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Molecular characterization of an *ABA insensitive 5* orthologue in *Brassica oleracea*

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ABSTRACT

ABI5 (ABA insensitive 5), a bZIP (Basic leucine zipper) transcription factor, has been shown to be a major mediator of plant ABA responses during seed germination. Although the molecular basis of ABI5-modulated processes has been well demonstrated in *Arabidopsis thaliana*, its identity and function in cabbage (*Brassica oleracea* var. capitata L.) remain elusive. Here, we describe our identification of *BolABI5* (an *ABI5* orthologue in *B. oleracea*) as a functional bZIP transcription factor in the modulation of plant ABA responses. Expression of *BolABI5* was dramatically induced by drought stress and exogenous ABA. Heterogeneous expression of BolABI5 rescued the insensitive phenotype of Arabidopsis *abi5-1* to ABA during seed germination. Subcellular localization and trans-activation assays revealed that BolABI5 was localized in the nucleus and possessed DNA binding and trans-activation activities. Deletion of the bZIP domain generated BolABI5ΔbZIP, which no longer localized exclusively in the nucleus and had almost no detectable DNA-binding or trans-activation activities. Overall, these results suggest that *BolABI5* may function as *ABI5* in the positive regulation of plant ABA responses.

1. Introduction

ABA (abscisic acid), one of the most important plant phytohormones, plays crucial roles in plant growth and development as well as in the response to several types of adaptive stress (reviewed in [1]). When challenged by drought stress and high salinity, the concentration of endogenous ABA has been observed to increase significantly, resulting in major changes in both stress-responsive gene expression and the adaptive physiological responses of plants [2–4]. Understanding the early molecular mechanism of ABA signaling has long been a major goal of plant research. Recently, it has been demonstrated that the double-negative regulatory mechanism is the core module of the early ABA signaling pathway [5,6].

In plants, there are several transcription factors involved in the regulation of seed germination [7–11]. Basic leucine zipper (bZIP) transcription factors comprise one of the largest transcription factor families involved in the regulation of this process. ABI5 (ABA insensitive 5), which has been shown to play crucial roles in controlling the expression of seed-specific and ABA-inducible genes, is one of the few bZIP transcription factors that has been thoroughly analyzed [12,13]. Genetic studies have revealed that *ABI5* loci, together with *ABI3* (*ABA insensitive 3*) loci, were involved in delaying the growth of germinating embryos during seed germination [14]. Similarly, orthologues of ABI5 and ABI3 have also been shown to participate in modulating the expression of embryogenesis-related genes in other plant cells [10,15].

In Arabidopsis, there are 75 bZIP transcription factors that have been identified and classified into 10 subfamilies by the sequence similarity of their bZIP regions. Thirteen members exhibiting high sequence similarity to ABI5 have been classified into the ABI5 subfamily [16,17]. However, besides ABI5, only a few members of this subfamily have been functionally analyzed. Molecular characterization has revealed that several members of this subfamily are specific in their binding of DNA sequences, dimerization and transcriptional activity [18]. Furthermore, many of these bZIP factors have been shown to be involved in ABA- or stress-signaling responses [16–18].

ABI5 subfamily members have been observed to bind the promoter regions of many stress-responsive genes, most of which contained at least two ABRE (ABA responsive element) *cis*-elements. The ABRE core sequence has been determined by various promoter assays [9,17]. In the past decade, a number of ABRE-binding bZIP transcription factors have been functionally characterized from different plants. Interestingly, several of these factors play a variety of roles in several essential plant life processes and ABA-dependent adaptive physiological responses [2,7,8,17,19,20].

Cabbage (*Brassica oleracea* var. capitata L.) is an important economic vegetable and oil resource [21]. Similar inhibitory effects of ABA on germination have been found previously in Arabidopsis and cabbage. Although cabbage exhibits a deep evolutionary homology with Arabidopsis, the early molecular mechanism of ABA signaling in cabbage has remained largely elusive [21]. In

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the present study, we describe the isolation and characterization of an ABI5 orthologue, *BolABI5*, from *B. oleracea* var. capitata L., "Qingan 80". Taken together, our data reveal that *BolABI5* can play the same role as *ABI5* in the modulation of plant ABA responses.

2. Materials and methods

2.1. Plant materials, growth conditions and stress treatments

Seeds of Wassilewskija (Ws-2), *abi5-1* and transgenic plants harboring *Flag-BolABI5* (*abi5-1::Flag-BolABI5*) were placed on MS or MS medium supplemented with 2 μM ABA, stored for 4 days at 4 °C in darkness for stratification and grown at 22 °C (16 h-light/8 h-dark cycle) for 7 or 12 days. Plants were grown in controlled-environment growth chambers under a 16 h-light (23 °C)/8 h-dark (20 °C) light cycle.

For abiotic stresses and exogenous hormone treatments, 11-day-old seedlings of cabbage were treated with abiotic stresses (300 mM NaCl, −1.7 MPa PEG-8000) and hormones (0.1 mM ABA, 10 μM IAA, 10 μM GA, or 1 μM BR), followed by sampling at 0, 4, 8 and 12 h. Drought stress was achieved by leaving the intact seedlings in the air without water supply, followed by sampling at 0, 4, 8 and 12 h.

2.2. Plasmid construction

To produce Flag-tagged BolABI5, the full-length coding sequence (CDS) of *BolABI5* was amplified by RT (reverse-transcription)-PCR with the following primers: BolABI5-*Bam*F, 5′-CGCGGATCCATGATGTCTGGACGAGAAGTAG-3′ and BolABI5-*Sal*R, 5′-ACGCGTCGACGAGAGGGCAACTAGGGTTCCTC-3′ (the *Bam*HI and *Sal*I sites are underlined). The sequence-confirmed, full-length CDS of *BolABI5* was then cloned into *Bam*HI and *Sal*I sites of the binary vector p1307-Flag.

To make GFP-tagged *BolABI5* (BolABI5-GFP), the CDS was removed from p1307-Flag-*BolABI5* and inserted into the Cam-35S-GFP vector between the *Bam*HI and *Sal*I sites, resulting in a C-terminal fusion to GFP.

To delete the bZIP motif, we used the BolABI5-*Bam*F and BolABI5-310*Sal*R 5′-ACGCGTCGACATCCACTTGCCCATGTCCTAGC-3′ (the *Sal*I site is underlined) primer pair for PCR amplification. The product was inserted into the Cam-35S-GFP vector between *Bam*HI and *Sal*I sites.

To express *ABI5* and *BolABI5* in yeast, the CDSs of *ABI5*, *ABI5ΔbZIP*, *BolABI5* and *BolABI5ΔbZIP* were cloned into the pPC86 vector between *Sal*I and *Eco*RI sites with the following primer pairs: for *BolABI5*, BolABI5-*Sal*F, 5′-ACGCGTCGACCATGATGTCTGGACGAGAAGTAG-3′ and BolABI5-*Eco*R, 5′-CCGGAATTCTCAGAGAGGGCAACTAGGGTTC-3′; for *BolABI5ΔbZIP*, BoABI5-*Sal*F and BolABI5-310*Eco*R, 5′-CCGGAATTCATCCACTTGCCCATGTCCTAGC-3′; for *ABI5*, ABI5-*Sal*F, 5′-ACGCGTCGACCATGGTAACTAGAGAAACGAAGTTG-3′ and ABI5-*Eco*R, 5′-CCGGAATTCTTAGAGTGGACAACTCGGGTTC-3′; for *ABI5ΔbZIP,* ABI5-*Sal*F and ABI5-320*Eco*R, 5′-CCGGAATTCTCCATCTGAAGACACCGGGCTTAAC-3′ (the *Sal*I and *Eco*RI sites are underlined).

To clone a hexamer of ABRE (6×ABRE), the ABRE oligonucleotides were synthesized with the sense strand sequence, 5′-GAAGTCCACGTGGAGGTGG-3′, and antisense strand sequence, 5′-TCCCACCTCCACGTGGACT-3′ (the core ABRE sequences are underlined). To clone a hexamer of mABRE (6×mABRE), the mABRE oligonucleotides were synthesized with the sense strand sequence, 5′-GAAGTAACATGTTCGGTGG-3′ and antisense strand sequence, 5′-TCCCACCGAACATGTTACT-3′ (the core mABRE sequences are underlined). The ssDNA phosphorylation, annealing and dsDNA phosphorylation were performed as previously described [20]. Two individual DNA fragments of approximately 120 bp around were extracted from an agarose gel and inserted into the pBluescript SK+ vector (Stratagene), resulting in the recombinant plasmids p6×ABRE and p6×mABRE, which contained an ABRE or mABRE hexamer, respectively. This result was confirmed by sequencing. ABRE/mABRE hexamers (6×ABRE/6×mABRE) were excised from the p6×ABRE and p6×mABRE plasmids and inserted into the pRS315-HIS vector between the *Bam*HI and *Xba*I sites, respectively.

2.3. Construction of BolABI5 transgenic plants

The p1307-Flag-BolABI5 construct was transformed into the *Agrobacterium tumefaciens* strain GV3101 and then infiltrated into *abi5-1* or Col-0 plants with the floral dip method. Seeds (T1) from infiltrated plants were selected on MS medium containing 25 μg/L hygromycin (Roche).

2.4. RT-PCR (reverse-transcription PCR) and qRT-PCR (real-time quantitative RT-PCR) analysis

Total RNA was extracted from seeds, 11-day-old seedlings, roots, young leaves, stems, heading leaves, flowers and samples after treatment with TRIzol reagent (Vigorous Biotechnology). Ten micrograms of total RNA was treated with RNase-free DNase (TaKaRa) to remove DNA, and 2 μg of treated RNA was used for reverse transcription with M-MLV reverse transcriptase (Promega) according to the manufacturer's protocol. For RT-PCR, the transcript-specific primers, 5′-TGTTTGTCTGCAGCTGGTAG-3′ and 5′-GCTCTCAACACATAAAAGCATCAAAG-3′ were used to examine the expression level of *BolABI5*. The cabbage 18S rRNA was amplified as an internal control with primers 18S-F, 5′-TAGGCGGTTCATCGGGTTG-3′ and 18S-R, 5′-AAGGGCTGGTAAGGTTCTGC-3′.

Real-time quantitative RT-PCR was performed using an IQ5 real-time PCR machine (Bio-Rad, Hercules, CA, USA) and SYBR Premix Ex Taq kit (TaKaRa) to monitor double-stranded DNA products [22]. The relative expression of *BolABI5* was normalized to the expression of cabbage *ACTIN* and expressed relative to the level in mock-treated seedlings. The following primer pairs were used: for *BolABI5*, 5′-GCTAATAACGGCGTTGGTGAG-3′ and 5′-TACACCCACCGAGAAAGAAGG-3′; for cabbage *ACTIN*, 5′-TCTCCTTGTACGCCAGTGGTC-3′ and 5′-CTCCATCTCCTGCTCGTAGTC-3′.

2.5. Western blot analysis

The total protein of transgenic seedlings was homogenized in IP buffer (10 mM Tris–HCl, pH 7.5, 0.5% Nonidet-P40, 2 mM EDTA, 150 mM NaCl, 1× protease inhibitor cocktail (Roche)) and cleared by centrifugation at 13,000*g* for 10 min at 4 °C. The resulting soluble protein was quantified by a Bradford protocol (TIANGEN Biotech), and 20 μg of protein was then separated by 12% SDS–PAGE and blotted onto a polyvinylidene difluoride membrane (Millipore). The blot was immediately blocked in 5% non-fat milk for 1 h. After washing three times with PBST buffer, the blot was probed with anti-Flag antibody (Sigma–Aldrich, 1:5000 dilutions) and then horseradish peroxidase-conjugated anti-mouse antiserum (Sigma–Aldrich, 1:5000 dilutions) as a secondary antibody. Signals of horseradish peroxidase-conjugated anti-mouse antiserum with ECL™ chemiluminescence substrate (GE Healthcare) were detected by film [22].

2.6. Subcellular localization

The Cam-35S-BolABI5-GFP and Cam-35S-BolABI5ΔbZIP-GFP vectors were introduced into the *A. tumefaciens* strain GV3101 and then infiltrated into 5- to 6-week-old *Nicotiana benthamiana* leaves for transient expression as previously described [23]. The

Agrobacterium strains were infiltrated at an OD_{600} of 0.5. For microscopic analyses, leaf disks were cut 3 days after infiltration. Cells from the lower epidermis were analyzed at room temperature with 20% glycine as the imaging medium. GFP-fluorescence signals were examined under an inverted Zeiss LSM 510 META fluorescence confocal microscope.

2.7. Yeast one-hybrid

The yeast strain yWAM2 was used to perform a yeast one-hybrid assay. Yeast transformation and growth assays were performed according to the Yeast Protocols Handbook provided by Clontech. Briefly, pPC86-BolABI5 combined with either pRS315-6×ABRE-HIS or pRS315-6×mABRE-HIS were transformed into the yeast strain yWAM2 with the lithium acetate/single-stranded carrier DNA/polyethylene glycol method. The transformed yeast cells were selected on synthetic complete medium lacking leucine and tryptophan (SC-LW). DNA binding and transactivation were determined by measuring the growth of serial dilutions of transformed yeast cells on synthetic complete medium lacking leucine, tryptophan and histidine (SC-LWH) for 2–3 days.

3. Results and discussion

3.1. Isolation and sequence analysis of BolABI5 in cabbage

A 1278 bp fragment of *BolABI5* was isolated from the flower tissue of cabbage via RT-PCR. *BolABI5* encodes a protein with 425

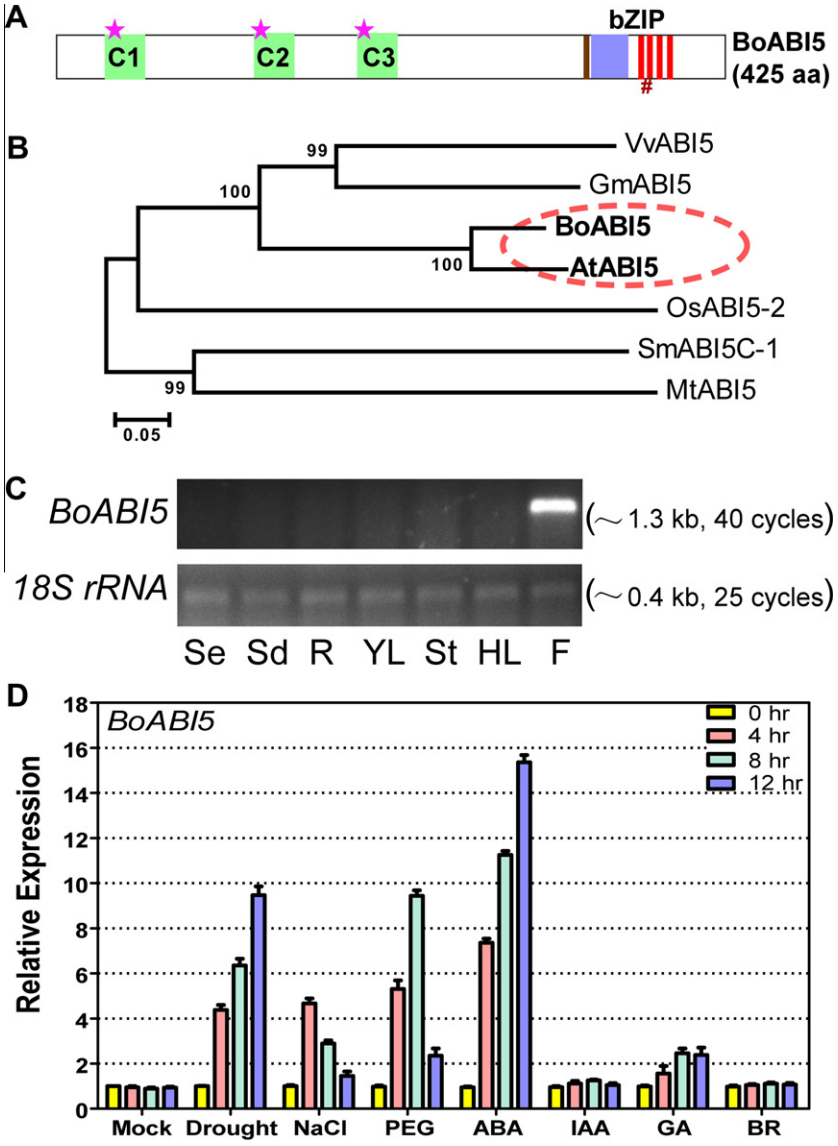

Fig. 1. Domain organization, sequence analysis and expression patterns of *BolABI5*. (A) Schematic diagram of domains in the BolABI5 protein. Three N-terminal conserved sequences (C1, C2 and C3) are shown in the green box, the basic domain is shown in the blue box, the bipartite nuclear localization signal is shown in the black brown rectangle and the Leu residues defining the Leu zipper are shown in the red rectangle. ☆, the conserved phosphorylation sites are shown in purple; #, the conserved sumoylation site is shown in brown. (B) Sequence relationships between BolABI5 and its orthologues. Deduced amino acid sequences of BolABI5 and its orthologues were aligned with MEGA 5.1 (http://www.megasoftware.net/). The dendrogram was generated by the neighbor-joining method, and evolutionary distances were computed using the Poisson method. VvABI5 (XP_002276783); GmABI5 (XP_003553595.1); BolABI5, *Brassica oleracea* ABI5-like protein; AtABI5, *Arabidopsis thaliana* abscisic acid insensitive 5 protein; OsABI5-2 (ABM90395); SmABI5C-1 (XP_002964990); MtABI5 (XP_003603049). (C) RT-PCR analysis of *BolABI5* expression in various tissues. Se, seeds; Sd, seedling; R, root; YL, young leaves; St, stem; HL, heading leaves; F, flower. (D) qRT-PCR analysis of the expression pattern of *BolABI5* under various environmental stress conditions and after treatment with exogenous hormones. Eleven-day-old seedlings were treated with abiotic stress (300 mM NaCl, −1.7 MPa PEG, drought) and hormones (0.1 mM ABA, 10 μM IAA, 10 μM GA or 1 μM BR), followed by sampling at 0, 4, 8 and 12 h. The relative expression of *BolABI5* was normalized to the expression of the cabbage *ACTIN* gene and expressed relative to the level in mock-treated seedlings.

amino acids (GenBank Accession No. JX870620). Amino acid analysis showed that BolABI5 contains nearly all of the conserved regions of ABI5 and ABI5-like proteins, such as the three putative phosphorylation sites at the N-terminus and an NLS (Bipartite nuclear localization signal), bZIP motif and putative sumoylation site at the C-terminus (Fig. 1A). Phylogenetic analysis revealed that BolABI5 shares an even deeper evolutionary homology with Arabidopsis ABI5 (Fig. 1B). ABI5 participated in the fine-tuning of ABA-mediated processes, such as seed germination, seedling growth and stress-responsive gene expression [9,12,13]. It was reported that *HvABI5* is indispensable for ABA induction of gene expression, which is an *ABI5* orthologue in barley [10]. In addition to modulating the adaptive stress response, *OsABI5* also has crucial roles in plant fertility [8,19]. Furthermore, *ABL1*, another rice *ABI5*-like gene, has been shown to be an important node in the cross-talk of ABA–auxin signaling [7]. BolABI5 and ABI5 were observed to be highly conserved (Fig. 1A and B), which led us to further investigate the role of BolABI5 in plants.

3.2. BolABI5 is mainly expressed in flowers and is induced by ABA and stress conditions

Accumulation of both *ABI5* transcripts and ABI5 protein upon exogenous ABA treatment has been determined by time-course analysis [12]. Several genes encoding members of the *ABI5* subfamily, such as *AREB1* and *AREB2*, also displayed ABA-, drought- and

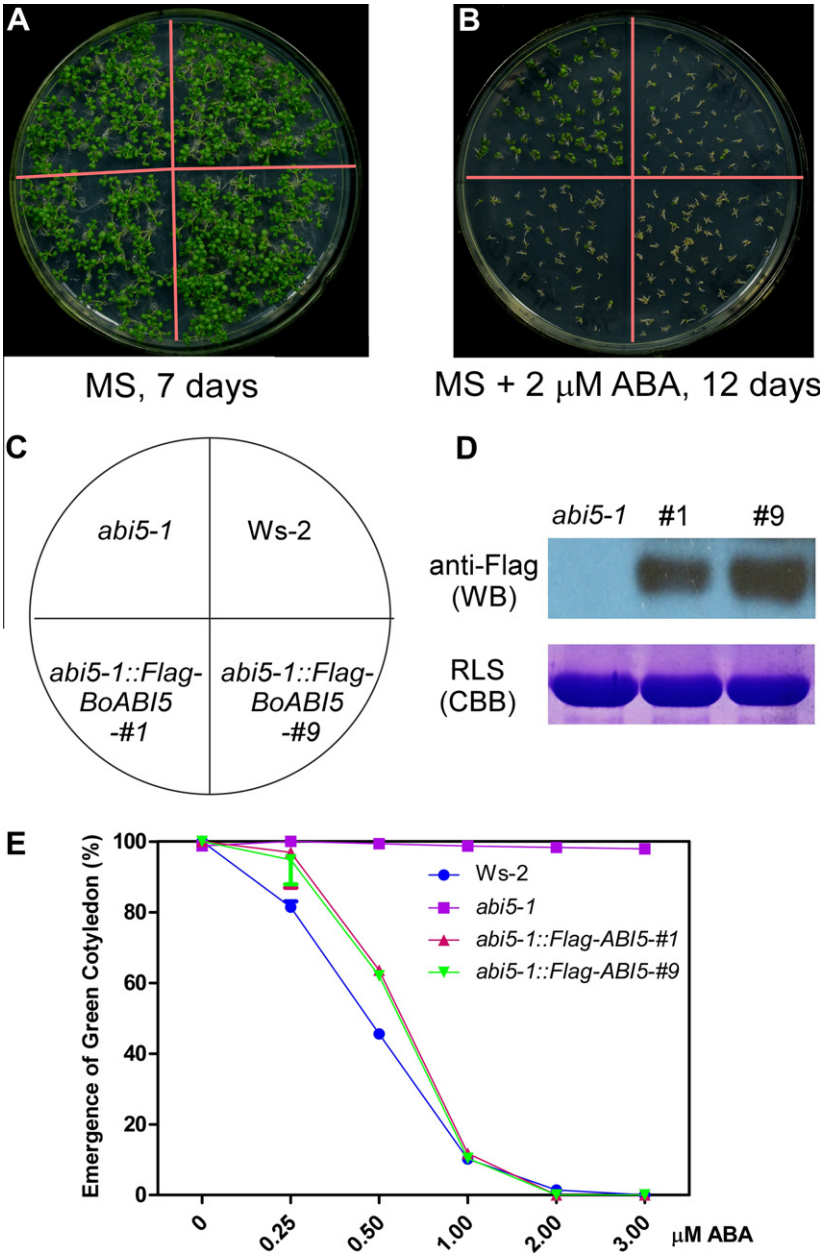

Fig. 2. Heterogeneous expression of *BolABI5* rescues the insensitivity of Arabidopsis *abi5-1* to ABA during seed germination. (A, B) Sensitivity of seeds to ABA. The seeds of Ws-2, *abi5-1*, and transgenic *abi5-1* lines carrying *Flag*-tagged *BolABI5* (*abi5-1*::*Flag-BolABI5*) were germinated on MS medium (A) and MS medium supplemented with 2 μM ABA (B) for the indicated days. The schematic of plants sown is shown in (C). (D) Western blots of Flag-BolABI5 protein levels in the transgenic *abi5-1* lines. Lane 1, transgenic *abi5-1* lines carrying the empty *Flag*-tagged vector; lanes 2 and 3, independent transgenic *abi5-1*::*Flag-BolABI5*. CBB (Coomassie Brilliant Blue) R250-stained Rubisco large subunit (RLS) served as a loading control. (E) The emergence rate of green cotyledons from Ws-2, *abi5-1* and *abi5-1*::*Flag-BolABI5*-#1/#9 transgenic seeds plated on MS supplemented with ABA. Approximately 150 seeds were used in each experiment. Error bars represent SD (seed number > 100).

high salinity-inducible expression profiles [16]. Recently, expression of *OsABI5* and *ABL1*, two *ABI5*-like genes in rice, was examined. Similar to other members of this subfamily, both genes were induced by ABA and stresses [7,8]. To further characterize the function of BolABI5, we first investigated the expression of *BolABI5* throughout its life cycle. As shown in Fig. 1C, transcripts of *BolABI5* were only detected in flower tissue, consistent with the easy isolation of *BolABI5* cDNA from flowers.

To further study the expression profiles of *BolABI5* under various environmental stress conditions and after treatment with exogenous hormones, we used qRT-PCR. The inducible expression of *BolABI5* was observed within 4 h after drought, NaCl, PEG and ABA treatments. Furthermore, the expression levels of *BolABI5* under drought, stress and ABA treatment were observed to significantly increase throughout a 12 h testing period (Fig. 1D), and the expression levels of *BolABI5* under high-salt and PEG treatments were observed to plateau at 4 and 8 h, respectively (Fig. 1D). However, expression of *BolABI5* was minimally stimulated by other plant hormones, such as IAA, GA and BR (Fig. 1D). Transcriptional reprogramming in response to certain types of stress often provides insight into the function of the changed genes [22]. Therefore, the stress-induced hyperexpression patterns of *BolABI5* suggested that it might be involved in plant ABA or drought responses.

3.3. BolABI5 can rescue the ABA-insensitive phenotype of abi5-1 during seed germination

The *abi5-1* mutant is insensitive to the ABA inhibition of seed germination in *Arabidopsis* [13]. To address whether *BolABI5* has a biologically significant role in the plant ABA response, we constructed *abi5-1* transgenic lines containing *Flag-BolABI5* (*abi5-1::Flag-BolABI5*). Two representative transgenic lines of the T3 generation were chosen to test seed germination in the presence of ABA. As previously reported, *abi5-1* showed a high germination frequency following 2 μM ABA treatments (Fig. 2A–C). In contrast with *abi5-1*, *abi5-1::Flag-BolABI5* transgenic lines were as sensitive to ABA as Ws-2 plants (Fig. 2A–C). We also determined the germination frequency of Ws-2, *abi5-1* and *abi5-1::Flag-BolABI5* under different ABA concentrations. As shown in Fig. 2E, *abi5-1::Flag-BolABI5* plants displayed similar percentages of seedlings with green cotyledons as Ws-2. The level of Flag-BolABI5 protein was also analyzed in transgenic lines (Fig. 2D). All of these results indicated that *BolABI5* was able to compensate for *abi5* deficiency during seed germination.

In Arabidopsis, overexpression of the *ABI5* subfamily members *ABF3* and *ABF4* has been shown to make plants hypersensitive to ABA during seed germination [24]. To further determine the biological significance of *BolABI5* in plant ABA signaling, we constructed plants overexpressing *BolABI5* and examined their responses to exogenous ABA. Surprisingly, we observed no significant difference in the sensitivity of Col-0::35S-*Flag-BolABI5* (Col-0 carrying the BolABI5 overexpression construct) transgenic plants to ABA during seed germination, although hyper-accumulation of BolABI5 protein was detected in the transgenic plants (data not shown). Phosphorylation has been previously reported to be essential for the full-level activation of ABI5 [12,25]. It is reasonable to believe that overexpression of BolABI5 alone may have a minor role in plant ABA responses. Combined with complementation and overexpression data, our results suggest that BolABI5 can play the same role as ABI5 in the modulation of plant ABA responses during seed germination.

3.4. Subcellular localization of the BolABI5-GFP fusion protein

An online motif scan tool (http://hits.isb-sib.ch/cgi-bin/PFSCAN) revealed that there is also an NLS at the C-terminus of BolABI5 (Fig. 1A), which has been shown to be a typical characteristic of many transcription factors. Consequently, we set out to determine the subcellular localization of BolABI5. To accomplish this, we observed the fluorescent signals of BolABI5-GFP and BolABI5ΔbZIP-GFP under a fluorescence microscope. As shown in Fig. 3A–F, the BolABI5-GFP fusion protein was observed to localize exclusively in the nucleus of the cell, whereas the BolABI5ΔbZIP-GFP fusion

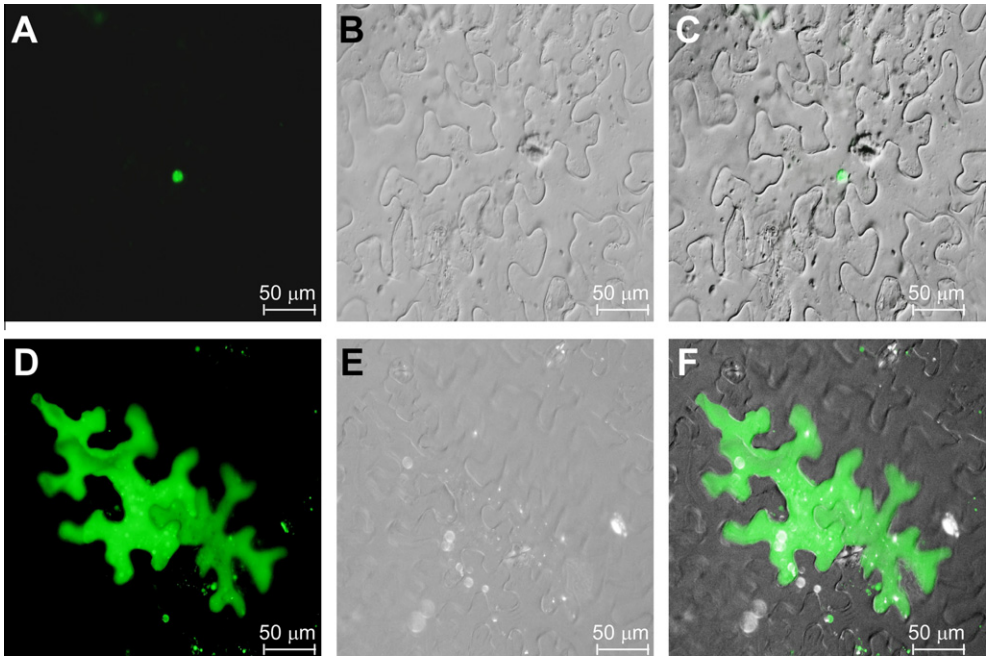

Fig. 3. Nuclear localization of the BolABI5 protein. (A–C) The GFP fluorescence signal of BolABI5-GFP. (D–F) The GFP fluorescence signal of BolABI5ΔbZIP-GFP. (A, D) Green fluorescence under dark field. (B, E) Cell morphology of the lower epidermis of a tobacco leaf under bright field. (C, F) Overlay of bright-field and green fluorescence signals. Bars = 50 μm. BolABI5ΔbZIP is a form of BolABI5 that carries a deletion of the intact C-terminal bZIP region.

protein was observed throughout the cell. These data suggest that BolABI5 is a nuclear-localized protein.

3.5. Transactivation and DNA-binding activity of BolABI5 to ABRE in yeast

ABI5 bZIP subfamily members are known to mainly bind ABRE/G-box elements [9,17,26]. The binding specificity of the OsABI5 splicing variants OsABI5-1 and OsABI5-2 to the G-box element was previously determined by a yeast one-hybrid assay [19]. Similarly, the binding of another rice ABI5-like protein, ABL1, to ABRE and ABRE-containing promoters was verified by yeast one-hybrid and EMSA (electrophoretic mobility shift assay), respectively [7]. To further analyze whether nuclear BolABI5 actually has DNA binding and transactivation activity, we used the yeast one-hybrid technique. The full-length coding regions of *BolABI5* and *ABI5* were introduced into the pPC86 vector, which contains a GAL4 activation domain (Fig. 4A). Hexamers of either ABRE or mutated ABRE fragments was introduced upstream of a *HIS* reporter gene in the pRS315-HIS vector (Fig. 4A). Combinations of the indicated plasmids were transformed into the yeast strain yWAM2, and growth of the transformants was observed. All of the transformants grew well on SC-LW medium (Fig. 4B, left panel). Nevertheless, only the yeast cells containing pPC86-BolABI5/pRS315-6×ABRE-HIS and pPC86-ABI5/pRS315-6×ABRE-HIS grew well on the SC-LWH selection medium (Fig. 4B). Low levels of DNA binding and transactivation activity were observed in the transformants containing constructs lacking an intact C-terminal bZIP region (pPC86-BolABI5ΔbZIP/pRS315-6×ABRE and pPC86-ABI5ΔbZIP/pRS315-6×ABRE, Fig. 4B, right panel, lanes 6 and 9). These results revealed that BolABI5 possesses similar DNA binding and transactivation activity to ABI5 and that the C-terminal bZIP domain is the primary contributor to these activities.

In this study, we succeeded in isolating and characterizing BolABI5, an ABI5 orthologue found in *Brassica oleracea* var. capitata L. Taken together, our data suggest that BolABI5 is a functional bZIP transcription factor that can play the same role as ABI5 in plant ABA responses during seed germination. As BolABI5 overexpression did not produce a significant difference in ABA sensitivity, a comprehensive investigation of its putative post-translational

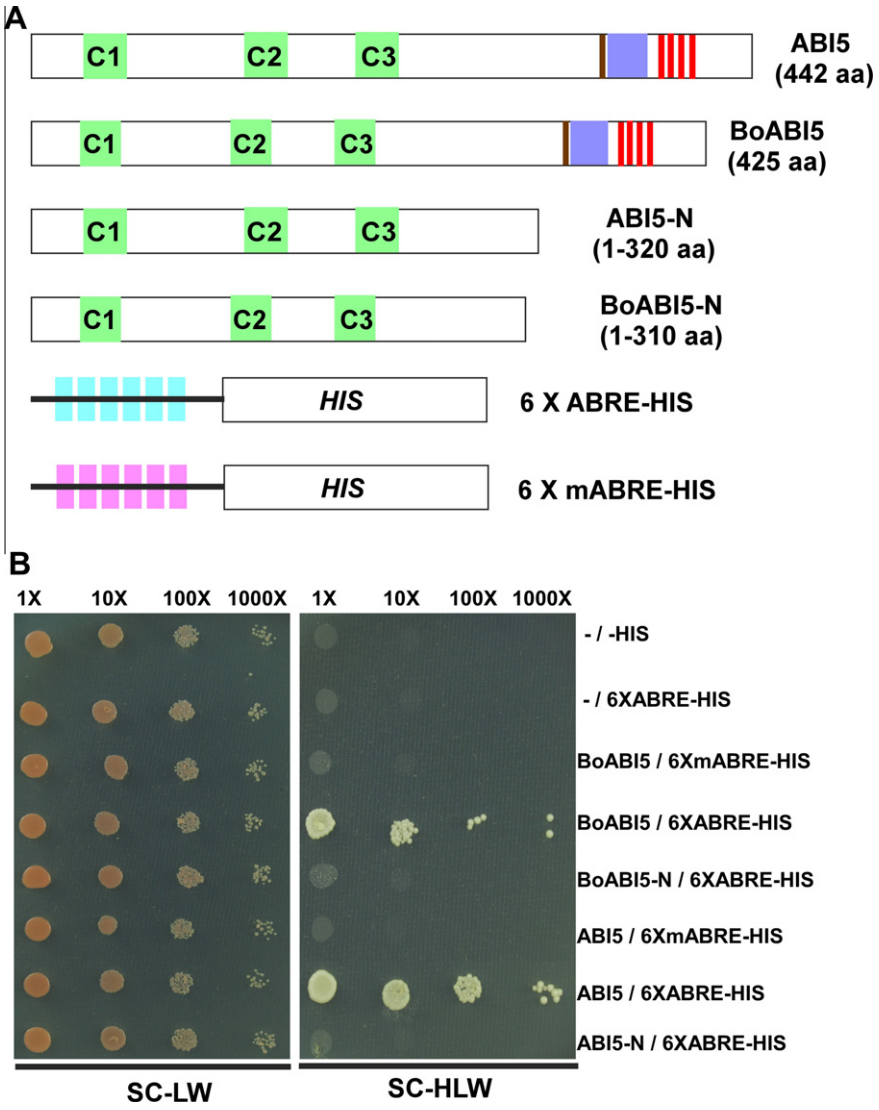

Fig. 4. Trans-activation and DNA-binding activity of the BolABI5 protein. (A) Schematics of the protein and the ABRE *cis*-element structure used in yeast one-hybrid experiments. (B) Trans-activation and DNA-binding analysis of the BolABI5 protein. ABI5/6×ABRE-HIS and -/-HIS (the empty pPC86 vector combined with the empty pRS315-HIS vector) were used as positive and negative controls, respectively. BolABI5ΔbZIP is a form of BolABI5 that carries a deletion of the intact C-terminal bZIP region; ABI5ΔbZIP is a form of ABI5 that carries a deletion of the intact C-terminal bZIP region.

modifications and modulating genes is required to completely understand the functional relationship between BolABI5 and its orthologues in future.

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