteins have been identified as regulators of drought tolerance in different plant species. *AtNF-YB1, ZmNF-YB2*, and *TaNF-YB2*, were reported to confer drought resistance in *Arabidopsis*, maize, and wheat, respectively, and to increase crop productivity under drought field tests (Nelson et al., 2007; Stephenson et al., 2007). *PdNF-YB7* was inferred to have the same effect on *Arabidopsis* because of the relatively close evolutionary relationship and genetic distance (Yan et al., 2012b). In addition, another NF-Y subunit family member, *AtNF-YA5*, plays a role in drought resistance in *Arabidopsis* (Li et al., 2008). Apart from these, other NF-YB family members, such as *AtNF-YB9* (*LEC1*) and *AtNF-YB6* (*LIL*), are essential factors controlling embryonic development and are phylogenetically and functionally distinct from other NF-YB family members, such as *AtNF-YB1* and *PtNF-YB7* (Kwong et al., 2003; Yamamoto et al., 2009; Yan et al., 2012b). Most of the above findings were reported for herbaceous plants such as *Arabidopsis* and maize. However, reports of NF-YBs in woody plants are still sparse. The *PdNF-YB7* gene studied here is the first reported NF-YB gene in fast-growing black poplar.

According to the phylogenetic analysis, *PdNF-YB7* shared high sequence similarity and clustered with a member of the...
Poplar NF-YB7 family genes. The conserved domain analysis of the multiple sequence alignment showed that PdNF-YB7, PtNF-YB7, and AtNF-YB3 are very highly conserved. In Arabidopsis, AtNF-YB3 plays an important role in the promotion of flowering specifically under inductive long-day photoperiodic conditions. Consistent with this, the overexpression of PdNF-YB7 in Arabidopsis caused earlier seedling germination time and enhanced the development of both vegetative and reproductive organs (Fig. 5F). Notably, in these experiments, the transcript levels of PdNF-YB7 were upregulated by drought stress, resembling those of AtNF-YB1 in Arabidopsis (Nelson et al., 2007). Different from AtNF-YB1, transcript levels of PdNF-YB7 were also affected by ABA treatment. Promoter expression analysis of PdNF-YB7 provided further support for its role in stress tolerance; GUS gene expression was enhanced by drought and was observed throughout the entire plant after stress treatment. Element analysis of the promoter indicated that several ABA-responsive elements were included in PdNF-YB7 promoter region (Supplementary Table S3). This is the same result of AtNF-YA5 in Arabidopsis; two ABA-responsive element sequences could be found in the promoter region of AtNF-YA5, which is proved to be involved in drought resistance (Li et al., 2008). Additionally, increasing evidence is being found for NF-YB/bZIP interactions, and bZIP proteins are well known to be involved in ABA signaling (Liu...
Interestingly, a tissue-specific expression analysis indicated another difference between PdNF-YB7 and AtNF-YB3. Previous research revealed that AtNF-YB3 is expressed more highly in flowers and young leaves, but is absent in roots (Siefers et al., 2009), while the current experiments suggested that PdNF-YB7 is highly expressed in root. This is consistent with AtNF-YA5, overexpression of which is proved to increase drought tolerance in Arabidopsis (Li et al., 2008).

To better understand the regulatory mechanisms of drought tolerance conferred by overexpressing PdNF-YB7, this study confirmed the expression patterns of genes that may potentially be regulated by NF-Ys according to previous research on AtNF-YB1 and AtNF-YA5. For the ABA pathway markers (CBF4, RD29B, and RAB19) or CBF pathway markers (COR15B, KIN1, and LEA76), none of them showed differences in expression between AtNF-YB1 overexpression plants and controls (Nelson et al., 2007). However, in the current study, RD29B and CBF4 showed significant and consistent differences expression in 35S::PdNF-YB7 plants, indicating that the ABA-dependent dehydration response was regulated by PdNF-YB7. Drought-inducible genes encoding functional proteins such as KIN1, LEA76, LTP, and GST were also highly expressed in transgenic plants, especially in response to water deficit, suggesting that PdNF-YB7 potentially increased the accumulation of protective proteins under drought conditions.

Transgenic Arabidopsis overexpressing poplar NF-YB7 showed significantly higher biomass under well-watered and drought conditions. Increased photosynthetic leaf area enhanced carbon assimilation, resulting in more biomass accumulation. Several studies have implicated NF-Y in controlling photosynthesis by regulating the chloroplast ATP synthase (Kusnetsov et al., 1999) and some nuclear-encoded photosynthesis genes such as RBCS and CAB (Miyoshi et al., 2003). Transgenic wheat with TaNF-YB3 has a significant enhancement in leaf chlorophyll content and photosynthesis rate (Stephenson et al., 2011).

Recent studies have shown that root growth is closely connected with drought tolerance (Pennisi, 2008). In the current study, overexpressing PdNF-YB7 in Arabidopsis increased primary root length that led to expansion of the root surface area. Ballif et al. (2011) also found that overexpressing AtNF-YB2 enhanced primary root elongation due to a faster cell division and/or elongation. Additionally, PdNF-YB7 overexpression transgenic plants also maintained higher leaf water potential than that of wild type under water-deficit conditions. Often during drought conditions, plants avoid...
low soil water potential by achieving a balance between water absorption and loss, for example, by decreasing the stomatal aperture while maintaining root growth (Verslues et al., 2006). Decrease of stomatal apertures resulted in decreased transpiration rate thus reduced water loss. Meanwhile, increased root length improved absorption of water and mineral solutes. Equality between uptake and loss of water and thereby maintenance of constant leaf water potential is assisted by stomatal changes, which appear to be in response to conditions in the root (Aston and Lawlor, 1979). Altogether, these physiological phenotypes conferred by PdNF-YB7 support that the gene could be potentially used in breeding drought-tolerant plants and promoting plant production under drought conditions. Further experiments are now needed to characterize the effects of PdNF-YB7 in poplar to elucidate its regulatory mechanisms in woody plants.

Supplementary material

Supplementary data are available at JXB online.

Supplementary Table S1. Primer sequences used for cloning of PdNF-YB7 cDNA.

Supplementary Table S2. Primers used for PCR and qPCR.
**Supplementary Table S3.** Putative elements of the promoter of *PdNF-YB7*.

**Supplementary Fig. S1.** The structural alignment result of the *PdNF-YB7* protein.

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