Genome-wide Identification of R2R3-MYB Gene Family and Response to Stress in Zingiber officinale Roscoe

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Research Article

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Abstract

Background: *Zingiber officinale* Roscoe (ginger), as a plant used for medicine and food, has a pivotal position all over the world. The R2R3-MYB transcription factor family is one of the largest, and it plays a significant role in plant growth, development, and stresses resistance. According to existing reports, the amounts of R2R3-MYB genes varies greatly among all kinds of plants. However, genome-wide discovery of *ZoMYBs* family and its corresponding stresses have not been reported in *Z. officinale*.

Results: The genome-wide analysis of R2R3-MYB genes in *Z. officinale* was carried out in this study. Protein analysis included phylogenetic analysis and conserved motifs analysis, while gene analysis included chromosome localization and gene replication analysis, gene structure analysis, cis-regulatory element analysis and gene expression pattern analysis under two different stresses. A total of 299 candidate *ZoMYB* genes were discovered in *Z. officinale*. Based on the grouping of R2R3-MYB genes in a model plant *Arabidopsis thaliana*, *ZoMYB*s was divided into 8 groups and distributed across 22 chromosomes at uneven densities. Through gene duplication analysis, a total of 28 segmental duplication were identified in ginger genome. In addition, the expression pattern of 10 chosen *ZoMYB*s in leaves of *Z. officinale* were detected under ABA and low temperature stress treatments. The result revealed that there were differences in gene expression of these ten MYB genes in ginger under ABA treatment and low temperature treatment.

Conclusions: Our results revealed the characteristics of *ZoMYB* genes family and provided valuable information for improving the ability of *Z. officinale* to cope with environmental changes during growth and development.

Background

Ginger, as the rhizome of perennial herb ginger (*Zingiber officinale* Roscoe), appears in front of human beings with traditional Chinese medicine and condiment [1]. Ginger is grown all over the world, mostly distributed in tropical and subtropical regions, with the most diverse species in tropical Asia [2]. Since ginger has regular homologous attributes of medication and food, its applications are increasing diverse [3]. Ginger is subjected to a variety of challenges throughout its growth and development, so the optimization of genetic engineering brings hope for the improvement of ginger crops.

Transcription factors (TFs) are proteins binding to certain cis-acting regions and regulating the target genes [4]. The binding domains of TFs in the promoters of the target genes can be divided into several families [5]. MYB TFs consist of 1–4 adjacent repeat sequences (R) with a highly conserved domain, which containing approximately 52 amino acids. MYB TFs belong to one of most large gene family of proteins that have homologs in animals, plants, and yeasts [6, 7]. The characteristics of the repeats are spaced tryptophan residues at interval, which cover two a-helices helix-turn-helix (HTH), and the third helix-bound DNA main groove [8]. According to the number of neighboring repetitions, MYB TFs are categorized into four types, 1R (R1/2, R3), 2R (R2R3), 3R (R1R2R3), and 4R, respectively [6, 9]. The proteins of 3R-MYB exist in animals with limited amounts as usual, while a bulk amounts of 2R-MYB proteins contained in plants. The proteins of R2R3-MYB have been further divided into 25 subgroups in *A. thaliana* ([7].

There were 126 *AtMYB* TFs discovered in *A. thaliana* [7], and 244 *GmMYB* TFs identified in *Glycine max* [10], 205 *GrMYB* TFs in *Gossypium raimondii* [11], 166 *MeMYB* TFs in *Manihot esculenta* [12], 157 *ZmMYB* TFs in *Zea mays* [13], and 122 *SlMYB* TFs in *Solanum lycopersicum* [14]. The most fully explored functions of R2R3-MYB TFs were their regulatory roles in four key plant-specific processes, involving the response to various stresses and regulation of plant defense [15, 16], primary and secondary metabolic processes [17, 18], developmental processes, and cell fate and identity [19, 20]. For example, *AtMYB41* from *A. thaliana* is induced when the plants were exposed to high salt, abscisic acid (ABA), drought, and cold [21]. *VcMYB* from *Vaccinium corymbosum* may be involved in the regulation of drought stress [22]. *AtMYB102* is dramatically up-regulated under osmotic stress and ABA treatment, either [23]. The expression level of *OsMYB55* increased gradually in rice treated by high temperature, which led to improving plant growth [24]. Moreover, *OsMYB91* contributes a lot to plant growth regulation and salinity stress response in rice [25]. Mechanisms of *JrMYB* transcription factors in

response to drought and ABA treatment in *Juglans regia* [26]. *TaMYB4* in *Triticum aestivum* responds to some biological stresses, such as salicylic acid, ABA, MeJA and ethylene [27]. *GuMYB* from *Glycyrrhiza uralensis* regulates flavonoid accumulation under MeJA induction [28]. *GbMYBFL*, also related to flavonoid biosynthesis accumulation in *Ginkgo biloba*, is the most strongly linked to R2R3-MYB TFs [29].

R2R3-MYBs have been identified clearly and their functions have been validated in most plants, which offered us a better view of the functional classification of the TF subfamily. In ginger, nevertheless, little is known about this gene subfamily. The identification and analysis of 299 *ZoMYB*s were performed at the genome-wide level of *Z. officinale* in this article. A comparison with data from known MYB TFs in *A. thaliana* was also conducted, which resulted in a systematic genome-wide identification and comprehensive evolutionary analysis of these R2R3-MYB TFs. In addition, comparative investigation with the MYB genes of *A. thaliana* predicted the roles of protein in every subcategory of the *ZoMYB* family. We used quantitative real-time reverse transcription (qRT-PCR) analysis to quantify and compare the expression level of chosen MYBs under two stress treatments of ABA (abscisic acid) and cold treatment.

Results

R2R3-MYB transcriptional factors in Z. officinale

Totally, 299 R2R3-MYBs were discovered in *Z. officinale* based on their similarity to the R2R3-MYBs of *A. thaliana* and the MYB consensus sequence existing as well as the HMM profile of MYB-DNA binding domain. They were named *ZoMYB1-ZoMYB299* (Table S1) successively. Chemical properties analysis of these *Z. officinale* R2R3 MYB proteins revealed that their length ranged from 103 to 1651 amino acids, molecular weight ranged from 11.87 kDa to 182.93 kDa, and their isoelectric point (PI) ranged from 4.44 to 10.67 (Table 1). The subcelluar localization of these MYBs were predicted, most of them were in nucleus (89.97%), while a few were in cytoplasmic (5.02%), mitochondrial (4.01%) and endoplasmic reticulum (1.00%) (Table S2).

Phylogenetic evolution and classification analysis of R2R3 MYBs in Z. officinale

For further analysis the phylogenetic evolutionary relationship of these R2R3-MYBs in ginger and other model plants, all *ZoMYB*s proteins discovered in ginger and 126 existing MYBs (*AtMYB*s) proteins in *A. thaliana* were chosen to construct a phylogenetic tree. The amino acid sequence alignment of R2R3-MYB proteins of the two species were analyzed by bootstrap at 1000 replicates. Through the comparative phylogenetic analysis of *Z. officinale* and *A. thaliana*, the evolutionary relationship in interspecies of the R2R3-MYB gene family of the two plants can be constructed, and the function of the unknown target gene in ginger can also be predicted. The resulting phylogenetic tree was divided into 8 groups according to the known clustering of *AtMYBs* from *A. thalian*, and that named as group G1 to Group G8 successively (Fig. 1). Seven of these groups contained R2R3 MYB from both ginger and *A. thaliana*. However, *ZoMYB086* was not clustered with all of other R2R3-MYBs, so we divided it into a group (G4). Among the groups containing two species, The G1 group had the largest number of members, with a total of 106, of which 84 members were identified from *Z. officinale* and 22 members were discovered from *A. thaliana*. In comparison, the G3 group had the second smallest number of members, with 17 members, including 4 from *A. thaliana* and 13 from *Z. officinale*. In the remaining groups, group G2, G5, G6, G7 and G8 contain 86, 18, 30, 74 and 93 R2R3-MYB members, respectively.

Gene duplication and chromosomal location of R2R3-MYBs in Z. officinale

The physical locations of these *ZoMYB* TF genes were localized to the chromosomes of *Z. officinale* (ZoChr) by Mapgene2chrom online tool (Fig. 2). Among the 299 R2R3-MYB genes discovered, 293 MYB genes were distributed on 22 chromosomes. ZoChr08A and ZoChr08B contained 21 *ZoMYB* genes, which is the largest number in chromosomes, followed by 20 *ZoMYB* genes on chromosome of ZoChr01A. Most of the chromosomes contained 10 to 19 R2R3 MYB TFs, except ZoChr22A and ZoChr10A, they were five and four this kind of TFs, respectively. The above results show that the

distribution of genes on chromosomes is uneven. Obviously, the majority of *ZoMYB* genes are found at the top and bottom of chromosomes, and less in the central position of chromosomes, such as ZoChr01A.

Gene duplication events play an important role in promoting plant evolution, that is, new gene functions are derived with plant evolution [30]. The gene family expands through the segmental and tandem duplication events mainly [30]. Here, collinearity of R2R3-MYB TF family in ginger genome was analyzed, as shown in Fig. 3 and Table 1. A total of 28 segmental duplication events were identified in ginger genome.

Table 1
Duplication models for *ZoMYB* gene pairs in *Z. officinale*.

Seq_1	Seq_2	Ka	Ks	Ka/Ks	Effective Length	Average S-sites	Average N-sites
ZoMYB007	*ZoMYB027*	0.024980233	0.0181278	1.37800908	867	195.4166667	671.5833333
ZoMYB019	*ZoMYB036*	0.03799668	0.0428178	0.887403642	918	216.25	701.75
ZoMYB048	*ZoMYB060*	0.038458337	0.1232118	0.31213203	615	143.75	471.25
ZoMYB066	*ZoMYB082*	0.088251239	0.1346333	0.655493596	831	195.4166667	635.5833333
ZoMYB069	*ZoMYB084*	0.015136163	0.0582714	0.259752685	753	163.5	589.5
ZoMYB091	*ZoMYB105*	0.067654496	0.0780992	0.866263133	615	128.0833333	486.9166667
ZoMYB093	*ZoMYB113*	0.014229676	0.1015634	0.140106374	276	63.16666667	212.8333333
ZoMYB094	*ZoMYB111*	0.02641193	0.0578003	0.456951786	786	188.75	597.25
ZoMYB095	*ZoMYB110*	0.008699532	0.0313362	0.27761968	741	162.9166667	578.0833333
ZoMYB097	*ZoMYB108*	0.075502439	0.1554003	0.48585789	942	223.8333333	718.1666667
ZoMYB115	*ZoMYB132*	0.016040386	0.0518246	0.309512718	2973	725.5833333	2247.416667
ZoMYB117	*ZoMYB133*	0.069518092	0.131514	0.528598424	1053	259.75	793.25
ZoMYB124	*ZoMYB138*	0.013781611	0.0175276	0.786281678	759	173.1666667	585.8333333
ZoMYB144	*ZoMYB156*	0.011592567	0.0588176	0.19709339	663	141.4166667	521.5833333
ZoMYB147	*ZoMYB159*	0.026184478	0.0224841	1.164576521	1527	361.1666667	1165.833333
ZoMYB150	*ZoMYB160*	0.01779943	0.0306339	0.581037658	690	149.9166667	540.0833333
ZoMYB153	*ZoMYB164*	0.069929281	0.1109741	0.630140699	1278	293.25	984.75
ZoMYB174	*ZoMYB192*	0.050252249	0.0629373	0.798449193	684	149.0833333	534.9166667
ZoMYB198	*ZoMYB218*	0.05024913	0.0888539	0.56552526	1191	287.4166667	903.5833333
ZoMYB204	*ZoMYB221*	0.05076618	0.1588057	0.319674818	615	136.25	478.75
ZoMYB207	*ZoMYB227*	0.073245268	0.0975543	0.750815495	681	160.3333333	520.6666667
ZoMYB208	*ZoMYB231*	0.054867763	0.1352741	0.405604428	483	119.1666667	363.8333333
ZoMYB209	*ZoMYB232*	0.062485297	0.0740966	0.843294525	1647	401.5833333	1245.416667
ZoMYB210	*ZoMYB230*	0.051619265	0.0622201	0.829623784	1359	326.5833333	1032.416667
ZoMYB210	*ZoMYB233*	0.039384048	0.0680599	0.578667794	1791	422.6666667	1368.333333
ZoMYB239	*ZoMYB254*	0.067049697	0.1004926	0.667210203	633	147.0833333	485.9166667
ZoMYB245	*ZoMYB259*	0.005828101	0.0083888	0.694750988	1101	239.75	861.25
ZoMYB266	*ZoMYB272*	0.086966689	0.1668916	0.521096878	879	189.9166667	689.0833333

Ks and Ka mutations occur in the open reading frame region after gene duplication, leading to new gene functions. Consequently, the rate of nonsynonymous (Ka) and synonymous (Ks) substitution (Ka/Ks) between target gene and its duplicated gene was determined whether there is selective pressure on the protein coding gene. The calculation results of Ka, Ks, and Ka/Ks for 28 pairs of repeated pairings were shown in Table 1. The Ka values of 28 *ZoMYB* duplicated genes

were at the range of 0.005828–0.088251 and their Ks value were at 0.0083888–0.1668916. The Ka/Ks value of 26 paralogous pairs of *ZoMYB* is < 1, which suggested that purifying selection with the segmental replication was the major driving force for the evolution of *ZoMYB*s. However, the Ka / Ks value of 2 paralogous pairs of *ZoMYB* is > 1, indicating that positive selection with the segmental duplication occurred during the evolution of *ZoMYB* family genes, for example, *ZoMYB007-ZoMYB027* and *ZoMYB147-ZoMYB159*.

Motifs of the R2R3 MYB TF genes in Z. officinale

All the amino acid sequences in full-length of 299 *ZoMYB*s were studied by the Multiple Em for Motif Elicitation (MEME Suite) program tool. There were 20 motifs identified in these *ZoMYBs*, and the length of them is between 6–50 amino acids (Figure S1). The results show that the number of conservative motifs in each *ZoMYB* is ranged from 1 to 10. Motifs 1, 2, 3, 7 and 20 are most likely to appear in *ZoMYBs*, and there is more than one same motif in most *ZoMYBs*. Except *ZoMYB063*, *ZoMYB188*, and *ZoMYB203*. As shown in Fig. 1 and Figure S1, most closed members belonging to the same subgroup exhibited similar motif compositions and exons position, which indicating that the members belonging to the same subgroup may perform the similar functions [11].

Exon–intron organization analysis of ZoMYB genes

The gene structure information is considered to provide a new source of evolutionary data for plant evolution [31]. In order to deeply comprehend the structural characteristics of genes encoding *ZoMYB*s, we studied the distribution of introns and exons of these 299 *ZoMYB* TF genes (Figure S2). R2R3 MYB had at least one exon in the DNA binding domain. Among these 299 *ZoMYB*s, 288 of them had 1–12 intron(s), accounting for 96.32%. In contrast, 11 *ZoMYB*s lacked introns. Among the 299 *ZoMYB* genes, 148 of them contain 3 exons and 2 introns. Obviously, *ZoMYB162* and *ZoMYB236* contain the largest number of introns and exons in their CDS, they all contain 13 exons and 12 introns. Remarkably, the gene structure analysis of these *ZoMYB*s indicated that those genes which were in the same subgroups commonly had nearly the same number of exon-intron pattern, with fully conserved position(s) of the intron(s). These results indicated the existence of a structure that is highly conserved within the *ZoMYB*s family and provided important evidence for the family nomenclature.

Stress-related elements in the candidate promoters of *ZoMYB* genes

Cis-regulatory elements are commonly restricted to the promoters of genes at 5' upstream areas. They are the DNA-binding sites of transcription factors, and they are responsible for regulating the target genes at transcriptional level [32]. Thus, the putative promoters of these 299 identified *ZoMYB* genes were used to excavate stress-related regulatory elements. As expected, there were abundant light-responsive elements in 298 of 299 *ZoMYB* gene promoters, except *ZoMYB298* (Figure S3). MeJA is one of the most important phytohormaones that plays a key role in plant responses to stress [33]. Interestingly, MeJA-responsive elements were the second largest element group of *ZoMYB* gene promoters, in which 256 *ZoMYB* genes respond to light response. The content of ABA- responsive elements in the whole *ZoMYB* genes was also very high.

ABA is considered as an important mediator in the plants answering to different adverse environmental conditions, including low temperature, drought, and salinity [34]. Among all promoters in these *ZoMYB* genes, 166 *ZoMYBs* contain ABA-responsive elements, followed by drought-responsive element and low-temperature-responsive element, 109 and 78, respectively.

Expression of ZoMYB genes under ABA and Cold stress in Z. officinale

There is always association between gene expression pattern and its function. MYB TFs have been claimed to regulate gene expression when plants suffered from environmental changes in previous studies [351, 36]. As have been reported, quite a lot MYB genes occupied a place in response to various abiotic stresses, for instance drought and salt stress [21, 23, 37, 38]. Ten *ZoMYB* genes, from 299 *ZoMYB*s, were selected to study their gene expression patterns under between abiotic

stresses and hormone treatments. These ten *ZoMYB*s were clustered together in the phylogenetic tree with known stress-related *AtMYB*. RT-qPCR was carried out at 5 time points: 0, 1, 3, 6 and 12 h after ABA (Figure. 4A) and low temperature (Figure. 4B) treatment.

As indicated in Figure. 4, the expression of many *ZoMYB* genes exhibited different trends after the treatments of ABA and low temperature. By treated with ABA, the transcriptional level of 5 genes (*ZoMYB26*, *ZoMYB46*, *ZoMYB61*, *ZoMYB84*, *ZoMYB157*) arrived at the peak value after 6 h treatment, and the relative expressional level of the remaining 5 MYB genes exhibited roughly increasing patterns during pulp development. Under low temperature treatment, there is significant difference among ten MYB genes in transcriptional level. For example, *ZoMYB46* and *ZoMYB148* reached the highest value at 12 h. However, *ZoMYB84* and *ZoMYB263* highly expressed at 6 h after treatment. The relative expression patterns of *ZoMYB61* and *ZoMYB157* tended to be the same at 3 h and 6 h.

Discussion

MYB TFs encoded by genes constitute a fairly large transcriptional factor family in higher plants. Of the four different types, type R2R3 is the more common type in the MYB family. Different plant species have different amounts of R2R3-MYB transcription factors in genome-wide. It is the first time to discover, identify and describe the 299 *ZoMYB* TF genes in *Z. officinale*, and which were categorized into 8 subfamilies (Fig. 1) in this study. Among them, the number of 299 *ZoMYBs* subfamily members in *Z. officinale* were far more than that the number of 126 *AtMYBs* members in *A. thaliana*, thus suggesting that the gene duplication occurring during genetic evolution may lead to the increase of R2R3-MYB gene abundance in ginger plants. Gene duplications are acted as the primary driving force underlying new gene functions [39, 40]. Here, the different expression patterns of ten *ZoMYB* genes under ABA stress and low temperature stress were observed, showing that this category of MYB transcription factors made a significant contribution in plant growth and development in *Z. officinale*.

Gene structure analysis of these 299 *ZoMYB* TF genes indicated that most coding sequences of *ZoMYB*s contained introns, except 11 genes (*ZoMYB002*, *ZoMYB023*, *ZoMYB096*, *ZoMYB109*, *ZoMYB117*, *ZoMYB141*, *ZoMYB184*, *ZoMYB228*, *ZoMYB242*, *ZoMYB270*, *ZoMYB289*) From Figure S2. The most of the *ZoMYB*s (96.32%) contained more than one intron, and the number of introns seems to be limited, which is similar to the corresponding gene structures of from *A. thaliana* [41]. It is worth noting that genes in the same subgroup usually showed similar gene structure profiles, including the position, distribution and phases of introns [10]. The main members of the same subfamily often have common conserved motifs and similar gene structure, but there are great differences among different subfamilies. In the same evolutionary branch, *ZoMYB*s and *AtMYB*s share same gene structures, and conserved motifs, and are classified in the same branch, strongly supported the result of our subfamily classification.

In present work, almost all *ZoMYB* TFs had many cis-acting elements connected to presses, such as light, ABA, drought, cold, MeJA, gibberellin, Auxin, salicylic acid, and wound stress, indicating that these *ZoMYB* genes TF from *Z. officinale* involved in various abiotic presses and hormone responses. All of 166 genes from this kind of MYB are regulated by ABA-responsive elements in their promoter regions, and 78 corresponding genes are regulated by low-temperature-responsive elements in their promoter regions to adapt low temperature stress (Figure S3). We selected ten genes to analyze their expression patterns under low temperature (cold) and hormone treatments (ABA) at five time points. Ten chosen genes were expressed in all samples (despite varying levels of expression), suggesting that these genes play a regulatory role in different abiotic stresses and hormone treatments by signal transduction pathway.

Conclusions

Our results laid a basic foundation for the identification and functional analysis of R2R3-MYB TFs on genome-wide in *Z. officinale*. A total of 299 *ZoMYB* TF genes were characterized from the genome of *Z. officinale* and analyzed by their

related proteins and genes, such as phylogenic relationship, conserved domain, gene structure analysis, collinearity analysis, etc. Furthermore, the potential functions of 299 *ZoMYB* TFs were predicted by Cis-regulatory Elements Analysis and gene expression patterns under various stresses were detected. These results revealed the characteristics of *ZoMYB* genes family and provided valuable information for improving the ability of *Z. officinale* to cope with environmental changes during growth and development.

Materials And Methods

Plant materials

The ginger raw materials were obtained from Laiwu city, Shandong province, and were cultivated in Anhui University of Chinese Medicine (Hefei, Anhui). The ginger samples were stored in the Herbarium of Anhui University of Chinese Medicine (code: 20211105). The seedlings of 45 days were chosen for related-stress and expression pattern analysis. For the ABA stress treating, the seedlings were suffered from 100 mM. For the treatment of low temperature treatment, the tested plants were kept in an incubator at 4°C. All ginger samples were gathered at 0, 1, 3, 6, 12 h after two types of treatment.

Identification of R2R3-MYBs in Z. officinale

The genomic database, protein database and their annotated documents of *Z. officinale* were extracted from the famous database of NCBI, which assembly accession is GCA-018446385.1 in GenBank. The R2R3-MYB TF genes of *A. thaliana* were identified subjecting to the Arabidopsis Information Resource, and the database is used as the query sequence for local BLASTp search, E-value is set to 0.00001. In addition, the hidden Markov model profile of MYB with accession number PF00249 [38](Finn et al., 2016) was downloaded from the Pfam database. These sequences were aligned and the producing file was used to build the HMM profile of R2R3-MYB domain using HMMER (http://hmmer.org/) and this profile was used to identify the R2R3-MYB domain containing protein sequences amongst these MYBs in *Z. officinale*. The resulting sequences were combined, and redundant sequences were deleted by CD-HIT online analysis software [43]. R2R3-MYB sequences were manually screened according to their structural characteristics. Chemical properties of these protein sequences were evaluated with the ProtParam tool of ExPASy [44] and protein subcellular localization predictions were made by the BUSCA online site (http://busca.biocomp.unibo.it/) [45].

Phylogenetic Evolution Analysis of R2R3-MYBs in Z. officinale

To analyze the evolutionary of conserved protein motifs, a phylogenetic tree was constructed based on the obtained protein sequences of R2R3-MYB TFs from ginger and *Arabidopsis* by MEGA X software [46]. The sequences were aligned by ClustalW and the neighbor-joining method was used to generate evolutionary tree, which bootstrap value was set at 1000.

R2R3-MYBs' gene duplication and chromosomal location in Z. officinale

The location information and chromosome length of the putative genes of R2R3-MYB were downloaded from the General Characteristic Format (GFF) file of ginger genome. Based on the result of Wang et al. [47], collinear analysis was carried out by the Multiple Collinear Scan Toolkit (MCScanX) and applied to analyze the duplication patterns of those acquired *ZoMYB* genes. The circular gene viewer software package of TBtools was used for integrating data and displaying the collinear relationship between the chromosome position of *ZoMYBs* and parahomologous MYBs in ginger. Synonymous (Ks) and nonsynonymous (Ka) substitution nucleotide ratios of *ZoMYB* gene pairs were also assessed by software of TBtools.

R2R3-MYBs' conserved motifs identification in Z. officinale

All full-length protein sequences of 299 *ZoMYB*s were analyzed by the Multiple Em for Motif Elicitation program (MEME; v4.9.0) to get conserved motifs except MYB repeats. The following parameters were set as follow: the motif sites are expected to be sequentially distributed, a contributing motif site per sequence; maximum number of motifs to find, 20; the

minimum width of motif and maximum width of motif are between 6 and 50 (to identify long R2R3 domains). The motif values less than E-20 were retained, and the default values were used for other options, and further analysis was conducted.

Gene Structure analysis of Z. officinale R2R3-MYB genes family

The whole genome sequence of *Z. officinale* was downloaded from the NCBI and the corresponding *ZoMYB* genes sequence were obtained from the genome sequence of *Z. officinale* were used for gene structure analysis. The gene structure schematic diagram is mapped through the Gene Structure Display Server (GSDS, http://gsds.gao-lab.org/index.php) after integrating genome and CDS sequence [48].

Cis-regulatory elements identification of *ZoMYB* genes promoter

Regulatory region sequence of *ZoMYB* genes at 1-K (Kilobase) upstream was extracted from ginger CDS sequence by TBtool software [49]. The software of PlantCARESearch for CARE website4. Plots was used to examine the cis-regulatory elements of the promoter sequence in TBtools software, and they are presented using the same software [50].

Real-time Florescence Quantitative Pcr (Rt-qpcr) Analysis

Total RNA from ginger was obtained using the TransZol kit (TransGen Biotech, Inc., Beijing, China). The obtained RNAs were synthesized into the cDNA using FastKing RT Kit (Tiangen, China) by reverse transcription reaction. The real-time quantitative PCR (RT-qPCR) was carried out on a LightCycle480 machine (Roche, Switzerland). SuperReal PreMix Plus (Tiangen, China) and a 20 μL reaction system were used [51]. Primer sequences were designed by software of Primer Premier. The primer sequences of genes of *ZoMYB*s were designed by primer design company (Sangon Biotech, Shanghai) (Table S3). And the data from qRT-PCR amplification were counted by the $2^{-\Delta\Delta CT}$ method with 'housekeeping' gene actin as the internal reference gene. Thermal cycling conditions were set as per the manufacturer's instructions for SYBR Green qPCR Master Mix (Vazyme). The cycling conditions included denaturation at 95°C for 30 seconds, 40 cycles of denaturation at 95°C for 5 seconds and annealing at 56°C for 30 seconds, and followed by a final melting curve analysis is performed. Each reaction was carried out in three independent biological replicates with a negative control group.

Abbreviations

ABA, abscisic acid; HTH, helix-turn-helix; Ka, nonsynonymous; Ks, Synonymous; LT, low temperature; MeJA, methyl jasmonate; NCBI, National Center for Biotechnology; qRT-PCR, quantitative real-time reverse transcription; TFs, Transcription factors.

Declarations

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Author contributions

SX, XY, CQ conceived, designed, and implemented the study; XY, XD, ZS, LW, SW analyzed the statistics; XY, SX, ZW, CQ and JZ performed the experiments; SX, XY, FM, XG, JW and XS drafted the manuscript; All authors participated in the editing of

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Availability of data and materials

The original contributions presented in the study included the article and Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics approval and consent to participate

This study include no human, animal or endangered plant samples, and the sample was legally collected in accordance with guidelines provided by the authors' institution and national or international regulations. Field studies was complied with local legislation. No ethical approval/permission is required in this study. The samples were stored in the Herbarium of Anhui University of Chinese Medicine (code: 20211105).

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Figures

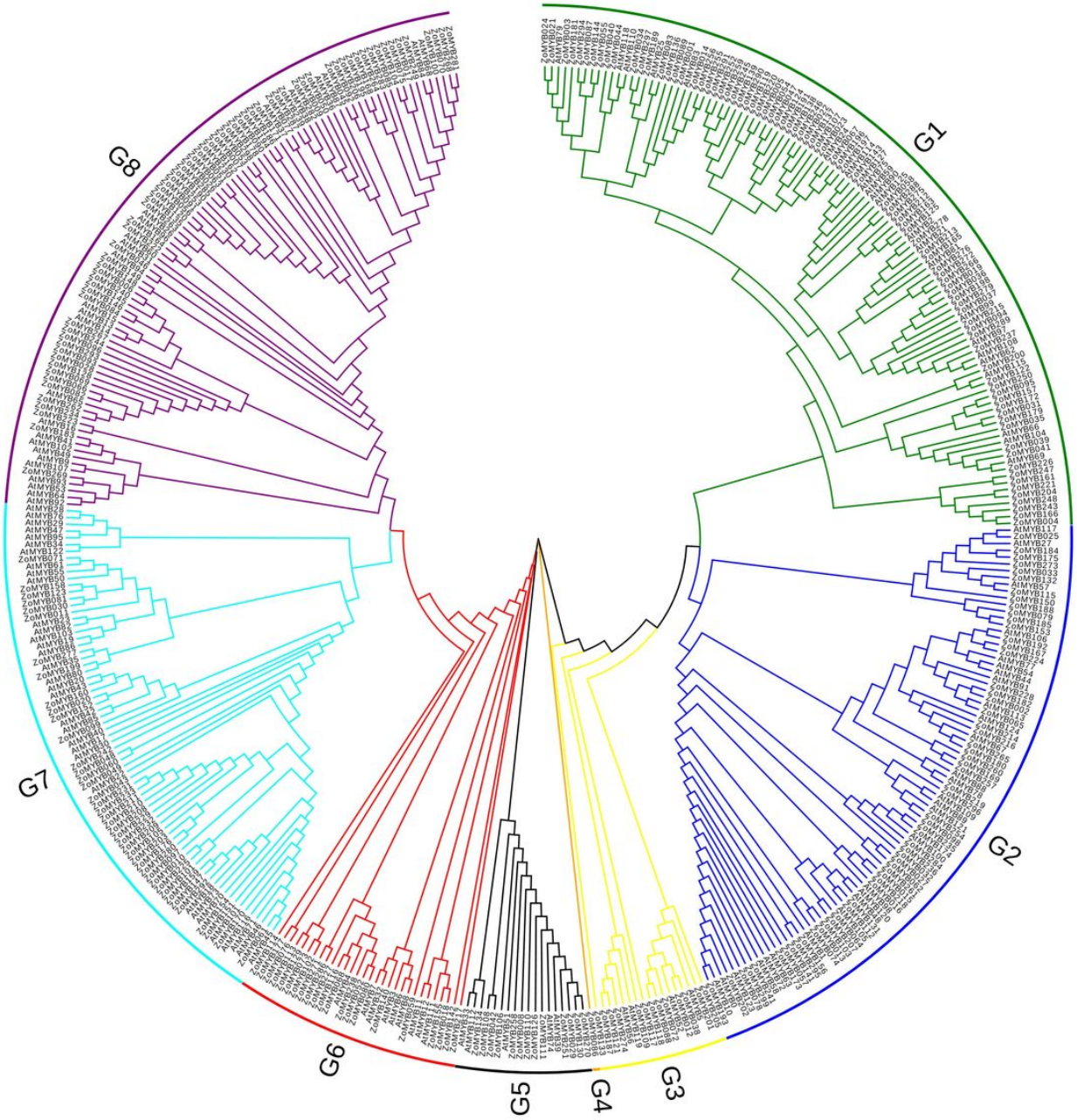

Figure 1

Phylogenetic tree of R2R3-MYBs in *Z. officinale* and *A. thaliana*. The sequences contain 299 *ZoMYB*s in *Z. officinale* and 126 *AtMYB*s in *A. thaliana*. All R2R3-MYBs were divided into 8 clusters (G1-G8). The picture was generated by using MEGA software coupled by Neighbor-Joining method with a bootstrap of 1000 replicates.

Figure 2

Distribution of 299 R2R3-MYB genes in chromosomes of *Z. officinale*. The physical position of each *ZoMYB* was mapped according to the *Z. officinale* genome. The chromosome number (ZoChr01A-11B) is indicated on the top of each chromosome.

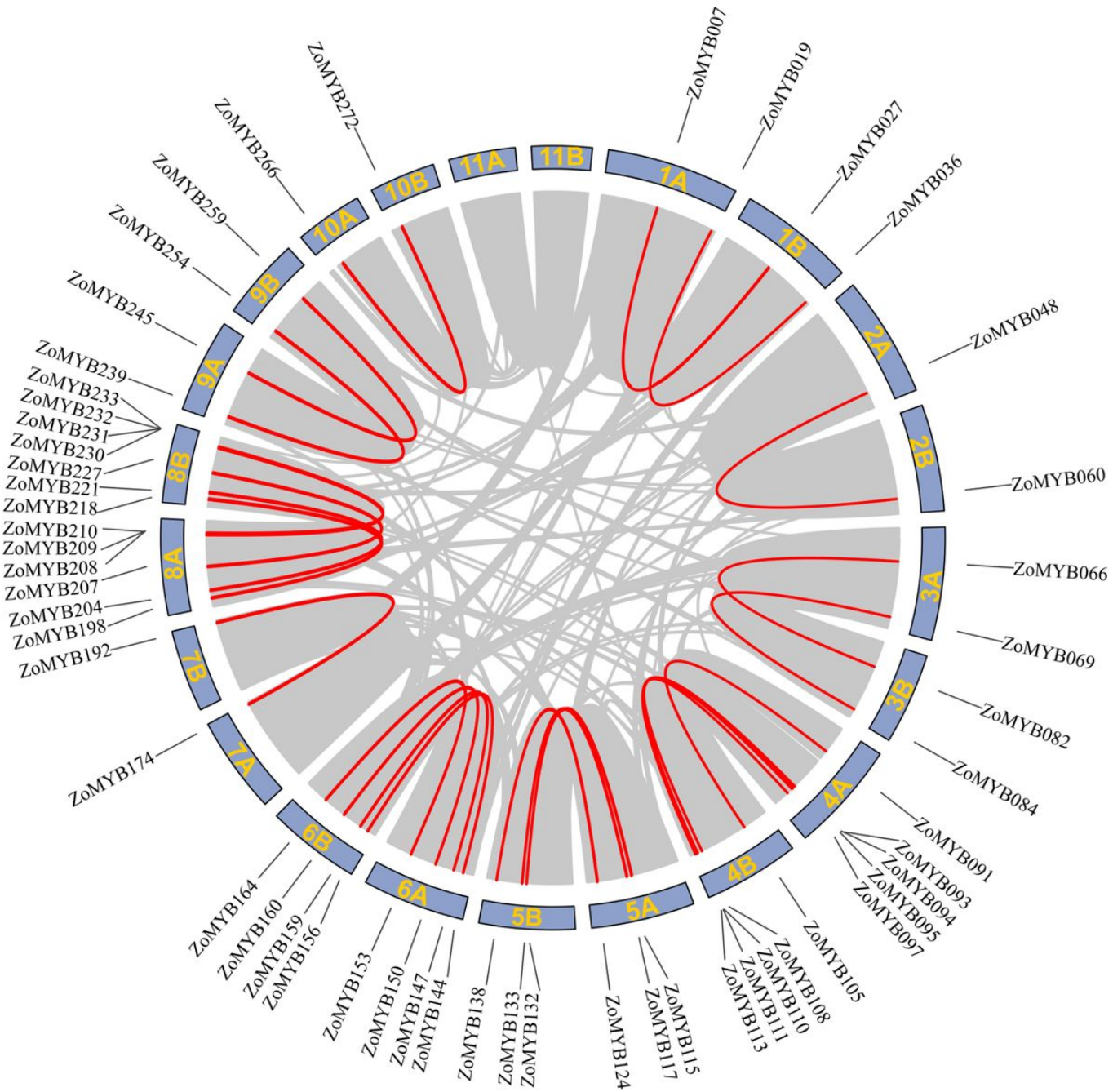

Figure 3

Schematic representations for the chromosomal distribution and inter chromosomal relationships of *ZoMYB* genes. Red lines in the middle indicate duplication gene pairs of *ZoMYBs*, while grey lines indicate genome duplication gene pairs.

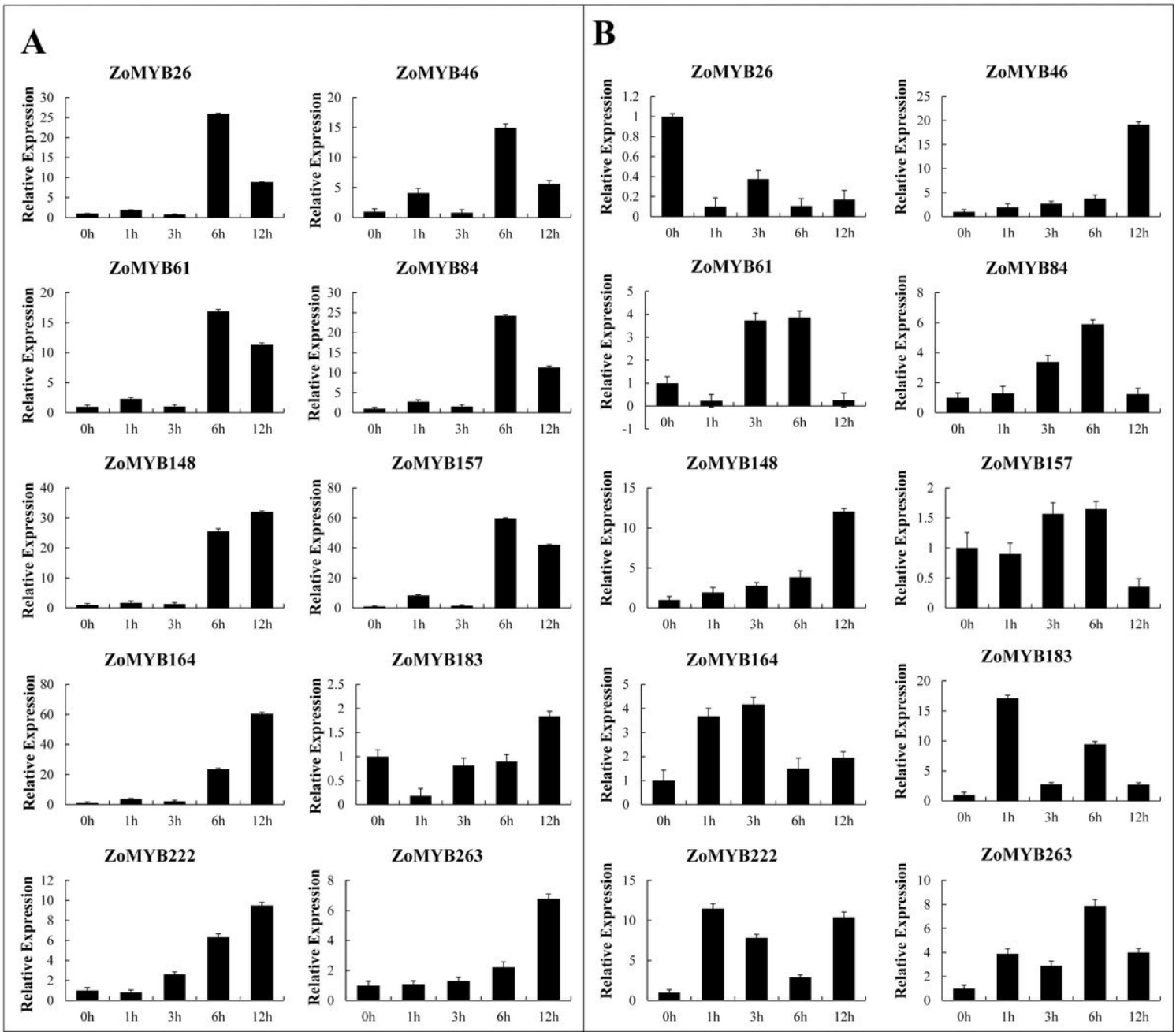

Figure 4

Expression level analysis of selected MYB genes using qRT-PCR of *ZoMYB*s. 0 h, 1 h,3 h, 6 h and 12 h correspond to five different gingers growth stages. A: under abscisic acid (ABA) treatments; B: under abiotic stress low temperature treatments. The x-axis represents the different stages and the y-axis the relative expression values. The standard deviations of three biological replicates are represented by the error bars.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS1.VisualizationoftheclassificationofZoMYBproteinsandthedistributionof20predictedmotifs.pdf
- FigureS2.PhylogeneticanalysisandstructuresofZoMYBgenesinZ.officinale.pdf
- FigureS3.Predictionofcisresponsiveelementsinthe1kupstreamregulatoryregionsofZoMYBgenes.pdf

- TableS1.Theaminoacidsequencesof299R2R3MYBfromZ.officinale.xlsx
- TableS2.ListofallZoMYBgenesidentifiedinZ.officinalegenome.xlsx
- TableS3.PCRprimersusedforquantitativerealtimePCRanalysis.xlsx