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The Effect of Light Intensity on the Chlorogenic Acid Biosynthesis Pathway in *Marsdenia tenacissima*

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Abstract: The goal of this study was to understand the effect of light intensity on the chlorogenic acid content and biosynthesis-related gene expression in *Marsdenia tenacissima*. In this study, *M. tenacissima* plants were treated with different light intensities; the chlorogenic acid content was determined by high-performance liquid chromatography; and transcriptome sequencing was performed. The amount of chlorogenic acid in the control was the highest and differed significantly from that under three different shading treatments. With a decrease in light intensity, the content of chlorogenic acid also showed a decreasing trend. A total of 1149 differentially expressed genes were identified by transcriptome sequencing, and most of the genes were down-regulated under the 90% shading treatment. A weighted gene co-expression network analysis identified the differentially expressed genes associated with light-induced chlorogenic acid biosynthesis. The different shading treatments down-regulated the expression of the chlorogenic acid biosynthesis pathway structural genes (*HCT*s). The MIKC family genes were the main transcription factors regulating light-induced chlorogenic acid biosynthesis, but the MYB and SBP family genes were also involved. In summary, combined physiological and transcriptome analysis, candidate structural genes, and transcription factors in the biosynthesis pathway of chlorogenic acid were identified in *M. tenacissima*.

Keywords: RNA-seq; KEGG enrichment; heatmap; *HCT* gene

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1. Introduction

Secondary metabolites are generally produced in the cytoplasm by multi-enzyme complexes through the pentose phosphate synthesis pathway, the shikimic acid synthesis pathway, and the phenylpropanoid synthesis pathway to form a series of derivatives, which are finally stored in vacuoles or cell walls. Chlorogenic acid, also known as coffee tannic acid, is a natural polyphenolic compound produced by plants during aerobic respiration and is the main component of the seventh major nutrient. The earliest report on chlorogenic acid was published by Robbiquet and Boutron in 1837. Subsequently, Payen formally defined this term in 1846, but the chemical structure of chlorogenic acid was still unclear. In 1932, chlorogenic acid was proved to be a conjugate formed by the dehydration and condensation of caffeic acid and quinic acid; therefore, it was also called 3-O-caffeoylquinic acid [1]. In recent years, chlorogenic acid has been highly praised, mainly because many studies in the medical community have found that chlorogenic acid has antioxidant, free radical scavenging, anti-inflammatory, antibacterial, and antiviral activities, among others. By studying the antioxidant capacity of polyphenols in mulberry leaves during air drying, it was found that chlorogenic acid plays an important role in the antioxidant activity of mulberry leaves [2]. The results of a population epidemiological investigation and intervention studies and animal experiments show that chlorogenic acid plays important roles in regulating glucose and lipid metabolism; improving insulin resistance; reducing the risk of diabetes and cardiovascular disease; protecting the nerves, liver, and other organs from oxidative and inflammatory damage; and assisting in

cancer prevention. Chlorogenic acid can scavenge reactive oxygen species (ROS) produced by a high-fat diet, inhibit the expression of inflammation, and lead to decreased insulin content, reduced fat accumulation, and weight loss, thereby preventing liver steatosis [3]. More and more scientific evidence shows that biological agents made from chlorogenic acid metabolites extracted from plants can significantly reduce blood pressure and are the most effective antihypertensive drugs [4]. In addition, chlorogenic acid has a variety of antibacterial effects and has efficient bactericidal effects on *Klebsiella pneumoniae*, *Helicobacter pylori*, *Escherichia coli*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*. It can be used as a natural molecule to preserve food products and is an ideal preservative and green food additive [5]. The chemical composition of mulberry leaf extract was analyzed by LC-MS, and it was found that it was mainly composed of chlorogenic acid and its derivatives and other polyphenols [6]. The effects of mulberry leaf extract and its main components, chlorogenic acid and neochlorogenic acid, on alcoholic steatohepatitis were investigated. It was found that mulberry leaf extract, chlorogenic acid, and neochlorogenic acid could up-regulate caveolin-1 expression and reduce EGFR/STAT3/iNOS signal transduction, which may help reduce liver lipid accumulation and peroxidation and inhibit pro-apoptotic cascades. It is also an excellent candidate for dietary supplements and functional food formulations [7]. As the main component of total phenols, chlorogenic acid plays a vital role in plant defense responses, immune regulation, and responses to biotic and abiotic stresses [8]. In trees, the enrichment of chlorogenic acid can significantly enhance the resistance and adaptability of plants, thereby improving the yield and quality of wood. In perennial trees, studies have shown that chlorogenic acid can significantly improve the cold resistance of peach trees [9], enhance the ability of *Populus trichocarpa* to cope with pathogen infection [10], and be used as an important indicator of poplar response to drought stress [11].

In nature, the growth and metabolism of plants are largely affected by the surrounding environment, including abiotic factors, such as temperature, moisture, light, atmosphere, salinity, and nutrients, and biological factors, such as herbivores and pathogenic microorganisms [12]. In order to improve the adaptability of plants to the ecological environment, on the one hand, plants can change their morphological structure, and on the other hand, they can change their physiology and biochemistry, and some secondary substances become the material basis of physiological and biochemical changes [13]. Light is an important environmental factor in the process of plant growth and plays a very important role in the synthesis of plants' active ingredients. In the process of plant growth, in order to adapt to the changes in light, some physiological characteristics of plants will be changed. Whether the effect of light intensity on the chlorogenic acid biosynthesis pathway in *Marsdenia tenacissima* is strong or weak, it will cause changes in plant morphology or some protection mechanisms in plants to adapt to light. Different light qualities affect the various stages of plant growth and development, thereby affecting plants' growth, metabolism, yield, and quality [14]. The study of the effect of light on plants has always been a problem explored by researchers. As early as the 1980s, researchers showed that shade can promote plant growth as long as it is limited to a certain period and extent. In 2010, a new research prospect was undertaken on the influence of light on plants, hoping to establish a faultless scientific evaluation system for light adaptability for plants in the future and to obtain a more objective evaluation of the light adaptability of plants. In 2017, research was undertaken on the effects of different light quality on woody plants at home and abroad, and the method of meta-analysis was used to evaluate the growth of woody plant seedlings. It was found that different varieties of woody plants had different light intensities and that not-full light was the best growth light. Only under the appropriate light intensity can woody plants grow better, and the appropriate shading conditions will not affect the growth of some varieties of woody plants. In a study of *Cerasus humilis*, it was found that the polyphenol content was the highest under full light conditions, and the synthesis of secondary metabolites decreased with the increase in shading intensity [15]. It has been reported that the activity of key enzymes involved in the phenolic metabolic pathway is related to light intensity [16].

M. tenacissima is a perennial vine of Asclepiadaceae, which is mainly distributed in the southwestern provinces of China, including Yunnan, Guizhou, Guangxi, and so on [17]. *M. tenacissima* has antitussive, antihypertensive, heat-clearing, and anti-inflammatory effects [18] and can effectively suppress malignant tumors [19,20]; its medicinal value has always been a primary research object. The 'Xiao-ai-ping' agent made from *M. tenacissima* received extensive attention after entering the market [21]. Many of the important pharmacological active ingredients of medicinal plants are secondary metabolites. The research on the relationship between environmental factors and the secondary metabolism of medicinal plants has attracted much attention, laying a theoretical foundation for analyzing the metabolic pathways of active ingredients in medicinal plants and revealing the formation mechanisms of medicinal quality. In this study, the content of chlorogenic acid in *M. tenacissima* was determined after treatment with different light conditions, and transcriptome sequencing was carried out. The accumulation characteristics of chlorogenic acid under different light conditions were found, and the important functional genes and transcription factors related to the biosynthesis of chlorogenic acid were identified, which provided theoretical data support for improving the accumulation of chlorogenic acid in *M. tenacissima* from the cultivation environment and then accelerated the research process of *M. tenacissima*.

2. Materials and Methods

2.1. Plant Materials and Treatments

In this study, 2-year-old *Marsdenia tenacissima* was used as the experimental material, and the experiment was carried out at the planting base of *M. tenacissima* in Shuitian Township, Mengzi City, Honghe Hani and Yi Autonomous Prefecture, Yunnan Province (115°60′36.15″, 39°32′87.35″, annual precipitation 1675 mm, annual mean temperature 22.1 °C, annual sunshine hours 1769 h). Three different light treatments were set up in the experiment, with shading nets fixed with bamboo, standing 2.5 m high. Natural light was used for illumination, and the shading rates were 50% (ZG50), 70% (ZG70), and 90% (ZG90), respectively. *M. tenacissima* under natural light and natural precipitation conditions was used as the control (CK). Each treatment set up three plots as three replicates, with each plot being 50 m^2. After 210 days of treatment, the maturing leaves at the top, middle, and bottom of *M. tenacissima* were collected for chlorogenic acid content determination and transcriptome sequencing.

2.2. Chlorogenic Acid Content

The leaves were dried at 105 °C for 30 min and then dried at 80 °C for 48 h. The dried leaves were crushed and then sieved through a 0.2 mm sieve to prepare leaf powder. Three plants per replicate were collected as one replicate, repeated three times. The leaf powder of *M. tenacissima* was weighed into 2 g samples and extracted with 50% methanol, and the material–liquid ratio was 1:25 (g:mL). After leaving it to stand for 12 h, the ultrasonic-assisted extraction method was used to extract for 30 min (120 W, 40 kHz), and then, the sample was centrifuged at 12,000 rpm for 15 min, and the supernatant was obtained as the test solution for the determination of chlorogenic acid content. A chromatographic column using YMCODS-H80 (4.6 mm × 250 mm, 4 μm) (Shenzhen Hengxusheng Scientific Instrument Co., Ltd., Shenzhen, China) was used, with a mobile phase of acetonitrile–water–glacial acetic acid (8:92:1), flow rate of 1.0 mL/min, detection wavelength of 327 nm, and injection volume of 10 μL.

2.3. Library Construction and Sequencing

Transcriptome sequencing was performed by Guangzhou Genedenovo Biotechnology Co., Ltd., Guangzhou, China Trizol was used to extract RNA from the plant samples. After the total RNA was extracted, eukaryotic mRNA was enriched by Oligo(dT) beads, while prokaryotic mRNA was enriched by removing rRNA using a Ribo-ZeroTM Magnetic Kit (Epicentre Biotechnology Company, Madison, WI, USA). Then, the enriched mRNA was fragmented into short fragments using a fragmentation buffer and reverse-transcribed into cDNA with random primers. Second-strand cDNA were synthesized by DNA polymerase I,

RNase H, dNTP, and buffer. Then, the cDNA fragments were purified with QiaQuick PCR extraction kit (QIAGEN, Dusseldorf, Germany), end-repaired, poly(A)-added, and ligated to Illumina (Illumina, Inc., San Diego, CA, USA) sequencing adapters. The ligation products were size-selected by agarose gel electrophoresis, PCR-amplified, and sequenced using Illumina HiSeqTM 4000 (Illumina, Inc., Diego, CA, USA).

2.4. Analysis of Transcription Factor (TF) and Differentially Expressed Genes (DEGs)

The protein coding sequences of unigenes were aligned by BLASTp to Plant TFdb (http://planttfdb.cbi.pku.edu.cn/) accessed on 10 June 2019 to predict TF families. To identify differentially expressed genes across samples or groups, the edgeR package 3.5.1 (http://www.r-project.org/) accessed on 12 June 2019 was used. We identified genes with a fold change ≥ 1 and a false discovery rate (FDR) < 0.05 in a comparison as significant DEGs.

2.5. Data Analysis

Microsoft Excel 2021 was used for data processing; Statistical Analysis System v. 9.2 was used for significance analysis (Duncan's test); and Origin 2021 and TBtools v2.095 [22] were used to draw figures. The software package and version used to analyze the Gene Ontology (GO) annotation, Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment, and Weighted Gene Co-expression Network Analysis (WGCNA) was R version 3.5.1, R version 4.2.2, performed using the Metware Cloud, a free online platform for data analysis (https://cloud.metware.cn) accessed on 15 Marh 2019 [23,24]. In the comparative analysis of transcriptome differences, three comparison groups were set up: CK compared with ZG50 (CK_VS_ZG50), CK compared with ZG90 (CK_VS_ZG90), and ZG50 compared with ZG90 (ZG50_VS_ZG90).

3. Results

3.1. Changes in Chlorogenic Acid Content in M. tenacissima Leaves Induced by Light Intensity

To explore the effect of light on the content of chlorogenic acid in *M. tenacissima*, the amount of chlorogenic acid in *M. tenacissima* under different light intensity treatments was analyzed. It can be seen in Figure 1 that the highest chlorogenic acid content was 712.44 μg/g under normal growth conditions (CK). Under the conditions with shading rates of 50%, 70%, and 90%, the content of chlorogenic acid decreased to different degrees compared with CK, and all of them were significantly different from CK ($p < 0.05$). At the same time, it can be found that with the increase in shading rate, the content of chlorogenic acid decreased gradually, but there was no significant difference among the three treatments.

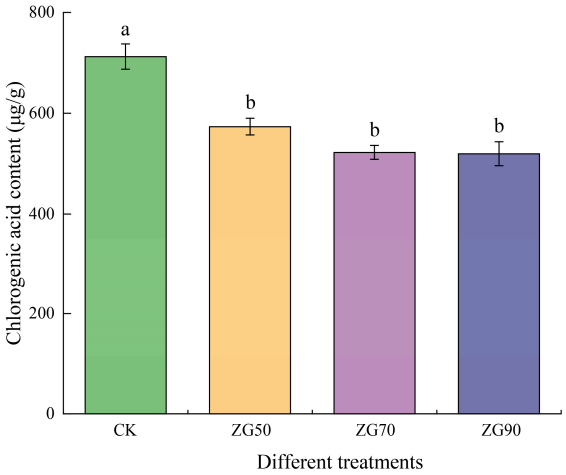

Figure 1. Chlorogenic acid content of *M. tenacissima* under different light intensity treatments. ZG50, ZG70, and ZG90 indicate illumination using natural light, with shading rates of 50%, 70%, and 90%, respectively. CK indicates control. Different letters indicate significant differences between different treatments ($p < 0.05$).

3.2. Overview of RNA Sequencing

Total RNA was extracted from *M. tenacissima* samples of CK, ZG50, and ZG90 for an RNA sequencing (RNA-seq) analysis. The number of raw reads for each library ranged from around 36.05 to 60.91 million. After the quality filtering process, the number of clean reads for each library ranged from around 36.00 to 60.80 million. At the same time, the Q20 and Q30 values for all libraries were more than 97.60% and 93.27%, respectively, confirming the high quality of the RNA and sequencing data that were used for further analyses. The total reference genome mapping rate varied from around 96.88% to 98.22%, with around 4.40–4.75% and around 81.35–82.49% of the reads multiple-mapped and uniquely mapped, respectively (Supplementary File S1, Table S1).

3.3. Analysis of Differentially Expressed Genes (DEGs)

A total of 1149 DEGs were identified in the three comparison groups. In total, 151, 88, and 690 DEGs were only expressed in CK_VS_ZG50, CK_VS_ZG90, and ZG50_VS_ZG90 comparison groups, respectively. There were DEGs in each of the two comparison groups, but there were no DEGs commonly expressed in the three comparison groups (Figure 2A). In the CK_VS_ZG50 comparison group, the expression levels of 143 and 110 genes were up-regulated and down-regulated, respectively. In the CK_VS_ZG90 comparison group, 111 and 143 gene expression levels were up-regulated and down-regulated, respectively. Furthermore, the expression levels of 323 and 539 genes were up-regulated and down-regulated, respectively, in the ZG50_VS_ZG90 comparison group (Figure 2B).

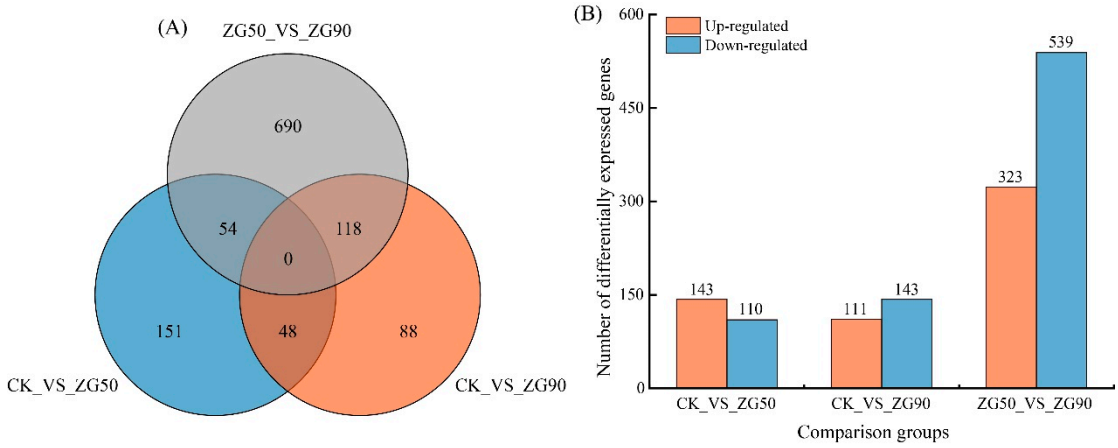

Figure 2. Analysis of differentially expressed genes in different comparison groups. (**A**) Venn diagram of differentially expressed genes. (**B**) The number of up-regulated and down-regulated differentially expressed genes in different comparison groups.

3.4. GO Annotation and KEGG Enrichment

The DEGs in the three comparison groups were functionally annotated based on the Gene Ontology (GO) database (Supplementary File S2, Figure S1). The DEGs in each comparison group were annotated into three major categories (biological process, cellular component, and molecular function), but there were some differences in the subcategories in each major category. In the CK_VS_ZG50 comparison group, more DEGs were annotated to gene expression, membrane, and structural molecule activity, with numbers of 15, 8, and 11, respectively. In the CK_VS_ZG90 comparison group, more DEGs were annotated to the primary metabolic process, intrinsic component of membrane, and purine ribonucleoside binding, at 5, 12, and 5, respectively. In the ZG50_VS_ZG90 comparison group, more DEGs were annotated to the phosphate-containing compound metabolic process, intrinsic component of membrane, and purine ribonucleoside binding, with the numbers of enriched genes being 17, 32, and 39, respectively. Additionally, the enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways among the DEGs were analyzed to elucidate the

biological pathways induced by the light treatment. The functional analysis revealed that the ribosome, oxidative phosphorylation; glycolysis/gluconeogenesis; and cutin, suberine, and wax biosynthesis pathways were significantly enriched in the two light treatments compared with the CK. In addition, oxidative phosphorylation and metabolic pathways were also significantly enriched between these two light treatments (Figure 3).

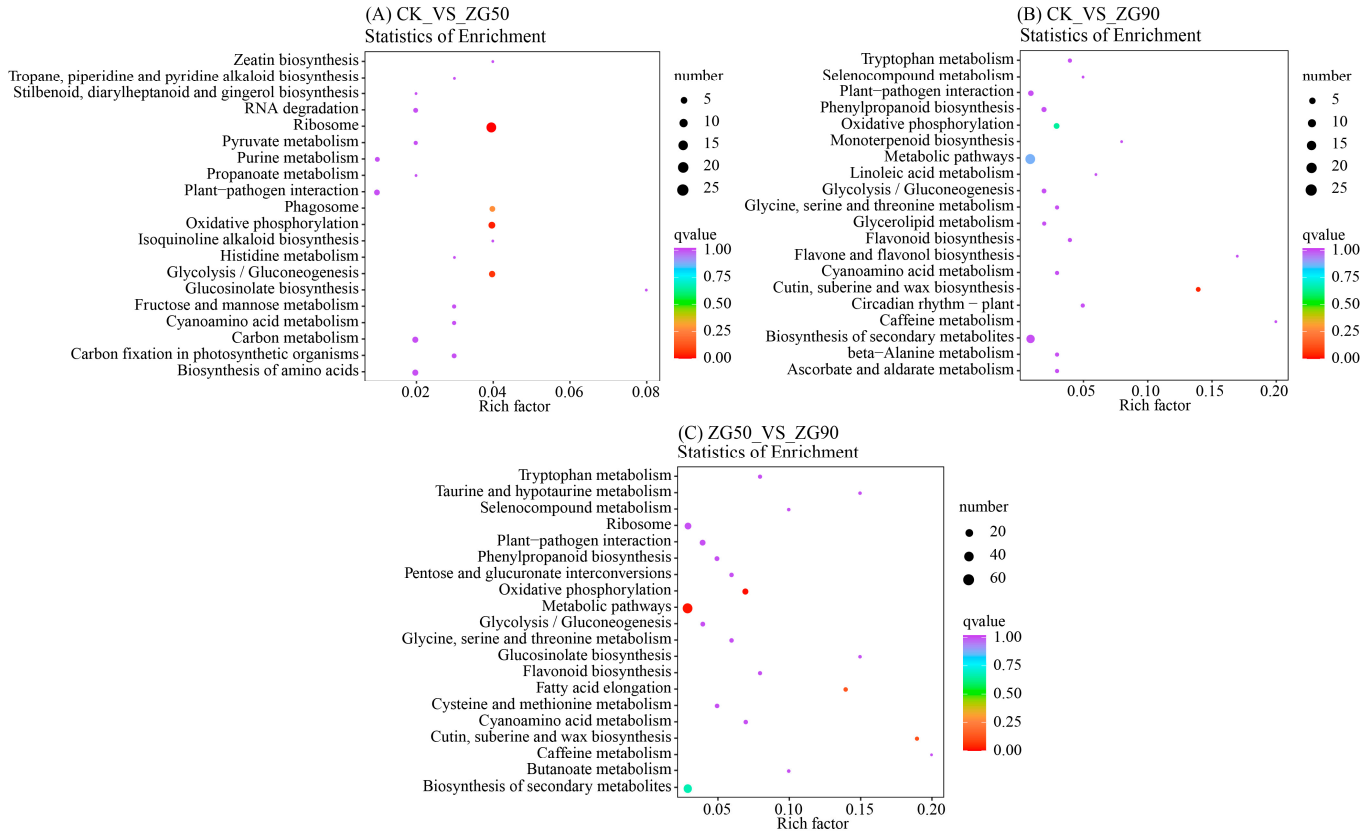

Figure 3. KEGG enrichment analysis of DEGs in different comparison groups. (**A**), CK_VS_ZG50, (**B**), CK_VS_ZG90, and (**C**), ZG50_VS_ZG90 comparison groups. Number indicates the gene number enriched in the KEGG pathway. Q-value indicates the corrected *p*-value to judge the enrichment significance.

3.5. Identification of WGCNA Modules Associated with Chlorogenic Acid Content

A gene co-expression network was constructed via a weighted gene co-expression network analysis (WGCNA) to identify the DEGs associated with chlorogenic acid content. The individual branches of the dendrogram represent the clusters of interconnected genes (i.e., modules). Hierarchical clustering identified eight modules (Figure 4A), including MEblue, MEturquoise, MEbrown, MEgreen, Mered, MEblack, MEyellow, and MEgrey. Among them, the number of DEGs clustered into the MEturquoise module was up to 515, and the number of DEGs clustered into the MEgrey module was at least 5. On the other hand, the correlation between each module and chlorogenic acid content was analyzed. An analysis of the module–trait relationships revealed that the MEyellow module was highly positively correlated with chlorogenic acid content ($r = 0.90$, $p = 0.00094$) (Figure 4B). The MEyellow module included 115 DEGs, and the overall expression patterns are shown in Figure 4C. It can be observed that basically all the genes have the highest expression in CK.

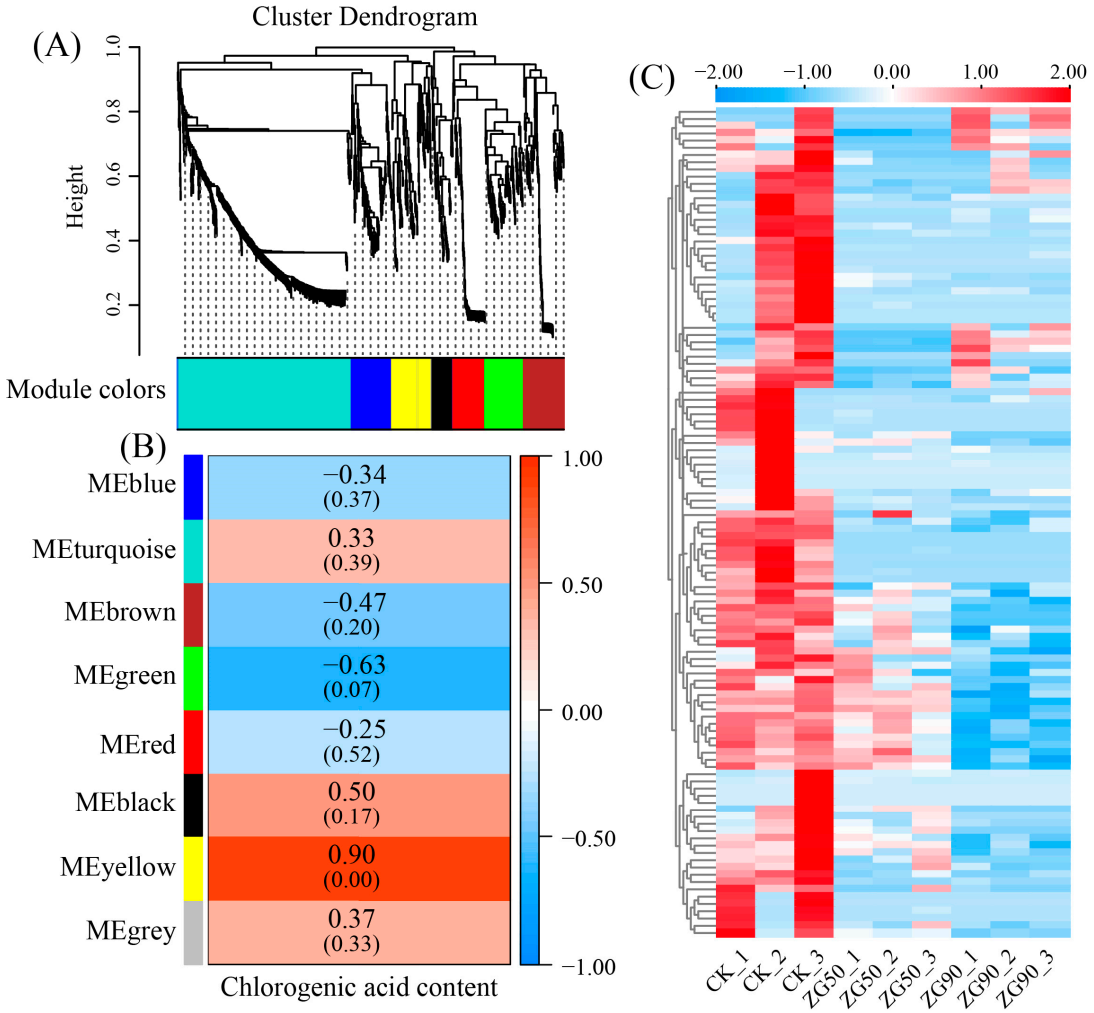

Figure 4. Weighted gene co-expression network analysis of the DEGs identified in the light treatment of *M. tenacissima*. (**A**) Dendrogram with co-expressed gene modules. (**B**) Module–trait correlations and *p*-values (in parentheses) with chlorogenic acid content. The color scale on the right presents the module–trait correlations from −1 (blue) to 1 (red). (**C**) Heatmap of DEGs in the MEyellow module. Height is the distance of merging similarity modules.

3.6. Chlorogenic Acid Biosynthesis Pathway

The biosynthesis pathway of chlorogenic acid was analyzed using transcriptome sequencing, and three branch pathways were drawn (Figure 5). They all started from phenyalanine; phenyalanine formed cinnamic acid under the catalysis of PAL and then catalyzed with UGCT to form cinnamoyl D-glucose and then formed caffeoyl D-glucose and finally formed chlorogenic acid under the catalysis of HCGQT. The other two branches were formed after phenyalanine formed p-coumaroyl-CoA under the actions of PAL, C4H, and 4CL, respectively. The p-coumaroyl-CoA formed p-coumaroyl quinic acid and caffeoyl-CoA under the actions of HCT, C3'H, and HQT, respectively, and further synthesized chlorogenic acid. Combined with WGCNA and DEGs analysis, a total of two *HCT* genes (Unigene0031284 and Unigene0019941) related to chlorogenic acid biosynthesis were selected as candidate genes for further analysis.

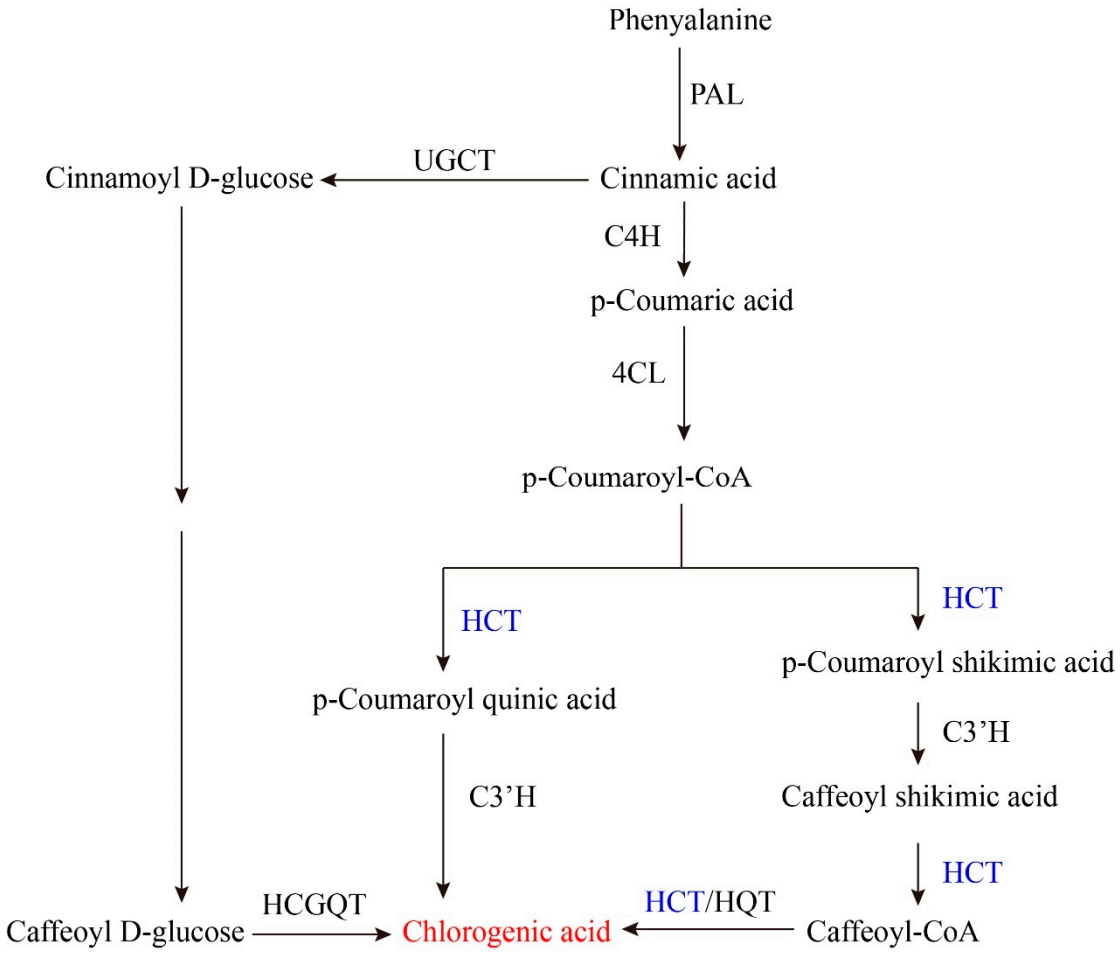

Figure 5. Chlorogenic acid biosynthesis pathway.

3.7. Transcriptional Regulation of Light-Intensity-Induced Chlorogenic Acid Biosynthesis

Transcription factors potentially involved in light-induced chlorogenic acid accumulation were analyzed, and the heatmap was drawn (Figure 6). A total of 31 differential transcription factors were identified, including 16 transcription factor families; among them, the 3 families of *bHLH*, *MIKC*, and *MYB* had the largest number of differential transcription factors, which was 4 for each. At the same time, the most differential transcription factors were highly expressed in CK and ZG50; the number of differential transcription factors highly expressed in ZG90 was lower; and most of the differential transcription factors had low expression in ZG90. Combined with WGCNA results (Figure 4), the correlation between gene expression and chlorogenic acid content was higher in the MEyellow module. In this module, four differential transcription factors were identified: Unigene0032680 (MYB), Unigene0000077 (SBP), Unigene0016214 (MIKC), and Unigene0016646 (MIKC). These four TFs were highly expressed in the CK, and the expression level decreased under different light intensity treatments.

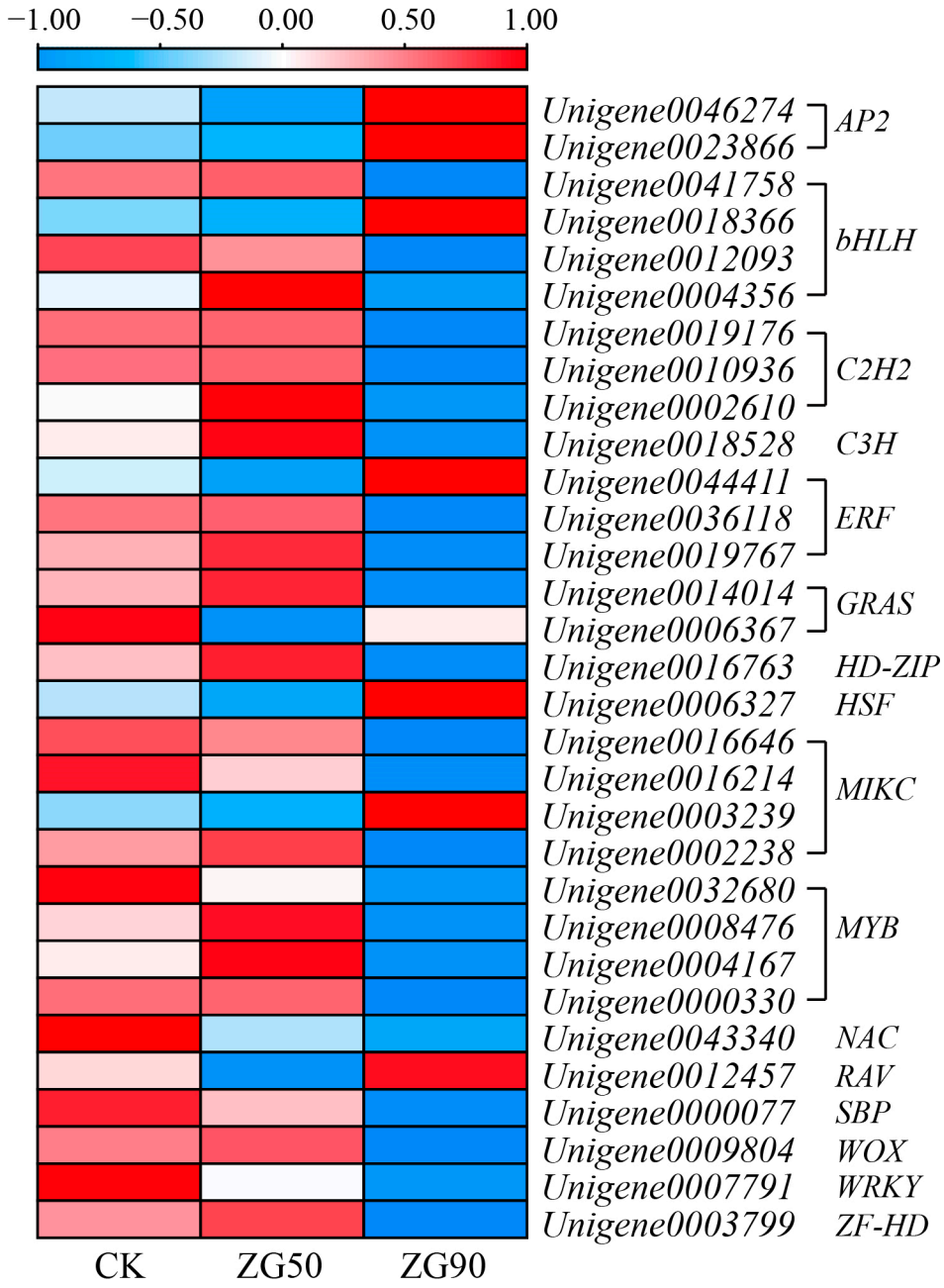

Figure 6. Heatmap of differential transcription factors.

4. Discussion

Chlorogenic acid is a kind of abundant secondary metabolite in the plant kingdom. It is widely distributed and has strong tissue specificity. It plays an important role in dealing with various biotic and abiotic stresses and improving plant adaptability. Chlorogenic acid is a very important antioxidant for plants and animals. It is synthesized by a series of enzymatic reactions of glucose in plants. It is of great significance for improving the content of chlorogenic acid in medicinal plants and crops and improving human life to explore the accumulation characteristics of chlorogenic acid and clarify the biosynthesis pathways of chlorogenic acid using modern biotechnology. The biosynthetic pathway of chlorogenic acid is complex, and many enzymes are involved in it, which are controlled by a multi-gene and multi-level genetic regulatory network. To date, three biosynthetic pathways of chlorogenic acid have been identified in some plant species, which involve the same type of enzymatic reactions, including esterification and hydroxylation [25]. Pathway 1 is caffeoyl

glucose as an active intermediate in the biosynthetic pathway of chlorogenic acid, which is catalyzed by hydroxycinnamoyl-D-glucose quinatehydroxycinnamoyltransferase (HCGQT) to generate chlorogenic acid from caffeoyl glucose and quinic acid [26,27]. Pathway 2 is hydroxycinnamoyl-CoA quinate hydroxycinnamoyltransferase (HQT), which catalyzes the condensation of quinic acid and caffeoyl CoA to generate chlorogenic acid [27]. Silencing the HQT gene in tomato significantly reduced the chlorogenic acid content. Overexpression of the HQT gene significantly increased the content of chlorogenic acid in leaves [28]. Pathway 3 is hydroxycinnamoyl-CoAshikimate/quinatehydroxycinnamoyltransferase (HCT), which catalyzes the reaction of coumaroyl CoA with quinic acid to produce p-coumaroylquinic acid, which is hydroxylated by coumaroylester 3′-hydroxylase (C3′H) to produce chlorogenic acid [29]. C3′H belongs to the CYP98A subfamily of cytochrome P450 monooxygenases. The conversion efficiency of p-coumaroylquinic acid to chlorogenic acid catalyzed by CYP98A22 is higher than that of p-coumaroylshikimic acid to caffeoylshikimic acid. The transient expression of the CYP98A22 gene in tobacco showed that the CYP98A22 gene could significantly increase the accumulation of chlorogenic acid in tobacco [30]. Quinic acid, as a precursor metabolite of pathway 1 and pathway 2, and an intermediate metabolite of pathway 3, plays an important role in regulating the final synthesis abundance of chlorogenic acid in plants. On the other hand, in the phenylpropanoid pathway of plants, caffeoyl-CoA can catalyze the reaction of quinic acid with feruloyl-CoA to produce feruloyl-quinic acid, a downstream metabolite of chlorogenic acid biosynthesis. At the same time, chlorogenic acid can also be synthesized under the catalysis of O-methyltransferase. This indicates that there may be a certain competition mechanism between chlorogenic acid and ferulic acid as well as quinic acid in this biosynthesis process. Based on the haploid analysis of the high-quality genome of *E. ulmoides* [31], it was found that the chlorogenic acid biosynthesis pathway in *E. ulmoides* only involved pathway 1 and pathway 2. This indicated that the chlorogenic acid biosynthesis pathways in herbaceous plants and woody plants may be different or that the importance of each biosynthesis pathway may be different. In this study, the biosynthesis pathway of chlorogenic acid in *M. tenacissima* was constructed by transcriptome sequencing, including three synthesis pathways studied by predecessors. After the further analysis of differentially expressed genes, it was found that only the *HCT* gene was differentially expressed after different light intensity treatments. At the same time, the expression level of the HCT gene was highly expressed in CK and less highly expressed in ZG90. Therefore, it was predicted that the difference in chlorogenic acid synthesis after light induction was mainly caused by p-coumaroyl quinic acid and the caffeoyl-CoA synthesis of chlorogenic acid. HCT is a key enzyme in the monomer synthesis pathway of lignin and plays a role in the hydroxylation of p-coumaroyl CoA to caffeoyl CoA; it is also a key enzyme in controlling the conversion of the lignin H. monomer to the G/S. monomer. HCT plays a regulatory role both upstream and downstream in catalyzing the hydroxylation of phenylpropanoids. It is an enzyme with dual functions. At the same time, it was found that HCT had dual activity in shikimate hydroxycinnamoyltransferase (CST) and quinate hydroxycinnamoyltransferase (CQT) [29]. Through the analysis of the function of the *Arabidopsis C3H* gene, the important role of *HCT* gene in lignin synthesis was further proved. Studies have shown that when the hydroxylation of the C3 position of phenylpropane occurs, it does not change at the level of free acid but with coumaroyl shikimic acid/quinic acid [32].

Transcription factors are widely involved in the regulation of plant phenylpropanoid metabolic pathways. The promoter region of many related enzyme genes for the synthesis of chlorogenic acid active substances contains binding sites. Transcription factors play an important regulatory role in the synthesis and synergistic expression of chlorogenic acid substances. MYB is currently one of the transcription factors that have been confirmed to be most closely related to chlorogenic acid content, and its biological function has been verified in a variety of model and non-model plants. In carrots, *DcMYB3* and *DcMYB5* can bind to the cis-element box-L and activate the transcription of *DcPAL1* and *DcPAL3*, resulting in high levels of chlorogenic acid accumulation [33]. The overexpression of AtPAP1 in

Arabidopsis increased the production of chlorogenic acid in the hairy roots of *Platycodon grandiflorum* [34]. In honeysuckle, *LmMYB15* binds to and activates the promoters of *4CL*, *MYB3*, and *MYB4*, thereby promoting the biosynthesis of chlorogenic acid [35]. In addition, some negative regulatory factors were also identified, such as the transcription factor genes *AmMYB308* and *AmMYB330*, which encode the goldfish grass transcription factor, and were transferred into tobacco to inhibit the synthesis of polyphenols, such as chlorogenic acid [36]. In addition, transcription factors such as WRKY [10], ERF [37], and bHLH [38] are also involved in the regulation of chlorogenic acid biosynthesis. In this study, four transcription factors related to the change in chlorogenic acid content under light induction were identified by the WGCNA method, including MYB, SBP, and MIKC. The two MIKC genes, Unigene0016214 and Unigene0016646, which were identified in this study, both had the highest expression levels in the control, and the expression level decreased under light induction and with the decrease in light intensity. It is speculated that the MIKC transcription factor in *Marsdenia tenacissima* may be changed by light-induced expression to regulate the synthesis of chlorogenic acid. MIKC is a conserved transcription factor family in the MADS-box protein family and is involved in regulating flowering time and floral organ development in plants. However, at the same time, some genes have multiple functions in apparently unrelated developmental stages. In coffee, three members of the MIKC-type MADS-box family are also involved in the biosynthesis of chlorogenic acid, including two positive regulators and one negative regulator [39].

In addition to the internal genetic factors, the external environment is also an important factor affecting the accumulation of chlorogenic acid in plants. Different light intensity levels, light time, and light quality can affect the chlorogenic acid content in plants [40]. The content of polyphenol metabolites in tobacco decreased significantly under low light conditions, and the content of these secondary metabolites increased correspondingly after increasing light intensity. After a bagging treatment for pears, it was found that the content of various polyphenol metabolites, including chlorogenic acid in pear peel, showed a decreasing trend [41]. In tartary buckwheat, red and blue light irradiation significantly increased the content of chlorogenic acid [42]. In lettuce, continuous blue light irradiation and elevated carbon dioxide concentration also increased the chlorogenic acid content [43]. In strawberry, blue light increased the synthesis of chlorogenic acid by inducing the expression of the *FvHCT* gene in the chlorogenic acid synthesis pathway [44]. In this study, different light intensities were used to treat *M. tenacissima* plants. It was found that the content of chlorogenic acid decreased to different degrees compared with the control. With the decrease in light intensity, the decrease in chlorogenic acid content was greater. At the same time, the decrease in light intensity also induced a decrease in *HCT* gene expression, which was also consistent with previous studies.

5. Conclusions

In this study, different light intensities were used to treat *M. tenacissima* plants; the chlorogenic acid content was determined; and transcriptome sequencing was carried out. It was found that a decrease in light intensity inhibited the biosynthesis of chlorogenic acid in *M. tenacissima* and also reduced the expression of related genes in the synthesis pathway. The accumulation characteristics of chlorogenic acid and the expression patterns of structural genes and transcription factors in the metabolic pathway induced by light intensity were preliminarily analyzed. In future studies, the function of the candidate genes selected can be analyzed in depth.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14051063/s1, Table S1: Detailed statistics of sequencing data. Figure S1: GO annotation in different comparison groups.

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