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Effects of Photoperiod on Anthocyanin Biosynthesis-Related Gene Expression and Enzymatic Activity in Purple-Leaf Tea Plants (*Camellia sinensis*)

Wei Li ^{1,2,3,*}, Jiacheng Huang ² and Qian Tang ^{2,3,*}

¹ Faculty of Agriculture, Forestry and Food Engineering, Yibin University, Yibin 644005, China

² College of Horticulture, Sichuan Agricultural University, Chengdu 611130, China

³ Tea Resources Utilization and Quality Testing Key Laboratory of Sichuan Province, Chengdu 611130, China

* Correspondence: liwei@yibinu.edu.cn (W.L.); 10668@sicau.edu.cn (Q.T.)

Abstract

Tea is a vital economic crop in China, with many anthocyanin-rich cultivars having been bred. Photoperiod is an important environmental factor that regulates anthocyanin production in plants. Nonetheless, the precise mechanisms by which photoperiod affects anthocyanin biosynthesis in tea plants remain unclear. In this study, the purple-leaf cultivar *Camellia sinensis* ‘Ziyan’ was exposed to three different photoperiods: 8 h/16 h (light/dark, short-day, SD), 14 h/10 h (light/dark, medium-day, MD), and 20 h/4 h (light/dark, long-day, LD). A comprehensive analytical approach, including transcriptomics, enzymology, and quantitative anthocyanin analysis, was used to uncover the molecular and biochemical processes regulating anthocyanin synthesis in purple-leaf tea plants grown under varying photoperiods. The results showed that the delphinidin, cyanidin, pelargonidin, and total anthocyanin contents in the long-day treatment were 178.10%, 92.37%, 50.40%, and 148.76% higher, respectively, than those in the short-day treatment. Under long-day conditions, all structural genes associated with anthocyanin synthesis were upregulated, and enzymatic activities related to anthocyanin synthesis were significantly increased. Furthermore, the regulatory genes (*MYB1*, *MYB73*, *MYB111*, *MYB48*, *MYB75*, *MYB113*, *MYB5*, *MYB12*, *MYB5a*, *MYB5b*, and *WRKY41*) were differentially expressed under short- and long-day treatments. These findings suggest that extended photoperiods activate the expression of structural genes through gene regulation and enhancement of enzymatic activity, thereby facilitating anthocyanin biosynthesis. This study provides novel insights into the photoperiodic regulation of anthocyanin biosynthesis in tea plants.

Keywords: *Camellia sinensis*; photoperiod; anthocyanin; transcriptomics; enzyme



Academic Editor: Xujun Zhu

Received: 12 January 2026

Revised: 6 February 2026

Accepted: 11 February 2026

Published: 15 February 2026

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1. Introduction

Anthocyanins are an important class of water-soluble flavonoid secondary metabolites that are ubiquitous in plants. They impart vivid red, purple, and blue hues to tissues, such as leaves, flowers, and fruits, thereby attracting pollinators and seed dispersers, while also serving as a key component of plant defense against diverse biotic and abiotic stresses. Specifically, anthocyanins mitigate ultraviolet radiation damage, scavenge reactive oxygen species (ROS), and enhance resistance to pathogens and herbivorous insects [1]. As effective antioxidants, anthocyanins have important nutritional and health benefits for human health [2]. In plants, the biosynthesis and accumulation of anthocyanins are regulated by multiple factors, including genetic factors such as transcriptional regulatory

networks and DNA methylation, as well as environmental conditions like light [3,4] and temperature [5–7]. Among the environmental factors, photoperiod is a key variable that significantly influences anthocyanin biosynthesis.

Photoperiod is defined as the relative duration of daylight and darkness within a 24 h cycle. In plants, it serves as a critical environmental signal for perceiving and adapting to seasonal changes. Plants regulate a series of physiological processes through the photoperiod perception system, including flowering time, dormancy cycle, and secondary metabolite synthesis. In recent years, studies have shown that photoperiod length significantly affects the accumulation pattern and anthocyanin content in plants [8–11]. The molecular mechanism of photoperiod-regulated anthocyanin synthesis involves a complex network of light signal perception and transduction pathways. Plants perceive photoperiod signals through photoreceptors, such as phytochromes (PHY), cryptochromes (CRY), and ultraviolet (UV) RESISTANCE LOCUS 8 (UVR8), and activate downstream transcriptional regulatory networks [12,13]. These photoreceptors modulate the stability of the transcription factor ELONGATED HYPOCOTYL5 (HY5) by influencing the activity of the COP1-SPA complex, which in turn governs the expression of structural genes within the anthocyanin biosynthesis pathway [3,14,15]. The output pathways of the circadian clock control anthocyanin biosynthesis by binding directly or indirectly to the promoters of structural genes or their upstream regulators, such as MYB transcription factors. This interaction entrains their expression to a circadian rhythm, thereby timing the efficient activation of the synthesis pathway to specific daily windows, such as at dusk [16].

Tea cultivation in China is primarily concentrated in regions located east of 102° E and south of 32° N. The climatic conditions in this geographical area, including temperature and precipitation, are highly conducive to the growth of tea plants. By 2025, the area allocated for tea cultivation in China had expanded to approximately 3.5 million hectares. Notably, there has been a significant increase in the development of anthocyanin-rich tea cultivars, such as ‘Ziyan’ and ‘Zijuan’, through breeding efforts, thereby enhancing the diversity and functional value of tea resources. Purple-leaf tea (*Camellia sinensis*) is a rare, antioxidant-rich cultivar characterized by a substantial accumulation of anthocyanins, a class of flavonoids responsible for its purple pigmentation. These compounds not only contribute to the distinctive leaf color of the tea plant but also result in a different color of the tea infusion compared to conventional green-leaf tea. Anthocyanin production in tea plants occurs through the conserved flavonoid metabolic pathway, in which a cascade of enzymes, encoded by the corresponding structural genes, catalyzes the sequential conversion of phenylalanine to diverse anthocyanin glycosides. The key enzymes in this cascade include PAL, CHS, CHI, DFR, and ANS [17]. The pivotal control point in this pathway is transcriptional activation, which is predominantly regulated by the MBW transcriptional complex. This complex acts as a master regulator, inducing the transcription of essential structural genes [18,19]. Notably, specific MYB family members in tea plants, including CsMYB75 and CsMYB5b, have been functionally validated as positive regulators of anthocyanin accumulation [20,21].

Current research on anthocyanin accumulation in tea plants has predominantly examined the inductive roles of temperature and light quality, particularly UV-B and blue light [22–24]. In contrast, photoperiodic regulation, a key environmental factor, has received limited and fragmented investigation. Therefore, the present study was designed to systematically uncover the molecular mechanisms underlying the photoperiodic control of anthocyanin biosynthesis in tea plants. These findings provide a theoretical basis and practical methods for developing high-anthocyanin specialty tea varieties through precise control of photoperiods.

2. Results

2.1. Analysis of Color Properties in New Shoots

The leaf coloration observed under short-day treatment was bright and yellow, whereas it appeared dark and purple under long-day treatment (Figure 1). The L , b , and h° values in the different photoperiod treatments followed the order: short-day > medium-day > long-day. Notably, significant variations in the L and h° values were observed across the treatments ($p < 0.05$). Specifically, leaf color brightness was highest under short-day conditions and darkest under long-day treatment (Figure 1D). Compared to the control, the a value increased significantly by 31.71% and 18.70% under medium- and long-day treatments, respectively, although no significant difference was found between these two treatments. Additionally, the C value under long-day treatment was 27.35% higher than that of the control, whereas the medium-day treatment showed no significant difference. These findings indicate that the color of leaves under short-day treatment was bright yellow, whereas under long-day treatment, it was dark purple (Figure 1).

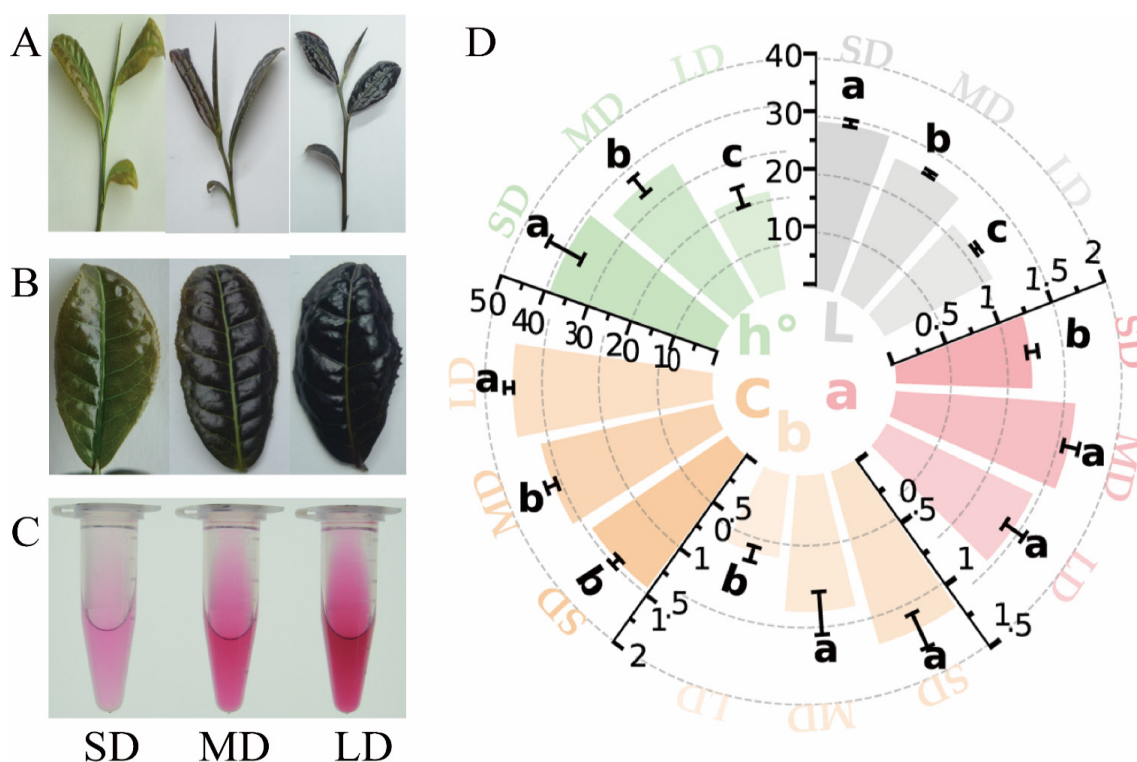


Figure 1. Phenotypes of new shoots, anthocyanin extracts, and color values under different photoperiods. (A) New shoots of 'Ziyan'; (B) second apical leaves of 'Ziyan'; (C) anthocyanin extract from young shoots of 'Ziyan'; (D) color values of tea leaves under different photoperiod treatments. SD, MD, and LD denote 8 h light/16 h dark, 14 h light/8 h dark, and 20 h light/4 h dark periods, respectively. Different lowercase letters on the error bars indicate significant differences ($p < 0.05$).

2.2. Anthocyanin Content in Different Treatments

The anthocyanin content in young shoots was significantly affected by the different photoperiod treatments (Figure 2). Anthocyanin accumulation in young shoots was significantly positively correlated with extended photoperiods ($p < 0.05$). Under long-day conditions, the contents of delphinidin, cyanidin, pelargonidin, and total anthocyanins increased by 178.10%, 92.37%, 50.40%, and 148.76%, respectively, compared to those of the control. Similarly, medium-day treatment elevated these components by 63.27%, 53.64%, 30.16%, and 58.92%, respectively, compared to the control. Consequently, the anthocyanin content in the new shoots of 'Ziyan' increased with prolonged light exposure.

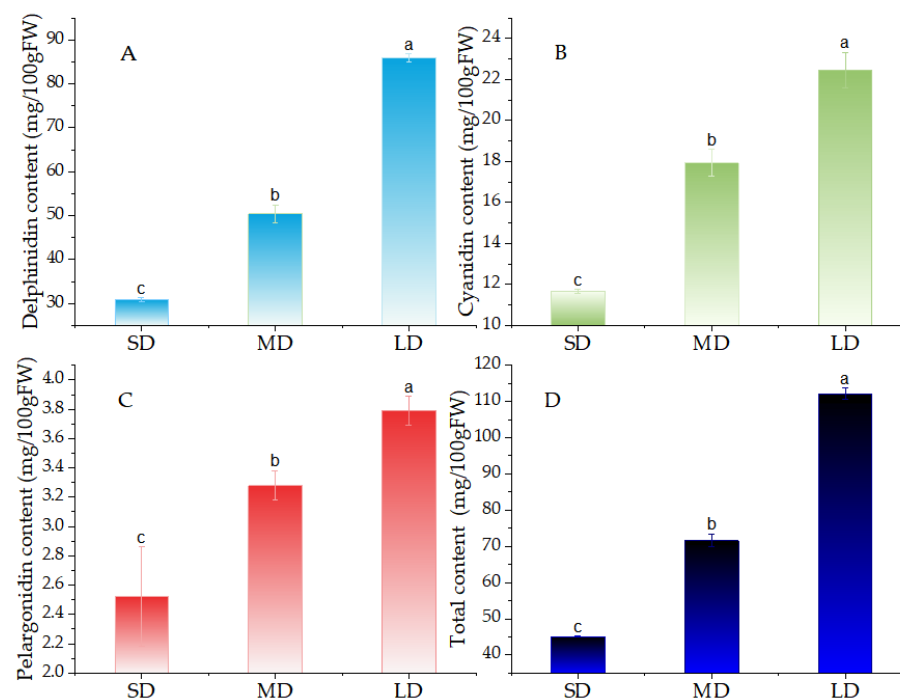


Figure 2. Anthocyanidin contents in different photoperiod treatments. (A) delphinidin content; (B) cyanidin content; (C) pelargonidin content; (D) total anthocyanins content. SD, MD, and LD denote 8 h light/16 h dark, 14 h light/8 h dark, and 20 h light/4 h dark periods, respectively. Different lowercase letters on the error bars indicate significant differences ($p < 0.05$).

2.3. Catechins Content in Young Shoots Treated by Different Photoperiods

To better understand how photoperiod affects anthocyanin biosynthesis, catechin content was measured (Table 1). Within each photoperiod treatment, the catechin component EGCG had the highest content, followed by EGC, and GCG had the lowest content. Compared to short- and medium-day treatments, long-day treatments significantly enhanced the contents of EGC, EC, GCG, ECG, C, and CG ($p < 0.05$). Specifically, these indicators under long-day treatment were increased by 209.47%, 285.71%, 455.56%, 186.79%, 13.64%, and 153.33%, respectively, compared to those under short-day treatment. Compared to the medium-day treatment, the increases in these indicators under the long-day treatment were 7.70%, 22.73%, 92.31%, 6.30%, 8.70%, and 40.74%, respectively. Conversely, compared to short- and long-day treatments, the GC, EGCG, and total catechin contents were significantly higher under medium-day treatment ($p < 0.05$) (Table 1). This analysis revealed that long-day treatment significantly increased catechin content.

Table 1. Catechin content in tea shoots under different photoperiods (% dry weight).

Catechins	SD	MD	LD
GC	0.15 ± 0.01 b	0.50 ± 0.01 a	0.17 ± 0.01 b
EGC	0.95 ± 0.01 c	2.73 ± 0.06 b	2.94 ± 0.07 a
C	0.44 ± 0.01 b	0.46 ± 0.02 b	0.50 ± 0.02 a
EC	0.07 ± 0.01 c	0.22 ± 0.01 b	0.27 ± 0.01 a
EGCG	4.34 ± 0.05 c	10.46 ± 0.39 a	8.06 ± 0.12 b
GCG	0.09 ± 0.01 c	0.26 ± 0.01 b	0.50 ± 0.02 a
ECG	0.53 ± 0.04 c	1.43 ± 0.01 b	1.52 ± 0.02 a
CG	0.30 ± 0.05 c	0.54 ± 0.01 b	0.76 ± 0.07 a
Total	6.60 ± 0.08 c	16.58 ± 0.35 a	14.18 ± 0.05 b

Different lowercase letters following the numbers in the same row indicate significant differences ($p < 0.05$).

2.4. Key Enzymatic Activities During Anthocyanin Biosynthesis

The activities of key enzymes in the anthocyanin biosynthesis pathway were significantly modulated by photoperiod (Figure 3). Notably, under long-day conditions, the activities of CHS and CHI enzymes were substantially enhanced compared to short- and medium-day conditions ($p < 0.05$) (Figure 3A,B). CHS activity increased by 25.75% and 8.46% under long-day treatment compared to short- and medium-day treatments, respectively. Similarly, CHI activity was enhanced by 22.69% and 15.34% under long-day conditions compared to short- and medium-day conditions, respectively. The F3H enzyme activity was significantly higher in both medium- and long-day conditions than in short-day conditions, with increases of 13.98% and 21.66%, respectively ($p < 0.05$) (Figure 3C). Photoperiod also markedly alters the activities of key biosynthetic enzymes. Under long-day treatment, F3'H activity increased by 37.14% relative to that in the control (Figure 3D). The activities of F3'5'H, DFR, and ANS were significantly enhanced under both medium- and long-day conditions ($p < 0.05$; Figure 3E–G). Specifically, long-day exposure increased these activities by 17.39%, 23.67%, and 22.22%, respectively; the corresponding increases under medium-day conditions were 9.32%, 9.44%, and 14.46%. LAR activity was highest under medium-day conditions, exceeding levels in short- and long-day treatments by 62.07% and 49.08%, respectively, although short- and long-day treatments did not differ significantly (Figure 3H). In contrast, ANR activity under long-day conditions was 25.26% lower than that in the control, with no significant differences observed between the short- and medium-day treatments (Figure 3I).

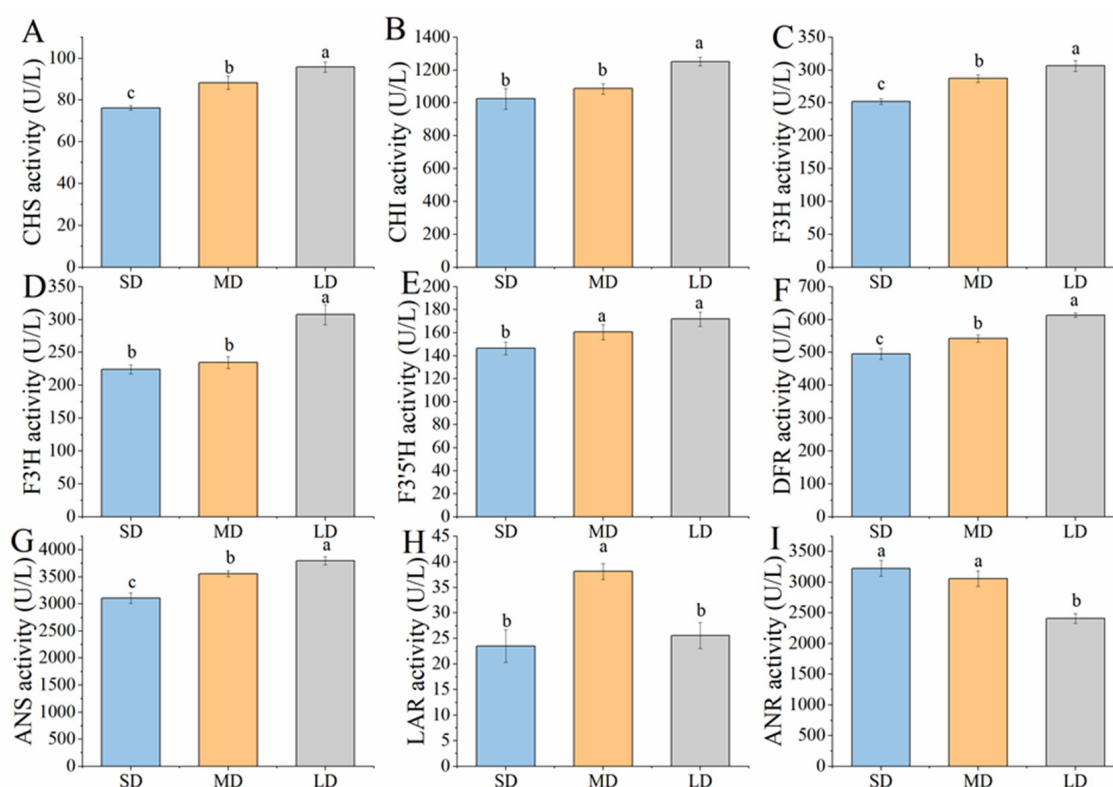


Figure 3. Effect of different photoperiods on the enzyme activity of anthocyanin biosynthesis. Panels (A–I) depict the activities of key anthocyanin pathway enzymes: chalcone synthase (CHS), chalcone isomerase (CHI), flavone-3-hydroxylase (F3H), flavonoid-3'-hydroxylase (F3'H), flavonoid-3',5'-hydroxylase (F3'5'H), dihydroflavonol-4-reductase (DFR), anthocyanidin synthase (ANS), leucoanthocyanidin reductase (LAR), and anthocyanidin reductase (ANR). Different lowercase letters on the error bars indicate significant differences ($p < 0.05$).

2.5. Transcriptome Sequencing and Gene Mapping

To elucidate the molecular mechanisms underlying photoperiod-regulated anthocyanin accumulation in the young shoots of 'Ziyan', high-throughput RNA sequencing was performed. Total RNA from the six samples was used to construct cDNA libraries, which were sequenced on the Illumina HiSeq 4000 platform, yielding 66.64 Gb of clean sequence data. The sequencing quality was high, with a GC content of >45% and Q30 of >92% for all libraries (Table S1). Clean reads were mapped to the tea plant reference genome with an alignment rate ranging from 87.22% to 89.24%. Furthermore, 12,655 novel genes were identified, and functional annotations were successfully assigned to 9435 of them through comparative analysis, alternative splicing prediction, and gene structure optimization. The results of the quantitative real-time PCR verification aligned with the expression patterns observed in the RNA-seq data, thereby confirming the reliability of the transcriptome data (Figure S1).

2.6. Analysis of Differentially Expressed Genes (DEGs)

To investigate photoperiod-responsive gene expression, differentially expressed genes (DEGs) between long-day (LD) and short-day (SD) treatments were identified using DESeq2, with thresholds set at a false discovery rate (FDR) < 0.01 and a fold change ≥ 2.0 . In the SD vs. LD comparison, a total of 2410 DEGs were detected, of which 1393 were upregulated and 1017 downregulated (Figure 4A). As illustrated in the cluster analysis (Figure 4B), the expression profiles showed high consistency among triplicate samples within each treatment, whereas clear opposing trends were observed between the two photoperiod conditions. These results indicate that young shoots undergo substantial transcriptomic reprogramming in response to day length. All identified DEGs were functionally annotated using several public databases, including KEGG, COG, Nr, KOG, Pfam, Swiss-Prot, GO, and eggNOG.

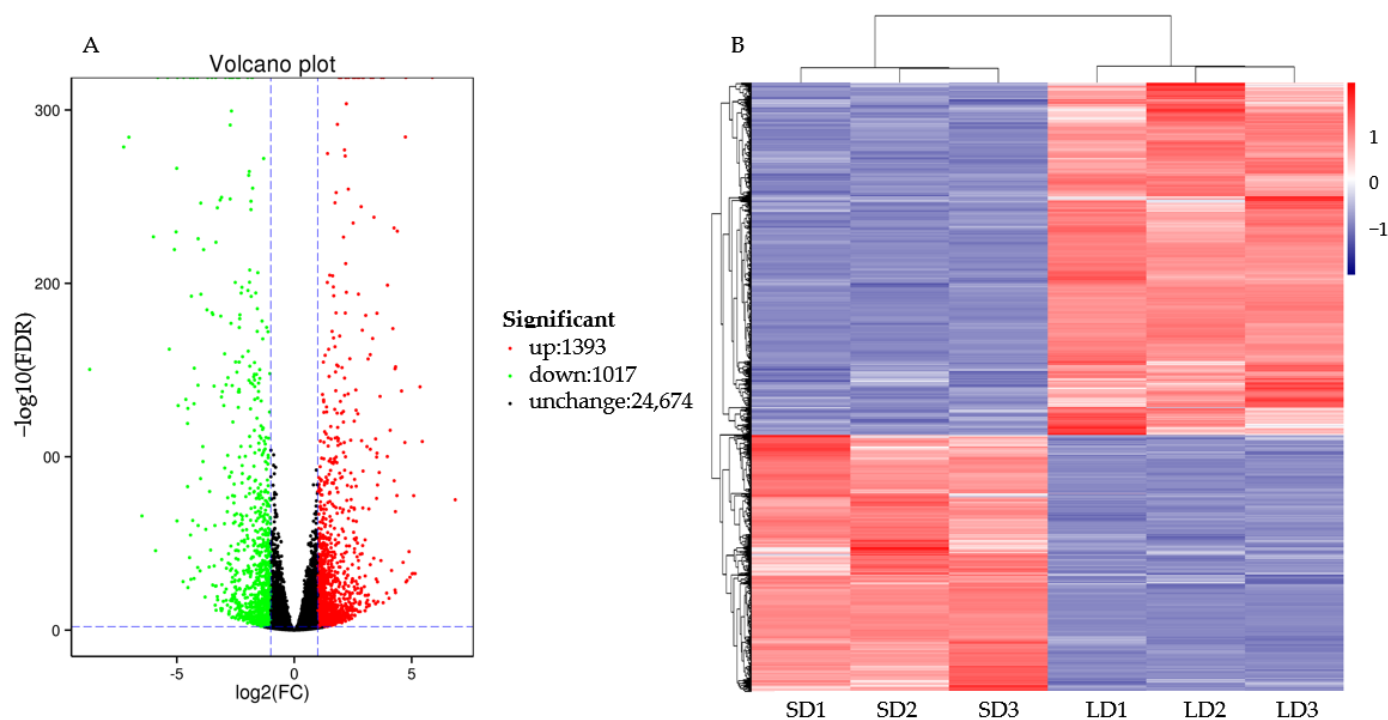


Figure 4. Volcano map and correlation heatmap of differential gene expression in comparisons of SD vs. LD. (A) Volcano plot of differentially expressed genes; (B) Correlation heatmap of differentially expressed genes.

Gene Ontology (GO) enrichment analysis was conducted to explore the functional significance of differentially expressed genes (DEGs) between the SD and LD groups. The DEGs were assigned to three standard GO domains: biological process, cellular component, and molecular function (Figure 4). Within the domain of biological processes, notable enrichments were identified for terms such as “metabolic process,” “cellular process,” “single-organism process,” “biological regulation,” and “response to stimulus.” Regarding cellular components, the DEGs were predominantly mapped to “cell part,” “cell,” “organelle,” “membrane,” “membrane part,” “organelle part,” and “macromolecular complex.” Regarding molecular function, the most enriched activities encompassed “catalytic activity,” “binding,” “transporter activity,” and “structural molecule activity” (Figure S2).

To delineate the key metabolic and signaling pathways involving the differentially expressed genes (DEGs), KEGG enrichment analysis was performed. The top 20 enriched pathways are shown in Figure 5. Notably, in the SD versus LD comparison, several pathways showed significant enrichment of DEGs: flavonoid biosynthesis encompassed 27 DEGs, glucosinolate biosynthesis contained 11 DEGs, plant circadian rhythm involved 15 DEGs, phenylpropanoid biosynthesis exhibited enrichment of 35 DEGs, phenylalanine metabolism included 16 DEGs, and starch and sucrose metabolism accounted for 36 DEGs (Figure 5).

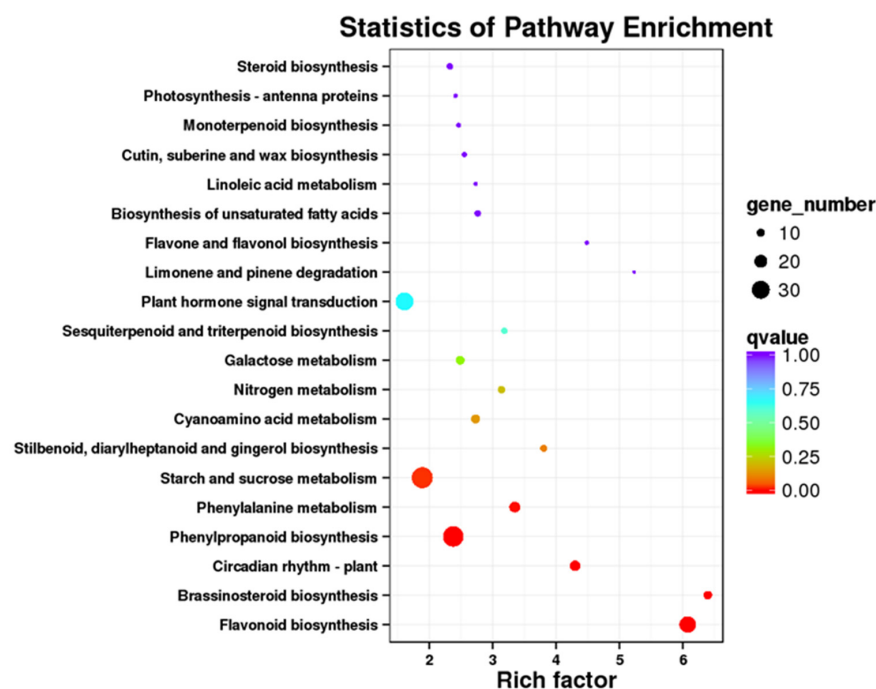


Figure 5. KEGG enrichment analysis comparing SD and LD. Each circle denotes a KEGG pathway. The y-axis lists the pathway names, and the x-axis represents the Enrichment Factor—the ratio of the proportion of differentially expressed genes annotated to a given pathway to that of all genes annotated to the same pathway. A larger Enrichment Factor indicates a higher enrichment level of differentially expressed genes in the pathway. Circle color reflects the q-value; smaller q-values correspond to more reliable enrichment significance. Circle size is proportional to the number of enriched genes within the pathway, with larger circles indicating more genes.

2.7. Transcription Factors (TFs) Analysis

Through the functional annotation of differentially expressed genes, 24 MYB, 10 bHLH, 7 WD40, 11 WRKY, and 11 NAC transcription factors were identified in the new shoots of tea plants grown under different photoperiods, exhibiting differential expression between short-day and long-day conditions (Figure 6). Most MYB family members were upregulated

under long-day conditions. Compared to short-day treatment, 8 MYBs were downregulated, whereas 16 MYBs were upregulated under long-day treatment. The WD40 family had two members with downregulated expression and five members with upregulated expression levels. Of the 10 bHLH family members, five were upregulated and the remaining five were downregulated. Only one member of the WRKY family was upregulated, whereas the other ten were downregulated. Three NAC family members were upregulated. These findings indicate that different photoperiod treatments significantly affect the transcription factor expression levels in tea shoots.

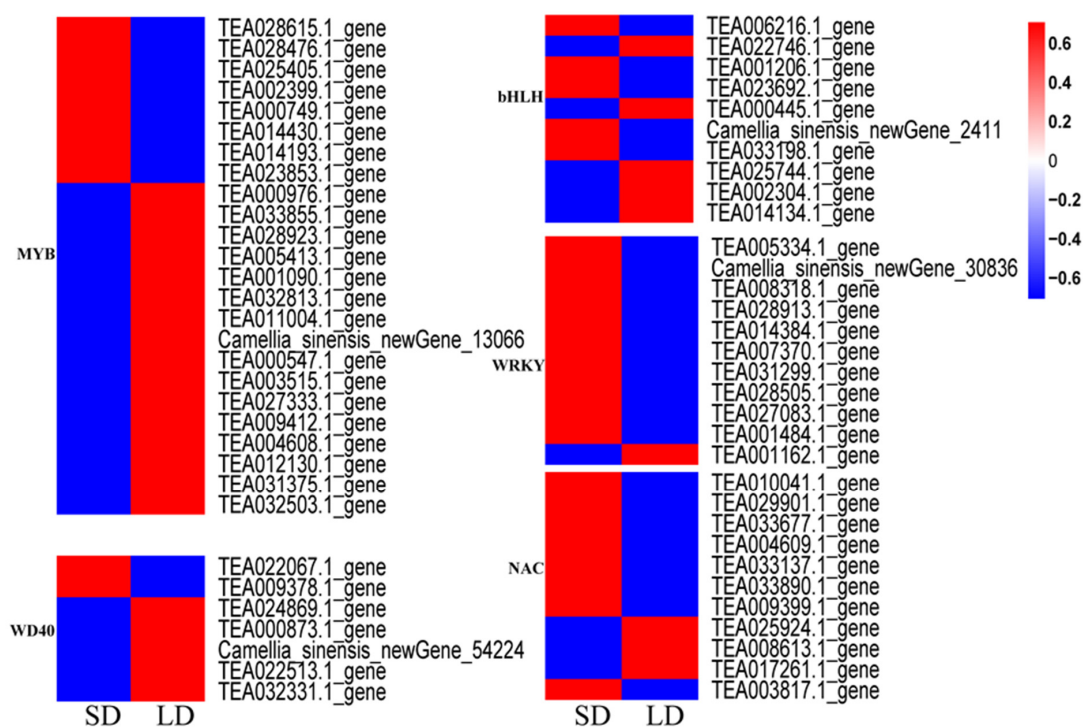


Figure 6. Transcription factor expression patterns in differentially expressed genes.

2.8. Expression Profiles of Anthocyanin Biosynthesis-Related Genes

We further examined the expression patterns of enzyme-encoding genes involved in this pathway under different photoperiod treatments. Except for *C4H* and *CHI*, all structural genes were represented by two or more family members (Figure 7). A notable expansion was observed in the *UFGT* gene family, which contains 29 members. Among these, only four (*TEA000079.1_gene*, *TEA016377.1_gene*, *TEA025143.1_gene*, and *TEA012478.1_gene*) were downregulated under long-day conditions, while all other structural genes in the anthocyanin biosynthetic pathway were upregulated. The expression of genes directing flux toward the catechin and flavonol branch pathways, namely *FLS*, *LAR*, and *ANR*, was also elevated under long-day treatment. Overall, extended light exposure promoted the expression of core anthocyanin biosynthesis genes (*PAL*, *CHS*, *F3H*, *F3'H*, *F3'5'H*, *DFR*, *ANS*, and *UFGT*) as well as branch-pathway genes (*FLS*, *LAR*, and *ANR*) (Figure 7). To better characterize the expression profiles of genes involved in anthocyanin biosynthesis across different photoperiods, quantitative real-time PCR was performed on 11 structural genes under short-, medium-, and long-day treatments. As shown in Figure 8, the expression levels of the selected genes generally increased with prolonged light exposure. Moreover, the expression of *FLS*, *LAR*, and *ANR*, which participate in the synthesis of flavonols and catechins, also increased with longer photoperiods. These findings suggest that the duration of light exposure has a considerable impact on the regulation of genes associated with anthocyanin biosynthesis.

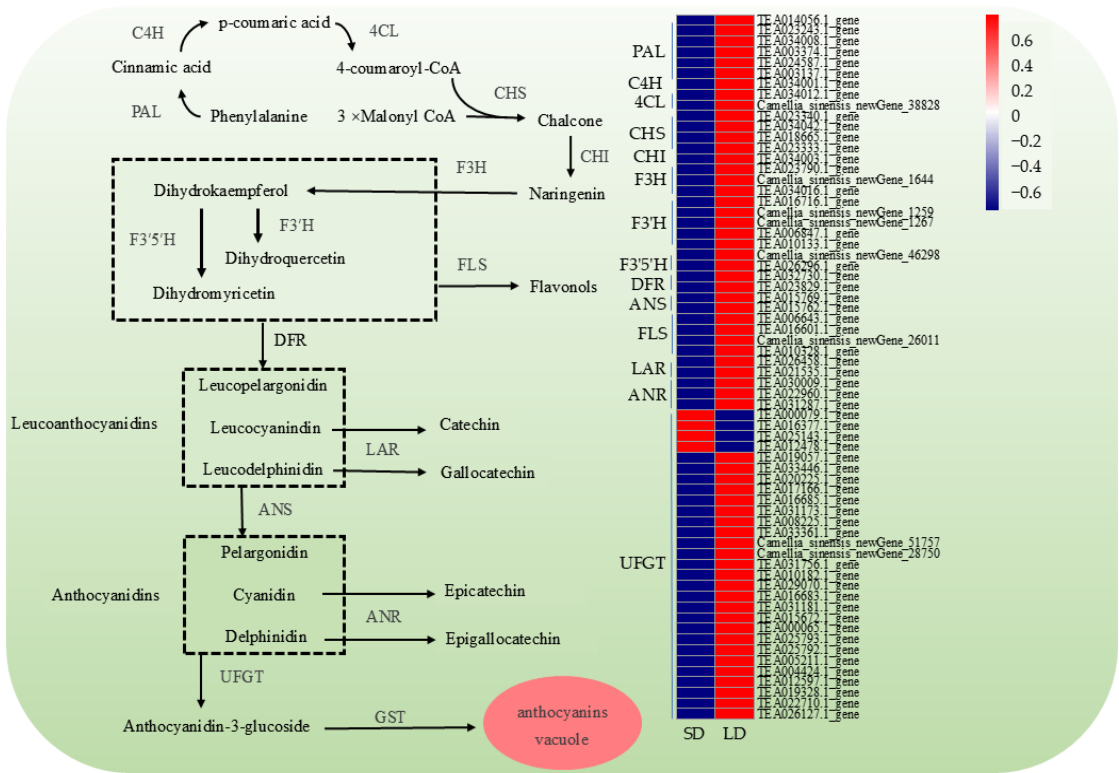


Figure 7. Expression patterns of structural genes in the anthocyanin synthesis pathway (RNA-Seq data) SD and LD represent photoperiods of 8 h light/16 h dark and 20 h light/4 h dark, respectively.

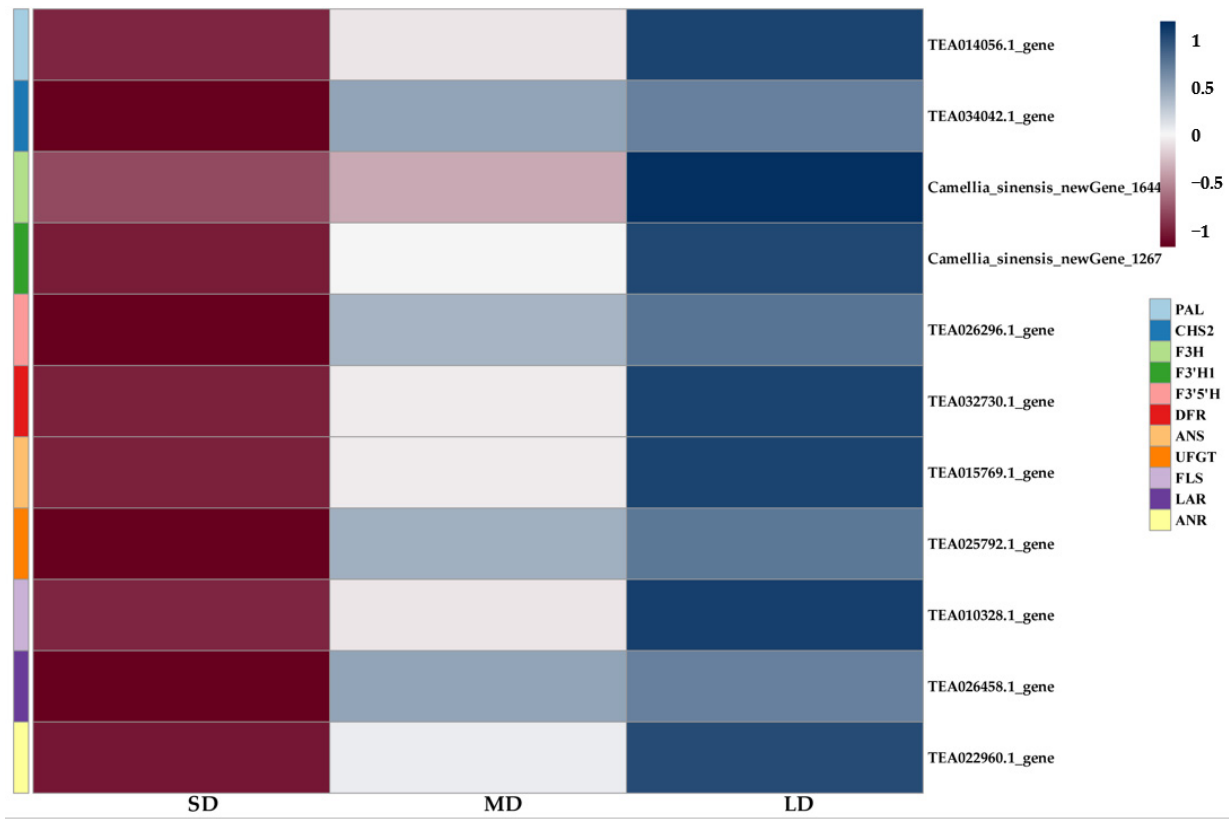


Figure 8. Expression patterns of structural genes in the anthocyanin synthesis pathway (qRT-PCR data). SD, MD, and LD represent photoperiods of 8 h light/16 h dark, 14 h light/8 h dark, and 20 h light/4 h dark, respectively.

3. Discussion

Anthocyanins in plant tissues and organs play a crucial role in protecting photosynthetic structures and scavenging free radicals [1]. The biosynthesis and accumulation of anthocyanins in plants generally require light. For instance, anthocyanin content in the peel can be increased under light exposure [25,26]. Photoperiod, a key light factor, significantly regulates the biosynthesis of secondary metabolites in plants [27]. Studies have found that photoperiod exerts varying effects on anthocyanin synthesis in different plant species [28], with extended daylight generally increasing anthocyanin content in leaves. The findings of this study demonstrated a significant increase in anthocyanin content in the new shoots of ‘Ziyan’ with prolonged light exposure (Figure 2). These findings are corroborated by reports of photoperiod-induced anthocyanin accumulation in potato tubers [29], Amaranth plantlets [30], and Tartary buckwheat sprouts [31].

Long-day treatment was associated with markedly elevated activities of key anthocyanin biosynthesis enzymes (CHS, CHI, F3H, DFR, and ANS) in tea plant shoots. Additionally, the activities of LAR and ANR, which are involved in catechin synthesis, were increased. The augmented activity of these enzymes facilitates the biosynthesis of anthocyanins and catechins, which aligns with the observed increase in their content. Concurrently, the analysis of anthocyanin component content in purple tea plant new shoots under varying photoperiod treatments revealed that photoperiod had a notably distinct influence on different anthocyanin components. For instance, the contents of delphinidin, cyanidin, pelargonidin, and total anthocyanins in new shoots subjected to long-day treatment were 178.10%, 92.37%, 50.40%, and 148.76% higher, respectively, than those under short-day period treatment. Therefore, during short-day seasons (e.g., spring and autumn), the implementation of artificial lighting, such as LED lights, to extend the photoperiod is a promising precision agriculture strategy. This technique can significantly boost the anthocyanin levels in tea leaves, thereby enhancing their quality and functional properties.

By combining transcriptome data with biochemical assays, this study demonstrated that long-day conditions concurrently upregulate key transcriptional regulators (MYB, WD40, and NAC) and enhance anthocyanin accumulation in tea shoots. This correlation suggests a direct role for these genes in the regulation of anthocyanin biosynthesis. Conversely, most WRKY family members were upregulated under short-day conditions, and there was no significant difference in bHLH gene expression between long-day and short-day treatments. Expression analysis revealed that nearly all structural genes in the anthocyanin pathway were upregulated under long-day conditions, consistent with the concomitant increase in biosynthesis activity. However, the activity of ANR did not correspond to its expression pattern. We hypothesized that a reduction in anthocyanin reductase activity may decrease anthocyanin degradation and provide feedback to ANR expression, thereby enhancing catechin synthesis and its accumulation. This is consistent with the highest anthocyanin and catechin contents observed under long-day conditions. He Ping [32] observed a correlation between anthocyanin levels in peach peels and the expression patterns of the *UFGT* gene. Similarly, this study revealed that an increase in anthocyanin content is associated with increased *UFGT* gene expression, underscoring the crucial role of *UFGT* in anthocyanin biosynthesis in tea plants.

4. Materials and Methods

4.1. Plant Materials and Experimental Treatments

Uniform and healthy one-year-old seedlings of the purple-leaf tea cultivar ‘Ziyan’ were selected for this study. Photoperiod treatments were conducted under controlled conditions of constant light intensity ($200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, white light), temperature ($25/18^\circ\text{C}$, light/dark), and relative humidity (80% RH). Three light regimes were ap-

plied: short-day (SD, 8 h light/16 h dark, control), medium-day (MD, 14 h light/10 h dark), and long-day (LD, 20 h light/4 h dark). The cultivation setup, growth conditions, and sampling procedures were performed as previously described [24,33].

4.2. Color Measurement

Leaf color was measured on the second top leaf of each sample using a CM-2600d spectrophotometer (Konica Minolta, Tokyo, Japan) under a D65 illuminant at a 45°/normal illumination angle. Five measurements were taken per leaf, avoiding the primary vein. Color was represented in the CIE *L a b* space, which provides the values of *L* (lightness; positive values indicate brightness, negative values indicate darkness), *a* (green–red axis; positive values indicate redness, negative values indicate greenness), *b* (blue–yellow axis; positive values indicate yellowness, negative values indicate blueness), chroma (*C*, saturation), and hue angle (*h°*).

4.3. Anthocyanin and Catechin Content Measurement by HPLC Method

To determine the anthocyanin and catechin content in new shoots, sample preparation, detection methods, and instrumentation were performed according to a previously established protocol [33,34].

4.4. Measurement of Anthocyanin Biosynthesis-Related Enzyme Activity

The methods for enzyme extraction, kit-based assays, and detection of key anthocyanin biosynthetic enzymes were consistent with those reported in Reference [33].

4.5. RNA Extraction, Library Construction, and RNA-Seq

Three samples from the SD and LD treatments were used to construct six cDNA libraries: SD_1, SD_2, SD_3, LD_1, LD_2, and LD_3. The procedures for RNA extraction, library preparation, and subsequent RNA-seq analysis were performed according to the methodology outlined in reference [33].

4.6. Transcriptome Analysis and Read Mapping

Raw sequencing reads underwent quality filtering using a Perl script to eliminate low-quality sequences, such as those consisting solely of adaptors, containing over 5% unknown nucleotides, or having a Q20 score below 20% (which corresponds to a base call error rate > 1%). The filtered clean reads were subsequently aligned to the *Camellia sinensis* reference genome (accessible at <https://tpia.teaplants.cn/download.html>, accessed on 12 January 2026) using Tophat2 [35]. Following alignment, potential PCR duplicates were removed from the BAM/SAM file. Finally, gene expression levels were calculated as fragments per kilobase of exon per million mapped fragments (FPKM) using Cufflinks [36].

4.7. Identification of Differentially Expressed Genes (DEGs)

Differential expression between the SD and LD groups was analyzed using DESeq2 [37]. The false discovery rate (FDR) was controlled to account for multiple testing, and genes with an absolute $\log_2$ (fold change) ≥ 1 and an FDR-adjusted *p*-value < 0.01 were identified as differentially expressed.

4.8. GO and KEGG Enrichment Analysis

Gene Ontology (GO) enrichment analysis for the identified DEGs was performed using the Goseq R package, which employs a Wallenius non-central hypergeometric distribution to correct for transcript length bias [38]. This method accounts for gene length bias in DEGs. We conducted KEGG pathway enrichment analysis of the differentially expressed genes utilizing KOBAS [39], based on the KEGG database [40].

4.9. Gene Expression Validation by qRT-PCR

The methodology applied for quantitative real-time PCR analysis is based on well-established research techniques [33]. Supplementary Table S2 provides a comprehensive list of gene-specific primers used in this study.

4.10. Statistical Analysis

All statistical analyses were performed using SPSS 20.0 (Statistical Product and Service Solutions, SPSS Inc., Chicago, IL, USA). Data are presented as mean $\pm$ standard deviation (SD) of three independent replicates ($n = 3$). Differences among treatments were assessed via one-way analysis of variance (ANOVA), followed by Duncan's multiple range test for post hoc comparisons. Statistical significance was set at $p < 0.05$.

5. Conclusions

This study revealed the correlation between anthocyanin production and photoperiod. Photoperiod can induce the expression of genes involved in anthocyanin production. Consequently, the activities of all enzymes involved in anthocyanin synthesis in new tea plant shoots increase with prolonged light exposure. It also suppresses the expression of anthocyanin reductase genes, thereby reducing enzyme activity. Long-day conditions effectively enhance anthocyanin synthesis in new purple tea plant shoots, significantly increasing the content of each component and promoting catechin synthesis.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/ijms27041867/s1>.

Author Contributions: Conceptualization, W.L. and Q.T.; formal analysis, W.L.; investigation, W.L. and J.H.; funding acquisition, W.L.; resources, Q.T.; writing and original draft, W.L.; writing, review, and editing, W.L. and Q.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Sichuan Science and Technology Program (grant number 2024NSFSC0394) and the high-level talent “QiHang” program of Yibin University (grant number 2021QH10).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available from the corresponding author upon reasonable request.

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

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