

Visible light induces the *PpPHYH* transcription to promote anthocyanin pigmentation in peach peel

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Abstract

Light is important for fruit coloration, but molecular mechanisms underlying the impact of visible light on anthocyanin pigmentation in peach peel remain elusive. Here, we investigated the influence of visible light, including red, green and blue light, on peel coloration in peach. Blue light had a better ability to induce anthocyanin pigmentation in peach peel than red and green light. A transcription factor, *PpPHYH*, was identified to be responsible for visible light-induced anthocyanin accumulation in peach peel. The *PpPHYH* gene was more highly expressed in peach peel exposed to blue light than in the peel exposed to red and green light. Moreover, *PpPHYH* had two alternative splicing variants, *PpPHYH-X1* and *PpPHYH-X2*, with the latter having a stronger ability to activate transcription of *PpMYB10.1*. Blue light had better ability to increase the ratio of *PpPHYH-X2* to *PpPHYH-X1* compared to green and red light. Thus, the role of blue light in peach peel anthocyanin pigmentation could be attributed to its ability to activate the transcription of *PpPHYH* and to ensure the relative abundance of the *PpPHYH-X2* transcript. Additionally, light was able to enhance the occurrence of alternative splicing variants in peach peel. Our results confirmed the important role of *PpPHYH* gene in light-induced anthocyanin pigmentation in peach peel.

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Introduction

Peach (*Prunus persica*) is one of the economically deciduous fruit trees in the temperate zone. In China, the growing area of peach and its production have increased slightly over the past decade, but the amount of peach consumption and trade has not increased significantly. To promote the sustainable development and growth of the peach industry in China, improvement of fruit quality is an urgent necessity. Skin color is an important trait of peach fruit quality that affects the purchase intention of consumers and the profitability of peach production. Therefore, the management of fruit coloration is an important aspect of the peach production process.

Fruit skin coloration is mainly ascribed to the accumulation of pigments, such as anthocyanin, carotenoid, and chlorophyll^[1,2]. Anthocyanin, a class of flavonoids, has strong antioxidant activity and is involved in preventing a variety of human diseases^[3,4]. The biosynthetic pathway of anthocyanin is conserved in plants. Synthesis of anthocyanin starts from 4-coumaroyl COA produced by phenylalanine metabolism and requires a series of enzymes, including chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), flavonoid 3'-hydroxylase (F3'H), dihydroflavone alcohol-4-reductase (DFR), leucoanthocyanidin dioxygenase (LDOX), and UDP glucose: flavonoid-3-O-glucosyltransferase (UFGT). Anthocyanin is synthesized in the cytoplasm and is then transported into the vacuole with the help of transporters to prevent its

degradation. In peach, anthocyanin pathway genes have been excavated^[5], GST (Glutathione-S-Transferase) and ABC (ATP Binding Cassette) transporters are involved in vacuolar transport of anthocyanins^[6,7]. The loss-function mutation of *PpGST* leads to the failure of anthocyanin accumulation in peach fruit^[8,9], while the overexpression of *PpABCC1* can increase anthocyanin accumulation in tobacco leaf and peach fruit^[10]. The expression of anthocyanin synthesis genes is transcriptionally regulated by the MBW complex formed by MYB, bHLH and WD40 transcription factors (TFs)^[11,12]. Three tandem-arranged R2R3-MYB genes on chromosome 3, *PpMYB10.1*, *PpMYB10.2* and *PpMYB10.3*, play important roles in regulating anthocyanin synthesis in peach fruit^[5,13]. Several studies indicate that the NAC TF *PpBL* as well as the bZIP TFs *PpPHYH* and *PpPHY5* are involved in the regulation of anthocyanin accumulation in peach fruit by activating the expression of the *PpMYB10.1* gene^[14–16].

Light is an important factor affecting fruit coloration. Numerous studies have found that light is involved in the regulation of anthocyanin accumulation by affecting the expression of MYBs or light-responsive genes. For example, light directly promotes the expression of *MdMYB1* gene, resulting in the synthesis of anthocyanin in apple fruit^[17]. Light promotes the expression of *PyBBX16* that activates the transcription of *PyMYB10* to induce anthocyanin accumulation in pear fruit^[18]. Upon light induction, *FvHY5* and *FvbHLH9* are activated to form heterodimer that synergistically promote the expression of

FvDFR gene, leading to anthocyanin accumulation in strawberry fruit^[19]. In addition, the influence of different light wavelengths on fruit coloration has been reported. Blue light promotes anthocyanin accumulation through both activating the CRY-COP1-HY5 regulation pathway and promoting the expression of *Pp4ERF24* and *Pp12ERF96* that function as positive regulators of anthocyanin biosynthesis^[20,21]. Ultraviolet B (UVB) promotes the expression of *MdBBX22* and *MdWRKY72*, leading to the accumulation of anthocyanin in apple peel^[22,23]. Although UV light, including UVA, UVB and UVC, is known to promote peach skin coloration^[7,24], the influence of visible light on peach skin coloration remains largely unclear.

In this study, we investigated the impact of red, green and blue light on peach peel coloration. The *PpHYH* gene, a key regulator involved in light-induced anthocyanin accumulation of peach peel, was variously expressed under different wavelengths of visible light, and its expression was positively correlated with anthocyanin accumulation. Our results provide further evidence that the *PpHYH* gene, rather than its homolog *PpHY5*, is the key gene responsible for light-induced anthocyanin pigmentation in the skin of peach fruit.

Materials and methods

Plant materials

Peach cultivar 'Jinxiu', which accumulates anthocyanins in the peel under the normal light conditions used in this study is maintained in Wuhan Botanical Garden of Chinese Academy of Sciences (Hubei province, China). Bagged fruits were collected at the early ripening stage and divided into four groups. The fruits in each group were treated at ambient temperature (25 °C) with the irradiation of visible light at different wavelengths (700 lux). Fruits incubated in darkness at ambient temperature were used as control. Fruit samples were collected at 0, 6, 24, 72, 120 and 168 h after treatment. The peel tissues were frozen immediately in liquid nitrogen and stored at -80 °C until use. Each treatment contained three biological replicates.

Anthocyanin extraction and measurement

The extraction and measurement of anthocyanin was conducted according to our previously reported method^[15]. Approximately 100 mg powder sample was added into a 2-mL centrifuge tube and immediately blended with 1 ml of 0.1% hydrochloric acid methanol. The mixture was refrigerated at 4 °C for 24 h and then centrifuged at 13,400 × g for 20 min at room temperature. The supernatants were transferred to a new 2-mL centrifuge tube, and the final volume was adjusted to 2 mL with 0.1% hydrochloric acid methanol. A total of 500 μL of the mixtures was transferred to a 1.5-ml centrifuge tube and then mixed with an equal volume of Buffer A (0.2 M HCl /0.2 M KCl, 67: 25, v/v) or Buffer B (0.2 M NaAc). After incubation in darkness for 4 h, the mixture containing Buffer A or B was subjected to measurement of the absorbance at 510 nm and 700 nm using multifunctional microplate reader (TECAN Infinite M200, Austria). Anthocyanin content was calculated using the following formula: [(A510-A700)_{Buffer A} - (A510-A700)_{Buffer B}]/g fresh weight (FW). Three biological replicates were performed for each sample.

Total RNA extraction and gene expression analysis

Total RNA was extracted using the HiPure HP Plant RNA Mini Kit (Magen, Guangzhou, China). The RNA concentrations of all

samples were measured using NanoDrop lite ultraviolet spectrophotometer (ND-LITE-PR, USA) and diluted with sterile double-distilled water (ddH₂O) to 500 ng/μl. The cDNA was synthesized using Hifair® III 1st Strand cDNA Synthesis Super-Mix for qPCR (Yeastar, Shanghai, China). Gene expression levels were analyzed using quantitative reverse transcriptase real-time PCR (qRT-PCR) with the StepOnePlus™ Real-Time PCR System (7300 Applied Biosystems™, USA). The qRT-PCR reaction system and program were carried out according to our previous report^[16]. The *PpGADPH* gene encoding glyceraldehyde-3-phosphate dehydrogenase was used as an internal reference control^[25]. Gene expression levels were calculated using the 2^{-ΔΔCT} method. Each sample contained three biological replicates and the primer sequences for the qRT-PCR are listed in Supplemental Table S1.

Transcriptome sequencing and data analysis

Total RNA was extracted from peel tissues of fruit samples with different lighting treatments as mentioned above. Each sample consisted of three biological replicates and each replicate contained three fruits. cDNA libraries and transcriptome sequencing were carried out by the Biomarker Technologies Co, Ltd (Beijing, China) with the Illumina HiSeq™ platform.

Clean data were obtained after removing useless data, including adapter sequence and reads with low quality and then mapped to the peach reference genome^[26]. Principal component analysis (PCA) and correlation coefficient for all sequenced samples were analyzed using the BMKCloud platform (www.biocloud.net). Expected number of Fragments Per Kilobase of transcript sequence per Millions base pairs sequenced (FPKM) was used to assess gene expression levels. The program DESeq2 was used to identify differentially expressed genes (DEGs) with the following parameters: false discovery rate (FDR) ≤ 0.01 and fold change (FC) ≤ 0.5 or ≥ 2. The Venn diagrams and Upset plots were performed using the TBtools software^[27].

Weighted Gene Co-expression Network Analysis (WGCNA)

WGCNA was carried out using the BMKCloud platform (www.biocloud.net), and anthocyanin content in peel tissues of fruit samples before light treatment (0 h) and after 72 h treatment with different wavelengths of visible light or without light (darkness) were used as trait data. Gene modules were identified with the following parameters: FPKM ≥ 1 and module similarity threshold ≥ 0.25.

Analysis of alternative splicing events

Alternative splicing (AS) events for all sequenced samples were analyzed using the rMATS software^[28]. Significant differences in AS frequency between different wavelengths of visible light and darkness were evaluated using Student's t-test at $p < 0.05$.

Results

Effects of visible light with different wavelengths on anthocyanin accumulation in peach peel

Peach cultivar 'Jinxiu' was selected to investigate the effect of visible light on peach peel coloration. Fruits at the early ripening stage were treated with red, green and blue light or under dark conditions. Patches of pale red pigmentation appeared on the peel of peach fruits at 72 h after irradiation with blue light,

Blue light promotes peach peel coloration

and the peel color changed from pale red to dark red after 120 h irradiation with blue light (Fig. 1a). In contrast, no red pigmentation was induced by the irradiation of red and green light although pale red blush occurred on the surface of peach fruits up to 120 h or 168 h of exposure. Moreover, anthocyanin content in fruit peel showed an increase during the light exposure process and blue light had the strongest positive effect on inducing anthocyanin biosynthesis (Fig. 1b). These results demonstrate that blue light, instead of red and green light, could significantly promote anthocyanin accumulation in peach peel.

Expression of anthocyanin-related genes in peach peel exposed to visible light with different wavelengths

To further clarify the impact of visible light on anthocyanin accumulation in peach peel, we conducted qRT-PCR analysis to investigate the expression of anthocyanin-related genes. The expression of anthocyanin structural genes *PpDFR* and *PpUFGT* as well as anthocyanin regulatory gene *PpMYB10.1* was significantly up-regulated at 6 h of exposure to red, green or blue light (Fig. 2a & b). However, their expression levels decreased thereafter and reached a plateau. Notably, all three genes exhibited higher activation in fruit peel exposed to blue light than in fruit peel exposed to red or green light. Overall, their expression patterns were in good agreement with the profiles of anthocyanin accumulation in peach peel exposed to visible light. In addition, the expression pattern of *PpGST*, the pivotal gene responsible for anthocyanin vacuolar transport, exhibited a biphasic curve, with a peak at 6 h of exposure, followed by a decrease and an increase again to a second peak at 72 h of exposure (Fig. 2c). Blue light had the strongest positive effect on the induction of the *PpGST* expression, with the highest level observed at 72 h of exposure. These findings suggest that anthocyanin accumulation in peach peel exposed to visible light is regulated at the transcriptional level.

Identification of candidate gene responsible for visible light-induced anthocyanin accumulation in peach peel

To investigate regulatory genes responsible for visible light-induced anthocyanin accumulation in peach peel, we conducted comparative transcriptome analysis between peel samples after 72 h of exposure to visible light and those after 0 and 72 h of treatment in dark. A total of 15 libraries were

constructed and sequenced, with Q30 average value of the 15 libraries being more than 91.5% (Supplemental Table S2). The numbers of clean reads for each library ranged from 39.0 to 54.5 million. Over 95.6% of clean reads were mapped to the v2.0 peach genome^[26]. Pearson correlation analysis revealed high consistency between biological replicates of samples after 72 h of exposure to red or blue light as well as the sample at 0 h of treatment in dark (Supplemental Fig. S1a). However, relatively low consistency was observed between biological replicates of samples after 72 h of exposure to green light or treatment in the dark. Moreover, principal component analysis (PCA) was conducted and the result was consistent with that of Pearson correlation analysis (Supplemental Fig. S1b).

We initially identified differentially expressed genes (DEGs) between fruit samples treated with 72 h of visible light irradiation and those at 0 and 72 h of treatment in dark. As a result, 744–1,820 DEGs were detected between visible light- and darkness-treated samples (Supplemental Table S3). Since pale red anthocyanin pigmentation occurred in the peel after 72 h of blue light irradiation, we focused on genes differently expressed between fruit samples treated with 72 h of blue light irradiation (B3) and those at 0 (D0) and 72 h (D3) of treatment in the dark. Totally, 583 and 814 DEGs were up-regulated in the D0 versus B3 and D3 versus B3 comparisons, respectively, with 246 commonly up-regulated (Supplemental Fig. S2a). Likewise, 343 and 1,006 DEGs were down-regulated in the D0 versus B3 and D3 versus B3 comparisons, respectively, with 92 commonly up-regulated. The Clusters of Orthologous Groups (COG) analysis revealed that the majority of commonly up- and down-regulated DEGs were enriched in carbohydrate transport and metabolism, as well as energy production and conversion pathways (Supplemental Fig. S2b). Of the 246 commonly up-regulated DEGs (Supplemental Table S4), three, i.e. *PpHYH* (Prupe.1G208500), *PpMYC2* (Prupe.2G195300) and *PpMYB29* (Prupe.8G270000), belonged to transcription factors (TFs). Similarly, three TFs, *PpERF053* (Prupe.3G157100), *PpRAV2* (Prupe.3G240000) and *PpJUB* (Prupe.4G106100), were included in the 92 commonly down-regulated DEGs.

In addition to blue light, red and green light could also induce anthocyanin accumulation in peach peel. Thus, we used an Upset plot to identify common DEGs in response to red, green, and blue light. As a result, 244 and 126 DEGs were

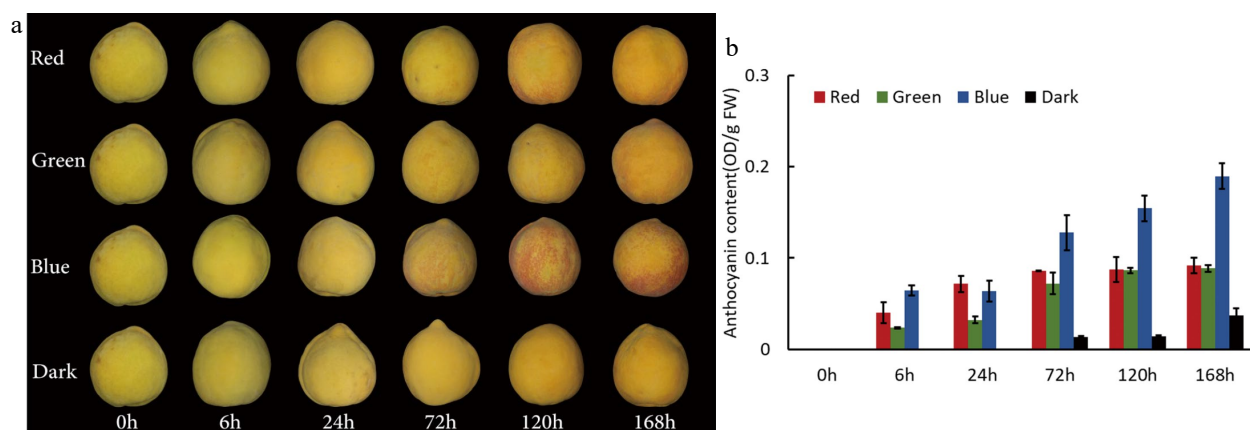


Fig. 1 Effects of visible light with different wavelengths on anthocyanin accumulation in peach peel. (a) The peel coloration of 'Jinxiu' treated with red, green and blue light or under dark conditions. (b) Anthocyanin accumulation of peach peel treated with red, green and blue light or under dark conditions. Error bars in (b) show the standard error (SE) of three biological replicates.

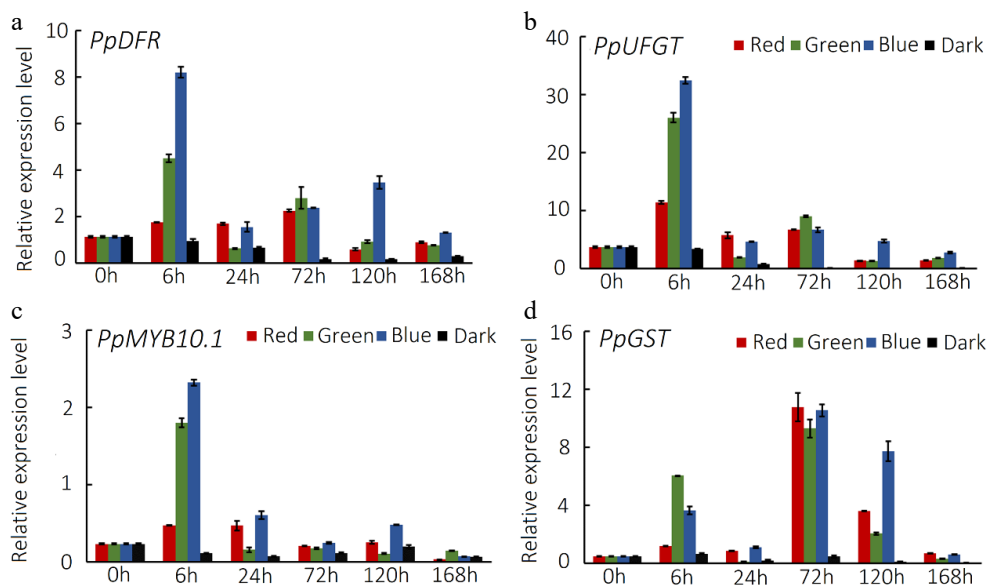


Fig. 2 Expression level of anthocyanin-related genes in peach peel exposed to visible light with different wavelengths. Expression profiles of anthocyanin synthesis genes (a) *PpDFR* and *PpUFGT*, (b) regulator gene *PpMYB10.1*, (c) transporter gene *PpGST* in peach peel treated with red, green and blue light or under dark conditions. Error bars show the \pm SE of three biological replicates.

commonly up-regulated and down-regulated, respectively, in the peel tissues treated with 72h of red light irradiation (R3) relative to the D0 and D3 samples (Fig 3a & b). Likewise, 98 and 29 DEGs were commonly up-regulated and down-regulated, respectively, in the peel tissues treated with 72 h of green irradiation (G3) relative to the D0 and D3 samples. Overall, 65 and 13 were commonly up-regulated and down-regulated, respectively, in the B3 versus D0, B3 versus D3, R3 versus D0, R3 versus D3, G3 versus D0, and G3 versus D3 comparisons. Of the 65 commonly up-regulated DEGs, only one TF *PpPHYH* was identified (Supplemental Table S5). However, no TFs were found in the 13 commonly down-regulated DEGs.

Finally, WGCNA analysis was performed by setting anthocyanin content under visible light with different wavelengths as the sole trait data to detect the modules of co-expressed genes. The WGCNA analysis yielded 13 distinct modules, and one termed MEgreen exhibited the highest correlation between gene expression and anthocyanin content ($r = 0.97$) (Fig. 4a). The MEgreen module consisted of 180 genes, including three TFs, *PpPHYH*, *PpPCL1* (Prupe.6G071400), and *PpMYC2* (Supplemental Table S6). COG analysis showed that the genes from the MEgreen module were primarily involved in secondary metabolite biosynthesis, lipid transport and metabolism, and defense mechanisms (Fig. 4b).

Taking the results of WGCNA and DEGs analysis together, we proposed that *PpPHYH* is a good candidate responsible for visible light-induced anthocyanin accumulation in peach peel.

Expression profiling of two alternative splicing variants of *PpPHYH* in peach peel exposed to visible light

Our recent study reported the important role of *PpPHYH* which has two alternative splicing variants *PpPHYH-X1* and *PpPHYH-X2* in light-induced anthocyanin accumulation in peach peel^[15]. *PpPHYH-X2* lacks an 84-bp fragment in the second exon when compared to *PpPHYH-X1* (Fig. 5a). The transcription of *PpPHYH-X1* and *PpPHYH-X2* was highly induced at 6 h of exposure to blue light, and then their expression levels remained relatively high (Fig. 5b). Whereas, the expression levels of *PpPHYH-X1*

and *PpPHYH-X2* showed an obvious increase at 72 h and 24 h of exposure to red or green light, respectively. To further explore the relationship between the expression of *PpPHYH-X2* gene and anthocyanin accumulation, we conducted Pearson's correlation analysis. The expression of *PpPHYH-X2* showed higher correlation with anthocyanin content than that of *PpPHYH-X1*. Moreover, there was a positive correlation between the expression of *PpPHYH-X2* and anthocyanin-related genes, including *PpDFR*, *PpUFGT*, *PpGST* and *PpMYB10.1* (Supplemental Fig. S3).

In addition, we investigated the expression profile of the *PpPHYH* homologous gene *PpPHY5* as it has been reported to regulate anthocyanin accumulation in peach peel exposed to UVA and UVB^[7]. The expression of *PpPHY5* was induced at 6 h of exposure to red, green or blue light, and showed a dramatic decrease at 24 h of visible light irradiation (Fig. 5c). Notably, the expression levels of *PpPHY5* in the peel after 24 h of irradiation were quite similar among red, green and blue light. Therefore, *PpPHY5* is unlikely the candidate gene responsible for visible light-induced anthocyanin accumulation in peach peel.

It has been reported that the relative abundance of different alternative splicing variants is often associated with phenotypic variation in plants^[29]. Therefore, we investigated the relative abundance of *PpPHYH-X1* and *PpPHYH-X2* in the peel exposed to visible light. Blue light had a better ability to increase the ratio of *PpPHYH-X2* to *PpPHYH-X1* compared to green and red light (Fig. 5d). The *PpPHYH-X2* variant has stronger activation on the transcription of *PpMYB10.1* compared to the *PpPHYH-X1* variant^[15]. Thus, the crucial role of blue light in peach peel anthocyanin pigmentation could be attributed to its ability to activate the transcription of *PpPHYH* and to ensure the relative abundance of the *PpPHYH-X2* transcript.

Identification and analysis of AS events under different light qualities

Since the ratio of *PpPHYH-X2* to *PpPHYH-X1* showed a variation between visible light with different wavelengths, we investigated the impact of visible light on alternative splicing (AS) events at the genome-wide level, including alternative 3' last

Blue light promotes peach peel coloration

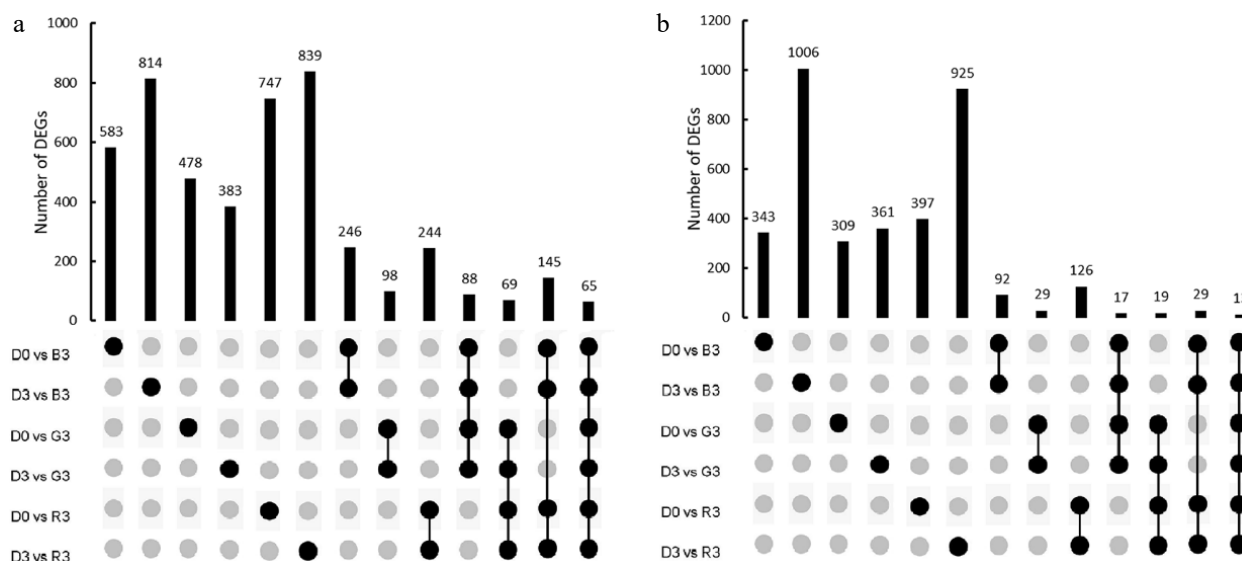


Fig. 3 Analysis of DEGs in peach peel samples treated with red, green and blue light irradiation. (a) The number of up-regulated genes in response to red, green, and blue light. (b) The number of down-regulated genes in response to red, green, and blue light.

exon (A3SS), alternative 5' first exon (A5SS), exon skipping (ES), retained intron (RI) and mutually exclusive exons (MXE). The frequencies of A5SS, A3SS, ES, RI and MXE in the peel at 72 h of visible light exposure were significantly higher than in the peel at 72 h of treatment in dark (Fig. 6). The frequency of different types of AS events had no significant difference between blue, green and red light. These results suggest that light could promote the occurrence of alternative splicing variants.

Discussion

Anthocyanins are a group of red to blue flavonoid pigments that may help plants cope with environmental stresses such as high light and nutrient deprivation^[30]. Light is a form of energy that travels as waves, and short-wavelength waves have more energy than long-wavelength waves. Blue (400–500 nm) and ultraviolet (300–400 nm) light with short wavelengths carry a tremendous amount of energy that induces damage to nuclear DNA^[31]. Under ultraviolet (UV) and blue light irradiation, plants naturally produce anthocyanins in epidermal cells to protect themselves from the damage of incoming energy^[32]. This is probably why UV and blue light have an important influence on the induction of anthocyanin pigmentation in the skin of various fruits, such as tomato^[33], pear^[20,21], strawberry^[34], grapevine^[35], and apple fruit^[22,36,37]. In peach, a recent study has revealed the positive effect of UV on anthocyanin pigmentation in the peel^[7]. In this study, we investigated the effect of visible light with different wavelengths on peach peel coloration and blue light was found to have a great effect on anthocyanin pigmentation. It is worth noting, however, that red and green light had the ability to induce anthocyanin accumulation but could not produce obvious red pigmentation in the skin of debagged fruits (Fig. 1a). This result demonstrated that red and green light treatments were less effective at increasing peach peel anthocyanin accumulation when compared to blue light treatment. In addition, anthocyanin content showed a slight increase in the peel of debagged fruits after 72 h of treatment in dark. Anthocyanin accumulation under dark

condition has been observed in the case of anthocyanin coloration in the peach flesh surrounding the stone (Cs). Given that the Cs trait occurs during the ripening process^[16], it is reasonable to speculate that anthocyanin accumulation in the peel of debagged fruits under dark conditions in this study could be due to the ripening behavior.

Previous studies have shown that light promotes anthocyanin accumulation in fruits by activating the expression of *HYS*, a key gene involved in the light signaling pathway, or increasing the *HYS* protein stability^[38,39]. In peach, our previous study shows that *PpHYH*, instead of *PpHYS*, plays an important role in the regulation of light-induced anthocyanin pigmentation in peach peel^[15]. In this study, our results indicated that *PpHYH* was more highly expressed in peach peel exposed to blue light than in peel exposed to green or red light. This finding further supported the crucial role of *PpHYH* in light-induced anthocyanin accumulation in peach peel. Moreover, *HYH* and its homolog *HYS* have no transcriptional activation domains and require interacting partners for transcriptional regulation. In pear and strawberry, light-activated genes such as *BBX* and *bHLH* can form heterodimeric complexes with *HY5* to promote anthocyanin accumulation^[18,19]. In peach, *PpBBX4* has been reported to act as an interacting partner of *PpHYH*^[15]. Here, *PpBBX4* was highly expressed in peach peel exposed to visible light with different wavelengths (Supplemental Fig. S4a). Thus, it seems that *PpHYH* forms a heterodimer with *PpBBX4* to promote anthocyanin pigmentation in peach peel exposed to visible light. Additionally, a previous study has revealed that *PpHYS* is highly activated in peach peel exposed to UVA and UVB^[7]. There is a difference between UV and visible light receptors^[40,41]. Thus, the mechanisms underlying anthocyanin accumulation in peach peel may be different between visible light and UV treatments.

Like *PpHYH*, *PpHYS* was highly expressed in peach peel exposed to blue light. Our recent study has domesticated that *PpHYH* can bind to the G-box element on the promoter of *PpMYB10.1* to activate its transcription^[15]. There was no G-box element in the *PpHYS* promoter, but two ACE-box elements at

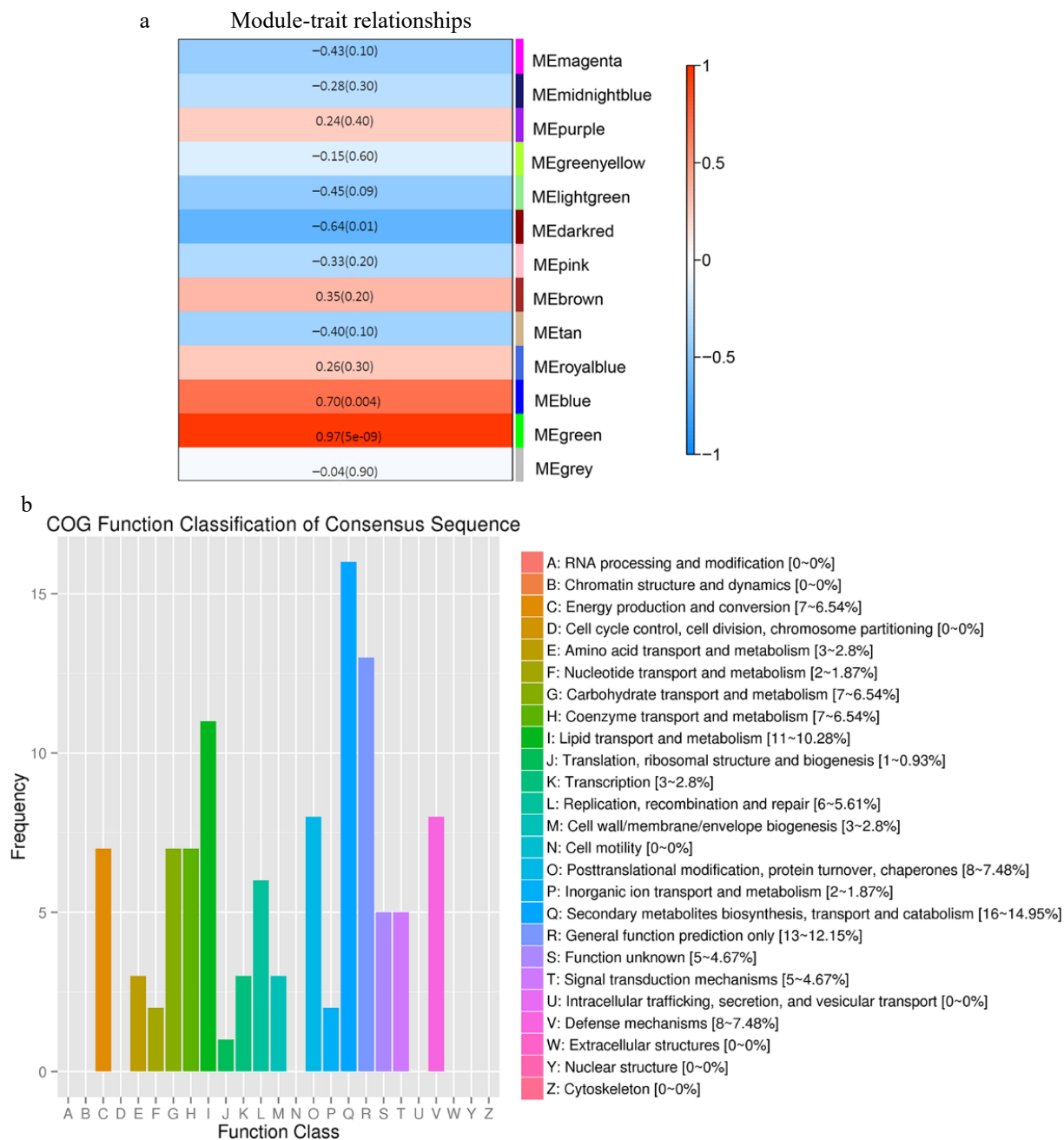


Fig. 4 Analysis of the WGCNA results. (a) Relationships between distinct modules and anthocyanin content. (b) Histogram of COG classification for the genes from MEgreen module.

79 bp and 1,042 bp upstream of the start codon were identified. The ACE-box element has been reported to be an important *cis*-element for HY5 protein binding^[42,43]. *PpHY5* plays an important role in activating expression of anthocyanin-related structural and regulatory genes^[7,15]. Thus, it is worthy of further studies to address whether the above mentioned *PpHYH/PpBBX4* complex could activate the transcription of *PpHY5*, which promotes anthocyanin accumulation in peach peel exposed to blue light.

The *PpHYH* gene had two alternative splicing variants, *PpHYH-X1* and *PpHYH-X2*. Compared with the *PpHYH-X1* variant, the *PpHYH-X2* variant showed higher expression in peach peel exposed to blue light. More importantly, the *PpHYH-X2* variant

has a stronger effect on promoting transcription of *PpMYB10.1* compared with the *PpHYH-X1* variant^[15]. Therefore, blue light-induced anthocyanin pigmentation in peach peel could be attributed to the strong action of *PpHYH-X2*. Notably, the *PpHYH-X1* protein is located in the nucleus, while the *PpHYH-X2* protein is located in the nucleus and cytoplasm^[15]. Previous studies have shown that the changes in the relative abundance of alternative splicing variants that show difference in subcellular localization can lead to phenotypic variations, including developmental processes^[44], signal transduction^[45], and flowering time^[29,46,47]. The ratio of *PpHYH-X2* to *PpHYH-X1* in peach peel showed a variation between visible light with different wavelengths. More studies are needed to clarify whether the

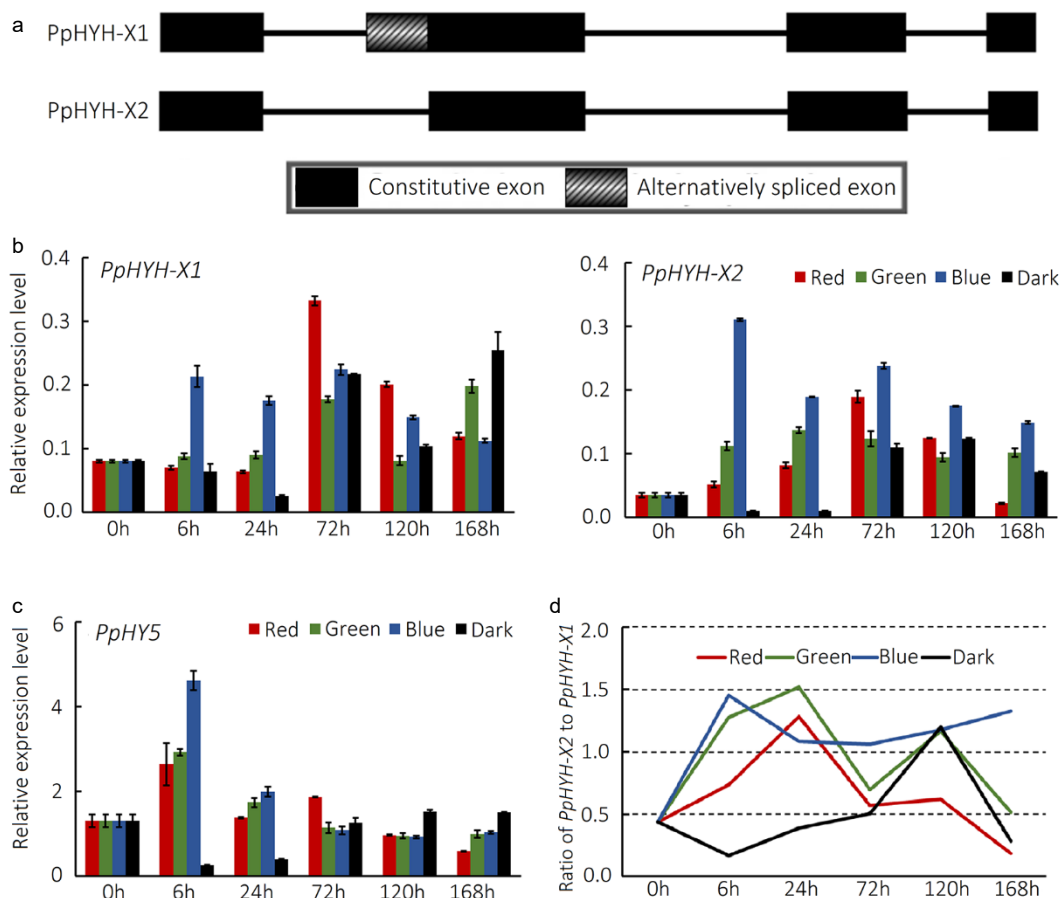


Fig. 5 Expression profiling of *PpHYH* and *PpHY5* genes in peach peel exposed to visible light. (a) Schematic diagram of the *PpHYH* gene. The solid boxes, striated boxes and solid lines represent constitutive exons, alternatively spliced exons and introns, respectively. (b) Relative expression level of the *PpHYH* gene treated with red, green and blue light or under dark conditions. (c) Relative expression level of the *PpHY5* gene treated with red, green and blue light or under dark conditions. (d) Ratio of *PpHYH-X2* to *PpHYH-X1* treated with red, green and blue light or under dark conditions. The error bars in (b) and (c) show \pm SE of three biological replicates.

changes in the *PpHYH-X2* to *PpHYH-X1* ratio have an impact on the accumulation of anthocyanins and other metabolites in peach peel.

COP1 is well known to target HY5/HYH for ubiquitination and subsequent 26S proteasome-dependent degradation. Under visible light conditions, light receptors, such as phytochromes (PHY), cryptochromes (CRY) and phototropins (PHOT), can interact with COP1 to inhibit its function^[48–53]. In this study, our results indicated that a visible light receptor *PpCRYD* (Prupe. 1G462400) was highly expressed in peach peel upon exposure to blue, green or red light (Supplemental Fig. S4b). However, no *PHY* and *PHOT* genes were found to show differential expression between visible light and darkness treatments. Moreover, *PpCOP1* showed high expression in peach peel exposed to visible light with different wavelengths (Supplemental Fig. S4c). An E3 ubiquitin ligase *PpCOP1* has been reported to interact with *PpHYH*^[15]. Therefore, *PpCRYD* seems to prevent *PpCOP1* from interacting with *PpHYH*, leading to anthocyanin accumulation in peach peel under visible light treatment.

External environmental factors such as temperature and light can promote the occurrence of alternative splicing events to improve the adaptability of plants^[54–58]. In this study, we found that red, green and blue light all significantly increased the frequency of alternative splicing events at the genome-wide

level. Previous studies have shown that phytochrome and splicing regulators play important roles in light-induced AS events in plants^[59–61]. Recent studies have shown that chloroplast signals are involved in the regulation of light-induced AS events in plants by influencing RNA polymerase II elongation^[56] and TOR kinase activity^[62]. In this study, 32 out of 65 DEGs that were up-regulated in peach peel upon exposure to visible light were chloroplast-related genes (Supplemental Table S5). This suggests that chloroplast signaling may play an important role in light-induced AS events in peach.

Conclusions

Our previous study has shown that light promotes peach peel coloration by activating the transcription of the *PpHYH* gene. In this study, we further demonstrated that visible light, including red, green and blue light, could induce anthocyanin accumulation in peach peel by promoting the expression of the *PpHYH* gene, instead of the *PpHY5* gene. Blue light had the strongest positive effect on inducing the *PpHYH-X2* alternative splicing variant, resulting in an increased ratio of *PpHYH-X2* variant to *PpHYH-X1* variant compared to green and red light. Thus, blue light had a stronger ability to induce peach peel coloration than red and green light. This study revealed the

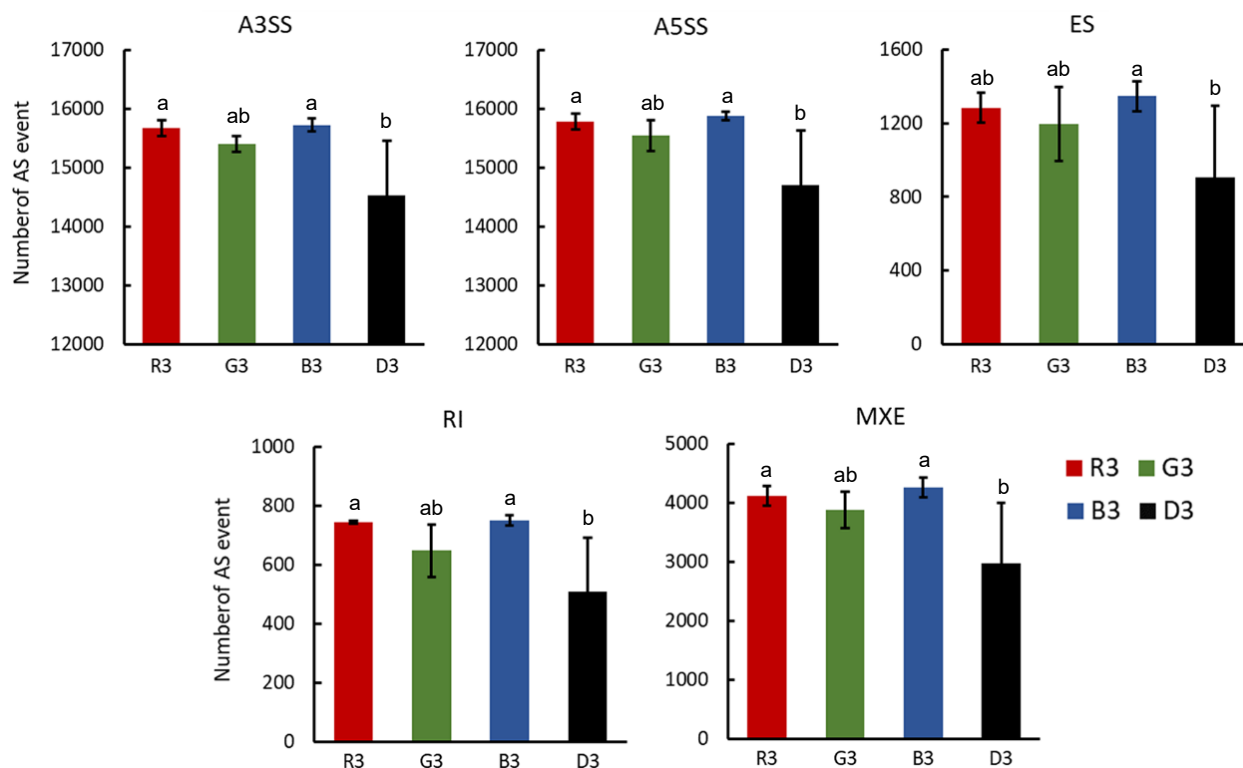


Fig. 6 Analysis of AS events of peel samples treated with red, green and blue light or under dark conditions. A3SS: alternative 3' last exon; A5SS: alternative 5' first exon; ES: exon skipping; RI: retained intron; MXE: mutually exclusive exons. R3, G3, B3 and D3 represent peel samples treated with 72 h of red, green, blue light irradiation and 72 h of treatment in the dark. The error bars show \pm SE of three biological replicates, and significant difference at $p < 0.05$ is indicated by different lowercase letters based on Fisher's Least Significant Difference (LSD) test.

mechanism of visible light-induced anthocyanin accumulation in peach peel, and confirmed the crucial role of the *PpHYH* gene in peach peel anthocyanin accumulation.

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Conflict of interest

The authors declare that they have no conflict of interest.

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