agronomy

MDPI

Article

Genome-Wide Identification of WRKY Transcription Factor Family and Its Expression Patterns in *Dalbergia odorifera* T. Chen

Qing Zhu [1,†], Feifei Chen [2,†], Xu Hu [1], Haoyue Zheng [1], Yi Liu [1], Chunchan Fu [1], Shangqian Xie [1], Dunxi Li [2,*] and Minqiang Tang [1,*]

[1] Key Laboratory of Genetics and Germplasm Innovation of Tropical Special Forest Trees and Ornamental Plants (Ministry of Education), School of Tropical Agriculture and Forestry, Hainan University, Haikou 570228, China; 22210907000001@hainanu.edu.cn (Q.Z.)
[2] Hainan Academy of Forestry (Hainan Academy of Mangrove), Haikou 571101, China
* Correspondence: lidxi@126.com (D.L.); tangminqiang@hainanu.edu.cn (M.T.)
† These authors contributed equally to this work.

Abstract: *Dalbergia odorifera* is a valuable material used in the crafting of luxury furniture, and the chemical constituents from heartwood possess significant medicinal value. The WRKY gene family, one of the most extensively studied gene families, plays an important role in plants. However, a comprehensive genome-wide identification of the WRKY gene family in *D. odorifera* has not been reported yet. In this study, a total of 99 WRKY genes were identified in *D. odorifera*. The DoWRKY genes were categorized into three primary groups with five subgroups. A collinearity analysis revealed 38 segmental duplications and 3 tandem duplications within the DoWRKY genes, indicating the pivotal role of segmental duplication in gene family expansion. Through the analysis of DoWRKY transcriptomic data across diverse tissues and under wounding stress, we found that only eight genes were universally expressed, while a subset displayed distinct tissue-specific patterns. Notably, *DoWRKY41* was exclusively expressed in leaves; *DoWRKY04* was solely in roots; and *DoWRKY17*, *DoWRKY28*, *DoWRKY47*, and *DoWRKY67* were uniquely in flowers. Furthermore, we identified 24 WRKY proteins that tightly respond to wounding stress (20 upregulated; 4 downregulated). This comprehensive investigation offered valuable insights into the WRKY gene family of *D. odorifera*, serving as a foundational resource for forthcoming explorations into the functional roles of these genes amid wounding stress.

Keywords: WRKY gene family; *D. odorifera*; transcriptome analysis; wounding stress

Citation: Zhu, Q.; Chen, F.; Hu, X.; Zheng, H.; Liu, Y.; Fu, C.; Xie, S.; Li, D.; Tang, M. Genome-Wide Identification of WRKY Transcription Factor Family and Its Expression Patterns in *Dalbergia odorifera* T. Chen. *Agronomy* **2023**, *13*, 2591. https://doi.org/10.3390/agronomy13102591

Academic Editor: Dilip R. Panthee

Received: 22 August 2023
Revised: 3 October 2023
Accepted: 8 October 2023
Published: 10 October 2023

1. Introduction

Dalbergia odorifera T. Chen, a valuable plant of the genus *Dalbergia*, is often referred to as "Huanghuali" in Chinese. *D. odorifera* is native to Hainan Province, China. Renowned for its exceptional resistance to insects and corrosion, it has become a highly precious species used in the production of luxurious furniture and exquisite crafts [1]. Moreover, the heartwood of *D. odorifera* possesses significant medicinal value and is referred to as "Jiang Xiang" in the Chinese pharmacopoeia. It is extensively utilized in the treatment of conditions related to blood stasis and ischemia [2]. Additionally, its unique fragrance renders it a valuable asset in the production of refined essential oils for perfumes [3]. Due to extensive exploitation, the wild *D. odorifera* trees, valued for their medicinal and commercial significance, have become scarce, prompting their inclusion in the IUCN Red List and underscoring the imperative for urgent protection and conservation efforts.

Transcription factors (TFs) are essential proteins that exert regulatory control over gene expression. This pivotal role enables them to influence a diverse array of biological processes [4]. Through an in-depth investigation of plant transcription factors, it is possible

to elucidate some intricate physiological regulatory mechanisms in plants. At present, the WRKY gene family stands as one of the most extensively investigated transcription factor families in plants [5], with its members unified by the highly conserved WRKY domain, a hallmark of the family. The most prominent feature within this highly conserved WRKY domain is the presence of an exceedingly conserved sequence (WRKYGQK) and zinc finger motifs (C2H2 or C2HC). These transcription factors serve crucial roles in governing gene expression through their interaction with specific DNA sequences, ultimately leading to the altered transcriptional activity of multiple target genes [6]. This DNA-binding domain specifically attaches to the W-box sequence located within the promoter region of the target gene [7].

WRKY gene families are usually classified into three major groups, considering the number of WRKY domains and the type of zinc-finger domains they contain. Members of Group I possess a dual presence of WRKY domains, while Group II and Group III constituents have a single WRKY domain [8]. Subsequently, we differentiated the second and third groups based on the type of zinc-finger domain. Members of Group II typically exhibited a C2H2-type zinc-finger domain, whereas those of Group III featured a C2HC-type zinc-finger domain. However, due to the excessive number of individuals in the second group, it is common practice to further categorize the Group II into five subgroups in accordance with the phylogenetic tree results. Group II is typically subdivided into two major branches, IIa + IIb and IId + IIe, while the remaining members belong to the IIc subgroup; in addition, Group II genes are not monophyletic [9]. In the last few years, significant advancements have been achieved in the identification of the WRKY gene family across numerous plant species. For instance, *Dendrobium catenatum* was found to possess 62 WRKY gene family members [10]; the number of WRKY genes in eggplant is 58 [11], and *Helianthus annuus* L. harbors a remarkable total of 90 WRKY genes [12]. However, a comprehensive genome-wide identification of the WRKY gene family in *D. odorifera* has not been reported yet.

Abundant studies indicated that WRKY TFs have the capacity to regulate numerous physiological activities in plants. These procedures cover a range of growth and development-related activities, including hormone signaling [13], senescence [14], and embryonic morphogenesis [15], as well as seed germination [16]. Furthermore, the WRKY gene family participates in responding to abiotic stressors, including drought and salt [2], as well as biotic stressors caused by fungi, bacteria, and viruses [17]. In cotton, *GhWRKY27* is capable of modulating leaf senescence through its interaction with other genes [18]. *OsWRKY29* directly downregulates the expression of *OsABF1* and *OsVP1*, resulting in repressing seed dormancy in rice [19]. Multiple stresses are tolerated by wheat through the actions of the *TaWRKY2* [20] and *TaWRKY44* [21]. By interacting with W-box elements of the *SbRD19* promoter, *SbWRKY30* controls the drought stress response gene in sorghum, thus increasing drought tolerance [22]. Low temperature and ABA both substantially activate the *BcWRKY46* gene in *Brassica campestris*, increasing the plant's resistance to low temperatures [23]. In terms of biotic stress, *AtWRKY3 and AtWRKY4* have been revealed to contribute positively to resistance against necrotrophic infections in *Arabidopsis* [24]. Greater sensitivity to both virulent and nonvirulent *Magnaporthe grisea* strains was seen in rice when the *OsWRKY22* gene was functionally lost, but greater resistance was seen in rice when *OsWRKY22* was overexpressed [25]. In several studies, it has been demonstrated that the WRKY gene family plays an important role in the regulatory response to physical damage in various plant species [26–28]. In cotton, we found that *GhWRKY40* overexpression affects the expression of defense-related genes in response to wounding [29]. To conclude, WRKY genes are widely acknowledged as key regulatory factors in the field of gene regulation, and they are capable of functioning as both beneficial and detrimental regulators.

A systematic genome-level analysis is required to fully comprehend the potential functions of the *D. odorifera*'s WRKY gene family. With the partial completion of genomic research on *D. odorifera*, we are now able to initiate further investigations into *D. odorifera* [30]. In this research, all WRKY gene family members of *D. odorifera* were carefully

determined, and then detailed investigations of their molecular properties, chromosomal distribution, gene structure, conserved domains, and motif composition were carried out. In addition, using transcriptomic data, we investigated the transcriptional expression levels of the WRKY genes in tissues and in response to wounding. Overall, this study provided a strong conceptual foundation for investigating WRKY genes in *D. odorifera.*

2. Materials and Methods

2.1. Identification of WRKY Genes in D. odorifera

The genomic and protein sequences of *D. odorifera* were retrieved from the GigaScience Database (http://gigadb.org/dataset/100760, accessed on 21 December 2022) for our investigation. We retrieved the concealed Markov model (HMM) profile for the WRKY DNA-binding domain (PF03106) from the PFAM protein family's database (http://pfam.xfam.org, accessed on 21 December 2022) and used HMMER 3.0 for the analysis to find WRKY genes in the *D. odorifera* genome ($E < 1 \times 10^{-5}$) [31]. Subsequently, all potential *D. odorifera* WRKYs were identified through the HMM searches and underwent further confirmation using the SMART (http://smart.emblheidelberg.de, accessed on 15 January 2023), CDD (https://www.ncbi.nlm.nih.gov/cdd, accessed on 15 January 2023), and InterProScan (http://www.ebi.ac.uk/interpro, accessed on 15 January 2023) databases. Only those sequences containing a conserved WRKY domain, as confirmed through a comparison with each respective database, were considered for subsequent analyses. The conserved heptapeptide sequence in the predicted WRKY domain's N-terminal region was then carefully checked for in each gene.

2.2. Phylogenetic Analysis and Classification of D. odorifera WRKY Gene Family

It was necessary to obtain previously categorized plants' WRKY proteins in order to construct the WRKY phylogenetic tree and divide them into groups. The *Arabidopsis* WRKY amino acid sequences in the TAIR database (http://www.arabidopsis.org, accessed on 21 December 2022) were downloaded. In this study, we used the MUSCLE algorithm with the default parameters to perform the alignment of multiple sequences of DoWRKY proteins and WRKY proteins isolated from *Arabidopsis*. The aligned sequences were then utilized to build a phylogenetic tree using the maximum likelihood approach in FastTree2 software [32]. The parameters used for tree construction included the JTT + CAT model and 1000 bootstrap replicates to ensure robustness and accuracy in the inferred relationships among the WRKY protiens. Subsequently, the generated phylogenetic tree was visualized using iTOL (https://itol.embl.de, accessed on 18 March 2023), a web-based tool [33]. Group I has two WRKY domains, while Groups II and III have one each. Group II members usually have a C2H2-type zinc-finger domain, while Group III members have a C2HC-type zinc-finger domain. The first step involves a preliminary classification into three major groups based on the number of WRKY domains and the types of zinc finger structures. In the second step, we construct a phylogenetic tree that includes both grouped AtWRKY and ungrouped DoWRKY genes, further distinguishing subgroups of the second group. The categorization method was used to separate all discovered *D. odorifera* WRKY genes into three major groups and five subgroups.

2.3. Protein Properties and Sequence Analysis

The amino acid number, isoelectric point (pI), and molecular weight of the DoWRKY proteins were predicted using ExPASy [34] (https://web.expasy.org/protparam, accessed on 15 January 2023). To predict the subcellular localization of the DoWRKY proteins, we utilized the Plant-mPLoc predictor, a web-based tool that is accessible at http://www.csbio.sjtu.edu.cn/bioinf/plant-multi, accessed on 18 March 2023. Furthermore, we conducted an in-depth analysis of conserved motifs within the WRKY proteins from *D. odorifera*, employing the online MEME tool available at http://meme-suite.org, accessed on 15 January 2023. The parameters were as follows: the number of motifs could have a maximum of only 20, and the ideal width of each motif could range from 6 to 100 residues [35].

Moreover, to investigate the gene structures, we obtained exon and intron positions from the gene annotation file of *D. odorifera*. To facilitate the visualization of the identified conserved motifs and gene structures, we employed the TBtools Gene Structure View tool, allowing for an intuitive and comprehensive representation of the findings [36].

2.4. Chromosomal Distribution and Gene Duplication

The WRKY family members of *D. odorifera* were given sequential numbers based on the positions of these genes on the chromosomes and some scaffolds. As the gene naming for *D. odorifera* abbreviation name, we chose a species-related word (Do), accompanied by the gene family acronym (WRKY), and a consecutive number. This standardized naming system enables the clear identification of specific genes within the WRKY family in this plant species [37]. Based on the physical position data from the file of the *D. odorifera* genome, all DoWRKY proteins were assigned to *D. odorifera* chromosomes, using the TBtools Gene Location Visualize tool. Gene density files on chromosomes were obtained by gene annotation files. With the default settings, the Multiple Collinearity Scan toolkit (MCScanX) was used to examine the gene duplication occurrences [38]. Using the One Step MCScanX tool in TBtools, the collinearity relationships and gene density were visualized. KaKs_Calculator 2.0 was used to determine the non-synonymous substitution (ka) and synonymous substitution (ks) of each duplicated WRKY gene pair [39].

2.5. Expression Pattern Analysis of WRKY Genes in D. odorifera

To investigate the transcript patterns of WRKY genes of *D. odorifera*, we used transcriptomics data of *D. odorifera*, spanning multiple tissues (PRJNA552194) and stem tissue under wounding stress (PRJNA612155) from the NCBI. The eight different tissues included in the dataset were flower, seed, leaf, root, stem, and three regions of vascular cambium. Additionally, the stem's damaged (D) and healthy (H) regions were used to obtain transcriptome data related to the injury response [40]. To ensure data quality, all RNA sequence datasets from *D. odorifera* underwent filtration using Fastp, with default parameters [41]. Subsequently, the HISAT2 program was then used to map the clean reads to the *D. odorifera* genome sequences [42]. The mapping quality parameter was set to 30, and the Samtools program was used to perform quality filtering and further sorting of the BAM result files [43]. To quantify gene expression, we used the StringTie program to calculate the normalized mapped reads for each gene as FPKM values, using the parameters "-B -e -G" [44].

3. Results

3.1. Identification and Analysis of Physicochemical Properties of the WRKY Genes in D. odorifera

WRKY domains (PF03106) in the *D. odorifera* genome were recognized using the HMMER algorithm, and the conserved domains in WRKY proteins were subsequently verified in the SMART and CDD databases. Finally, given the physical position in chromosomes, a total of 99 WRKY genes were identified and given the names *DoWRKY01* to *DoWRKY99* (Supplementary File S1). We examined the physicochemical characteristics of the 99 discovered WRKY proteins by using the ExPaSy program. The results revealed that these proteins ranged in length from 142 to 763 amino acids, exhibiting molecular weights ranging from 16.7 to 83.17 kDa. The mean theoretical isoelectric point (pI) of all WRKY proteins was calculated to be 6.9, with a range spanning from 4.8 to 9.9. Remarkably, 59 WRKY proteins exhibited pI values below 7, classifying them as acidic, while the remaining 40 WRKY proteins had pI values above 7, classifying them as alkaline. Except for *DoWRKY79*, which was shown to be potentially dispersed in both the cell membrane and nucleus, all the proteins' subcellular localization was anticipated to be in the nucleus (Supplementary Table S1). This detailed examination of the physicochemical characteristics and subcellular localization of the identified WRKY proteins contributes to a deeper understanding of their significance in *D. odorifera*.

3.2. Characterization and Phylogenetic Study of WRKY Genes in D. odorifera

Phylogenetic trees of WRKY genes from *D. odorifera* and *Arabidopsis thaliana* were constructed using the ClustalW and FastTree2 programs. The heptapeptide WRKYGQK structural domain and the zinc-finger domain serve as the WRKY family's distinguishing characteristics. However, each group has unique distinctions in the two elements. According to Eulgem et al.'s classification criteria in Arabidopsis, we performed a phylogenetic analysis and revealed the result that *D. odorifera* WRKY genes might be classified into three main groups. The findings indicated that, out of the 99 DoWRKY proteins, there were 12 in Group I, 70 in Group II, and 17 in Group III (Figures 1 and 2). Group I had 12 genes with two WRKY domains. These two conserved DNA-binding domains are located at the C- and N-terminal of the WRKY protein sequence. Group II possess a WRKY domain and a C2H2-type zinc finger structure. Moreover, through a systematic phylogenetic analysis, Group II can be divided into five subgroups, namely IIa, IIb, IIc, IId, and IIe, each comprising 7, 16, 28, 9, and 10 DoWRKY members, respectively. In comparison to Group II, the 17 DoWRKY proteins in Group III differ primarily in their zinc-finger domains. The zinc-finger domain found in Group III proteins is of the C2HC-type (Figure 2).

3.3. D. odorifera's WRKY Gene Duplication and Chromosomal Location

The DoWRKY proteins were located on their appropriate chromosome by scanning the publicly available genomic information of *D. odorifera*. In our investigation, the 99 DoWRKY proteins were distributed across 10 chromosomes and 3 scaffolds, with chromosome 8 containing the most DoWRKY genes (19) (Figure 3). To investigate the gene expansion processes of the DoWRKY family, we utilized MCScanX to analyze fragment duplication events. Our analysis revealed 38 pairs of segmentally duplicated (SD) genes and 3 pairs of tandem duplications (TDs) among the 99 DoWRKY proteins, indicating that the gene expansion of DoWRKY proteins was significantly influenced by segmental duplications (Figures 3 and 4). Red lines and the inner circle (Figure 4) showed the gene density on the chromosome. Circle 3 (Figure 4) indicated the chromosomal positions of the WRKY genes.

We estimated the Ka/Ks ratios for the duplicated gene pairs to obtain additional information into the adaptive evolution of DoWRKY proteins. It is a usual practice to measure the selection pressure on genes that code for proteins using the Ka/Ks ratio. Purifying (negative) selection is indicated by a Ka/Ks value below 1, neutral evolution is suggested by a Ka/Ks value of 1, and adaptive (positive) selection is indicated by a Ka/Ks value higher than 1 [45]. Remarkably, the Ka/Ks ratios of all duplicated gene pairs in our analysis were below 1, showing that negative selection was likely a factor in the development of these duplicated genes. However, for four pairs of segmentally duplicated genes, we found it difficult to determine their Ka/Ks values (Table 1). Our findings contribute to an improved comprehension of the mechanisms driving the diversification and functional evolution of this significant gene family by shedding light on the evolutionary dynamics and selection forces impacting the growth of the DoWRKYs in *D. odorifera*.

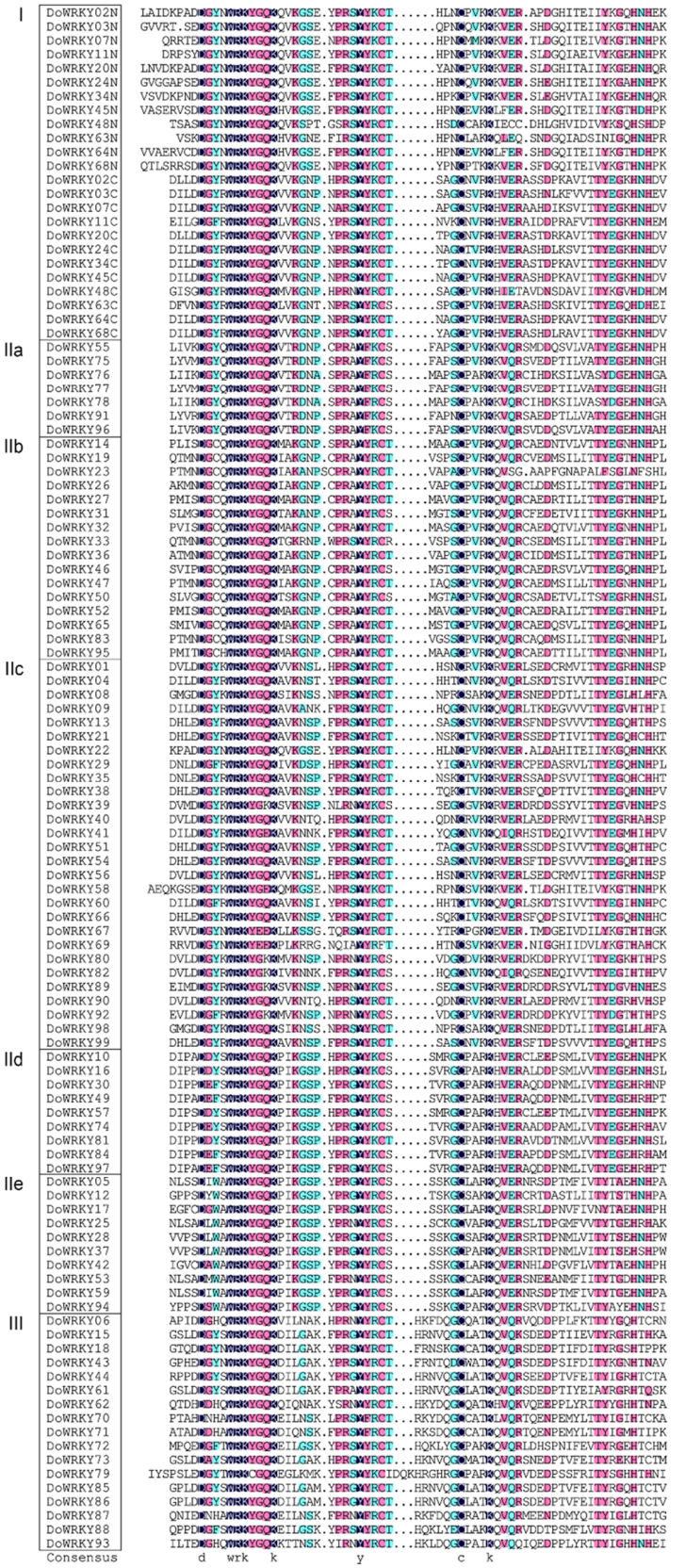

Figure 1. Multiple sequence alignments of DoWRKY proteins. (Dark blue regions indicate the highest consensus, followed by pink regions and light blue regions.)

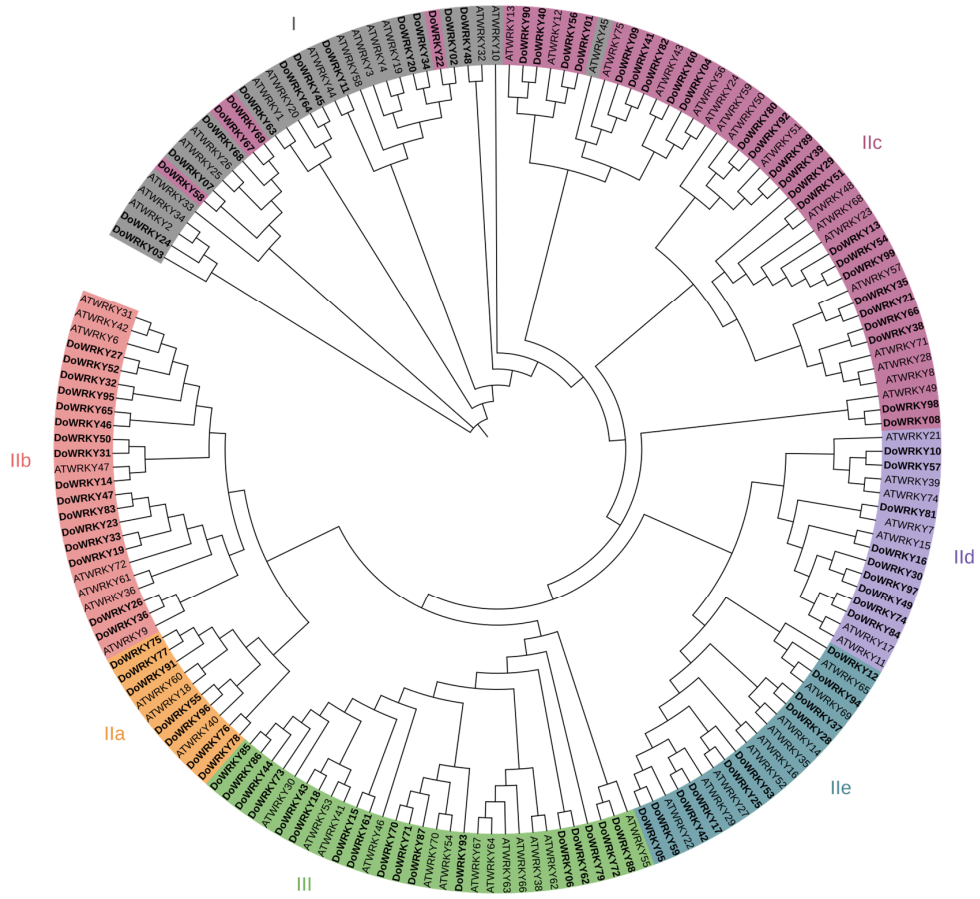

Figure 2. Phylogenetic tree of WRKY proteins sequences in *D. odorifera* and *Arabidopsis* (different colored shaded areas are used to differentiate distinct groups or subgroups, and bold text indicates WRKY proteins of *D. odorifera*).

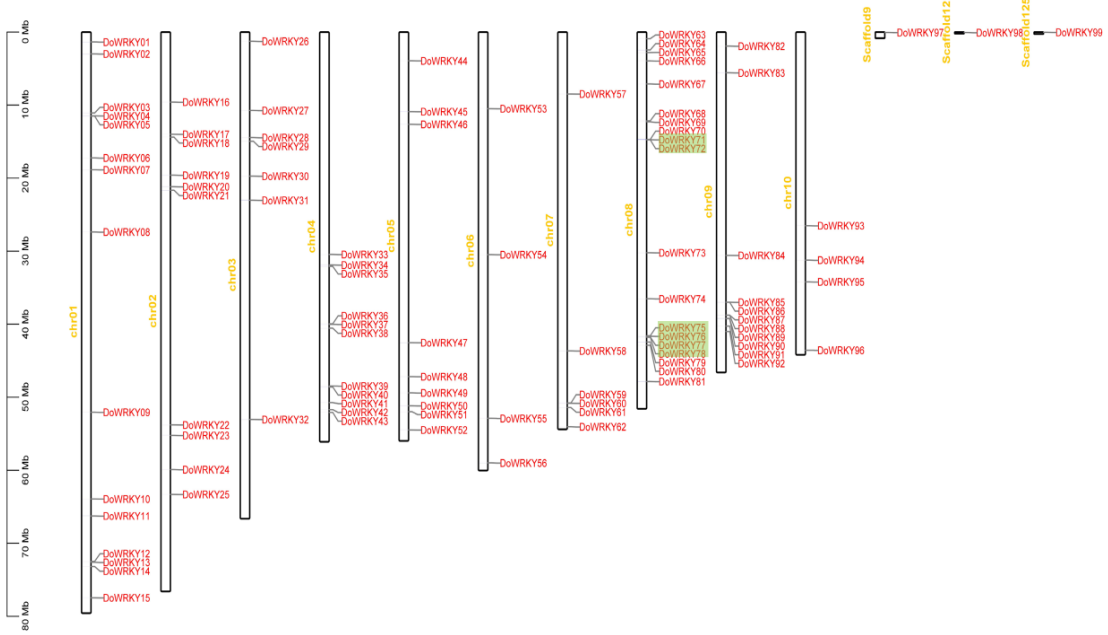

Figure 3. Chromosomal locations of the 99 DoWRKYs. Those with green shading represent tandemly duplicated genes.

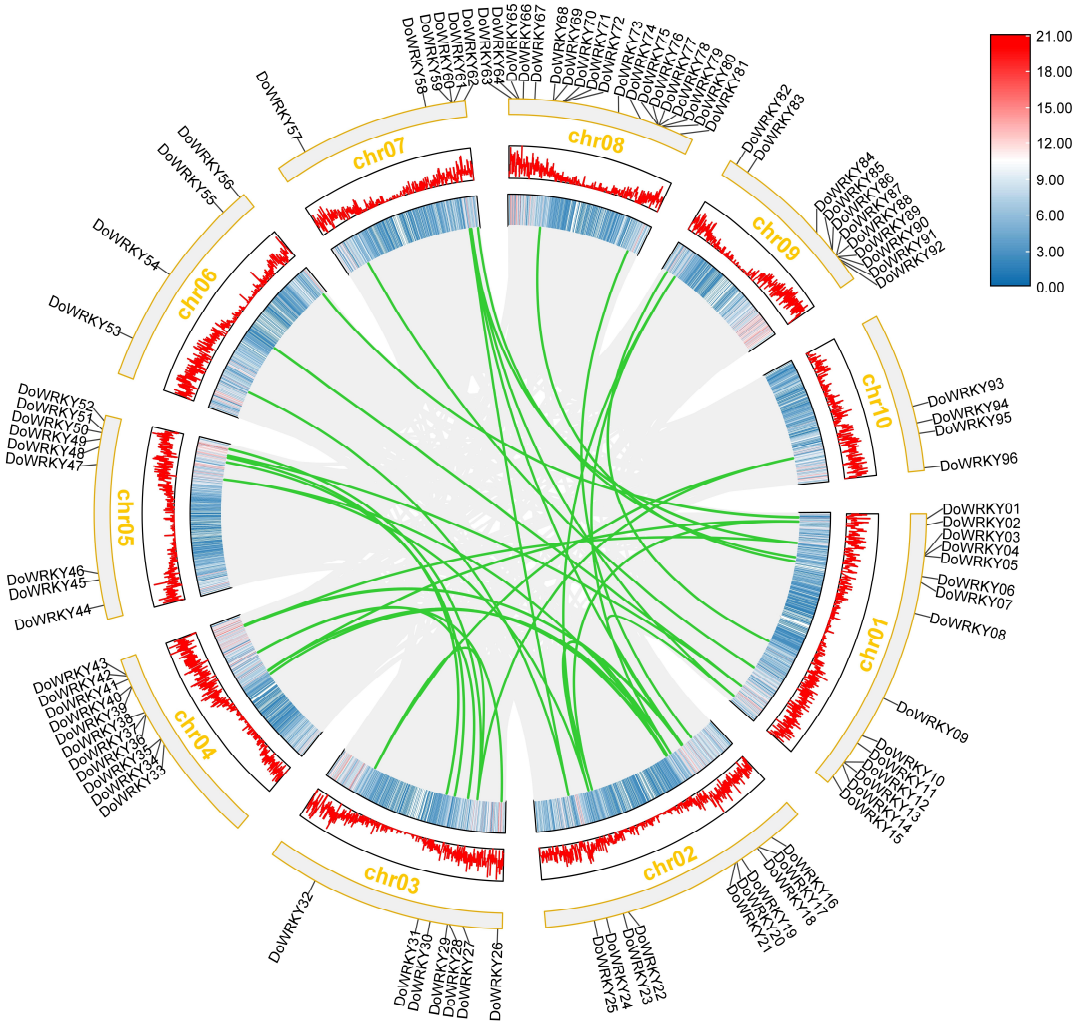

Figure 4. The intrachromosomal segmental duplication map of the DoWRKYs. Green lines are the syntenic gene pairs of DoWRKYs. Gray lines are collinear blocks in *D. odorifera.* Red lines show the gene density on the chromosome.

Table 1. Ka, Ks, and Ka/Ks of syntenic gene pairs of the WRKY gene family in *D. odorifera*.

Duplicated Gene Pairs	Non-Synonymous (Ka)	Synonymous (Ks)	Ka/Ks	Duplicated Type
DoWRKY97&DoWRKY30	0.14	1.04	0.13	segmental
DoWRKY97&DoWRKY31	0.00	0.02	0.16	segmental
DoWRKY15&DoWRKY18	0.50	2.17	0.23	segmental
DoWRKY02&DoWRKY22	0.17	0.66	0.25	segmental
DoWRKY02&DoWRKY34	0.32	1.98	0.16	segmental
DoWRKY05&DoWRKY42	0.52	2.02	0.26	segmental
DoWRKY13&DoWRKY51	0.43	-	-	segmental
DoWRKY13&DoWRKY54	0.16	0.59	0.27	segmental
DoWRKY01&DoWRKY56	0.11	0.69	0.16	segmental
DoWRKY10&DoWRKY57	0.08	0.54	0.15	segmental
DoWRKY15&DoWRKY61	0.19	0.44	0.44	segmental
DoWRKY04&DoWRKY60	0.21	1.11	0.19	segmental
DoWRKY05&DoWRKY59	0.23	0.88	0.26	segmental
DoWRKY06&DoWRKY62	0.32	0.88	0.37	segmental

Table 1. *Cont.*

Duplicated Gene Pairs	Non-Synonymous (Ka)	Synonymous (Ks)	Ka/Ks	Duplicated Type
DoWRKY07&DoWRKY68	0.34	1.64	0.21	segmental
DoWRKY09&DoWRKY82	0.37	-	-	segmental
DoWRKY19&DoWRKY23	0.56	1.68	0.33	segmental
DoWRKY20&DoWRKY22	0.35	2.55	0.14	segmental
DoWRKY19&DoWRKY33	0.25	0.58	0.43	segmental
DoWRKY20&DoWRKY34	0.18	0.71	0.25	segmental
DoWRKY21&DoWRKY35	0.29	0.78	0.37	segmental
DoWRKY17&DoWRKY42	0.29	0.75	0.38	segmental
DoWRKY18&DoWRKY43	0.23	0.51	0.45	segmental
DoWRKY19&DoWRKY47	0.53	1.88	0.28	segmental
DoWRKY23&DoWRKY57	0.47	2.45	0.19	segmental
DoWRKY25&DoWRKY53	0.36	1.00	0.36	segmental
DoWRKY25&DoWRKY59	0.65	-	-	segmental
DoWRKY16&DoWRKY81	0.36	-	-	segmental
DoWRKY19&DoWRKY83	0.48	1.28	0.38	segmental
DoWRKY23&DoWRKY83	0.48	2.48	0.19	segmental
DoWRKY27&DoWRKY32	0.37	2.28	0.16	segmental
DoWRKY26&DoWRKY36	0.21	0.70	0.29	segmental
DoWRKY28&DoWRKY37	0.24	0.70	0.34	segmental
DoWRKY27&DoWRKY52	0.16	0.75	0.21	segmental
DoWRKY30&DoWRKY49	0.14	1.06	0.13	segmental
DoWRKY31&DoWRKY50	0.23	0.55	0.42	segmental
DoWRKY32&DoWRKY95	0.20	0.71	0.28	segmental
DoWRKY27&DoWRKY95	0.32	3.01	0.11	segmental
DoWRKY77&DoWRKY75	0.01	0.02	0.43	tandem
DoWRKY78&DoWRKY76	0.01	0.03	0.34	tandem
DoWRKY70&DoWRKY71	0.15	0.32	0.47	tandem

3.4. DoWRKY Genes' Structure and Conserved Motifs

Similar patterns were observed for family members in the same group, suggesting that they have related functions. Utilizing the online MEME tool, the conserved motifs of DoWRKY proteins were examined in order to further comprehend the similarities and variety of their motifs. The WRKYGQK domain, which was thought to be a fundamental feature of the WRKY family, was present in both Motif 1 and Motif 9. As shown, both Motif 1 and Motif 9 were present in the DoWRKYs of Group I, whereas only Motif 1 was present in the other DoWRKYs (Figure 5A,B). We displayed the exon–intron configurations of the 99 DoWRKYs to examine the variety of gene architectures (Figure 5C). The number of exons varied among the 99 DoWRKYs, ranging from 1 to 6. We specifically found 11 genes containing six exons, 14 genes containing five exons, 11 genes containing four exons, 52 genes containing three exons, 10 genes containing two exons, and 1 gene containing one exon. Notably, *DoWRKY69* was exceptional, featuring a unique gene structure consisting of only one exon. The accuracy of the group classifications is substantially supported by the conserved motif and comparable gene architectures of the WRKY genes in the same group.

3.5. Expression Patterns of DoWRKY Proteins Based on Transcriptome Data

The WRKY gene family is recognized for its pivotal roles in a wide array of response pathways. Determining gene function can be achieved by quantifying transcript levels produced in different tissues. For a more complete insight into the physiological roles of WRKY genes in *D. odorifera*, we conducted an in-depth analysis of the transcriptome data of DoWRKY across diverse tissues and under conditions of wounding stress. Depending on the FPKM values from samples, the transcript levels of the 99 DoWRKY genes were determined (Figure 6). The FPKM values of DoWRKY genes were grouped systematically and shown in a heat map. Few DoWRKYs were discovered to be expressed in all tissues, according to the expression profiles, whereas more than half of the genes were not present

in any tissue. A selected group of genes, including *DoWRKY02*, *DoWRKY03*, *DoWRKY16*, *DoWRKY48*, *DoWRKY55*, *DoWRKY57*, *DoWRKY63*, and *DoWRKY64*, exhibited expression across diverse tissue types, suggesting their potential significance in plant growth and organ development. Notably, *DoWRKY16* and *DoWRKY55* were shown to be present in stems and leaves, indicating their potentially crucial roles in these tissues. *DoWRKY57*, on the other hand, was present in all tissues, but its expression was particularly pronounced in distinct regions of the vascular cambium. This finding suggested that it is likely involved in vascular functions. *DoWRKY41* was exclusively expressed in leaves, emphasizing its importance in leaf-related processes, while *DoWRKY04* was present only in root samples, indicating a possible role in root functions. Moreover, *DoWRKY17*, *DoWRKY28*, *DoWRKY47*, and *DoWRKY67* were present in flowers, suggesting their involvement in flower development or flowering-related processes. Particularly intriguing, *DoWRKY53* was present in both the roots and vascular cambium tissue samples, with notably high expression levels in the vascular cambium samples, further highlighting its potential regulatory role (Figure 6A,B).

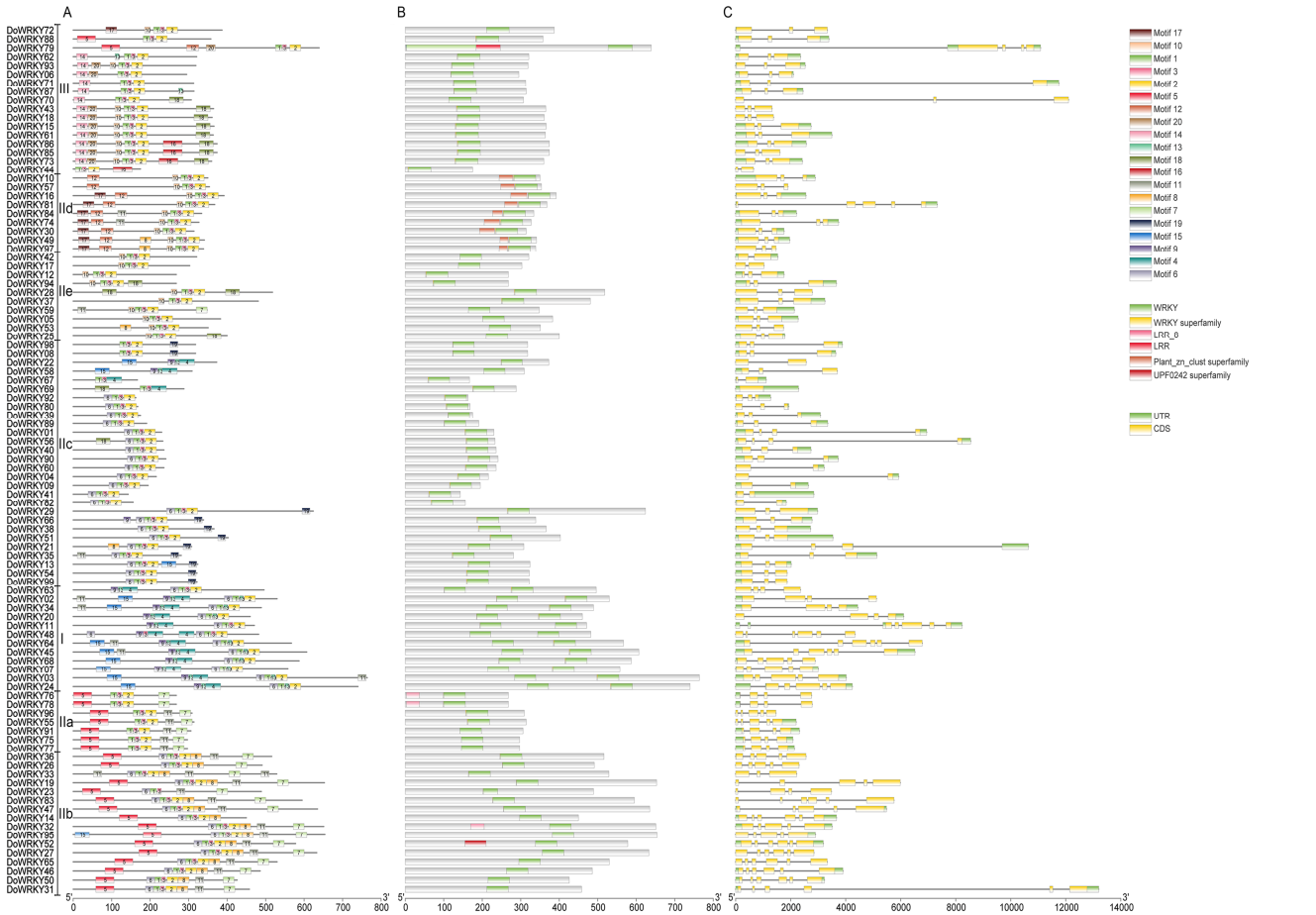

Figure 5. Conserved motifs, conserved domains, and gene structures of the DoWRKYs. (**A**) Conserved motifs in DoWRKYs. (**B**) The conserved domains in the DoWRKYs. (**C**) The exon–intron structure of DoWRKYs.

Mechanical injury can stimulate *D. odorifera* to synthesize elevated levels of flavonoid compounds, which are renowned for their multifaceted biological and pharmacological activities [46], encompassing anti-inflammatory, antioxidant, antitumor, and antimicrobial effects [47]. We investigated the changes in WRKY gene transcripts in response to injury by utilizing transcriptomic data. The results revealed that approximately half of the genes showed no expression before or after the treatment, while some genes exhibited a significant increase in expression following the injury. The expression levels of *DoWRKY04*,

DoWRKY06, *DoWRKY07*, *DoWRKY09*, *DoWRKY14*, *DoWRKY27*, *DoWRKY36*, *DoWRKY38*, *DoWRKY47*, *DoWRKY52*, *DoWRKY53*, *DoWRKY55*, *DoWRKY60*, *DoWRKY66*, *DoWRKY67*, *DoWRKY81*, *DoWRKY91*, *DoWRKY92*, *DoWRKY93*, and *DoWRKY95* showed a remarkable upregulation after mechanical injury. It is noteworthy that almost all of the aforementioned genes are expressed at almost negligible levels before wounding occurs. It was surprising to observe that four genes (*DoWRKY01*, *DoWRKY12*, *DoWRKY40*, and *DoWRKY85*) exhibited downregulation after pruning (Figure 6C and Supplementary Table S2).

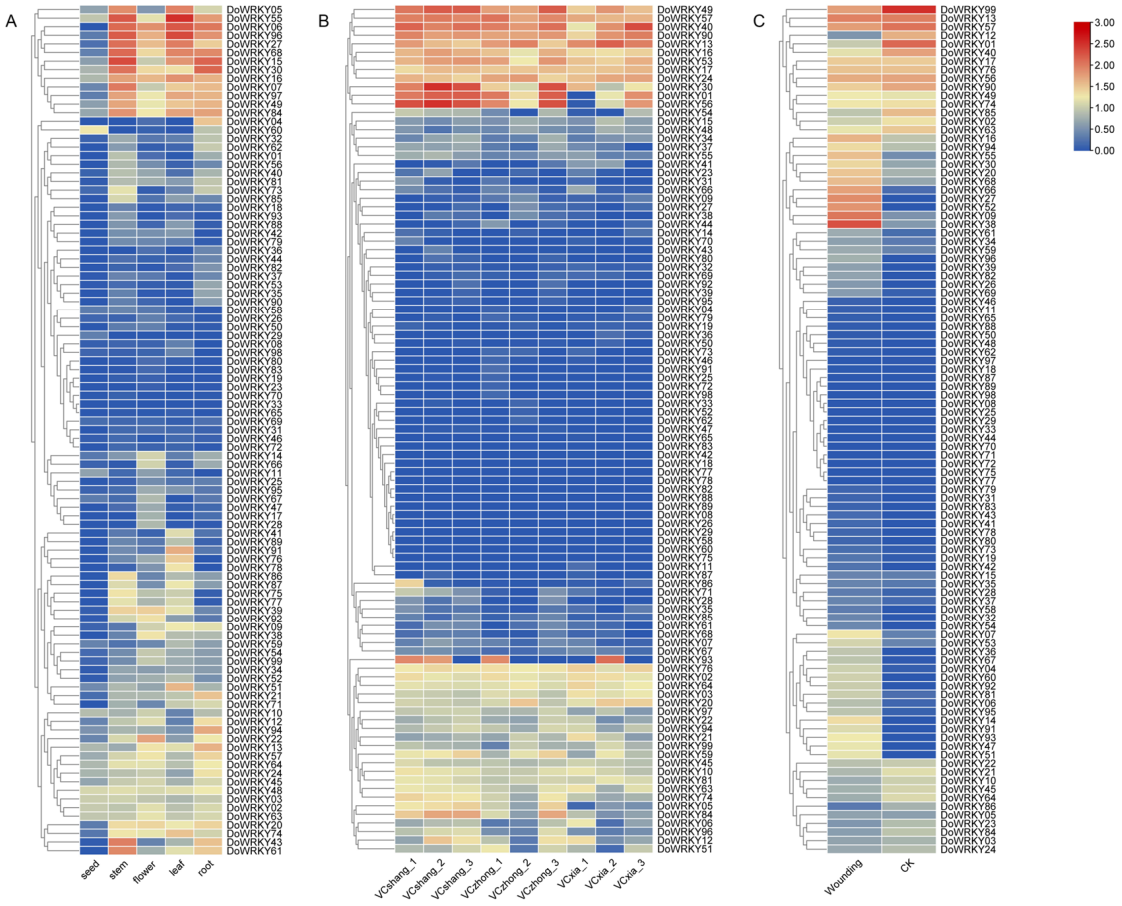

Figure 6. Expression of DoWRKYs in tissues and under stress. (**A**) The expression of DoWRKYs in seed, stem, flower, leaf, and root. (**B**) The expression of DoWRKYs in different parts of vascular cambium, top (VCshang), middle (VCzhong), and bottom (VCxia). (**C**) The expression of DoWRKYs in stems after wounding. Expression profiles were normalized to $\log_{10}$(FPKM).

4. Discussion

By comprehensively analyzing the functional, genomic, and structural features of these gene families, researchers can unravel the underlying mechanisms regulating plant growth, development, and response to environmental cues [48]. Furthermore, studying the characteristics of gene families enables us to gain insights into how plants cope with biotic and abiotic stresses, such as drought [49], salinity [50], and pathogen attacks [51]. Identifying relevant gene families and conducting meticulous annotation work becomes imperative for identifying genes with potentially specialized functions. Recent research endeavors have primarily focused on conducting genome-wide analyses of plant gene families, with particular emphasis on the WRKY gene family [52]. In this study, we utilized recently available genomic data of *D. odorifera* to conclude the identification of 99 WRKY gene members in *D. odorifera* following bioinformatics analysis.

In the DoWRKY gene family, some members displayed mutations in the WRKYGQK heptapeptide structure, changing it to WRKYGKK or WRKYGEK. Similar variations in

this region have been documented in other plant species, such as Chinese jujube (*Ziziphus jujuba*) [53] and *Oryza nivara* [54]. The WRKYGQK domain's capability to bind to w-box cis-elements containing a core sequence of C/TTGACC/T has been previously established, thereby activating downstream genes. The observed variations in the WRKYGQK domain have the potential to influence DNA-binding specificity, leading to certain WRKY genes lacking this motif potentially binding to other specific DNA sequences instead [55]. Notably, tobacco's *NtWRKY12* has shown a distinct binding affinity to the WK-box through its WRKYGKK structural domain [56]. Variations in conserved WRKY sequences could influence binding specificity with downstream target genes, thereby affecting gene function [57]. Further investigation into these variations will contribute to a comprehensive understanding of the intricate regulatory processes orchestrated by WRKY transcription factors in plants.

In this study, with the exception of a small number of WRKY proteins characterized by partial domain absence or mutations, the rest of the members possess intact and accurate structural components. Some motifs are extremely conserved, as shown by a careful examination of the conserved structural domains of *D. odorifera* WRKY proteins (Figure 5A). Notably, all WRKY proteins encompass C-terminal Motif 1, while Group I proteins concurrently feature N-terminal Motif 4. Motif 5 was identified in both IIa and IIb, while Motif 10 was consistently present in both IId and IIe. This underscores the pronounced structural conservation among members of the *D. odorifera* WRKY gene family.

In *D. odorifera*, upon grouping the confirmed 99 DoWRKYs members, it was observed that the IIc subgroup exhibited the highest member count, a trend similar to that seen in other leguminous plants [58]. In comparison to the model species *Arabidopsis thaliana* (72 AtWRKYs), there was a notable increase in member count. Groups II and III both saw a gain in members, with the exception of Group I, which had a drop of two members. Notably, Group II displayed a substantial augmentation, with the addition of 25 members (IIa, 4; IIb, 8; IIc, 10; IId, 2; and IIe, 1), while Group III gained 4 members. These findings imply that WRKY members in Group II expanded during the course of evolution, whereas Group I may have experienced gene loss events. Moreover, in line with the majority of research findings, the expansion of the DoWRKY gene family in *D. odorifera* seems to be primarily influenced by segmental duplications [59]. It is notable that, within Group IIc, *DoWRKY58*, *DoWRKY67*, and *DoWRKY69* exhibit a closer phylogenetic relationship with Group I. An oat study [60] showed a similar observation. There is evidence that both Groups IIc and I evolved from a IIc-like ancestral WRKY. Therefore, we could speculate that Group IIc and Group I may have come from the same ancestor, or these three genes wanted to evolve into Group I.

Meng validated *HIS2* as the most stable reference gene in *D. odorifera* via RT-PCR method, enhancing the accuracy of qRT-PCR data normalization [61]. From the sequence transfer analysis, it was found that part of the DNA originated from the mitochondrial and chloroplast genomes [62]. Most of the transfer events have taken place relatively recently, indicating a possible role in environmental adaptation [63]. Ruoke discovered 126 members within the R2R3-MYB transcription factor family of *D. odorifera*. The research highlighted that segmental duplication events played an important role in expanding the R2R3-MYB family, implicating *DodMYB32*, *DodMYB55*, and *DodMYB89* as potential participants in the regulatory process of heartwood [64]. Wang identified 24 CCR gene family members, and a synteny analysis showed that segmental and tandem duplications were crucial in the expansion of the CCR family [65].

The WRKY genes expression in *D. odorifera* indicus was found to be organ-specific, with *DoWRKY41* being expressed only in leaves; *DoWRKY04* in roots; and *DoWRKY17*, *DoWRKY28*, *DoWRKY47*, and *DoWRKY67* in flowers. *DoWRKY16* and *DoWRKY55* were present in stems and leaves, indicating their potentially crucial roles in these tissues. *DoWRKY57* was present in all tissues, with particularly pronounced expression in specific regions of the vascular cambium, suggesting its likely involvement in vascular functions. Additionally, 20 DoWRKY genes showed a substantial increase in expression following

wounds, suggesting that they may be involved in the regulation of damage stress in *D. odorifera*. *DoWRKY95* showed a remarkable upregulation after mechanical injury. Observing downregulation after pruning in four genes (*DoWRKY01*, *DoWRKY12*, *DoWRKY40*, and *DoWRKY85*) was surprising. As a result, our thorough examination of the DoWRKYs provides a useful foundation for the further functional genomic characterizations of these genes. More WRKY genes will likely be discovered in different additional plant species as the number of fully sequenced genomes rises. These findings will enable a deeper investigation of the WRKY gene family's functional properties and further contribute to a more thorough knowledge of the genesis and development of the WRKY gene family. Overall, the WRKY gene family's evolutionary processes and functional diversity across several plant species are critically illuminated by this research.

5. Conclusions

In this research, 99 DoWRKY proteins were identified from the *Dalbergia odorifera* genome. The comprehensive analyses, including phylogenetic relationships, gene structure, conserved motifs, chromosomal locations, and transcriptome expression patterns, were conducted. We further explored the evolutionary expansion of the WRKY gene family in *D. odorifera*, primarily resulting from segmental duplications. The functional annotation and expression profiling of DoWRKY proteins revealed their active involvement in diverse aspects of *D. odorifera* growth, development, and stress responses. The findings provide significant and broad perspectives in the study of DoWRKY proteins and may inspire further research in this area.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy13102591/s1, Supplementary File S1: The protein sequences of 99 WRKY genes in *D. odorifera*; Supplementary Table S1: The detailed information of WYKY genes in *D. odorifera*; Supplementary Table S2: The expression levels of WRKY genes in different tissues and under wound stress.

Author Contributions: M.T. and D.L. designed this study and revised the manuscript; Q.Z. carried out the study and wrote the manuscript; F.C. revised the manuscript and provided constructive suggestions; X.H., H.Z., C.F., Y.L. and S.X. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Key Science and Technology Program of Hainan Province (ZDKJ2021031), the National Natural Science Foundation of China (32201624), the Hainan Provincial Natural Science Foundation of China (321RC469), and the Hainan Province Science and Technology Special Fund (ZDYF2023XDNY078).

Data Availability Statement: The original contributions presented in this study are included in the article and Supplementary Materials. The RNA-seq datasets can be downloaded from the NCBI database under BioProject accession Nos. PRJNA552194 and PRJNA612155.

Acknowledgments: We are sincerely thankful to Daping Xu (Chinese Academy of Forestry), who published and uploaded the genome data of *D. odorifera*. We would like to thank the reviewers for their constructive comments.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. López-Sampson, A.; Page, T. History of Use and Trade of Agarwood. *Econ. Bot.* **2018**, *72*, 107–129. [CrossRef]
2. Kang, T.H.; Tian, Y.-H.; Kim, Y.-C. Isoliquiritigenin: A Competitive Tyrosinase Inhibitor from the Heartwood of *Dalbergia odorifera*. *Biomol. Ther.* **2005**, *13*, 32–34.
3. Lianhe, Z.; Xing, H.; Li, W.; Zhengxing, C. Physicochemical Properties, Chemical Composition and Antioxidant Activity of *Dalbergia odorifera* T. Chen Seed Oil. *J. Am. Oil Chem. Soc.* **2012**, *89*, 883–890. [CrossRef]
4. Riechmann, J.L.; Heard, J.; Martin, G.; Reuber, L.; Jiang, C.-Z.; Keddie, J.; Adam, L.; Pineda, O.; Ratcliffe, O.J.; Samaha, R.R.; et al. Arabidopsis Transcription Factors: Genome-Wide Comparative Analysis Among Eukaryotes. *Science* **2000**, *290*, 2105–2110. [CrossRef]

5. Ülker, B.; Somssich, I.E. *WRKY* Transcription Factors: From DNA Binding towards Biological Function. *Curr. Opin. Plant Biol.* **2004**, *7*, 491–498. [CrossRef]
6. Yang, P.; Chen, Z. A Family of Dispersed Repetitive DNA Sequences in Tobacco Contain Clusters of W-Box Elements Recognized by Pathogen-Induced *WRKY* DNA-Binding Proteins. *Plant Sci.* **2001**, *161*, 655–664. [CrossRef]
7. Yamasaki, K.; Kigawa, T.; Watanabe, S.; Inoue, M.; Yamasaki, T.; Seki, M.; Shinozaki, K.; Yokoyama, S. Structural Basis for Sequence-Specific DNA Recognition by an Arabidopsis *WRKY* Transcription Factor. *J. Biol. Chem.* **2012**, *287*, 7683–7691. [CrossRef] [PubMed]
8. Eulgem, T.; Rushton, P.J.; Robatzek, S.; Somssich, I.E. The *WRKY* Superfamily of Plant Transcription Factors. *Trends Plant Sci.* **2000**, *5*, 199–206. [CrossRef] [PubMed]
9. Rushton, P.J.; Somssich, I.E.; Ringler, P.; Shen, Q.J. *WRKY* Transcription Factors. *Trends Plant Sci.* **2010**, *15*, 247–258. [CrossRef] [PubMed]
10. Zhang, T.; Xu, Y.; Ding, Y.; Yu, W.; Wang, J.; Lai, H.; Zhou, Y. Identification and Expression Analysis of *WRKY* Gene Family in Response to Abiotic Stress in *Dendrobium catenatum*. *Front. Genet.* **2022**, *13*, 800019. [CrossRef]
11. Yang, Y.; Liu, J.; Zhou, X.; Liu, S.; Zhuang, Y. Identification of *WRKY* Gene Family and Characterization of Cold Stress-Responsive *WRKY* Genes in Eggplant. *Peerj* **2020**, *8*, e8777. [CrossRef] [PubMed]
12. Li, J.; Islam, F.; Huang, Q.; Wang, J.; Zhou, W.; Xu, L.; Yang, C. Genome-Wide Characterization of *WRKY* Gene Family in *Helianthus annuus* L. and Their Expression Profiles under Biotic and Abiotic Stresses. *PLoS ONE* **2020**, *15*, e0241965. [CrossRef] [PubMed]
13. Xie, Z.; Zhang, Z.-L.; Zou, X.; Yang, G.; Komatsu, S.; Shen, Q.J. Interactions of Two Abscisic-Acid Induced *WRKY* Genes in Repressing Gibberellin Signaling in Aleurone Cells. *Plant J. Cell Mol. Biol.* **2006**, *46*, 231–242. [CrossRef] [PubMed]
14. Robatzek, S.; Somssich, I.E. Targets of *AtWRKY6* Regulation during Plant Senescence and Pathogen Defense. *Genes Dev.* **2002**, *16*, 1139–1149. [CrossRef]
15. Lagacé, M.; Matton, D.P. Characterization of a *WRKY* Transcription Factor Expressed in Late Torpedo-Stage Embryos of *Solanum chacoense*. *Planta* **2004**, *219*, 185–189. [CrossRef] [PubMed]
16. Wang, H.; Chen, W.; Xu, Z.; Chen, M.; Yu, D. Functions of WRKYs in Plant Growth and Development. *Trends Plant Sci.* **2023**, *28*, 630–645. [CrossRef]
17. Baillo, E.H.; Kimotho, R.N.; Zhang, Z.; Xu, P. Transcription Factors Associated with Abiotic and Biotic Stress Tolerance and Their Potential for Crops Improvement. *Genes* **2019**, *10*, 771. [CrossRef]
18. Gu, L.; Dou, L.; Guo, Y.; Wang, H.; Li, L.; Wang, C.; Ma, L.; Wei, H.; Yu, S. The *WRKY* Transcription Factor *GhWRKY27* Coordinates the Senescence Regulatory Pathway in Upland Cotton (*Gossypium hirsutum* L.). *BMC Plant Biol.* **2019**, *19*, 116. [CrossRef]
19. Zhou, C.; Lin, Q.; Lan, J.; Zhang, T.; Liu, X.; Miao, R.; Mou, C.; Nguyen, T.; Wang, J.; Zhang, X.; et al. *WRKY* Transcription Factor *OsWRKY29* Represses Seed Dormancy in Rice by Weakening Abscisic Acid Response. *Front. Plant Sci.* **2020**, *11*, 691. [CrossRef]
20. Niu, C.-F.; Wei, W.; Zhou, Q.-Y.; Tian, A.-G.; Hao, Y.-J.; Zhang, W.-K.; Ma, B.; Lin, Q.; Zhang, Z.-B.; Zhang, J.-S.; et al. Wheat *WRKY* Genes *TaWRKY2* and *TaWRKY19* Regulate Abiotic Stress Tolerance in Transgenic *Arabidopsis* Plants. *Plant Cell Environ.* **2012**, *35*, 1156–1170. [CrossRef]
21. Wang, X.; Zeng, J.; Li, Y.; Rong, X.; Sun, J.; Sun, T.; Li, M.; Wang, L.; Feng, Y.; Chai, R.; et al. Expression of *TaWRKY44*, a Wheat *WRKY* Gene, in Transgenic Tobacco Confers Multiple Abiotic Stress Tolerances. *Front. Plant Sci.* **2015**, *6*, 615. [CrossRef] [PubMed]
22. Yang, Z.; Chi, X.; Guo, F.; Jin, X.; Luo, H.; Hawar, A.; Chen, Y.; Feng, K.; Wang, B.; Qi, J.; et al. *SbWRKY30* Enhances the Drought Tolerance of Plants and Regulates a Drought Stress-Responsive Gene, SbRD19, in Sorghum. *J. Plant Physiol.* **2020**, *246–247*, 153142. [CrossRef] [PubMed]
23. Wang, F.; Hou, X.; Tang, J.; Wang, Z.; Wang, S.; Jiang, F.; Li, Y. A Novel Cold-Inducible Gene from Pak-Choi (*Brassica campestris* Ssp. *chinensis*), *BcWRKY46*, Enhances the Cold, Salt and Dehydration Stress Tolerance in Transgenic Tobacco. *Mol. Biol. Rep.* **2012**, *39*, 4553–4564. [CrossRef] [PubMed]
24. Lai, Z.; Vinod, K.; Zheng, Z.; Fan, B.; Chen, Z. Roles of *ArabidopsisWRKY3* and *WRKY4* Transcription Factors in Plant Responses to Pathogens. *BMC Plant Biol.* **2008**, *8*, 68. [CrossRef]
25. Abbruscato, P.; Nepusz, T.; Mizzi, L.; Del Corvo, M.; Morandini, P.; Fumasoni, I.; Michel, C.; Paccanaro, A.; Guiderdoni, E.; Schaffrath, U. *OsWRKY22*, a Monocot *WRKY* Gene, Plays a Role in the Resistance Response to Blast. *Mol. Plant Pathol.* **2012**, *13*, 828–841. [CrossRef]
26. Chen, W.; Provart, N.J.; Glazebrook, J.; Katagiri, F.; Chang, H.S.; Eulgem, T.; Mauch, F.; Luan, S.; Zou, G.; Whitham, S.A.; et al. Expression profile matrix of *Arabidopsis* transcription factor genes suggests their putative functions in response to environmental stresses. *Plant Cell* **2002**, *14*, 559–574. [CrossRef]
27. Srivastava, S.; Pandey, S.P.; Singh, P.; Pradhan, L.; Pande, V.; Sane, A.P. Early wound-responsive cues regulate the expression of *WRKY* family genes in chickpea differently under wounded and unwounded conditions. *Physiol. Mol. Biol. Plants* **2022**, *28*, 719–735. [CrossRef]
28. Sun, P.W.; Xu, Y.H.; Yu, C.C.; Lv, F.F.; Tang, X.L.; Gao, Z.H.; Zhang, Z.; Wang, H.; Liu, Y.; Wei, J.H. *WRKY44* represses expression of the wound-induced sesquiterpene biosynthetic gene *ASS1* in *Aquilaria sinensis*. *J. Exp. Bot.* **2020**, *71*, 1128–1138. [CrossRef]
29. Wang, X.; Yan, Y.; Li, Y.; Chu, X.; Wu, C.; Guo, X. *GhWRKY40*, a multiple stress-responsive cotton *WRKY* gene, plays an important role in the wounding response and enhances susceptibility to *Ralstonia solanacearum* infection in transgenic *Nicotiana benthamiana*. *PLoS ONE* **2014**, *9*, e93577. [CrossRef]

30. Hong, Z.; Li, J.; Liu, X.; Lian, J.; Zhang, N.; Yang, Z.; Niu, Y.; Cui, Z.; Xu, D. The Chromosome-Level Draft Genome of *Dalbergia odorifera*. *GigaScience* **2020**, *9*, giaa084. [CrossRef]
31. Finn, R.D.; Clements, J.; Eddy, S.R. HMMER Web Server: Interactive Sequence Similarity Searching. *Nucleic Acids Res.* **2011**, *39*, W29–W37. [CrossRef] [PubMed]
32. Price, M.N.; Dehal, P.S.; Arkin, A.P. FastTree2-Approximately Maximum-Likelihood Trees for Large Alignments. *PLoS ONE* **2010**, *5*, e9490. [CrossRef] [PubMed]
33. Letunic, I.; Bork, P. Interactive Tree Of Life (ITOL) v5: An Online Tool for Phylogenetic Tree Display and Annotation. *Nucleic Acids Res.* **2021**, *49*, W293–W296. [CrossRef] [PubMed]
34. Gasteiger, E.; Gattiker, A.; Hoogland, C.; Ivanyi, I.; Appel, R.D.; Bairoch, A. ExPASy: The Proteomics Server for in-Depth Protein Knowledge and Analysis. *Nucleic Acids Res.* **2003**, *31*, 3784–3788. [CrossRef] [PubMed]
35. Bailey, T.L.; Boden, M.; Buske, F.A.; Frith, M.; Grant, C.E.; Clementi, L.; Ren, J.; Li, W.W.; Noble, W.S. MEME SUITE: Tools for Motif Discovery and Searching. *Nucleic Acids Res.* **2009**, *37*, W202–W208. [CrossRef] [PubMed]
36. Chen, C.; Chen, H.; Zhang, Y.; Thomas, H.R.; Frank, M.H.; He, Y.; Xia, R. TBtools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data. *Mol. Plant* **2020**, *13*, 1194–1202. [CrossRef]
37. Carocha, V.; Soler, M.; Hefer, C.; Cassan-Wang, H.; Fevereiro, P.; Myburg, A.A.; Paiva, J.A.P.; Grima-Pettenati, J. Genome-Wide Analysis of the Lignin Toolbox of Eucalyptus Grandis. *New Phytol.* **2015**, *206*, 1297–1313. [CrossRef]
38. Wang, Y.; Tang, H.; Debarry, J.D.; Tan, X.; Li, J.; Wang, X.; Lee, T.; Jin, H.; Marler, B.; Guo, H.; et al. MCScanX: A Toolkit for Detection and Evolutionary Analysis of Gene Synteny and Collinearity. *Nucleic Acids Res.* **2012**, *40*, e49. [CrossRef]
39. Wang, D.; Zhang, Y.; Zhang, Z.; Zhu, J.; Yu, J. KaKs_Calculator 2.0: A Toolkit Incorporating Gamma-Series Methods and Sliding Window Strategies. *Genom. Proteom. Bioinform.* **2010**, *8*, 77–80. [CrossRef]
40. Sun, Y.; Gao, M.; Kang, S.; Yang, C.; Meng, H.; Yang, Y.; Zhao, X.; Gao, Z.; Xu, Y.; Jin, Y.; et al. Molecular Mechanism Underlying Mechanical Wounding-Induced Flavonoid Accumulation in *Dalbergia odorifera* T. Chen, an Endangered Tree That Produces Chinese Rosewood. *Genes* **2020**, *11*, 478. [CrossRef]
41. Chen, S.; Zhou, Y.; Chen, Y.; Gu, J. Fastp: An Ultra-Fast All-in-One FASTQ Preprocessor. *Bioinformatics* **2018**, *34*, i884–i890. [CrossRef] [PubMed]
42. Kim, D.; Paggi, J.M.; Park, C.; Bennett, C.; Salzberg, S.L. Graph-Based Genome Alignment and Genotyping with HISAT2 and HISAT-Genotype. *Nat. Biotechnol.* **2019**, *37*, 907–915. [CrossRef] [PubMed]
43. Danecek, P.; Bonfield, J.K.; Liddle, J.; Marshall, J.; Ohan, V.; Pollard, M.O.; Whitwham, A.; Keane, T.; McCarthy, S.A.; Davies, R.M.; et al. Twelve Years of SAMtools and BCFtools. *GigaScience* **2021**, *10*, giab008. [CrossRef] [PubMed]
44. Pertea, M.; Pertea, G.M.; Antonescu, C.M.; Chang, T.-C.; Mendell, J.T.; Salzberg, S.L. StringTie Enables Improved Reconstruction of a Transcriptome from RNA-Seq Reads. *Nat. Biotechnol.* **2015**, *33*, 290–295. [CrossRef]
45. Hurst, L.D. The Ka/Ks Ratio: Diagnosing the Form of Sequence Evolution. *Trends Genet.* **2002**, *18*, 486. [CrossRef]
46. Yang, Z.-H.; Mei, C.; He, X.-H.; Sun, X.-B. Advance in studies on chemical constitutions, pharmacological mechanism and pharmacokinetic profile of dalbergiae odoriferae lignum. *Chin. J. Chin. Mat. Med.* **2013**, *38*, 1679–1683.
47. Lee, D.-S.; Kim, K.-S.; Ko, W.; Li, B.; Keo, S.; Jeong, G.-S.; Oh, H.; Kim, Y.-C. The Neoflavonoid Latifolin Isolated from MeOH Extract of *Dalbergia odorifera* Attenuates Inflammatory Responses by Inhibiting NF-KB Activation via Nrf2-Mediated Heme Oxygenase-1 Expression. *Phytother. Res. PTR* **2014**, *28*, 1216–1223. [CrossRef]
48. Wang, W.; Gao, T.; Chen, J.; Yang, J.; Huang, H.; Yu, Y. The Late Embryogenesis Abundant Gene Family in Tea Plant (Camellia Sinensis): Genome-Wide Characterization and Expression Analysis in Response to Cold and Dehydration Stress. *Plant Physiol. Biochem.* **2019**, *135*, 277–286. [CrossRef] [PubMed]
49. Jaimes-Miranda, F.; Chávez Montes, R.A. The Plant MBF1 Protein Family: A Bridge between Stress and Transcription. *J. Exp. Bot.* **2020**, *71*, 1782–1791. [CrossRef] [PubMed]
50. Ferreira, M.D.G.; Castro, J.A.; Silva, R.J.S.; Micheli, F. HVA22 from Citrus: A Small Gene Family Whose Some Members Are Involved in Plant Response to Abiotic Stress. *Plant Physiol. Biochem.* **2019**, *142*, 395–404. [CrossRef]
51. Yu, Y.; Wang, Z.; Xu, Y.; Ma, B.; Chen, X. Genome-wide identification of SUN gene family in *Fragaria vesca* and stresses-response analysis. *Chin. J. Biotechnol.* **2023**, *39*, 724–740. [CrossRef]
52. Ma, Z.; Li, W.; Wang, H.; Yu, D. *WRKY* Transcription Factors *WRKY12* and *WRKY13* Interact with SPL10 to Modulate Age-Mediated Flowering. *J. Integr. Plant Biol.* **2020**, *62*, 1659–1673. [CrossRef]
53. Chen, X.; Chen, R.; Wang, Y.; Wu, C.; Huang, J. Genome-Wide Identification of *WRKY* Transcription Factors in Chinese Jujube (*Ziziphus Jujuba* Mill.) and Their Involvement in Fruit Developing, Ripening, and Abiotic Stress. *Genes* **2019**, *10*, 360. [CrossRef]
54. Xu, H.; Watanabe, K.A.; Zhang, L.; Shen, Q.J. *WRKY* Transcription Factor Genes in Wild Rice *Oryza nivara*. *DNA Res.* **2016**, *23*, 311–323. [CrossRef]
55. Ciolkowski, I.; Wanke, D.; Birkenbihl, R.P.; Somssich, I.E. Studies on DNA-Binding Selectivity of *WRKY* Transcription Factors Lend Structural Clues into *WRKY*-Domain Function. *Plant Mol. Biol.* **2008**, *68*, 81–92. [CrossRef] [PubMed]
56. Van Verk, M.C.; Pappaioannou, D.; Neeleman, L.; Bol, J.F.; Linthorst, H.J.M. A Novel *WRKY* Transcription Factor Is Required for Induction of *PR-1a* Gene Expression by Salicylic Acid and Bacterial Elicitors. *Plant Physiol.* **2008**, *146*, 1983–1995. [CrossRef]
57. Xie, T.; Chen, C.; Li, C.; Liu, J.; Liu, C.; He, Y. Genome-Wide Investigation of *WRKY* Gene Family in Pineapple: Evolution and Expression Profiles during Development and Stress. *BMC Genom.* **2018**, *19*, 490. [CrossRef] [PubMed]
58. Song, H.; Sun, W.; Yang, G.; Sun, J. *WRKY* Transcription Factors in Legumes. *BMC Plant Biol.* **2018**, *18*, 243. [CrossRef]

59. Cannon, S.B.; Mitra, A.; Baumgarten, A.; Young, N.D.; May, G. The Roles of Segmental and Tandem Gene Duplication in the Evolution of Large Gene Families in *Arabidopsis thaliana*. *BMC Plant Biol.* **2004**, *4*, 10. [CrossRef] [PubMed]
60. Liu, K.; Ju, Z.; Jia, Z.; Liang, G.; Ma, X.; Liu, W. Genome-Wide Identification and Characterization of the Oat (*Avena sativa* L.) *WRKY* Transcription Factor Family. *Genes* **2022**, *13*, 1918. [CrossRef] [PubMed]
61. Meng, H.; Yang, Y.; Gao, Z.; Wei, J. Selection and Validation of Reference Genes for Gene Expression Studies by RT-PCR in *Dalbergia odorifera*. *Sci. Rep.* **2019**, *9*, 3341. [CrossRef] [PubMed]
62. Liu, Y.; Huang, P.; Li, C.H.; Zang, F.; Zheng, Y. Characterization of the complete chloroplast genome of *Dalbergia cultrata* (Leguminosae). *Mitochondrial DNA Part B Resour.* **2019**, *4*, 2369–2370. [CrossRef] [PubMed]
63. Hong, Z.; Liao, X.; Ye, Y.; Zhang, N.; Yang, Z.; Zhu, W.; Gao, W.; Sharbrough, J.; Tembrock, L.; Xu, D.; et al. A complete mitochondrial genome for fragrant Chinese rosewood (*Dalbergia odorifera*, Fabaceae) with comparative analyses of genome structure and intergenomic sequence transfers. *BMC Genom.* **2021**, *22*, 672. [CrossRef] [PubMed]
64. Ma, R.; Luo, J.; Wang, W.; Song, T.; Fu, Y. Function of the R2R3-MYB Transcription Factors in *Dalbergia odorifera* and Their Relationship with Heartwood Formation. *Int. J. Mol. Sci.* **2023**, *24*, 12430. [CrossRef]
65. Wang, Y.; Xu, J.; Zhao, W.; Li, J.; Chen, J. Genome-wide identification, characterization, and genetic diversity of CCR gene family in *Dalbergia odorifera*. *Front. Plant Sci.* **2022**, *13*, 1064262. [CrossRef]