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CaHY5 Mediates UV-B Induced Anthocyanin Biosynthesis in Purple Pepper

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Abstract: Anthocyanins are important flavonoid compounds in plants that are associated with the color formation and antioxidant activity of flowers, fruits, and other organs. Ultraviolet B radiation (UV-B) is one of the key environmental factors that influence anthocyanin accumulation in plants and *HY5* is involved in plant photomorphogenesis. However, the molecular mechanism of the signal network of UV-B regulating anthocyanin biosynthesis in capsicum via *HY5* remains unclear. In this study, we identified the transcription factor *CaHY5*, which mediates UV-B signaling, and demonstrated its regulatory role in anthocyanin biosynthesis in purple pepper (*Capsicum annuum* L.). The results showed that there were photoresponsive and hormone-responsive elements on the *CaHY5* promoter that responded to UV-B, indoleacetic acid, salicylic acid, 6-benzyladenine, abscisic acid, and melatonin treatments. UV-B treatment induced the expression of *CaHY5* and anthocyanin structural genes. *CaHY5* gene-silenced positive plants showed different degrees of the yellowing phenomenon, which affected the expression of the anthocyanin biosynthesis structural gene. The expression levels of anthocyanin biosynthesis-related genes in *CaHY5*-silenced positive plants increased considerably. This study provides insights into the role of *CaHY5* in UV-B-induced anthocyanin biosynthesis in purple pepper.

Keywords: *Capsicum annuum* L.; *CaHY5* gene; abiotic stress; anthocyanin biosynthesis

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1. Introduction

Various tissues of purple pepper (*Capsicum annuum* L.) exhibit a purple or purplish-black color due to the substantial accumulation of anthocyanins in the tissues [1]. Using high-performance liquid chromatography (HPLC) and other approaches, Taylor [2] discovered that the leaves of purple pepper contain a high concentration of a single anthocyanin, delphinidin-3-p coumaroylrutinoside-5-glucoside. This component confers a distinctive phenotype on capsicum [3].

Anthocyanins fall under the category of flavonoid compounds. The fundamental framework of anthocyanins consists of two benzene rings linked by a C3 unit, namely, the 2-phenylbenzopyrane structure, featuring hydroxyl and methoxy groups at different positions. Based on their distinct substitution positions, plant anthocyanins can be classified

into six types, namely delphinidin, peonidin, cyanidin, petunidin, malvidin, and pelargonidin [4–6]. In plants, anthocyanins are extensively distributed as secondary metabolites, which endow plants with blue, red, and purple hues, and have high ornamental and economic values. Anthocyanins not only attract pollinators and seed dispersal agents for reproduction, but also protect plants against various abiotic and biotic stresses [7–9].

Anthocyanins are predominantly accumulated in diverse plant organs, such as stems, leaves, flowers, and fruits, with the primary accumulation site being the vacuole [6,10]. Anthocyanins are also crucial pigments that confer various colors to plants. Phenylalanine serves as the direct precursor for anthocyanin biosynthesis. Anthocyanin uses phenylalanine as the substrate within plant cells and ultimately forms anthocyanin components through a succession of enzymatic reactions [11]. Genes influencing anthocyanin metabolism are categorized into structural and regulatory genes. The structural genes directly encode the enzymes necessary for the biosynthesis of anthocyanins, including *PAL*, *CHS*, *CHI*, *F3H*, *F3′5′H*, *DFR*, *ANS*, and *UFGT* [12]. Anthocyanin permeability enzyme, ANP and glutathione transporter enzyme, GS are also requisite for anthocyanin biosynthesis, transportation, and enrichment in vacuoles [13].

Presently, the regulatory genes associated with anthocyanin biosynthesis primarily include *R2R3-MYB*, *BHLH*, and *WD40* transcription factors [14–16]. MYB transcription factors co-regulate the expression of related structural genes by interacting with bHLH and WD40 to form a MBW complex. According to Tang [17], *CaANT1*, *CaANT2*, *CaAN1*, and *CaTTG1* are involved in anthocyanin accumulation in purple pepper and they can activate anthocyanin accumulation by forming new MBW transcription complexes. In addition to the MBW complex, transcription factors, such as *PIF3*, *HY5*, *COP1*, *WRKY*, *WIP*, *MADS-box*, *NAC*, and *SPL* are involved in transcriptional regulation of anthocyanin biosynthesis [18–20].

As one of the key environmental factors, light affects anthocyanin biosynthesis in plants [21]. The structural genes encoding enzymes associated with the anthocyanin biosynthetic pathway are predominantly regulated by light and their expression is upregulated under a high light intensity, but decreased or not expressed under low light or dark conditions [22,23]. Ultraviolet (UV) light, which is a component of the light spectrum, can induce anthocyanin biosynthesis in plants and because the ozone layer absorbs UV light below 280 nm, only UV-A and UV-B are of biological importance [24]. UV-B can directly promote anthocyanin accumulation by inducing the expression of plant anthocyanin biosynthesis structural genes, such as *CHS*, *ANS*, *UFGT*, among others [25,26]. UV-B can regulate phenylalanine ammonia-lyase (PAL) enzyme activity, affect anthocyanin biosynthesis in apple peels, and enhance apple coloring [27,28]. In *A. thaliana*, UV-B radiation can induce the expression of the *CHS* gene, promote the synthesis of phenylpropane substances, and enhance UV-B radiation absorption by plants, thereby reducing the damage caused by UV-B radiation to plants [29]. The expression of *CHS* and *CHI* genes involved in the flavonoid biosynthetic pathway is substantially upregulated in maize leaves treated with UV-B [30–32].

The transcription factor, HY5 belongs to the basic leucine zipper family (bZIP transcription factor), which is closely associated with plant photomorphogenesis. *HY5* occurs in different states when plants are under different darkness and light conditions. *HY5* is retained in the plant nucleus in the form of a small amount of phosphorylated *HY5* under dark conditions, and *COP1* interacts with and degrades *HY5. COP1* is transferred to the cytoplasm when plants are exposed to light, leading to the accumulation of HY5 [33,34]. Furthermore, *HY5* promotes photomorphogenesis and regulates CHS in anthocyanin biosynthesis. In *A. thaliana*, *HY5* inhibits *MYBL2* expression by controlling *MYBD* to promote anthocyanin accumulation [35].

In the UV-B signal transduction pathway, *HY5* can directly bind to the anthocyanin synthase structural gene and *cis*-acting elements of the transcription factor promoter to regulate anthocyanin biosynthesis [36]. In eggplant (*Solanum melongena*), SmHY5 can directly bind to the G-box element in the promoter region of *SmMYB35* gene to regulate the expression of *SmMYB35* [37] (Li et al., 2022). HY5 has been shown to regulate anthocyanin biosynthesis through transcriptional activation of *PAP1*. HY5 can recognize and bind to the *MYBL2* promoter, and inhibit its expression. miR858a inhibits the expression of *MYBL2* and is a negative regulator of anthocyanin biosynthesis. HY5-MIR858a-MYBL2 complex regulates phytoanthocyanin biosynthesis through transcriptional and post-transcriptional regulation in response to light and other environmental factors [37]. UV-B induces *BBX31* expression to promote flavonoid accumulation and HY5 can directly bind to the *BBX31* promoter and regulate its transcription level [38]. Anthocyanin accumulation in eggplants is regulated through light-mediated interaction of CRY1/CRY2-COP with SmHY5 and SmMYB1. PIF3 and HY5 directly bind to different regions of the same anthocyanin biosynthesis gene promoter to activate it and positively regulate anthocyanin biosynthesis, but are not directly associated with photochrome-mediated signaling [39].

To investigate the regulation of genes associated with anthocyanin biosynthesis in purple pepper by UV-B and *HY5*, this study analyzed the mechanisms of the transcription factor, *CaHY5* in regulating anthocyanin biosynthesis in purple pepper under different durations of UV-B treatment. In addition, the expression of *HY5* and anthocyanin biosynthesis-related genes, and changes in anthocyanin content in purple pepper under different durations of UV-B treatment were evaluated.

2. Materials and Methods

2.1. Experimental Materials

The purple pepper (*C. annuum* L.) used in this study was obtained from the Key Laboratory of Vegetable Biology of Yunnan Province, College of Landscape and Horticulture, Yunnan Agricultural University, Kunming, China. Under high light intensity, the exposed part of purple pepper appeared purple or purplish-black. The experimental materials were seeded and planted in cultivation pots with a diameter of 24.6 cm and a depth of 16 cm in late April of the same year. The inflorescences were artificially pollinated after they unfolded and a follow-up experiment was performed after 25 days of fruit development.

2.2. CaHY5 TFs Cloning and Bioinformatics Analysis

Total RNA was extracted from purple pepper using an RNA extraction kit (Huayueyang Biotech Co., Ltd., Beijing, China) and the first strand cDNA was synthesized using PrimeScript reverse transcription kit (Takara Bio Inc., Kusatsu, Japan). A novel rapid plant genomic DNA extraction kit (BioTeke Corporation, Beijing, China) was used to extract total DNA from purple pepper. Using cDNA as template, CaHY5 RT-PCR specific primers were designed using Primer Premier 6.0 (FOR: GGTGCTTATTCATTCCATCAG; REV: AATCAGAACAAGACAGTCCA) to clone CaHY5. The 2000 bp DNA sequences upstream of *CaHY5* gene coding sequences in the Zunla genome database were used as the reference sequence. Primers were designed (FOR: ACAACGGGTATGGCAAATCTTA; REV: GGAAGCAGCCTGATGGAATG), cloned, and sequenced the upstream promoter sequence of *CaHY5* using total DNA extracted from purple pepper as the template. The upstream *cis*-acting elements were predicted using PlantCARE (https://bioinformatics.psb.ugent.be/webtools/plantcare/html/ (accessed on 6 December 2021)). The cis-acting elements were screened and sorted using Microsoft Excel 365 (Microsoft Corp., Redmond, WA, USA), and visualized using Tbtools. The cloning reaction system and procedures used in the study are described in detail in Supplementary Table S1.

Bioinformatics analysis of *CaHY5* gene and protein sequences was performed according to a previously described method [40].

2.3. Subcellular Localization of CaHY5

The pAN580-GFP vector was double-digested with XbaI and BamHI restriction enzymes (New England Biolabs, Ipswich, MA, USA), and the full-length *CaHY5* gene sequence was used as the insertion fragment. The inserted fragment and linearized vector were recombined into a pCaHY5-AN580 recombinant vector using In-Fusion HD Cloning kit (Takara Bio Inc., Kusatsu, Japan), according to the manufacturer's instructions. Subcellular localization of *CaHY5* was analyzed according to a previously described method [40].

2.4. Hormone Treatments

The concentration of treated hormone was determined by reference to relevant literature and pre-experiment [41]. Aqueous solutions of indole-3-acetic acid (IAA, 10 μM), 6-benzyladenine (6-BA, 1 μM), melatonin (MT, 100 μM), salicylic acid (SA, 100 μM), and abscisic acid (ABA, 1 μM) were prepared(Sigma-Aldrich, MO, USA). Uniformly grown and sized purple pepper fruits were selected and 30 biological replicates were set for each treatment. Distilled water was used as the control. The plant hormone solutions of each concentration were evenly sprayed on the surface of purple pepper plants. Purple pepper fruit samples were collected at 0, 0.5, 1, 3, and 6 h. The treated and control fruits were stored at −80 °C after quick freezing in liquid nitrogen or used directly to conduct subsequent tests.

2.5. Light Treatments

After 25 days of fruit development, purple pepper plants were subjected to UV-B treatment by placing a UV-B lamp (313 nm, irradiance range was less than or equal to 0.8 W/m^2) 30 cm away from the plants [42]. Under the same conditions, the purple pepper plants were placed in an incubator with 100% light to simulate white light and 0% light to simulate dark treatment. The temperature of the incubator was set to 28 °C for all treatments. The leaves, stems, and fruits of purple pepper plants treated with UV-B, white light, and dark conditions over the same period (25 days after flowering) were sampled at 0, 0.5, 1, 3, 6, 12, and 24 h, frozen in liquid nitrogen, and stored at −80 °C for subsequent analysis.

2.6. Anthocyanin Content Determination

Purple pepper samples (0.5 g) were mixed with liquid nitrogen and ground into powder in a mortar. Thereafter, 95% ethanol–1.5 $mol \cdot L^{-1}$ hydrochloric acid (85:15, v/v) mixture was added to the samples and ultrasonic extraction was performed for 30 min, followed by centrifugation at 4 °C and 5000 rpm for 5 min. The supernatant was collected as a liquid and used to determine anthocyanin content by HPLC (Agilent 1260; Agilent Technologies, Santa Clara, CA, USA), according to the method described by [43].

2.7. Analysis of Relative Gene Expression

The cDNA was synthesized from untreated purple pepper organs using the method described in the preceding section. Quantitative real-time (qRT-PCR) was performed using Hieff qPCR SYBR Green Master Mix kit (Yeasen, Biotechnology Co., Ltd., Shanghai, China), according to the manufacturer's instructions. qRT-PCR reaction was performed on QuantStudio5 and the relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method. The primers used are listed in Supplementary Table S2.

2.8. Virus-Induced Gene Silencing

For gene silencing, *CaHY5* fragment was silenced using the SGN VIGS tool (https://vigs.solgenomics.net/ (accessed on 10 April 2021)). The pTRV2 plasmid was double-digested with EcoR1 and BAMH1 restriction enzymes (New England Biolabs, Ipswich, MA, USA), and the pTRV2::CaHY5 silencing plasmid was reconstructed. pTRV1, pTRV2::PDS, and pTRV2::CaHY5 were transformed into the Agrobacterium strain GV3101. GV3101 pTRV1 with an OD600 = 0.8 was mixed with pTRV2::HY5, pTRV2::PDS, and pTRV2::00 bacterial solutions in a ratio of 1:1. The bacterial solution was gradually injected on the back of the two purple pepper cotyledons after they unfolded using a syringe. The expression levels of *CaHY5* and *ABGs* related genes were determined by qRT-PCR. The positive silenced plants were exposed to white light, darkness, and UV-B, and the expression levels of related genes were determined after 3 h of treatment. Three biological replicates were set for each test [40]. The primers used are listed in Supplementary Table S3.

2.9. Transient Expression of CaHY5 in Purple Pepper

Full-length cDNAs of *CaHY5* were cloned into pCambia1300: GUS [43]. The Agrobacterium tumefaciens strain GV3101 harboring pCambia1300-CaHY5: GUS or pCambia1300-GUS (used as control) was cultured overnight in an induction medium and the infiltrated tissue was collected three days after inoculation for further analysis [43]. Agrobacterium tumefaciens strain GV3101 harboring each construct was injected on the back of the purple pepper leaves. The expression levels of *CaHY5* and *ABGs* related genes were determined by qRT-PCR. The positive silenced plants were exposed to white light, darkness, and UV-B, and the expression levels of related genes were determined after 3 h of treatment. Three biological replicates were set for each test. The primers used are listed in Supplementary Table S3.

2.10. Statistical Analyses

Data collection and sorting were conducted using Microsoft Excel 365 (Microsoft Corp., Redmond, WA, USA). IBM SPSS Statistics 23.0 software was used to conduct Duncan's multiple range tests for one-way ANOVA. Significant differences were indicated when $p < 0.05$. Least significant difference test was used to determine significant differences between treatments. Graphs were plotted using GraphPad Prism 9 (GraphPad Software Inc., La Jolla, CA, USA).

3. Results

3.1. Anthocyanin Content in Purple Pepper After Exposure to Different Light Treatments

After 24 h of UV-B continuous treatment, there was no significant difference in the phenotype of purple peppers. Large purple spots were observed on purple pepper leaves after 20 days of UV-B treatment. The color of purple pepper leaves after UV-B treatment was purple, while the color of purple pepper leaves exposed to the dark treatment was yellow (Figure 1). Delphinin was only detected in purple pepper plants exposed to UV-B and its content was 248.56 $\mu g \cdot g^{-1}$.

3.2. Expression Levels of Transcription Factors and Anthocyanin Biosynthesis Genes in Purple Pepper Fruits Under Different Light Treatments

The results showed that UV-B treatment had varying effects on purple pepper fruits when compared to white light and dark treatments (Figure 2). Based on gene expression, the transcription factors *CaHY5* and *CaMYB113* were clustered together with anthocyanin biosynthesis structural genes *CaDFR*, *CaUFGT*, *CaF3H*, *CaC4H*, and *Ca4CL*, with similar expression patterns being observed. The anthocyanin biosynthesis structural genes, *CaPAL* and *CaF3′5′H* exhibited high expression levels, especially under UV-B treatment.

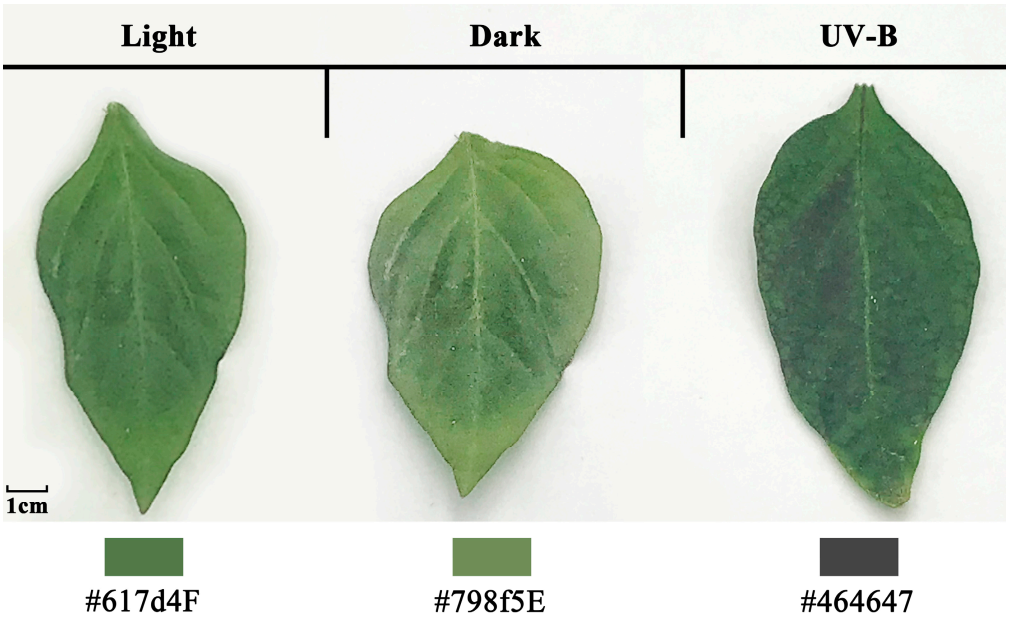

Figure 1. Effects of different treatments on leaf phenotype of purple pepper. The leaves in the figure show the phenotype of pepper leaves under different treatments. The bottom pair of the leaf should visualize the color block and hexadecimal color code of the leaf color.

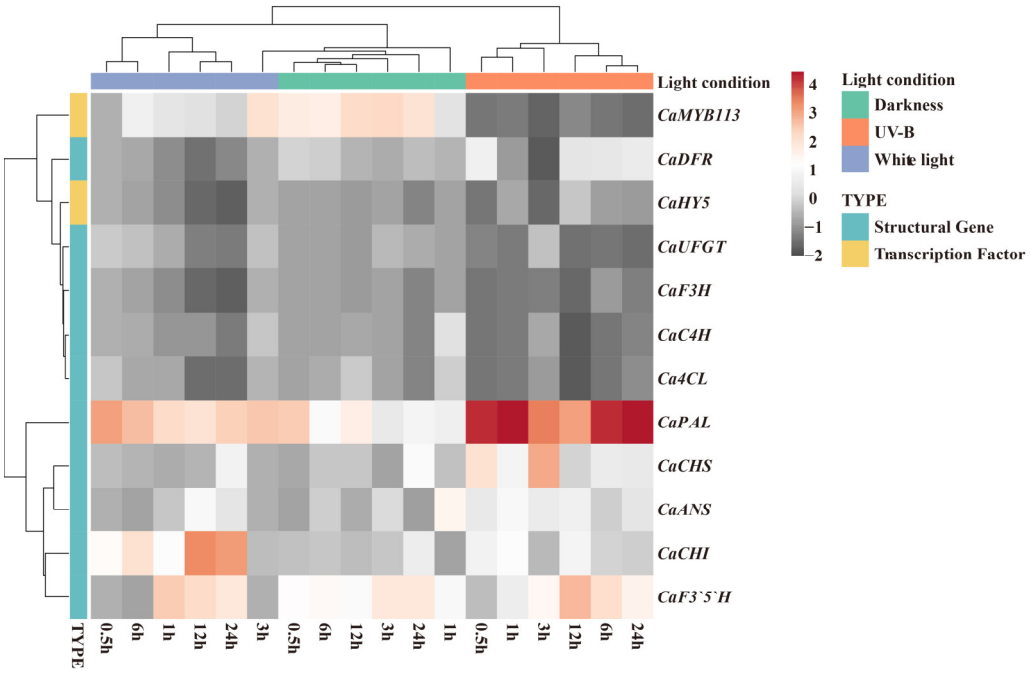

Figure 2. Heat map clustering of TFs and ABGs in pepper under different light conditions.

3.3. CaHY5 Cloning and Bioinformatics Analysis

The CaHY5 gene in purple pepper was isolated by homologous cloning method. The gel electrophoresis of CaHY5 gene amplification is shown in Supplementary Figure S1. Sequencing of specific amplification products showed that the RT-PCR product was 788 bp in length, contained an open reading frame of 477 bp, and encoded 158 amino acid residues (Supplementary Figure S2). The HY5 gene sequence obtained was homologous to the HY5 sequence in the NCBI database (GeneID: 107838859); therefore, the HY5 in purple pepper was named CaHY5. The molecular formula of CaHY5 protein was $C_{715}H_{1216}N_{240}O_{251}S_4$ and its molecular weight was 1.73 kDa. The CaHY5 protein had a theoretical isoelectric

point of 9.69, was fat-soluble, hydrophilic, and unstable. Moreover, the CaHY5 protein had no signal peptide, no transmembrane structure, and had 24 phosphorylation sites, including 7 T-type, 17 S-type, and no Y-type phosphorylation site.

The transcription factor CaHY5 belongs to the leucine zipper or bZIP family. Their site for protein structure domain: (ENKRLKRLLRNRVSAQQARERKKAYLIDLEARVKELETK-NAELEERLSTLQNENQMLRHILKNT) 86–149. The amino acid sequence of CaHY5 of purple pepper was compared by BLAST and 15 significant conserved motifs were obtained by performing the motif significance test (Figure 3A). Motifs 8, 3, 7, 5, 2, and 1 had six different conserved motifs, which are the predicted motifs in plants, and they influence the function of these proteins. Combined with the prediction results of CaHY5 protein domain, motifs 2 and 1 cover the bZIP domain, which is the specific motif for this type of protein.

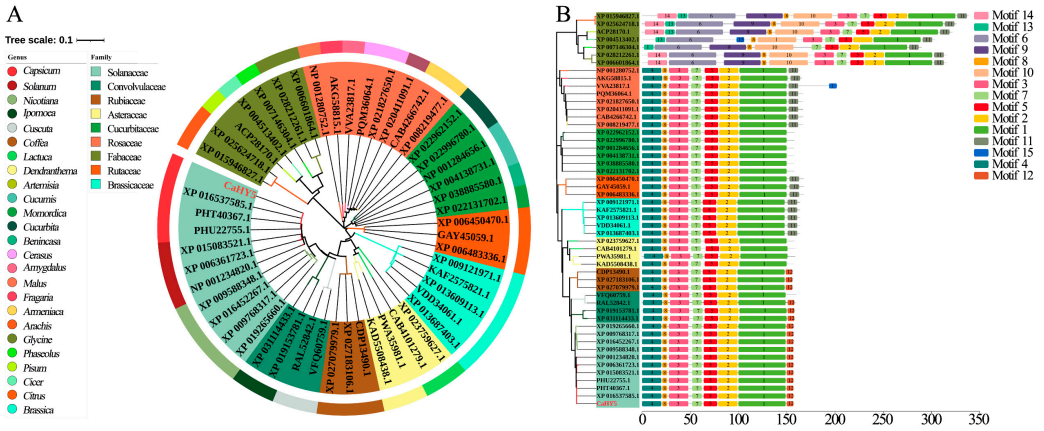

Figure 3. Phylogenic tree of HY5 transcription factors (TFs)and motif analysis of amino acids in HY5 of different species. (**A**) Phylogenetic tree comprising CaHY5 in purple pepper and 50 HY5 TFs in plant. (**B**) Motif analysis of HY5 protein in purple pepper and 50 other plants.

The prediction results of the secondary structure of proteins showed that the CaHY5 protein consisted of 77 α helices (48.73%), 71 random crimps (44.94%), nine β corners (5.70%), and one extended chain (0.63%) (Supplementary Figure S2). The tertiary structure of the CaHY5 protein was predicted based on I-TASSER. The predicted CaHY5 ligand structure C value was 0.20, and the binding site residues were 96, 99, 100, and 103 (Supplementary Figure S2). A phylogenetic tree was constructed using the adjacency matrix method with a total of 51 HY5 amino acid sequences from nine families selected (Figure 3B). The results showed that CaHY5 had the farthest relationship with legumes (Fabaceae) and tobacco (Solanaceae), but had the closest relationship with capsicum (Solanaceae).

3.4. Subcellular Localization of CaHY5 in Purple Pepper

The pCaHY5-AN580-GFP recombinant vector (Figure 4A) was transferred into the protoplasts of A. thaliana using an empty pAN580-GFP vector as a control. According to the results, pAN580-GFP exhibited green fluorescence in the protoplast nucleus, cytoplasm, and cell membrane of A. thaliana, while the fusion protein pCaHY5-AN580-GFP exhibited green fluorescence in the protoplast nucleus (Figure 4B), indicating that the CaHY5 protein was located in the nucleus.

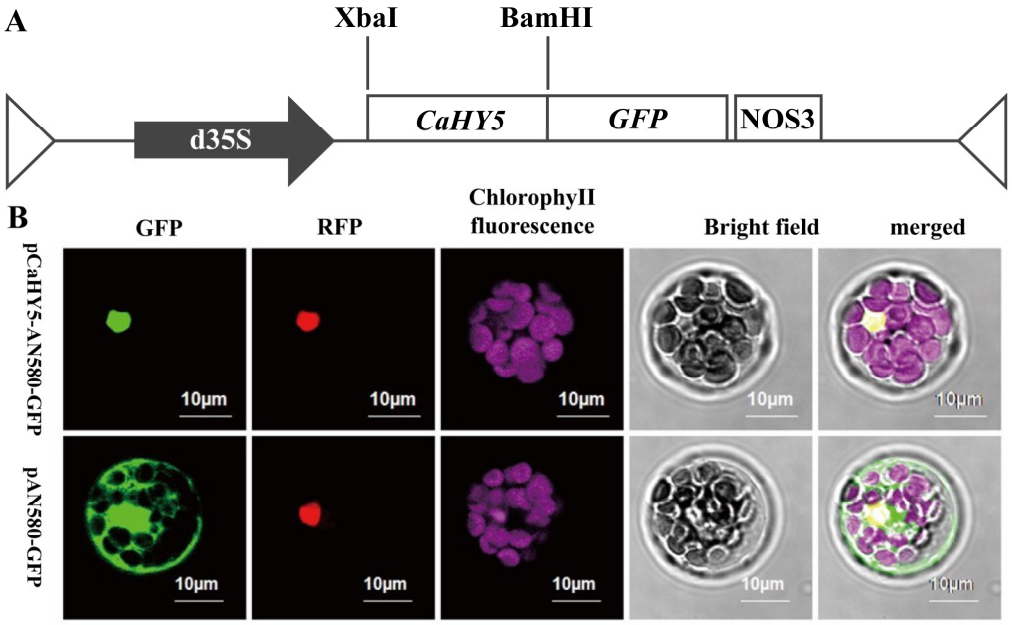

Figure 4. Subcellular localization of CaHY5 in purple pepper. (**A**) It was the schematic diagram of pCAHY5-AN580-GFP vector construction. (**B**) Green fluorescent protein (GFP) signals indicated that CaHY5 was localized to nucleus. GFP fluorescence signals were detected using a Zeiss lsm710 confocal laser scanning microscope.

3.5. Upstream Cis-Element Analysis of CaHY5 and Heat Map of CaHY5 Expression Under Different Exogenous Factors

The upstream promoter of CaHY5 gene in purple pepper was cloned using total DNA of purple pepper as template, and its length was 1935 bp after sequencing (Supplementary Figure S3). PlantCARE prediction results showed that the CaHY5 promoter of purple pepper differed from that of Zunla. CaHY5 in purple pepper had defense against stress and hormone response cis-elements, such as ABA and SA (Figure 5A), indicating that CaHY5 was regulated by stress and plant hormones. To verify whether different plant hormones affect CaHY5 expression in purple pepper, water was used as a control in this study and quantitative fluorescence PCR was used to determine the expression level of CaHY5 under five different plant hormones (IAA, SA, 6-BA, ABA, and MT). The results showed that IAA, SA, 6-BA, ABA, and MT affected CaHY5 expression after 0.5–6 h of plant hormone treatments and the expression patterns of CaHY5 varied under different plant hormone treatments. CaHY5 expression was considerably affected by IAA and MT treatments, with its expression reaching a peak 1 h after IAA treatment. CaHY5 expression was substantially upregulated under MT treatment, although its expression after 1 h of treatment was low (Figure 5B).

3.6. Downregulation of CaHY5 Decreases Genes of Anthocyanin Biogenesis Pathway Expression

The anthocyanin biogenesis pathway and virus-induced gene silencing (VIGS) vector are shown in Figure 6A,B. The results showed that the CaPDS-silenced purple pepper plants at the cotyledon stage were bleached 21 days after inoculation (Figure 6C), indicating that the silencing system was precise. The leaves of CaHY5-silenced plants exhibited different degrees of bleaching, although the change in leaf color was weaker than that of CaPDS-silenced plants. The qPCR was used to determine the expression levels of CaHY5 gene and anthocyanin biosynthesis-related genes in CaHY5-silenced plants. The results showed that expression level of the CaHY5 gene was downregulated to varying degrees. CaHY5 expression in CaHY5-silenced plants was significantly different from that in control and pTRV2::00 plants, indicating that CaHY5 gene silencing was successful. The expression

levels of all anthocyanin biosynthesis-related genes were downregulated to varying degrees and differences in their expression levels were significant ($p < 0.05$). Among the anthocyanin biosynthesis-related genes, CaHY5 had the greatest effect on CaF3H gene after silencing and its expression level was only 0.41% when compared to that of the control ($p < 0.05$) (Figure 6D).

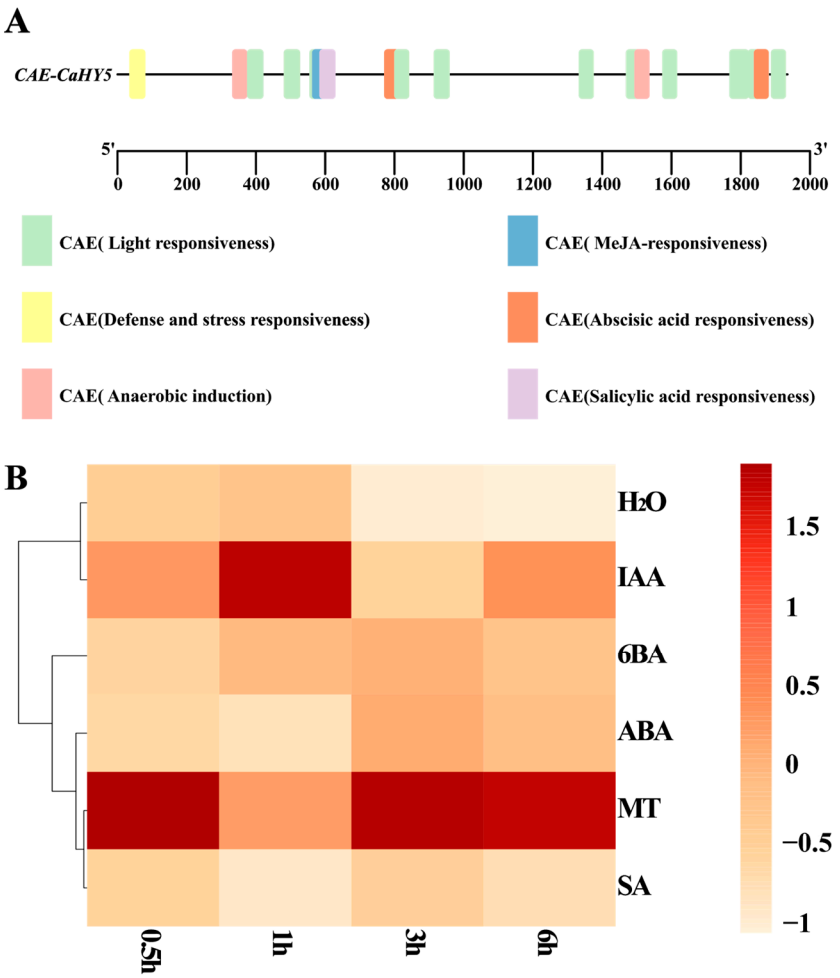

Figure 5. Upstream cis-element analysis of CaHY5 and heat map of CaHY5 expression under different exogenous factors. (**A**) Cis-element analysis of 2000 bp upstream of CaHY5 in purple pepper. (**B**) The expression levels of CaHY5 TFs after 0.5–6 h treatment with IAA, SA, 6BA, ABA, and MT.

Regarding the effect of light on gene expression, the results showed that anthocyanin biosynthesis-related genes in purple pepper plants exposed to different light conditions were upregulated to varying degrees (Figure 6E). The expression level of the *CaHY5* gene was significantly higher in positive silenced plants exposed to white light and UV-B treatments than that in untreated silenced plants ($p < 0.05$), indicating that white light and UV-B induced *CaHY5* gene expression, with UV-B exerting the strongest effect ($p < 0.05$). The expression of the *CaCOP1* gene was upregulated to a certain extent under dark and UV-B treatments. White light had the greatest effect on *CaMYB113*, followed by UV-B treatment ($p < 0.05$). UV-B treatment exerted the strongest effect on structural genes associated with anthocyanin biosynthesis ($p < 0.05$), with the expression levels of *CaPAL*, *CaC4H*, and *Ca4CL* increasing substantially. However, the expression levels of *CaCHS*, *CaF3H*, and *CaF3′5′H* were slightly increased ($p < 0.05$).

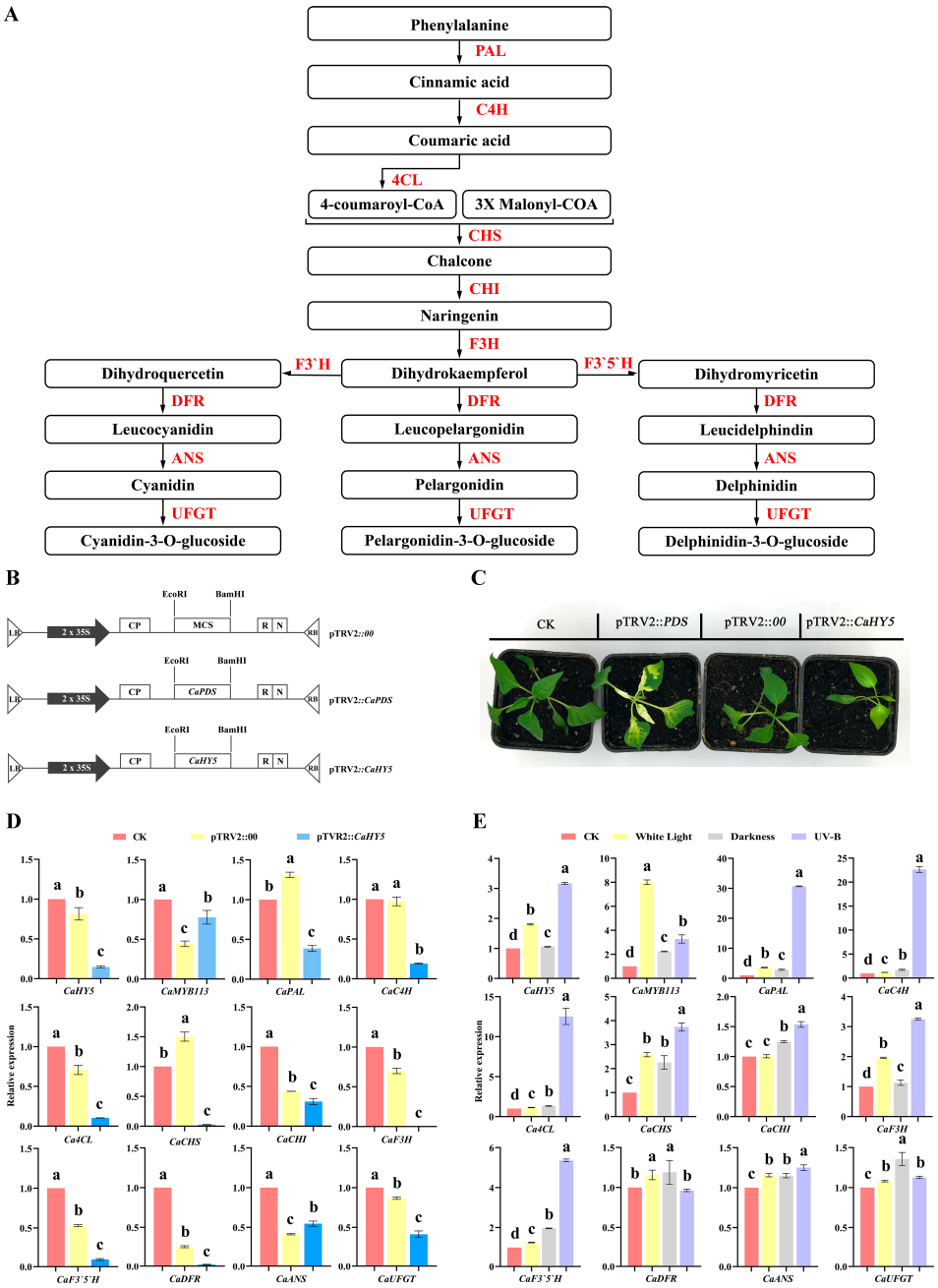

Figure 6. Anthocyanin biosynthetic pathway, VIGS vector, silenced plant phenotype and related gene expression level. (**A**) Schematic diagram of anthocyanin biosynthesis pathway. (**B**) VIGS vector construction diagram. (**C**) Pepper's phenotype of VIGS. CK is pepper without inoculation; pTRV2::00 was inoculated with pTRV2 empty vector strain. pTRV2:: CaHY5 was a silent plant of CaHY5. (**D**) Expression levels of CaHY5 TFs and anthocyanin biosynthesis-related genes after CaHY5 silencing. (**E**) Effects of different light conditions on genes related to anthocyanin biosynthesis in silenced pepper. The same letters represent no significant difference ($p \geq 0.05$), while different letters represent significant difference ($p < 0.05$).

3.7. Transient Overexpression of CaHY5 in Purple Pepper

GUS staining results showed that both 35S:GUS and 35S:CaHY5-GUS leaves stained blue (Figure 7A), indicating successful CaHY5-GUS expression. The results of qPCR showed that the expression level of *CaHY5* in purple pepper leaves treated with 35S: CaHY5-GUS increased significantly by 111-fold that in leaves treated with 35S:GUS ($p < 0.05$) (Figure 7B). The transcription factor *CaMYB113* was upregulated together with *CaC4H*, *CaF3H*, *CaF3′5′H*,

CaDFR, and *CaANS* to varying degrees. Overexpression of *CaHY5* had a significant effect on the expression of *CaF3H* and *CaDFR* genes ($p < 0.05$). The results suggested that *CaHY5* regulated anthocyanin biosynthesis in purple pepper by regulating MYB113 and anthocyanin biosynthesis genes.

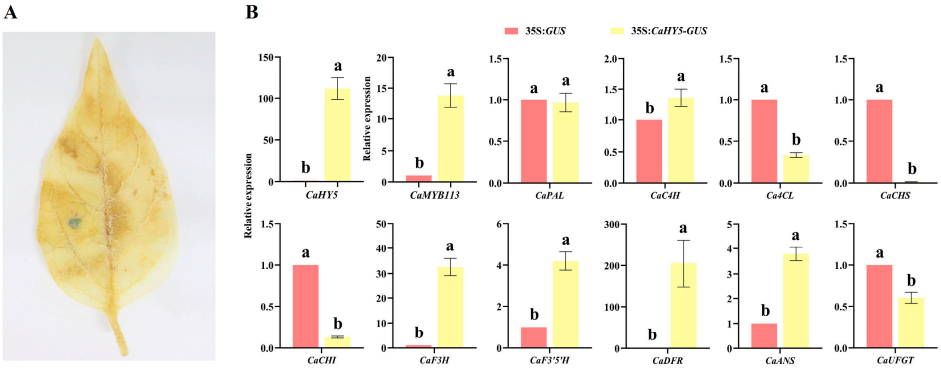

Figure 7. GUS staining of pepper tissues and related gene expression level. (**A**) Staining of 35S:GUS and 35S:CaHY5-GUS pepper tissues. (**B**) Expression levels of TFs and ABGs after CaHY5 transient overexpression. The same letters represent no significant difference ($p \geq 0.05$), while different letters represent a significant difference ($p < 0.05$).

4. Discussion

4.1. CaHY5 Gene Is Regulated by Several Factors

As key transcription factors involved in photomorphogenesis, 15 *cis*-elements related to photoresponse, as well as plant hormone and anaerobic responses were identified at 2000 bp upstream of the *HY5* gene in purple pepper. In addition to participating in photomorphogenesis, HY5 facilitates the regulation of plant hormones during the growth of purple pepper. According to previous studies, HY5 is involved in the signaling pathways of gibberellins, cytokinins, and other plant hormones [44]. The *CaHY5* gene is a key factor influencing responses to abiotic stress, reactive oxygen species, and other signaling pathways. In this study, the expression of *CaHY5* gene was upregulated to varying degrees under different exogenous factors, suggesting that it regulated hormone signaling and response in purple pepper. In addition, the results showed that MT had the greatest effect on the up-regulation of *CaHY5* expression, indicating that *CaHY5* gene was involved in MT signal response.

4.2. Effect of Different Light Conditions on Anthocyanin Biosynthesis in Purple Pepper

Light is one of the key environmental factors affecting plant growth and development. *HY5*, a member of the *ZIP* transcription factor family, regulates plant growth and development, as well as pigment accumulation in a light-dependent manner [44,45]. The results of this study revealed significant differences in the expression patterns of *CaHY5* and *CaCOP1* genes in purple pepper under white light and dark treatments. Specifically, the expression levels of *CaHY5* and *CaCOP1* genes under UV-B treatment, particularly in purple pepper fruits increased considerably. The results indicate that *CaHY5* and *CaCOP1* genes are involved in regulating the response of purple pepper to UV-B.

With regard to structural genes involved in anthocyanin biosynthesis, the expression levels of *CaPAL*, *CaCHI*, *CaF3′5′H*, and *CaANS* were significantly upregulated by 122.3~2183.1-fold under white light treatment. The expression levels of *CaPAL*, *CaCHS*, *CaCHI*, *CaF3′5′H*, *CaANS*, and other genes were substantially affected by UV-B treatment, with their expression levels being upregulated by 158.2~6271.6-fold. Studies have shown that almost all genes involved in anthocyanin biosynthesis are affected by light [46]. However, UV-B can regulate PAL enzyme activity and directly induce the expression of *CHS*,

CHI, *ANS* and other anthocyanin biosynthesis genes in plants, which is consistent with the results of this study [25,26,30,31]. Therefore, structural genes involved in anthocyanin biosynthesis in purple pepper can be regulated indirectly by the transcription factor, *CaHY5* or directly in response to UV-B. Although the phenotypic changes in purple pepper were not evident after 24 h of continuous treatment, large areas of purple plaques were observed on the surfaces of leaves after 20 days of UV-B treatment. The color of purple pepper leaves turned purple after UV-B treatment. However, the color of purple pepper leaves turned yellow after dark treatment (Figure 1). The results suggested that UV-B treatment induced anthocyanin biosynthesis and accumulation, but the accumulation occurred after a certain duration of time.

Notably, some anthocyanin biosynthesis genes were also upregulated to varying degrees under dark conditions, but the overall upregulation was minimal [3,6]. Zhou [47] found that the predominant anthocyanin in pepper was delphinidin. A small amount of delphinidin (3-O-glucoside) and a substantially low amount of pelargonidin (3-O-galactoside) are still synthesized. Furthermore, pelargonidin has been shown to accumulate only under dark conditions. *DFR* and *F3′5′H* genes regulate pelargonidin biosynthesis in larkspur flower (*Delphinium* sp.) [48]. In this study, *CaDFR* and *CaF3′5′H* and their downstream synthetic genes were up-regulated to varying degrees in the leaves and fruits of dark treated purple pepper. It is shown that different types of anthocyanins such as anthocyanins under light and dark treatment, although the content is low, but still accumulate.

The bZIP transcription factor, *HY5* is a major hub for optical signal cascades under visible and UV-B light [49]. The results of this study showed that the *CaHY5*-silenced plants exhibited varying degrees of yellowing and white spots on leaves. The expression levels of *CaHY5*, *CaCOP1*, and capsaicin biosynthesis genes were not downregulated, whereas those of *CaC4H*, *CaCHS*, *CaF3H*, *CaF3′5′H*, and *CaDFR* were substantially downregulated. In *A. thaliana*, HY5 and LZF1 regulated anthocyanin and chlorophyll accumulation, and played a positive regulatory role in the deyelization process in *A. thaliana* [21,50]. Therefore, the change in leaf color observed in *CaHY5*-silenced plants could be because of the decreased expression of anthocyanin and chlorophyll biosynthesis-related genes. In addition, *CaPDS* gene expression decreased after *CaHY5* silencing (Figure 6). *PDS* is a key gene involved in carotenoid biosynthesis.

4.3. UV-B Induces Expression of Anthocyanin Biosynthesis Genes to Promote Anthocyanin Accumulation

Previous studies have shown that UV-B can directly promote anthocyanin accumulation by inducing the expression of *CHS*, *ANS*, *UFGT*, and other structural genes involved in anthocyanin biosynthesis [25,26]. Under UV-B treatment, *CaPAL*, *CaC4H*, and *Ca4CL* were responsive to anthocyanin biosynthesis genes and the expression of *CaCHS*, *CaF3H*, and *CaF3′5′*H was upregulated. The results suggested that UV-B directly induced the expression of structural genes associated with anthocyanin biosynthesis in purple pepper, which is consistent with the findings of previous studies.

4.4. CaHY5 Directly Regulates CaF3H Expression to Promote Anthocyanin Accumulation in Purple Pepper

Previous studies have shown that the transcription factor, HY5 can directly recognize and bind upstream *cis*-elements of structural genes associated with anthocyanin biosynthesis in tomato (*Solanum lycopersicum*), such as G-box and ACGT elements in *CHS1*, *CHS2*, and *DFR* promoters [51]. An analysis of the upstream promoter of capsaicin biosynthesis-related genes revealed that CaHY5 regulated capsaicin biosynthesis by recognizing and binding to the G-box and ACGT elements in the upstream promoter region of capsaicin biosynthesis-related genes. *CaHY5* silencing had the greatest effect on the *CaF3H* gene

in *CaHY5*-silenced plants. Analysis of *cis*-elements in the upstream promoter region of the *CaHY5* gene revealed the presence of G-box *cis*-elements upstream of the *CaF3H* gene. Therefore, anthocyanin biosynthesis genes in purple pepper was directly regulated by UV-B and indirectly regulated by CaHY5.

The intensity of UV-B radiation is very important for plants, and appropriate radiation dose can promote the establishment of plant photomorphology and improve plant tolerance to adversity, while excessive radiation dose will lead to plant damage [52]. However, due to the decrease of ozone level, the dose of UV-B radiation in the environment is increasing year by year, so it has great potential threat to the growth and development of plants [53]. Therefore, this study explains how purple pepper responds to UV-B and promotes anthocyanin biosynthesis, which has certain guiding significance in pepper production. Meanwhile, because purple pepper has high ornamental value, research on its anthocyanin biosynthesis will help us better understand its regulatory mechanism and help to cultivate purple pepper varieties.

5. Conclusions

This study showed that anthocyanin biosynthesis in purple pepper under UV-B treatment was regulated through various mechanisms. UV-B directly regulated structural genes involved in anthocyanin biosynthesis. The transcription factor CaHY5 also indirectly regulated the expression of anthocyanin biosynthesis-related genes, such as *CaCHS*, *CaF3H*, *CaF3′5′H*, thereby enhancing anthocyanin accumulation in purple pepper.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy15010028/s1, Figure S1: *CaHY5* gel electrophoresis; Figure S2: CaHY5 protein structure; Figure S3: Gel electrophoresis of *CaHY5* gene promoter clone; Table S1: PCR reaction system and procedure; Table S2: The primer table of qRT-PCR; Table S3: Recombinant primers for plasmid construction.

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References

1. Li, N.; Liu, Y.; Yin, Y.; Gao, S.; Wu, F.; Yu, C.; Wang, F.; Kang, B.C.; Xu, K.; Jiao, C. Identification of *CaPs* locus involving in purple stripe formation on unripe fruit, reveals allelic variation and alternative splicing of R2R3-MYB transcription factor in pepper (*Capsicum annuum* L.). *Front. Plant Sci.* **2023**, *14*, 1140851. [CrossRef] [PubMed]
2. Taylor, C.L. Purple Pepper Plants, an Anthocyanin Powerhouse: Extraction, Separation and Characterization. Ph.D. Thesis, University of Maryland, College Park, MD, USA, 2014.
3. Chen, T.-T.; Liu, H.; Li, Y.-P.; Yao, X.-H.; Qin, W.; Yan, X.; Wang, X.-Y.; Peng, B.-W.; Zhang, Y.-J.; Shao, J. *AaSEPALLATA1* integrates jasmonate and light-regulated glandular secretory trichome initiation in *Artemisia annua*. *Plant Physiol.* **2023**, *192*, 1483–1497. [CrossRef]

4. Castañeda-Ovando, A.; De Lourdes Pacheco-Hernández, M.; Páez-Hernández, M.E.; Rodríguez, J.A.; Galán-Vidal, C.A. Chemical studies of anthocyanins: A review. *Food Chem.* **2009**, *113*, 859–871. [CrossRef]
5. An, J.P.; Xu, R.R.; Wang, X.N.; Zhang, X.W.; You, C.X.; Han, Y. *MdbHLH162* connects the gibberellin and jasmonic acid signals to regulate anthocyanin biosynthesis in apple. *J. Integr. Plant Biol.* **2024**, *66*, 265–284. [CrossRef] [PubMed]
6. Li, J.; Zhang, C.; Xu, X.; Su, Y.; Gao, Y.; Yang, J.; Xie, C.; Ma, J. A MYB family transcription factor *TdRCA1* from wild emmer wheat regulates anthocyanin biosynthesis in coleoptile. *Theor. Appl. Genet.* **2024**, *137*, 208. [CrossRef] [PubMed]
7. Gould, K.S. Nature′s Swiss Army Knife: The Diverse Protective Roles of Anthocyanins in Leaves. *BioMed Res. Int.* **2004**, *2004*, 314–320. [CrossRef]
8. Liu, Y.; Tikunov, Y.; Schouten, R.E.; Marcelis, L.F.; Visser, R.G.; Bovy, A. Anthocyanin biosynthesis and degradation mechanisms in Solanaceous vegetables: A review. *Front. Chem.* **2018**, *6*, 52. [CrossRef]
9. Mazza, G. *Anthocyanins in Fruits, Vegetables, and Grains*; CRC Press: Boca Raton, FL, USA, 2018.
10. Ni, J.; Wang, S.; Yu, W.; Liao, Y.; Pan, C.; Zhang, M.; Tao, R.; Wei, J.; Gao, Y.; Wang, D. The ethylene-responsive transcription factor *PpERF9* represses *PpRAP2. 4* and *PpMYB114* via histone deacetylation to inhibit anthocyanin biosynthesis in pear. *Plant Cell* **2023**, *35*, 2271–2292. [CrossRef] [PubMed]
11. Menconi, J.; Perata, P.; Gonzali, S. In pursuit of purple: Anthocyanin biosynthesis in fruits of the tomato clade. *Trends Plant Sci.* **2024**, *29*, 589–604. [CrossRef]
12. Jaakola, L. New insights into the regulation of anthocyanin biosynthesis in fruits. *Trends Plant Sci.* **2013**, *18*, 477–483. [CrossRef] [PubMed]
13. Aza-Gonzalez, C.; Herrera-Isidrón, L.; Núñez-Palenius, H.; Martínez De La Vega, O.; Ochoa-Alejo, N. Anthocyanin accumulation and expression analysis of biosynthesis-related genes during chili pepper fruit development. *Biol. Plant.* **2013**, *57*, 49–55. [CrossRef]
14. Koes, R.; Verweij, W.; Quattrocchio, F. Flavonoids: A colorful model for the regulation and evolution of biochemical pathways. *Trends Plant Sci.* **2005**, *10*, 236–342. [CrossRef] [PubMed]
15. Saito, K.; Yonekura-Sakakibara, K.; Nakabayashi, R.; Higashi, Y.; Yamazaki, M.; Tohge, T.; Fernie, A.R. The flavonoid biosynthetic pathway in Arabidopsis: Structural and genetic diversity. *Plant Physiol. Biochem.* **2013**, *72*, 21–34. [CrossRef] [PubMed]
16. Chen, J.; Wu, W.; Gao, S.; Jahan, M.S.; Xiao, J.; Guo, T.; Chen, C.; Li, B.; Luo, C.; He, X. Integrated transcriptome and metabolome analyses reveal anthocyanin biosynthesis in red and green mango pericarps under light and shade conditions. *Sci. Hortic.* **2024**, *338*, 113617. [CrossRef]
17. Tang, B.; Li, L.; Hu, Z.; Chen, Y.; Tan, T.; Jia, Y.; Xie, Q.; Chen, G. Anthocyanin accumulation and transcriptional regulation of anthocyanin biosynthesis in purple pepper. *J. Agric. Food Chem.* **2020**, *68*, 12152–12163. [CrossRef] [PubMed]
18. Das, P.K.; Shin, D.H.; Choi, S.-B.; Park, Y.-I. Sugar-hormone cross-talk in anthocyanin biosynthesis. *Mol. Cells* **2012**, *34*, 501–507. [CrossRef] [PubMed]
19. Zhou, H.; Lin-Wang, K.; Wang, H.; Gu, C.; Dare, A.P.; Espley, R.V.; He, H.; Allan, A.C.; Han, Y. Molecular genetics of blood-fleshed peach reveals activation of anthocyanin biosynthesis by *NAC* transcription factors. *Plant J.* **2015**, *82*, 105–121. [CrossRef] [PubMed]
20. Lu, B.-Y.; Gong, Z.-H. Research Progress of Anthocyanin Biosynthesis and Regulation from Purple Pepper (*Capsicum annuum* L.). In Proceedings of the 2017 2nd International Conference on Biological Sciences and Technology (BST 2017), F, Zhuhai, China, 17–19 November 2018; Atlantis Press: Amsterdam, The Netherlands, 2018.
21. Zhao, Y.; Min, T.; Chen, M.; Wang, H.; Zhu, C.; Jin, R.; Allan, A.C.; Lin-Wang, K.; Xu, C. The photomorphogenic transcription factor *PpHY5* regulates anthocyanin accumulation in response to UVA and UVB irradiation. *Front. Plant Sci.* **2021**, *11*, 603178. [CrossRef]
22. Alabd, A.; Ahmad, M.; Zhang, X.; Gao, Y.; Peng, L.; Zhang, L.; Ni, J.; Bai, S.; Teng, Y. Light-responsive transcription factor *PpWRKY44* induces anthocyanin accumulation by regulating *PpMYB10* expression in pear. *Hortic. Res.* **2022**, *9*, uhac199. [CrossRef]
23. Kaur, S.; Sharma, N.; Kapoor, P.; Chunduri, V.; Pandey, A.K.; Garg, M. Spotlight on the overlapping routes and partners for anthocyanin transport in plants. *Physiol. Plant.* **2021**, *171*, 868–881. [CrossRef]
24. Ni, J.; Liao, Y.; Zhang, M.; Pan, C.; Yang, Q.; Bai, S.; Teng, Y. Blue light simultaneously induces peel anthocyanin biosynthesis and flesh carotenoid/sucrose biosynthesis in mango fruit. *J. Agric. Food Chem.* **2022**, *70*, 16021–16035. [CrossRef] [PubMed]
25. Ubi, B.E.; Honda, C.; Bessho, H.; Kondo, S.; Wada, M.; Kobayashi, S.; Moriguchi, T. Expression analysis of anthocyanin biosynthetic genes in apple skin: Effect of UV-B and temperature. *Plant Sci.* **2006**, *170*, 571–578. [CrossRef]
26. Zhang, D.; Yu, B.; Bai, J.; Qian, M.; Shu, Q.; Su, J.; Teng, Y. Effects of high temperatures on UV-B/visible irradiation induced postharvest anthocyanin accumulation in 'Yunhongli No. 1'(*Pyrus pyrifolia Nakai*) pears. *Sci. Hortic.* **2012**, *134*, 53–59. [CrossRef]

27. Yue, P.; Jiang, Z.; Sun, Q.; Wei, R.; Yin, Y.; Xie, Z.; Larkin, R.M.; Ye, J.; Chai, L.; Deng, X. Jasmonate activates a CsMPK6-CsMYC2 module that regulates the expression of β-citraurin biosynthetic genes and fruit coloration in orange (*Citrus sinensis*). *Plant Cell* **2023**, *35*, 1167–1185. [CrossRef]
28. Qian, M.; Wu, H.; Yang, C.; Zhu, W.; Shi, B.; Zheng, B.; Wang, S.; Zhou, K.; Gao, A. RNA-Seq reveals the key pathways and genes involved in the light-regulated flavonoids biosynthesis in mango (*Mangifera indica* L.) peel. *Front. Plant Sci.* **2023**, *13*, 1119384. [CrossRef]
29. Christie, J.M.; Jenkins, G.I. Distinct UV-B and UV-A/blue light signal transduction pathways induce chalcone synthase gene expression in Arabidopsis cells. *Plant Cell* **1996**, *8*, 1555–1567.
30. Rius, S.P.; Grotewold, E.; Casati, P. Analysis of the P1 promoter in response to UV-B radiation in allelic variants of high-altitude maize. *BMC Plant Biol.* **2012**, *12*, 92. [CrossRef] [PubMed]
31. Contreras-Avilés, W.; Heuvelink, E.; Marcelis, L.F.; Kappers, I.F. Ménage à trois: Light, terpenoids, and quality of plants. *Trends Plant Sci.* **2024**, *29*, 572–588. [CrossRef] [PubMed]
32. Wang, Y.; Xiao, Y.; Sun, Y.; Zhang, X.; Du, B.; Turupu, M.; Yao, Q.; Gai, S.; Tong, S.; Huang, J. Two B-box proteins, PavBBX6/9, positively regulate light-induced anthocyanin accumulation in sweet cherry. *Plant Physiol.* **2023**, *192*, 2030–2048. [CrossRef]
33. Li, C.; Pei, J.; Yan, X.; Cui, X.; Tsuruta, M.; Liu, Y.; Lian, C. A poplar B-box protein PtrBBX23 modulates the accumulation of anthocyanins and proanthocyanidins in response to high light. *Plant Cell Environ.* **2021**, *44*, 3015–3033. [CrossRef] [PubMed]
34. Bursch, K.; Toledo-Ortiz, G.; Pireyre, M.; Lohr, M.; Braatz, C.; Johansson, H. Identification of BBX proteins as rate-limiting cofactors of HY5. *Nat. Plants* **2020**, *6*, 921–928. [CrossRef] [PubMed]
35. Nguyen, N.H.; Jeong, C.Y.; Kang, G.H.; Yoo, S.D.; Hong, S.W.; Lee, H. MYBD employed by HY5 increases anthocyanin accumulation via repression of MYBL 2 in Arabidopsis. *Plant J.* **2015**, *84*, 1192–1205. [CrossRef]
36. Bai, S.; Tao, R.; Yin, L.; Ni, J.; Yang, Q.; Yan, X.; Yang, F.; Guo, X.; Li, H.; Teng, Y. Two B-box proteins, PpBBX18 and PpBBX21, antagonistically regulate anthocyanin biosynthesis via competitive association with *Pyrus pyrifolia ELONGATED HYPOCOTYL 5* in the peel of pear fruit. *Plant J.* **2019**, *100*, 1208–1223. [CrossRef]
37. Li, L.; Li, S.; Ge, H.; Shi, S.; Li, D.; Liu, Y.; Chen, H. A light-responsive transcription factor *SmMYB35* enhances anthocyanin biosynthesis in eggplant (*Solanum melongena* L.). *Planta* **2022**, *255*, 12. [CrossRef] [PubMed]
38. Jiang, M.; Ren, L.; Lian, H.; Liu, Y.; Chen, H. Novel insight into the mechanism underlying light-controlled anthocyanin accumulation in eggplant (*Solanum melongena* L.). *Plant Sci.* **2016**, *249*, 46–58. [CrossRef]
39. Shin, J.; Park, E.; Choi, G. PIF3 regulates anthocyanin biosynthesis in an HY5-dependent manner with both factors directly binding anthocyanin biosynthetic gene promoters in Arabidopsis. *Plant J.* **2007**, *49*, 981–994. [CrossRef] [PubMed]
40. Wen, J.; Lv, J.; Zhao, K.; Zhang, X.; Li, Z.; Zhang, H.; Huo, J.; Wan, H.; Wang, Z.; Zhu, H. Ethylene-inducible *AP2/ERF* transcription factor involved in the capsaicinoid biosynthesis in Capsicum. *Front. Plant Sci.* **2022**, *13*, 832669. [CrossRef]
41. Liu, F.; Yu, H.; Deng, Y.; Zheng, J.; Liu, M.; Ou, L.; Yang, B.; Dai, X.; Ma, Y.; Feng, S. PepperHub, an informatics hub for the chili pepper research community. *Mol. Plant* **2017**, *10*, 1129–1132. [CrossRef] [PubMed]
42. Wang, Y.; Liu, S.; Wang, H.; Zhang, Y.; Li, W.; Liu, J.; Cheng, Q.; Sun, L.; Shen, H. Identification of the regulatory genes of UV-B-induced anthocyanin biosynthesis in pepper fruit. *Int. J. Mol. Sci.* **2022**, *23*, 1960. [CrossRef] [PubMed]
43. Lightbourn, G.J.; Griesbach, R.J.; Novotny, J.A.; Clevidence, B.A.; Rao, D.D.; Stommel, J.R. Effects of anthocyanin and carotenoid combinations on foliage and immature fruit color of *Capsicum annuum* L. *J. Hered.* **2008**, *99*, 105–111. [CrossRef]
44. Gangappa, S.N.; Botto, J.F. The multifaceted roles of *HY5* in plant growth and development. *Mol. Plant* **2016**, *9*, 1353–1365. [CrossRef]
45. Xu, D. COP1 and BBXs-HY5-mediated light signal transduction in plants. *New Phytol.* **2020**, *228*, 1748–1753. [CrossRef]
46. Yadav, A.; Singh, D.; Lingwan, M.; Yadukrishnan, P.; Masakapalli, S.K.; Datta, S. Light signaling and UV-B-mediated plant growth regulation. *J. Integr. Plant Biol.* **2020**, *62*, 1270–1292. [CrossRef] [PubMed]
47. Zhou, Y.; Mumtaz, M.A.; Zhang, Y.; Yang, Z.; Hao, Y.; Shu, H.; Zhu, J.; Bao, W.; Cheng, S.; Zhu, G. Response of anthocyanin biosynthesis to light by strand-specific transcriptome and miRNA analysis in *Capsicum annuum*. *BMC Plant Biol.* **2022**, *22*, 79. [CrossRef]
48. Ma, Y.; Ma, X.; Gao, X.; Wu, W.; Zhou, B. Light induced regulation pathway of anthocyanin biosynthesis in plants. *Int. J. Mol. Sci.* **2021**, *22*, 11116. [CrossRef] [PubMed]
49. Yadav, A.; Bakshi, S.; Yadukrishnan, P.; Lingwan, M.; Dolde, U.; Wenkel, S.; Masakapalli, S.K.; Datta, S. The B-Box-Containing MicroProtein miP1a/BBX31 Regulates Photomorphogenesis and UV-B Protection. *Plant Physiol.* **2019**, *179*, 1876–1892. [CrossRef]
50. Chang, C.-S.-J.; Li, Y.H.; Chen, L.T.; Chen, W.C.; Hsieh, W.P.; Shin, J.; Jane, W.N.; Chou, S.J.; Choi, G.; Hu, J.M. LZF1, a HY5-regulated transcriptional factor, functions in Arabidopsis de-etiolation. *Plant J.* **2008**, *54*, 205–219. [CrossRef] [PubMed]
51. Liu, C.-C.; Chi, C.; Jin, L.J.; Zhu, J.; Yu, J.-Q.; Zhou, Y.-H. The bZip transcription factor HY5 mediates CRY1a-induced anthocyanin biosynthesis in tomato. *Plant Cell Environ.* **2018**, *41*, 1762–1775. [CrossRef]

52. Chen, M.; Gu, H.; Wang, L.; Shao, Y.; Li, R.; Li, W. Exogenous ethylene promotes peel color transformation by regulating the degradation of chlorophyll and synthesis of anthocyanin in postharvest mango fruit. *Front. Nutr.* **2022**, *9*, 911542. [CrossRef] [PubMed]
53. Yao, J.-W.; Ma, Z.; Ma, Y.-Q.; Zhu, Y.; Lei, M.-Q.; Hao, C.-Y.; Chen, L.-Y.; Xu, Z.-Q.; Huang, X. Role of melatonin in UV-B signaling pathway and UV-B stress resistance in *Arabidopsis thaliana*. *Plant Cell Environ.* **2021**, *44*, 114–129. [CrossRef]

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