A comprehensive expression analysis of the expansin gene family in potato (Solanum tuberosum) discloses stress-responsive expansin-like B genes for drought and heat tolerances

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Abstract

Expansin is a type of cell wall elongation and stress relaxation protein involved in various developmental processes and stress resistances in plant. In this study, we identified 36 potato (Solanum tuberosum L.) genes belonging to the expansin (StEXP) gene family from the genome reference. These genes included 24 α-expansins (StEXPAs), five β-expansins (StEXPBs), one expansin-like A (StEXLA) and six expansin-like B (StEXLBs). The RNA-Seq analysis conducted from a variety of tissue types showed 34 expansins differentially expressed among tissues, some of which only expressed in specific tissues. Most of the StEXPAs and StEXPB2 transcripts were more abundant in young tuber compared with other tissues, suggesting they likely play a role in tuber development. There were 31 genes, especially StEXLB6, showed differential expression under the treatments of ABA, IAA and GA3, as well as under the drought and heat stresses, indicating they were likely involved in potato stress resistance. In addition, the gene co-expression analysis indicated the StEXLBs likely contribute to a wider range of stress resistances compared with other genes. We found the StEXLA and six StEXLBs expressed differently under a range of abiotic stresses (salt, alkaline, heavy metals, drought, heat, and cold stresses), which likely participated in the associated signaling pathways. Comparing with the control group, potato growing under the drought or heat stresses exhibited up-regulation of the all six StEXLB genes in leaves, whereas, the StEXLB3, StEXLB4, StEXLB5 and StEXLB6 showed relatively higher expression levels in roots. This suggested these genes likely played a role in the drought and heat tolerance. Overall, this study has shown the potential role of the StEXP genes in potato growth and stress tolerance, and provided fundamental resources for the future studies in potato breeding.
Introduction

Expansins, a class of pH-dependent protein family, play a role in cell wall proliferation and growth [1,2]. Generally, it is believed that expansin binds to glucan-coated cellulose in cell wall causing reversible disruption of hydrogen bond between cellulose microfibrils and glucan matrix, which results in cell expansion or elongation through increasing cell wall extensibility [1,3–5]. The typical expansins (containing 250–275 amino acids and two conserved domains) are divided into four subfamilies: α-expansins (EXPA), β-expansins (EXPB), expansin-like A (EXLA), and expansin-like B (EXLB) [6].

A variety of expansin genes have been identified from a range of species. Among all these genes, the functions of EXPA and EXPB have been mostly studied, which are found to be involved in multiple processes of plant development through regulating the roles of cell walls [7,8]. For example, they are found to contribute to cell wall loosening in rice coleoptile [9,10], Arabidopsis petiole growth [11], tomato fruit softening [12], rose petal expansion [13], soybean root system architecture [14], cotton fiber elongation [15], and tobacco leaf enlargement and internode growth [16]. Expansins are also involved in cell expansion and cell wall changes induced by phytohormones such as gibberellin (GA), abscisic acid (ABA), auxin, and ethylene, as well as biotic and abiotic stresses including heat, drought, salt and heavy metals [7,17,18]. In specific, the overexpression of rose expansin gene RhEXPA4 in Arabidopsis enhances plant tolerance to drought stress, salt stress, and ABA content [19,20]. The overexpression of wheat expansin genes TaEXPB2 and TaEXPB23 increases the transgenic tobacco tolerance to drought [21], high salt and high temperature [22], oxidative stress [16,23,24], and water stress [25]. Some expansin genes are involved in the plant resistance to cadmium (Cd). For example, the heterologous expression of TaEXPA2 can increase the Cd resistance of tobacco [26]. Eleven expansin genes are involved in the response to Cd stress in the Cd hyperaccumulator of Phyto-lacca americana [27]. The roles of expansin genes playing in plant development and stress-resistance have provided opportunities in plant breeding for regulating leaf size, fruit growth, root development, biotic and abiotic stress resistance, etc. [28].

Parts of expansin genes have been identified in potato (Solanum tuberosum L.), but still largely restricted to those genes involved in its growth and development, and abiotic stresses. Specifically, nine StEXPAs have been recently found to be involved in the growth and development of tubers and stems, and StEXPA1, StEXPA4 and StEXPA5 are also hormone-regulated [29]. Two StEXP genes (PGSC0003DMG40029331 and PGSC0003DMG40009951) homologous to the Arabidopsis expansin11 (AT1G20190) showed expression increase under the cold plate-treatment, whereas significant decrease under the heat [30]. Although these results have been obtained, the research on StEXP family is still very limited. Potato is the third most important food crop in the world and often suffering from drought, heat, salt and some other environmental stresses. Several reports have shown that expansins participate in resistance to these stresses [18,28]. However, it is not clear which expansins are involved in which kinds of stresses in potato.

In this study, we identified potato expansins and their corresponding genes (StEXP) from the genome and transcriptomes, and then analyzed their phylogenetic relationships, gene and protein structures. The expression patterns of StEXPs in different organs as well as under different hormone and abiotic stress treatments are studied. Quantitative real-time PCR experiment was also performed to investigate the roles of seven StEXLs in multiple abiotic stress, such as salt, alkaline, heavy metals, drought, heat, and cold stresses.
Materials and methods

Genome-wide identification of expansin proteins and genes

A total of 130 expansin amino acid sequences from *Arabidopsis thaliana*, poplar (*Populus trichocarpa*) and rice (*Oryza sativa*) were used to search sequence homologs in the potato genome published on Phytozome v12 using BLAST program (https://phytozome.jgi.doe.gov/pz/portal.html#search?show=BLAST). Moreover, the keyword “expansin” was used to obtain expansin information from the Phytozome (https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Stuberosum) and the Spud DB Potato Genomics Resource (http://solanaceae.plantbiology.msu.edu/) databases. All the target amino acid sequences were downloaded and their conserved domains were analyzed at the Conserved Domain Database (CDD) (https://www.ncbi.nlm.nih.gov/cdd) with expect value < 0.05. After the repeated sequences and the sequences without pfam 03330 and pfam 01357 domains [7] were excluded from the target amino acid sequences, the remained were considered as candidate expansins. All the candidate expansins were then confirmed with online BLASTP (https://blast.ncbi.nlm.nih.gov/) and those without best hit being expansins were discarded.

StEXPs structure, conserved domain, motif, and phylogenetic analysis

Gene structure was obtained through aligning each expansin gene coding sequence (CDS) to the genomic DNA sequences and displayed using the online Gene Structure Display Server (GSDS) 2.0 (http://gsds.cbi.pku.edu.cn/). The Multiple Expectation Maximisation for Motif elicitation (MEME) tool (http://meme-suite.org/index.html) was used to identify conserved protein domain and motif. Multiple sequence alignments of *Arabidopsis*, rice, poplar, and potato expansins were performed using ClustalW within MEGA7 [31], and then the phylogenetic tree was constructed by MEGA7 (neighbor-joining method; Poisson correction model; 1,000 bootstrap tests).

Chromosomal localization of StEXP

StEXPs were mapped on potato chromosome and displayed by MapInspect software (http://mapinspect.apponic.com/) according to the potato expansin gene positions in the Spud DB database. The segmental duplicated and tandem repeated genes were determined through the ClustalW alignment comparison of all expansins with a threshold of similarity > 75% and their genomic locations, and tandem duplicated genes are restricted within the range of 100 kb distance [32].

Expression profiling of StEXP

The RNA-Seq data used for generating gene expression levels were downloaded from the Spud DB. These data were sequenced from many tissues of the heterozygous diploid potato (RH89-039-16 (RH)) or the doubled monoploid potato (Group Phureja clone DM1-3 (DM)) under various treatments. The sequenced tissues included tuber, root, stem, flower, petiole, stolon, tuber pith, tuber peels, and tuber cortex, and treatment condition covered 50 μmol L⁻¹ abscisic acid (ABA), 10 μmol L⁻¹ indole-3-acetic acid (IAA), 50 μmol L⁻¹ gibberellin A3 (GA3) and 10 μmol L⁻¹ 6-benzylaminopurine (BAP) for 24 h, and biotic and abiotic stresses such as 150 mmol L⁻¹ NaCl, 260 μmol L⁻¹ mannitol, 35°C high temperature for 24 h, and 2 days water stress, *Phytophthora infestans*, 2 mg ml⁻¹ BABA (DL-β-aminobutyric), and 10 μg ml⁻¹ BTH (benzo (1, 2, 3)-thiadiazole-7-carbothionic acid-S-methyl ester) [33]. Gene expression profiling was produced using MeV v4.9 [34]. The FPKM = 0 was replaced by FPKM = 0.01 and then
all the FPKM data were undergone log₂FPKM transformation. The fold change of gene differential expression was calculated as: log₂(FPKM_{Treatment} / FPKM_{Control}).

**Weighted gene co-expression network analysis (WGCNA) of StEXP**

WGCNA was performed to deduce the highly co-expressed gene clusters using the WGCNA program in R package [35]. An unsigned type of topological overlap matrix (TOM) was constructed with β = 16 and then the correlation between the potato expansin genes and the selected differentially expressed genes were analyzed. The resulted co-expression network was visualized using Cytoscape 3.6.1 [36] and analyzed using Network Analyzer in Cytoscape.

**Quantitative real-time PCR (qRT-PCR) analysis of StEXLs**

The hydroponic seedlings of tetraploid potato ‘Cooperation-88’ were transplanted to Pearl Rock Medium and cultured at 25°C (16 h light/8 h dark). These seedlings were firstly irrigated by 1/4 Hoagland’s nutrient solution for three times within 15 days. Then The Pearl Rock Medium of seedlings were overflowed thrice by 1/4 Hoagland’s nutrient solution containing 150 mmol L⁻¹ NaCl, 10 mmol L⁻¹ NaHCO₃, 5 mmol L⁻¹ ZnSO₄, 20% PEG6000, or 1/4 Hoagland’s solution, respectively. NaCl, NaHCO₃, ZnSO₄ and PEG6000 treated seedlings were cultured at 25°C for 24h. 1/4 Hoagland’s flowed seedlings were respectively placed at 35°C, 4°C and 25°C for 24h, as the heat, low temperature stress and control. All the seedlings were given the same photoperiod (16 h light/8 h dark). The root and leaf samples were collected for qRT-PCR analysis. Total RNA was isolated from all samples using Trizol (Invitrogen, USA) method and then reverse-transcribed into cDNA using PrimeScript RT reagent Kit with gDNA Eraser (Takara, China). qRT-PCR was performed on Roche LightCycler 96 Real Time PCR System (Roche, Switzerland) with a final volume of 20 μl containing 2 μl of a 1/10 diluted cDNA template, 10 μl of the 2× TB Green Premix Ex Taq II (Takara, China) and 1.5 μl (5 mM) of gene-specific forward and reverse primers. The specific primers were designed with Primer Premier 5.0 software (PREMIER Biosoft, USA) based on the conserved part of CDS sequences, all the primer sequences used in the qRT-PCR were listed in Supplement S1 Table. The qRT-PCR program was set to a 30s preincubation at 95°C, 2 step amplification of 45 cycles at 95°C for 5s and 60°C for 5s, following a 60°C to 97°C melting curve analysis at the final step. Three independent biological repetitions and three parallel reactions were conducted in qRT-PCR. The relative expression level of target genes was analyzed using the 2⁻^ΔΔCT method [37] with *S. tuberosum* elongation factor-1a (EF1α) used as the reference gene [38].

**Results**

**Expansin and corresponding genes**

A total of 36 candidate StEXPs were identified and shown in Table 1. According to the evolutionary analysis of amino acid sequences (Fig 1), 36 StEXP genes were divided into 4 subfamilies, StEXPA, StEXPB, StEXLA, and StEXLB, that contain 24, 5, 1 and 6 member(s), respectively (Table 1). The expansins encoded by these genes had 199–279 amino acids and their molecular weights were between 21.45 and 30.28 kD. In addition, the theoretical pl (isoelectric point) of these StEXPs proteins ranged from 4.68 to 9.87. Specifically, the pl of StEXPA6s and StEXPBs (except StEXPB5) were all more than 7.0, while that of the StEXLBs (except for StEXLB2) were below 7.0. As the averaged value of hydropathicity (GRAVY) of these proteins (except for StEXPA6, StEXPA10, StEXPA18, StEXPB4, and StEXLB1) were negative, most of the StEXPs were hydrophilic proteins. The instability coefficients of these expansins
were between 17.79 and 50.85 (only two expansins being more than 40), that is, most of these expansins were stable.

### Table 1. Description of expansin's genes identified from potato genome.

| Gene     | Encoding amino acid no. | Molecular weight (kD) | Theoretical pI | GRAVY  | Instability index | Aliphatic index |
|----------|-------------------------|-----------------------|----------------|--------|-------------------|-----------------|
| StEXPA1  | 241                     | 25.96                 | 9.49           | -0.076 | 25.39             | 72.86           |
| StEXPA2  | 256                     | 28.05                 | 9.38           | -0.120 | 36.09             | 70.51           |
| StEXPA3  | 259                     | 27.99                 | 9.43           | -0.052 | 19.09             | 71.20           |
| StEXPA4  | 257                     | 28.06                 | 9.39           | -0.012 | 30.41             | 78.13           |
| StEXPA5  | 239                     | 25.54                 | 9.36           | -0.031 | 29.06             | 69.00           |
| StEXPA6  | 260                     | 28.28                 | 9.32           | 0.026  | 36.26             | 72.08           |
| StEXPA7  | 266                     | 28.60                 | 9.10           | -0.029 | 23.60             | 65.34           |
| StEXPA8  | 258                     | 27.63                 | 8.56           | -0.132 | 23.91             | 68.49           |
| StEXPA9  | 261                     | 28.56                 | 9.55           | -0.013 | 29.59             | 76.25           |
| StEXPA10 | 250                     | 26.88                 | 8.45           | 0.038  | 26.13             | 69.84           |
| StEXPA11 | 257                     | 27.68                 | 8.97           | -0.016 | 27.26             | 64.98           |
| StEXPA12 | 256                     | 28.72                 | 9.87           | -0.250 | 50.85             | 66.25           |
| StEXPA13 | 267                     | 28.96                 | 8.60           | -0.151 | 34.97             | 70.90           |
| StEXPA14 | 247                     | 26.46                 | 7.52           | -0.097 | 30.41             | 61.30           |
| StEXPA15 | 249                     | 26.57                 | 9.14           | -0.133 | 34.34             | 64.62           |
| StEXPA16 | 263                     | 28.68                 | 9.48           | -0.048 | 23.81             | 74.18           |
| StEXPA17 | 257                     | 27.55                 | 9.03           | -0.170 | 30.26             | 62.26           |
| StEXPA18 | 269                     | 29.48                 | 9.26           | 0.052  | 25.30             | 61.71           |
| StEXPA19 | 257                     | 28.26                 | 9.23           | -0.163 | 25.63             | 68.29           |
| StEXPA20 | 265                     | 28.75                 | 8.56           | -0.075 | 35.62             | 76.98           |
| StEXPA21 | 199                     | 21.45                 | 8.61           | -0.221 | 30.98             | 72.91           |
| StEXPA22 | 240                     | 26.75                 | 8.74           | -0.123 | 27.71             | 78.33           |
| StEXPA23 | 244                     | 27.18                 | 8.55           | -0.417 | 27.05             | 65.90           |
| StEXPA24 | 259                     | 28.72                 | 8.72           | -0.179 | 28.42             | 71.51           |
| StEXPB1  | 262                     | 28.64                 | 9.87           | -0.066 | 30.71             | 82.67           |
| StEXPB2  | 279                     | 30.28                 | 8.74           | -0.092 | 37.47             | 75.13           |
| StEXPB3  | 267                     | 28.95                 | 8.76           | -0.052 | 39.48             | 71.20           |
| StEXPB4  | 257                     | 27.23                 | 8.48           | 0.018  | 29.05             | 74.05           |
| StEXPB5  | 256                     | 27.61                 | 5.35           | -0.134 | 30.34             | 73.48           |
| StEXLA1  | 260                     | 28.33                 | 8.39           | 0.021  | 29.87             | 79.88           |
| StEXLB1  | 253                     | 28.25                 | 5.96           | -0.108 | 18.96             | 77.83           |
| StEXLB2  | 251                     | 27.97                 | 8.47           | -0.230 | 17.79             | 78.09           |
| StEXLB3  | 255                     | 27.91                 | 4.68           | -0.248 | 40.85             | 73.80           |
| StEXLB4  | 253                     | 27.76                 | 4.87           | -0.206 | 35.28             | 80.20           |
| StEXLB5  | 251                     | 27.47                 | 6.42           | -0.139 | 38.23             | 78.84           |
| StEXLB6  | 248                     | 27.20                 | 6.88           | -0.229 | 27.75             | 76.61           |

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Phylogenetic analysis of expansins

Phylogenetic tree was constructed from 36 potato StEXPs, 36 Arabidopsis AtEXPs, 36 poplar PtEXPs, and 58 rice OsEXPs. These expansins were grouped into four clades (EXPA, EXPB, EXLA, and EXLB) based on species (Fig 1), indicating that expansins were highly conserved among species (Fig 1). The sequence similarities among EXPB, EXLA, and EXLB were more than that between them and EXPA, so EXLA and EXLB could be considered as a part of EXPB.
clade. In addition, the phylogenetic analysis showed expansins were most likely present before the differentiation of monocotyledon and dicotyledon, suggesting that expansins were evolved from the same ancestor (Fig 1).

The potato expansin phylogenetic tree divided 36 StEXP proteins into five clusters. All the StEXPB, StEXLA, and StEXLB proteins formed into one clade, while the 24 StEXPA proteins were divided into four clades, and one of them contained 19 proteins (Fig 2A).
Gene structure of StEXPs

Each of these 36 StEXPs contained 1–4 introns (Fig 2B). Specifically, StEXLA1, StEXLB3, and StEXLB4 each contained 4 introns, StEXPs, StEXPB, StEXLB1, StEXLB2, StEXLB5, and StEXLB6 each contained 3 introns, while the others each contained 1 or 2 intron(s). Among the 24 StEXAs, nine of them (StEXPA7, StEXPA8, StEXPA9, StEXPA13, StEXPA20, StEXPA21, StEXPA22, StEXPA23, and StEXPA24) each contained one intron, while the rest each had two introns. The genes within one subfamily were the same type due to they have similar length and similar intron, exons and motif structures (Fig 2B and 2C).

MEME analysis revealed that genes in the subfamilies of StEXPA, StEXPB and StEXL (StEXLA and StEXLB) had common motif and unique motifs. For example, each of the StEXPA3, StEXPA4, StEXPA6, StEXPA9, StEXPA16 and StEXPA19 had an additional motif (Motif 12) at N-terminals compared with the other StEXPs. Comparing the members of StEXPBs, StEXPB5 lacked Motif 4, StEXPB2 had an additional motif (Motif 14) at N-terminal, and StEXLB3 and StEXLB4 each had an additional motif (Motif 16) at C-terminal (Fig 2C, S1 Fig).
Chromosomal distribution of StEXP genes

The 36 StEXP genes were distributed on 11 of 12 chromosomes (chr. 1- chr. 10 and chr.12) of potato genome. chr. 3 and chr. 8 each contained the most seven StEXP genes. Six StEXPAs and 1 StEXPB were located on chr.3, and 2 StEXPAs and 5 StEXLBs were present on chr. 8. In comparison, only one StEXP was present on chr. 4 and chr. 12 (Fig 3). The 5 StEXPAs (StEXPA10 and StEXPA 21- StEXPA24) were located within a 29.0-kb region on chr. 3. And the 4 genes of StEXPA21-StEXPA24 were closely adjacent and their sequence similarity were more than 75%. Moreover, they were clustered together in phylogenetic tree. The four StEXLB genes on chr.8 (StEXLB1-StEXLB3 and StEXLB5) were located within a short region, had higher sequence similarity, and were clustered together in phylogenetic tree. The closely linked genes on chr. 3 or chr. 8 might be tandem repeated genes (Fig 3).

Moreover, among the 36 StEXP genes, there were four paralogous pairs, StEXPA8-StEXPA14, StEXPA11-StEXPA17, StEXPA13-StEXPA20, and StEXLB3-StEXLB4, that were dispersed segmental duplications.

Tissue-preferential expression of potato expansin

The gene members of StEXP showed significantly different expression levels. StEXPB2 transcript was the most abundant among StEXPs. It had the FPKM value of 852.8 in young tuber, while was absent in root, stem, flower, and other tissues (Fig 4A). This suggested that StEXPB2 played an important role during tuber development. StEXPA11, StEXPA16, StEXPA4, StEXPA14, and StEXLA1 transcripts also showed relatively high abundance in most tissues and their average FPKM values were 76.3, 56.3, 38.9, 32.56 and 23.65, respectively. However, StEXPA21 and StEXPB3 transcripts were absent in all tissues. Different StEXP genes are expressed differently among tissues. The average FPKM values of all StEXPs were 28.5 in roots and 20.0 in leaves, while it was only 1.3 in tuber peel.

Differential expression of StEXP after phytohormone treatment

Thirty-one of 36 StEXP genes responded to ABA, IAA, GA3, and BAP induction in different ways (Fig 4B, Table 2). Among them, there were 8, 7, 8, and 3 StEXP genes showed up-regulation under ABA, IAA, GA3, and BAP treatments, respectively (Fig 4B, Table 2). And all the StEXLB DEGs induced by ABA and GA3 were up-regulated. Specially, StEXPA7 and StEXLB6 were remarkably up-regulated by several hormones. StEXPA7 and StEXPA18 were up-
regulated by the three types of hormone (IAA, GA3 and BAP). Besides, five StEXP genes (StEXPA2, StEXPA8, StEXLB2, StEXLB5 and StEXLB6) were up-regulated by two of the four hormones (ABA, IAA, GA3 and BAP), and another 11 StEXP genes were up-regulated by one hormone. These results not only show the different expression patterns of potato expansin gene in response to different hormones but also reveal similar functions within the same expansin gene group.

**Induced expression of StEXP exposure to biotic and abiotic stresses**

Most of the identified StEXP genes were up- or down-regulated when exposed to different biotic and abiotic stresses (Fig 4C, Table 2). Specifically, StEXPs responded to NaCl and
mannitol treatments similarly. The number of differentially expressed genes (Log2 fold change >1) under NaCl and mannitol treatments was the same, with eight genes were up-regulated and nine were down-regulated. And StEXPA8, StEXPA19, StEXPB2 were up-regulated, while StEXPA4 and StEXLB4 were down-regulated under both treatments. There were 23 StEXP genes in response to water stress, with 14 of them being up-regulated and 9 of them down-regulated. Among the up-regulated genes, StEXPA4, StEXPA15, StEXLB1, StEXLB5 and StEXLB6 were up-regulated. StEXPA8, StEXPA19, and StEXPB2 were up-regulated, while StEXPA4 and StEXLB4 were down-regulated under both treatments. There were 23 StEXP genes in response to water stress, with 14 of them being up-regulated and 9 of them down-regulated. Among the up-regulated genes, StEXPA4, StEXPA15, StEXLB1, StEXLB5 and StEXLB6 showed 20-fold more transcript abundance than the control, and among the down-regulation genes, the transcription levels of StEXPA5, StEXPA11, StEXPA12, and StEXPA14 were decreased by nearly 95%. The expression levels of 18 genes were changed under high temperature stress, and seven of them (StEXPA7, StEXPA8, StEXPA18, StEXPA20, StEXPB4, StEXLB5 and StEXLB6) were up-regulated. StEXLB6 showed the highest expression levels under both drought and high temperature stresses, and its transcription levels under the two stresses were similar. While StEXPB2 was down-regulated the most by high temperature stress.

The effects of P. infestans and disease resistant inducer BABA on StEXP genes were very similar, but the effect was significantly different from that of BTH. Gene expression patterns (Fig 4C) showed that 14 StEXP genes were transcribed in similar ways when they were induced by P. infestans or BABA, whereas 10 of them were transcribed in an opposite way when induced by BTH (Fig 4C, Table 2).

In summary, most of the StEXPs showed more complex expression patterns in response to biotic and abiotic stresses than to hormones. Five genes (StEXPA1, StEXPA21, StEXPA23, StEXPA24 and StEXPB5) did not show significant transcription changes under either biotic and abiotic stresses or hormones. It was likely due to they had low expression level in tissues, because a small number of reads were detected from RNA-Seq data.

**Weighted gene co-expression network analysis (WGCNA) of StEXPs**

In the WGCNA, four StEXPs (StEXPA7, StEXPA18, StEXPA21 and StEXLB2) were found to be involved in the co-expression networks with other genes (Fig 5, S2 Fig). Specifically, StEXPA7 and StEXPA18 were involved in the same co-expression network and interacted with 409 genes. The directly adjacent genes of StEXPA7 were mainly associated with the development of cell wall and the formation of cytoskeleton. And the genes directly adjacent to StEXPA18 were involved in cell wall development, nutrient uptake and transport, and stress resistance. StEXPA21 was co-expressed only with a gene with unknown function. StEXLB2 and other 289 genes constituted a co-expression network. In this network, StEXLB2 was directly neighboring 18 genes, half of which had unknown functions and the other half were related to biotic and abiotic resistances (Table 3).

**Expression patterns of StEXLs and co-expression network involved genes under abiotic stresses as determined by qRT-PCR**

Our analysis above indicated that StEXLB genes contributed to the resistances of a wide range of abiotic stresses. qRT-PCR results (Fig 6) confirmed that six StEXLs (StEXLB1, StEXLB3, StEXLB4, StEXLB5, and StEXLB6) and StEXLA1 were significantly up-regulated in roots and leaves under drought stress. And among the seven up-regulated genes, the transcription levels of StEXLB3, StEXLB4, StEXLB5 and StEXLB6 in roots changed the most, which were 56.0, 28.4, 70.1 and 21.2 folds higher than that of control, respectively. StEXLB1-6 genes were up-regulated under the heat treatment, in which, the StEXLB3, StEXLB4, StEXLB5 and StEXLB6 transcription levels in roots were the highest four, which were 11.7, 9.6, 94.3 and 56.4 folds greater than that of control, respectively. The genes StEXLB2-SteXLB4 were up-regulated under the ZnSO_{4} stress and their transcription levels were significantly increased in roots. And
among them, StEXLB4 were up-regulated the most, with 6.4 folds greater of that in control. Although the four genes (StEXLB3-StEXLB6) showed mild expression level under NaCl, NaHCO₃ and cold treatments, they were involved in a wide range of plant resistance.

The qRT-PCR analyses of 4–5 genes within the co-expression network of StEXPA7, StEXPA18 and StEXLB2 were also be performed. StEXPA7 and StEXPA18 which were co-expressed in a same network (S2 Fig) showed similar expression patterns. They both were significantly induced under drought, NaCl and heat stresses in root, and cold induced in leaf (Fig

Table 2. The expression levels of potato expansin genes (StEXPs) under hormone and stress treatments.

| Gene   | ABA   | IAA | GA3 | BAP | Salt | Mannitol | 35°C | Water stress | P. infestans | BABA | BTH |
|--------|-------|-----|-----|-----|------|----------|------|--------------|--------------|------|-----|
| StEXPA1|       |     |     |     |      |          |      |              |              |      |     |
| StEXPA2| 2.77  | 1.14|     | -1.70|      | -1.99    |      | -1.74        | -10.19       |      |     |
| StEXPA3| -2.84 | -1.06|    | -2.96|      | -1.99    |      |              |              |      |     |
| StEXPA4| -1.55 | -1.41| -2.64| -2.34|  2.19|  4.23     |      |              |              |      |     |
| StEXPA5| -1.79 |     | -3.76| -3.25|  1.28|  3.11     |      |              |              |      |     |
| StEXPA6| -2.49 |     |      |  1.71| -1.38|  2.03     |      |              |              |      |     |
| StEXPA7| 11.23 | 11.12| 11.41|  3.08|      |          |      |              |              |      |     |
| StEXPA8| 2.57  | 1.51|  3.98|  2.57|  1.47|  1.35     |      |              |              |      |     |
| StEXPA9| -1.83 |     |      |  1.56|      |          |      |              |              |      |     |
| StEXPA10| -3.04 |     |      | -1.12|  2.14|          |      |              |              |      |     |
| StEXPA11| -2.80 |     |      | -4.08| -2.44| -5.53     |  2.70|              |              |      |     |
| StEXPA12| 1.26  |     |      | -2.59| -2.82|          |      |              |              |      |     |
| StEXPA13| -1.52 |     |      |      |      |          |      |              |              |      |     |
| StEXPA14| -2.95 |     |      | -3.25| -2.56| -3.47     |  2.43|              |              |      |     |
| StEXPA15| -1.45 |     |      | -1.34|  1.76| -1.33     | -4.25|  1.50        |              |      |     |
| StEXPA16| 1.62  |     | -1.66|     | -1.05|          |      |              |              |      |     |
| StEXPA17| 1.91  |     | -1.07|  1.15|      |          |      |              |  11.89       |     |  2.36|
| StEXPA18| -2.09 |  1.84|  2.39|  2.25|      |      |      |              |              |      |     |
| StEXPA19| 2.07  |     |  1.57|  1.51|      |  7.47    |      |              |              |      |     |
| StEXPA20| 1.08  |     |      |      |      |          |      |              |  1.93        |     |     |
| StEXPA21|       |     |      |      |      |          |      |              |  7.47        |     |     |
| StEXPA22| 1.34  |     |      |      |      |          |      |              |              |      |     |
| StEXPA23|       |     |      |      |      |          |      |              |              |      |     |
| StEXPA24|       |     |      |      |      |          |      |              |              |      |     |
| StEXPB1| -2.14 |     | -2.87| -1.21| -1.27| -3.31     |      |              |              |      |     |
| StEXPB2| -3.01 |  1.11|  1.88|  1.34| -10.38|  4.77    |      |              |              |      |     |
| StEXPB3| -1.70 |     | -2.02|      |      |  5.32    |      |              |              |      |     |
| StEXPB4| -4.61 |  2.02|  2.20|  1.37|  5.83| -1.97     |  3.58|              |              |      |     |
| StEXPB5|       |     |      |      |      |          |      |              |              |      |     |
| StEXLA1|       |     | -1.04|      |      |          |      |              | -1.07        |      |     |
| StEXLB1| 2.49  |     | -3.04| -1.21|  6.75|  1.63     |  2.54|              |              |      |     |
| StEXLB2| 3.77  |  1.07|     | -1.72|      |          |  2.92| -2.99        |              |      |     |
| StEXLB3| -3.20 |     |      | -4.32| -1.60| -2.80     |      |              |              |      |     |
| StEXLB4| 4.21  |     | -2.02| -1.59| -2.55| -1.43     | -5.25|              |              |      |     |
| StEXLB5| 7.86  | -1.83|  2.44|  1.92|  3.54|  7.90     | -3.34| -14.33       |              |      |     |
| StEXLB6| 17.98 | 11.41|     | 13.90| 14.70| -2.03     | -9.05|              |              |      |     |
|        | (8)   |     | (2) | (8) | (17) | (5) | (45) | (7) | (11) | (40) | (2) | (11) | (10) |

Note: The data is the log₂(Fold change) >1. The number in front of and in bracket are the gene No. of up- and down-regulated, respectively.

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In the co-expression network of StEXA7, 3 direct adjacent genes were analyzed by qRT-PCR, of which, EXT1 (PGSC0003DMG400011599) and ADF2 (PGSC0003DMG400029916) were similar to StEXPA7 (Fig 7), with up-regulation under drought, NaCl and heat stresses in root. And the expression of them were significantly correlated (Table 4). In the co-expression network of StEXPA18, POE1 (PGSC0003DMG400030033) and PME (PGSC0003DMG400018037) were significantly correlated to StEXPA18 (Table 5). The most obvious response of StEXLB2 was the up-regulation under ZnSO$_4$ treatment in root (Fig 6). ERF (PGSC0003DMG400013401), APOD (PGSC0003DMG400022342), CP (PGSC0003DMG400018037) and miraculin (PGSC0003DMG400015219), these co-expressed genes also exhibited a response to ZnSO$_4$ (Fig 7). The abiotic responsive correlations of ERF, APOD and CP to StEXLB2 were significantly (Table 6).

**Discussion**

Expansins have been recently found in many plant species. For example, there were 52 expansins (36 EXPAs, 6 EXPBs, 3 EXLAs, and 7 EXLBs) identified in tobacco [7]. In tomato, 38 expansins were found, which include 25 EXPAs, 8 EXPBs, 1 EXLA, and 4 EXLBs [8]. In this study, we identified a total of 36 potato expansins, including 24 EXPAs, 5 EXPBs, 1 EXLA, and 6 EXLBs. The difference in gene copies in expansin family and subfamily among species is likely due to biological evolution resulting from varied requirements in growth and development of plant and environmental adaptation [8]. In addition, the varied motif structures
among different subfamilies of expansins indicate their possible differences in action and function. For example, of the 11 cadmium-responded differential expression expansins in *P. americana*, EXPA was down-regulated while EXPB was up-regulated [27]. In potato, all StEXPBs were differential expression under ABA treatment (Fig 4B). Whether the genes in one subfamily show similar functions in potato need to be validated.

Gene expression pattern can provide insights into gene function. That expansins were involved in root or root hair development and stress tolerance have been reported in many species, such as *A. thaliana* [60,61], grapevine [62], and Tibetan wild barley [63]. The potato expansin genes, such as *StEXPAS*, *StEXPA11*, *StEXPA14*, and *StEXPA16*, had higher expression levels in root, leaf and stem than in other tissues, indicated that they might take effects in plant development. They also expressed in high levels under IAA and GA3 treatments. In Jung’s report [29], these 4 expansin genes were involved in tuber development and etiolated stem elongation, and also be induced in varying degrees under IAA treatment. Expansin genes also participated in the development of tuber in some species, such as *Rehmannia glutinosa*, *Smallanthus sonchifolius* [64,65]. Simultaneously, expansins are pleiotropic and play multiple roles during plant growth and development as well as stress resistance. For example, the over-expression of *TaEXPA2* and *TaEXPB23* from wheat not only contributed to the drought resistance ability of transgenic tobacco, but also increased its seed number, and *TaEXPB23* was also involved in leaf area development and internode length [16]. Many potato *StEXPs* were found to be involved in plant growth and stress resistance too. Most of the adjacent genes of *StEXPA7*

| Label | Annotation | Function | Reference |
|-------|------------|----------|-----------|
| SAUR | SAUR^a^ family protein | Regulate plant growth and development, promote cell expansion. | [39] |
| GT | Glycosyltransferase | Involved in the biosyntheses of cell-wall polysaccharides. | [40] |
| EXT1 | Extensin Ext1 | Involved in building and maintaining the growing primary cell wall. | [41] |
| ADF2 | Pollen specific actin-depolymerizing factor 2 | Reorganizing the actin cytoskeleton. | [42] |
| POE1 | Pollen en e 1 allergen and extensin family protein | Developmental regulators in plant tissues. | [43] |
| POD44 | Peroxidase 44 | Tolerant stress, biosynthesis and degradation of lignin in cell walls, auxin catabolism, etc. | [44] |
| PME | Pectinesterase | Involved in cell wall stiffening. | [45] |
| PEPT | Oligopeptide transporter | Involve in amino acids, nitrogen, or carbon transport. | [46] |
| OR | Oxidoreductase | Redox activity. | [47] |
| NPT | Inorganic phosphate transporter | Acquisition of Phosphorus in roots. | [48] |
| CPOD1 | Cationic peroxidase 1 | Biotic and abiotic stress. | [49] |
| AAT | Anthocyanin acyltransferase | Anthocyanin synthesis. | [50] |
| P450 | Cytochrome P450 | | |
| Miraculin | Miraculin | Biotic and abiotic stress. | [51,52] |
| LRR | Leucine-rich repeat protein | Endoplasmic reticulum retention. | [53] |
| KDEL1 | KDEL^b^ motif-containing protein 1 | Biotic and abiotic stress. | [54] |
| ERF | Ethylene-responsive transcription factor | Biotic and abiotic stress. | [55,56] |
| CP | Cysteine protease | Involved in suberization of tuber development. | [57] |
| ANP | Anthranilate N-benzoyltransferase protein | Disease resistance. | [58] |
| UPF0497 | UPF^c^0497 membrane protein | Response to abiotic stress. | [59] |
| unknown | Gene of unknown function | | |

^a^SAUR Small auxin-up RNAs.  
^b^KDEL motif: Lys-Asp-Glu-Leu.  
^c^UPF uncharacterized protein family.
or StEXPA18 in their co-expression network were related with the development of cell wall (Fig 5, Table 3), and StEXPA7 and StEXPA18 could also be induced by abiotic stresses (Fig 7).

![Expression profiles of potato expansin-like genes under various abiotic stresses.](https://doi.org/10.1371/journal.pone.0219837.g006)
Expansins expression in potato discloses drought and heat responsive expansin-like B genes

A

SIEXPA7

Relative expression level

0.6
0.5
0.4
0.3
0.2
0.1
0.0
Control Drought NaCl ZnSO4 35°C 4°C

Leaf Root

EXT1

Relative expression level

0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
Control Drought NaCl ZnSO4 35°C 4°C

Leaf Root

ADF2

Relative expression level

0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
Control Drought NaCl ZnSO4 35°C 4°C

Leaf Root

UN

Relative expression level

0.5
0.4
0.3
0.2
0.1
0.0
0
0
0
0
0
0
0
0
0
0
0
Control Drought NaCl ZnSO4 35°C 4°C

Leaf Root

B

SIEXPA18

Relative expression level

0.3
0.24
0.2
0.16
0.12
0.08
0.04
0
Control Drought NaCl ZnSO4 35°C 4°C

Leaf Root

POE1

Relative expression level

0.05
0.04
0.03
0.02
0.01
0
Control Drought NaCl ZnSO4 35°C 4°C

Leaf Root

PME

Relative expression level

0.2
0.15
0.1
0.05
0
Control Drought NaCl ZnSO4 35°C 4°C

Leaf Root

CPD1

Relative expression level

0.04
0.03
0.02
0.01
0
Control Drought NaCl ZnSO4 35°C 4°C

Leaf Root

C

ERF

Relative expression level

0.06
0.055
0.05
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
Control Drought NaCl ZnSO4 35°C 4°C

Leaf Root

APD

Relative expression level

0.2
0.15
0.1
0.05
0
Control Drought NaCl ZnSO4 35°C 4°C

Leaf Root

CP

Relative expression level

2.5
2
1.5
1
0.5
0
Control Drought NaCl ZnSO4 35°C 4°C

Leaf Root

Miracinulin

Relative expression level

1.0
0.8
0.6
0.4
0.2
0
Control Drought NaCl ZnSO4 35°C 4°C

Leaf Root
In comparison with StEXPA7 and StEXPA18, StEXLB2 was associated with more biotic and abiotic stresses related genes in the co-expression network (Fig 5, Table 3). The qRT-PCR analysis and the co-expression network deduced by the expression in diploid potato were showed a similar correlation implied potato expansins act in common modes among different genotypes.

ABA is a stress signal [66], which could up-regulate eight potato expansin genes. Of these eight genes, there were 5 StEXLBs (Table 2). StEXLB4, StEXLB5, and StEXLB6 showed changed expression levels under ABA, high temperature, and water stresses (Fig 4B and 4C; Fig 5), indicated that they also work in a wide range of abiotic resistances. In addition, it had been reported that the overexpression of PtEXPA8 in Populus tomentosa and AstEXPA1 in Agrostis stolonifera enhanced the transgenic plants tolerance to many stresses [67,68]. It also indicates that expansin has potential of resistance to wide abiotic stresses. The qRT-PCR confirmed the above results, all the 6 StEXLB genes could be induced by one or more stress treatments. In tomato, the closely related species of potato, there were three of four SlEXLB genes inducible by stress treatments [8]. More specificity of the pleiotropic roles of EXLB in tolerance to abiotic stresses. Furthermore, StEXLBs were mainly distributed on chromosome 8 (Fig 3), and StEXLB3 and StEXLB4 were the potential duplicated gene pairs, suggesting a selective advantage exists for retaining these gene copies [69]. Therefore, we speculate that the EXLB subfamily in potato may also play important roles in plant adaptability [69,70].

The expansin genes can loosen cell walls, and the loosened cell walls can lead to vulnerable cells that are easy to be damaged by biotic invaders [71]. We predicted that the up-regulations of StEXPA5, StEXPB3, and StEXLB1 were likely to increase cell wall loosening, thus increase the chance of P. infestans invasion. The down-regulations of StEXPA2, StEXPA6, StEXPA11, StEXPA15, StEXPB4, StEXLB4, StEXLB5, and StEXLB6 were likely to improve the potato resistance to disease. The induction mechanisms of disease resistance inducers BTH and BABA are different [72], which could be indicated by the different responsible patterns of StEXP genes. The inducers can work much efficiently only when the induction of disease resistance by inducers is similar to the way that plant responses. The way that StEXPs responded to P. infestans is the same as that of BABA induction, therefore BABA likely induced the resistance to P. infestans in potato through activating expansins.

Conclusions

In this study, 36 putative expansin genes in potato were identified and analyzed. The StEXP gene family was divided into four groups based on phylogenetic analysis, indicating that

Table 4. Pearson’s correlation coefficient of StEXPA7, EXT1, ADF2 and an unknown function gene.

|         | StEXPA7 | EXT1  | ADF2 |
|---------|---------|-------|------|
| EXT1    | 0.889** |       |      |
| ADF2    | 0.871** | 0.945** |      |
| UN      | 0.028   | 0.257 | 0.113|

Pearson’s correlation coefficients were calculated using PROC CORR of SAS 9.4.

**" indicates P<0.01

https://doi.org/10.1371/journal.pone.0219837.t004
**Table 5. Pearson’s correlation coefficient of StEXPA18, POE1, PME and CPOD1.**

|          | StEXPA18 | POE1  | PME  | CPOD1 |
|----------|----------|-------|------|-------|
| POE1     | 0.759**  |       |      |       |
| PME      | 0.679*   | 0.450 |      |       |
| CPOD1    | 0.456    | 0.772** | 0.282 |

Pearson’s correlation coefficients were calculated using PROC CORR of SAS 9.4.

**”** indicates P<0.05

**”** indicates P<0.01.

https://doi.org/10.1371/journal.pone.0219837.t005

**Table 6. Pearson’s correlation coefficient of StEXLB2, ERF, APOD, CP and miraculin.**

|          | StEXLB2 | ERF   | APOD  | CP    |
|----------|----------|-------|-------|-------|
| ERF      | 0.947**  |       |       |       |
| APOD     | 0.740**  | 0.851** |       |       |
| CP       | 0.621*   | 0.594* | 0.369 |       |
| Miraculin| 0.463    | 0.434 | 0.226 | 0.910** |

Pearson’s correlation coefficients were calculated using PROC CORR of SAS 9.4.

**”** indicates P<0.05

**”** indicates P<0.01.

https://doi.org/10.1371/journal.pone.0219837.t006

StEXP genes showed a high level of functional divergence. StEXP genes exhibited tissue-specific expression patterns and distinctly modulated by exogenous hormones, biotic or abiotic stress conditions. The preferential expression of StEXPB2 in young tubers indicated its role in tuber development. Many of the StEXP genes, especially the StEXLB subfamily members, were significantly up-regulated under water stress, high temperature, and other abiotic stress conditions. The tissue-specific expression patterns of expansin genes would provide insights for their functional characterization in potato. These results were valuable for understanding the biological functions of expansins during the growth and development of potato, especially tuber development.

**Supporting information**

S1 Fig. Motifs of potato expansins.
(TIF)

S2 Fig. The co-expression network involved in potato expansins genes (StEXPs).
(TIF)

S1 Table. Primers for qRT-PCR analysis.
(DOCX)

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