Genome-Wide Identification and Analysis of the MYB Transcription Factor Gene Family in Chili Pepper (Capsicum spp.)

Magda L. Arce-Rodríguez 1*, Octavio Martínez 2,* and Neftalí Ochoa-Alejo 1,*

Abstract: The MYB transcription factor family is very large and functionally diverse in plants, however, only a few members of this family have been reported and characterized in chili pepper (Capsicum spp.). In the present study, we performed genome-wide analyses of the MYB family in Capsicum annuum, including phylogenetic relationships, conserved domain, gene structure organization, motif protein arrangement, chromosome distribution, chemical properties predictions, RNA-seq expression, and RT-qPCR expression assays. A total of 235 non-redundant MYB proteins were identified from C. annuum, including R2R3-MYB, 3R-MYB, atypical MYB, and MYB-related subclasses. The sequence analysis of CaMYBs compared with other plant MYB proteins revealed gene conservation, but also potential specialized genes. Tissue-specific expression profiles showed that CaMYB genes were differentially expressed, suggesting that they are functionally divergent. Furthermore, the integration of our data allowed us to propose strong CaMYBs candidates to be regulating phenylpropanoid, lignin, capsaicinoid, carotenoid, and vitamin C biosynthesis, providing new insights into the role of MYB transcription factors in secondary metabolism. This study adds valuable knowledge about the functions of CaMYB genes in various processes in the Capsicum genus.

Keywords: Capsicum; chili pepper; MYB transcription factor; expression profiles; secondary metabolism; capsaicinoids; carotenoids; phenylpropanoids; lignins; vitamin C

1. Introduction

Transcription factors (TFs) are sequence specific DNA binding proteins that recognize specific cis-elements in the promoters of target genes to activate or repress their expression in response to endogenous or exogenous stimuli and in order to control biochemical and physiological processes. TFs can be divided into many multigene families according to their DNA-binding domains [1]. MYB transcription factors are one of the largest TF families, and they are present in all eukaryotes. The oncogene v-MYB was the first MYB transcription factor identified in avian myeloblastosis virus [2]. MYB proteins are widely distributed in plants and they have been implicated in the regulation of many biological processes, such as primary and secondary metabolism, plant growth and development, circadian clock control, cell cycling development, response to biotic and abiotic stresses, and plant defense [3]. The first plant MYB gene identified was COLORED1 (C1) from Zea mays, involved in the regulation of anthocyanin biosynthesis in the aleurone and scutellum tissues of the kernel [4].

MYB TFs are characterized by containing a MYB DNA-binding conserved domain, which is approximately 50–55 amino acid residues in length, with three spaced tryptophan residues, forming a helix-turn-helix (HTH) fold. Conversely, the amino acid sequence...
outside the conserved MYB domain is highly divergent and responsible for the diverse regulatory activity of the MYB proteins. MYB TFs are classified into four classes depending on the number of MYB repeats: 1R-MYB, R2R3-MYB, 3R-MYB, and 4R-MYB, containing one, two, three, or four imperfect MYB repeats. The 1R-MYBs are also referred to as MYB-related, which typically, but not always, contain a single or partial MYB repeat. MYB-related proteins comprise a collection of several protein subgroups, including CIRCADIAN CLOCK ASSOCIATED1 (CCA-1)-like, I-box-binding factor-like, DIVARICATA (DIV)-like, Telomeric DNA-binding protein (TPB)-like, CAPRICE (CPC)-like, Late Elongated Hypocotyl (LHY)-like, KANADI (KAN)-like, TRYPTYCHON (TRY)-LIKE, Enhancer of TRY and CPC (ETC)-like, Phosphate Starvation Response (PHR)-like, RADIALIS (RAD)-like, Golden1/2 (GLK)-like, Early Flowering MYB (EFM)-like, HRS1 Homolog 5 (HHO5)-like, MYBC1-like, MYR1/2-like, KUODA1 (KUA1)-like, Early Phytochrome Responsive1 (RVE)-like, and Salt-Related MYB1 (SRM1)-like [5–14]. MYB family TFs have been identified in numerous plant species, with 100–250 members, for example, in Chinese pear 129 PbMYBs were identified [15], rice genome contained 233 OsMYBs [16], Arabidopsis genome included 198 AtMYBs [17], in pineapple, a total of 184 AcMYBs were identified [18], in the tomato genome, 127 SlMYBs were found [19], 245 HaMYBs were identified in sunflower [20], and 253 StMYBs were reported in potato [21].

Chili pepper fruit is a major vegetable and spice crop worldwide, being *Capsicum annuum* the most widely grown in the world [22]. The genome size of *C. annuum* is 3.48 Gb [23,24], in which 2153 TFs were identified (6.25% of the total genes) spanning 80 gene families [23]. A few CaMYB genes have been shown to play important roles in the regulation of plant development and secondary metabolism in the *Capsicum* genus. For instance, CaA an R2R3-MYB gene is involved in the control of anthocyanin biosynthesis [25,26]. CaMYB31, CaMYB108, and CaMYB48 have been shown to participate in the regulation of capsaicinoid biosynthesis [27–29], and additionally CaMYB108 is involved in stamen development [28]. CaBLIND was found to regulate axillary meristem initiation and flowering transition [30]. Recently, 108 members of the R2R3-MYB gene subfamily were identified in chili pepper, of which six genes were proposed as candidates to be regulating the synthesis of capsaicinoids [31].

The present study was focused on the genome-wide analysis of the MYB family in *C. annuum* and a comparison to other known MYB plant proteins, including database searches, phylogenetic relationships, gene structure organization, chromosome distribution, conserved domain analysis, and motif protein composition. Furthermore, the co-expression analysis during chili pepper fruit development and their validation by the RT-qPCR analysis identified CaMYB genes that may play important roles in the regulation of phenylpropanoid, lignin, capsaicinoid, carotenoid, and vitamin C biosynthetic pathways. This work provided deep insights into the function of CaMYBs, allowing the identification of gene resources for breeding and engineering of *Capsicum* spp. and possibly for other plant species.

2. Results

2.1. Identification of MYB Family Genes in *Capsicum annum*

The identification of MYB family genes in *C. annum* genome (cultivar Zunla-1) was carried out with a Blastp search using as query the Pfam domains: PF00249 and PF13921. We identified 345 proteins with at least one MYB domain, which were localized in 235 loci, representing almost 11% of TFs in *Capsicum*. The presence of the MYB domain was verified using the Pfam database [32]. In total, 116 R2R3-MYBs, five 3R-MYBs, 92 MYBs-related, and two atypical MYB (CaMYB5R and CaMYBDC) were identified in *C. annum*. Interestingly, we did not identify any 4R-MYB, but a single gene with five MYB repeats was detected. In addition, the domain analysis showed the presence of proteins with other conserved family domains alongside the MYB-repeat, including: nine response regulator receiver domain, five linker-histone, two zinc finger zz type, and four SWIRM domains. The identified CaMYB genes ranged from 237 to 3465 pb, and the corresponding proteins ranged...
from 78 to 1154 amino acids in length. The protein mass ranged from 8.96 to 183.49 kDa, while the isoelectric point (pI) values ranged from 4.34 to 9.36. Additionally, we predicted the subcellular localization of CaMYB proteins, of which 90.2% presented a unique Nuclear Localization Signal (NLS), 7.65% in addition to NLS signals for mitochondrial, extracellular, cytoplasmic, plasma membrane, or chloroplast localization were identified. Only five of 235 CaMYB proteins did not present NSL. Table S1 shows the protein list with at least one MYB repeat, and includes protein id, protein length, number of exons, NCBI annotation, id transcriptomic data, Pfam family domains, MYB classification, locus id, chromosome localization, strand orientation, isoelectric point, protein mass, and subcellular localization.

2.2. Phylogenetic Analysis of the MYB Family

We constructed a phylogenetic tree with 301 MYB proteins using the Neighbor joining method, based on the alignment of the MYB domain using the MEGA7 software, with a bootstrap test of 1000 replicates (Figure 1). We analyzed 126 CaMYBs, including all R2R3-MYB, 3R-MYB, atypical proteins (5R-MYB and CDC5-like), and three MYB-related proteins compared with the MYB family of Arabidopsis thaliana and 25 functionally characterized proteins from other plant species (Table S2). The phylogenetic tree was consistent with the subgroups designated for A. thaliana MYB proteins [33,34]. Based on the phylogenetic tree topology, we grouped the CaMYB proteins in 46 clades (C1–C46), of which 37 clades were present both in C. annuum and A. thaliana. None of the analyzed CaMYBs was grouped with R2R3-MYB genes of the clades C31, C34, C36, and C42 of Arabidopsis. Conversely, clades C24, C25, C32, and C40 were grouped with only CaMYB proteins. The presence or absence of specie-specific MYBs could have resulted in functional divergence. In this vein, MYBs clustered in the same clade may have a conserved biological function. Based on the phylogenetic relationship of CaMYB proteins with known proteins of other species, we assigned putative functions to each subgroup (Table S3). For instance, clade C1 comprised only 3R-MYB proteins of C. annuum, A. thaliana, and Oryza sativa, which were associated with the cell cycle process and stress response [35,36]. Clade C38 included several R2R3-MYBs: four from C. annuum, three from A. thaliana, and one from Solanum lycopersicum, all of which were related with the flavonol biosynthesis [37,38]. Clade C46 included three MYB-related genes from C. annuum and one of Antirrhinum majus, which was related to the flower development [39]. The 5R-MYB was found also in other Capsicum species (i.e., C. chinense and C. baccatum), but not in other Solanaceae genomes (i.e., Solanum tuberosum, Solanum lycopersicum, and Nicotiana tabacum). However, the CaMYB5R protein was clustered with 4R-MYB proteins whose functions are unknown.

2.3. Chromosomal Localization of the MYB Family

Based on the starting and ending position of MYB genes within the chromosomes, 213 genes with at least one MYB-domain were distributed among the 12 chromosomes of C. annuum, whereas 23 MYB genes stayed on as yet unmapped scaffolds (Figure 2 and Table S1). Most of the CaMYB genes were concentrated at both extremes of the respective chromosome, with fewer exceptions of genes located in the middle section of the chromosome. Chromosome 3 encompassed the highest density of CaMYB genes (27), followed by chromosome 6 (26). Conversely, chromosome 8 (10) contained the lowest density of MYB genes. The distribution of CaMYB genes was not congruent with the MYB classification subgroups, except for a few gene pairs that had a close proximity localization on the chromosome. For example, CaMYB36 and CaMYB37 from Clade C16 were located only 0.009 Mb apart on chromosome 12. CaMYB90 and CaMYB91, members of the clade C35, were located with a separation of 0.02 Mb on chromosome 11. Moreover, CaMYB81 and CaMYB82, both belonging to clade C32, were localized on chromosome 1 separated by only 0.08 Mb.
Figure 1. Phylogenetic relationship of CaMYB proteins with other plant MYB transcription factors. A phylogenetic tree was constructed using the Neighbor-joining method based on the MYB domain alignment using the MEGA7 software, with 1000 bootstrap replicates. The tree shows the 46 clades (C1–C46) with a high bootstrap value (highlighted with colored squares for each clade). Bootstrap values >50 are indicated on the nodes. Ca: Capsicum annuum; Cb: Capsicum baccatum; Cc: Capsicum chinense; At: Arabidopsis thaliana; Sl: Solanum lycopersicum; St: Solanum tuberosum; Nt: Nicotiana tabacum; Am: Antirrhinum majus; Os: Oryza sativa; Mm: Mus musculus; Hv: Hordeum vulgare; Zm: Zea mays; Ph: Petunia hybrida; Vv: Vitis vinifera. Accession numbers for all protein sequences are listed in Tables S1 and S2.
CaMYB82, both belonging to clade C32, were localized on chromosome 1 separated by only 0.08 Mb.

Figure 2. Chromosomal distribution of CaMYB genes. The chromosomal position was mapped according to the Capsicum annuum genome. Only 213 MYBs were mapped to the 12 chromosomes of C. annuum. The chromosome number is indicated at the top of each chromosome. The scale on the left is in megabases (Mb).

2.4. Gene Structure Analysis

To explore the structural diversity of CaMYB genes, their exon-intron organization was analyzed. Most CaMYBs clustered in the same clade exhibited similar exon-intron structures (Figure 3), particularly regarding the number of exons, and in some cases the length of the exons and introns was also consistent. For instance, the members of clade C8 displayed a similar gene structure without introns and a similar exon size, or clade C21 in which all the elements showed three exons of similar size. Conversely, 3R-MYB gene members of clade C1 presented multiple exons ranging 7–11 exons. The number of exons ranged from one to thirteen. The 39.83% of the CaMYB genes were organized by three exons, while 53.76% were organized with two or more than three exons, and 6.35% were intron-less.
Figure 3. Cont.
Figure 3. Exon-intron structures of CaMYB genes. Red bars represent exons and solid lines correspond to introns. Upstream and downstream regions are indicated by the blue boxes. The scale on the bottom is in kilobases (Kb). Ca: Capsicum annuum; Cb: Capsicum baccatum; Cc: Capsicum chinense; At: Arabidopsis thaliana; Sl: Solanum lycopersicum; St: Solanum tuberosum; Nt: Nicotiana tabacum; Am: Antirrhinum majus; Os: Oryza sativa; Mm: Mus musculus; Hv: Hordeum vulgare; Zm: Zea mays; Ph: Petunia hybrida; Vv: Vitis vinifera. The protein IDs are listed in Tables S1 and S2.
2.5. Motif-Detection Analysis

The MEME motif-detection software [40] was used to analyze the diversification of the CaMYB proteins. We observed that the motif structure of MYB proteins was consistent with the classification of the clades based on the phylogenetic tree topology (Figure 4). Closer proteins revealed a better match in the motif arrangement. However, members of the same clade showed slight differences in the presence or absence of motifs outside the MYB domain, which may be related to whether or not these genes share a biological function in specific conditions. For example, in clade C25, CaMYB31 did not display motifs 7 and 9 unlike other MYBs in solanaceae, all of which exhibited one or both of these motifs, suggesting that the lack of motifs 7 and 9 could be related to the participation of CaMYB31 gene in the capsaicinoid biosynthetic regulation given that this is a pathway unique to the Capsicum genus.

Additionally, MYB-related and MYB proteins with other domains (histones, ZZ, ARR, and SWI/SNF) were classified in subgroups based on their exon-intron structure and motif protein organization. MYB-related proteins were divided into 13 subgroups: DIVARICATA-like, RADIALIS-like, ETC-like, RVE/LHY/CCA-like, KAN-like, EFM/HH05-like, MYBC1/ PCL-like, MYB1R/KUA-like, GLK-like, Atg14600-like, TRF-like, MYB-CC, and unknown genes (Table S3). Each MYB-related subgroup shared a similar gene structure (Figure S1) and protein organization (Figure S2). For example, all DIVARICATA-like genes presented a gene structure composed of two exons, and a common MYB-binding domain with the SHAQKY consensus. Moreover, MYBC1/PCL-like shared an intron-less gene structure, and the MYB-binding domain included the SHLQKYR consensus. Furthermore, we classified MYB proteins with other domains in MYB-histone, MYB-ARR, MYB-ZZ, and MYB-SWI/SNF, which were highly conserved between C. annuum and A. thaliana species. Moreover, considering the conserved relationship between MYB proteins among plant species, we suggested a putative function for each subgroup (Table S3).

2.6. Co-Expression Analysis

We generated RNA-seq data from flowers (0 dpa; days post-anthesis) and chili pepper fruits at 10, 20, 30, 40, 50, and 60 dpa to explore gene expression profiles (Figure 5). During the transcriptome assembly every gene received an identification number (ID) that represents the expression profile (Table S1). We found that 218 of 235 CaMYB genes were expressed in at least one of the analyzed tissues. Fifteen of those CaMYB genes presented more than one ID, and some of them showed a distinct expression pattern (Figure 6). Additionally, 10.5% of CaMYB genes (CaMYB7, CaMYB23, CaMYB28, CaMYB31, CaMY39, CaMYB44, CaMYB49, CaMYB50, CaMYB61, CaMYB62, CaMYB71, CaMYB78, CaMYB88, CaMYBR4, CaMYBR7, CaMYBR8, CaMYBR12, CaDIV8, CaDIV11, CaDIV13, CaDIV14, CaARR8, and CaMYB3R) did not show expression in flower, while 4.1% (CaMYB30, CaMYB37, CaMYB39, CaMYB66, CaMYB91, CaMYB99, CaMYBR3, CaMYBR5, and CaRL1) were exclusively expressed in flower tissue. Furthermore, the co-expression analysis revealed thirteen main clusters of genes depending on their expression pattern through the development time points (Figure 6). For several genes, we found a correlation between its expression profile and its putative function. For example, CaMYB17 exhibited a high expression in flower tissue, and showed phylogenetic proximity to AtMYB125 that regulates pollen sperm cell differentiation [41] (Table S3). Moreover, CaMYB19, CaMYB20, CaMYB21, and CaMYB22, all of them members of clade C11, were related to anther and tapetum development genes (subgroup S18 of Arabidopsis) [42,43], and displayed a high expression in flower tissue. However, we also found cases where the expression pattern did not correlate with any putative function, for example, CaMYB12 and CaMYB13 were related to subgroup S23 of Arabidopsis which were functionally related to pollen development [44], and they showed their highest expression at 50 dpa, with a lower expression in flower tissue.

Furthermore, we looked for CaMYB genes that showed a positive correlation with key genes of important metabolic pathways, such as the phenylpropanoid, lignin, capsaicinoid, carotenoid, anthocyanin, and vitamin C biosynthetic pathways. Based on the
profile expression, capsaicinoid and carotenoid biosynthetic genes were clustered by the metabolic pathway. AT3 (acyltransferase), Kas (ketocycl-ACP synthase), pAmT (aminotransferase), and BCKDH (branched-chain amino acid transferase), specific genes from the capsaicinoid biosynthetic pathway, presented the characteristic known expression profile that was null in flowers, low at 10 dpa, increased to a maximum at 20 dpa, decreased at 30–40 dpa, and null between 50–60 dpa. These genes were clustered with CaMYB31, previously functionally characterized as the capsaicinoid biosynthesis regulator [27,45], and also co-expressed with CaRVE4, CaARR4, CaMYB115, and CaMYB103. Surprisingly, CaMYB48 and CaMYB108 recently identified as regulators of capsaicinoid biosynthesis [28,29] did not exhibit a positive correlation with the capsaicinoid structural genes. CCS (capsanthin-capsorubin synthase), BCH (β-carotene hydroxylase), and PSY (phytoene synthase), carotenoid biosynthetic genes, showed a lower or even null expression between 0–40 dpa, with a sudden increase between 50–60 dpa. Interestingly, these genes were clustered with six MYB-related genes (CaDIV1, CaDIV3, CaMYB13, CaTRF2, CaMYB1, and CaPHR9) and an atypical MYB (CaMYB5R). Contrariwise, structural genes of the anthocyanin [DFR (dihydroflavonol 4-reductase), F3′5′H (flavonoid 3′, 5′-hydroxylase), and CHS (chalcone synthase)], phenylpropanoid [PAL (phenylalanine ammonia lyase), 4CL (4-coumarate-CoA ligase) and C4H (cinnamic acid 4-hydroxylase)], vitamin C [GLDH (L-galactono-1,4-lactone dehydrogenase), GalDH (L-galactose-1-dehydrogenase) and GME (GDP-D-mannose-3′,5′-epimerase)], and lignin [CAD (cinnamyl alcohol dehydrogenase), CCR (cinnamoyl CoA reductase) and POD (peroxidase)] biosynthetic pathways were not clustered by the metabolic pathway. Nevertheless, the expression profile of these genes was highly correlated with at least one CaMYB gene. F3′5′H was grouped with CaMYB54, CaMYB67, CaMYB69, and CaMYB90, all of them R2R3-MYB genes highly expressed at 10 dpa. DFR was highly expressed at 40 dpa and clustered with two R2R3-MYB genes (CaMYB25 and CaMYB46), and one DIVARICATA-like gene (CaDIV11). PAL, CHS, and POD presented the highest expression in flowers, and suddenly decreased during the fruit development. These genes co-expressed with a large cluster of R2R3-MYB genes (CaBLIND, CaMYB11, CaMYB24, CaMYB30, CaMYB32, CaMYB33, CaMYB34, CaMYB37, CaMYB57, CaMYB59, CaMYB66, CaMYB75, CaMYB85, CaMYB89, CaMYB91, CaMYB99, CaMYB102, and CaMYB105) and MYB-related genes (CaKAN7, CaKUA4, CaRL1, CaRL2, CaMYB34, CaMYB46, and CaPHR5). 4CL displayed its highest expression in flower, and gradually decreased during the fruit development along with CaARR7 and CaKUA3. C4H exhibited its highest expression in flower that was slowly decreasing during the fruit development, and it was co-expressed with CaDIV4. Regarding the expression of genes involved in vitamin C biosynthesis, GLDH displayed a steady expression throughout the fruit development, and it correlated closely to CaA. GalDH showed the highest expression between 10–30 dpa, along with CaMYB1, CaMYB43, and CaMYB91. GME (vitamin C biosynthesis) and CAD (lignin biosynthesis) showed the highest expression in flower tissue, then their expression suddenly decreased between 10–40 dpa, and slightly increased at 50–60 dpa. Both genes were closer to CaMYB36, CaMYB64, CaMYB80, CaMYB98, CaMYB108, CaHY, CaDIV7, CaDIV10, CaSWI3B, CaKAN6, and CaKUA2 expression. CCR started accumulating at 0–10 dpa, peaked at 20 dpa, and abruptly decreased at 30–40 dpa, and again its expression increased at 50–60 dpa. The CCR expression showed a positive correlation with CaMYB37 and an isoform of CaLHY.
Figure 4. Cont.
Figure 4. Cont.
Figure 4. Schematic representation of motif composition of CaMYB subgroups. Each subgroup was analyzed using the MEME motif-detection software. The length of the solid line represents the length of the protein sequences. Colored boxes represent different motifs. Asterisks (*) indicate motifs containing the conserved MYB domain sequence. Ca: Capsicum annuum; Cb: Capsicum baccatum; Cc: Capsicum chinense; At: Arabidopsis thaliana; Sl: Solanum lycopersicum; St: Solanum tuberosum; Nt: Nicotiana tabacum; Am: Antirrhinum majus; Os: Oryza sativa; Mm: Mus musculus; Hv: Hordeum vulgare; Zm: Zea mays; Ph: Petunia hybrida; Vv: Vitis vinifera. The protein IDs are listed in Tables S1 and S2.

Figure 5. Serrano “Tampiqueño 74” fruits throughout developmental stages. Flower (0 dpa; days post-anthesis) and chili pepper fruits at 10, 20, 30, 40, 50, and 60 dpa.
Figure 6. Expression profiles of CaMYB genes in flowers and during chili pepper fruit development. The heat map shows the expression pattern of CaMYB genes in flower (0 dpa; days post-anthesis).
and in chili pepper fruit at 10, 20, 30, 40, 50, and 60 dpa in FPKM (fragments per kilobase of transcript sequence per millions base pairs sequenced). Red color represents the highest relative expression level. The number extension (0.2, 0.3, and 0.4) indicates CaMYB genes with more than one expression profile. Capsaicinoid biosynthetic genes: AT3 (acyltransferase), Kas (ketoacyl-ACP synthase), pAmt (amino-transferase), and BCKDH (branched-chain amino acid transferase); carotenoid biosynthetic genes: CCS (capsanthin-capsorubin synthase), BCH (β-carotene hydroxylase), and PSY (phytoene synthase); anthocyanin biosynthetic genes: DFR (dihydroflavonol 4-reductase), F3′5′H (flavonoid 3′, 5′-hydroxylase), and CHS (chalcone synthase); phenylpropanoid biosynthetic genes: PAL (phenylalanine ammonia lyase), 4CL (4-coumarate-CoA ligase), and CAH (cinnamic acid 4-hydroxylase); vitamin C biosynthetic genes: GLDH (L-galactono-1,4-lactone dehydrogenase), GalDH (L-galactose-1-dehydrogenase), and GME (GDP-D-mannose-3′,5′-epimerase); lignin biosynthetic genes: CAD (cinnamyl alcohol dehydrogenase), CCR (cinnamoyl CoA reductase), and POD (peroxidase).

2.7. RT-qPCR Analysis

To identify CaMYB genes as regulators of important secondary metabolic pathways, we analyzed the expression profile of AT3 (capsaicinoid biosynthesis), CCS (carotenoid biosynthesis), PAL (phenylpropanoid biosynthesis), CAD (lignin biosynthesis), and GLDH (vitamin C biosynthesis) structural genes compared with their possible CaMYB regulators throughout the chili pepper fruit development (Figure 7). The transcript FPKM expression was similar to the quantitative relative expression. The AT3 expression was not detected in flower tissue, very low at 10 dpa, increased to a maximum between 20–30 dpa, decreased at 40 dpa, and was undetectable at 50 and 60 dpa. We verified that the CaMYB31 expression positively correlated with AT3. Moreover, the expression profile of CaMYB103, CaMYB115, and CaDIV14 positively correlated with that of AT3 and CaMYB31, suggesting that these transcription factors could be involved in the regulation of the capsaicinoid biosynthesis pathway. The CCS expression pattern was low between 0 and 40 dpa, and highly expressed at 50 and 60 dpa. The CaDIV1 and CaMYB3R-5 expression profile was correlated with that of CCS, being good candidates to regulate the CCS transcription. PAL showed the highest expression level at 0 dpa, and gradually decreased during the fruit development. Based on the RNA-seq data, several CaMYB genes were positively correlated with PAL, of which CaMYB32, CaMYB33, and CaMYB93 expression pattern was investigated. These three CaMYB genes showed the highest expression at 0 dpa, whereas the CaMYB93 expression gradually decreased during the fruit development, CaMYB32 and CaMYB33 presented a very low or even null expression during the fruit development. The CAD expression was highest at 0 dpa, drastically decreased between 10–20 dpa, diminished a little more between 30–40 dpa, and increased moderately between 50–60 dpa. We verified the expression profile of two possible candidates to regulate the expression of CAD, CaMYB98, and CaMYB108, and both presented a positive correlation with CAD. The GLDH expression showed the highest value between 0–10 dpa, diminished at 20 dpa, and remained constant until 60 dpa. CaMYB16 presented an expression pattern similar to GLDH.
Figure 7. RT-qPCR of the expression profile of AT3 (capsaicinoid pathway), PAL (phenylpropanoid pathway), CCS (carotenoid pathway), CAD (lignin pathway), and GLDH (vitamin C pathway) structural genes and their putative CaMYB regulators. The relative expression level was analyzed in flower (0 dpa; days post-anthesis) and in chili pepper fruit at 10, 20, 30, 40, 50, and 60 dpa. AT3: Aeryltransferase; PAL: Phenylalanine ammonia lyase; CCS: Capsanthin-capsorubin synthase; CAD: Cinnamyl alcohol dehydrogenase; GLDH: L-galactono-1,4-lactone dehydrogenase. The data points represent the means of three biological replicates ± SD. See Table S4 for primers sequence details.

3. Discussion

The MYB family, one of the largest transcription factor families, has been implicated in diverse important biological process in plants such as primary and secondary metabolism, developmental processes, biotic and abiotic stress response, cellular and organ morphogenesis, and cell cycle control [3,8]. MYB transcription factors have been identified in several plant species such as Arabidopsis, rice, potato, pineapple, and tomato [17–19,21,34]. In the current study, we performed a wide analysis of CaMYB genes family in chili pepper in comparison to the MYB gene family of A. thaliana and other known plant MYB genes.
3.1. The 235 CaMYB Genes Were Identified in Capsicum annuum Genome

A total of 235 non-redundant genes with at least one MYB repeat were identified in the C. annuum genome. These genes were divided into five subfamilies, including 116 R2R3-MYB, five 3R-MYB, 92 MYB-related, two atypical MYBs (CaMYB5R and CaMYBCDC), and 20 MYB proteins with other conserved domains (Table S3). Recently, Wang et al. [31] identified 108 R2R3-MYBs in chili pepper, five genes less than in our study, probably since our strategy for the identification and classification of CaMYBs was also based on the comparative analysis with other known MYB plant proteins. Consistent with our results, the R2R3-MYB subfamily has been reported as the largest subfamily of the MYB family in other plant species [15,17,46], with some exceptions [21,47]. The total number of CaMYB genes was higher than the number of MYB genes identified in Arabidopsis (198), tomato (127), and pineapple (184), but lower than those in potato (253), sunflower (245), and sesame (287) [17–21,47], suggesting a distinct degree of evolutionary expansion of the MYB family among plant species. The molecular weight and isoelectric points play important roles in determining the molecular and biochemical function [48]. We investigated CaMYB protein sizes and their pI, which presented a clear variation, probably due to their roles across different environments, contributing to a great functional diversity in MYB proteins. A typical TF contains a DNA-binding region, an oligomerization site, a transcription-regulation domain, and a nuclear localization site [1]. To inspect whether all CaMYBs presented a NLS, we predicted the subcellular localization of CaMYB proteins, of which almost 98% exhibited nuclear localization. Those genes that did not have an NLS were two MYB-related genes (CaPHR4 and CaRL3) and three MYBs with other conserved domains (CaSWI3A, CaMYBH4, and CaMYBH5). Transcriptional regulatory proteins without NLS may be imported into the nucleus by dimerization with proteins that do contain NLS [49]. CaMYBs were distributed throughout all twelve chromosomes of chili pepper, but their distribution seemed to be uneven, showing the highest density on the top and bottom of the chromosomes (Figure 2). This MYB gene distribution was similar to previous studies in other Solanaceae members, such as tomato [19] and potato [21].

3.2. CaMYB Family Relationships with Other Plant MYB Proteins

A phylogenetic tree was constructed with the R2R3-MYB, 3R-MYB, and atypical proteins of C. annuum compared with the MYB family of Arabidopsis and other known function MYB proteins (Figure 1). The phylogenetic analysis was congruent with previous reports for Arabidopsis [33,34]. Genes grouped in the same clade may have a common ancestor and thus share conserved functions. Most of the CaMYBs were clustered with AtMYBs, providing interesting insights on the roles of CaMYB genes (Table S3). For example, CaMYB97, CaMYB98, CaMYB99, and CaMYB100 grouped together with AtMYB11, AtMYB12, AtMYB111, and SIMYB12 could be implicated in the regulation of flavonoid biosynthesis [37,38,50]. Clade C15 was constituted by CaMYB32 and CaMYB33 of C. annuum and the well-known proteins AtMYB21, AtMYB24, AtMYB57, AmMYB305, and AmMYB340 that have been implicated in the control of PAL gene and stamen filament elongation [51,52]. Conversely, some CaMYBs did not cluster with any AtMYB proteins, suggesting evolutive events of the gain or loss of genes. The biochemical and physiological differences between C. annuum and A. thaliana may hint at the existence of species-specific MYBs with specialized functions. For instance, CaMYB31 did not exhibit orthologous genes in Arabidopsis, probably since CaMYB31 regulates the capsaicinoid biosynthetic pathway, which is specific to the Capsicum genus. Likewise, clade C34 did not include CaMYBs, but only AtMYBs, which are involved in the glucosinolate biosynthetic process, which is predominant in the Brassicaceae family.

A subgroup classification was highly supported by the gene structure (Figure 3) and motif protein arrangement analysis (Figure 4). Consistent with previous studies [53,54], most of the MYB genes within the same subgroup shared a similar exon-intron structure, showing that MYB genes are highly conserved among species. MYB proteins comprise the conserved MYB domain that recognizes the promoter of the target gene, and a highly
variable region responsible for the regulatory activity [3,8]. The motif analysis revealed that CaMYB proteins belonging to the same subgroup displayed common motifs in the amino acid sequence outside the MYB domains, suggesting that they might share similar functions. Clade C33, which included four CaMYB genes along with subgroup S1 of Arabidopsis, shared three exons of very similar size and they have been implicated in salt-tolerance and suberin biosynthesis [55,56]. In addition, these proteins showed conserved motifs in the C-terminal region (i.e., motifs 5 and 6) indicating common functions. However, they also displayed exclusive motifs for CaMYBs (i.e., motif 8) and AtMYBs (i.e., motif 10).

Furthermore, we classified and assigned putative functions to MYB-related and MYBs with other conserved domains based on their intron-exon organizations and motif protein composition, which was highly conserved (Table S3 and Figures S1–S4). The subgroup KAN-like contained seven CaMYB-related genes that shared a MYB-domain encompassing the SHLQMYR consensus. The exon-intron organization and motif protein arrangement revealed that CaKAN1, CaKAN2, CaKAN3, and CaKAN4 exhibited similarities with AtKAN2 suggesting that they may be involved in lateral organ formation [57]. Conversely, CaKAN5, CaKAN6, and CaKAN7 were more distant to AtKAN2, thus suggesting they may be playing other roles in Capsicum. The EFM/HHO5-like subgroup included four CaMYB-related genes together with AtEFM and AtHHO5 genes, in which all presented a MYB-domain with the SHLQKYR consensus. Outside the MYB-domain, those proteins exhibited motifs 2 and 6, implying common possible functions in the flowering process [12,14].

3.3. CaMYB Gene Expression Profiles during the Chili Pepper Fruit Development

The transcriptome annotation of the CaMYB family provides insights into their functions. A comparative expression analysis has allowed identifying numerous regulatory genes important for the control of different biological processes in Capsicum [25,27,30]. We performed the co-expression analysis of CaMYB genes using transcriptome data of flower (0 dpa) and chili pepper fruit throughout different developmental stages (10–60 dpa). We detected transcripts for 218 CaMYB genes (92.7%) in at least one of the tissues tested, and they showed different tissue-specific expression patterns, suggesting that these genes might be implicated in the control of diverse biological processes related to the specific expression pattern that they showed in the corresponding tissues. Most of the CaMYB genes in the same subgroup exhibited dissimilar expression profiles suggesting that these genes may have similar roles in the distinct tissues or under different environmental conditions. For the heat map construction, in addition to including the CaMYB genes, we also analyzed key structural genes of the phenylpropanoid, lignin, capsaicinoid, carotenoid, anthocyanin, and vitamin C biosynthetic pathways to identify CaMYB genes as regulator candidates of such pathways. The co-expression analysis showed that at least one CaMYB gene positively correlated with the biosynthetic genes analyzed (Figure 6). Moreover, the expression profile for some of these candidates was verified by RT-qPCR (Figure 7), reinforcing that they could be strong candidates to regulate these metabolic routes.

The phenylpropanoid metabolism generates a great range of secondary metabolites that contribute to numerous biological processes, such as plant growth and development, and biotic and abiotic response [58]. PAL is responsible for the first step in the synthesis of phenylpropanoid-derived compounds. Among all the CaMYB genes that positively correlated with the PAL expression profile, we corroborated that CaMYB32, CaMYB33, and CaMYB93 expression patterns effectively co-expressed with the PAL gene. Unlike the RNA-seq analysis, the RT-qPCR study showed that the PAL gene presented the highest expression in flowers (0 dpa), and then gradually decreased throughout the fruit development, probably due to the wide diversification of the phenylpropanoid pathway (i.e., flavonoid, lignin, capsaicinoid, etc.), which is also carried out in the different developmental stages of chili pepper fruit. The CaMYB93 expression correlated quite well with the PAL expression profile throughout the fruit development, and based on the phylogeny tree, CaMYB93 grouped with subgroup S1 implicated in secondary metabolism, plant development, and stress response [59–63]. Therefore, the CaMYB93 gene may be regulating PAL transcript-
tion throughout the fruit development. Contrarily, CaMYB32 and CaMYB33 showed the highest expression in flower tissue, very low in chili pepper fruit at 10–20 dpa, and undetectable at 30–60 dpa. These two TFs were the R2R3-MYB type and phylogenetically related to AmMYB305, which has been proposed to activate the expression of phenylpropanoid biosynthetic genes in flowers [51], and with AtMYB21, which has been reported to be required for the activation of PAL [64]. Additionally, it is known that members of the subgroup S19 are expressed primarily in flowers [65]. Based on these results, both R2R3-MYB TFs are strong candidates to regulate the PAL gene expression in both flower tissue and early chili fruit developmental stages. Moreover, these genes shared a highly conserved motif structure in the C-terminal region with the S19 of Arabidopsis and AmMYB305 and AmMYB340 proteins, supporting the fact that they play a common role in the phenylpropanoid regulation.

The most widely known characteristic of chili pepper fruits is their capacity to produce capsaicinoids which are responsible for the pungency sensation [66]. To date, three MYBs transcription factors (CaMYB31, CaMYB48, and CaMYB108) have been reported as regulators of the capsaicinoid biosynthesis pathway [27–29]. In a recent work, six candidate CaR2R3-MYB genes were proposed to be regulating the synthesis of capsaicinoids: Capana08g001690, Capana02g003351, Capana08g000900, Capana02g000906, Capana01g000495, and Capana07g001604 [31] which correspond to CaMYB47, CaMYB87, CaMYB73, CaMYB92, CaMYB74, and CaMYB64, respectively (Table S3). In our co-expression analysis, only the CaMYB31 expression profile correlated positively with the expression profiles of capsaicinoid biosynthetic marker genes. To identify more CaMYB gene candidates possibly involved in the regulation of the capsaicinoid biosynthetic pathway, AT3 and CaMYB115 expression was quantified and compared with two R2R3-MYB (CaMYB103 and CaMYB115) and one MYB-related (CaDIV14) genes, resulting in a positive correlation, suggesting that they are strong candidates to regulate the capsaicinoid accumulation. These CaMYB genes were phylogenetically grouped in different clades, thus they could be involved in different functional activities necessary for the capsaicin production, such as molecular, biochemical, or physiological processes. CaMYB103 did not show any orthologous genes in Arabidopsis, indicating perhaps a specialized role in Capsicum, such as the control of capsaicinoid biosynthesis. CaMYB115 was grouped with genes related to the phenylpropanoid pathway [67], thus this gene may be implicated in the regulation of early genes of capsaicinoid biosynthesis. Conversely, CaDIV14 was clustered with DiVARICATA-like genes that have been characterized for their participation in the flower development [39]. Since CaDIV14 did not show an expression in flower tissue, but it did exhibit the characteristic expression pattern for capsaicinoid biosynthetic genes, it is likely that this gene could be associated with the blister (capsaicin deposit) development process, which has been linked to the capsaicinoid accumulation [68,69].

Lignin is one of the main components of plants cell wall and contributes to plant growth, tissue/organ development, and response to various stresses [70]. The regulation of the lignin biosynthesis pathway has not been well described in Capsicum. The content of lignin in different developmental stages of chili pepper fruit was determined, resulting in a gradual decrease in lignin content from 14 until 42 dpa [71,72]. Studies have demonstrated that the lignin biosynthesis is regulated by MYB transcription factors [73,74]. To explore possible MYB regulators of the lignin biosynthetic pathway, we analyzed the relative expression of the CAD gene, responsible for the last step in the biosynthesis of lignins. We confirmed that CaMYB98 and CaMYB108 genes co-expressed with the CAD gene expression during the development of chili pepper fruit. Interestingly, the expression profile was opposite to that of capsaicinoid biosynthetic genes, probably due to the precursor competition between the capsaicinoid and lignin production [71,72]. Unexpectedly, the CaMYB108 expression profile was not consistent with previous studies that have suggested that this gene regulates the capsaicinoid biosynthesis pathway [28]. Both TFs were phylogenetically related to MYB proteins involved in flavonol biosynthesis, overall growth, and response to biotic and abiotic stresses, which is consistent with some biological roles of lignins.
Chili pepper fruits also synthesize and accumulate carotenoid pigments, which are responsible for the yellow, orange, and red colors. The CCS gene is responsible for the last biochemical step of capsanthin and capsorubin biosynthesis, which confer the red color [75]. The CCS gene was used here as a reference to identify CaMYB genes candidates involved in the regulation of the carotenoid biosynthesis pathway. CaDIV1 and CaMYB3R-5 genes shared a similar expression profile during the fruit development as well as with the CCS gene. CaDIV1 belongs to the DIVARICATA-like genes classification (R-R type MYB domain) that to date have been characterized by their participation in plant development and stress response [13]. CaMYB3R-5 was classified as 3R-MYB, whose function has been related to the cell cycle process and abiotic stress response [76]. The temporal and spatial regulation of cell proliferation impacts on the shape and size of a plant organ in response to specific environmental conditions or developmental stages [77]. CaDIV1 and CaMYB3R-5 genes might be involved in the regulation of organelle structures biogenesis for carotenoid accumulation.

The L-ascorbic acid (vitamin C) is the most abundant antioxidant in plant cells, which participates in diverse biological processes, including cell expansion, environment response, photoprotection, and photosynthesis, etc. [78]. GLDH is responsible for the last step in producing vitamin C through the L-galactose pathway, and it was used here as a reference gene to find CaMYB genes candidates possibly involved in the control of vitamin C biosynthesis. The CaMYB16 expression profile correlated positively with both the GLDH expression pattern and content of vitamin C throughout the chili pepper fruit development [78]. The constant production of vitamin C during the chili pepper fruit development can be necessary either for protection purposes or development. Based on the phylogenetic analysis, CaMYB16 was closer to the subgroup S22 of Arabidopsis, whose elements are implicated in biotic and abiotic responses, and plant growth and development, which are considered biological functions of vitamin C in plants. Taking it all together, this study might contribute as an important resource to propose new MYB gene candidates for the regulation of diverse biological processes in Capsicum spp.

4. Materials and Methods

4.1. Plant Material and Growth Conditions

Chili pepper (Capsicum annuum) “Tampiqueño 74” (Serrano type) were grown under greenhouse conditions and fertilized with a Long Ashton solution every 2 weeks. Flowers (0 dpa) and whole chili pepper fruits at 10, 20, 30, 40, 50, and 60 dpa were harvested from at least 12 plants, immediately frozen in liquid nitrogen, and stored at −80°C. These materials were used for the RNA-seq library construction and RT-qPCR expression analysis.

4.2. Identification of MYB Proteins

The genome database of chili pepper cultivar Zunla-1 (C. annuum) was used in this study [24]. The identification of MYB genes was carried out with a Blastp search using as query the Pfam domains: PF00249 and PF1392. To verify the presence of the significant MYB domain, all the sequences were examined using the Pfam database (https://pfam.xfam.org, accessed on 5 January 2021) [32]. The protein, coding and genome sequences, and chromosome localization were downloaded from the RefSeq_protein National Center for Biotechnology Information database. Three hundred and forty-five proteins with at least one MYB domain were identified, which were localized in 235 loci. Therefore, 235 non-redundant CaMYBs were identified in C. annuum.

4.3. Phylogenetic Analysis and Sequence Analysis

For the phylogenetic tree construction, we selected 126 non-redundant CaMYB proteins, including all R2R3-MYB, 3R-MYB, atypical proteins (CaMYB5R and CaMYBCDC), and three MYB-related proteins. CaMYBs were compared with the MYB family of Arabidopsis thaliana and 25 MYB proteins were functionally characterized from other plant species (Tables S1 and S2). The sequences were aligned with ClustalW using default pa-
rameters, and it was manually adjusted. Based on the alignment of the MYB domain, a phylogenetic tree was constructed with the Neighbor-joining method, model JTT+G, and a bootstrap test of 1000 replicates using the MEGA7 software. The isoelectric point and protein molecular weight were predicted using the isoelectric point calculator (IPC) software (http://isoelectric.org/index.html, accessed on 5 January 2021) [79]. To predict the subcellular localization of CaMYBs, the CELLO v.2.5: Subcellular localization predictor (http://cello.life.nctu.edu.tw, accessed on 5 January 2021) [80] was used. MEME version 5.2.0 (http://meme-suite.org/tools/meme, accessed on 5 January 2021) [40] was used to discover conserved motifs outside the MYB domain. The exon-intron structures of CaMYB genes were schemed by the Gene Structure Display Server version 2.0 (GSDS) (http://gsds.gao-lab.org, accessed on 5 January 2021) [81] comparing the CDS and genome sequences. The chromosomal distribution of CaMYB genes was mapped using the Map Gene 2 Chromosome (MG2C) server version 2.0 (http://mg2c.iask.in/mg2c_v2.0/, accessed on 5 January 2021) [82]. The CaMYB subfamily classification was carried out according to the topology of the phylogenetic tree, motif protein composition, and exon-intron organization.

4.4. RNA-Seq Library Construction and Processing

Total RNA was extracted from flowers (0 dpa) and whole chili pepper fruits at 10, 20, 30, 40, 50, and 60 dpa using a NucleoSpin™ RNA plant kit (MACHEREY-NAGEL, Bethlehem, PA, USA) following the manufacturer’s indications. The extraction was performed in duplicate for each sample. Flower samples were collected from 6–12 different plants, and fruit samples included 3–6 different plants. The RNA quality was verified by 1% agarose gel electrophoresis and the RNA integrity number (RIN) for each sample was determined. For library construction, sequencing and mapping to a reference genome, the Novogene company (Sacramento, CA, USA) services were used. Libraries were prepared and sequenced using the Illumina Platform to obtain 6G raw paired-end reads of 250–300 pb per sample. These reads were mapped to the Capsicum reference genome, identified by the protein product, and annotated with gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) terms.

4.5. Co-Expression Analysis

The heat map was generated in R Studio version 1.2.5019 using the pheatmap package version 1.0.12 with the FPKM expression data. The genes were clustered based on the Pearson correlation analysis.

4.6. Quantitative PCR Assays

Total RNA was extracted, purified, and treated with DNase I (MACHEREY-NAGEL, Bethlehem, PA, USA) according to the manufacturer’s instructions. The RNA extraction from flowers and whole chili pepper fruits at different developmental stages was performed in triplicate experiments for each sample. The cDNA was synthesized with SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA, USA) and adjusted to 100 ng µL⁻¹. The PCR primers were designed to avoid the conserved region and to amplify products of 90 to 142 bp (Table S4). We analyzed the expression of eleven CaMYB genes and five key structural genes of the phenylpropanoid, lignin, capsaicinoid, carotenoid, and vitamin C biosynthesis pathways. The EF1-a elongation was used as the normalization reference gene. RT-qPCR assays were performed as reported by Arce-Rodríguez and Ochoa-Alejo [83].

5. Conclusions

The characterization and classification of gene families is a crucial first step for functional studies. A total of 235 CaMYB genes were identified and classified, comprising R2R3-MYB, 3R-MYB, atypical MYBs, and MYB-related genes. These genes were unevenly distributed on the twelve chromosomes of C. annuum. Based on the phylogenetic relationships, most of the CaMYBs presented possible orthologous in Arabidopsis, indicating
common evolutionary origins. Furthermore, a computational analysis revealed that CaMYB genes might play roles in diverse biological processes, both conserved and specialized functions. The co-expression analysis in flower and fruits at different developmental stages showed that CaMYB genes were differentially expressed in all the tissues analyzed, supporting the idea that CaMYBs are functionally divergent. The integration of our results allowed us to propose some strong MYB candidates that might be involved in the regulation of phenylpropanoid, lignin, capsaicinoid, carotenoid, and vitamin C biosynthesis, providing new insights into the role of MYB transcription factors in secondary metabolism. Further functional characterization of CaMYB genes is needed for a better understanding of the role and regulatory mechanisms of the MYB family in Capsicum spp.

Supplementary Materials: The following materials are available online at https://www.mdpi.com/1422-0067/22/5/2229/s1.

Author Contributions: Conceptualization, M.L.A.-R. and N.O.-A.; visualization, M.L.A.-R.; methodology, M.L.A.-R. and O.M.; validation, M.L.A.-R.; investigation, M.L.A.-R. and O.M.; resources, N.O.-A. and O.M.; supervision, M.L.A.-R., N.O.-A. and O.M.; writing—original draft preparation, M.L.A.-R.; writing—review and editing, M.L.A.-R., N.O.-A. and O.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Consejo Nacional de Ciencia y Tecnología (CONACYT), Mexico, project 1570.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is available at the Gene Expression Omnibus (GEO) of the NCBI (https://www.ncbi.nlm.nih.gov/geo/, accessed on 5 January 2021). The accession number is GSE165448.

Acknowledgments: We thank M.C. Fernando Hernandez, Andrés Guzman, Angie Zambrano, Fernanda Peñaranda, Carolina Elias, Oscar Nieves, Alonso Garduño, and Marissa Sauceda for technical assistance in sampling and processing plant materials. Thanks to Antonio Cisneros for taking the photos of chili pepper fruits at different developmental stages.

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

References
1. Liu, L.; Michael, J.W.; MacRase, T.M. Transcription factors and their genes in higher plants functional domains, evolution and regulation. *Eur. J. Biochem.* 1999, 262, 247–257. [CrossRef]
2. Klemplauer, K.H.; Gonda, T.J.; Bishop, J.M. Nucleotide sequence of the retroviral leukemia gene v-myb and its cellular progenitor c-myb: The architecture of a transduced oncogene. *Cell* 1982, 31, 453–463. [CrossRef]
3. Ambawat, S.; Sharma, P.; Yadav, N.R.; Yadav, R.C. MYB transcription factor genes as regulators for plant responses: An overview. *Physiol. Mol. Biol. Plants* 2013, 19, 307–321. [CrossRef]
4. Paz-Ares, J.; Ghosal, D.; Wienand, U.; Peterson, P.A.; Saedler, H. The regulatory c1 locus of *Zea mays* encodes a protein with homology to myb proto-oncogene products and with structural similarities to transcriptional activators. *EMBO J.* 1987, 6, 3553–3558. [CrossRef] [PubMed]
5. Kuno, N.; Möller, S.G.; Shinomura, T.; Xu, X.; Chua, N.H.; Furuya, M. The novel MYB protein EARLY-PHYTOCHROME-RESPONSIVE1 is a component of a slave circadian oscillator in *Arabidopsis*. *Plant Cell* 2003, 15, 2476–2488. [CrossRef] [PubMed]
6. Corley, S.B.; Carpenter, R.; Copsey, L.; Coen, E. Floral asymmetry involves an interplay between TCP and MYB transcription factors in *Antirrhinum*. *Proc. Natl. Acad. Sci. USA* 2005, 102, 5068–5073. [CrossRef]
7. Wang, S.; Hubbard, L.; Chang, Y.; Guo, J.; Schiefelbein, J.; Chen, J.G. Comprehensive analysis of single-repeat R3 MYB proteins in epidermal cell patterning and their transcriptional regulation in *Arabidopsis*. *BMC Plant Biol.* 2008, 8, 81. [CrossRef]
8. Dubos, C.; Stracke, R.; Grotewold, E.; Weisshaar, B.; Martin, C.; Lepiniec, L. MYB transcription factors in *Arabidopsis*. *Trends Plant Sci.* 2010, 15, 573–581. [CrossRef]
9. Zhai, H.; Bai, X.; Zhu, Y.; Li, Y.; Cai, H.; Ji, W.; Ji, Z.; Liu, X.; Liu, X.; Li, J. A single-repeat R3-MYB transcription factor MYBC1 negatively regulates freezing tolerance in *Arabidopsis*. *Biochem. Biophys. Res. Commun.* 2010, 394, 1018–1023. [CrossRef] [PubMed]
10. Zhao, C.; Beers, E. Alternative splicing of Myb-related genes MYR1 and MYR2 may modulate activities through changes in dimerization, localization, or protein folding. *Plant Signal. Behav.* 2013, 8, e27325. [CrossRef] [PubMed]
11. Lu, D.; Wang, T.; Persson, S.; Mueller-Roeders, B.; Schippers, J.H. Transcriptional control of ROS homeostasis by KUODA1 regulates cell expansion during leaf development. *Nat. Commun.* 2014, 5, 3767. [CrossRef] [PubMed]

12. Yan, Y.; Shen, L.; Chen, Y.; Bao, S.; Thong, Z.; Yu, H. A MYB-domain protein EFM mediates flowering responses to environmental cues in Arabidopsis. *Dev. Cell* 2014, 30, 437–448. [CrossRef]

13. Wang, T.; Tohge, T.; Ivakov, A.; Mueller-Roeders, B.; Fernie, A.R.; Mutwil, M.; Schippers, J.H.; Persson, S. Salt-related MYB1 coordinates abscisic acid signaling and stress responses during salt stress in Arabidopsis. *Plant Physiol.* 2015, 169, 1027–1041. [CrossRef]

14. Moreau, F.; Thévenon, E.; Blanvillain, R.; Lopez-Vidriero, I.; Franco-Zorrilla, J.M.; Dumas, R.; Parcy, F.; Morel, P.; Trehin, C.; Carles, C.C. The Myb-domain protein ULTRAPETALAL1 INTERACTING FACTOR 1 controls floral meristem activities in Arabidopsis. *Development* 2016, 143, 1108–1119. [CrossRef]

15. Cao, Y.; Han, Y.; Li, D.; Lin, Y.; Cai, Y. MYB transcription factors in Chinese pear (*Pyrus bretschneideri* Rehd.). Genome-wide identification, classification, and expression profiling during fruit development. *Front. Plant Sci.* 2016, 7, 577. [CrossRef] [PubMed]

16. Smita, S.; Katayri, A.; Chinnusamy, V.; Pandey, D.M.; Bansal, K.C.Transcriptional regulatory network analysis of MYB transcription factor family genes in rice. *Front. Plant Sci.* 2015, 6, 1157. [CrossRef]

17. Yanhui, C.; Xiaoyuan, Y.; Kun, H.; Meihua, L.; Jiagang, L.; Zhaofeng, G.; Zhiquiang, L.; Yunfei, Z.; Xiaoxiao, W.; Xiaoming, Q.; et al. The MYB transcription factor superfamily of Arabidopsis: Expression analysis and phylogenetic comparison with the rice MYB family. *Plant Mol. Biol.* 2006, 60, 107–124. [CrossRef] [PubMed]

18. Liu, C.; Xie, T.; Chen, C.; Luan, A.; Long, J.; Li, C.; Ding, Y.; He, Y. Genome-wide organization and expression profiling of the R2R3-MYB transcription factor family in pineapple (*Ananas comosus*). *BMC Genom.* 2017, 18, 503. [CrossRef]

19. Li, Z.; Peng, R.; Tian, Y.; Han, H.; Xu, J.; Yao, Q. Genome-wide identification and analysis of the MYB transcription factor superfamily in *Solanum lycopersicum*. *Plant Cell Physiol.* 2015, 56, 1657–1677. [CrossRef]

20. Li, J.; Liu, H.; Yang, C.; Wang, J.; Yan, G.; Si, P.; Bai, Q.; Lu, Z.; Zhou, W.; Xu, L. Genome-wide identification of MYB genes and expression analysis of *MYB* gene family in *Helianthus annuus*. *L. Ind. Crop. Prod.* 2020, 143, 111924. [CrossRef]

21. Li, Y.; Lin-Wang, K.; Liu, Z.; Allan, A.C.; Qin, S.; Zhang, J.; Liu, Y. Genome-wide analysis and expression profiles of the StR2R3-MYB transcription factor superfamily in potato (*Solanum tuberosum L.*). *Int. J. Mol. Sci.* 2020, 148, 817–832. [CrossRef]

22. Jarret, R.L.; Barboza, G.E.; Costa Batista, F.; Berke, T.; Chou, Y.; Hulse-Kemp, A.; Ochoa-Alejo, N.; Tripodi, P.; Veres, A.; Garcia, C.C.; et al. *Capsicum*—an abbreviated compendium. *J. Am. Soc. Hortic. Sci.* 2019, 144, 3–22. [CrossRef]

23. Kim, S.; Park, M.; Yeom, S.I.; Kim, Y.M.; Lee, J.M.; Lee, H.A.; Seo, E.; Choi, J.; Cheong, K.; Kim, K.T.; et al. Genome sequence of the hot pepper provides insights into the evolution of pungency in *Capsicum* species. *Nat. Genet.* 2014, 46, 270–278. [CrossRef]

24. Qin, C.; Yu, C.; Shen, Y.; Fang, X.; Chen, L.; Min, J.; Cheng, J.; Zhao, S.; Xu, M.; Luo, Y.; et al. Whole-genome sequencing of cultivated and wild peppers provides insights into Capsicum domestication and specialization. *Proc. Natl. Acad. Sci. USA* 2014, 111, 5135–5140. [CrossRef]

25. Borovskyy, V.; Oren-Shamir, M.; Ovadia, R.; De Jong, W.; Paran, I. The A locus that controls anthocyanin accumulation in pepper encodes a MYB transcription factor homologous to *Anthocyanin2* of Petunia. *Theor. Appl. Genet.* 2004, 109, 23–29. [CrossRef]

26. Aguilar-Barragán, A.; Ochoa-Alejo, N. Virus-induced silencing of MYB and WD40 transcription factor genes affects the accumulation of anthocyanins in chilli pepper fruit. *Plant Biotechnol.* 2014, 58, 567–574. [CrossRef]

27. Arce-Rodriguez, M.L.; Ochoa-Alejo, N. An R2R3-MYB transcription factor regulates capsaicinoid biosynthesis. *Plant Physiol.* 2017, 174, 1539–1570. [CrossRef] [PubMed]

28. Sun, B.; Zhuo, Z.; Chen, C.; Chen, G.; Cao, B.; Chen, C.; Lei, J. Jasmonate-inducible R2R3-MYB transcription factor regulates capsaicinoid biosynthesis and stamen development in *Capsicum*. *J. Agric. Food Chem.* 2019, 67, 10891–10903. [CrossRef] [PubMed]

29. Sun, B.; Zhou, X.; Chen, C.; Chen, C.; Chen, M.; Liu, S.; Chen, G.; Cao, B.; Cao, F.; et al. Coexpression network analysis reveals an MYB transcriptional regulator involved in capsaicin biosynthesis in hot peppers. *Hortic. Res.* 2020, 7, 162. [CrossRef] [PubMed]

30. Jeifetz, D.; David-Schwartz, R.; Borovskyy, Y.; Paran, I. CaBLIND regulates auxillary meristem initiation and transition to flowering in pepper. *Planta* 2011, 234, 1227–1236. [CrossRef]

31. Wang, J.; Liu, Y.; Tang, B.; Dai, X.; Xie, L.; Li, L.; Liu, F.; Zou, X. Genome-wide identification and capsaicinoids-related expression analysis of the R2R3-MYB gene family in *Capsicum annuum* L. *Front. Genet.* 2020, 21, 598183. [CrossRef]

32. El-Gebali, S.; Mistry, J.; Bateman, A.; Eddy, S.R.; Luciani, A.; Potter, S.C.; Qureshi, M.; Richardson, L.J.; Salazar, G.A.; Smart, A.; et al. The Pfam protein families database in 2019. *Nucleic Acids Res.* 2019, 47, 427–432. [CrossRef]

33. Kranz, H.D.; Denekamp, M.; Greco, R.; Jin, H.; Levyva, A.; Meissner, R.C.; Petroni, K.; Urzainqui, A.; Bevan, M.; Martin, C.; et al. Towards functional characterisation of the members of the R2R3-MYB gene family from *Arabidopsis thaliana*. *Plant J.* 1998, 16, 263–276. [CrossRef]

34. Stracke, R.; Werber, M.; Weisshaar, B. The R2R3-MYB gene family in *Arabidopsis thaliana*. *Curr. Opin. Plant Biol.* 2001, 4, 447–456. [CrossRef]

35. Dai, X.; Xu, Y.; Ma, Q.; Xu, W.; Wang, T.; Xue, Y.; Chong, K. Overexpression of an R1R2R3 MYB gene, OsMYB3R-2, increases tolerance to freezing, drought, and salt stress in transgenic *Arabidopsis*. *Plant Physiol.* 2007, 143, 1739–1751. [CrossRef]
36. Haga, N.; Kobayashi, K.; Suzuki, T.; Maeo, K.; Kubo, M.; Ohtani, M.; Mitsuda, N.; Demura, T.; Nakamura, K.; Jürgens, G.; et al. Mutations in MYB3R1 and MYB3R4 cause pleiotropic developmental defects and preferential down-regulation of multiple G2/M-specific genes in Arabidopsis. *Plant Physiol.* **2011**, *157*, 706–717. [CrossRef]

37. Ballester, A.R.; Moltoff, J.; De Vos, R.; Hekkert, B.; Orzaez, D.; Fernández-Moreno, J.P.; Tripodi, P.; Grandillo, S.; Martin, C.; Heldens, J.; et al. Biochemical and molecular analysis of pink tomatoes: Deregulated expression of the gene encoding transcription factor SIMYB12 leads to pink tomato fruit color. *Plant Physiol.* **2010**, *152*, 71–84. [CrossRef] [PubMed]

38. Stracke, R.; Jahns, O.; Keck, M.; Töhge, T.; Niehaus, K.; Fernie, A.R.; Weissing, B. Analysis of production of flavonol glycosides-dependent flavonol glycoside accumulation in *Arabidopsis thaliana* plants reveals MYB11-, MYB12- and MYB111-dependent flavonol glycoside accumulation. *New Phytol.* **2010**, *188*, 985–1000. [CrossRef] [PubMed]

39. Galego, L.; Almeida, J. Role of *DIVARICATA* in the control of dorsoventral asymmetry in *Antirrhinum* flowers. *Genes Dev.* **2002**, *16*, 880–891. [CrossRef]

40. Bailey, T.L.; Bodén, M.; Buske, F.A.; Frith, M.; Grant, C.E.; Clementi, L.; Ren, J.; Li, W.W.; Noble, W.S. MEME SUITE: Tools for motif discovery and searching. *Nucleic Acids Res.* **2009**, *37*, W202–W208. [CrossRef]

41. Borg, M.; Brownfield, L.; Khatab, H.; Sidorova, A.; Lingaya, M.; Twell, D. The R2R3 MYB transcription factor DUO1 activates a male germline-specific regulon essential for sperm cell differentiation in *Arabidopsis*. *Plant Cell* **2011**, *23*, 534–549. [CrossRef] [PubMed]

42. Millar, A.A.; Gubler, F. The Arabidopsis GAMYB-like genes, MYB33 and MYB65, are microRNA-regulated genes that redundantly facilitate anther development. *Plant Cell* **2015**, *17*, 705–721. [CrossRef] [PubMed]

43. Aya, K.; Ueguchi-Tanaka, M.; Kondo, M.; Hamada, K.; Yano, K.; Nishimura, M.; Matsuoka, M. Gibberellin modulates anther development in rice via the transcriptional regulation of GAMYB. *Plant Cell* **2009**, *21*, 1453–1472. [CrossRef]

44. Reń, N.; Kubo, M.; Ohtani, M.; Mitsuda, N.; Demura, T.; Nakamura, K.; Jürgens, G.; Heldens, J.; et al. Biochemical and molecular analysis of pink tomatoes: Deregulated expression of the gene encoding transcription factor SIMYB12 leads to pink tomato fruit color. *Plant Physiol.* **2010**, *152*, 71–84. [CrossRef] [PubMed]

45. Jiang, C.K.; Rao, G.Y. Insights into the diversification and evolution of R2R3-MYB transcription factors in plants. *Plant Cell Physiol.* **2012**, *53*, 1973–1986. [CrossRef]

46. Qing, J.; Dawei, W.; Jun, Z.; Yulan, X.; Bingqi, S.; Fan, Z. Genome-wide characterization and expression analyses of the *AtMYB41*-like R2R3-MYB transcription factor family in *Arabidopsis* reveals essential roles in plant development and responses to drought and waterlogging in *Arabidopsis*. *Plant Cell Physiol.* **2011**, *52*, 1219–1233. [CrossRef] [PubMed]

47. Mmadi, M.A.; Dossa, K.; Wang, L.; Zhou, R.; Wang, Y.; Cisse, N.; Sy, M.O.; Zhang, X. Functional characterization of the versatile MYB transcription factor MYB11 regulates very-long-chain fatty acid biosynthesis for activation of the hypersensitive cell death response in tomato. *Physiol. Plant.* **2013**, *147*, 174–182. [CrossRef] [PubMed]

48. Kosma, D.K.; Murmu, J.; Razeq, F.M.; Santos, P.; Bourgault, R.; Molina, I.; Rowland, O. Functional characterization of the versatile MYB transcription factor MYB11 regulates very-long-chain fatty acid biosynthesis for activation of the hypersensitive cell death response in tomato. *Physiol. Plant.* **2013**, *147*, 174–182. [CrossRef] [PubMed]

49. Heldens, J.; et al. Biochemical and molecular analysis of pink tomatoes: Deregulated expression of the gene encoding transcription factor SIMYB12 leads to pink tomato fruit color. *Plant Physiol.* **2010**, *152*, 71–84. [CrossRef] [PubMed]

50. Reń, N.; Kubo, M.; Ohtani, M.; Mitsuda, N.; Demura, T.; Nakamura, K.; Jürgens, G.; Heldens, J.; et al. Biochemical and molecular analysis of pink tomatoes: Deregulated expression of the gene encoding transcription factor SIMYB12 leads to pink tomato fruit color. *Plant Physiol.* **2010**, *152*, 71–84. [CrossRef] [PubMed]

51. Sablowski, R.W.; Moyano, E.; Culianez-Macia, F.A.; Schuch, W.; Martin, C.; Bevan, M. A flower-specific Myb protein activates G2/M-specific genes in *Arabidopsis* gametophyte development in *Arabidopsis*. *Sex. Plant Reprod.* **2012**, *25*, 39–60. [CrossRef]

52. Cheng, H.; Song, S.; Xiao, L.; Soo, H.M.; Cheng, Z.; Xie, D.; Peng, J. Gibberellin acts through jasmonate to control the expression of *AtMYB12* and *AtMYB24*, and *AtMYB57* to promote stamen filament growth in *Arabidopsis*. *Plant Cell* **2012**, *24*, 106. [CrossRef] [PubMed]

53. Wu, X.; Zheng, J.; Zhou, Z.; Wang, L.; Sun, J.; Liu, L.; Huang, Y.B.; Tang, Y.X. Genome-wide analysis of the MYB transcription factor superfamily in soybean. *BMC Plant Biol.* **2012**, *12*, 106. [CrossRef] [PubMed]

54. Jiang, C.K.; Rao, G.Y. Insights into the diversification and evolution of R2R3-MYB transcription factor families in plants. *Plant Physiol.* **2020**, *183*, 637–655. [CrossRef]

55. Kosma, D.K.; Murmu, J.; Razeq, F.M.; Santos, P.; Bourgault, R.; Molina, I.; Rowland, O. *AtMYB41* activates ectopic suberin synthesis and assembly in multiple plant species and cell types. *Plant J.* **2014**, *80*, 216–229. [CrossRef] [PubMed]

56. Zhang, P.; Wang, R.; Yang, X.; Ju, Q.; Li, W.; Li, S.; Tran, L.P.; Xu, J. The R2R3-MYB transcription factor *AtMYB49* modulates salt tolerance in *Arabidopsis* by modulating the cuticle formation and antioxidative defence. *Plant Cell Environ.* **2020**, *43*, 1925–1943. [CrossRef]

57. Eshed, Y.; Baum, S.F.; Perea, J.V.; Bowman, J.L. Establishment of polarity in lateral organs of plants. *Curr. Biol.* **2001**, *11*, 1251–1260. [CrossRef]

58. Liu, J.; Osbourn, A.; Ma, P. MYB transcription factors as regulators of phenylpropanoid metabolism in plants. *Mol. Plant* **2015**, *8*, 689–708. [CrossRef] [PubMed]

59. Vailleur, F.; Daniel, X.; Tronchet, M.; Montillet, J.L.; Triantaphylides, C.; Roby, D. A R2R3-MYB gene, *AtMYB30*, acts as a positive regulator of the hypersensitive cell death program in plants in response to pathogen attack. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 10179–10184. [CrossRef] [PubMed]

60. Raffaele, S.; Vailleur, F.; Léger, A.; Joubès, J.; Miersch, O.; Huard, C.; Blée, E.; Mongrand, S.; Domergue, F.; Roby, D. A MYB transcription factor regulates very-long-chain fatty acid biosynthesis for activation of the hypersensitive cell death response in *Arabidopsis*. *Plant Cell* **2008**, *20*, 752–767. [CrossRef] [PubMed]
61. Galbiati, M.; Matus, J.T.; Francia, P.; Rusconi, F.; Cañón, P.; Medina, C.; Conti, L.; Cominelli, E.; Tonelli, C.; Arce-Johnson, P. The grapevine guard cell-related VvMYB60 transcription factor is involved in the regulation of stomatal activity and is differentially expressed in response to ABA and osmotic stress. BMC Plant Biol. 2011, 11, 142. [CrossRef]

62. Lee, H.G.; Lee, K.; Seo, P.J. The Arabidopsis MYB96 transcription factor plays a role in seed dormancy. Plant Mol. Biol. 2015, 87, 371–381. [CrossRef]

63. Lee, S.B.; Kim, H.U.; Suh, M.C. MYB94 and MYB96 additively activate cuticular wax biosynthesis in Arabidopsis. Plant Cell Physiol. 2016, 57, 2300-2311. [CrossRef] [PubMed]

64. Shin, B.; Choi, G.; Yi, H.; Yang, S.; Cho, I.; Kim, J.; Lee, S.; Paek, N.C.; Kim, J.H.; Song, P.S.; et al. AtMYB21, a gene encoding a flower-specific transcription factor, is regulated by COP1. Plant J. 2002, 30, 23–32. [CrossRef]

65. Jackson, D.; Cullinan-Macia, F.; Prescott, A.G.; Roberts, K.; Martin, C. Expression patterns of myb genes from Antirrhinum flowers. Plant Cell 1991, 3, 115-125. [CrossRef]

66. Arce-Rodríguez, M.L.; Ochoa-Alejo, N. Biochemistry and molecular biology of capsaicinoid biosynthesis: Recent advances and perspectives. Plant Cell Rep. 2019, 38, 1017–1030. [CrossRef] [PubMed]

67. Borevitz, J.O.; Xia, Y.; Blount, J.; Dixon, R.A.; Lamb, C. Activation tagging identifies a conserved MYB regulator of phenylpropanoid biosynthesis. Plant Cell 2000, 12, 2383–2394. [CrossRef]

68. Stewart, C., Jr.; Mazourek, M.; Stellari, G.M.; O’Connell, M.; Jahn, M. Genetic control of pungency in Capsicum annuum via the PaNC locus. J. Exp. Bot. 2007, 58, 979–991. [CrossRef]

69. Sánchez-Segura, L.; Téllez-Medina, D.I.; Evangelista-Lozano, S.; García-Armenta, E.; Alamilla-Beltrán, L.; Hernández-Sánchez, H.; Jiménez-Aparicio, A.R.; Gutiérrez-López, G.F. Morpho-structural description of epidermal tissues related to pungency of Capsicum species. J. Food Eng. 2015, 152, 95–104. [CrossRef]

70. Liu, Q.; Luo, L.; Zheng, L. Lignins: Biosynthesis and biological functions in plants. Int. J. Mol. Sci. 2018, 19, 335. [CrossRef]

71. Estrada, B.; Bernal, M.A.; Díaz, J.; Pomar, F.; Merino, F. Fruit development in Capsicum annuum: Changes in capsaicin, lignin, free phenolics, and peroxidase patterns. J. Agric. Food Chem. 2000, 48, 6234–6239. [CrossRef]

72. Díaz, J.; Pomar, F.; Bernal, A.; Merino, F. Peroxidases and the metabolism of capsaicin in Capsicum annuum L. Phytochem. Rev. 2004, 3, 139–157. [CrossRef]

73. Zhou, J.; Lee, C.; Zhong, R.; Ye, Z.H. MYB58 and MYB63 are transcriptional activators of the lignin biosynthetic pathway during secondary cell wall formation in Arabidopsis. Plant Cell 2009, 21, 248–266. [CrossRef]

74. Geng, P.; Zhang, S.; Liu, J.; Zhao, C.; Wu, J.; Cao, Y.; Fu, C.; Han, X.; He, H.; Zhao, Q. MYB20, MYB42, MYB43, and MYB85 regulate phenylalanine and lignin biosynthesis during secondary cell wall formation. Plant Physiol. 2020, 182, 1272–1283. [CrossRef] [PubMed]

75. Gómez-Garcia, M.R.; Ochoa-Alejo, N. Biochemistry and molecular biology of carotenoid biosynthesis in chili peppers (Capsicum spp.). Int. J. Mol. Sci. 2013, 14, 19025–19053. [CrossRef]

76. Kobayashi, K.; Suzuki, T.; Iwata, E.; Nakamichi, N.; Suzuki, T.; Chen, P.; Ohtani, M.; Ishida, T.; Hosoya, H.; Müller, S.; et al. Transcriptional repression by MYB3R proteins regulates plant organ growth. EMBO J. 2015, 34, 1992–2007. [CrossRef]

77. Hепworth, J.; Lenhard, M. Regulation of plant lateral-organ growth by modulating cell number and size. Curr. Opin. Plant Biol. 2013, 17, 36–42. [CrossRef] [PubMed]

78. Gómez-Garcia, M.R.; Ochoa-Alejo, N. Predominant role of the L-galactose pathway in L-ascorbic acid biosynthesis in fruits and leaves of the Capsicum annuum L. chili pepper. Braz. J. Bot. 2016, 39, 157–168. [CrossRef]

79. Kozłowski, L.P. IPC—Isoelectric Point Calculator. Biol. Direct 2016, 11, 55. [CrossRef] [PubMed]

80. Yu, C.S.; Chen, Y.C.; Lu, C.H.; Hwang, J.K. Prediction of protein subcellular localization. Proteins 2006, 64, 643–651. [CrossRef] [PubMed]

81. Hu, B.; Jin, J.; Guo, A.Y.; Zhang, H.; Luo, J.; Gao, G. GSDS 2.0: An upgraded gene feature visualization server. Bioinformatics 2015, 31, 1296–1297. [CrossRef] [PubMed]

82. Jiangtao, C.; Yingzhen, K.; Qian, W.; Yube, S.; Daping, G.; Jing, L.; Guanshan, L. MapGene2Chrom, a tool to draw gene physical map based on Perl and SVG languages. Yi Chuan 2015, 37, 91–97. [CrossRef] [PubMed]

83. Arce-Rodríguez, M.L.; Ochoa-Alejo, N. Silencing AT3 gene reduces the expression of pAmt, BCAT, Kas, and Acl genes involved in capsaicinoid biosynthesis in chili pepper fruits. Biol. Plant. 2015, 59, 477–484. [CrossRef]