Phyton-
International Journal of
Experimental Botany

Tech Science Press

DOI: 10.32604/phyton.2023.045389

ARTICLE

Identification and Molecular Characterization of the Alkaloid Biosynthesis Gene Family in *Dendrobium catenatum*

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Received: 25 August 2023 Accepted: 22 November 2023 Published: 26 January 2024

ABSTRACT

As one of the main active components of *Dendrobium catenatum*, alkaloids have high medicinal value. The physicochemical properties, conserved domains and motifs, phylogenetic analysis, and cis-acting elements of the gene family members in the alkaloid biosynthesis pathway of *D. catenatum* were analyzed by bioinformatics, and the expression of the genes in different years and tissues was analyzed by qRT-PCR. There are 16 gene families, including 25 genes, in the *D. catenatum* alkaloid biosynthesis pathway. The analysis of conserved domains and motifs showed that the types, quantities, and orders of domains and motifs were similar among members of the same family, but there were significant differences among families. Phylogenetic analysis indicated that the gene family members showed some evolutionary conservation. Cis-acting element analysis revealed that there were a large number of light-responsive elements and MYB (v-myb avian myeloblastosis viral oncogene homolog)-related elements in these genes. qRT-PCR showed that expressions of gene family members involved in alkaloid synthesis were different in different years and tissues of *D. catenatum*. This study provides a theoretical basis for further exploration of the regulatory mechanisms of these genes in the alkaloid biosynthesis of *D. catenatum*.

KEYWORDS

Dendrobium catenatum; gene family; alkaloid biosynthesis

Abbreviations

DXS	1-deoxy-D-xylulose 5-phosphate synthase
DXR	1-deoxy-D-xylulose 5-phosphate reductoisomerase
MCT	2-C-methyl-D-erythritol-4-phosphate cytidylyl-transferase
CMK	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase
MDS	2-C-methyl-d-erythritol 2,4-cyclodiphosphate synthase
HDS	1-hydroxy-2-methyl-2-butenyl 4-diphosphate synthase
HDR	1-hydroxy-2-methyl-2-butenyl 4-diphosphate reductase
FPPS	Farnesyl diphosphate synthase
AACT	Acetoacetyl-CoA thiolase

HMGS	3-hydroxy-3-methylglutaryl-CoA synthase
HMGR	3-hydroxy-3-methylglutaryl-CoA reductase
MVK	Mevalonate kinase
PMK	Phosphomevalonate kinase
MPDC	Mevalonate diphosphosphate decarboxylase
IPPI	Isopentenyl diphosphate isomerase
GGPPS	Geranylgeranyl pyrophosphate synthase

1 Introduction

D. catenatum is a perennial herb of the orchid family *Dendrobium*, mainly distributed in Southeastern, Southern, and Southwest China [1]. *Dendrobium* plants have high medicinal value and are widely used in traditional Chinese medicine [2]. *Dendrobium* has special pharmacological effects on resisting aging [3] and treating gastritis [4], diabetes [5], and cancer [6]. It has been used as a folk medicine in China for more than 2,000 years [7]. The main active components of *Dendrobium* include alkaloids [8], polysaccharides [9], and polyphenolic compounds [10], of which alkaloids are the main medicinal components, with significant antioxidant [11], anticancer [12], analgesia [13], neuroprotection [14], and other effects. *D. catenatum* is one of the most famous *Dendrobium* species in China but is now on the brink of extinction due to its widespread use in healthcare [15–17] and has been listed as one of the rare medicinal plants in China [18].

Alkaloids have been said to be important biomarkers because of their complex chemical structure and variety [19]. These include pyrrole, indolizidine, terpenoid alkaloids, organic amine alkaloids, indole, quinazoline, and others [20]. Most of the alkaloids in *D. catenatum* are terpenoid indole alkaloids [21], which are concentrated in the leaves. The amount of alkaloids in the leaves is between 0.0291% and 0.0421% [22], which is less than the total alkaloid content of related Dendrobium species [23]. However, the alkaloids produced by *D. catenatum* are of higher quality than other related species [24]. The upstream biosynthetic pathway of *D. catenatum* alkaloids consists of the conserved MVA pathway and MEP pathway, providing the basic skeleton for terpenoid alkaloids [25]. There are a series of P450 monooxygenases and aminotransferases following strictosidine in the downstream of Dendrobium alkaloid synthesis pathway [23]. Cytochrome P450 is involved in oxidation and hydroxylation reactions, and aminotransferases convert amino acids to form alkaloids [26].

In recent years, with the publication of the complete gene sequence of *Dendrobium* [27,28], there have been many studies on genes related to alkaloid biosynthetic pathways in *Dendrobium*. For instance, Yuan et al.'s transcriptome analysis of *Dendrobium huoshanense* revealed numerous differentially expressed genes involved in the production of alkaloids in various samples, and they also confirmed the expression patterns of the five important enzyme genes involved in terpenoid pathway in different tissues [29]. Transcriptome analysis revealed related genes and genetic markers in alkaloid biosynthesis pathways in *D. catenatum*, including 25 alkaloid backbone biosynthesis genes, P450 genes, transaminase genes, methyltransferase genes, multidrug resistance protein transporter protein genes, and transcription factor genes [23]. By using MeJA, alkaloid biosynthesis was induced and abundant MeJA-induced transcription factor-encoding genes were found, indicating that the genetic network that affect the metabolism of terpenoid alkaloids in *D. catenatum* is very complex [30]. By analyzing transcripts from four organs of *D. catenatum*, genes associated with putative upstream elements of the alkaloid biosynthesis pathway in *D. catenatum* were identified [31]. Song et al. introduced the biosynthetic pathways and associated gene clusters for each class of alkaloids in *Dendrobium* [32]. Wang et al. analyzed the correlation between gene expression levels and metabolite content from comparative transcriptome sequencing of different organs of *D. catenatum*, identifying putative genes for enzymes involved in alkaloid biosynthesis [33].

These studies have made great contributions to the further development and application of *Dendrobium* alkaloids, but there is a lack of studies on the structure and function of gene families related to the alkaloid biosynthesis of *Dendrobium*, and their roles in complex regulatory networks are not well understood. Therefore, in this article, we conducted a more in-depth understanding and mining of related gene families in the alkaloid biosynthesis of *D. catenatum*.

In this study, we identified 16 types of gene family members in the alkaloid biosynthesis pathway by using *D. catenatum* genome data as a resource platform and using relevant bioinformatics methods. The physicochemical properties, gene structure, phylogeny, and cis-acting elements of the encoded protein were analyzed. Quantitative analysis of *D. catenatum* in different years and different tissues was performed using qRT-PCR to verify the differential regulation of structural genes in the alkaloid biosynthesis pathway. Our research will help to further interpret the molecular mechanism of *D. catenatum* alkaloid biosynthesis and regulation and can also provide references for alkaloid research in other *Dendrobium* species.

2 Materials and Methods

2.1 Plant Materials

The *D. catenatum* stems were grown in the greenhouse of Jiangsu Yangzhou Urban Forest Ecosystem National Observation and Research Station, China. Half-strength Murashige and Skoog (MS) culture media supplemented with 6-BA 0.1 mg·L^{-1}, NAA 0.5 mg·L^{-1} and 1% additives (30 g·L^{-1} sucrose + 4 g·L^{-1} agar + 20% potato) were used for seed germination and protocorm-like body. The plants were cultured at 25°C ± 2°C and the photoperiod was 12/12 h (day/night, 30 μmol·m^{-2}·S^{-1}). After 18 months, the plants were transplanted into a greenhouse pot, the environmental conditions were 25°C–27°C, the day and night light was 12/12 h and the relative humidity was 60%–70%. They were sampled at one, two, three and four years of age. Annual, biennial, triennial, and quadrennial *D. catenatum* stems were collected and then frozen quickly in liquid nitrogen for RNA extraction.

2.2 Data Mining and Identification of Alkaloid Biosynthetic Genes

By the *A. thaliana* Information Resource (TAIR) database, sequences of *Arabidopsis thaliana AtDXS*, *AtDXR*, *AtMCT*, *AtCMK*, *AtMDS*, *AtHDS*, *AtHDR*, *AtAACT*, *AtHMGS*, *AtHMGR*, *AtMVK*, *AtPMK*, *AtMPDC*, *AtIPPI*, *AtFPPS*, and *AtGGPPS* were downloaded (Supplementary file 1 Table S1). By using BLAST program (E-value < 1e-5) in TBtools (v1.098745), the protein sequence for identifying homologous genes in the *D. catenatum* genome was inquired. The sequences obtained were compared with NCBI database by BLASTp program and corrected manually. The conserved domains of obtained candidate protein sequences were predicted by the Pfam database (v35.0) (http://pfam.xfam.org/) and manually removed incomplete conserved structural domains. Simple Modular Architecture Research Tool (SMART) database (http://smart.embl-heidelberg.de/) was used to verify conserved domains. Finally, 25 protein sequences with conserved structural domains corresponding to 16 gene families were obtained and named.

2.3 Analysis of Physicochemical Properties and Gene Structure

ExPASy (http://web.expasy.org/protparam/) was used to predict proteins' physical and chemical properties, such as length, molecular weight, isoelectric point, instability index, fat index, and hydrophilic average. The online tool WOLF PSORT (http://wolfpsort.hgc.jp/) was used to predict the subcellular localization of proteins encoded by genes in the alkaloid biosynthesis pathway of *D. catenatum*. The program SOPMA secondary structure prediction (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl/page=npsa_sopma.html) was used for predicting the secondary structure of members of gene families, including α-helix, β-fold, extended chain, and irregular crimp ratio.

2.4 Analysis of the Domain and Conservative Motif

Pfam was used to predict protein domains of members of gene families and the protein domain map was created. Conserved motifs of proteins encoded by all members of the gene family were predicted by MEME (http://meme.nbcr.net/meme/). It was set to 15 as the maximum motif number and the rest of the values were set to default. TBtools was used to visualize the results of the analysis in conjunction with the phylogenetic tree [34].

2.5 Multiple Sequence Alignment and Phylogenetic Analysis

As part of the analysis of evolution within and between gene families of alkaloid biosynthesis in *D. catenatum*, the protein sequences of 32 genes involved in alkaloid biosynthesis have been downloaded from the TAIR database and we downloaded the protein sequences of 131 genes from 47 species from the NCBI database. The obtained gene precursor sequences were compared using ClustalX2.1 online software, and the results were entered into MEGAX [35,36]. Based on the adjacency method (Nearby-joining, NJ), phylogenetic trees of gene families were constructed for the *D. catenatum* genome and complex multi-species. We set the Bootstrap value to 1000, and the other parameters were set to the system defaults.

2.6 Prediction of Cis-Acting Elements

The promoter sequences for gene family members (2000 bp upstream of transcriptional initiation sites) were obtained from the genome of *D. catenatum*. Plant CARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) was used for predicting cis-acting elements and visually mapped by using TBtools [34].

2.7 Expression Analysis of Alkaloid Biosynthesis Gene Family

The alkaloid biosynthesis gene family was resolved using tissue-specific and different growth years analysis with the online database NCBI, then we downloaded the raw data from the NCBI database (PRJNA476016 and PRJNA776680), and the expression level of these genes was extracted [37,38]. To estimate the gene expression based on the Fragments Per Kilobase of the gene model per Million fragments mapped (FPKM), RSEM (v1.2.15) with default parameters was employed [39].

2.8 RNA Isolation and Reverse Transcription Quantitative PCR (qRT-PCR) Analysis

Omni Plant RNA kit (CWBIO, China) was used to check the purity of total RNA extracted from *D. catenatum* stems. cDNA was converted from two hundred ng Poly(A)+ mRNA from different samples at 42°C in a 20 μL reaction volume by AMV Reverse Transcriptase. Gene-specific primers were designed by Oligo 7 (Supplementary file 1 Table S2). Amplification conditions of all PCRs were performed as follows: 10 min at 95°C, followed by 40 cycles of 10 s at 95°C, 15 s at 50°C, and 30 s at 72°C. Based on the expression level of the Actin gene (GenBank accession number: KC831582.1) in *D. catenatum* as the reference gene [40]. The expression level of the target gene was calculated by the $2^{-\Delta\Delta CT}$ method [41]. The qRT-PCR was performed by using the ABI 7500 real-time PCR system (Applied Biosystems, USA).

3 Results

3.1 Identification and Characterization of the Alkaloid Biosynthetic Gene Family in D. catenatum

Sixteen gene families in the alkaloid synthesis pathway of *D. catenatum* were identified and 25 enzyme genes were obtained in this study. “Dc” was used as a prefix for the genes in the *D. catenatum* alkaloid synthesis pathway and sorted the genes according to their chromosome position. The number of these 16 gene family members in *D. catenatum* and *A. thaliana* were compared (Fig. 1). It was found that there were 13 GGPPS gene family members in *A. thaliana*, while only 3 in *D. catenatum*. The remaining 15 gene family members in *D. catenatum*, except for *GGPPS*, were similar in number to those in *A. thaliana*.

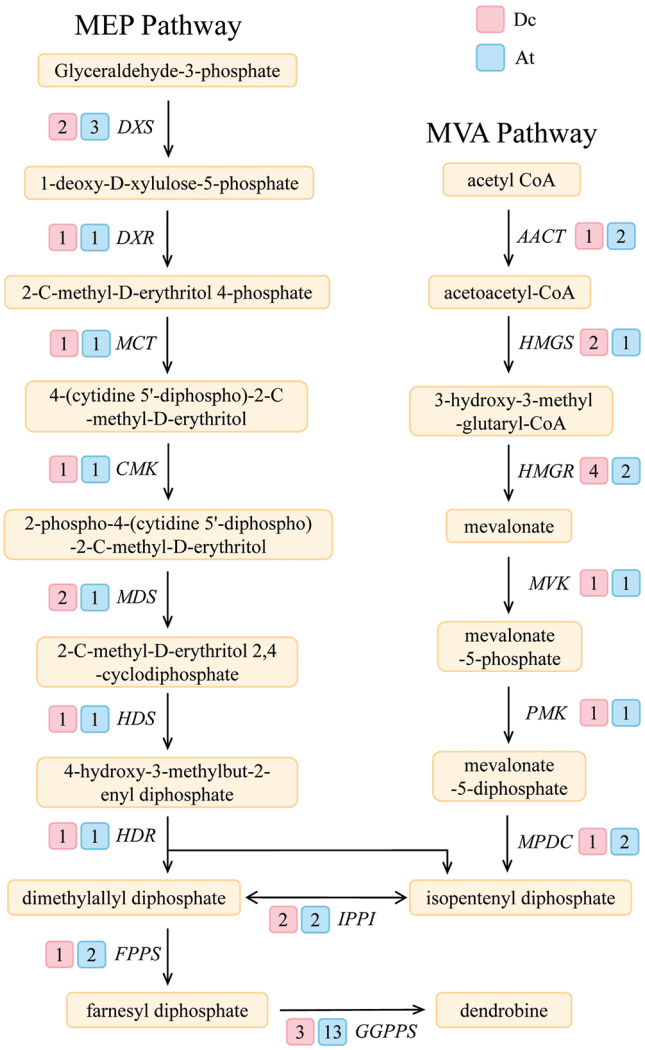

Figure 1: Comparative analysis of gene numbers in families in the alkaloid biosynthesis pathway in *D. catenatum* and *A. thaliana*. The pink and blue boxes indicate the number of genes in *D. catenatum* and *A. thaliana*, respectively

In addition, the physicochemical properties, subcellular localization, and secondary structure of these 25 proteins were analyzed and compared, which ranged from 137 (*DcMDS1*) to 745 (*DcHDS*) amino acids in length, with an average length of 407. The grand average of hydropathicity for these proteins ranged from −0.440 (*DcHDR*) to 0.307 (*DcHMGR3*), and there were 16 hydrophilic proteins and 9 hydrophobic proteins (Supplementary file 1 Table S3). Subcellular localization showed that 16 genes were localized in chloroplasts, 4 in cytoplasmic stroma, 2 in mitochondria, 1 in endoplasmic reticulum, 1 in plasma membrane, and 1 in chloroplasts and endoplasmic reticulum (Supplementary file 2). These proteins have four types of structures, including alpha-helix, beta-turn, random coil, and extended strand. The major secondary structure types of these proteins were alpha helix and random coil, accounting for 24.82%–63.51% and 27.24%–53.28%, respectively. Beta turn accounted for 2.30%~10.15%, and extended strand accounted for 5.19%~25.89% (Supplementary file 3).

3.2 Structural Analysis and Protein Domain Prediction of Genes Related to Alkaloid Biosynthesis

In order to clarify the structure and function of gene families related to the *D. catenatum* alkaloid biosynthesis pathway, the conserved domains of 25 genes in these gene families were identified and compared, and the domain composition was explored (Fig. 2). It was found that members of the same protein family share the same conserved domains which are not specific to one family. For example, *DcFPPS* and *DcGGPPS* both contain polyprenyl_synt; *DcCMK*, *DcMVK*, and *DcPMK* all contain GHMP_kinases_C; *DcCMK*, *DcMVK*, *DcPMK* and *DcMPDC* all contain GHMP_kinases_N. In addition, motifs of proteins in gene families were analyzed the conserved. It was found that proteins have multiple conserved motifs with similar types, numbers, and order in the same gene family (Fig. 3).

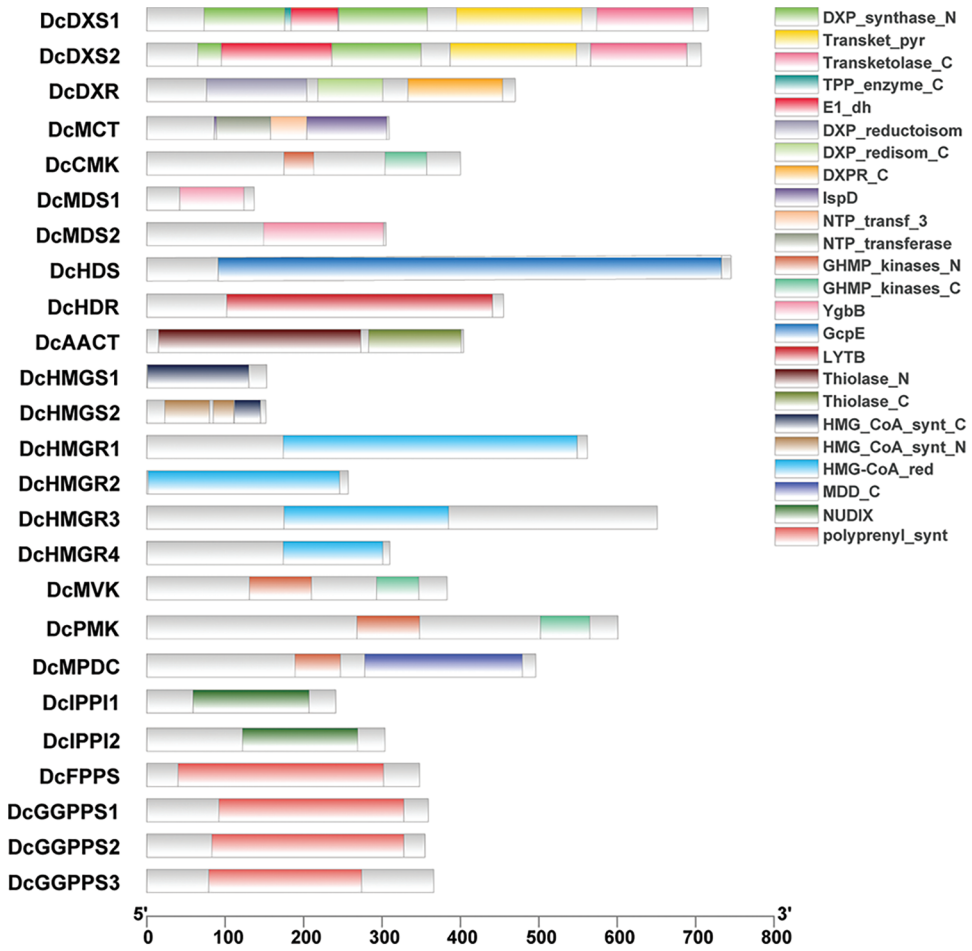

Figure 2: Protein domain analysis. Different colors represent different structural domains

3.3 Phylogenetic Analysis of Gene Families

A phylogenetic tree of each gene family was constructed to clarify the evolutionary relationships of these 16 gene family members between *D. catenatum* and other species (Fig. 4, Supplementary file 1 Figs. S1 and S2). Notably, the two members of the *DcDXS* family are evolutionarily distant. In the *DcHMGR* family, unlike *DcHMGR1* and *DcHMGR2*, *DcHMGR3* and *DcHMGR4* are evolutionarily distant from the *HMGR* in other plants. In the *DcGGPPS* family, *DcGGPPS3* is located in a separate small evolutionary branch, away from the genes of other species. All family members of *DcDXR*, *DcPMK*, and *DcIPPI* are on a small evolutionary branch.

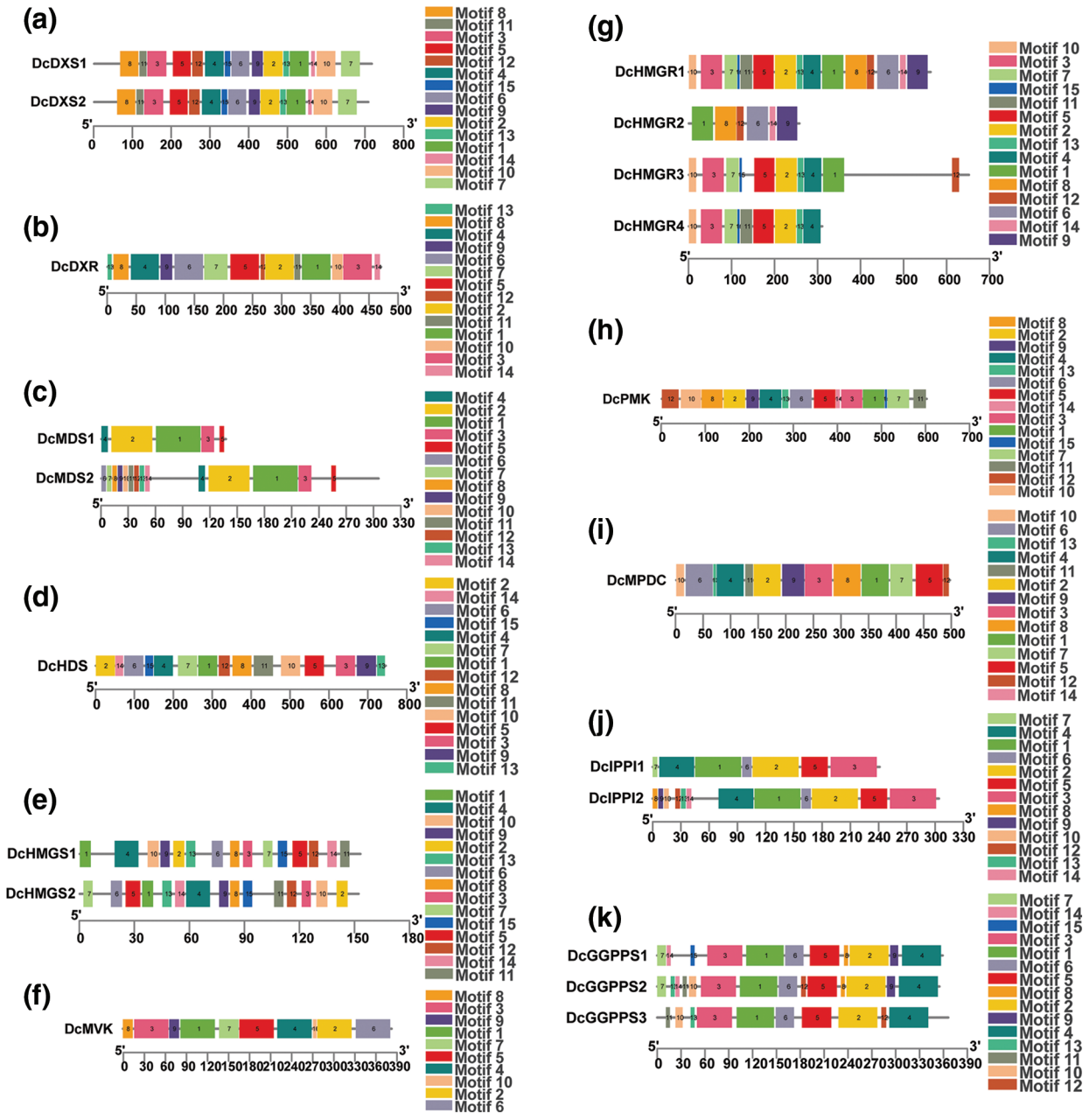

Figure 3: Analysis of conserved motifs of proteins. Different colored squares represent different motifs. (a) *DcDXS*, (b) *DcDXR*, (c) *DcMDS*, (d) *DcHDS*, (e) *DcHMGS*, (f) *DcMVK*, (g) *DcHMGR*, (h) *DcPMK*, (i) *DcMPDC*, (j) *DcIPPI*, (k) *DcGGPPS*

3.4 Promoter Cis-Acting Element Analysis

To determine the potential biological roles of these genes in *D. catenatum*, cis-acting elements were identified in the promoter regions of these genes (Fig. 5). Except *DcMDS1*, a total of 43 cis-acting elements in the promoters of other genes were detected. The promoters of these 24 genes contain 5-55 cis-acting elements and with an average of 32. *DcHDR* had the most cis-acting elements, while *DcHMGS1* contained the fewest cis-acting elements. These cis-acting elements are associated with plant

growth and development, hormone response, and stress response. For example, MRE is related to flowering, CAT-box is related to shooting and root meristem tissue expression; MBS is related to drought induction, LTR is related to low temperature stress; ABRE is related to abscisic acid, and TGA-element related to growth hormone.

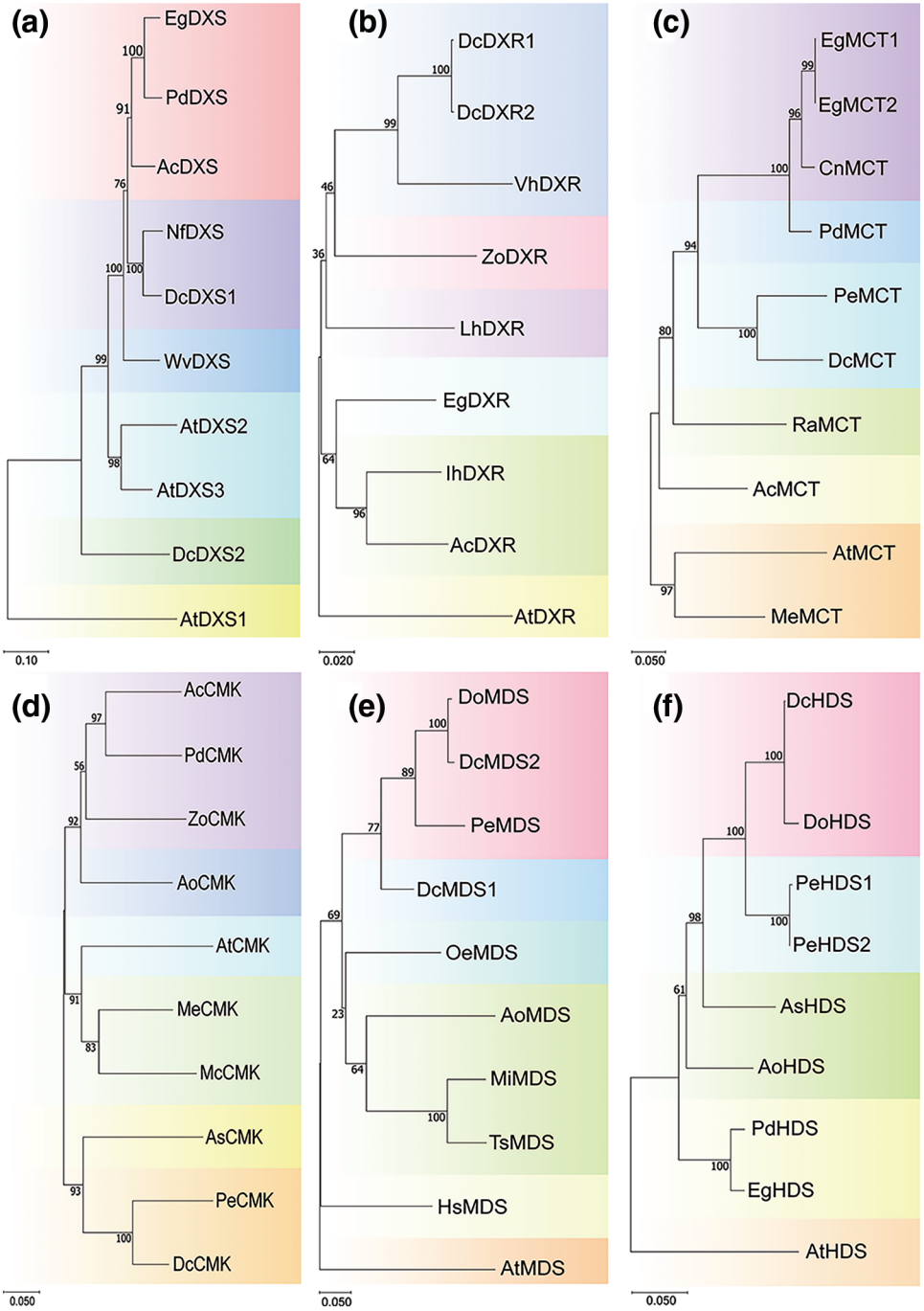

Figure 4: Phylogenetic analysis of genes in different species. (a) *DXS*, (b) *DXR*, (c) *MCT*, (d) *CMK*, (e) *MDS*, (f) *HDS*

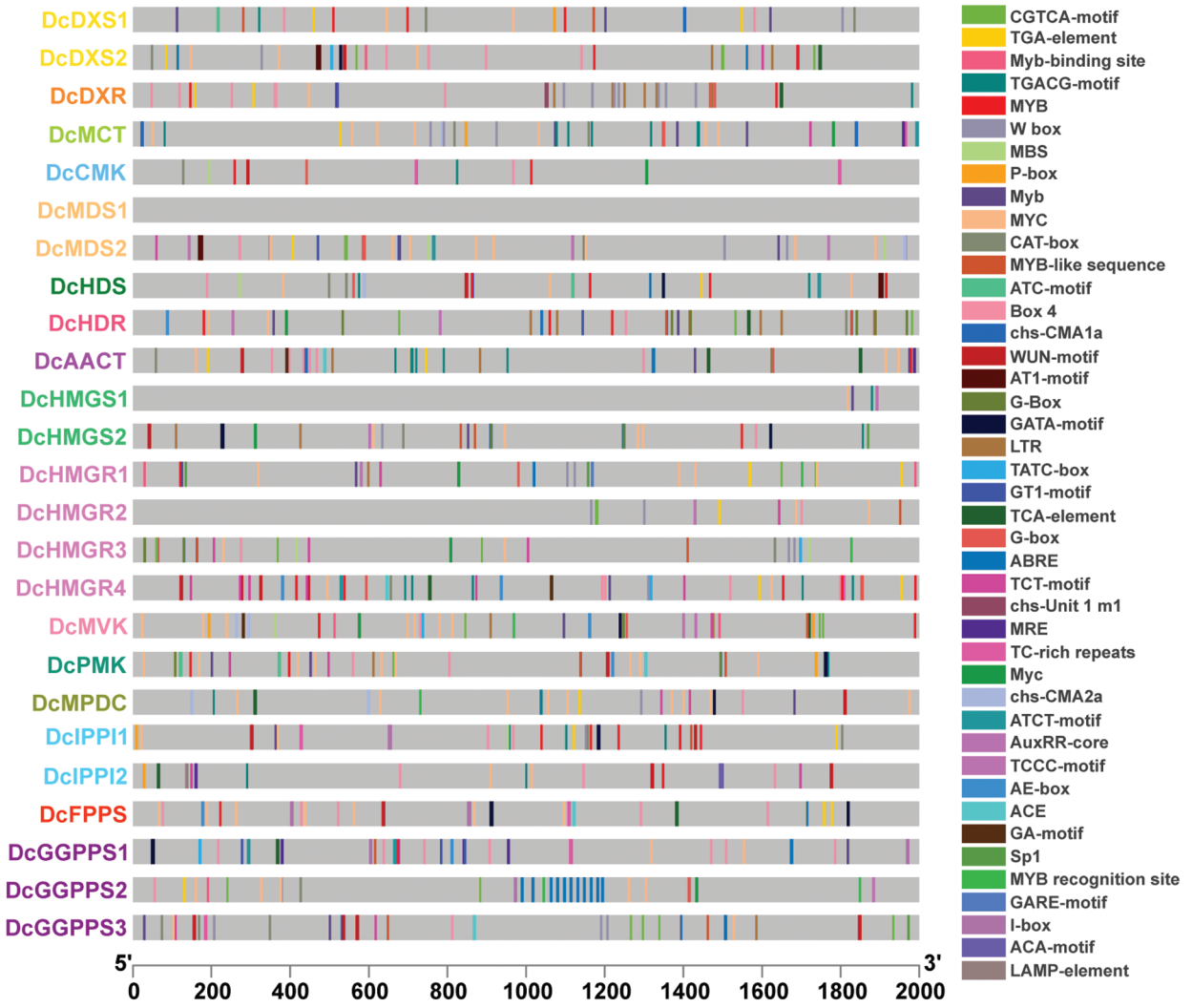

Figure 5: *Cis*-acting element analysis of promoters. Different colored squares represent different *cis*-acting elements

The occurrence of 14 cis-acting elements in each gene were counted and analyzed (Fig. 6). In the heatmap, the frequency of cis-acting elements was similar for these 25 genes. The light-responsive element was the most prevalent among almost all genes, especially in *DcHDR*, *DcAACT*, *DcHMGR*, *DcMVK*, *DcPMK*, *DcFPPS*, and *DcGGPPS* families. MeJA-responsive element, MYC-related element, and MYB-related element also appeared more frequently. In addition, the ABA-response element was more abundant in *DcHDR* and *DcGGPPS2*, but very few or even absent in other genes. In addition to the root-specific element, the remaining eight elements were detected, but in very small numbers and distributed only in individual gene families.

3.5 Gene Expression Analysis of Different Samples of D. catenatum

To explore the regulatory functions of genes in *D. catenatum*, we compared the expression of these genes in different tissues of biennial *D. catenatum* (Fig. 7a). Interestingly, the expression of these genes was generally low in the aboveground parts. *DcAACT*, *DcGGPPS1*, *DcHMGS1*, *DcMPDC*, *DcHMGS2*, *DcHMGR4*, *DcHMGR1*, and *DcFPPS* were highly expressed in the stem but lowly in the root, leaf, and

aboveground parts, suggesting that they may play a regulatory role in the stem. *DcDXS2* was highly expressed in the roots and lowly in the stems, leaves, and aboveground parts, indicating that it may play a regulatory role in the roots.

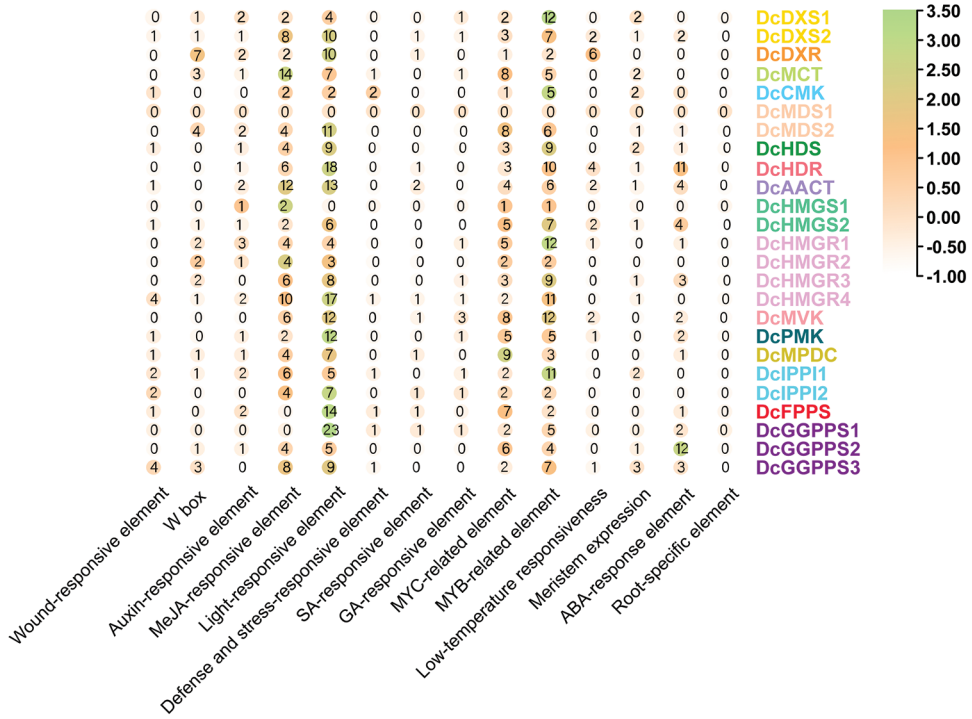

Figure 6: The number of heatmap clustering occurrences of *cis*-acting elements in the promoter sequences

In addition, the expression of 25 genes was analyzed in four different years in the stem of *D. catenatum* (annual, biennial, triennial, and quadrennial, respectively) (Fig. 7b). The results showed that the expression levels of these genes were highly variable among the samples of different years, but there was some similarity. *DcHMGR1* and *DcGGPPS1* were highly expressed in biennial *D. catenatum* and lowly expressed in other years. *DcDXS1*, *DcDXR*, *DcAACT*, *DcHMGS2*, *DcIPPI1*, and *DcGGPPS3* genes were highly expressed, which indicated that these genes may play an important regulatory role in the alkaloid biosynthetic pathway. We also found that the expression levels of different members of the same family differed in different years. For example, *DcMDS1* was expressed at low levels in annual and biennial *D. catenatum* and at higher levels in triennials and quadrennials, while *DcMDS2* was the opposite.

To further investigate the expression of alkaloid biosynthetic pathway gene family members in different years of *D. catenatum*, we selected 10 genes and determined their expression in four different years of plants using qRT-PCR. The results showed that the expression was relatively high in annual and biennial samples, and the expression of different genes in the triennial and quadrennial samples had large differences (Fig. 8). The highest expression of *DcMDS1* and the lowest expression of *DcDXS2* were found in the triennial samples. Therefore, we speculate that genes related to *D. catenatum* alkaloid biosynthesis have different functional and regulatory effects in different years.

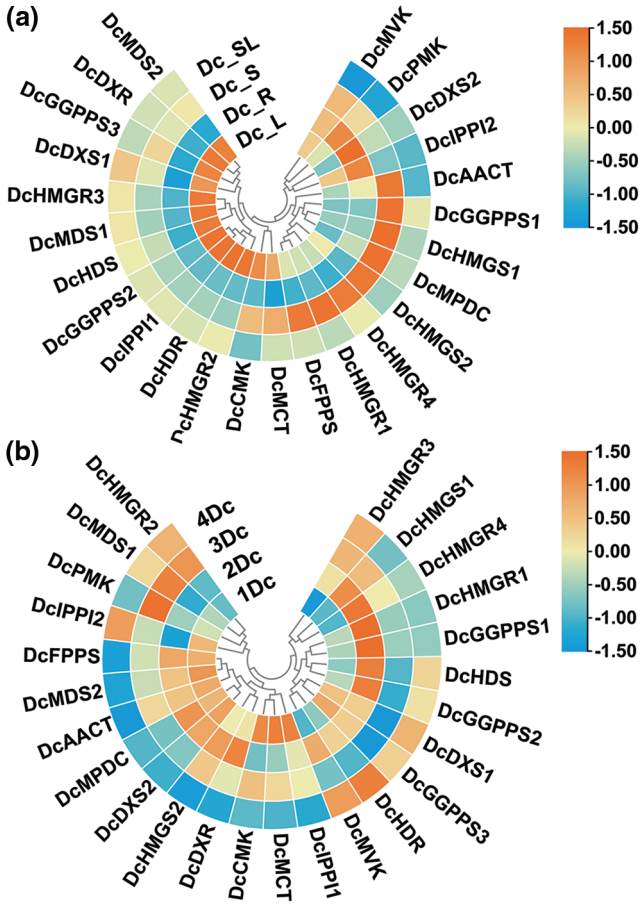

Figure 7: Heatmap of gene expression. (a) Heat map of gene expression in different tissues of biennial *D. catenatum*. (b) Heatmap of gene expression at different years of stem. Expression levels are indicated using a color scale from blue (low expression) to red (high expression)

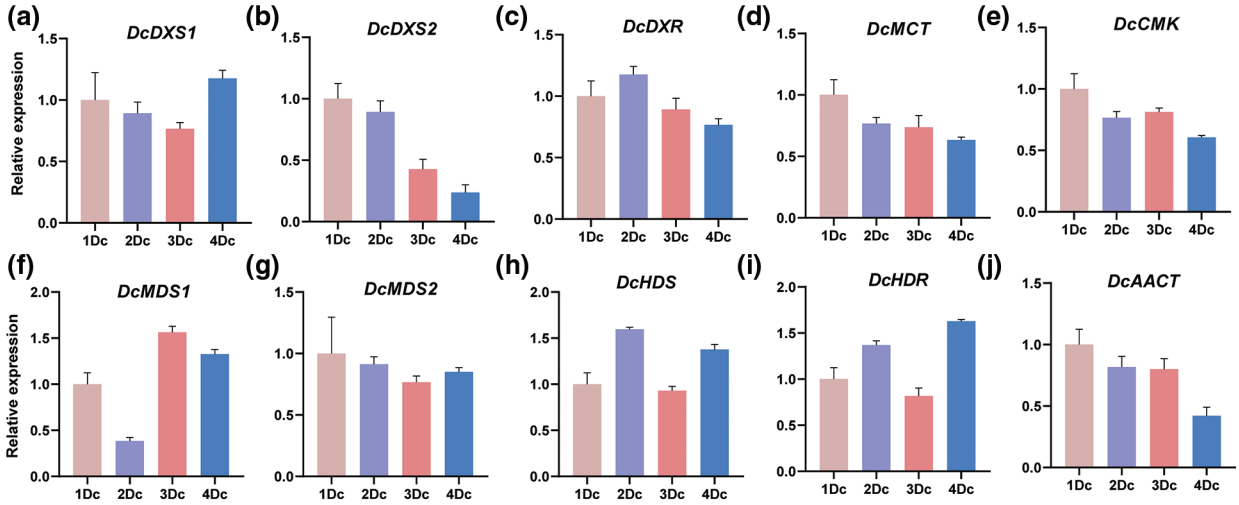

Figure 8: qRT-PCR of *D. catenatum* in different years. 1Dc indicates annual *D. catenatum*, 2Dc indicates biennial *D. catenatum*, 3Dc indicates triennial *D. catenatum* and 4Dc indicates quadrennial *D. catenatum*. The error bars indicate the mean ± SD (n = 3)

4 Discussion

The alkaloid extracted from *D. catenatum* is a natural product with significant biological activity, with neuroprotective, anti-inflammatory, and anti-tumor activity effects [8]. Genes related to alkaloid biosynthesis have been identified in other species, such as the model plants of alkaloid biosynthesis *Nicotiana tabacum* [42], *Catharanthus roseus* [43], *Sophora flavescens* [44], and *Papaver somniferum* [45]. Currently, studies on secondary metabolites of *D. catenatum* have focused on phenylpropanoids such as flavonoids [46] and anthocyanins [47]. The distribution of terpenoids in *D. catenatum* was determined by Zhan et al. [48]. It was found that *Dendrobium* alkaloid biosynthesis could be induced by MeJA treatment [30] and mycorrhizal fungal infestation [49]. AgNP applications significantly increased the indole alkaloid indirubin production in the shoot cultures of *Isatis tinctoria* and *Isatis ermenekensis* [50]. 14 potential genes associated with *D. catenatum* alkaloid biosynthesis were identified by Chen et al. which are similar to our identification [30]. There are few gene family analyses based on genome-wide identification of entire secondary metabolites, and all gene families on the flavonoid biosynthesis have been identified only in *Ginkgo* [51] and *Salvia* [52]. However, systematic analysis of all gene families in alkaloid biosynthesis has not been studied. In this study, bioinformatic analysis of 25 genes in 16 gene families of alkaloid biosynthesis in *D. catenatum* has laid the foundation for studying the gene regulatory network of *D. catenatum* alkaloids.

By structural analysis of these 25 genes, we found that the conserved structural domains exhibited significant differences among different family members. However, in the same family, there may also be differences in the composition of the conserved structural domains. For example, in the *DcDXS* family, compared to *DcDXS2, DcDXS1* has an additional conserved structural domain TPP_enzyme_C. In the conserved motif analysis, there were also differences between members of the same family, indicating that these genes are likely to also perform certain specific functions. The analysis of conserved domains and motifs revealed the structural diversity of gene family members in *D. catenatum*, which laid an important foundation for the subsequent exploration of the functional diversity of related gene families. We also constructed a multispecies evolutionary tree to screen for genes of other species with similar evolutionary relationships to functional genes. In constructing the evolutionary tree, we found that two family members of *DcHMGS* could not be made in the same evolutionary tree, and the remaining genes and homologs were in a large evolutionary branch. However, each of the two members of *DcHMGS* can form an evolutionary tree with the *HMGS* of other species. Therefore, we speculate that the *HMGS* gene was broken into two genes due to problems in genome sequencing or analysis. Phylogenetic analysis showed that members of the gene family were mostly clustered together, while a few genes were loosely distributed. The *DcDXR* family is evolutionarily closely related to *VhDX*R. Previous studies have shown that the transcription level of *DXR* is higher in florescence and full florescence of *Vanda* and regulates the generation of odor [53]. We guess that the *DXR* family in *D. catenatum* plays a role in regulating the generation of floral odor. *DcPMK* and *AtPMK* are not in the same branch and are far apart in an evolutionary relationship, which indicates that the *PMK* family plays different regulatory roles in these two species. Our results show that members of different gene families have different functions in the evolutionary process, which provides a way to predict the relationship between gene functions and specific biological processes.

Cis-acting elements are important molecular switches that participate in the transcriptional regulation of gene activity dynamic networks and control various biological processes, including abiotic stress responses, hormone responses, and developmental processes [54]. Through cis-acting element analysis, we found that except for *DcMDS1* and *DcHMGS1*, light-responsive elements appeared in a large number of other genes, so we speculated that these genes participated in and responded to plant photomorphogenesis. In addition, the MeJA-responsive element appears frequently in the gene. It has been shown that MeJA treatment can induce *D. catenatum* alkaloid biosynthesis [30]. We identified a large number of hormone response elements and

stress response elements in different quantities and types and speculated that *D. catenatum* alkaloid biosynthesis-related genes can respond to stimulation and hormone signal transduction.

To sum up, 16 gene families in *D. catenatum* alkaloid biosynthesis were identified in this study, and 25 genes were obtained. The protein structure, physicochemical properties, subcellular localization, evolutionary relationship, cis-acting elements of the promoter, and expression level of the gene were analyzed. By analyzing the expression level of gene family members in the alkaloid biosynthesis in different years and tissues of *D. catenatum*, it was found that the gene expression levels varied greatly in different years and different tissues. For example, the expression level of *DcHMGR1* in the stems of biennial *D. catenatum* was significantly higher than that in other years. Therefore, our future research on plant genetics and stress resistance will pay more attention to the expression level of specific genes in specific years and tissues of *D. catenatum*. These gene family members not only play an important role in alkaloid biosynthesis, but also participate in stress and hormone response, and play an important regulatory role in plant growth and development. This study revealed the characteristics of 16 gene families in alkaloid synthesis, which is helpful to further explore the structure and function of these family members.

Acknowledgement: This project funded by the Forestry Science and Technology Innovation and promotion Project of Jiangsu Province 'Long-Term Research Base of Forest and Wetland Positioning Monitoring in Jiangsu Province' (Grant No. LYKJ [2020]21), Natural Science Foundation of Jiangsu Province, China (Grant No. BK20210800), the National Natural Science Foundation of China (Grant Nos. 32001341 and 32202523) and Jiangsu Agriculture Science and Technology Innovation Fund (Grant No. CX (21)3047).

Funding Statement: Not applicable.

Author Contributions: L. Yang: conceptualization; data curation; resources; software; visualization; writing—original draft; writing—review and editing. X. Wan: conceptualization; data curation; investigation; resources; visualization; writing—review and editing; writing—review and editing. R. Zhou: visualization; writing—review and editing. Y. Yuan: conceptualization; funding acquisition; resources; supervision; validation; writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Availability of Data and Materials: The original contributions presented in the study are included the article and in Supplemental Materials. Further inquiries should be directed to the corresponding authors.

Ethics Approval: This article does not contain any studies with animals or humans performed by any of the authors.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

Supplementary Materials: The supplementary material is available online at https://doi.org/10.32604/phyton.2023.045389.

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