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Characterization and expression quantitative trait loci analysis of *TaABI4*, a pre-harvest sprouting related gene in wheat

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Abstract

Pre-harvest sprouting (PHS) induced by the absence of seed dormancy causes a severe reduction in crop yield and flour quality. In this study, we isolated and characterized *TaABI4*, an ABA-responsive transcription factor that participates in regulating seed germination in wheat. Sequence analysis revealed that *TaABI4* has three homologues, located on chromosomes 1A/1B/1D. *TaABI4* contains a conserved AP2 domain, and AP2-associated, LRP and potential PEST motifs. Putative *cis*-acting regulatory elements (CE1-like box, W-box, ABRE elements and RY elements) were identified in the *TaABI4* promoter region that showed high conservation in 17 wheat cultivars and wheat-related species. Expression profiling of *TaABI4* indicated that it is a seed-specific gene accumulating during the middle stages of seed development. Transcript accumulation of *TaABI4* in wheat cultivar Chuanmai 32 (CM32, PHS susceptible) was 5.07-fold and 1.39-fold higher than that in synthetic hexaploidy wheat SHW-L1 (PHS resistant) at 15 and 20 DPA, respectively. Six expression quantitative trait loci (eQTL) of *TaABI4* on chromosomes 2A, 2D, 3B and 4A were characterized based on the accumulated transcripts of *TaABI4* in SHW-L1 and CM32-derived recombinant inbred lines. These QTLs explained 10.7 to 46.1% of the trait variation with 4.53–10.59 of LOD scores, which contain genes that may affect the expression of *TaABI4*.

Introduction

Pre-harvest sprouting (PHS) is the germination of grain prior to ripening in the spike when there is excessive moisture before harvest. PHS has become a recurring worldwide problem since it causes a severe reduction in crop yield and flour quality due to starch and protein degradation (Olaerts et al., 2016). Seed dormancy accounts for up to 60% of the variation in PHS tolerance, and PHS in wheat is mainly caused by the lack of adequate seed dormancy (DePauw and McCaig, 1991; Li et al., 2004). The level of wheat grain dormancy partly depends on abscisic acid (ABA) sensitivity before and after the grain reaches physiological maturity (Gubler et al., 2005; Sun et al., 2005; Shu et al., 2016). One well-characterized positive regulator of ABA signalling, *ABSCISIC ACID-INSENSITIVE 4* (*ABI4*), was initially identified in screens for mutants exhibiting ABA-resistant germination (Finkelstein, 1994). *ABI4* is a member of the APETALA 2 (AP2/ERF) transcription factor family and can activate or repress gene expression by binding to specific *cis*-elements in gene promoters via its AP2 DNA-binding domain (Wind et al., 2013). It has been documented that *ABI4* interacts with target genes to regulate seed dormancy and germination. For example, *ABI4*-dependent temporal regulation of *PTR2* expression influences water status during seed germination, promoting the germination of imbibed grain (Choi et al., 2020). *ABI4* is indispensable for inhibiting the expression of three family members of Arabidopsis response regulators (ARRs), namely, *ARR6/7/15*, which are involved in seed dormancy (Huang et al., 2017). Moreover, *ABI4* is a primary positive regulator of *ABI4*, *ABI5* and *starch branching enzyme 2.2* (*SBE2.2*), activating transcription by binding the CACCG-box (CE1-like) in the promoter regions during seed development (Bossi et al., 2009). Apart from *ABI4* itself, various transcription factors may regulate *ABI4* transcription, including several WRKY transcription factors that can bind to the W-box sequence in the *ABI4* promoter region (Shang et al., 2010; Antoni et al., 2011; Liu et al., 2012). MYELOBLASTOSIS 96 (MYB96) induces *ABI4* expression by binding to its promoter during seed germination and seedling development (Lee and Seo, 2015).

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In addition to *ABI4*, two other transcription factors (*ABI3/VP1* and *ABI5*) have been characterized that regulate ABA response during seed development (Finkelstein, 1994; Finkelstein and Lynch, 2000; Osa et al., 2003). It has been reported that some cross-regulation of expression existed among *ABI3*, *ABI4* and *ABI5*, which function in a combinatorial network, rather than a regulatory hierarchy, controlling seed development and ABA response (Soderman et al., 2000). Moreover, *ABI3*, *ABI4* and *ABI5* have similar effects on seed dormancy and the expression of maturation-specific seed proteins (Finkelstein, 1994). However, *ABI4* is a focal point in the signal transduction pathways of ABA (Niu et al., 2002). Orthologues of *ABI4* have been reported in many other plant species, including maize, rice and lotus (Niu et al., 2002; Ming et al., 2013; Wang et al., 2015). In maize, *ZmABI4* is seed specific, reaching maximum expression at 20 days post-anthesis (DPA) (Niu et al., 2002). In the rice database, a single sequence shares significant homology with the *AtABI4* AP2 domain, indicating that a single *ABI4* homologue exists in rice (Yu et al., 2002). However, there is limited information available for *ABI4* orthologues in wheat.

Synthetic hexaploid wheat SHW-L1 obtained from the hybridization of *Triticum turgidum* and *Aegilops tauschii* is a useful genetic resource and shows significant tolerance to PHS (Yang et al., 2014). To investigate the regulatory factors that interact with *TaABI4* and the role of *TaABI4* in the ABA-induced seed dormancy pathway, we performed a conservation analysis on *ABI4* in wheat ancestral species and modern cultivars and subsequently cloned this gene. We analysed the expression pattern of *TaABI4* at different grain developmental stages. Furthermore, we carried out expression QTL analysis (eQTL) to detect regions regulating the expression of *TaABI4* in recombinant inbred lines (RILs). Finally, candidate genes were also predicted and evaluated in the eQTL interval, providing further insight into the role of *TaABI4* in ABA signal transduction pathways and into the regulatory framework that controls seed germination in wheat.

Materials and methods

Plant material

Chuanmai32 (CM32, PHS susceptible), synthetic hexaploid wheat (SHW-L1, PHS-resistant) and their derived RILs (138 lines) were grown under glasshouse conditions (16 house light at 22°C, 8 h dark at 12°C, 70% relative humidity). Days to flowering was measured for each spikelet based on the anther extrusion at 50% of the spike. Developing grains from 5 to 30 DPA were collected at 5-d intervals from the centre florets for subsequent gene expression profiling. Young leaves of SHW-L1 and CM32 were used for DNA extraction. Each sample had biological replicates and was immediately frozen into liquid nitrogen and stored at −80°C for RNA extraction.

Sequence characterization and in silico promoter analysis

Based on the results of BLASTP searches, we obtained coding sequences of *TaABI4* in Chinese Spring using EnsemblPlants (http://plants.ensembl.org/index.html). Protein domains of genes were predicted using the SMART tool (http://smart.embl-heidelberg.de/). The coding sequences of *TaABI4* were used to query the target database (ViroBLAST, http://202.194.139.32/blast/viroblast.php, The Wheat 'Pan Genome', http://www.10wheatgenomes.com/data-repository/, and The *Aegilops tauschii* genome, http://aegilops.wheat.ucdavis.edu/ATGSP/data.php) to download homologous genes and 2 kb upstream sequences from translational initiation codon in 17 wheat cultivars and 3 wheat ancestors (Altschul et al., 1997; Luo et al., 2017; Ling et al., 2018; Zhu et al., 2019; supplementary Table S1).

Amino-acid sequences were aligned using DNAMAN (Version. 5.2.10, Lynnon Biosoft, Quebec, Canada). Putative *cis*-acting regulatory elements located in the promoter regions were predicted using PLANTCARE (Lescot et al., 2002; http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) and PLACE (Higo et al., 1999; http://www.dna.affrc.go.jp/PLACE/). An analysis of conserved motifs in 17 wheat cultivars was obtained using the MEME suite (Bailey et al., 2009; http://meme-suite.org/tools/meme). This program was used to search for the top five *cis*-motifs with consensus patterns of 6–50 base width and *E*-value < 0.01, on the forward strand of the input sequences only.

Prediction of proteins and PEST motifs

Generated coding sequences were translated to predicted proteins using DNAMAN with default parameters. Searches for potential PEST sequences were performed using the ePESTfind (http://www.bioinformatics.nl/cgi-bin/emboss/epestfind). We used the input parameters in all cases and defined that a score above zero denoted a possible PEST sequence (Gregorio et al., 2014).

PCR amplification

According to the *TaABI4* nucleotide sequences of Chinese Spring, specific primers for the gene were designed online (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and shown in supplementary Table S2. Genomic DNA was isolated from SHW-L1/CM32 young leaves using the CTAB method (Zhang et al., 2013) and was used as templates to amplify the DNA sequences of *TaABI4*. PCR was performed using high-fidelity Prime STAR Polymerase (TaKaRa, Dalian, China) under the following conditions: 98°C for 3 min, 35 cycles of 98°C for 50 s, 60–65°C for 50 s and 72°C for 90 s, followed by a final extension step of 72°C for 10 min. The PCR amplification products were ligated into the pEASY-blunt Cloning Vector (TransGen, Beijing, China), and the resulting ligation mixtures were transformed into *E. coli* Trans1-T1 chemically competent cells (TransGen, Beijing, China) to obtain positive clones for sequencing.

RNA extraction and expression analysis

Primer pairs in the relevant conserved exon regions of *TaABI4* among A, B and D genomes in SHW-L1 and CM32 were used to amplify 151 bp amplicons (supplementary Table S2). The expression level of *TaABI4* was measured in the parents at six seed development stages (5, 10, 15, 20, 25 and 30 DPA). RNA was extracted from each sample using the total RNA extraction kit (Biofit, China), and genomic DNA was removed with DNaseI.

Three seed-developing stages (10, 20 and 30 DAP) of SHW-L1/CM32 were selected to carry out RNA sequencing (RNAseq). RNA quantity and quality were assessed using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Fremont CA, USA) and checked for integrity on an Agilent 2100 bioanalyser (Agilent Technologies, Palo alto CA, USA) by denaturing agarose gel electrophoresis with ethidium bromide staining. Equimolar amounts of the libraries were constructed and sequenced by BerryGenomics (Beijing, China) using the Illumina HiSeq-2000 and HiSeq X Ten platform

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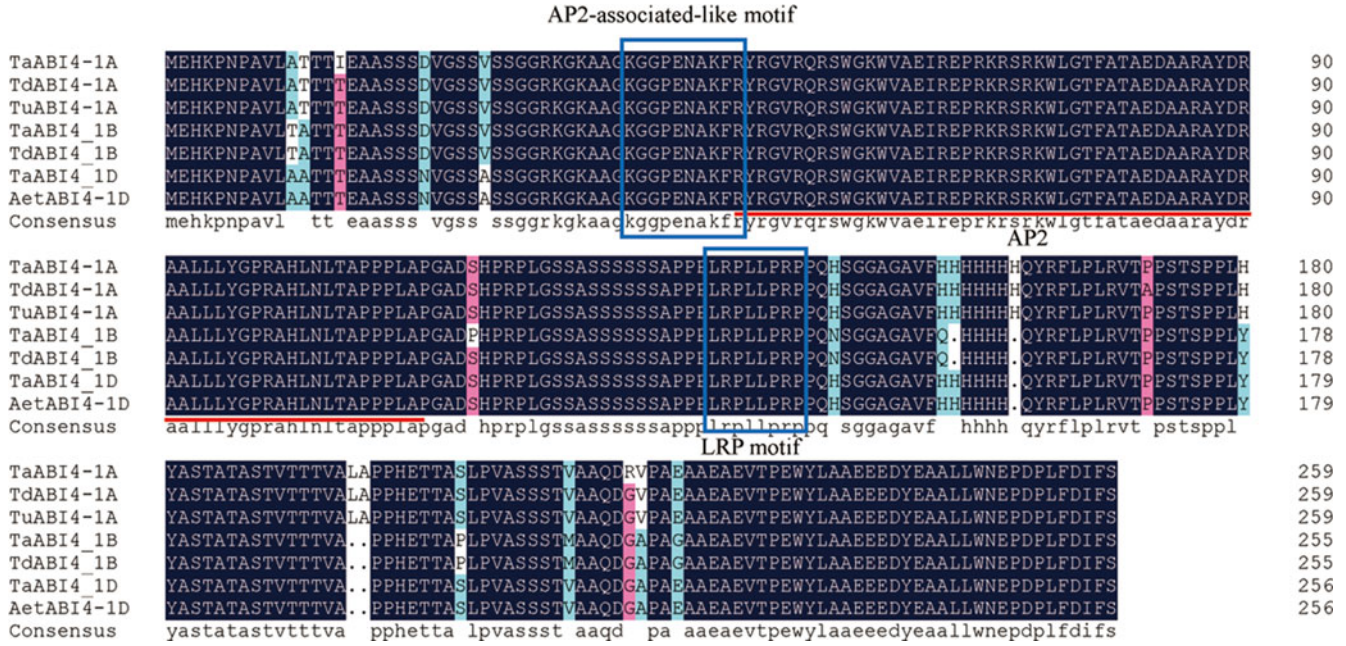

Fig. 1. The alignment of *ABI4* proteins of Chinese spring and wheat ancestors. Sequences including *TaABI4-1A*/*TaABI4-1B*/*TaABI4-1D* from Chinese spring, *TdABI4-1A* and *TdABI4-1B* from *T. dicoccoides* cv. Zavitan, *TuABI4-1A* from *T. Urartu* and *AetABI4-1D* from *Ae. tauschii*. The AP2 region is indicated by the red lines at the bottom; LRP and AP2-associated-like motifs were boxed.

(Illumina, Hayward CA, USA). Gene transcript levels were estimated using transcripts per million (TPM; Zhao et al., 2020).

First-strand cDNA was synthesized using a PrimeScriptTM 1st Strand cDNA Synthesis Kit (TaKaRa, Dalian, China). cDNA sampling was performed in duplicate and SsoFast™ EvaGreen® Supermix (Bio-Rad, Hercules CA, USA) was used for real-time quantitative PCR (RT-qPCR) (CFX96 Touch™ Real-Time PCR Detection System, Bio-Rad, USA). Each reaction contained approximately 50 ng first-strand cDNA, 0.5 µl, 10 µmol l^{-1} gene-specific primers and 10 µl real-time PCR SYBR Green (TIANGEN, Beijing, China). Amplification conditions were 5 min at 95°C, followed by 40 cycles of 30 s at 95°C, 30 s at 60°C, 40 s at 72°C and a final extension of 10 min at 72°C. Seven 1/10 dilutions of the recombinant plasmid cDNA template were used to make a standard curve for amplification efficiency (*E*) calculation. Three housekeeping genes, *TaActin*, *Ta.14126.1* and *Ta.7894.3.a1_at*, were used as internal controls (Long et al., 2010). Gene expression data were analysed using the Bio-Rad CFX Manager (Bio-Rad, Hercules CA, USA) software. The expression profile of the target gene was normalized to that of the internal control genes, and the geometric mean was calculated. The relative gene expression quantity of each sample was calculated using the $E^{-\Delta\Delta Ct}$ method (Pfaffl, 2001).

Expression QTL (eQTL) analysis

In order to characterize regions that regulate *TaABI4* expression levels, we conducted eQTL mapping analysis within the RIL population using the previously constructed high-density genetic map (Yang et al., 2019). eQTL analysis was achieved using the WinQTLcart2.5 software (North Carolina State University, Raleigh, NC, USA) with the composite interval mapping (CIM) method (Wang and Basten, 2007). The analysis was done by setting the control parameters to model 6 (standard model), forward regression, 10-cm windows and five makers as the control. The threshold was set at 4.0 to detect eQTLs. The wheat reference genome 'Chinese Spring', IWGSC RefSeq v1.0 (International Wheat Genome Sequencing Consortium, 2018), was used to query marker positions using the blastn2.2.26+ package (Camacho et al., 2009). On the basis of eQTL intervals, the gene annotation was conducted using Wheat Gmap (http://www.wheatgmap.org.cn/tools/gene/information/). Subsequent candidate genes were validated by querying all of the predictions against the Nr-NCBI (http://www.ncbi.nlm.nih.gov/) and EnsemblPlants (http://plants.ensembl.org). Genes relating to the ABA signalling pathway were compared and mapped to the genome reference sequence of Chinese Spring v1.0 to identify candidate genes that may underlie eQTL. The CORREL function in Excel was used to calculate the correlation coefficient between the expression pattern of *TaABI4* and RNAseq data of candidate genes in SHW-L1 and CM32. The correlation coefficient was used to measure the strength of the relationship between two variables.

Results

Sequence characterization of TaABI4

The DNA sequence of *ABI4* (AT2G40220) from *Arabidopsis* was used as a query sequence to carry out BLAST searches in EnsemblPlants. Three homologues of *TaABI4* were identified on the A, B and D sub-genomes of 18 wheat cultivars (*TaABI4-1A*, *TaABI4-1B* and *TaABI4-1D*). All the 50 *TaABI4* sequences were found to be represented by a single exon. The coding sequences (CDS) of three homologues of Chinese Spring were conserved with 97.53% nucleotide identity. Compared with *TaABI4-1A*, *TaABI4-1B* and *TaABI4-1D* had two 3–6 bp deletions as well as 28 single-nucleotide polymorphisms (SNPs), 13 SNPs of which caused non-synonymous mutations (supplementary Fig. S1). The three homologue-encoded proteins with 260, 256 and 257 amino-acid residues, respectively. The proteins have highly conserved AP2 domains that were also found in previously annotated *AtABI4* in *Arabidopsis* and *ZmABI4* in maize (*Zea mays*) (Fig. 1).

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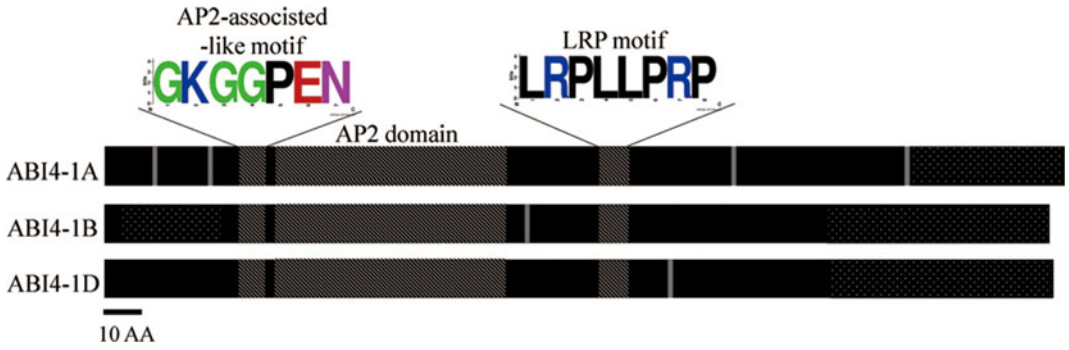

Fig. 2. Protein structure schematic diagram of *ABI4* in wheat cultivars. The grey boxes indicate the polymorphism of amino-acid sequences and the black boxes are highly conserved amino-acid sequences. The boxes filled with twill are conserved domains and motifs, and the boxes filled with dots are potential PEST motifs.

Table 1. Conservation of putative PEST sequences in *ABI4* proteins from wheat cultivars

Name	Length	Score value	Position (N/C-termini)	Identity (%)	Motif logo
ABI4-1A	21AA	0.44	219–260(C)	99.8	
ABI4-1B	20AA	2.08	196–256(C)	100	
		3.52	4–32(N)	100	
ABI4-1D	19AA	3.13	197–257(C)	100	

In addition, ten amino acids (KGGPENAKFR) were contiguous to the AP2 domain (designated as the AP2-associated motif). Additionally, a stretch of eight amino acids (LRPLLPRP) identified as the LRP motif was located nearby (Fig. 1). *TaABI4* proteins revealed 100% identity in these common regions, while TaABI4-1A contained three additional amino acids, His_{171}, Leu_{196} and Ala_{197} (Fig. 1). Putative proteins were predicted from *Ae. tauschii*, *T. dicoccoides* cv. Zavitan and *T. urartu*. The protein sequence of AetABI4 obtained from *Ae. tauschii* showed 100% identity with the TaABI4-1D sequence. TuABI4-1A obtained from *T. urartu* showed 99.23% amino acid identity with TaABI4-1A. TdABI4-1B of *T. dicoccoides* cv. Zavitan shared 98.08% identity with TaABI4-1B (Fig. 1).

ABI4 proteins and putative motif analysis in wheat cultivars

The AP2 domains, the AP2-associated motifs and the LRP motifs were conserved in 50 putative ABI4 homologous proteins in terms of their position and sequence identity (Fig. 2). Putative PEST degradation signals at the terminus of wheat ABI4 proteins with a positive probability value (>0) were detected using the PEST-find program (Rice et al., 2000), which were in agreement with a previous report (Gregorio et al., 2014). This demonstrated that potential PEST sequences were detected in all of these proteins, with probability scores ranging from +0.44 (ABI4-1A) to +3.52 (ABI4-1B) (Table 1). For ABI4-1B and ABI4-1D proteins, one PEST sequence was detected at the C-terminal with a length of 60AA. For ABI4-1A proteins, a shorter PEST motif of 41 amino acids was detected, sharing 99.8% identity among the 17 cultivars in addition to another PEST sequence predicted at the N-terminus that was also identified in TaABI4-1B. Although some variant amino acids were detected in the proteins of each genome, as shown in grey boxes in Fig. 2, they did not locate in the region of crucial motifs. This demonstrates that the ABI4 proteins are conserved in their protein architecture, coinciding with their central role in wheat hormone signalling.

Potential cis-acting regulatory elements of ABI4 promoters in wheat ancestors

The presence of potential *cis*-regulatory elements in the upstream (≥2000 bp) region of *TaABI4* homologues from wheat cultivar Chinese Spring was analysed. Eleven types of potential *cis*-acting regulatory elements were identified in the upstream region (Fig. 3). This region was also isolated from *T. dicoccoides* cv. Zavitan, *T. urartu* and *Ae. tauschii*. A putative TATA-box was detected 190 bp upstream of the start codon. A binding site (CE1-like motif, CACCGCCCC) was present immediately downstream from a putative W-box (TTGACY). In addition, RY elements with CATGCATG involved in seed-specific regulation were predicted. ABRE elements known to be involved in ABA response, with CACGTG core motif, were recognized nearby the 5′-termini. ARE elements with an AAACCA core motif that are essential for the anaerobic induction also existed in all ABI4 proteins. Additionally, conserved motifs such as CAAT-box, CAT-box and A-box were detected. One Myb and one Myc

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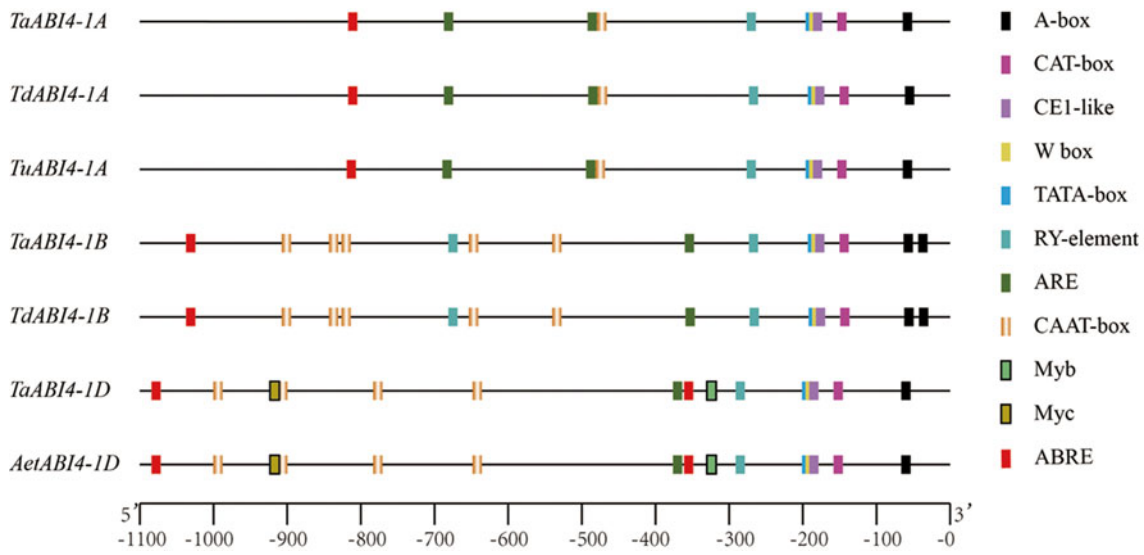

Fig. 3. Potential *cis*-acting regulatory elements in the upstream regions of *ABI4* genes from wheat and wheat ancestors. The coloured boxes represent different *cis*-regulatory elements.

element, known to be involved in ABA signalling (Lin, 2009), were predicted in the *TaABI4-1D* and *AetABI4* promoter regions. The detected *cis*-acting regulatory elements were conserved among the wheat and its ancestral species.

The putative motif analysis of ABI4 *genes in wheat cultivars*

The top five motifs identified by this analysis were found in almost all of the *ABI4* genes in wheat cultivars and were highly conserved in terms of number and position (Fig. 4). Although motif 2 did not exist in the A sub-genome of Kronos, it shared 99.4% identity among 50 upstream regions and was regarded as a novel *cis*-motif with no current description in the PLACE database (Table 2). As shown in Table 2, motif 1 with a W-box as its core element was also conserved in all sequences with 100% identity. Although there were some variable SNPs in motif 3, motif 4 and motif 5, they did not exist in the core region of each motif. Overall, putative motifs within the upstream of *ABI4* genes were almost completely conserved in wheat cultivars.

Cloning and qRT-PCR analysis of TaABI4 *in SHW-L/CM32 developing seeds*

The *TaABI4* sequences were cloned from SHW-L and CM32, which were highly conserved in these two cultivars (supplementary Fig. S2). According to RNAseq analysis, the expression level of *TaABI4* in CM32 was higher than that in SHW-L1 at each detected stage (Fig. 5A). Then, RT-qPCR assays were performed using cDNA from five time points (5, 15, 20, 25 and 30 DPA) to detect the expression level variation of *TaABI4* between SHW-L1 and CM32. During seed development, *TaABI4* expression began as early as 10 DPA, increasing between 10 and 15 DPA as the transition from growth to storage phase of grain development (starting after 12 DPA) took place, and peaked at 20 DPA, with a decline in expression until 30 DPA. The expression of *TaABI4* in CM32 was higher than that in SHW-L1 in most of the measured stages. Two significant differences in relative expression were detected at 15 DPA (5.07-fold) and 20 DPA (1.39-fold) (Fig. 5B).

eQTL mapping

The significant difference between CM32 and SHW-L1 in the expression levels of *TaABI4* at 15 and 20 DPA enabled the detection of eQTLs. Based on the consensus genetic map and corresponding SNP marker positions, six significant eQTLs ($P < 0.05$, LOD > 4) were identified (Table 3 and Fig. 6). One eQTL detected on chromosome 2A at 15 DAP was designated as *eQABI4.15DPA.2A.1*, with LOD scores at 4.53. Two eQTL regions located on chromosome 2D designated as *eQABI4.20DPA.2D.1* and *eQABI4.20DPA.2D.2* were detected at 20 DAP, showing 9.63 and 6.38 LOD scores, respectively. *eQABI4.20DPA.4A.1* and *eQABI4.20DPA.4A.2* were located on chromosome 4D with negative alleles from SHW-L1. They explained 38.2 and 46.1% of the phenotypic variation, respectively. The physical mapping of 3B eQTL was designated as *eQABI4.20DPA.3B.1*, showing that the corresponding interval location was Chr.3B: 667902308-669428443. All identified eQTLs had negative additive effects, indicating that eQTLs that could reduce the expression of *TaABI4* were derived from synthetic wheat SHW-L1. Genes involved in regulatory processes, including hormone response and biosynthesis, signal transduction, protein phosphorylation, starch and sucrose metabolism, were selected in those eQTL regions. Subsequently, genes expressed in seeds and ABA related were highlighted, resulting in five candidate genes being identified (Table 4). Further correlation coefficient analysis of gene expression was carried out (Table 4). Correlation coefficients between the expression of *TaABI4* and candidate genes ranged from 0.61 to 0.9. *TaABI4* expression positively correlated with the expression of TraesCS2A02G089400, TraesCS2A02G099400, TraesCS4A02G094300 and TraesCS4A02G114400, while it negatively correlated with the expression of TraesCS4A02G093600.

Discussion

In this study, we presented the characterization of the wheat *ABI4*, a gene involved in ABA responsiveness during seed development and germination. TaABI4 proteins from three wheat sub-genomes

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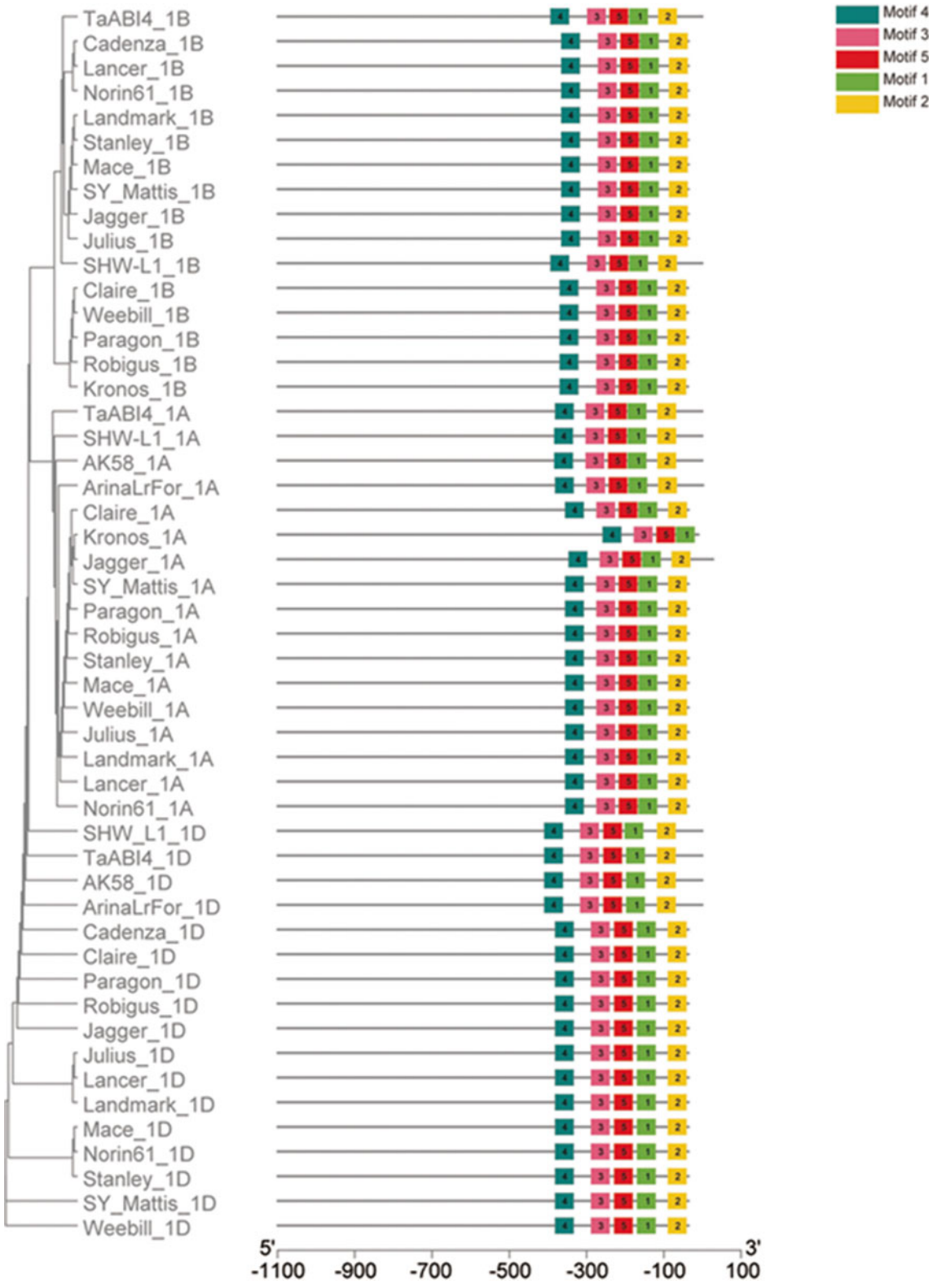

Fig. 4. Schematic representation of conserved *cis*-motifs (obtained using MEME) in the upstream regions of *ABI4* genes from wheat cultivars. Different motifs are represented by boxes of different colours.

were conserved with an AP2 domain required for nuclear localization (AP2-associated motif), as well as regions for transcriptional activation (LRP motif). The conserved domains are used as hallmarks to identify ABI4 orthologues in different species (Gregorio et al., 2014). Although the protein sequences for the three homologues had slight polymorphisms, the overall identity was high (96.9%). Our results suggested that the AP2 proteins presented in wheat are the orthologues of the *Arabidopsis* ABI4

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Table 2. Conserved *cis*-motifs found in the upstream promoter regions of *ABI4* genes in wheat cultivars. llr means log likelihood ratio

MEME-generated motifs	llr	*E*-value	Identity (%)	Core elements	Motif Logo
GCCCTTTATATTTGACCACCGCCCCTCTCCACCAAGCACATCCTCCTCGG	3736	5.0×10^{-1093}	100.0	WBOX	motif1
TCTTCATCGGTCTCCCCAACGCACAGCGAGCTAGGTATCCCTCTCTCTTC	3690	7.9×10^{-1098}	99.4	Novel motif	motif2
TACCTGCATGAGAGCATGACCTTTCACTGCATTGCATGCATGTCCGTTCC	3753	1.2×10^{-1100}	98.0	WBOX	motif3
TAAAACAAGCAGAAAACGTAGGCACATCGCTACAGAAGATAAACCACGCC	3705	3.6×10^{-1081}	97.1	MYB1AT	motif4
TCTCTCACTCACTCACTCCTGCTCGCTGTACTCCACTGTGCACGCTCTCT	3465	2.6×10^{-986}	95.0	CACTFTPPCA1	motif5

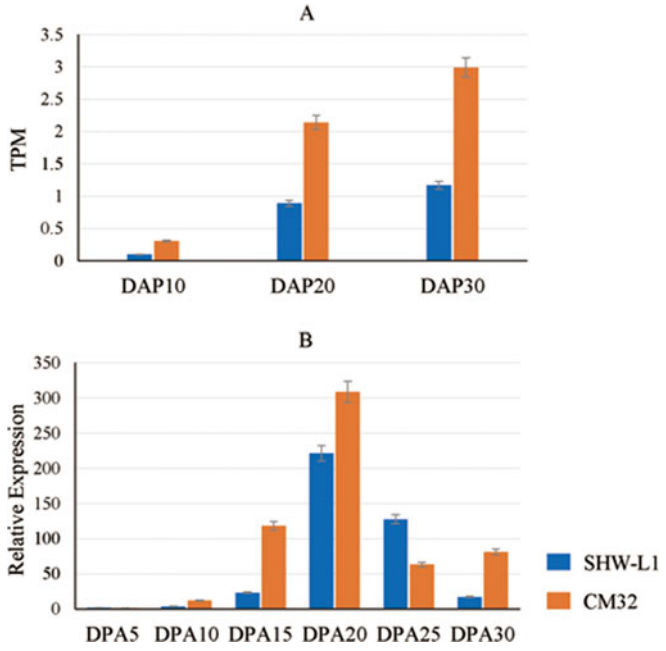

Fig. 5. The expression pattern of *TaABI4* in SHW-L1 and CM32. (A) The expression assays using RNAseq. The *y* axis denotes TPM (transcripts per kilobase million). (B) The expression assays using qRT-PCR.

and should be considered as TaABI4-1A, TaABI4-1B and TaABI4-1D.

Compared with the ABI4 from *T. urartu*, *Ae. tauschii* and *T. dicoccoides*, the amino-acid variation existed only in TaABI4-1A (Thr_{15}/Leu_{15}, Gly_{218}/Arg_{218}) and TaABI4-1B (Ser_{108}/Pro_{108}) and was not located in core regulatory regions (Fig. 1). This indicated that TaABI4 was highly conserved during the polyploidization and domestication processes of wheat. In *Arabidopsis*, the low accumulation of ABI4 resulted from both post-transcriptional and post-translational regulation (Finkelstein et al., 2011). PEST sequences are degradation motifs that can affect protein stability (Gregorio et al., 2014) and are characterized by regions enriched in the amino-acid proline, glutamic acid, serine and threonine (Rogers et al., 1986). Based on the available pan-genome data, we analysed 50 putative ABI4 proteins from 18 wheat cultivars to predict potential PEST motifs. Most of the possible PEST sequences were located in the N-terminal region of the protein and were longer than AtABI4 (Table 1). These differences may cause divergence in post-translational mechanisms compared with *Arabidopsis*. In fact, ZmABI4 also has two PEST motifs located in the N-terminus and C-terminus, showing score values of +3.04 and +0.68, respectively (Gregorio et al., 2014).

The discovery of *cis*-acting regulatory elements in the promoter regions is essential to understanding the spatial and temporal expression patterns of *ABI4* genes. The six *cis*-acting regulatory elements were conserved in terms of position and sequence identity (Fig. 3). TATA-box is regarded as the core promoter element, and transcription factors bind to TATA-proximal regions (W-box, CE-1 like) having been shown to regulate downstream gene transcription (Heins et al., 1992; Busk et al., 1997; Phukan et al., 2016). Additionally, A-box and RY element are *cis*-acting regulatory elements, and CAT-box is related to meristem expression in *Arabidopsis* (Sakata et al., 2010). ABRE (ABA-responsive elements) motifs are known to participate in response to ABA (Sarkar and Lahiri, 2013). *TaABI4-1D* contained

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Table 3. eQTL mapping results of *TaABI4* in SHW-L1 and CM32

eQTLs	Chr.	Position(cM)		LOD	Flanking markers	Additive	R^2	Phenotype	Physical location (bp)
eQABI4.15DPA.2A.1	2A	241.49	4.53	AX-111636801	AX-110609678	−6.4226	0.223	15 DAP	48661724–48805480
eQABI4.20DPA.2D.1	2D	179.81	9.63	AX-108856494	AX-110515525	−9.3612	0.382	20 DAP	613440336–616018344
eQABI4.20DPA.2D.2	2D	181.66	6.38	AX-110515525	AX-111690676	−8.8654	0.348	20 DAP	616018344–618242947
eQABI4.20DPA.3B.1	3B	243.92	4.34	AX-110503866	AX-95660238	−4.9156	0.107	20 DAP	667902308–669428443
eQABI4.20DPA.4A.1	4A	20.97	7.16	AX-110465181	AX-86175059	−8.5772	0.382	20 DAP	104670825–140756768
eQABI4.20DPA.4A.2	4A	31.15	10.59	AX-109911754	AX-110113739	−9.5375	0.461	20 DAP	89088986–103724030

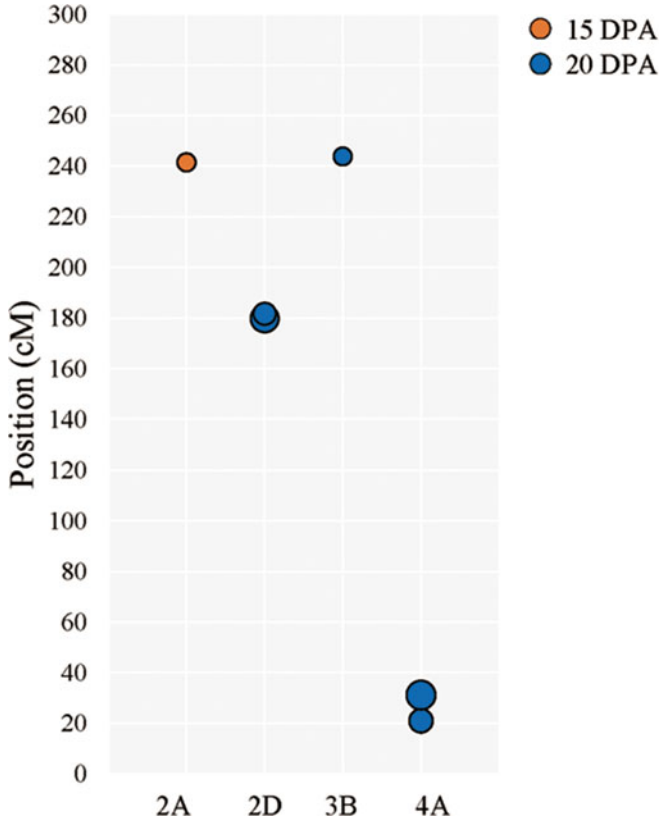

Fig. 6. eQTL genetic locations in the genetic map. The size of the circles means LOD values. The *x* axis denotes different chromosomes. The yellow/blue circles indicate eQTLs for *TaABI4* at 15 DPA/20 DPA.

two classical ABRE elements that are necessary to constitute an active ABA-responsive complex because a single ABRE is not sufficient to confer ABA responsiveness (Hobo et al., 1999; Zhang et al., 2005; Ganguly et al., 2011). The identification of conserved *cis*-acting regulatory elements in *ABI4* promoters of wheat revealed that other transcription factors might regulate those homologues.

The expression pattern of *TaABI4* was variable between modern wheat cultivar CM32 and synthetic wheat SHW-L1. It is noteworthy that *TaABI4* showed a higher transcript accumulation in weakly dormant material (CM32) than in dormant material (SHW-L1) during most periods of seed development (Fig. 5). By contrast, seeds of the *Arabidopsis abi4* mutant germinated significantly more quickly than wild type (Shu et al., 2013), indicating that the presence of functional *ABI4* is important for resistance to PHS. The expressions of both *TaABI3* and *TaABI5* in SHW-L1 were significantly higher than those in CM32 (Zhou et al., 2016). These results are consistent with the corresponding research results in *Arabidopsis* and maize, finding that *ABI3* and *ABI5* are positive regulators of seed dormancy (McCarty et al., 1991; Hoecker et al., 1995; Finkelstein and Lynch, 2000). The gene expression patterns of *TaABI3* and *TaABI5* were similar to that of *TaABI4* in the early and middle stages of seed development (5–15 DPA), signifying that *TaABI4* associated the ABA biosynthetic pathway with *TaABI3* and *TaABI5* as found in *Arabidopsis* (Lopez-Molina et al., 2002). From these results, other regulatory factors interacting with *TaABI4* are required to complete our understanding of the gene networks involving seed germination.

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Table 4. Candidate genes expressed in seeds and ABA-related genes in eQTL interval

Gene ID	Chromosome location	Orthologous genes	Description in Wheat Gmap	r^a
TraesCS2A02G089400	2A:42494117–42495524	*PYL4* (AT2G38310)	Abscisic acid receptor	0.61
TraesCS2A02G099400	2A:52391814–52394012	*OsbZIP62* (Os07g0686100)	bZIP transcription factor	0.90
TraesCS4A02G093600	4A:101402669–101406532	*OsPP1* (Os03g0268000)	Serine/threonine-protein phosphatase	−0.74
TraesCS4A02G094300	4A:102445872–102447774	*OsPP2C30* (Os03g0268600)	Protein phosphatase 2C	0.83
TraesCS4A02G114400	4A:138832030–138833256	*OsPYL* (Os03g0297600)	Abscisic acid receptor	0.71

[a]Correlation coefficient (*r*) was calculated between the expression pattern of *TaABI4* and RNAseq data of candidate genes in SHW-L1 and CM32. A correlation of −1.0 shows a perfect negative correlation, while a correlation of 1.0 shows a perfect positive correlation.

Fig. 7. The locations of QTL associated with the pre-harvest sprouting and seed dormancy and eQTL of *TaABI4* were mapped on a physical map of Chinese spring. The location numbers and the corresponding locations can be found in Tables 3 and 4.

eQTL mapping is an efficient approach to identify genetic loci controlling complex crop traits (Chen et al., 2010; Motomura et al., 2013). In this study, we chose 15 and 20 DPA, which are the middle periods of seed development, to identify six significant eQTLs associated with *TaABI4* expression variation on chromosomes 2A, 2D, 3B and 4A (Table 3), suggesting that the observed differences in *TaABI4* expression in the RIL population were regulated in part by *trans*-acting factors (Doss et al., 2005). Several previous studies mapped the major QTLs for seed dormancy and PHS tolerance to chromosomes 4A (Mares et al., 2005; Torada et al., 2005; Chen et al., 2008). In this study, two major eQTLs located on chromosome 4A accounted for 38.2 and 46.1% of the phenotypic variance. This result further confirmed that the chromosome 4A harbours QTL, and eQTL associated with the PHS resistance is important for wheat. The eQTL regions detected in this study may provide candidate genes that play potential roles in regulating PHS through effects on *TaABI4* expression. Thus, eQTLs detected in this study suggested that unidentified genes or indirect regulation genes would affect *TaABI4*, which causes the different expression patterns of *TaABI4* compared with *Arabidopsis*.

Five putative candidate genes were detected in eQTL internals, and the correlation between the expression of each candidate gene and the expression of *TaABI4* was analysed according to the available RNAseq database of SHW-L1 and CM32. In this study, the eQTL for *TaABI4* at 15 DPA was located close to TraesCS2A02G089400, the orthologues of Pyrabactin resistance 1-like (*PYL*) abscisic acid receptors 4 (*PYL4*), approximately 6.16 Mb (Fig. 7). TraesCS2A02G099400 was regarded as an orthologue of *OsbZIP62*, which was involved in ABA signalling pathways (Yang et al., 2019). In particular, TraesCS2A02G 099400 displayed a high expressional correlation coefficient with *TaABI4* (+0.90), indicating the involvement of this gene in up-regulating *TaABI4* expression. Two genes located in the region of *eQABI4.20DPA.4A.2* were related to protein phosphatase. As an orthologue of TraesCS4A02G094300, *OsPP2C30* is involved

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in ABA signalling pathway during seed germination (Kim et al., 2012). TraesCS4A02G114400 located in the internals of *eQABI4.20DPA.4A.2* was the orthologue of *OsPYL*, which positively regulated the ABA response during the seed germination (Tian et al., 2015). Together, these results suggested that five candidate genes may have a regulatory relationship with *TaABI4*.

In this study, the characterization of *TaABI4*, including its conserved protein domains and *cis*-acting regulatory elements analysis, provides information on the critical nucleotide and amino-acid residues of this gene. Meanwhile, high conservation was found in the amino-acid sequences and promoter regions, but the different expression level of *TaABI4* in two wheat cultivars drove us to identify regions linked to candidate genes that function upstream of *TaABI4* transcripts. Six potential eQTL regions that may regulate the expression of *TaABI4* were detected. Five potential upstream candidate genes that may influence the expression of *TaABI4* were also detected. These results can be utilized for future *TaABI4* studies on interactions with other transcription factors in response to ABA and the establishment of the co-expressed networks relating to seed germination, which will successfully boost the efficiency of wheat breeding with sufficient seed dormancy to prevent PHS.

Supplementary material. To view supplementary material for this article, please visit: https://doi.org/10.1017/S0960258521000015.

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References

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W and Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**, 3389–3402.

Antoni R, Rodriguez L, Gonzalez-Guzman M, Pizzio GA and Rodriguez PL (2011) News on ABA transport, protein degradation, and *ABFs/WRKYs* in ABA signaling. *Current Opinion in Plant Biology* **14**, 547–553.

Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, Ren J, Li WW and Noble WS (2009) MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Research* **37**, W202–W208.

Bossi F, Cordoba E, Dupré P, Mendoza MS, Román CS and León P (2009) The *Arabidopsis* ABA-INSENSITIVE (ABI) 4 factor acts as a central transcription activator of the expression of its own gene, and for the induction of *ABI5* and *SBE2.2* genes during sugar signaling. *Plant Journal* **59**, 359–374.

Busk PK, Jensen AB and Pagès M (1997) Regulatory elements in vivo in the promoter of the abscisic acid responsive gene *rab17* from maize. *Plant Journal* **11**, 1285–1295.

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K and Madden TL (2009) BLAST+: architecture and applications. *BMC Bioinformatics* **10**, 421.

Chen C, Cai S and Bai G (2008) A major QTL controlling seed dormancy and pre-harvest sprouting resistance on chromosome 4A in a Chinese wheat landrace. *Molecular Breeding* **21**, 351–358.

Chen X, Hackett CA, Niks RE, Hedley PE, Booth C, Druka A, Marcel TC, Vels A, Bayer M, Milne I, Morris J, Ramsay L, Marshall D, Cardle L and Waugh R (2010) An eQTL analysis of partial resistance to *Puccinia hordei* in barley. *PLoS ONE* **5**, e8598.

Choi M-G, Kim EJ, Song J-Y, Choi S-B, Cho S-W, Park CS, Kang C-S and Park Y-I (2020) Peptide transporter2 (PTR2) enhances water uptake during early seed germination in *Arabidopsis thaliana*. *Plant Molecular Biology* **102**, 615–624.

DePauw RM and McCaig TN (1991) Components of variation, heritabilities and correlations for indices of sprouting tolerance and seed dormancy in *Triticum* spp. *Euphytica* **52**, 221–229.

Doss S, Schadt EE, Drake TA and Lusis AJ (2005) *cis*-acting expression quantitative trait loci in mice. *Genome Research* **15**, 681–691.

Finkelstein RR (1994) Mutations at two new *Arabidopsis* ABA response loci are similar to the *abi3* mutations. *Plant Journal* **5**, 765–771.

Finkelstein RR and Lynch TJ (2000) The *Arabidopsis* abscisic acid response gene *ABI5* encodes a basic leucine zipper transcription factor. *Plant Cell* **12**, 599–609.

Finkelstein R, Lynch T, Reeves W, Petitfils M and Mostachetti M (2011) Accumulation of the transcription factor ABA-insensitive (ABI)4 is tightly regulated post-transcriptionally. *Journal of Experimental Botany* **62**, 3971–3979.

Ganguly M, Roychoudhury A, Sarkar SN, Sengupta DN, Datta SK and Datta K (2011) Inducibility of three salinity/abscisic acid-regulated promoters in transgenic rice with gusA reporter gene. *Plant Cell Reports* **30**, 1617–1625.

Gregorio J, Hernandez-Bernal AF, Cordoba E and Leon P (2014) Characterization of evolutionarily conserved motifs involved in activity and regulation of the ABA-INSENSITIVE (ABI) 4 transcription factor. *Molecular Plant* **7**, 422–436.

Gubler F, Millar AA and Jacobsen JV (2005) Dormancy release, ABA and pre-harvest sprouting. *Current Opinion in Plant Biology* **8**, 183–187.

Heins L, Frohberg C and Gatz C (1992) The Tn10-encoded Tet repressor blocks early but not late steps of assembly of the RNA polymerase II initiation complex in vivo. *Molecular and General Genetics* **232**, 328–331.

Higo K, Ugawa Y, Iwamoto M and Korenaga T (1999) Plant *cis*-acting regulatory DNA elements (PLACE) database: 1999. *Nucleic Acids Research* **27**, 297–300.

Hobo T, Asada M, Kowyama Y and Hattori T (1999) ACGT-containing abscisic acid response element (ABRE) and coupling element 3 (CE3) are functionally equivalent. *Plant Journal* **19**, 679–689.

Hoecker U, Vasil IK and McCarty DR (1995) Integrated control of seed maturation and germination programs by activator and repressor functions of viviparous-1 of maize. *Genes and Development* **9**, 2459–2469.

Huang X, Xiaoyan Z, Zhizhong G, Shuhua Y and Yiting S (2017) *ABI4* represses the expression of type-A ARRs to inhibit seed germination in *Arabidopsis*. *Plant Journal* **89**, 354–365.

International Wheat Genome Sequencing Consortium (2018) Shifting the limits in wheat research and breeding through a fully annotated and anchored reference genome sequence. *Science* **361**, eaar7191.

Kim H, Hwang H, Hong JW, Lee YN, Ahn IP, Yoon IS, Yoo SD, Lee S, Lee SC and Kim BG (2012) A rice orthologue of the ABA receptor, OsPYL/RCAR5, is a positive regulator of the ABA signal transduction pathway in seed germination and early seedling growth. *Journal of Experimental Botany* **63**, 1013–1024.

Lee HG and Seo PJJPJ (2015) The MYB96-HHP module integrates cold and abscisic acid signaling to activate the CBF-COR pathway in *Arabidopsis*. *Plant Journal* **82**, 962–977.

Lescot M, Déhais P, Thijs G, Marchal K, Moreau Y, Van de Peer Y, Rouzé P and Rombauts S (2002) PlantCARE, a database of plant *cis*-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucleic Acids Research* **30**, 325–327.

Li C, Ni P, Francki M, Hunter A, Zhang Y, Schibeci D, Li H, Tarr A, Wang J, Cakir M, Yu J, Bellgard M, Lance R and Appels R (2004) Genes controlling seed dormancy and pre-harvest sprouting in a rice-wheat-barley comparison. *Functional and Integrative Genomics* **4**, 84–93.

Lin Y (2009) Isolation and analysis of glucose-6-phosphate dehydrogenase (G6PDH) promoter from poplar. *Genomics and Applied Biology* **28**, 445–449.

Ling HQ, Ma B, Shi X, Liu H, Dong L, Sun H, Cao Y, Gao Q, Zheng S, Li Y, Yu Y, Du H, Qi M, Li Y, Lu H, Yu H, Cui Y, Wang N, Chen C, Wu H, Zhao Y, Zhang J, Li Y, Zhou W, Zhang B, Hu W, van Eijk MJT, Tang J,

https://doi.org/10.1017/S0960258521000015 Published online by Cambridge University Press

Witsenboer HMA, Zhao S, Li Z, Zhang A, Wang D and Liang C (2018) Genome sequence of the progenitor of wheat A subgenome *Triticum urartu*. *Nature* **557**, 424–428.

Liu Z, Yan L, Wu Z, Mei C, Lu K, Yu Y, Liang S, Zhang X, Wang X and Zhang D (2012) Cooperation of three WRKY-domain transcription factors WRKY18, WRKY40, and WRKY60 in repressing two ABA-responsive genes *ABI4* and *ABI5* in *Arabidopsis*. *Journal of Experimental Botany* **63**, 6371–6392.

Long XY, Wang JR, Ouellet T, Rocheleau H, Wei YM, Pu ZE, Jiang QT, Lan XJ and Zheng YLJPMB (2010) Genome-wide identification and evaluation of novel internal control genes for Q-PCR based transcript normalization in wheat. *Plant Molecular Biology* **74**, 307–311.

Lopez-Molina L, Mongrand S, McLachlin DT, Chait BT and Chua NH (2002) *ABI5* acts downstream of *ABI3* to execute an ABA-dependent growth arrest during germination. *Plant Journal* **32**, 317–328.

Luo MC, Gu YQ, Puiu D, Wang H, Twardziok SO, Deal KR, Huo N, Zhu T, Wang L, Wang Y, McGuire PE, Liu S, Long H, Ramasamy RK, Rodriguez JC, Van SL, Yuan L, Wang Z, Xia Z, Xiao L, Anderson OD, Ouyang S, Liang Y, Zimin AV, Pertea G, Qi P, Bennetzen JL, Dai X, Dawson MW, Müller HG, Kugler K, Rivarola-Duarte L, Spannagl M, Mayer KFX, Lu FH, Bevan MW, Leroy P, Li P, You FM, Sun Q, Liu Z, Lyons E, Wicker T, Salzberg SL, Devos KM and Dvořák J (2017) Genome sequence of the progenitor of the wheat D genome *Aegilops tauschii*. *Nature* **551**, 498–502.

Mares DJ, Mrva K, Cheong J, Williams K, Watson B, Storlie E, Sutherland M and Zou Y (2005) A QTL located on chromosome 4A associated with dormancy in white- and red-grained wheats of diverse origin. *Theoretical and Applied Genetics* **111**, 1357–1364.

McCarty DR, Hattori T, Carson CB, Vasil V, Lazar M and Vasil IK (1991) The viviparous-1 developmental gene of maize encodes a novel transcriptional activator. *Cell* **66**, 895–905.

Ming R, Vanburen R, Liu Y, Mei Y and Shen-Miller JJGB (2013) Genome of the long-living sacred lotus (*Nelumbo nucifera Gaertn.*). *Genome Biology* **14**, R41.

Motomura Y, Kobayashi F, Iehisa JCM and Takumi S (2013) A major quantitative trait locus for cold-responsive gene expression is linked to frost-resistance gene *Fr-A2* in common wheat. *Breeding Science* **63**, 58–67.

Niu X, Helentjaris T and Bate NJ (2002) Maize ABI4 binds coupling element1 in abscisic acid and sugar response genes. *Plant Cell* **14**, 2565–2575.

Olaerts H, Roye C, Derde LJ, Sinnaeve G, Meza WR, Bodson B and Courtin CM (2016) Impact of preharvest sprouting of wheat (*Triticum aestivum*) in the field on starch, protein, and arabinoxylan properties. *Journal of Agricultural and Food Chemistry* **64**, 8324–8332.

Osa M, Kato K, Mori M, Shindo C, Torada A and Miura H (2003) Mapping QTLs for seed dormancy and the Vp1 homologue on chromosome 3A in wheat. *Theoretical and Applied Genetics* **106**, 1491–1496.

Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT–PCR. *Nucleic Acids Research* **29**, e45–e45.

Phukan UJ, Jeena GS and Shukla RK (2016) WRKY transcription factors: molecular regulation and stress responses in plants. *Frontiers in Plant Science* **7**, 760.

Rice P, Longden I and Bleasby A (2000) EMBOSS: the European molecular biology open software suite. *Trends in Genetics* **16**, 276–277.

Rogers S, Wells R and Rechsteiner M (1986) Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. *Science* **234**, 364–368.

Sakata Y, Nakamura I, Taji T, Tanaka S and Quatrano RS (2010) Regulation of the ABA-responsive Em promoter by *ABI3* in the moss *Physcomitrella patens*: role of the ABA response element and the RY element. *Plant Signalling Behaviour* **5**, 1061–1066.

Sarkar AK and Lahiri A (2013) Specificity determinants for the abscisic acid response element. *FEBS Open Bio* **3**, 101–105.

Shang Y, Yan L, Liu Z, Cao Z, Mei C, Xin Q, Wu F, Wang X, Du S, Jiang T, Zhang X, Zhao R, Sun H, Liu R, Yu Y and Zhang D (2010) The Mg-chelatase H subunit of Arabidopsis antagonizes a group of WRKY transcription repressors to relieve ABA-responsive genes of inhibition. *Plant Cell* **22**, 1909–1935.

Shu K, Zhang H, Wang S, Chen M, Wu Y, Tang S, Liu C, Feng Y, Cao X and Xie Q (2013) *ABI4* regulates primary seed dormancy by regulating the biogenesis of abscisic acid and gibberellins in Arabidopsis. *PLoS Genetics* **9**, e1003577.

Shu K, Liu XD, Xie Q and He ZH (2016) Two faces of one seed: hormonal regulation of dormancy and germination. *Molecular Plant* **9**, 34–45.

Soderman EM, Brocard IM, Lynch TJ and Finkelstein RR (2000) Regulation and function of the *Arabidopsis* ABA-insensitive4 gene in seed and abscisic acid response signaling networks. *Plant Physiology* **124**, 1752–1765.

Sun G, Zhang X and Xiao S (2005) Maternal effect on sensitivity of embryo to ABA and grain dormancy in wheat. *Journal of Triticeae Crops* **25**, 37–41.

Tian X, Wang Z, Li X, Lv T, Liu H, Wang L, Niu H and Bu Q (2015) Characterization and functional analysis of pyrabactin resistance-like abscisic acid receptor family in rice. *Rice* **8**, 28.

Torada A, Ikeguchi S and Koike M (2005) Mapping and validation of PCR-based markers associated with a major QTL for seed dormancy in wheat. *Euphytica* **143**, 251–255.

Wang S and Basten CJ (2007) *Windows QTL cartographer 2.5*. Department of Statistics, North Carolina State University, Raleigh, NC.

Wang L, Liu K, Mao S, Li Z, Lu Y, Wang J, Liu Y, Wei Y and Zheng Y (2015) Large-scale screening for *Aegilops tauschii* tolerant genotypes to phosphorus deficiency at seedling stage. *Euphytica* **204**, 571–586.

Wind JJ, Peviani A, Snel B, Hanson J and Smeekens SCJ (2013) ABI4: versatile activator and repressor. *Trends in Plant Science* **18**, 125–132.

Yang J, Liu Y, Pu Z, Zhang L, Yuan Z and Chen G (2014) Molecular characterization of high pI α-amylase and its expression QTL analysis in synthetic wheat RILs. *Molecular Breeding* **34**, 1075–1085.

Yang S, Xu K, Chen S, Li T, Xia H, Chen L, Liu H and Luo L (2019) A stress-responsive bZIP transcription factor OsbZIP62 improves drought and oxidative tolerance in rice. *BMC Plant Biology* **19**, 260.

Yang J, Tan C, Lang J, Tang H, Hao M, Tan Z, Yu H, Zhou, Y, Liu Z, Li M, Zhou Y, Cheng M, Zhang L, Liu D and Wang J (2019) Identification of *qPHS.sicau-1B* and *qPHS.sicau-3D* from synthetic wheat for pre-harvest sprouting resistance wheat improvement. *Molecular Breeding* **39**, 132–143.

Yu J, Hu S, Wang J, Wong GK, Li S, Liu B, Deng Y, Dai L, Zhou Y, Zhang X, Cao M, Liu J, Sun J, Tang J, Chen Y, Huang X, Lin W, Ye C, Tong W, Cong L, Geng J, Han Y, Li L, Li W, Hu G, Huang X, Li W, Li J, Liu Z, Li L, Liu J, Qi Q, Liu J, Li L, Li T, Wang X, Lu H, Wu T, Zhu M, Ni P, Han H, Dong W, Ren X, Feng X, Cui P, Li X, Wang H, Xu X, Zhai W, Xu Z, Zhang J, He S, Zhang J, Xu J, Zhang K, Zheng X, Dong J, Zeng W, Tao L, Ye J, Tan J, Ren X, Chen X, He J, Liu D, Tian W, Tian C, Xia H, Bao Q, Li G, Gao H, Cao T, Wang J, Zhao W, Li P, Chen W, Wang X, Zhang Y, Hu J, Wang J, Liu S, Yang J, Zhang G, Xiong Y, Li Z, Mao L, Zhou C, Zhu Z, Chen R, Hao B, Zheng W, Chen S, Guo W, Li G, Liu S, Tao M, Wang J, Zhu L, Yuan L and Yang H (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*). *Science* **296**, 79–92.

Zhang Y, Zhao F and Zhao J (2005) Effects of exogenous ABA on the seed germination of rice (*Oryza sativa* L.) and the expression of relative genes. *Journal of Wuhan Botanical Research/Plant Science Journal* **23**, 203–210.

Zhang L, Wang B, Pan L and Peng J (2013) Recycling isolation of plant DNA, a novel method. *Journal of Genetical Genomics* **40**, 45–54.

Zhao S, Ye Z and Stanton R (2020) Misuse of RPKM or TPM normalization when comparing across samples and sequencing protocols. *RNA* **26**, 903–909.

Zhou K, Yang J, Wang Z and Wang J (2016) Sequence analysis and expression profiles of *TaABI5*, a pre-harvest sprouting resistance gene in wheat. *Genes & Genomics* **39**, 161–171.

Zhu T, Wang L, Rodriguez JC, Deal KR, Avni R, Distelfeld A, McGuire PE, Dvorak J and Luo MC (2019) Improved genome sequence of wild emmer wheat Zavitan with the aid of optical maps. *G3 (Bethesda)* **9**, 619–624.

https://doi.org/10.1017/S0960258521000015 Published online by Cambridge University Press