

Genome-wide identification of R2R3-MYB Family Genes and their Response to Stress in *Dendrobium nobile*

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Abstract

R2R3-MYB is one of the largest and most important gene families, participating in the regulation of plant growth and development and response to abiotic stresses. However, the function of *R2R3-MYB* genes in *Dendrobium nobile* remains largely unknown. Here, a comprehensive genome-wide analysis of *D. nobile* R2R3-*DnMYBs* was performed, investigating phylogenetic relationships, gene structures, motif composition, chromosomal locations, collinearity analysis, and cis-acting elements. Totally, 125 *DnMYB* genes were identified in the *D. nobile* genome, and they could be subdivided into 26 groups by further divided through phylogenetic analysis. Most genes with similar exon-intron structures and motif compositions in each subgroup exhibited similar functions. All of *DnMYB* genes were mapped on 19 chromosomes with the collinearity relationship. Moreover, their expression patterns were analyzed in various tissues and abiotic stresses. The results showed that *DnMYBs* were significantly differential expressed in different tissues, following abiotic stresses and phytohormone treatments, indicating their possible roles in biological processes and some abiotic stress tolerance and adaptation. This work provides a comprehensive understanding of the R2R3-MYB family of *D. nobile*, and lays a foundation for future research on the potential function of *DnMYB* gene in the growth and development of *D. nobile*.

1 Introduction

Dendrobium nobile Lindl is a plant of the *Dendrobium* genus of Orchidaceae, which is a precious and high-grade Chinese medicinal material in China. *Dendrobium* has a medicinal history of more than 1500 years[1]. It is mainly distributed in the subtropical regions south of the Yangtze River such as Guizhou, Yunnan and Guangxi. *D. nobile* in Chinese Medicine was listed as the primary source of medicinal *dendrobium* in the Chinese Pharmacopoeia (2010). *D. nobile* contains several classes of interesting bioactive secondary metabolites including polysaccharides, alkaloids, flavonoids, coumarins, phenols, sesquiterpenes, etc. [2]. *D. nobile* comprises medicinal, nutritional, and ornamental functions, so it has great prospects for further developments.

Transcription factors are located in the nucleus and can recognize and bind cis-acting elements in the promoter region of eukaryotic genes, thereby regulating the expression of downstream genes. Such as the SBP-box family[3], MYB[4, 5], and BLH[6, 7]. MYB TFs (transcription factors) are part of one of the largest families of TFs in eukaryotes, which contains a highly conserved DNA binding domain (R) in N-terminus[8]. According to the number and position of R sequences, the MYB transcription factor family can be divided into four categories, namely R1-MYB, R2R3-MYB, R1R2R3-MYB and 4R-MYB[9, 10]. Among them, R2R3-MYB is the largest subfamily in the MYB family, which is widely involved in various physiological and biochemical processes, such as biotic and abiotic stress, metabolism, growth and development of plants[11]. To date, MYB TFs has been identified in many plants, including the orchid *Dendrobium*[8]. 101 *DoMYB1* and 99 *PaMYB* *R2R3-MYB* genes were identified in the genomes of *Dendrobium officinale* and *Phalaenopsis aphrodite*, respectively[12]. A total of 99 R2R3-MYB proteins were identified in *Dendrobium catenatum*[13]. However, there is no report on the *R2R3-MYB* gene family in *D. nobile*.

In this study, we conducted a genome-wide analysis of the *R2R3-MYB* gene family in *D. nobile*, a total of 125 R2R3-MYB proteins from *D. nobile* were identified and analyzed, including phylogenetic analysis, chromosome

localization, gene duplication analysis, conserved domains and gene structures. We used real-time quantitative PCR (RT-qPCR) experiments to compare and analyze three stress treatments (cold, ABA and MeJA) with a control treatment. Our study will provide a foundation for future research into the molecular function of *D. nobile* *R2R3-MYB* genes.

2 Materials And Methods

2.1 Plant growth and treatment

D. nobile were cultivated at the Anhui University of Chinese Medicine (Hefei, Anhui, China). *D. nobile* samples are stored in the Herbarium of Anhui University of Chinese Medicine (code: 20220601). It was identified as *D. nobile* by Professor Yang Qingshan of Anhui University of Traditional Chinese Medicine. In this study, the root, stem, leaf, flower of *D. nobile* were tested for tissue specificity. Each tissue was collected from three different plants, and the collected samples were immediately stored at -80°C . For the stress treatment, the plants were treated with 100 μM abscisic acid (ABA), 100 μM methyl jasmonate (MeJA), and 4°C . And the leaves were then collected and frozen in liquid nitrogen at different time points (0, 1, 3, 6, 12, and 24 h) after treatment.

2.2 Identification and sequence analysis

The genomes of *D. nobile* (<https://www.ncbi.nlm.nih.gov/genome/?term=dendrobium+nobile>) were downloaded to identify the candidate *R2R3-MYB* genes. We used the hidden Markov model (HMM) profile of the MYB domain (PF00249) from the Pfam database to search the protein sequences[14, 15]. The putative DnMYB sequences were checked by the National Center for Biotechnology Information (NCBI). Redundant sequences were removed using CD-HIT online analysis (<http://www.bioinformatics.org/CD-HIT/>). The remaining sequences were submitted to HMMscan online analysis (<https://www.ebi.ac.uk/Tools/hmmer/search/hmmscan>) to determine the MYB domain and quantity, *R2R3-MYB* sequences were screened for structural features. A total of 126 *R2R3-MYB* transcription factor sequences of *Arabidopsis* were obtained from the TAIR (The *Arabidopsis* Information Resource) database (<https://www.arabidopsis.org/>).

The physicochemical properties of the protein sequences were analyzed with the ProtParam tool of ExPASy (<https://web.expasy.org/protparam/>). TMHMM software was used to determine the number of transmembrane domains. The subcellular localization of MYB proteins was predicted using the PSORT tool.

2.3 Multiple Alignment and Phylogenetic Analysis

The MEGA X software was used for sequence alignment, and the Neighbor-Joining method was used to construct the phylogenetic tree of *D. nobile* and *Arabidopsis* *R2R3-MYB* proteins. The 126 *R2R3-MYB* proteins of *Arabidopsis* was used as references, bootstrap analysis of 1000 repetitions were performed to compute the reliability of the phylogenetic tree.

2.4 Gene Structure and Conserved Motif Analysis

For gene structure analysis, the corresponding genome sequences of DnMYB genes were obtained from the genome sequences of *D. nobile* which were downloaded from NCBI. The Gene Structure Display Server 2.04

(GSDS) (<http://gsds.gao-lab.org/>) was employed to analyze the exons-introns structures of the obtained full sequences. For conserved motif analysis, The MEME suite (Multiple Expectation Maximization for Motif Elicitation, version 5.4.1) (<https://meme-suite.org/meme/tools/meme>) was used to analyze the conserved motif[16, 17].

2.5 Chromosomal Locations and Synteny analysis

The physical position of the *DnMYB* genes on the chromosome was mapped by MG2G online software (http://mg2c.iask.in/mg2c_v2.1/)[18]. The synteny correlation analysis of *DnMYB* genes between the homologs in *Dendrobium chrysotoxum* and *Arabidopsis thaliana* was verified and visualized using TBtools software[19].

2.6 Analysis of the cis-regulatory elements in the promoters of *DnMYB* genes

The upstream 2 kb regulatory regions (from the translation start site) of *DnMYB* genes were obtained from the *D. nobile* genome database, and the analysis was then performed using PlantCARE software (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>).

2.7 Real-time Quantitative PCR (RT-qPCR) Analysis

RT-qPCR analysis was performed using a SuperReal PreMix Plus SyBr Green PCR kit (Qiagen) on a Cobas z480 Real-Time PCR System as the method described by Wang et al. Candidate primers were designed using Primer 5.0 (Supplementary Table 1). Reactions contained 2.0 μ L of diluted cDNA, 0.6 μ L of each primer, 10 μ L of 2 $\times$ SuperReal PreMix Plus, and 6.8 μ L of RNase-free double-distilled water (ddH₂O). All RT-qPCRs were performed as follows: denaturation at 95°C for 15 min, followed by 45 cycles of 95°C for 10 s, 58°C for 20 s, and 72°C for 30 s. Each sample contained three biological replicates, a housekeeping gene (18S) was used as a reference, and the relative expression level of each gene was calculated using the $2^{-\Delta\Delta C_t}$ approach[20].

3 Results

3.1 Identification and Classification of *R2R3-DnMYB* Genes in *D. nobile*

To identify MYB proteins in *D. nobile*, the genomic sequence was downloaded from NCBI. After searching through the Biolinux system using PF00249 as a seed sequence to perform HMMER alignment, the redundant sequences were removed by the CD-Hit online tool analysis, totally 276 candidate *MYB* genes were obtained. The number of MYB domains in the sequence of candidates were analyzed by HMMscan online analysis software, and 125 sequences were screened out which matched the characteristics of *R2R3-MYB* genes. The *R2R3-MYB* genes of *D. nobile* were terminated *DnMYB1* to *DnMYB125* (Supplementary Table 2). These *DnMYBs* were classified into four distinct groups: R1-MYB family (123 proteins, 44.57%), R2R3-MYB family (125 proteins, 45.29%), 3R-MYB (27 protein, 9.78%), and 4R-MYB families (1 protein, 0.36%), of which the R2R3-MYB family contains the most *DnMYB* genes, this result is consistent with previous reports, the R2R3-MYB subfamily is the most abundant transcription factor subfamily in plants [21]. The physicochemical

properties of 125 *R2R3-MYB* genes, including gene locus, protein length, MW, pI, number of transmembrane regions, and subcellular localization of MYB proteins, are listed in Supplementary Table 2. The proteins encoded by these genes ranged from 88 (DnMYB50) to 2424 amino acids (DnMYB64), with an average number of 348 amino acids. The protein molecular weight (MW) and isoelectric point (pI) of predicted DnMYB proteins ranged from 10.32 to 74.13 kD and 4.88–9.01. All predicted DnMYB are not transmembrane proteins. Subcellular localization results show that all predicted DnMYB proteins are localized in the nucleus, which is consistent with the fact that transcription factors must act in the nucleus in order to regulate their expression [22].

3.2 Phylogenetic analysis of the R2R3-MYB Proteins in *D. nobile*

In order to explore the evolutionary relationship among these 125 R2R3-MYB proteins, a phylogenetic tree was constructed based on 125 and 126 MYB proteins from *D. nobile* and *A. thaliana*, respectively (Fig. 1). The results showed most of the DnMYB in *D. nobile* were classified into different subclasses, which were supported by strong bootstrap. The phylogenetic tree of 125 DnMYB were divided into 26 subgroups with the number of members ranging from 1 to 26. The R2R3-MYB proteins has the most members in the S21 subfamily, including 14 DnMYBs. This result is consistent with the classification results of *D. officinale* and *P. aphrodite*. The S14 and S18 subfamily contained 7 DnMYBs respectively, followed by 5 DoMYBs were both divided into S4 and S17 subfamilies. The S13, S22, and S25 subfamilies contained 4 DnMYBs. The S1 and S11 subfamily contained 3 DnMYBs. Four subfamilies, including S2, S6, S10, S20, contained 2 DnMYBs. The remaining subfamilies, including S5, S9, S19, S23 and S24, contained only one DnMYBs. Interestingly, no R2R3-MYB proteins were classified into the S12 subfamilies. The *Arabidopsis* R2R3-MYB transcription factor contains 25 subclasses, of which the S1, S2, S20, and S22 subfamilies are involved in regulating plant responses to abiotic stresses[23]. The S4, S5 and S6 subfamilies of the *Arabidopsis* R2R3-MYB transcription factors are involved in plant anthocyanin synthesis[23]. Since some AtMYB proteins with similar functions are clustered in the same clade, this may indicate that DnMYB proteins in the same clade have similar functions[24]. However, the functions of these identified transcription factors need to be further studied.

3.3 Gene Structure Analysis of 125 *DnMYB* genes

Exon/intron analysis showed that 125 *DnMYB* genes had introns in their coding regions. The 125 *DnMYB* genes have a high degree of diversity in the number and relative positions of introns and exons. As we know, genetic structural diversity may explain the evolution of polygenic families[24]. The gene structure map of these 125 *DnMYB* genes was constructed, and the results showed that the clustering pattern of 125 *DnMYB* genes was not clearly consistent with the exon/intron structure (Fig. 2) (Supplementary Table 3). Interestingly, most genes in the same subclass showed similar exon/intron structure. This phenomenon is similar to that mentioned in *Liriodendron*[24]. The number of exons ranged from 1 to 13, and most of these genes contained two exons (27 genes) and three exons (67 genes) based to the 125 *DnMYB* gene structure.

3.4 Motif composition of 125 DnMYB proteins

There are many conserved sequences in MYB transcription factors and these conserved sequences may combine with some parts of DNA and play a role in expression regulation[25]. In order to deeply study the potentially conserved motifs in 125 DnMYB proteins of *D. nobile*, we systematically studied the evolutionary relationship of 125 DnMYB proteins, and analyzed the motif composition of 125 MYB-encoded proteins by MEME online analysis software. A total of 10 motifs were identified, of which motif 3 is the N-terminal domain of DnMYB proteins, which is present in almost all DnMYBs. Compared with the N-terminus, the C-terminus has a higher variability of motifs and different sequences, and these specific motifs indicate that different subgroups may have different functions[26] (Fig. 2). Diversity in the composition of DnMYB motifs explains the diversity of its functions. Moreover, most DnMYB had motifs 1, 2, 3, 4 and 6, and most members in the same subgroup contained more than one identical motif. Motifs 3, 6, and 2 constitute the complete R2 domain, of which the three tryptophan (W) residues are highly conserved. Motif 1 contains the complete R3 domain, the first tryptophan (W) residue is often replaced by phenylalanine (F), and the remaining two tryptophan (W) residues are highly conserved, and 19 amino acids are separated between the two adjacent tryptophan residues. These are consistent with the characteristics of MYB domain identified in soybean[26], Ginkgo biloba[27], *Arabidopsis*[28] and other species. Interestingly, the results also show that most members of the same subclass have similar motif composition.

3.5 Chromosomal location and gene duplication of *R2R3-MYB* gene family

In order to understand the distribution of R2R3-DnMYB genes in the genome, MG2G online software was used to locate the 125 *R2R3-MYB* genes on the corresponding chromosomes. *D. nobile* has 19 chromosomes, shown ranging from Chr1 to Chr19 (Supplementary Table 4). *DnMYBs* were present on all chromosomes, while the arrangement and density of *DnMYBs* on every chromosome were uneven (Fig. 3). The maximal number of *DnMYBs* (12%) was located on chromosome 9, whereas the minimum number of *DnMYBs* (3.2%) was located on chromosome 4. There was a high density of *DnMYBs* located on chromosomes 6, 11, 12, 13, 17.

Collinearity diagrams among *DnMYBs* were analyzed using gene duplication analysis.

A total of 46 pairs of gene duplications were identified among *DnMYBs* (Fig. 4). The *DnMYB* genes were homologous to genes in other plants, and syntenic conservation was observed among *D. chrysotoxum* (174 orthologous gene pairs dispersed on all chromosomes). 28 collinear gene pairs between *D. nobile* and *A. thaliana* (Fig. 5). The number of orthologous events of *D. nobile-D. chrysotoxum* was far greater than that of *D. nobile-Arabidopsis*, and the closer evolutionary distance between *D. nobile* and *D. chrysotoxum* was confirmed.

3.6 Prediction of Cis-Acting Elements in the Promoters of 125 *DnMYB* Genes

Promoter is a DNA sequence recognized and combined by RNA polymerase and starts the transcription of downstream genes. The cis-acting element in promoter can bind transcription factors and play an important role in the regulation of gene expression[29]. To analyze the cis-acting elements located in the 2000bp

sequence upstream of the promoter region, we obtained 125 *DnMYB* promoter sequences and predicted them by PlantCARE online tool (Supplementary Table 5). In addition to the basic elements, these cis-acting elements could be divided into three groups, including hormone-responsive elements (ABA, salicylic acid, gibberellin, MeJA, auxin), abiotic stress elements (low temperature-responsive elements and drought-responsive elements), metabolism elements (regulation flavonoid biosynthetic) (Supplementary Fig. 1). MeJA-responsive element is the most abundant element, containing 350 instances. As we all know, Methyl jasmonic acid regulates physiological processes such as plant growth and development, especially the response of plants to biotic and abiotic stresses[30]. *DnMYB23* contained 24 MeJA-responsive elements in its 2-k upstream regulatory region. In addition, hormone-related elements included 210 ABA-responsive elements, 87 gibberellin-responsive elements, 64 auxin-responsive elements, 58 salicylic acid-responsive elements. Abiotic stress elements contained 60 low temperature responsive elements, of which *DnMYB100* contained 3 low temperature responsive elements. Flowed by 80 drought responsive elements, *DnMYB34*, *DnMYB37*, *DnMYB58*, *DnMYB6*, *DnMYB74* and *DnMYB83* all harbored 3 drought responsive elements. Moreover, 14 regulation flavonoid biosynthetic-responsive elements in its 2-kb upstream regulatory region were identified. These results suggest that the expressions of *DnMYB* genes are controlled by complex regulatory networks.

3.7 Expression pattern of candidate *DnMYB* genes in different tissues

MYB genes in different subgroups may perform different functions during the growth and development of *D. nobile*. To investigate the spatial expression levels of MYB genes in *D. nobile*, the expression levels of 8 genes from each type across four tissues, roots, stems, leaves, and flower, were analyzed by RT-qPCR. *DnMYB8*, *DnMYB14*, *DnMYB26*, *DnMYB27*, *DnMYB81*, *DnMYB90*, *DnMYB108*, *DnMYB116* belong to the S22, S2, S21, S1, S7, S4, S19, S20 subfamilies, respectively. As shown in Fig. 6, 8 genes were expressed in all tissues. The results indicated that *DnMYB8*, *DnMYB14*, *DnMYB26* and *DnMYB81* shared similar expression patterns and were predominantly expressed in root. *DnMYB27* had higher expression in the flower if compared with that in other tissues. Moreover, *DnMYB108* showed tissue-specific expression patterns, with 30-fold higher expression levels in flowers than in other tissues. *DnMYB90* was principally expressed in leaf and stem. *DnMYB116* was classified in the S20 subfamilies with relatively high expression levels in leaf and/or stem. The results suggested that these genes had a possible role in the growth and development of *D. nobile*.

3.8 Expression pattern of candidate *DnMYB* genes in response to abiotic stress

Based on the reported functions of R2R3-MYB in other plants, we screened out 8 *DnMYBs* belonging to eight subfamilies to study the role of R2R3-MYB in the regulation of secondary metabolism and the responses to abiotic stress for *D. nobile*. We performed the expression level of these genes by RT-qPCR after treatment with cold (4°C), ABA, and MeJA. Six *DnMYB* genes, *DnMYB8*, *DnMYB14*, *DnMYB26*, *DnMYB27*, *DnMYB90*, and *DnMYB116* showed increased expression levels at different times under the low temperature, ABA, and MeJA treatment (Fig. 7). While *DnMYB81* was the most responsive to low temperature, and showed downregulated expression patterns under ABA and MeJA stress. *DnMYB108* showed downregulated expression patterns

under either cold or MeJA stress. The gene expression of *DnMYB108* plummeted at the lowest value at 12 h and reached its peak at 24 h under ABA stress.

4 Discussion

4.1 Evolutionary Analysis of the *DnMYB* Gene Family

R2R3-MYB transcription factors have been identified in many plants, including *Arabidopsis*[28], maize[31], soybean[26], etc. To date, there is no full analysis of the *R2R3-DnMYB* gene family and most functions remain unclear. In this study, 125 R2R3-MYB transcription factors were identified in the *D. nobile* genome and divided into 23 subfamilies. Gene structure, cis-acting element analysis, chromosomal distribution, phylogenetic analysis, gene duplication events, and expression level analysis were determined. This study offers new insights for future investigators to identify functional differences in the *R2R3-DnMYB* family genes. This study provides new insights for future researchers to determine the functional differences of *R2R3-MYB* family genes in *D. nobile*.

4.2 *DnMYB* Genes Play Crucial Roles in *D. nobile* Growth and Development and Response to Phytohormone and Abiotic Stresses

Low temperature, drought and abiotic stress seriously affected the yield of *D. nobile*. Secondary metabolites in plants, including flavonoids and anthocyanins, can help plants adapt to harsh environments. Meanwhile, flavonoid biosynthesis can be induced under a wide range of abiotic stresses. Therefore, the stress resistance of *D. nobile* can be improved by regulating the genes related to secondary metabolites, thereby increasing the yield. MYB gene can regulate the development of trichomes and root hairs, and plays a crucial role in regulating the synthesis of plant secondary metabolites such as flavonoids and anthocyanins and helping plants resist abiotic stress. In order to screen *DnMYB* genes that may be involved in regulating the growth, development and secondary metabolic synthesis of *D. nobile*, this study selected a total of 8 genes from different subgroups and measured their expression levels in different tissues and different stresses. Gene functions are closely related to tissue-specific expression. As the major pigments in plant, flavonoids contain various important bioactivities and have gained a lot of attention. It reported that flavonoids biosynthesis in plants could be induced under a wide range of abiotic stresses. The effects of these stresses on the contents of flavonoids have been investigated mainly in some crops and model plants. For example, in wheat, the key enzymes in the flavonoids' biosynthesis pathway were induced by drought stress[32]. The S4 subfamily *AtMYB4*, and *AtMYB7* were related to the flavonol synthesis pathway[33]. The S7 subfamily *AtMYB11*, *AtMYB12* and *AtMYB111* were involved in regulating flavonol biosynthesis in *Arabidopsis*[34]. Genes with high homology in the same branch of a phylogenetic tree generally have high sequence similarity and may also have similar functions. *DnMYB90* also belonged to S4 subfamily, which is a homologous gene of *AtMYB4*, *AtMYB7*. RT-qPCR showed that *DnMYB90* was expressed in all tissues of *D. nobile*, but the highest expression level was found in leaf. *DnMYB90* was also expressed at very high levels under phytohormonal and abiotic stresses. *DnMYB90* exhibited an elevated expression level under cold, ABA and MeJA stress. And it was the most responsive to low temperature. *DnMYB81* belonging to the S7 subfamily had close homology with *AtMYB11*, *AtMYB12*, and *AtMYB111*. *DnMYB81* belonging to the S7 subfamily had close homology with *AtMYB11*, *AtMYB12*, and *AtMYB111*. The RT-qPCR showed that *DnMYB81* expressed in all tissues of *D. nobile*,

but the highest expression level was found in the root. *DnMYB81* was also responsive to phytohormonal and abiotic stresses. *DnMYB81* was the most responsive to low temperature, and showed downregulated expression patterns under ABA and MeJA stress. In the future, we can verify whether *DnMYB81* and *DnMYB90* can regulate flavonoid synthesis through more in-depth experiments.

It has been reported that several *R2R3-MYB* genes are involved in regulating responses to biotic and abiotic stresses. For example, overexpression of the S1 subfamily *AtMYB94*, *AtMYB96* gene enhances drought tolerance in plants[35]. The S2 subfamily *AtMYB14* regulates cold tolerance in *Arabidopsis*[36]. Under phosphate starvation conditions, The S2 subfamily *AtMYB20*, which plays a role in plant responses to salt and drought stress, directly regulates the expression of miR399f [37]. Overexpression of the S21 subfamily *AtMYB52* confers ABA hypersensitivity and drought tolerance[38]. The S22 subfamily *AtMYB44* was tested in response to salt, MeJA and drought[39]. In order to explore *DnMYB* genes involved in hormonal and abiotic stresses, we focused on the expression levels of *DnMYB* genes from S1, S2, S20, S21, and S22 under low temperature, ABA, and MeJA stress. Using RT-qPCR, we found that *DnMYB27* of S1, *DnMYB14* of S2, *DnMYB116* of S20, *DnMYB26* of S21, and *DnMYB8* of S22, which were homologous to stress response genes, were expressed in all tissues. To further identify candidate genes involved in hormone signaling pathways or abiotic stress responses, we measured their expression levels under low temperature, ABA and MeJA stress. The results showed that the five genes showed increased expression levels at different times under different stress, which was consistent with the cis-acting element in its promoter (Fig. 2, Fig. 7). Interestingly, the expression level of the S19 subfamily *DnMYB108* gradually decreased after treatment with cold and MeJA stress. These results may suggest that members of the S19 subfamily play an important role in the response of *D. nobile* to abiotic stress. Some of the *DnMYB* genes screened in this study may be involved in abiotic stresses such as low temperature, ABA and MeJA stress, but the specific mechanism is still unclear and needs to be further verified in future experiments.

Abbreviations

TFs: transcription factors; RT-qPCR: real-time quantitative PCR; ABA: abscisic acid; MeJA: methyl jasmonate; HMM: hidden Markov model; NCBI: National Center for Biotechnology Information; MW: molecular weight; pI: isoelectric point.

Declarations

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Statement

Our experimental research on *Dendrobium nobile* was in accordance with the relevant national/institutional guidelines.

Author contributions

Conceived, designed, and implemented the study: LW, LZ, and SX; Statistics analysis: LW, JF, WJ, LZ, and XS; Reagents/materials/analysis tools: ZS, DP, and SX; Drafted the manuscript: LW, XS, WR, YD, YW, and SX; All authors participated in the editing of the manuscript and agreed to submit the final version.

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Data availability statement

All data generated or analyzed in this study are included in this article (Supplementary file). The genome sequences of *D. nobile*, *D. chrysotoxum* and *Arabidopsis* were downloaded from the <https://www.ncbi.nlm.nih.gov/genome/?term=dendrobium+nobile>, <https://www.ncbi.nlm.nih.gov/genome/?term=Dendrobium+chrysotoxum>, and <https://www.arabidopsis.org/>.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

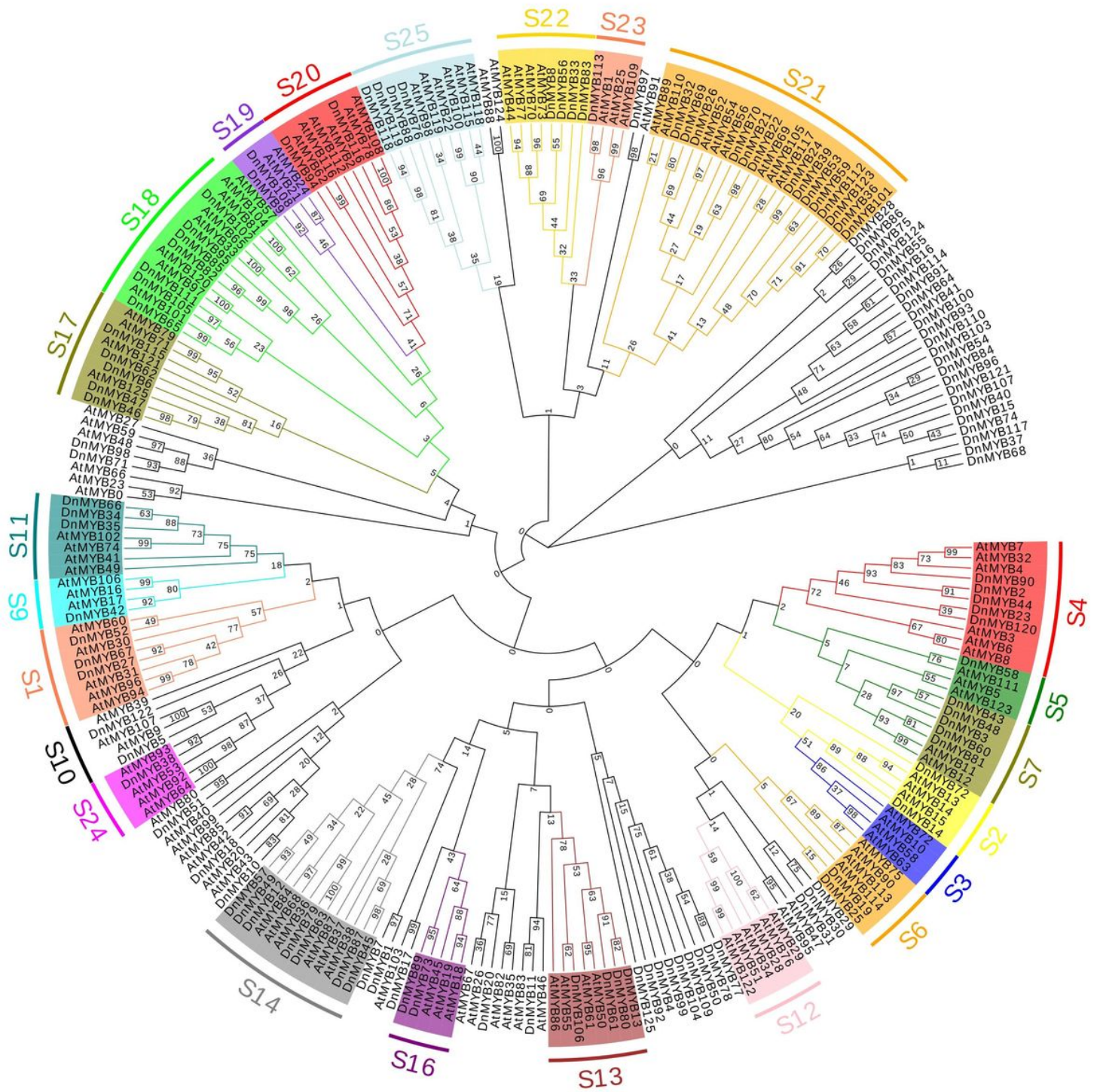


Figure 1

Phylogenetic tree of R2R3-MYBs in *D. nobile* and *A. thaliana*. The figure was generated by using MEGA-software coupled with a neighbor-joining method and a bootstrap of 1,000 replicate.

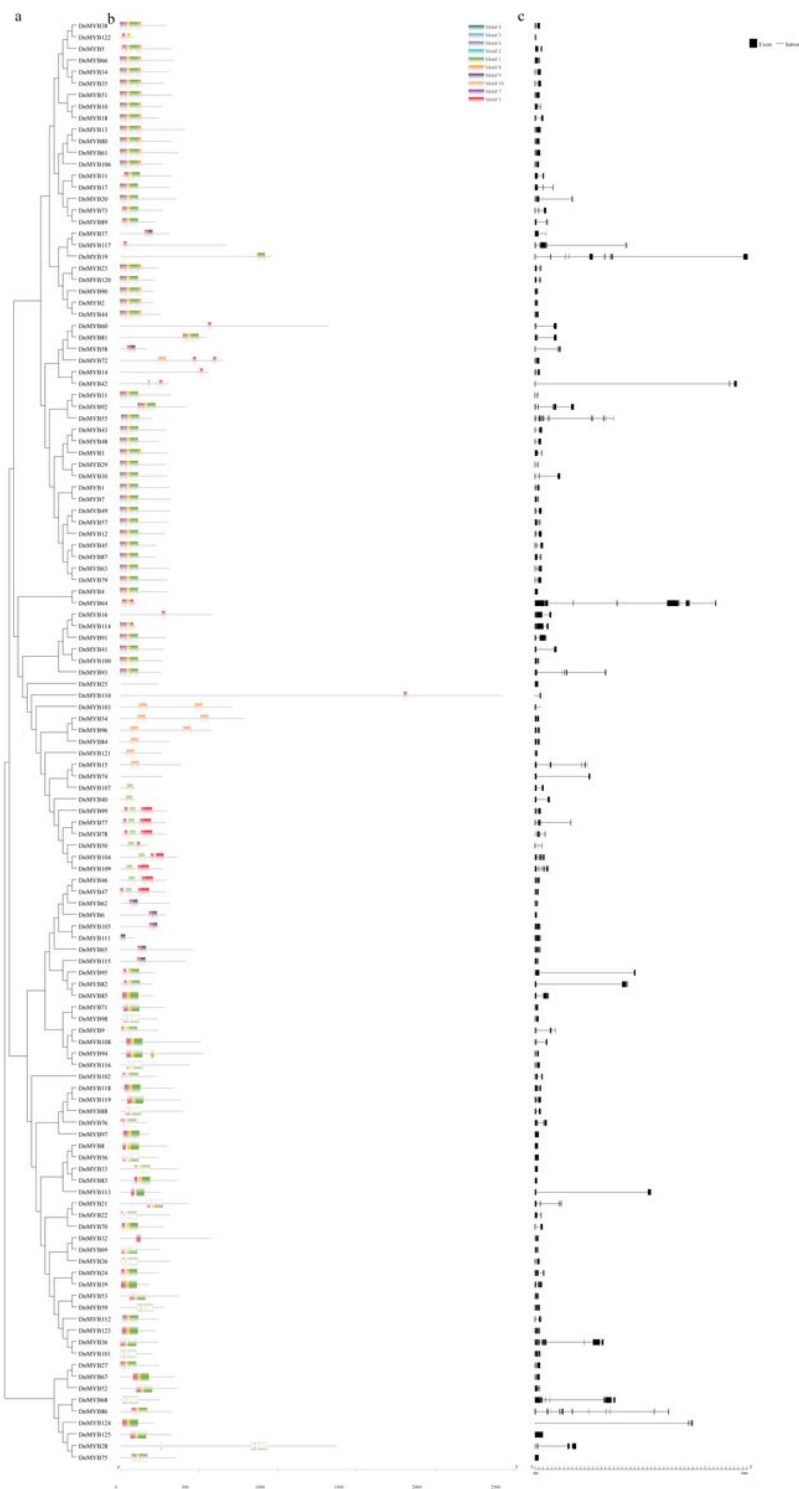


Figure 2

Phylogenetic relationships, conserved motifs, and gene structures of R2R3-DnMYBs family member. (a) The phylogenetic tree was constructed based on the full-length sequences of 125 R2R3-DnMYB proteins using the neighbor-joining method in Mega X, with 1000 bootstrap replicates. (b) Conserved motifs of DnMYB proteins. Different motifs are represented by various colored boxes. (c) Exon/intron structures of *DnMYB* genes. Exon(s), and intron(s) are represented by black boxes and black lines, respectively.

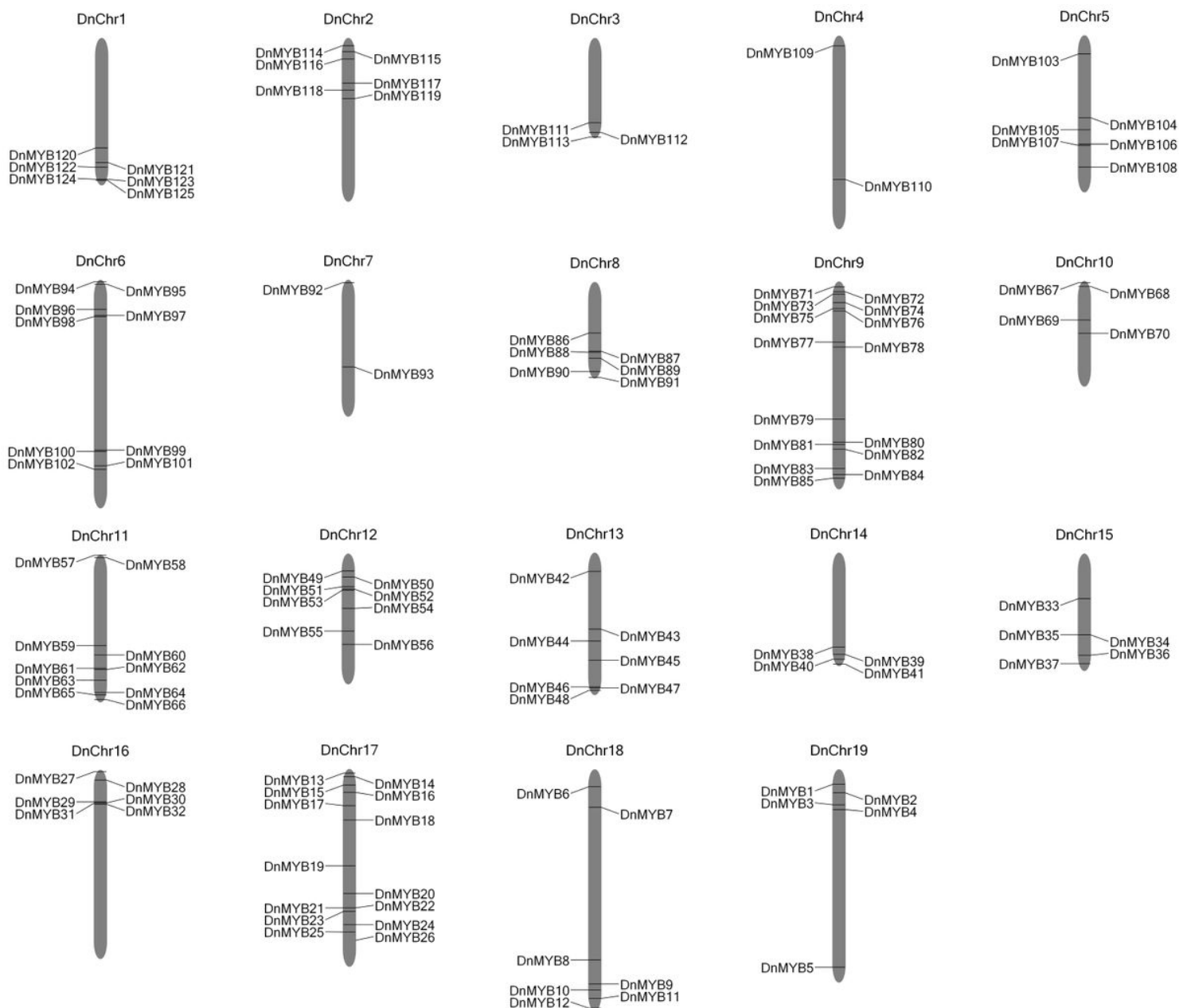


Figure 3

Distribution of 125 R2R3-DnMYB genes on chromosomes of *D. nobile*.

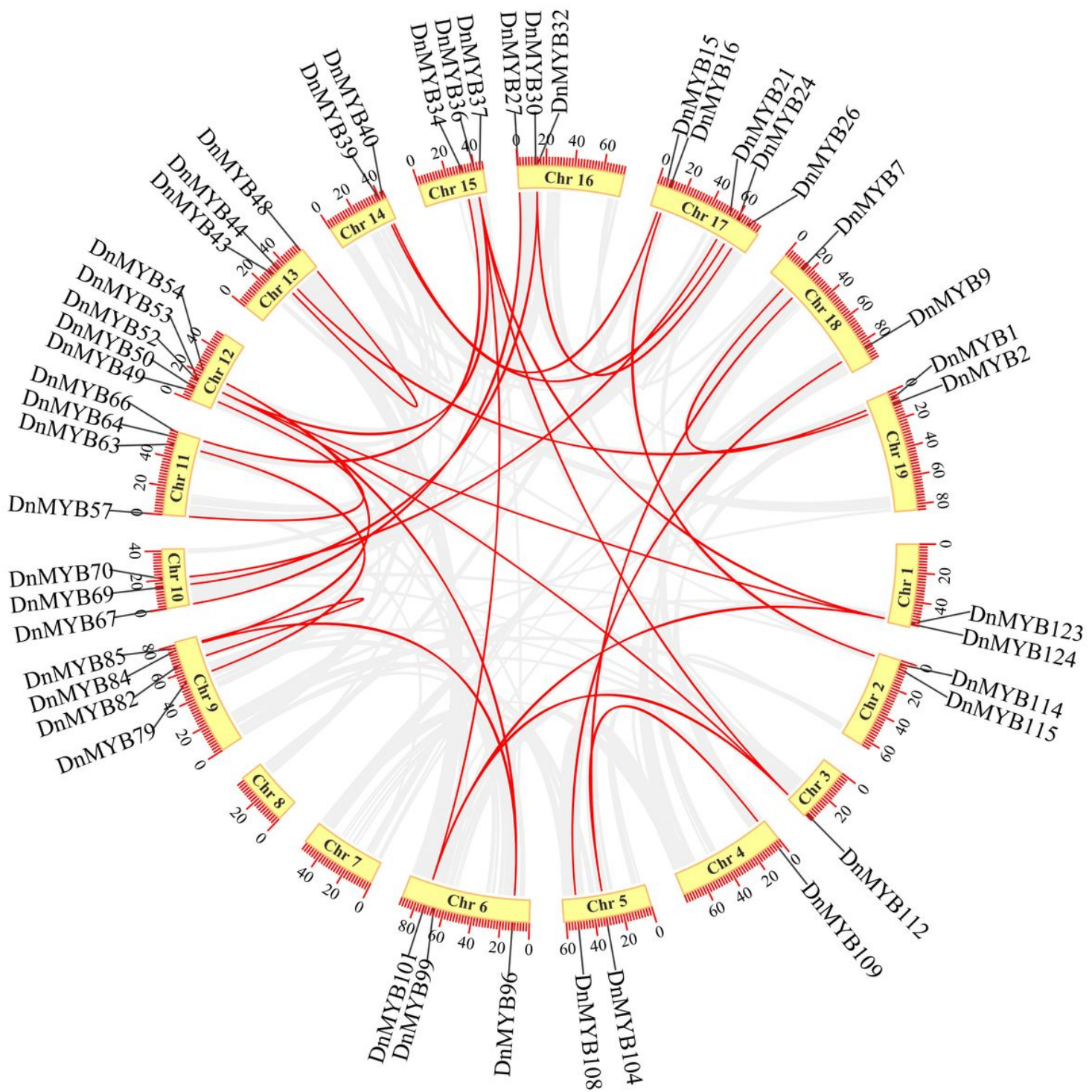


Figure 4

Synteny analysis of interchromosomal relationships of *DnMYB* genes. Red lines indicate duplicated gene pair.

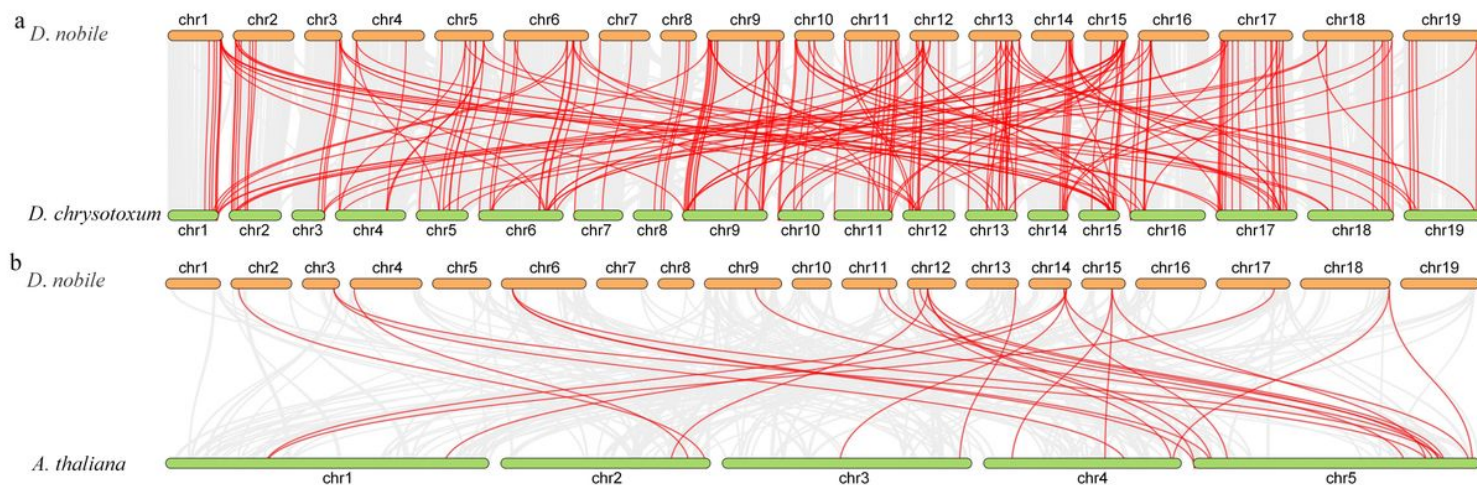


Figure 5

Synteny analysis of *DnMYB* genes in the genomes between *D. nobile* and *D. chrysotoxum* *A. thaliana*. (a) *D. nobile* and *D. chrysotoxum*; (b) *D. nobile* and *A. thaliana*. The gray lines show collinear blocks. The red and blue lines indicate the syntenic gene pairs between *D. nobile* and *D. chrysotoxum* *A. thaliana*, respectively.

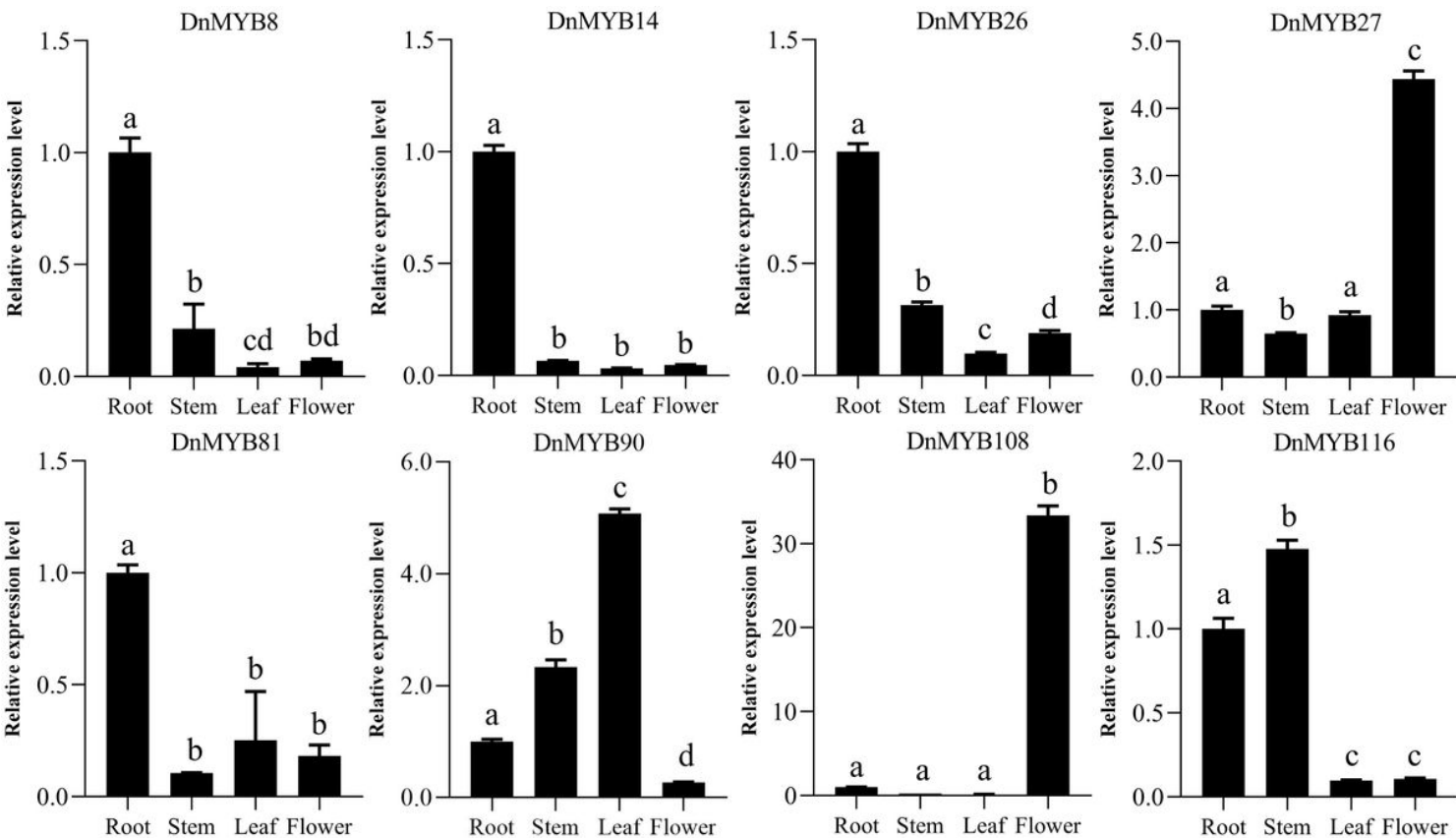


Figure 6

Relative expression levels of 8 selected R2R3-DnMYB genes in different tissues. Error bars indicate the mean $\pm$ SE of three independent replicates. Different lowercase letters (a, b, and c) are significantly different (p -value < 0.05).

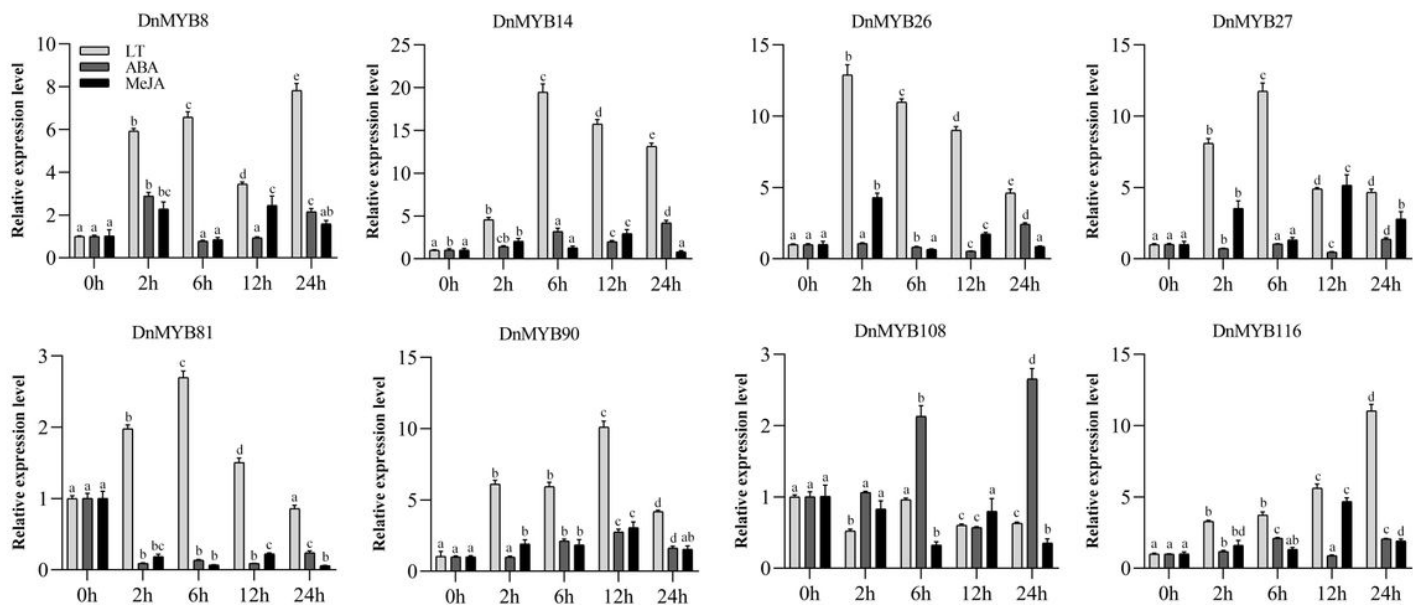


Figure 7

Expression of 8 R2R3-DnMYB genes at 0, 2, 6, 12, and 24 h under various abiotic stresses. Error bars indicate the mean $\pm$ SE of three independent replicates. Different lowercase letters (a, b, and c) are significantly different (p -value < 0.05).

Supplementary Files

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- [SupplementaryFigureS1ThecisactingelementsanalysisofDnMYBgenespromoters.jpg](#)
- [SupplementaryTableS1PrimersusedinrealtimequantitativePCRRanalysis..xlsx](#)
- [SupplementaryTableS2PhysicochemicalpropertiesofDnMYBproteins..xlsx](#)
- [SupplementaryTableS3ExonsandintronsstructureofallDnMYBs..xlsx](#)
- [SupplementaryTableS4LengthandchromosomallocationoftheDnMYBs..xlsx](#)
- [SupplementaryTableS5Thecisactingelements2000bpsequenceupstreamofDnMYBspromoterregion..xlsx](#)