Gene Family in Cucurbitaceae

YABBY

Article

Xiaofeng Chen 2, Ying Wang 3, Xingwang Liu 1,4 and Huazhong Ren 1,4,*

1. Introduction

The YABBY gene family, which belongs to the zinc finger protein superfamily, is plant-specific transcription factors (TFs). All YABBY members share two highly conserved domains that are characterized by a N-terminal C2C2 zinc finger domain and a helix–loop–helix motif (called the YABBY domain) at their C-terminus [1,2]. The first YABBY gene family was described and six members were identified in Arabidopsis, including YABBY1 (YAB1)/FILAMENTOUS FLOWER (FIL), CRABS CLAW (CRC), INNER NO OUTER (INO), YABBY2 (YAB2), YABBY3 (YAB3), and YABBY5 (YAB5) [1–3]. It has been reported that the YABBY genes are unique to seed plants [4,5] and genome-wide identification studies of the YABBY gene family have been performed in various plant species, such as tomato (9) [6], Chinese cabbage (12) [7], rice (8) [8], and maize (13) [9].

Based on evolutionary relationships, the angiosperm YABBY genes can be classified into five subfamilies—YAB1, CRC, INO, YAB2, and YAB5 [10,11]. Substantial evidence has demonstrated that the YABBY genes play important roles in many aspects of plant development, and phytohormone, stress, and light responses. Importantly, YABBY genes exhibited organ-specific patterns in expression in cucumber. Furthermore, a gene CsaV3_6G038650 was constitutively expressed at higher levels at different fruit development stages and might play a crucial role in cucumber fruit development. Collectively, our work will provide a better understanding for further function identifications of YABBY genes in Cucurbitaceae.

**Keywords:** Cucurbitaceae; YABBY genes; evolution; expression pattern; cucumber

**Abstract:** YABBY transcription factors play important roles in plant growth and development. However, little is known about YABBY genes in Cucurbitaceae. Here, we identified 59 YABBY genes from eight cucurbit species, including cucumber (C. sativus L.), melon (C. melon L.), watermelon (C. lanatus), wax gourd (B. hispida), pumpkin (C. maxima), zucchini (C. pepo L.), silver-seed gourd (C. argyrosperma), and bottle gourd (L. siceraria). The 59 YABBY genes were clustered into five subfamilies wherein the gene structures and motifs are conserved, suggesting similar functions within each subfamily. Different YABBY gene numbers in eight cucurbit species indicated that gene loss or duplication events exist in an evolutionary process across Cucurbitaceae. The cis-acting elements analysis implied that the YABBYs may be involved in plant development, and phytohormone, stress, and light responses. Consequently, the YABBY genes exhibit organ-specific patterns in expression in cucumber. Furthermore, a gene CsaV3_6G038650 was constitutively expressed at higher levels at different fruit development stages and might play a crucial role in cucumber fruit development. Collectively, our work will provide a better understanding for further function identifications of YABBY genes in Cucurbitaceae.
growth and development, such as lateral organ development, establishment of polarity, and reproductive organ development in angiosperms [12,13]. In Arabidopsis, four genes—FIL, YAB2, YAB3, and YAB5—are involved in vegetative tissues development [13–15]. FIL, YAB2, and YAB3 are specifically expressed in all lateral organ primordia derived from the apical and flower meristems [2,14,16]. FIL and YAB3 are responsible for leaf development in a redundant manner with YAB2 and YAB5 [2,14,17], and FIL also contributes to the establishment of floral meristem identity and flower development [2,16,18,19]. While CRC and INO regulate reproductive organ development [1,3,13]. CRC is required for carpel and nectary development [1,20,21]. INO promotes the formation and asymmetric growth of ovule outer integument [3,22]. Furthermore, in monocots, the biological functions of YABBY genes have been well studied in rice. For example, OsDL, the homolog of CRC, affects the development of both flowers and leaves [23–25]. During rice domestication, the YABBY genes OsSh1 and OsSh3 are required for seed shattering [26,27]. OsYAB1 controls meristem development and the maintenance of stamens and carpels [28]. OsYAB3 (TOB3), OsYAB4 (TOB2), and OsYAB5 (TOB1) are enriched in lateral organ primordia and play a crucial role in rice spikelet development [29,30]. These results suggest that YABBY genes have diverse roles in plant growth and development; meanwhile, the functions of each subgroup are both differentiated and conserved.

The Cucurbitaceae are widely distributed in the tropics and subtropics [31]. The major cucurbit crops have global economic importance, such as cucumber (C. sativus L.), melon (C. melo L.), watermelon (C. lanatus), and squash/pumpkin (C. maxima) [31]. These cucurbit crops mainly consume their fruits. For example, cucumber and watermelon are consumed fresh fruits [32–34] and melon mainly contributes to diets as a sweet and fleshy dessert [35]. Many cucurbit crops contain important nutrients, such as sugars and lycopene in watermelon [36], and vitamins and flavonoids in wax gourd [37]. Importantly, the metabolites derived from cucurbit crops can function as medicines, for instance, the amino acids citrulline, arginine, and glutathione from watermelon promote cardiovascular health [38,39], the wax gourd metabolites can be used in treating various disorders [40,41], the cucurbitacins produced by cucurbit plants play a vital role in cancer therapy [42,43]. Although the YABBY gene family has been characterized in many plant species, little is known of YABBY gene characteristics in Cucurbitaceae. There are only two reports on the YABBY gene functions in cucumber. Liu et al. (2018) found that CsYAB1, CsYAB3, and CsINO are involved in the integument of ovules by interacting with CsSPL [44]. The other report suggested that CsYAB5 regulates leaf morphology, and vascular and fruit development [45].

Here, we identified and characterized the YABBY genes in Cucurbitaceae, including cucumber (C. sativus L.), melon (C. melo L.), watermelon (C. lanatus), wax gourd (B. hispida), pumpkin (C. maxima), zucchini (C. pepo L.), silver-seed gourd (C. argyrosperma), and bottle gourd (L. siceraria). We systematically performed gene structure, conserved motifs, chromosomal location, cis-acting elements, and phylogenetic analysis of 59 YABBY genes in the eight cucurbit species. The evolutionary relationship of YABBY genes between cucumber and other cucurbit species was explored. Finally, the expression patterns of YABBY genes in cucumber were investigated in various organs/tissues and different development stages of ovary/fruit. Our results will provide valuable clues for the function identifications of YABBY genes in Cucurbitaceae.

2. Materials and Methods
2.1. Gene Identification and Chromosomal Locations

The YABBY domain (PF04690) was downloaded from the Pfam protein families database [46] (http://pfam.xfam.org/) (accessed on 6 October 2021)) and was used to identify YABBY genes in eight cucurbit species by HMMER 3.0 software (E-value < 1 × 10−10). All predicted YABBY sequences were further manually examined to confirm the conserved C2C2 zinc finger domain at N-terminus and YABBY domain at C-terminus using CDD [47] (https://www.ncbi.nlm.nih.gov/cdd (accessed on 6 October 2021)) and SMART [48]
The YABBY genes of each species were mapped on chromosomes by the online software MapGene2Chrom [49] (http://mg2c.iask.in/mg2c_v2.0/ (accessed on 6 October 2021)). The physicochemical properties, including molecular weights (MW) and isoelectric points (pI), were predicted with the ProtParam tool on the ExPASy server [50] (https://web.expasy.org/protparam/ (accessed on 6 October 2021)). The genome data for eight species in Cucurbitaceae were downloaded from Cucurbit Genomics Database (http://cucurbitgenomics.org/ (accessed on 6 October 2021)) and Arabidopsis protein data (Araport11 protein lists) was downloaded from TAIR (https://www.arabidopsis.org/ (accessed on 6 October 2021)).

2.2. Phylogenetic and Gene Duplication Analysis

Multiple sequence alignment of all identified YABBYs in Cucurbitaceae was carried out using ClustalW, and a phylogenetic tree was generated by neighbor-joining (NJ) method with default parameters: bootstrap method setting to 1000, Poisson model, and complete deletion in MEGA 11. The tree was visualized and optimized via Interactive Tree Of Life (iTOL) (https://itol.embl.de/ (accessed on 7 October 2021)). To explore the gene duplication events, the collinearity analysis was performed with Multiple Collinearity Scan toolkit (MCScanX) [51].

2.3. Gene Structure Analysis and Conserved Motif Identification

All of the identified YABBY gene structures were analyzed by Gene Structure Display Server (GSDS) [52] (http://gsds.gao-lab.org/ (accessed on 7 October 2021)). The MEME online program [53] (https://meme-suite.org/meme/tools/meme (accessed on 7 October 2021)) was employed to predict the motifs within the 59 Cucurbitaceae YABBY protein sequences.

2.4. Cis-Regulatory Elements Analysis

The promoter sequences (2000 bp upstream of ATG) of 59 YABBY genes were extracted from genome sequences of eight cucurbit species by TBtools software [54]. The cis-regulatory elements in promoter region were analyzed using the online PlantCARE database [55] (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/ (accessed on 8 October 2021)).

2.5. Plant Materials

The cucumber cultivar (“Xintaimici”) was grown in a greenhouse in Beijing, China. Roots and tender stems of two-week-old seedlings, the third true leaves, tender tendrils, male and female buds at 8 DBF (days before flowering), and ovaries/fruits at different growth stages were selected as samples, frozen in liquid nitrogen, and stored at −80 °C.

2.6. RNA Extraction and qRT-PCR Analysis

The sample RNA was extracted using the Quick RNA Isolation Kit (Huayueyang, Beijing, China). The FastKing gDNA Dispelling RT SuperMix (TianGen Biotech, Beijing, China) was applied to synthesize the first-strand cDNA with the extractive RNA template. qRT-PCR was performed using the UltraSYBR Mixture (Low ROX) (Cwbio, Beijing, China) on an Applied Biosystems 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). The UBIQUITIN EXTENSION PROTEIN (UBI-EP) gene [56] was used as a reference gene. Three biological and three technical replicates were carried out for expression dynamics analysis. The significant differences were analyzed by Student’s t-tests (p ≤ 0.05). The primers were listed in Table S6.

2.7. Transcriptome Analysis of YABBY Genes in Cucumber

For the expression patterns of CsYABBY genes at 4 DBF, ovaries from two near isogenic lines with different fruit lengths were obtained from publicly available transcriptomic data, which were downloaded from Gene Expression Omnibus (GEO) and analyzed to reveal
the genes and gene networks that regulate fruit length in cucumber (GSE60346) [57]. Clean tags were remapped to the cucumber v3 genome sequence (http://cucurbitgenomics.org (accessed on 20 October 2021)) by Hisat2, and the TPM values were recalculated. Every line had two biological replicates. The $p$-value $\leq 0.05$ and fold-change $\geq 1.5$ were used to define differential expression genes. The expression pattern of the YABBY genes was shown on a heatmap using TBtools software [54].

3. Results

3.1. Identification of YABBY Genes in Eight Cucurbit Species and Their Chromosomal Distribution

To identify YABBY members in Cucurbitaceae, we performed a Hidden Markov Model (HMM) search using YABBY domain (PF04690) across eight cucurbit species. Based on a further confirmation of conserved C2C2 domain and YABBY domain by CDD and SMART analysis, 59 YABBYs were finally obtained in Cucurbitaceae, including 8 from cucumber (C. sativus L.), 4 from melon (C. melon L.), 9 from watermelon (C. lanatus), 5 from wax gourd (B. hispida), 11 from pumpkin (C. maxima), 9 from zucchini (C. pepo L.), 10 from silver-seed gourd (C. argyrosperma), and 3 from bottle gourd (L. siceraria) (Tables 1 and S1). These gene coding sequence lengths varied from 471 to 1179 bp and the encoded protein length ranged from 156 to 392 amino acids along with the predicted protein molecular weight (MW) ranging from 17.05 to 43.64 KDs. The isoelectric point (pI) values of the YABBYs were 4.23 to 9.62 (Table S2).

Table 1. Classification of the YABBY gene family in eight cucurbit species.

| Species            | Group | Total |
|-------------------|-------|-------|
|                   | YAB1 | CRC  | INO | YAB2 | YAB5 |
| C. sativus L.     | 2    | 1    | 2   | 1    | 2    | 8    |
| C. melo L.        | 0    | 1    | 1   | 1    | 1    | 4    |
| C. lanatus        | 2    | 2    | 2   | 1    | 2    | 9    |
| B. hispida        | 2    | 1    | 0   | 1    | 1    | 5    |
| C. maxima         | 3    | 1    | 2   | 1    | 4    | 11   |
| C. pepo L.        | 2    | 0    | 2   | 1    | 4    | 9    |
| C. argyrosperma   | 2    | 1    | 2   | 1    | 4    | 10   |
| L. siceraria      | 1    | 0    | 1   | 0    | 1    | 3    |

Next, we mapped the YABBY genes on chromosomes of seven cucurbit species (except silver-seed gourd with a lower quality genome draft), respectively. The YABBY genes of each cucurbit species were randomly distributed on their chromosomes and distribution results were shown in Figure S1. For example, the chromosome 1, 2, 3, 5, and 6—but not chromosome 4 and 7—harbored the eight YABBY genes in cucumber, the nine YABBYs were distributed on chromosome 1, 2, 5, 6, 8, 10, and 11 other than chromosome 3, 4, 7, and 9 in watermelon (Figure S1).

3.2. Evolutionary Relationship and Synteny Analysis of YABBYs in Cucurbitaceae

To better explore the evolutionary relationship of YABBYs, six Arabidopsis YABBYs, eight rice YABBYs and 59 YABBY members in Cucurbitaceae were used to construct an evolutionary tree using MEGA 11 with ClustalW and NJ methods. As reported in Arabidopsis, the Cucurbitaceae YABBYs were also divided into five subfamilies, YAB1, YAB2, CRC, INO, and YAB5 (Figure 1 and Table 1). The YAB1 and YAB5 subfamilies had the larger numbers of YABBYs, in which the YAB1 subfamily contained 14 members while YAB5 subfamily contained 19 members. The two subfamilies of YAB2 and CRC shared the smallest YABBYs with seven members, respectively. The four cucurbit species cucumber, watermelon, pumpkin, and silver-seed gourd YABBYs could be divided into all five subfamilies. However, the other four cucurbit species lack one or two subfamilies, such as melon lacking YAB1 subfamily; wax gourd lacking INO subfamily; zucchini lacking CRC subfamily, and bottle gourd lacking CRC and YAB2 subfamilies (Figure 1 and Table 1).
Taken together, these results suggested that there are evolutionary splits and diversifications of YABBYs among different cucurbit species.

Figure 1. Phylogenetic tree of the YABBY proteins from Arabidopsis, rice, and eight cucurbit species. Red star, red star filled with pink color, yellow star, green star filled with light purple color, black star filled with dark purple color, bluish grey star, red star filled with blue color, black star, black triangle filled with red color and black circle filled with green color represent the YABBYs from cucumber, melon, watermelon, wax gourd, pumpkin, zucchini, silver-seed gourd, bottle gourd, Arabidopsis, and rice, respectively. Cs, cucumber (C. sativus L.); MELO, melon (C. melo L.); Cla, watermelon (C. lanatus); Bhi, wax gourd (B. hispida); Cma, pumpkin (C. maxima); Cp, zucchini (C. pepo L.); Carg, silver-seed gourd (C. argyrosperma); Lsi, bottle gourd (L. siceraria); AT, Arabidopsis (Arabidopsis thaliana); Os, rice (Oryza sativa L.).

Generally, gene duplications contribute to novel gene function generation and gene family expansion [58]. Here, we performed a synteny analysis of YABBYs between cucumber and other cucurbit species (except silver-seed gourd with a lower quality genome draft) by MCScanX. The collinear gene pairs between cucumber and melon, wax gourd, watermelon, bottle gourd, pumpkin, and zucchini were 10, 9, 14, 14, 19, and 21, respectively (Figure 2 and Table S3). The all eight CsyABBYs showed a syntenic relationship (at least two syntenic gene pairs of each CsyABBY gene) with zucchini YABBYs, 75% of CsYABBYs (except CsaV3_5G033460 and CsaV3_6G038650 possessing one syntenic gene pair, respectively) shared two, or more than two, syntenic pairs with the YABBYs in watermelon, pumpkin, and bottle gourd (Figure 2 and Table S3), indicating that the YABBY genes in cucumber, watermelon, pumpkin, bottle gourd, and zucchini evolved from the same ancestral gene. In addition, six YABBY genes had only one syntenic gene pair between cucumber and melon, respectively. However, CsaV3_1G030340 and CsaV3_2G002960 were sisters to two melon YABBY genes, respectively. The same case was observed between cucumber and wax gourd (only CsaV3_2G002960 and CsaV3_2G024750 have two sisters in wax gourd, respectively).
(Figure 2 and Table S3), suggesting that there are no apparent gene family expansion events between cucumber and melon/wax gourd. Notably, when we identified \textit{YABBY} genes, the truncated genes, lacking either a C2C2 domain and/or a YABBY domain, were excluded. This is possibly responsible for why the final number of \textit{YABBY} genes of each cucurbit species (except cucumber and watermelon) is less than that obtained by synteny analysis (Table S3).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{Synteny analysis of \textit{YABBY} genes between cucumber and melon, watermelon, wax gourd, pumpkin, zucchini, and bottle gourd. The synteny gene pairs are highlighted in the red lines.}
\end{figure}
3.3. Gene Structure and Conserved Motifs Analysis of YABBYs in Cucurbitaceae

The evolutionary history of gene families can be reflected by gene structural diversity [59]. Thus, we analyzed the gene structure that are characterized by the exon–intron organization of YABBYs open reading fame (ORF) from eight cucurbit species to explore the YABBYs evolution in Cucurbitaceae. An unrooted evolutionary tree was conducted by MEGA 11 and used for distinguishing different YAB subfamilies. As shown in Figure 3 and Table S2, the 59 YABBYs contained exon numbers ranging from 5 to 12 and intron number varying from 4 to 11. Among them, all YAB2 subfamily members had six exons and five introns, most members of the CRC subfamily (except Carg17317 with nine exons and eight introns in silver-seed gourd) shared seven exons and six introns (Figure 3 and Table S2). However, we found that the INO subfamily and the two larger subfamilies, YAB1 and YAB5, harbor different numbers of exon and intron, 5 to 11 exons and 4 to 10 introns in the
YAB1 subfamily, 5 to 9 exons and 4 to 8 introns in the YAB5 subfamily, and 6 to 12 exons and 5 to 11 introns in the INO subfamily (Figure 3 and Table S2).

The MEME analysis was carried out to predict the conserved motifs of protein sequence to better understand the conservation and diversification of YABBYs in Cucurbitaceae. We observed that the YABBYs in same subfamily share highly similar motif compositions (Figures 3 and S2). For instance, motif 9 was unique to YAB1 subfamily, whereas motif 5 was lacking in YAB2 subfamily. Specially, motif 10 only existed in a subgroup of INO subfamily (Figure 3). These results suggested the possible functional diversification of YABBYs across Cucurbitaceae. Finally, given that the YABBYs are plant-specific proteins containing conserved C2C2 domain and YABBY domain, we aligned the 59 YABBY protein sequences. The alignment results exhibited that all 59 YABBYs contain the two conserved domains (Figure 4), indicating the conservation in Cucurbitaceae YABBYs evolution.

![Figure 4](image-url). The conserved C2C2 domain and YABBY domain of 59 YABBY proteins from eight cucurbit species. The typical amino acid residues within C2C2 domain are indicated with red asterisks.

### 3.4. Cis-Acting Elements Analysis of Cucurbitaceae YABBY Genes Promoter Regions

The 2000 bp upstream sequences from the transcription start site of 59 YABBYs were extracted by TBtools from the genome of the eight cucurbit species and used to analyze the cis-acting elements in these promotors using the online PlantCARE database. The predicted cis-acting elements were mainly associated with phytohormone response, plant development, stress, and light responses (Figure 5 and Table S4). We noted that the light response elements exist in most YABBYs promotors. The phytohormone response elements mainly involved in abscisic acid (ABRE) and methyl jasmonate (CGTCA-motif, TGACG-motif) were identified, while fewer response elements associated with auxin (TGA-element), cytokinin (O2-site), gibberellin (P-box), and salicylic acid (TCA-element) were discovered. Moreover, the stress response elements—such as TC-rich repeats for defense and stress, MBS for drought stress, and LTR for low temperature stress—were also found. Notably, we detected some development-related elements but with fewer numbers, including CAT-
Genes 2022, 13, 467

box (meristem), circadian (circadian control), GCN4_motif (endosperm), MSA-like (cell cycle), and RY-element (seed-specific) (Figure 5 and Table S4). Taken together, these results suggested possible roles of YABBYs in plant development, stress, and phytohormone responses across Cucurbitaceae.

Table 1. Numbers of cis-acting elements in promoters of five genes across Cucurbitaceae.

| Element Type       | YAB5 | YAB2 | CRC | YAB1 | INO |
|--------------------|------|------|-----|------|-----|
| AuxRR-core         |      |      |     |      |     |
| TGA-element        |      |      |     |      |     |
| TGA-box            |      |      |     |      |     |
| G-box              |      |      |     |      |     |
| GARE-motif         |      |      |     |      |     |
| Cis-AT             |      |      |     |      |     |
| P-box              |      |      |     |      |     |
| TAC-box            |      |      |     |      |     |
| GCCTA-motif        |      |      |     |      |     |
| TGCAGS-motif       |      |      |     |      |     |
| TARE               |      |      |     |      |     |
| TCA-element        |      |      |     |      |     |
| CAT-box            |      |      |     |      |     |
| circadian-motif    |      |      |     |      |     |
| GCN_motif          |      |      |     |      |     |
| MSA-like           |      |      |     |      |     |
| RY-element         |      |      |     |      |     |
| ARE                |      |      |     |      |     |
| DRE                |      |      |     |      |     |
| LTR                |      |      |     |      |     |
| NBS                |      |      |     |      |     |
| CCGG repeats       |      |      |     |      |     |
| WUN-motif          |      |      |     |      |     |
| W box              |      |      |     |      |     |
| Box 4              |      |      |     |      |     |
| G-box              |      |      |     |      |     |
| GT1-motif          |      |      |     |      |     |
| MRE                |      |      |     |      |     |
| TCCG-motif         |      |      |     |      |     |
| TCT-motif          |      |      |     |      |     |

Figure 5. The cis-acting elements analysis of 59 YABBY genes promoters across Cucurbitaceae. The numbers of cis-acting elements are shown in a heatmap.

3.5. Expression Pattern of YABBYs in Cucumber

To explore the detailed expression profiles of YABBYs in Cucurbitaceae, we selected cucumber as a representative species to examine YABBY gene expression patterns in different organs including root, stem, leaf, tendril, male bud, female bud, and different development stages of ovary/fruit. As shown in Figure 6, the CsYABBYs exhibited various expression patterns in test materials. The four genes CsaV3_1G030340, CsaV3_2G002960, CsaV3_3G003040, and CsaV3_5G003950 had high expression levels in leaf. We noticed that CsaV3_2G002960 also expressed higher in tendrils and CsaV3_2G024750 shows high expression level in stems. Moreover, we found that CsaV3_5G003950 and CsaV3_5G033400 are highly expressed in floral organs. In addition, CsaV3_2G002960, CsaV3_3G003040, and CsaV3_6G038650 also had higher expression levels in male and female buds. However, the expression of CsaV3_5G031440 was mostly undetectable in all organs (Figure 6).

Given the higher expression levels of CsaV3_2G002960, CsaV3_3G003040, CsaV3_5G003950, CsaV3_5G033400 and CsaV3_6G038650 in floral organs, we further explore the expression patterns of these five genes in different development stages of ovary/fruit. We found that CsaV3_2G002960, CsaV3_3G003040, CsaV3_5G003950, and CsaV3_5G033400 are mainly expressed at all ovary development stages (12, 9, 6, 3, and 0 DBF) but not fruit development stages, whereas CsaV3_6G038650 shows high expression at all fruit development stages.
(3, 6, and 12 DAF) other than ovary development stages (Figure 7A). The similar expression patterns were also observed in an RNA-seq data wherein a transcriptome analysis of 4 DBF ovary from two near-isogenic lines 408 and 409 was performed [57] (Figure 7B and Table S5). Four YABBY genes (except CsaV3_2G002960 with no differential expression) were higher expressed in line 408 which is characterized by long fruit compared to the line 409 which has a short fruit [57] (Figure 7B and Table S5). Hence, the results indicated that the YABBYs may play various and important roles during plant growth and development in cucumber, such as leaf, tendril, stem, male flower, and ovary/fruit development.

![Figure 6](image-url)

**Figure 6.** The expression patterns of CsyABBY genes in different tissues of cucumber. (A–H) Tissues-specific of CsyABBY expression was examined in cucumber by qRT-PCR. Values are means ± SD of three biological replicates.
Figure 7. The expression patterns of CsYABBY genes in different development stages of ovary/fruit of cucumber. (A) The relative expression patterns of CsYABBYs at different development stages of ovary/fruit. (B) The TPM values of CsYABBYs at different development stages of ovary/fruit. DBF: days before flowering. Values are means ± SD of three biological replicates.

4. Discussion

The YABBYs, plant-specific transcription factors, play significant roles during different plant development processes [12,13]. So far, it is well known that Cucurbitaceae—such as cucumber, melon, watermelon, and pumpkin—are important horticultural crops with global economic value [31]. However, little is known about the characteristics and functions of YABBYs across Cucurbitaceae, except some studies of cucumber [44,45].

Recently, the whole-genome sequences of eight cucurbit species—including cucumber, melon, watermelon, wax gourd, pumpkin, zucchini, silver-seed gourd, and bottle gourd—were released or updated [34,60–66], providing a useful strategy to deepen our understanding of genome-wide identification of YABBY family in Cucurbitaceae. In this study, we identified and characterized the YABBY genes in Cucurbitaceae based on these whole-genome sequences. Totally, we obtained 3, 4, 5, 8, 9, 9, 10, and 11 YABBY genes in bottle gourd, melon, wax gourd, cucumber, watermelon, zucchini, silver-seed gourd, and pumpkin, respectively (Tables 1 and S1). Like Arabidopsis YABBY genes [10,11], we found that the YABBY genes in eight cucurbit species are also divided into five subfamilies, YAB1, YAB2, CRC, INO, and YAB5 (Figure 1 and Table 1). Notably, compared to Arabidopsis which has six YABBY genes [1–3], the number of YABBY genes in bottle gourd, melon, and wax gourd was less than six but more than six in the other five cucurbit species (Tables 1 and S1). We speculated that the differences of YABBY gene numbers among different cucurbit species may be explained by gene duplication or loss during evolutionary process, as the segment and tandem duplications contribute to the expansion of gene family [58]. The synteny analysis results showed that there are many more syntenic gene pairs between cucumber and watermelon, bottle gourd, pumpkin, and zucchini (Figure 2 and Table S3). In addition, we observed that an obvious expansion of INO and YAB5 subfamilies among Cucurbitaceae, which possibly is owing to much more orthologs within the two subfamilies in watermelon, pumpkin, and zucchini except bottle gourd (Figure 1 and Table 1). These results suggested that gene duplication may contribute to the more YABBY genes in these three cucurbit species except bottle gourd. However, for melon and
wax gourd which had less YABBY genes (Tables 1 and S1), we noticed that 75% (6 of 8) of CsYABBY genes share only one syntenic gene pair between cucumber and melon/wax gourd (Figure 2 and Table S3), and the loss events also exist in melon and wax gourd, including no YAB1 subfamily in melon and no INO subfamily in wax gourd (Figure 1 and Table 1). Furthermore, although more syntenic gene pairs between cucumber and bottle gourd were found (Figure 2 and Table S3), bottle gourd contained only three YABBY genes and lacked CRC and YAB2 subfamilies (Figure 1 and Table 1). Since the YABBY gene contains a conserved C2C2 domain and a conserved YABBY domain, the truncated genes, which lack either a C2C2 domain and/or a YABBY domain, were excluded when we identified the YABBY genes, which might account for the final number of identified YABBY genes in Cucurbitaceae (except cucumber and watermelon) being lower than that obtained by synteny analysis and one and/or two YABBY subfamilies are lacking in melon, wax gourd, zucchini, and bottle gourd. Possibly due to the low quality of silver-seed gourd genome draft, we could not perform the synteny analysis between cucumber and silver-seed gourd. Hence, further studying is recommended to explore whether lower quality of genome draft or gene loss events during evolutionary process are the cause of fewer YABBY genes and the lacking of some subfamilies in these cucurbit species.

To better understand the conservation and diversification of YABBYs in Cucurbitaceae, we performed an analysis of gene structures and conserved motifs/domains. The 59 YABBYs shared a conserved C2C2 domain and a conserved YABBY domain (Figure 4 and Table S1). However, there were some differences among different subfamilies. For instance, all YAB2 subfamily members had six exons and five introns, whereas CRC subfamily (except Carg17317 with nine exons and eight introns in silver-seed gourd) members contained seven exons and six introns. YAB2 and CRC subfamilies commonly contained motif 1, motif 2, motif 4, and motif 6; meanwhile, the YAB2 subfamily had motif 8 but lost motif 5, whereas the CRC subfamily had motif 5 but lacked motif 8 (Figure 3). These results hinted that the similar characteristics of YABBY genes within the same subfamily may indicate conserved function, and the differences of YABBY gene characteristics among various subfamilies possibly contribute to functional differentiation across Cucurbitaceae. Generally, transcription factors bind to specific cis-acting elements of targeted genes to regulate their expression. The cis-acting elements in promoters of the 59 YABBY genes suggested that they may be involved in plant development, phytohormone, stress, and light responses in Cucurbitaceae.

Importantly, the expression patterns of CsYABBY genes in cucumber were analyzed to explore the potential functions of different YABBY subfamilies across Cucurbitaceae. Previous reports found that YAB1 (FIL)/YAB3, YAB2, and YAB5 are associated with leaf and floral organ development in Arabidopsis and rice [2,14,16,18,19,29,30,67], the similar higher expression levels of their homologous genes (except YAB2 homolog) CsaV3_5G003950, CsaV3_3G003040, CsaV3_2G002960, and CsaV3_1G030340 in leaf and floral organs suggested the functional conservation of these genes between cucumber, Arabidopsis, and rice (Figure 6). Meanwhile, the special expression of CsaV3_5G033400, the homolog of CRC which functions in carpel development in Arabidopsis [1,20,21] and rice [23], in female bud hinted the underlying role in carpel development of cucumber (Figure 6). However, we observed that the YAB2 homolog CsaV3_2G002960 was also highly expressed in tendrils. Additionally, there are two homologs of INO in cucumber. According to the tissue-specific feature with low levels in various tissues of INO expression in Arabidopsis [3], we detected almost no expression of CsaV3_5G0314400 in cucumber tissues. However, the other gene CsaV3_2G024750 were expressed in different tissues, especially in stem (Figure 6), although CsaV3_2G024750 (gene ID in paper: Csa011583) was reported in involved with integument of ovules but not with functional verification [44]. Thus, the different or specific expression patterns of these four genes suggested their function differentiations in cucumber. Further studies are needed to verify our speculation by their functional identifications.
Furthermore, four genes—CsaV3_2G002960, CsaV3_3G003040, CsaV3_5G003950, and CsaV3_5G033400—showed higher expression levels at all ovary development stages but not in fruits (Figure 7A), suggesting their possible roles in ovary development of cucumber. Particularly, CsaV3_5G003950—the homolog of YAB1—had very high transcriptional level and thus might mainly regulate ovary development of cucumber, this is in line with the function of YAB1 which is involved in abaxial cell type specification in leaves and fruits in Arabidopsis [2,14,16]. In rice, the YABI clade genes—TOB1, TOB2, and TOB3—are required to maintain proper function of the spikelet and branch meristems [29,30]. It is likely that the function of YAB1 clade genes has been conserved between cucumber, rice, and Arabidopsis. For the CRC homolog in cucumber, CsaV3_5G033400, was significantly expressed in the early development stage of ovary at 12DBF and 9DBF (Figure 7A), indicating its possible role in carpel development of cucumber and is similar with CRC function involved in carpel development in Arabidopsis [1,20,21] and rice [23]. Interestingly, we noted that the YAB2 homolog CsaV3_6G038650 is more highly expressed in fruit than ovary (Figure 7A). Although YAB2 is involved in polarity development of leaf and flower [2], the function in regulating fruit development is not proved in Arabidopsis. The specific expression pattern of CsaV3_6G038650 in fruit implied its prominent function in cucumber fruit development. Coincidentally, fas—a YABBY-like transcription factor homologous to Arabidopsis YAB2—regulated fruit development by controlling carpel number in tomato, resulting in an extreme fruit size [68]. Additionally, in cereals, SH1 or ObSH3—which are closely related to Arabidopsis YAB2—is required for seed shattering during domestication [26,27]. These results suggested the functional differentiation of YAB2 gene between cucumber, tomato, cereals, and Arabidopsis, and CsaV3_6G038650 is a possibly major regulatory factor of fruit development in cucumber. Taken together, the results implied that the YABBY genes possibly play vital roles in many aspects of plant growth and development in cucumber—such as leaf, tendril, and ovary/fruit—which will contribute to the applications of YABBY genes in breeding of cucumber, even Cucurbitaceae.

5. Conclusions

In this study, 59 YABBY genes were identified from eight cucurbit species. A systematic characterization study was performed for chromosomal location, gene structure, conserved motifs, cis-acting elements, evolutional relationship, and gene duplication. The evolutionary relationship showed that the YABBY genes from Cucurbitaceae are classified into five subfamilies. Gene duplication events occurred between cucumber and watermelon, bottle gourd, pumpkin, and zucchini, which contributes to YABBY gene family expansions in these four cucurbit species. However, we have not confirmed whether or not low quality of genome draft or gene loss events are the cause of fewer YABBY genes in bottle gourd, melon, and wax gourd—further verification is required. The expression patterns of most YABBY genes in cucumber were similar with that in Arabidopsis and rice, indicating the function conservation of these YABBY genes among Cucurbitaceae. However, the different expression patterns of several CsaYABBY genes are needed to illuminate by their function verifications. Importantly, we identified CsaV3_6G038650 as a potential regulatory factor of fruit development in cucumber. In conclusion, our study provided a foundation for further research on YABBY gene functions which will facilitate breeding in Cucurbitaceae.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/genes13030467/s1. Figure S1: Chromosomal locations of YABBY genes in the genome of seven cucurbit species; Figure S2: Analysis of conserved motifs in YABBY proteins of eight cucurbit species; Table S1: List of YABBY genes identified in cucumber, melon, watermelon, wax gourd, pumpkin, zucchini, silver-seed gourd, and bottle gourd; Table S2: Information of YABBY genes identified in eight cucurbit species; Table S3: Orthologous relationships of YABBY genes between cucumber and other six cucurbit species; Table S4: Cis-acting elements of YABBY genes promoters in cucumber, melon, watermelon, wax gourd, pumpkin, zucchini, silver-seed gourd, and bottle gourd; Table S5: The TPM values of YABBY family genes in cucumber by RNA-Seq analysis; Table S6: Primers of cucumber YABBY genes used in qRT-PCR.
Author Contributions: S.Y., X.L. and H.R. conceived, designed, and assessed the experiments; E.S.B., R.W., H.Y., C.L., X.C. and Y.W. participated in the design of the experiments. S.Y. and S.L. performed the experiments; S.Y. and Y.G. analyzed the data; S.Y., X.L. and H.R. wrote the manuscript. All authors reviewed and modified the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by Sanya institute of China Agricultural University (SYND-2021-18; SYND-2022-20), Yantai institute of China Agricultural University (Z202206), and the Construction of Beijing Science and Technology Innovation and Service Capacity in Top Subjects (CEFF-PXM2019_014207_000032).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data used in this study are presented in the article or supplementary information.

Acknowledgments: We sincerely thank Chunhua Chen for the checking of the manuscript.

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

References
1. Bowman, J.L.; Smyth, D.R. CRABS CLAW, a gene that regulates carpel and nectary development in Arabidopsis, encodes a novel protein with zinc finger and helix-loop-helix domains. Development 1999, 126, 2387–2396. [CrossRef] [PubMed]
2. 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]
3. Villanueva, J.M.; Broadhurst, J.; Hauser, B.A.; Meister, R.J.; Schnetz, K.; Gasser, C.S. INNER NO OUTER regulates abaxial-adaxial patterning in Arabidopsis ovules. Genes Devol. 1999, 13, 3160–3169. [CrossRef] [PubMed]
4. Nishiyama, T.; Fujita, T.; Shin, I.T.; Seki, M.; Nishide, H.; Uchiyama, I.; Kamiya, A.; Carninci, P.; Hayashizaki, Y.; Shinozaki, K.; et al. Comparative genomics of Physcomitrella patens gene families and expression analysis of Arabidopsis thaliana: Implication for land plant evolution. Proc. Natl. Acad. Sci. USA 2003, 100, 8007–8012. [CrossRef]
5. Floyd, S.K.; Bowman, J.L. The Ancestral Developmental Tool Kit of Land Plants. Int. J. Plant Sci. 2007, 168, 1–35. [CrossRef]
6. Huang, Z.; Houten, J.V.; Gonzalez, G.; Xiao, H.; Knaap, E.v.d. Genome-wide identification, phylogeny and expression analysis of YABBY gene family in tomato. Mol. Gen. Genom. MGG 2013, 288, 111–129. [CrossRef]
7. Hou, H.; Wu, P.; Gao, L.; Zhang, C.; Hou, X. Characterization and expression profile analysis of YABBY family genes in Pak-choi (Brassica rapa ssp. chinensis) under abiotic stresses and hormone treatments. Plant Growth Regul. 2019, 87, 421–432. [CrossRef]
8. 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. Genom. Genom. MGG 2007, 277, 457–468. [CrossRef]
9. Cao, Y.; Lang, Z.H.; Wang, L. Characteristics and Expression Analysis of Transcription Factor YABBY Family in Maize. J. Agric. Sci. Technol. 2015, 17, 32–41.
10. Bowman, J.L. The YABBY gene family and abaxial cell fate. Curr. Opin. Plant Biol. 2000, 3, 17–22. [CrossRef]
11. Yamada, T.; Yokota, S.y.; Hirayama, Y.; Imaichi, R.; Kato, M.; Gasser, C.S. Ancestral expression patterns and evolutionary diversification of YABBY genes in angiosperms. Plant J. 2011, 67, 26–36. [CrossRef] [PubMed]
12. 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] [PubMed]
13. 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]
14. Sarojam, R.; Sappi, P.G.; Goldshmidt, A.; Efroni, I.; Floyd, S.K.; Eshed, Y.; Bowman, J.L. Differentiating Arabidopsis shoots from leaves by combined YABBY activities. Plant Cell 2010, 22, 2113–2130. [CrossRef] [PubMed]
15. Eckardt, N.A. YABBY genes and the development and origin of seed plant leaves. Plant Cell 2010, 22, 2103. [CrossRef]
16. Sawa, S.; Ito, T.; Shimura, Y.; Okada, K. FILAMENTOUS FLOWER controls the formation and development of Arabidopsis inflorescences and floral meristems. Plant Cell 1999, 11, 69–86. [CrossRef] [PubMed]
17. Stahle, M.I.; Kuehlich, J.; Staron, L.; Armim, A.G.v.; Golz, J.F. YABBYs and the transcriptional corepressors LEUNIG and LEUNIG_HOMOLOG maintain leaf polarity and meristem activity in Arabidopsis. Plant Cell 2009, 21, 3105–3118. [CrossRef]
18. Chen, Q.; Atkinson, A.; Otsuga, D.; Christensen, T.; Reynolds, L.; Drews, G.N. The Arabidopsis FILAMENTOUS FLOWER gene is required for flower formation. Development 1999, 126, 2715–2726. [CrossRef]
19. Watanabe, K.; Okada, K. Two discrete cis elements control the Abaxial side-specific expression of the FILAMENTOUS FLOWER gene in Arabidopsis. Plant Cell 2003, 15, 2592–2602. [CrossRef]
20. Alvarez, J.; Smyth, D.R. CRABS CLAW and SPATULA, two Arabidopsis genes that control carpel development in parallel with AGAMOUS. Development 1999, 126, 2377–2386. [CrossRef]
21. Lee, J.-V.; Baum, S.F.; Alvarez, J.; Patel, A.; Chitwood, D.H.; Bowman, J.L. Activation of CRABS CLAW in the nectaries and carpels of Arabidopsis. Plant Cell 2012, 17, 25–36. [CrossRef] [PubMed]

22. Gallagher, T.L.; Gasser, C.S. Independence and interaction of regions of the INNER NO OUTER protein in growth control during ovule development. Plant Physiol. 2006, 147, 306–315. [CrossRef] [PubMed]

23. 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] [PubMed]

24. Ohmori, Y.; Toriba, T.; Nakamura, H.; Ichikawa, H.; Hirano, H.-Y. Temporal and spatial regulation of DROOPING LEAF gene expression that promotes midrib formation in rice. Plant J. 2011, 65, 77–86. [CrossRef]

25. Nagasawa, N.; Miyoshi, M.; Sano, Y.; Satoh, H.; Hirano, H.; Sakai, H.; Nagato, Y. SUPERWOMAN1 and DROOPING LEAF genes control floral organ identity in rice. Development 2003, 130, 705–718. [CrossRef]

26. Lin, Z.; Li, X.; Shannon, L.M.; Yeh, C.-T.; Wang, M.L.; Bai, G.; Peng, Z.; Li, J.; Trick, H.N.; Clemente, T.E.; et al. Parallel domestication of the Shattering1 genes in cereals. Nat. Gen. 2012, 44, 720–724. [CrossRef]

27. Lv, S.; Wu, W.; Wang, M.; Meyer, R.S.; Ndjonjop, M.-N.; Tan, L.; Zhou, H.; Zhang, J.; Fu, Y.; Cai, H.; et al. Genetic control of seed shattering during African rice domestication. Nat. Plants 2018, 4, 331–337. [CrossRef]

28. Jang, S.; Hur, J.; Kim, S.-J.; Han, M.-J.; Kim, S.-R.; An, G. Ectopic expression of OsYAB1 causes extra stamens and carpels in rice. Plant Mol. Biol. 2004, 56, 133–143. [CrossRef]

29. Tanaka, W.; Toriba, T.; Ohmori, Y.; Yoshida, A.; Kawai, A.; Mayama-Tsuchida, T.; Lchikawa, 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]

30. 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]

31. Schaefer, H.; Renner, S.S. Phylogenetic relationships in the order Cucurbitales and a new classification of the gourd family (Cucurbitaceae). TAXON 2011, 60, 122–138. [CrossRef]

32. Huang, S.; Li, R.; Zhang, Z.; Li, L.; Gu, X.; Fan, W.; Lucas, W.J.; Wang, X.; Xie, B.; Ni, P.; et al. The genome of the cucumber, Cucumis sativus L. Nat. Gen. 2009, 41, 1275–1281. [CrossRef] [PubMed]

33. Pan, Y.; Wang, Y.; McGregor, C.; Liu, S.; Luan, F.; Gao, M.; Weng, Y. Genetic architecture of fruit size and shape variation in cucurbits: A comparative perspective. Theor. Appl. Gen. 2020, 133, 1–21. [CrossRef]

34. Guo, S.; Zhang, J.; Sun, H.; Salse, J.; Lucas, W.J.; Zhang, H.; Zheng, Y.; Mao, L.; Ren, Y.; Wang, Z.; et al. The draft genome of watermelon (Citrullus lanatus) and resequencing of 20 diverse accessions. Nat. Gen. 2013, 45, 51–58. [CrossRef]

35. Burger, Y.; Paris, H.S.; Cohen, R.; Katzir, N.; Tadmor, Y.; Lewinsohn, E. Genetic diversity of Cucumis melo. Horticult. Rev. J. Am. Soc. Horticult. Sci. 2009, 36, 165–198. [CrossRef]

36. Perkins-Veazie, P.; Collins, J.; Davis, A.R.; Roberts, W. Carotenoid Content of 50 Watermelon Cultivars. J. Agric. Food Chem. 2006, 54, 2593–2597. [CrossRef]

37. Han, X.-N.; Liu, C.-Y.; Liu, Y.-L.; Xu, Q.-M.; Li, X.-R.; Yang, S.-L. New triterpenoids and other constituents from the fruits of Benincasa hispida (Thunb.) Cogn. J. Agric. Food Chem. 2013, 61, 12692–12699. [CrossRef]

38. Hayashi, T.; Julié, P.A.R.; Matsu-Hirai, H.; Miyazaki, A.; Fukatsu, A.; Funami, J.; Iiguchi, A.; Ignarro, L.J. L-Citrulline and L-arginine supplementation retards the progression of high-cholesterol-diet-induced atherosclerosis in rabbits. Proc. Natl. Acad. Sci. USA 2005, 102, 13681–13686. [CrossRef]

39. Collins, J.K.; Wu, G.; Perkins-Veazie, P.; Spears, K.; Claypool, P.L.; Baker, R.A.; Clevendon, B.A. Watermelon consumption increases plasma arginine concentrations in adults. Nutrition 2007, 23, 261–266. [CrossRef]

40. Grover, J.K.; Adiga, G.; Vats, V.; Rathi, S.S. Extracts of Benincasa hispida prevent development of experimental ulcers. J. Ethnopharmacol. 2001, 78, 159–164. [CrossRef]

41. Gu, M.; Fan, S.; Liu, G.; Guo, L.; Ding, X.; Lu, Y.; Zhang, Y.; Ji, G.; Huang, C. Extract of Wax Gourd Peel Prevents High-Fat Diet-Induced Hyperlipidemia in C57BL/6 Mice via the Inhibition of the PPARy Pathway. Evid.-Based Complement Alt. Med. 2013, 2013, 432451. [CrossRef] [PubMed]

42. Thoenissen, N.H.; Iwanski, G.B.; Doan, N.B.; Okamoto, R.; Lin, P.; Abbassi, S.; Song, J.H.; Yin, D.; Toh, M.; Xie, W.D.; et al. Cucurbitacin B induces apoptosis by inhibition of the JAK/STAT pathway and potentiates antiproliferative effects of gemcitabine on pancreatic cancer cells. Cancer Res. 2009, 69, 5876–5884. [CrossRef] [PubMed]

43. Chen, X.; Bao, J.; Guo, J.; Ding, Q.; Lu, J.; Huang, M.; Wang, Y. Biological activities and potential molecular targets of cucurbitacins: A focus on cancer. Anti-Cancer Drugs 2012, 23, 777–787. [CrossRef]

44. Liu, X.; Ning, K.; Che, G.; Yan, S.; Han, L.; Gu, R.; Li, Z.; Weng, Y.; Zhang, X. CsSPL functions as an adaptor between HD-ZIP III and CsWUS transcription factors regulating anther and ovule development in Cucumis sativus (cucumber). Plant J. 2018, 94, 535–547. [CrossRef] [PubMed]

45. Yan, S.; Ning, K.; Wang, Z.; Liu, X.; Zhong, Y.; Ding, L.; Zi, H.; Cheng, Z.; Li, X.; Shan, H.; et al. CsVFP functions in vasculature development and downy mildew resistance in cucumber. PloS Biol. 2020, 18, e3000671. [CrossRef] [PubMed]

46. Mistry, J.; Chuguransky, S.; Williams, L.; Qureshi, M.; Salazar, G.A.; Sonnhammer, E.L.L.; Tosatto, S.C.E.; Paladini, L.; Raj, S.; Richardson, I.J.; et al. Pfam: The protein families database in 2021. Nucl. Acids Res. 2021, 49, D412–D419. [CrossRef]

47. Lu, S.; Wang, J.; Chitsaz, F.; Derbyshire, M.K.; Geer, R.C.; Gonzales, N.R.; Gwadz, M.; Hurwitz, D.I.; Marchler, G.H.; Song, J.S.; et al. CDD/SPARCLE: The conserved domain database in 2020. Nucl. Acids Res. 2020, 48, D265–D268. [CrossRef]
