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Overexpression of a wheat MYB transcription factor gene, *TaMYB56-B*, enhances tolerances to freezing and salt stresses in transgenic Arabidopsis

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ABSTRACT

The MYB proteins play central roles in the stress response in plants. Our previous works identified a cold stress-related gene, *TaMYB56*, which encodes a MYB protein in wheat. In this study, we isolated the sequences of *TaMYB56* genes, and mapped them to the wheat chromosomes 3B and 3D. The expression levels of *TaMYB56-B* and *TaMYB56-D* were strongly induced by cold stress, but slightly induced by salt stress in wheat. The detailed characterization of the Arabidopsis transgenic plants that overexpress *TaMYB56-B* revealed that TaMYB56-B is possibly involved in the responses of plant to freezing and salt stresses. The expression of some cold stress-responsive genes, such as *DREB1A*/*CBF3* and *COR15a*, were found to be elevated in the *TaMYB56-B*-overexpressing Arabidopsis plants compared to wild-type. These results indicate that TaMYB56-B may act as a regulator in plant stress response.

1. Introduction

Plant growth and agricultural production are greatly constrained in response to environmental stresses, such as low temperatures and high salt levels. To survive and adapt in such adverse conditions, plants have evolved appropriate regulatory mechanisms that act at the cellular, molecular, physiological and biochemical levels, in these processes, a variety of stress-inducible genes play important roles (Ahuja et al., 2010; Hirayama and Shinozaki, 2010). The products of stress-inducible genes can be classified as either functional or regulatory proteins (Kreps et al., 2002; Seki et al., 2002). Regulatory proteins act as early responders to environmental signals, and their functions can be gradually amplified through signal transduction cascades. Therefore, regulatory proteins have become the important topic in the study of responses and tolerances of plants to abiotic stresses.

Transcriptional factors (TFs) are important regulatory proteins that are able to regulate the expression of target genes by specifically binding to the *cis*-acting elements of interactional genes. Based on differences in the DNA-binding domains and the overall structures, the TFs can be classified into different families. In plants, the MYB TFs compose one of the largest of these families (Pabo and Sauer, 1992; Riechmann et al., 2000). The members of the MYB family are characterized by the possession of a MYB domain that is located near the amino terminus and is composed of different numbers of imperfect tandem repeats (MYB repeats). The MYB gene family is divided into different types according to the number of repeat(s) in the MYB domain: 4R-MYB has four repeats, 3R-MYB (R1R2R3-MYB) has three repeats, R2R3-MYB has two repeats and the MYB-related type usually but not always has a single repeat (Dubos et al., 2010; Jin and Martin, 1999; Rosinski and Atchley, 1998). The MYB repeat consists of a region of 50 to 53 amino acids and contains three regularly distributed tryptophan (or phenylalanine) residues. Typically, each repeat is capable of forming three α-helices; the two that are located at the C terminus adopt a variation of the helix-turn-helix (HLH) conformation that recognizes and binds to the DNA major groove at the specific recognition site. The three tryptophan (or phenylalanine) residues together form a hydrophobic core that participates in protein–DNA interactions (Ogata, 1998; Ogata and Nishimura, 1995). In plants, the MYB transcription factors are involved in the control of several biological functions, including the regulation of primary and secondary metabolisms, the control of cell development and the cell cycle, participation in the biotic and abiotic stress responses and hormone synthesis and signal transduction (Du et al., 2009; Dubos et al., 2010; Feller et al., 2011; Stracke et al., 2001).

Increasing evidence have indicated that numerous plant MYB genes are involved in responses to diverse abiotic stresses. AtMYB2 activates the expression of the ABA-inducible gene under drought stress conditions (Abe et al., 2003; Urao et al., 1996). The overexpression of *AtMYB15* in Arabidopsis results in an enhanced sensitivity to abscisic acid (ABA) and improved drought tolerance (Ding et al., 2009). AtMYB44/AtMYBR1 regulates ABA-mediated stomatal closure in response to abiotic stresses

Abbreviations: ABA, abscisic acid; CS, Chinese Spring; NT, nulli-tetrasomic; DREB, dehydration-responsive element-binding; GFP, green fluorescent protein; OP, osmotic potentials; WT, wild type.

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(Jung et al., 2008), and AtMYB60 regulates stomatal and root growth under drought stress (Oh et al., 2011). AtMYB62 is involved in the response to phosphate starvation (Devaiah et al., 2009), and AtMYB96 mediates ABA signaling during pathogen resistance and the drought stress response (Seo et al., 2009, 2011; Seo and Park, 2010). In rice, OsMYB4 enhances the cold and freezing tolerances of transgenic plants (Laura et al., 2010; Park et al., 2010; Pasquali et al., 2008; Vannini et al., 2004). The overexpression of *OsMYB3R-2* improves cold stress tolerance in rice and confers resistance to multiple abiotic stresses in Arabidopsis (Dai et al., 2007; Ma et al., 2009). In wheat, TaMYB1 is involved in responses to abiotic and ABA stresses (Lee et al., 2007). TaMYB2A confers enhanced tolerances to multiple abiotic stresses in transgenic Arabidopsis (Mao et al., 2011). *TaPIMP1* encodes a R2R3 MYB protein that enhances resistances to biotic and abiotic stresses in transgenic tobacco (Liu et al., 2011).

From a project of large-scale sequencing and functional study of wheat full-length cDNAs, a cold stress-related gene, named *TaMYB56*, was identified (Zhang et al., 2012). To investigate the roles of *TaMYB56* in the plant stress response, we functionally characterized this gene. Herein, we demonstrate that the expression of *TaMYB56* is strongly induced by cold stress and slightly induced by salt stress in wheat. The detailed characterization of *TaMYB56-B* overexpression in Arabidopsis suggested that it is involved in the responses of plants to freezing and salt stresses. We also performed a comparison of the expression profiles of eight cold stress-responsive genes in the wild-type (WT) and *TaMYB56-B*-overexpressing Arabidopsis plants under normal conditions and following exposure to freezing stress; from this comparison, we identified two *TaMYB56-B* activated genes.

2. Materials and methods

2.1. Plant materials

Wild and cultivated wheat lines of different ploidy levels were used to amplify the genomic and cDNA sequences of *TaMYB56*. The *Triticum urartu* accession UR206 (original code No.1010015) was generously provided by Mr. Reader from the John Innes Centre, Norwich, UK. The *Aegilops tauschii* accession Y2282 (original code AL78/8) was kindly provided by Dr. Mingcheng Luo, UC Davis. The *Aegilops speltoides* accession Y2006 and Chinese Spring (CS) were from our laboratory.

The CS nulli-tetrasomic (NT) lines were used for the chromosomal locations and the wheat cv. Chinese Spring (CS) was used for the low temperature treatments. Wheat cv. Chadianhong (salt resistance) was used for the salt stress treatment. The *Arabidopsis* (*Arabidopsis thaliana*) Columbia-0 was used for overexpressing of *TaMYB56-B*.

2.2. Cold and salt treatment of wheat

The wheat seedlings were grown in Hogland liquid culture at 22 °C under a 16 h light/8 h dark photoperiod. Ten-day-old seedlings were transferred from 22 °C to 4 °C for the cold treatment, and samples were harvested at 0 h, 1 h, 3 h, 6 h, 12 h, 24 h, 48 h and 72 h. For the salt treatment, 10-day-old seedlings were treated with 250 mM NaCl, and the samples were collected at 0 h, 1 h, 3 h, 6 h, 12 h, 24 h, 48 h and 72 h.

2.3. Cloning the sequences of TaMYB56 members

The following TaMYB56FL primers were designed according to the cDNA sequences of *TaMYB56* (JF951939) to cover the open reading frame (ORF) and were used to amplify the genomic and cDNA sequences in UR206 (AA genome), Y2006 (SS genome), Y2282 (DD genome) and CS (ABD genome): MYB56FL-F, 5′-CCGCACCACCAACATCT-3′ and MYB56FL-R, 5′-AGTAGCCTATTATACATTGCTAAAA-3′. The PCR products were cloned into pEASY-T1 vectors (TransGen) and sequenced with an ABI 3730XL 96-capillary DNA analyzer (Applied Biosystems).

2.4. Chromosomal locations of TaMYB56 members

The gene-specific primers were designed based on the nucleotide sequence polymorphisms of *TaMYB56-B* and *TaMYB56-D* (MYB56-B-F, 5′-ATCTTCCGTGTTCACATCTCC-3′ and MYB56-B-R, 5′-GAACCACGCCAGCTTCA-3; MYB56-D-F, 5′-CAGCTCCTCCTCTTCAACGAT-3′ and MYB56-D-R, 5′-CCCACAGCGAATCCGAG-3′) and employed to distinguish the homologous genes from different genomes. The templates for the PCR amplifications were genomic DNA samples that were prepared from CS NT lines and CS. The PCR parameters were as follows: 95 °C for 5 min; followed by 32 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 40 s; and a final step at 72 °C for 5 min. The amplified products were separated using 3% agarose gel electrophoresis.

2.5. Quantitative real-time PCR

Total RNA was extracted from wheat or Arabidopsis seedlings using TRIZOL reagent and treated with DNase I. For each sample, 10 μg of total RNA was used to synthesize first-strand cDNA using the SuperScript™ II Reverse Transcriptase (Invitrogen). For quantitative real-time PCR, each reaction contained 10 μL of 2× SYBR® Premix Ex Taq™ (TaKaRa), 3 μL of gene-specific primers (2.0 μM), 0.4 μL of 50× ROX Reference Dye and 2.0 μL of cDNA in a final volume of 20 μL. The PCR parameters were as follows: 95 °C for 30 s; followed by 40 cycles of 95 °C for 5 s and 60 °C for 31 s; and a dissociation at 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s. Wheat tubulin (U76558.1) and Arabidopsis actin (NM_179953) genes were used as reference genes. The reactions were performed using the ABI Prism 7300 real-time PCR system (Applied Biosystems) and repeated three times. A quantitative analysis was performed using the $2^{-\Delta\Delta CT}$ method. The primers used for quantitative real-time PCR are listed in Supplementary Table S1.

2.6. Subcellular location of TaMYB56-B-GFP fusion protein

The full-length coding sequence of *TaMYB56-B* that did not contain the stop codon was amplified and cloned adjacent to cauliflower mosaic virus (CaMV) 35S promoters in pJIT163-GFP vectors to express the 35S::TaMYB56-B-GFP fusion protein. The construct was confirmed by sequencing and transformed into onion epidermal cells via a Gene Gun (Bio-Rad). Transformed onion cells were observed under a confocal microscope (Nikon).

2.7. Transactivation assay in yeast cells

The yeast strain AH109 *Saccharomyces cerevisiae*, containing the *HIS3*, *ADE2* reporter genes with GAL4 binding elements in the promoters, and the vector pDEST32 (ProQuest™ Two-Hybrid System with Gateway® Technology, Invitrogen, Cat. 10835), containing the GAL4 DNA-binding domain (BD) and *LEU2* were used in this assay. The coding sequences of *TaMYB56* were obtained by PCR using the following gene-specific primers containing attB-sites: 5′-GGGGACAAGTTTGTACAAAAAAGCAGGCTGGATGAGCCCCGACGAGGA-3 and 5′-GGGGACCACTTTGTACAAGAAAGCTGGGTATCATGAGGCACCGGCGAC-3 (attB sites are underlined). The purified PCR products fused in-frame with BD domain were cloned into pDEST32 vectors by the Gateway method. The construct *pDEST32-TaMYB56* and the negative control *pDEST32* vector alone were transformed into AH109 respectively according to the protocol of the manufacturer in the Leu-medium.

About 2 days, the positive transformants verified by PCR were dropped on Leu- and Leu-His-Ade-medium respectively. The transcriptional activation activities were evaluated according to their growth status.