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Ectopic expression of a wheat MYB transcription factor gene, TaMYB73, improves salinity stress tolerance in Arabidopsis thaliana

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Received 6 September 2011; Revised 3 November 2011; Accepted 7 November 2011

Abstract

MYB transcription factors (TFs) play pivotal roles in the abiotic stress response in plants, but their characteristics and functions in wheat (Triticum aestivum L.) have not been fully investigated. A novel wheat MYB TF gene, TaMYB73, is reported here based on the observation that its targeting probe showed the highest salinity-inducibility level among all probes annotated as MYB TFs in the cDNA microarray. TaMYB73 is a R2R3 type MYB protein with transactivation activity, and binds with types I, II, and IIG MYB binding motifs. The gene was induced by NaCl, dehydration, and several phytohormones, as well as some stress-, ABA-, and GA-responsive cis-elements present in its promoter region. Its over-expression in Arabidopsis enhanced the tolerance to NaCl as well as to LiCl and KCl, whereas it had no contribution to mannitol tolerance. The over-expression lines had superior germination ability under NaCl and ABA treatments. The expression of many stress signalling genes such as AtCBF3 and AtABF3, as well as downstream responsive genes such as AtRD29A and AtRD29B, was improved in these over-expression lines, and TaMYB73 can bind with promoter sequences of AtCBF3 and AtABF3. Taken together, it is suggested that TaMYB73, a novel MYB transcription factor gene, participates in salinity tolerance based on improved ionic resistance partly via the regulation of stress-responsive genes.

Key words: Ionic stress, MYB transcription factor, salt stress, Triticum aestivum.

Introduction

Both grain yield and quality of wheat are downgraded by adverse environmental conditions, among which soil salinity, drought, and extreme temperatures cause the greatest economic losses. Thus, improving its level of abiotic stress tolerance has become a key objective in many wheat breeding programmes. Genetic variation for stress tolerance is rather limited in the domesticated gene pool, but a number of wild relatives of wheat have been proved to process notable high-level tolerance (Zamir, 1995); thus one approach to enhance stress tolerance genetically is to create alien gene introgression lines via somatic hybridization. The salinity-tolerant cultivar Shanrong No. 3 (SR3) was obtained in this way; it was selected among the derivatives of a somatic hybrid between the conventional, salinity-sensitive bread wheat cultivar and the highly tolerant wild species Thinopyrum ponticum (Peng et al., 2009). Cytogenetic and molecular analysis showed that a few chromatin fragments were introgressed into the genome of SR3 (Xia et al., 2003; Wang et al., 2005), and dramatic genomic variation arises, suggesting that SR3 is a genetic reservoir for salinity tolerance associated gene mining.

Stress tolerance in plants can be enhanced via both ABA-dependent and ABA-independent pathways (Barrero et al., 2009; Ergen et al., 2009). In the former, AREB/ABF transcription factors are thought to play an important role while, in the latter, DREB/CBF transcription factors are...
heavily responsible for signal transduction. The *Arabidopsis* genome contains >1600 transcription factors, equivalent to ~6% of the total number of genes present, of which the MYB family has more members than any other (Riechmann et al., 2000). MYB proteins are recognized by their DNA-binding domains, comprising one to four imperfect repeats, each of which form a helix-turn-helix structure of ~50 residues (Dubos et al., 2010). As a prototype, three repeats of c-Myb are referred to as R1, R2, and R3, and repeats from other MYB proteins are named according to their similarity to R1, R2, or R3. Four MYB subfamilies have been recognized according the repeats that they possess: MYB-related (containing a single or partial R1, R2, or R3 domain), R2R3, R1R2R3, and 4R (containing four R1 and/or R2 domains) (Dubos et al., 2010). MYB proteins were found to be implicated in various developmental and physiological processes, especially in abiotic and biotic stress responses (Dubos et al., 2010). For instance, *AtMYB2* functions in the *ABA*-mediated drought-stress response (Abe et al., 2003); Over-expression of *AtMYB44* can reduce the rate of water loss and improve drought and salinity tolerance (Jung et al., 2008); *Arabidopsis* lines engineered to over-express *AtMYB96* are more drought-tolerant than the wild type (Seo et al., 2009); *AtMYB15* accounts for tolerance to drought and salt stresses (Agarwal et al., 2006; Ding et al., 2009); over-expression of *OsMYB4* has been shown to increase the chilling and freezing tolerance of *Arabidopsis* (Vannini et al., 2004); *OsMYB3R-2* contributes to tolerance to cold (Ma et al., 2009) and resistance to multiple abiotic stresses in *Arabidopsis* (Dai et al., 2007).

In recent years, the identification of wheat MYB TF genes and their roles in abiotic stress tolerance have been attempted. *TaMYB10* was proved to be responsible for the coat pigmentation of red wheat grain (Himi et al., 2005). *TaWR52* has been shown to suppress the expression of *KNOX*, and hence affects lateral organ initiation and meristem differentiation (Morimoto et al., 2009). *TaMYB1* is involved in the response to hypoxia (Lee et al., 2007). *TaMyb2*, *TaMyb4*, and *TaMyb1* were elevated when treated with 30% PEG, but their function(s) have not been fully described to date (Chen et al., 2005). Ectopic expression of *TaMYB2A* confers enhanced tolerance to multiple abiotic stresses in *Arabidopsis* (Mao et al., 2011). *TaMYBsd1* has higher expression in salt-tolerant wheat cultivars than in salt-sensitive cultivars under saline and drought conditions, and is predicted to be involved in wheat adaptation to both salt and drought stresses (Rahaie et al., 2010). However, not much is known about MYB proteins in wheat, and more attempts for isolating MYB genes and characterizing their functions are needed.

In order to identify a MYB gene that possibly plays an important role in the abiotic stress response from wheat, a transcriptomic analysis of the wheat introgression line SR3 was performed using the wheat gene microarray, in which a set of probes annotated as MYB TF genes appeared to be either over- or under-expressed under salt stress (Wang et al., 2008). In this study, a new R2R3 type MYB gene, *TaMYB73*, for SR3 was cloned, whose referring probes showed the most salinity-induced pattern among all the MYB probes. It was found that *TaMYB73* over-expression in *Arabidopsis* enhanced tolerance to salinity, while it had no positive effect on coping with osmotic stresses, suggesting that *TaMYB73* may play special roles in response to ionic stresses.

**Materials and methods**

**Wheat growing conditions and treatment**

The wheat introgression line SR3 with high salt and drought tolerance was used as the wheat material in this work. Seedlings of SR3 were grown hydroponically in half-strength Hoagland’s solution under a 16/8 h light/dark, 22/20 °C, 60% humidity regime to the three-leaf stage and were then treated in half-strength Hoagland’s solution with or without 200 mM NaCl, 200 μM abscisic acid (ABA), 200 μM salicylic acid (SA), 200 μM 1-aminoacyclopropane-1-carboxylic acid (ACC), 200 μM gibberellic acid (GA) or 200 μM jasmonic acid (JA) for 0–24 h. Dehydration stress was imposed by removing the seedlings from liquid culture and leaving them on a sheet of Whatman No. 5 filter paper in the dark at room temperature for various periods of time. All samples were selected at the same time to avoid the influence of photo-period on gene expression. Mock analysis of NaCl and dehydration was carried out by leaving the seedlings in half-strength Hoagland’s medium, and that of phytohormone treatment by transferring the seedlings into half-strength Hoagland medium and adding an equivalent volume of the corresponding solution for solving these phytohormones. All of the treatments were repeated three times.

**Cloning and sequence analysis**

Based on the data of transcriptomic analysis of SR3 seedlings with or without salt stress that was conducted using our customized 60-mer oligo-DNA microarray containing 15 172 probes (the probes harbouring 15 170 genes were designed according to the EST sequences either from the SR3 cDNA library or from the public wheat EST database), the expression pattern of probes annotated as wheat MYB TFs (*TaMYBs*) under salinity/drought stress were fully analysed. Among them, one fragment, *TaMYB73*, was induced to the greatest extent under stress. To obtain the full-length cDNA of *TaMYB73*, its 5′ segment was amplified from the SR3 cDNA library using the 3′ terminal specific reverse primer, Ta.S405.As, in combination with the NT3 primer targeting the pBluescript (+) vector sequence at the 5′ end of the inserts, and its 3′ segment was amplified using the 5′-terminal specific forward primer, Ta.S405.S, and the NT7 primer targeting vector sequence at the 3′ end of the inserts. Two segments were sequenced and assembled into a full-length cDNA. According to the assembled sequence, the *TaMYB73* open reading frame (ORF) was cloned for further analysis. Its phylogeny was constructed from the peptide sequences of *TaMYB73* and various heterologous MYB proteins using a Neighbor–Joining method within CLUSTAL X and MEGA3 software (Kumar et al., 2004; Larkin et al., 2007). SNAT domains of *TaMYB73* were aligned with those of other MYBs using CLUSTAL X software (Larkin et al., 2007).

**Promoter isolation and analysis**

The promoter sequence was isolated using the site-finding PCR method (Tan et al., 2005) (the primers are listed in Supplementary Table S1 at *JXB* online). Promoter cis-elements were identified using the algorithm developed by Higo et al. (1999) and are available at www.dna.afrc.go.jp/PLACE/index.html.

**RT-PCR and real-time PCR**

Total RNA was extracted from both *Arabidopsis* and wheat seedlings with the Trizol reagent (Takara) and treated with...
RNase-free DNase (Promega). The cDNA first strand was synthesized using an M-MLV kit (Invitrogen), following the manufacturer’s protocol. *TaActin* (AB181991) and *AtActin* (GI:145338402) were used as internal reference genes for assessing expression levels in wheat and *Arabidopsis*, respectively, and primers for these two genes are both specific to amplify a single gene. All RT-PCR experiments described in this section were reproduced at least three times using independent cDNA preparations. LightCycler® Fast-Start DNA Master SYBR Green I (Roche) was used for real-time PCR. Each 10 μl real-time PCR was subjected to a cycling regime of 94 °C for 10 s, 60 °C for 10 s, and 72 °C for 10 s. After the PCR program, data were quantified using the comparative C_{T} method based on C_{T} values (Livak and Schmittgen, 2001). Each RT-PCR was repeated four times, and each real-time PCR was run in triplicate. Primer sequences for RT-PCR and real-time PCR were listed in Supplementary Table S1 at *JXB* online.

**Subcellular localization and transactivation assay of TaMYB73**

The *TaMYB73* sequence, lacking its stop codon, was PCR cloned (see Supplementary Table S1 at *JXB* online) from SR3 cDNA and ligated into the XbaI and BamHI sites of the 326-GFP vector (Lee et al., 2003). The positive construct or the 326-GFP vector alone was transformed into onion epidermal cells (Lee et al., 2003) and *Arabidopsis* mesophyll protoplasts (Yoo et al., 2007), respectively. After a period of 16 h at 22 °C in the dark, these cells were analysed by bright field and fluorescence microscopy. The full-length *TaMYB73* ORF and a series of truncated ORF fragments were amplified using specific primers with EcoRI and PstI restriction digestion sites that flanked both terminals of each full-length/truncated sequence (see Supplementary Table S1 at *JXB* online), and these amplified sequences were inserted into the EcoRI and PstI sites of the pGBKTK7 vector (Clontech). Each of the positive plasmids and an insert-less vector were transformed separately into the yeast AH109 strain (Clontech), according to the supplier’s protocol. Yeast colonies growing in SD-Trp medium were transferred to either SD-Trp-His-Ade or fresh SD-Trp medium after 2 d of culture at 30 °C. The β-galactosidase assay was conducted using O-nitrophenyl-β-D-galactopyranoside (ONPG) as the substrate following the manufacturer’s specifications (Clontech).

**Yeast two-hybrid and one-hybrid assays**

Among TaMYB73’s homologous proteins, AtMYB15 from *Arabidopsis* was found to bind to MYB binding cis-elements in the AtCBF3 promoter and to interact with AtICE1 (Zhu et al., 2007). In order to know whether TaMYB73 can bind to the promoter of AtCBF3 and interact with AtICE1 as well, a yeast one-hybrid assay and a yeast two-hybrid assay were conducted respectively. For the yeast one-hybrid assay, MYB binding motif sequences were synthesized; different fragments containing different MYB binding cis-elements of promoter sequences of *Arabidopsis* AtCBF3 and AtABF3 were cloned using primer pairs with restriction digestion sites that flank each of the fragments with genomic DNA as the template (see Supplementary Table S1 at *JXB* online). These sequences were ligated into the EcoRI and SacI sites of the pHIS2 vector (Clontech). The pGADTK7 with full-length *TaMYB73* ORF and pHIS2 either with or without the MYB binding motif sequences/AtCBF3 or AtABF3 promoter fragments were co-transformed into the yeast strain Y187 (Clontech), and transformants were selected by exposure to SD-Trp-Leu medium at 30 °C for 2 d, after which surviving colonies were transferred to SD-Trp-Leu-His medium containing either 10 mM or 30 mM 3-AT for 2 d. For the yeast two-hybrid assay, a full-length *TaMYB73* ORF was PCR cloned (see Supplementary Table S1 at *JXB* online) into the pGADTK7 vector (Clontech); the full-length AtICE1 ORF was PCR cloned from *Arabidopsis* cDNAs and ligated into pGBKTK7 vector (Clontech). These vectors were transformed into the yeast AH109 strain (Clontech) for yeast two-hybrid analysis. Transformants were selected by growing on a SD-Trp-Leu medium at 30 °C. Surviving colonies were transferred to SD-Trp-Leu and SD-Trp-Leu-His-Ade medium for a further 2 d before observation.

**Generation of transgenic Arabidopsis plants**

*TaMYB73* was introduced into the BannH1 and Sall restriction endonuclease sites of the pSTART vector to construct a *TaMYB73* over-expressor driven by the cauliflower mosaic virus (CaMV) 35S promoter (De Amicis et al., 2007) using the primers listed in Supplementary Table S1 at *JXB* online, and then transformed into *Arabidopsis* Col-0 by the Agrobacterium tumefaciens-mediated floral dip method (Clough and Bent, 1998). Transformed *Arabidopsis* plants were grown in soil under a 16/8 h light/dark, 22/20°C, 60% relative humidity regime. Transformed lines were selected by growing them on one-half Murashige and Skoog (MS) agar plates containing 60 μg ml⁻¹ kanamycin under the same growth regime, and homozygous T_{3} progeny, bred from a T_{2} population segregating into three kanamycin-resistant to one kanamycin-sensitive, were used for the phenotypic analysis. *Arabidopsis* Col-0 transformed with the empty pSTART vector was used as the control.

**Response to applied stress of transgenic Arabidopsis**

Seeds harvested from both homozygous transgenic *Arabidopsis* carrying *TaMYB73* (OE lines) and the pSTART empty vector (VC line) were surface-sterilized by a 5 min immersion in 70% ethanol, followed by a 10 min treatment with 5% w/v NaOCl and finally rinsed several times in sterile water. They were then germinated for 72 h in the dark at 22 °C by placing on sterile, solidified half-strength MS medium. Uniformly germinated seeds were then transferred to half-strength MS plates with various concentrations of NaCl, LiCl, KCl, and mannitol and the plates were placed vertically and kept under a 16/8 h light/dark, 22/20 °C, 60% relative humidity regime for one week. Two-week-old seedlings of VC and OE lines grown in soils were watered with 100 mM NaCl every 2 d for 2 weeks.

**Germination assay of transgenic Arabidopsis**

VC and OE seeds were surface-sterilized as above, laid on solidified half-strength MS medium containing various concentrations of ABA (0–2 μM) or NaCl (0–200 mM), and held at 4 °C for 2 d in the dark before being transferred to 22 °C under a 16/8 h light/dark regime. The germination rate of seedlings producing open cotyledons was expressed as a percentage of the total number of seeds plated.

**Results**

**Analysis of TaMYB73 gDNA, cDNA, and upstream sequences**

In order to identify a MYB transcription factor gene that may be closely involved in the salinity response in wheat, probes were screened targeting a set of unigenes with annotation as MYB transcriptional factors in the wheat gene microarray in our previous transcriptomic analysis on SR3 (Wang et al., 2008). According to these unigenes, more than 40 MYB genes were cloned (data not shown). Of them, the gene (*TaMYB73*) harboured three probes, which were induced to the greatest extent in salinity-stressed SR3 (Table 1). In roots, transcript abundance rose by about 6-fold within 0.5 h of the imposition of stress, while at 24 h, the transcript level was still higher than that in non-stressed plants (Table 1). The 1200 bp cDNA sequence of *TaMYB73*...
Table 1. Three probes referring to TaMYB73 in a cDNA microarray

Expression of TaMYB73 in the roots of SR3 in the presence and absence of 200 mM NaCl, as determined by hybridization patterns with three probes lying within the TaMYB73 sequence. N0.5 ratio: log2 (transcript level 0.5 h after the imposition of salinity stress/transcript level in the absence of salinity stress). N24 ratio: log2 (transcript level 24 h after the imposition of salinity stress/transcript level in the absence of salinity stress). 1, 2, 3: three independent biological replicates. P values calculated from these three replicates.

<table>
<thead>
<tr>
<th>Probe</th>
<th>N0.5 ratio</th>
<th>N24 ratio</th>
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<tr>
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<td>1  2  3  Mean</td>
<td>1  2  3  Mean</td>
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<tr>
<td>ta_02121</td>
<td>2.41 2.24 2.85 2.50</td>
<td>2.63e-3</td>
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<tr>
<td>ta_02124</td>
<td>1.84 1.59 2.10 1.84</td>
<td>3.18e-3</td>
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<tr>
<td>ta_13116</td>
<td>2.43 2.31 2.64 2.46</td>
<td>7.89e-4</td>
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Comprised a 149 bp 5'-UTR, a 765 bp ORF, and a 286 bp 3'-UTR (Fig. 1a). A comparison with the genomic sequence showed that no intron was present (data not shown). The 254 residue predicted polypeptide included two conserved SANT domains at the N terminus (Fig. 1b). Phylogenetic analysis indicated that it clustered with R2R3 type MYB proteins of different plants (Fig. 1c), of which AtMYB15 has been confirmed as an important transcriptional factor involved in the salt- and drought-response signal transduction pathways in Arabidopsis (Agarwal et al., 2006; Ding et al., 2009). The TaMYB73 R2 and R3 SANT domains were conserved when compared with other plant MYB proteins (Fig. 1d). In the 847 bp upstream sequence of TaMYB73, ABA-responsive cis-elements, ACGT and ABRE, gibberellin-responsive cis-elements, GARE and MYC, as well as light-responsive G and E boxes were identified (see Supplementary Table S2 at JXB online).

TaMYB73 localizes in nuclei and has transactivation activity

The transient expression assay using onion epidermis cells and Arabidopsis mesophyll protoplasts both showed that TaMYB73 was localized in the nucleus (Fig. 2a). The transactivation assay indicated that all transformants grew well on the transformant selective SD/Trp plates (Fig. 2b), whereas only transformants of pGBK7 that was fused with full-length TaMYB73 ORF (covering 1–254 aa) and truncated TaMYB73 ORF fragments expressing C terminal amino acid sequences (covering 65–254 aa, 121–254 aa, and 179–254 aa, respectively) grew on the transactivation selective SD/Trp/Ade/His plates (Fig. 2b), showing that these transformants activated the transcription of reporter genes Ade and His in the genome of the yeast AH109 strain. Consistently, β-galactosidase activity analysis indicated that the β-galactosidase units of the transformant with pGBK7-TaMYB73 (covering 1–254 aa) were higher by about 2-fold than that of pGBK7, and those of the pGBK7s fused with truncated fragments (covering 65–254 aa, 121–254 aa, and 179–254 aa, respectively) were also significantly improved (Fig. 2c), demonstrating that the full-length and truncated TaMYB73 fragments containing C terminal 179–254 residues also activated the reporter gene LacZ in the genome of AH109, so that these transformants had higher β-galactosidase activity to catalyse more substrate OPNG. These two assays both indicated that TaMYB73 has transactivation activity, which is attributed to the C terminal 179–254 residues. In addition, the TaMYB73 protein can bind types I, II, and IIG MYB binding motifs (Fig. 2d).

TaMYB73 is induced by various abiotic stresses

RT-PCR profiling indicated that TaMYB73 expression was increased in SR3 roots between 0.5 h and 24 h after exposure to 200 mM NaCl, while in the leaf, its transcript level was somewhat lower than in the non-stressed controls (Fig. 3a). Under dehydration stress, an increase in the TaMYB73 transcript level was observed within 0.5 h of the imposition of the treatment (Fig. 3b). The expression of TaMYB73 was induced during the whole course (0.5–24 h) of exogenous JA treatment (Fig. 3c). However, the response to exogenous supply of other phytohormones (GA, ABA, ACC, and SA) was an increase in TaMYB73 expression within 0.5 h, but the transcript level returned to its background level thereafter.

TaMYB73 transgenic Arabidopsis shows enhanced salinity tolerance

In total, seven homozygous transgenic lines over-expressing TaMYB73 (OE) were obtained, and their TaMYB73 expression levels (transcription abundance of TaMYB73 to that of the internal reference gene AtActin) showed differences using real-time PCR. The vector control line (VC) had no detectable TaMYB73 transcript; by comparison with the transgenic line with the lowest expression level (6-13) as the standard (the relative expression level was 1), three other OE lines (3-21, 4-21, and 6-10) had the highest relative expression levels (the ratio of their expression levels to the that of the standard), and were selected for further analysis (Fig. 4a). When cultured in soil in the greenhouse, the plant phenotype, as well as developmental and reproductive ability such as flowering period and seed production, showed no significant difference among TaMYB73 OE and VC lines (upper panel in Fig. 4c; data not shown). When grown on solidified medium, the root length of OE plants was similar to that of VC plants (Fig. 4b). However, in the presence of 50 mM NaCl, leaf growth in VC plants was

...
retarded, and root elongation was poorer than that in OE plants (Fig. 4b). Root growth remained superior in OE plants as the NaCl concentration was raised to 100, 150, and 200 mM (Fig. 4b). Similarly, OE lines grown in soils irrigated with 100 mM NaCl showed stronger development capacity than the VC line (Fig. 4c). The germination rate of OE seeds was higher than that of VC seeds in the presence of various concentrations of NaCl (Fig. 4d). A similar result was also found with treatment with different concentrations of ABA and, in the presence of 2 μM ABA, the germination of VC seeds was abolished, but some OE seeds continued to germinate (Fig. 4e).

**TaMYB73 transgenic Arabidopsis is specifically tolerant to ionic stress**

When stressed by 5–15 mM LiCl, the OE lines appeared to be more tolerant than VC line (Fig. 5b, c). Roots of the OE seedlings also grew better than those of VC seedlings in the presence of 100 mM KCl (Fig. 5d). However, no obvious
difference between VC and OE seedlings could be observed under 100–300 mM mannitol stress (Fig. 5e, f).

TaMYB73 promotes transcription of a range of stress response genes in Arabidopsis

Under the control conditions, *AtABF3*, *AtMYB2*, and *AtRD29B* participating in the ABA-dependent pathway were all induced in OE lines; ABA-independent genes *AtRAB18* and *AtCBF3* were induced in OE lines, but *AtDREB2A* and *RD29A* showed inconsistent changes among the three OE lines (Fig. 6). Except for *AtDREB2A*, all of these genes had higher transcriptional levels in the OE lines than in the VC line under salt stress. This increase in the OE lines was minor in comparison with the VC line under both control and saline stress conditions except for *AtABF3* in two OE lines (3-21 and 4-21), the increased levels in the OE lines under control conditions were significantly lower than the induction level by salt stress in the VC line (Fig. 6). It should be noted that there was a strong variation of expression among three OE lines for *AtRAB18*, *AtCBF3*, *AtRD29A*, *AtABF3* and *AtRD29B* (Fig. 6), indicating that the induction strength of their expression can be correlated to the transcription abundance of transgenic TaMYB73.

TaMYB73 binds to promoters of *AtABF3* and *AtCBF3*

A yeast one-hybrid assay was conducted to detect whether or not TaMYB73 is a putative transcription factor directly to regulate expression of *AtABF3* and *AtCBF3*. The result showed that TaMYB73 can bind the upstream sequences of *AtCBF3* and *AtABF3* containing different MYB binding sequences such as MYBCORE and MYBCOREATCYB1.
indicating that AtABF3 and AtCBF3 are possibly direct targets of TaMYB73. This is consistent with AtMYB15 (Agarwal et al., 2006), directly regulating the expression of these two genes (Zhu et al., 2007). Apart from this similarity, the yeast two-hybrid assay showed that, unlike AtMYB15 which interacts with AtICE1 to accomplish its direct regulation on AtABF3 and AtCBF3 (Zhu et al., 2007), TaMYB73 did not interact with AtICE1 (Fig. 7b).

**Discussion**

**TaMYB73 encodes a novel salinity response transcription factor**

The MYB superfamily is the largest transcription factor family in plants. Sublocalization, β-galactosidase and transactivation assays, and yeast one-hybrid analyses indicate that TaMYB73 is a transcription factor (Figs 2, 7a). TaMYB73 clusters with R2R3 type MYB proteins of other plants (Fig. 1c), indicating that TaMYB73 is a R2R3 type MYB transcription factor. TaMYB73 can be identified on the basis of their conserved SANT domains, which are typically located in the N terminal region and critical for DNA binding activity, while SANT domains of different MYB proteins have their specificities for distinct types of binding motifs. As R2R3 type MYBs, AtMYB15 binds preferentially to types II and IIG, rather than type I (Agarwal et al., 2006), whereas TaMYB73 binds equally well to types I, II, and IIG (Fig. 2d). This indicates that TaMYB73 is a novel MYB TF gene of plants.

Various plant transcription factors are up- or down-regulated in response to abiotic stress (Yamaguchi-Shinozaki and Shinozaki, 2006), and the response is typically quite rapid (Tran et al., 2004; Liu et al., 2007). An increase in TaMYB73 expression in SR3 roots occurred within 0.5 h of the imposition of either dehydration or salinity stress (Fig. 3a, b), which was consistent with the report that Tamyb2 was rapidly induced under osmotic stress for 1 h (Chen et al., 2005), showing the important roles of MYB transcription factors in rapid response to abiotic stresses in plants. By contrast, in the SR3 leaf, the TaMYB73 transcript level was slightly reduced at 0.5 h after the imposition of salinity stress (Fig. 3a). The Arabidopsis MYB

![Fig. 3. Expression of TaMYB73 in SR3, as assessed by RT-PCR. (a) Three-leaf stage seedlings exposed to 200 mM NaCl and dehydration. (b) Response of three-leaf stage seedlings to exogenously supplied phytohormone. GA, gibberellic acid; ABA, abscisic acid; ACC, 1-aminocyclopropane-1-carboxylic acid; SA, salicylic acid; JA, jasmonic acid. Three repetitions were performed for each PCR analysis.](https://academic.oup.com/jxb/article-abstract/63/3/1511/474916/1517)

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transcription factor *AtMYB44* behaves in a very similar fashion (Jung et al., 2008). Because salt first enters the plant via the root (not via the leaf), any response is bound to occur earlier in the root than in the leaf. Similarly for moisture stress: the leaf can conserve its moisture by stomatal closure while this mechanism is not available to the root (Berthomieu et al., 2003). Therefore, the different temporal response of the leaf and root to stress suggests that *TaMYB73* expression simply reflects the extent of localized stress—the greater the stress, the more the gene is expressed. Apart from its rapid response to abiotic stress, *TaMYB73* is also induced by the exogenous application of the phytohormones ABA, JA, ACC, GA, and SA (Fig. 3b, c). Of these phytohormones, the effect of ABA and GA is partially attributed to the presence of the ABA- and GA-responsive cis-elements ACGT, ABRE, GARE, and MYC in the promoter sequence of *TaMYB73* (see Supplementary Table S2 at JXB online). More interestingly, unlike other phytohormones, the induction effect of JA is constitutive during the whole course of treatment. JA participates in biotic and abiotic stresses in plants, and has been found to exhibit antagonistic effects against ABA (Anderson et al., 2004; Moons et al., 1997), and to induce reactive oxygen species (ROS), nitric oxide, calcium influx, and mitogen-activated protein kinase (MAPK) and some salt-responsive genes such as *RD26* (Fujita et al., 2006). Thus, it appears that this transcription factor can be activated by a number of different signals, so that it represents a node integrating...
several signalling pathways. Similarly, the expression of the Arabidopsis MYB transcription factor gene *AtMYB102* has been documented to integrate signals derived from both wounding and osmotic stress (Denekamp and Smeekens, 2003).

**TaMYB73** plays a role in the response to salinity

MYB proteins are involved in the response to stress in plants which, in turn, facilitate the ability to cope with these stresses. For example, salt- and drought-induced *TaMYB2A* promotes tolerance to these stresses in *Arabidopsis* (Mao et al., 2011). Here, the over-expression of *TaMYB73* increased the tolerance of *Arabidopsis* to NaCl stress (Fig. 4b, c). It is concluded that the response to salt and drought stress is performed via both ABA-dependent and ABA-independent pathways. In transgenic *Arabidopsis* expressing *TaMYB73*, the ABA-independent pathway genes *AtRAB18* and *AtCBF3*, as well as the ABA-dependent genes *AtABF3* and *AtRD29B*, had more transcripts under both the control and salt-stressed conditions (Fig. 6). All of these genes have been reported to be induced under salt stress, and they also play positive roles in salt tolerance (Yamaguchi-Shinozaki and Shinozaki, 2006). For example, the over-expression of *AtABF3* in rice improves drought tolerance without stunting growth (Oh et al., 2005). The combined results of induced transcription of *TaMYB73* within 0.5 h of the ABA treatment (Fig. 3c) and the binding of TaMYB73 to the promoter sequences of *AtCBF3* and *AtABF3* (Fig. 7a) indicate that *TaMYB73* is a component modulating both the ABA-dependent and ABA-independent pathways. Moreover, although TaMYB73 and AtMYB15 both bind to promoters of *AtCBF3* and *AtABF3* (Agarwal et al., 2006), TaMYB73 does not interact with AtICE1 (Fig. 7b), a crucial interacting protein that interacts with AtMYB15 to decrease, but not increase, the expression of these two genes (Zhu et al., 2007), demonstrating the common and specific characteristics among different MYB proteins. Furthermore, the ABA-independent pathway gene *AtDREB2A* remained constant between the VC and OE lines under salt stress (Fig. 6), whereas over-expression of other MYB factors have been documented to promote the expression of *AtDREB2A* rather than *AtCBF3* (Dai et al., 2007; Liao et al., 2008). This shows the novel role of *TaMYB73* in the response to salinity stress.

The salinity tolerance enhancement is possibly achieved by coping with ionic stress

Interestingly, OE lines had an increased tolerance to high concentrations of LiCl and KCl, yet showed no osmotic tolerance (mannitol treatment) (Fig. 5). The ionic-stress-specific response was also found in *Arabidopsis sos4* and *nks1* mutants: the former is hypersensitive to NaCl, LiCl, and KCl (Shi et al., 2002), and the latter is hypersensitive to NaCl and KCl (Choi et al., 2011). Salinity is thought to trigger programmed cell death (PCD) in plants largely through ionic (rather than osmotic) stress (Huh et al., 2002), suggesting that *TaMYB73* functions to prevent salt-induced PCD. Given that salt stress comprises ionic and osmotic stresses (Munns and Tester, 2008), the salinity tolerance conferred by *TaMYB73* appears to be mainly based on an improved ionic tolerance rather than osmotic tolerance. However, this seems to conflict with the higher transcription levels of the above detected marker genes in OE lines, for these genes are reported to be induced by
drought and osmotic stress and their over-expression enhances the tolerance to osmotic and drought stresses. The possible cause is that most of these genes were increased in OE lines under both the control and saline stress conditions whereas the increase under the control conditions is only minor compared with the levels determined following salt treatment (Fig. 6), and therefore TaMYB73 is most likely to be a secondary player in the regulation of these stress-responsive genes in ABA-dependent and ABA-independent pathways, or this protein might undergo a post-translational modification under stress conditions which increase its potency as a transcription factor. Alternatively, it is proposed that the principal regulatory mechanism underlying the specific role of TaMYB73 in ionic stress may be beyond ABA-dependent and ABA-independent pathways. It has been found that the perception of the salt (ionic) stress signal as well as ionic absorption and intracellular compartmentation is fulfilled through both the SOS pathway (Yamaguchi-Shinozaki and Shinozaki, 2006) and the SOS-independent pathway (e.g. ER-associated endomembrane system) (Choi et al., 2011); however, in which pathway TaMYB73 participates is a question yet to be answered.

Taken together, the novel characteristic of TaMYB73 implies the complex roles of MYB transcription factors in salt response as well as their difference between Arabidopsis and wheat. TaMYB73’s specific role in tolerance to ionic stress provides an entrance to elucidate the difference in mechanisms underlying tolerance to salinity and osmotic stresses.

**Supplementary data**

Supplementary data can be found at JXB online.

**Supplementary Table S1.** Primer sequences used in this study.
Fig. 7. Binding ability of TaMYB73 with AtICE1 as well as promoters of AtABF3 and AtCBF3. (a) DNA binding activity of TaMYB73 in a yeast one-hybrid system. -1, -2, and -3 mean different fragments of the promoter sequences of AtCBF3 and AtABF3. (b) Interaction with AtICE1 in the yeast two-hybrid system.

Supplementary Table S2. Cis-elements present in the promoter of TaMYB73.

Acknowledgements

This work was supported by the National Basic Research 973 Program of China (2009CB118300 and 2012CB114200), the funds of the Major Program of the Natural Science Foundation of China (No. 30530480), and the National Transgenic Project (Grants 2009ZX08009-082B and 2008ZX08002-002). GenBank accession number JN969051.

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