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ORIGINAL ARTICLE

Genome-wide identification and expression analysis of the expansin gene family in tomato

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Abstract Plant expansins are capable of inducing pH-dependent cell wall extension and stress relaxation. They may be useful as targets for crop improvement to enhance fruit development and stress resistance. Tomato is a major agricultural crop and a model plant for studying fruit development. Because only some tomato expansins have been studied, a genome-wide analysis of the tomato expansin family is necessary. In this study, we identified 25 *SlEXPAs*, eight *SlEXPBs*, one *SlEXLA*, four *SlEXLBs*, and five short homologs in the tomato genome. 25 of these genes were identified as being expressed. Bioinformatic analysis showed that although tomato expansins share similarities with those from other plants, they also exhibit specific features regarding genetic structure and amino acid sequences, which indicates a unique evolutionary process. Segmental and tandem duplication events have played important roles in expanding the tomato expansin family. Additionally, the 3-exon/2-intron structure may form the basic organization of expansin genes. We identified new expansin genes preferentially expressed in fruits (*SlEXPA8*, *SlEXPB8*, and *SlEXLB1*), roots (*SlEXPA9*, *SlEXLB2*, and *SlEXLB4*), and floral organs. Among the analyzed genes those that were inducible by hormone or stress treatments, including *SlEXPA3*, *SlEXPA7*, *SlEXPB1–B2*, *SlEXPB8*, *SlEXLB1–LB2*, and *SlEXLB4*. Our findings may further clarify the biological activities of tomato expansins, especially those related to fruit development and stress resistance, and contribute to the genetic modification of tomato plants to improve crop quality and yield.

Keywords Expansin · Tomato · Abiotic stress · Plant hormone · Gene expression

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Y. Lu and L. Liu contributed equally to this work.

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Introduction

Expansins are proteins that can induce pH-dependent cell wall extension and stress relaxation and are commonly found in plants (Cosgrove et al. 2002). These proteins have diverse biological roles, but most are related to cell wall modification, including leaf initiation and development (Goh et al. 2012), root hair growth (Cho and Cosgrove 2002), fiber development (Harmer et al. 2002), stem and pollen tube elongation (Cho and Kende 1997; Tabuchi et al. 2011), fruit development and ripening (Nardi et al. 2015; Brummell et al. 1999), seed germination (Yan et al. 2014; Chen et al. 2001), mycorrhiza and root nodule formation (Balestrini et al. 2005; Flemetakis et al. 2004), abscission (Tsuchiya et al. 2015; Belfield et al. 2005), and biotic and abiotic stress resistance (Abuqamar et al. 2013; Lü et al. 2013). Because of their important functions in plant development and stress resistance, researchers have considered expansins as potential targets for crop improvement (Choi et al. 2006).

Expansins are encoded by a large multigene family. A typical expansin contains 250–275 amino acids and is made up of two domains (domain 1 and domain 2) preceded by a signal peptide consisting of 20–30 amino acids

(Li et al. 2003). Domain 1 shares some key structural features with the catalytic domain of glycoside hydrolase family 45 proteins, including a series of conserved cysteines and a histidine-phenylalanine-aspartate (HFD) motif. Domain 2 is homologous to group-2 grass pollen allergens, which have been hypothesized to form a binding strip for cell wall polysaccharides (Cosgrove et al. 1997). Plant expansins can be phylogenetically divided into four groups, designated as EXPANSIN A (EXPA), EXPANSIN B (EXPB), EXPANSIN-LIKE A (EXLA), and EXPANSIN-LIKE B (EXLB) (Kende et al. 2004). Expansin-like proteins (EXLA and EXLB) also possess domains 1 and 2 and a signal peptide, but the amino acid sequences are different from those of EXPAs and EXPBs (Choi et al. 2006). Some EXPAs and EXPBs are capable of inducing rapid cell wall extension or stress relaxation (Cosgrove et al. 1997), whereas no biological or biochemical function has been established for any EXLAs or EXLBs (Sampedro and Cosgrove 2005).

Proteins with homology to only one of the expansin domains also exist in plants, including grass secreted proteins, whose functions are unclear, and citrus tree p12 proteins, which are speculated to function as peptide hormones to maintain water and solute balance (Ludidi et al. 2002). Expansin orthologs were also identified in non-plant organisms such as bacteria and fungi. These proteins may function in plant cell wall digestion to facilitate pathogen invasion (Cosgrove et al. 2002).

Tomato (*Solanum lycopersicum*) is a major crop plant worldwide and biotic and abiotic stresses are seriously threatening its production. Expansins may play significant roles in different stress resistance processes. Tomato is also a model system for the study of fruit development. Expansins may cooperatively function in every fruit development stage. Studies on tomato expansins will benefit tomato production and provide valuable information regarding how fruits develop in other crops. The tomato expansin family is composed of many members. Although several related studies have been reported (Keller and Cosgrove 1995; Rose et al. 1997), to the best of our knowledge, there have been no systematic studies conducted on the whole family despite the fact the genome was sequenced over 2 years.

In this study, we identified 38 expansin family members and five short homologs in tomato. We systematically analyzed the phylogenetic relationships, gene structure, and protein architecture of the identified members. The transcription patterns of some expressed expansin genes in different organs, and their expression patterns following different hormone and abiotic stress treatments were investigated. We identified new expansin genes with organ-specific or preferential expression patterns, as well as those with stress- and/or hormone-responsive properties. Our findings may advance our understanding of the roles of different expansins in tomato growth, development, and responses to environmental stresses. This study may provide important information for future investigations of the biological functions of expansins.

Materials and methods

Identification of the tomato expansin gene family

To identify all tomato expansin genes, eight previously reported tomato expansin gene cDNA sequences were extracted from a National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/nucleotide/) and subjected to BLASTN searches using the SOL Genomics Network (SGN; http://solgenomics.net/) and KaFTom (http://www.pgb.kazusa.or.jp/kaftom/) databases (Brummell et al. 1999; Chen et al. 2001). We also searched the databases for homologs using 'expansin' as a keyword. Redundant sequences were removed after a similarity comparison. The open reading frames were identified by ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Expansin protein sequences from other species were retrieved from the databases of the National Center for Biotechnology Information, the Arabidopsis Information Resource (www.arabidopsis.org/), the Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/), and the Expansin Central website (http://www.personal.psu.edu/fsl/ExpCentral/index.html).

Sequence analysis and phylogenetic tree construction

Deduced protein sequences were aligned using Clustal X 1.83 with the following parameters: gap opening, 4.0; gap extension, 0.2; delay divergent sequences, 30 %; DNA transition weight, 0.5; and use negative matrix, off (Larkin et al. 2007). Putative motifs were analyzed using the MEME program 4.10.2 with the following parameters: number of repetitions, any; maximum number of motifs, 20; and Motif width, 20–100 (http://meme-suite.org/tools/meme, Bailey et al. 2009). A phylogenetic tree was constructed using the maximum likelihood method and MEGA 6 software with default parameters (Tamura et al. 2013). The exon–intron structures were generated using GSDS software 2.0 (http://gsds.cbi.pku.edu.cn/, Hu et al. 2015).

Duplication analysis of tomato expansin genes

We completed duplication analysis of the tomato expansin gene family using MCScanX software (http://chibba.pgml.uga.edu/mcscan2/, Wang et al. 2012). All tomato expansin

and expansin-like genes were compared with themselves and others using the BLASTp program, with an *E* value <1E−5. The BLAST search outputs were imported into MCScanX software using the default criterion. Diagrams were generated with Circos software, version 0.63 (http://circos.ca/, Krzywinski et al. 2009).

Plant growth during hormone and stress treatments

Tomato seeds (*S. lycopersicum* cv. Ailsa Craig) were germinated and grown in a greenhouse under a photosynthetic photon flux density of approximately 120 μmol/m^2/s with a 12-h light/dark photoperiod. The temperature and relative humidity were maintained at 25 ± 2 °C and 60 %, respectively. Five-week-old seedlings were used to investigate the effects of hormones and abiotic stresses on expansin genes. Seedlings were sprayed with solutions containing 100 μM 6-benzylaminopurine (6-BA), indole-3-acetic acid (IAA), ethephon, abscisic acid (ABA), and gibberellic acid (GA). Control plants were treated with water. Seedling shoots were collected 0, 1.5, 3.0, 6.0, 12, and 24 h after spraying. Heat and cold stresses were applied by exposing the seedlings to 42 and 4 °C, respectively. Shoots were harvested after 1.5, 3.0, 6.0, 12, and 24 h. Drought stress was induced by placing the seedlings on a clean bench after removal from the soil. Shoots were collected after 0.5, 1.0, 1.5, 2.0, and 2.5 h. Untreated plants served as controls. To investigate organ-specific expression profiles, sixteen kinds of organ samples were collected from adult plants. All samples were quickly frozen in liquid nitrogen and stored at −70 °C. For all experiments, at least two biological replicates were used for further analysis.

Real-time quantitative reverse transcription (qRT)-PCR analysis

Total RNA was extracted using TRIzol reagent. First-strand cDNA was synthesized using M-MLV reverse transcriptase (Invitrogen) with 3 μg RNA, the product was finally diluted to a volume of 90 μl. Real-time qRT-PCR was performed using the LightCycler 480 system (Roche Diagnostics). Each reaction contained 5 μl SYBR Premix Ex Taq (Takara), 0.5 μl cDNA, and 0.4 μl 10 μM gene-specific primers in a final volume of 10 μl. The PCR cycle was as follows: 95 °C for 30 s, 40 cycles of 95 °C for 5 s and 60 °C for 25 s. Data were collected during the extension step. Three technical replicates were performed for each sample. The tomato actin gene (Solyc04g011500) was used as an internal control (Supplementary Table 1). Data were analyzed using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

Results

Identification of tomato expansin genes

By removing redundant sequences from databases, 38 expansin genes and five short homologs were identified in the tomato genome. Among these, 25 were identified based on expressed sequence tag data (Table 1). Some of the genes were identified and named previously while others have never been reported. According to the nomenclature rules for expansin superfamily genes and proteins suggested by Kende et al. (2004), genes expressing proteins with only one expansin domain were not classified as expansins. For convenience, we first constructed a phylogenetic tree using the deduced sequences of proteins with both expansin domains (Supplementary Fig. 1). Phylogenetic analysis showed that these proteins can be divided into four subfamilies, EXPA, EXPB, EXLA, and EXLB. Newly identified expansins and expansin-like genes were named according to their locations in the phylogenetic tree and their relationships with *Arabidopsis thaliana* homologs. Three members that were homologous to only domain 1 were designated as *SlPNPL1–L3*. Two members that were homologous to only domain 2 were designated as *SlG2A1* and *SlG2A2* (Table 1).

The expansin genes were unevenly distributed on 11 chromosomes. Some of the genes may have evolved from gene duplications (Supplementary Fig. 2). On chromosome 3, six expansin genes (*SlEXPA7* and *SlEXPA19–A23*) were clustered in a small 25.4 kb region. These genes were also classified in a small group in the phylogenetic tree, suggesting their close evolutionary relationship. Of these genes, only *SlEXPA7* was identified as being expressed (Supplementary Fig. 1).

Gene structure and phylogenetic analysis

Similar to those of other plants (Lee et al. 2001), tomato expansin gene exon–intron organizations were diverse (Fig. 1). However, members of a subfamily showed a similar structure. Among the 25 *SlEXPAs*, 15 had a 3-exon/2-intron structure, nine had a 2-exon/1-intron structure, and only one member had a 4-exon/3-intron structure. As shown in Fig. 1, other structures likely originated from an intron loss or intron gain in a 3-exon/2-intron structure. The structures of *SlEXPA7*, *SlEXPA19*, and *SlEXPA20–A22* may have resulted from the loss of the second intron (generally called intron B), while the structures of *SlEXPA1*, *SlEXPA3*, and *SlEXPA24* may be due to the loss of the first intron (intron A). Conversely, the 4-exon/3-intron structure of *SlEXPA13* may have been the result of an intron-gain (intron E) in the first exon. All *SlEXPB* family members

Table 1 Description of tomato expansin family genes

Gene name	Locus name	Best hit	cDNA length (bp)	AA	pIs	MW
SlEXPA12	Solyc05g007830.2.1	SGN-U585093	1002	249	9.12	26.58
SlEXPA5	Solyc02g088100.2.1	SGN-U581279	1184	239	9.34	25.61
SlEXPA10	Solyc03g115890.2.1	SGN-U584567	767	250	8.65	26.82
SlEXPA13	Solyc01g010890.2.1	Not found	1072	265	9.46	28.80
SlEXPA14	Solyc09g018020.2.1	SGN-U585925	1090	255	9.39	27.98
SlEXPA15	Solyc05g052250.2.1	Not found	942	258	8.56	27.67
SlEXPA2	Solyc06g049050.2.1	SGN-U581296	1194	247	8.39	26.47
SlEXPA16	Solyc00g017230.1.1	Not found	789	262	9.53	28.27
SlEXPA1	Solyc06g051800.2.1	SGN-U578473	1474	261	9.46	28.69
SlEXPA18	Solyc06g076220.2.1	SGN-U580471	1263	260	9.39	28.31
SlEXPA9	Solyc06g005560.2.1	SGN-U580110	1144	257	9.46	28.13
SlEXPA4	Solyc09g010860.2.1	SGN-U580310	1068	263	9.48	28.70
SlEXPA6	Solyc10g086520.1.1	SGN-U581571	636	211	9.61	23.12
SlEXPA11	Solyc04g081870.2.1	SGN-U562920	1176	257	9.17	27.73
SlEXPA8	Solyc12g089380.1.1	SGN-U563017	747	257	9.02	27.60
SlEXPA17	Solyc08g080060.2.1	Not found	1004	269	9.46	29.40
SlEXPA19	Solyc03g115270.1.1	Not found	780	259	8.86	28.68
SlEXPA7	Solyc03g115300.1.1	SGN-U599547	780	259	8.06	28.61
SlEXPA20	Solyc03g115310.1.1	Not found	780	259	8.33	28.91
SlEXPA21	Solyc03g115350.1.1	Not found	777	258	8.59	28.95
SlEXPA22	Solyc03g115320.1.1	Not found	783	260	8.98	28.47
SlEXPA23	Solyc03g115340.1.1	Not found	534	177	10.13	19.44
SlEXPA24	Solyc02g081210.2.1	Not found	1291	264	8.60	28.68
SlEXPA3	Solyc03g031840.2.1	SGN-U576599	1117	267	8.56	28.90
SlEXPA25	Solyc07g063210.2.1	Not found	832	256	10.12	28.71
SlEXPB1	Solyc07g054170.2.1	SGN-U602406	1182	267	8.90	28.99
SlEXPB3	Solyc10g008440.2.1	SGN-U601312	1123	262	9.85	28.57
SlEXPB4	Solyc05g052330.1.1	Not found	807	268	4.82	29.75
SlEXPB5	Solyc07g049540.1.1	Not found	774	257	5.89	27.71
SlEXPB6	Solyc01g090820.1.1	Not found	774	257	8.99	27.13
SlEXPB7	Solyc10g018500.1.1	Not found	798	265	5.13	28.26
SlEXPB8	Solyc01g090810.2.1	SGN-U577727	1192	275	6.79	29.04
SlEXPB2	Solyc03g093390.2.1	SGN-U562660	1127	285	9.11	31.03
SlEXLA1	Solyc01g112000.2.1	SGN-U583056	1032	261	8.00	28.31
SlEXLB1	Solyc08g077330.2.1	SGN-U586328	983	255	4.67	27.71
SlEXLB2	Solyc08g077900.2.1	SGN-U582695	1092	253	6.15	28.23
SlEXLB3	Solyc06g060970.1.1	Not found	747	248	5.98	27.15
SlEXLB4	Solyc08g077910.2.1	SGN-U577404	1019	251	8.63	27.97
SlPNPL1	Solyc08g007780.2.1	SGN-U579858	794	127	5.74	13.45
SlPNPL2	Solyc08g061060.1.1	Not found	390	129	8.68	14.05
SlPNPL3	Solyc08g067390.2.1	SGN-U584557	1034	142	4.72	15.07
SlG2A1	Solyc08g007090.1.1	SGN-U586326	207	68	4.76	7.68
SlG2A2	Solyc10g084780.1.1	Not found	372	123	10.25	14.06

AA protein length (number of amino acid), *pIs* theoretical isoelectric point, *MW* molecular weight (kDa)

consisted of the 4-exon/3-intron structure, which is likely because of an intron-gain in the second or third exon of a 3-exon/2-intron structure (intron C and F).

To explore the phylogenetic relationship among plant expansins, we constructed a phylogenetic tree using all of the reported expansin and expansin-like protein sequences

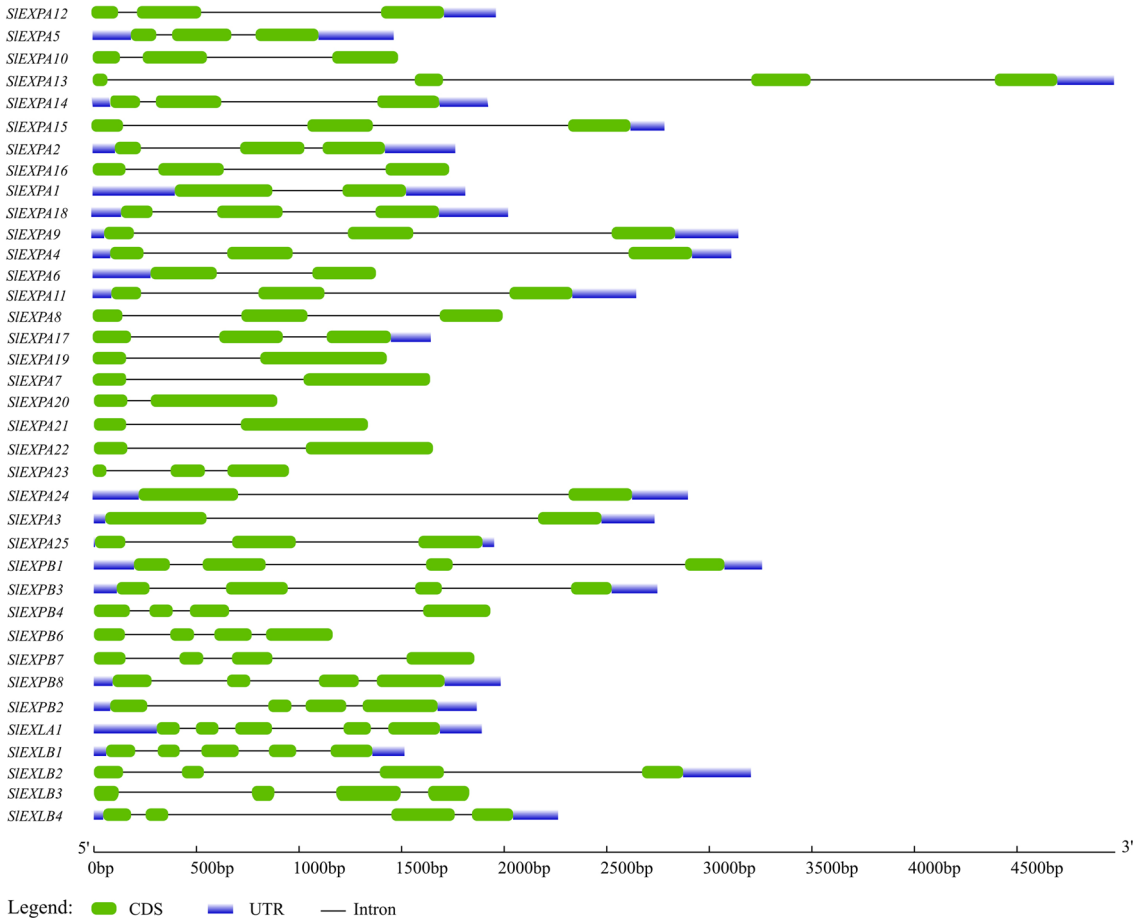

Fig. 1 Exon–intron organization of tomato expansin family genes. Gene structures were generated with GSDS 2.0 (http://gsds.cbi.pku.edu.cn/)

of tomato, *A. thaliana*, and rice (Fig. 2). These expansins were grouped into four major classes, namely EXPA, EXPB, EXLA and EXLB. In all species, EXPA was the largest subfamily. As previously reported, among the EXPB members, there are more monocots (e.g., rice) than eudicots (e.g., *A. thaliana* and tomato). The EXLA and EXLB groups are small subfamilies. There are three and four EXLA members in rice and *A. thaliana*, respectively, but only one in tomato. Four EXLB members were identified in tomato, while only one was found in rice and *A. thaliana*.

All expansin and expansin-like genes are believed to have evolved from a common ancestor. This conclusion was also supported by our phylogenetic analysis, as some members, which come from different plant species, like *SlEXPA24*, *AtEXPA13*, and *OsEXPA10*, can be clustered in one small subfamily (Fig. 2). Differences in the expansins between monocots and eudicots, as well as those between two dicotyledonous species can also be distinguished in the phylogenetic tree, suggesting that the expansin gene family has evolved separately in each plant species.

Sequence alignment and putative motif identification

To obtain detailed information regarding the tomato expansin superfamily, we completed multiple sequence alignments of the deduced protein sequences (Fig. 3). Most of the expansins and expansin-like proteins consisted of three parts, a 20–30 amino acid signal peptide, domain 1, and domain 2. The amino acid sequence of domain 1 was more conserved than that of domain 2, especially among EXPB members. As reported by Li et al. (2002), the EXPA and EXPB subfamilies can be easily distinguished based on two types of insertions. The α-insertions existed only in the EXPA subfamily and consisted of about 14 residues, with most insertions containing four highly conserved residues at the 3′ end, WCNP (Trp-Cys-Asn-Pro). The β-insertions were present among EXPB, EXLA, and EXLB members. In EXPB members, the β-insertion was about seven residues long and contained a conserved G residue. The β-insertions among the EXLA and EXLB members consisted of 7–9 residues but were relatively diverse with no obvious conserved residues. The HFD motif was conserved

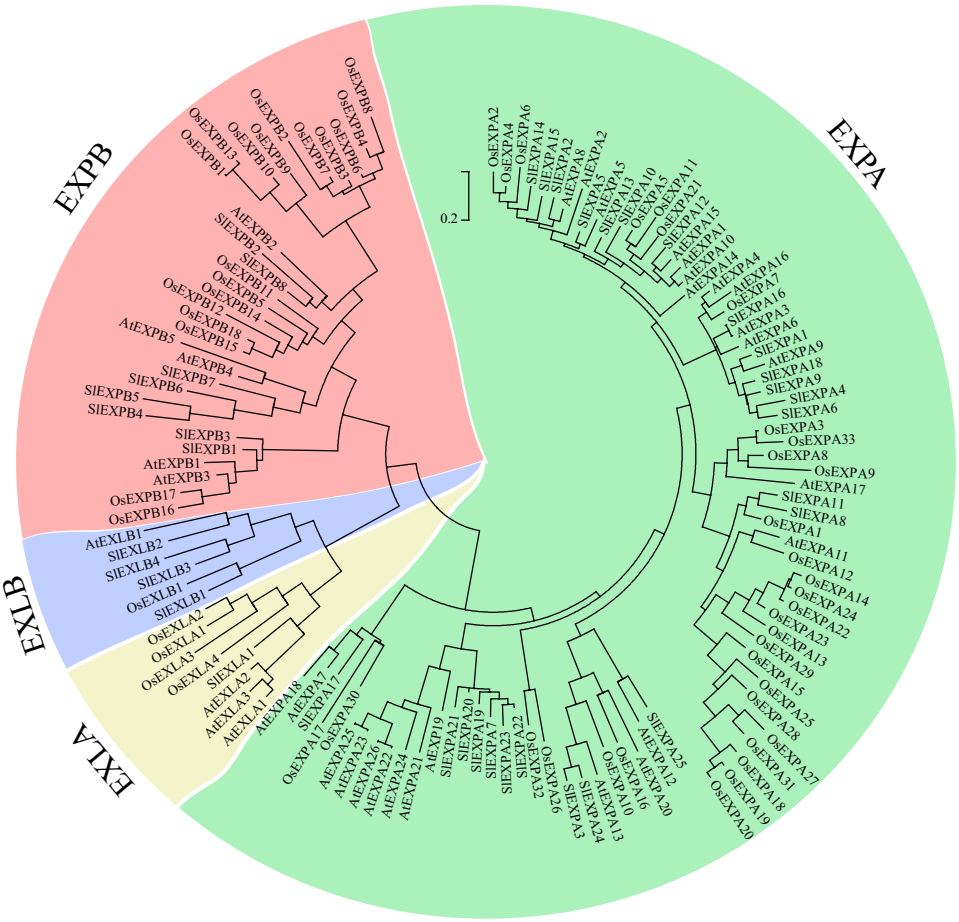

Fig. 2 Phylogenetic analysis of the expansin family in tomato, *A. thaliana*, and rice. The phylogenetic tree was generated using the maximum likelihood method in MEGA 6. Rice and *A. thaliana* expansin sequences were retrieved from the databases of Arabidopsis Information Resource (www.arabidopsis.org/), the Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/), and the Expansin Central website (http://www.personal.psu.edu/fsl/ExpCentral/index.html)

among all EXPB and the majority of EXPA members, while members with either absent (i.e., SlEXPA23) or incompletely conserved (i.e., SlEXPA3, SlEXPA24, and all SlEXLA and SlEXLB members) HFD motif were also found. A series of conserved Cys (N-terminal) and Trp (C-terminal) residues reported in other plant expansins were also present in tomato expansins. The Cys residues were strictly conserved in all members, while the Trp residues were not as conserved, especially in SlEXLAs and SlEXLBs. These expansin properties were also reflected in the MEME analysis (Supplementary Figs. 3, 4).

Organ-preferential expression of tomato expansin genes

For a more comprehensive understanding of tomato expansin gene expression, we analyzed the transcription profiles of 23 members, including 15 *SlEXPAs*, four *SlEXPBs*, one *SlEXLA*, and three *SlEXLBs* using real-time qRT-PCR. All selected members were identified as expressed genes in SGN database except for *SlEXPA24*. The results showed that the tested genes were expressed differently in tomato parts (Fig. 4).

First, we observed differences in expression levels. Three members (*SlEXPA1*, *SlEXPA11*, and *SlEXPA12*) were highly expressed in several organs with expression levels more than 4-fold higher than that of the reference gene. Six members (*SlEXPA6*, *SlEXPA14*, and four *SlEXPB* genes) were expressed at a very low level (<0.1 relative to the expression of the reference gene) in all examined organs. The other tested genes exhibited a relative expression level of 0.1–1.5 times the expression of the reference gene in at least one organ.

Second, there were differences in expression patterns. Most genes showed an organ-preferential expression pattern. Some genes were expressed more in the roots, including *SlEXPA3*, *SlEXPA9*, *SlEXPA18*, *SlEXPB1*, *SlEXPB2*, *SlEXPB8*, *SlEXLB2*, and *SlEXLB4*. Among these genes, *SlEXPB2* and *SlEXLB4* exhibited a root-specific expression pattern. Genes that were expressed more in fruits included *SlEXPA1*, *SlEXPA3*, *SlEXPA5*, *SlEXPA6*, *SlEXPB8*, and *SlEXLB1*. Among these, *SlEXPA1* was expressed specifically in yellow and red ripe fruit, *SlEXPB8* was expressed preferentially in yellow ripe fruit, *SlEXLB1* was expressed preferentially

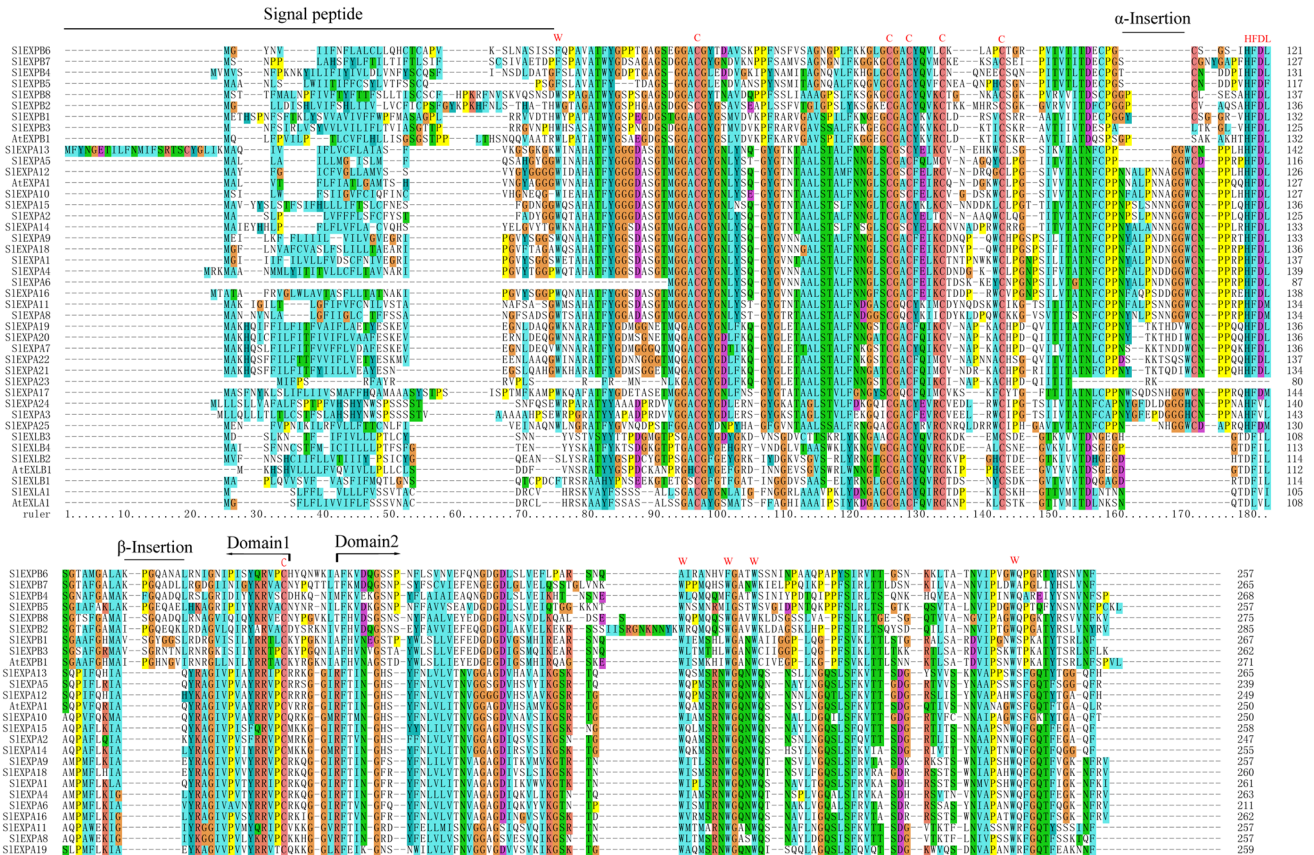

Fig. 3 Multiple sequence alignment of tomato expansins. Sequence alignments of tomato expansins and three *A. thaliana* expansins were prepared using the Clustal X software. *Colored shading* indicates identical and conversed amino acid residues. Conserved features such as signal peptides, expansin domains, HFD motif, and conserved Cys and Trp residues are also indicated

during the breaker stage, and *SlEXPA3*, *SlEXPA5*, and *SlEXPA6* were preferentially expressed in immature or mature green fruit.

Some genes were expressed more in floral organs, including *SlEXPA2* (alabastrum, sepal, and petal), *SlEXPA4* (petal), *SlEXPA10* (alabastrum), *SlEXPA8* (flower axis and alabastrum), *SlEXPA11* (petal), *SlEXPA14* (sepal), *SlEXPA18* (alabastrum), *SlEXPA24* (sepal), *SlEXPB3* (alabastrum), *SlEXLB1* (sepal), *SlEXPA4* (stigma or ovary), and *SlEXLB2* (stigma or ovary). Additionally, *SlEXPA7* was preferentially expressed in mature leaves. We also observed that *SlEXPA12* was constitutively expressed in every tested organ except for the stamen in which it was expressed at very low levels.

Differential expression of expansin genes after phytohormone treatment

The expression of some expansin genes can be regulated by phytohormones. We investigated the expression of selected expansin genes in shoots in response to the application of exogenous hormones (Fig. 5). The expression of most of the analyzed genes was up-regulated. Seven genes (*SlEXPA2*, *SlEXPA3*, *SlEXPA5*, *SlEXPA8*, *SlEXPA11*, *SlEXPB2*, and *SlEXPB8*) were up-regulated by 6-BA, ten (*SlEXPA1*, *SlEXPA3*, *SlEXPA5–A9*, *SlEXPA12*, *SlEXPB8*, and *SlEXLB2*) were up-regulated by IAA, four (*SlEXPA2*, *SlEXPA10*, *SlEXPA11*, and *SlEXLB2*) were up-regulated by GA, eight (*SlEXPA4–A6*, *SlEXPA11*, *SlEXPA18*, *SlEXPB8*,

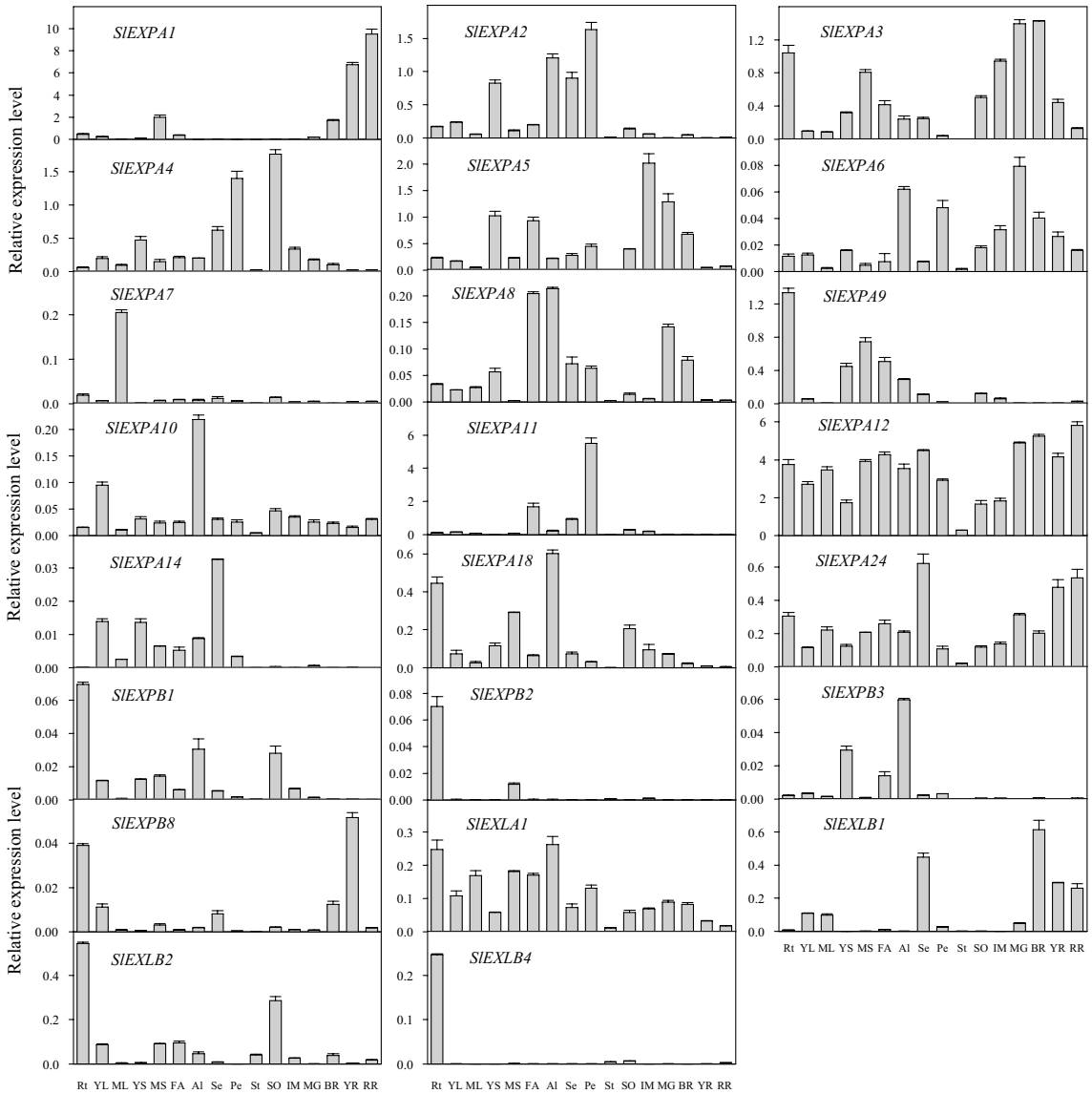

Fig. 4 Expression profiles of tomato expansin genes in various organs. The *y*-axis represents the expression levels of expansin or expansin-like genes relative to that of *SlActin*. The *x*-axis represents different organs. *Rt* root, *YS* young stem, *MS* mature stem, *FA* flower axis, *YL* young leaf, *ML* mature leaf, *Al* alabastrum, *Se* sepal, *Pe* petal, *St* stamen, *SO* stigma and ovary, *IM* pericarp of immature fruit, *MG* pericarp of mature green fruit, *BR* pericarp of breaker fruit, *YR* pericarp of yellow ripe fruit, *RR* pericarp of red ripe fruit. *Error bars* represent standard deviations for three replicates

SlEXLB2, and *SlEXLB4*) were up-regulated by ethephon, and eight (*SlEXPA1*, *SlEXPA3*, *SlEXPA5–A7*, *SlEXPA9*, *SlEXLB1*, and *SlEXLB2*) were up-regulated by ABA. The expressions of some expansin genes were up-regulated by multiple hormones. For example, *SlEXPA5* was up-regulated by all hormones except for GA, while *SlEXPA3* was up-regulated by 6-BA, IAA, and ABA. However, *SlEXPB2* and *SlEXLB4* were up-regulated by only one hormone. Some members were markedly up-regulated by one or several hormones and exhibited higher expression levels than the control, including *SlEXPB2*, *SlEXPB8*, *SlEXLB1*, *SlEXLB2*, and *SlEXLB4*.

Induced expression of expansin genes during exposure to abiotic stress

Most of the analyzed genes were up- or down-regulated following exposure to stress (Fig. 6). Genes that were up-regulated in response to drought stress included *SlEXPA4*, *SlEXPA10*, *SlEXPA12*, *SlEXPA14*, *SlEXPB2*, *SlEXPB8*, *SlEXLA1*, *SlEXLB1*, and *SlEXLB4*. Of these genes, the transcription levels for *SlEXPA10*, *SlEXPA14*, *SlEXPB8*, *SlEXLA1*, *SlEXLB1*, and *SlEXLB4* were 4-, 14-, 120-, 12-, 24-, and 20-fold higher than that of the control, respectively. Genes down-regulated by drought treatment

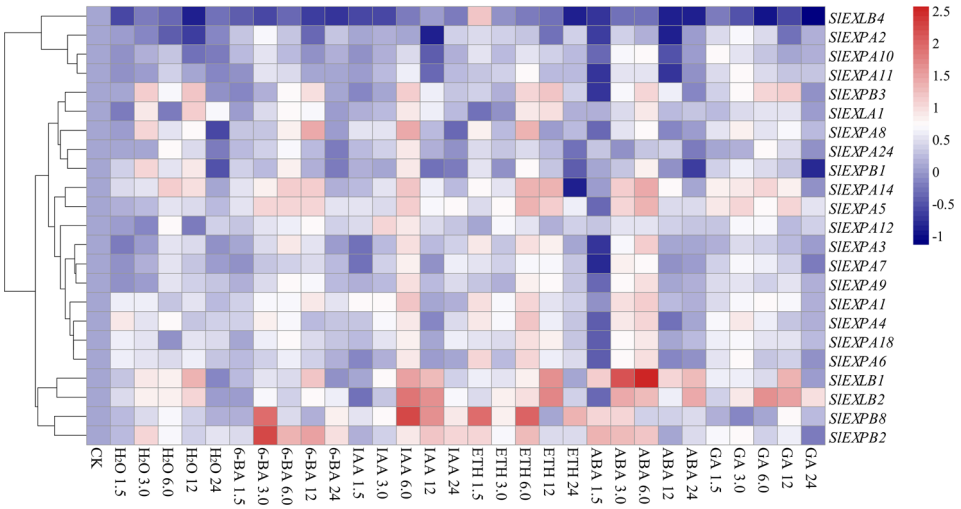

Fig. 5 Expression patterns of tomato expansin genes following exogenous phytohormone treatments. The heatmap was constructed based on the expression level of each expansin gene in the shoot relative to that of *SlActin*. *Blue* and *red boxes* indicate lower and higher expression levels, respectively. The *scale bar* represents the fold change (log2 value). H_2O, ABA, GA, SA, IAA, and ETH represent seedlings sprayed with water, or 100 μM ABA, GA, SA, IAA, or ethephon, respectively. The numbers 1.5, 3, 6, 12, and 24 represent the time (h) after treatment. CK represents untreated seedlings at the start of the experiment (i.e., time zero)

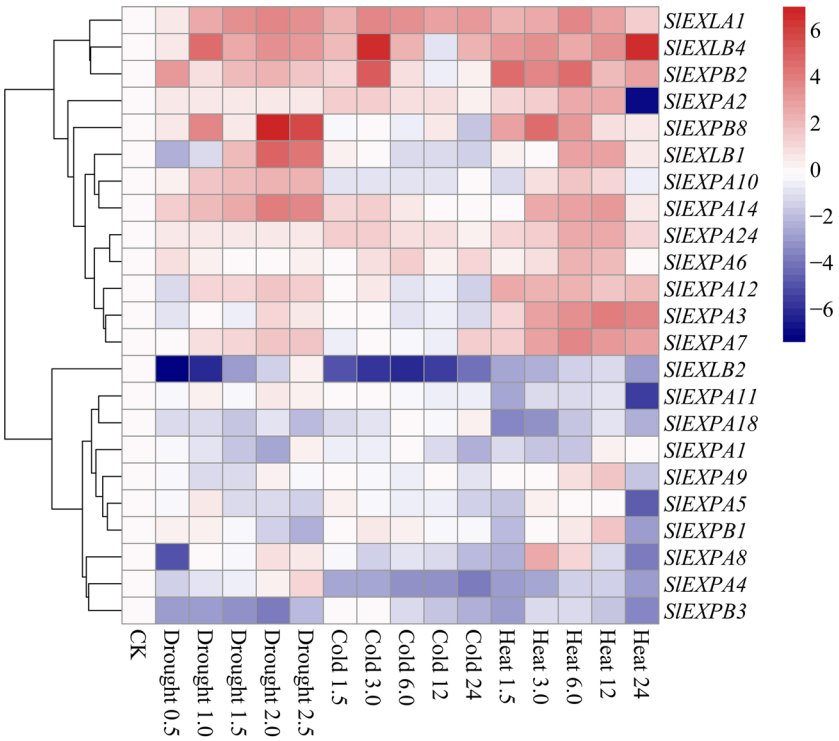

Fig. 6 Expression patterns of tomato expansin genes following exposure to different abiotic stresses. The heatmap was constructed based on the expression level of each expansin gene in the shoot relative to that of *SlActin*. *Blue* and *red boxes* indicate lower and higher expression levels, respectively. The *scale bar* represents the fold change (log2 value). Drought 0.5–2.5 represent samples collected 0.5, 1.0, 1.5, 2.0, and 2.5 h after exposure to drought stress. Cold and heat 1.5–24 represent samples collected 1.5, 3.0, 6.0, 12, and 24 h after cold (4 °C) or heat (42 °C) stress treatments. CK represents untreated seedlings at the start of the experiment (i.e., time zero)

included *SlEXPA1*, *SlEXPA18*, and *SlEXPB3*, and the *SlEXPA1* and *SlEXPB3* transcription levels were decreased by nearly 80 %.

Following cold stress, the transcription levels of *SlEXPB2*, *SlEXLA1*, and *SlEXLB4* were nearly 30-, 12-, and 100-fold higher than that of the control, respectively. Conversely, the expression levels of *SlEXPA4*, *SlEXPA5*, *SlEXPA10*, and *SlEXLB2* were significantly decreased. There were no significant expression level changes for the other genes following cold stress treatment.

The expression levels of more than half of the analyzed genes were affected by heat stress. Up-regulated genes included *SlEXPA2*, *SlEXPA3*, *SlEXPA6–A9*, *SlEXPA12*, *SlEXPA14*, *SlEXPA24*, *SlEXPB1*, and *SlEXLB4*.

Down-regulated genes included *SlEXPB3* and *SlEXLB2*. Among the up-regulated genes, *SlEXLB4* exhibited the highest expression level change, with an 80-fold increase over the control.

Discussion

Previous studies have revealed that EXPAs and EXPBs existed when vascular plants and mosses were diverging, whereas EXLAs and EXLBs can only be traced back to the last angiosperm and gymnosperm ancestors (Sampedro and Cosgrove 2005). The expansin family has more recently continued to grow and diversify in different species. The last common ancestors of eudicots and monocots are believed to have 12 EXPAs, two EXPBs, one EXLA, and two EXLBs (Sampedro and Cosgrove 2005), and the number of genes has almost doubled or tripled in "modern" plants. In this study, we identified 38 tomato expansin and expansin-like genes, including 25 *EXPAs*, eight *EXPBs*, one *EXLA*, and four *EXLBs* (Table 1). These findings are more consistent with those of *A. thaliana* (26 *EXPAs*, six *EXPBs*, three *EXLAs*, and one *EXLB*) than with those of rice (34 *EXPAs*, 19 *EXPBs*, four *EXLAs*, and one *EXLB*). Rice and maize contain more *EXPB*s than eudicots (e.g., tomato and *A. thaliana*) (Zhang et al. 2014), which may be because of the differences in their cell wall composition and their evolutionary differences (Cosgrove et al. 2002).

The growth of the plant expansin superfamily is mostly due to gene duplications (Zhu et al. 2014). Our results indicate that segmental and tandem duplication play key roles in the growth of the tomato expansin gene family (Supplementary Fig. 2). A cluster of expansin genes that had evolved from tandem duplications was present in tomato and *A. thaliana* (Li et al. 2002), but not in rice. These expansins were grouped together in the phylogenetic tree (including *SlEXPA7*, *SlEXPA19–A23*, *AtEXPA19*, and *AtEXPA21–A26*) (Fig. 2), suggesting this duplication may have occurred after the eudicots and monocots diverged, but before the divergence of tomato and *A. thaliana*.

It has been assumed that EXPA and EXPB members have similar functions, but on different cell wall polymers (Cosgrove et al. 2002). Differences in substrate preferences may be mainly attributed to compositional diversity in domain 2 (Fig. 3). This domain was suggested as the polysaccharide (cellulose)-binding domain because several aromatic (e.g., Trp) and polar residues on the expansin surface are located in this region (Cosgrove 2000). It is reasonable that different sequences in this domain exhibit diverse substrate affinity. SlEXLA and SlEXLB members consist of the same motifs as SlEXPB members. However, there are sequence differences, especially related to the HFD motif (Fig. 3), which greatly decreased the possibility these proteins would have cell wall loosening activity in vivo.

In addition to the fruit-specific expansin gene *SlEXPA1*, five other *SlEXPA*s expressed in the fruit, *SlEXPA3–A7*, were identified by Brummell et al. (1999). Unlike *SlEXPA1*, these expansin genes are expressed more during cell division and/or cell expansion. *SlEXPA3* is also highly expressed during the breaker stage. The previously described fruit expression patterns of *SlEXPA1* and *SlEXPA3–A6* were confirmed in this study, while our findings indicated that *SlEXPA7* expression levels were higher only in mature leaves (Fig. 4). Additionally, *SlEXPA8*, *SlEXPB8*, *SlEXLB1*, and *SlEXLB2* were determined as genes that were preferentially expressed in the fruit. Diverse fruit expression patterns of these expansin and expansin-like genes may mean they have diverse functions during fruit development. They may have coordinated functions in cell division and enlargement stages in which cell wall modifications occur. Fruit-specific or preferential expression patterns have also been reported in other plants such as strawberry (Nardi et al. 2015), pear (Hiwasa et al. 2003), and grapevine (Santo et al. 2013).

Expansins involved in root or root hair development have been widely reported in different plant species, including *A. thaliana* (Cho and Cosgrove 2002), maize (Wu et al. 2001), grapevine (Santo et al. 2013), and rice (Cho and Kende 1997). In this study, eight expansin genes preferentially expressed in the root were identified, including three *EXPAs*, three *EXPBs*, and two *EXLBs* (Fig. 4). Because the root is the main organ responsible for water and nutrient acquisition, it has always been speculated that expansins expressed in the root play important roles in water and nutrient absorption. Overexpression of the wheat expansin gene, *TaEXPB23*, enhanced drought tolerance in transgenic tobacco (Han et al. 2014). Overexpression of *GmEXPB2* in soybean stimulated root growth and improved phosphate use efficiency in phosphate-limited conditions (Zhou et al. 2014). Therefore, the tomato expansin genes identified in this study as being specifically or preferentially expressed in the root may also have similar effects.

Phytohormones play critical roles in regulating plant growth, development, and adaptation to environmental changes. Expansins are regarded as the mediators of hormone activities. Actively expressed expansin genes are always observed in growing tissues with high levels of growth-promoting hormones, including GA and IAA (Lee and Kende 2002; Cho and Cosgrove 2004). We also identified expansin genes preferentially or specifically expressed in rapidly growing organs (Fig. 4), including young leaves (*SlEXPA10*), young shoots (*SlEXPA2* and *SlEXPA5*), and immature fruits (*SlEXPA3* and *SlEXPA5*). Preferential or specific expression of these expansin genes in these organs may be induced by endogenous hormones. The expression of most of these genes can be induced in shoots by exogenous cytokinin, GA, or IAA (Fig. 5).

Ethylene can induce fruit maturation and leaf senescence. The expression of the tomato ripening-specific expansin gene, *SlEXPA1*, can be considerably induced by exogenous ethylene (Rose et al. 1997), which can also stimulate root hair formation. Expression of two *A. thaliana* expansin genes, *AtEXP7* and *AtEXP18*, which are tightly linked to root hair initiation, is also stimulated by exogenous ethylene (Cho and Cosgrove 2002). In our study, the expression of four expansin genes specifically or preferentially expressed in the root, *SlEXPA3*, *SlEXPB8*, *SlEXLB2*, and *SlEXLB4*, were also induced by exogenous ethylene applied to the shoots. Of these genes, *SlEXLB4* was specifically expressed in roots and was induced only by exogenous ethylene (Fig. 5).

Various stresses, including salinity, drought, and extreme temperatures, can induce ABA production to activate genes needed for plant stress resistance. The relationship between expansins and ABA is still unclear. Maize can adapt to relatively low water potentials. This adaptation has been associated with the increased expression and activities of three expansins present in the root. Roots treated with the ABA biosynthesis inhibitor did not exhibit these adaptive features. However, the application of exogenous ABA to well-watered roots failed to increase expansin gene expression levels to those induced by drought stress (Wu et al. 2001). Cho and Cosgrove (2004) proposed that ABA may be required to induce root responses, but drought-modulated expansin gene expression may be regulated by an ABA-independent pathway. In our study, the expression of most expansin genes in shoots was induced by at least one stress treatment (Fig. 6). Some genes were also induced by the application of exogenous ABA, such as *SlEXPA3*, *SlEXPA7*, *SlEXPA9*, and *SlEXLB1* (Fig. 5). This suggests their expression may depend on ABA. However, the expression of some genes was not induced by ABA, such as *SlEXPA8*, *SlEXPA12*, *SlEXPA24*, *SlEXPB1*, and *SlEXPB8*, which suggests their expression may be regulated by an ABA-independent pathway.

In conclusion, we identified 25 *SlEXPAs*, eight *SlEXPBs*, one *SlEXLA*, four *SlEXLBs*, and five short homolog sequences in the tomato genome. Twenty-five of these genes were identified being expressed. These expansins exhibited some specific features in terms of gene structure and amino acid sequences, suggesting that they underwent unique evolutionary processes. Segmental and tandem duplication events contributed to the expansion of the tomato expansin family. The 3-exon/2-intron pattern may represent the basic genetic structure, with other structural patterns arising from intron loss/gain events. Additionally, we identified three previously unreported expansin genes, *SlEXPA8*, *SlEXPB8*, and *SlEXLB1*, which were preferentially expressed in the fruit. We also identified expansin genes preferentially expressed in the root (*SlEXPA9*, *SlEXLB2*, and *SlEXLB4*), hormone and/or stress inducible expansin genes (*SlEXPA3*, *SlEXPA7*, *SlEXPB1–B2*, *SlEXPB8*, *SlEXLB1–LB2*, and *SlEXLB4*), and expansin genes preferentially expressed in floral parts. We believe our study findings may advance our understanding of the biological roles of tomato expansins. A more comprehensive understanding may be important for future crop improvement efforts involving genetic engineering.

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