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Research article

A R2R3-MYB transcription factor gene, *FtMYB13*, from Tartary buckwheat improves salt/drought tolerance in Arabidopsis

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ABSTRACT

Abiotic stress causes various negative impacts on plants, such as water loss, reactive oxygen species (ROS) accumulation and decreased photosynthesis. R2R3-MYB transcription factors (TFs) play crucial roles in the response of plants to abiotic stress. However, their functions in Tartary buckwheat, a strongly abiotic and resistant coarse cereal, haven't been fully investigated. In this paper, we report that a R2R3-MYB from Tartary buckwheat, FtMYB13, is not an activator of transcriptional activity but is located in the nucleus. Moreover, compared to the wild type (WT), transgenic Arabidopsis overexpressing *FtMYB13* had a lower sensitivity to ABA and caused improved drought/salt tolerance, which was attributed to the higher proline content, greater photosynthetic efficiency, higher transcript abundance of some stress-related genes and the smaller amount of reactive oxygen species (ROS) and malondialdehyde (MDA) in the transgenic lines compared to WT. Consequently, our work indicates that *FtMYB13* is involved in mediating plant responses to ABA, as well as salt and drought.

1. Introduction

High salinity and drought are two harsh environmental problems affecting crop production and worldwide plant growth (Agarwal et al., 2012; Flowers, 2004). To resist extreme and complicated environments, plants have evolved numerous mechanisms to adapt to these environmental problems. Various genes encode structural and functional protectants, including stress-tolerance-conferring proteins, antioxidants and osmolytes, are involved in the processes of plant resistance to abiotic stresses (Wang et al., 2011). Many proteins and genes in the above metabolic networks are regulated by multiple TF families, for instance, the NAC, WRKY, bHLH, and MYB families. Among these TFs, the MYB family has been proven to be indispensable for responding to environmental stresses (Søren et al., 2013).

MYB is the largest family of TFs in plants and has been subdivided into four subfamilies according to the number and position of repeats: 4R-MYB, R1R2R3-MYB, 1R-MYB, and R2R3-MYB (Dubos et al., 2010). The R2R3-MYB TFs have been studied in many plants. More than 100 R2R3-MYB TFs have been identified in Arabidopsis, and many of them have been demonstrated to participate in the transcriptional regulation of numerous vital activities related to growth and development, along with responses to various stresses. For instance, in *Arabidopsis thaliana* (At), *AtMYB20* enhances salt stress tolerance by downregulating the expression of PP2Cs (Cui et al., 2013). *AtMYB52* conferred ABA-hypersensitivity during seedling growth and enhanced drought tolerance of seedlings (Min et al., 2011); *AtMYB15* enhanced salt/drought tolerance in Arabidopsis (Ding et al., 2009). In wheat, overexpression of *TaMYBsm1*, *TaMYB19* and *TaMYB30*-B have been shown to improve the drought tolerance in Arabidopsis (Li et al., 2016; Zhang et al., 2012, 2014). In rice, *OsMYB2P-1*, *OsMYBS3*, *OsMYB3R-2*, *OsMYB4* and *OsMYB2* were involved in various stress responses (Baldoni et al., 2015). Although many R2R3-MYB TFs have been identified in model plants and crops, the function of many R2R3-MYBs is still unknown in other plants.

Tartary buckwheat (*Fagopyrum tataricum*), which is an important coarse cereal, grows in drought, cold, and strong ultraviolet conditions in mountainous areas (Wang and Claytong, 2007; Zhou et al., 2015). Genomic data indicates that its' high tolerance to living in adverse environments is attributed to the expression of multiple gene families involved in gene regulation, signal transduction, and membrane transport (Zhang et al., 2017). For instance, transgenic Arabidopsis overexpressing *FtMYB12* enhances cold tolerance (Zhou et al., 2015). Eight stress-related MYB genes were identified in previous research (Gao et al., 2016b). Further researches have shown that *FtMYB10* and *FtMYB9* act as negative and positive regulators of salt/drought response in transgenic Arabidopsis, respectively (Gao et al., 2016a, 2017).

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Table 1
The qPCR primers used in this study.

Gene	Forward primer (5′→3′)	Reverse primer (5′→3′)
FtMYB13	CCGCTGGCAGAGGTGGCAACTA	TCTTTCCTTATCATCTCCTTCATAGCCCC
DREB2A	GGTAAAGGAGGACCAGAGAATAGCC	AGACGAGCCAAAGGACCATACATAG
RD17	CGTGGATTGTTTGATTTCTTGGG	CTTGTTCTCCTCATCTTCCTCGGTC
RD22	CACTCCCATTCCCAACTCTCTCCAT	CTTTTCCGCTGCCAACGCTCAC
RD29A	GTCTGCCGTGACGACGAAGTTAC	TCCTTCTTCTCTTCTTCTCCTCCAA
RD29B	TCCGACAAGAGGTGATGTGAAAGTAG	ACTGCTCTGTGTAGGTGCTTGGTTT
CBF1	TGTCTCAACTTCGCTGACTCGGC	ACCTTCGCTCTGTTCCGGTGTATAA
CBF2	GGTTTCCTCAGGCGGTGATTACAGT	TCAGCGGTTTGGAAAGTCCCGAGCC
CBF3	GGAATCAACTTGCGCTAAGGACATCCAA	CCAACAAACTCGGCATCTCAAACATC
COR15A	GGTAAAGCAGGAGAGGCTAAGGATG	AAGAATGTGACGGTGACTGTGGATA
P5CS1	TCTTATGGCTTACTATGAGACTATGTTTGAC	TATGGGGCTCTTCGGGTGCTAATAG
ERD10	ACCAGGGGGTGATGATGTACCAGTC	AATCGGTGTTGCTGTTTCAACCAGC
KIN1	GGACCAACAAGAATGCCTTCCAAGC	CGCTGCCGCATCCGATACACT
Actin2	CTGGAATGGTGAAGGCTGGTT	CGATTGGATACTTCAGAGTGAGGAT
ABA1	GATTCTGGAGATAAGGTTACTGTGG	CCGTGTAACAAGTGTAGCCTGAAT
ABA2	GCTTGTCTGTGTCAATAGTGAGGCA	GATCAGAGGAAAAAGTTGGAGAAAG
ABI1	AGAAACCGATGCTCTGCGATGGTGATAC	GTTAGCGACGAAGATGTGAGACGGG
HAB1	TAAGGTTCTTGAGGCTGTTGCGTCT	GCATATTCATCCTCTCTATCTGGTTTG
HAB2	TGGTGAGAATAAACGGCTGGTG	TTCCAAACCTCAACTGGAGCAA
PP2CA	TCTGAGAATCATCATTTCTACGGTGT	CTCGTTGGCTAACTTCTTTATCCATT
H3	AATTCGCAAGTACCAGAAG	CCAACAAGGTATGCCTCAGC

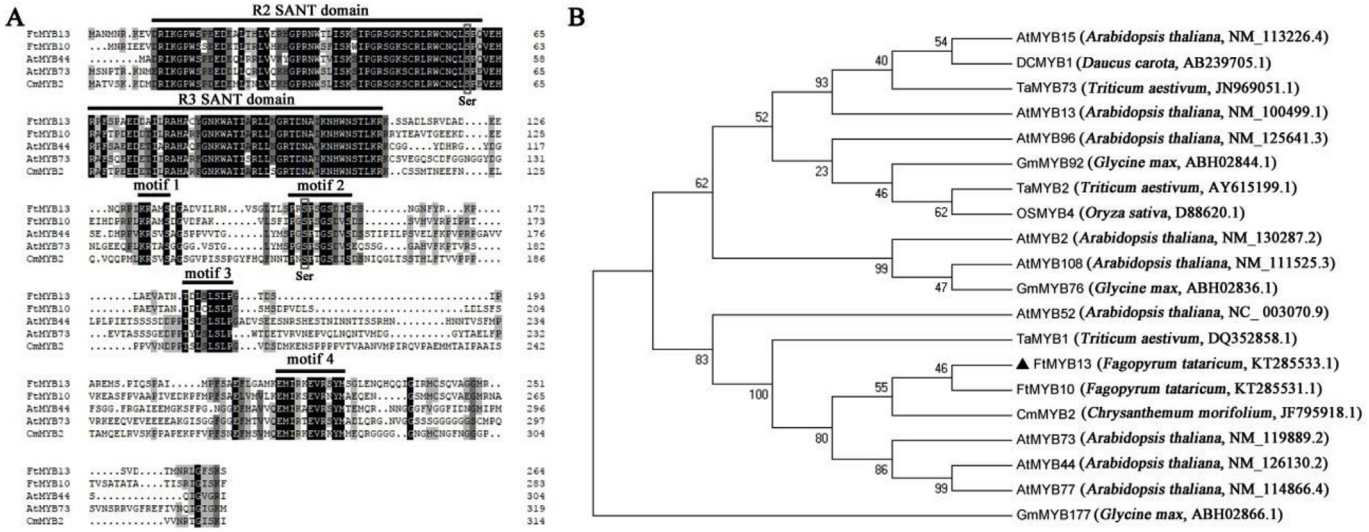

Fig. 1. Molecular identification of MYB13. A. Sequence alignment of FtMYB13. The R2 and R3 SANT domains and four specific motifs are indicated by underlining. Potential serine phosphorylation sites have been marked with a box. B. The phylogenetic tree of FtMYB13. The GenBank accession numbers are shown to the right of the protein names. FtMYB13 is marked with a dark triangle.

Although a few MYB proteins have been reported to play a crucial role in various stress responses in Tartary buckwheat, genomic data shows that Tartary buckwheat has more than 150 MYB TFs (Zhang et al., 2017). Therefore, it is urgent to examine further the role of other MYB TFs involved in environmental stress responses of Tartary buckwheat to clarify the regulation mechanisms of this highly stress-tolerant plant.

In this research, we are committed to further studying the role of *FtMYB13* in stress response. Our work showed that ectopic expression of *FtMYB13* could improve drought/salt tolerance by upregulating the transcription of several stress-related genes and activating the partial antioxidant system in Arabidopsis.

2. Materials and methods

2.1. Plant materials and treatment

Seeds of Tartary buckwheat ("Xiqiao NO.2") were germinated with 16 h light/8 h dark. The 14-day-old Tartary buckwheat sprouts were then transferred into new 1/2 Hoagland's solution supplied with 30% PEG 6000, 150 mM NaCl, 100 μM ABA. The samples were immediately stored at −80 °C for RNA isolated. Each sample contained eight seedlings, and three replicates were measured for each sample. *Arabidopsis thaliana* ecotype Col-0 was used as subsequent experiments.

2.2. Subcellular localization of FtMYB13

To investigate its exact sub-cellular localization, the ORF of *FtMYB13*, lacking its stop codon, was PCR-amplified using the following primers: 5′-GGGGTACCATGGCGAACATGAACAGGAA-3′ (*Kpn*I site underlined) and 5′-CTGGTACCATCACTCTTGCTAAATCCTA-3′ (*Kpn*I site underlined). Then, the ORF was inserted into the HBT95-GFP vector (Yao et al., 2017). Plasmids with *FtMYB13*-GFP, *NLS*-RFP and HBT95-GFP were introduced through polyethylene glycol-mediated transference into the Arabidopsis protoplasts, and cells were cultured at 24 °C for 24 h in the dark. They were photographed under a laser confocal scanning microscope.