Responses of Secondary Metabolites and Transcriptomes in Tea Cultivar ‘Zhong Ming 6’ (*Camellia Sinensis*) under Blue Light and Red Light

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Abstract

Light is one of the most prestigious environmental sign, which stimulates plant metabolite consequences. Zhong Ming 6 (ZM6) is a green tea (*Camellia sinensis*) cultivar with highly accumulating TGGP. Here, three kinds of supplemental light wavelengths including blue light (BL, 200 µmol m$^{-2}$ s$^{-1}$), red light (RL, 200 µmol m$^{-2}$ s$^{-1}$), and White Light (WL/CK, 200 µmol m$^{-2}$ s$^{-1}$) were applied to explore their effects on the transcriptomes and metabolomics of young shoots (one bud and two leaves) in tea plants. Interestingly, artificial BL and RL significantly affect the secondary metabolites and transcriptome factors of tea plants. Here, BL extensively dominated the multiple physiological actions with secondary metabolism. In addition, RL could induce plant growth, development, and photosynthesis. Thoroughly, the identification of eight structural genes and 34 transcription factors (TFs) significantly correlated with total catechin (TC) and anthocyanin. Due to the upregulations of the *CsGSTF1* gene in the flavonoid biosynthesis pathway, anthocyanin production was maximum under BL. Then, *CsMYB75*, *bHLH-MYC*, and other *R2R3-MYBs* were highly upregulated in BL to increase the accumulation of TC and anthocyanins in tea plants. Again, the *CsMYB4* gene was highly significant and positively correlated with TC and anthocyanin accumulation under RL. We indicated that BL is more feasible due to the number of functional metabolites (gallic acid, caffeine, TC, TGGP (1, 2, 6-tri-O-galloyl-β-D-glucopyranose), and anthocyanin) being supreme. For taste, quality, and dynamic indigenous mechanism of the tea plant, RL is also suitable that increase Chlorophyll content and tea yield.

1. Introduction

Tea (*Camellia sinensis*) is the globally most effective non-alcoholic beverage for its' beneficial effects in human health (Bai et al., 2021) as well as adding GDP in many countries (Fu et al., 2015). The extracts of green pertained from *Camellia sinensis* species under Theaceae family bearing several kinds of secondary metabolites, for example, catechins, anthocyanin (Liao et al., 2021), caffeine (Parvez et al., 2021), Gallic acids (Jovanovic et al., 2021), etc. Flavan 3-ols, namely catechins, is also known as the principal element of polyphenols in the young shoot of tea plants (Guo et al., 2017) with high anti-oxidant and reduced the high level of blood glucose, various kinds of renal function, hepatic and lipid parameters in animals (Nazir et al., 2021). By adding extra anti-oxidant substances, the human body uses them safely and has extracted them from food and plants (Bae et al., 2020). The phenylpropanoid and flavonoid biosynthetic pathways are the essential pathways for catechins and anthocyanin production (Zhang et al., 2018).

Again, 1, 2, 6-tri-O-galloyl-β-D-glucopyranose is also known as 1, 2, 6-TGGP, naturally producing compound in green tea (*Camellia sinensis*) with very low (0 to 6.6mgg$^{-1}$) availability (Wei et al., 2019). It was first recognized in *Cornus officinalis* by cell cultures (Yazaki and Okuda, 1989) and also highly beneficial against various multidrug-resistant bacteria and medical science (Kim et al., 2009) by using synergistic combination and conventional antibiotics (Bag et al., 2013).
During growing period, light affects the tea plant as an environmental factor which, effortlessly stimulates the secondary metabolites (catechins, anthocyanin, etc.) accumulation to enhance the tea quality (Liao et al., 2021). Particularly, BL and RL increased these secondary metabolites’ activity (Jin et al., 2021). Plant received certain types of photoreceptor under different kinds of light wavelength to regulated secondary metabolites (Wang et al., 2020; Zheng et al., 2019). It is also a crucial signaling factor to the production of yield (Wang et al., 2021; Ye et al., 2021). In addition, photosynthetic ability is very essential, as it directly contributes to plant growth and productivity (Sperdouli et al., 2021). However, photosynthetic pathways have been detrimentally affected in light stress than other abiotic oppressions (Nouri et al., 2015).

Firstly, the benefits of BL and RL having 470 nm and 660 nm respective single-wavelengths investigated by (Fu et al., 2015). Again, the metabolic content and growth rates of greenhouse or indoor vertical farms' plants mainly perform in LEDs (Sng et al., 2021). It can be replaced both broad-spectrum WL and narrow wavelengths of LED lights that were particularly significant to increase plant production (Liu et al., 2018). Physiological processes of plants stomatal opening, photosynthesis, leaf development, photo morphogenesis, and flowering time has been regulated under BL (Li et al., 2019; Tian et al., 2021). Oppositely, compared with the BL, chlorophyll content was higher in RL (Tian et al., 2019) of 'Huangjinya' (novel light-sensitive albino tea cultivar).

TFs of MYB genes act as principal regulators that stimulated crop yield and are inhibited by regulating proposed genes correlated to metabolism and compound synthesis (Song et al., 2019). A recent study showed that employing high-intensity supplemental blue light (BL) is involved in the molecular regulation of secondary metabolites. BL help to induce the biosynthesis of the functional metabolite such as long embryogenic calli, catechins, rutin, anthocyanin, etc., and MYB, HY5, MYC2, bHLH, and PIF4 are the principal regulators (Li et al., 2019; Wang et al., 2020). This BL was also better than RL to promote the biosynthesis of carotenoids by the upregulation of correlated genes in tea plants (Tian et al., 2021). Again, after nine hours withering under RL (630–640 nm), the catechins synthesis genes inhibited the TC for their down-regulating expression but increased the partial catechins (EGC and GCG) content in black tea leaves (Li et al., 2021).

Anthocyanin, a pink-purple color group of secondary metabolites (Gould et al., 2000), is another principal compound of flavonoid biosynthesis pathways in plants that works as an anti-oxidant in the plant and reduced several diseases in human body (Wang et al., 2020). It also used as stress-reducing, color creator, (Liao et al., 2021) and delay senescence of plants under various biotic and abiotic conditions (Agati et al., 2012). Light also stimulates the anthocyanin synthesis in plant metabolic pathways (Wang et al., 2020).

Besides, RL (630 nm) could accumulates more anthocyanin to increase the aroma and tea quality to compare with natural light (260mn) (Lin et al., 2021). Nevertheless, the signal network and mechanism in light for regulating the anthocyanin synthesis still keep unclear (Li et al., 2020). However, we observed, there is no exact investigation of the relationship between BL and RL yet on the secondary metabolites (Catechin and anthocyanin) of ZM6. In current time, LEDs have been used rapidly as substitute light
sources (Bantis et al., 2018). It has high quality, quantity, adjustability, durability, and is used as energy-saving, and has successfully applied to such crops as cucumber (Wang et al., 2021), pepper (Brown et al., 1995), banana (Nhut et al., 2002), tomato (Li et al., 2017), and grape (Poudel et al., 2008)

We observed that the regulating factors of different polyphenols, molecular mechanisms, and phenotypes changes in both BL and RL are still unknown. In this study, to determine the RNA-Seq analysis and secondary metabolites in different LED wavelengths, the young shoots of tea (ZM6) were selected. So, the genes involved in secondary metabolites were identified under BL and RL. Furthermore, how different wavelengths of LED light affect the biosynthetic pathways to determine the chlorophyll content, secondary metabolites, and plant growth in tea plants.

2. Materials And Methods

2.1. Plant Materials and Light Treatments

The experiment was carried out in the growth chamber of Tea Research Institute, CAAS, Hangzhou, to investigate the different phenotypic traits and molecular mechanisms of ZM6. It was derived from a Camellia sinensis (green tea cultivar) under artificial LEDs (light-emitting diodes) light. One and half-year-old tea plants (30cm in length) were cultivated for seven days in grown culture under white LED light. Tea plants were from the germplasm collection garden to use as sample selection materials. They were grown in Plastic Pots (30×30×20cm) containing a soil matrix in an environment adjusting growth chamber. The growth conditions of this experiment were at 25 ± 2°C with a 14 L/10 D photoperiod, and the RH (relative humidity) was at 70 ± 5%. It subjected the LED lamps horizontally above the plant canopy inside the chamber. The average PPF (photosynthetic photon flux) at the plant canopy level of each treatment was at 200 µmolm−2·s-1 by adjusting the number of LED lamps and the distance between the LED lamps and tea plants. Then it placed in a normal condition for one day due to acclimatizing before light quality treatments. After that, they were transferred to three controlled plastic boxes (each light) for ten days under WL (Control/CK), BL, and RL LED wavelength of 200-µmol m–2 s–1 during the 14-h photoperiod (Additional file S6). After ten days, we collected the young shoots (one bud and two leaves) of each treatment for transcriptome and metabolome analyses and executed under three biological replicates.

2.2. HPLC analysis

The estimation of catechins contents of HPLC was narrated by (Wei et al., 2015). In this study, we expelled the tea samples (0.2g, fresh weight) with 5ml 70% (v/v) methanol in a water bath.

Then placed it for 10 min at 70°C. Then shaken after a 5 min interval. After that centrifuged this liquid at 12000r/min for 10 min and then taken into a 10-ml volumetric flask. Then repeated these steps with a final volume of 10ml. The extracts were then filtered through 0.45µm Millipore filters before the injection. The measurement of catechins, caffeine, and TGGP (1, 2, 6-tri-O-galloyl-β-D-glucopyranose) was completed in a Waters HPLC system analysis with a Phenomenex C12, 4.6mm×250 mm, 5µm (reversed-
phase column) at 40°C. One % (v/v) formic acid (A) and acetonitrile (B) with a linear gradient, 0–42min: 4–18.7% B; 42–43min: 18.7–4% B and water determined the mobile phase. These supernatants were bunk at 1mlmin⁻¹ ow volume and observed at 280nm. The each sample made by independently extracted replications. Triplicates of TC formed the Std. (standard deviation) error bars. Both one-way ANOVA with DMRT (Duncan's multiple range test) (P≤0.05) determined the significant difference of this analysis.

2.3. Analysis of RNA Sequencing

RNA prep pure Plant Kit (Tiangen, Beijing, China) is used to extract the total RNA sequencing from the young shoot (bud and two leaves) of ZM6(*Camellia sinensis*). Here, by using agarose gel (1%), it confirmed the RNA quality. NanoDrop 2000 (Thermo Scientific, DE, USA) determined the concentration. Nine samples were selected from RNAs into the Illumina Nova Seq. 6000 to sequence RNA following the method narrated by (Wei et al., 2015b). The reads of this sequence were classified due to the reference of the tea genome applying Hierarchical Indexing to Splice Array of Transcripts (HISAT2, v. 2.0.4) (Kim et al., 2015; Wei et al., 2018).

2.4. Identification of DEGs (differential gene expressions)

The levels of tea samples’ gene expression were normalized and estimated following (FPKM) fragments per kilobase of transcript per million reads) values. Two groups (Group BL vs WL and Group RL vs WL) of samples that compared by the PCA (principal component analysis). DEGs were identified using Cuffdiff with blind dispersion processes and maintained by (FDR≤0.01) false discovery rate (Trapnell et al., 2013). The key DEGs that acquired with a p-value≤0.05 and a minimum 2-folder change in expression over the groups. Besides, blastx annotated the KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis of DEGs

2.5. Quantitative Real-Time PCR (qRT-PCR) Validation for DEGs

Here, we selected eighteen genes to confirm the RNA-seq results for qRT-PCR (real-time PCR) analysis. Then analyzed the data by using three biological replications with means ± SD (n =3). The performance of qRT-PCR and RNA extraction precisely followed by (Zhang et al., 2018). In this study, we applied statistical software (SPSS 16.0) to determine the analysis of variance. Again, we used ChemoHS qPCR Mix (MonAmp) as a kit, and a Light Cycler 480 qRT-PCR instrument derived the whole reactions according to the parameters: 95°C for 30 s, 40 cycles at 95°C for five s, 60°C for 30 s. For endogenous control, applied triplicate biological samples and GAPDH for qRT-PCR analysis. QRT-PCR analysis delivered the CT values and used the 2−ΔΔCT method to estimate the relative fold change values. Thirty-six primers were applied and shown in additional file S2.

2.6. Correlation Analysis of Gene Expression and Catechins Accumulation
To bivariate correlation analysis, used SPSS (Statistical Package for the Social Sciences) Statistics 17.0 to calculate the key genes having FPKM values, and catechins concentrations. Here, we considered the Pearson correlation to display the correlation analysis. Here, significant values means P-values < 0.05 and highly significant values means p-values < 0.01.

3. Results

3.5. Phenotypical Expression and Chlorophyll Content of the Tea Young Shoot (C. sinensis) in Different Light Qualities:

Zhong Ming 6 (ZM6) was derived from a green tea cultivar of Camellia sinensis in China. We mainly focused on young shoots (a bud with two leaves) for phenotypical supervision under three LED wavelengths. After ten days of light quality treatments, we found unique phenotypes among the CK, BL, and RL in the second leaf of the tea plant. It observed that a crimpy phenotype with comparatively reducing leaf area was in the new leaf under BL (Fig. 1a). In contrast, the newly grown shoots of tea plants provided the spacious phenotype with maintaining growth under supplemental RL (Fig. 1a). Although the seeding of ZM6 produced enough leaf growth in CK, they displayed a lower photosynthetic capacity. Oppositely, chlorophyll content was higher under RL than BL (Fig. 1b). Our results reported that supplemental RL has a positive outcome for the growth and development of young leaves in ZM6 compared to BL.

3.2. Transcriptional Variation to the reference genome under Selected Light Qualities:

Different wavelengths of LED light indicated the various molecular regulation in this research due to performing transcriptome sequencing on a young shoots of tea plants under WL, BL, and RL. We found that 41.93–49.39 million raw reads, and Q20 and Q30 were greater than 98% and 95%, respectively, showing the maximum yields and quality. To the tea plant genome, this study mapped about 83.08.89–89.41 percentage of clean reads data. Compared with white light (CK), the global gene transcript abundance was increased due to increasing the BL intensity (Fig. 4). The numbers of differentially expressed genes (DEGs) observed under BL vs WL and RL vs WL groups and shown in Fig. 4. Interestingly, compared to CK, the total number of DEGs in blue light (BL, 200 µmol m−2 s−1), and Red light (RL, 200 µmol m−2 s−1) was 2938 and 1942, respectively, indicating that the RL affected on the tea plant shoots was significantly limited.

3.3. Quantitative Real-Time PCR for Validation of Transcriptome Data

FPKM values were used to measure the level of gene expression in the tea plant. By confirming the results of RNA-Seq, the selection of eighteen genes derived from flavonoid biosynthesis, regulation, and
transformation for validation of qRT-PCR analysis. This study showed the qRT-PCR and RNA-Seq results in Fig. 4. It was clear that the tea genes' expression patterns were estimated by qRT-PCR and highly compatible with the RNA-seq (Additional file S4). The results of RNA-seq and qRT-PCR data confirmed that the methods of RNA-seq analysis provided feasible data. The correlation analysis was positively and significantly correlated \((r = 0.78**)\) in this study. The data identified the principal genes involved in flavonoids biosynthesis in tea plants.

3.4. DEGs (Differentially Expressed Genes) in *C. sinensis* under Various Light Qualities

The application of each LQ treatment with CK was estimated the DEGs by using the DESeq2 R package. Here, various LQ were used to explore the molecular comparison in tea plants. There were some DEGs under BL (2938), while the DEGs in RL (1942) was comparatively lower (Fig. 4). Interestingly, samples clustering were derived from the different LQ that was similar to our results of catechin distribution (Fig. 2). It suggested that samples' difference in the light quality played a dominant role. Moreover, leaf samples under BL vs WL were far from the RL vs WL groups, revealing a significant difference between them. Thus, the nine groups were divided into three groups, namely group CK (CK-1, CK-2, and CK-3), BL (BL-1, BL-2, and BL-3), and RL (RL-1, RL-2, and RL-3). The various expression of genes were analyzed between-group BL vs WL, group RL vs WL. Total 2938 DEGs were identified between-group BL and WL, 1920 with genes up regulated and 1018 down regulated. Similarly, 1942 DEGs were identified in the group of RL vs WL (Fig. 4). The enrichment analysis of the KEGG pathway was estimated the putative DEGs function. The comparative group of BL vs WL and RL vs WL were obtained 762 and 526 DEGs to reference responsible pathways (Fig. 3). Both phenylpropanoid biosynthesis (vvi00940) and flavonoid biosynthesis (vvi00941) were placed among these delegate pathways in two pairs.

Because of the enrichment of DEGs between BL vs CK and RL vs CK, we further analyzed the global metabolic pathways of DEGs. Additional files (S1 and S3), most DEGs were annotated to Photosynthesis, Ribosome, carbon metabolism, Phenylpropanoid biosynthesis, flavonoid metabolism, Plant hormone signal transduction, and amino acid metabolism. Among these DEGs, photosynthesis, amino acid, and Ribosome in energy metabolism were significantly high that representing the energy metabolism of tea plant shoots under BL and RL. In the enrichment analysis of the KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway, 336 DEGs annotated in the 10 most significant pathways (FDR < 0.05). It included the flavone and flavonol biosynthesis, Phenylpropanoid biosynthesis, flavonoid metabolism, Plant hormone signal transduction, amino acid metabolism, photosynthesis, Ribosome, Oxidative phosphorylation; Protein processing in the endoplasmic reticulum, Ubiquitin mediated proteolysis, Glycolysis or Gluconeogenesis under BL (Additional file S1 and S3).

On the other hand, the enrichment analysis of the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, 296 DEGs were annotated in the ten most significant pathways (FDR < 0.05). It included Photosynthesis, Phenylpropanoid biosynthesis, flavonoid metabolism, Plant hormone signal transduction, amino acid metabolism, and Photosynthesis - antenna proteins, Carbon fixation in
photosynthetic organisms, Ribosome, MAPK signaling pathway – plant, Carbon metabolism under RL (Additional file S1 and S3).

Overall, many DEGs showed that the distinct expression patterns between CK and BL were related to photosynthesis and flavonoid metabolism. Interestingly, the 32 DEGs annotated to Catechin biosynthesis were upregulated significantly in BL (Fig. 7). Oppositely, PSI-H genes from Photosystem further suggested that the high-energy RL regulated energy metabolism in tea plants (Fig. 1b). Twenty-three functional genes associated with catechins biosynthesis and 34TFs were identified in the DEGs with the expression patterns of BL > RL.

Moreover, the key functional genes generally had high expression levels when their bio-product contents were significant, and obtained 17 potentially important functional genes. They included PAL (two genes), 4CL (1), FHT (1), F3H (1), F3’H (1), F3’5’H (1), FLS (3), DFR (2), UFGT (2), GSTF12 (1), 3GT (1) and LAR (1).

3.5. Correlation Analysis within Candidate Genes and Catechins Groups

HPLC analysis provided the determination of catechins shown in Fig. 2A and Fig. 2B. This analysis showed the six categories of catechins, namely GC, EGC, C, EC, EGCG, and ECG. We found, TC content significantly varied among the different wavelengths of light with the sequence of light > BL > RL. The TC contents in RL were remarkably lower than BL. For individual catechins, EGCG accounted (224mg/g or about 58%) of the TC contents in BL of leaves, followed by EGC. Both catechins come from trihydroxylated catechins. It indicated that the synthesis of trihydroxylated catechins added a remarkable contribution under BL and RL in the tea plant. While EGCG, EGC, and ECG (trihydroxylated catechins) were the dominant catechin components in BL (totally about 92%) and red light (90%). The results provided a significant change for the trihydroxylated catechins biosynthesis under BL that significantly more than RL. Again, this HPLC analysis also determined the accumulation of GA (gallic acid), CAF (caffeine), Gallocatechin gallate (GCG), and TGGP (1, 2, 6-tri-O-galloyl-β-D-glucopyranose) that significantly more in BL than RL (Fig. 2A and S5).

3.6. Analysis of DEGs Involved in Flavonoid Biosynthesis under particular light qualities

Our results have shown those maximum metabolic products such as anthocyanins, catechins, and flavonols were derived from the flavonoid biosynthesis pathway. The most enzymatic genes of this pathway significantly induced by BL, such as 4CL, PAL, C4H, CHI, F3H, F3’H, F3’5’H, FLS, DFR, GST, FGS, LDOX, and UFGT (Fig. 5b). For the simultaneous application of RL, the expression level of maximum enzymatic genes (PAL, 4CL, F3’H, F3’5’H, FLS, GST, LDOX, LAR, and UFGT) in the pathway significantly decreased (Fig. 5b). On the other hand, phenylalanine provided a relatively low variation reduction with all LQ treatments. The production level of anthocyanins and catechins such as (catechin (C), epicatechin (EC), gallocatechin (GC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin
gallate (EGCG)) levels significantly changed in LQ treatments. They displayed a higher accumulation level under BL than RL (Fig. 2B).

Almost 23 structural genes of flavonoid biosynthesis were visualized in both BL and RL (Fig. 5b), including 5PAL, 1 (4CL), 1 FHT, 1F3’5’H, 1F3H, 2 DFR, 2 F3’H, 4 FLS, 1LAR, 1LODX, 3UFGT, 1GST, and 1(3GT) genes. Surprisingly, all structural DEGs were significantly upregulated in BL except LAR following the varying degrees (Fig. 5a). Oppositely, these PAL, CHI, FLS, FHT, F3’5’H, LODX, GSTF12, UFGT, and LAR were downregulated under RL. RL comparatively inhibited the flavonoid metabolism due to the downregulation of PAL and LAR in the tea plant (Fig. 5c).

3.7. Anthocyanin Contents in Tea Young Shoot of Selected Light Qualities

Total 1F3’5’H, 2DFR, 1GSTF12, 1(3GT), and 3UFGT genes were upregulated under BL but down regulated under RL in flavonoid biosynthesis pathway for synthesis and degraded the anthocyanin production in RL. Moreover, the young shoots of the tea plant inhibited the anthocyanin synthesis due to the downregulated expression of DEGs by the effects of RL whereas increased in BL (Fig. 2C).

3.8. Analysis of DETFs (Differentially Expressed Transcription Factors)

TFs are essential regulatory factors that regulated the plant growth and yield. Again, for the accumulation of plant flavonoids, the bHLH and MYB TFs genes were played a significant part in this study. According to our RNA-seq data, the selected 34 DETFs (differentially expressed transcription factors) under twelve families from the BL vs WL and RL vs WL group (Fig. 6b and Fig. 7). Among these DETFs, the most exorbitant TF families were MYB (9, 28.13%) and bHLH (8, 25%) followed by (6, 18.75%), WRKY (3, 9.38%), UNE (3, 9.38%), and TCP (3, 9.38%). Interestingly, maximum genes of MYB and bHLH were significantly upregulated in BL and balanced with related structural DEGs expression involving flavonoid biosynthesis (Fig. 7). The regulatory genes were expressed by the TFs. Here, TFs played significant roles for catechins regulation. Among them, CsMYB75 (Cha01g017900) showed to be highly similar to CsMYB75 (114319222) (Wei et al., 2019) a key regulator of anthocyanin production! Moreover, in the RL wavelength, a CsMYB4 (Cha08g011300) may be a homologous gene of MYB4 in Arabidopsis thaliana (Wang et al., 2020). For BL wavelength, bHLH-MYC and R2R3-MYB (Cha04g003640) were also played an important role for catechin and anthocyanin accumulation that significantly similar to R2R3-MYBs gene (Zheng et al., 2019) also identified in these DETFs. Further research of these TFs would condense our intelligence of regulatory system for catechin and anthocyanin biosynthesis (Fig. 7).

4. Discussion

Till today, numerous studies have concentrated various modes in plants that stimulate the different artificial LED light wavelengths in several crops involving metabolic analysis such as Arabidopsis(Xu et al., 2021), tomato (Li et al., 2017), lettuce(Sng et al., 2021), strawberry(Yunting et al., 2018) including tea
plants (Tian et al., 2019). Our study was schematic to explore the effective of a selected application of BL and RL to form plant growth and development with different kind of secondary metabolism. Even though, a comparative investigation of which DEGs respond to BL and RL in catechins and anthocyanin metabolic pathways of tea plants. For this region, we focused on the transcriptional and metabolic changes and key processes elicited in tea plant shoots in response to BL and RL wavelengths. We also expressed that the supplemental light qualities aggravated the phenotypical differences (Fig. 1a) and various metabolic factors (Fig. 2) in tealeaves under BL and RL. It also assisted due to elucidating which intensity of LED light was more reacted between these two light wavelengths (Fig. 5).

The HBL (high blue light) at 200 µmol m$^{-2}$ s$^{-1}$ on the growth and metabolism, such as flavonoid (catechins and anthocyanin) were maximum than other low condition light (Wang et al., 2020). Our study revealed that the numbers of DEGs under BL in differential metabolic pathways were higher than RL in the young shoot of the tea plant (Fig. 3 and Table 1). This result reported that TFs and metabolic pathways were extensively enhanced under BL compared to RL. BL also accumulated the secondary metabolism more than RL in the tea plants.

### Table 1

The RNA-Seq Data sets' Summary of ZM6 (C. sinensis)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of reads (million)</th>
<th>Total base (Gb)</th>
<th>Q20% (%)</th>
<th>Q30% (%)</th>
<th>Total mapped (million)</th>
<th>Mapping rate (%)</th>
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<tbody>
<tr>
<td>CK1</td>
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<td>98.52</td>
<td>95.29</td>
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</table>

Plant growth, development, and chlorophyll content stimulated under optimum/low light conditions to improve the photosynthesis capacity in Zhonghuang 3 (ZH3) tea plant (Zheng et al., 2019). On the other hand, for plant photosynthesis, BL and RL were used as the light energy source that recognized for antibacterial activity (Lin et al., 2021). Our research has shown that the chlorophyll content and area of the second leaf (one bud and two leaves) in the tea plant were maximum under RL (Fig. 1b). Though wavebands (200-µmol m$^{-2}$ s$^{-1}$) are the same in both two lights, BL might be provided more light intensity than RL (Additional file S3). Leaf area, and plant height were comparatively reduced in BL than in RL (Liang et al., 2021). It has concluded that BL could only manage the normal plant production. This
result is significantly and positively correlated in cucumber and tomato (Liang et al., 2021) but negatively correlated in grafted watermelon (Moosavi-Nezhad et al., 2021). In BL, leaf area was increased by 94% as compared to WL in Pakchoi (Brassica campestris) (Fan et al., 2019). In ‘Huangjinya’ (albino tea) leaves, RL might be promoted more chlorophyll content due to gene upregulation than BL (Tian et al., 2021).

The accumulation of catechin in the young shoots was maximum in BL because of gene expression in the biosynthetic pathway (Zhang et al., 2018). The HLPC analysis of our research displayed that the peak of the chromatogram of catechin composition, e.g., EGCG, EGC, ECG, EC, was significantly high compared to other lights (Fig. 2A) and the total content of catechins was also maximum (Fig. 2B). FtANR and FtLAR1 genes have been cloned to the formation of (+) C and EC were upregulated in BL but downregulated in RL in Tartary buckwheat (Fagopyrum tataricum) (Jiang et al., 2018). In Red Raspberry Leaves, the quantitative amount of (-)-epicatechin and (+)-catechin were increased under BL by expression of CHS (chalcone synthase) gene that analyzed by HLPC (Kobori et al., 2019). In addition, epicatechin was also promoted by BL in longan embryogenic calli (Li et al., 2018) but epigallocatechin gallate (EGCG) was highest in Gongmei white tea plant under RL (Huang et al., 2021).

Caffeine (1, 3, 7-trimethylxanthine) (Zhang et al., 2020) and Gallic acid were used for the fermentation and manufacturing of tea leaves and also beneficial for human health (Trevisanato and Kim, 2000). In this study, HPLC analysis also showed that the highest accumulation of caffeine and Gallic Acid were found in BL than RL in the young shoot of ZM6 (Fig. 2A and S5). BL also induced accumulation of caffeine content in cucumbers (Palma et al., 2021). Though caffeine accumulation was maximum under RL and WL that significantly reduced the flavonoids in Gongmei white tea plant (Huang et al., 2021). Again, RL also reduced the content of Gallic Acid in Protea cynaroides L as an ornamental plant.

TGGP maybe also work as a microbicide that prevents the transmission of sexual HIV in the human body (Sun et al., 2008). Our study also found that TGGP accumulation was maximum under BL by HPLC analysis (Additional file S5).

Quantitative and qualitative changes in anthocyanin can be occurred by light in the plant (Zhang et al., 2018). Light duration, quality, and intensity regulated the anthocyanin biosynthetic process by influencing various light receptors such as PHYs, PHOTs, CRYs, and UVR8. These photoreceptors affected the PIFs (phytochrome-interacting factors), COP1, and HY5. WDR, bHLH, and MYB used as a promoter to activate or inhibit the genes expression (Wang et al., 2020). BL enhanced the maximum anthocyanin accumulation in leaf vacuole (Fig. 7) of the tea plant (ZM6) by upregulating the expression of CsGSTF12 and CsMYB75 genes in this study (Fig. 2C and Fig. 5). Again, PHOT2 was recognized in BL by strawberry fruits (Kadomura-Ishikawa et al., 2013) and tea plants as a photoreceptor that contributes to anthocyanin synthesis (Zheng et al., 2019).

PAL, CHS, F3H, F30H, ANR1, and UFGT were involved in Flavonoid biosynthesis, which downregulated in low light conditions and significantly reduced the TC and TFG (Transcriptome factors ‘Gene) in tea leaves (Wang et al., 2012). The expression of MYB and HY5 group members belong to the candidate TFs gene families that significantly correlated with definite flavonoids upregulation under BL (Ye et al., 2021).
The Synthesis of flavonoids was affected by both BL and RL (Palma et al., 2021) and significantly depended on plant species (Taulavuori et al., 2018). Again, the products of flavonoid biosynthesis could be enhanced effectively by BL in *Eruca salvia* (arugula) but have no response from the *Ocimum basilican* (basil) and *Rumex sanguineus* (bloody dock) species (Taulavuori et al., 2018). On the other hand, the activity of flavonoid glycoside transferase affected by the RL, but the accumulation of flavonoids was reduced in the tobacco plant. In addition, flavonoids productivity could be enhanced under BL by upregulating of *CHS* gene in *Cyclocarya paliurus* leaves (Liu et al., 2018)

Our result revealed that a total number of structural and TFs genes were 25 and 34 respectively included in flavonoid productions. But maximum genes structural (Fig. 5b) and TFs (Fig. 6) were upregulated in BL vs WL and downregulated in RL vs WL that’s why total catechin content (Fig. 2B) and anthocyanin accumulation (Fig. 2C) was higher in BL as compared to RL. The expression of the structural genes (*CsPAL, Cs4CL, CsFHT, CsFLS, CsDFR1, CsUFGT* and *CsUFGT1*) was upregulated in flavonoid biosynthesis pathways under BL vs WL. For this region, most of the catechin compositions, for example, GC, EC, EGCG, GCG, and ECG accumulated extensively in BL (Additional file S5). Meanwhile, we found that most of the TFs members, including MYB (8/9).WRKY (3), and bHLH (7/8) were upregulated highly in BL (Fig. 7). Especially, *CsMYB75 (Cha01g017900)* may be a homologous gene of *CsMYB75* (114319222) in *Arabidopsis thaliana* (Wei et al., 2019) was significantly and positively correlated with catechin and anthocyanin accumulation (Fig. 5) in both cytosol and vacuole of tea leaves (Fig. 7).

On the contrary, the structural candidate genes expression (*CsPAL, Cs4CL, CsFHT, CsFLS, CsDFR, CsUFGT, and LAR*) downregulated in flavonoid biosynthesis pathways under RL vs WL. TC and anthocyanin content were also positively correlated under RL by upregulating of *CsMYB4* (Cha08g011300) gene. It may be the homologous gene of *MYB4* in *Arabidopsis thaliana* that inhibited flavonoid accumulation (Wang et al., 2020), an important TFs gene in flavonoid biosynthesis pathways, significantly upregulated in ZM6 (Fig. 5c and Fig. 7).

In addition, we observed from the RNA seq. analysis, *CsGSTF12 (Cha08g011300)* may be a homologous gene of *CsGSTF1* (114322623) in *Arabidopsis thaliana*, and *Cs3GT (ChaUn26415.1)* were upregulated significantly in BL vs WL group but down regulated in RL vs WL group (Fig. 6). According to the previous study, *CsMYB5a, CsMYB5e, CsMYB* (TEA001045) (hub gene), *CsCHS* (TEA018665), and *CsADH* (TEA029314) genes positively and significantly correlated for regulating the flavonoids productions in tea plant under BL (Jiang et al., 2018; Wang et al., 2020).

However, Chlorophyll content were enhanced but the major catechins, including C, EC, GC, and EGC were decreased under light efficiency. Oppositely, extensive light intensity regulated the genes expression involving in flavonoid biosynthesis pathways (Liu et al., 2018). However, we concluded that BL might be more compatible for accumulating the secondary metabolites by regulating the gene expression than RL. On the contrary, RL could be provided maximum chlorophyll content and yield production in tea plants. According to our observation, different conditions such as season, time, and plant species can change the results due to the gene expression in flavonoid biosynthesis pathways.
Conclusions

Eventually, two types of LED light (blue and red) were used in our study to compare the effectiveness of secondary metabolism and its’ gene activity identification in tea plants. The young tea shoots were analyzed by following transcriptome data under these light treatments. The correlation analysis were analyzed by expression patterns of 57 candidate genes that belong to catechin and anthocyanin biosynthesis in this study. Here, eight structural genes, including Cs4CL (cha08g00420), CsFHT (Cha09g001340), CsFLS (ChaUn4699.1), CsDFR1 (Cha04g001890), CsUFGT (Cha01g00830), CsUFGT1 (ChaUn13756.1), CsGSTF12 (Cha03g041090), and Cs3GGT (ChaUn26415.1) were significantly and positively related to TC, and anthocyanin accumulation. It suggests that they might played a vital role in catechins and anthocyanin biosynthesis. In addition, LAR also significantly and positively related to Catechin biosynthesis under RL. According to the transcriptome data, we found BL mainly responsible for increasing the gallic acid, caffeine, and flavonoid (flavonols, anthocyanin, and catechin) content, consistent with the annotation of the significantly changed metabolites. Nevertheless, chlorophyll content, plant growth, and development were maximum in RL. Again, we further identified that CsWRKY (Cha01g001420), CsMYB75 (Cha01g017900), bHLH-MYC (Cha04g003640), and R2R3-MYB (Cha04g003640) affected by BL that might be involved in anthocyanin and catechins metabolism. Again, the CsMYB4 (Cha08g011300) gene may be responsible for inhibiting the catechin accumulation as it was induced by RL. This study provided a valuable message for studying the biosynthetic and metabolic pathways of secondary metabolites. It would also facilitate the functional analysis of genes involved in light response in woody plants, especially tea (Camellia sinensis).

Abbreviations
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tbody>
<tr>
<td>BL</td>
<td>Blue Light</td>
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<tr>
<td>RL</td>
<td>Red light</td>
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<tr>
<td>WL</td>
<td>White Light</td>
</tr>
<tr>
<td>EGC</td>
<td>Epigallate-catechin</td>
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<tr>
<td>TF</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>EC</td>
<td>Epicatechin</td>
</tr>
<tr>
<td>LEDs</td>
<td>Light emitting diodes</td>
</tr>
<tr>
<td>ECG</td>
<td>Epicatechin gallate</td>
</tr>
<tr>
<td>DEGs</td>
<td>Differentially expressed genes</td>
</tr>
<tr>
<td>EGCG</td>
<td>Epigallocatechin gallate</td>
</tr>
<tr>
<td>KEGG</td>
<td>Kyoto Encyclopedia of Genes and Genomes</td>
</tr>
<tr>
<td>TCCP</td>
<td>1, 2, 6-tri-O-galloyl-β-D-glucopyranose</td>
</tr>
<tr>
<td>DETFs</td>
<td>Differentially Expressed Transcription Factors</td>
</tr>
<tr>
<td>TC</td>
<td>Total catechin</td>
</tr>
<tr>
<td>LQ</td>
<td>Light Quality</td>
</tr>
<tr>
<td>PAL</td>
<td>Phenylalanine ammonia-lyase</td>
</tr>
<tr>
<td>ANR</td>
<td>Anthocyanidin reductase</td>
</tr>
<tr>
<td>F3H</td>
<td>Flavanone 3-hydroxylase</td>
</tr>
<tr>
<td>ANS</td>
<td>Anthocyanidin synthase</td>
</tr>
<tr>
<td>F3'H</td>
<td>Flavanone 3'-hydroxylase</td>
</tr>
<tr>
<td>C</td>
<td>Catechins</td>
</tr>
<tr>
<td>F3'5'H</td>
<td>Flavonoid 3'5'-hydroxylase</td>
</tr>
<tr>
<td>4CL</td>
<td>4-coumarate CoA ligase</td>
</tr>
<tr>
<td>FDR</td>
<td>False discovery rate</td>
</tr>
<tr>
<td>CHI</td>
<td>Chalcone isomerase</td>
</tr>
<tr>
<td>CHS</td>
<td>Chalcone synthase</td>
</tr>
<tr>
<td>DFR</td>
<td>Dihydroflavonol 4-reductase</td>
</tr>
<tr>
<td>FLS</td>
<td>Flavonol synthase</td>
</tr>
<tr>
<td>FPKM</td>
<td>Fragments per kb per million</td>
</tr>
<tr>
<td>PCA</td>
<td>Principal component analysis</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>qRT-PCR</td>
<td>Quantitative real-time PCR</td>
</tr>
<tr>
<td>LAR</td>
<td>Leuco-anthocyanidin reductase</td>
</tr>
<tr>
<td>GSTF12</td>
<td>Glutathione S-transferase F12</td>
</tr>
<tr>
<td>NCBI</td>
<td>Non-redundant protein database</td>
</tr>
<tr>
<td>3GT</td>
<td>3-O-glucoside-2''-O-glucosyltransferase</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
</tr>
<tr>
<td>UFGT</td>
<td>UDP-glucose:chalcone2'-O-glucosyltransferase</td>
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**Declarations**

**Ethical statements**

The authors declare that they have no conflict of interest. All authors read this paper and confirm the context.

**Author contribution**
Shirin Aktar, Wei kang, Liyuan Wang, Hao Cheng conceived and designed the experiments. Shirin Aktar, Kang Wei, Li Ruan, Yazhen Zhang, Peixian Bai, Liyun Wu, Xun Hanshuo, and Wang Yongxin helped to data collection and performed the experiments. Shirin Aktar and Kang Wei analyzed the data. Kang Wei, Liyuan Wang, Li Ruan and Hao Cheng contributed reagents, materials analysis tools. Shirin Aktar and Wei Kang wrote the paper.

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Figures

Figure 1

Effects of three light qualities on tea plant growth and photosynthetic capacity. (a) The phenotypes and (b) photosynthetic rate of control (CK)/white light and plants supplied with Blue light (BL) and Red light (RL) for 10 days. Error bars represent standard error, n = 13

Figure 2

Total Catechin and Anthocyanin Analysis. A) Chromatograms of typical tea young shoot samples under particular Light treatments. a. White light; b. Blue Light; c. Red light; 1. GA (Gallic Acid); 2. (−)-gallocatechin (GC); 3. (−)-epigallocatechin (EGC); 4. (+)-catechin (C); 5. Caffeine; 6. Caffeine 7. (−)-epicatechin (EC); 8. (−)-epigallocatechin gallate (EGCG); 9. (−)-Gallocatechin gallate (GCG); 10. 1, 2, 6-tri-O-galloyl-β-D-glucopyranose (TGGP); 11. (−)-epicatechin gallate (ECG). B) Catechin accumulations of newly grown tea shoots under particular light qualities by HPLC (Mean ± standard deviation, n=3). Means showing effective difference (P < 0.05) are labeled with different letters based on one-way ANOVA. C) Anthocyanin accumulations of young tea shoot in particular light qualities (Mean ± standard deviation, n=3)

Figure 3

Differentially expressed genes under BL vs WL and RL vs WL groups of different lights
Figure 4

Comparison analysis for 18 selected genes as estimated by RNA-Seq and (qRT-PCR) Real Time-PCR. The means of qRT-PCR analysis data are the ± SD (n=3)

Figure 5
Putative flavonoid biosynthetic pathway in ZM6 (*Camellia sinensis*). (a,c) Pathway mentioned the anthocyanin and catechin biosynthesis under selected Light Qualities. Enzyme abbreviations: phenylalanine ammonia lyase (PAL); Cinnamate 4-hydroxylase(C4H); 4-coumarate CoA ligase(4CL); Chalcone synthase(CHS); Chalcone isomerase(CHI); flavonoid 3′-hydroxylase (F3′H); flavonoid 3′,5′-hydroxylase (F3′5′H); Flavanone 3-hydroxylase (F3H); Flavonol synthase (FLS); dihydroflavanol-4-reductase (DFR); UDP-glucose:chalcone2'-O-glucosyltransferase(UFGT); 3-O-glucoside-2'-O-glucosyl transferase (3GT); Glutathione S-transferase F12(GSTF12); Leucocyanidin reductase(LAR); Anthocyanidin synthase (ANS); Anthocyanidin reductase(ANR). (b) The expression levels of unigenes related to anthocyanin and catechins biosynthesis. Green and red colors are applied to display the low-to-high expression levels, and color scales correspond to the mean significant FPKM values.

**Figure 6**

Expression levels of potential TFs (transcription factors) for catechins regulation under selected light qualities. The heat map was originated from the log2-fold change (log2FC) and mean value calculated from three replicates of RNA-Seq data. The color shows the fold change of DEGs under BL, RL, and WL, as displayed on the Right side.

**Figure 7**

Depict the flavonoid biosynthetic pathway of *Camellia sinensis* within Cell under blue (a) and red (b) LED light.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

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- AdditionalfileS3.xlsx
- AdditionalfileS4.xlsx
- AdditionalfileS5.docx
- AdditionalfileS6.docx