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Characterization of 33 *HbbZIP* Gene Family Members and Analysis of Their Expression Profiles in Rubber Tree in Response to ABA, Glyphosate and Powdery Mildew Treatment

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Abstract: Plant bZIP transcription factors play important roles in nearly all biological processes. Elucidation of the potential functions of these proteins in rubber trees will help to improve breeding and cultivation techniques. Here, we identified 33 *HbbZIP* family genes based on genomic data and named them according to their homologs in *Arabidopsis thaliana*. The genes were divided into 10 subgroups, namely, A to I. All the proteins had three motifs that varied in the different subgroups. The exons and introns were also analyzed on the basis of DNA sequence analysis. Expression analysis revealed that the 33 *HbbZIPs* were expressed primarily in the flowers, followed by the leaves and roots, while the lowest expression was detected in the latex and bark. In response to ABA treatment, the genes were significantly differentially expressed. The highest *HbbZIP*38 level increased by approximately 21-fold, and the lowest *HbbZIP*56 level decreased by 21-fold. In response to powdery mildew infection, most *HbbZIPs* were upregulated at 6 h after treatment; however, *HbbZIP*58 and *HbbZIP*47 were downregulated at this time point. In response to glyphosate, the expression of only *HbbZIP*21 and *HbbZIP*38 decreased, while that of the other HbbZIP proteins increased. Taken together, these results suggested that members of the different *HbbZIP* subgroups have specific functions. Overall, this study lays a solid foundation for further exploration of the potential roles of *HbbZIPs* in rubber trees.

Keywords: *HbbZIPs*; *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg.; ABA; glyphosate; powdery mildew

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1. Introduction

bZIP transcription factor (TF) family members participate in plant signaling in response to light, growth and development, pathogen defense, stress responses through the abscisic acid (ABA) signaling pathway, etc. [1–3]. bZIPs have a conserved bZIP domain that consists of 60–80 amino acids and is surrounded by two functionally different domains: a basic domain and a leucine zipper [4]. The basic domain comprises approximately 16 amino acid residues that bind to specific DNA sequences, and the leucine zipper domain is tightly bound to the alkaline domain [5]. bZIP proteins preferentially bind to ACGT-containing DNA sequences, especially G-box (CACGTG), C-box (GACGTC) and A-box (TACGTA) sequences [6,7]. When bZIPs bind to DNA, the N-terminus of the basic domain comes into contact with the C-terminus of the double-stranded DNA leucine zipper domain and mediates dimerization to form a superposed coiled helix [8,9]. Members of the bZIP TF family have been identified in plant species such as *Arabidopsis thaliana* [10] cassava [11],

poplar [12] and tobacco [13]. The members of the bZIP TF-encoding gene families in *Arabidopsis thaliana* [14] and cassava [15] are divided into 10 subfamilies, while those in rice, cucumber [16] and potato [17] are divided into eleven, eight and nine subgroups, respectively. The members of different subgroups have different functions, of which subgroup A is the most widely studied and whose members are categorized as ABA-responsive element binding proteins (ABREs) or ABRE-binding factors (ABFs) [14,18–20]. Members of subgroup A are involved in the regulation of the response to stress and ABA signaling. Plant responses to ABA signals and various stresses are achieved mainly by inducing the expression of a series of genes through ABREs. ABRE/ABF TFs can widely bind gene promoters containing ABREs, thus initiating the expression of downstream genes. In addition, ABA and various stresses can induce the expression of ABRE/ABF TFs, and ABREs/ABFs are activated via ABA-induced phosphorylation [14]. The subgroup A BI5 (*AtbZIP*39) gene can be induced in response to ABA and stresses such as drought and high salinity and plays an important role in plant stress resistance [16]. In addition, it has been found that genes of subgroup C and subgroup S are involved in the stress response and that the genes of subgroup F are expressed in response to zinc deficiency stress [21].

The rubber tree (*Hevea brasiliensis*) is a perennial tropical plant species that originated in the Amazon rainforest. Rubber trees are an important source of natural rubber (NR; cis 1,4-polyisoprene) [22,23]. Rubber trees produce relatively large amounts of NR and have unique physical properties [24]. At present, rubber tree cultivation is often affected by stresses such as drought [25,26], phytotoxicity [27] and leaf disease [28,29]. These stresses reduce NR production and result in economic losses. Spraying glyphosate on rubber trees can cause adverse effects such as new leaf deformities, chloroplast structural changes and soluble sugar content reductions. The causal agent of powdery mildew, which affects rubber trees, is transmitted through the air and can severely hinder NR production [30]. Natural rubber is an indispensable raw material for the transportation, medicine and national defense industries, and has great economic value. Rubber trees are the main source of natural rubber and make great contributions to China's economic construction and social development. The Reyan73397, bred from PR107 × RRIM600, is one of the varieties of rubber trees, has excellent rubber production performance and is one of the rubber tree varieties with a large planting area in the rubber planting area of China. ABA regulates plant growth and is involved in plant abiotic stress tolerance, and plants synthesize ABA in response to stress. On the basis of the function of *bZIP* genes associated with resistance in banana (*Musa acuminata* L.) [31], cotton (*Gossypium hirsutum* L.) [4] and cabbage (*Brassica oleracea* L.) [32], we speculated that *bZIP* genes in *Hevea brasiliensis* also have different functions in stress resistance. Therefore, in this study, both bioinformatics analysis of 33 *HbbZIP* family genes and expression analysis were used to study the mechanism of *bZIP* genes in response to ABA, glyphosate treatment and powdery mildew infection. These results serve as a theoretical basis for studying the response mechanism of *HbbZIP* genes and other candidate genes for use in pesticide and disease control.

2. Methods

2.1. Plant Material

Rubber tree plant material was collected from the teaching station of the Danzhou Campus of the School of Plant Protection, Hainan University. The samples were collected from Reyan73397 healthy tapping trees, and tissue culture-generated seedlings were used for subsequent assays. First, leaves, roots, bark, latex and flower tissues were collected from 18-year-old rubber trees for tissue analysis to measure the transcript levels in different tissues. Second, the experimental group consisted of 2-year-old tissue culture-generated seedlings treated with 200 μmol·L^{-1} ABA, and the plants of the control group were sprayed with distilled water. The leaves of the plants of two groups were collected after 0, 0.5, 2, 6, 12 and 24 h. The tissue culture-generated seedlings were sprayed with 200 μmol·L^{-1} glyphosate. The leaves of plants of the two groups were collected after 0, 0.5, 3, 6, 12 and 24 h. Third, two groups of 1-year-old tissue culture-generated seedlings were selected as

the experimental group and the control group, with six seedlings in each group. After we treated the plants with sulfur powder for one week, the leaves were washed, the sulfur powder was removed and the leaves were dried. The experimental group was inoculated with the powdery mildew isolate HO-73, and the control group was not treated and strictly isolated. Then, the treated rubber seedlings were incubated at 18–22°C, 70%–90% RH and via a light and dark cycle of 12 h:12 h. The leaves of the plants of two groups were collected at 0, 3, 6, 12 and 24 h after powdery mildew infection [29]. All of the above materials were used for RNA extraction and real-time quantitative PCR (qRT-PCR) quantification experiments after reverse transcription. Each sample included three independent biological replicates, and all the harvested samples were immediately frozen in liquid nitrogen and stored at –80 °C.

2.2. Gene Sequence Acquisition

The sequences of *bZIPs* of model plant species such as maize, *Arabidopsis thaliana* and rice were downloaded from the NCBI protein database, *Arabidopsis thaliana* database and rice database, respectively. Arabidopsis cDNA sequences were used to search the Hevea genomic database [33]. The sequences obtained were analyzed and compared using BLAST with the default settings, and redundant sequences were manually removed. The ORFs of the genes were analyzed via NCBI ORFfinder, and the amino acid sequences of the candidate genes were predicted via searches of the NCBI Conserved Domains Database. The physical and chemical information of the proteins, such as their molecular weight (MW) and isoelectric point (pI), was predicted via ExPASy. The relevant website information is listed in Table 1.

Table 1. Bioinformatics tools and websites.

Name	Website
NCBI ORF Finder	https://www.ncbi.nlm.nih.gov/orffinder/ (1 January 2020)
ExPASy ProtParam	http://web.expasy.org/protparam/ (1 January 2020)
NCBI Conserved Domains Database	http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi (1 January 2020)
Program SCANPROSITE	http://prosite.expasy.org/scanprosite/ (1 January 2020)
SWISS-MODEL	
TMHMM Server 2.0	https://services.healthtech.dtu.dk/service.php?TMHMM-2.0 (1 January 2020)
SignalP 5.0 Server	https://services.healthtech.dtu.dk/service.php?SignalP-5.0 (1 January 2020)
MEME	http://meme-suite.org/tools/meme (1 January 2020)
WoLF PSORT prediction	http://psort.hgc.jp/form.html (1 January 2020)
PlantCARE	http://bioinformatics.psb.ugent.be/ (1 January 2020)
ClustalW	http://www.clustal.org/ (1 January 2020)
GSDS	gsds.gao-lab.org (1 January 2020)
NCBI database	http://www.ncbi.nlm.nih.gov/guide/ (1 January 2020)
TAIR	http://www.arabidopsis.org/ (1 January 2020)
Rice Genome Annotation Project	http://rice.plantbiology.msu.edu/analyses_search_locus.shtml (1 January 2020)

2.3. Structural and Functional Analyses

The NCBI Conserved Domains Database and Pfam database were used to analyze conserved domains encoded by the genes, and Wolf PSORT prediction was used to predict the subcellular localization of the proteins encoded by the genes. The cis-acting elements within the promoter region were also analyzed, and the sequence 1 kb upstream of the start codon was obtained from the genome sequence. The cis-acting elements were searched via PlantCARE software to determine whether the promoter region contained any cis-acting elements. NCBI online BLAST was used to analyze similarities among the gene sequences.

The exon–intron structure of the genes was determined via alignment between the coding sequence and genome sequence, and the exon–intron structure was mapped by the Gene Structure Display Server (GSDS).

Protein motifs were predicted using MEME 4.9.1 with an optimal width of 16–100 residues.

2.4. Sequence Alignment and Phylogenetic Analysis

Multiple Sequence Alignment (MUSCLE) in Molecular Evolutionary Genetic Analysis (MEGA X) was used for the obtained amino acid sequences, with the default parameters. Phylogenetic analysis was performed using the neighbor-joining tree (NJ) method, and the parameters were verified with 1000 bootstraps. A phylogenetic tree was subsequently constructed after ambiguous branches were removed.

2.5. Expression Analysis of 33 HbbZIPs via qRT-PCR

Specific primers for the 33 *HbbZIPs* (Supplementary Table S2) were used for qRT-PCR amplification, and *HbActin* (GenBank accession: HO004792) was used as a housekeeping gene. A PCR was performed in a 20 µL reaction mixture consisting of 1× SYBR Premix Ex Taq (TaKaRa Bio, Inc. Dalian, China). After the reaction was complete, the cycle threshold (C_T) value was calculated, after which the $\Delta\Delta C_T$ algorithm was used to calculate the relative expression levels of the target genes [34]. All 3 biological replicates were analyzed via one-way ANOVA, and multiple comparisons were performed via the Tukey's test at $p < 0.05$ with OriginPro 2018 (OriginLab Corporation, Northampton, MA, USA).

3. Results

3.1. Identification and Analysis of bZIP Gene Family Members in Rubber Tree

The sequences of bZIPs in *Arabidopsis thaliana* and rice were used as queries for searching rubber tree bZIP family TF sequences in the rubber tree genome and transcriptome databases. After removing repetitive genes, 84 candidate rubber tree bZIP family members were identified, and their bZIP domains were analyzed (Supplement Table S1). The size and sequence of the 84 *HbbZIP* genes varied widely, and their encoded proteins, physicochemical properties and positions of bZIP-related domains in proteins also differed. The 84 HbbZIP proteins ranged from 81 to 767 amino acids. EXPASY analysis indicated that the relative molecular mass of the HbbZIPs ranged from 9887.37 to 83,007.31 Da, and the isoelectric points ranged from 5.04 to 10.16 (Supplement Table S1). We named the rubber tree *bZIP* genes based on the homologs in *Arabidopsis thaliana* and selected 33 *HbbZIPs* from subgroups A–S for further study (Figure 1).

3.2. Phylogenetic Tree of 33 HbbZIP Family Genes and AtbZIP Family Genes

A comprehensive phylogenetic analysis was performed to understand the evolutionary significance of the domains in the HbbZIP proteins and the bZIP protein sequences in Arabidopsis. All bZIP proteins of both species were classified into 10 groups, which were named as the A to I subgroups and the S subgroup. There were 5, 3, 3, 5, 1, 2, 2, 4 and 8 members of the HbbZIP family in the A, B, C, D, E, G, H, I and S subgroups, respectively (Supplement Table S1). The A and S subgroup contain at least five members. From the phylogenetic tree, we determined that there is a parallel evolutionary relationship between the *bZIP* genes among the clusters in rubber tree and Arabidopsis. The distribution of *bZIP* genes among the subgroups in rubber tree is approximately the same as that in Arabidopsis, rice and cassava.

3.3. Analysis of the Gene Structure and Function of the bZIP Family Members in Rubber Tree

To gain insight into the function of HbbZIPs, we analyzed the structure of 33 *HbbZIP* genes and proteins. The thirty-three HbbZIP proteins have a specific bZIP domain, although the position of the bZIP domain within the HbbZIP protein varies widely. In addition to searching for the bZIP domain, Multiple EM for Motif Elicitation (MEME) software was used to search for three conserved motifs and map them. Most of the HbbZIP proteins that clustered along the same branch shared one or more conserved motif or structure (Figure 2). Most motif-encoding sequences were present in the genes that composed the same subgroup, meaning that these family members may have the same function. The D subgroup had three motifs, and the A, B, C, D, E, G, H, I and S subgroups had one motif. Among the members of the nine subgroups, those of the D subgroup had the most motifs,

and each D subgroup member had three motifs. Taken together, these results suggest that members of subgroup D have multiple functions (Figure 2).

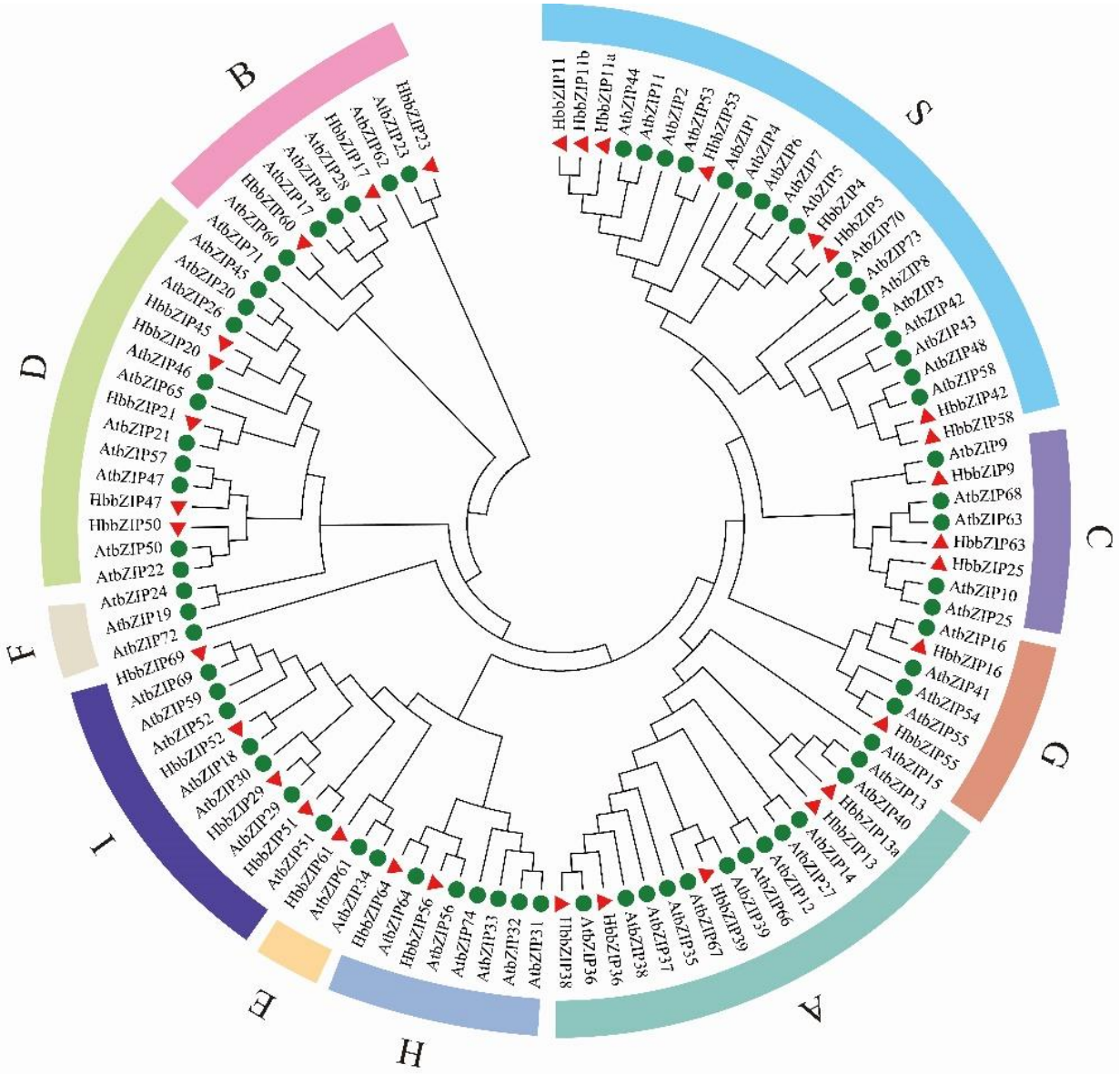

Figure 1. Phylogenetic tree of bZIP family genes among 33 *HbbZIPs* and 74 *AtbbZIPs*.

The genes in the same subgroup have similar exon–intron structures and conserved motif distributions, which have certain evolutionary importance. The members of the *bZIP* gene family in the rubber tree can be divided into two types: those enriched in introns and those with few introns. Among the members, eight S subgroup ones have no introns, and members of the B subgroup *HbbZIP*17 and A subgroup *HbbZIP*13 have only one intron. Members of the H subgroup have three introns, those of I subgroups *HbbZIP*52 and *HbbZIP*69 have two introns, and those of *HbbZIP*29 and *HbbZIP*51 have three introns (Figure 3).

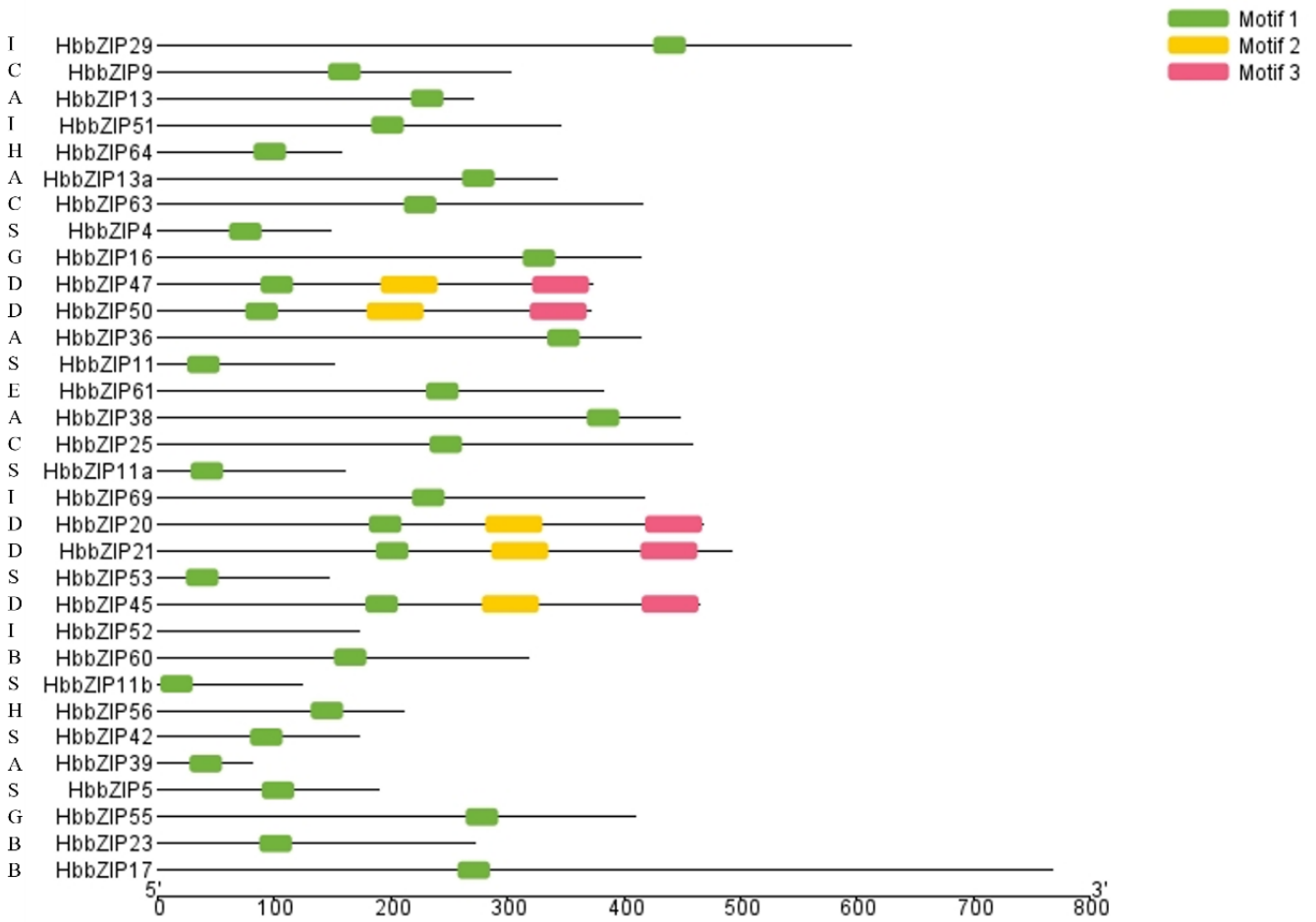

Figure 2. Motif structure of 33 *HbbZIPs* in rubber trees.

3.4. Analysis of HbbZIP Expression in Different Tissues in Response to ABA, Powdery Mildew and Glyphosate Treatment

To identify differentially expressed genes, the expression of 33 *HbbZIP*s in different tissues in response to ABA, glyphosate and powdery mildew treatment was analyzed. As shown in Figure 4, overall, *HbbZIP*s were highly expressed in the flowers, followed by the leaves and roots, and were expressed the lowest in the latex and bark. Specifically, *HbbZIP*13 and *HbbZIP*29 were highly expressed in the flowers, and *HbbZIP*11*a* and *HbbZIP*11 were highly expressed in the flowers, leaves and roots, suggesting that these genes may play an important role in these tissues (Figure 4). To further explore the functions of *HbbZIP*s in response to abiotic stress, we analyzed the expression of *HbbZIPs* in rubber tree leaves at 24 h after ABA treatment. Most *HbbZIP* family members were downregulated or upregulated in the early stage of ABA treatment. Among the A subgroup members, *HbbZIP*13*a*, *HbbZIP*139 and *HbbZIP*138 were expressed at the highest level at 2, 24 and 12 h after ABA treatment, respectively. The B subgroup member *HbbZIP*17 was induced by ABA, and its expression continued to increase until it was 4.2 times that at 24 h compared to the control level. The expression of *HbbZIP*25, *HbbZIP*63 and *HbbZIP*9 was the highest in the C subgroup at 12 h after ABA treatment. Among the D subgroup members, *HbbZIP*21 was downregulated after ABA treatment, whereas the other members were upregulated. The expression of both the E subgroup and H subgroup members increased after ABA treatment. The other two genes of subgroup G were downregulated at 0.5 h and 24 h after ABA treatment. The expression of subgroup I members *HbbZIP*29 and *HbbZIP*51 decreased in response to ABA treatment whereas that of *HbbZIP*69 increased, and the expression increased 8-fold at 24 h. Most members of the S subgroup were also upregulated in response to ABA treatment (Figure 5).

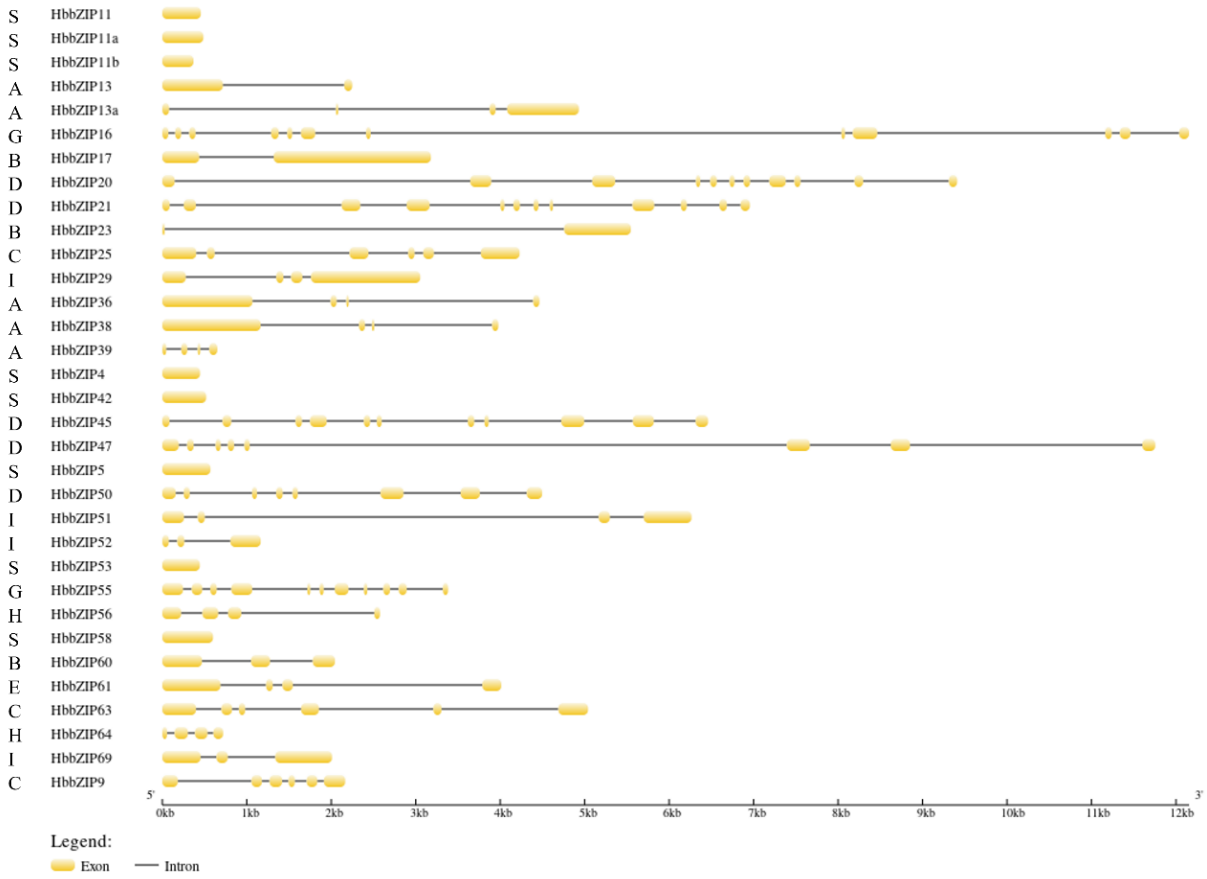

Figure 3. Exon–intron structure of 33 *HbbZIP* genes in rubber trees.

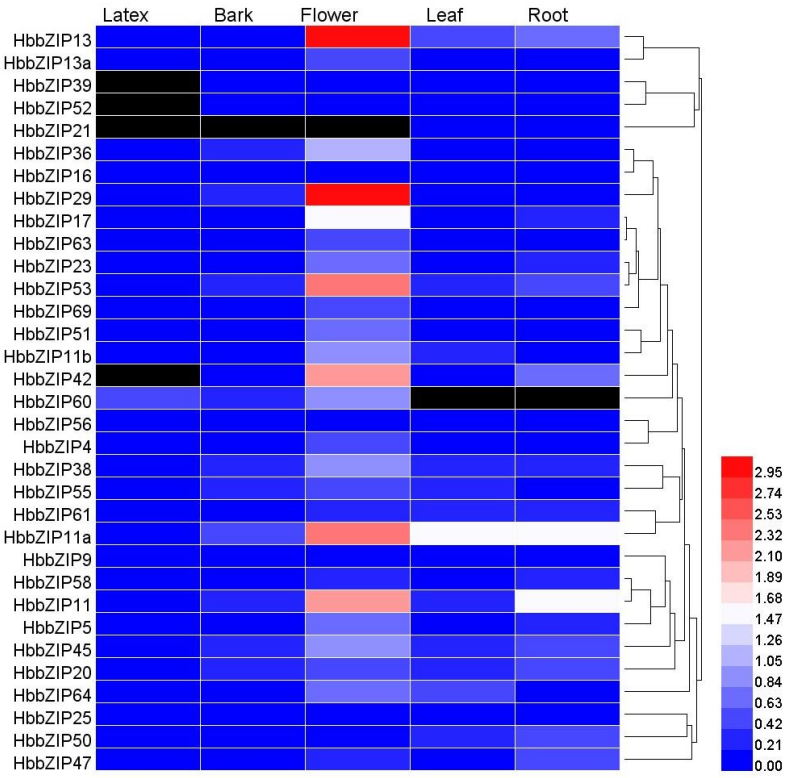

Figure 4. Expression of 33 *HbbZIPs* in different tissues in rubber trees.

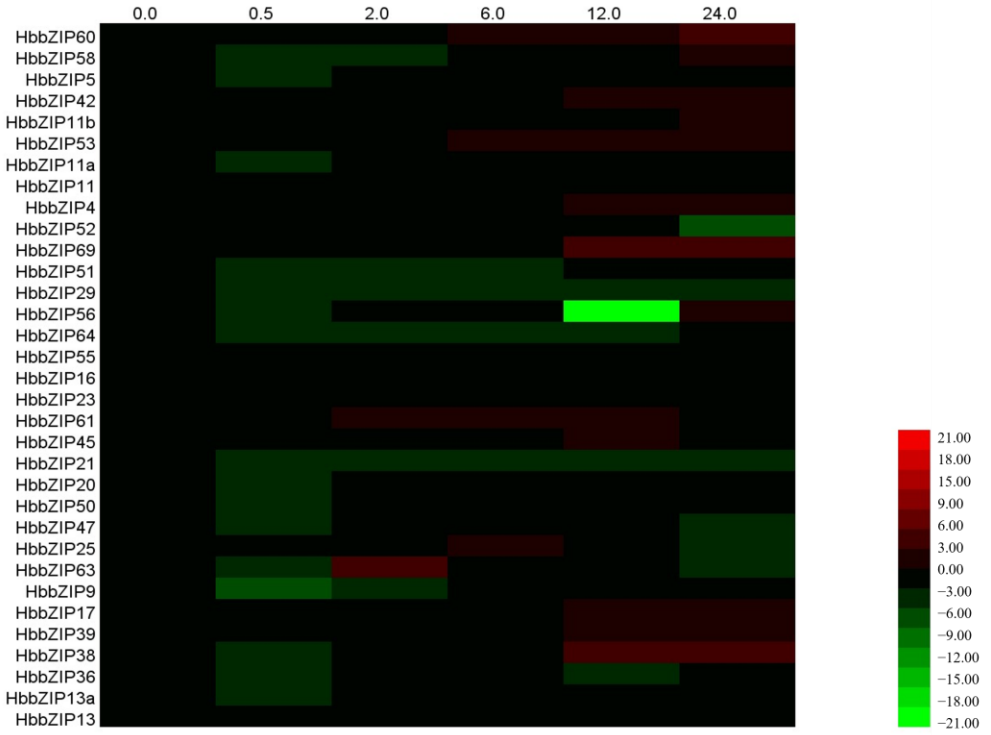

Figure 5. Expression of 33 *HbbZIPs* in rubber trees in response to ABA treatment.

For biotic stress, we analyzed the expression of *HbbZIP*s in rubber tree leaves for 0–24 h after powdery mildew infection. The results showed that *HbbZIP*s were differentially expressed after powdery mildew infection. For example, *HbbZIP*60 in the B subgroup, *HbbZIP*11 in the S subgroup and *HbbZIP*13 and *HbbZIP*13*a* in the A subgroup were significantly upregulated by approximately 15–21-fold. In contrast, *HbbZIP*4 in the S subgroup and *HbbZIP*47 in the D subgroup were significantly downregulated (Figure 6). We also analyzed the expression of *HbbZIP*s in rubber tree leaves at 24 h after glyphosate treatment. The expression fold change for all genes of subgroup A in response to glyphosate increased at 0 h at 12 h, although the expression of *HbbZIP*38 was downregulated. The B subgroup *HbbZIP*17 gene was upregulated in response to glyphosate treatment. At 0.5–12 h after glyphosate treatment, the levels increased by approximately 10-fold. Notably, in the C subgroup, the *HbbZIP*25 gene was not expressed, and in the D subgroup, *HbbZIP*9 and *HbbZIP*63 were expressed at 0 h and 24 h after glyphosate, and *HbbZIP*47 was upregulated at 24 h (15.5-fold increase in expression). The E subgroup gene *HbbZIP*61 was upregulated after glyphosate treatment, and the gene expression fold change decreased to almost 0 at 24 h. The *HbbZIP*55 and *HbbZIP*16 genes of the G subgroup were upregulated in response to glyphosate, whereas the *HbbZIP*56 and *HbbZIP*64 genes in the H subgroup were barely expressed at 24 h. Several genes other than *HbbZIP*29 in the I subgroup were also upregulated in response to glyphosate until the expression fold change nearly peaked, which occurred at 6 h after treatment. The S subgroup genes *HbbZIP*53, *HbbZIP*4, *HbbZIP*58 and *HbbZIP*5 were upregulated in response to glyphosate after 0.5–12 h, and the *HbbZIP*42 gene was also upregulated at 12 h after glyphosate treatment, with a 12-fold maximum expression fold change (Figure 7).

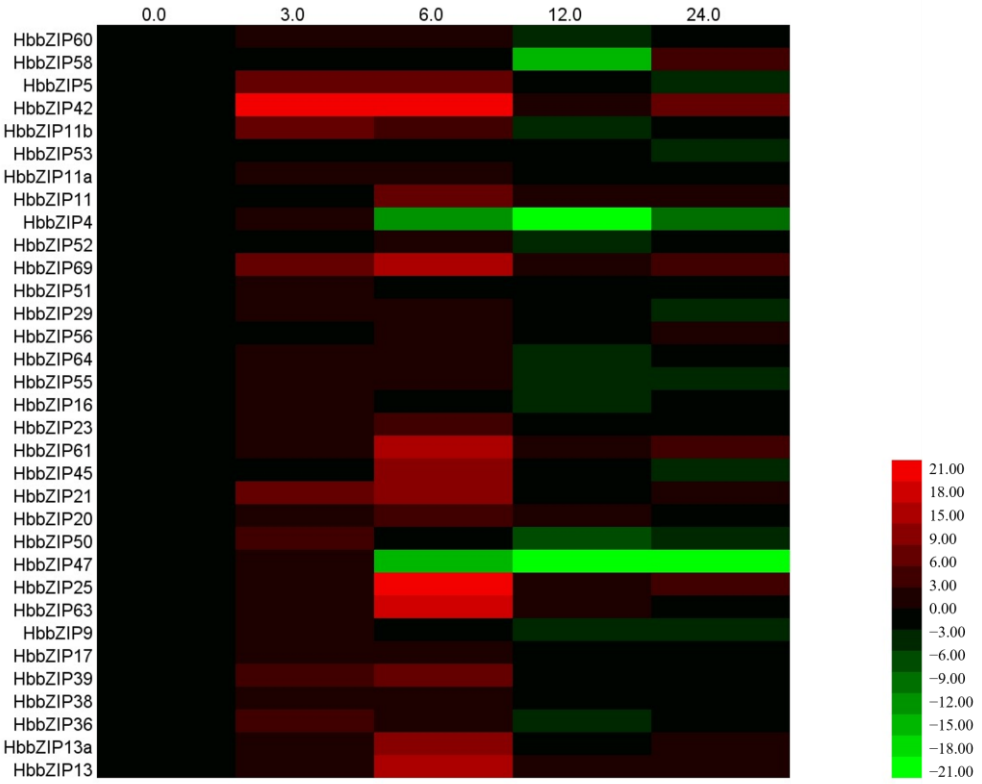

Figure 6. Expression of 33 *HbbZIPs* in rubber trees in response to powdery mildew infection.

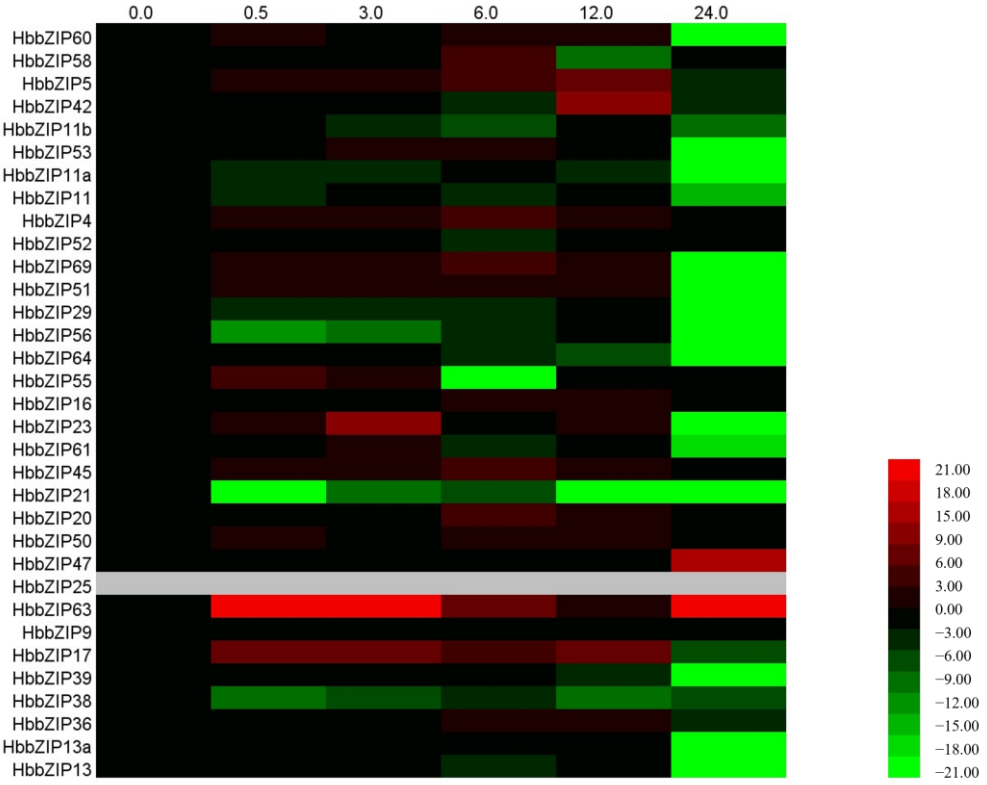

Figure 7. Expression of 33 *HbbZIPs* in rubber trees in response to glyphosate treatment.

4. Discussion

Owing to the importance of bZIP TFs, numerous studies on these proteins have been conducted on model plant species [35]. In Arabidopsis, 77 members of the *bZIP* gene family have been identified [20,36]. However, researchers found that the sequences of some of these genes encoded incomplete bZIP domains and ultimately identified 72 *bZIP* gene members in *Arabidopsis thaliana*. Members of the rice *bZIP* gene family have previously been identified at the genome-wide level [5], and a detailed comparison between rice *bZIP* genes and sorghum *bZIP* family genes revealed only 88 *bZIP* genes in the former [37]. It has been reported that the castor bean [38], maize [39] and poplar [20] genomes encode 49, 125 and 89 members, respectively, of the *bZIP* gene family. In this study, 85 *HbbZIP* genes were identified via bioinformatics methods for the first time in rubber trees. To analyze the evolution of the *HbbZIP* gene family in rubber trees, we constructed an evolutionary tree and found that the *HbbZIP* gene family could be divided into nine subgroups (Figures 1–3); this number of subgroups was similar to those in tomato and bean, three more than that in cucumber, two more than that in sorghum and one fewer than those in rice and maize [5,39]. Phylogenetic analysis revealed that most branches contained bZIP proteins in Arabidopsis and rubber trees, suggesting that at least some of the bZIP TFs appeared before the divergence of monocotyledons and dicotyledons. The evolutionary tree also shows that homologous bZIP TF-encoding genes exist. Therefore, it can be inferred that the structure and function of *HbbZIPs* have been conserved across species during evolution.

The genomic structure of each gene can be considered an imprint of the record of the key to evolution and thus provides insight into the emergence and evolution of a particular gene or a particular family of genes. To understand the structural evolution of the *HbbZIP* gene, we analyzed the structure of each *HbbZIP* family gene and predicted its function. The structure of the *HbbZIP* genes in each subgroup was similar, and the structural differences among genes in the different subgroups were readily apparent, indicating that there were large differences among members of the *HbbZIP* family. Members of the banana G and D subgroups have more than nine exons, while members of the other subgroups have fewer introns [15]. Moreover, the number of introns in the D and G subgroups of maize varies greatly [39]. The number of introns in subgroup D ranged from five to twelve, while the number of introns in subgroup G ranged from three to fourteen. The number of introns in the other subgroup members varied less, mainly from one to three. Similar to the findings associated with the banana *bZIP* gene structure [31], the exons of the D and G subgroups were the most abundant in rubber trees, while the exons of the genes in the other subgroups were less abundant than those in the D and G subgroups. The exon numbers of the genes in the D subgroup and G subgroup in rubber trees were stable at 8–12 and 10–12, respectively. The number of exons in the genes in the other subgroups was also high, with only a few subgroup genes having 1–3 exons. The exon–intron structure bears the imprint of the evolution of gene families, and according to previous studies, the rate of intron loss after gene fragment replication is higher than the rate of intron gain [40,41]. Therefore, it can be concluded that subgroups G and D may contain the original genes, while the other genes were derived through gene duplication and subsequently lost introns. Eight *HbbZIP* gene members of the S subgroup had no introns (Figure 3). Among the members with introns, the number that were within the open reading frame (ORF) ranged from one to eleven, indicating that the *HbbZIP* gene family members varied widely in terms of their intron number. Similarly, the number of exons in the genes in subgroups A and B varied greatly, ranging from 1–7 to 2–8, respectively. The number of introns in the other groups varied little, and there were five exons in the genes in subgroup C (Figure 3). The locations at which introns are present within the ORFs vary greatly, and the splicing sites have different phases; however, the positions and phases of introns in the alkaline region of the bZIP domain and the leucine zipper domain are highly conserved. In grape [42], banana [31] and other plant species, the *bZIPs* in the same group have a similar exon–intron organization and similar conserved motifs, but there are differences among the members

of the different groups, indicating that *bZIPs* in the same group are closely related to the process of gene evolution.

According to studies on this gene family in other plant species, the expression of different *bZIP* genes under stress differs greatly, and the expression of genes in the same subgroup or the same gene in response to different treatments also differs. The expression of two *BrABI*5 genes in Chinese cabbage under drought treatment was completely opposite [32]: *BrABI*5*a* was downregulated at 0–12 h and upregulated at 12–24 h, and the expression level of *BrABI*5*b* peaked at 12 h (five times that before drought treatment) [32]. In the present study, the *HbbZIP*25 gene was not expressed in response to glyphosate but was expressed in response to ABA treatment. *HbbZIP*23 and *HbbZIP*55 were induced in response to glyphosate treatment but not ABA treatment. The expression of the *HbbZIP*38 gene in subgroup A strongly increased in response to glyphosate treatment but decreased in response to ABA treatment. It was found that the expression levels of five *CabZIP* genes in the leaves of cucumber continuously decreased under drought treatment [16]. After 12 days of drought stress in grape, 7 of the 45 *bZIP* genes were downregulated, and the expression of 25 of the upregulated genes was significant after 8–12 days of drought stress [42]. Like in other plant species, only partial and key bZIP TFs play roles in the response to drought stress, for example, carrot DcABF3 [43], *Camellia sinensis* CsbZIP18 [10] and *Brassica napus* BnaA10. ABF2 [44] play a role in response to drought stress and in the ABA signaling pathway. In the present study, we found that *HbbZIP*60, *HbbZIP*58 and other *HbbZIP*s responded to ABA treatment, suggesting that they potentially function in response to drought stress in rubber trees.

Interestingly, we found that most *HbbZIPs* differentially responded to powdery mildew infection (Figure 6). Usually, rubber tree resistance to powdery mildew relies on key genes such as *HbMLO* [45,46]. Studying pepper [47] and rice [48], researchers have also found that *CabZIP*2 and *RF*2*b* can increase disease resistance. These findings expand our knowledge about rubber tree disease resistance. Overall, we found that most *HbbZIPs* except *HbbZIP*58, *HbbZIP*4 and *HbbZIP*47 were upregulated in response to powdery mildew infection.

Furthermore, we found that *HbbZIPs* are expressed in response to glyphosate treatment. In NR production, glyphosate was found to be harmful to both tapping trees and rubber tree seedlings. After its applied, glyphosate as well as its metabolites can persist in the soil or water, leading to the production of damaging compounds [49,50]. In addition to mutations in the glyphosate target gene 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which can increase plant resistance to glyphosate [51,52], our results showed competition between glyphosate and ABA for the induction of *HbbZIP* expression. We found that, at 7 days after treatment with glyphosate, the leaves of rubber trees were completely yellow and began to fall off. However, bud-grafted seedlings sprayed with exogenous ABA gradually turned yellow, and no leaves fell off. These results suggested that exogenous ABA can alleviate glyphosate damage to rubber trees. These findings may be related to the induction of *HbbZIPs* in response to ABA. In summary, thirty-three *HbbZIPs* from nine subgroups were characterized, and their potential roles in biotic and abiotic stress responses were determined. Taken together, the results of this study serve as useful references for predicting and determining the biological functions of *bZIPs*.

5. Conclusions

In the research, 33 HbbZIP family genes were characterized and their potential roles in biotic and abiotic stress responses were identified. There were significant differences in the expression of different *HbbZIP* genes under stress. The highest (lowest) *HbbZIP* gene level increased (decreased) 21-fold after ABA treatment, respectively. The majority of *HbbZIPs* expression was upregulated after 6 h of powdery mildew infection and glyphosate treatment. These results imply members of different HbbZIP subgroups have specific functions that may be related to drought stress response, powdery mildew resistance response and relief from glyphosate damage in rubber trees. The results of this study

provide a foundation for further exploration of the potential role of *HbbZIPs* in rubber trees, which will facilitate the advancement of rubber tree breeding and cultivation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14030556/s1, Supplemental Table S1 Basic information concerning the 84 *HbbZIP* gene family members in rubber tree; Supplemental Table S2 qRT-PCR primers used for 33 *HbbZIPs* and *HbActin* for expression analysis.

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