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Genome-wide identification of expansin genes in *Brachypodium distachyon* and functional characterization of *BdEXPA27*

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ABSTRACT

Plant expansin belongs to a group of cell wall proteins and functions in plant growth and development. However, limited data are available on the contributions of expansins in *Brachypodium distachyon*. In the present study, a total of 38 expansins were identified in *B. distachyon* genome. Phylogenetic analysis divided the expansins into four groups, namely EXPA, EXPB, EXLA, and EXLB. Chromosomal distribution showed that they were unevenly distributed on 4 chromosomes. A total of six tandem duplication pairs and four segmental duplication pairs were detected, which contributed to the expansion of the *B. distachyon* expansin gene family. Expansins in the same group shared similar gene structure and motif composition. Three types of *cis*-elements, development-related, hormone-related, and abiotic stresses-related elements were found in the *B. distachyon* expansin gene promoters. Expression profiles indicated that most of *B. distachyon* expansin genes participate in plant development and abiotic stress responses. Overexpression of *BdEXPA27* increased seed width and length, root length, root hair number and length in *Arabidopsis* and showed higher germination rate in transgenic lines. This study establishes a foundation for further investigation of *B. distachyon* expansin genes and provides novel insights into their biological functions.

1. Introduction

Expansins are a group of loosening proteins located in the plant cell wall [1]. According to previous study, plant expansin superfamily includes four subfamilies: α-expansin (EXPA), β-expansin (EXPB), expansin-like A (EXLA), and expansin-like B (EXLB) [2]. The molecular weight of mature expansin proteins is between 25 − 30 kDa, corresponding to 250–300 amino acids [3]. Expansins are characterized by two conserved domains: one is a six-stranded double-psi beta-barrel (DBPP) with His-Phe-Asp motif and some conserved polar residues; the other is Pollen_allerg domain, which contains conserved aromatic amino acids [4].

Expansins are involved in plant growth and development processes such as root hair growth [5], seed size [6], germination [7], leaf growth [8], and respond to abiotic stresses [9]. In EXPA subfamily, *AtEXPA2* responds to salt and osmotic stresses in *Arabidopsis* [7]; *AtEXPA7* and *AtEXPA18* are specifically expressed in root hairs and play important roles in root hair initiation and elongation [10,11]; overexpression of *TaEXPA2* improves seed production, salt and drought stress tolerance in transgenic tobacco [6,12], and oxidative stress tolerance in transgenic *Arabidopsis* [13]. In EXPB subfamily, *OsEXPB2* involves in rice root system architecture and promotes growth [14]; In EXLA and EXLB subfamilies, overexpression of *Arabidopsis EXLA2* decreases the wall strength in hypocotyls [15]; overexpression of soybean *GmEXLB1* gene improves phosphorus acquisition in *Arabidopsis* by altering root architecture [16].

Till now, genome-wide analyses of expansin genes have been reported in several species, for example, there are 31 in *Arabidopsis*, 40 in rice [17], 88 in maize [18], 52 in tobacco [19], 92 in sugarcane [20], 75 in soybean [21]. However, no systemic analysis of expansin genes was reported in *Brachypodium distachyon*, a good model plant for functional genomic studies in plants [22]. In this study, we identified and analyzed expansin genes at genome-wide, and characterized the functions of *BdEXPA27* in plant growth. Our study lays a foundation for further study of expansin genes.

2. Material and methods

2.1. Genome-wide identification of expansins in Brachypodium distachyon

To identify the expansins in *B. distachyon*, two methods were performed. Firstly, used the DPBB_1 (PF03330) and Pollen_allerg_1 domain

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(PF01357) from Pfam database (http://pfam.xfam.org/) and searched against the *B. distachyon* genome protein sequences with a threshold e < 1e^−5. Secondly, used the rice and *Arabidopsis* expansin protein sequences from previous study [23] to build a Hidden Markov Model (HMM) and searched against the sequences of results in the first step. After that, a manual correction was performed to remove the alternative splicing events and redundancy. Finally, the NCBI-CDD database (NCBI Conserved Domain Database) and SMART database (Simple Modular Architecture Research Tool) were used to confirm the putative expansins. To verify the existence of expansins in *B. distachyon*, we performed BLASTN to search for ESTs (Expressed Sequence Tags) using the CDS (Coding sequences) of expansins in NCBI database (https://www.ncbi.nlm.nih.gov/). The ExPASy webserver [24] was used to predict the theoretical isoelectric point and molecular weight of expansins. The CELLO web server [25] was used to predict the subcellular localization of expansins.

Sequences of genome DNA, CDS, cDNA, protein, and upstream 2-kb genomic DNA of *B. distachyon* (version 3.0) were downloaded from the Ensembl Plant database (http://plants.ensembl.org/index.html). The protein sequences of *Arabidopsis* and rice were downloaded from Phytozome database (https://phytozome.jgi.doe.gov/pz/portal.html).

2.2. Chromosome distribution, gene duplication, and phylogenetic analyses

The chromosome distribution information of *B. distachyon* expansins was obtained from the Ensembl Plants database. The gene duplication information was obtained from the Plant Genome Duplication database [26]. Chromosome distribution and gene duplication were visualized using Circos (v0.69) program [27]. An un-rooted neighbor-joining (NJ) tree was constructed by MEGA 7.0 software [28] with 1000 bootstrap replications based on full-length protein sequence alignment.

2.3. Cis-elements, gene structures, and motifs analyses

The upstream 2-kb genomic DNA sequences of *B. distachyon* expansin genes were submitted to PlantCARE database, a database of plant *cis*-acting regulatory DNA elements [29], to predict the *cis*-acting elements. The exon-intron structure of *B. distachyon* expansin genes was graphically displayed by the GSDS 2.0 (Gene Structure Display Server) using the CDS and genome sequences. The MEME Suite (Motif-based sequence analysis tools) [30] was used to identify conserved motifs with the *B. distachyon* expansins with the following parameters: optimum motif width set to ≥6 and ≤200, maximum number of motif was 10. The phylogenetic tree, gene structure, and motifs were visualized by Evolview [31].

2.4. Brachypodium distachyon growth and stresses treatment

B. distachyon Bd-21 was planted in an artificial climate chamber at 24/22℃ (day/night) with a photoperiod of 16/8 h (day/night). For different tissue analyses, roots, stems, leaves, inflorescences were collected at the heading stage (8-week-old). For abiotic stresses, 2-week-old seedling plants were subjected to heat (42℃), cold (4℃), NaCl (200 mM), drought (20 % PEG6000), and ABA (100 μM) for 2 h, the whole plant were immediately frozen in liquid nitrogen and then stored at -80℃ for RNA isolation.

2.5. RNA isolation and quantitative RT-PCR

Total RNA was isolated using the TRIZOL reagent (TAKARA) and treated with RNase-free DNase I (TAKARA) followed the manufacturer's instruction. The cDNA were synthesis by Transcriptor First Strand cDNA synthesis Kit (Roche). The quantitative RT-PCR (qRT-PCR) was performed in triplicate and 15 μl reaction systems containing 7.5 μl SYBR Premix Ex Taq (TAKARA), 0.15 μl ROX reference Dye (50×), 0.5 μl cDNA (5.0 ng/μl), 0.75 μl (10 pmol/μl) each of forward and reverse primers, and 5.35 μl ddH_2O. The qRT-PCR conditions were 50℃ for 2 min, 95℃ for 10 min, followed by 40 cycles of 95℃ for 15 s, 60℃ for 1 min in the PCR stage and 95℃ for 15 s, 60℃ for 1 min, 95℃ for 15 s in the melt curve stage. Data acquisition were performed using the QuantiStudio 7 Flex Real-Time PCR System (ThermoFisher Scientific). Relative expression of target genes were designed by OLIGO 7 software [32] and the primers were listed in Table S1. Data were normalized with the expression of *GAPDH* in *B. distachyon* [33] and *Arabidopsis* [34]. The relative expression level was calculated using the $2^{-\Delta\Delta Ct}$ analysis method [35]. High-throughput sequencing data of *B. distachyon* were obtained from the ArrayExpress database (http://www.ebi.ac.uk/arrayexpress) under accession number E-MTAB-4401.

2.6. Vector construction and plant transformation

The *BdEXPA27* was amplified through PCR using primers as follows: 5′-ggtggtTCTAGAATGGCGCCGGCTCCAGCTCA-3′ (*XbaI* site underlined) and 5′-ggtggtGGTACCTTAATTGAACTGTACTTTG-3′ (*KpnI* site underlined). Subsequently subcloned into the pCambia-1300 plant expression vector harboring the *CaMV35S* promoter. The transformation of the obtained recombinant vector into wild type (*Col*-0) *Arabidopsis* via *Agrobacterium tumefaciens* (GV3101) mediated floral dip method [36].

2.7. Phonotype analysis in Arabidopsis

The *Arabidopsis Col*-0 was used to generate *BdEXPA27* transgenic plants. Transgenic lines were screened by 40 mg/L Hygromycin B solution and then confirmed by PCR. Seeds from T3 generation of the transgenic and WT plants were sterilized with 75 % alcohol for one min and cleaned three times with sterilized distilled water, then sterilized with 10 % NaClO for 10 min and cleaned six times with sterilized distilled water. The seeds were germinated in 1/2-strength Murashige-Skoog (MS) medium in long-day conditions of 22/20℃(day/night) with a photoperiod of 16/8 h (day/night). For each plant, root length was measured, on the seventh day. Four-leaf-stage seedlings were transplanted into soil. At the mature fruit stage, fruits were counted.

For the germination assays, the seeds of *Col*-0 and transgenic lines were surface sterilized and kept at 4℃ for 72 h in the dark before germination. About 50 seeds of every genotype were sown on the same plate containing 1/2 MS medium at 22/20℃ (day/night) with a photoperiod of 16/8 h (day/night) for 7 days. Each day germinated seeds with protruded radicles were counted.

2.8. Seeds of light microscopy, and observation of root hair number and length

Seeds from the siliques located in the basis of a major inflorescence were selected for observation. Mature seeds from wild type and transgenic lines were randomly selected, observed, and photographed using an OLYMPUS SZ 61 stereomicroscope. Root hair number and length were measured using a stereomicroscope after sowing for 7 days and according to Cho and Cosgrove [10] with some modifications.

2.9. Statistical analysis

The length of roots, length and width of seeds were counted by ImageJ software [37]. Data were analyzed and graphs were drawn using Excel 2013 (Microsoft Corporation, USA). In all graphs, error bars indicate standard deviation, and significant differences are indicated with "*" ($P < 0.05$) or "**" ($P < 0.01$).

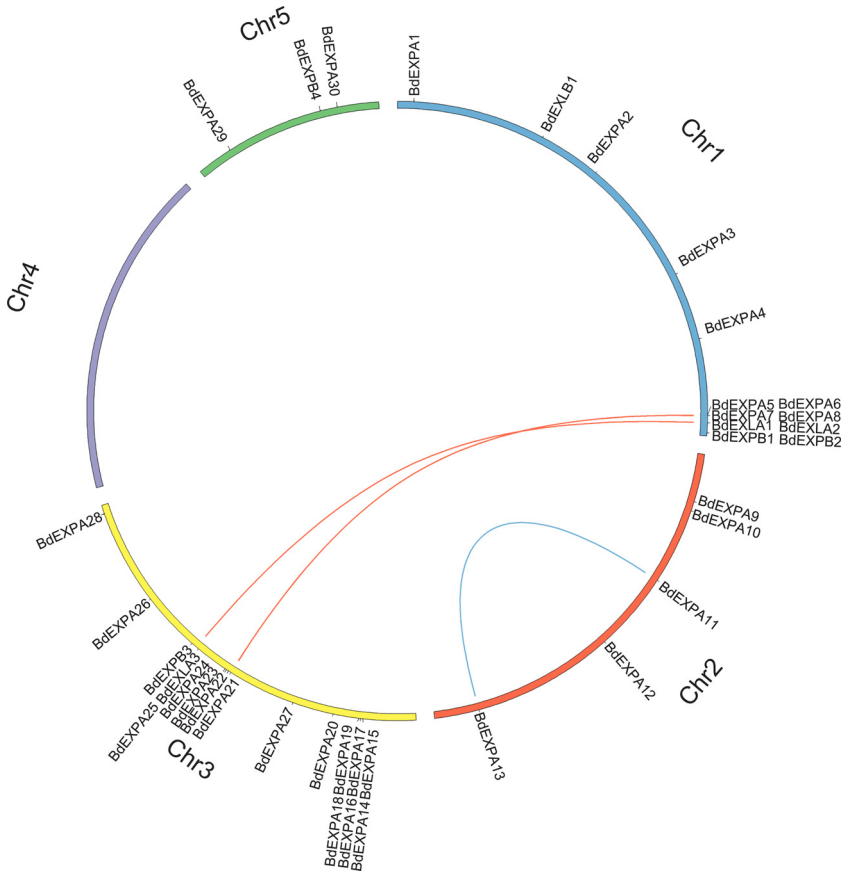

Fig. 1. Chromosome location, and segmentally duplicated gene pairs of *B. distachyon* expansins. Different chromosomes were showed in different colors. The gene pairs were indicated by lines.

3. Results

3.1. Identification of Brachypodium distachyon expansins

Overall, 38 putative expansins were identified in *B. distachyon* genome, accounting for 0.1213 % of annotated *B. distachyon* genes. Of them, 30 were validated by expressed sequence tags (ESTs) in the NCBI database. The predicted length of these *B.distachyon* expansin proteins varies from 244 (BdEXPA4) to 316 (BdEXPB1) amino acids; the predicted molecular weight (MW) ranges from 25.60 kDa (BdEXPA10) to 34.15 kDa (BdEXLB1). The detailed information is listed in Table S2.

3.2. Chromosome distribution and gene duplication

Those 38 expansin genes were unevenly distributed on four chromosomes (Fig. 1): 17 on Chr 3, 13 on Chr 1, 5 on Chr 2, and 3 on Chr 5. There is no expansin gene on Chr 4. To investigate gene duplication, tandem duplication and segmental duplication events were also identified. As results, 11 tandem duplication genes were identified and formed 6 tandem duplication pairs; 4 segmental duplication pairs were generated from 6 BdEXPA and 2 BdEXLA members (Table S3). To explore the evolutionary process of *B. distachyon* expansins, genome synteny among *Arabidopsis*, rice, maize, and sorghum was also investigated using Plant Genome Duplication Database [26]. Results showed that 4, 27, 29, and 22 *B. distachyon* expansins had homologous genes in *Arabidopsis*, rice, maize, and sorghum, respectively (Table S4).

3.3. Phylogenic tree, gene structures, and conserved motifs

To study the evolutionary relationships of *B. distachyon* expansins, we constructed an un-rooted Neighbor-Joining phylogenic tree with MEGA 7 software based on multiple alignment of 128 expansins from *B. distachyon*, rice, and *Arabidopsis*. According to the clade support values and the classification of orthologs in rice and *Arabidopsis*, *B. distachyon* expansins could be classified into four subfamilies EXPA, EXPB, EXLA, and EXLB (Fig. 2), which were proposed by Kende et al. [2] with high confidence values. Among them, 30, 4, 3, and 1 belong to the EXPA, EXPB, EXLA, and EXLB subfamily, respectively (Figs. 2 and 3A).

The gene structure of the *B. distachyon* expansin genes was also examined (Fig. 3B). The number of exons ranges from 1 to 5. The EXPA members contained two or three exons except for *BdEXPA21* and *BdEXPA14*, which contained only one exon. All *EXPB*, *EXLA*, and *EXLB* genes contained more than 2 exons. In addition, 10 conserved motifs were identified (Fig. 3C). Among them, motif 2 is highly conserved in all expansin proteins, motif 1, 3, 4, 5, 6, and 9 are EXPA specific. On contrary, motifs 7, 8, 10 are excluded from EXPA members. Previous study showed that the DBPP and Pollen_allerg domain are main components of expansins [4]. In EXPA group, motifs 1, 2, 6, and 9 constitute the DBPP domain, while motifs 3 and 4 constitute the Pollen_allerg domain. In the EXPB, EXLA, and EXLB groups, motifs 2 and 7 form the DBPP domain, while motifs 8 and 10 form the Pollen_allerg domain.

3.4. Cis-elements

We also identified the *cis*-element in the 2-kb promoters of *B. distachyon* expansin genes by using the PlantCARE database. As shown in Fig. 3D, three types of *cis*-elements, including development-related, hormone-related, and abiotic stresses-related were identified. *Cis*-elements related to development include light-response elements Sp1 (GGGCGG) [38], root regulation *cis*-element G-box (CACGTC) [39], the metabolism regulation related *cis*-element O2-site [40], and the meristem expression related *cis*-element CAT-box [41]. *Cis*-elements related

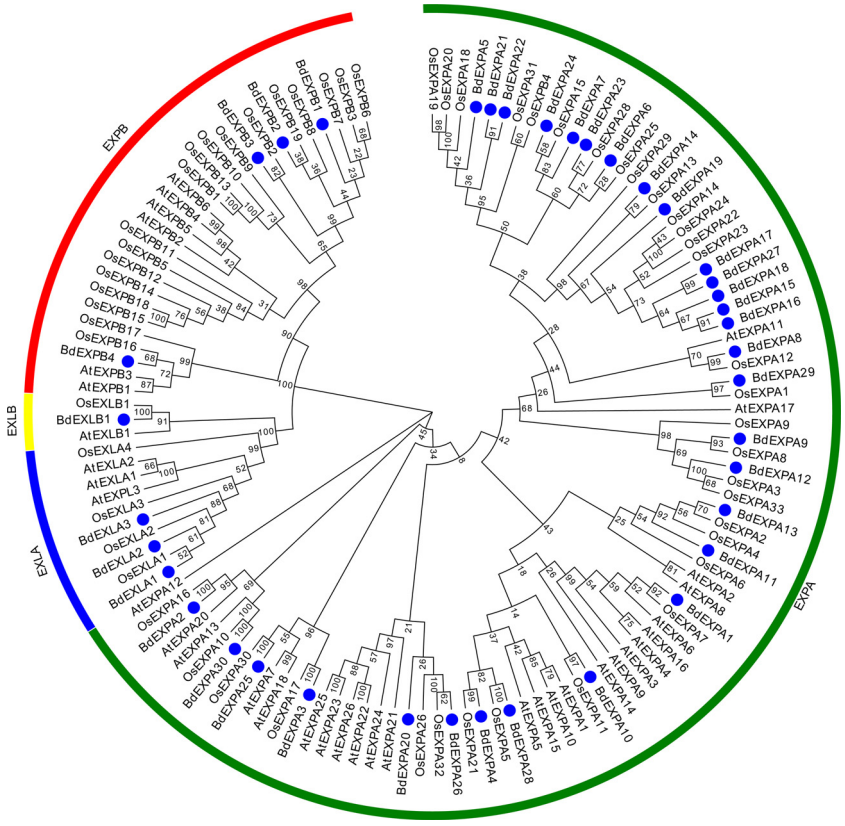

Fig. 2. Phylogenetic tree of expansins in *B. distachyon*, rice, and *Arabidopsis*. *B. distachyon* expansins were marked by blue circle dots. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

to hormone stress include methyl jasmonate (MeJA)-related *cis*-element CGTCA-motif [42], abscisic acid (ABA)-responsive related *cis*-element ABRE (ACGTG) [43], gibberellin (GA)-responsive related *cis*-element GARE-motif [44], and auxin-responsive *cis*-elements AuxRR-core (GGTCCAT) and TGA-element (CCATCTTTTT) [45,46]. *Cis*-elements related to abiotic stresses include anoxic specific inducibility element GC-motif (A/CGCCGCGCA) [47], drought-inducibility *cis*-element MBS (CAACTG) [48], and low-temperature responsive *cis*-element LTR (CCGAAA) [49]. Among these *cis*-elements, G-box, CGTCA-motif, and ABRE were found in most cases.

3.5. Expression profiles

To obtain insight into the temporal and spatial expression patterns of *B. distachyon* expansin genes, we used high-throughput data to analyze the expression patterns in different tissues by integrating the published data. There are 26 *B. distachyon* expansin genes were mainly expressed in roots, and 12 showed high expression in flowers (Fig. S1).

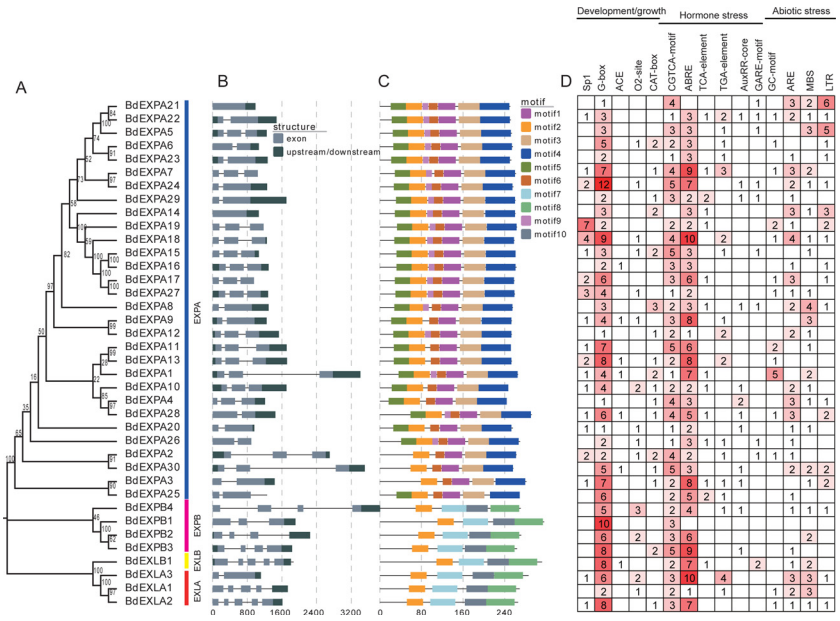

Fig. 3. Phylogenetic relationships, gene structures, motifs, and main *cis*-elements of *B. distachyon* expansins. (A) *B. distachyon* expansins were classified into four groups according to bootstrap values; (B) Gene structures of *B. distachyon* expansins. Exons and introns were indicated by boxes and lines respectively; (C) Different motifs of *B. distachyon* expansins. Different boxes indicated different motifs. (D) Three types cis-elements identified in the promoters.

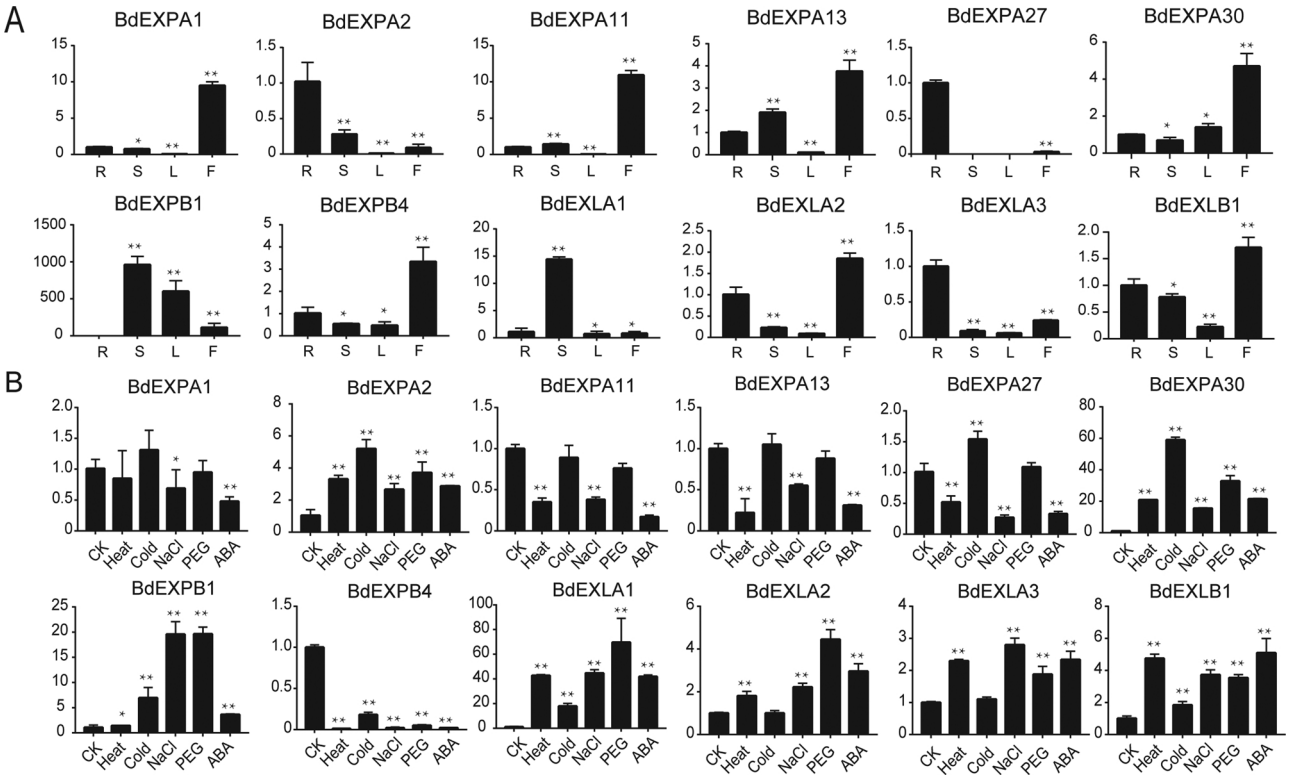

Fig. 4. The expression patterns of 12 *B. distachyon* expansin genes in different tissues (A), and in seedlings with different treatments (B). R, S, L, F in (A) indicate roots, stems, leaves, and inflorescences respectively. CK in (B) indicates control. Error bars represent standard deviations, and statistically significant differences are indicated: *, $P < 0.05$; **, $P < 0.01$ (Student's t-test).

We randomly selected 6 *EXPA*, 2 *EXPB*, 3 *EXLA*, and 1 *EXLB* genes to further analyze the expression profiles by performing qRT-PCR (Fig. 4). 10 (except for *BdEXPA27* and *BdEXPB1*) are expressed in all tissues; 7 (*BdEXPA1*, *BdEXPA11*, *BdEXPA13*, *BdEXPA30*, *BdEXPB4*, *BdEXLA3*, and *BdEXLB1*) are highly expressed in inflorescences; and 3 (*BdEXPA2*, *BdEXPA27*, and *BdEXLA3*) are highly expressed in roots (Fig. 4A).

We also analyzed the expression of these genes under different abiotic stresses in 2-week old seedlings (Fig. 4B). Of 12 genes, the expression of *BdEXPA2*, *BdEXPA30*, *BdEXPB1*, *BdEXLA1*, *BdEXLA2*, and *BdEXLB1* was obviously up-regulated by different treatments, and the expression of *BdEXPB4* is down-regulated by different treatments (Fig. 4B).

3.6. Phenotypes of transgenic Arabidopsis overexpressing BdEXPA27

BdEXPA27 is specifically expressed in roots (Fig. 4A), indicating that it might play a role in root development. To verify this prediction, we overexpressed this gene in *Arabidopsis*. We selected lines OE-2 and OE-3 with high expression levels for further analyses (Fig. 5A). Compared with WT plants, transgenic *Arabidopsis* showed quicker germination (Fig. 5B and C). Furthermore, the seed size of the transgenic *BdEXPA27* plants were markedly larger than the control. Seeds of transgenic lines displayed significant differences in width and length, compared to the control (Fig. 5D–G). The 1000-grain weight of the two *BdEXPA27* transgenic lines increased by 18.18 % and 17.36 %, compared to the control plants (Fig. 5H).

To explore the changes in root architecture on transgenic *Arabidopsis*, microscopic observations and measurements of roots were performed in this study. Seven days after sewing, transgenic seedlings exhibited longer roots than WT plants (Fig. 6A and B). And *BdEXPA27* overexpression lines significantly increased the number and length of root hairs (Fig. 6C–E).

4. Discussion

4.1. Extent function of expansin genes

Expansins are proteins that can loosen the cell wall in plants [50]. However, little is known about expansins in *B. distachyon*, a new model plant of grasses [51]. Previous studies showed that many expansin genes are specifically expressed in roots and regulate root development. For example, rice *OsEXPB5* and barley *HvEXPB1* are expressed in root hair specifically [52,53], wheat *α*-expansin gene *TaEXPA6* and *β*-expansin gene *TaEXPB8* are predominantly expressed in roots [54,55]. Overexpression of *OsEXPA8* and *TaEXPA2* result in root elongation [6,56]. In this study, high-throughput sequencing data showed that 68.42 % (26/38) *B. distachyon* expansin genes were highly expressed in roots, and 31.58 % (12/38) mainly expressed in flowers. qRT-PCR results also showed that the selected genes were expressed in all tissues, overexpression of *BdEXPA27* increased the root length when compare to the WT (Fig. 5D), implying their multi-functions in plant growth and development.

Up- and down-regulated expression under different abiotic stresses suggests that expansin genes also participate in the response to abiotic stresses. For example, the qRT-PCR results showed that the expression of the randomly selected *B. distachyon* expansin genes were up-regulated by cold (10), drought (8), and NaCl (7) (Fig. 4B). In addition, there are many *cis*-elements related to abiotic stresses in the promoter of *B. distachyon* expansin genes, such as drought-inducibility *cis*-element MBS (CAACTG) [48], and low-temperature responsive *cis*-element LTR (CCGAAA) [49]. Most studies also showed that expansin genes are involved in response to abiotic stresses, such as salt, drought, and cold responses [6,12,57,58]. For example, overexpression of wheat *TaEXPA2* improves the salt and drought tolerance in transgenic tobacco [6,12]; overexpression *TaEXPA8* improves low-temperature tolerance in *Arabidopsis* [59]. Taken together, these results clearly showed the function

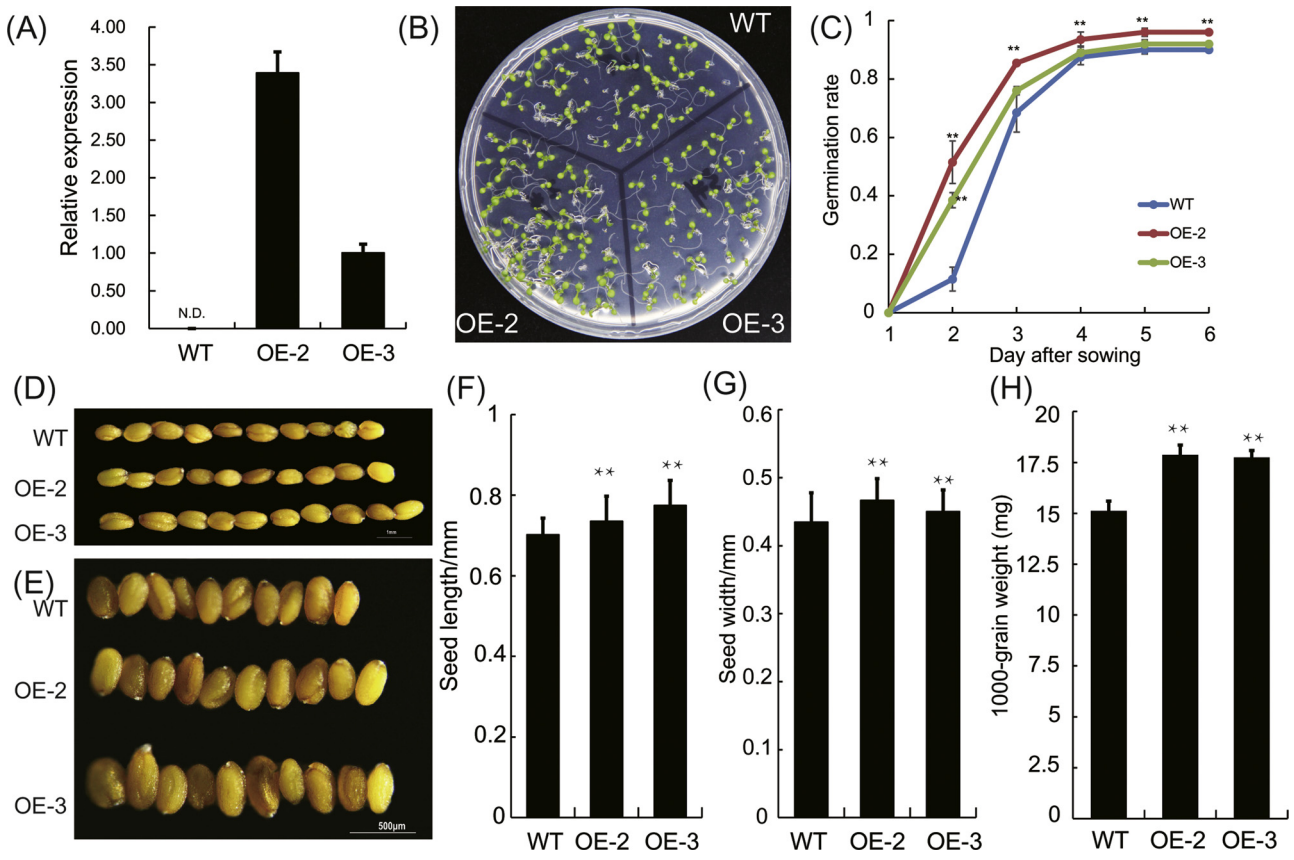

Fig. 5. Phenotypes of WT and transgenic *Arabidopsis* overexpressing *BdEXPA27*. (A) The expression level of *BdEXPA27* in T3 transgenic *Arabidopsis* lines. Bars indicate standard deviations of three biological replicates. (B) WT and *BdEXPA27* transgenic 5-day seedlings. (C) Seed germination rate. (D) and (F) Seed length of WT and transgenic *Arabidopsis* lines (bar = 1 mm, n > 40). (E) and (G) Seed width of WT and transgenic *Arabidopsis* lines (bar = 500 μm, n > 40). (H) 1000-grain weight. Error bars represent standard deviations. Statistically significant differences are indicated: *, P < 0.05; **, P < 0.01 (Student's t-test).

in response to abiotic stresses.

In addition, expansin genes in the same subfamily share similar exon-intron organization and motif composition, suggesting that expansin genes within subfamily are highly conservative. Different subfamilies contained different motifs, the motifs 3 and 4 constituted the Pollen_allerg domain in the EXPA members, while motifs 8 and 10 formed the Pollen_allerg domain in the EXPB, EXLA, and EXLB members. Previous studies showed that different group members function

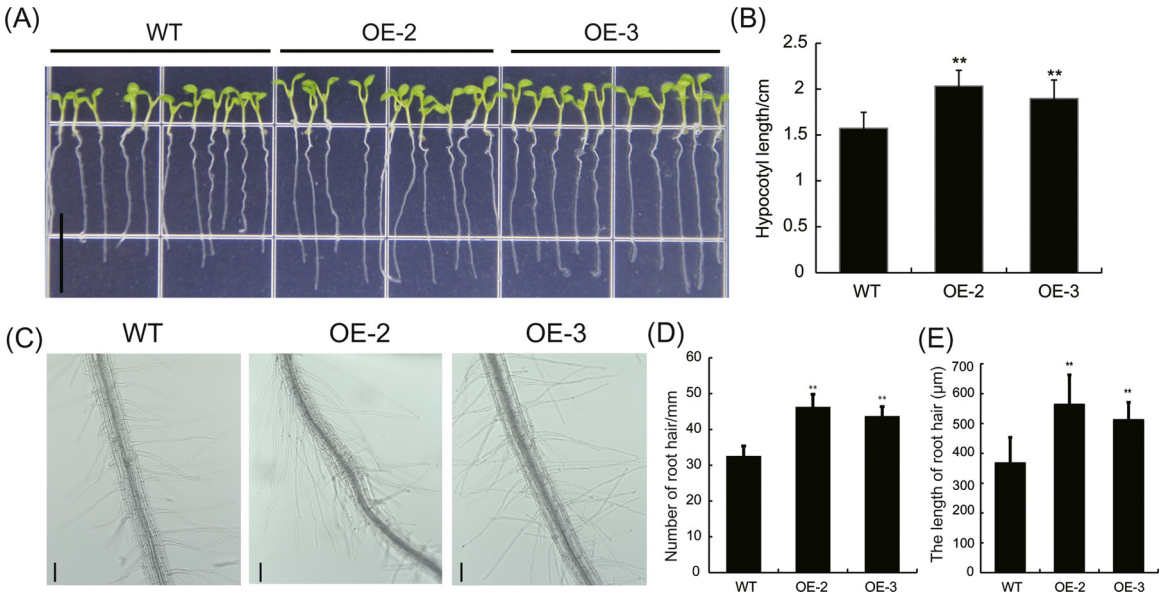

Fig. 6. Root phenotypes of WT and *BdEXPA27* transgenic seedlings. (A) and (B) Root length of 7-day seedlings (bar = 1 cm). (C) Microscopic observation of root hairs in mature zone. Bars = 100 μm. (D) Number of root hairs in root mature zone within 1 mm. (E) The length of root hair in the mature zone. Error bars represent the standard deviations. Statistically significant differences are indicated: *, P < 0.05; **, P < 0.01 (Student's t-test).

extensively. EXPA members have extensive function in plant growth and development, such as root hair elongation, leaf and stem initiation and growth. For example, *OsEXPA17* and *OsEXPA30* have been reported to regulate root hair elongation [60,61], *OsEXPA2* and *OsEXPA4* are involved in stem elongation [62], while *AtEXPA10* and *PnEXPA1* are significantly affect plant leaf cell sizes resulting in larger leaves when overexpressed [63]. EXPB members are predominately found in grasses [64] and function in root hair development, such as *OsEXPB2*, *ZmEXPB1*, *HvEXPB1*, and *HvEXPB7* are found to be root-specific and associated with root hair and cell wall formation [14,52,65–67]. Although little research on *EXLA* and *EXLB* genes, genome-wide expression analysis of expansin family genes in wheat, tobacco, and soybean show that *EXLA* and *EXLB* genes are preferentially expressed in mature or senescent tissues, especially in leaves and stems [21,68,69]. These results indicated that expansin genes function extensively.

4.2. BdEXPA27 regulates plant growth and development

Many studies have showed that *Arabidopsis* expansin genes function in plant growth and development. For example, *AtEXPA1*, *-7*, *-18* are expressed in root hair and associated with root hair initiation [10,11,70], *AtEXPA2*, *-4*, *-8*, *-9*, *-14*, and *-17* are mainly involved in lateral root development [7,71–74]. These results clearly showed their function in root development. Compared to the progresses in other species, little is known about the functions of expansins in *B. distachyon.* In the present study, *BdEXPA27* was specifically expressed in roots, indicating its role in root development. Overexpression of *BdEXPA27* in *Arabidopsis* also improved root development, it increased the length of the root, as well as the length and number of root hairs, indicating the functional similarity.

Moreover, the transgenic lines showed quicker germination than WT. In plants, some reports have reported that expansin genes function directly in seed germination [75,76]. For example, *Arabidopsis exp2* mutant shows delayed germination [7]. Pervious study showed that overexpression of wheat *TaEXPA2* in tobacco exhibited increased seed production by altering seed size [6]. Similarly, overexpression of *BdEXPA27* increased seed width and length, as well as the 1000-grain weight. *B. distachyon* is one relative species of wheat, and both of them belongs to pooideae. The function of *BdEXPA27* in seed size and root development indicated it can be used to molecular breeding in cereal crops, especially, in wheat.

Ethical statement

There is no ethical statement to declare.

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Author contributions

HL planed the experiments. YL, GW, and CF performed the experiments and analyzed the data. SC and HL wrote the manuscript. All authors discussed the results and comment on the manuscript. All authors gave final approval for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

There are no acknowledgments to declare.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.plantsci.2020.110490.

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