

# Ectopic Expression of *Fagopyrum tataricum* *FtMYB12* Improves Cold Tolerance in *Arabidopsis thaliana*

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**Abstract** MYB transcription factors play important roles in the abiotic stress response in plants, but their characteristics and functions in buckwheat (*Fagopyrum tataricum*) have not been fully investigated. Here, a novel R2R3-type MYB gene, designated *FtMYB12*, was isolated from the cultivated tartary buckwheat *F. tataricum*. Using quantitative real-time PCR, we found that the *FtMYB12* was greatly induced by low temperature. Sub-localization and yeast transactivity assay demonstrated that the *FtMYB12* gene encodes a nuclear transcription activator. Overexpression of *FtMYB12* in transgenic *Arabidopsis* plants resulted in enhanced cold tolerance. The *FtMYB12* overexpressing *Arabidopsis* lines showed higher root length and had elevated levels of proline content and lower

levels of malondialdehyde under cold stress conditions compared to the wild-type plants. The results revealed that *FtMYB12* may play an essential role in regulation of cold stress-responsive signaling in *F. tataricum*.

**Keywords** Buckwheat · Cold stress · *COR15a* · Malondialdehyde · R2R3-type transcription factor

## Introduction

Buckwheat is an ancient dicotyledonous crop belonging to the Polygonaceae family. The most widely cultivated buckwheat species include *Fagopyrum esculentum* (common buckwheat) and *Fagopyrum tataricum* (tartary buckwheat), constituting raw material for the production of buckwheat tea, groats, flour, and noodles (Zhang and others 2012a, b). *Fagopyrum tataricum* is an important crop in the high mountain areas of western China and in the Himalayan hills (up to 4,500 m high-altitude areas) because of its cold resistant nature. In addition, it has a higher nutritional value, especially in respect to a high antioxidant activity in terms of its flavonoid content compared to common buckwheat (Wang and Campbell 2007; Zhang and others 2012a, b; Zhou and others 2013). It has been reported that rutin content in the seeds of tartary buckwheat is approximately 100 times higher than that found in common buckwheat (Fabjan and others 2003). Thus, more researchers focus on tartary buckwheat as a beneficial health crop for the production of nutraceutical products and functional foods (Zhou and others 2013).

Low temperature presents a major challenge in our quest for sustainable food production as it reduces the potential yields in crop plants. Surviving low temperatures leads plants to acquire mechanisms by which they can sensitively

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perceive freezing tolerance and regulate their physiology accordingly over a long evolutionary scale. Deciphering the mechanism by which plants perceive low temperature is of critical importance for the development of rational breeding and transgenic strategies (Zhou and others 2010a, b). Transcription factors (TFs), including the MYB family, which have been widely studied in plants, play essential roles in regulating gene expression in response to low temperature (Zhou and others 2010a, b). MYB proteins contain the conserved SANT (for SWI3, ADA2, N-CoR, and TFIIB) DNA-binding domain (Rosinski and Atchley 1998; Jin and Martin 1999). According to the number of tandem repeats of the SANT domains, MYB proteins can be classified into three subfamilies: MYB-like proteins (MYB1R), R2R3-type MYB factors, and R1R2R3 MYB (MYB3R) factors, with one, two, and three repeats, respectively (Rosinski and Atchley 1998).

Several plant MYB factors involved in the responses to abiotic stresses such as drought, salt, and cold have been studied. In *Arabidopsis thaliana*, AtMYB2, AtMYB44, AtMYB102, AtMYB60, AtMYB96, and AtMYB14 function in drought or salt-stress response (Abe and others 2003; Denekamp and Smeekens 2003; Cominelli and others 2005; Agarwal and others 2006; Jung and others 2008; Seo and others 2009; Ding and others 2009), while AtMYBC1 and AtMYB14 play important roles in the plant response to cold stress (Zhai and others 2010; Chen and others 2013). Over-expression of *OsMYB4*, *OsMYB3R-2*, and *OsMYB2* have been shown to increase the freezing and multiple abiotic stress tolerance of *Arabidopsis* (Vannini and others 2004; Dai and others 2007; Ma and others 2009; Yang and others 2012). In wheat, several MYB TFs have also been found to play important roles in response to abiotic stresses. TaMYB1 is involved in the response to hypoxia (Lee and others 2007). Over-expression of *TaMYB2A* and *TaMYB56-B* have been shown to increase the salt, drought, and cold stress tolerance of *Arabidopsis* (Zhang and others 2012a, b). TaMYBsdu1, TaMYB73, TaMYB3R1 and TaMYB4 function in drought and salt tolerance (Rahaie and others 2010; Cai and others 2011; He and others 2012; Al-Attala and others 2014). Recently, the identification of MYB factor genes from other plants and their roles in abiotic stress tolerance have been attempted. Over-expression of chrysanthemum *CmMYB2* in *Arabidopsis* significantly increased tolerance to drought (Shan and others 2012). Over-expression of sheepgrass *LcMYB1* in *Arabidopsis* improved salt tolerance (Cheng and others 2013). In addition, over-expression of apple *MdMYB121* and *MdSIMYB1* remarkably enhanced the tolerance to high salinity, drought, and cold stress in transgenic plants (Cao and others 2013; Wang and others 2014). However, not much is known about MYB factors involved in abiotic

stress from buckwheat, and more attempts at isolating MYB genes and characterizing their functions are needed.

In the present study, we isolated and investigated the function of a new R2R3-MYB TF FtMYB12 from *F. tataricum*. We characterized its gene expression patterns, sub-cellular localization, and trans-activity. Transgenic *Arabidopsis* plants over-expressing *FtMYB12* were evaluated for cold stress tolerance. Our data indicate that FtMYB12 may play an important role in the cold stress signaling pathway in buckwheat.

## Materials and Methods

### Transcriptomic Data Analyses and Isolation of the FtMYB12 Gene

The publicly available, annotated set of *F. tataricum* genes expressed in the flower and inflorescence was collected (Logacheva and others 2011). The conserved MYB domain DNA sequence (At5g35550 and FtMYB123L) search was employed to confirm the candidate sequences as MYB genes (Zhou and others 2013). To confirm the full length of putative cDNA sequence, *FtMYB12* cDNA was amplified using the primers FtMYB12F 5'-ATG AGG AAT CCG GCG GTA-3' and FtMYB12R 5'-TTA GAT TTC TGA TGG GAT CAA AG-3'. The amplification conditions were as follows: 94 °C for 5 min, followed by 30 cycles of 94 °C for 45 s, 57 °C for 45 s, 72 °C for 2 min, followed by a final extension step of 72 °C for 10 min. The PCR products were cloned into the pMD19-T vector and sequenced. The nucleotide and amino acid sequences were analyzed by the DNAMAN software and compared with those released in GeneBank databases by using the BLAST program.

### Phylogenetic Tree

Protein sequences were aligned by clustalW and a tree was constructed with the MEGA version 5 program with the neighbor-joining (NJ) method (Saitou and Nei 1987; Tamura and others 2011).

### Plant Materials and Stress Treatments

Two-week-old *F. tataricum* seedlings were treated under different stress conditions. Cold treatment was conducted by transferring the young trees to a growth chamber set at 4 °C under a 16 h light/8 h dark cycle (2,500 lux). For salt, drought, and UV stress treatments, seedlings were grown in soil irrigated with 100-mM NaCl, 30 % PEG6000 and exposed to UV-B (302 nm, 0.1 mW/cm<sup>2</sup>), respectively. The length of time for the above treatments was 0, 12, and 24 h. The control plants were mock-treated with water.

Tissue-specific expression of the *FtMYB12* genes was analyzed in 3-week-old seedlings that were separated into root, stem, and leaf tissues. All samples were quickly frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until use.

#### Subcellular Localization and Transcriptional Activity Assay

To determine its exact sub-cellular location, FtMYB12 was combined with the N-terminal of green fluorescent protein (GFP) to yield a fused FtMYB12-GFP protein. The entire coding region of the target gene amplified by PCR was inserted into the *SalI* and *BamHI* sites of vector p163-GFP. Protoplasts were isolated from *Arabidopsis* cell suspension ecotype Col-0, and 10- $\mu\text{g}$  plasmid DNA was introduced by polyethylene glycol (PEG)-mediated transfection as previously described (Schirawski and others 2000). The transformed cells were cultured on Murashige-Skoog (MS) medium at  $28^{\circ}\text{C}$  for 24 h in the dark and observed under a laser confocal scanning microscope (Leica DM IRBE).

To investigate the transcriptional activity of FtMYB12, the full length coding region was inserted into the *EcoRI* and *SalI* sites of the yeast expression vector pBridge containing the binding domain (BD) of GAL4. The plasmids were introduced into the yeast strain AH109 with the reporter gene *His3* and *LacZ* following the manufacturer's instructions (Clontech, Palo Alto, CA, USA). Yeast cells containing pBridge empty vector were used as negative control. The colony-lift filter  $\beta$ -galactosidase assay was carried out according to the Yeast Protocols Handbook (Clontech Laboratories, Inc.).

#### Generation of Transgenic *Arabidopsis*

The FtMYB12 open reading frame (ORF) was PCR-amplified from *F. tataricum* cDNA using the primer set 5'-CGG GATC CAT GAG GAA TCC GGC GGT ATC-3' and 5'-CGG AAT TCG ATT TCT GAT GGG ATC AAA G-3', digested with *BamHI* and *EcoRI* and cloned into pRT101 (Töpfer and others 1987). For the construction of transgenic lines constitutively overexpressing FtMYB12, the cauliflower mosaic virus (CaMV) 35S cassette containing the *FtMYB12* ORF was digested from pRT101 with *SphI* and cloned in pCAMBIA1300 digested with *SphI*. The binary vector pCAMBIA1300-*FtMYB12* was introduced into the *Agrobacterium tumefaciens* strain LBA4404. *Arabidopsis* ecotype Col-0 plants were transformed using the floral dip method. Seeds were surface-sterilized by incubation for 1 min in 70 % ethanol, 15 min in 50 % bleach, and five rinses with sterile water. Surface-sterilized seeds were grown on plates containing MA medium supplemented with 0.6 % w/v agar. Transgenic plants were selected on solid MA medium containing 100 mg/L timentin and 50 mg/L hygromycin. Transgenic plants from T2 generations were

selected on MA medium (Masson and Paszkowski 1992) containing only 50-mg/L hygromycin. Following stratification for 3 days at  $4^{\circ}\text{C}$ , seeds were incubated at  $25^{\circ}\text{C}$  in a growth chamber under the 16 h light/8 h dark cycle (2,500 lux). Positive homozygous lines were test for expression of *FtMYB12* by RT-PCR.

#### Determination of Physiological and Biochemical Properties

The malondialdehyde (MDA) content was determined as previously described (Zhou and others 2012). The proline content was calculated as described by Bates and others (1973).

#### Quantitative Real-Time PCR

Total RNA was extracted from pulverized frozen tissue by phenol/chloroform extraction followed by overnight precipitation with 2 M lithium chloride, washed with 70 % ethanol, and resuspended in water. Reverse transcription was carried out using the M-MuLV Reverse Transcriptase (Revert Aid<sup>TM</sup> First Strand cDNA Synthesis Kit, Fermentas) according to the manufacturer's protocol. The quantitative PCR (Q-PCR) amplification was carried out as previously described (Zhou and others 2010a, b). The following primer sets for Q-PCR were used: 5'-CGT CTC CGA TGG ATG AAC TA-3' and 5'-TGC CAG CTA TGA GAG ACC AC-3' for *FtMYB12*; 5'-CGC AAG TAC CAG AAG AGC AC-3' and 5'-GAG AGC AGA CAC AGC AGA GC-3' for *FtH3* (histone H3); 5'-CAA GGA AGG TAT CCC ACC G-3' and 5'-TTA GAA TCC ACC ACG AAG ACG-3' for *Arabidopsis* polyubiquitin gene. The primers of some genes (*COR15a*, *DREB2A*, *RD29A*, *CBF1*, *CBF2*, *CBF3*, and *COR47*) in *Arabidopsis* were previously described (Dai and others 2007). The genes encoding *FtH3* and polyubiquitin were used as reference genes in buckwheat and *Arabidopsis*, respectively. Relative gene expression was quantified using the comparative threshold cycle (CT) method (Zhou and others 2010a, b).

#### Statistical Analysis

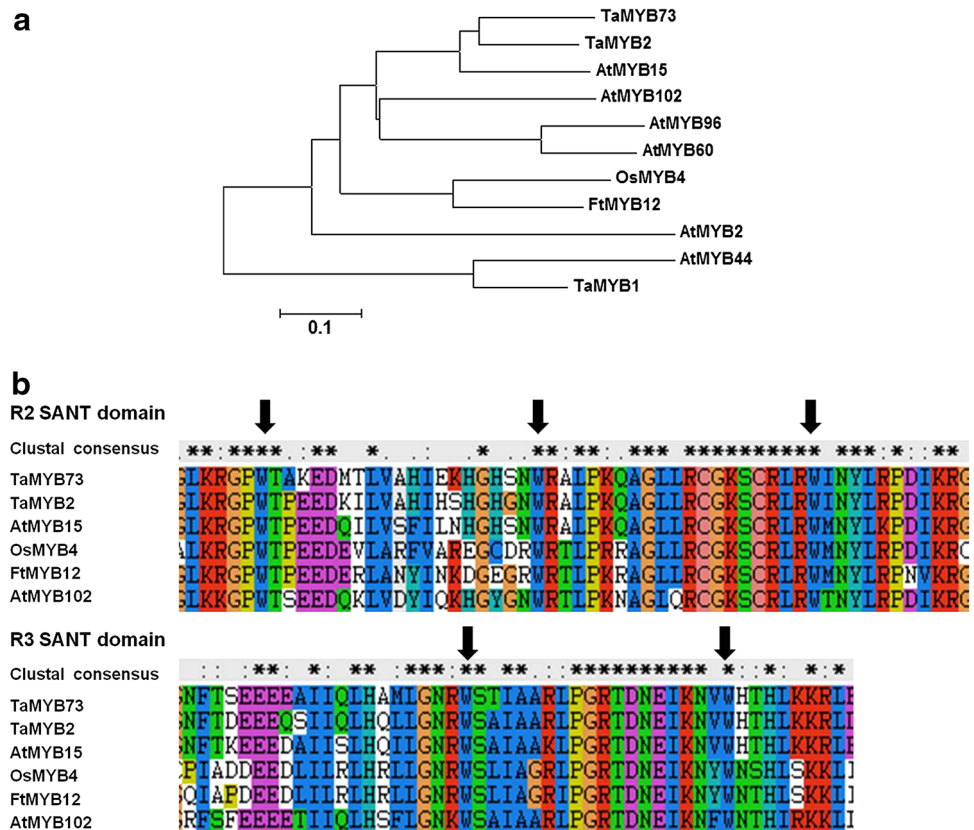
Data were analyzed using the Student's *t* test. A *P* value of  $<0.05$  was considered to be significant.

## Results

#### Identification and Phylogenetic Analysis of FtMYB12

To identify putative *MYB* genes that may be closely involved in the cold response in buckwheat, two *MYB* genes *At5g35550* and *FtMYB123L* as probes were screened

**Fig. 1** Phylogeny and alignment of FtMYB12. **a** Phylogenetic tree shows the relationships of FtMYB12 to MYB factors from other plants, the tree is based on alignment of complete protein sequences (AtMYB2 (AT2G47190), AtMYB44 (AT5G67300), AtMYB96 (AT5G62470), AtMYB15 (AT3G23250), AtMYB60 (AT1G08810), AtMYB102 (AT4G21440), OsMYB4 (D88620), TaMYB73 (JN969051), TaMYB1 (DQ353858), and TaMYB2 (AY615199)). **b** Alignment of R2 and R3 SANT domains. Identical amino acid residues are shown by *stars* and conserved amino acid residues of SANT domain motifs are indicated by *arrows*



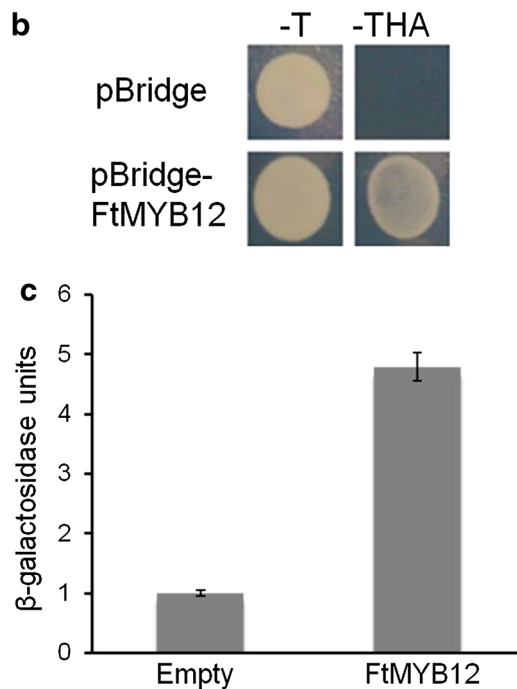
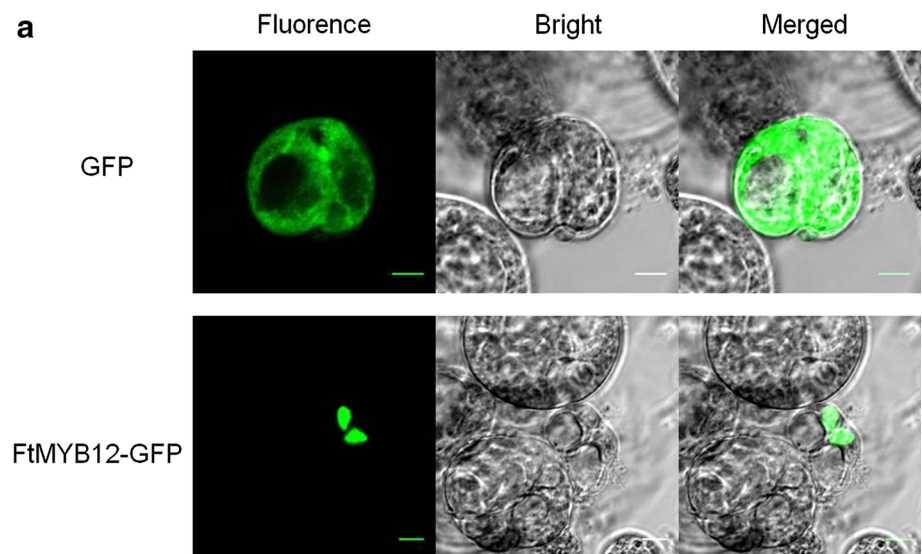
in publicly available transcriptomic data (Logacheva and others 2011). According to a set of unigenes with annotation as MYB TFs, more than 5 full length MYB genes were cloned (data not shown). Of them, the gene (*FtMYB12*) encoded R2R3-type MYB factor, which clusters with the OsMYB4 factor involved in freezing tolerance in rice (Fig. 1a) (Vannini and others 2004). The *FtMYB12* gene encodes a protein of 171 amino acid residues with a predicted molecular mass of 19.46 kDa and a calculated pI of 10.32. *FtMYB12* has an open reading frame of 516 bp (GenBank Accession No. KJ586579, Supplemental Fig. 1). Genomic PCR products amplified by primers designed from the 5' and 3' untranslated region revealed that *FtMYB12* had 1 intron of 88 bp. Analyses of the primary structure revealed that FtMYB12 contains two imperfect MYB repeats within its N-terminal region that correspond approximately to the R2 and R3 MYB repeats (Fig. 1b). The highly conserved W residues, which are thought to be involved in the folding of the DNA-binding domain, are present in FtMYB12. In addition, the short linker sequence between the two MYB repeats also displays amino acid conservation (Zhou and others 2013).

### FtMYB12 Localizes in Nuclei and has Trans-Activation Activity

The transient expression assay using *Arabidopsis* protoplasts showed that FtMYB12 fused to the GFP was localized in the nucleus (Fig. 2a). The control GFP protein was distributed throughout the cell, as expected (Fig. 2a). These results confirm that FtMYB12 is a nuclear protein. The transactivation assay indicated that all transformants grew well on the transformant selective SD/Trp plates (Fig. 2b), whereas only transformants of pBridge that were fused with FtMYB12 grew on the transactivation selective SD/Trp-/Ade-/His plates (Fig. 2b), showing that FtMYB12 activated the transcription of reporter genes Ade and His in the genome of the yeast AH109 strain. Consistently, the β-galactosidase activity assay indicated that the β-galactosidase units of the transformant with pBridge-FtMYB12 were higher by about fivefold than that of pBridge (Fig. 2c), demonstrating that FtMYB12 also activated the reporter gene *LacZ* in the genome of AH109. These results demonstrate that FtMYB12 is a nuclear transcription activator.



**Fig. 2** Subcellular localization and transactivation assay of FtMYB12. **a** Transiently expressed FtMYB12 in *Arabidopsis* protoplasts shows its subcellular localization. Bars 10  $\mu\text{m}$ . **b** Transactivation assay of FtMYB12. The full length of FtMYB12 was fused with pBridge, and transformed yeasts were selected on both SD-Trp and SD-Trp-His-Ade media, respectively. **c**  $\beta$ -galactosidase activity assay (Miller units). The mean value is from three independent measurements, and error bars indicate the standard variation. pBridge was used as a negative control



### Expression of *FtMYB12* is Activated by Cold Stress

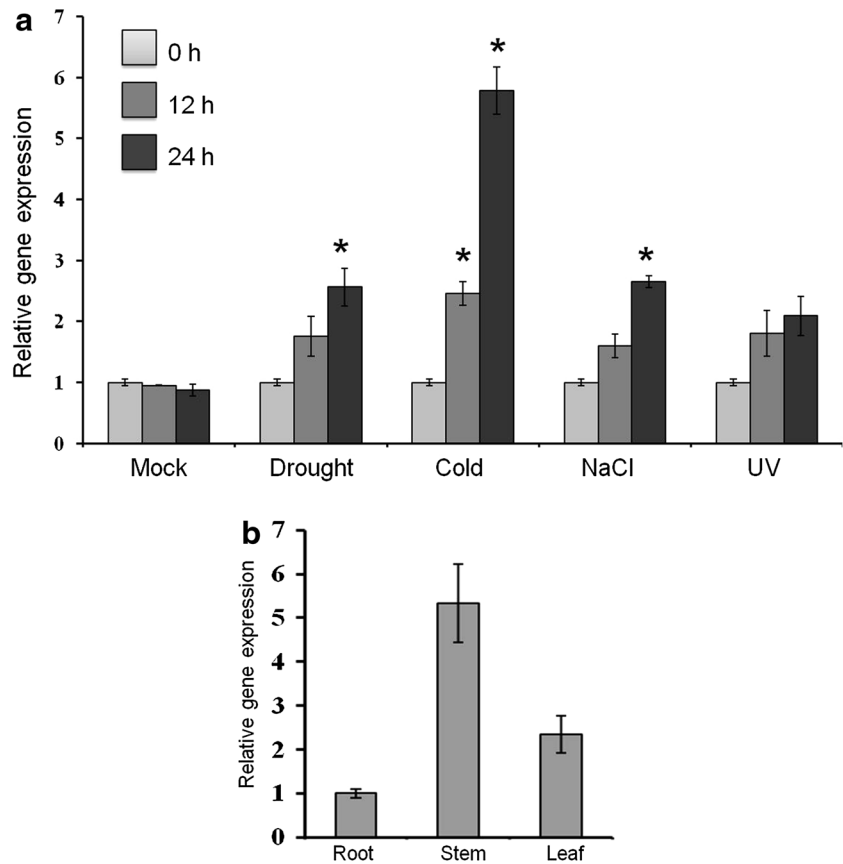
The regulation of *FtMYB12* expression by various abiotic stresses was investigated by subjecting two-week-old buckwheat seedlings to drought (30 % PEG6000), cold (4  $^{\circ}\text{C}$ ), salt (100 mM NaCl), and UV-B treatments. The *FtMYB12* mRNA accumulated quickly in response to cold and reached its maximal level at 12 h (Fig. 3a). In addition, expression of *FtMYB12* was also induced by PEG and salt treatment, but the highest levels of transcription occurred at 12 h (Fig. 3a). To further study *FtMYB12* gene expression patterns, we used QRT-PCR analysis with RNA from roots, stems, and leaves. The result indicated that *FtMYB12* was

found to be ubiquitous in all tissues but the highest level was in young stems (Fig. 3b). Taken together, these results suggest that *FtMYB12* is induced under cold, salt, and drought stimulation, which suggests that it functions during these stresses.

### Cold Tolerance of Transgenic *Arabidopsis*

Since *FtMYB12* was mainly induced by cold, its role in cold tolerance in *Arabidopsis* was explored by the gain-of-function approach. Transgenic *Arabidopsis* plants were developed with the *FtMYB12* gene under the control of the CaMV35S promoter. Overall, nine lines of transgenic

**Fig. 3** Expression of *FtMYB12* as assessed by QRT-PCR. **a** The expression of *FtMYB12* in response to various stresses. **b** The expression pattern of *FtMYB12* in buckwheat root, stem, and leaf. *FtH3* was used as an internal control. The data were normalized to wild-type or root (set at 1) using the comparative threshold cycle method. Error bars represent the standard deviation of triplicate runs for QRT-PCR



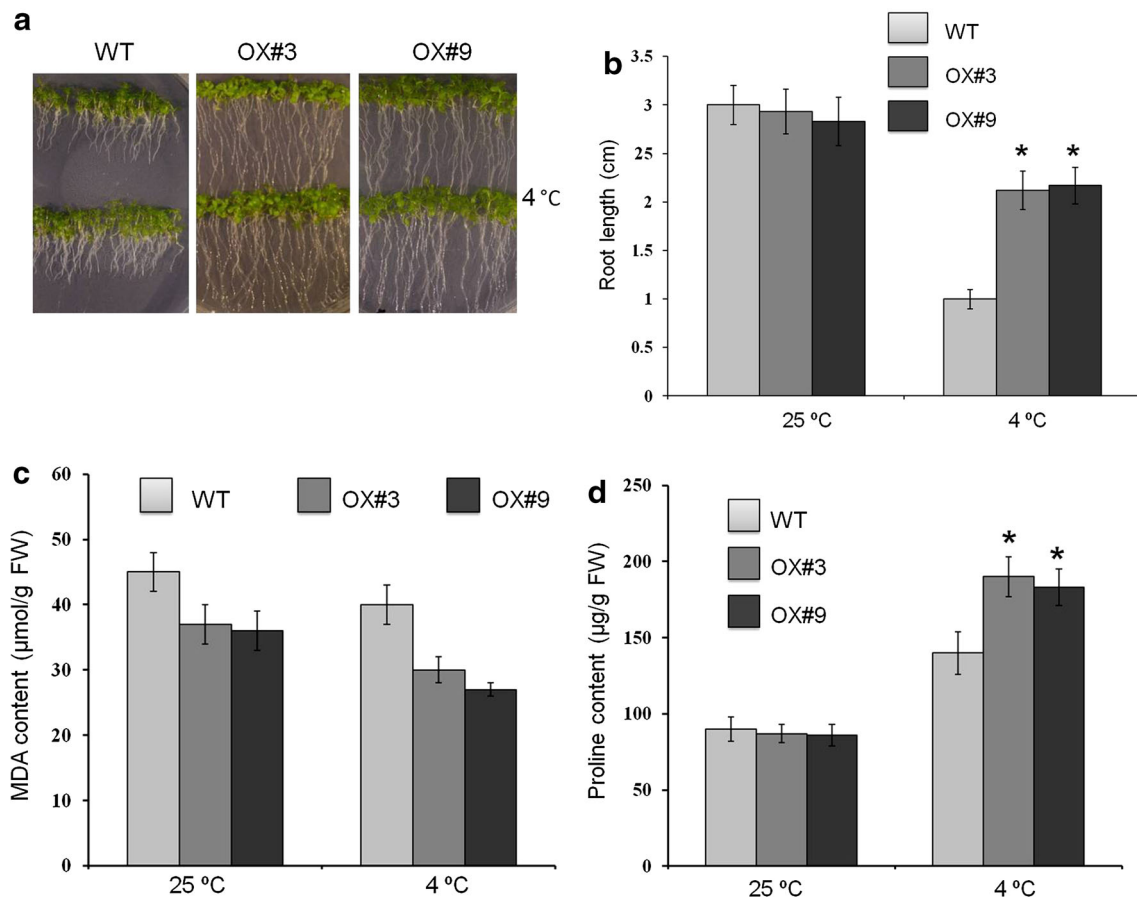
*Arabidopsis* were positively confirmed by PCR (data not shown). From the nine transgenic lines, we selected two higher *FtMYB12* expression lines (OX#3 and OX#9) for further analysis (Supplemental Fig. 2). The transgenic plants (T2) showed no morphological difference in the vegetative and floral tissues, as compared to wild-type (WT) plants (data not shown). To examine the freezing tolerance of transgenic *Arabidopsis*, we tested the effect of low temperature on seedling root growth. The primary root lengths of the WT and transgenic lines were relatively consistent at room temperature (25 °C) (Fig. 4a, b). However, when seedlings were grown at low temperature (4 °C), the root length of the WT lines was significantly retarded compared to transgenic lines (Fig. 4a, b). The cold treatment caused root growth retardation in both WT and transgenic plants but the effect was more pronounced in WT seedlings. These results suggested that overexpression of *FtMYB12* can improve cold tolerance in transgenic plants.

To cope with low temperature, the primary effects of stress are a reduction in cellular membrane damage and accumulation of proline content. We discovered that cold stress decreased MDA content and increased proline content. The levels of MDA content and proline content in lines *FtMYB12* overexpressing *Arabidopsis* plants did not

show much difference as compared with those in control plants under normal growth conditions (Fig. 4c, d). However, when seedlings were grown at low temperature (4 °C), after 14 days of cold stress, the MDA content in WT plants was higher than that in transgenic plants (Fig. 4c), whereas the proline content of the WT lines was significantly retarded compared to transgenic lines (Fig. 4d). The result suggested that the cold tolerance of the transgenic line was enhanced effectively because of the expeditious accumulation of the substance content compared to control plants (Zhou and others 2012).

#### *FtMYB12* Promotes Transcription of *COR15a* in *Arabidopsis*

To elucidate the molecular mechanism of *FtMYB12* in the cold response, we monitored the expression of cold-responsive genes identified in the regulated pathways by QRT-PCR analysis. Under cold treatment for 14 days, the tested marker genes, including *COR15a*, *DREB2A*, *RD29A*, *CBF1*, *CBF2*, *CBF3*, and *COR47* (Fig. 5), showed significant induction in both WT and transgenic plants which is consistent with previous studies (Stockinger and others 1997; Dai and others 2007). However, under room temperature (25 °C), the expression of *COR15a* in *FtMYB12*



**Fig. 4** Stress response of *Arabidopsis* transgenic plants overexpressing *FtMYB12*. **a** and **b** Phenotypic response to low temperature, three-day-old seedlings grown at room temperature (25 °C) and at low temperature (4 °C), 14 days before the images shown were taken and primary root growth was detected. **c** and **d** MDA and proline contents

were at room temperature and low temperature. Each experiment was repeated three times. *Error bars* represent the standard error of triplicate analyses. **b**, **c**, **d** The significance of difference between indices of WT and each OX was calculated using Student's *t* test (\**P* < 0.05)

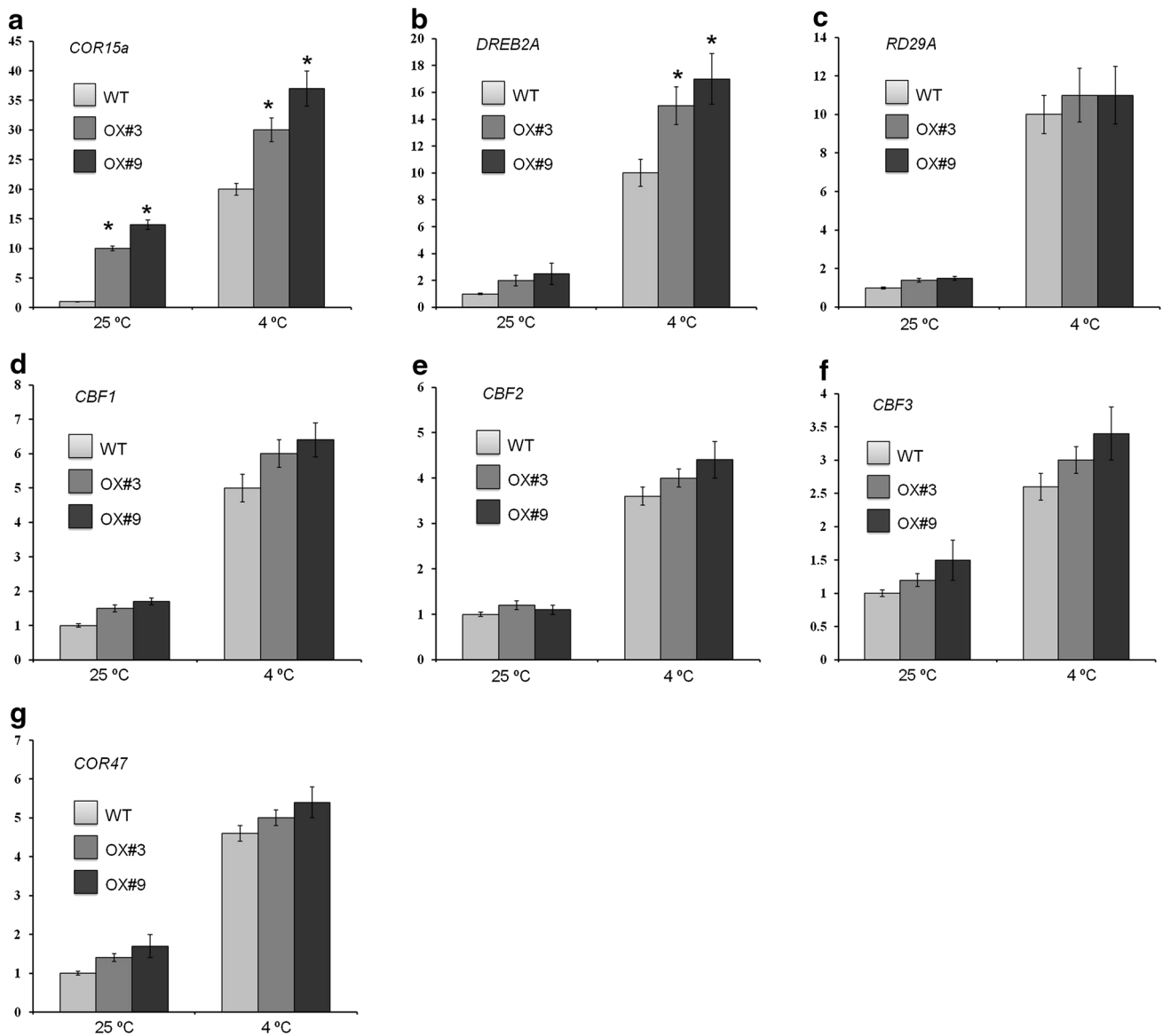
overexpressed transgenic plants was substantially higher than that in wild-type plants (Supplemental Fig. 3), whereas there was no significant induction in expression of *DREB2A*, *RD29A*, *CBF1*, *CBF2*, *CBF3*, and *COR47* in transgenic or wild-type plants (Fig. 5). These results indicate that the induction strength of the *COR15a* gene expression can be correlated to the transcription abundance of transgenic *FtMYB12* (Supplemental Fig. 3).

## Discussion

In plants, TF including the MYB family function in various pathways to confer stress tolerance. Several plant MYB factors involved in the responses to cold stress have been studied, such as *AtMYB1* (Zhai and others 2010), *AtMYB14* (Chen and others 2013), *OsMYB4* (Vannini and others 2004), and *OsMYB3R-2* (Dai and others 2007). In this study, we identified a cold-inducible R2R3

MYB TF, *FtMYB12*, from cold-tolerance buckwheat. Sub-localization,  $\beta$ -galactosidase, and transactivation assays indicate that *FtMYB12* is a transcription activator (Fig. 2). *FtMYB12* clusters with R2R3 type MYB proteins of other plants (Fig. 1a), indicating that *FtMYB12* is a R2R3 type MYB transcription factor. In addition, *FtMYB12* can be identified on the basis of conserved SANT domains, which are typically located in the N-terminal region and critical for DNA-binding activity (Fig. 1b) (He and others 2012). This domain generally consists of up to four imperfect amino acid sequence repeats (R) of about 52 amino acids, each forming three  $\alpha$ -helices, and form a helix–helix–turn–helix motif, and are involved in transcriptional regulation mechanisms (Dubos and others 2010).

Usually, TFs are induced rapidly during the early phase of the response to abiotic stresses, reach maximal induction at several hours, and then decrease in expression level (Dai and others 2007). For example, *CBF1* and *CBF3* showed



**Fig. 5** Expression patterns of stress-responsive genes in WT and transgenic *Arabidopsis* using QRT-PCR. Total RNA was extracted from 17-d-old plants grown at room temperature (25 °C) and low temperature (4 °C) for 14 days, respectively. Transcript levels

measured by QRT-PCR of *COR15a* (a), *DREB2A* (b), *RD29A* (c), *CBF1* (d), *CBF2* (e), *CBF3* (f), and *COR47* (g). The polyubiquitin gene was used as an internal control. Data represent means and standard error of three replicates

peak induction at 6 h in wild-type plants and *CBF2* showed peak induction at 3 h (Gong and others 2002), whereas *TaMYB73* showed peak induction at 0.5 h during stress treatment (He and others 2012). However, the transcript level of *FtMYB12* increases after cold treatment for 12 h and gradually accumulates within 24 h. Furthermore, *FtMYB12* is induced by drought and salt stress (Fig. 3a), which is dissimilar to *OsMYB4* involved in cold stress in rice that was induced only by cold stress (Vannini and others 2004). This is the first report, to our knowledge, showing that an R2R3- type MYB from buckwheat is involved in cold, drought, and salt stress.

TFs are involved in the response to stress in plants which, in turn, facilitate the ability to cope with these stresses. For example, cold-, salt-, and drought-induced *OsMYB3R-2* promote tolerance to these stresses in *Arabidopsis* (Dai and others 2007), salt- induced *TaMYB73* promotes tolerance to this stress in *Arabidopsis* (He and others 2012). Here, the over-expression of *FtMYB12* increased the tolerance of *Arabidopsis* to cold stress (Fig. 4). Previous reports showed that *COR15a* and *DREB2A* are involved in cold stress signaling by CBF/ DREB1 pathways (Artus and others 1996; Liu and others 1998). However, our results reveal that *FtMYB12* only



activates the expression of *COR15a* but not *DREB2A* and *CBFs* (Fig. 5). Thus, overexpression of *FtMYB12* increases expression of *COR15a* which is involved in plant tolerance by the CBF/DREB1-independent pathways. In addition, we found that MDA content decreased under cold stress (Fig. 4c). Leaf oxidative damage to lipids was expressed as equivalents of MDA. The increases in MDA content suggested that cold-stressed plants encountered cellular damage and lipid peroxidation. After 14 days of cold stress, the MDA content in transgenic plants was lower than that in WT plants (Fig. 4c). It has been reported that increased proline content is positively correlated with freezing tolerance (Ma and others 2009). Under normal growth conditions (25 °C), the levels of cellular free proline did not differ between WT and transgenic plants (Fig. 4d). In contrast, after cold treatment the levels of free proline in *FtMYB12* overexpressing transgenic lines increased substantially compared with WT plants. These results were similar to the alterations observed in the transgenic rice overexpressing the freezing resistant *OsMYB3R-2* (Ma and others 2009). Taken together, these results indicate that the FtMYB12 protein may play an important role under cold stress through changing a variety of physiological and biochemical processes.

Low temperature lowers plant production worldwide. The responses of plants to cold stress have evolved a variety of alterations in physiological, biochemical, and gene expression processes. Here, we cloned and characterized a novel R2R3-type MYB protein FtMYB12 localized at the nucleus and induced by cold, drought, and salt stress. The enhanced freezing tolerance of CaMV35S-*FtMYB12 Arabidopsis* plants reveals that FtMYB12 could mediate low-temperature signal transduction. Taken together, this report provides beneficial information for understanding of the mechanism of cold regulation in buckwheat.

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