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ORIGINAL ARTICLE

The soybean gene, *GmMYBJ2*, encodes a R2R3-type transcription factor involved in drought stress tolerance in *Arabidopsis thaliana*

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Abstract *MYB* genes are extensively distributed in higher plants and constitute one of the largest transcription factors (TFs) families. These TFs have been proved to be implicated in the regulation of plant growth, development, metabolism, and multiple abiotic stress responses. In the present study, a new soybean *MYB* gene, denoted *GmMYBJ2*, was isolated and its function was characterized. The *GmMYBJ2* cDNA is 1428 bp in length with an open reading frame (ORF) of 960 bp encoding 319 amino acids. Sequence and yeast one-hybrid analyses showed GmMYBJ2 contains two MYB domains and belongs to R2R3-MYB protein with transactivation activity. Transient expression analysis using the *GmMYBJ2-GFP* fusion gene in onion epidermal cells showed GmMYBJ2 protein is targeted to the nucleus. *GmMYBJ2* was induced by drought, cold, salt, and exogenous abscisic acid (ABA). *Arabidopsis* overexpressing *GmMYBJ2* exhibited a higher seed germination rates (GRs), a notable increase in the soluble sugar content under water-deficit stress, and a lower water loss rate (WLR) when water is sufficient. These results indicated the overexpression of *GmMYBJ2* make transgenic *Arabidopsis* more tolerant to drought stress than wild-type (WT) plants, and *GmMYBJ2* may be useful for improving drought stress tolerance in transgenic plant breeding.

Keywords Soybean · MYB transcription factor · Transgenic *Arabidopsis* · Drought tolerance

Abbreviations

TF(s)	Transcription factor(s)
ORF	Open reading frame
ABA	Abscisic acid
WT	Wild-type
PEG	Polyethylene glycol
β-tubulin	Beta-tubulin
CaMV35S	Cauliflower mosaic virus 35S
3-AT	3-Amino-1, 2, 4-triazole

Introduction

Plants are frequently confronted with adverse environmental conditions, for instance, drought, salt, and extreme temperature, which may seriously affect their growth and development. These adverse conditions have always resulted in decreased crop yields and economic losses. Therefore, cultivating the plant species tolerant to abiotic stresses has become a main objective of many crop breeders. In recent years, overexpression or knockdown of some key genes that mediate plant responses to environmental stress (e.g., drought stress) provides an effective method to enhance tolerance to water-deficit stress

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(Valliyodan and Nguyen 2006). The regulatory proteins translated from these pivotal genes often act as responders to various signals, resulting in expression modifications of stress-related genes. Moreover, the function of regulatory proteins can also be amplified and strengthened via some signal transduction cascades. Thus, some researchers working on plant stress response have focused on regulatory proteins (Kreps et al. 2002; Ahuja et al. 2010). TFs are pivotal regulatory proteins regulating its downstream target genes, and it has been proven that some of these TFs are implicated in stress responses in plants (Hu et al. 2008). In terms of differences in their DNA-binding domains, these TFs can be divided into diverse families, for instance, MYB, NAC, AP2, and WRKY (Dubouzet et al. 2003; Mare et al. 2004; Song et al. 2011).

In plants, MYB proteins comprise a large, functionally various family of TFs (Riechmann et al. 2000). These proteins have a structurally conserved DNA-binding domain, namely the MYB domain, which is made up of approximately 53 amino acid residues, each of which often forms a helix-turn-helix structure. In terms of the number of imperfect repeats (one, two, three, or four) in their MYB domain, MYB proteins can be classified into different subfamilies, such as MYB-related (with a partial or a single MYB repeat), R2R3-MYB (2R-MYB), R1R2R3-MYB (3R-MYB), and 4R-MYB (Dubos et al. 2010). MYB-related proteins are implicated in the regulation of cellular morphogenesis (Simon et al. 2007; Pesch and Hulskamp 2009) and secondary metabolism (Dubos et al. 2008; Matsui et al. 2008). Genes encoding 3R-MYB proteins have been observed in most eukaryotic genomes and participate in cell cycle control (Ito 2005; Haga et al. 2007). The 4R-MYB group is the smallest class, and in contrast with other groups, very little is known regarding the functions of these proteins in plants. It is worth noting that a majority of plant *MYB* genes encode 2R-MYB proteins and are confirmed to be concerned in cell fate and identity, regulation of primary and secondary metabolism, developmental processes, and especially responses to various stresses (Du et al. 2009). For instance, AtMYB2 plays a role in drought stress response in an ABA-dependent manner (Abe et al. 2003). *Arabidopsis AtMYB41* is induced by drought, ABA and salt, and presents up-regulated expression pattern (Cominelli et al. 2008; Lippold et al. 2009). Overexpressed *OsMYB4* has been demonstrated that it could enhance *Arabidopsis* tolerance to freezing stress (Vannini et al. 2004). TaMYB33, a novel member of MYB TF family from wheat, improves *Arabidopsis* tolerance to drought and salt stresses (Qin et al. 2012).

Soybean [*Glycine max* (L.)] is a vital crop that provides humans with abundant proteins and vegetable oil. However, with the rapid development of society and global climate change, its growth and development are increasingly affected by diverse environmental stresses. In soybean, some genes, whose expressions were induced by various stresses, have been isolated and their functions have been discussed (He et al. 2002; Luo et al. 2005; Wang et al. 2005; Liao et al. 2008a, b; Zhai et al. 2012, 2013). Some soybean *MYB* genes have been reported. For example, the expression of *GmMYB76*, *GmMYB92*, and *GmMYB177* is induced by NaCl, cold, or drought whereas not by ABA treatment (Liao et al. 2008b). Li et al. (2013) reported that GmMYB12B2 exhibits transcriptional activity and is involved in plant flavonoid biosynthesis. Su et al. (2014) reported that *Arabidopsis* overexpressing *GmMYBJ1* exhibited high resistance to drought and cold stresses. There are 252 *MYB*-encoding genes in soybean genome, including 244 typical 2R-, six 3R-, and two 4R-like MYB proteins (Du et al. 2012). Nevertheless, in contrast with other plant species, few soybean *MYB* genes participating in environmental stresses have been characterized. An in silico analysis looking for the *Arabidopsis* gene homologous to soybean is an effective way to predict gene function, and can provide extensive information regarding the gene which will be cloned. Based on in silico analysis, a soybean *R2R3-MYB* gene, which is designated *GmMYBJ2*, was isolated from soybean in this study, and its role in response to drought stress was characterized in *Arabidopsis* through the generation of transgenic plants overexpressing *GmMYBJ2*.

Materials and methods

Isolation and sequence analysis of GmMYBJ2 from soybean

Through screening the gene expression profiles of soybean Jilin32, together with the NCBI blast tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi), we found a cDNA clone exhibiting high homology with other plant *MYB* genes from the differently expressed genes. Total RNA was extracted from soybean Jilin32 and cDNA was synthesized using PrimeScript™ 1st Strand cDNA Synthesis Kit (Takara, China). Subsequently, reverse transcription polymerase chain reaction (RT-PCR) was carried out to obtain this clone. The *MYB* gene cloned was designated *GmMYBJ2* (GenBank accession number: KC759158). The primer pairs used for the isolation of *GmMYBJ2* were 5′-GA ACCTTGTCTCTGCTTCACCA-3′ (forward) and 5′-CA CACACGCACGCATACTCTTT-3′ (reverse). The specific PCR product was inserted into the pMD18-T cloning vector (Takara, China) and sequenced for its accuracy. The conserved MYB domain of the GmMYBJ2 protein was analyzed using online SMART software (Letunic et al. 2006), and phylogenetic analysis was performed using the

Neighbor-Joining (NJ) algorithm of MEGA 5.1 (Tamura et al. 2011).

Plant materials and stress treatments

For soybean seedlings treatment, about 24 plants (~8 plants per pot, each pot is considered as an independent repeat) for each treatment were used as starting material. Seedlings of the soybean cultivar Jilin32 were cultivated in Hoagland's solution under 16-h light/8-h dark cycles at 25 °C and 70 % humidity for 2 weeks (four-leaf stage) and then maintained in same solution with or without 150 mM NaCl, 100 μM ABA and 10 % polyethylene glycol (PEG) 8000. Regarding cold treatment, the seedlings were kept in an incubator at 4 °C. For sample preparation, ~0.2 g plant leaves were randomly collected from each pot (~8 plants) and then pooled as one sample. The collection time were 0, 1, 3, 6, 9, 12, and 24 h (0 h was the non-treated control) from the initiation of the treatments and immediately frozen in liquid nitrogen for total RNA extraction.

For transgenic *Arabidopsis* treatment, transgenic seeds from *Arabidopsis* plants were sterilized, planted in MS medium supplemented with different concentrations (treatment) of mannitol and incubated under normal conditions for germination. The germination rates (GRs) were scored on the 6th day after sowing. All of the treatments were repeated three times, and the data were assessed from the results of three independent experiments. For dehydration treatment in soil, the WT and transgenic plants were exposed to stress for 19 days by withholding water, and the phenotype was observed.

Quantitative RT-PCR (qRT-PCR) analysis

Total RNA from soybean leaves was extracted using RNAiso Plus (Takara, China) in accordance with the manufacturer's instruction, and converted to cDNA using an M-MLV kit (Takara, China). The qRT-PCR system and reaction program were carried out following the protocol described in Su et al. (2014). Each reaction was composed of 10 μL of SYBR Green I, 2 μL of the cDNA (~25 ng/μL) samples, 0.4 μL of ROX reference Dye II, 0.4 μL of 10 μM gene-specific primers, and finally added distilled water to a total volume of 20 μL. The primer pairs 5′-TCGGTTCCCACTAATACAGGTTT-3′ (forward) and 5′-ATAGTTGGTCCATCTGAGTCTGC-3′ (reverse) were used for *GmMYBJ2* amplification. *β-tubulin* (GenBank accession number: GMU12286) was amplified and used as an internal reference to standardize the data. The fold changes were determined using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). All qRT-PCR reactions were run in triplicate.

Subcellular localization and transactivation assay of GmMYBJ2

The *GFP* fusion vector was constructed as described in Su et al. (2014) to generate pBI121-*GmMYBJ2-GFP* construct controlled by CaMV 35S promoter. The recombinant construct was then introduced into *A. tumefaciens* strain EHA105 and subsequently transformed into onion epidermal cells. After 24 h of incubation at 25 °C in the dark, the GFP fluorescence was detected under a confocal microscope (Olympus, Japan). To explore the transactivation activity, the full-length ORF of *GmMYBJ2* was cloned into pGBKT7 vector (Invitrogen), which was in advance digested with the *Eco* RI and *Sal* I restriction enzymes. The positive plasmid pGBKT7-*GmMYBJ2* was subsequently transformed into yeast AH109 strain, and the transformants were selected on SD medium lacking Trp (SD/-Trp) at 30 °C for 3 days. After 2 days of culture at 30 °C, the yeast colonies were shifted from SD/-Trp medium to SD/-Trp-His-Ade medium, which 10 mM 3-amino-1, 2, 4-triazole (3-AT) was included. Furthermore, the β-galactosidase assay was conducted for examination of transactivation ability within 8 h.

Plasmid construction and *Arabidopsis* transformation

The GATEWAY clone technology was used to construct a plant expression vector based on the detailed method described by Karimi et al. (2002) and Su et al. (2014). The pCB35SR1R2-GFP-*GmMYBJ2* resultant was electroporated into *A. tumefaciens* EHA105 and the method transforming *Arabidopsis* Col-0 was in accordance with the description of Clough and Bent (1998). The transformed *Arabidopsis* plants were selected on MS medium containing 4 mg L^{-1} glufosinate-ammonium (Sigma). The homozygous T_3 progeny was confirmed by PCR and RT-PCR prior to further analysis.

Determination of excised-leaf water loss rate (WLR) and soluble sugar contents

Leaves from WT and overexpressed plants were detached at the rosette stage and weighed immediately at room temperature to obtain the initial weight (~0.5 g). Subsequently, the weight of the excised leaf was measured and recorded at different time points. Three replicates were used for each transgenic line and WT. The total soluble sugar contents were measured following the methods described by Bailey (1958), Song et al. (2011) and Su et al. (2014).

Analysis of genes regulated by GmMYBJ2 using qRT-PCR

Total RNAs from WT and transgenic *Arabidopsis* were extracted as mentioned above. The gene-specific primers for qRT-PCR are listed in Supplementary Table S1. The *Arabidopsis actin* gene (GenBank accession number: NM_112764) was chosen as an internal control.

Statistical analysis

Statistical analyses were performed using the SPSS 13.0 program. The differences were considered significant at $P < 0.05$ or $P < 0.01$.

Results

Isolation and sequence analysis of GmMYBJ2

Soybean Jilin32 is an improved variety with better tolerance to stress (e.g., drought and salt) and better disease resistance (Fu 1995). Based on an analysis of the gene expression profiles of soybean Jilin32 immature embryos (20, 30, and 50 d), we found six unknown cDNA clones that were homologous to other plant MYB TFs from the differentially expressed genes. These clones contained the full-length ORF, and their predicted translation product contained two conserved MYB domains. qRT-PCR was conducted to determine the expression pattern of these *GmMYB*s in response to abiotic stresses (not published). One of these showed significant modifications under abiotic stresses, and this gene was designated *GmMYBJ2*. Based on this finding and the results of phylogenetic analyses, this gene was picked for further functional analysis. The ORF of *GmMYBJ2* was 960 bp in length and encodes a protein with 319 amino acids with a calculated molecular mass of 36.09 kDa and a theoretical pI of 5.72. The alignment results between cDNA and soybean GmGDB database (http://www.plantgdb.org/GmGDB/cgi-bin/blastGDB.pl) demonstrated that *GmMYBJ2* contains two introns and is located on chromosome 4. The predicted GmMYBJ2 protein contains two conserved MYB domains, as determined by SMART analysis (Fig. 1), and the phylogenetic analysis revealed that it clustered with *Arabidopsis* AtMYB60, which belongs to the MYB protein family and participates in plant drought tolerance (Cominelli et al. 2005) (Fig. 2).

GmMYBJ2 is located in the nucleus and has transactivation activity in yeast

The transient expression assay using onion epidermis cells showed that the GmMYBJ2-GFP fusion protein was specifically localized in the nucleus, whereas the GFP fluorescence was visualized in the whole cells transformed with the *GFP* control vector (Fig. 3). MYB proteins usually play roles as a transcriptional activator or repressor. To verify our presumption, a yeast assay system was used for the analysis. The transactivation assay showed that all of the transformants could grow well on the selective SD/-Trp medium (Fig. 4a). However, only the transformants of pGBKT7 fused with the full-length ORF of *GmMYBJ2* could grow normally on the selective SD/-Trp/-Ade/-His medium and exhibited β-galactosidase activity when X-gal was added to the Whatman filter paper (Fig. 4b, c). The results confirmed that GmMYBJ2 is a transcriptional activator that activates the transcription of the reporter genes *Ade*, *His* and *LacZ*.

Expression patterns of GmMYBJ2 under abiotic stress

To further elucidate the role of *GmMYBJ2* in plants, its expression patterns in soybean seedlings treated with drought, salt, cold stresses, and ABA were monitored by qRT-PCR (Fig. 5), and photographs of the soybean seeds and non-treated seedlings are shown in Supplementary Fig. S1. A clear increase in the *GmMYBJ2* transcript was observed at 6 and 9 h after initiation of PEG treatment, and the transcript then rapidly decreased. A similar trend was observed with ABA treatment. For cold and salt treatments, the transcripts showed a gradual increase, and both peaked 24 h after initiation of the treatments. *GmMYBJ2* was induced by salt, ABA, cold, and drought, but the transcripts increased more obviously under PEG treatment to levels nearly 16-fold higher compared with those observed in the control.

Monitoring of seed germination and the changes in physiological parameters of GmMYBJ2-overexpressing Arabidopsis under stress conditions

Due to the marked change in the expression of *GmMYBJ2* under drought stress, we inferred that this gene may be implicated in drought tolerance in plants. Four transgenic lines, which were positive plants, as verified by PCR and RT-PCR, were used in these experiments. Transgenic seeds harvested from overexpressed *GmMYBJ2* and WT plants were sowed on MS medium supplemented with various concentrations of mannitol for germination, and the GRs were measured (Fig. 6). The seeds from all of the transgenic lines displayed higher GRs in contrast with the WT when the mannitol concentration was 200 and 300 mM. For instance, the WT seed GRs were approximately 73.85 and 53.85 % with mannitol concentrations of 200 and 300 mM, respectively, whereas approximately 86 and

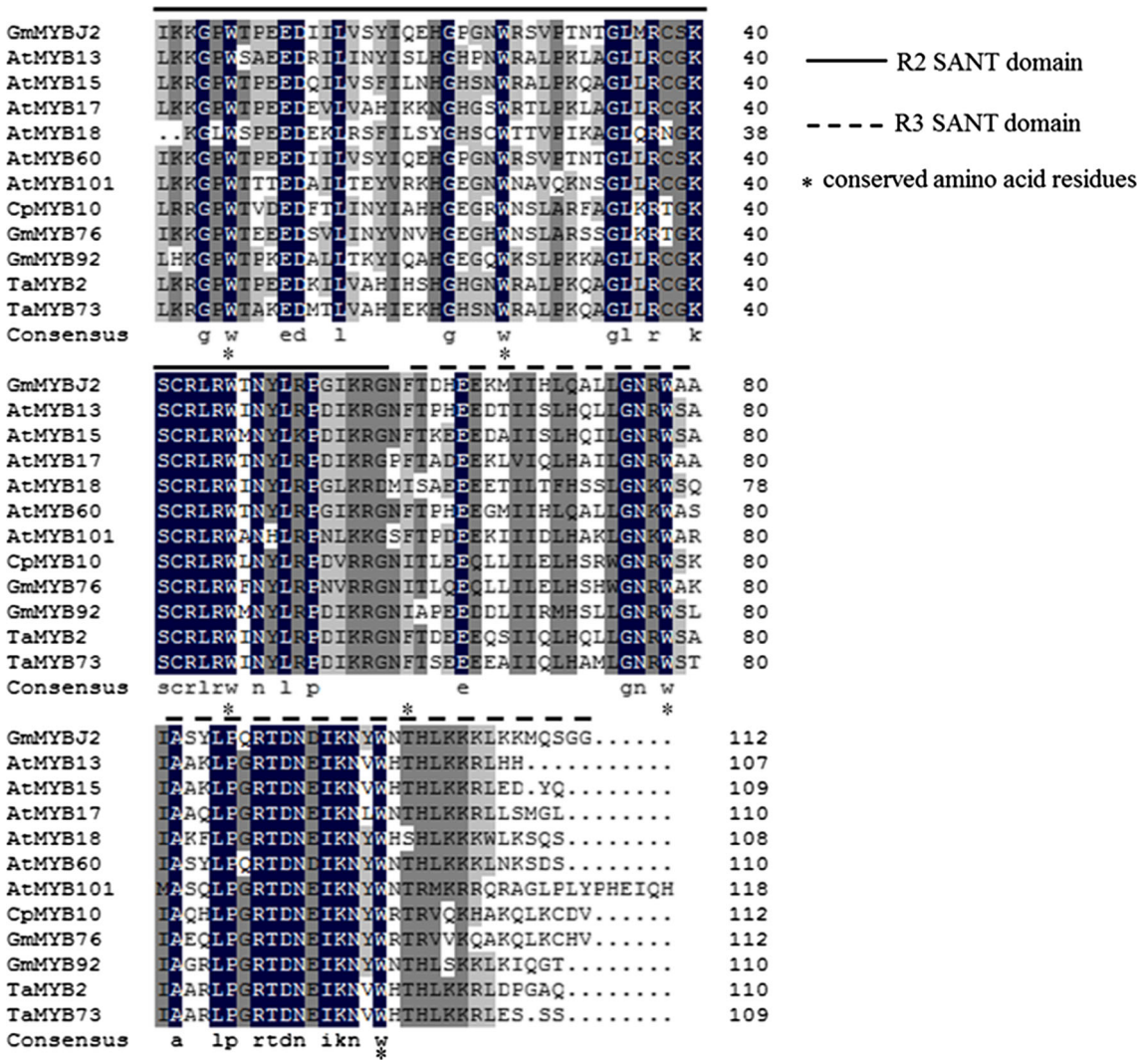

Fig. 1 Alignments of the deduced amino acids of GmMYBJ2 with those of other plant MYBs: TaMYB2 (AAT37168.1), TaMYB73 (AEW23186), AtMYB13 (NP_172108), AtMYB17 (XP_002876601), GmMYB92 (ABH02844.1), AtMYB60 (NP_973795.1), GmMYBJ2 (AGN96216.1), CpMYB10 (AAM43912.1), GmMYB76 (ABH02836.1), AtMYB18 (XP_002869667), and AtMYB101 (AEC08688.1)

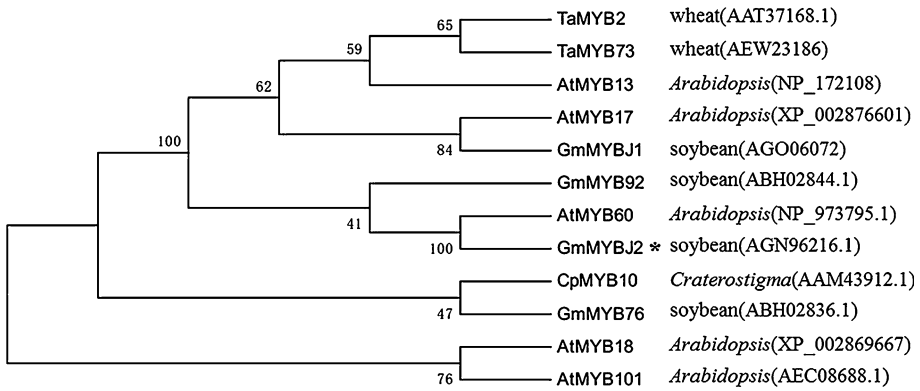

Fig. 2 Neighbor-joining tree of the predicted GmMYBJ2 (*starred*). The bootstrap values (1000 replicates) are indicated in the branches. The plant sources and GenBank accession numbers are indicated

78.5 % of the transgenic seeds could germinate at the corresponding mannitol concentrations. The seedlings in normal MS medium were then transferred into soil for normal growth (Fig. 7a). After 7 days, all of the plants were subjected to water withholding for 19 days. The results indicated that the WT plants presented smaller

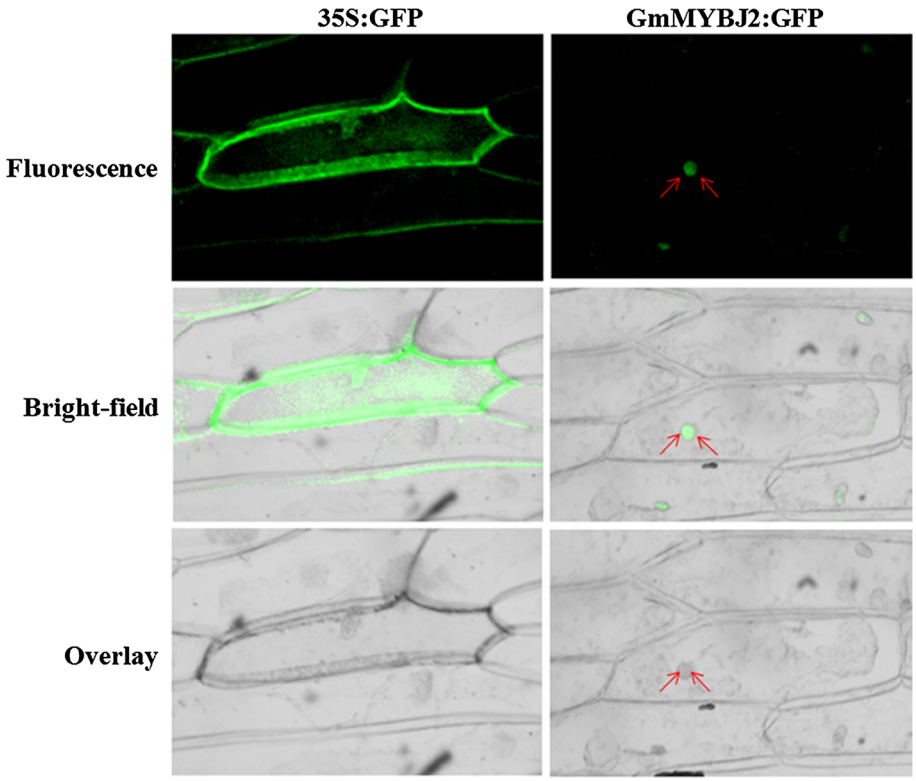

Fig. 3 Nuclear localization of GmMYBJ2. p35S:GFP (as a control) and p35S:GFP-GmMYBJ2 were transiently expressed in onion epidermal cells

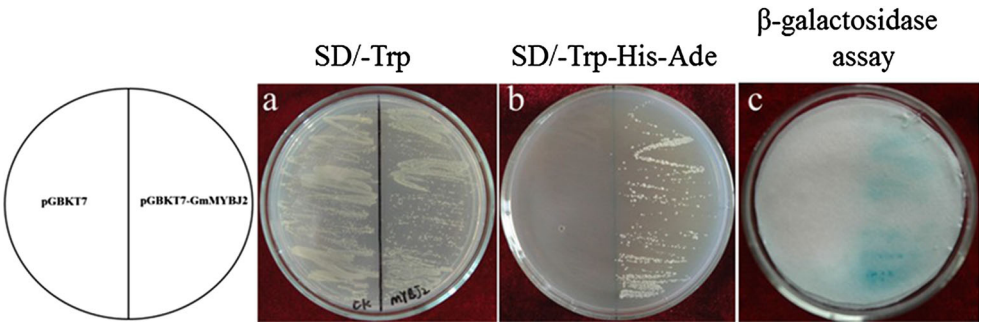

Fig. 4 Transactivation analysis of *GmMYBJ2* in the yeast AH109 strain. The fusion protein of the GAL4 DNA-binding domain and *GmMYBJ2* were expressed. The empty pGBKT7 vector was expressed as a control. **a** Growth of the transformants on an SD plate lacking tryptophan (SD/-Trp plus 10 mM 3-AT). **b** Growth of the transformants on an SD plate lacking tryptophan, histidine and adenine (SD/-Trp-Ade-His plus 10 mM 3-AT). **c** β-galactosidase activity assay

leaves with obvious etiolation and wilting, whereas the transgenic line showed better growth (Fig. 7b).

The physiological traits of plants are often altered under stress conditions. To explore the physiological mechanism when plants were under stress conditions, the changes in water loss and soluble sugar contents in overexpressed lines and WT were assessed. The fresh weights of the detached leaves from transgenic and WT plants were used for assessment of the water loss at the designated time points. The four transgenic lines displayed lower WLR than the WT plants within 10 h after leaf detachment (Fig. 8). Soluble sugar is an osmotic regulation substance. Alterations in the soluble sugar content are reported to be connected with the plant response to unfavorable conditions, and its accumulation is considered as a very important adaptive mechanism for coping with stress. No significant difference was observed in soluble sugar content between the transgenic and WT plants when plants were normally incubated. However, its content in the transgenic plants displayed obviously higher levels than that in the WT plants after drought stress treatment (Fig. 9).

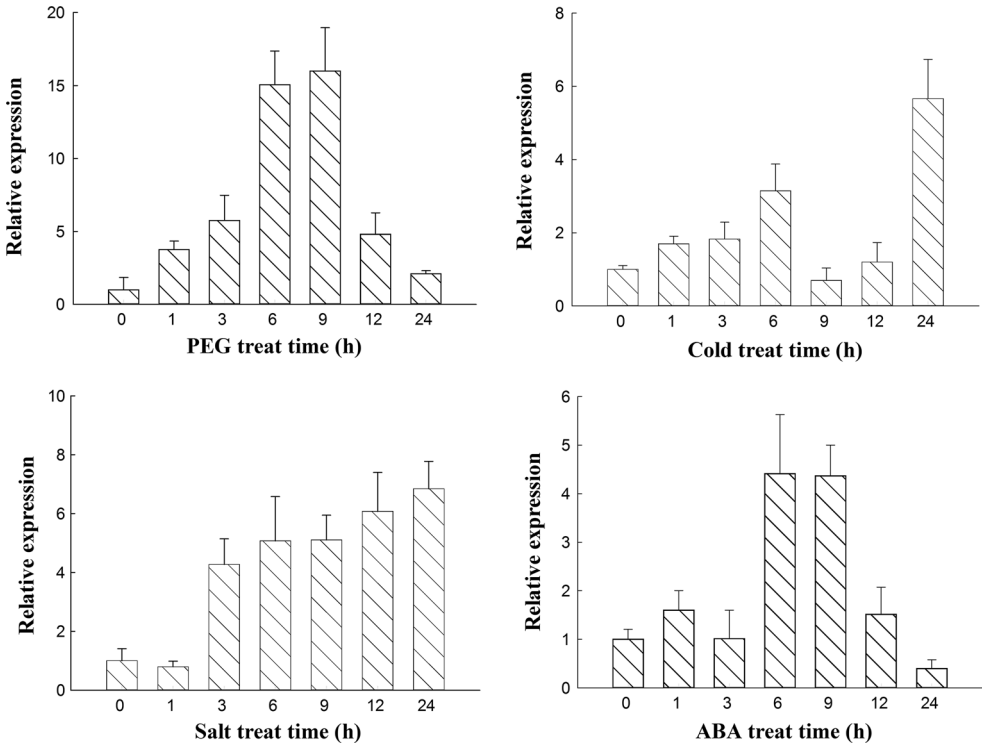

Fig. 5 Quantitative real-time PCR analysis of *GmMYBJ2* transcripts under PEG, cold, salt, and ABA treatments. Samples were collected 0, 1, 3, 6, 9, 12, and 24 h after initiation of the treatments. The data represent the mean ± SD of three biological repeats

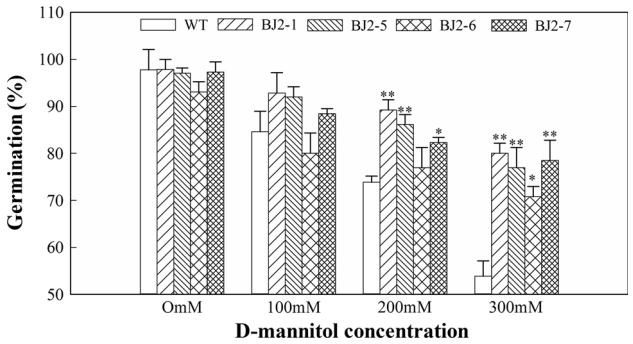

Fig. 6 Comparison of the germination rates of transgenic seeds under drought stress. BJ2-1, BJ2-5, BJ2-6, and BJ2-7 represent the transgenic lines. The data represent the average of three independent experiments ± SD. * and ** indicate significant ($P < 0.05$) and highly significant differences ($P < 0.01$) between the transgenic lines and the WT

GmMYBJ2 altered the expression of abiotic stress-responsive genes

The overexpression of *GmMYBJ2* enhanced plant resistance to drought stress. To further elucidate the molecular mechanism, the expression levels of six downstream stress-responsive genes, which were chosen according to Liao et al. (2008a) and Qin et al. (2012), in *GmMYBJ2*-overexpressed lines grown under non-stressed conditions were examined by qRT-PCR. In contrast with WT plants, *ERD10*, *DREB2A*, *COR15a*, *RD29A*, and *RD29B* were shown to be up-regulated to varying degrees in transgenic plants (Fig. 10). Among these genes, the expression level of *RD29B* increased by approximately 14-fold in the BJ2-1 transgenic line compared with the WT plant and by almost six-fold in the other three lines. However, no obvious expression changes in *RD17* were observed.

Discussion

TFs play a very important role in regulating gene expression when plants suffer from adverse conditions. The plant MYB family is large and these regulatory proteins are frequently exhibits multiple functions (Dubos et al. 2010). Recent studies on *MYB* genes in rice, wheat, *Arabidopsis*, and other plants have demonstrated that numerous MYB proteins are implicated in stress response (He et al. 2012; Qin et al. 2012; Yang et al. 2012). In the present study, a new member of the soybean *MYB* gene family, denoted *GmMYBJ2*, was isolated, and its function was analyzed.

Sequence analysis using some databases and software packages is a primary and effective way to predict gene function. Analyses of the amino acid sequence and phylogenetic tree of GmMYBJ2 showed that it contains two highly conserved MYB domains and clusters with R2R3-MYB proteins from other plants (Figs. 1, 2), indicating that GmMYBJ2 is an R2R3-MYB TF. A majority of MYB proteins are presumed to be transcriptional activators with

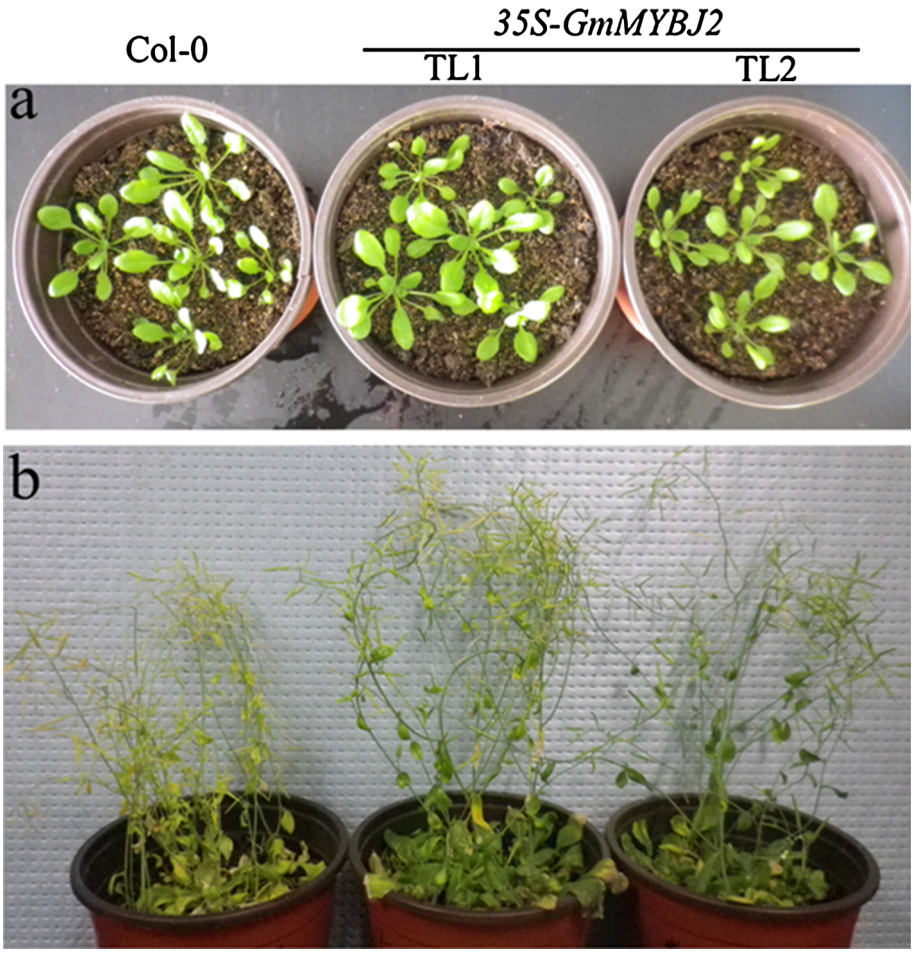

Fig. 7 Effects of drought stress on the transgenic plants overexpressing *GmMYBJ2*. **a** Phenotypic comparison of plant growth before treatment. **b** Phenotypic comparison of plants **a** after 7 days of normal growth followed by water withholding for 19 days. Col-0 indicates the WT plants, and TL1 and TL2 represent the transgenic lines

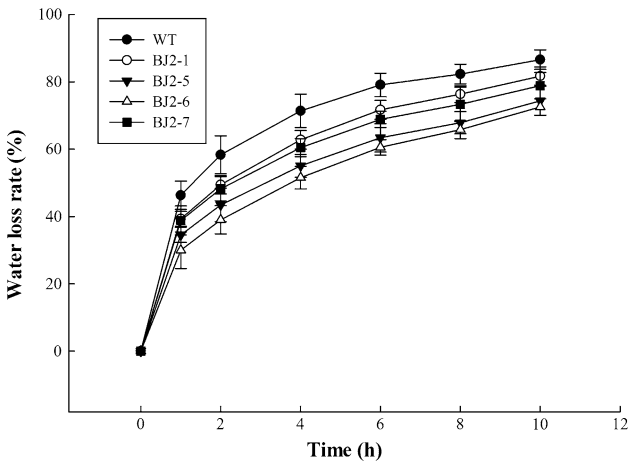

Fig. 8 Comparison of the water loss rate of the detached leaves from the WT and transgenic *Arabidopsis* plants. BJ2-1, BJ2-5, BJ2-6, and BJ2-7 represent the transgenic lines overexpressing *GmMYBJ2* used in the analysis. The data represent the means of three replicates ± SD

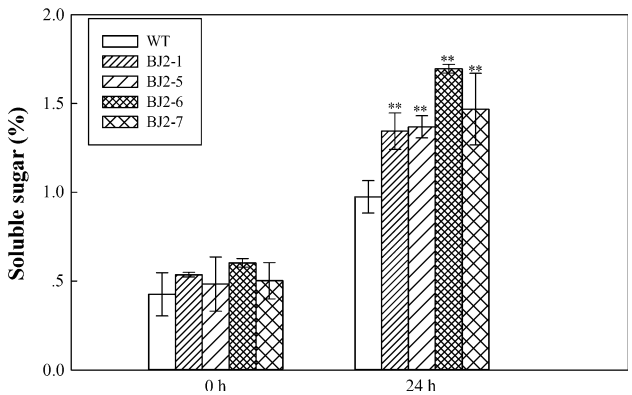

Fig. 9 Soluble sugar content in the *GmMYBJ2*-overexpressing transgenic plants, and the transgenic lines BJ2-1, BJ2-5, BJ2-6, and BJ2-7 were used for the analysis. The data represent the means from three independent tests ± SD. ** indicates that the differences between the transgenic lines and WT are highly significant ($P < 0.01$)

activation domains (ADs) in the C-terminal region (Weston 1998). It is well-known that N-terminal region of MYB proteins are conserved, however, the sequences in the C termini are generally not, and not all MYB TFs function as activators in the nucleus. The sub-localization of GmMYBJ2 showed that it is a nucleus-localized protein (Fig. 3), which is in agreement with its role as a TF. β-

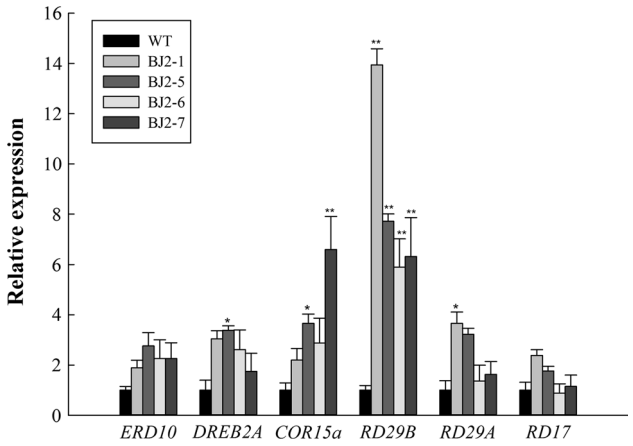

Fig. 10 Expression levels of stress-responsive genes in the WT and *GmMYBJ2* transgenic plants under normal growth conditions, and BJ2-1, BJ2-5, BJ2-6, and BJ2-7 represent the transgenic lines. The data represent the means of three replicates. The *error bars* indicate the SD. * and ** indicate significant ($P < 0.05$) and highly significant differences ($P < 0.01$) between the transgenic lines and the WT

galactosidase and transactivation assays using a yeast one-hybrid system pointed that it has transactivation activity and may act as an activator in the yeast assay system (Fig. 4). This observation needs to be further examined in a plant system.

In plants, such as *Arabidopsis*, wheat and rice, many MYB TFs have been depicted to taken part in stress response. AmMYB1, a single-repeat MYB TF, is powerfully induced by diverse stresses, such as salt, light and ABA, and confers NaCl tolerance to tobacco (Ganesan et al. 2012). He et al. (2012) described that *TaMYB73* is induced by NaCl, dehydration and several phytohormones and that ectopic expression of *TaMYB73* improves plant resistance to salinity. Zhang et al. (2012) reported that *TaMYB30-B* is a PEG stress-induced gene, and overexpression of the gene uncovered TaMYB30-B protein could improve stress tolerance to drought during the germination and seedling stages. *OsMYB2* is induced by cold, salt, drought, and ABA and enhances the tolerance of plants to salinity, chilling, and dehydration in rice (Yang et al. 2012). In soybean, Liao et al. (2008b) reported that *GmMYB76*, *GmMYB92*, and *GmMYB177* was induced by salt, cold, or drought but not influenced by ABA treatment, and overexpressed *Arabidopsis* displayed higher resistance to salt and freezing. *GmMYBJ1* was remarkably induced by cold and drought stresses (Su et al. 2014). The present study showed the *MYB* gene, *GmMYBJ2*, from soybean could be induced by diverse stresses, particularly drought, at the seedling stage (Fig. 5), inferring that *GmMYBJ2* may play important roles in multiple signaling transduction pathways responsive to abiotic stress.

To further investigate the role of *GmMYBJ2* in plant systems, overexpressed plants were generated. The seed GRs were compared between transgenic and WT seeds (Fig. 6). An increase in the mannitol concentration resulted in decreases in the GRs of WT and transgenic seeds, whereas a markedly lower GR was observed in the WT seeds. After 19 days of withholding water, the transgenic lines displayed better growth in comparison with WT plants (Fig. 7). These findings inferred that *GmMYBJ2* is implicated in regulating the plant response to water-deficit stress and may be used for plant improvement. In addition, these results are also partly consistent with the function of AtMYB60, which clusters with GmMYBJ2 (Fig. 2). It has been reported that *AtMYB60* exhibits guard-cell-specific expression and participates in the tolerance of plants to drought by regulating stomatal movements. Moreover, ABA usually participates in the process by strongly down-regulating the expression of *AtMYB60* (Cominelli et al. 2005). In response to drought stress, stress response hormone ABA was usually accumulated, resulting in rapid closing of the stomata, to restrain water loss by transpiration (Cominelli et al. 2005; Gray 2005). Hamanishi et al. (2012) also found that drought exposure results in decreases in stomatal conductance and an alteration in stomatal development in *Populus balsamifera*, and some genes that could strengthen drought-responsive changes in stomatal development were found to be altered in poplar. Further studies are required to elucidate whether a similar mechanism occurs with *GmMYBJ2*, which regulates the stomatal movements of plants and consequently enhances their tolerance to drought. Previous studies have demonstrated that numerous genes responding to drought stress, including the *MYB* genes, could be induced by the application of exogenous ABA (Nakashima et al. 2009). Zhu et al. (2014) reported that the *EsWAX1* gene from *E. salsugineum*, whose product is a 2R-MYB protein, is induced rapidly by exogenous ABA and drought stress. Overexpression of *EsWAX1* improves *Arabidopsis* tolerance to drought. In the present study, *GmMYBJ2* was also found to be induced rapidly by exogenous ABA and drought stress (Fig. 5); thus, we inferred that *GmMYBJ2* may be positively correlated with the ABA signaling pathways and may regulate ABA-mediated gene expression when plants undergone environmental stress. More experiments will be required to verify our presumption in the future.

Various physiological responses can be induced in plants when suffering from drought stress (Seki et al. 2007). The WLR and soluble sugar content are considered as important indicators of drought resistance and are widely used in this research field (Dedio 1975; Vinocur and Altman 2005). In our present study, in contrast with WT, the WLR was found to be lower in the detached leaves from overexpressed lines (Fig. 8), which is in concert with the results reported for the soybean *ERF*, soybean *MYB*, wheat *MYB* and *WRKY* gene family (Zhang et al. 2012; Niu et al. 2012; Zhai et al. 2013;

Zhu et al. 2014; Su et al. 2014). The results suggest that the transgenic plants may exhibit better water retention abilities. Soluble sugar is an important osmolyte for facilitating osmoregulation, thereby protecting plants from dehydration due to osmotic stress by reducing the cell water potential. Previous studies have confirmed the correlation between the existence of exceptional soluble sugars and the acquisition of stress tolerance (Kerepesi and Galiba 2000; Cominelli et al. 2005; Song et al. 2011; Zhang et al. 2012; Zhai et al. 2012; Su et al. 2014). In this study, more soluble sugars were accumulated in overexpressed lines compared with WT plants when drought stress was presented (Fig. 9), supporting the hypothesis that a greater accumulation of soluble sugar contributes to plant resistance to stress. The transactivation assay results showed that GmMYBJ2 may be an activator (Fig. 4). Some downstream genes, including *AtERD10*, *AtDREB2A*, *AtCOR15a*, *AtRD29B*, and *AtRD29A*, were activated and up-regulated to different levels in the transgenic plants (Fig. 10). It has been confirmed that these genes are implicated in multiple stress responses (Kang et al. 2002; Sakuma 2006). *DREB2A,* a TF containing AP2 domain, is induced by salt and osmotic stress. Overexpression of *DREB2A* could induce feeble expression of its downstream genes in the case of unstressed situations (Liu et al. 1998). DRE or related motifs are existed in the promoter regions of *RD29A*, *RD17,* and *COR15a*, and they are induced by cold, salt, and dehydration stresses (Narusaka et al. 2003; Simpson et al. 2003). The *RD29B* gene could slowly make response to dehydration stress and is probably induced by endogenous ABA produced under dehydration conditions (Yamaguchi-Shinozaki and Shinozaki 1993). Kiyosue et al. (1994) reported that *ERD10* could make rapidly response to water-deficit stress in *Arabidopsis,* and its expression could be induced when ABA was applied. Thus, we reasoned that *GmMYBJ2* may confer drought stress tolerance through the up-regulation of downstream genes. Additionally, some research indicated that overexpression of some genes in plant can cause severe growth retardation, a stunted phenotype or a never-produced seed (Dai et al. 2007; Onate-Sanchez et al. 2007). In our study presented here, the *GmMYBJ2*-overexpressing lines exhibited normal growth under normal conditions (Fig. 7a).

Collectively, *GmMYBJ2*, an *R2R3-MYB* gene, was isolated from soybean. *GmMYBJ2* encodes a nucleus-localized protein and functions as a transcriptional activator, and its transcript was found to be rapidly induced by ABA and drought stress. Overexpression of *GmMYBJ2* in *Arabidopsis* improved plant tolerance to dehydration stress, and the enhanced tolerance maybe resulted from the activation of stress-responsive genes and alterations in some physiological traits. The results provide insights into the soybean MYB TFs activated in response to environmental stresses and suggest this gene could be a candidate for crop improvement.

Author contribution statement L. T. Su and Q. Y. Wang designed the research. L. T. Su and Y. Wang performed the majority of the experiments and wrote most of the paper. D.Q. Liu provided assistance during the research study and analyzed the gene expression profile. X. W. Li and Y. Zhai provided guidance and advice on experimental techniques and performed the yeast one-hybrid and subcellular localization assays. All of the statistical analyses were performed by X. Sun and J. W. Li, and these authors performed the sample collection for qPCR and the transformation of *Arabidopsis*. X. Y. Li and Y.J. Liu contributed technical assistance and insightful discussions. Q.Y. Wang supervised the study. All of the authors have read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest There are no conflicts of interest.

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