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Identification and Expression Profiling of the bZIP Gene Family Throughout the Genome in Phoebe bournei under Abiotic Stress

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Abstract

The *bZIP*gene family plays a crucial role in supporting plant life as one of the most important transcription factors. Its involvement in various processes such as light signaling, seed maturation, flower development, and cell elongation has been established, but limited to perennial and agricultural crops. This study focused on the classification of 71 *bZIP* genes across 12 chromosomes based on their evolutionary relationships. Each group exhibited distinct gene structures and variations in the number of conserved motifs. The presence of multiple cis-acting elements suggested that the *PbbZIP* gene family played a vital role in hormonal and regulatory signaling, which were essential for plant growth, development, and resilience against abiotic stress. RNA and qPCR analysis revealed expression levels of *PbbZIP*32, *PbbZI*P14, *PbbZIP*26, *PbbZIP*67, and *PbbZIP*69 under salt, drought, and temperature stress conditions. This research contributed to a deeper understanding of the underlying mechanisms governing stress resistance, growth, and development processes mediated by the *bZIP* gene family. It also can serve as a foundation for future investigations on the *bZIP*gene family in forestry species.

1. Introduction

Abiotic stress has a direct impact on the growth and development of plants (Zhang et al. 2023). When plants are exposed to an environment with harsh conditions that they are not able to tolerate, their normal growth and development will be affected. *Phoebia bournei*, as a plant under harsh conditions such as high temperature, salinity, and drought, will be affected in its physiological and biological activities(Ojasvini Ahluwalia a, and Ranjana Bhatia 2021). Other plants have mechanisms to adjust themselves to tolerate various stresses. For instance, the *Mangifera indica* undergoes wilting during drought to protect the plants from water loss(Zhang et al. 2017). Similarly, desert plants develop thorns to reduce water loss to adopt drought environment(Asif Iqbal, et al.). In addition, different plants develop deep and advanced roots to allow the plant to tolerate the anaerobic respiration conditions caused by high water content(Zhou et al. 2020). The same applies to *Phoebia bourneia*, which is involved in different biological processes including organ and tissue differentiation, energy metabolism, synthesis of storage proteins, and the regulation of genes involved in responding to biotic stress in order to maintain its homeostasis(Yiming Ma et al.). Plants respond to biotic stress by engaging in various processes to maintain their normal functioning(Nawaz et al. 2023). One of the process involves activation of crucial chemical reactions, including the production of antioxidant enzymes. These enzymes play a vital role in neutralizing harmful free radicals before they can cause damage to the plant's cellular components. Furthermore, plants undergo transcription processes to facilitate gene expression and signal pathways(Kapoor et al. 2019).

The transcriptional process in plants is controlled by transcription factors. These transcription factors are proteins that bind to DNA to bring a response to various signals such as growth factors, development factors and environmental stress signals like extremely temperature, drought and salinity(Wang et al. 2015). During stressful conditions, the transcription factors provide a response to the plant, accelerating the expression of stress-tolerant genes that control gene expression in plant(Javed et al. 2020). The structure of a transcription factor consists of a DNA binding domain (N-× 7-R/K-× 9), which is highly conserved. The Basic leucine zipper family (*bZIP*) plays an important role in responding to abiotic stress. Several studies have shown the strong correlation between abscisic acid (ABA) and *bZIP* genes(Lina Yao, and Hongli Cao 2020). The upregulation of *StbZIP*-65 in potatoes in response to salinity stress indicates the potential role of *StbZIP*-65 in enhancing salt tolerance within the gene family of *Solanum tuberosum L*. supported by research demonstrating the upregulation of *StbZIP-65* under salinity stress conditions(Joo et al. 2021), among one of the Arabidopsis representative group revealed the function of *bZIP* in ABA activities and drought stress response (Joo et al. 2021). Furthermore, bZIP TFs serve as essential controllers in plant development and growth. The *bZIP* gene in poplar plants shows different expression of *bZIP* in various tissues such as roots, stem, and leaves, which demonstrates secondary metabolite biosynthesis and secondary metabolic involvement(Zhao et al. 2021). The function of *bZIP* in abiotic stress in herbaceous perennial plants has been revealed by several studies(Liu et al. 2023; Zhou et al. 2023), but there are very few studies on the significance of *bZI*P in wood plants like *Phoebe bournei* (Azeem et al. 2020).Compared to herbaceous perennials, wood plants are more vulnerable to long-term harm from intricate environmental stress because of their extended life cycle, and it is extremely difficult to improve their genetic makeup(Liao et al. 2023). Therefore, it is imperative to know the roles of the *bZIP* gene family in *P. bournei*.

Phoebia bournei is a crucial plant in china as it plays significant role in forestry ecosystem,in old times the *P.bournei* was used for manufacture of furniture due to its super characteristic of providing a strong wood (Wang et al. 2013; Xiong et al. 2023). *Phoebia*

bournei has risen up the timber industry in china and across the Asian continent. *Phoebe bournei* have encounter many challenges to the environment which imposed by human activities but also natural environment (Li et al. 2017), although establishment of most advanced and good seedling with agronomic characteristics but the survival and resistivity of of *phoebia bournei* remain challenge, under environmental factors like high temperature,salinity and drought (Da 2001). Different studies have shown that the *phioebia bournei* are more sensitive to biotic stress (Li et al. 2022; Wei et al. 2023). To ensure the survival and development of the *Phoebea bournei* population, studying the biochemical and molecular response of *Phoebea bournei bZIP* under different abiotic stress is very important. This will help gain a deep understanding of the changes in gene expression and molecular level, which will aid in deciphering the genetic breeding engineering and establishing the basis for future research on *Phoebea bournei* growth and development under various abiotic stress(Su et al. 2014; Zhang et al. 2022).

2. Results

2.1. *PbbZIP* Gene Identification and Physicochemical Characteristics

The hidden Markov model of the conserved Mem_trans domain of the *bZIP* (PF03547) attained from website (http://pfam.xfam.org/), by application of TBtools version 1.108 was employed to check for the bZIP sequences. From attained outcome, 71 *PbbZIP* gene families were found and renamed from *PbbZIP1* to *PbbZIP71* based on the gene distribution of *P. bournei*'s 12 chromosomes. Among them, the physical and chemical properties were integrated, as presented in Table 1, which shows that the amino acid number ranged from 74 (*PbbZIP* 15) to 839 (*PbbZIP* 71), while the relative molecular weight varied between 8813.28 (*PbbZIP* 15) and 88864.94 (*PbbZIP* 71). The theoretical isoelectric point (PI) was between 4.78 (*PbbZIP* 26) and 11.45 (*PbbZIP* 48). Herein, based on the principle that amphiphilic proteins have a hydrophilicity index between − 0.5 and + 0.5 (negative values of GRAVY indicate hydrophilicity and positive values indicate hydrophobicity), Based on the physicochemical characteristics outlined in Table 1, it is evident that the entire *PbbZIP* gene family consists of amphiphilic proteins with negative values.

2.3. Phylogenetic and Collinearity Analysis of the *PbbZIP* Gene Family.

P. bournei's bZIP gene family members were categorized into 12 major subgroups (Fig. 2) using a well-established classification system based on *Arabidopsis*: K(1) *PbbZIP* 03, B(1) *PbbZIP* 25, D(13) *PbbZIP* 24, *PbbZIP* 10, *PbbZIP* 07, *PbbZIP* 16, *PbbZIP* 32, *PbbZIP* 21, *PbbZIP* 45, *PbbZIP* 06, *PbbZIP* 13, *PbbZIP* 38, *PbbZIP* 02, *PbbZIP* 01, *PbbZIP* 08, F(3) *PbbZIP* 20, *PbbZIP* 43, *PbbZIP* 28, A(14) *PbbZIP* 33, *PbbZIP* 71, *PbbZIP* 47, *PbbZIP* 46, *PbbZIP* 11, *PbbZIP* 17, *PbbZIP* 67, *PbbZIP* 65, *PbbZIP* 62, *PbbZIP* 58, *PbbZIP* 29, *PbbZIP* 22, *PbbZIP* 44, *PbbZIP* 68, G(6) *PbbZIP* 23, *PbbZIP* 09, *PbbZIP* 41, *PbbZIP* 55, *PbbZIP* 63, *PbbZIP* 70, J(1) *PbbZIP* 34, S(12) *PbbZIP* 35, *PbbZIP* 14, *PbbZIP* 59, *PbbZIP* 37, *PbbZIP* 12, *PbbZIP* 49, *PbbZIP* 39, *PbbZIP* 60, *PbbZIP* 53, *PbbZIP* 15, *PbbZIP* 04, *PbbZIP* 56, C(3) *PbbZIP* 19, *PbbZIP* 61, *PbbZIP* 50, E(7) *PbbZIP* 66, *PbbZIP* 48, *PbbZIP* 26, *PbbZIP* 51, *PbbZIP* 40, *PbbZIP* 31, *PbbZIP* 52, I(7)*PbbZIP* 54, *PbbZIP* 30, *PbbZIP* 69, *PbbZIP* 18, *PbbZIP* 27, *PbbZIP* 42, *PbbZIP* 64 H(3) *PbbZIP* 36, *PbbZIP* 05, and *PbbZIP* 57. Additionally, to gain more insight (Fig. 2) 11 of the 12 chromosomes identified in *P. bournei* were found to be collinear with the genes identified on *A. thaliana* chromosomes at positions 003076.8 to 003071.7 on the genomic sequence.

2.4. Motif Analysis of *PbbZIP* Gene and Exon–Intron Structure of the *PbbZIP* Gene.

In this study highlighted that individuals belonging to the same subgroup displayed uniform motif composition and sequential organization (refer to Fig. 3a). Out of the 71 members identified, it was observed that all group members in the *PbbZIP* gene possess motif 7, with the exception of subfamily G, wherein *PbbZIP* 55 does not contain motif 7. All subfamily members in-group D have a high number of conserved motifs compared to other members, which have 5 to 11 motifs, followed by group A, which has 2 to 6 motifs, while other subfamily members have 3 to 4 motifs. This includes groups S, C, E, H, K, B, and I. The subfamily, group K, has only two motifs (motif 7 and motif 8). In plants, introns regulate gene expressions (Yang et al. 2019; Brito et al. 2020). Therefore, elucidating gene function through the analysis of the intron–exon structure is important. The gene architecture of *PbbZIP* family members (Fig. 3b) reveals that the number of exons range from 2 upon 12 and the number of introns s range from 3–15, whereas all of the *PbbZIP* genes have a 5' UTR. The UTR of a gene sequence plays a crucial role in mRNA stability.

Furthermore, there is extensive variation in the position and number of exons, as well as the length of introns, among the different members of the family.

2.5. *Cis*-Acting Element Analysis of the *PbbZIP* Gene Family

Numerous functional elements and sites are involved in regulating the various activities of bZIP proteins identified by previous experimental validation and data analysis. To validate these results, we performed multiple sequence comparisons (Fig. 4). The results demonstrated that the majority of elements in the *bZIP* gene are associated with growth and development, hormones, pathogenic defense, and light responsiveness. These elements primarily consist of those that are responsive to hormones, gibberellins, MeJA, light, auxin, anaerobic induction, zein metabolism regulation, abscisic acid, low temperature, and light stimulation, MYB binding site responsiveness to light and drought inducibility, circadian control, salicylic acid responsiveness, auxin responsiveness, endosperm expression, the MYBHv1 binding site, phytochrome downregulation expression, anoxic specific inducibility, MYB binding site-flavonoid biosynthesis genes regulation, and part of a conserved DNA module array (CMA3). Each *PbbZIP* gene family contain one or two elements or bind site. This implies that *PbbZIP* expression might be connected to abiotic stressors.

2.6. Multiple Sequence Alignment Analysis of the *PbbZIP* Gene Family.

We identified 71 proteins from the *bZIP* gene in this study through application of HMMER bioinformatics tool with an E-value less than 1×10^{-5}. Next, we used the SMART and Pfam databases to confirm our findings (Letunic et al. 2015; El-Gebali et al. 2019). The 71 *P. bournei* proteins shared the *bZIP* domain, as our Pfam and SMART analyses revealed, which was consistent with our hypothesis. Consequently, we performed multiple sequence alignments after extracting each member's amino acid sequences from the *bZIP*-conserved domain (Zhou et al. 2017), as displayed in Fig. 5. A fundamental DNA-binding region and a nearby leucine zipper structure make up the *bZIP* domain. The N-X7-R/K motif is consistently present in the basic region. A heptapeptide repeat of leucine (L) or a related hydrophobic amino acid makes up the leucine zipper domain.

2.7. Examination Expression pattern of *PbbZIP Genes* in Different Tissues

In order to gain a deeper insight into *PbbZIP*s in *P. bournei* with regards to abiotic stressor response, growth and development process, we analyzed the expression pattern of the 71 *PbbZIP* genes (refer to Fig. 6). Analysis of the heat maps showed gene patterns specific to different tissues. These expression patterns allowed *PbbZIP* to be divided into five different branches. *PbbZIP* 33, *PbbZIP* 49, *PbbZIP* 71, *PbbZIP* 61, *PbbZIP* 57, *PbbZIP* 54, *PbbZIP* 4, *PbbZIP* 58, *PbbZIP* 30, *PbbZIP* 63, *PbbZIP* 41, *PbbZIP* 55, *PbbZIP* 60, *PbbZIP* 36, and *PbbZIP* 66 comprised the first branch. These genes showed elevated expression levels in leaves, indicating a relationship between gene expression and leaf growth. Significantly, every one of the nine genes in the second branch *PbZIP* 6, *PbZIP* 64, *PbZIP* 1, *PbbZIP* 2, *PbbZIP* 20, *PbbZIP* 67, *PbbZIP* 15, *PbbZIP* 33, and *PbbZIP* 21 shows high expression in the stem bark, whereas the third branch *PbbZIP* 17, *PbbZIP* 9, *PbbZIP* 47, *PbbZIP* 34, *PbbZIP* 45, *PbbZIP* 32, *PbbZIP* 16, *PbbZIP* 24, *PbbZIP* 37, *PbbZIP* 12, *PbbZIP* 50, and *PbbZIP* 8 shows prominent expression in the roots and root bark (Fig. 6). *PbbZIP* 39, *PbbZIP* 18, *PbbZIP* 44, *PbbZIP* 14, *PbbZIP* 27, *PbbZIP* 42, *PbbZIP* 52, *PbbZIP* 29, *PbbZIP* 31, *PbbZIP* 38, *PbbZIP* 26, *PbbZIP* 48, *PbbZIP* 46, and *PbbZIP* 51 were included in the fourth branch. On the other hand, very few or no *PbbZIP*s were expressed in the root xylem.

2.8. Examination of *PbbZIP* Genes Expression Patten under Various Abiotic Stress.

To examine how the *PbbZIP* gene responds to abiotic stressors like temperature, salt, and drought, five genes in Table 2 (*PbZIP*32, *PbZIP*14, *PbZIP*26, *PbZIP*67, and *PbZIP*69) selected from five representative *PbZIP* genes based on selection done by(Chang et al. 2023; Ziyuan et al.). The five *PbZIP* genes originate from four distinct subfamilies, each reflecting the subfamily's reaction to abiotic stress. Moreover, the cis-acting elements found in these genes encompass a greater number of stress-related elements. Additionally, the transcriptional analysis confirms varying levels of transient expression in response to different adverse stresses. The expression patterns suggested that, at 40°C, the *PbbZIP* genes were up- and downregulated (Fig. 7). To be more precise, *PbZIP*32, *PbZIP*14, and *PbZIP*26 peaked at 6 hours, 12 hours, and 24 hours, respectively, and then started to decline. Surprisingly, all five genes showed a cyclical pattern of "rise–fall–rise–fall", with notably high peaks at 6 hours, 12 hours, and 24 hours when

exposed to 10% PEG-induced drought stress. On the other hand, under 10% NaCl-induced salt stress, all five genes showed the greatest significant upregulation in contrast to the other two treatments.

3. Materials and Methods

3.1. Plant Material, Data Sources, and Conditions for Growth

Dr. Kehui Chang provided assistance in the provision of seedlings of *Phoebia bournei* and allowed them to grow in an area with a humidity of 77%. The average temperature ranged from 16–20°C and the total precipitation ranged from 900-2100mm. The sequence data of *Phoebia bournei* used in this study were accessed from a publicly available source(Chen et al. 2020), the protein sequence encoded by the *bzip* gene, were obtained from the *Arabidopsis* database (https://www.arabidopsis.org) and allowed to be identified as the query sequence for genetic screening and identification. The overall data was accessed on April 5, 2023.

3.2. *PbbZIP* Gene Identification and Physicochemical Characteristics

We obtained the HMM model of the conserved domain of the *bZIP* gene from the Pfam library (http://pfam.xfam.org/), using the simple HMM search function of tbtools. The protein sequences of *Phoebe bournei* were searched and compared. From the Plant Transcription Factor Database (http://planttfdb.gao-lab.org/index.php), 75 Arabidopsis ZIP protein sequences were downloaded. In March 2023, blast analysis was performed on the gene group of Phoebe bournei using tbtools. Genes with e-values over 10 − 5 were extracted from the blast analysis, and 71 members of the final *bZIP* gene were obtained. To confirm whether these genes are members of the *bZIP* gene, we used NCBI (NCBI-CDD) (https://www.ncbi.nlm.nih.gov/cdd/) and SMART (http://smart.embl-heidelberg.de/) to screen them further, renaming the genes in line with convention and previous research. Using the ExPASy website (http://www.expasy.org/tools), the isoelectric point, amino acid length, and molecular weight of the protein.

3.3. Motif Analysis and Gene Structure of the *PbbZIPs.*

We analyzed the motif of the protein sequences of the *bZIP* gene family members in *P. bournei* using MEME online tool (http://meme-suite.org/). The maximum target value of the motif was set to 25, using the ZOOPS (Zero or One Occurrence Per Sequence) model(Bailey et al. 2006). The distribution of introns and exons of b*ZIP* gene family members in *P. bournei* was determined by using the gene annotation files of *P. bournei*. Tbtools was used to visualize the gene structure and motif. (Brito et al. 2020).

3.4. Construction and Analysis of the Evolutionary Tree

We used MEGA7 software to analyze the *bZIP* gene of phoebe *bournei* and *Arabidopsis thaliana* and carried out 1000 bootstrap analyses to evaluate the accuracy of the phylogenetic tree. Before drawing the tree, we used the Cluster W algorithm of MEGA7 to perform multiple sequence alignments, alignment, phylogenetic analysis, and then for visualization, we used the Evolview web platform to beautify (http://www.evolgenius.info/evolview).

3.5. Examination of *Cis*-Elements in the Promoters of the *PbbZIP* Genes

To determine the *Cis*-acting element of the *Phoebe bournei bZIP* gene, the analysis of region of stretched DNA which is predicted to be responsible to control gene expression was done by accessing the PlantCare website (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) and through the application of Tbtools website (https://www.omicstudio.cn) on August 28, 2023 the display of the results was carried.2.6.

3.6.*PbbZIP* Gene Chromosomal Distribution and Collinearity Analysis

Gene assignment was done by allocating of phoebe bournei gene to their genome, this was done by comparison their relationship in their chromosome location .followed employing of the TBtools software to assess their relatedness by utilizing the genome database of two species (phoebia bournei and Arabidopsis thaliana) to facilitate the determination of related shared characters of the homologous *bZIP* gene present in both *P. bournei* and *A. thaliana*. Subsequently, generation of visualizing related characters by application of TBtools software. And lastly application of the Ka/Ks_Calculator tool within Tbtools was utilized to calculate the non-synonymous substitution rate (Ka), synonymous substitution rate (Ks), and Ka/Ks to examine selection pressures

The expression of the pbzip gene from various tissues was obtained from the NCBI database. Heatmap analysis was conducted using the Tbtools website to represent the expression level and pattern. Seed treatments and cultivation were performed in a climax box. The seedlings of phoebe bournei were allowed to be cultivated for one year after being provided by the Fujian Academy of Forestry. Different treatments, including simulated drought, salt, and temperature conditions, were applied to ensure consistent growth potential. The seedlings with similar characteristics were collected. They were then divided into a control group of 30 individuals and a stress treatment group of 3 individuals. Sample collection involved taking leaves and storing them at -80 ℃ for RNA extraction. Real-time fluorescence quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) was used to control targeted gene expression. A heat map based on Spearman correlation was created to visually illustrate the connections among gene expression.

3.8. Abiotic stresses treatment.

To maintain the continuous growth of *Phoebe bournei*, the selection was done based on seedlings with uniform features. For each time, 30 individuals were chosen for the treatment group, while three individuals were chosen for the control group. The treatment group of 30 individuals was first put in a nutrient solution with 10% PEG, while distilled water was used to treat the control group by soaking the *Phoebe bournei* seedlings. The second treatment involved temperature, where by the *Phoebe bournei* seedlings were treated with a temperature of 45°C, while the controlled group was kept under room conditions in an incubator with a temperature of 25°C and a humidity of 75%. Finally, the salinity treatment carried by soaking the seedlings of *Phoebe bournei* seedlings in a solution of 10% NaCl nutrient, while the controlled group was soaked in distilled water.

3.9. RNA extraction and qRT-PCR analysis.

(1)first RNA extraction was done using the RNA Extraction Kit (Omega Bio-TEK, Shanghai, China). for all controlled phoebea bournei seedling samples and treatment samples. (2) followed by formation of cDNA using EasyScript One-step gDNA Removal and cDNA Synthesis SuperMix (Transgen, Beijing, China) (3). The Quantitative RT-PCR was conducted using TransStart top green qPCR SuperMix. (4) The selection of the reference gene, PbEF1α, was done (Yang et al. 2019) (5). The preparation of the RT-qPCR Reaction Mixture involved mixing 10 μL of SYBR Premix Ex TaqTM II, 1 μL of cDNA, 7 μL of ddH2O, and 2 μL of specific primers. (6)followed by the reaction process of RT-qPCR, which includes pre-degeneration, denaturation, 40 cycles of annealing and extension steps under various temperatures: 95 ℃ for 30 s, followed by 95 ℃ for 5 s, 60 ℃ for 30 s, and 95 ℃ for 5 s, 60 ℃ for 60 s, and 50 ℃ for 30 s, respectively. (7) The data then analyzed to determine the relative expression of bZIP genes using the 2-ΔΔCt method. (8) the Duncan multiple comparison tests, together with analysis of variance (ANOVA), are performed using the SSPS software for multiple data comparison.(9)finally applications of GraphPad Prism 8.0 was employed for data visualization.

4. Discussion

The basic laucena zipper becomes the core to multifunctional activities. Previous, the bZIP transcriptional factor in Eukaryotes has reported in various studies as biologically and physiologically a driving force of plant toward growth and bring about a successive plant development(Huang et al. 2019). The bZIP trasncriptional factor in poplar plant shows different expressions pattern in various tissues such as roots, stem, and leaves, which demonstrates directly involvement in metabolite biosynthesis and secondary metabolic activities, resulting in the growth and development of *poplar*(Zhao et al. 2021). Additionally, the bZIP transcription factor is reported to play a important role in controlling biotic stress(Wang et al. 2021). For instance, the upregulation of *StbZIP*-65 in potatoes in response to salinity is a direct postive results of bZIP trascnription factor on abiotic stress signals (Zhao et al. 2020). Furthermore, among one of the *Arabidopsis* representative groups specifically group A, the function of bZIP trascription factor in ABA activities and drought stress response has been revealed((Joo et al. 2021). Although the genomic activities and expression profile of the bZIP transcription factor in forestry species still have uncertain(Jing et al. 2022), here in our study we aim to highlight the shade region and paving a way for new knowledge. We have looked into various biochemical and molecular activities of the bZIP transcription factor in response to abiotic stress together with responsible process for growth and development in *Phoebe bournei*.

We analyzed the *bZIP* genes in *P. bournei* using whole-genome identification and systematic bioinformatics techniques. The *P. bournei* genome database was used to identify and thoroughly investigate 71 members of the *PbbZIP* gene family. The theoretical isoelectric point (PI) of all the *PbbZIP*s, except *PbbZIP* 21, is 4.78. A few members, namely *PbbZIP* 1, *PbbZIP* 2, *PbbZIP* 5, *PbbZIP*

10, *PbbZIP* 11, *PbbZIP* 18, *PbbZIP* 19, *PbbZIP* 23, *PbbZIP* 24, *PbbZIP* 25, *PbbZIP* 31, *PbbZIP* 32, *PbbZIP* 37, *PbbZIP* 59, *PbbZIP* 62, *PbbZIP* 63, *PbbZIP* 65, *PbbZIP* 69, and *PbbZIP* 71, have PIs ranging from 5.89 to 6.4; the remaining members have PIs greater than 7, signifying that they are alkaline proteins made up of basic amino acids (Table 1). Moreover,we examining conserved motifs offers a theoretical foundation for investigating the roles and categorization of the *PbbZIP* gene family. After a prediction of the PbbZIP protein sequence motifs using MEME, 25 related conserved motifs were discovered. All members of the gene family shared the conserved motif 7 of the PbbZIP domain, according to a schematic diagram of the full-length PbbZIP protein have distinct conserved motifs (Fig. 3a). The variation in motif number indicates that there may have been a splicing error in the annotation or that the sequence of these genes was lost or deleted during the gene's long-term evolution, which could provide more evidence for the functional of *PbbZIP* gene in various subfamilies. According to additional examination of the intron–exon structure, every gene has both exons and introns, suggesting some degree of structural conservation. Variations exist in the locations, counts, and lengths of exons and introns among the various *PbbZIP* gene members (Fig. 3b), indicating that the family has essential biological fuction such as signal transduction and gene re*g*ulation which is essential for organism to survive(Gao et al. 2021). The unequal distribution of exons and introns; these results showed that the *PbbZIP* family's divergent functions are linked to the intron and exon structures' divergence(Sawada and Mitaku 2011; Manzoor et al. 2021). To bolster our theory regarding the close relationship between *Phoebe bournei* and *A. thaliana*, an ancestral tree was constructed. Of these, 71 *PbbZIP* gene families and 75 *AtbZIP* gene families were found, with the *PbbZIP* in *P. bournei* being divided into 12 groups. The findings demonstrated how closely these two species have evolved. In particular, *A. thaliana* is more closely related to *PbbZIP* groups S, A, D, I, and E than it is to *PbbZIP* gene family groups F, G, C, and A. Furthermore, 90% of the genes seemed to form gene clusters, and it was hypothesized that *PbbZIP's* function was conserved throughout evolution. This hypothesis is supported by the finding that *P. bournei* and *A. thaliana* have a high degree of gene homology, as determined by a collinear analysis of the localization of genes in chromosomes. *P. bournei* shared approximately 11 of its 12 chromosomes with *A. thaliana* genes, with chromosome positions ranging from 003076.9 to 00307.1 in the genomic sequence. This suggested that *P. bournei* is widespread throughout species and originated before the formation of the species. Every *PbbZIP* gene family member is physically mapped onto each of the 12 chromosomes(Fig. 1). The greatest number of *PbbZIP*s are found on chromosome 5, while the fewest *PbbZIP*s are found on chromosomes 7 and 8, which have two *PbbZIP* genes. The distribution of *PbbZIP* genes across chromosomes showed that certain regions had a higher frequency of gene clusters than others. On chromosomes 3 and 4, the *PbbZIP* gene family are uniformly distributed throughout the chromosome, whereas *PbbZIP* genes on chromosomes 1, 2, and 5 appear to congregate at the lower and upper ends of the arms. Moreover, it was shown that *PbbZIP* gene family members duplicated in tandem. Genes are tandemly duplicated on chromosomes 1, 4, 5, and 6. Only four pairs of tandem duplicates have been found overall, which suggests that tandem duplication has a small role in the expansion of gene families(Wang et al. 2011; Baloglu et al. 2014). These genes are separated by a distance ranging from 25 to 140 megabases (Mb). Next, we identified segmentally duplicated *bZIP* genes in *P. bournei* genome. Twelve segmentally duplicated *P. bournei bZIP* genes in total were found; these comprise approximately sixteen percent (12/71) of *PbbZIP* genes. An important mechanism thought to be responsible for the expansion of gene families is segmental duplication in which indicate that gene is heavly coserved. Furthermore, a few *cis*-acting elements were found to relate to abiotic stress hormone regulation, growth and development, for example, auxin, abscisic acid, gibberellin, and jasmonate-responsive elements. Abiotic stress response required a number of *cis*-acting elements, while the encompassed elements—such as the high-temperature response—were crucial to the defense and stress responses. Related elements like light-responsive elements, salicylic-acid-response elements, and seed-specific regulatory response elements are necessary for plant growth and development. A more recent study discovered that an MYB gene could attach to the *PbbZIP* genes. This gene regulates the synthesis of flavonoids, as well as light and drought tolerance in plants. It is feasible that the MYB gene and the PbbZIP protein interact to create a regulatory network (Mitra 2018; Jiang et al. 2022).

Lastly, the *PbbZIP* gene family in leaves were determined using qRT-PCR. The *PbbZIP* gene family exhibited a significant peak at various time points (0, 6, 12 and 24 h). Further analysis was conducted on the *PbZIP* expression intensity in various tissues (Fig. 6). The findings show that the genes.were expressed in the leaves, root xylem, root, stem bark, and stem xylem. The *PbbZIP* gene family, however, is highly expressed in the stem and roots and, except for a few members, poorly expressed in the stem, xylem, and leaves. High expression was found in the leaves for *PbbZIP* 33, *PbbZIP* 49, *PbbZIP* 71, *PbbZIP* 61, *PbbZIP* 57, *PbbZIP* 54, *PbbZIP* 40, *PbbZIP* 58, *PbbZIP* 30, PbbZIP 63, *PbbZIP* 41, *PbbZIP* 55, *PbbZIP* 60, *PbbZIP* 36, and *PbbZIP* 66. This suggested that *P. bournei's* growth and development depend heavily on the *bZIP* gene family (Wang et al. 2022; Dong et al. 2023). Additionally, the average length of the core conserved structure domain in PbbZIP members reveals the amino acid residues

constituting the PbbZIP domain. The basic domain comprises 25 amino acids, characterized by numerous arginine and lysine residues. Its primary structure, N-R-X8-R/K-X-R/K, is situated within the site region 10–25, as depicted in (Fig. 5). Furthermore, within the leucine zipper structure domain, delineated by the site region 26–65 in (Fig. 5), there exist 41 amino acid residues, including leucine (X6-L).The basic region has a distinctive motif (N-X7-R/K-X9) that is important for nuclear localization and DNA binding. It also deviates from bZIP's overall distribution of amino acids. However, as previously reported, the leucine zipper forms an amphipathic surface that meditates on particular recognition and dimerization (Li et al. 2016). Such variation may alter this protein's DNA binding specificity and subcellular localization(Dröge-Laser et al. 2018). These predictions align with the PbbZIP protein's variable subcellular localization signal in plants, which may be important for activation (Jin et al. 2014). Thus, phylogenetic analysis, transcriptomic data, and RT-qPCR all showed that five PbbZIP transcriptomic factors (*PbbZIP* 14, *PbbZIP* 26, *PbbZIP* 32, *PbbZIP* 67, and *PbbZIP* 69) are quickly activated and play key roles in response to abiotic stress (Fig. 7). In this study, we observed upregulation of these representative genes after exposure to a high temperature of 40°C and salt stress. Under salt stress, *PbbZIP* 14, *PbbZIP* 67, and *PbbZIP* 69 showed rapid induction and reached peak expression levels at 6 and 12 hours. This suggests their potential involvement in mechanisms contributing to salinity tolerance of *P. bournei*. The *PbbZIP* gene family is also upregulated when the temperature is high, with peak expression at 6 and 12 hours (mostly *PbbZIP* 14, *PbbZIP* 67, and *PbbZIP* 69). However, no clear patterns were seen when the plant was under drought stress; as we observed previously, each gene showed varying degrees of upregulation or downregulation(Ramakrishna et al. 2018). These findings provide preliminary evidence of the important functions of the five *PbbZI*P genes in adapting to abiotic stressors such as heat, drought, and salinity.

5. Conclusions

To establish a foundation for future research on the vital biological functions of the *P. bournei bZIP* gene family in regulating abiotic stress, growth, and development, we have identified the genomic information and located 71 bZIP gene family members. Additionally, we have investigated the physiological and chemical characteristics of the protein motifs, analyzed the genes' structure and chromosomal arrangements, and assessed their evolutionary trajectory. Moreover, we have examined the cis-acting elements and epistasis expression patterns of the genes in response to stressors such as drought, salinity, and temperature. The outcomes of this study significantly improve our understanding of the biological and physiological mechanisms involved in growth and development. Furthermore, they highlight key genes that could be utilized in P. bournei plant cultivation to enhance the plants' resilience to temperature fluctuations, drought, and salinity. Furthermore, the obtained results provide valuable insights for breeders to identify the optimal target gene for genetic improvement, which could be manipulated to create a new lineage with superior resistance to abiotic stress, as well as exceptional growth potential and development.

Declarations

Author Contributions: Conceptualization, S.C.; Software and data curation, H.G.; Methodology ,Data curation and validation,A.B. , J.L.; Resources, J.Y.and S.C.; Drawing , C.H.; Writing original draft prep-aration, A.B. ; Assist in publishing papers , Q.L.; Revising and Editing, C.H. ,J.Z.and Z.T., All authors contributed to the article and approved the submitted version.

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Data Availability Statement:The genome sequence data and annotation information of P. bournei were downloaded from the Sequence Archive of China National GeneBank Data-base (CNSA) with accession number CNP0002030.

Conflicts of Interest: The study, according to the authors, conducted in a financial corre-spondence that may be interpreted as a latent disagreement.

Ethical approval: This article does not censor any research conducted by other researchers using humans or animals.

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Tables

Table 1. Detailed information on the 71 *PbbZIP* genes of *P. bournei* and their encoded proteins.

Gene Accession	Gene ID	Size/aa[1]	MW[2]/Da	Theoretical pI[3]	Aliphatic Index	GRAVY[4]
OF19974-RA	*PbbZIP1*	399	*41600.65*	*5.89*	*51.93*	*-0.67*
OF19973-RA	*PbbZIP2*	144	*16506.95*	*6.28*	*82.64*	*-0.585*
OF19914-RA	*PbbZIP3*	136	15566.31	9.52	55.96	-1.396
OF10270-RA	*PbbZIP4*	231	25909.15	8.5	58.74	-0.698
OF18209-RA	*PbbZIP5*	198	22954.6	6.71	72.47	-0.855
OF02523-RA	*PbbZIP6*	141	16414.99	7.8	82.34	-0.684
OF11694-RA	*PbbZIP7*	159	18344.72	10.29	76.73	-0.76
OF11818-RA	*PbbZIP8*	325	36545.42	9.25	69.05	-0.674
OF11930-RA	*PbbZIP9*	199	23154.87	6.05	79.4	-0.855
OF28301-RA	*PbbZIP10*	144	16675.1	6.19	75.83	-0.704
OF28225-RA	*PbbZIP11*	447	49195.23	6.28	78.68	-0.46
OF04812-RA	*PbbZIP12*	327	36606.14	8.68	64.68	-0.878
OF04669-RA	*PbbZIP13*	368	41352.8	6.73	80.92	-0.451
OF06905-RA	*PbbZIP14*	322	35902.66	7.96	79.97	-0.577
OF08770-RA	*PbbZIP15*	74	8813.28	10.34	59.46	-1.295
OF03422-RA	*PbbZIP16*	373	41747.12	6.88	63.06	-0.914
OF22080-RA	*PbbZIP17*	169	19451.17	9.23	70.89	-0.337
OF12918-RA	*PbbZIP18*	374	41217.7	5.51	70.43	-0.613
OF09866-RA	*PbbZIP19*	387	42269.25	6.38	61.4	-0.716
OF05546-RA	*PbbZIP20*	433	45888.29	9.01	64.09	-0.659
OF25842-RA	*PbbZIP21*	368	40364.2	8.74	57.61	-0.713
OF24005-RA	*PbbZIP22*	365	41331.5	8.37	83.64	-0.368
OF23680-RA	*PbbZIP23*	277	31093.66	5.51	76.03	-0.716
OF23482-RA	*PbbZIP24*	496	55948.95	6.42	81.47	-0.393
OF23448-RA	*PbbZIP25*	461	52301.17	5.78	76.68	-0.558
OF10424-RA	*PbbZIP26*	339	38460.28	4.78	81.36	-0.556
OF01790-RA	*PbbZIP27*	117	13424.64	6.72	90.85	-0.318
OF01865-RA	*PbbZIP28*	135	15157	6.19	67.19	-0.977
OF01905-RA	*PbbZIP29*	324	35867.17	7.19	65.12	-0.762
OF01159-RA	*PbbZIP30*	419	45724.98	7.83	74.61	-0.562
OF00429-RA	*PbbZIP31*	244	26831.46	5.51	67.99	-0.641
OF29773-RA	*PbbZIP32*	374	39382.74	5.96	47.83	-0.74
OF27741-RA	*PbbZIP33*	470	51343.31	6.89	76.47	-0.514
OF15721-RA	*PbbZIP34*	337	37502.44	7.03	68.31	-0.788
OF15812-RA	*PbbZIP35*	217	24620.4	7.03	76.45	-0.745

OF11162-RA	*PbbZIP36*	597	65168.15	8.83	56.52	-0.898
OF10986-RA	*PbbZIP37*	280	29704.17	5.45	67.93	-0.514
OF10903-RA	*PbbZIP38*	121	14142.05	8.54	57.27	-1.001
OF09410-RA	*PbbZIP39*	139	16192.43	6.83	73.74	-0.831
OF09226-RA	*PbbZIP40*	363	39949.87	6.19	74.71	-0.599
OF07945-RA	*PbbZIP41*	389	42749.74	5.97	68.71	-0.666
OF05157-RA	*PbbZIP42*	284	31775.42	6.51	69.75	-0.744
OF05208-RA	*PbbZIP43*	468	52189.65	6.52	74.49	-0.58
OF05212-RA	*PbbZIP44*	416	45110	8.78	59.59	-0.779
OF19397-RA	*PbbZIP45*	407	46959.1	8.7	63.51	-0.926
OF19394-RA	*PbbZIP46*	177	20625.17	6.44	80.96	-0.801
OF18693-RA	*PbbZIP47*	167	18448.37	9.74	60.78	-1.117
OF18548-RA	*PbbZIP48*	150	17606.19	11.45	94.87	-0.687
OF28795-RA	*PbbZIP49*	453	50318.03	6.55	78.9	-0.482
OF26318-RA	*PbbZIP50*	151	17332.69	6.59	79.47	-0.643
OF26807-RA	*PbbZIP51*	143	16152.73	9.71	83.15	-0.45
OF24581-RA	*PbbZIP52*	235	25001.1	9.56	57.74	-0.487
OF06079-RA	*PbbZIP53*	584	64394.12	6.65	58.18	-0.9
OF29662-RA	*PbbZIP54*	273	29723.97	6.55	62.89	-0.756
OF16038-RA	*PbbZIP55*	169	19301.84	9.62	61.83	-0.872
OF02992-RA	*PbbZIP56*	376	42234.62	7.68	60.51	-0.903
OF03083-RA	*PbbZIP57*	431	46862.33	9.61	63.34	-0.757
OF03232-RA	*PbbZIP58*	262	29182.16	9.59	47.33	-0.919
OF01374-RA	*PbbZIP59*	430	46845.64	5.57	64.02	-0.78
OF10634-RA	*PbbZIP60*	170	20349.2	7.74	68.82	-1.127
OF29018-RA	*PbbZIP61*	403	43556.63	6.61	56.23	-0.771
OF28905-RA	*PbbZIP62*	358	39138.26	6.5	62.23	-0.794
OF18055-RA	*PbbZIP63*	265	29261.84	6.38	60.75	-0.774
OF20655-RA	*PbbZIP64*	348	38339.97	5.89	62.61	-0.615
OF20635-RA	*PbbZIP65*	419	45891.11	5.39	63.56	-0.692
OF14232-RA	*PbbZIP66*	260	28706.32	6.82	71.69	-0.58
OF17740-RA	*PbbZIP67*	366	41515.21	8.39	80.25	-0.485
OF17491-RA	*PbbZIP68*	180	19918.45	8.68	71.56	-0.411
OF12212-RA	*PbbZIP69*	409	43379.93	6.18	49.24	-0.838
OF07610-RA	*PbbZIP70*	448	48779.58	7.18	78.04	-0.485
OF26090-RA	*PbbZIP71*	839	88864.94	6.41	68.1	-0.464

Table 2. A list of the internal reference genes and primers used.

Gene Name	Forward Primer (5′–3′)	Reverse Primer (5′–3′)
PbZIP14	GCAGCCTGGTGAGGTAGC	GTGGACGTGGGGTAAGGC
PbZIP26	AGAGGTCCGAGTCCGCAA	CGTTCAACCCTTCCGCCT
PbZIP32	GACGAGCACCACAGGCAT	TCGGACTTGGCGGCAATT
PbZIP67	TCGGCATGCCTGATGGTG	TGACTCAGAGTCCGCGGA
PbZIP69	CATGGCCCCTGCAAGTGT	GGCACCACTCCACTTGCT
PbEF1α	CATTCAAGTATGCGTGGGT	ACGGTGACCAGGAGCA

Figures

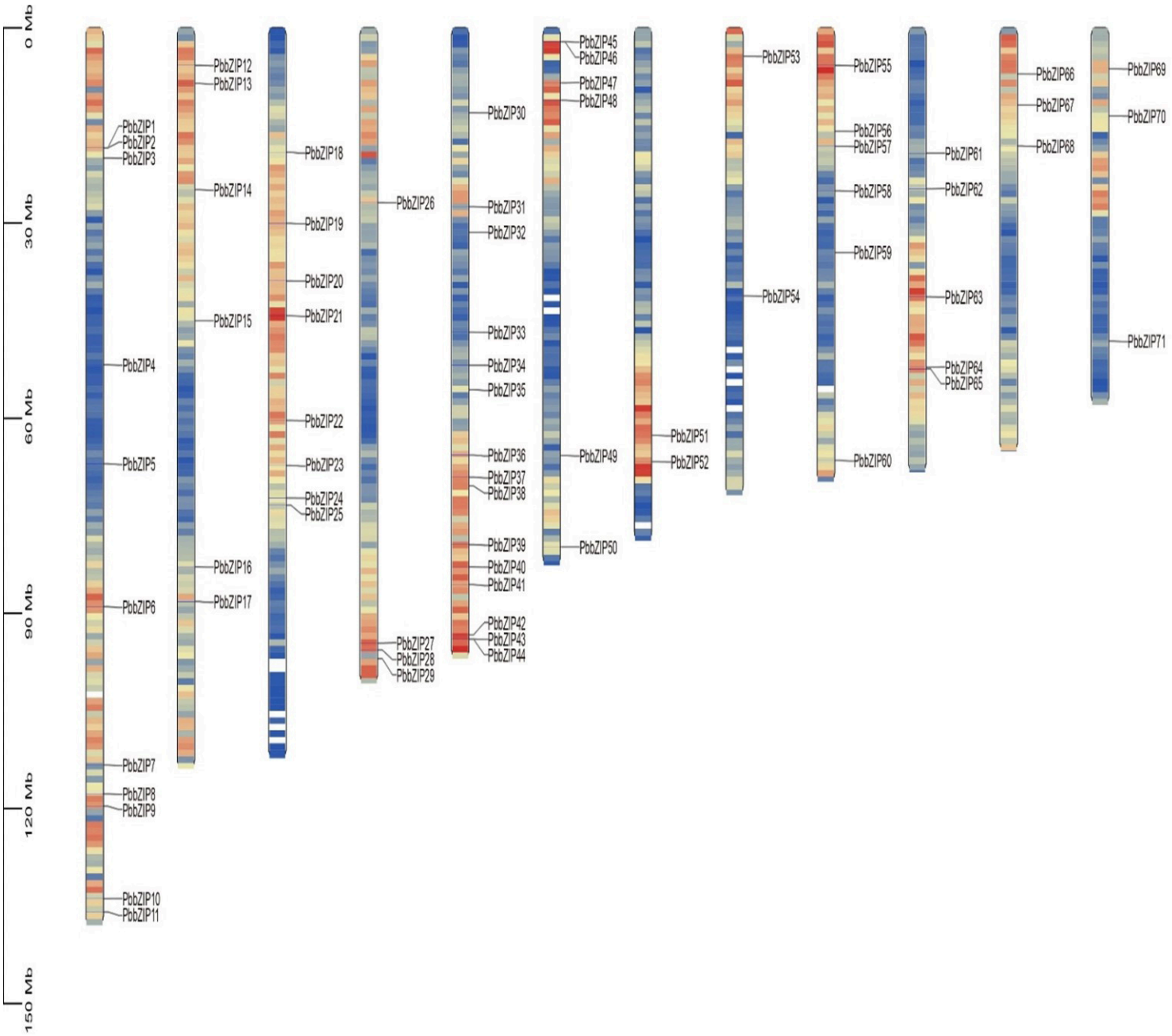


Figure 1

Chromosomal location and distribution of *PbbZIP* genes. The black line at the right side imprise 71 mapped *PbbZIP*genes meausure in a mega base scale. Chromosome numbers are counted horizontaly from left to right side of the chromosomes.

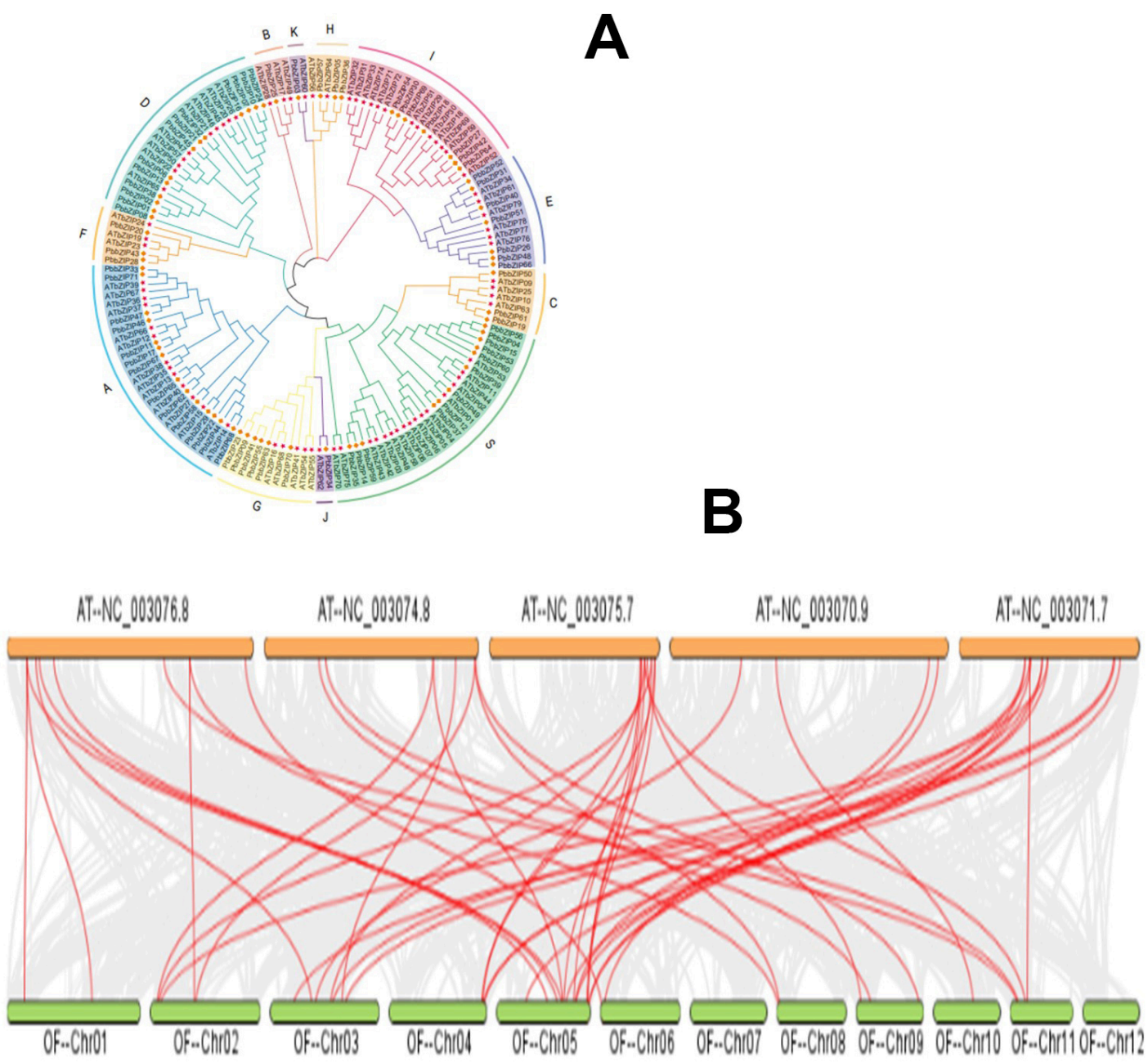


Figure 2

Phylogenetic tree showing the categorization of *P. bournei bZIP* gene family members into 12 major subgroups based on multiple sequence alignments of 75 *A. thaliana*bZIP proteins and 71 Phoebe bournei bZIP proteins. The subgroups are labeled as K, B, D, F, A, G, J, S, C, E, and I. The collinearity analysis indicates that 11 of the 12 chromosomes identified in *P. bournei* are collinear with genes identified on A. thaliana chromosomes at positions 003076.8 to 003071.7 on the genomic sequence.

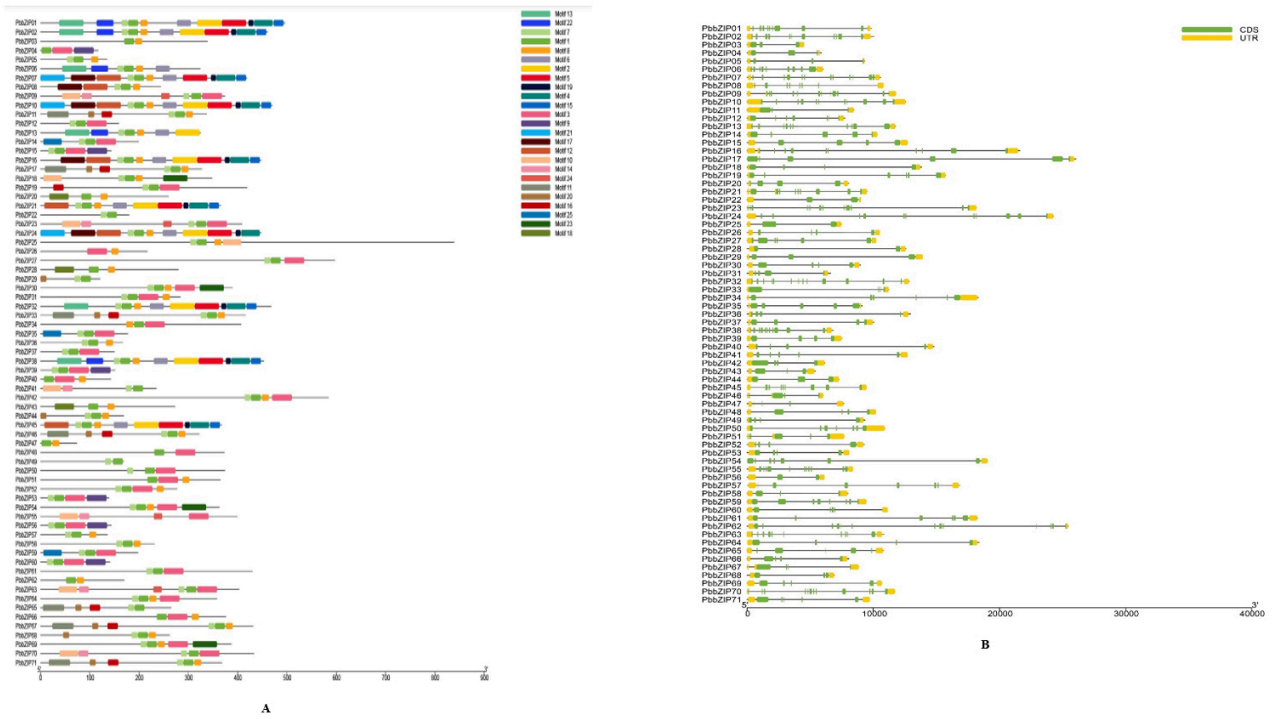


Figure 3

(a) The arrangement of the protein motifs of the PbbZIP; (b) Collectively examination of *PbbZIP* gene structure. Green boxes indicate exons (CDS), black lines indicate introns, and yellow boxes indicate 5’ and 3’ untranslated regions.

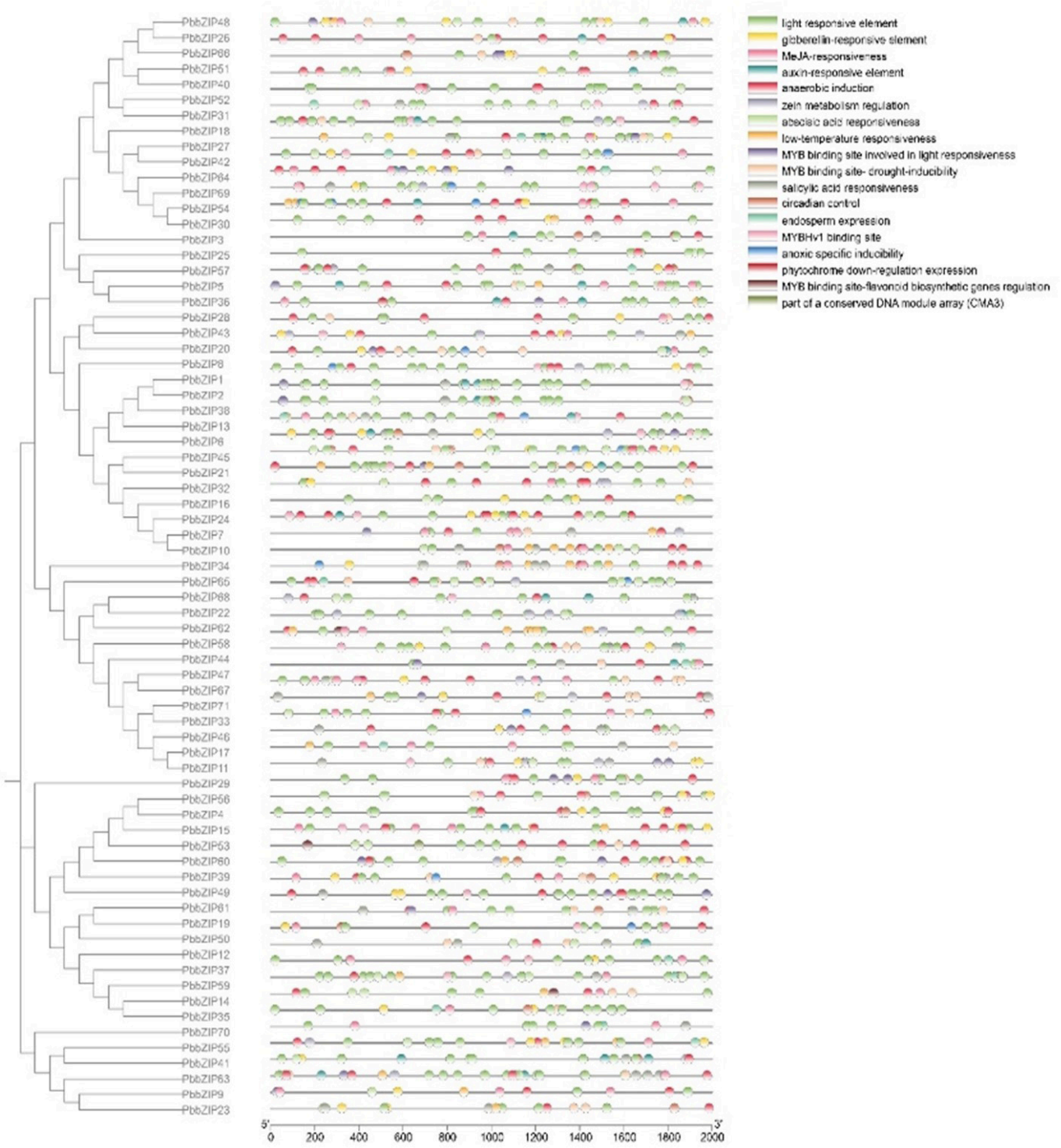


Figure 4

The promoter regions of *PbbZIP* genes were analyzed to predict the presence of cis-acting elements. The promoter position (-2000 bp) is indicated on the left. These cis-acting regulatory elements were classified into 22 types, each represented by a different color. The quantity of each cis-acting element is shown on the lower axis.

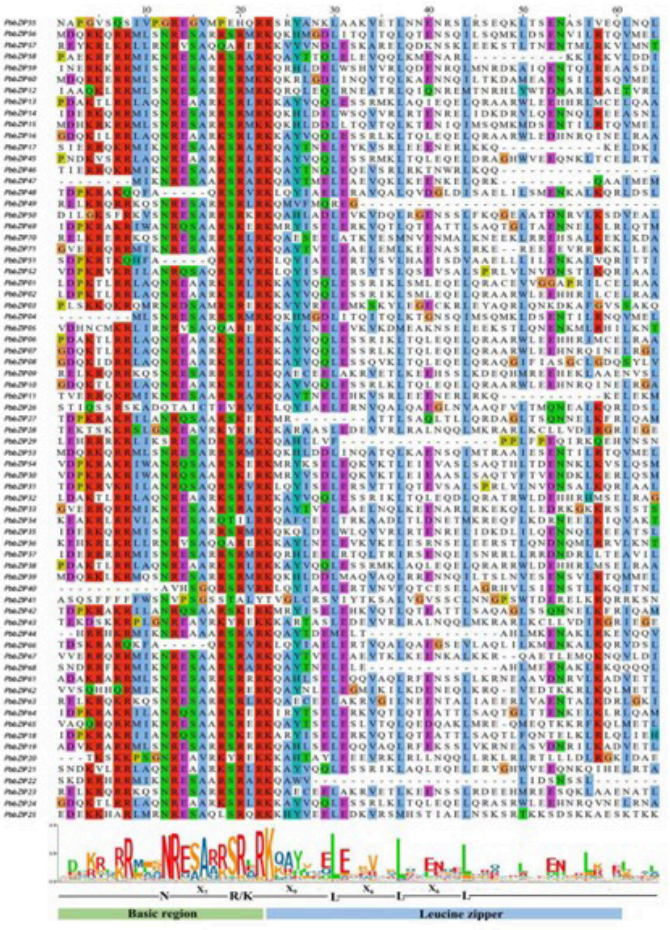


Figure 5

The multiple sequence alignment of the DNA binding domain in the PbbZIP protein sequence. The consensus sequence logo PbbZIPs conserved motifs is shown at the bottom.

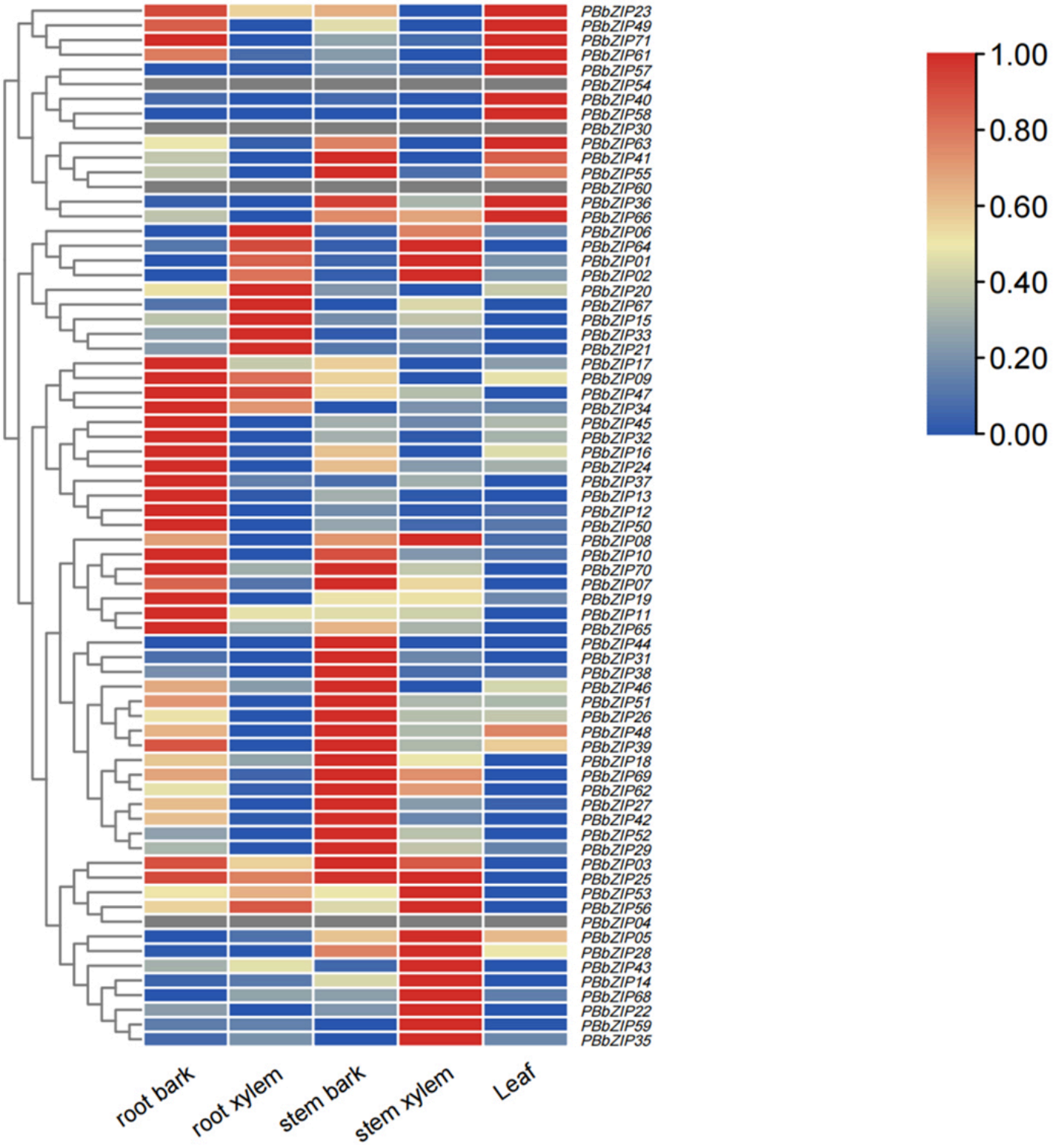


Figure 6

The expression patterns of 71 *PbbZIP*genes in various tissues are examined. These tissues include root bark, root xylem, stem bark, stem xylem, and leaves. The high transcript abundance is represented by the color red, while the low transcript abundance is represented by the color blue.

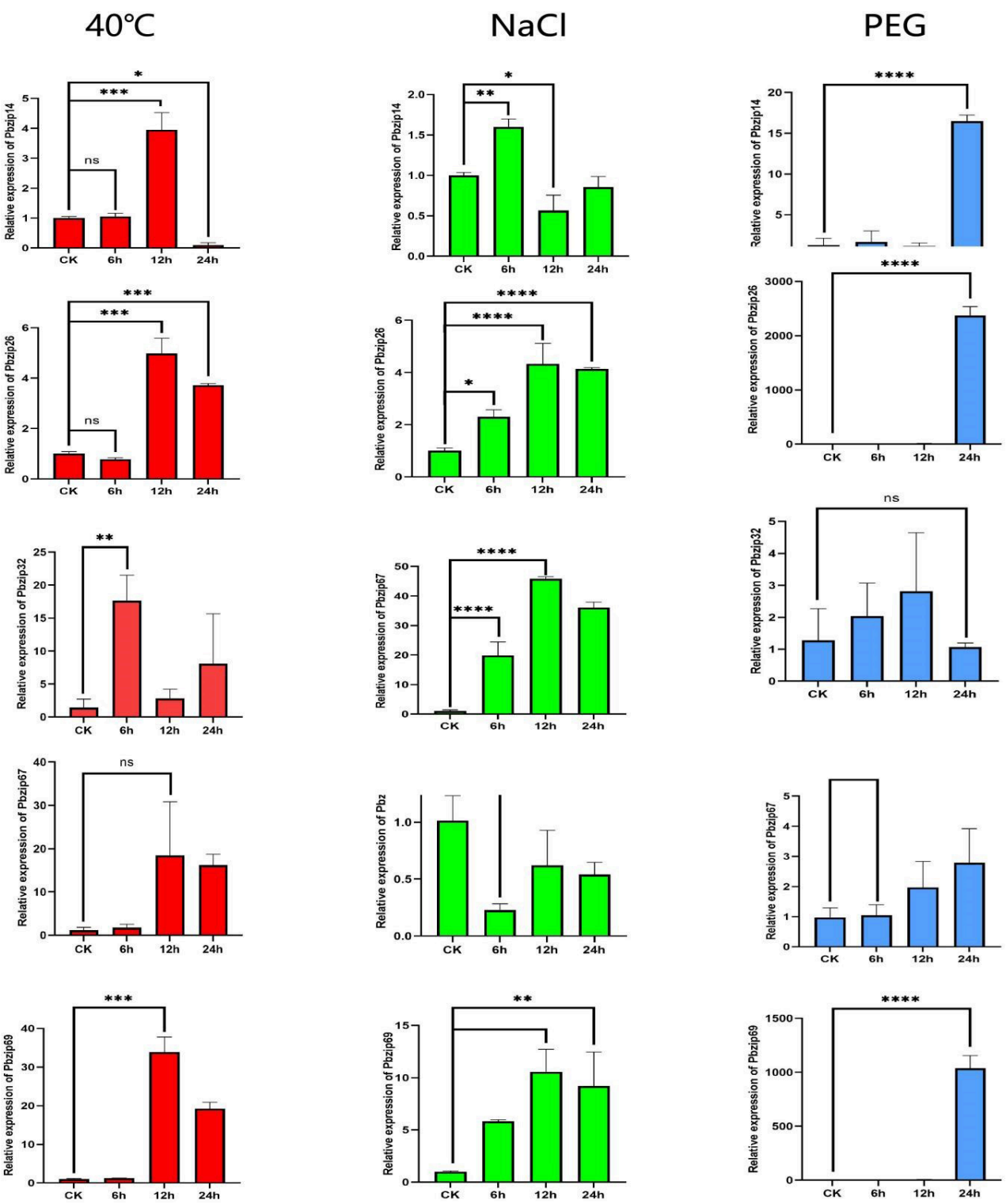


Figure 7

The relative expression levels of *PbbZIP*genes responding to abiotic stress using qRT-PCR. Red (CK) denotes high temperature, green denotes high salt, and blue denotes drought stress. Each treatment was run for the same time period (0, 6, 12, 24 h).