Basic Leucine zipper (bZIP) transcription factor genes and their response to drought stress in ginseng, *Panax ginseng* C.A. Meyer

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1 **Basic Leucine zipper (bZIP) transcription factor genes and**
2 **their response to drought stress in ginseng, *Panax ginseng***
3 **C.A. Meyer**

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Abstract

Background: As a famous and important medicinal herb in the world, ginseng contains numerous bioactive components that are remarkable for mankind's health. The basic leucine zipper (bZIP) transcription factor genes play important roles in many biological processes and plant response to abiotic and biotic stresses. Nevertheless, these genes remain unknown in ginseng.

Results: Here, we report 91 *bZIP* genes, designated as *PgbZIP* genes, identified from ginseng. These *PgbZIP* genes were alternatively spliced into 273 transcripts. Phylogenetic analysis grouped the *PgbZIP* genes into ten groups, including A, B, C, D, E, F, G, H, I and S. Gene Ontology (GO) categorized the *PgbZIP* genes into a number of functional categories, suggesting that they have substantially diversified in functionality, even though their putative proteins share a number of conserved motifs. These 273 *PgbZIP* transcripts expressed quite differentially across 14 different tissues, the roots of different aged, and the roots of different cultivars. However, the expression of these transcripts was coordinated as they formed a co-expression network. Furthermore, we studied their response to drought stress in ginseng using five representatives of the *PgbZIP* genes, including *PgbZIP25*, *PgbZIP38*, *PgbZIP39*, *PgbZIP53* and *PgbZIP54*. The results showed that these *PgbZIP* genes all responded to drought stress in ginseng, but the magnitudes of their response to drought stress varied.

Conclusions: These results provide knowledge and resources for deeper functional analysis of *PgbZIP* genes and molecular tools for enhanced drought tolerance

breeding in ginseng.

Keywords: *Panax ginseng*, bZIP transcription factor, Phylogenetic analysis, Functional differentiation, Drought stress

Background

As one member of the order Apiales in Araliaceae, ginseng (*Panax ginseng* C.A. Meyer) is an important medicinal herb in East Asia. In China, the cultivation of ginseng has a long history and was mainly cultivated in Jilin Province, which was known as Jilin ginseng. Ginsenosides, presenting in most tissues of ginseng, are recognized as the most valuable active components in ginseng [1, 2]. Research showed that ginseng has brought a lot of benefits for human, such as improving relieving pain, brain function, and antitumor activity, etc. [3-5]. However, ginseng is a perennial herb which suffers from different biotic or abiotic stresses frequently. Therefore, the ginseng production is greatly threatened by these challenges. To surmount such difficulties, it is urgent to analyze and utilize the defense-related genes in ginseng.

Various transcription factors (TFs) have received more and more attention in plant defense as they play a vital role in various biotic or abiotic stresses resistance. Among the TFs families, the basic leucine zipper (bZIP), contained a conserved bZIP domain composed of 60-80 amino acids, was known as one of the most conserved and largest families [6, 7]. The conserved bZIP domain was composed by two important functional regions, i.e., one basic region and one leucine zipper, linked by one hinge

region [8, 9]. The basic region usually contained an invariant N-x7-R/K motif (approximately sixteen amino acid), which is responsible for both nuclear localization and DNA binding. In contrast, the leucine zipper regions mediate the homo- and/or heterodimerization as they contained a less conserved dimerization motif [10-13].

Considerable evidence demonstrated that many bZIP genes play a vital role in various biological processes, including the differentiation of many tissues and organs, vascular development, initiation in plants [14, 15], embryogenesis [16], and seed maturation [17]. However, there is increasing evidence has indicated that bZIP protein can also be used as key components in plant regulation of biotic and abiotic stresses, e.g., pathogen defense [18, 19], osmosis [20, 21], high salt environment [22, 23], cold [13, 24], and drought [25, 26]. For example, it has been reported that *AtbZIP28* was activated by thermal impact, and then the expression of heat-responsive genes increased to protect the plants from heat [27]. In rice, *bZIP23* and *bZIP72* were reported to play a critical role in attenuating drought treatment by activating ABA signaling [28, 29]. Knockout *slbZIP1* genes and *slAREB1*, belonging to subfamily A of bZIP TF, increased salt stress tolerance, while overexpression of *slbZIP1* and *slAREB1* decreased salt stress tolerance in *Solanum lycopersicum* [30, 31].

The bZIP TF families have been analyzed in various plant species, varying among plants. For example, there were 75 bZIP genes were identified in Arabidopsis [10], 69 genes in tomato [32] and 89 members in rice [7]. However, there is currently no research on the bZIP family in ginseng. Herein, the bZIP genes from ginseng were identified, and then the phylogenetic analysis, conserved protein motifs, and predicts

functional analysis, as well as expression characteristics were performed. Due to drought stress is an important factor for restricting the growth and development of plants [33], the expression level of five *bZIP* genes in drought stress was also quantized by quantitative real-time PCR (qRT-PCR) to verify their role in drought resisting.

Materials and methods

Database resources

The Jilin ginseng transcriptome database developed from 14 different tissues of Jilin ginseng of four years and the roots of different years old were both used in this study [34]. In addition, the transcriptomes derived from the four-year-old plants root of 42 different farmers' cultivars of ginseng in Jilin province, China (named from S1 to S42) [35, 36] were also consulted.

Genetic differences of ginseng in different regions

In this study, we first downloaded the sequence of bZIP transcription factor from Ginseng Genome Database (<http://ginsengdb.snu.ac.kr/index.php>) [37]. Above bZIP transcription factor sequences were aligned with bZIP transcription factor identified from Jilin ginseng transcriptome database. The sequences with identity $\geq 95\%$, alignment length ≥ 240 bp (bZIP maximum domain length) were identified as the same gene in this study.

Identification and phylogenetic analyses of *bZIP* gene family in ginseng

Firstly, the local Hidden Markov Model (HMM) search was carried out on the basis of HMM profile of the bZIP domain (including PF07716, PF00170, PF03131 and PF012498) (<http://pfam.sanger.ac.uk/>) using HMMER3.0 (HMMER: <http://hmmer.wustl.edu/>). Then, BLAST searches were carried out with a threshold of 1.0E-06 to authenticate the predicted *bZIP* genes in ginseng transcriptome database. Then, all the candidate nucleic acid sequences were filtered by the Online Conserved Domains (CDS) search and the resulting genes were identified as *PgbZIP* gene.

The whole bZIP protein sequences from *Arabidopsis thaliana* (Arabidopsis), *Solanum lycopersicum* (tomato) and *Oryza sativa* (rice) were obtained from the plant TF database (PlantTFDB, v3.0) [38], Sol Genomics Network (SGN; <http://solgenomics.net/>) and Rice Genome Annotation Project RGAP (<http://rice.plantbiology.msu.edu/>) databases [32], respectively. Then, all these full proteins encoded by bZIP genes of Arabidopsis, rice, tomato and ginseng were subjected to muscle to perform multiple sequence alignments. Finally, the phylogenetic tree was constructed using MEGA version 7.0 (<http://www.megasoftware.net>), and the parameter is the neighbor-joining (NJ) algorithm, 1000 replicates, pairwise deletion of gaps and p-distance method.

Conserved motifs analyses

The full length of the *PgbZIP* transcripts were subjected to the online ORF Finder at NCBI (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>) to search their open reading frames (ORFs). Then, the conserved motifs of the *PgbZIP* protein were searched

using the Motif Elicitation tool (version 4.9.1, <http://meme.sdsc.edu/meme/cgi-bin/meme.cgi>) [39] with a maximum number of 20. The minimum width, maximum width of the conserved motifs was 10 and 50, respectively and other parameters were used as default [40].

Functional categorization of the *PgbZIP* transcripts

The 273 *PgbZIP* transcripts were submitted to Blast2GO software (Version 4.1.5) to perform GO analysis. After predict the functional differentiation of *PgbZIP* transcripts, the enrichment of these functional subcategories were analyzed [41]. In the enrichment analysis, the GO function classification of 273 unknown ginseng gene transcripts randomly selected throughout the Jilin ginseng transcriptome database was used as a control (level 2). The significance test between the number of *PgbZIP* transcripts and 273 unknown ginseng transcripts classified into each functional subcategory were determined by the Chi-square test.

Expression and network analysis of the *PgbZIP* transcripts

The expression of 273 *PgbZIP* transcripts was extracted from the following transcriptome databases: the 14 tissues in one four-year-old ginseng, the roots of 5-, 12-, 18- and 25-year-old ginseng [34], as well as the four-year-old roots of 42 ginseng genotypes in Jilin, China [35]. We used the R programming language and software to construct the expression heatmaps of the 273 *PgbZIP* transcripts, and the BioLayout Express3D software (Version 3.2) to construct the gene coexpression networks [42].

Plant growth and stress treatments

The ginseng seeds were obtained from Jilin Province, China, and were grown in the pots containing nutritional soil for 4 weeks. Then, the four-week-old ginseng seedlings were treated by 20% PEG-6000 to simulate the drought stress treatments. After treated with PEG-6000 for 3 h, 6 h, 12 h 24 h and 48 h, the entire ginseng seedlings were harvested and weighed straightway (fresh weight) at indicated time points. The seedlings were placed in a vial containing 4 °C distilled water for 24 h, and the saturated weight was measured. Then, these seedlings dried at 70 °C for 72 h, and the dry weight was measured. Finally, the relative water content (RWC) of the control and drought stress treated seedlings was measured using the following formula: $[(\text{fresh weight} - \text{dry weight}) / (\text{saturated weight} - \text{dry weight})] \times 100$ [43].

RNA isolation and quantitative real-time PCR analysis

After treat with PEG -6000 for 3 h, 6 h, 12 h 24 h and 48 h, the total RNA of ginseng seedlings were isolated by TRIzol reagent (Biotake, Beijing, China) following the manufacturer's instructions and the first-strand cDNAs were also synthesized by the PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, Tokyo, Japan) according to the manufacturer's instructions. Ubiquinol-cytochrome C reductase gene (QCR) of ginseng was selected as marker genes for drought treatments [44]. For the *PgbZIP* genes, the specific primers were also designed and synthesized and the primers were listed in Additional file 1: Table S1. qRT-PCR was conducted using an Applied Biosystems 7500 Real Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) and SYBR Premix Ex Taq™ II (TaKaRa, Tokyo, Japan). The PCR conditions were 30 s at 95 °C, and followed by forty cycles of 5 s at 95 °C, 34 s at 60 °C, and

one cycle of 15 s at 95 °C, 60 s at 60 °C and finally 15 s at 95 °C. Three biological replicates were set and the genes relative expression was calculated using the $2^{-\Delta\Delta Ct}$ formula.

Results

Identification of *PgbZIP* genes

After searching from the 248,993 unigenes in the ginseng transcriptome database created in our previous study, 1957 gene sequences were characterized from ginseng. However, as the CDS of 1684 gene sequences were incomplete or outside the ORFs, the left 273 transcripts containing complete bZIP domain inside their ORFs were identified from Jilin ginseng. These 273 transcripts had a sequence of 210 to 3,651 bp, with an average number of 1449 bp (Additional file 2: Table S2). By Trinity software, these 273 transcripts were defined to derive from 91 *bZIP* genes, which were further named *PgbZIP 01* to *91* [45]. The multiple transcripts derived from one *PgbZIP* gene were suffixed with another Arabic number, e.g., *PgbZIP11-1* and *PgbZIP11-2*. Among the 273 *PgbZIP* transcripts, 190 contained full-length open-reading frames (ORF), which were derived from 62 *PgbZIP* genes (Additional file 3: Table S3). The encoded full-length proteins by 190 *PgbZIP* gene transcripts contained amino acids varying from 46 (*PgbZIP84*) to 785 (*PgbZIP63-1*), with an average of 294. Comparing to the sequences of Korean ginseng bZIP from Ginseng Genome Database (<http://ginsengdb.snu.ac.kr/index.php>), 45 (49%) Jilin ginseng bZIP genes identified in this study were similar ($\geq 95\%$) to 111 (76%) Korean ginseng bZIP, which could

be supposed to the same genes (Additional file 4: Table S4). However, 46 (51%) Jilin ginseng bZIP genes identified in this study were assigned as newly discovered bZIP genes, as their sequences were quite different from Korean ginseng bZIP from Ginseng Genome Database (Additional file 5: Figure S1).

Motif identification and phylogeny of the *PgbZIP* gene family

To study the structural characteristics of *PgbZIPs*, 20 conserved motifs of *PgbZIP* proteins encoded by 62 *PgbZIP* genes were predicted using online MEME software. The distribution of each motif was shown in Fig. 1a. Motif 1, which was annotated as bZIP domain, presented in all *PgbZIP* members. Besides, most *PgbZIP* proteins of one subfamily usually exhibited similar motif features (Fig. 1a). For instance, most *PgbZIP* members of subfamily A contained collective motifs of 12, 15, 16 and 17; subfamily D harbors the same motifs of 2, 4, 6, 7, 8, 13 and 20; subfamily G shares motif 11. Additionally, multiple subfamilies can share the same motif. For instance, subfamily G and S possess motifs 10; and motifs 14 appeared in subfamily F and G. These results indicated that *PgbZIP* TFs in the same groups showed similar motif characteristic, suggesting their functional similarities.

Then, the longest transcript of the 62 genes containing full-length ORF was used as a representative to construct the phylogenetic tree. Fifty six *bZIP* genes, which were identified from Arabidopsis, rice and tomato (Additional file 6: Table S5), were obtained from a previous study and constructed phylogenetic tree together with the

PgbZIP gene. As shown in Fig. 1b, these 62 *PgbZIPs* could be clustered into ten clades, together with the outgroup bZIPs from Arabidopsis, tomato and rice. The ginseng *PgbZIP* family still has same number of clades with Arabidopsis, rice, tomato and maize [8], but contains more clades than castor bean [46], cucumber [47] and sorghum [48]. Comparing with these outgroup species, groups A contained 11 members in Jilin ginseng, possessing the biggest subfamily in ginseng, whereas subfamily H only have one gene (*PgbZIP13*), suggesting the function differentiation among different members of *PgbZIP* gene family.

Functional differentiation of the *PgbZIP* gene family

The functional differentiation of these 273 transcripts derived from 91 *PgbZIP* genes were functionally categorized by the in silico Gene Ontology (GO) terms. *PgbZIP* transcripts were annotated into different functional categories (Fig. 2 and Additional file 7: Table S6). Specifically, 235 *PgbZIP* transcripts were categorized to BP, while 249 *PgbZIP* transcripts has functions in MF and only 2 *PgbZIP* transcripts were annotated to cellular CC, respectively (Fig. 2a). At Level 2, the transcripts were categorized into 5 subcategories, including two BP subcategories: transcription, DNA-templated and regulation of gene expression; two MF subcategories: nucleic acid binding transcription factor activity and DNA binding; and one CC subcategories: cytosol (Fig. 2b). Furthermore, among these 5 subcategories, the 4 subcategories except cytosol were significantly enriched relative to the 273 unknown ginseng transcripts randomly selected throughout the transcriptome database ($P \leq 0.01$).

The functions of the *PgbZIP* gene transcripts expressed in the roots of 4 differently aged plants (Fig. 3a), 14 tissues (Fig. 3b) and 42 farmers' cultivars (Fig. 3c), were also categorized respectively. Generally, these transcripts were also categorized into the above mentioned 5 subcategories, but the numbers of the *PgbZIP* gene transcripts in these 5 subcategories (Level 2) varied greatly among the different age, tissues and cultivars. The above results also demonstrated the potential functional diversity of these *PgbZIP* superfamily genes but also confirmed their functional consistency among different developmental stages, tissues and genotypes.

Expression characteristics of the *PgbZIP* gene transcripts in ginseng

To understand the possible roles of *PgbZIP* genes, the expression profiles of the *PgbZIP* transcripts in different-aged plants, 14 different tissues, and 42 different farmers' cultivars were investigated (Additional file 8: Table S7). The expression profile was analyzed and the expression heatmap were constructed using *PgbZIP* transcripts because different transcripts of one gene probably different biological functions [49]. The expression profile of *PgbZIP* transcript varied dramatically among different-aged plant roots, different tissues, and different cultivars, ranging from silent to 178.53 TPM, 307.66 TPM and 169.85 TPM, respectively.

Specifically, 248 *PgbZIP* transcripts expressed in at least one tissue, and most *PgbZIP* genes usually expressed in two or more tissues of Jilin ginseng (Fig. 4a, Fig. 5 and Additional file 9: Figure S2a). Among the 248 expressed *PgbZIP* transcripts, only 68

PgbZIP transcripts, i.e., 25%, expressed in all tissues. Besides, 37 *PgbZIP* gene transcripts, accounting for 14%, showed tissue-specific expression as they specifically expressed in one tissue. On the other hand, a total of 208 *PgbZIP* transcripts were found to express in at least one farmer's cultivars in Jilin province (Fig. 4b). As for each farmer's cultivars, the numbers of the expressed transcripts varied from 106 to 150, which were relatively consistent (Additional file 9: Figure S2b). Finally, 167 *PgbZIP* transcripts expressed at least in one of 5-, 12-, 18- and 25-year-old ginsengs roots (Fig. 4c), while 131, 120, 125 and 130 *PgbZIP* transcripts were separately expressed in above four years-old roots (Additional file 9: Figure S2c), which were also relatively consistent. To analyze the expression pattern of ginseng bZIP, we constructed a heat map based on the ginseng transcriptome database. Fig. 5 showed the expression heatmap of the *PgbZIP* gene superfamily in different tissues, different cultivars, and different years-old ginsengs. Generally, the expression of *PgbZIP* transcripts varied greatly among different tissues, cultivars, and developmental stages.

The network of *PgbZIP* genes expression

To detect the expression association among different members of the *PgbZIP* genes, the co-expression networks of 273 *PgbZIP* transcripts were constructed basing on their expression profile in 14 different tissues and 42 different farmers' cultivars, respectively, at a value of $P \leq 5.0E-02$ (Additional file 10: Figure S3 and Fig. 6). Meanwhile, 273 unknown transcripts of ginseng genes were randomly extracted from the 248,993 ginseng unigenes and used for negative controls. In 14 different tissues of ginseng, the expression profile of 248 *PgbZIP* transcripts expressed in at least one

tissues formed a co-expression network with 248 nodes, 2,429 edges (Additional file 10: Figure S3a), and 20 clusters (Additional file 10: Figure S3b, Additional file 11: Table S8). Comparing with the negative control, *PgbZIP* transcripts contained fewer edges. However, *PgbZIP* transcripts possessed evidently more nodes than negative controls at P-values from 1.0E-02 to 1.0E-03 (Additional file 10: Figure S3c-f). In the 42 different farmers' cultivars, 208 *PgbZIP* transcripts tended to expressed connectedly and form a co-expression network composed of 207 nodes, 1994 edges (Fig. 6a), and 18 clusters (Fig. 6b, Additional file 12: Table S9). Statistical analysis showed that these *PgbZIP* transcripts were tended to form a closer co-expression network much farther than the randomly extracted transcripts in 42 ginseng cultivars (Fig. 6c-f).

***PgbZIP* genes actively respond to drought**

To further speculate the function of *PgbZIP* genes in drought stresses response, the expression patterns of 5 *PgbZIPs* randomly selected from several large subfamilies, i.e. *PgbZIP25*, *PgbZIP38*, *PgbZIP39*, *PgbZIP53* and *PgbZIP54*, were analyzed by q-RT PCR. To simulate drought stresses, ginseng seedlings were exposed with 20% (w/v) PEG-6000, for 3, 6, 12, 24 and 48 h and then the RWC were determinated. As a result, the RWC of seedlings treated with PEG-6000 for 6 h were significantly reduced comparing with the control plants. However, there was no significant difference in RWC at other time points (Additional file 13: Figure S4). On the other hand, the expressions of these five *PgbZIP* genes in the ginseng seedlings were mainly up-regulated by PEG-6000 treatment. Specifically, the expression of

PgbZIP25, *PgbZIP38*, *PgbZIP39*, *PgbZIP53* and *PgbZIP54* genes treated with PEG-6000 for 3h were significantly up-regulated by 4.11, 12.62, 8.62, 4.74 and 18.93 times comparing to the untreated plants ($P < 0.01$). However, with time going on, the expression patterns of these five genes are different from each other. For instance, the expression of *PgbZIP25* reached to the crest after drought stress treated for 6 h while showed no significant difference with the control plants after treated for 12 h, 24 h and 48 h. *PgbZIP38* gene was continuously up-regulated by PEG-6000 treated for 3h-24h but returned to normal level at 48h. Noteworthy, except 3 h, the expression of *PgbZIP53* in ginseng seedling exposed with PEG-6000 for other time points showed no significant difference with the control. On the other hand, the left two genes, i.e. *PgbZIP39* and *PgbZIP54*, showed irregular variation (Fig. 7).

Discussion

To date, due to the significant role in regulate various biological processes, the bZIP TFs has been attractive and identified in various plants, e.g. Arabidopsis (75 members) [10], maize (125 genes) [8], soybean (160 genes) [13], grapevine (55 genes) [50], castor bean (49 members) [46], cucumber (64 members) [47], sorghum (92 genes) [48], barley (89 genes) [12] and *Brachypodium distachyon* (96 members) [9], respectively. Herein, we have identified 91 bZIP genes from Jilin ginseng. Comparing with the members of bZIP in other plants, the number of bZIP gene family in ginseng was similar to that in rice, barley, sorghum and *Brachypodium distachyon*, but expanding to Arabidopsis, castor bean (49), and cucumber (64), and grapevine (55), whereas shrunk to maize (125) and soybean (160).

Phylogenetic tree showed that the *PgbZIPs* could be divided into 10 subfamilies, which has the same evolutionary classification as bZIPs of Arabidopsis, rice, tomato and maize, suggesting similar evolutionary trajectories between ginseng and these plants (Fig. 1b). Notably, most clades contained both bZIPs of ginseng, Arabidopsis, tomato and rice, suggesting that at least some members of the bZIPs have been existed before the differentiation of monocots and dicots. The interspecies clustering also suggested that some homologous *bZIP* genes were existed between ginseng, Arabidopsis, rice and tomato. Therefore, by phylogenetic analyses, bZIPs were supposed to have a relatively conservative structure and function among different plant species when evolution. Additionally, conserved motif analysis revealed that the typical bZIP domain was found in almost all the *PgbZIPs* and different subfamilies usually had some distinguishable motifs. However, sometimes some groups shared the same special motifs (Fig. 1a), which were consistent with the features of bZIPs in grapevine [50].

Numerous bZIP proteins were reported to involve in various biological processes, e.g., the drought/osmotic stress response [9, 47, 48], growth and development, cell elongation [51, 52], organ and tissue differentiation [53] and the gene regulation of seed storage protein [17]. By GO terms in silico, *PgbZIPs* were functionally classified into 5 subcategories, including transcription, DNA-templated, regulation of gene expression, nucleic acid binding transcription factor activity, DNA binding and cytosol. However, the number of the functional differentiation of *bZIPs* gene family was a lot less than the *RLK* gene family (23 subcategories) [54] and *NBS* gene family

(36 subcategories) [35] in ginseng. Our results indicate that the encoded bZIP proteins in ginseng might mainly worked as TFs by binding to the promoter region of specific genes and different members probably perform similar functions in some specific biological processes.

Furthermore, transcriptional patterns of *PgbZIP* transcripts were analyzed using three transcriptome databases [34] of Jilin ginseng reported previously. The heatmap based on three transcriptome databases suggested that most genes expressed in seeds, roots, stems, leaves and flowers, which was in consistent with the studies in *Arabidopsis* [10]. However, these *PgbZIP* genes exhibited significant variations in expression across different tissues, also similar to the other plants, e.g., studies in rice and maize [7, 8]. Sometimes, different transcripts of the same gene exhibited different expression patterns, which might be resulted from the alternatively splicing of pre-RNA.

Unexpectedly, despite of the structure, functional differentiation and expression activity among *PgbZIP* transcripts exhibited a noteworthy diversity, the majority of the *PgbZIP* transcripts remained to correlatively express and form a co-expression network by analyzing their expression association. Besides, the networks among *PgbZIP* transcripts was markedly stronger than that in unknown the transcripts randomly extracted from ginseng transcriptome databases, suggesting that *PgbZIP* transcripts probably somehow interact with each other or remain functionally coordinated (Additional file 13: Figure S4). On the other side, the numerous clusters formed by the *PgbZIP* gene family in the network further confirmed the functional

differentiation among different *PgbZIP* transcripts.

As a perennial herb, ginseng has been frequently suffering from various environmental stresses. However, to date, the mechanisms of the resistance of ginseng to environmental stress were not clearly clarified. Herein, to excavate potential *bZIP* genes resisting to the drought stress in ginseng, the PEG were selected to simulate drought treatment and exposed to ginseng as it was reported that PEG could significantly change the relative water content in plants [43]. It was reported that the group A of *PgbZIPs* play a significant role in the drought and high salinity stress [55-57]. e.g., overexpression of *OsbZIP46*, a member of group A, could significantly improve both drought resistance and osmotic resistance in rice [58]; *ThbZIP1* gene of *Tamarix hispida*, can increase the tolerance to salt and drought stress in Arabidopsis [59]. Herein, the expression level of five randomly selected *PgbZIP* genes, including a group A, i.e., *PgbZIP39*, under drought stresses were analyzed by qPCR. As a result, *PgbZIP39* was significantly up-regulated (8.62 times comparing to the untreated plants) by PEG-6000 treated. Besides, the other four genes, (*PgbZIP25*, *PgbZIP38*, *PgbZIP53* and *PgbZIP54* genes), randomly selected from the S, G, S and I groups, also showed significantly transcriptional changes ($p < 0.01$) after drought stress treatments at least one time point. Therefore, it can be speculated that besides group A, other groups of *PgbZIP* genes, probably also play an essential role in responding drought stresses. The above results could provide valuable information for the functional verification of *PgbZIP* genes and identifying new drought resistance genes in ginseng. However, it is still far from unraveling the exact biological functions of

PgbZIPs, which needs to be further investigated.

Conclusions

91 bZIP genes were identified from ginseng and systematically analyzed by phylogenetic analyses, conserved motif and functionally categorizing in silico. Besides, the expression characteristics and network were also analyzed. The function of *PgbZIP* genes were obviously differentiated, the expression of *PgbZIP* genes are also varied in spatial and temporal in ginseng. Simultaneously, under drought stress treatments, the *PgbZIP* genes expression levels suggested that several groups of *PgbZIP* were involving in drought signaling. Together, this study showed some new characteristics of the bZIP TFs gene family in Jilin ginseng, which would not only provided some basis for further understanding the evolution, expression and functional prediction of the *PgbZIPs*, but also increased our understanding the role of *PgbZIP* genes in the regulation of drought resistance in ginseng.

Figure Captions

Fig. 1 Conserved motifs and Phylogenetic relationship of the *PgbZIP* proteins. **a**

Conserved motifs of *PgbZIP* proteins according to the evolutionary relationship. The conserved motifs in the *PgbZIP* proteins were identified with MEME software. Each motif is indicated by a colored box numbered at the top. **b** Phylogenetic analysis of ginseng , Arabidopsis , tomato and rice bZIP proteins.

Fig. 2 In silico functional categorization and GO term enrichment of the *PgbZIP* gene

transcripts. **a** Venn diagram of numbers of the *PgbZIP* transcripts involved in the biological process (BP) (235 genes), molecular function (MF) (249

genes) and cellular component (CC) (2 genes) categories. **b** Subcategories in which the *PgbZIP* transcripts are involved at Level 2 and their enrichments. The GO terms of the transcripts expressed in 14 tissues of the four-year-old plant used for identification of the *PgbZIP* genes as the background control for the enrichment analysis. **, significant at $P \leq 0.01$; the remaining GO terms are not significant at $P \leq 0.05$.

Fig. 3 Variation of the functional categories of the *PgbZIP* transcripts. **a** Variation of the functional categories of the *PgbZIP* transcripts among 14 tissues of a 4-year-old plant. **b** Variation of the functional categories of the *PgbZIP* transcripts among the 4-year-old roots of 42 ginseng cultivars from Jilin, China. **c** Variation of the functional categories of the *PgbZIP* transcripts among the roots of differently aged plants.

Fig. 4 Expression of the *PgbZIP* gene transcripts in ginseng. **a** Expression of the *PgbZIP* gene transcripts in different numbers of tissues. The percentages of each part of the pie indicate the percentage of the 273 *PgbZIP* gene transcripts; the number behind the percentage in each part of the pie indicates the number of the 14 tissues in which the gene transcripts expressed. **b** Expression of the *PgbZIP* gene transcripts in four-year-old roots of different genotypes. The percentages of each part of the pie indicate the percentage of the 273 *PgbZIP* gene transcripts; the number behind the percentage in each part of the pie indicates the number of the four-year-old roots of different genotypes in which the gene transcripts expressed. **c** Expression of the *PgbZIP* gene transcripts in differently-aged roots. The percentages of each part of the pie indicate the percentage of the 273 *PgbZIP* gene transcripts; the number behind the percentage in each part of the pie indicates the number of the

number of four aged roots in which the gene transcripts expressed.

Fig. 5 Expression of the *PgbZIP* transcripts categorized into the immune system process. **a** Expression of the *PgbZIP* transcripts in 14 tissues. **b** Expression of the *PgbZIP* transcripts in 42 cultivars. **c** Expression of the *PgbZIP* transcripts in four different year-old roots.

Fig. 6 Network analysis of the *PgbZIP* transcripts expressed in the 4-year-old roots of 42 farmers' cultivars. **a** The co-expression network constructed from 273 *PgbZIP* transcripts at $P \leq 5.0E-02$. It consists of 207 nodes and 1994 edges. **b** 18 clusters in the network. **c** Tendency that *PgbZIP* genes form a network using the randomly-selected ginseng unknown genes as a control: variation in number of nodes. **d** Tendency that *PgbZIP* genes form a network using the randomly-selected ginseng unknown genes as a control: variation in number of edges. The networks shown in (**c** and **d**) were constructed from 273 transcripts with no replicate. **e** Statistics of variation in number of nodes in the *PgbZIP* network. **f** Statistics of variation in number of edges in the *PgbZIP* network. Capital letters, significant at $P \leq 0.01$; Error bar, the standard deviation for 20 bootstrap replications.

Fig. 7 *PgbZIP* gene expression levels in ginseng seedlings after 3, 6, 12, 24 and 48 h of 20% PEG-6000 treatment. The values are expressed as the means of 3 replicates. *: $P < 0.05$. **: $P < 0.01$.

Additional files

Additional file 1: Table S1. Primers used in qRT-PCR analysis. (XLSX 57 kb)

Additional file 2: Table S2. The *PgbZIP* genes identified in this study and their transcript sequences. (XLSX 132 kb)

457 **Additional file 3: Table S3.** The PgbZIP genes and their putative protein sequences.
 458 (XLSX 30 kb)

459 **Additional file 4: Table S4.** Sequence Alignment between bZIP transcription factor
 460 from Ginseng Genome Database and bZIP transcription factor identified from Jilin
 461 ginseng transcriptome database. (XLSX 13 kb)

462 **Additional file 5: Figure S1.** Comparison of bZIP transcription factor from Ginseng
 463 Genome Database and bZIP transcription factor identified from Jilin ginseng
 464 transcriptome database. (TIFF 4471 kb)

465 **Additional file 6: Table S5.** The published *bZIP* genes used as outgroup for
 466 classification and phylogenetic analysis of the *PgbZIP* gene superfamily. (XLSX 47
 467 kb)

468 **Additional file 7: Table S6.** Annotation and GO categorization of the 273 PgbZIP
 469 transcripts. (XLSX 25 kb)

470 **Additional file 8: Table S7.** Expressions of the *PgbZIP* transcripts in the
 471 four-year-old roots of 42 farmers' cultivars, 14 tissues of a four-year-old plant and 4
 472 different year-old roots (TPM). (XLSX 97 kb)

473 **Additional file 9: Figure S2.** Expression of the *PgbZIP* transcripts in ginseng. **a**
 474 Number of cultivars in the four-year-old roots of which the *PgbZIP* transcripts
 475 expressed. **b** Number of tissues in which the *PgbZIP* transcripts expressed. **c** Venn
 476 diagram of numbers of the *PgbZIP* transcripts expressed in differently-aged roots.
 477 (TIFF 854 kb)

478 **Additional file 10: Figure S3.** Network of the *PgbZIP* transcripts expressed in 14
 479 tissues of a four-year-old ginseng plant. **a** The co-expression network constructed

from 440 *PgbZIP* transcripts at $P \leq 5.0E-02$. It consists of 248 nodes and 2429 edges. **b** 20 clusters in the network. **c** Tendency that *PgbZIP* genes form a network using the randomly-selected ginseng unknown genes as a control: variation in number of nodes. **d** Tendency that *PgbZIP* genes form a network using the randomly-selected ginseng unknown genes as a control: variation in number of edges. The networks shown in (**c** and **d**) were constructed from 273 transcripts with no replicate. **e** Statistics of variation in number of nodes in the *PgbZIP* network. **f** Statistics of variation in number of edges in the *PgbZIP* network. Capital letters, significant at $P \leq 0.01$; Error bar, the standard deviation for 20 bootstrap replications. (TIFF 3663 kb)

Additional file 11: Table S8. Matrix of expression correlation coefficients between the *PgbZIP* gene transcripts in 14 tissues of a fouryearold plant used for coexpression network construction of the *PgbZIP* genes in different tissues. (XLSX 673 kb)

Additional file 12: Table S9. Matrix of expression correlation coefficients between the *PgbZIP* gene transcripts in the roots of fouryearold plants of 42 cultivars used for coexpression network construction of the *PgbZIP* genes in the roots of different cultivars. (XLSX 606 kb)

Additional file 13: Figure S4. Relative water content of ginseng seedlings subjected to PEG stress. The control plants were maintained under normal water irrigation conditions. The values are expressed as the means of 3 replicates. *: $P < 0.05$. **: $P < 0.01$. (TIFF 295 kb)

Abbreviations

Ginseng: *Panax ginseng* C.A. Meyer; bZIP: basic leucine zipper; *PgbZIP*: *Panax ginseng* bZIP; ORF: Open reading frame; CDS: Conserved Domains; MEME: Multiple EM for motif elicitation; GO: gene ontology; MF: molecular function; BP:

504 biological process; CC: cellular component; HMM: Hidden Markov Model; RWC:

505 Relative water content

506 **Ethics approval and consent to participate**

507 Not applicable

508 **Consent for publication**

509 Not applicable

510 **Availability of data and materials**

511 Not applicable

512 **Competing interests**

513 The authors declare that the research was conducted in the absence of any commercial
514 or financial relationships that could be construed as a potential conflict of interest.

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517 **Authors' contributions**

518 MPZ and YW planned and designed this study; HL performed the work and wrote the
519 manuscript; HL, QZ and YH performed drought stress and conducted qRT-PCR; CS,
520 KW, YFW, PC and JL prepared the tables and figures. JC and MPZ revised the
521 manuscript. All the authors read and approved the final version of the manuscript.

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Figures

Figure 1

Conserved motifs and Phylogenetic relationship of the PgbZIP proteins. a Conserved motifs of PgbZIP proteins according to the evolutionary relationship. The conserved motifs in the PgbZIP proteins were identified with MEME software. Each motif is indicated by a colored box numbered at the top. b Phylogenetic analysis of ginseng , Arabidopsis , tomato and rice bZIP proteins.

Figure 2

In silico functional categorization and GO term enrichment of the PgbZIP gene transcripts. a Venn diagram of numbers of the PgbZIP transcripts involved in the biological process (BP) (235 genes), molecular function (MF) (249 genes) and cellular component (CC) (2 genes) categories. b Subcategories in which the PgbZIP transcripts are involved at Level 2 and their enrichments. The GO terms of the transcripts expressed in 14 tissues of the four-year-old plant used for identification of the PgbZIP genes as the background control for the enrichment analysis. **, significant at $P \leq 0.01$; the remaining GO terms are not significant at $P \leq 0.05$.

Figure 3

Variation of the functional categories of the PgbZIP transcripts. a Variation of the functional categories of the PgbZIP transcripts among 14 tissues of a 4-year-old plant. b Variation of the functional categories of the PgbZIP transcripts among the 4-year-old roots of 42 ginseng cultivars from Jilin, China. c Variation of the functional categories of the PgbZIP transcripts among the roots of differently aged plants.

Figure 4

Expression of the PgbZIP gene transcripts in ginseng. a Expression of the PgbZIP gene transcripts in different numbers of tissues. The percentages of each part of the pie indicates the percentage of the 273 PgbZIP gene transcripts; the number behind the percentage in each part of the pie indicates the number of the 14 tissues in which the gene transcripts expressed. b Expression of the PgbZIP gene transcripts in four-year-old roots of different genotypes. The percentages of each part of the pie indicates the percentage of the 273 PgbZIP gene transcripts; the number behind the percentage in each part of the pie indicates the number of the four-year-old roots of different genotypes in which the gene transcripts expressed. c Expression of the PgbZIP gene transcripts in differently-aged roots.. The percentages of each part of the pie indicates the percentage of the 273 PgbZIP gene transcripts; the number behind the percentage in each part of the pie indicates the number of the number of four aged roots in which the gene transcripts expressed.

Figure 5

Expression of the PgbZIP transcripts categorized into the immune system process. a Expression of the PgbZIP transcripts in 14 tissues. b Expression of the PgbZIP transcripts in 42 cultivars. c Expression of the PgbZIP transcripts in four different year-old roots.

Figure 6

Network analysis of the PgbZIP transcripts expressed in the 4-year-old roots of 42 farmers' cultivars. a The co-expression network constructed from 273 PgbZIP transcripts at $P \leq 5.0E-02$. It consists of 207 nodes and 1994 edges. b 18 clusters in the network. c Tendency that PgbZIP genes form a network using the randomly-selected ginseng unknown genes as a control: variation in number of nodes. d Tendency that PgbZIP genes form a network using the randomly-selected ginseng unknown genes as a control:

variation in number of edges. The networks shown in (c and d) were constructed from 273 transcripts with no replicate. e Statistics of variation in number of nodes in the PgbZIP network. f Statistics of variation in number of edges in the PgbZIP network. Capital letters, significant at $P \leq 0.01$; Error bar, the standard deviation for 20 bootstrap replications.

Figure 7

PgbZIP gene expression levels in ginseng seedlings after 3, 6, 12, 24 and 48 h of 20% PEG-6000 treatment. The values are expressed as the means of 3 replicates.*: $P < 0.05$. **: $P < 0.01$.

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