Molecular cloning and functional analysis of *MbWRKY3* involved in improved drought tolerance in transformed tobacco

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**ABSTRACT**

Plant-specific WRKY transcription factors were involved in stress responses and ABA signaling. In the present study, a WRKY gene is isolated from *Malus baccata* (L.) Borkh and designated as *MbWRKY3*. Subcellular localization revealed that *MbWRKY3* was localized onto nucleus. The *MbWRKY3* expression levels were up-regulated by salinity, drought, and ABA treatments in *M. baccata*. When *MbWRKY3* was introduced into tobaccos, it improved drought stress tolerance in transgenic plants. Compared to WT, the transgenic tobaccos had the higher levels of relative water content, and proline and chlorophyll contents, decreased levels of electrolyte leakage, MDA, and H_{2}O_{2}, increased activities of the reactive oxygen species-related enzymes (SOD, CAT, and POD), and greater up-regulations of the corresponding genes (*NtSOD*, *NtCAT*, and *NtPOD*), especially when dealt with drought stress. These results suggest that *MbWRKY3* gene plays a positive regulatory role in drought stress response.

**Introduction**

As sessile organisms, plants are frequently exposed to variable environmental stresses, such as drought, salt, heat, chilling, pathogen attack, and nutrient deprivation, which adversely affect plant growth, development, and productivity (Gong & Liu 2013). Environmental stresses are perceived and transduced through a chain of signaling molecules that ultimately affect regulatory element of stress-inducible genes to initiate the synthesis of different classes of protein including transcription factors, enzymes, molecular chaperons, ion channels, and transporters or alter their activities (Mukhopadhyay et al. 2004). To avoid such deficiencies, plants have developed adaptable mechanisms to perceive external signaling networks and to manifest adaptive responses with appropriate physiological, cellular, and molecular changes (Liu et al. 2014). These environmental stimuli usually disrupt the cellular homeostasis of reactive oxygen species (ROS), leading to the oxidative stresses (Mittler, 2002). In these stresses, drought is the most severe threat to crop yield worldwide. Plants have evolved complex strategies to reduce potential damage of drought stress (Wang et al. 2009).

Among the numerous stress-induced genes, many transcription factors have been identified and studied, such as WRKY, NAC, and bZIP, which can interact with cis-elements present in the promoter regions of various abiotic stress-related genes and thus regulate the expression of many genes resulting in imparting tolerance to abiotic stresses (Agarwal et al. 2006). Among TFs, the WRKY genes received much attention in past decade. The WRKY proteins, which contain one or two conserved WRKY domains consisting of 60 amino acid region with a conserved WRKYGQK motif in its N-terminus followed by a C_{2}H_{2} or C_{2}HC zinc-finger motif, represent a large family of plant-specific TFs (Rushton et al. 2010). There are over 70 WRKY genes in *Arabidopsis* (Eulgem et al. 2000; Dong et al. 2003; Sáenz-Mata et al. 2014). Studies on WRKY genes predominantly point to an involvement in salicylic acid (SA) signaling and disease responses (Eulgem et al. 2000; Asai et al. 2002). In addition, WRKY genes are involved in plant responses to drought, heat, cold, salinity (Zhou et al. 2008; Liu et al. 2014; Sáenz-Mata et al. 2014; Cheng et al. 2017; Ye et al. 2017; Han et al. 2018), freezing (Huang & Duman 2002), wounding (Hara et al., 2000), and oxidative stress (Rizhsky et al. 2004; Cui et al. 2018). But these studies mostly focused on model plants or crops, and the roles of the WRKY genes in stress responses of *Malus* plant were less well known.

*Malus baccata* is widely used as an apple rootstock in northern China, but is also grown elsewhere as an ornamental tree and is highly resistant to low temperatures and drought (Xiao et al. 2008). To better understand the roles of WRKY genes involved in drought stress tolerance and to provide potentially genetic resources for the improvement of the drought resistance of *Malus* plant, a novel drought-responsive WRKY transcription factor was isolated from *M. baccata* and designated as *MbWRKY3*. Moreover, the over-expression of *MbWRKY3* in transgenic tobacco increased the tolerance to drought stress.

**Materials and methods**

**Plant material and growth conditions**

In vitro grown seedlings of *M. baccata* were propagated and rooted on MS medium, and transferred to Hoagland’s solution for acclimation and growing for 50 d (Han et al. 2018). When the seedlings had 8–9 mature leaves (fully
expanded), they were exposed to air on filter paper with 20% relative humidity for dehydration and drought treatment. For ABA and salt treatments, seedlings were immersed in 0.1 mM ABA or 250 mM NaCl solution, respectively (Liu et al. 2014).

**Isolation, phylogenetic analysis and qRT-PCR expression analysis of MbWRKY3**

Total RNA was respectively extracted from root, new leaf (partly expanded), mature leaf (fully expanded), and stem, using the CTAB method (Han et al. 2017). The samples of all control and roots of treated plants were sealed after treatments of 0, 3, 6, 9, 12 h, respectively, frozen immediately in liquid nitrogen, and then stored at −80°C for RNA extraction. First strand cDNA was synthesized with 1 μg total RNA and 1 μL super script II enzyme (Invitrogen, USA) according to the manufacturer protocol. PCR was performed to obtain a whole sequence of MbWRKY3 by using the first strand cDNA of *M. baccata* as a template. A pair of primers (F1, 5′-ATGGGCTACCTTAGAAGGGACCTTAGC-3′ and R1, 5′-CTATGAAATTTGGCTATTTGGAGTC-3′) was designed according to the homologous regions of *Malus domestica* WRKY gene 3 (MDP0000263961) to amplify the full-length cDNA sequence. The full-length cDNA of MbWRKY3 gene was isolated from *M. baccata* using PCR with F1 and R1 as primers. The obtained DNA fragments were gel purified and cloned into the pMD18-T vector (Takara, Dalian, China) and sequenced (Invitrogen, Beijing, China).

Sequence similarity analyses were performed using the BLAST program against the NCBI database. To investigate the evolutionary relationship among plant WRKY TFs, the amino acid sequences of nine WRKY TFs from different species (MbWRKY3, MdWRKY3 (MDP0000263961), BhWRKY1 (FJ222453.1), VpWRKY3 (JF500755.1), FvWRKY40 (XP_004303242.1), MeWRKY79 (AMO00447.1), PbWRKY40 (XP_009349633b.1), TcWRKY40 (EOY26052.1), and ZjWRKY40 (XP_01586938.1) were analyzed by DNAMAN (v.6.0).

The expression level of MbWRKY3 to abiotic stress was analyzed by real-time (RT)-PCR method in *M. baccata* and conducted according to Han et al. (2015). The primers for MbWRKY3 were designed for qRT-PCR from partial cDNA sequence. The full-length cDNA of MbWRKY3 gene was isolated from *M. baccata* using PCR with F1 and R1 as primers. The obtained DNA fragments were gel purified and cloned into the pMD18-T vector (Takara, Dalian, China) and sequenced (Invitrogen, Beijing, China).

**Subcellular localization of the MbWRKY3 protein**

The MbWRKY3 ORF was cloned into the *SalI* and *BamHI* sites of the pSAT6-GFP-N1 vector. This vector contains a modified red shifted green fluorescent protein (GFP) at *SalI-BamHI* sites. The MbWRKY3-GFP construct was transformed into onion epidermal cells by particle bombardment as described earlier (Han et al. 2015). The transient expression of the MbWRKY3-GFP fusion protein was observed under confocal microscopy.

**Vector construction and agrobacterium-mediated tobacco transformation**

To construct an expression vector for transformation of tobacco, restriction enzyme cut sites of *SmaI* and *SacI* were added into MbWRKY3 cDNA at both 5′ and 3′ ends by PCR. To construct the pBI121-MbWRKY3 vector, the products of PCR and pBI121 were digested by *SmaI* and *SacI*, and linked together through the replacing of *GUS* gene. The MbWRKY3 gene driven under the CaMV 35S promoter was introduced into *Nicotiana tabacum* cv. Xanthi ecotype tobacco by Agrobacterium-mediated GV3101 transformation (An et al. 1986). Transformants were selected on MS medium containing 50 mg dm⁻³ Kanamycin. T₂ generation transgenic tobaccos were used for further analysis. Expression levels of the MbWRKY3 in tobacco transgenic lines were analyzed by semi-quantitative RT-PCR. The primers of MF and MR were used for RT-PCR detection of leaves of tobacco, with the *NiUbiquitin* gene (U66264.1) as reference gene (*NiUbFi*, 5′-TTAACACATGGAAGTCCGACG-3′ and *NiUbR1*, 5′-GAGACCTCAGTAGACAAGACATC-3′). PCR was performed for 30 and 25 amplification cycles for *MbWRKY3* and *NiUbiquitin*, respectively.

**Drought tolerance analysis of transgenic tobaccos**

Seeds (T₂ generation) of transgenic plants (OE-4 and OE-13) were sown and germinated on the germination and culture medium (nutrient soil: vermiculite ratio is 4:1) with normal management in a growth chamber at 25 ± 1°C under a 16 h light (50 µmol/m²/s)/8 h dark regime in parallel with WT seeds. Seedlings were grown for 3 weeks with regular irrigation prior to drought stress. Drought stress experiments were conducted by withholding water for 12 d. Then the tobaccos were rewatered for 6 d to determine the survival rate. The experiments were performed twice with three independent leaves for each treatment at each time point. During the whole growth process, all tobacco seedlings were observed and recorded with photograph on drought stress for 6 d (D₆d), 12 d (D₁₂d), and rewatered for 6 d (R₆d).

**Measurement the levels of relative water content and electrolyte leakage**

Three-week-old transgenic tobacco (OE-4 and OE-13) and WT tobacco plants above were conducted by withholding water for 6 d, and the leaves of all samples (before drought and after drought) were collected for measurement. Leaf relative water content (RWC) was estimated according to RWC (%) = (fresh weight − dry weight)/(turgid weight − dry weight) × 100 (Wang et al. 2009). Electrolyte leakage (EL) in tobacco leaves was determined following the protocols described by Xing et al. (2011).

**Measurement of the contents of proline, chlorophyll, MDA, and H₂O₂**

The leaves (including samples of before drought and after drought for 6 d) of 3-week-old transgenic and WT
tobaccos above were collected for measurement. Proline contents were assayed following the method of Irigoyen et al. (1992). Chlorophyll contents were measured according to the method of Aono et al. (1993). MDA contents in tobacco leaves were measured by the method described previously (Dong et al. 2003). H₂O₂ contents in tobacco leaves were determined by the method of Alexieva et al. (2001).

**Measurement of activities of SOD, CAT, and POD**

The SOD, CAT, and POD activities of all the tobacco leaf samples of before treatments and exposed to drought stresses for 6 d above were also measured. SOD (EC 1.15.1.1) activities were measured using the protocols described by Beuchamp & Fridovich (1971). CAT (EC 1.11.1.6) activities were measured following the methods used previously (Zhang et al. 2011). POD (EC 1.11.1.7) activities were measured following Ranieri et al. (2000). Relative quantification relates the enzyme activity in the transgenic lines (OE-4 and OE-13) to that of the WT.

**Gene expression analyses of NtSOD, NtCAT, NtPOD in transformed and WT tobaccos**

Three-week-old tobaccos were exposed to drought treatments for 5 d, and the leaf samples above were collected. Expressions of the ROS-related genes and stress-responsive genes in tobaccos (transgenic lines and WT) were analyzed by qRT-PCR. The qRT-PCR was performed as described above. The primers were for NtSOD gene (AB093097.1), 5'-AGTCTACATGACCGCATTTCC-3' and 5'-CCCTGTTAGAAGCACACTTCC-3'; for NtCAT gene (U93244.1), 5'-AAGAATGGCTCCAGAATTTA-3' and 5'-GGCTAGCATTCCAGAGTG-3'; for NtPOD gene (AB178953.1), 5'-GCTTGTCAGAGGTGTAACAG-3' and 5'-CTCTGGTGAGTGTGTGTTGAG-3'; for NtUbiquitin gene (U66264.1), 5'-TCCAGGACAAAGGAGGT-3' and 5'-CATCAACACAGGCACCT-3'; The qRT-PCR analysis was performed as described above. The ROS-related genes and stress-responsive genes expression levels were normalized against the NtUbiquitin gene expression level. Relative quantification relates the PCR signal of the ROS-related genes expression levels in the transformed lines (OE-4 and OE-13) to that of the WT plants.

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**Figure 1.** Nucleotide and deduced amino acid sequences of MbWRKY3. (a) The WRKY domain and (b) The C₃H₂ zinc-finger motif.
Statistical analysis

Duncan multiple range tests were performed by using SPSS 13.0 program. Statistical differences were referred to as significant when \( P < .05 \).

Results

Isolation of MbWRKY3 gene from M. baccata

Sequence analysis showed that the MbWRKY3 cDNA has a complete ORF of 1059 bp, the predicted MbWRKY3 comprises...
352 amino acids (Figure 1) with a theoretical isoelectric point of 8.53 and a predicted molecular weight of 39.5 kDa. MbWRKY3 protein contains one WRKY domain (Figure 1(a)) and one C2H2 zinc-finger motif (C-X5-C-X23-H-X-H) (Figure 1(b)).

**Phylogenetic relationship of MbWRKY3 with other WRKY transcription factors**

As shown in Figure 2, all the deduced amino acid sequence of WRKY TFs include a WRKY domain (WRKYGQK, Figure 2(a)) and one conserved C2H2 zinc-finger motif (Figure 2(b)). Comparing the amino acid sequences of MbWRKY3 with other WRKY TFs, we found that MbWRKY3 has a high identity to the WRKY TFs.

Additionally, a phylogenetic tree (neighbor-joining) was constructed with the amino acid residues (Figure 3) by MEGA program (v.4.1). Phylogenetic analysis demonstrated that the majority of 24 isolated proteins belong to 7 different subgroups of II-a, II-b, II-d, II-c, I, II-e, and III. MbWRKY3 was clustered into II-a subgroup, and more closely related to the VpWRKY3 and BhWRKY1.

**MbWRKY3 was localized in nucleus**

As shown in Figure 4, the MbWRKY3-GFP fusion protein is targeted into nucleus with 4′,6-diamidino-2-phenylindole (DAPI) staining, whereas the control GFP alone is distributed throughout the cytoplasm. These results showed that the MbWRKY3 is a nucleus localization protein.

**Expression patterns of MbWRKY3 under abiotic stresses in M. baccata**

The spatial-specific expression of MbWRKY3 in different tissues of M. baccata was determined by qRT-PCR. The result shows that MbWRKY3 mRNA is more abundant in root and new leaf than in stem and mature leaf (Figure 5(a)). The results showed that MbWRKY3 increased in root under high salinity, drought, and ABA treatments (Figure 5(b–d)) by the qRT-PCR method. For high salinity stress, the expression level of MbWRKY3 increased rapidly and reached the maximum at 9 h and maintained the stable high level for 12 h (Figure 5(b)). Under drought stress, the expression levels of MbWRKY3 began to increase after 3 h dehydration treatment and increased gradually until the experiment was concluded at 12 h (Figure 5(c)). The expression level of MbWRKY3 increased rapidly and peaked at 9 h, and then decreased at 12 h in response to ABA treatment (Figure 5(d)).

**Over-expression of MbWRKY3 confers enhanced tolerance to drought stress**

In order to investigate the role of MbWRKY3 in response to drought stress in plants, we generated transgenic tobacco with over-expression of MbWRKY3 under the control of the CaMV 35S promoter. Among 25 transformed lines, 6 of them (OE-3, OE-4, OE-9, OE-10, OE-13, and OE-23) were confirmed by using RT-PCR analysis with WT as control (Figure 6(a)). As shown in Figure 6(b), no significant difference existed in appearance between WT and MbWRKY3-OE (OE-4 and OE-13, randomly selected) lines after 3 weeks of growth on culture matrix with normal conditions, both types of tobaccos grew well (Unt: untreated). However, when dealt with drought stress (withholding water), improved drought tolerance in transformed plants was observed (D6d, D12d). The higher survival rates of transgenic plants after rewatering for 6 d (R6d) were found, 83.3% (20/24) for OE-4; 80.8% (21/26) for OE-13, compared to WT, only 25.9% (7/27).

**Over-expression of MbWRKY3 in transformed tobaccos conferred higher levels of RWC, proline, and chlorophyll under drought stress**

In order to study the reasons why the transgenic tobaccos had the better appearances under the drought stress, the RWC,
proline, and chlorophyll levels of all lines (both transgenic tobaccos OE-4, OE-13, and WT line) under normal irrigation and drought stress were measured. When dealt with drought stress for 6 d, the RWC, Pro and Chl levels in leaves of transgenic tobaccos were significantly higher relative to WT (Figure 7(a–c)). However, these are no significant difference in the levels of RWC, Pro, and Chl between the MbWRKY3-over-expression tobaccos and WT line under normal conditions.

**MbWRKY3-over-expression tobaccos accumulated less EL, MDA, and H2O2 under drought stress**

We also measured the EL, MDA, and H2O2 levels of all lines (OE-4, OE-13, and WT) under normal irrigation and drought stress. The EL, MDA, and H2O2 levels in leaves of transgenic tobaccos were significantly lower relative to WT, especially when dealt with drought stress for 6 d (Figure 7(d–f)). When dealt with drought stress, the levels of EL, MDA, and H2O2 in WT tobaccos were about respectively 1.6-, 1.7-, and 1.8-fold than transgenic lines, which indicated that the WT line had more severe membrane damage. These results indicate that the over-expression of MbWRKY3 in transgenic tobaccos confers greater tolerance to the oxidative stress associated with drought stress.

**Over-expression of MbWRKY3 confers to enhanced antioxidant enzyme activities**

Aim to research the reason why WT tobaccos had more severe membrane damage relative to transgenic lines, the activities of ROS-scavenging enzymes such as SOD, CAT, and POD in both transgenic tobacccos (OE-4 and OE-13) and WT line before and after drought stress were also measured. Under normal water management, the activities of SOD, CAT, and POD in the transgenic lines were 1.4-, 1.2-, and 1.3-fold higher than WT line, respectively. When dealt with drought stress, the activities of SOD, CAT, and POD increased in all lines approximately 1.7-, 1.6-, and 1.8-fold than WT, respectively (Figure 7(g–i)). The change tendency of activities of ROS-scavenging enzymes between the WT and transgenic lines remained more or less the same. Compared to WT line, the transgenic tobaccos had the higher ROS-scavenging enzymes activities so that they can remove more reactive oxygen radicals and protect integrity of the membrane.

**Over-expression of MbWRKY3 confers to enhanced expression levels of ROS-related genes**

In order to research what caused the higher antioxidant enzyme activities (SOD, CAT, and POD) in transgenic tobaccos (OE-4 and OE-13) than in WT line, the expression levels of NtSOD, NtCAT, and NtPOD in all lines were also measured by qRT-PCR method. The result showed that the expression levels of three genes were 2.4-, 1.9-, and 2.5-fold than WT, respectively (Figure 8(a–c)).

**Discussion**

The WRKY transcription factors have been proved to play important functions in the regulation of transcriptional reprogramming related to plant biotic and abiotic stress responses (Chen et al. 2012). In the present study, a new WRKY gene was isolated from *M. baccata* and designated as *MbWRKY3*. Sequence analysis showed that *MbWRKY3* transcription factor contains one WRKY domain and one C2H2 zinc-finger motif (C-X7-C-X23-H-X-H) (Figure 1).

All the WRKY TFs include a WRKY domain (WRKYGQK) and one conserved C2H2 or C2HC zinc-finger motif (Liu et al. 2014). These results showed that the WRKY TFs family was highly conserved during evolution (Rinerson et al. 2015). Previous reports have indicated that WRKY TFs genes are widely distributed in *Arabidopsis*, rice, soybean, maize, cotton, strawberry, orange, grape, and peach, which are known to be involved in abiotic (Ramoomooorthy et al. 2008; Han et al. 2018) and biotic stress responses (Pandey & Somssich 2009), seed development
and germination (Johnson et al. 2002), and leaf senescence (Miao et al. 2004). The MbWRKY3 was structurally similar to VpWRKY3 and BhWRKY1, which were isolated from Vitis pseudoreticulata (Zhu et al. 2012) and Boea hygrometrica (Wang et al. 2009) under high-drought stress. Phylogenetic analysis demonstrated that MbWRKY3 was clustered into II-a subgroup and more closely related to the VpWRKY3 and BhWRKY1. These results indicate that MbWRKY3 is a novel member of the WRKY transcription factor family (Figure 3).

Certain WRKY TFs were reported to localize also in nucleus (Miao et al. 2004; Ramamoorthy et al. 2008; Liu et al. 2014). Subcellular localization experiment has revealed that MbWRKY3 is preferentially localized in nucleus (Figure 4), which is consistent with previous research.

The expression of MbWRKY3 was much enriched in root and new leaf than in stem and mature leaf (Figure 5(a)),

![Figure 7. Analysis of relative water content (RWC), proline (Pro), chlorophyll (Chl), oxidants, electrolyte leakage (EL), and antioxidant enzymes between WT and transgenic T2 tobaccos (OE-4 and OE-13) under normal water management (Before drought) or withheld irrigation for 6 d (After drought), respectively. (a) RWC, (b) Pro, (c) Chl, (d) EL, (e) MDA, (f) H2O2, (g) SOD, (h) CAT, (i) POD. Data represent means and standard errors of three replicates. Different letters above columns indicate (P < .05) significance using Duncan’s multiple range test differences between treatments.](image)

![Figure 8. Expression of ROS-related genes in WT and transgenic T2 tobaccos (OE-4 and OE-13). (a–c) The relative transcript levels of NtSOD, NtCAT, and NtPOD under normal water management or withheld irrigation for 6 d in WT and transgenic tobaccos, respectively. Data represent means and standard errors of three replicates. Different letters above columns indicate (P < .05) significance using Duncan’s multiple range test differences between treatments.](image)
which indicated that \textit{MbWRKY3} may play an important role in active organs. When treated with salt and drought stresses, the transcript levels of \textit{MbWRKY3} in roots were markedly increased by drought and salt stress (Figure 5(b,c)). The expression of \textit{MbWRKY3} was markedly affected by ABA treatment, which increased in the first stage, and then decreased (Figure 5(d)). ABA has been proved to mediate many stress responses through regulating the expression levels of the stress defense genes (Chinnusamy et al. 2004). In this study, the expression analysis showed that \textit{MbWRKY3} substantially induced by the treatment of salt, drought, and ABA, may be involved in the abiotic stress response via the ABA-dependent signaling pathway. These results above suggested that \textit{MbWRKY3} gene had participated in stress responses in \textit{M. baccata}.

The 35S:\textit{MbWRKY3} transgenic tobacco exhibited a markedly increased tolerance to drought. Over-expression of \textit{BlhWRKY1} also enhanced the tolerance to dehydration stress in transgenic tobacco (Wang et al. 2009). The over-expression of stress-responsive WRKY genes TaWRKY2 and TaWRKY19 in \textit{Arabidopsis} have been reported to enhance the tolerance to drought and salt (Niu et al. 2012). The chlorophyll content and RWC were usually used as markers for severity of drought stress (Paknejad et al. 2007; Wang et al. 2009). Proline has been associated with the general stress response (Toka et al. 2010), which may also be cryoprotective, since proline overproducers display an enhanced drought tolerance (Van Rensburg et al. 1993). When dealt with drought treatment, the 35S:\textit{MbWRKY3} transgenic tobaccos had the higher RWC level and contents of proline and chlorophyll than WT. EL reflects membrane injury severity after abiotic stresses (Xing et al. 2011). MDA is the organic compound for lipid peroxidation (Caradonna & Mauro 2016). Under drought stress, the \textit{MbWRKY3}-over-expression transgenic tobaccos had the lower EL levels and generated smaller amounts of MDA and H2O2, and then in WT (Figure 7(d–f)). Drought stress can cause the accumulation of ROS and induce lipid peroxidation, which can damage cytomembrane structure and lead to oxidative stress (Huda et al. 2013). In this study, it was found that \textit{MbWRKY3}-over-expression transgenic plants possess higher activities of ROS-scavenging enzymes (SOD, CAT, and POD) in comparison with WT line under normal water management and drought stress (Figure 7(g–i)). These results suggest that over-expression of \textit{MbWRKY3} in transgenic tobacco results in the higher activities of ROS-scavenging enzymes. Consequently, higher protective enzyme activities lead to the suppression of ROS accumulation in order to suffer less from oxidative damage under drought stress.

The results suggest that over-expression of \textit{MbWRKY3} in transgenic tobaccos stimulates the enhanced expressions of the ROS-scavenging enzyme genes, including \textit{NiSOD}, \textit{NiCAT}, and \textit{NiPOD} (Figure 8(a–c)), and results in the higher activities of ROS-scavenging enzymes. Consequently, higher protective enzyme activities lead to the suppression of ROS accumulation in order to suffer less from oxidative damage under drought stress. These were the reasons why over-expression of \textit{MbWRKY3} in transgenic tobaccos confer enhanced tolerance (Figure 6) and have the higher survival rate to drought stress.

In conclusion, a WKRY transcription factor encoding \textit{MbWRKY3}, which was induced by salt and drought stresses, and ABA treatment, was isolated from \textit{M. baccata}. Over-expression of \textit{MbWRKY3} in tobacco resulted in enhanced tolerance to drought stress. This was partially correlated with the activation of ROS-related antioxidant genes/enzymes, leading to less accumulation of ROS under drought stress. More importantly, over-expression of \textit{MbWRKY3} in tobacco was achieved without affecting their phenotypes under normal conditions. Therefore, \textit{MbWRKY3} provides a potentially excellent genetic resource for improving drought tolerance in plants.

Disclosure statement
No potential conflict of interest was reported by the authors.

Funding
This project was supported by the National Natural Science Foundation of China (31301757), Natural Science Foundation of Heilongjiang Province (C2015015), Academic Backbone Project of Northeast Agricultural University (15XG06) and Postdoctoral Scientific Research Development Fund of Heilongjiang Province, China (LBH-Q16020).

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