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EXPRESSION AND FUNCTION ANALYSIS OF WHEAT EXPASIN GENES *EXPA2* AND *EXPB1*

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Expansins are a group of plant cell wall loosening proteins that play important roles in plant growth and development. In this study, we performed the first study on the molecular characterization, transcriptional expression and functional properties of two wheat expansin genes *TaEXPA2* and *TaEXPB1*. The results indicated that *TaEXPA2* and *TaEXPB1* genes had typical structural features of plant expansin gene family. As a member of α-expansins, TaEXPA2 is closely related to rice OsEXPA17 while the β-expansin member TaEXPB1 has closely phylogenetic relationships with rice OsEXPAB4. The genetic transformation to Arabidopsis showed that both TaEXPA2 and TaEXPB1 were located in cell wall and highly expressed in roots, leaves and seeds. Overexpression of *TaEXPA2* and *TaEXPB1* genes showed similar functions, causing rapid root elongation, early bolting, and increases in leaves number, rosette diameter and stems length. These results demonstrated that wheat expansin genes *TaEXPA1* and *TaEXPB2* can enhance plant growth and development.

Keywords: expansins, expression, function, *TaEXPA2, TaEXPB1*, wheat

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide, which accounts for about 35% of the world's staple food (PAUX *et al*., 2008). Wheat also serves as main protein source of human food. Hexaploid wheat contains A, B, and D genomes with about 17 Gb in genome size, five times larger than that of humans (FELDMAN *et al*., 2005, PAUX *et al*., 2008). The cell growth is closely related with cell wall, which must withstand the internal turgor pressure and also ensure the extension of the cell during cell growth. The expansins are a group of plant cell wall loosening proteins that play an important role in cell wall modification, which is critical in cell enlargement. Expansins have been found to participate in a variety of development processes such as leaf growth (KULUEV *et al*., 2013; KULUEV *et al*., 2014), root hair initiation and growth (YU *et al*., 2011; LIU *et al*., 2018), fruit softening and ripening (PERINI *et al*., 2017), and pollen tube growth (COSGROVE *et al*., 1997; PEZZOTTI *et al*., 2002). In addition, expansins also contribute to nutrient-uptake efficiency (LU *et al.,* 2013) as well as various biotic and abiotic stress tolerances (LI *et al*., 2011; ZHOU *et al*., 2014; HAN *et al*., 2019).

Expansins were firstly found in the study of acid-induced cell wall elongation in cucumber hypocotyls, and then identified, isolated and purified from the hypocotyls of cucumber seedlings (MCQUEEN-MASON *et al*., 1992). Subsequently, plenty of studies focused on investigation of homologous expansin genes in a series of plants, including oat coleoptiles (LI *et al*., 1993), rice (TAN *et al*., 2018), cotton fiber (LI *et al*., 2016), soybean (ZHU *et al*., 2013), and tobacco (KULUEV *et al*., 2013). Expansin proteins normally contain 200-250 amino acids and correspond to a molecular weight of 25-30 kDa (SYNAN *et al*., 2014). They all have two conserved domains, domain I at C- terminal with 120 to 135 amino acid residues and domain II at C- terminus with 90 to 120 amino acid residues (SYNAN *et al*., 2014). The domain I, termed glycoside hydrolase-like family 45 (GH45-like), is considered as an important catalytic domain sharing a high homology with the conserved catalytic domain of GH45 family. The domain II contains a series of conserved tryptophans, which is generally regarded as a binding region with about 50% similarity to Group-II pollen allergen protein (G2A family) (SAMPEDRO *et al*., 2005). In addition, a signal peptide of 20-30 amino acid residues is present at the N-terminus, which is important for expansin expression (LI *et al*., 2003; SAMPEDRO *et al*., 2005).

According to the phylogenetic analysis, superfamily of plant expansin can be divided into four subfamilies: α-expansin (EXPA), β-expansin (EXPB), expansin-like A (EXLA) and expansin-like B (EXLB) (*KENDE et al*., 2004). The α-expansin is mainly found in dicotyledonous and the family poaceae of monocotyledonous plants, while β-expansin is predominantly present in other monocotyledonous plants. Studies showed that the α-expansin and β-expansin gene subfamilies already existed before the disorganization of vascular plants and bryophytes, and the recent ancestral era of expansin-like A and expansin-like B subfamily can be traced back to gymnosperms and angiosperms (LI *et al*., 2002; SCHIPPER *et al*., 2002). A lot of evidence indicated that EXPA and EXPB proteins are required for cell expansion and biological processes involving cell wall modification (DAL *et al*., 2013). Interestingly, both EXLA and EXLB have two typical domains of expansin proteins, but few experimental reports showed the cell relaxation activity of these two family members, therefore their functions are still not clear (SAMPEDRO *et al*., 2005; DAL *et al*., 2013).

Considerable work indicated that the expression of expansin genes has obvious tissue specificity. In soybean, the expansin gene *GmEXP1* specifically expressed in the roots (LEE *et*

al., 2003). Through *in situ* mRNA hybridization and immunohistochemical analysis, rice expansin genes showed high expression levels in the growing internodal epidermis, differentiating vascular bundles of internodes, lateral root primordia and emerging leaf primordia (CHO *et al.*, 2010). In wheat, the transcript of *TaExpA6* was found in the pericarp during early growth in grain development and, subsequently, in both endosperm and pericarp (LIZANA *et al.*, 2010). Meanwhile, the expression of expansin genes were affected by various environmental stresses such as oxidative stress (CHEN *et al.*, 2018), cold stress (ZHANG *et al.*, 2018), salinity stress (CHEN *et al.*, 2017), drought stress (CHEN *et al.*, 2016) and cadmium toxicity stress (REN *et al.*, 2017).

Recent reports have showed that at least 30 α-expansins and 65 β-expansins were present in wheat genome (ZHAO *et al*., 2016; CHEN *et al*., 2016). Until now, functional studies have revealed that wheat expansins are closely associated with grain size and weight (LIZANA *et al*., 2010; KUMAR *et al*., 2017), male gametophyte development (JIN *et al*., 2006), phosphorus absorption efficiency (HAN *et al*., 2014) and various abiotic stress tolerance (ZHAO *et al*., 2012; CHEN *et al*., 2018; REN *et al*., 2018). Wheat expansin genes *TaEXPA2* has been reported to improve seed production and tolerance in Cd toxicity (REN *et al*., 2018), salt stress (CHEN *et al*., 2017) and drought stress (CHEN *et al*., 2016), while poor functional studies is performed on wheat expansin genes *TaEXPA2*. In the current study, to discover functional properties of *TaEXPA2* and *TaEXPB1* genes in plant growth and development, we performed the study on the molecular characterization, transcriptional expression and functional properties of two wheat expansin genes *TaEXPA2* and *TaEXPB1*. Our results expand our knowledge of wheat expansins and provide new evidence for further understanding the structure and more functions of plant expansin gene family.

MATERIALS AND METHODS

Plant materials and seedling culture

Common wheat (*Triticum aestivum L., AABBDD, 2n=6x=42)* Chinese Spring (*CS)* was used as material. The seeds with similar size were surface-sterilized with 70% ethanol and 10% sodium hypochlorite and germinated on the wet sterile filter paper in sterilized Petri dishes for 48 h at room temperature. Following germination, seedlings were spread out in buckets to conduct hydroponic cultures under the condition of 16/8 h light/dark cycle, 20ºC temperature and 70% relative humidity. Hoagland nutrient solution (at a 1/2 dilution) was renewed every 3 d. At two-leaf stage, all tissues of seedling were harvested and frozen in liquid nitrogen prior to use.

Sequences retrieval and identification of wheat expansins

A total of 13 expansin sequences from Arabidopsis and rice were firstly obtained from EXPANSIN CENTRAL website (http://www.personal.psu.edu/fsl/ExpCentral/), then these sequences were used for BLAST searches in the rice, common wheat, and Arabidopsis proteome database in Phytozome v12.1 (https://phytozome.jgi. doe.gov/pz/portal.html) and NCBI (https://www.ncbi.nlm.nih.gov/). The online tools Pfam (http://pfam.xfam.org/) and SMART (http://smart.embl-heidelberg.de/) were used to screen expansin proteins. Those with only one of two domains or without a complete open reading frame were removed.

BLASTp tool was used to search for homologs of wheat expansin proteins in *Arabidopsis thaliana* and *Oryza sativa* genome database, Phytozome v9.0 (http://www.phytozome.net). Multiple sequence alignments of homogeneous protein sequences were performed using

COBALT (https://www.ncbi.nlm.nih.gov/ tools/cobalt/), and phylogenetic tree was constructed using the MEGA5.0 software with Maximum likelihood method (ML) analysis (TAMURA *et al.*, 2011).

mRNA extraction, cDNA synthesis and qRT-PCR

Total RNA was extracted using TRIZOL Reagent (Invitrogen) according to the manufacturer's instructions. Genomic DNA was removed by digesting each sample (20-50 μg of total RNA) with DNase I (Promega). Then reverse transcription reactions were performed with the PrimeScript® RT Reagent Kit with gDNA Eraser (TaKaRa, Shiga, Japan) according to the manufacturer's instructions. Gene-specific primers were designed using online Primer3Plus (http://www.primer3plus.com/) according to UNTERGASSER *et al.*, (2012). ADP-ribosylation factor gene was used as reference for normalization. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed in 20 μL volumes containing 10μL 2.5 × RealMaster Mix/20 × SYBR solution, 2 μL cDNA, 0.5 μL of each gene-specific primer and 8 μL ddH_2O. PCR conditions were: 95°C for 3 min, 39 cycles of20 s at 95°C, 58°C for 15 s and 72°C for 20 s, a melt curve of 65°C to 95°C. Reactions were conducted on a CFX96Real-time PCR Detection System (Bio-Rad). All data were analyzed with CFX Manager Software (Bio-Rad) (ANDREAS *et al.*, 2007).

Molecular cloning, plasmid construction, Arabidopsis transformation and identification

The full-length of *TaEXPA2* and *TaEXPB1* genes were cloned by PCR using wheat seed cDNA as template, and then recombined into pUC18 vector for sequencing. The specific-primer sequences designed were: CE-TaEXPA2-F: 5'-ggatcttccagagatATGG AGACGAGACGTCCAGCGGTTTCC-3', CE-TaEXPA2-R: 5'-ctgccgttcgacgatTCCA TCCGTCTTGTATTACAGGTC-3' and CE-TaEXPB1-F: 5'-ggatcttccagagatATGGCT CCTCTTTCGTC-3', CE-TaEXPB1-R: 5'-ctgccgttcgacgatTCAGCTGTACTGGACG AT-3'. DNA polymerase KOD-Plus-Neo was purchased from TOYOBO (Japan). PCR reaction in a 20 μL volume with cDNA, dNTPs and buffer was performed in a S1000TM thermal cycler (Bio-Rad, USA) with the following program: an initial step of 94ºC for 5 min, 34 cycles of 94ºC for 1 min, 62ºC for 45 s and 72ºC for 1 min 30 s, and a final step of 10 min at 72ºC. Five recombined DNA clones for each gene were sequenced by Sangong Company (Shanghai) to avoid possible errors.

Arabidopsis thaliana Col-0 ecotype seeds were surface-sterilized with 75% ethanol for 1 min, followed by 10% NaClO for 10 min, and then washed with sterile distilled water at least 5 times. After stratification at 4°C in dark for 3 days, the seeds were germinated and grown on Murashige and Skoog (MS) medium at 22°C with 16 h light/8 h dark cycle. After one week, the seedlings were transferred to vermiculite for following transformation assays. The full length cDNA fragments without stop codon were amplified by PCR using specific primers (Table S3). The coding sequence (CDS) fragments were recombined into pCAMBIA1302 vector to construct 35S::*EXPA2*:GFP and 35S::*EXPB1*:GFP. The recombined plasmids were transformed into *Arabidopsis* Col-0 ecotype using floral dip method (ZHANG *et al.*, 2006) and transgenic lines were obtained by screening progeny for hygromycin resistance. Seeds of first-generation transgenic lines from transformed plants were germinated on 1/2 MS medium containing 100 mg/L kanamycin to select for the positive seedlings. Transgenic lines were obtained from each transformation after performing at least two generations of resistance screening.

The stable transgenic Arabidopsis plants with overexpression of *TaEXPA2* and *TaEXPB1* were further confirmed by PCR with genomic DNA as template. The transcriptional level of the transgenes was detected by qRT-PCR with cDNA of leaves, roots (20, 30, 40 and 50 day) and seeds (seed-green, seed-yellow, seed-brown and seed-maturity) as template.

Subcellular location

To analysis the location of TaEXPA2 and TaEXPB1 proteins in plant cell, we performed a detection of green fluorescent protein (GFP) signal from transgenic plants. Seeds of the homozygous transgenic lines were surface-sterilized with 75% ethanol and 10% NaClO, and then placed at 4°C in dark for 3 days. Following stratification, the seeds were germinated and grown on MS medium at 22°C with 16 h light/8 h dark cycle. After one week, the roots of transgenic seedlings were cut and observed by using Zeiss LSM 780 fluorescence confocal microscopy.

Observation of growth and development of transgenic plants

For observing the growth and development of transgenic Arabidopsis plants, wild-type (WT) and transgenic Arabidopsis seeds were both germinated on MS medium, and then cultivated the 14-day-old seedlings under strictly same growth conditions. In the process of growth, bolting time and phenotypic characters including principal root length, average leaf number, rosette diameter, and stem height in both WT and transgenic plants were measured. Each measurement was performed with thirty plants to minimize experimental errors.

RESULTS

Molecular characterization of wheat TaEXPA2 and TaEXPB1 genes

Through BLASTp online tool, we found that wheat *TaEXPA2* (AAS48871) and *TaEXPB1* (AAT99292) genes had typical structural characterization of plant expansin gene family. *TaEXPA2* consisted of 777 bp encoding 258 amino acid residues with deduced molecular mass 27.8 kDa and isoelectric point 8.10. *TaEXPB1* contained 795 bp encoding 265 amino acid residues and the deduced molecular mass was 28.7 kDa with isoelectric point 4.90 (Fig. S1 and Fig. S2). The multiple sequence alignment of expansin proteins from wheat (TaEXPA2 and TaEXPB1), Arabidopsis (ATEXA1, ATEXPB2), rice (OsEXPA4, OsEXPB17) and Maize (ZmEXPA1, ZmEXPB1) showed that wheat expansins had similar structural features with those from Arabidopsis and rice (Fig. 1A). Same to the previously characterized expansin genes (LU *et al.*, 2016), both *TaEXPA2* and *TaEXPB1* shared three distinct domains: one short signal peptide, one *DPBB_1* domain and one *pollen_allerg_1* domain. In addition, the α-insertion was present in the TaEXPA2 in front of conserved His-Phe-Asp (HFD) motif with five conserved amino acids WCNPP. The β-insertion was found in TaEXPB1 behind conserved HDF motif with only one conserved amino acid glycine (Fig. 1A).

Phylogenetic tree of 16 expansin proteins from different plant species was constructed, including two from wheat (TaEXPA2 and TaEXPB1), eight from Arabidopsis (ATEXPA7, ATEXPA18, ATEXPB1, ATEXPB2, ATEXPB3, ATEXPB4, ATEXPB5, and ATEXPB6), and six from rice (OsEXPA17, OsEXPB2, OsEXPB4, OsEXPB5, OsEXPB10, and OsEXPB16). The results revealed that TaEXPA2 was closely related to rice OsEXPA17, and TaEXPB1 showed a close relationship with rice OsEXPAB4 (Fig. 1B).

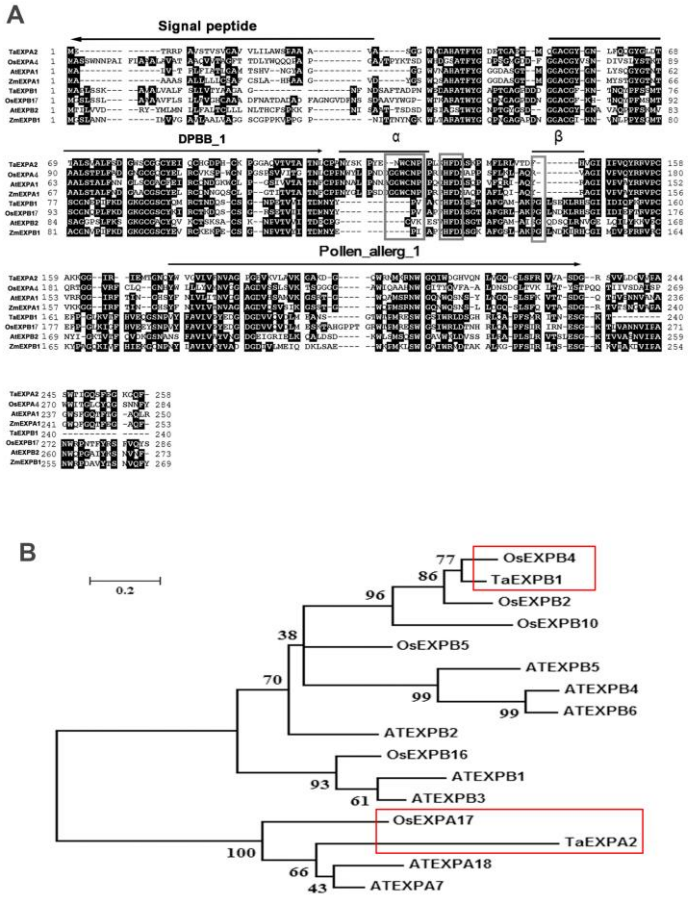

Fig. 1. Sequence alignment and phylogenetic analysis of wheat expansins TaEXPA2 and TaEXPB1 and other 14 expansin members from rice and Arabidopsis. (A) Alignments of the essential domains signal peptide, *DPBB_1* and *pollen_allerg_1* domain present in plant expansin proteins. The α-insertion and β-insertion were labeled with bold overlining, and conserved domains and amino acids were marked with grey boxes. (B) Phylogenetic relationships between TaEXPA2 and TaEXPB1 proteins and the related expansin proteins from rice and Arabidopsis. Numbers on the main branches indicate boot strap percentages for 1,000 replicates.

Expression of TaEXPA2 and TaEXPB1 genes in different wheat tissues and organs

qRT-PCR was used to detect the expression profiles of *TaEXPA2* and *TaEXPB1* in different wheat tissues and organs, including seed, leaf, and root (Fig. 2). Specific primers for *TaEXPA2* and *TaEXPB1* genes were designed and listed in Table S3. The results showed that *TaEXPA2* had an abundant expression in wheat seeds, and a lower expressional level in both leaves and

roots (Fig. 2A). *TaEXPB1* had a high expression level in both seeds and leaves and a lower expression level in roots (Fig. 2B).

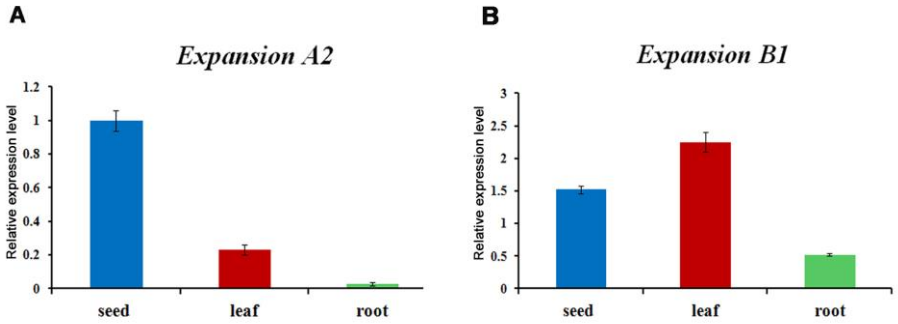

Fig. 2. Transcriptional expression profiles of *TaEXPA2* and *TaEXPB1* genes in different wheat tissues and organs. (A) Expression of *TaEXPA2* in seed, leaf and root of wheat. (B) Expression of *TaEXPB1* in seed, leaf and root of wheat. Error bars indicate standard errors of three biological replicates.

Genetic transformation and identification of TaEXPA2 and TaEXPB1 genes

To analyze function of *TaEXPA2* and *TaEXPB1* in plant growth and development, the overexpressed transgenic lines OE-TaEXPA2 and OE-TaEXPB1 were obtained by hygromycin screen and genomic PCR confirmation (Fig. 3A). Then, we conducted an observation on the roots of transgenic seedlings by using Zeiss LSM 780 fluorescence confocal microscopy to perform the subcellular location of TaEXPA2 and TaEXPB1 proteins. The result showed that the GFP signals of the fusion proteins of both TaEXPA2 and TaEXPB1 located in cell wall (Fig. 3B).

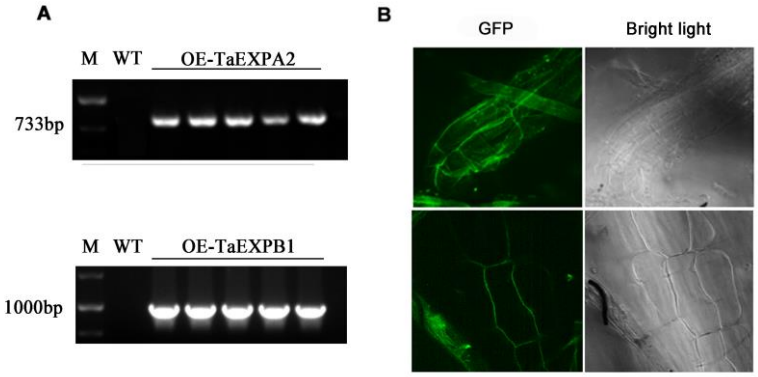

Fig. 3. PCR identification and subcellular location of Arabidopsis transgenic lines OE-TaEXPA2 and OE-TaEXPB1. (A) The genomic PCR identification of transgenic Arabidopsis. (B) The GFP signals of fusion proteins detected by Fluorescence scanning in transgenic plants.

Transcriptional expression profiling of TaEXPA2 and TaEXPB1 in wild type and transgenic Arabidopsis seedling

qRT-PCR was used to detect the transcriptional expression profiling of *TaEXPA2* and *TaEXPB1* genes at different tissues/organs and growth stages of wild type and transgenic Arabidopsis plants (Fig. 4). The results showed that *TaEXPA2* had a continually high expressional level in both leaves and roots at 20, 30, 40 and 50 days. Interestingly, the expression of *TaEXPA2* gradually increased along with seed development, and reached to a high level in mature seeds (Fig 4A). *TaEXPB1* displayed a high expression at 20 d and 50 d of leaves, but with a very low expression level at 30d and 40d in leaves. During the development of root, *TaEXPB1* highly expressed at 20 d, but had a low expression level at 30 d and almost no expression at 40 d. In addition, *TaEXPB1* also showed a high expression in mature seeds whereas almost no expression occurred in the developing yellow and brown seeds (Fig. 4B).

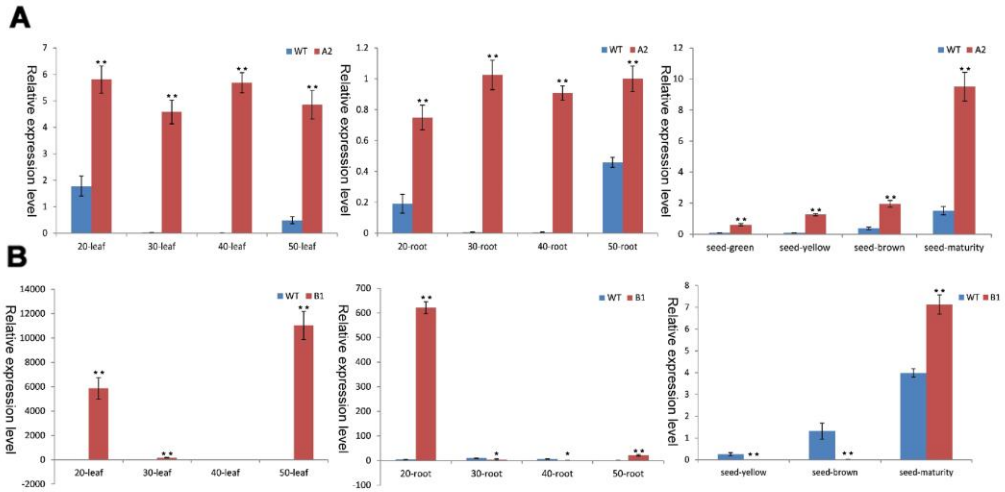

Fig. 4. Transcriptional expression profiles of *TaEXPA2* and *TaEXPB1* in the different organs and developmental stages of wild type and Arabidopsis transgenic plants detected by qRT-PCR. A. OE-TaEXPA2 (A2); B. OE-TaEXPB1 (B1). WT, wild type. Error bars indicate standard errors of three biological replicates. Statistically significant differences between wild type and transgenic plant were calculated by independent Student's *T*-tests: *$p < 0.05$; ** $p < 0.01$.

Comparison of wild type and Arabidopsis transgenic plants

The growth and development features of the wild type and transgenic lines were observed to estimate the biological function of *TaEXPA2* and *TaEXPB1* genes, including bolting time, principal root length, average leaf number, rosette diameter, and stem height (Fig. 5, Table 1). We found that the phenotypic characters of the transgenic lines OE-TaEXPA2 and OE-TaEXPB1 were similar, and the growth and development of both lines were significantly

enhanced at all stages. Compared with wild type, the seedlings at 14 d in both transgenic lines showed much longer principle roots, and at 20 d, the rosette diameter of OE-TaEXPA2 and OE-TaEXPB1 was 0.7 and 0.9 cm longer than wild type, respectively. At 30 d, the rosette diameter between wild type and transgenic plants maintained a clear difference of 0.7 cm, and slight difference in average leaf number. The difference of the rosette diameter at 35 d between wild type and transgenic plants increased from 0.7 cm to 1.4 cm, and OE-TaEXPA2 and OE-TaEXPB1 lines had two and three leaves more than wild type plants, respectively. Meanwhile, the stem high of OE-TaEXPA2 and OE-TaEXPB1 plants was 1.8 and 1.4 times higher than that of wild type plants, respectively (Table 1). At 40 d, the difference in average leaf number between OE-TaEXPA2 and OE-TaEXPB1 was still significant, but the gap of the rosette diameter between wild type and transgenic plants was decreased to 0.2 cm. In addition, the stem height of both transgenic lines was approximately two times longer than the wild type. At maturity stage, the stem height of OE-TaEXPA2 and OE-TaEXPB1 plants was respectively 3.7 cm and 1.2 cm higher than the wild type. Notably, the bolting time of OE-TaEXPA2 and OE-TaEXPB1 was significantly expedited with 4 and 3 days faster than wild type plants (Table 1).

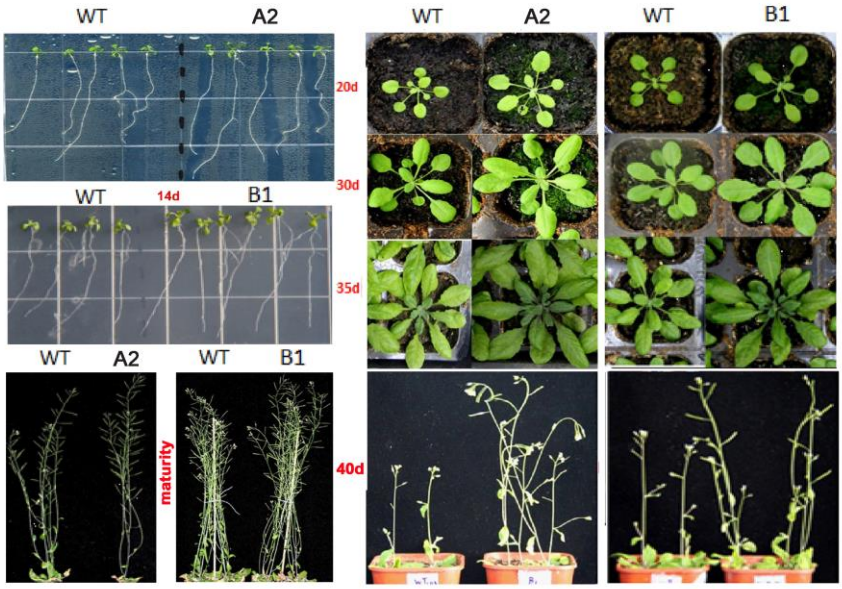

Fig. 5. Comparison of *WT*, *TaEXPB1* and *TaEXPA2* Arabidopsis transgenic plants at different growth and development stages. WT, wild type; A2, OE-TaEXPA2; B1, OE-TaEXPB1. Statistically significant differences between wild type and transgenic plant were calculated by independent Student's *T*-tests: *$p < 0.05$; ** $p < 0.01$.

Table 1. Comparison of plant growth and developmental characters between wild type (WT) and the transgenic Arabidopsis lines OE-TaEXPA2 and OE-TaEXPB1.

Growth Stages (days)	Genotypes	Length of pricinple root (cm)	Average number of leaves	Rosette diameter (cm)	Stem length (cm)	Bolting time (days)
14	WT	3.13±0.15				
	OE-TaEXPA2	4.24±0.16**				
	OE-TaEXPB1	3.71±0.11**				
20	WT		8.11±0.13	2.01±0.01		
	OE-TaEXPA2		8.08±0.11	2.89±0.02**		
	OE-TaEXPB1		9.02±0.13*	2.7±0.02**		
30	WT		12.02±0.07	3.9±0.03		
	OE-TaEXPA2		12.01±0.05	4.62±0.09*		
	OE-TaEXPB1		13.01±0.11*	4.61±0.08*		
35	WT		16.02±0.12	6.21±0.09	0.81±0.02	
	OE-TaEXPA2		19.01±0.11**	7.61±0.08**	2.22±0.02**	
	OE-TaEXPB1		18.02±0.12**	7.61±0.07**	1.91±0.01**	
40	WT		18.03±0.11	7.22±0.08	4.21±0.05	
	OE-TaEXPA2		21.01±0.13**	7.71±0.10*	8.52±0.13**	
	OE-TaEXPB1		20.01±0.12**	7.42±0.11	8.52±0.12**	
Maturity	WT		-	-	28.41±0.21	36.01±0.23
	OE-TaEXPA2		-	-	32.11±0.23**	32.11±0.21**
	OE-TaEXPB1		-	-	29.32±0.18**	33.02±0.18**

*$p < 0.05$, **$p < 0.01$.

DISCUSSION

Plant cells enclosed themselves within a complex polysaccharide wall, which represents a critical determinant in plant architecture and is of fundamental importance in plant growth and development (COSGROVE, 2005). Expansins have their unique ability with inducing immediately cell wall extension *in vitro* and cell expansion *in vivo*. In this study, we performed a sequence alignment and phylogenic analysis and found that wheat expansin genes *TaEXPA2* and *TaEXPB1* showed high similarity and closely phylogenetic relationships with rice expansin genes *OsEXPA17* (NC_029261) and *OsEXPB4* (NC_029265), respectively. Both *OsEXPA17* and *OsEXPB4* were found to be closely associated with plant growth and development (LEE *et al*., 2001; YU *et al*., 2011). Thus, we speculate that *TaEXPA2* and *TaEXPB1* might have similar biological function with *OsEXPA17* and *OsEXPB4*.

The overexpression of *TaEXPA2* and *TaEXPB1* in Arabidopsis caused pleiotropic morphological changes, including rapid root elongation, leaf and plant growth, corresponding to their high transcriptional expression levels at cell wall of both roots and leaves, particularly at the early stages of plant growth. Similar results were also obtained in the previous study in which high expression level of soybean expansin gene *GmEXP1* occurred in the seedling roots and further ectopic expression of *GmEXP1* in tobacco (*Nicotiana tabacum*) seedlings caused the acceleration in the root growth (LEE *et al*., 2003).

Early study showed that local transient induction of expansin expression on the flank of developing primordia leads to the induction of the entire process of leaf development (PIEN *et al*., 2001). Both OE-TaEXPA2 and OE-TaEXPB1 transgenic lines had more leaves than wild type plants, which might be caused by bulging of the apical meristem induced by overaccumulation of expansin proteins (CHOI *et al*., 2003). Our results also showed that the overexpression of two wheat expansin genes led to significant increase in rosette diameters, suggesting a role of

TaEXPA2 and *TaEXPB1* in the induction of leaf formation and development. The most striking observation of the transgenic plants overexpressing *TaEXPA2* or *TaEXPB1* genes exhibited a rapid stem elongation and significant increase in plant height, consistent with the previous studies in rice and aspen (CHOI *et al*., 2003; GRAY-MITSUMUNE *et al*., 2008). Therefore, the overaccumulation of TaEXPA2 and TaEXPB1 proteins could promote cell growth by loosening the cell wall, resulting in the acceleration of plant growth and development in the transgenic plants.

CONCLUSION

Wheat expansin genes *TaEXPA2* and *TaEXPB1* showed a high sequence similarity and typical structural features with those from Arabidopsis and rice, which were closely related to rice *OsEXPA17* and *OsEXPAB4*, respectively. Genetic transformation to Arabidopsis showed that both genes were located in cell wall and had a high expression level in roots, leaves and seeds. Overexpression of *TaEXPA2* and *TaEXPB1* showed a similar function and led to early bolting, rapid root elongation and increases in leaves number, rosette diameter and stems length in Arabidopsis. The results demonstrated that wheat expansin genes *TaEXPA1* and *TaEXPB2* could enhance plant growth and development.

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EXPRESIONA I FUNKCIONALNA ANALIZA EKSPANZIN GENA *EXPA2* I *EXPB1* KOD PŠENICE

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Izvod

Ekspansini su grupa biljnih proteina, koji se nalaze u ćelijskom zidu i imaju važne uloge u rastu i razviću biljke. U ovom radu, urađeno je prvo proučavanje molekularne karakterizacije, transkripcione ekspresije i funkcionalnih osobina dva ekspanzin gena pšenice *TaEXPA2* i *TaEXPB1*. Rezultati su pokazali da oba gena imaju tipične strukturne karakteristike familije ekspanzin gena kod biljaka. Kao član α -ekspanzina, *TaEKSPA2* je blisko povezan sa genom *OsEKSPA17* pirinča, dok je β-ekspanzijski član *TaEKSPB1* blisko filogenetski povezan *OsEKSPAB4* pirinča. Genetska transformacija kod Arabidopsis-a pokazala je da su i *TaEKSPA2* i *TaEKSPB1* locirani u ćelijskom zidu i visoko izraženi u korenu, lišću i semenu. Prekomerna ekspresija gena *TaEKSPA2* i *TaEKSPB1* pokazala je slične funkcije, uzrokujući brzo izduživanje korena, rani porast i povećanje broja listova, prečnika rozete i dužine stabljika. Ovi rezultati su pokazali da geni ekspanzini pšenice *TaEKSPA1* i *TaEKSPB2* mogu poboljšati rast i razvoj biljaka.

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