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Overexpression of a tartary buckwheat R2R3-MYB transcription factor gene, *FtMYB9*, enhances tolerance to drought and salt stresses in transgenic Arabidopsis

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ABSTRACT

Tartary buckwheat (*Fagopyrum tataricum*) is a traditional coarse cereal that exhibits strong plasticity in its adaptation to harsh and complicated environmental stresses. In an attempt to study the strong tolerance of tartary buckwheat, the *FtMYB9* gene, which encodes an R2R3-MYB transcription factor protein, was functionally investigated. *FtMYB9* expression was rapidly and strongly induced by ABA, cold, salt, and drought treatments in the seedling stage. A yeast one-hybrid system assay indicated that FtMYB9 is an activator of transcriptional activity, consistent with its roles as a transcription factor. Its overexpression in plants resulted in increased sensitivity to ABA at the germination and seedling stages compared to wild type. The overexpression of *FtMYB9* increased tolerance to drought and salt stresses by the activation of some stress-related genes from both ABA-independent and ABA-dependent pathways in transgenic Arabidopsis. Furthermore, enhanced proline content and the activation of the *P5CS1* gene implied that *FtMYB9* may be involved in proline synthesis in plants. Collectively, these results suggest that FtMYB9 functions as a novel R2R3-MYB TF which plays positive roles in salt and drought tolerance by regulating different stress-responsive signaling pathways.

1. Introduction

Environmental stresses, including high salinity, drought, and extreme temperatures, affect the development and growth of crop plants, which results in the reduction of food supplies (Abe et al., 2003; Yu et al., 2015a). To address these stresses, plants have evolved complex mechanisms at the morphological, physiological, metabolic, and molecular levels to build stronger tolerance or resistance (Zhu, 2002). At early stages of the responses to complicated environmental stresses, many transcription factor genes are activated in plant cells via signal perception and subsequent signal transduction (Tran et al., 2007). Transcription factor (TF) families, including NAC, MYC, MYB, WRKY, DREB/CBF, and ERF, have been found to act as nodes in regulation of complicated signal transduction networks, resulting in the accumulation of a variety of stress-related proteins that have been found to play key roles in the adaptation to abiotic stress in plants (Ma et al., 2009). Therefore, increases in stress tolerance could be potentially achieved by overexpression of these transcription factors in plants (Yuan et al., 2015).

MYB is the largest transcription factor family in plants (Dubos et al., 2010a,b), in which all members share a MYB domain. MYB proteins can be divided into four subfamilies, 1R-MYB, R2R3-MYB, R1R2R3-MYB, and 4R-MYB, depending on the numbers of imperfect repeats in the MYB domain (Zhang et al., 2011). Members of the R2R3-MYB subfamily play important roles in diverse processes, including secondary metabolism, hormonal signaling, cell cycle control, developmental control, and response to environmental stresses in plants (Dubos et al., 2010a,b; Liao et al., 2008; Stracke et al., 2001). In Arabidopsis, *AtMYB2*, *AtMYB14*, *AtMYB15*, *AtMYB96*, *AtMYB70*, *AtMYB44*, *AtMYB77*, and *AtMYB73* have been widely reported to play central roles in cold, salt, and/or drought responses (Abe et al., 2003; Baldoni et al., 2015; Chen et al., 2013; Jung et al., 2008; Katiyar et al., 2012; Kim et al., 2013; Mengiste et al., 2003). Similarly, in rice, *OsMYB2*, *OsMYB4*, *OsMYB3R-2, OsMYBS3,* and *OsMYB2P-1* have also been found to be involved in the stress response process (Dai et al., 2012; Ma et al., 2009; Su et al., 2010; Vannini et al., 2004; Yang et al., 2012). Although

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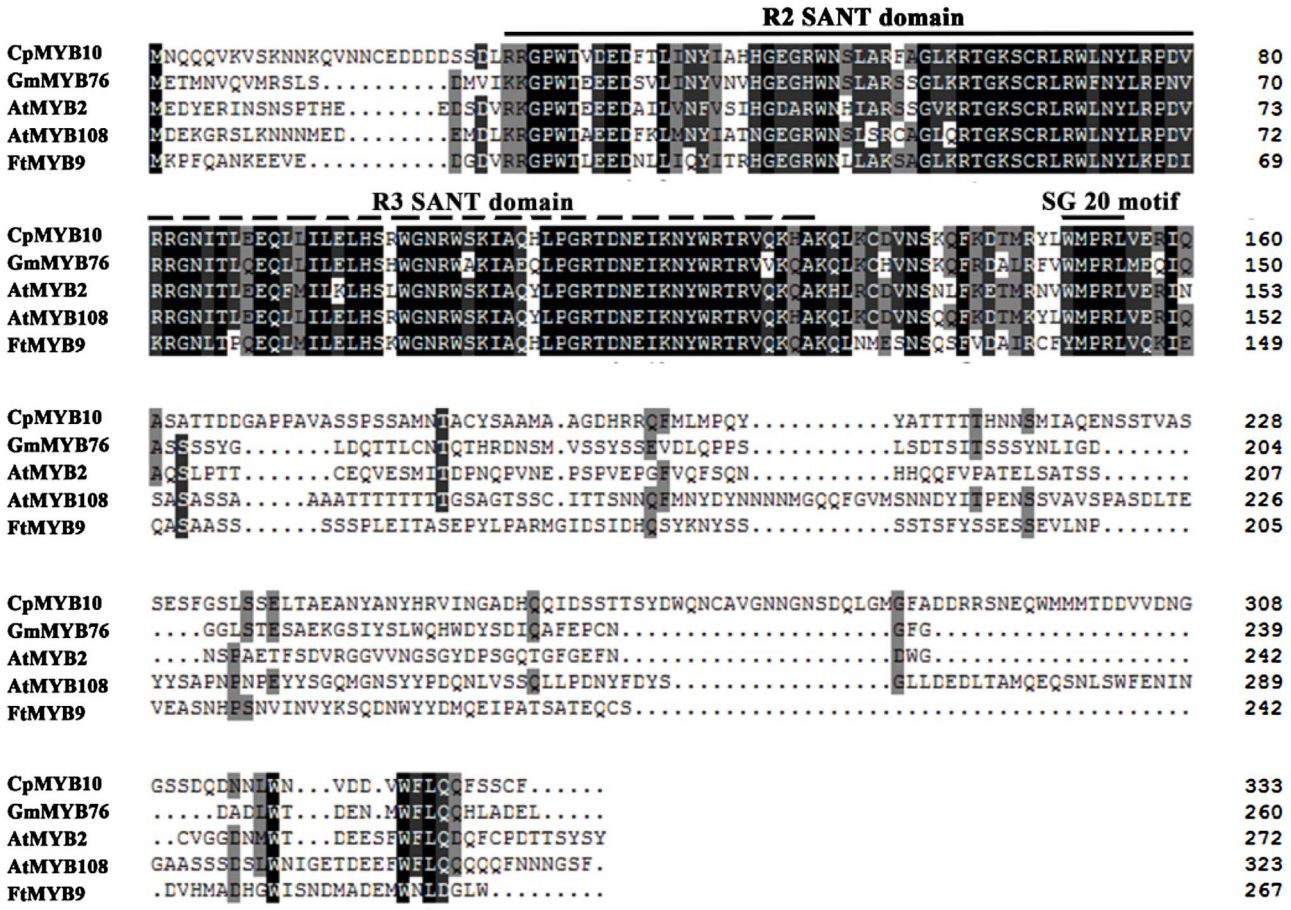

Fig. 1. Alignment of FtMYB9 with other MYB proteins. Identical amino acids are shaded in black, and similar amino acids are shaded in gray. Cp: *Craterostigmaplantagineum*, Gm: *Glycine max*, At: *Arabidopsis thaliana*. The GenBank accession numbers: CpMYB10: AAM43912.1, GmMYB76: ABH02836.1, AtMYB2: NM_130287.2, AtMYB108: NM_111525.3.

a number of efforts have been made in the investigation of the functions of different R2R3-MYB TFs in abiotic stress responses, the specific functions of each R2R3-MYB TF are still largely unknown. Thus, further attempts at the isolation and functional characterization of R2R3-MYB TF genes are needed.

Tartary buckwheat (*Fagopyrum tataricum*) is a traditional coarse cereal in the *Polygonaceae* family that contains high flavonoid content and other nutrients. It is widely planted in mountainous areas in Asian countries, such as China, Japan, Korea, Nepal, and India (Park et al., 2011). After a long period of evolution, tartary buckwheat shows strong resistance to complex and harsh environments, such as cold, strong ultraviolet radiation, and drought conditions in high altitude mountainous areas (Wang and Campbell, 2007; Zhou et al., 2015). However, only a handful of tartary buckwheat R2R3-MYB genes involved in stress responses have been previously reported (Gao et al., 2016a,b; Zhou et al., 2015). To determine the putative functions of MYB family genes in abiotic stress, eight stress-related R2R3-MYB genes were isolated from tartary buckwheat in a previous study (Gao et al., 2016b). Of them, *FtMYB9* expression was substantially induced by salt, drought, and ABA treatments compared with the other seven genes. Thus, in this study, we sought to further investigate the potential function of FtMYB9 in plant stress tolerance. We found that overexpression of *FtMYB9* in Arabidopsis could increase both salt and drought tolerance by promoting the expression of several genes belonging to various stress-inducible pathways, indicating that *FtMYB9* is an ideal candidate gene for genetic breeding of stress-tolerant crops.

2. Materials and methods

2.1. Plant materials and treatment

Seeds of tartary buckwheat cultivar Xi-Qiao 2 were germinated at 28 °C for 24 h and grown in 1/2 Hoagland's solution in a growth chamber at 25 °C with a 12-h photoperiod for 12 days. The 12-day-old tartary buckwheat seedlings were then treated in 1/2 Hoagland's solution with 100 μM ABA, 200 mM NaCl, and 20% PEG 6000, respectively. The 12-day-old seedlings were transferred to a growth chamber at 4 °C for the cold treatment. For all the treatments, seedlings were collected at 0, 1, 3, 6, 12, and 24 h (0 h was a non-treated control). After preparation, all samples were quickly frozen in liquid nitrogen and then stored at −80 °C for RNA isolation.

2.2. Transcriptional activity assay of the FtMYB9 protein

The ORF of *FtMYB9* was inserted into the *Eco*RI and *Bam*HI restriction sites of the pBridge vector, which contains the GAL4 DNA-binding domain, to obtain pBridge-*FtMYB9*. The pBridge-*FtMYB9* vector, pGAL4 vector (positive control), and pBridge vector (negative control) were introduced into the yeast strain AH109, respectively, with the *His3* and *LacZ* reporter genes. Transformed yeast strains were cultured in SD/-His-Trp medium. The colony-lift filter *b*-galactosidase assay was conducted following the Yeast Protocol Handbook (Clontech Laboratories, Inc.).

2.3. Arabidopsis transformation

To generate *FtMYB9*-overexpressing plants, the ORF of *FtMYB9* was