agronomy

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Genome-Wide Identification and Characterization of *YABBY* Gene Family in *Juglans regia* and *Juglans mandshurica*

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Abstract: The *YABBY* gene family is a plant transcription factor that exists in all seed plants. *YABBY* family members have been studied extensively in various plants and were to play significant roles in plant growth and development. *Juglans*, especially walnuts, are important economic tree species that are widely distributed worldwide. However, the identification and related research of YABBY in *Juglans* have not been reported to date. In this study, we identified 19 *YABBY* genes from two *Juglans* species, namely, *J. regia* and *J. mandshurica*. Ten *JrYABBY* genes and nine *JmYABBY* genes were divided into five subfamilies (YAB1/3, YAB2, INO, CRC, and YAB5). Sequence analysis revealed that all encoded YABBY protein sequences had a highly conserved YABBY and C2C2 zinc-finger domains. An analysis of the assumed cis-acting elements revealed that *JrYABBY* and *JmYABBY* genes were deeply involved in phytohormone and light responses. Further, gene expression pattern analysis suggested that most walnut *YABBY* genes were likely involved in peel and flower development and responses to biotic stress. This study not only suppled novel insights into the evolutionary basis of *YABBY* gene families in *Juglans*, but also provided clues for the further functional verification and investigation of *YABBY* genes in other tree species.

Keywords: *Juglans regia*; *Juglans mandshurica*; evolution; *YABBY* genes family

Citation: Liu, H.; Ye, H.; Wang, J.; Chen, S.; Li, M.; Wang, G.; Hou, N.; Zhao, P. Genome-Wide Identification and Characterization of *YABBY* Gene Family in *Juglans regia* and *Juglans mandshurica*. *Agronomy* **2022**, *12*, 1914. https://doi.org/10.3390/agronomy12081914

Academic Editors: Yin Li, Guangxiao Yang, Yongfang Wan, Jian Zeng and Yaqiong Wang

Received: 15 July 2022
Accepted: 11 August 2022
Published: 14 August 2022

1. Introduction

The *YABBY* transcription factor has a prominent role in regulating plant growth and developmental activities, such as the plant developmental, flowering, abiotic, and biotic responses [1–5]. *YABBY* proteins are comprised of two highly conserved DNA-binding domains, namely the zinc finger-like domain (C2C2) and helix–loop–helix domain (*YABBY*), respectively [2]. In important crops, *YABBY* genes have been examined in their related genomes, including 21 in *Wheat* [3], 17 in *soybean* [4], 8 in rice [5] and 30 in *Cucumber* [6]. In woody plants, *YABBY* genes have been examined in their related genomes, namely, 55 genes in seven species of *Magnoliids* [7].

The development of plants depends on the function of the shoot apical meristem (SAM), which is established during embryogenesis [8,9]. Following the determination of the fates of apiece cells in differentiated organs, the development of lateral organ primordia is still regulated by the meristem through undetermined signals [10]. Recent impalement studies in *Arabidopsis thaliana* indicated that the activities of *YABBY* genes were involved in this signal transduction [11].

As specific types of transcription factor family members related to plant morphogenesis, *YABBY* genes play critical roles in the regulation of various developmental processes in eudicots such as the establishment of ad axial–abaxial polarity, lamina expansion, and floral development [9,12,13]. This is due to their significant functionalities, genomic characteristics, and expression patterns, which have been extensively studied in various plants [14,15].

Although the *YABBY* gene family is deeply involved in plant life processes, its expressions are not conserved and its functions have not been well investigated to date [16]. According to previous studies, the *YABBY* gene family is divided into five distinct groups in angiosperms, which present different expression patterns in leaves and flowers [4,17].

In *Arabidopsis*, *YABBY* genes play significant roles in the establishment of dorsal ventral polarity, leaf expansion, and flower development [18,19]. The *Arabidopsis YABBY* gene family contains six members, namely, *FIL* (*FILAMEN-TOUS FLOWER*), *CRC* (*CRAB-SCLAW*), *INO* (*INNER NO OUTER; YABBY4*), *YAB2* (*YABBY2*), *YAB3* (*YABBY3*), and *YAB5* (*YABBY5*) [20–22]. Phylogenetic analysis has shown that *FIL* and *YAB3* are closely related, which may be due to gene replication. The expression of these two genes was observed in the abaxial cells of the initial primordium of the developing organ, which determined the fate of abaxial cells [23]. *FIL* genes participated in the development of flowers and leaves [24], whereas *CRC* genes were transcribed in tissues such as the abaxial carpel, placentas, and nectarium. Furthermore, *INO* and its role in ovule development were mainly characterized in the model plant species *Arabidopsis*. *Arabidopsis INO* was restrictively expressed in the furthermost cell layer of the outer integument in ovules to promote its growth [7,25,26]. *YAB3*, *YAB2*, and *YAB5* are referred to as the "vegetative *YABBY* genes" of *Arabidopsis*, with *YAB2* and *YAB3* expressed in the abaxial domains of all leaf-derived organs, including cotyledons, leaves, and floral organs [9,27,28].

YABBY genes have also been identified in numerous other species. In *Nelumbo nucifera*, *NnYABBY* genes were highly expressed during plant growth, but not in mature tissues, which indicated that *YABBY* was a considerable regulatory factor in *Nelumbo* growth [29]. A total of 17 *YABBY* genes were identified in the soybean genome [4], and it was found that the survival rate of wild-type seeds was higher than that of GM seeds (*YABBY10*). The results revealed that this gene might have negative regulatory effects on environmental stress resistance [29]. Moreover, there were eight *YABBY* members found in *Oryza sativa*, and expression analysis showed that they might play significant roles in floral development and hormone responses [5,30].

The genus *Juglans* belongs to the Juglandaceae family, which is an important economic tree species worldwide with important material uses, in addition to having food, medicinal and artistic values [2]. However, *YABBY* genes, as important regulatory factors that affect plant growth and development, have rarely been studied in *Juglans*. Thus, the identification and characterization of this gene family in *Juglans* species is warranted, in addition to extensive directly corresponding studies. In recent years, the publication of the reference genomes of *J. regia* [31] and *J. mandshurica* [32] provided valuable opportunities to investigate important functional genes involved in plant development and environmental adaption. The *WAK*, *WRKY*, and *NAC* gene families have been identified in walnut; thus, these studies have provided an effective molecular basis for the genetic improvement and breeding of walnut. In this study, for the first time, we identified and analyzed the *YABBY* gene family in *J. regia* and its wild related species *J. mandshurica*. Specifically, the conserved domain characteristics, gene structure analysis, chromosome localization, gene duplication identification, phylogenetic relationships, and transcriptome expression profiles of *YABBY* members were elucidated for these two *Juglans* species [33]. Our study initially revealed the genetic and evolutionary origins of the *YABBY* gene family in the *Juglans* species, and laid the foundation for the further in-depth verification of *YABBY* gene functionality in other agronomic tree species.

2. Materials and Methods

2.1. Identification of YABBY Transcription Factors in J. regia and J. mandshurica

To identify the potential *YABBY* gene candidates for the two walnut species, *AtYABBY* reference protein sequences (https://www.arabidopsis.org/ (accessed on 10 February 2022)) were employed as query sequences, and the genomic sequences of *J. regia* (https://www.ncbi.nlm.nih.gov/genome/ (accessed on 10 February 2022)) and *J. mandshurica* were used as databases for blastp with an E-value $< 1 \times 10^{-5}$. Subsequently, the protein domains

of all candidates were analyzed to determine the final *YABBY* members. Specifically, the candidate members that did not contain "C2C2" domains and "*YABBY*" or "*YABBY_super*" family domains were removed by searching the Conserved Domain Database (CDD) in NCBI [33]. Furthermore, hmmersearch analysis was also performed via the Pfam online tools (http://pfam.xfam.org/ (accessed on 10 February 2022))) to remove the candidate sequences without the *YABBY* domain (pf04690).

2.2. Characteristics and Phylogenetic Relationships of Identified YABBYs

The physicochemical properties of all identified *YABBY* properties were predicted from the EXPASY online tools (http://www.expasy.org/tools/protparam.html (accessed on 10 February 2022))). Subsequently, the subcellular locations of all identified members were predicted using the Wolf PSORT website (https://wolfpsort.hgc.jp/ (accessed on 10 February 2022))).

The chromosomal locations of *YABBY* genes were found using TBtools software [34]. The collinearity analysis of gene replication patterns was conducted using MCScanX software [35]. KaKs_Calculator 2.0 software [36] was employed to calculate the *Ka*/*Ks* ratio.

The maximum likelihood (ML) tree was reconstructed for the *YABBY* members in *J. regia* and *J. mandshurica*, where the *YABBY* genes of *Arabidopsis Thaliana* and *Oryza sativa* were adopted as the outgroups. IQTREE software was used to construct the ML tree, whereas JTT+G4 was selected as the best-fit substitution model according to the BIC scores with 1000 ultra-fast bootstraps.

2.3. Cis-Acting Element Prediction and Gene Expression Analysis

The prediction of cis-acting elements was performed using 2000 bp sequences upstream of the *YABBY* genes in PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/ (accessed on 10 February 2022)). The walnut transcriptome sequencing data under pathogen stress were downloaded from NCBI (accession number GSE147083: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE147083 (accessed on 10 February 2022)) [37]. The original data were filtered using FASTP [38], followed by sequence alignments using HISAT2 [39]. Next, gene expressions were calculated using FeatureCounts software. Finally, TBtools [34] was employed to visualize the heatmaps of these candidate genes.

3. Results

3.1. Genome-Wide Identification and Phylogenetic Analysis of YABBY Gene Family in J. regia and J. mandshurica

YABBY gene members were identified in *J. regia* and its wild relative species *J. mandshurica*. By screening candidate protein domains, 10 genes in *J. regia* and nine genes in *J. mandshurica*, encoding for *YABBY* proteins, were identified. Akin to *YABBY* proteins found in other plants, all *JrYABBY* and *JmYABBY* contained two conserved domains corresponding to zinc C2C2 FNGER and *YABBY*. To facilitate analysis, we renamed all the members according to the order in which they were located in the chromosomes. The gene names and protein sequences of all identified *YABBY* members are shown in Table S1.

A maximum likelihood phylogenetic tree was constructed using the protein sequences of *Arabidopsis thaliana*, *Oryza sativa*, *J. regia*, and *J. mandshurica* (Figure 1). Members of the *YABBY* family were divided into five major clades. The largest group was YABBY1/YABBY3, which contained four *J. regia YABBYs (JrYABBY2, JrYABBY3, JrYABBY5*, and *JrYABBY9)*, three *J. mandshurica YABBYs* (*JmYABBY5*, *JmYABBY6*, and *JmYABBY7*), and three *Oryza sativa YABBYs* (*OsYABBY3*, *OsYABBY4*, and *OsYABBY5*), which were clustered together with two *YABBY* proteins in *Arabidopsis* (*AtYABBY1* and *AtYABBY3*). The group I (INO) contained one *YABBY* member each in *J. regia, J. mandshurica, Oryza sativa*, and *Arabidopsis thaliana*. Group III (*YABBY5*) consisted of two *YABBYs* from *J. regia* (*JrYABBY1* and *JrYABBY6*), two *YABBYs* (*JmYABBY1* and *JmYABBY2*) from *J. mandshurica,* and one *YABBY* (*AtYABBY5*) from *Arabidopsis*. The IV (CRC) group contained *YABBY* members (*JrYABBY7*

and *JmYABBY4*) of the four species. Group V (YABBY2) was the second largest group with two *J. regia YABBYs* (*JrYABBY4* and *JrYABBY10*), two *J. mandshurica YABBYs* (*JmYABBY8* and *JmYABBY9*), three *O. sativa YABBYs* (*OsYABBY1*, *OsYABBY2*, and *OsYABBY6*), and one *A. thaliana YABBY* (*AtYABBY2*).

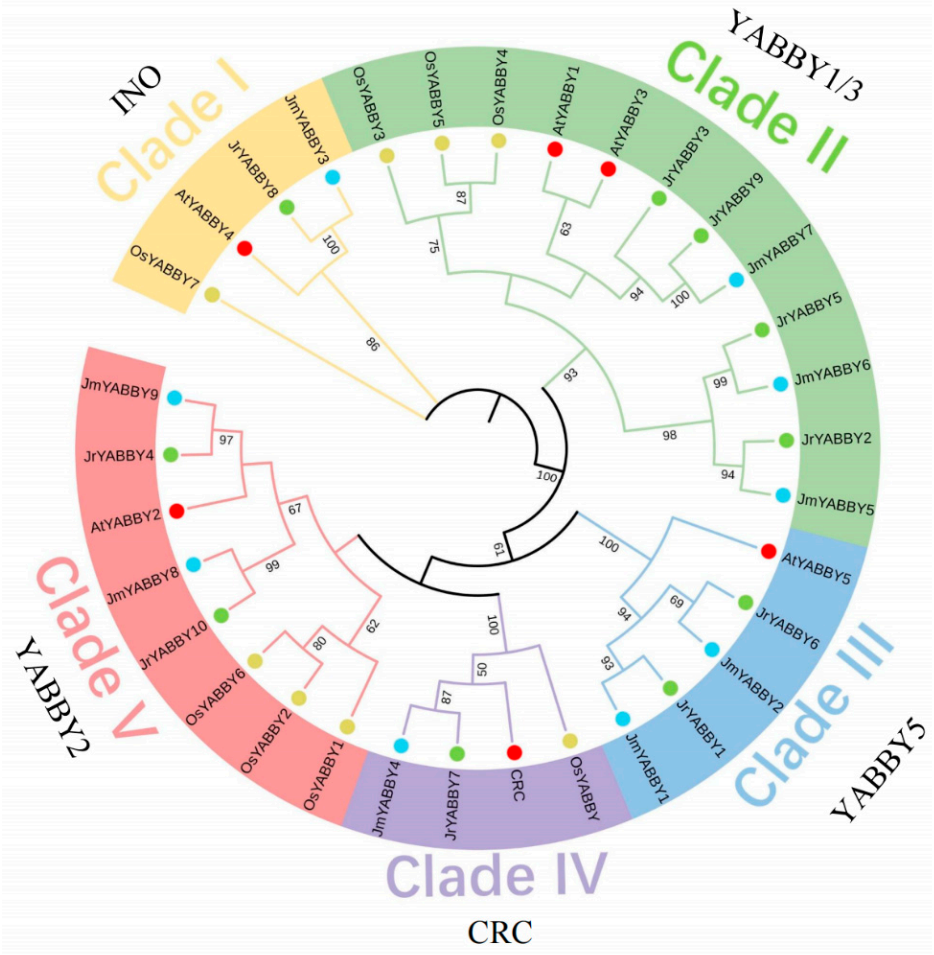

Figure 1. Maximum likelihood phylogenetic (ML) tree of *YABBY* proteins of *Arabidopsis thaliana*, *Oryza sativa*, *Juglans regia*, and *Juglans mandshurica*.

3.2. Physicochemical Properties and Subcellular Localization Analysis of YABBY Proteins in J. regia and J. mandshurica

The lengths of the *YABBY* proteins in *J. regia* ranged from 168 amino acids (aa) (*JrYABBY7*) to 215 aa (*JrYABBY5*), with an average length of 194 aa (Table 1). In contrast, the *YABBY* proteins in *J. mandshurica* were shorter, ranging from 122 aa (*JmYABBY4*) to 215 aa (*JmYABBY6*), with an average length of 180 aa. The molecular weights (MWs) of the *JrYABBY* proteins ranged from 19.75 kDa (*JrYABBY4*) to 23.91 kDa (*JrYABBY5*), with an average of 21.50 kDa. The MWs of *JmYABBY* proteins were lower than those of *JrYABBY*, ranging from 13.40 kDa (*JmYABBY4*) to 23.88 kDa (*JmYABBY6*), with an average of 19.90 kDa. Furthermore, four *YABBY* proteins were acidic (isoelectric point < 7) in *J. regia* (*JrYABBY8*) and *J. mandshurica* (*JmYABBY3*, *JmYABBY4*, and *JmYABBY6*), respectively. There were a total of four *YABBY* proteins with instability index values of >50 in *J. regia* and *J. mandshurica*. Almost all the identified *YABBY* proteins had negative grand average of hydropathicity (GRAVY) values (with only one exception (*JmYABBY3*)) for these two species, indicating that they were hydrophilic. As expected, all identified *YABBY* genes resided in the nucleus (Table 1).

Table 1. Predicted protein data of *YABBY* in *J. regia* and *J. mandshurica*.

Gene Name	No. of Amino Acids	Mol. Wt (Da)	Isoelectric Point (pI)	Instability Index (II)	Aliphatic Index	Grand Average of Hydropathicity (GRAVY)	Subcellular Localization [a]
JrYABBY1	192	21,332.24	8.83	37.17	72.71	−0.354	nucl
JrYABBY2	211	23,466.72	7.71	49.57	77.58	−0.315	nucl
JrYABBY3	213	23,467.82	8.58	50.22	77.37	−0.299	nucl
JrYABBY4	177	19,751.41	8.48	48.15	68.93	−0.494	nucl
JrYABBY5	215	23,918.36	7.71	47.33	79.81	−0.304	nucl
JrYABBY6	187	20,819.69	8.76	37.96	76.26	−0.306	nucl
JrYABBY7	168	18,543.27	9.26	41.79	72.56	−0.39	nucl
JrYABBY8	186	20,892.41	5.68	71.33	71.83	−0.492	nucl
JrYABBY9	207	22,630.76	8.25	48.02	75.89	−0.293	nucl
JrYABBY10	181	20,172.9	7.64	52.41	65.25	−0.532	nucl
JmYABBY1	202	22,227.28	8.65	35.78	76.83	−0.240	nucl
JmYABBY2	182	20,381.32	8.34	40.92	79.34	−0.196	nucl
JmYABBY3	186	20,887.85	5.86	59.77	93.28	0.022	nucl
JmYABBY4	122	13,404.34	6.80	42.39	82.30	−0.191	nucl
JmYABBY5	211	23,466.72	7.71	49.57	77.58	−0.315	nucl
JmYABBY6	215	23,887.33	8.25	45.82	82.98	−0.262	nucl
JmYABBY7	207	22,700.9	8.25	48.81	78.21	−0.259	nucl
JmYABBY8	138	15,184.45	6.98	44.67	74.13	−0.204	nucl
JmYABBY9	155	17,011.65	8.99	45.46	80.52	−0.168	nucl

[a] Note:plas: chlo: chloroplast; nucl: nucleus.

3.3. Protein Domain and Gene Structure Distribution Analysis of YABBY Genes in J. regia and J. mandshurica

The reconstructed ML tree based on the two species under study (Figure 2a) presented similar topologies to those using four species (Figure 1). Gene structural analysis revealed that the *YABBY* proteins were relatively conservative, and all *YABBY* genes contained at least one conserved domain (Figure 2b). Moreover, each *JrYABBY* and *JmYABBY* gene contained the C2C2 zinc finger and YABBY domains (Figure 2d).

Among the 10 *YABBY* genes in *J. regia*, eight contained six introns and two contained five introns. Among the nine *YABBY* genes in *J. midthoracic*, five contained six introns, three contained four introns, and one contained three introns. The numbers of exons were different between *J. regia* and *J. mandshurica* (Table S2, Figure 2c), where *JmYABBY8* had only four exons, whereas *JmYABBY3*, *JmYABBY4*, *JmYABBY9*, and *JrYABBY4* had five. The other *YABBY* genes in *J. regia* and *J. mandshurica* contained seven exons. These results indicated that the main structural characteristics in *YABBY* genes included six introns and seven exons. Interestingly, it was worthy of note that several *YABBY* members possessed relatively long introns, particularly *JrYABBY4*, *JrYABBY10*, *JmYABBY8*, and *JmYABBY9*, which belonged to the YABBY II subgroup (Figure 1).

3.4. Chromosomal Distribution and Duplication Mode Analysis of YABBY Gene Family in J. regia and J. mandshurica

To investigate chromosomal localizations, *JrYABBY* and *JmYABBY* genes were obtained and mapped to seven different corresponding chromosomes. For *J. regia*, except for two *JrYABBY* members on chr5, chr13, and chr14 chromosomes, there was only one *JrYABBY* member on the other chromosomes. For *J. mandshurica*, there were two *JmYABBY* members on chr5 and chr13 chromosomes, with only one *JmYABBY* member on other chromosomes.

The numbers of *YABBY* members identified in *J. regia* (ten) and *J. mandshurica* (nine) were nearly doubled in contrast to *Arabidopsis*. Notably, the results of gene duplication analysis showed that there were no tandem repeats between these identified *YABBY* genes. Similar results were also obtained from other species such as maize, cotton, and *Oryza sativa*. In contrast, whole-genome duplication (WGD) and dispersed duplication (DSD)

may be the driving forces behind *YABBY* gene duplication, with 10 *YABBY* genes subject to WGD followed by DSD (nine genes) (Table 2, Figure 3).

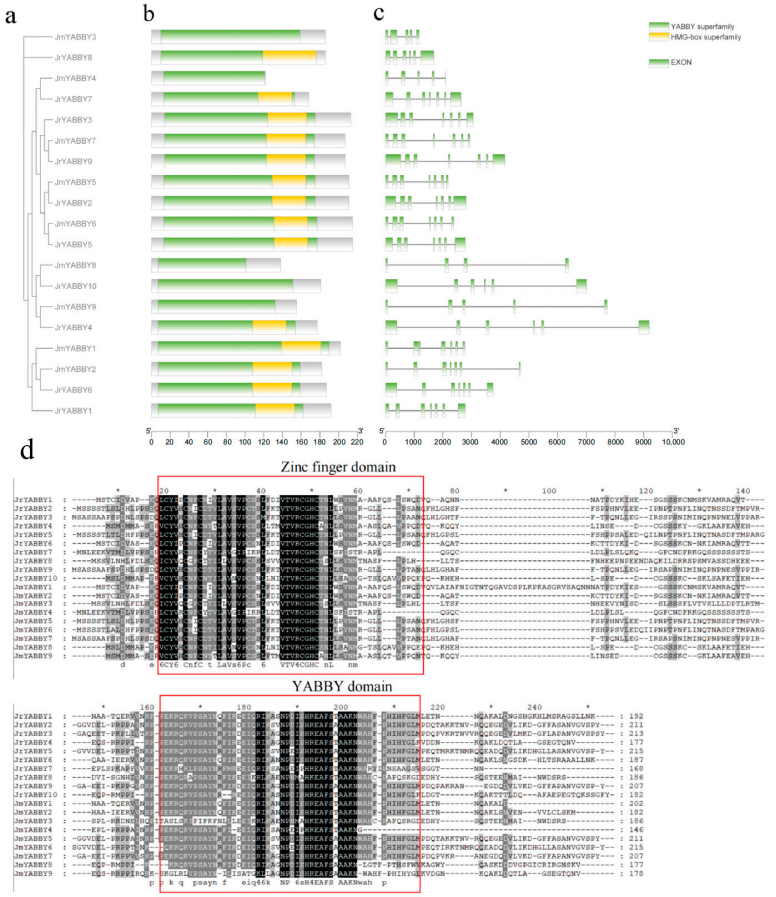

Figure 2. Gene structures and protein domains of *YABBY* gene members. (**a**) Maximum likelihood phylogenetic tree of *YABBY* in two *Juglans* species. (**b**) Protein domains of *YABBYS* in two *Juglans* species. Various domains are represented by different colored boxes. (**c**) Gene structures of *YABBYS* in two *Juglans* species. Green boxes indicate exons, and gray lines indicate introns. (**d**) Multiple sequence alignment of the *YABBY* family genes from two *Juglans* species.

Table 2. Four duplicated types of *YABBY* genes in *J. regia* and *J. mandshurica*.

Gene Name	Whole Genome Duplication (WGD)	Tandem Duplication (TD)	Dispersed Duplication (DSD)	Proximal Duplication (PD)
JrYABBY1	√			
JrYABBY2	√			
JrYABBY3	√			
JrYABBY4			√	
JrYABBY5	√			
JrYABBY6	√			
JrYABBY7			√	
JrYABBY8			√	
JrYABBY9			√	
JrYABBY10	√			
JmYABBY1	√			
JmYABBY2	√			
JmYABBY3			√	
JmYABBY4			√	
JmYABBY5	√			
JmYABBY6	√			
JmYABBY7			√	
JmYABBY8			√	
JmYABBY9			√	

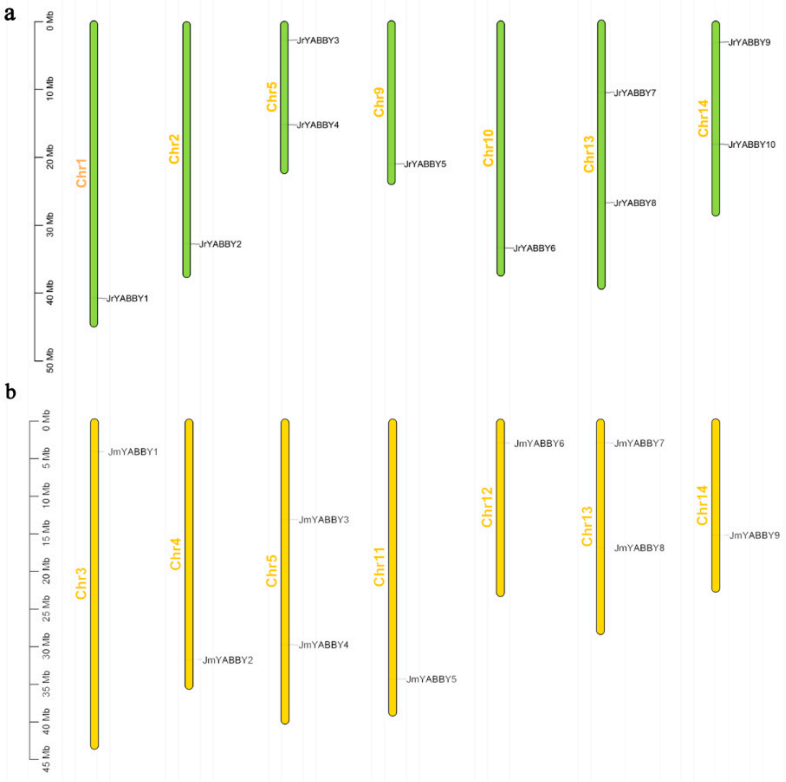

Figure 3. Chromosomal distribution of *YABBY* genes. (**a**) Chromosomal distribution of *YABBY* genes in *J. regia*. (**b**) Chromosomal distribution of *YABBY* genes in *J. mandshurica*. Chromosome numbers are shown below each chromosome. Black, *JrYABBY* genes, *JmYABBY* genes. Green, *J. regia* chromosomes; Yellow, *J. mandshurica* chromosomes.

3.5. Collinearity and Selective Pressure Analysis of YABBY Members in J. regia and J. mandshurica

Collinearity analysis revealed that there were 12 and eight *YABBY* paralogous gene pairs in *J. regia* and *J. mandshurica*, respectively, and 18 *YABBY* orthologous gene pairs between the two *Juglans* species (Figure 4). It was clear that the number of orthologous gene pairs identified between the two species was greater than that of paralogous gene pairs within the given species (Table S3). To investigate the selective pressure of these homologous gene pairs, their *Ka/Ks* values were calculated. The *Ka/Ks* ratio of most homologous *YABBY* pairs was lower than one, suggesting that they underwent purifying selection and may have evolved at a relatively low rate.

3.6. Analysis of Cis-Acting Elements in J. regia and J. mandshurica

To investigate the potential functions of *YABBY* genes in *J. regia* and *J. mandshurica*, we analyzed the cis-acting elements in their upstream promoter regions. The results revealed that the upstream promoter regions of the *YABBY* genes contained the greatest number of cis-acting elements in the two species. Further, the identified cis-acting elements were associated with four types of life activities: plant development and growth, phytohormone responses, abiotic stress responses, and light responses (Figure 5). Moreover, stress response elements such as MBS under drought stress and LTR under low temperature stress were also found. It is worth noting that development-related elements were also found, including CAT-box (meristem), circadian (circadian control), and the GCN4_motif. These results suggested that *YABBYs* may play a role in plant development, in addition to stress and phytohormone responses in *J. regia* and its wild related species *J. mandshurica.*

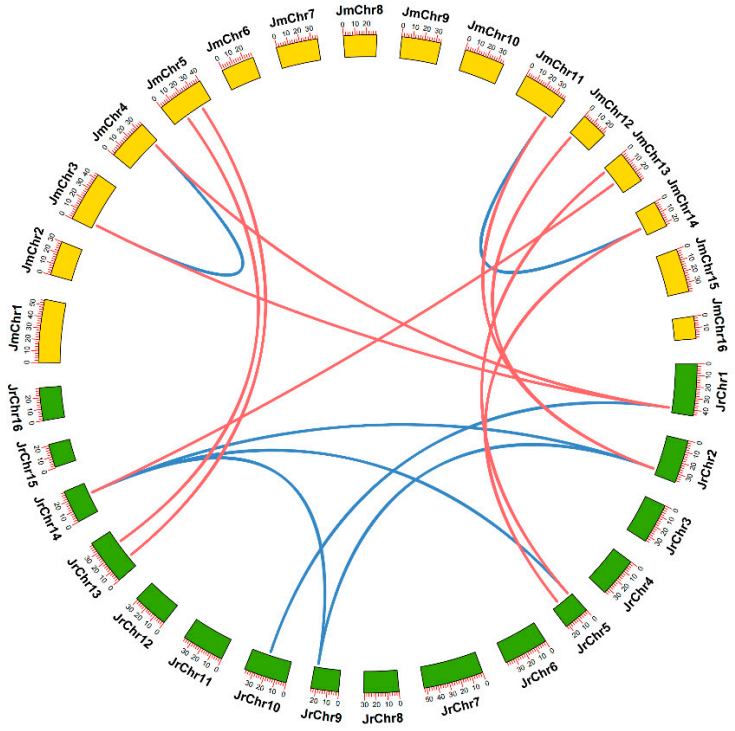

Figure 4. Genome-wide synteny analysis of *YABBY* genes for *J. regia* and *J. mandshurica*. Orthologous and paralogous *YABBY* genes were mapped onto the chromosomes and linked by each other. Red lines indicate orthologous gene pairs, blue lines indicate paralogous gene pairs.

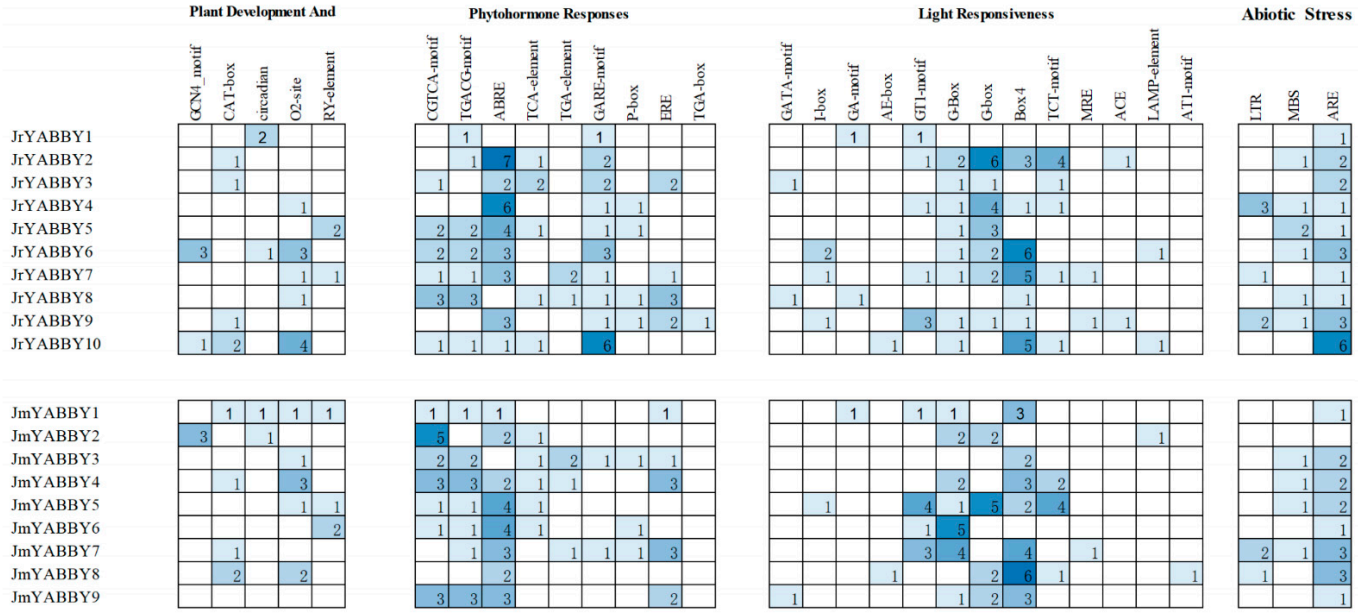

Figure 5. Cis-acting elements in promoter regions of *YABBY* genes in two *Juglans* species. Based on functional annotations, the cis-acting elements were categorized into four major classes: plant development and growth, phytohormone responses, abiotic stress responses, and light responsive cis-acting elements. Numbers in the colored boxes represent the number of cis-acting elements.

3.7. Gene Expression Profile Analysis of YABBY Genes in J. regia and J. mandshurica

To determine the expression profiles of tissue-specific *YABBY* in *J. regia* and its wild related species *J. mandshurica*, we analyzed the transcriptome data of female flowers, male flowers, leaves, and green fruit peels of these two species (Figure 6; Table S4). For *J. regia*,

the *JrYABBY3* gene exhibited relatively low expression levels in all studied tissues. Most *JrYABBY* genes were highly expressed in green pericarp, except for *JrYABBY1*, 6, and 8. *JrYABBY 1* and 6 were primarily expressed in leaves, whereas *JrYABBY 7* and 8 were found to be significantly expressed in female and male flowers, respectively. For *J. mandshurica*, most *JmYABBY* genes exhibited high expression levels in the tissues under study, except for *JmYABBY 4* and 6 in *J. regia* and *JmYABBY4* and *JmYABBY6* in *J. mandshurica*. The above results revealed that *YABBY* genes in *J. regia* and *J. mandshurica* were related to the growth and development of flowers, leaves, and fruits.

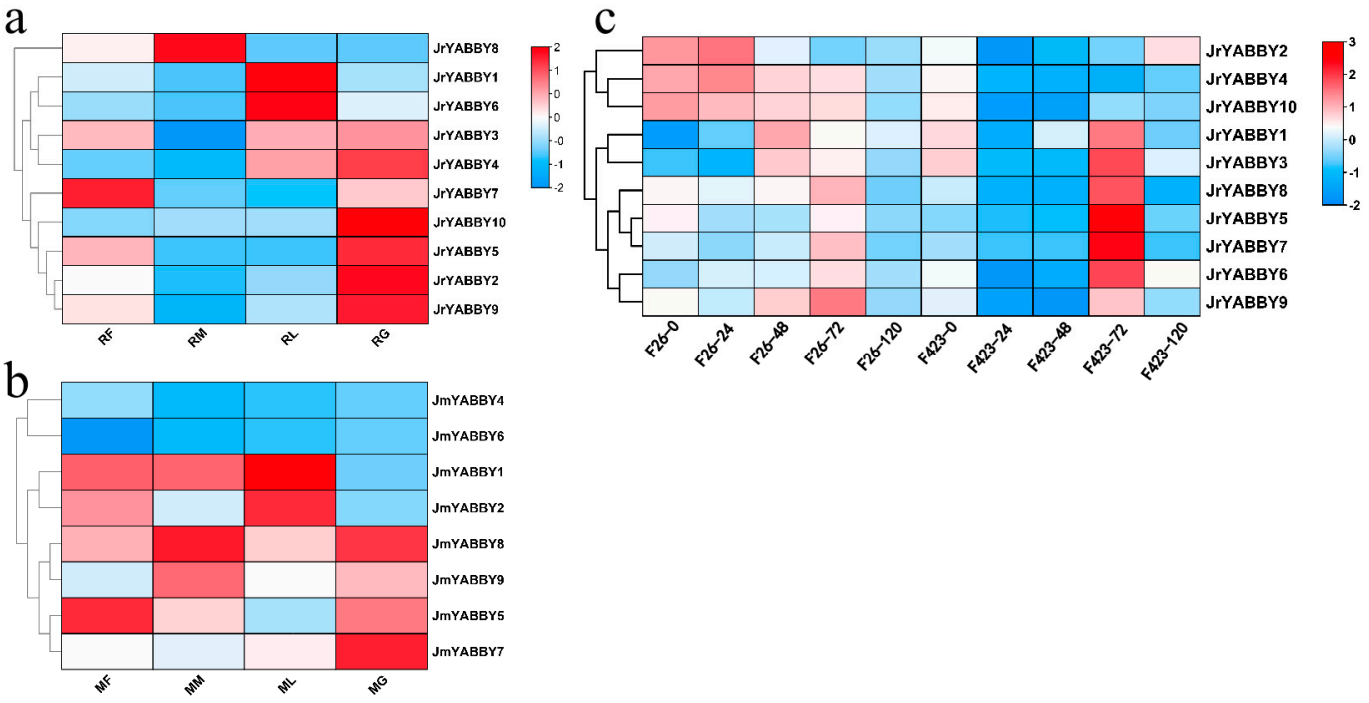

Figure 6. Expression patterns of *YABBY* genes in *J. regia* and *J. mandshurica*. (**a**) Expression patterns of identified *YABBYs* in female flowers, male flowers, leaves, and green pericarps of *J. regia*.; RF, female flowers of *J. regia*; RM, male flowers of *J. regia*; RL, leaves of *J. regia*; RG, green pericarp of *J. regia*. (**b**) Expression patterns of identified *YABBYs* in female flowers, male flowers, leaves, and green pericarps of *J. mandshurica*. MF, female flowers of *J. mandshurica*; MM, male flowers of *J. mandshurica*; ML, leaves of *J. mandshurica*; MG, green pericarps of *J. mandshurica*. (**c**) Expression patterns of identified *YABBY* genes in *J. regia* under biotic stress. F26 indicates anthracnose-resistant varieties, F423 indicates anthracnose-susceptible varieties. Numbers after '-' represent time after infection (unit: hour). Colored scale reflects gene expression levels.

To further investigate the roles of *JrYABBY* in *J. regia* stress responses, we analyzed gene expression patterns in different varieties of walnuts under biotic stress. In summary, the expression levels of *JrYABBY* genes in anthracnose-resistant cultivars (F26) were higher than those in anthracnose-susceptible cultivars (F423), which suggested that *JrYABBY* may play a role in anthracnose resistance in walnut. The *YABBY* genes typically presented low expression levels in the early-stage cultivars (F423); however, *JrYABBY2* 4 and 10 were highly expressed in the early-stage anthracnose-resistant cultivars (F26). This signified the important roles of the *YABBY* genes in defending against early-stage biotic stress.

4. Discussion

As economic forest species, the common walnut (*J. regia*) and its wild relative (*J. mandshurica*), have significant economic, nutritional, and medicinal value [40]. However, knowledge regarding the characteristics and functions of *YABBY* genes across *J. regia* and its wild related species *J. mandshurica* is negligible. With the recent completion of whole-

genome sequencing, comparative studies of the key gene families for these two species have become possible [33,40].The *YABBY* gene family is a class of unique transcription factor in seed plants that contain the C2C2 zinc finger and *YABBY* domains [4,12], and plays critical roles in a variety of biological processes, such as the development of abaxial-paraxial polarity in plant leaves, reproductive organ formation and development, plant hormone signal responses, plant biotic and abiotic stresses, leaf extension, and agricultural production [41,42].

In this study, the *YABBY* genes in *J. regia* and *J. mandshurica* were identified and analyzed, and it was found that walnut had more *YABBY* members than *Arabidopsis*. Further, genetic structural analysis indicated that the *YABBY* genes in walnut exhibited more diversity and variations. This may have been related to the participation of *YABBY* gene family members in additional evolutionary biological regulation processes. For this study, there were ten *YABBY* genes identified in *J. regia* and nine in *J. mandshurica*. To elucidate the evolutionary relationships between *JrYABBY* and *JmYABBY*, we constructed a phylogenetic tree that included *YABBY* proteins in four species (Figure 1). According to the phylogenetic relationships, *JrYABBY* and *JmYABBY* genes were divided into five different clades, which was consistent with the classification of *YABBY* genes in *Arabidopsis* and *tomato* [23,43]. The expression of *CRC* and *INO* subfamilies in *Arabidopsis* was related to the reproductive organs of plants, whereas the *FIL*, YAB2, YAB3, and YAB5 subfamilies were expressed in both leaves and flowers. *FIL* regulates *Arabidopsis* flower development, positively regulates genes involved in plant development, and participates in anthocyanin accumulation through two-way activation and repression [21].

We renamed the *YABBY* genes according to their locations on various chromosomes. All identified *JrYABBY* and *JmYABBY* genes contained C2C2 zinc finger and *YABBY* domains (Figures 1–3). Genes of the same subfamilies (e.g., *JrYABBY2* and *JrYABBY5*, *JmYABBY5* and *JmYABBY6*) exhibited similar motif and exon–intron structures. This apparently recent evolution meant that these pairs of genes may have similar functions (Figure 2, Table S2). Conversely, the evolutionary differentiation of genetic structures implied variations in gene function [44]. To investigate the selective pressures of these homologous gene pairs, their *Ka/Ks* values were calculated. The *Ka/Ks* ratios of most *YABBY* homologous gene pairs were lower than 1, suggesting that they had undergone purifying selection and may have evolved at a relatively low rate (Table S3).

Gene replication and differentiation events are considered to be major contributors to the momentum of evolution. The number of *YABBY* genes in *J. regia* and *J. mandshurica* was greater than in *Arabidopsis*; thus, we conjectured that the differences in the number of *YABBY* genes between species may be due to gene duplication or loss during evolutionary processes. Fragmentation and tandem duplications contribute to the expansion of gene families [45]. Therefore, we explored the main extended *YABBY* gene family replication types in walnut and *J. mandshurica*. However, in this study, no tandem repeat gene events were discovered in the *YABBY* gene family. In contrast, the WGD may be the major force leading to *YABBY* duplication and expansion in walnut species (Table 2).

The expressions of genes are primarily the result of the interactions between cis-acting elements and trans-acting factors. By analyzing the cis-acting elements of promoters and their upstream sequences, the functions of genes can be predicted. Various cis-acting elements in gene promoters may be correlated with different gene functions [19]. This study found that *YABBY* genes contained significant quantities of cis-regulatory elements associated with phytohormones (e.g., gibberellin, abscisic acid, and jasmonic acid) and light responses, along with endosperm expression-related active elements; thus, *JrYABBY* and *JmYABBY* genes were likely closely related to these aspects (Figure 5).

It was speculated that *JrYABBY* and *JmYABBY* genes may be related to leaf and fruit development in *J. regia* and *J. mandshurica*. To probe the expression patterns of *YABBY* in *J. regia* and *J. mandshurica*, we performed an analysis based on the transcriptome data of female flowers, male flowers, leaves, and green pericarps between these two species (Figure 6, Table S4). The results indicated that *JrYABBY* and *JmYABBY* genes were significantly

upregulated in lateral organs such as fruits and leaves. This further verified that *JrYABBY* and *JmYABBY* genes were similar to the *Arabidopsis YABBY* gene, which had specific effects on the development of lateral organs and leaves. Further detailed elucidation of how *JrYABBY* and *JmYABBY* genes induce fruit development in *J. regia* and *J. mandshurica* will require comprehensive and systematic molecular biological experiments and analysis [46]. The transcriptome profile revealed that *JrYABBY2*, *JrYABBY3*, *JrYABBY5*, and *JrYABBY10* were significantly expressed in fruit peel, which may play similar roles as the *FIL* gene in *Arabidopsis* (Figure 6); it was speculated that they may be related to the accumulation of anthocyanin. It was clear in mature flowers that the expression of *JrYABBY2*, belonging to the YAB1/YAB3 subfamily, regulated the development of flowers and fruits, which was consistent with previous research [10,17].

In this study, the *YABBY* gene family members in *J. regia* and *J. mandshurica* were systematically identified using bioinformatics methods. Simultaneously, the physicochemical properties, conserved domains, chromosomal localization, evolutionary relationships, cis-acting elements, and expression patterns of the *YABBY* gene family members, which were relatively conserved in *J. regia* and *J. mandshurica*, were analyzed. Many WGD events occurred during the expansion of the *YABBY* gene family. Furthermore, transcriptome profiles showed distinct expression patterns in different tissues, which suggested the specific functions of these identified *YABBY* genes. This study provides theoretical support and references for the functional characterization of the *YABBY* gene family in *Juglans*.

5. Conclusions

For this study, we identified ten *JrYABBY* and nine *JmYABBY* genes in *J. regia* and *J. mandshurica*. Phylogenetic analysis revealed that the *YABBY* genes could be divided into five groups and, akin to other angiosperms/basic angiosperms, they possess unique sequence characteristics beyond the conserved amino acid domains. Phylogenetic and synchronic analyses revealed that the *YABBY* gene family was evolutionarily conservative. Although *YABBY* genes were not evenly distributed across chromosomes, they were consistent across homologous chromosomes, which suggested that they were relatively conserved across subgenomes. Expression Profile Analysis indicated that the *YABBY* gene family determined significant functions in the development and growth of green pericarps, flowers, and fruits. Further exploration is required to determine whether *JrYABBY* and *JmYABBY* genes may play various roles at different stages of development of other lateral organs such as flowers and leaves.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12081914/s1, Table S1. Protein sequences of all YABBY genes of two *Juglans* species; Table S2. Number of exons and introns of YABBY genes in *Juglans regia* and *J. mandshurica*; Table S3. Estimated Ka/Ks ratios of duplicated YABBY gene pairs in *Juglans regia* and *J. mandshurica*; Table S4. FPKM values of all YABBY genes of two *Juglans* species.

Author Contributions: H.L. and H.Y. analyzed and interpreted the raw data. H.L. H.Y. S.C. and J.W. wrote the first draft. M.L., N.H., G.W. and J.W. collected the samples. N.H. and P.Z. finished the work of language editing. P.Z. conceived the main concepts for this study. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the National Natural Science Foundation of China (32070372 and 31860215), by the Bridging Project for the Enterprises and the Local walnut R&D Groups in Guizhou Province (grant number [2015] 4010 and [2019] 5643), by innovation and application of Guizhou walnut germplasm (Qianlin ke [2022] 17) and Study on the Vitality and Stigma of Guizhou Wuren Walnut Pollen (Qianlin J [2022] No. 05).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data were downloaded from the SRA database under accession number (GSE147083).

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

References

1. Liu, X.; Liao, X.-Y.; Zheng, Y.; Zhu, M.-J.; Yu, X.; Jiang, Y.-T.; Zhang, D.-Y.; Ma, L.; Xu, X.-Y.; Liu, Z.-J.; et al. Genome-Wide Identification of the YABBY Gene Family in Seven Species of Magnoliids and Expression Analysis in Litsea. *Plants* **2021**, *10*, 21. [CrossRef]
2. Yang, Z.; Gong, Q.; Wang, L.; Jin, Y.; Xi, J.; Li, Z.; Qin, W.; Yang, Z.; Lu, L.; Chen, Q.; et al. Genome-Wide Study of YABBY Genes in Upland Cotton and Their Expression Patterns under Different Stresses. *Front. Genet.* **2018**, *9*, 33. [CrossRef]
3. Buttar, Z.A.; Yang, Y.; Sharif, R.; Nan Wu, S.; Xie, Y.; Wang, C. Genome Wide Identification, Characterization, and Expression Analysis of YABBY-Gene Family in WHEAT (*Triticum aestivum* L.). *Agronomy* **2020**, *10*, 1189. [CrossRef]
4. Shamimuzzaman, M.; Vodkin, L. Genome-wide identification of binding sites for NAC and YABBY transcription factors and co-regulated genes during soybean seedling development by ChIP-Seq and RNA-Seq. *BMC Genom.* **2013**, *14*, 477. [CrossRef]
5. Toriba, T.; Harada, K.; Takamura, A.; Nakamura, H.; Ichikawa, H.; Suzaki, T.; Hirano, H.-Y. Molecular characterization the YABBY gene family in Oryza sativa and expression analysis of OsYABBY1. *Mol. Genet. Genom.* **2007**, *277*, 457–468. [CrossRef] [PubMed]
6. Luo, Y.; Niu, Y.; Gao, R.; Wang, C.; Liao, W. Genome-Wide Identification and Expression Analysis of SnRK Gene Family under Abiotic Stress in Cucumber (*Cucumis sativus* L.). *Agronomy* **2022**, *12*, 1550. [CrossRef]
7. di Rienzo, V.; Imanifard, Z.; Mascio, I.; Gasser, C.S.; Skinner, D.J.; Pierri, C.L.; Marini, M.; Fanelli, V.; Sabetta, W.; Montemurro, C.; et al. Functional conservation of the grapevine candidate gene INNER NO OUTER for ovule development and seed formation. *Hortic Res.* **2021**, *8*, 29. [CrossRef]
8. Finet, C.; Floyd, S.K.; Conway, S.J.; Zhong, B.; Scutt, C.P.; Bowman, J.L. Evolution of the YABBY gene family in seed plants. *Evol. Dev.* **2016**, *18*, 116–126. [CrossRef]
9. Tanaka, W.; Toriba, T.; Hirano, H.-Y. Three TOB1-related YABBY genes are required to maintain proper function of the spikelet and branch meristems in rice. *New Phytol.* **2017**, *215*, 825–839. [CrossRef]
10. Xia, J.; Wang, D.; Peng, Y.; Wang, W.; Wang, Q.; Xu, Y.; Li, T.; Zhang, K.; Li, J.; Xu, X. Genome-Wide Analysis of the YABBY Transcription Factor Family in Rapeseed (*Brassica napus* L.). *Genes* **2021**, *12*, 981. [CrossRef]
11. Eckardt, N.A. YABBY Genes and the Development and Origin of Seed Plant Leaves. *Plant Cell* **2010**, *22*, 2103. [CrossRef] [PubMed]
12. Xiang, J.; Liu, R.Q.; Li, T.M.; Han, L.J.; Zou, Y.; Xu, T.F.; Wei, J.Y.; Wang, Y.J.; Xu, Y. Isolation and characterization of two VpYABBY genes from wild Chinese Vitis pseudoreticulata. *Protoplasma* **2013**, *250*, 1315–1325. [CrossRef]
13. She, Z.; Huang, X.; Aslam, M.; Wang, L.; Yan, M.; Qin, R.; Chen, Y.; Qin, Y.; Niu, X. Expression characterization and cross-species complementation uncover the functional conservation of YABBY genes for leaf abaxial polarity and carpel polarity establishment in *Saccharum spontaneum*. *BMC Plant Biol.* **2022**, *22*, 124. [CrossRef] [PubMed]
14. Cong, B.; Barrero, L.S.; Tanksley, S.D. Regulatory change in YABBY-like transcription factor led to evolution of extreme fruit size during tomato domestication. *Nat. Genet.* **2008**, *40*, 800–804. [CrossRef] [PubMed]
15. Soundararajan, P.; Won, S.Y.; Park, D.S.; Lee, Y.-H.; Kim, J.S. Comparative Analysis of the YABBY Gene Family of Bienertia sinuspersici, a Single-Cell C4 Plant. *Plants* **2019**, *8*, 536. [CrossRef] [PubMed]
16. Yin, S.; Li, S.; Gao, Y.; Bartholomew, E.S.; Wang, R.; Yang, H.; Liu, C.; Chen, X.; Wang, Y.; Liu, X.; et al. Genome-Wide Identification of YABBY Gene Family in Cucurbitaceae and Expression Analysis in Cucumber (*Cucumis sativus* L.). *Genes* **2022**, *13*, 467. [CrossRef]
17. Tanaka, W.; Toriba, T.; Ohmori, Y.; Yoshida, A.; Kawai, A.; Mayama-Tsuchida, T.; Ichikawa, H.; Mitsuda, N.; Ohme-Takagi, M.; Hirano, H.-Y. The YABBY gene TONGARI-BOUSHI1 is involved in lateral organ development and maintenance of meristem organization in the rice spikelet. *Plant Cell* **2012**, *24*, 80–95. [CrossRef]
18. Zhang, T.; Li, C.; Li, D.; Liu, Y.; Yang, X. Roles of YABBY transcription factors in the modulation of morphogenesis, development, and phytohormone and stress responses in plants. *J. Plant Res.* **2020**, *133*, 751–763. [CrossRef]
19. Zhao, Y.; Liu, C.; Ge, D.; Yan, M.; Ren, Y.; Huang, X.; Yuan, Z. Genome-wide identification and expression of YABBY genes family during flower development in *Punica granatum* L. *Gene* **2020**, *752*, 144784. [CrossRef]
20. Eshed, Y.; Baum, S.F.; Bowman, J.L. Distinct mechanisms promote polarity establishment in carpels of Arabidopsis. *Cell* **1999**, *99*, 199–209. [CrossRef]
21. Villanueva, J.M.; Broadhvest, J.; Hauser, B.A.; Meister, R.J.; Schneitz, K.; Gasser, C.S. INNER NO OUTER regulates abaxial- adaxial patterning in *Arabidopsis ovules*. *Genes Dev.* **1999**, *13*, 3160–3169. [CrossRef]
22. Chen, Y.-Y.; Hsiao, Y.-Y.; Chang, S.-B.; Zhang, D.; Lan, S.-R.; Liu, Z.-J.; Tsai, W.-C. Genome-Wide Identification of YABBY Genes in Orchidaceae and Their Expression Patterns in Phalaenopsis Orchid. *Genes* **2020**, *11*, 955. [CrossRef]
23. Siegfried, K.R.; Eshed, Y.; Baum, S.F.; Otsuga, D.; Drews, G.N.; Bowman, J.L. Members of the YABBY gene family specify abaxial cell fate in Arabidopsis. *Development* **1999**, *126*, 4117–4128. [CrossRef] [PubMed]
24. Sawa, S.; Watanabe, K.; Goto, K.; Liu, Y.G.; Shibata, D.; Kanaya, E.; Morita, E.H.; Okada, K. FILAMENTOUS FLOWER, a meristem and organ identity gene of Arabidopsis, encodes a protein with a zinc finger and HMG-related domains. *Genes Dev.* **1999**, *13*, 1079–1088. [CrossRef]

25. Bartholmes, C.; Hidalgo, O.; Gleissberg, S. Evolution of the YABBY gene family with emphasis on the basal eudicot Eschscholzia californica (Papaveraceae). *Plant Biol.* **2012**, *14*, 11–23. [CrossRef]
26. Hou, H.; Lin, Y.; Hou, X. Ectopic Expression of a Pak-choi YABBY Gene, BcYAB3, Causes Leaf Curvature and Flowering Stage Delay in *Arabidopsis thaliana*. *Genes* **2020**, *11*, 370. [CrossRef]
27. Bowman, J.L.; Smyth, D.R.; Meyerowitz, E.M. Genes directing flower development in Arabidopsis. *Plant Cell* **1989**, *1*, 37–52. [CrossRef]
28. Lu, Y.-H.; Alam, I.; Yang, Y.-Q.; Yu, Y.-C.; Chi, W.-C.; Chen, S.-B.; Chalhoub, B.; Jiang, L.-X. Evolutionary Analysis of the YABBY Gene Family in Brassicaceae. *Plants* **2021**, *10*, 2700. [CrossRef]
29. Xu, Y.; Qiao, Z.; Zhao, L.; Zhao, W.; Chen, L.; Hu, Z. Genome-wide and Transcriptome Analysis of YABBY Family Gene in *Lotus (Nelumbo nucifera Gaertn.)*. *Mol. Plant Breed.* 2022. Available online: https://kns.cnki.net/kcms/detail/46.1068.S.20210326.1013.006.html (accessed on 14 July 2022).
30. Yamaguchi, T.; Nagasawa, N.; Kawasaki, S.; Matsuoka, M.; Nagato, Y.; Hirano, H.-Y. The YABBY gene DROOPING LEAF regulates carpel specification and midrib development in *Oryza sativa*. *Plant Cell* **2004**, *16*, 500–509. [CrossRef]
31. Marrano, A.; Britton, M.; Zaini, P.A.; Zimin, A.V.; Workman, R.E.; Puiu, D.; Bianco, L.; Pierro, E.A.D.; Allen, B.J.; Chakraborty, S.; et al. High-quality chromosome-scale assembly of the walnut (*Juglans regia* L.). *reference genome. GigaScience* **2020**, *9*, giaa050. [CrossRef]
32. Yan, F.; Xi, R.-M.; She, R.-X.; Chen, P.-P.; Yan, Y.-J.; Yang, G.; Dang, M.; Yue, M.; Pei, D.; Woeste, K.; et al. Improved de novo chromosome-level genome assembly of the vulnerable walnut tree *Juglans* mandshurica reveals gene family evolution and possible genome basis of resistance to lesion nematode. *Mol. Ecol. Resour.* **2021**, *21*, 2063–2076. [CrossRef] [PubMed]
33. Li, M.; Ma, J.; Liu, H.; Ou, M.; Ye, H.; Zhao, P. Identification and Characterization of Wall-Associated Kinase (WAK) and WAK-like (WAKL) Gene Family in *Juglans regia* and Its Wild Related Species *Juglans mandshurica*. *Genes* **2022**, *13*, 134. [CrossRef] [PubMed]
34. 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] [PubMed]
35. Wang, Y.; Tang, H.; Debarry, J.D.; Tan, X.; Li, J.; Wang, X.; Lee, T.-h.; 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]
36. Zhang, Z.; Li, J.; Zhao, X.-Q.; Wang, J.; Wong, G.K.-S.; Yu, J. KaKs_Calculator: Calculating Ka and Ks Through Model Selection and Model Averaging. *Genom. Proteom. Bioinform.* **2006**, *4*, 259–263. [CrossRef]
37. Feng, S.; Fang, H.; Liu, X.; Dong, Y.; Wang, Q.; Yang, K.Q. Genome-wide identification and characterization of long non-coding RNAs conferring resistance to Colletotrichum gloeosporioides in walnut (*Juglans regia*). *BMC Genom.* **2021**, *22*, 15. [CrossRef]
38. Chen, S.; Zhou, Y.; Chen, Y.; Gu, J. fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **2018**, *34*, i884–i890. [CrossRef]
39. Kim, D.; Langmead, B.; Salzberg, S.L. HISAT: A fast spliced aligner with low memory requirements. *Nat. Methods* **2015**, *12*, 357–360. [CrossRef]
40. Martínez-García, P.J.; Crepeau, M.W.; Puiu, D.; Gonzalez-Ibeas, D.; Whalen, J.; Stevens, K.A.; Paul, R.; Butterfield, T.S.; Britton, M.T.; Reagan, R.L.; et al. The walnut (*Juglans regia*) genome sequence reveals diversity in genes coding for the biosynthesis of non-structural polyphenols. *Plant J.* **2016**, *87*, 507–532. [CrossRef]
41. Zhao, S.-P.; Lu, D.; Yu, T.-F.; Ji, Y.-J.; Zheng, W.-J.; Zhang, S.-X.; Chai, S.-C.; Chen, Z.-Y.; Cui, X.-Y. Genome-wide analysis of the YABBY family in soybean and functional identification of GmYABBY10 involvement in high salt and drought stresses. *Plant Physiol. Biochem.* **2017**, *119*, 132–146. [CrossRef]
42. Romanova, M.A.; Maksimova, A.I.; Pawlowski, K.; Voitsekhovskaja, O.V. YABBY Genes in the Development and Evolution of Land Plants. *Int. J. Mol. Sci.* **2021**, *22*, 4139. [CrossRef] [PubMed]
43. Huang, Z.; Van Houten, J.; Gonzalez, G.; Xiao, H.; van der Knaap, E. Genome-wide identification, phylogeny and expression analysis of SUN, OFP and YABBY gene family in tomato. *Mol. Genet. Genom.* **2013**, *288*, 111–129. [CrossRef] [PubMed]
44. Wan, T.; Liu, Z.-M.; Li, L.-F.; Leitch, A.R.; Leitch, I.J.; Lohaus, R.; Liu, Z.-J.; Xin, H.-P.; Gong, Y.-B.; Liu, Y.; et al. A genome for gnetophytes and early evolution of seed plants. *Nat. Plants* **2018**, *4*, 82–89. [CrossRef] [PubMed]
45. 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]
46. Azam, S.M.; Liu, Y.; Rahman, Z.U.; Ali, H.; Yan, C.; Wang, L.; Priyadarshani, S.V.G.N.; Hu, B.; Huang, X.; Xiong, J.; et al. Identification, Characterization and Expression Profiles of Dof Transcription Factors in Pineapple (Ananas comosus L). *Trop. Plant Biol.* **2018**, *11*, 49–64. [CrossRef]