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Impact of Blue Light Treatment on Strawberry Plant Flowering Regulation

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ABSTRACT

In this paper we explored the Impact of Blue Light Treatment on Strawberry Plant Flowering Regulation. Strawberry plants are sensitive to blue light, which controls blooming. Seedlings of cultivated strawberry (Fragaria ananassa Duch.) 'Benihoppe' were exposed to a white light treatment (WL) and a blue light treatment (BL) till blooming to uncover the mechanism of early flowering under blue light treatment at the transcriptional level. A transcriptome study based on RNA-Seq was conducted to identify gene expression patterns in response to BL. The findings revealed a total of 6875 differentially expressed genes (DEGs) that reacted to BL, with 3138 (45.64%) being downregulated and 3737 (54.36%) being upregulated. Based on gene function annotation, these DEGs were highly enriched into 98 GO keywords and 71 KEGG pathways. Under BL, the expression levels of genes involved in light signaling (PhyB, PIFs, and HY5) as well as circadian rhythm (FKF1, CCA1, LHY, and CO) were changed in plants. The BL-responsive BBX transcription factors were also discovered. The findings suggested that FaBBX29, a strawberry BBX family gene, may be involved in flowering control. Our findings offer a timely, complete perspective of blooming control under various light quality, as well as a trustworthy reference data resource for future research.
KEYWORD: Impact, Blue Light, Treatment, Strawberry, Plant, Flowering, Regulation

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INTRODUCTION

One of the most significant developmental changes in the plant life cycle is the transition from vegetative to reproductive growth, which includes blooming and subsequent seed production [1]. Flowering is required for the development of seeds and fruits that will be harvested later [2], thus flowering period is an important agronomic characteristic in crop breeding and research. Multiple environmental signals, including as temperature, stress, and light, influence flowering induction [3, 4]. Plants are constantly monitoring the strength of light, as well as its length, spectrum, and direction, in order to modify their growth and development. Photoperiodic signals are used by many plants to regulate flowering induction. In addition, various light spectra may have distinct impacts on flowering induction [5]. Plants have been studied extensively for their photoperiodic blooming mechanism. The external coincidence hypothesis was suggested in the twentieth century and is backed by a known photoperiodic response molecular mechanism in long-day plants like Arabidopsis thaliana and short-day plants like rice [6]. In Arabidopsis, a number of genes are involved in the molecular processes that control the photoperiodic blooming pathway. By expressing a protein that is a major component of florigen in the companion cells of the phloem inside leaves and is carried to the shoot apex, the AtFT (FLOWERING LOCUS T) gene regulates flowering timing [7, 8]. The AtCO (CONSTANS) protein integrates circadian rhythm and light signal inputs by regulating AtFT gene expression in leaves through a CCT (CONSTANS, CONSTANS-like, and TOC1) conserved domain that binds to the upstream promoter of the AtFT gene [9, 10]. Gene expression as well as protein stability control limits the amount of AtCO protein produced. By binding to the CDF binding sites at the transcription start site, the AtCDF1 (CYCLING DOF FACTOR 1), a CDF family member, acts as a morning repressor of AtCO gene expression. Other CDF family members may similarly inhibit AtCO and AtFT expression redundantly, delaying Arabidopsis flowering time [11, 12]. Furthermore, the fundamental components of the circadian clock control AtCDF1 gene expression. AtCCA1 (Circadian Clock Associated 1) and AtLHY (LATE ELONGATED HYPOCOTYL) increase AtCDF production in the morning, while PRR (PSEUDORESPONSE REGULATOR), another circadian clock component protein, represses AtCDF transcription in the afternoon [13–15]. Both AtFKF1 (FLAVIN-BINDING, KELCH REPEAT, F-BOX 1) and AtGI (GIGANTEA) form a complex in Arabidopsis that regulates the ubiquitin-dependent degradation

of the AtCDF1 protein in a blue light-dependent way. The interaction between AtGI and AtCDF1 is required for AtFKF1 function [16, 17]. In a long-day situation, the AtFKF1 interacts with AtCO via its LOV domain and stabilizes the AtCO protein in the afternoon [18]. AtGI also interacts with the AtFKF1 homologs AtZTL (ZEITLUPE) and AtLKP2 (LOV KELCH PROTEIN 2), degrading CDF2 protein in a synergistic manner with AtFKF1. Furthermore, AtGI has been shown to stabilize AtFKF1 and AtZTL [17]. As a critical regulator in the light signaling system, the AtCOP1 (CONSTITUTIVE PHOTOMORPHOGENIC 1) protein, an E3 ubiquitin ligase, is involved in both the plant circadian clock and flowering time regulation. During the night under both long-day and short-day circumstances, the AtCO protein is allegedly degraded by a protein complex produced by AtCOP1, AtSPA1 (SUPPRESSOR OF PHYA), AtSPA3, and AtSPA4 [19]. Recent study has shown that AtFKF1 serves as an upstream negative regulator of AtCOP1, regulating AtCO stability and photoperiodic flowering via interacting with AtCOP1 and reducing AtCOP1 activity in a day-length-dependent manner [20].

Plants have various sets of photoreceptors for detecting different wavelengths of light, ranging from near-UVB (280–315 nm) to far-red (750 nm). Different light quality stimulate or delay blooming in plants [21, 22]. Photoreceptors and key genes in the light signalling system are involved in floral initiation in Arabidopsis, and Cryptochromes (CRYs) are blue light receptors capable of binding a FAD chromophore in plants [23]. The mutant plants (cry2) of AtCRY2 bloomed later than wild-type plants in an early Arabidopsis research [24]. AtCRY2 has been demonstrated in Arabidopsis to promote AtFT expression in response to blue light by inhibiting AtCO protein degradation. In the flowering process, AtCRY2 interacts with the AtCOP1-AtSPA complex under blue light, suppressing the COP1-dependent proteolysis of AtCO [25, 26]. The function of AtCIB (CRY2-interacting HLH) proteins in regulating the AtCRY2 promoting flowering pathway has just recently been discovered. The AtCIBs are engaged in the AtCRY2 signaling pathway, and the expression of related AtCIBs is particularly controlled by blue light. AtCIB1, for example, promotes AtFT expression by interacting with the AtFT gene's chromatin DNA [27]. Another Arabidopsis CRY, AtCRY1, interacts with the AtCOP1-AtSPA complex and increases the AtCRY2-AtCOP1-AtSPA interaction, which helps regulate flowering [25]. In Arabidopsis, the LOV (light, oxygen, or voltage, a subfamily of PAS domains) domain of the AtFKF1 protein performs a function as a blue light receptor, in which AtFKF1 interacts with AtGI to create a complex by absorbing blue light via its LOV domain. The AtFKF1-AtGI complex regulates AtCO expression by degrading AtCDF protein and forming an AtFKF1-AtGI-AtCDF1 complex that acts on the AtCO promoter [28, 29]. According to more recent study, AtFKF1 can regulate a robust At FT mRNA induction through various feed-forward processes. AtFKF1 stabilizes AtCO by interacting with it, and blue light enhances this connection. Simultaneously, the AtFKF1-AtGI complex removes the AtCDF1 protein from the At FT gene promoter [18]. In Arabidopsis, blue-light-activated AtFKF1 interacts with AtCOP1 and inhibits homodimerization of AtCOP1, allowing it to regulate flowering time [20].

Strawberry is a model plant in the Rosaceae family, as well as an important fruit crop. Strawberry flowering is a critical breeding characteristic that is influenced by genetic background and a variety of environmental variables [5, 30–33]. Many physiological features of strawberry have been found to be affected by blue light irradiation, including anthocyanin accumulation in fruits, flowering induction, and in vitro plantlet development [32, 34, 35]. The results of a recent study of woodland strawberry (*Fragaria vesca*) seedlings exposed to various light quality treatments revealed that FvFT1 is involved in flowering induction; FvFT1 is strongly activated by FR light but weakly activated by blue light, and FvFT1 mediated the promotion of flowering in the perpetual flowering accession under blue and FR light treatments. However, the molecular mechanism by which variable light quality regulates the induction of flowering in farmed strawberry (*Fragaria ananassa* Duch.) remains unclear and needs to be elucidated.

Given that blue light stimulates strawberry blooming through changed gene expression [5, 36], an overview aiming at identifying flowering induction under blue light treatment at the transcriptome level is required. The impact of blue light (BL) and white light (WL) on flowering induction in farmed strawberry plants was investigated in this research. RNA-Seq technology was used to establish a transcriptome profile for strawberry leaves collected from seedlings under various light quality treatments. The transcription factors BBX that react to blue light were later discovered. The findings suggest that FaBBX29, a strawberry BBX family gene, may play an essential role in flowering control. Our findings, taken combined, offer a complete picture of transcriptional control of blooming in response to blue light therapy. This study may also be used as a reference data source in future research of blue light flowering regulation.

MATERIAL AND METHODOS

- *Extraction of RNA from Plant Materials*

The leaves of the seedlings of cultivated strawberry were tested at a period when all of the seedlings had bloomed under a particular light quality treatment. The experiment was carried out three times, yielding samples for three different repetitions (at least 10 leaves per sample). For downstream analysis, all collected materials were promptly frozen in liquid nitrogen and kept at –80°C.

Chen et al. [37] developed a modified CTAB (cetyltrimethylammonium bromide) technique for isolating total RNA from each sample. Electrophoresis on a 1% agarose gel was used to determine the integrity of 3 RNA, and RNA Nanodrop 2000 was used to determine the amount of RNA.

- *Measurements of Flowering Time and Statistical Analysis*

Flowering time was tracked from the start of each treatment. Every seedling's blooming period was calculated as the number of days from the start of the treatment to the first bloom [5]. R software was used to display the blooming time data (v3.6.3).

- *Illumina Sequencing and cDNA Library Preparation*

The mRNA was isolated from total RNA using poly-T oligo-attached magnetic beads, and then fragmented using divalent cations in a NEBNext First Strand Synthesis Reaction Buffer at high temperatures (5x). A random hexamer primer and RNase H were used to make the first strand cDNA, and a buffer, dNTPs, DNA polymerase I, and RNase H were used to make the second strand cDNA. Following that, the library fragments were purified using a QIAquick PCR kit and eluted with EB buffer before being repaired, A-tailed, and an adaptor was inserted. The library was completed after retrieving the targeted products and performing the PCR amplification. Finally, the Illumina Hi-Seq X platform was used to sequence the cDNA libraries, yielding 150 bp paired-end reads. The suffixes '_R1' and '_R2' denote corresponding data pairs, which are used to differentiate data produced from opposite ends of the same cDNA pool.

- *Genome-Guided Read Mapping and RNA-Seq DataFiltering*

Trimoraic software (v0.32) [38] filtered raw data from the sequencing platform to eliminate adaptor sequence and low-quality reads, resulting in a clean data set was used. The Q30 contents of raw and clean data were computed at the same time. Because high-quality whole-genome sequencing data for grown strawberries were recently released [39], the HISAT2-Stringtie pipeline [40] was used to conduct a genome-guide assembly based on cultivated strawberry genome data. After that, the clean data was mapped back to the reference genome. The TPM (Transcripts Per Kilobase Million) technique was used to standardize both the mapping rate and the expression level of transcripts. The HISAT2 and String tie analysis pipelines were run using their default programmer parameter settings.

- *Analysis of Differential Expression*

The R package 'DESeq2' is a statistical procedure that uses a model based on the negative binomial distribution to perform differential expression analysis of digital gene expression data [41]. A matrix of read counts mapped to transcripts was created from the String tie output data for this differential expression analysis with DESeq2 using a python script (https://github.com/gpertea/ stringtie/ blob/master/prep DE.py) [40].

- *Strawberry FaBBX Transcription Factor Family Study*

We performed a survey of the FaBBX family using our RNA-Seq assembly data and annotations from the Pfam database. The proteins that include the zf-B box protein domain (PF00643) were formerly classified as FaBBX proteins. The Pfam database (http://pfam.xfam.org/) annotated the domains of FaBBX proteins [46]. TBtools [47] was used to display the domain distribution map of the proteins.

RESULTS AND DISCUSSIONS

- *Flowering Induction in Cultivated Strawberry Is Promoted by Blue Light*

Under two light quality treatments, the blooming period of strawberry seedlings was examined. The blue light treatment substantially accelerated strawberry blooming time, as seen in Figure 1. Only 50% of the strawberry seedlings under the blue light treatment had flowered by the 46th day after treatment (DAT), while all of the seedlings under the white light therapy had bloomed by this time (Figure 2). At the time (DAT=46), we took leaf samples from strawberry seedlings.

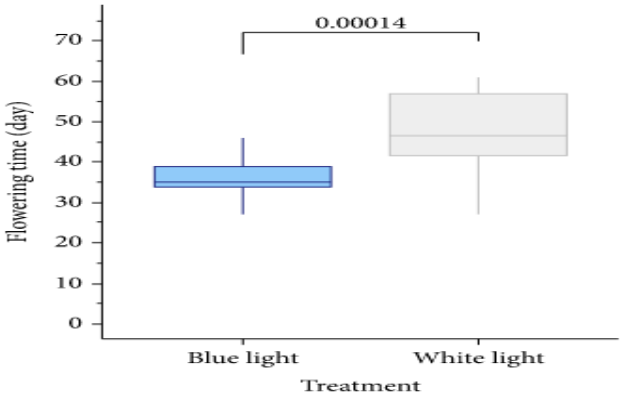

Figure 1 Flowering period of strawberry seedlings under blue and white light treatments in boxplots

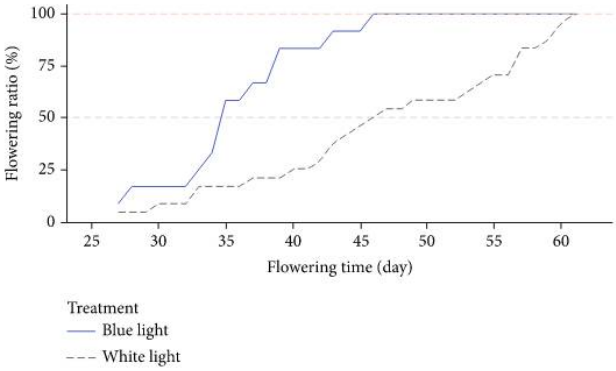

Figure 2 Flowering period of strawberry seedlings after being exposed to blue and white light. A line plot showing the proportion of flowering seedlings observed is shown

- *DEG Identification and Transcriptome Assembly*

Transcriptional regulation is one of the most important ways to control blooming. Six cDNA libraries of leaf samples from the 46th DAT were produced for RNA sequencing on the Illumina platform to get a worldwide knowledge of the molecular mechanism underlying the control of flowering time as influenced by light quality. Table 1 shows the statistics for the sequencing data. After data filtering, a collection of raw data (42.41 Gbp) was produced, from which clean data (37.33 Gbp) with high-quality reads was retrieved. More than 92.95 percent of these clean readings received a Q30 quality score (error rate 0.001), indicating that the data was reliable for further analysis.

Table 1 Statistics on the sequencing data in a nutshell

Data name	Raw data (Gbp)	Clean data (Gbp)	Clean sequence	Q30 (%)	Mapping rate (%)
WL1_R1	3.49	3.07	23286017	94.23	92.50
WL1_R2	3.49	3.07	23286017	93.21	
WL2_R1	3.57	3.14	23803445	94.20	92.40
WL2_R2	3.57	3.14	23803445	92.95	
WL3_R1	3.64	3.20	24249787	94.33	92.17
WL3_R2	3.64	3.20	24249787	93.04	
BL1_R1	3.51	3.09	23416747	94.18	92.05
BL1_R2	3.51	3.09	23416747	93.58	
BL2_R1	3.40	2.99	22645767	94.20	90.18
BL2_R2	3.40	2.99	22645767	93.36	
BL3_R1	3.60	3.17	24011737	94.21	92.19
BL3_R2	3.60	3.17	24011737	93.25	

Notes: Q30: percentage of bases with a ; WL1, WL2, WL3 and BL1, BL2, BL3 represent three biological replication samples from white light and blue light treatments, respectively; the prefixes _R1 and _R2 indicate Illumina paired-end sequencing paired data. Gbp stands for giga base pair.

In the next study, more over 90.18 percent of clean reads were successfully mapped back to farmed strawberry reference genome data, which was recently published [37]. The high mapping rate of all sequencing data, as shown in Table 1, showed the trustworthiness of our sequencing data. Furthermore, this finding showed that grown strawberry genome data was suitable for our subsequent transcriptome study. For genome-guided transcriptome assembly, all clean reads were processed via the HIASAT2-Stringtie workflow. A total of 152 031 transcripts were acquired this way.

TPM was used to calculate and normalize gene expression levels. Between the various samples, Pearson's correlation coefficient was computed. To verify the trustworthiness of biological replications' data, a PCA (principal components analysis) of various sample data was utilized. A study of DEGs was conducted to determine the genes responding to light quality interventions (using the DESeq2 package in R). As a consequence, a total of 6765 genes were identified as DEGs between the white and blue light treatments, with 3737 (54.36 percent) upregulated genes and 3138 (45.64 percent) downregulated genes (Figure 3,). TMP>0 was also used as a criteria for determining whether DEGs were uniquely expressed. Under the white light treatment, 601 (8.7%) DEGs were expressed, whereas under the blue light treatment, TMP=0 DEGs were expressed. For the blue light treatment, 790 (11.5 percent) DEGs were identified as uniquely expressed genes, with TMP=0 under the white light treatment (Figure 4). Under the two treatments, the DEGs were hierarchically grouped based on their expression patterns. These findings revealed a comparable gene expression pattern for biological replications of the same treatment, with DEGs from BL and WL clustering together, respectively (Figure 5).

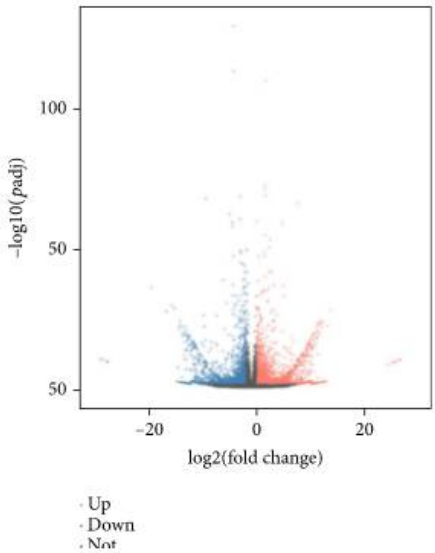

Figure 3 Differentially expressed genes (DEGs) that reacted to the blue light therapy in a volcano plot (BL). The -log10(Padj) value is shown on the y-axis, while the log2 (fold change) is plotted on the x-axis. The red dots represent transcripts that have seen substantial upregulation (|fold change|>1 andPadj0.05) under BL, whereas the blue points represent transcripts that have experienced significant downregulation. The grey dots represent transcripts that haven't changed much.

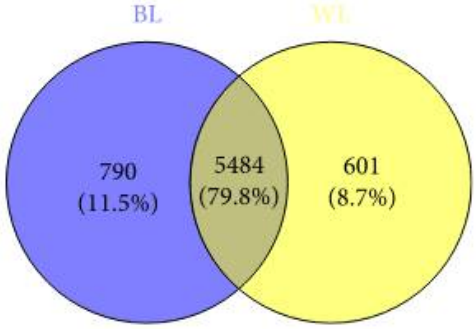

Figure 4 Genes with a unique expression pattern that respond to blue light therapy (BL). A Venn diagram of genes expressed exclusively in BL or WL (white light therapy) and those expressed in both is shown.

The number of such genes under BL is shown in blue; the number of such genes under WL is shown in yellow; and the number of DEGs expressed both in BL and WL is shown in grey.

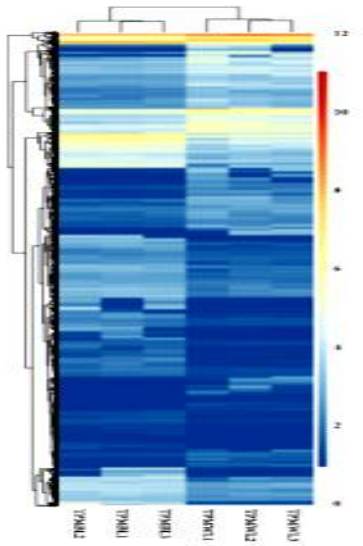

Figure 5 The expression profiles of DEGs are shown as a heatmap. The TPM technique was used to standardize the expression level, and the color scale bar reflects the Log10(TPM+1). Under the blue light treatment, the TPMBL1, TPMBL2, and TPMBL3 correspond to three biological replications. The three biological replications under white light therapy are TPMWL1, TPMWL2, and TPMWL3.

- *DEG Gene Function Enrichment Analysis*

We performed a comprehensive functional annotation of all assembled transcripts of these DEGs and their enrichment analysis to better understand the gene functions of these DEGs. The DEGs were substantially enriched in three major GO categories: 'cellular component,''molecular function,' and 'biological process,' according to the GO enrichment study. The 'biological process' category was the most well-represented, with 73 GO words, followed by the 'molecular function' category, which had 18 GO terms, and the 'cellular component' category, which had just 5 GO terms.

The top three enriched GO keywords in the 'biological process' category (Figure 6, Table S2) were 'response to red light' (GO: 0010114), 'flavonoid biosynthetic process' (GO: 0009813), and 'anthocyanin-containing compound biosynthetic process' (GO: 0009718). (q value). The GO keywords 'response to blue light' (GO: 0010114), 'response to far-red light' (GO: 0010218), 'red or far-red light signaling pathway' (GO: 0010017), 'red light signaling pathway' (GO: 0010161), and 'response to UVB' were all enriched in our study's DEGs, as anticipated (GO: 0010224). Moreover, many GO keywords linked to secondary metabolism, such as 'regulation of anthocyanin biosynthetic process' (GO: 0031540) and 'flavonoid metabolic process' (GO: 0031540), were enhanced (GO: 0009812). Two GO keywords related in the blooming process, 'negative regulation of long-day photoperiodism, flowering' (GO: 0048579) and 'long-day photoperiodism, flowering' (GO: 00485 74), were also enriched in our study (Figure 6)

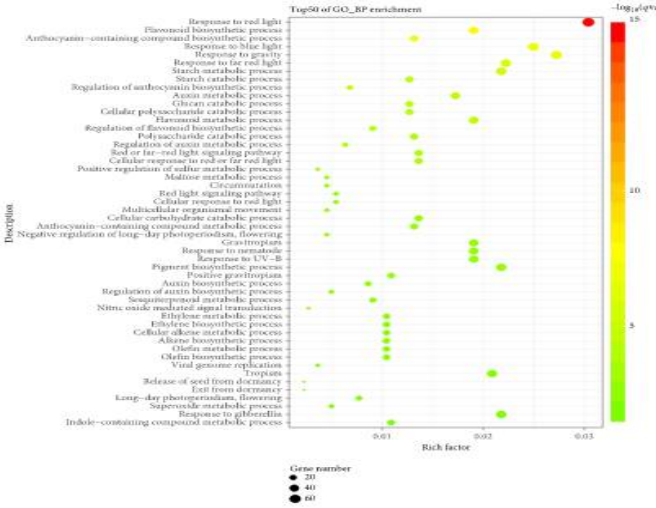

Figure 6 DEG domain enrichment study using conserved domains. The top 25 protein conserved domain enrichments discovered, as determined by the Pfams database, are shown. The names of the conserved domains are shown on the y-axis. The amount of DEGs to the total number of genes in the annotation cluster is the rich factor. The number of

DEGs in the annotation cluster is the gene number. The –log10 (-q-value) is shown by the color scale bar; the q-value is the P-value corrected using the 'BH' technique.

- *Gene Expression Changes Associated with Light Signal Response*

Many plant physiological systems are influenced by light as a key environmental signal. The model plant Arabidopsis has been used to identify the different light-signal transduction pathways. The DEGs were substantially enriched in eight GO keywords relevant to the perception and transduction of light signals, as shown by our GO enrichment findings (GO:0010114,''GO:0009637,''GO:0010218,' 'GO:0010017,''GO:0071489,''GO:0010161,''GO:0071491,' and 'GO:0071482'). We investigated the expression data of 147 DEGs exposed to the aforementioned eight GO keywords and their functional annotation based on the Uniref90 database to learn more about the gene expression patterns and functions of DEGs implicated in the light response. Under the blue light therapy, 72 (48.97%) transcripts were found to be upregulated, whereas 75 (51.02%) transcripts were found to be downregulated.

Light receptor proteins detect the external light environment and activate downstream light signal transduction in plants. Cryptochromes (CRYs), phytochromes (Phys), phototropism (PHOTs), and ultraviolet-B receptors are only a few of the many types of light receptors that plants have (UVR8s). The blue light therapy had an effect on two kinds of light receptors in our research (Figure 9). Blue light therapy substantially increased the transcript encoding a Phi protein (MSTRG.33392.2) as well as three transcripts encoding UVR8 (maker-Fvb1-4-augustus-gene-152.27-mRNA-1, MSTRG.5465.2, and MSTRG.3549.1). The expression of transcripts encoding light receptors involved in other kinds of light signal pathways in plants, on the other hand, did not differ significantly between the two treatments.

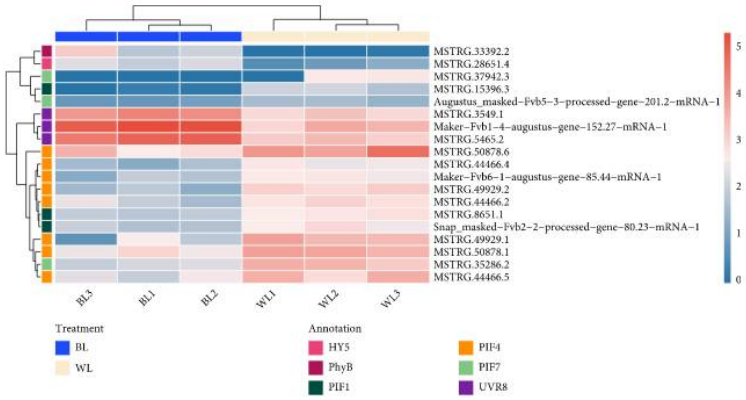

Figure 7 Genes involved in light perception and transduction are shown by a heatmap of their expression. The log10(TPM+1) is shown by the color scale bar. Different colors are used to annotate the transcripts. The three biological replications under blue light therapy are BL1, BL2, and BL3. The three biological replications under white light therapy are WL1, WL2, and WL3.

The zip transcription factor protein ELONGATED HYPOCOTYL5 (HY5) has been identified as a critical hub in plants' light signal transduction network. The blue light therapy increased the expression level of a transcript (MSTRG.28651.4) encoding a homologue of HY5 in our research (Figure 9). In addition, phytochrome-interacting components in this research reacted to blue light. We discovered four transcripts encoding PIF1 proteins (MSTRG.8651.1, MSTRG.15396.3, MSTRG.8651.1, and snap masked-Fvb2-2-processed-gene-80.23-mRNA-1) and eight transcripts encoding PIF4 proteins (MSTRG.50878.1, MSTRG.44466.2, MSTRG.44466.4, MSTRG.44466.5, MSTRG.4992 The blue light treatment reduced the expression of transcripts encoding PIFs (Figure 7).

- *Floral Induction Genes Involved in the Circadian Rhythm*

The blooming period of the experimental strawberry seedlings was clearly influenced by blue light exposure in this research. The blue light treatment altered 66 transcripts whose expression levels could be categorized into two GO keywords ('GO:0048579' and 'GO:0048574') and one KEGG pathway, according to our enrichment analysis findings based on GO and KEGG annotations (ko04712). In all, 20 transcripts (30.30%) were found to be downregulated by blue light, whereas 46 transcripts (69.69%) were found to be upregulated.

The FvCO (CONSTANT) protein plays an essential role in wild strawberry floral induction [33]. Three transcripts (maker-Fvb6-2-augustus-gene-317.47-mRNA-1, maker-Fvb6-3-augustus-gene-0.33-mRNA-1, and maker-Fvb6-1-augustus-gene-48.60-mRNA-1) that encode CO protein homologues in strawberry were identified in this study from DEGs that showed upregulated expression patterns under blue light treatment. Similarly, we discovered that the blue light treatment increased the expression levels of three

additional transcripts (maker-Fvb4-3-augustus-gene-20.38-mRNA-1, maker-Fvb4-1-augustus-gene-185.40-mRNA-1, and maker-Fvb4-2-augustus-gene-18.64-mRNA-1) expressing Adagio protein 3, a homologue of FKF1. Our RNA-Seq findings also revealed the downregulation of 16 genes implicated in floral induction pathways in the circadian cycle. The transcript MSTRG.64658.6 encodes a homologue of the CCA1 (Circadian Clock Associated 1) protein, whereas the other 15 transcripts code for LHY (LATE ELONGATED HYPOCOTYL) proteins (Figure 8).

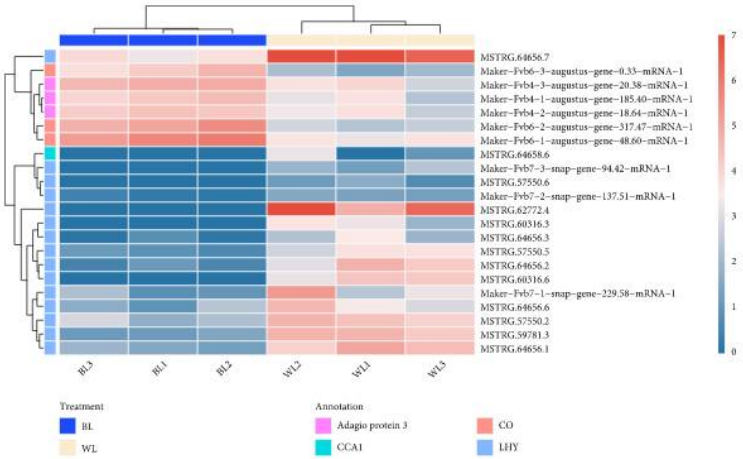

Figure 8 Genes involved in the circadian rhythm pathway are shown by a heatmap of their expression. The log10(TPM+1) is shown by the color scale bar. Different colors are used to annotate the transcripts. The three biological replications under blue light therapy are BL1, BL2, and BL3. The three biological replications under white light therapy are WL1, WL2, and WL3.

- *Factors Affecting BBX Transcription*

Many transcription factors have been found to have a role in light signaling and flowering control. The BBX protein family may play a key role in floral induction under blue light, according to our enrichment study utilizingPfams database annotations. To find out more, we used Pfams annotations and RNA-Seq assembly data to perform a thorough survey of BBX transcription factors. These findings revealed a total of 72 BBX protein-coding transcripts, each of which has at least one zinc finger B-box conserved domain (Figure 9).

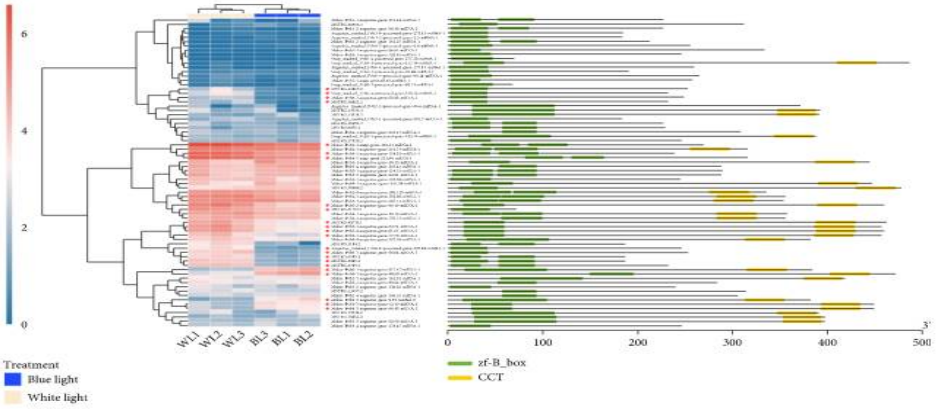

Figure 9 The BBX transcription factor family has been studied. The expression levels of the BBXs are shown in the heatmap on the left. The log10(TPM+1) is shown by the color scale bar in the bottom left corner. Based on the current RNA-Seq study, the red dots before the transcript names indicate the instance of substantial differential expression. The domain distribution of BBX proteins encoded by transcripts is shown in the diagram on the right. The B-box domain and the CCT domain are represented by the green and yellow blocks, respectively.

Under blue light, there were 23 significant differentially expressed transcripts encoding a BBX protein, 18 of which were downregulated and 5 of which were upregulated. The 23 proteins were found to be homologues of AtCO, AtBBX15, AtBBX19, AtBBX24, and AtBBX29, according to the TAIR database (https://www.arabidopsis.org/) (Table S6). The transcript maker-Fvb4-4-snap-gene-165.32-mRNA-1, which encodes a homologue of AtBBX24, has the greatest expression level among these transcripts. With a Pad value of 2.33E-114, the transcript MSTRG.2819.2, which encodes a homologous protein of AtBBX19, exhibited the highest statistically significant differential expression. With a log2 (fold change) value of –

4.41, the transcript snap masked-Fvb6-4-processed-gene-318.22-mRNA-1 encoding a homologue protein of AtBBX29 experienced the most substantial change in expression level.

DISCUSSION

- *Impact of Blue Light on Strawberry*

Light is an important environmental element that influences many aspects of plant development. LED (light-emitting diodes) are now widely used to provide the primary light source or as supplementary illumination, in addition to ambient light, as a tool for fine-tuning the light conditions of the plant growth environment, thanks to the widespread use of plastic greenhouses and growth chambers in protected cultivation [48, 49]. Using a blue light treatment in the growth chamber, we were able to substantially enhance total anthocyanin content and alter the anthocyanin profile of strawberry fruits in earlier research [50]. Because improving light quality may enhance breeding timelines by speeding plant development, LED light resources with high light quality are often utilized in breeding systems. The red-to-blue light ratio is critical for blooming [51]. When used in an LED system, blue light has been proven to stimulate blooming in forest strawberries [5]. Cultivated strawberry seedlings were exposed to blue or white light in this research, using LED as the light source. When compared to the white light treatment, the blue light treatment substantially stimulates blooming in farmed strawberry plants. This result is consistent with previous studies on woodland strawberry and petunia [52].

Aside from encouraging floral initiation, light quality may have an impact on other physiological processes in plants. The chlorophyll content of grape leaves, for example, was substantially greater in plantlets produced under blue light than in plantlets cultivated under white light [53]. In addition, gene expression levels of transcripts producing proteins containing the chloroa b binding domain were altered by blue light, according to enrichment analysis based on the Pfams database annotation for the conserved protein domain.

Blue light boosts the synthesis of secondary metabolites including phenolics and flavonoids in Stevia rubidian callus cultures, according to research. Previous study in our lab showed that various light quality treatments had an impact on the secondary metabolism of strawberry fruit [54]. The DEGs were substantially enriched in GO keywords and KEGG pathways in this research, suggesting that the blue light treatment may have a comparable impact on seedling leaves. The levels of phytohormone in plants have been found to be affected by light. The indoleacetic acid (IAA) content of Norway spruce (PiceaAbies (L.) Karst.) tree seedlings illuminated with blue light LEDs was significantly higher than that of those illuminated with red light, and their transcriptome findings revealed that blue light altered gene expression involved in auxin-response transduction [55]. Similarly, we discovered several DEGs linked to auxin metabolism that were substantially enriched in strawberry after an annotation and enrichment analysis. As a consequence, we found that strawberry seedlings may react to blue light via the mediation of auxin metabolism in our research. However, whether this altered auxin metabolism is linked to strawberry blooming would need to be investigated further in future research.

- *Involved in the Light Transduction Network in Response to Blue Light*

As previously said, light is an important element in plants that regulates a variety of activities. A complete signalling network related with light signal detection and transduction has been developed based on studies using the model plant Arabidopsis. According to our findings, blue light may influence flowering time through photoreceptors and signal transduction components, whose changed activity causes changes in downstream gene expression.

- *The Functions of Circadian Rhythm Genes in Floral Induction*

By processing various light wavelengths, intensities, and photoperiodic duration for the internal clock-setting mechanism, the circadian clock offers important timing information to guarantee plants' optimum development to external environmental circumstances [79]. Some of the DEGs responding to blue light treatment were substantially enriched in the plant circadian rhythm pathway, as shown by our KEGG enrichment analysis.

- *The BBX Family's Roles in Flowering Time Regulation*

Transcription factors regulate gene expression in the light transduction and floral induction pathways, allowing plants to react appropriately to external environmental stimuli. The DNA-binding domains of transcription factors in plants may be divided into various groups. Too far, the Pfams and PlantTFDB databases have categorized over 300,000 transcription factors from 165 plant species into 64 families based on their annotation. We used the Pfams database to do a conserved domain annotation of DEGs as well as an enrichment analysis. The transcription factors with a zf B-box conserved domain were substantially enriched among the DEGs discovered, according to our findings.

The function of AtBBX29, on the other hand, is uncertain. Under the blue light treatment, the expression level of FaBBX29, which encodes a strawberry homologue of AtBBX29, was significantly reduced. As a

result, we hypothesis that FaBBX29 is a negative regulator of blooming in response to various light quality treatments. However, further research into the functional aspects of FaBBX29 in the processes of blooming time control is needed, preferably using a combination of genetic and biochemical methods.

CONCLUSION

This was concluded that the blue light treatment enhanced the blooming of farmed strawberry seedlings in this research. We also looked at the global transcriptome of their leaves under two distinct light quality conditions to get a better idea of how blue light regulates flowering at the gene expression level. Blue light quality may activate the light signal transduction pathway, according to the identification and annotation of DEGs. The control of blooming time is aided by changing gene expression of BBX transcription factors. The FaBBX29 gene, which belongs to the FaBBX family, may play a key role in the mechanism that controls the blooming period of this important crop.

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