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Transcriptome-wide characterization, evolutionary analysis, and expression pattern analysis of the NF-Y transcription factor gene family and salt stress response in *Panax ginseng*

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Abstract

Jilin ginseng (*Panax ginseng* C. A. Meyer) has a long history of medicinal use worldwide. The quality of ginseng is governed by a variety of internal and external factors. Nuclear factor Y (NF-Y), an important transcription factor in eukaryotes, plays a crucial role in the plant response to abiotic stresses by binding to a specific promoter, the CCAAT box. However, the NF-Y gene family has not been reported in *Panax ginseng*. In this study, 115 *PgNF-Y* transcripts with 40 gene IDs were identified from the Jilin ginseng transcriptome database. These genes were classified into the *PgNF-YA* (13), *PgNF-YB* (14), and *PgNF-YC* (13) subgroups according to their subunit types, and their nucleotide sequence lengths, structural domain information, and amino acid sequence lengths were analyzed. The phylogenetic analysis showed that the 79 *PgNF-Y* transcripts with complete ORFs were divided into three subfamilies, NF-YA, NF-YB, and NF-YC. *PgNF-Y* was annotated to eight subclasses under three major functions (BP, MF, and CC) by GO annotation, indicating that these transcripts perform different functions in ginseng growth and development. Expression pattern analysis of the roots of 42 farm cultivars, 14 different tissues of 4-year-old ginseng plants, and the roots of 4 different-ages of ginseng plants showed that *PgNF-Y* gene expression differed across lineages and had spatiotemporal specificity. Coexpression network analysis showed that *PgNF-Ys* acted synergistically with each other in Jilin ginseng. In addition, the analysis of the response of *PgNF-YB09*, *PgNF-YC02*, and *PgNF-YC07-04* genes to salt stress treatment was investigated by fluorescence quantitative PCR. The expression of these genes increased after salt stress treatment, indicating that they may be involved in the regulation of the response to salt stresses in ginseng. These results provide important functional genetic resources for the improvement and gene breeding of ginseng in the future.

Conclusions: This study fills a knowledge gap regarding the *NF-Y* gene family in ginseng, provides systematic theoretical support for subsequent research on *PgNF-Y* genes, and provides data resources for resistance to salt stress in ginseng.

Keywords: *Panax ginseng*, NF-Y transcription factor, Phylogeny, Expression pattern, Salt stress

Background

Nuclear factor Y (NF-Y) family transcription factors are important regulators of plant development and physiology [1]. Nuclear factor Y transcription factors (also known as heme-associated proteins [HAPS] and CCAAT box binding factors [CBFs]) are sequence-specific

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transcription factors with histidine-like subunits that are uniquely characterized by their binding to DNA at the CCAAT site as a heterotrimeric complex consisting of NF-YA, NF-YB and NF-YC, three individual subunits of the protein family [2]. To date, members of the *NF-Y* gene family have been identified in a variety of plants, including *Arabidopsis thaliana* [3], *Oryza sativa* [4], *Prunus persica* [5], and *Populus* [6]. Studies have shown that the *NF-Y* gene family influences flowering in plants [7], improves photosynthetic capacity [8], regulates embryogenesis in *Arabidopsis* [9], and assists in abiotic stress resistance [10–12]. NF-Y family members are involved in plant responses to abiotic stresses, and *ZmNF-YB16* functions by regulating the expression of photosynthesis-related genes to improve the antioxidant capacity of cells and thus achieve drought resistance [13]. In *Lycopersicon esculentum*, *SINFYA10* negatively regulates the AsA (ascorbic acid) biosynthetic pathway by binding to the CCAAT box in response to oxidative stress [14]. Overexpression of the *GmNFYA5* gene in soybean enhances drought resistance [15]. Although the *NF-Y* gene family has been extensively studied in other species, it has not been reported in ginseng.

Jilin ginseng (*Panax ginseng* C.A. Meyer) is a perennial herb in the *Araliaceae* family that has a cultivation history of at least 4,000 years. The Chinese "Sheng Nong's Herbal Classic" records in detail that ginseng tastes sweet, can be used as a tonic for the five organs, calms the spirit, fixes the soul, stops panic and palpitations, removes evil spirits, brightens the eyes, makes the mind happy, educates the mind, lightens the body and prolongs life when taken for a long time [16]. Ginseng is not currently used by consumers as a single medicinal plant but as a food and health product [17]. The quantity of wild ginseng can hardly meet the social demand for ginseng products, while the quality of artificially cultivated ginseng is constrained by various factors. Some members of the NF-Y gene family respond to salt stress. We obtained the *NF-Y* gene family members in response to salt stress treatment by screening the adventitious roots of ginseng, which will provide genetic resources for the subsequent improvement of salt stress resistance in ginseng cultivars.

In this study, 40 *PgNF-Y* genes were identified in the Jilin ginseng transcriptome database and classified according to their structural domain information (NF-YA, NF-YB, and NF-YC). Subsequently, we analyzed the evolutionary relationships of the *PgNF-Y* gene family, and conserved motifs and annotated them with GO functions. In addition, expression pattern analysis and coexpression network analysis were performed based on the *PgNF-Y* gene expression data. Finally, the response of *PgNF-Y* family members to different concentrations of salt was explored. The results of this experiment provide

important theoretical information and experimental data on the *NF-Y* gene family for the subsequent study of functional genes in ginseng.

Materials and methods

Identification of the *NF-Y* genes in *Panax ginseng*

To maximize data integrity and reliability, we adopted three different approaches to screen members of the *NF-Y* gene family in the Jilin ginseng database containing 248,993 transcripts [18]. First, the hidden Markov model of the NF-Y gene family was downloaded from the PFAM protein family database (<http://pfam.xfam.org/>), and using PFAM IDs (PF02045, PF00808) as the interrogated sequences, tBlastn was performed in the Jilin ginseng transcriptome. Second, the coding and protein sequences of NF-YA, NF-YB, and NF-YC were downloaded from the Korean Ginseng Genome website (<http://ginsengdb.snu.ac.kr/pathway.php>); these sequences were used as the interrogation sequences for Blastn and tBlast of the Jilin ginseng transcriptome database at an e-value of 1×10^{-6} . Finally, 10 protein sequences from the NF-Y family with verified functions were downloaded from GenBank (<https://www.ncbi.nlm.nih.gov/>) and used as interrogation sequences for tBlastn of the transcriptome of Jilin ginseng. These 10 sequences were from *Arabidopsis thaliana* [10, 19], *Triticum aestivum* [20, 21], *Oryza sativa* [22], *Glycine max* [19], *Solanum tuberosum* [8], *Nicotiana tabacum* [23], and *Zea mays* [11]. Subsequently, the results obtained by the three methods were combined, and after removing duplicate values, the files were used for our preliminary investigation. To exclude some spurious comparison information, we submitted the results to iTAK (<http://itak.feilab.net/cgi-bin/itak/index.cgi>) for online analysis while maintaining the sequence information regarding NF-Y structural domains. Based on the results of iTAK, all the obtained NF-Y transcripts were named *PgNF-Ys*. Finally, the obtained transcripts were verified one by one for structural domains by the SMART online tool (<http://smart.embl-heidelberg.de/>).

Phylogenetic evolutionary analysis and conserved motifs of the *PgNF-Y* gene family in *Panax ginseng*

We identified 79 transcript sequences with complete open reading frames from 115 *PgNF-Y* gene transcripts based on ORF Finder in NCBI (the remaining 36 transcripts contained only the structural domain information of NF-Y). Based on these results, 12 nucleic acid sequences in the NF-YA, NF-YB, and NF-YC subgroups from *Lycopersicon esculentum*, *Arabidopsis thaliana*, *Oryza sativa*, and *Helianthus annuus* were downloaded from the NCBI database as outgroups for the phylogenetic tree. These nucleic acid sequences were translated into protein sequences. In MEGA-X software [24], the

maximum-likelihood (ML) method was chosen to obtain the evolutionary tree of the *PgNF-Y* genes with 1,000 bootstrap replicates to further illustrate the evolutionary relationships between the *PgNF-Y* genes in different species. Finally, the evolutionary tree was optimized by Evolview (<https://www.evolgenius.info/evolview/#/treeview>). To determine the conserved sequence patterns in the *NF-Y* gene family members in Jilin ginseng, we analyzed the motifs of *PgNF-Y* transcription factors by the MEME online tool (https://meme-suite.org/meme/doc/cite.html?man_type=web). Finally, the obtained results were visualized by TBtools [25].

GO (Gene Ontology) annotation and functional categorization of *PgNF-Y* gene transcripts

We used Blast2GO version 6.0.3 [26] to annotate the identified *NF-Y* transcripts based on Gene Ontology (GO) functional annotation into three major categories: BP (Biological Process), CC (Cellular Component), and MF (Molecular Function). The enrichment of all the nodes where the genes were located was determined by the *Chi-square* test at Level 2. The functions of 109,781 transcripts that were annotated by previous authors were used as reference information [18].

Expression pattern and network analysis of *PgNF-Y* gene transcripts

Since gene expression is subject to a variety of conditions, we obtained data on the expression of *PgNF-Ys* in 42 farm cultivars, 14 different tissues of 4-year-old ginseng, and 4-year-old ginseng roots, and we plotted the heatmap in R. Thus, the expression pattern of the *PgNF-Y* genes in Jilin ginseng in time and space was determined. To further investigate the interrelationship between *PgNF-Y* gene expression in 42 farm cultivars, Spearman's correlation coefficients of *PgNF-Y* gene expression were calculated using R, and BioLayout Express^{3D} version 3.3 software was used to form a visual network of the obtained results.

Analysis of the response of *PgNF-Y* genes to salt stress

In this experiment, the adventitious roots of Jilin ginseng (*Panax ginseng* C.A. Meyer) were treated with different concentrations of salt in B5 medium (0, 70, 80, 90, and 100 mM NaCl), and the treated adventitious roots were incubated under dark conditions at 22 °C for 30 days.

Plant materials, RNA isolation, and quantitative real-time PCR analysis

Adventitious root material (0.1 g) treated with different salt concentrations was weighed separately, and total RNA was extracted from ginseng adventitious root tissue using TRIzol (BioTeke, Beijing, China). The HiFiScript

gDNA Removal cDNA Synthesis Kit (CW BIO, Beijing, China) was used to reverse transcribe the extracted RNA into cDNA. Actin 1 was selected as the internal reference gene, and according to the instructions for the UltraSYBR One-step RT-qPCR Kit (Low ROX) (CW BIO, Beijing, China), a 7500 Real Time PCR System was used to perform the reaction. The total reaction system was 10 μ L, which included UltraSYBR Mixture (Low ROX) at 5 μ L, upstream and downstream primers at 0.2 μ L each, template at 1 μ L, and ddH₂O at 3.6 μ L. qPCR was performed in a thermal cycling system with the following conditions: 95 °C for 10 min and 40 cycles of 95 °C for 15 s, and 60 °C for 60 s. To ensure the accuracy of the results obtained for each treatment, we set up 3 biological replicates and 3 technical replicates. The final results were obtained using the $2^{-\Delta\Delta Ct}$ analysis method.

Results

Identification of *PgNF-Y* gene family transcripts

A total of 2,266 transcripts remained after removing duplicates from the *NF-Y* gene family transcripts obtained by three different methods for interrogating the Jilin ginseng transcriptome database. These transcripts were submitted to iTAK and queried for structural domains by SMART. Finally, 115 transcripts containing the structural domain of the *NF-Y* gene family were obtained. We collected basic information on the *PgNF-Y* gene family members of Jilin ginseng, including transcript ID, gene ID, mRNA sequence information, the number of transcripts with a length ranging from 240–2,624, and the number of amino acids with complete open reading frames (ORFs) ranging from 70–336 (Table S1).

Motif prediction and phylogenetic analysis of *PgNF-Y* genes

Since ginseng originated approximately 100 million years ago in the Cretaceous period, it has a long evolutionary history. We further investigated the evolutionary characteristics of *PgNF-Y* gene family members in Jilin ginseng based on 79 *NF-Y* gene family members with complete ORFs (the remaining 36 transcripts contained only structural domain information of *NF-Y*). We explored the evolutionary relationships of the *PgNF-Y* gene family from the perspectives of closely related species, model plants, monocotyledons, and dicotyledons. We selected *Solanum lycopersicum*, *Arabidopsis thaliana*, *Oryza sativa*, and *Helianthus annuus* as outgroups for phylogenetic analysis via the maximum likelihood method (Table S2). Phylogenetic tree analysis showed that the transcripts of the *PgNF-YA*, *PgNF-YB*, and *PgNF-YC* subgroups were all concentrated in specific subclades (except *PgNF-YC04* and *PgNF-YC10*), and the *NF-Y* members that were distributed in the same subclass had functional

similarity. Based on the evolutionary relationship between outgroups and PgNF-Y transcripts, NF-Y members in these species have an evolutionary origin from a common ancestor (Fig. 1A).

To understand the sequence characteristics of the PgNF-Y protein, its conserved structural domains were analyzed by the online tool MEME. The results showed that among the 10 motifs analyzed, the number of motifs contained in different subfamily members ranged from 1 to 5 (Fig. 1B). Motif 3, motif 6, and motif 7 were only present in the PgNF-YA subfamily; motif 4, motif 5, and motif 8 were only present in the NF-YC subfamily; and motif 2, motif 9, and motif 10 were commonly found in the NF-YB subfamily. The differences in the number and type of motif in different subfamilies demonstrate that the NF-Y gene family is functionally diverse and structurally different.

GO annotation of PgNF-Y gene transcripts

Genes often have more than one function in organisms. To further characterize the properties of PgNF-Y genes and gene products, GO annotation of 115 PgNF-Y transcripts showed (Fig. 2A, Table S3) that 95 of the 115 transcripts were annotated to any at least one of the three major functions: 9 were annotated to Biological Process, 91 to Cellular Component and 67 to Molecular Function. There were 30 transcripts annotated to only one function, 61 transcripts annotated to two major functions, and 5 transcripts annotated to three major functions (*PgNF-YB08-05*, *PgNF-YB08-12*, *PgNF-YB08-15*, *PgNF-YB08-19*, and *PgNF-YB09*). At Level 2, 4 sublevels (cellular process, metabolic process, regulation of biological process, and biological regulation) were enriched in BP, 2 sublevels (cellular anatomical entity and protein-containing complex) were enriched

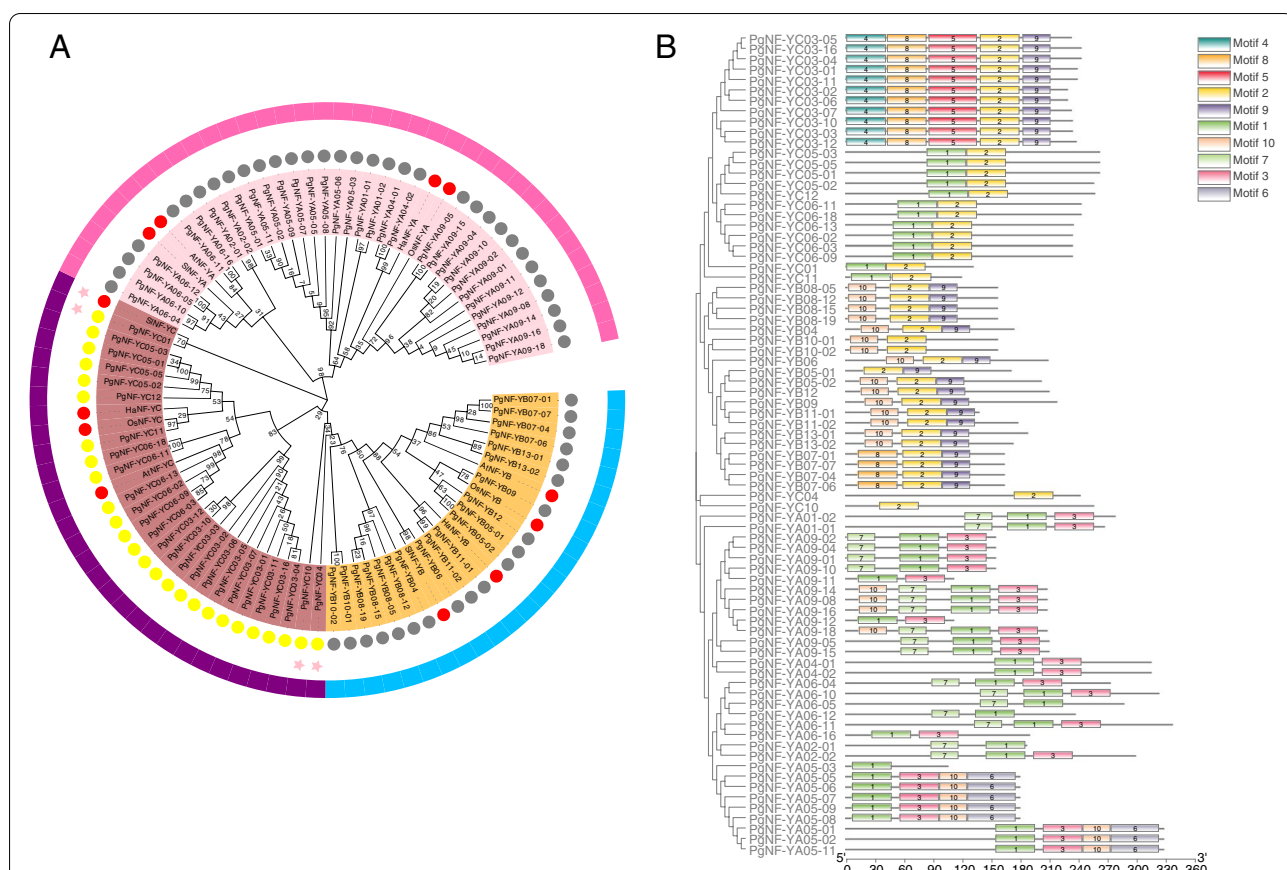
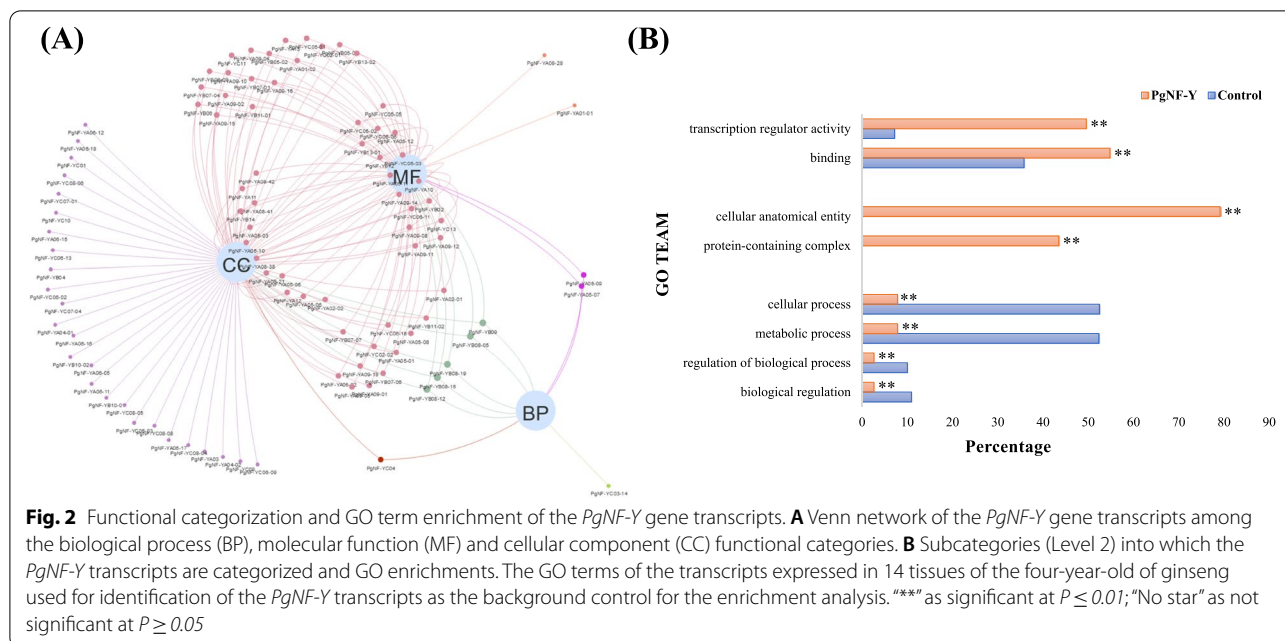


Fig. 1 Phylogenetic relationship and conserved motifs of the PgNF-Y proteins. **A** Phylogeny of the PgNF-Y gene family. A total of 79 PgNF-Y transcripts that had complete ORFs and the longest length among the transcripts alternatively spliced from a gene were used as representatives to construct the phylogeny of the PgNF-Y gene family (Table S3). A total of 12 PgNF-Y genes from *Arabidopsis thaliana* (At), *Oryza sativa* (Os), *Solanum lycopersicum* (Sl) and *Helianthus annuus* (Ha) were used as outgroup (Table S4). The subfamilies to which the PgNF-Y genes belong are indicated by numbers highlighted by red color. The numbers for the branches of the tree are bootstrap confidence out of 1000 replications. **B** Conserved motifs of PgNF-Y proteins according to their evolutionary relationship. The conserved motifs of the PgNF-Y proteins are indicated by colored boxes. The capital letters indicate the groups of the PgNF-Y gene family



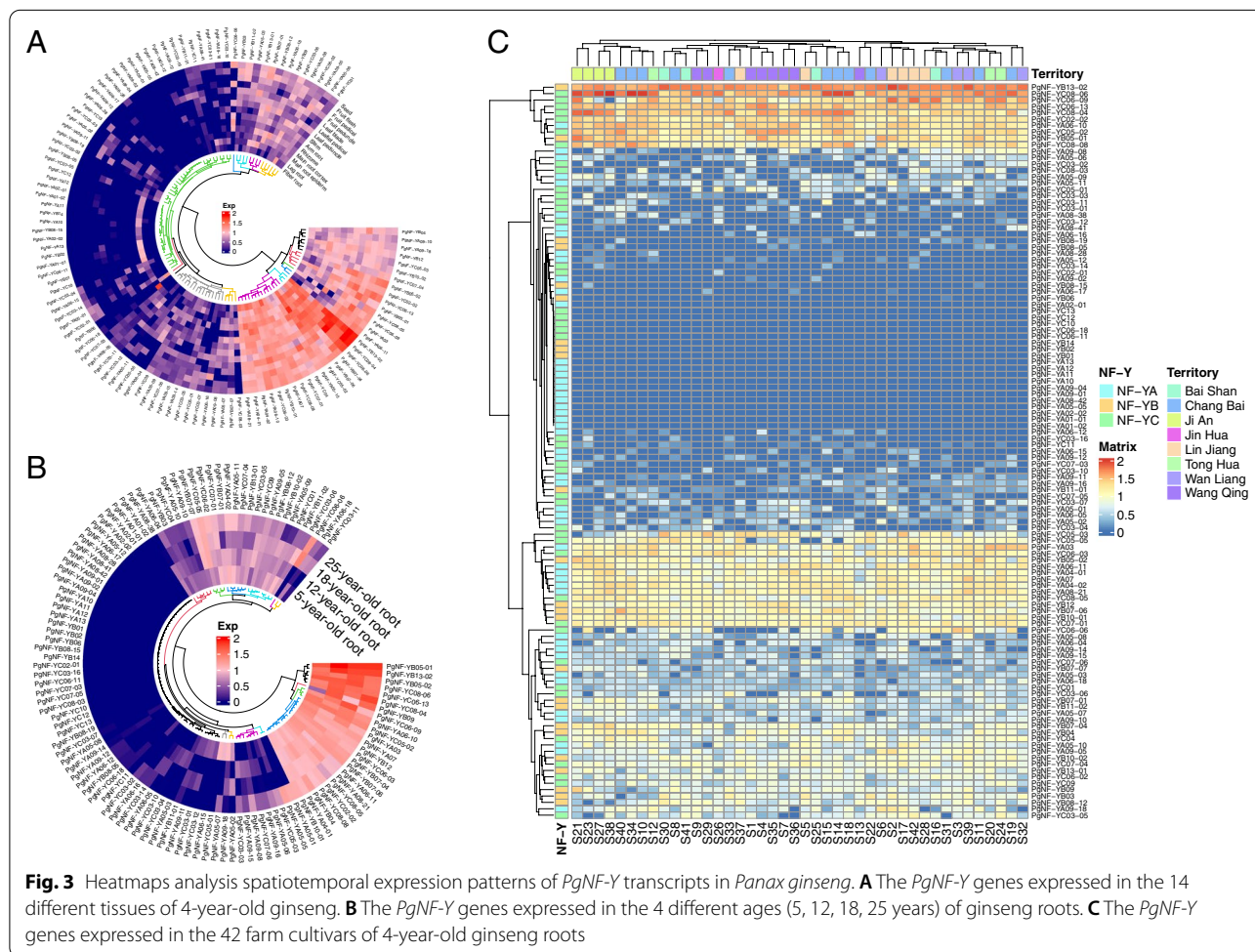
in CC, and 2 sublevels (transcription regulator activity and binding) were enriched in MF. The results of 109,781 previously annotated transcripts from the Jilin ginseng transcriptome data were used as a reference, and 115 *PgNF-Y* transcripts were interrogated. The obtained results were subjected to the *chi-square* test in SPSS version 23.0 software, and the results showed that all eight sublevels obtained from interrogated gene enrichment were significantly different from those for reference genes ($P \leq 0.05$). The cellular anatomical entity and the protein-containing complex were not annotated in the reference genes (Fig. 2B).

Expression characteristics of the *PgNF-Y* gene transcripts

To investigate the expression pattern of the *PgNF-Y* gene family from a temporal and spatial perspective, we retrieved *PgNF-Y* gene expression data from 4-year-old Jilin ginseng Damaya roots from the database containing 42 farm cultivars (S1–S42) and 14 different tissues (stem, fiber root, fruit peduncle, main root epiderm, fruit pedicel, rhizome, leaf peduncle, arm root, leaflet pedicel, leg root, leaf blade, fruit flesh, main root cortex, and seed), as well as four ginseng root samples of various ages (5, 12, 18, and 25 years) of *PgNF-Y* gene expression data (Table S4). The results of the heatmap of the roots of 42 farm cultivars showed that 96 *PgNF-Y* transcripts (85%) were expressed in at least one cultivar and 36 transcripts (31%) were expressed in all 42 cultivars (Fig. 3C). *PgNF-YB13-02* and *PgNF-YC08-06* had higher gene expression in 42 farm cultivar, and 18 transcripts were

not expressed in any of the cultivar. The heatmap results of 14 tissue from four-year-old ginseng showed that 110 *PgNF-Y* transcripts (96%) were expressed in at least one tissue, and 41 transcripts (36%) were expressed in all 14 tissues (Fig. 3A). *PgNF-YB13-02*, *PgNF-YC08-04*, and *PgNF-YC08-06* were expressed at relatively high levels, and only 5 transcripts were not expressed in any tissue. The heatmap of gene expression in roots of plants at four different ages showed that 85 *PgNF-Y* transcripts (74%) appeared in at least one age stage and 48 *PgNF-Y* transcripts (42%) were expressed in all four age stages (Fig. 3B). Among them, *PgNF-YB05-01*, *PgNF-YB13-02*, *PgNF-YB05-02*, *PgNF-YC08-06*, *PgNF-YC06-13*, and *PgNF-YC08-04* were highly expressed, and 30 transcripts were not expressed across the four different ages.

To further understand the expression trends of the *PgNF-Y* genes in Jilin ginseng, the expression of *PgNF-Y* transcripts was analyzed in the roots of 42 farm cultivars, 14 different tissues, and the roots of plants at 4 different ages. In the bar graphs showing the results for the roots of plants at four different ages (Fig. 4A), *PgNF-Y* tended to be more highly expressed in 5-year-old ginseng roots and less expressed in 12-year-old and 18-year-old ginseng roots. In the bar graph of the 14 different tissues within ginseng (Fig. 4B), the *PgNF-Y* transcripts tended to be expressed mostly in the fruit pedicel, while the leaf blade showed the lowest amount of *PgNF-Y* expression. Among the 42 farm cultivars of ginseng, the number of *PgNF-Y* transcripts expressed accounted for 53.04%–63.48% of the total *PgNF-Y* transcripts.

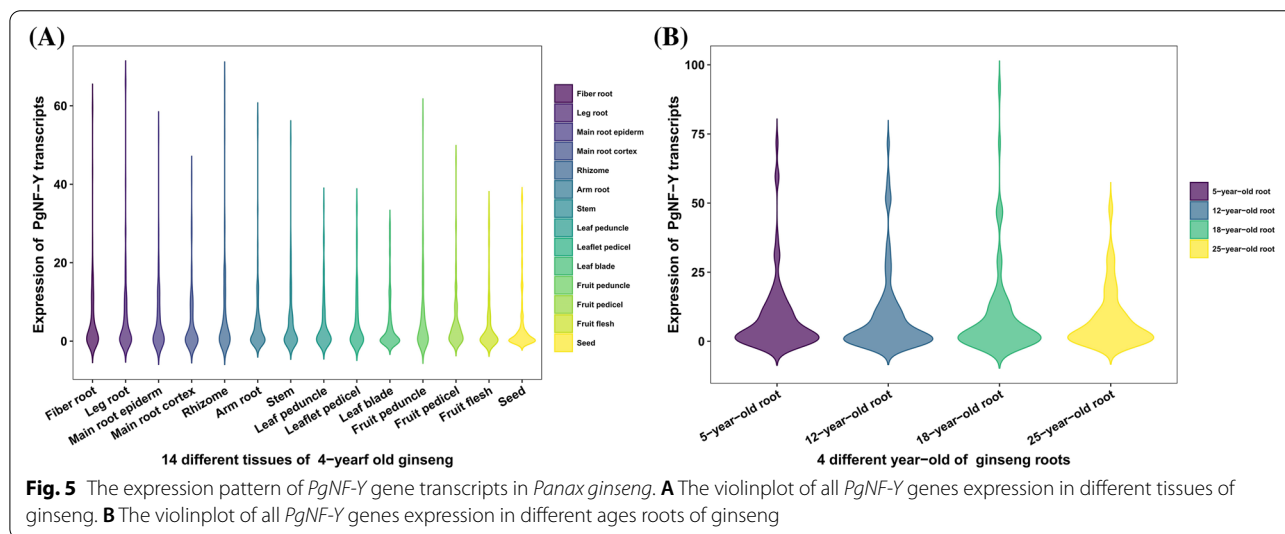
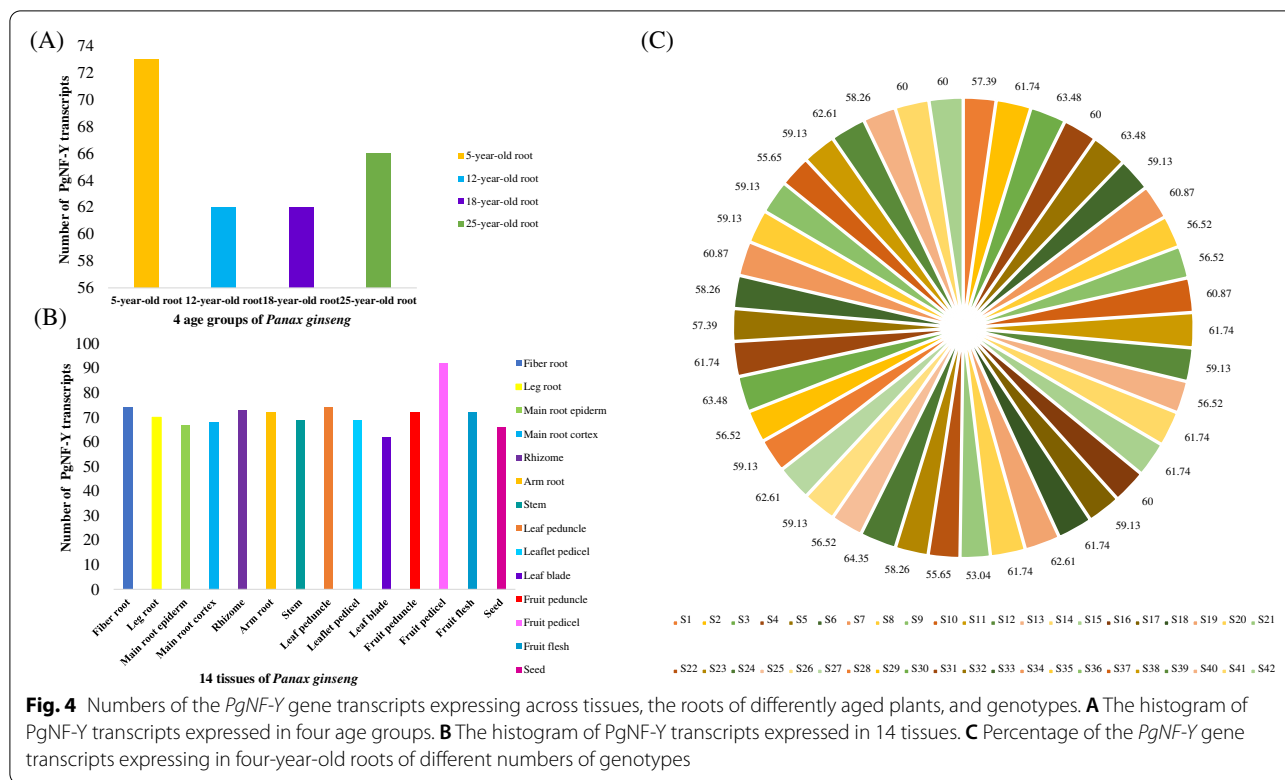


To better understand the general level of *PgNF-Y* gene expression in 14 different tissues and 4 different ages, violin plots of gene expression versus tissue and age were plotted (Fig. 5A and B). The results showed that *PgNF-Y* gene expression was lower in the main root cortex and leaf blade and higher in the fruit peduncle and fruit pedicel; the expression of *PgNF-Y* genes was relatively consistent across the four different ages. Based on this analysis of the expression pattern of *PgNF-Y* transcripts, we found that the expression of *PgNF-Y* genes in Jilin ginseng was influenced by geographical factors, tissues and plant age.

Coexpression network of *PgNF-Y* gene transcripts

To investigate whether there is a relationship between *PgNF-Y* transcripts among different genotypes, we performed a coexpression network analysis of *PgNF-Y* transcripts from 42 farm cultivars. We selected 96 of these transcripts for coexpression network analysis (the remaining 19 transcripts were not expressed in any of

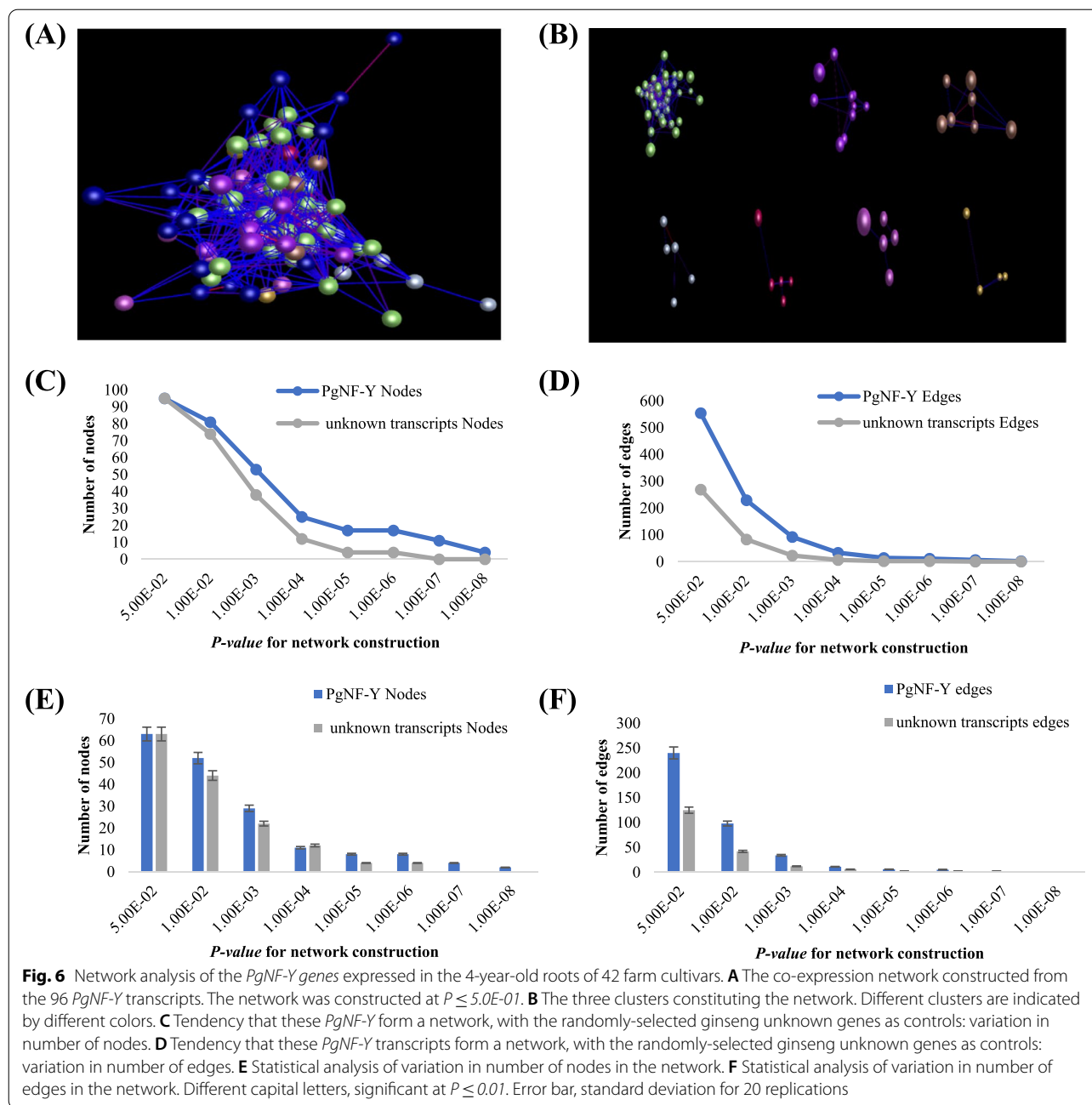
the 42 farm cultivars). The coexpression network results showed that at $P \leq 5.0E-02$, the 96 transcripts formed a coexpression network containing 95 nodes with 554 edges (Fig. 6A and B). The network contains 7 clusters. As the P value gradually reduced to $1.0E-08$, the *PgNF-YA09-16* gene remained strongly correlated with *PgNF-YA09-08*, *PgNF-YA09-14*, and *PgNF-YA09-15*. To explore the closeness of the formed networks, we randomly selected 96 transcripts in database A as negative controls and constructed a coexpression network. The results showed (Fig. 6C and D) that the *PgNF-Y* transcripts formed 95 nodes and 269 edges compared to the randomly selected transcripts at $P \leq 5.0E-02$, and the number of nodes and edges of the unknown transcripts was 0 when the P value gradually decreased to $1.00E-07$. Therefore, we determined that the *PgNF-Y* transcripts form a tighter regulatory network than the negative controls. To further determine the rigor of the regulatory network, we selected two-thirds of the randomly selected *PgNF-Y* transcripts (65) to construct the regulatory network and



randomly selected another 65 transcripts as the negative control (Fig. 6E and F). At $P \leq 5.0E-02$, the 65 *PgNF-Y* transcripts formed a regulatory network with 63 nodes, and at $P \leq 1.0E-08$, the *PgNF-Y* transcripts formed 2 nodes and 1 edge; the number of nodes and edges was 0 for the unknown transcripts at $P \leq 1.00E-07$. Thus, the *PgNF-Y* transcripts are more likely to form a coexpression network than the randomly selected transcripts.

Analysis of the response of *PgNF-Y* genes under salt stress treatment

Ginseng grown in the wild is subject to a variety of factors, and gene expression in a specific environment is often used to predict gene function. To verify the response of *PgNF-Y* genes to salt stress, we downloaded the known salt tolerance-related NF-Y nucleic acid sequences from the NCBI database and used local BLAST to initially screen three

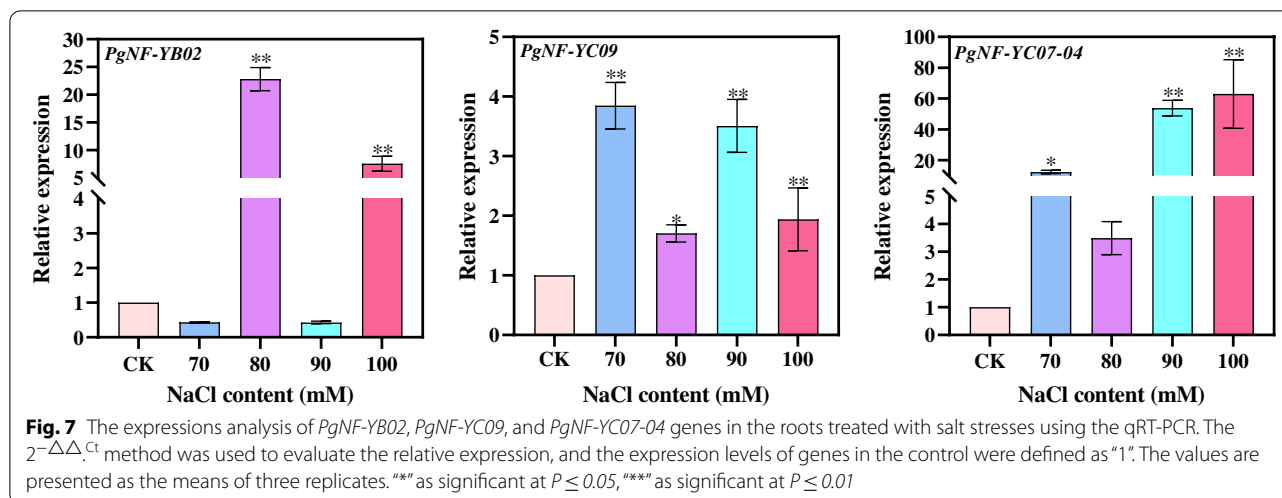


salt resistance-related genes (*PgNF-YB02*, *PgNF-YC09*, and *PgNF-YC07-04*) from 115 *PgNF-Y* gene transcripts. The results of fluorescence quantitative PCR showed that all three *PgNF-Y* genes were upregulated in ginseng adventitious roots under different salt stress treatments (Fig. 7). Under salt stress, the expression of the *PgNF-YC07-04* gene was significantly increased in ginseng adventitious roots compared with the control. This result further indicates that the *PgNF-YC07-04* gene has a strong response to salt stress and plays an important role in the biological process

of salt stress resistance in ginseng. This result also provides genetic resources for the study of salt stress resistance and gene breeding in Jilin ginseng.

Discussion

The NF-Y gene family is prevalent in plants. Therefore, the NF-Y transcription factor family has been extensively studied in plants, including *Arabidopsis thaliana* [27], *Glycine max* [7], *Triticum aestivum* [28], and *Lotus japonicus* [29]. The functional diversity of NF-Y gene



families has been well demonstrated by targeting specific genes in the *NF-Y* gene family. In this study, 40 *NF-Y* gene family members were identified in Jilin ginseng, compared to 36 *NF-Y* members in *Arabidopsis*, 19 *NF-Y* members in *Citrullus lanatus*, 24 *NF-Y* members in *Prunus persica*, and 52 *NF-Y* members in *Populus simonii*. We found that the number of *NF-Y* gene family members in plants does not seem to differ between herbaceous and woody plants. Polyploidy or whole genome duplication (WGD) is a common phenomenon in angiosperms [30], and the *NF-Y* transcription factor family in ginseng may have expanded as a result.

Genome sequencing has shown that most of the genes specifying core biological functions are common to all eukaryotes [26]. Multiple potential functions exist for *PgNF-Y* transcripts. Previous studies have shown that *NF-Y* transcription factors are involved in the regulation of biological processes such as flowering [31], photomorphogenesis [32], and abiotic stress [10] in *Arabidopsis* by binding to the CCAAT box [33]. According to the GO annotation results, 67 *PgNF-Y* transcripts were annotated to molecular functions (binding and transcription regulator activity); therefore, these genes are likely to bind the CCAAT box of other genes to regulate the growth and development of Jilin ginseng. Nine *PgNF-Y* transcripts were annotated to biological processes, and 91 *PgNF-Y* transcripts were annotated to cellular components, suggesting that *PgNF-Y* genes act not only as a functional gene but also as structural genes in Jilin ginseng. In summary, the *PgNF-Y* transcripts are functionally diverse in Jilin ginseng.

Clarifying the expression pattern of *PgNF-Y* transcripts is helpful for exploring the function of these transcripts. From the analysis of the expression levels of *PgNF-Y* transcripts in the 42 farm cultivars, the relatively close

expression patterns of 115 *PgNF-Y* transcripts suggest that they are widely expressed in Jilin ginseng. The *PgNF-YB13-02* and *PgNF-YC08-06* genes had high expression levels in the 42 farm cultivars; therefore, these two genes may be housekeeping genes in Jilin ginseng and thus maintain the basic life activities of the plant. The expression of *PgNF-Y* genes in 14 different tissues of 4-year-old ginseng showed that their expression was tissue specific, and *PgNF-YA12*, *PgNF-YA13*, *PgNF-YB01*, *PgNF-YB08-15*, *PgNF-YC10*, and *PgNF-YC12* were expressed only in the fruit pedicel, suggesting that these five *PgNF-Y* transcripts may be involved in the development of ginseng fruit pedicels. The *PgNF-YC13* gene was expressed only in leg roots and may be related to their development in ginseng. The median expression levels of the *PgNF-Y* gene in the leaf blade and fruit pedicel were higher than that in other tissues, indicating that *PgNF-Ys* are likely to be involved in ginseng fruit and flower development. In ginseng roots of plants at different ages, approximately 42% of the *PgNF-Y* transcripts were expressed at different times, and approximately 13% of the *PgNF-Y* transcripts were expressed only in a specific year. The median expression levels of *PgNF-Y* genes across the four ages was fairly consistent, indicating that the expression levels of the *PgNF-Y* gene family members did not increase with growth year. The specific expression of *PgNF-Y* transcripts at four different ages suggested that not all *PgNF-Y* transcripts were constitutively expressed in Jilin ginseng, and some transcripts might be induced to be expressed in response to changes in external conditions such as soil and climate.

The *NF-Y* transcription factor is a heterotrimer formed by evolutionarily conserved subunits: *NF-YA*, *NF-YB*, and *NF-YC* [34]. *NF-YB* and *NF-YC* contain a histone folding domain (HFD), and *NF-YA* binds to the *NF-YB*/

NF-YC dimer [35]. There are 30 predicted NF-Y members in the Arabidopsis genome; in theory, this could result in approximately 1,000 heterotrimeric combinations [36]. In the coexpression network of 42 farm cultivars, we found that PgNF-Y formed some small clusters with close relationships at $P \leq 5.0E-02$, suggesting that such heterologous trimers may also be formed in Jilin ginseng. However, this result needs to be verified using yeast two-hybrid and yeast three-hybrid techniques.

The cultivation time for both garden and forest ginseng is usually 5–7 years. During the long cultivation cycle, the soil environment is one of the key factors controlling the quality of ginseng [37]. A recently identified salt tolerance gene, *NF-YC13*, was found in indica rice [38]; the *TaNF-YA10-1* gene was isolated from the salt-tolerant wheat variety SR3; and overexpression of the *NF-YA10* gene in *Arabidopsis thaliana* regulates the response of *Arabidopsis* to salt stress [21]. In this paper, our fluorescence quantitative PCR results showed that all three transcripts, *PgNF-YB02*, *PgNF-YC09*, and *PgNF-YC07-04*, in ginseng responded to salt stress. This result also indicates that the *NF-Y* gene family is prevalent in plants responding to salt stress.

Conclusion

In this study, we screened 40 *PgNF-Y* genes from Jilin ginseng and analyzed their evolution, structure, function, expression pattern and coexpression network to verify the response of *PgNF-Y* genes to salt stress treatment. The results of the above analysis showed that *PgNF-Y* gene family members are functionally diverse and exhibit tissue specificity, time specificity, and interspecies differences in their expression patterns. *PgNF-Y* gene family members act synergistically with each other in the plant. Moreover, the *PgNF-Y* gene family plays an important role in the plant response to salt stress. This study further demonstrates the role of the *NF-Y* gene family in the response to salt stress and provides a theoretical basis for the genetic breeding of ginseng.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12870-022-03687-6>.

Additional file 1: Table S1. Basic information of the PgNF-Y gene family

Additional file 2: Table S2. The identified genes used as evolutionary controls for the PgNF-Y gene phylogenetic analysis.

Additional file 3: Table S3. The classification, annotation and GO functional categorization of the PgNF-Y gene transcripts

Additional file 4: Table S4. The expressions of the PgNF-Y gene transcripts in 14 tissues, 42 farm cultivar roots and 4 ages roots (TPM).

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Authors' contributions

Meiping Zhang and Yi Wang designed the experiments of the study. Mingming Liu and Kangyu Wang wrote and revised the main manuscript. Zhaoxi Pan, Jie Yu, Lei Zhu, Mingzhu Zhao, Yanfang Wang, Ping Chen, Chang Liu, Jian Hu, and Tao Liu performed the experiments and contributed to data analysis. All authors reviewed and approved the final manuscript.

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Availability of data and materials

All data analyzed during this study are included in the supplementary information files, and genotypic data have been deposited in the Sequence Read Archive (<https://www.ncbi.nlm.nih.gov/sra>) to NCBI under BioProject RJNA302556.

Declarations

Ethics approval and consent to participate

During the experiment, all plant materials were used in accordance with national and international standards and local laws and regulations. The use of all plant materials does not pose any risk to other species in nature. No specific permission is required for the collection of all *Panax ginseng* samples described in this study. The planting area of *Panax ginseng* samples is the cooperative base of our laboratory, and the field collection does not involve endangered or protected species. All *Panax ginseng* samples (14 tissues, 42 farm cultivar roots and 4 ages roots) and ginseng adventitious root materials were stored in Jilin Agricultural University. These ginseng samples materials can be accessed at the request of the corresponding author.

Consent for publication

Not applicable.

Competing of interests

The authors declare that there is no conflict of interest.

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