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ARTICLE

Comprehensive Genome Identification Analysis of *EXO70* Gene Family in *Panax ginseng* and *PgEXO70* Gene Expression under Methyl Jasmonate Regulation

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ABSTRACT: As an important medicinal plant in China, *Panax ginseng* is rich in secondary metabolites and is widely used in immune regulation, antitumor, anti-aging applications. The exocyst complex is a crucial protein complex that regulates vesicle trafficking in plants and plays key roles in cellular secretion, polar growth, and stress responses. Methyl jasmonate (MeJA) is a widely occurring derivative of jasmonic acid (JA) in plants and belongs to the category of endogenous signaling molecules in plants, which significantly regulates plant defense and secondary metabolism. MeJA is a key signaling molecule that significantly regulates plant defense and secondary metabolic pathways and has been shown to induce ginsenoside synthesis in *P. ginseng*. Despite the absence of reports on the effects of the *EXO70* gene family in ginseng under MeJA treatment, this study comprehensively screened, identified, and systematically analyzed the *EXO70* gene family in ginseng. Additionally, *PgEXO70* candidate genes that exhibited significant responses to MeJA were identified, and an extensive investigation of *PgEXO70* gene expression patterns was conducted. Utilizing ginseng genome and transcriptome data, we identified 58 members of the *EXO70* gene family in ginseng. We conducted the analysis of their phylogenetic relationships, gene structures, chromosomal distributions, cis-regulatory elements, co-expression network, and gene expression patterns. In addition, we treated ginseng adventitious roots with MeJA and investigated the expression trends of the four *PgEXO70* genes containing the highest number of MeJA elements. All four genes responded to MeJA treatment, but the expression of *PgEXO70-18* was significantly elevated at all treatment times. These results provide the genetic resource support for further exploration of the functions of *EXO70* genes in *P. ginseng* and their roles in plant hormone regulation and secondary metabolism.

KEYWORDS: *Panax ginseng*; exocyst complex; *EXO70* gene family; methyl jasmonate regulation; gene expression pattern

1 Introduction

The vesicle transport system is essential for intracellular material transport and signal transduction in vascular plants. The precise collaboration of membrane-bound proteins, motor proteins, and the exocyst complex enables the orderly transport of signaling molecules, proteins, and metabolites between organelles [1]. The exocyst complex is a key protein complex that controls vesicle docking and fusion at the plasma membranes. Initially discovered in yeast and animals, it consists of eight highly conserved subunits: SEC3, SEC5, SEC6, SEC8, SEC10, SEC15, EXO70, and EXO84 [2]. In plants, EXO70 is the most significantly expanded subunit of this complex and has undergone extreme proliferation in land plants. It interacts

with small G proteins and membrane lipids, participating in the assembly of the complex at the target membrane [3]. In land plants, there are multiple copies of EXO70 in the genome, a phenomenon unique to EXO70 compared to other subunits of the exocyst complex [4]. While most exocyst subunits are encoded by one or a few genes, the gene encoding EXO70 is the most duplicated. For example, there are 23 *EXO70* genes in *Arabidopsis thaliana* [5], and varying numbers of *EXO70* members have been found in *Oryza sativa* [6], *Setaria italica* [7], *Haynaldia villosa* [8], *Gossypium hirsutum* [9], and other species. The exocyst complex in plants plays a crucial role in various biological processes, such as cell growth, wall formation, and division [10]. The *EXO70* gene family members are integral to various physiological processes, such as cell polarity establishment, cell wall synthesis, and pollen tube elongation, and have also been proven to participate in the regulation of plant responses to both abiotic stresses and biotic stresses [11]. Exocytosis, a cellular mechanism facilitating the transport of membrane-bound vesicles from the cell interior to the plasma membrane, thereby releasing their contents into the extracellular environment, which is crucial in plant-microbe interactions [12].

Panax ginseng has been used as a traditional Chinese medicinal material for over two thousand years, with its roots being the primary medicinal part. Since ancient times, ginseng has been a precious traditional medicinal material [13] and has held an important position in traditional medicine in East Asia, especially in China, South Korea, and Japan. The roots of ginseng contain a wealth of bioactive components, including ginsenosides, volatile oils, and ginseng polysaccharides, which possess various pharmacological activities, such as anti-fatigue, anti-aging, immune regulation, and antitumor effects [13,14]. These properties make it irreplaceable in modern pharmaceutical research and clinical settings. With the development of functional genomics and metabolic engineering technologies, molecular biological research on *P. ginseng* has gradually deepened, especially in the field of secondary metabolite synthesis regulation, where a series of breakthroughs have been achieved [15]. However, there is still a lack of systematic analysis and functional elucidation of the transport mechanisms that play a key role in these physiological processes, especially the gene families related to vesicle-mediated exocytosis. Vesicular transport mediated by the *EXO70* gene family is a rate-limiting step in the secretion of specific metabolites; therefore, it is necessary to study this process in *P. ginseng*.

Methyl jasmonate (MeJA) is a widely occurring derivative of jasmonic acid (JA) in plants and belongs to the category of endogenous signaling molecules in plants. MeJA primarily regulates plant immune responses, wound reactions, and the accumulation of secondary metabolites via the COI1-JAZ-MYC signaling pathway [16]. However, the mechanisms by which MeJA interacts with the vesicle transport system and indirectly affects metabolic pathways by regulating specific transport-related factors remain unclear.

A comprehensive review of current domestic and international research findings reveals that studies on ginseng metabolism, especially in the areas of ginsenoside synthases and transcription factors, began relatively early and have yielded abundant results. However, the role of vesicle transport in the regulation of signaling molecules has been less explored. Research on the *EXO70* gene family members in *A. thaliana*, such as *EXO70B1*, *EXO70B2*, and *EXO70H1* [17], in immune regulation has gradually deepened; however, similar studies on ginseng have not yet been conducted. A thorough genome-wide identification and analysis of the expression regulation of the *EXO70* gene family in ginseng, particularly under hormone-induced conditions, is necessary to identify key targets for subsequent functional validation and metabolic engineering studies.

2 Materials and Methods

2.1 Ginseng Plants Materials

The ginseng plant materials consisted from Jilin Province (1) 4 different ages (5, 12, 18, 25 years-old) of ginseng roots; (2) 14 different tissues of 4-year-old ginseng (stem, fibrous root, fruit pedicel, main root epidermis, rhizome, petiole, lateral petiolule, lateral root, branch root, leaf, fruit flesh, main root cortex, and seed); (3) 42 cultivars of 4-year-old ginseng roots (S1–S42). All plant materials were sourced from the collaborative research base of the Jilin Engineering Research Center for Ginseng Genetic Resources Development and Utilization.

2.2 Genome and Transcriptome Database Resources

In this study, ginseng genome database is *P. ginseng* vT2T, PRJCA022032 for NCBI [18]; ginseng transcriptome database is our laboratory publicly available RNA-Seq data: PRJNA302556 for NCBI [19].

2.3 Identification of the PgEXO70 Genes from P. ginseng

The nucleotide sequences of each *EXO70* gene family member from monocotyledonous plants (*O. sativa*), dicotyledonous plants (*S. lycopersicum*), Araliaceae plants (*P. notoginseng* and *Panax japonicus*), and the model plant *A. thaliana* were downloaded from database. These sequences were then compared with the ginseng transcriptome database using Blastn. The hidden Markov model (HMM) of the conserved domain EXO70 (PF03081) was downloaded from the Pfam website (http://pfam.xfam.org/) and compared with protein database using the HMMER version 3.2 software [20]. All obtained sequences were merged, screened, and removed to obtain the *EXO70* gene family sequences in ginseng. Subsequently, conserved domain analysis of the obtained sequences was performed using the NCBI's Conserved Domain Database (CDD) version 3.20 search and SMART tools online. *EXO70* gene family members were identified and renamed in the format *PgEXO70* + gene number + transcript number.

2.4 Chromosomal Localization and Collinearity Analysis of PgEXO70 Gene Family Members

We investigated the localization of the *PgEXO70* gene family members within the ginseng genome. The BLASTN version 2.14.0 software facilitated comparison of the *PgEXO70* gene family sequences, requiring a sequence consistency of at least 95%, an alignment length of no less than 300 bp, and an E-value < $1.0E^{-100}$. Subsequently, the MG2C online tool was utilized to visualize the chromosomal positioning of the *PgEXO70* genes. Collinearity among *PgEXO70* members within the ginseng chromosomes was depicted using R language.

2.5 Conserved Domain and Phylogenetic Analysis of PgEXO70 Gene Family Members

The online NCBI ORF Finder tool was used to identify *PgEXO70* genes with intact and conserved domains. These sequences were uploaded to MEME version 5.5.9 software for motif conservation analyses. Subsequently, the conserved domain characteristics of *PgEXO70s* were illustrated using TBtools-II [21].

Phylogenetic analysis was conducted using MEGA software version 11 [22]. A total of *PgEXO70* gene sequences with complete domains were subjected to multiple sequence alignment. Additionally, orthologous genes from dicotyledonous plants (*S. lycopersicum*), monocotyledonous plants (*O. sativa*), Araliaceae plants (*P. notoginseng* and *P. japonicus*), and the model plant *A. thaliana* were selected as exogenous plant species to construct a multispecies phylogenetic tree. The deduced amino acid sequences were aligned using the MEGA version 11 software for multiple sequence alignment [22]. A phylogenetic tree was constructed using

the maximum-likelihood (ML) method, employing the WAG + G model, with bootstrap values calculated from 1000 replicates. Following the construction of the phylogenetic tree, visual enhancements were applied using the iTOL online tool (https://itol.embl.de/).

2.6 Gene Expression Pattern Analysis of PgEXO70 Genes in Ginseng

The expression levels of *PgEXO70* genes in the 4 different ages (5, 12, 18, and 25 years old) of ginseng roots, 14 different tissues of 4-year-old ginseng, and 42 farmer cultivars were extracted using the R language. Subsequently, TBtools II software [21] was used to create a heatmap and analyze the expression pattern of this gene family across the three databases, revealing the spatiotemporal expression characteristics and features of the different genotypes of *PgEXO70* genes.

2.7 PgEXO70 Genes Co-Expression Interaction Network Analysis in Ginseng

Based on the TPM expression database of *PgEXO70* genes, the Spearman correlation coefficient was calculated using the R language. Subsequently, BioLayout Express ^{3D} software created a *PgEXO70* genes co-expression network ($p < 0.01$) [23].

2.8 Cis-Acting Element Prediction Analysis of PgEXO70 Genes

To explore the potential biological roles of the *PgEXO70* gene family, promoter sequences upstream of the initial codon were selected based on the chromosomal localization of the *PgEXO70* genes in ginseng genome. These sequences underwent analysis using the PlantCARE online tool (https://bioinformatics.psb.ugent.be/webtools/plantcare/html/) to identify cis-acting elements. The analysis revealed cis-acting elements associated with hormone response functions. Subsequently, the Plant-CARE Result Classify module, a plugin available in TBtools-II (version 2.0), was utilized to visualize these components [21], following the plugin's dedicated tutorial.

2.9 Candidate PgEXO70 Genes Expression after MeJA Treatment

1.0 g of ginseng adventitious roots was inoculated into 250 mL triangular flasks containing 150 mL of liquid MS medium and cultured on a shaker at 22°C and 110 rpm for 21 days. On day 22, 200 μM MeJA was added to the flasks, and the treatment times were set at 0 h (as control), 6, 12, 24, 36, 48, 72, 96, and 120 h, with three biological replicates at each time point. The ginseng samples collected were rapidly frozen in liquid nitrogen and subsequently stored at −80°C for future experiments.

We collected 1.0 g of ginseng adventitious root material from each treatment times and extracted total RNA using the TRIZOL method. The RNA concentration was measured, and reverse transcription into cDNA was conducted using the RevertAid™ first-strand cDNA Synthesis Kit (MBI Company, USA). *Actin 1* gene served as the internal reference gene, and all primer sequences are listed in Table 1. The fluorescence quantitative PCR (qRT-PCR) was executed on the 7500 real-time fluorescence quantitative PCR system, adhering to the UltraSYBR one-step qRT-PCR kit (Low ROX) (CWBIO, Beijing, China). The qRT-PCR conditions were as follows: 95°C for 10 minutes, 95°C for 15 seconds, 60°C for 60 seconds, over 40 cycles. To ensure result accuracy, each experiment included three biological and three technical replicates, and data analysis was performed using the $2^{-\Delta\Delta Ct}$ method.

Table 1: Genes primers sequences used in this study.

Primer Name	Fluorescence Quantification Primer Sequence	Product Size
Action 1-F	TGGCATCACTTTCTACAACG	150 bp
Action 1-R	TTTGTGTCATCTTCTCCCTGTT	
PgEXO70-18-F	CAGACTCCGACGAATCTCCG	164 bp
PgEXO70-18-R	AATGACCCTTGCGACGATGT	
PgEXO70-1002-F	TACAGCAAGTTAGCGGTGCA	176 bp
PgEXO70-1002-R	GTGTGGTCCGTGGGTTGTAT	
PgEXO70-1101-F	GCTCCGAAGACAGTTCACCA	135 bp
PgEXO70-1101-R	TGCTGGACAGTTCGATTGCT	
PgEXO70-1716-F	GTGGAGCCTCTGGAAACCTC	100 bp
PgEXO70-1716-R	GAATCCTCTTCCCGAGCGAG	

3 Statistical Analysis

A one-way ANOVA was performed on gene expression levels at different time points, using the untreated group as control (CK). Differences between MeJA-treated and control group were analyzed by *t*-test ($p < 0.05$) and one-way ANOVA with Tukey's post hoc test ($p < 0.01$) for time-course expression.

4 Results

4.1 Screening of the PgEXO70 Genes from P. ginseng

Nucleotide sequences of the *EXO70* gene family were compared with the ginseng genome and transcriptome database using three different mining methods: native species comparison, closely related species comparison, and the hidden Markov model (HMM). Initially, 224 *EXO70* gene sequences were screened from ginseng. Subsequently, 58 *EXO70* transcripts in ginseng containing conserved domains were obtained through conserved domain analysis, with each transcript containing at least one *EXO70* conserved domain. The subcellular localization of the PgEXO70 protein is in the cytoplasm. These 58 *PgEXO70* genes were renamed in the format *PgEXO70* + gene number + transcript number (Table S1).

4.2 Chromosomal Localization and Collinearity Analysis of PgEXO70 Gene Family Members

58 *PgEXO70* genes were mapped onto the ginseng chromosomes. As shown in Fig. 1a, *PgEXO70* genes were unevenly distributed across 15 chromosomes, with the highest concentrations on chromosomes chr6, chr10, chr13, chr19, and chr22, chr6 and chr10 had the most genes, with eight genes each. The remaining genes were distributed on chromosomes chr1, chr2, chr3, chr4, chr5, chr11, chr14, chr17, chr20, and chr24. Gene localization results indicate that certain transcripts of the same gene were found at identical positions. Despite originating from the same gene, these transcripts exhibited structural variations, likely due to alternative splicing post-transcription. These transcripts have the potential to encode distinct proteins with specific biological functions. Fig. 1b presents the collinearity analysis of *PgEXO70s*, where 21 pairs of *PgEXO70* gene family sequences are linked by red lines. This connection implies the presence of sequence repeats on the chromosomes. Such repeats may have arisen from biological processes including gene duplication, gene fragment repetition, transposition, reverse transposition, and chromosomal crossover during evolutionary events.

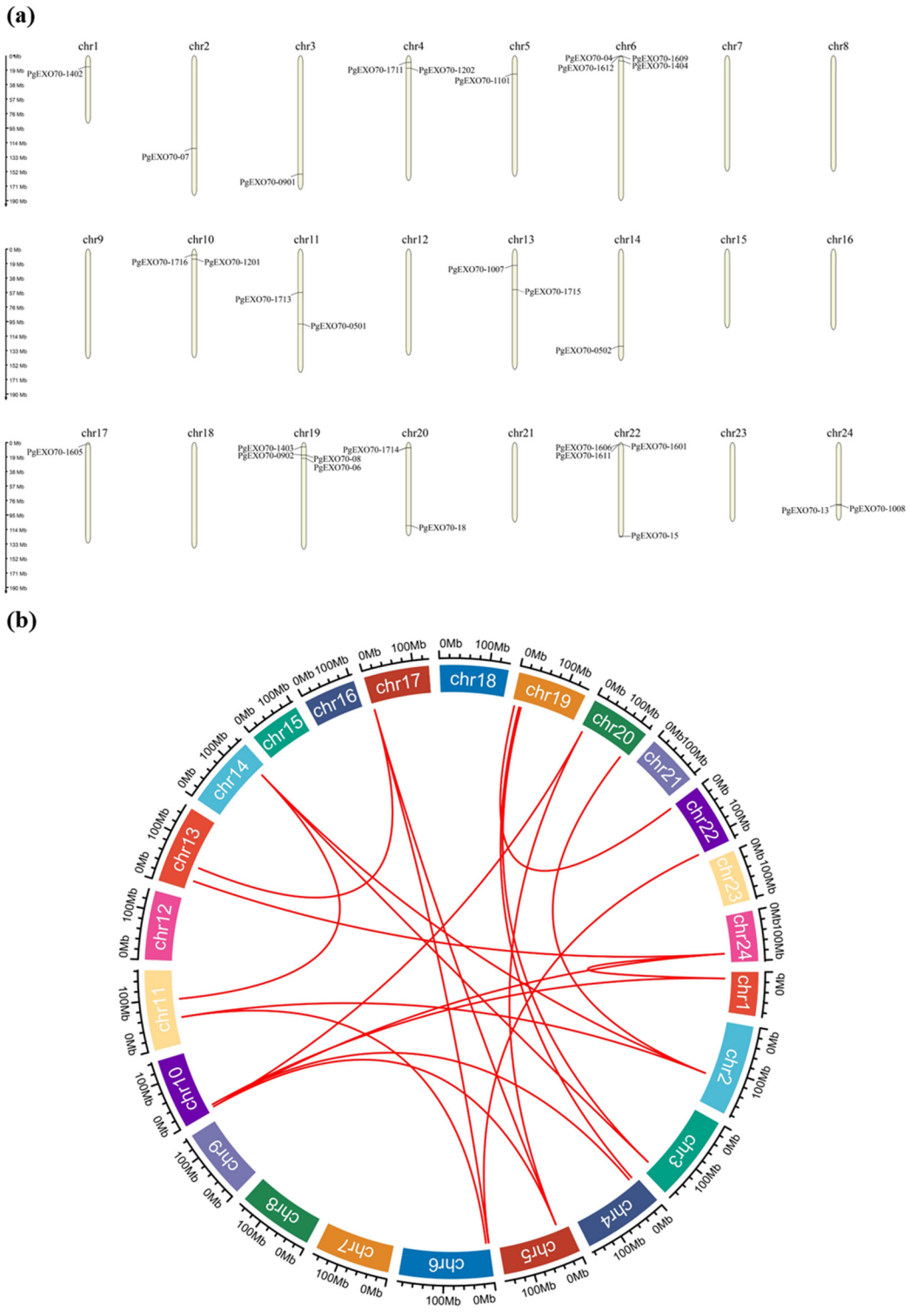

Figure 1: *PgEXO70* genes localization and collinearity analysis in the ginseng genome. (**a**) Chromosomes that show gene localization are shown in yellow. (**b**) The arc points to a paraphyletic pair resulting from *PgEXO70* gene duplication. The termini of the red arcs indicate parallel pairs resulting from gene duplication. The colored squares denote the chromosomes of ginseng, while the scale external to the chromosome indicates its length.

4.3 Conserved Domain Analysis and Phylogenetic Analysis of PgEXO70 Genes

Using the online tool NCBI ORF Finder to identify open reading frames (ORFs) in the *PgEXO70* genes revealed that the ORF lengths varied significantly among the genes. Specifically, 13.8% of the genes had ORFs shorter than 300 bp, 56.9% had ORFs between 300 and 600 bp, and 29.3% had ORFs longer than 600 bp. The longest ORF was found in *PgEXO70-1101* at 785 bp, whereas the shortest ORF was found in *PgEXO70-1702* at 251 bp (Table S1).

Conserved domain analysis of *PgEXO70s* using the MEME online tool showed that different members contained varying numbers of motifs, ranging from one to eight (Fig. 2). As shown in Fig. 2, motif 1 was present in the majority of the members. Most members in the first branch of the phylogenetic tree shared the same conserved motifs (motifs 1–6). However, each *PgEXO70* family member contained at least one highly conserved EXO70 domain. Moreover, members of the same branch of the phylogenetic tree tended to have similar types of conserved domains, indicating that sequences within the same family share similar motif structural features.

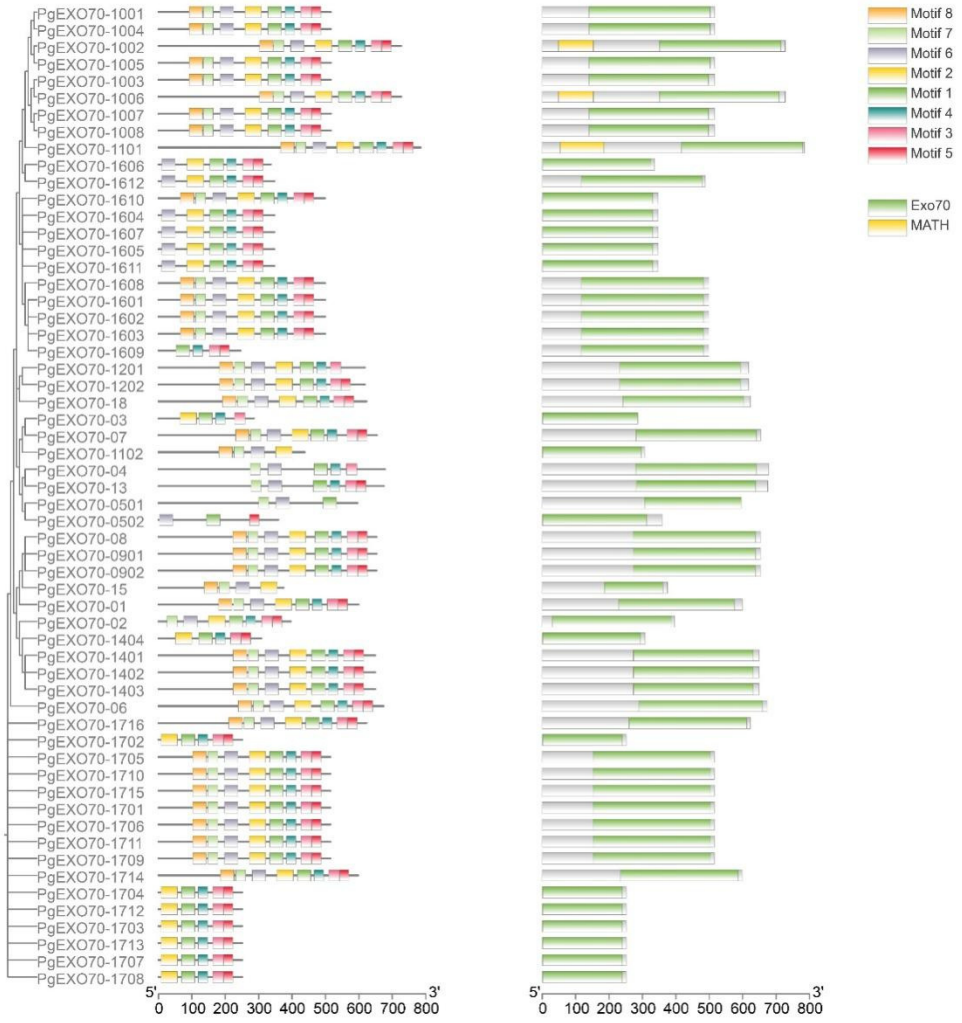

Figure 2: PgEXO70 protein sequences analysis of conserved motifs and structural domains. Fonts of varying colors denote distinct subfamilies, while boxes of different colors indicate conserved motifs and structural domains.

The ML phylogenetic tree of EXO70 protein sequences was constructed from *P. ginseng* and five exogenous species. As shown in Fig. 3, The members can be categorized into four branches according to their relevance to the topics. Clade I comprises the fewest members, totaling 12, whereas Clade IV contains the most members, with a total of 34. Key members of the *EXO70* gene family formed tight clusters with closely related species, such as *PjEXO70* and *PnEXO70*, indicating their high conservation. The *PgEXO70* gene family is divided into four clades in ginseng, with members distributed evenly and exhibiting a high degree of homology with *PgEXO70* gene members from exogenous species in terms of phylogenetic development.

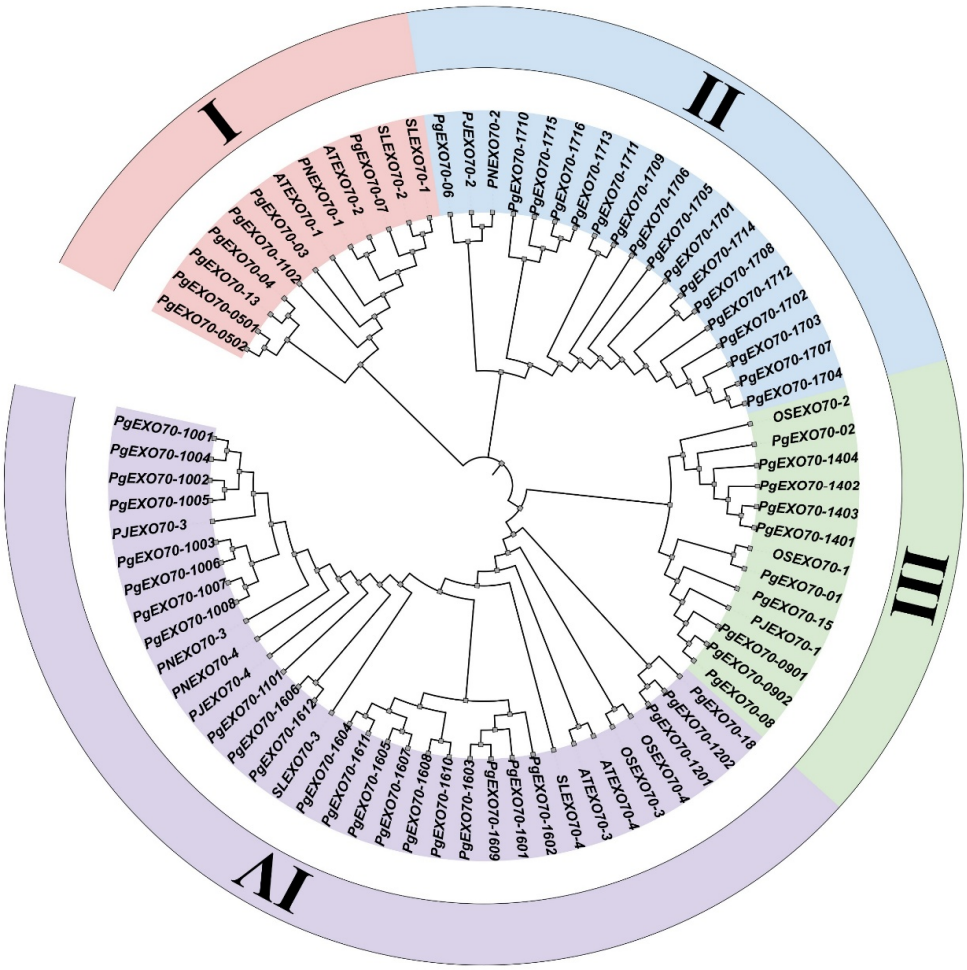

Figure 3: The ML phylogenetic tree of the *PgEXO70* gene family. The EXO70 protein sequences were from *P. ginseng* (*Pg*), *A. thaliana* (*At*), *P. notoginseng* (*Pn*), *P. japonicus* (*Pj*), *S. lycopersicum* (*Sl*), and *O. sativa* (*Os*).

4.4 Gene Expression Pattern Analysis of PgEXO70 Genes

To investigate the expression patterns of *PgEXO70* genes in ginseng, heatmaps of their expression levels were generated for ginseng roots of four different ages, 14 different tissues of 4-year-old ginseng, and 42 farmer cultivars (Fig. 4, Table S2). (1) *PgEXO70* genes expression pattern in 4 different ages: The expression patterns of *PgEXO70* genes varied among roots of different ages. Fourteen *PgEXO70* genes were not expressed in any of the four age groups, whereas 28 were expressed in all age groups. Among these 28 genes, *PgEXO70-1101* exhibited the highest expression level. *PgEXO70-1714* was only expressed in

the main roots of 12-year-old ginseng plants. Some genes showed a gradual decrease in expression with increasing age, with *PgEXO70-1711* being the most notable (Fig. 4a). (2) Expression patterns of *PgEXO70* genes in 14 different tissues: Of the 52 *PgEXO70* genes expressed in the 14 tissues of 4-year-old ginseng, 27 were expressed in all tissues, and six were not expressed in any tissue. The highest number of expressed genes was found in the leaves, with 43 genes expressed, followed by the fibrous roots, stems, main root cortex, and fruit flesh, each with 41 genes expressed. *PgEXO70-1101* exhibited the highest expression levels in all five tissues. Some genes were expressed only in specific tissues; for example, *PgEXO70-01* and *PgEXO70-03* were expressed only in pedicels (Fig. 4b). (3) Expression patterns of *PgEXO70* genes in 42 different farmer cultivars: Among the 54 *PgEXO70* genes expressed in the 42 cultivars, four genes were not expressed. Twenty genes were found to be highly expressed in all the cultivars. Five genes were expressed only in specific local farmer cultivars, possibly in response to environmental changes (Fig. 4c).

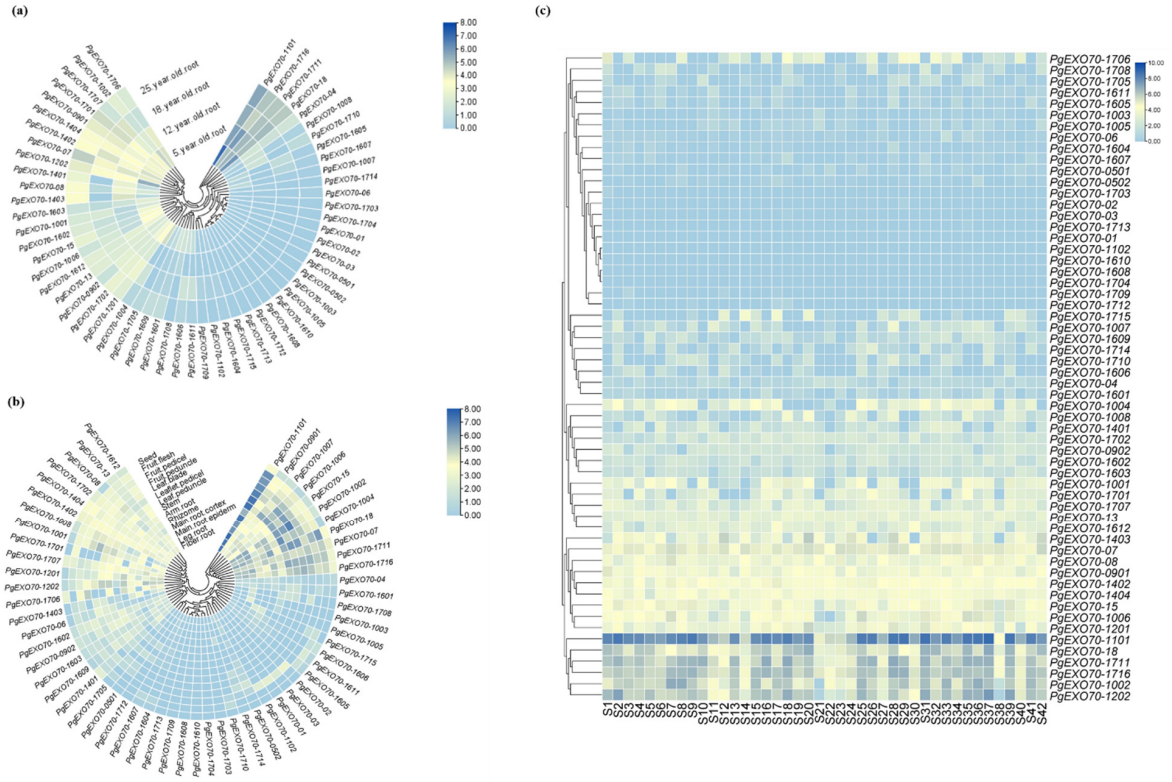

Figure 4: *PgEXO70* genes spatiotemporal expression patterns heatmap analysis in *P. ginseng*. (**a**) *PgEXO70* gene expression in ginseng roots at four different ages; (**b**) *PgEXO70* gene expression in 14 different tissues of 4-year-old ginseng; (**c**) *PgEXO70* gene expression in 42 cultivars roots of 4-year-old ginseng.

4.5 PgEXO70 Genes Co-Expression Networks Analysis

The co-expression interaction network of *PgEXO70* genes in the 42 farmer cultivars is presented in Fig. 5. A total of *PgEXO70* genes formed an interaction network, and a co-expression network was constructed at $p < 0.01$ (Fig. 5a). This network consisted of four clusters (Fig. 5b), and individual *PgEXO70* genes could interact with multiple *PgEXO70* genes, indicating potentially strong interactions among *PgEXO70* members. Members of the *PgEXO70* gene family form an interactive network through cooperative interactions, thereby performing various biological functions.

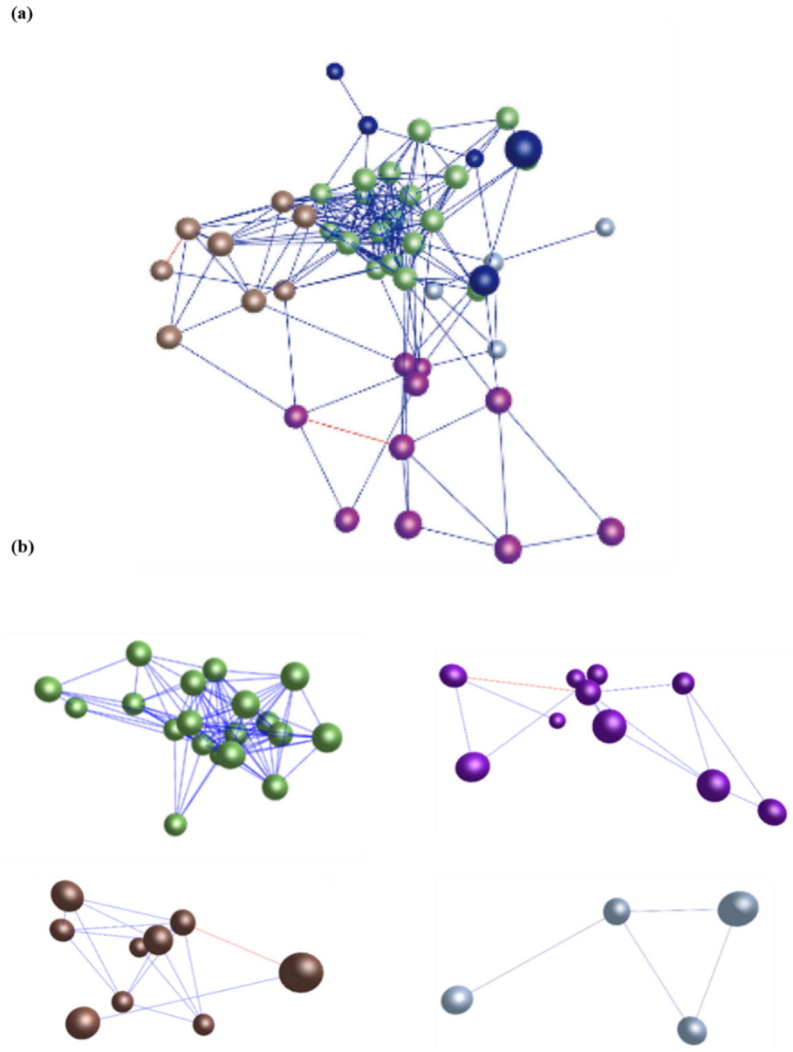

Figure 5: *PgEXO70* genes co-expression interaction network analysis of 42 ginseng local farmer cultivars roots. (**a**) The co-expression network derived from *PgEXO70* genes was constructed with a significance level of $p < 0.01$. (**b**) The four clusters that make up *PgEXO70* genes network.

4.6 Cis-Acting Elements Promoter Analysis of the PgEXO70 Genes Members

The functions of genes are typically determined by cis-acting elements. The *PgEXO70* genes in ginseng possess common cis-acting elements, including the TAAT-box and CAAT-box, as illustrated in Fig. 6. These genes also contain light response signals and various response elements related to low temperature, drought, defense, and adversity. Additionally, they are rich in hormone response-related elements. This suggests that the *EXO70* gene family may participate in hormone signal transduction in ginseng, playing a significant role in this process.

The hormone response elements were categorized into four groups: auxin reactivity, gibberellin reactivity, abscisic acid reactivity, and MeJA reactivity. Among these, 34 *PgEXO70* genes contained MeJA response elements. Specifically, the *PgEXO70-18* gene included 14 MeJA cis-acting elements, while *PgEXO70-1716* and *PgEXO70-1101* each contained four, and *PgEXO70-1002* contained two. These four genes exhibited the highest number of MeJA response elements, and they were selected for analysis of *PgEXO70* gene expression under MeJA regulation.

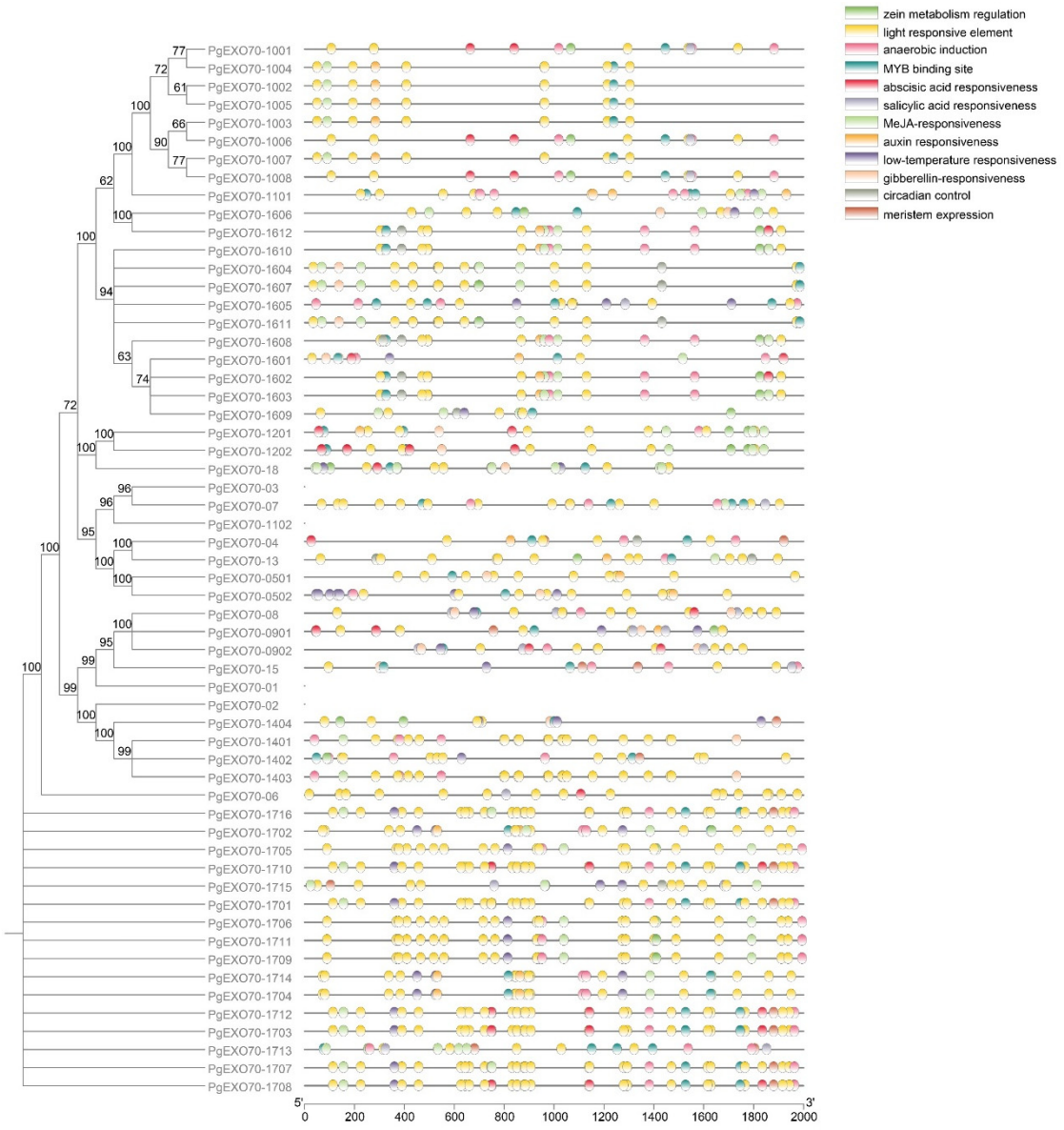

Figure 6: Cis-acting elements promoter analysis of *PgEXO70* gene family members. The circles of varying colors denote cis-acting elements, each serving distinct functions.

4.7 PgEXO70 Candidate Genes Expression Analysis under MeJA Treatment

Four *PgEXO70* candidate genes, which characterized by numerous MeJA regulatory elements, and were selected due to their high expression levels in ginseng. Following MeJA treatment, we analyzed the expression of four *PgEXO70* genes (*PgEXO70-18*, *PgEXO70-1002*, *PgEXO70-1101*, and *PgEXO70-1716*) in ginseng adventitious roots at different time points. The results (Fig. 7) showed that under MeJA treatment, the expression levels of the three *PgEXO70* genes (*PgEXO70-18*, *PgEXO70-1002*, and *PgEXO70-1101*) exhibited a significant upward trend over time compared with the control group. Concurrently, the expression patterns of these three *PgEXO70* genes followed a pattern of first increasing, then decreasing, and then increasing again, with 72 h marking the inflection point in gene expression. This indicates that MeJA treatment of ginseng adventitious roots induces changes in vesicular biology through the expression of

EXO genes within the cells. After 72 h of MeJA treatment, the cells stabilized and resumed their normal physiological functions without the influence of stress. In contrast, the expression pattern of the *PgEXO70-1716* gene followed a sequence of first decreasing, then increasing, and finally decreasing again, with 72 h also serving as the inflection point for gene expression. We also found that *PgEXO70-18* exhibited the highest number of MeJA-responsive elements and was the most sensitive to MeJA treatment, with gene expression significantly elevated at all nine treatment time points. Consequently, the selection of *PgEXO70-18* as a primary candidate *EXO* gene in ginseng establishes a significant theoretical basis for future investigations into the function and mechanisms of the exocyst complex in ginseng.

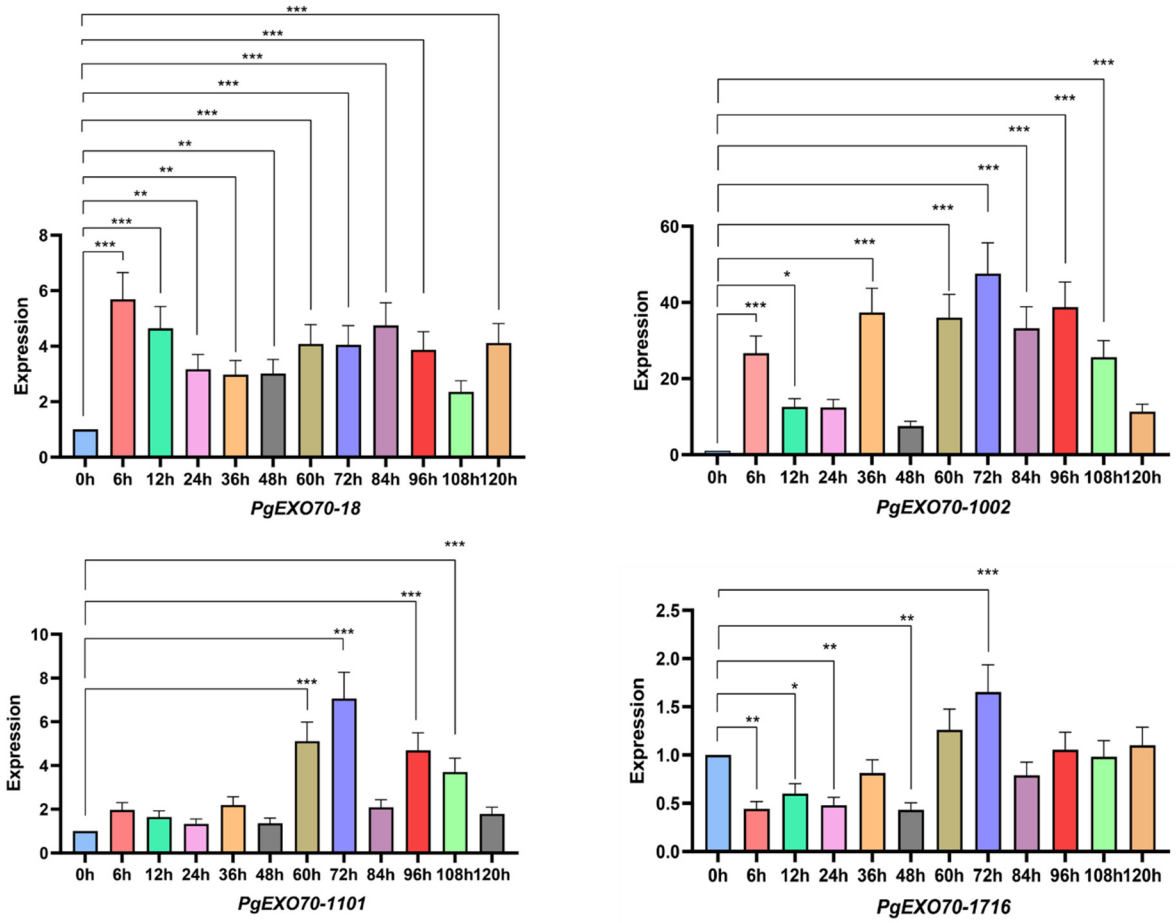

Figure 7: The expression analysis of *PgEXO70* candidate genes were detected after MeJA-treated. Significance analysis plot of the expression levels of *PgEXO70-18*, *PgEXO70-1002*, *PgEXO70-1101*, and *PgEXO70-1716* genes (mean ± standard deviation). The relative expression levels of the four *PgEXO70* genes were calculated with the gene expression level set to "1" at 0 h. The value is the average of the three replicates of the experiment. A significant difference is denoted by * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$.

5 Discussion

We identified the *EXO70* gene family in ginseng and conducted a comprehensive analysis of its structure, function, evolution, and interaction networks. Specifically, this study focused on the *EXO70* gene family in ginseng and systematically examined its structural, functional, evolutionary, and interactional characteristics. In addition to its involvement in light signal transduction in plants, the functions of the

EXO70 gene family in plant growth and development have been elucidated in *A. thaliana*. This gene family is integral in regulating various physiological processes in plants, including hormone signaling and responses to both biotic and abiotic stresses. Compared with other crops, such as the model plants *A. thaliana* (23) [5], *Cucumis sativus* (18) [24], and *Vitis vinifera* (14) [25], *P. ginseng* (58) possesses a greater number of *EXO70* gene family members. This increase may be attributed to the tetraploid nature of ginseng and the occurrence of gene duplication events throughout its evolutionary history. Chromosomal localization analysis revealed that *PgEXO70* gene family transcripts were unevenly distributed across ginseng chromosomes. Gene duplication events are pivotal for gene family expansion and are the primary drivers of family expansion, particularly in monocots, where they give rise to lineage-specific subtypes that may confer unique immune regulatory functions [4,10].

The *PgEXO70* family exhibited significant spatiotemporal specificity in expression across different developmental stages and in different tissues of ginseng. In the main roots of different ages, there were marked differences in the expression levels of different members, with some members likely participating in functions specific to certain developmental stages. Tissue expression analysis revealed that some members were actively expressed in multiple organs, whereas others had very low or specific expression. The family likely regulates key physiological processes in ginseng through the vesicle transport pathway. *EXO70* gene family members are primarily localized to the cell membrane and dispersed sites within the cytoplasm. For example, different subtypes in tobacco pollen tubes are localized to distinct membrane domains (e.g., EXO70A1a is localized to exocytotic sites, whereas EXO70B1 is localized to endocytic regions), suggesting that they regulate the specificity of membrane transport by forming different subtypes of exosome complexes [4,17]. Furthermore, the differential expression of *EXO70* in ginseng can modulate the activity and localization of exosome complexes, further enhancing their functional diversity.

The analysis of cis-acting elements has demonstrated that members of the *EXO70* gene family exhibit a diverse array of biological functions in ginseng. The promoter regions of *PgEXO70* are rich in a variety of cis-acting elements related to hormone response and stress, especially the TGACG- and CGTCA-motifs related to jasmonic acid response, which are distributed in more than one-seventh of the family members examined. This suggests that *PgEXO70* genes may be involved in the jasmonic acid signal transduction pathway and respond to endogenous or exogenous MeJA signal regulation in plants. In grapes, MeJA significantly induces the expression of *EXO70* genes by activating the defense signaling pathways. After 24 h of MeJA treatment, the expression of all 14 *VvEXO70* genes was significantly upregulated. Among them, *VvEXO70-02* showed a 361-fold increase in expression under PEG stress, suggesting a synergistic response to osmotic stress and hormonal signals [25]. In wheat, *EXO70E1-V* genes show enhanced expression following MeJA treatment and interact with pathogen response genes to jointly regulate immune responses [8]. Simultaneously, MeJA activates EXO70 transcription by regulating cis-acting elements in the promoter. It has been no reported that *EXO70* interacts with components of the JA signaling pathway and may regulate the secretion of defense compounds by influencing vesicular transport. The *EXO70* genes were identified in response to MeJA treatment in ginseng still have potential benefits, which need to be verified through future experiments.

In this study, ginseng adventitious roots were subjected to treatment with MeJA. Subsequently, the expression changes analysis of the four genes possessing the highest number of MeJA-responsive elements in these roots were assessed at various treatment durations. Some genes, such as *PgEXO70-18*, *PgEXO70-1002*, and *PgEXO70-1101*, were rapidly upregulated in the early stage (3–12 h) and were typical “early response” genes. These genes may play a role in the signal response at the beginning of MeJA stimulation through mechanisms such as mediating vesicle fusion and transport of secretions. The expression of *PgEXO70-18*

demonstrated the most significant alteration, showing an increased expression relative to the control in all instances. This gene is a key candidate for further research into the molecular mechanisms of the *PgEXO70* gene family under the regulation of the MeJA mechanism in *P. ginseng*.

6 Conclusion

Identification and comprehensive analysis of 58 *PgEXO70* genes in ginseng, and *PgEXO70* genes expression analysis in response to MeJA. Additionally, dynamic expression analysis of four *PgEXO70* genes with a higher number of MeJA response elements was performed using qRT-PCR following treatment with 200 μM MeJA. The findings indicated that all four genes responded to MeJA treatment, with the expression of *PgEXO70-18* significantly elevated at all treatment intervals. In the future, we will conduct in-depth research on the differentially expressed *PgEXO70-18* under MeJA treatment to elucidate the molecular mechanisms of ginsenoside biosynthesis. Specifically, functional validation of *PgEXO70-18* through overexpression or gene-editing technologies approaches could be conducted to confirm their definitive role in regulating ginsenoside biosynthesis.

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Availability of Data and Materials: Transcriptome databases of 14 ginseng tissues, 42 landrace cultivar roots, and four root ages are available in the PRJNA302556 for NCBI. The data and materials that support the findings of this study are available from the Corresponding Author, [Kangyu Wang], upon reasonable request.

Ethics Approval: Not applicable.

Conflicts of Interest: Author Bo Sui is affiliated with Jilin Likang Cell Regeneration Medicine Co., Ltd., a for-profit enterprise. All authors declare no conflicts of interest.

Supplementary Materials: The supplementary material is available online at https://www.techscience.com/doi/10.32604/phyton.2026.085307/s1. Table S1. Basic information of *PgEXO70* gene family in ginseng. Table S2. The gene TPM expressions of the *PgEXO70* genes in 42 cultivars roots, 14 tissues, and 4 aged roots in ginseng.

References

1. Cui L, Li H, Xi Y, Hu Q, Liu H, Fan J, et al. Vesicle trafficking and vesicle fusion: Mechanisms, biological functions, and their implications for potential disease therapy. Mol Biomed. 2022;3(1):29. [CrossRef].
2. Mei K, Guo W. The exocyst complex. Curr Biol. 2028;28(17):R922–5. [CrossRef].
3. Zhu Y, Wu B, Guo W. The role of Exo70 in exocytosis and beyond. Small Gtpases. 2019;10(5):331–5. [CrossRef].
4. Žárský V, Sekereš J, Kubátová Z, Pečenková T, Cvrčková F. Three subfamilies of exocyst EXO70 family subunits in land plants: Early divergence and ongoing functional specialization. J Exp Bot. 2020;71(1):49–62. [CrossRef].
5. Li S, van Os GM, Ren S, Yu D, Ketelaar T, Emons AM, et al. Expression and functional analyses of EXO70 genes in *Arabidopsis* implicate their roles in regulating cell type-specific exocytosis. Plant Physiol. 2010;154(4):1819–30. [CrossRef].
6. Wang T, Zhou K, Yang B, Lefebvre B, He G. OsEXO70L2 is required for large lateral root formation and arbuscular mycorrhiza establishment in rice. J Integr Agric. 2025;24(6):2035–45. [CrossRef].

7. Jiang Z, Dai Y, Jing Q, Jiang M. Genome-wide identification of Exo70 genes in foxtail millet: implications for salt tolerance assessment and utilization of genetic resources. Genet Resour Crop Evol. 2026;73(1):36. [CrossRef].
8. Zhao J, Zhang X, Wan W, Zhang H, Liu J, Li M, et al. Identification and characterization of the EXO70 gene family in polyploid wheat and related species. Int J Mol Sci. 2018;20(1):60. [CrossRef].
9. Zhu YQ, Qiu L, Liu LL, Luo L, Han XP, Zhai YH, et al. Identification and comprehensive structural and functional analyses of the EXO70 gene family in cotton. Genes. 2021;12(10):1594. [CrossRef].
10. De la Concepcion JC, Duverge H, Kim Y, Julian J, Xu HD, Watt MN, et al. Electrostatic changes enabled the diversification of an exocyst subunit via protein complex escape. Nat Plants. 2025;11(11):2350–67. [CrossRef].
11. Xue Y, Cui S, Lou Y, Ma L, Liang J, Ren J, et al. Characterization of the OsEXO70 gene family and their response to salt stress in rice. BMC Genom. 2026;27(1):513. [CrossRef].
12. Nielsen E. Plant exocytosis: Weaving distinct pathways to the plant plasma membrane. Mol Plant. 2022;15(3):382–4. [CrossRef].
13. Li XK, Kang J. The medicinal value of ginseng: Phytochemistry, pharmacological actions, and translational applications. Tradit Med Res. 2026;11(11):76. [CrossRef].
14. Li Y, Wang J, Li L, Song W, Li M, Hua X, et al. Natural products of pentacyclic triterpenoids: From discovery to heterologous biosynthesis. Nat Prod Rep. 2023;40(8):1303–53. [CrossRef].
15. Ye XW, Li CS, Zhang HX, Li Q, Cheng SQ, Wen J, et al. Saponins of ginseng products: A review of their transformation in processing. Front Pharmacol. 2023;14:1177819. [CrossRef].
16. Yu X, Zhang W, Zhang Y, Zhang X, Lang D, Zhang X. The roles of methyl jasmonate to stress in plants. Funct Plant Biol. 2019;46(3):197–212. [CrossRef].
17. Wang W, Liu N, Gao C, Cai H, Romeis T, Tang D. The *Arabidopsis* exocyst subunits EXO70B1 and EXO70B2 regulate FLS2 homeostasis at the plasma membrane. New Phytol. 2020;227(2):529–44. [CrossRef].
18. Song Y, Zhang Y, Wang X, Yu X, Liao Y, Zhang H, et al. Telomere-to-telomere reference genome for *Panax ginseng* highlights the evolution of saponin biosynthesis. Hortic Res. 2024;11(6):uhae107. [CrossRef].
19. Wang K, Jiang S, Sun C, Lin Y, Yin R, Wang Y, et al. The spatial and temporal transcriptomic landscapes of ginseng, *Panax ginseng* C. A. Meyer. Sci Rep. 2015;5:18283. [CrossRef].
20. Mistry J, Chuguransky S, Williams L, Qureshi M, Salazar GA, Sonnhammer ELL, et al. Pfam: The protein families database in 2021. Nucleic Acids Res. 2021;49(D1):D412–9. [CrossRef].
21. Chen C, Wu Y, Li J, Wang X, Zeng Z, Xu J, et al. TBtools-II: A "one for all, all for one" bioinformatics platform for biological big-data mining. Mol Plant. 2023;16(11):1733–42. [CrossRef].
22. Tamura K, Stecher G, Kumar S. MEGA11: Molecular evolutionary genetics analysis version 11. Mol Biol Evol. 2021;38(7):3022–7. [CrossRef].
23. Theocharidis A, van Dongen S, Enright AJ, Freeman TC. Network visualization and analysis of gene expression data using BioLayout Express3D. Nat Protoc. 2009;4(10):1535–50. [CrossRef].
24. Liu L, Gu C, Zhang J, Guo J, Zhang X, Zhou Z. Genome-wide analysis of exocyst complex subunit Exo70 gene family in cucumber. Int J Mol Sci. 2023;24(13):10929. [CrossRef].
25. Wang H, Ma ZH, Mao J, Chen BH. Genome-wide identification and expression analysis of the EXO70 gene family in grape (*Vitis vinifera* L.). PeerJ. 2021;9:e11176. [CrossRef].