A R2R3 MYB transcription factor from ash positively regulates salt response in tobacco

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1. Introduction

High salinity usually causes osmotic stress and ion injury in plant cells, which seriously affects the growth and development of crops and trees (Barnabas et al., 2008). After a long period of biological evolution, plants have developed a variety of mechanisms to adapt to this environmental stress (Jaradat et al., 2013). Many genes have been found to be involved in this regulation process. Transcription factors (TFs) usually regulate a large number of downstream genes that take part in different stress conditions through action on the specific cis-acting elements (Nakashima et al., 2009). MYB proteins comprising the largest transcription factor family in plants are characterized by their highly conserved MYB domains. Based on the numbers of MYB DNA-binding repeats, MYB proteins are divided into at least four different classes in plants, among which MYB proteins with two-repeat forms constitute the largest subfamily (Dubos et al., 2010).

In this family, R2R3 type genes have been characterized to be involved in many developmental and physiological processes in plants, including determination of cell fate, regulation of metabolism, control of growth processes, and response to stresses (Ambawat et al., 2013). For instance, MtMYB2 controls proanthocyanidin (PA) expression patterns in Medicago truncatula, while AtMYB30, AtMYB60, AtMYB96, and AtMYB112 function in responses to various environmental stresses (Cominelli et al., 2005; Raffaele et al., 2008; Li et al., 2009; Jun et al., 2015; Lotkowska et al., 2015). In addition, GmMYBs expression patterns in soybean under pathogen stress have been investigated (Aoyagi et al., 2014). The roles of R2R3-MYB in salt stress have been studied in many plants, including A. thaliana, wheat (Triticum aestivum), sheepgrass (Leymus chinensis), soybean (Glycine max), and Papaver somniferum (Zhang et al., 2012; Cheng et al., 2013; Kim et al., 2013; Su et al., 2014; Kakeshpour et al., 2015; Wang et al., 2015). However, the functions of MYB TFs in resistance in tree species need to be further studied, compared to the model plants, such as A. thaliana, Thellungiella halophila (Wang et al., 2013), and rice (Oryza sativa) (Wang et al., 2015).

Fraxinus velutina (F. velutina), commonly referred to as ash, is famous among tree species for its salt and drought tolerance. It is well suited for afforestation and widely cultivated in landscaping large yards, parks, and roadsides. Here, we isolated the R2R3 MYB gene FvMYB2 and overexpression of FvMYB2 in tobacco increased salinity resistance.

Abstract: The roles of MYB transcription factors are diverse and important in regulating environmental stress in plants. In this study, the R2R3 MYB gene FvMYB2 (GenBank Accession No. KY767843) and its promoter region were cloned from ash (Fraxinus velutina Torr.). Phylogenetic analysis revealed that FvMYB2 was clustered with VvMYB60 from Vitis vinifera. PlantCARE software analysis showed that its promoter contained different cis elements involved in diverse abiotic stresses. The expression patterns of FvMYB2 were investigated under different abiotic stress conditions. FvMYB2 subcellular localization was mainly localized in the nucleus. Overexpression of FvMYB2 in tobacco showed enhanced salt resistance and upregulation of stress- and ABA-related genes after NaCl treatment. These results indicated that FvMYB2 may play a positive role in salt stress regulation mediated by ABA-dependent signaling mechanisms.

Key words: Ash, MYB transcription factor, transcription, salt stress.
2. Materials and methods

2.1. Plant materials and stress treatments

*F. velutina* seeds were grown in sand and then were put into a culture room with constant temperature (25 ± 0.5 °C) and a 12 h light/12 h dark cycle. Healthy seedlings were used for abiotic stress treatments, including 200 mM NaCl, 20% PEG6000, and 100 μM ABA treatments for 0.5 h, 6 h, 12 h, and 24 h, respectively.

2.2. Cloning of *FvMYB2* gene and its promoter

A cDNA (CL7606) sequence showing homology to the R2R3 MYB was obtained from the *F. velutina* transcriptome database (data unpublished). Analysis with the ORF finder showed that the 5’ sequence of the gene was complete, and the gene-specific primers RACE-outer (5’-GCTCTCTGCTGTGATAAAGTCCGTT-3’) and RACE-inner (5’-A CCGAAAAACTCTAATTCGA TATATGCCT-3’) were used for obtaining 3’ end sequence using a 3’-Full RACE kit (Takara Bio, Kusatsu, Japan). The *FvMYB2* full-length cDNA was then amplified with primers *FvMYB2*-S (5’-GACTATAGACACAGAGGAAGGA-3’) and *FvMYB2*-A (5’-TGGTAAAGAGAGACCATGGTCA-3’). The PCR procedure was: Pre-denaturation at 94 °C for 4 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 2 min, and a final extension at 72 °C for 10 min. In addition, the genomic sequence of the *FvMYB2* gene from *F. velutina* was amplified by primers *FvMYB2*-S and *FvMYB2*-A.

Then the upstream region of *FvMYB2* was isolated using genome walking with nested primers of *FvMYB2*-outer (5’-CTAATGCGCAATTTCGCCGAGCATGT TCCTTT-3’) and *FvMYB2*-inner (5’-CTTGCAGGGACTCATG GGTTCCTTTCTTGATAC-3’). The PCR procedure was: Pre-denaturation at 94 °C for 4 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 2 min, and a final extension at 72 °C for 10 min. In addition, the genomic sequence of the *FvMYB2* gene from *F. velutina* was amplified by primers *FvMYB2*-S and *FvMYB2*-A.

2.3. Expression analysis of *FvMYB2* under stresses

*F. velutina* seedlings after NaCl, PEG, and ABA treatments were used for expression analysis. The synthesized cDNA were used as templates for semiquantitative RT-PCR. The *actin* gene amplified by specific primers ActinS (5’-TCCTCCAGCTTCTT-3’) and ActinA (5’-CTCTTCTGCTGCTACTGCA-3’) was used as internal reference. The specific primers RTS (5’-GAGGTGAGAGTAGATT G-3’) and RTA (5’-ACATTATGGCAGGGATT-3’) were used for the amplification of the *FvMYB2* partial fragment. The 20 μL reaction system included TaqMix 10.0 μL, cDNA template 2.0 μL, specific primers RTS and RTA 0.5 μL, and ddH2O 7.0 μL. The PCR program was as the followings: pre-denaturation at 94 °C for 4 min, then 25 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 10 min.

2.4. Subcellular localization of *FvMYB2*

The primer *FvMYB2*-BSK-S (5’-CGCGGATCCAGAG GAGGAAGGAATGG-3’) with a BamHI site and *FvMYB2*-BSK-A (5’-TCTAGAGAATATTGTTGGAAGTTCC-3’) with an XbaI site were used for the amplification of *FvMYB2*. The *FvMYB2* fragment was introduced into the pBSK-3SS-GFP vector. The gene gun method was used to achieve transient expression of *FvMYB2*-GFP fusion protein in onion cells and images were captured with the fluorescence microscope (Nikon, Minato, Japan).

2.5. Vector recombination and tobacco transformation

The 936 bp coding sequence of *FvMYB2* was amplified with the primers Full-S (5’-GCTCTCTAGAA CAGAGGAAAGAATGGGAAG-3’) and Full-A (5’-CGCGGATCCTCAAT GAGCTAGAAATTTGGTTGA-3’) with a BamHI site. The amplified fragment was introduced into the pROKII vector driven by the CaMV35S promoter. The freeze–thaw and leaf disc methods were used as described by Holsters et al. (1978) and Horsch et al. (1985). RT-PCR was used to ensure transgenic integration using the primers of *FvMYB2*-S (5’-GACTATAGACAGAGAGGAAG-3’) and *FvMYB2*-A (5’-TGGTAAAGAGAGACCATG GGTTCCTTTCTTGATAC-3’).

2.6. Salt resistance analysis of transgenic lines

To test the salt resistance of the *FvMYB2* overexpression lines, the seeds from transgenic tobaccos and WT were sown on 1/2 MS medium with NaCl (0, 100, and 200 mM) for 10 days and the germination rate was investigated. The root growth was measured after 7 days of germination.

POD, SOD, and CAT activities and MDA content in *FvMYB2* transgenic lines under NaCl treatments for 2 days were measured as previously described (Miao et al., 2010; Song et al., 2011).

2.7. qPCR for the expression of stress and ABA-related genes

The transcript levels of stress-responsive genes and ABA-related genes in *FvMYB2* transgenic tobaccos and WT under 200 mM NaCl stress were investigated by qPCR according to the procedures detailed in the SYBR Premix Ex TaqII (Takara Bio). The thermal cycling consisted of 95 °C for 20 s, 40 cycles of 95 °C for 5 s, and 60 °C for 20 s. The melting curve analysis temperature was performed from 60 °C to 97 °C. The 2^−ΔΔCT method was used for relative quantitative analysis. *NtActin* was used as the internal reference. Primers used for downstream genes were as follows: *NtActinF*: 5’-CCCT CCCACATGCTATTCTT-3’, *NtActinR*: 5’-AGAGCCTCCAATCCAGACA-3’, *NtRD29AF*: 5’-TGGTTACACAGGCCATA-3’, *NtRD29AR*: 5’-CCCTTCTTTGTTTGTTTT-3’, *NtERD10CF*: 5’-AACGGGAGGCTACAGAATGG-3’, *NtERD10CR*: 5’-GTTCCCT CTTGGGCATTGGTTGA-3’, *NtPP2CF*: 5’-AGCCGATGCACTGCCCATACGA-3’.
3. Results

3.1. Cloning and sequence analysis of FvMYB2 gene

A pair of gene-specific primers was used for 3’ RACE. Finally, a full-length cDNA of FvMYB2 (GenBank Accession No. KY767843) was obtained from F. velutina (Figure 1). FvMYB2 is 1201 bp in length and encodes 311 aa (Figure 2) with a protein mass of 35.0 kDa. Sequence analysis indicated that FvMYB2 protein contains two MYB repeats. The genomic sequence of FvMYB2 was 1476 bp in length, with three exons (135 bp, 128 bp, and 676 bp) and two introns (140 bp and 135 bp) (Figure 3). The first repeat R2 MYB DNA-binding domain was located between aa 13 and 63 and spaced by 19 amino acids. The R3 MYB DNA-binding domain was located between aa 66 and 114. The first tryptophan of R3 repeat could be replaced by a phenylalanine, which is also found in other plants. Thus the conserved tryptophan sites retained in FvMYB2 showed that the FvMYB2 protein is a stereotypical R2R3-MYB protein.

BLAST in NCBI revealed that FvMYB2 showed the highest homology with CsMYB from Cucumis sativus (ABC33923) with 55.0% overall identity and 97.4% identity in conserved domains. FvMYB2 also displayed high identities with other MYB proteins whose functions are unknown. This indicates that FvMYB2 belongs to the R2R3 type subfamily (Figure 4). The similarities of the R2R3 domain between FvMYB2 and MtMYB, CmsMYB, and EsMYB4 were about 97.4%, indicating that this domain is strongly conserved among different species. In addition, FvMYB2 showed the specific PHEEG signature, including two highly conserved Glu residues in the loop, which linked the R2 and R3 domains. However, the identities of the whole protein sequence of FvMYB2 were limited, compared to proteins in Medicago truncatula (XP003624757, 54%), Cucumis melo subsp. (ADN33980, 55%), and Epimedium sagittatum (AFH03056, 48%). Hence, the N-terminal region containing the R2R3 domain showed a higher similarity and the C-terminal region displayed more diversity. In addition, phylogenetic tree analysis revealed that FvMYB2 belonged to subgroup 1 (Figure 5), together with MtMYB, CmsMYB, CsMYB, VvMYB60, EsMYB4, and AtMYB60 (Kranz et al., 1998).

3.2. Regulatory elements in the FvMYB2 gene promoter

Several known stress-associated, cis-acting elements were identified in the upstream sequence of FvMYB2 (Figure 6), including the ARE motif, which has been found to be essential for anaerobic induction (TGGTTT); fungal-responsive element Box-W1 motif; low-temperature–responsive element LTR motif (TTTCG); heat-stress–responsive element HSE motif (CGAATTTTGT); and stress-responsive elements like TC-rich, salt stress motif (GT1GSCAM4) and drought-responsive motif MBS. One 5’UTR Py-rich stretch cis-acting element conferring high transcription levels in plants was also found in the FvMYB2 promoter. In addition, there were several plant-hormone–responsive motifs, such as abscisic acid response elements (ABREs), salicylic acid response elements (TCAs), ethylene-responsive elements (EREs), as well as auxin- and gibberellin-response elements (TGAs and GAREs). Interestingly, three ABRE motifs were found in the FvMYB2 promoter, implying that FvMYB2 might be involved in ABA response.
Figure 2. *FvMYB2* nucleotide sequence. R2 and R3 MYB domains are underlined.

Figure 3. *FvMYB2* gene organization.
Figure 4. FvMYB2 amino acid sequence alignment and the R2R3-MYB sequences of other plants. The line above indicates the R2 and R3 MYB repeats. The conserved motif PHEEG is boxed. Black squares correspond to positions of conserved Trp and Phe residues.
3.3. FvMYB2 transcript level under abiotic stress
To rationalize FvMYB2 gene expression patterns, the potential involvement of FvMYB2 in abiotic stress-induced responses (NaCl, PEG, and ABA) was studied by semiquantitative RT-PCR (Figure 7). Compared to the WT, FvMYB2 gene transcription in leaves increased after ABA stress treatment for 6 h, 12 h, and 24 h, and there were no significant differences in the expression of the three time periods, which indicated that FvMYB2 may be involved in the ABA signaling pathway. After 20% PEG treatment, FvMYB2 expression was increased to a certain extent at both 6 h and 12 h, with the highest expression appearing at 6 h. Under the 200 mM NaCl treatment, FvMYB2 expression all increased with increasing treatment time, and the highest expression appeared at 6 h, while the expression was still significantly higher than the control at 24 h.

3.4. Nuclear localization of FvMYB2
Recombinant construct p35S-FvMYB2-GFP was transformed into onion cells using a gene gun. The subcellular localization of FvMYB2 was investigated with a fluorescence microscope. The 35S-FvMYB2-GFP fusion protein was localized in the nucleus, while the green fluorescence of 35S-GFP as a control was observed in the whole cell (Figure 8).

3.5. FvMYB2 transgenic lines showed improved salt traits
Transgenic T2 tobacco plants were used to examine the function of FvMYB2 in salt stress response (Figure 9a). The germination rates and the root length were measured to evaluate growth under salt stress. The results indicated that there were no obvious differences in germination rates between the transgenic plants and WT under normal conditions. However, in the media with NaCl treatments, the transgenic lines showed significantly higher germination rates than WT. For example, after 100 mM and 200 mM of NaCl treatments, the germination rates of the transgenic lines were 2.3-fold and 3.1-fold higher than those of WT, respectively (Figures 9b and 9c). Furthermore, the root lengths of the transgenic plants increased more remarkably than those of the WT, about 1.8-fold and 2.9-fold, respectively (Figures 9d and 9e). These data demonstrated that the overexpression of FvMYB2 in tobacco enhanced salt resistance during seed germination and growth.

Figure 5. Construction of phylogenetic tree between FvMYB2 and MYB proteins from various plant species. The accession numbers were as below: FvMYB2 from F. velutina; XP 003624757 from Medicago truncatula; ADN33980 from Cucumis melo subsp.; ABC33923 from Cucumis sativus; ACF21938 and ABK59040 from Vitis vinifera; AHF03056 from Epimedium sagittatum; NP201053, NP190344, NP177603, NP189533, NP172358, NP172108, NP180676, NP188966, and NP172425 from Arabidopsis thaliana; and AEV91147 from Triticum aestivum. Subgroup 1 are clustered by Kranz et al. (1998).
Figure 6. Cis elements in the FvMYB2 gene promoter.
Figure 7. Expression analysis of FvMYB2 response to abiotic stress.

Figure 8. Nuclear localization of the FvMYB2-GFP fusion protein.
Figure 9. NaCl hypersensitivity measurement of FvMYB2 transgenic lines. (a) RT-PCR analysis of transgenic tobaccos. (b) Seed germination of FvMYB2 overexpression and WT seeds on 1/2 MS medium containing NaCl. (c) Calculation of the germination rates of transgenic and WT seeds. (d) Seedling development and (e) root length in transgenic lines and WT. (f) Effects of salt stress on SOD, POD, and CAT activities and MDA content of transgenic lines and WT.
POD, SOD, and CAT activities and MDA content, which have been widely used as important indicators for the response to salt stress in plants, were analyzed to determine the effects of FvMYB2 in salt resistance. The results showed that the activities of these enzymes in transgenic lines were significantly higher than in WT after salt stress (Figure 9f). For example, SOD, POD, and CAT activities in transgenic lines were 1.70, 2.46, and 2.05 times those of WT, respectively. In addition, MDA content was 52.6% lower than the control after salt stress. These results indicate that FvMYB2 can enhance antioxidant enzyme activities and reduce MDA content.

3.6. Expression analysis of ABA-related and stress-responsive genes under salt stress
To further investigate the function of FvMYB2 in salt stress resistance in plants, the expression of stress- and ABA-related genes was detected with qPCR in WT and transgenic lines after 200 mM NaCl treatment. The genes NtRD29A and NtERD10C are involved in stress defense, and NtNCED, NtAREB, NtPP2C, and NtSnRK2.1 function in ABA biosynthesis and signaling. The results showed that mRNA levels of most genes, except NtPP2C, were upregulated in plants after exposure to salt stress with higher expression levels in transgenic lines than in WT (Figure 10). Therefore, the increased salt tolerance in FvMYB2-overexpressing plants might be related to the increased expression of some stress-responsive and ABA-related genes.

4. Discussion
Two-repeat (R2R3) MYB proteins comprise the biggest subgroup of the MYB transcription factor family in plants, with more than 100 R2R3-MYB genes in A. thaliana (Kranz et al., 1998). Although TFs are a hot research issue in plants, the function of MYB in tree species has been less investigated (Preston et al., 2004). Recently, some MYB families have been determined to play important roles in wood formation and development in some tree species. For instance, AaMYB2 is involved in anthocyanin biosynthesis in anthurium spathes and leaves, while PtMYB4 from Pinus taeda was reported to regulate phenolic acid biosynthesis (Hao et al., 2016; Li et al., 2016). In addition, an R2R3 MYB TF MYB134 was found to be coinduced with PA biosynthetic genes in poplar after several kinds of stress treatments (Mellway et al., 2009). Some papers reported that MYB TFs in A. thaliana are involved in response to salt, drought, or cold conditions (Xu et al., 2015; Yu et al., 2016). However, knowledge of the function of the MYB genes involved in environmental stress resistance in tree species is still limited.

Here, we cloned and characterized the MYB gene FvMYB2 in F. velutina. FvMYB2 contains two imperfect repeats of 53 aa in the N-terminal with high similarity with other plant MYB proteins, suggesting that FvMYB2 belongs to the R2R3-MYB subfamily. Comparing to other available MYB proteins, FvMYB2 showed the highest similarity to CsMYB, a Myb-like cucumber protein that was found to play roles in activating chrC promoter in Cucumis sativus (Tzfira et al., 2005). Further phylogenetic analysis revealed that FvMYB2 is located in subgroup 1, including VvMYB60 of Vitis vinifera and AtMYB60 of Arabidopsis, which are involved in the guard-cell–related drought resistance and in environmental stress in plants (Li et al., 2009; Galbiati et al., 2011). These results indicate that FvMYB2 from F. velutina may play important roles in salt- and drought-related stress conditions.

TF gene expression patterns are different in space and time. Studies have shown that most of the MYB genes can be expressed in the various organs of plants (vegetative and reproductive organs), and they belong to the constitutive expression. For example, in wheat, the tissue expression patterns of MYB genes in root, stem,
leaf, pistil, and stamen were studied by semiquantitative RT-PCR. The results indicated there are nearly 60 kinds of tissue-specificity expression patterns in the MYB genes of wheat, and half of the expressions showed high levels in all the test tissues (Zhang et al., 2012). Our previous study has shown via semiquantitative RT-PCR that FvMYB2 was expressed in all the test organizations (root, cotyledon, stem, and leaf), and the expression levels of FvMYB2 in these tissues showed no obvious differences (Li et al., 2013). The constitutive expression of these MYB genes suggested that they play important roles in all aspects of the physiological and developmental process in plants. In addition, the expression of MYB genes has tissue specificity, such as MdMYBA in apple, PtoMYB216 in poplar, and AtMYB93, which is only expressed in the endodermis (Ban et al., 2007; Tian et al., 2013; Gibbs and Coates, 2014). Gene specificity expression has important physiological significance because a number of important genes in selective expression in a particular organization usually involve some special physiological and developmental process of plants. Meanwhile, there is time-specificity in TF gene expression patterns. For example, StMYB1R-1 expression can respond to NaCl only after 2 h, while AmMYB1 expression still increased after salt stress for 10 days (Shin et al., 2011; Ganesan et al., 2012). Here, FvMYB2 transcript levels were increased in the transgenic tobacco leaf within several hours of NaCl stress, which was consistent with the previous reports that the reaction of various plant TFs to stress are typically quite rapid, implying the essential and rapid regulation abilities of MYB TFs when encountering environmental stresses (Chen et al., 2005).

In order to adapt to salt stress, plants have developed their own adaptation mechanisms, including salt ion damage control and osmotic stress regulation (Knight et al., 1997). During this progress, many MYB TFs play roles through ABA-dependent and ABA-independent pathways (Yang et al., 2011). In our previous study, the MYB-related gene FvMYB1 was isolated and its expression was upregulated by salt stress but not by ABA (Li et al., 2016). Here, our results showed that the expression of FvMYB2 was not only induced by salt and drought stress, but also increased after the exogenous application of ABA. The different response patterns of FvMYB1 and FvMYB2 to ABA imply that they regulate salt stress by different signaling pathways. Most MYB TF promoters that play roles in the ABA-dependent pathway usually contain ABREs, and the number of the regulation motifs usually affects the expression level of the target gene (Agarwal and Jha, 2010; Qin et al., 2012). For example, two ABREs functioned more strongly than one ABRE coupled with a DRE in RD29A expression in embryos, and one ABRE motif was not sufficient to respond to stress in Arabidopsis (Uno et al., 2000; Nakashima et al., 2006). The cis-acting elements analysis of FvMYB1 and FvMYB2 promoters found that there were three ABREs in the FvMYB2 promoter, while only one ABRE in the FvMYB1 promoter. The results indicate that the different expression patterns of FvMYB2 and FvMYB1 to ABA are due to the differences in their promoters. It is well known that regulation of osmotic stress through stomatal closure under salt stress environment is one of the main events at the cell level in plants, and ABA has been identified to play important roles in stomatal aperture control (Campos et al., 2016).

In this study, the increased FvMYB2 expression after ABA treatment implies that the ABA signaling pathway may play a vital role in FvMYB2-regulated salinity stress in F. velutina. It is similar to other MYB genes that function in stress response with the ABA-dependent pathway, but different from the MYB-related gene FvMYB1 that plays a role in the ABA-independent way. In plants, AtMYB44, OsMYB48-1, OsMYB2, and TaMYB73 expressions all were reported to be induced by NaCl and ABA, and ABA signaling was regarded as important in regulating plant stress resistance (He et al., 2012; Yang et al., 2012; Jaradat et al., 2013; Xiong et al., 2014).

Growing studies have indicated that MYB TFs can respond to salt stress, such as TaMYB2A, TaMYB73, and SbMYB15, and that the overexpression of these genes enhances NaCl stress tolerance (Mao et al., 2011; He et al., 2012; Shukla et al., 2015). In this study, the overexpression of FvMYB2 in tobacco promoted salt tolerance. MYB TFs that function in improving stress abilities often regulate a number of stress-related genes to affect the physiological and metabolic activities of transgenic plants (Meng et al., 2014). In our previous study, overexpression of FvMYB1 mainly induced the upregulation of some stress-related genes, such as SOD, PCS, and DREB (Li et al., 2016). Among them, most DREB genes have been identified to play roles in an ABA-independent way under stress (Chen et al., 2008), and so we suspected that FvMYB1 enhanced salt stress tolerance mainly through the ABA-independent way. Here, ectopic expression of FvMYB2 increased the expression of stress-related genes NtERD10C and NtRD29A, which or whose homologues have been reported to play important roles in preventing water loss in cells; overexpression of these genes conferred stress tolerance (Huo et al., 2016). In addition, multiple genes that have been reported to be involved in the process of ABA signal transduction were also up- or downregulated. For example, overexpression of NCED and AReB could improve drought and salt stress tolerance in transgenic plants (Xian et al., 2014; Yoshida et al., 2015). The overexpression of FvMYB2 increases the transcripts of
the ABA synthesis gene *NtNCED* and ABA signaling transduction gene *NtAREB* in tobacco under salt stress, indicating that *FvMYB2* may be a positive regulator of ABA signaling and confers salt stress tolerance at least partially by ABA signal pathway. Previous reports indicated that SnRKs were central switches in abiotic stress signaling in plants, while PP2C-family genes were usually regarded as negative regulators of ABA signaling (Dong et al., 2012; Fuchs et al., 2013). Given the upregulation of *NtSnRK2.1* and downregulation of *NtPP2C* in transgenic plants, we inferred that the salt tolerance of *FvMYB2* seems to be achieved partially by affecting the ABA signaling pathway.

Taken together, the MYB TF *FvMYB2* regulated some stress-related genes in plants under NaCl treatment, and *FvMYB2* overexpression lines showed strengthened salt tolerance by enhancing the capacity of antioxidant enzyme in the ABA-dependent way. *FvMYB2* characterization provides useful information for understanding the function of MYB genes in salinity tolerance.

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**References**


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