Comparative transcriptome analysis reveals the molecular regulation underlying the adaptive mechanism of cherry (Cerasus pseudocerasus Lindl.) to shelter covering

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Abstract

Background Rain-shelter covering is widely applied during cherry fruit development in subtropical monsoon climates with the aim of decreasing the dropping and cracking of fruit caused by excessive rainfall. Under rain-shelter conditions, the characteristics of the leaves and fruit of the cherry plant may adapt to the changes in the microclimate. However, the molecular mechanism underlying such adaptation remains unclear, although clarifying it may be helpful for improving the yield and quality of cherry under rain-shelter conditions.

Results To better understand the regulation and adaptive mechanism of cherry under rain-shelter covering, 38,621 and 3,584 differentially expressed genes were identified with the combination of Illumina HiSeq and single-molecule real-time sequencing in leaves and fruits, respectively, at three developmental stages. Among these, key genes, such as those encoding photosynthetic-antenna proteins (Lhca and Lhcb) and photosynthetic electron transporters (PsbP , PsbR , PsbY , and PetF), were upregulated following the application of rain-shelter covering, leading to increased efficiency of light utilization. The mRNA levels of genes involved in carbon fixation, namely, rbcL and rbcS , were clearly increased in comparison to those under shelter-free conditions, giving rise to improved CO 2 utilization. Furthermore, the transcription levels of genes involved in chlorophyll (hemA , hemN , and chlH) and carotenoid synthesis (crtB , PDS , crtISO , and lcyB) in the sheltered leaves peaked earlier than those in the unsheltered leaves, thereby promoting organic matter accumulation in leaves. Remarkably, the expression levels of key genes involved in the metabolic pathways of phenylpropanoid (PAL , C4H , and 4CL) and flavonoid (CHS , CHI , F3’H , DFR , and ANS) in the sheltered fruits were also upregulated earlier than those in the shelter-free fruits, conducive to an increase in anthocyanin content in the fruits.

Conclusions According to the physiological indicators and transcriptional expression levels of related genes, the adaptive regulation mechanism of cherry plants was systematically revealed. These findings can help understand the effect of rain-shelter covering on Chinese cherry cultivation in rainy regions.

Background

Cherry is a popular spring–summer fruit because of its early maturity and delicious taste, along with its antioxidant properties [1]. Cherry trees prefer environments that are sunny and without stagnant water [2]. During fruit development, they are vulnerable to the weather, such as rain and hail, which are the main causes of the severe dropping and cracking of fruit [3].

Colorless rain-shelter coverings for fruit crops such as grapes [4] and apples [5] are increasingly being used worldwide, which might significantly enhance fruit yield [6], while having no obvious adverse effect on fruit quality [7]. However, rain-shelter covering may

unavoidably alter the microclimate of the canopy, leading to a reduction of photosynthetically active radiation (PAR) [8], which is regarded as a key constraint for vegetative growth and fruit development [9]. Therefore, flexible adjustment between light energy utilization and photoprotection is critical for plant performance and field adaptability under changing light conditions [10].

Recently, the adaptability to weak light and shade conditions of plants was documented chiefly in terms of physiological adjustment in many species, such as rubber tree [11], cotton [12], and sweet cherry [13]. It is generally accepted that the shift in physiological adaptability of plants to adverse conditions can ultimately be attributed to alterations at the molecular level. To date, the adaptive regulatory mechanisms of plants under variable light and/or temperature conditions have been analyzed using transcriptomic strategies in many crops. For example, differentially expressed genes (DEGs) in maize were shown to be mainly involved in most assimilation processes (photosynthesis and carbon fixation pathways), namely, photosynthetic light capture via the modification of chlorophyl biosynthesis, suggesting the complex regulatory mechanisms and interactions between cold and light signaling processes [14]. Under low-light conditions, the expression of photosystem I and II complex and electron transport-related genes increased in low-light-tolerant rice varieties; thus, the accelerated expression of photosynthesis-related genes under low-light conditions contributed to the maintenance of rice yield [15]. The available evidence indicates that photosynthesis and the synthesis of photosynthetic pigments play major roles upon exposure to shaded conditions.

Under shelter covering, previous studies focused on the interaction between fruit anthocyanin contents and the transcription of related genes in grape [16], as well as between the biosynthesis of nutritional ingredients and DEG profiles in citrus [17]. To date, however, the molecular regulation of photosynthetic metabolism under shelter covering has not yet been elucidated, although this may lead to substantial changes in cherry growth and development.

Chinese cherry (*Cerasus pseudocerasus* Lindl.) is widely grown in southwest China, which is characterized as a region with a subtropical monsoon, with the weather featuring frequent rain and hail in spring; this severely constrains the expansion of the cherry industry. Previously, the use of shelter covering was justified by it substantially contributing to the total Pn accumulation and elevating fruit yield, while having no obvious adverse effects on the vegetative growth and fruit quality of cherry [18]. To fully understand the adaptability and regulatory mechanism of cherry upon microclimatic changes under shelter covering, the present study was established with the following objectives: 1) to discover the DEGs upon shelter covering via transcriptomic analysis; 2) to identify the key genes involved in growth and development; and 3) to reveal the regulatory network involved in light harvesting, photosynthetic electronic transport, and the antioxidant system, among others, which can strongly facilitate the innovative improvement of plantation systems in this fruit crop.

Results

- **Overview of the SMRT sequencing**

To obtain the gene expression profiles under sheltered and unsheltered conditions, SMRT sequencing and Illumina RNA-Seq were carried out for leaves and fruits at three developmental stages (DAF35, 45, 55). A total of 1,048,866 post-filter polymerase reads (21.55G) were scanned. The subreads from the same polymerase read sequence generated a circular consistent sequence (CCS), which yielded 685,339 CCS sequences. Among them, 542,795 full-length non-chimera (FLNC) sequences with 5′-primer, 3′-primer, and poly-A were obtained, including 79.20% of all CCSs being FLNCs (300–22,293 bp). This proportion varied slightly between the two tissues, at 34.96% in the leaf data set and 44.25% in the fruit data set. The mean length of FLNC reads is shown in Table 1, and the length distribution of FLNC is shown in Figure S1. FLNC length of leaf and fruit libraries >2 kb accounted for 63.95% and 65.21% of the corresponding FLNC, respectively. Comparison between the protein-coding gene transcripts and those of FLNC revealed strong

concordance, which exhibited better recovery of large transcripts than previous Illumina RNA-Seq data for gene model prediction, particularly in the 2,500–4,000-bp size range [27].

Table 1 Summary of PacBio Sequel real-time sequencing

Library	Polymerase N50 length[a]	Subreads N50 length[b]	Mean number of passes[c]	Number of CCS[d]	Number of FLNC[e]	Mean length of FLNC
SL and UL[1]	41,750	4,031	10.11	69,562	52,291	3,468
SL and UL[2]	43,250	4,009	11.26	21,950	17,739	3,549
SL and UL[3]	36,750	2,808	10.73	278,447	231,786	2,886
SL and UL[4]	45,750	4,117	12.06	1,692	1,355	3,631
SF and UF	39,750	2,742	11.49	313,688	239,624	2,834

The four rows of “SL and UL[1-4]” represent the leaf library, and the row “SF and UF” represent the fruit library. [a]Polymerase, the original read generated by PacBio Sequel; [b]Subreads, Post-filter polymerase reads; [c]Sequencing times of insert; [d]Number of circular consensus sequences; [e]Number of full-length non-chimeric.

- **Annotation and functional classification of unigenes**

The functions of unigenes were annotated by BLAST comparison and were predicted by comparative analysis with five databases. Among the 45,825 unigenes distributed to each of the databases, 45,747 (97.35% of the total) for Nr, 28,481 (60.61%) for COG, 38,629 (82.20%) for SwissProt, 18,202 (38.73%) for KEGG, and 26,065 (55.46%) for GO were investigated (Figure 1a).

Analysis of the Nr database indicated that the highest homologies of cherry were with *Prunus mume* or with *P. persica*, with 22,919 (50.09%) and 18,545 (40.53%) unigenes annotated, respectively. In total, 28,481 annotated unigenes were classified into 37 functional groups of the three GO main categories: nine groups for molecular function (MF), 18 for biological process (BP), and 10 for cellular component (CC) (Figure 1b). The top three GO terms for the classified genes were “protein binding” (4,812), “ATP binding” (3,907), and “protein kinase activity” (2,272) for MF; “protein phosphorylation” (2,273), “oxidation-reduction process” (1,833), and “signal transduction” (1,255) for BP; and “membrane” (1,656), “integral component of membrane” (1,571), and “nucleus” (748) for CC (Supplementary Table S2).

A total of 18,202 unigenes were distributed into 265 KEGG database pathways. The top three KEGG pathways were “Metabolism, Carbohydrate Metabolism” (1,719); ‘Environmental Information Processing, Folding, Sorting, and Degradation” (1,563); and ‘Genetic Information Processing, Translation” (1,491) (Figure 1c, Supplementary Table S3).

- **Functional classification of DEGs under sheltered covering**

After filtering the low-quality reads, 17.25 billion clean reads were acquired by Illumina RNA-Seq (Table S4). According to the Illumina data, pair-wise comparisons (UL *vs*. SL, UF *vs*. SF) of gene expression among the three stages were performed. In response to

microclimatic change with low-PAR conditions, in total, 38,621 (UL35 *vs*. SL35, UL45 *vs*. SL45, and UL55 *vs*. SL55) and 3,584 (UF35 *vs*. SF35, UF45 *vs*. SF45, and UF55 *vs*. SF55) DEGs were detected from the GO database. Additionally, 38,621 DEGs for UL45 *vs*. SL45 and 2,871 DEGs for UF45 *vs*. SF45 were acquired (Figure 2a). Throughout the three developmental stages, a total of 6,911 and 1,755 DEGs were detected from the KEGG database in leaves and fruits; 6,868 and 1,552 DEGs for UL45 *vs*. SL45 and UF45 *vs*. SF45 were obtained, respectively (Figure 2b).

Compared with UL and UF, the upregulated genes from GO terms of SL were significantly enriched in “catalytic activity,” “biological process,” “oxidoreductase activity,” and “oxidation-reduction process,” among others (Figure 2c). The upregulated genes of SF were primarily associated with “catalytic activity,” “biological process,” “oxidoreductase activity,” and “oxidation-reduction process,” among others, at DAF45 (Figure 2d). Upregulated genes from KEGG terms of RL in contrast to UL included those associated with “circadian rhythm-plant,” “glyoxylate and dicarboxylate metabolism,” “porphyrin and chlorophyl metabolism,” and “carbon fixation in photosynthetic organisms,” among others (Figure 2e); moreover, the upregulated genes of RF were particularly associated with “biosynthesis of amino acids,” “phenylpropanoid biosynthesis,” “phenylalanine metabolism,” and “flavonoid biosynthesis,” among others, at DAF45 (Figure 2f).

Common expression patterns were employed to further analyze the DEGs between UL *vs.* SL and UF *vs*. SF at three stages; overall, 7,244 (leaf) and 1,707 (fruit) DEGs were placed into four clusters (Figure 3). Most of the candidate DEGs was categorized into either leaf Cluster 1 (3796 genes) or fruit Cluster 1 (1271 genes). For the top six enriched pathways in leaf Cluster 1, the DEGs exhibited peak expression at DAF45 of SL. For the top five accumulated pathways in leaf Cluster 2, the DEGs showed peak expression at DAF45 of UL (Figure 3a). Meanwhile, for the top four enriched pathways in fruit Cluster 1, the DEGs showed peak expression at DAF35 of SF. Finally, for the top five enriched pathways in fruit Cluster 2, the DEGs showed peak expression at DAF55 of SF (Figure 3b).

Transcriptional results indicated that the adaptability of cherry to low PAR began at DAF35. The adaptability of leaves to the microclimate was primarily attributed to the regulation of photosynthetic characteristics, assimilation, antioxidant status, as well as circadian rhythm. The genes implicated in the biosynthesis of anthocyanins and sugars in the sheltered fruits were upregulated at DAF35 until DAF55, reflecting the prior accumulation of nutrition in comparison to that under shelter-free conditions.

To confirm their authenticity, 12 DEGs were randomly selected to analyze their expression profiles by qRT-PCR. The results of qRT-PCR analysis showed that the expression profiles of the 12 DEGs were similar to those obtained through high-throughput sequencing (Supplementary Table S2). These results confirmed the reliability of the genome-wide transcriptome profiling analysis.

To verify the authenticity of the RNA-Seq results, 12 DEGs were randomly selected and their expression profiles were analyzed by qRT-PCR. The results of qRT-PCR analysis showed that the expression profiles of these DEGs were similar to those of RNA-Seq (Figure 4), confirming the reliability and accuracy of our RNA-Seq data.

- **Expression of genes involved in photosynthetic system in sheltered leaves**

To clarify the molecular adaptability of cherry trees to the microclimatic change during fruit development upon exposure to the shelter covering, genes involved in environmental sensitivity were screened out from the filtered DEGs for further investigation (Figure 4). Most of the DEGs encoding antenna proteins, or proteins involved in electron transport, reaction center in photosystem I (PSI) and photosystem II (PSII), as well as components of CO_2 fixation, were highly expressed in SL compared with UL at DAF45 (Figure 4a).

More than 70 genes were annotated to three metabolic pathways: photosynthesis-antenna proteins (ko00196), photosynthesis (ko00195), and carbon fixation in photosynthetic organisms (ko00710). Therefore, we focused on the transcriptional levels of those genes closely related to photosynthetic efficiency (Figure 5a). All annotated DEGs of light-harvesting chlorophyll a/b binding protein complex I and II (*LHCs*), namely, 17 genes encoding the chlorophyl a/b binding protein complex I (*Lcha1*, *Lcha2*, *Lcha3, Lcha4*, *Lcha5*) and 20 genes encoding chlorophyl a/b binding protein complex II (*Lchb1*, *Lchb2*, *Lchb3*, *Lchb4*, *Lchb5*, *Lchb6*, *Lchb7*), were upregulated in SL at DAF45 (Figure 6a). Moreover, 13 genes encoding proteins involved in reaction center and electron transport in photosynthesis, including, PSI reaction center subunit X (*PsaK*), reaction center subunit VI (*PsaH*), PSI reaction center subunit *PsaN* (*PsaN*), PSII oxygen-evolving enhancer protein 2 (*PsbP*), PSII 10 kDa protein (*PsbR*), PSII repair protein *Psb27-H1* (*Psb27*), ferredoxin of photosynthetic electron transport (*PetF*), and H^+/Na^+-transporting *ATPase* subunit beta (*AtpF*), were upregulated under sheltered conditions (Figure 6b), whereas one gene of cytochrome f complex (*PetA*) and one gene of H^+/Na^+-transporting *ATPase* subunit alpha (*ATPF1AI*) had lower transcription levels. Within the pathway of carbon fixation, D-ribulose 1,5-bisphosphate (RuBP) and CO_2 produced 3-phosphate-glycerate (3-PGA) under the action of ribulose-bisphosphate carboxylase large chain (*rcbL*) and ribulose-bisphosphate carboxylase small chain (*rcbS*); then, 3-PGA was reduced to glyceraldehyde-3P (3-PGAld) by glyceraldehyde 3-phosphate dehydrogenase (*GADPH*) and glyceraldehyde-3-phosphate dehydrogenase (*GAPA*), which completes the energy storage process of photosynthesis and increases the production and accumulation of photosynthate; among them, one gene of *rbcL*, nine genes of *rbcS*, one gene of *GADPH*, and seven genes of *GAPA* were significantly upregulated under sheltered conditions. Notably, the expression of *rbcL* and *GAPA* was upregulated more than 10-fold (Figure 6c).

In combination with the PAR-Pn and CO_2-Pn curves, the Pn of sheltered leaves was lower, but there were no significant differences in the first two stages of fruit development

(Figure 7). The AQY and ACE of sheltered leaves visibly increased by 13.0% and 23.5%; meanwhile, the LCP and CCP parameters decreased to 13.87 and 75.62 μmol·m^{-2}·s^{-1} at DAF45 (Table S5). In general, combined with photosynthetic characteristics and transcriptomic results of leaves, the findings illustrated that sheltered leaves had stronger abilities to capture and utilize the weak light, while also maintaining stable efficiency of CO_2 utilization. This indicated the possibility of good adaptation to the weak light conditions under the sheltered covering in a short time.

- **Expression of genes encoding photosynthetic pigments in sheltered leaves**

The adaptability of plants to weak light is inextricably related to the photosynthetic pigmentation synthesis pathway [28]. In this study, 34 genes involved in porphyrin and chlorophyl metabolism (ko00860) and 1 gene related to each of terpenoid backbone biosynthesis (ko00900) and carotenoid biosynthesis (ko00906) in sheltered leaves were upregulated at DAF45 (Figure 5b). Regarding the DEGs annotated to chlorophyl synthesis and carotenoid biosynthesis, one gene encoding geranylgeranyl diphosphate reductase (*CHLP*), one gene encoding oxygen-independent coproporphyrinogen III oxidase (*hemN*), two genes encoding glutamyl-tRNA reductase (*hemA*), 23 genes encoding magnesium chelatase subunit H (*ChlH*), and two genes encoding magnesium chelatase subunit I (*ChlI*), among others, were upregulated under sheltered conditions at DAF45 (Figure 6d). Additionally, four genes of 15-cis-phytoene synthase (*crtB*), two genes of 15-cis-phytoene desaturase (*PDS*), one gene of prolycopene isomerase (*crtISO*), and two genes of lycopene beta-cyclase (*lcyB*) were upregulated under sheltered conditions at DAF45 (Figure 6e). The contents of photosynthetic pigments Chl a, Chl b and Car tended to increase under sheltered conditions. Moreover, the contents were higher than those under Cont; for example, Chl a increased by 14% to 16.7%, Chl b by 13.6% to 24%, and Car consistently increased by 22% under sheltered conditions (Figure 8). The transcription levels of genes

related to chlorophyl and carotenoid synthesis in the sheltered leaves also fully confirmed that they would not be adversely affected by the sheltered covering (Figure 5b); on the contrary, sheltered covering would enhance the gene transcription levels, thereby increasing the pigment content.

- **Expression of genes involved in antioxidant systems in sheltered leaves**

ROS scavenging enzymes such as *SOD*, catalase (*CAT*), peroxidase (*POD*), and ascorbate peroxidase (*APX*), as well as nonenzymatic antioxidants (glutathione, carotenoids, etc.), are essential for ROS detoxification [29]. Malondialdehyde (MDA) can be used as an indicator of lipid peroxidation under different stress conditions. Transcription levels of multiple genes relating to antioxidant capacity including peroxisome (ko04146), phenylpropanoid biosynthesis (ko00940), glutathione metabolism (ko00053), and carotenoid biosynthesis (ko00906) fluctuated slightly with the microclimatic conditions (Figure 5c). The expression of most antioxidant-related genes was clearly higher in SL than in UL at DAF45. For example, eight genes involved in peroxisome (encoding SOD, CAT), 32 genes involved in phenylpropanoid biosynthesis [encoding trans-cinnamate 4-monooxygenase, *CYP*; phenylalanine ammonia-lyase, *PAL*; 4-coumarate-CoA ligase, *4CL*; cinnamyl-alcohol dehydrogenase, *CAD*; caffeic acid 3-O-methyltransferase, *COMT*; 5-O-(4-coumaroyl)-D-quinate-3′-monooxygenase, *C3′H*; caffeoyl-CoA O-methyltransferase, *CCoAOMT*; *POD*]; eight genes involved in glutathione metabolism (encoding glutathione synthetase, *GSS*; glutathione reductase, *GSR*; glutathione S-transferase, *GST*); and 16 genes involved in carotenoid biosynthesis (encoding zeaxanthin epoxidase, *ZEP*; violaxanthin de-epoxidase, *VED*; beta-carotene 3-hydroxylase, *CrtZ*; beta-ring hydroxylase, *LUT5*; lycopene beta-cyclase, *lcyB*; abscisic acid 8′-hydroxylase, *CYP707A*) showed markedly higher expression in SL than in UL at DAF45 (Figure 5c).

The antioxidant enzyme activities and MDA content in leaves also changed with the change of microclimate associated with sheltered conditions. At DAF35, there was no remarkable difference in the activities of *SOD*, *POD*, and *CAT* of SL and UL; however, the MDA content was higher under sheltered conditions. Moreover, the activities of antioxidant enzymes were higher than under shelter-free conditions at DAF45, while the MDA content was lower than in UL, with the *POD*, *SOD*, and *CAT* activities increasing by 1.7-, 1.9-, and 1.3-fold, respectively (Figure 9). These results showed that the activities of antioxidant enzymes in leaves increased during RSC, which maintained the ability of plants to scavenge ROS, and prevented the damage of membrane lipid peroxidation to leaves, thus preserving photosynthetic efficiency. The results showed the same trend as the results of gene expression levels.

- **Expression of genes associated with anthocyanin synthesis in sheltered fruits**

The anthocyanin content of mature cherry fruits under sheltered conditions increased significantly compared with that under unsheltered conditions [18]. The upregulated genes of fruits were mainly involved in phenylpropanoid biosynthesis (ko00940) and flavonoid biosynthesis (ko00941). The transcriptional levels of more than 25 genes in sheltered fruits began to increase from the DAF35 stage until fruit ripening (Figure 5d); these included 12 genes involved in phenylpropanoid biosynthesis, with one gene of PAL, six genes of C4H, and five genes of 4CL. Among these, the expressions of *C4H* and *4CL* were upregulated more than 8.5- and 6.1-fold, respectively. Moreover, for 13 genes involved in flavonoid biosynthesis encoding chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3'-hydroxylase (F3'H), dihydro-flavonol 4-reductase (DFR), and anthocyanidin synthase (ANS), the expressions were remarkably higher in the sheltered fruits at DAF45 (Figure 10). The results showed that the synthesis of anthocyanin could be accelerated and the accumulation cycle could be prolonged in the sheltered fruits.

Discussion

- **Regulation highlighted photosynthetic adaptation under shelter covering**

The alteration of the physiological characteristics of leaves/fruits may be involved in adaptive responses to changes in microclimate, and the transcriptional regulation of genes is the molecular basis for plant adaptation to shifts in environmental factors such as light and temperature. Photosynthesis is pivotal for plant growth and yield [30] as well as fruit quality; therefore, agronomic techniques are generally particularly focused on improving photosynthesis. Under low-light intensity, the photosynthetic efficiency of plants mainly depends on the adaptability of light-harvesting apparatus to the microclimatic conditions, in which PSI/PSII represents the adaptability of plants to low light [31]. *LHCs* in PSI and PSII play important roles in light harvesting and light protection [32]. *Lhcb* (PSII) was reported to be transcriptionally amplified under low-light intensity to enhance photon capture [33]. The differentially expressed transcripts of plant *LHCs* in response to low-light stress might play collective roles in maintaining the normal metabolic activities under low-light intensity. For example, the genes encoding Lchbs from the low-light-tolerant rice cultivar were upregulated under low-light intensity [15]; in the current study, all the DEGs associated with the members of *LHCs*, such as *Lcha* and *Lchb*, were upregulated in cherry leaves under shelter covering. A total of 37 genes were involved in the regulation of light capture protein expression (Figure 5a), and compared with the unsheltered leaves, nine genes encoded Lcha2 and nine genes encoded Lchb1 were upregulated, and the highest expression multiple increased by 6-fold (Lcha2) and 11-fold (Lchb1). In addition, *Psb27*, which is essential for energy metabolism and effective recovery of photodamaged PSII complex [34], was also upregulated in the sheltered leaves. It was documented that *PsbS* might stabilize the PSII–LHCII supercomplex structure and could improve electron transmission efficiency [35]; in the current study, *PsbS* was upregulated in the sheltered leaves (Figure 5a). During the light reaction of photosynthesis, genes encoding LCHs were

upregulated; among these, the absorption and transmission of light energy under low-light conditions were promoted by *Lchbs*, and the light capture area and light capture efficiency were also improved. In addition, the photosynthetic characteristics of sheltered leaves showed that LCP decreased and AQY increased (Table S4). It is speculated that these genes play important roles in regulating leaf adaptation to the low-light environment associated with the rain-shelter conditions.

Rubisco (*rbcL* and *rbcS*) is a bifunctional enzyme located in the chloroplast stoma that catalyzes photosynthetic CO_2 fixation to form ribulose-1,5-bisphosphate (RuBP) [36]; the transcriptional levels of *rbcL* and *rbcS* genes could directly reflect the photosynthetic efficiency of leaves [37]. Studies have shown that the activation status of *Rubisco* is affected by light intensity [38]; therefore, the upregulation of *rbcL* and *rbcS* genes in cherry leaves under sheltered conditions plays a crucial role in CO_2 fixation (Figure 6c). During the CO_2 assimilation in photosynthesis, genes encoding Rubisco (*rbcL* and *rbcS*) were found to be upregulated; among them, the gene expression of *rbcL* increased by 10.8-fold, while nine genes of *rbcS* were also upregulated (Figure 5c). Moreover, in terms of the photosynthetic characteristics of sheltered leaves, it was shown that CCP decreased and ACE increased (Table S5). These results indicated that the assimilation of leaves under low-light intensity could be improved by regulating their CO_2 fixation ability. These factors play a significant role in the adaptation and regulation of rain-sheltered leaves in low-light intensity.

Previous studies indicated that the mechanism of chlorophyl synthesis plays a crucial role in adaptation to low-light conditions [11]. Chlorophyll synthesis directly affects photosynthetic efficiency [39], and Chl b is a prerequisite for the stable existence of light-collecting complex protein (LHCP) [40]. The increase of Chl b was found to be beneficial for increasing LHCP and the absorption of short-wavelength light, thus effectively improving the illumination ability of plants under low-light conditions [11]. The results obtained here are similar to those of previous studies [41]; in the current case, the levels of Chl b and Chl a also increased significantly, with the rate of increase of Chl b being faster

than that of Chl a (Figure 7). Furthermore, the research demonstrated that a large increase of Chl b in rice (low-light-tolerant varieties) was beneficial to capture light energy and drive electron transfer under low light, thereby maintaining a high photosynthetic rate [42]. In plants, ChlH is a multifunctional protein with roles in plastid-to-nucleus and plant hormone signal transduction pathways. ChlH accelerated the catalytic activity of ChlM, which catalyzed the conversion of Mg-Proto to Mg-protoporphyrin IX monomethyl ester; this is the key enzyme for chlorophyl synthesis, finally resulting in the synthesis of chlorophyl [43]. In the chlorophyl synthesis pathway of sheltered leaves, the expression of genes encoding HemA, HemN, ChlH, and ChlL was upregulated in rain-sheltered leaves. In addition, 23 genes encoding ChlH were upregulated (Figure 5d). These results indicate that the leaves under rain-sheltered conditions could increase their chlorophyl content by enhancing the expression of genes encoding ChlH involved in chlorophyl synthesis and metabolism; this would enable adaptation to the low-light microenvironment.

- **Regulation of antioxidant capacity under shelter covering**

ROS are produced by plants under stress, which can lead to photoinhibition and photooxidative damage of PSII and PSI [44]. ROS accumulation was shown to be regulated by a series of antioxidant enzymes; the expression of genes involved in the antioxidant system in plants was found to be diversely inhibited under light stress [45]. The antioxidant enzymes (*SOD*, *CAT*, *POD*, etc.) and nonenzymatic antioxidants (lutein, lignin, glutathione, anthocyanins, etc.) play various roles in the response to abiotic stress [45]. Under low-light conditions, low-light-tolerant rice varieties were shown to maintain their carbohydrate production level by maintaining an effective photosynthetic rate and oxygen resistance [15]. Likewise, the activity of antioxidant enzymes in cherry leaves under sheltered conditions was higher, which could avoid membrane lipid peroxidation and help maintain photosynthetic efficiency (Figure 9). Meanwhile, one gene for *SOD*, two genes for *CAT*, and six genes for *POD* were found to be upregulated, while five genes were inhibited in cherry leaves under the sheltered conditions (Figure 5). β-carotene participates in the energy-

transfer process and plays an essential role in scavenging singlet oxygen in the photosynthetic reaction centers [46]. In the carotenoid synthesis pathway, nine genes encoding crtB, PDS, crtISO, and lcyB were upregulated in rain-sheltered leaves (Figure 4b), and the level of carotene was higher in the sheltered leaves (Figure 7). This result is similar to that of tea [29]. Lutein has the photoprotective function of quenching triplet chlorophyl and excited singlet chlorophyl in light-harvesting antenna systems [47], which provides antioxidant activity by scavenging oxides, thus preventing lipid peroxidation [48]. In the current study, five genes for LUT5, crtZ, and lcyB that synthesize lutein were upregulated in sheltered leaves. The genes for *PAL*, *4CL*, and *CYP* were previously reported to be upregulated in the phenylpropanoid metabolism of spinach under heat stress [45], similar to the finding that 14 genes for *PAL*, *4CL*, and *CYP* were upregulated under sheltered leaves in this study (Figure 5c). Glutathione (GSH) can be used as an electron donor to inactivate free radicals, or as a cofactor of several antioxidant enzymes [49] for the detoxification of hydrogen peroxide produced under different stress conditions [50]; in this study, genes for *GSS* and *GSR* were upregulated in cherry leaves under the sheltered conditions (Figure 5c). Therefore, the antioxidant ability of leaves under shelter covering is highly dependent on carotenoids and phenolics, so as to attenuate the negative effects of low-light intensity.

- **Regulation of anthocyanin biosynthesis under shelter covering**

Solar radiation, air humidity, and air temperature in the sheltered conditions were reported to be affected by a rain-shelter facility [51]. Anthocyanin accumulation was also influenced by microenvironmental changes [52]. Meanwhile, the light quality could affect the synthesis of anthocyanin by regulating the expression patterns of related genes [53]. Therefore, anthocyanin content is governed by the differential expression of corresponding genes in a metabolic network, which was ascribed to the change of environmental factors [54]. Available evidence shows that *VvPAL* plays a key role in the phenylpropane biosynthesis pathway (pathway upstream of anthocyanin synthesis) by catalyzing

phenylalanine to cinnamic acid [55]. Structural genes involved in anthocyanin biosynthesis in *Arabidopsis thaliana* shown to be enzymes expressed in the initial and late stages of the regulation of the flavonoid biosynthesis pathway (pathway downstream of anthocyanin synthesis), such as *CHS* and *CHI* (initial stage), and *DFR* and *ANS* (late stage) are essential for anthocyanin biosynthesis [56]. In addition, *CHS* catalyzes the initial steps of the phenylpropanoid pathway branching to the flavonoid pathway, which is particularly important for the synthesis and accumulation of fruit anthocyanin [57]. In sweet cherry, DEGs including *PAL*, *4CL*, *CHS*, *CHI*, *F3'H*, *DFR*, and *ANS* were confirmed to be involved in anthocyanin synthesis [58]. With the progression of cherry fruit ripening, the genes involved in anthocyanin synthesis were presumably upregulated under unsheltered conditions [59]. In another study, the levels of phenolic compounds in grape peel under shelter covering were found to be decreased in comparison with those under shelter-free conditions [60]. In addition, a previous study proved that, in grapes under shading, the transcript abundance of VvF3'5'H and VvF3'H was significantly reduced [61], which contrasts with the results of the current study. In this study, these DEGs involved in phenylpropanoid and flavonoid biosynthesis, such as *PAL*, *C4H*, *4CL*, *CHS*, and *CHI*, were upregulated under covered conditions (Figure 5d). Among these, the expression of three genes encoding 4CL increased over 3.5-fold, and that of one gene encoding C4H increased 8.5-fold. In the flavonoid biosynthesis pathway, compared with the case for fruit grown under shelter-free conditions (DAF45), DEGs encoding CHS, CHI, F3'H, DFR, and ANS were upregulated, of which the expression levels of five genes encoding CHS increased more than 3.5-fold (Figure 9). Strong evidence showed that, compared with uncovered sweet cherry, the average level of phenols in covered sweet cherry increased by 14%. The results demonstrated that phenolics present under covered conditions can be explained by the larger temperature fluctuations and moderate but not excessive heat stress under the lower-light conditions under the cover [9]. Previous research also showed that anthocyanin synthesis was restricted by upstream metabolites (such as sugar) during fruit development, which proved that anthocyanin accumulation is closely related to photo-assimilates [62]. Furthermore, the results for grapes proved that the increase in anthocyanin content was attributable to a prolonged life of functional leaves, an increase in assimilative products of

leaves, and an increase in soluble solids of fruits under sheltered conditions; ultimately, the content of anthocyanin was also increased [16]. From the results of transcriptomic regulation in the current study, the anthocyanin synthesis of fruit under sheltered conditions increased the anthocyanin content of fruit by synergizing with key enzymes in upstream/downstream pathways. The synthesis and accumulation of anthocyanins could be increased by increasing the upregulation of genes involved in phenylpropane biosynthesis and flavonoid biosynthesis. Of course, this process was also inseparable from the adaptation of leaves to weak light, which provided assimilation products for the synthesis of fruit anthocyanins.

Collectively, cherry trees could improve photosynthesis and antioxidant capacity by regulating the genes involved in light capture (*LCHs*), carbon dioxide fixation (*Rubisco*), photosynthetic pigment synthesis (*ChlH*), and the antioxidant system (carotenoids and phenolics), and ultimately enhance their adaptability to low-light intensity under sheltered conditions.

Conclusion

During this research study, we produced some new transcriptome information for “Manaohong” from PacBio and Illumina sequencing in cherry leaves and fruits at three developmental stages under control and rain-shelter conditions. Moreover, the adaptive regulation of cherry leaves and fruits under the sheltered condition was mainly associated with their physiological characteristics (such as LCP, CCP, and Chl content) and transcriptome expression profiles. The results confirmed that the mechanisms of leaf adaptation to low light intensity included the following metabolic pathways: photosynthesis (antenna proteins), photosynthetic electron transport, CO_2 fixation, photosynthetic pigment synthesis, and antioxidant system. Compared with the unsheltered leaves, the sheltered leaves maintained a higher ability of light capture under low light intensity by actively regulating the synthesis of *Lcha* and *Lchb*, promoted the expressions of *rbcL* and *rbcS* to

enhance CO_2 fixation, and enhanced the expression of *chlH*, thereby promoting chlorophyll synthesis. Furthermore, combined with the antioxidant system to enable leaves produce enough carbohydrates under the sheltered condition, the regulation of leaf adaptation provides a material basis for improving fruit quality. In addition, the genes related to the upstream and downstream pathways of anthocyanin biosynthesis were significantly enriched and upregulated in the fruits under the rain-shelter condition, which promoted the biosynthesis and accumulation of anthocyanin in the fruits. The present study and its findings can serve as valuable resources for future genomic studies on Chinese cherries. The DEG data may also provide useful candidate genes to elucidate the adaptation mechanism of “Manaohong” cherries and other varieties to low-light condition and other abiotic stress.

Methods

- **Plant materials**

The trials were conducted from 2016 to 2018 in an orchard located at Fuquan, Guizhou Province, P.R. China (latitude 26°70′ N, longitude 107°51′ E), which is characterized by a subtropical monsoon climate. At the site, the average temperature is 14°C, the relative humidity is 88%, and the annual total precipitation is 1,220 mm. Five-year-old Chinese cherry trees (“Manaohong” cultivar) were spaced with 3 m between each row and each tree, with the open-center model of trunks and branches. All trees were subjected to management under the same integrated technique. The trials were conducted with covering by colorless polyethylene film (semi-covered to ensure ventilation), with a transmission rate of approximately 70%, which was applied from before blooming until fruit harvest. The length, width, and height of the steel frame shelter were 30 m, 10 m, and 4 m (above the ground), respectively. Unsheltered trees were set as a control (Cont). Three trees each under sheltered and unsheltered conditions were labeled for sample collection on the 35^{th},

45^{th}, and 55^{th} days after flowering (DAF35, DAF45, and DAF55, respectively). Samples of unsheltered leaves (UL35, 45, and 55), unsheltered fruits (UF35, 45, and 55), sheltered leaves (SL35, 45, and 55), and sheltered fruits (SF35, 45, and 55) were collected, immediately frozen in liquid nitrogen, and stored at −80°C for physiological indicator measurement and RNA isolation.

- **Physiological characteristics of leaves under rain-shelter covering**

The photosynthesis light-response (PAR-Pn) and CO_2-response (CO_2-Pn) curves under shelter covering and shelter-free conditions were measured between 9:00 a.m. and 11:30 a.m. on a sunny day using a portable infrared gas analyzer (Li-6400XT; Li-Cor, Inc., Lincoln, NE, USA). The photosynthetic PAR-response and CO_2-response curves were fitted by the Farquhar mathematical model (Prioul and Chartier, 1977). The AQY and CE were obtained by linear regression under a PAR range of 0–200 $\mu mol \cdot m^{-1} \cdot s^{-1}$ and CO_2 concentration range of 0–200 $\mu mol \cdot mol^{-1}$; in addition, the LCP and CCP were calculated. Two new southern branches were selected for each tree (three trees per sheltered condition), and the fifth mature leaf was selected from the base of the shoot.

Thirty mature leaves per tree were sampled to quantify the following parameters, and three biological replicates were performed for each of the sheltered and unsheltered conditions. To minimize the positional effect of the canopy, leaves were harvested from four directions: east, south, west, and north. The contents of chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids (Car) were determined according to the method described by Shi et al. (2013). The amount of malondialdehyde (MDA) and activity of superoxide dismutase (*SOD*, E.C.1.15.1.1) were measured as documented by Wen et al. (2011). The activities of peroxidase (POD, E.C. 1.11.1.7) and catalase (CAT, E.C. 1.11.1.6) were determined using a spectrophotometer following a previously described method (Sharma et al., 2016). All absorbances of extracts were measured using a microplate spectrophotometer (Thermo Scientific, MA, USA).

- **Total RNA extraction, PacBio and Illumina library construction and sequencing**

A total of 36 samples [two sheltered coverings (sheltered and unsheltered) × three stages (DAF35, 45, 55) × two tissues (leaves and fruits) × three biological replicates] were prepared, and the total RNAs were separately obtained using the QIAGEN RNeasy Plus Mini Kit (Cat. No. 74134). The purity and concentration of RNA were determined using a Nanodrop 2000 spectrophotometer and QUBIT Fluorometer (Life Technologies), and the integrity of RNA was assessed using an Agilent 2100 bioanalyzer (Agilent Technologies).

The qualified RNAs of leaves (UL and SL) and fruits (UF and SF) from three stages (DAF35, 45, and 55) were equivalently mixed into two corresponding libraries. cDNA was synthesized using a SMART PCR cDNA Synthesis Kit (Clontech, Cat. No. 639206). PCR was optimized using a KAPA HiFi PCR Kit; different cDNA fractions (0.5–2 kb, 2–3 kb, and >3 kb) were classified using the BluePippin size selection system (Sage Science, Beverly, MA, USA). The PCR products were amplified and constructed using SMRTbell Template Prep Kit 1.0 (part.100-259-100) following the vendor's protocol. The library was sequenced on the PacBio Sequel real-time nanopore sequencer using PacBio Sequel V2.1 with five cells (one cell for fruits and four cells for leaves); 0.5–6 kb mixed libraries were sequenced. A total of 36 qualified RNA samples were used for Illumina sequencing. The RNA samples were used for poly(A)$^+$ selection using oligo(dT) magnetic beads, while constructing RNA-Seq libraries. The cDNA libraries were sequenced using PacBio Sequel and HiSeq X Ten at Wuhan Nextomics Biosciences Co., Ltd.

- **Data processing and gene function annotation**

Sequence data of PacBio Sequel were collected, and the high-quality consensus transcript sequences were obtained using smrtlink 5.0. Subsequently, the iterative clustering for error correction (ICE) algorithm was used to remove redundancy and improve the accuracy of the full-length transcripts. Raw data of Illumina HiSeq were

processed through inner Perl scripts. Clean data were obtained by removing reads containing adapters, reads containing poly-N regions, and low-quality reads from the raw data. Meanwhile, the Illumina HiSiq reads were used to correct all full-length transcripts of single-molecule real-time (SMRT) Squel by Lordec [19]. The proofread-corrected sequences after removal of the redundant sequences using CD-HIT-EST [20] were used as reference sequences for further analyses.

Gene functions were searched against the databases of NCBI Nonredundant Protein Sequences (NR), Clusters of Orthologous Groups of Proteins (COG), a manually annotated and reviewed protein sequence database (Swiss-Prot), Kyoto Encyclopedia of Genes and Genomics (KEGG), and Gene Ontology (GO).

- **Differential expression, GO, and KEGG enrichment analysis**

Clean reads of all samples were counted and normalized into fragments per kilobase of transcript per million fragments mapped reads (FPKM) value using RSEM (v1.1.12) [21]. DEGs between different treatments were determined using the DESeq package [22]. A p value ≤0.01 and an absolute value of log2FoldChange ≥1 were used to identify genes that were significantly differentially expressed. The GO functional classification statistics of DEGs used the WEGO software [23]. Metabolic pathway assignments of DEGs were implemented based on the KEGG orthology database (http://www.genome.ad.jp/kegg/) using the KAAS system (http://www.genome.jp/tools/kaas/). The different expression patterns of DEGs among the leaves and fruits were assessed using the R language, Cluster, Fpc, Ggplot2, and Reshape2 packages; the clustering method was K-means clustering.

- **Quantitative real-time PCR (qRT-PCR) analysis**

The expression levels of samples under the different conditions, including leaves and fruits, were validated by qRT-PCR using PowerUp™ SYBR Green Master Mix

(ThermoFisher, Chongqing, China) in a volume of 10 µL, which contained 5 µl of SYBR Green Master Mix, 150 ng (leaf)/100 ng (fruit) of cDNA template, and 0.4 µM of each of the forward and reverse primers. The qRT-PCR amplification was performed as follows: 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s. Three cherry actin genes (*UBCE*, *CYP2*, and *ACT2*) were used as reference genes, as previously reported [24, 25], the primer sequences are listed in Table S1. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method [26] with the CFX Connect™ Real-Time PCR Detection System (Bio-Rad Laboratories, CA, USA). All validations were performed in three biological and technical replicates.

- **Statistical analysis**

All data were assessed for significant differences using Duncan's and Tukey's tests using the SPSS 21.0 statistics package (Chicago, IL, USA). All data are presented as the mean and standard deviation (SD) of at least three replicates. The graphs were constructed with Origin 9.0 (Origin Lab, Northampton, MA, USA).

Declarations

Ethics approval and consent to participate

The experiments did not utilize transgenic technology or involve protected species. "Manaohong" used in this study were obtained from an orchard in Fuquan, which was a demonstration base of Guizhou University, China. It does not require specific permits.

Consent for publication

Not applicable.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

XPW designed the research. TT carried out the experiments with the help of GQ, ZW, BD, ZLQ, and HY. TT collected the experimental data and drafted the manuscript. XPW and GQ reviewed the manuscript and part of the data analysis. All authors read and approved the final manuscript.

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References

1. Acero N, Gradillas A, Beltran M, García A, Mingarro DM: Comparison of phenolic compounds profile and antioxidant properties of different sweet cherry (Prunus avium L.) varieties. Food Chem 2019, 279:260-271. 2. Muccillo L, Colantuoni V, Sciarrillo R, Baiamonte G, Salerno G, Marziano M, Sabatino L, Guarino C: Molecular and environmental analysis of Campania (Italy) sweet cherry (Prunus avium L.) cultivars for biocultural refugia identification and conservation. Sci Rep-Uk 2019, 9(1):6796. 3. Kafle GK, Khot LR,

Zhou J, Bahlol HY, Si Y: Towards precision spray applications to prevent rain-induced sweet cherry cracking: Understanding calcium washout due to rain and fruit cracking susceptibility. Sci Hortic-Amsterdam 2016, 203:152-157. 4. Gao Y, Li XX, Han MM, Yang XF, Li Z, Wang J, Pan QH: Rain-Shelter Cultivation Modifies Carbon Allocation in the Polyphenolic and Volatile Metabolism ofVitis viniferaL. Chardonnay Grapes. Plos One 2016, 11(5):e156117. 5. Ozkan Y, Yıldız K, KüçÜker E, ÇEkìÇ C, Özgen M, AkçA Y: Early performance of cv. Jonagold apple on M.9 in five tree training systems. Hortic Sci 2012, 39(4):158-163. 6. Yan W, Long LE: Physiological and biochemical changes relating to postharvest splitting of sweet cherries affected by calcium application in hydrocooling water. Food Chem 2015, 181:241-247. 7. Faniadis D, Drogoudi PD, Vasilakakis M: Effects of cultivar, orchard elevation, and storage on fruit quality characters of sweet cherry (Prunus avium L.). Sci Hortic-Amsterdam 2010, 125(3):301-304. 8. Aikio S, Taulavuori K, Hurskainen S, Taulavuori E, Tuomi J: Contributions of day length, temperature and individual variability on the rate and timing of leaf senescence in the common lilac Syringa vulgaris. Tree Physiol 2019, 39(6). 9. Overbeck V, Schmitz M, Tartachnyk I, Blanke M: Identification of light availability in different sweet cherry orchards under cover by using non-destructive measurements with a Dualex™. Eur J Agron 2017, 93:50-56. 10. Schneider T, Bolger A, Zeier J, Preiskkowski S, Matsubara S: Fluctuating Light Interacts with Time of Day and Leaf Development Stage to Reprogram Gene Expression. Plant Physiol 2019. 11. Wang LF: Physiological and molecular responses to variation of light intensity in rubber Tree (Hevea brasiliensis Muell. Arg.). Plant Physiology & Biochemistry 2014, 83(2):243-249. 12. Echer FR, Rosolem CA: Cotton yield and fiber quality affected by row spacing and shading at different growth stages. Eur J Agron 2015, 65:18-26. 13. Overbeck V, Schmitz M, Blanke M: Targeted forcing improves quality, nutritional and health value of sweet cherry fruit. J Sci Food Agric 2017, 97(11):3649-3655. 14. Szalai G, Majláth I, Pál M, Gondor OK, Rudnóy S, Oláh C, Vanková R, Kalapos B, Janda T: Janus-Faced Nature of Light in the Cold Acclimation Processes of Maize. Front Plant Sci 2018, 9:850. 15. Sekhar S, Panda D, Kumar J, Mohanty N, Biswal M, Baig MJ, Kumar A, Umakanta N, Samantaray S, Pradhan SK et al: Comparative transcriptome profiling of low light tolerant and sensitive rice varieties induced by low light stress at active tillering stage. Sci Rep-Uk 2019, 9(1):5753. 16. Li T, Liu L, Jiang C, Liu Y, Shi L: Effects of mutual shading on the regulation of photosynthesis in field-grown sorghum. J Photoch Photobio B 2014, 137(SI):31-38. 17. Jin L, Guo D, Ning D, Hussain SB, Liu Y: Covering the trees of Kinokuni tangerine with plastic film during fruit ripening improves sweetness and alters the metabolism of cell wall components. Acta Physiol Plant 2018, 40(10):1-10. 18. Tian T, Qiao G, Deng B, Wen Z, Hong Y, Wen X: The effects of rain shelter coverings on the vegetative growth and fruit characteristics of Chinese cherry (Prunus pseudocerasus Lindl.). Sci Hortic-Amsterdam 2019, 254:228-235. 19. Leena S, Eric R: LoRDEC: accurate and efficient long read error correction. Bioinformatics 2014, 30(24):3506-3514. 20. Xu Z, Peters RJ, Weirather J, Luo H, Liao B, Zhang X, Zhu Y, Ji A, Zhang B, Hu S: Full-length transcriptome sequences and splice variants obtained by a combination of sequencing platforms applied to different root tissues of Salvia miltiorrhiza and tanshinone biosynthesis. Plant J 2015, 82(6):951-961. 21. Bo L, Dewey CN: RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. Bmc Bioinformatics 2011, 12(1):323. 22. Likun W, Zhixing F, Xi W, Xiaowo W, Xuegong Z: DEGseq: an R package for identifying differentially expressed genes from RNA-seq data. Bioinformatics 2010, 26(1):136-138. 23. Jia Y, Lin F, Hongkun Z, Yong Z, Jie C, Zengjin Z, Jing W,

Shengting L, Ruiqiang L, Lars B: WEGO: a web tool for plotting GO annotations. Nucleic Acids Res 2006, 34(Web Server issue):W293-W297. 24. Xia Y, Zhang F, Tao Y, Song S, Fang J: Reference gene selection for quantitative real-time PCR normalization in different cherry genotypes, developmental stages and organs. Sci Hortic-Amsterdam 2015, 181:182-188. 25. Zhu YY, Wang Y, Zhang H, Shao X, Li YQ, Guo WD: Selection and Characterization of Reliable Reference Genes in Chinese Cherry (Prunus pseudocerasus) Using Quantitative Real- time PCR (qRT-PCR). Journal of Agricultural Biotechnology 2015, 23(05):690-700. 26. Schmittgen TD, Livak KJ: Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc 2008, 3(6):1101-1108. 27. Liu X, Mei W, Soltis PS, Soltis DE, Barbazuk WB: Detecting Alternatively Spliced Transcript Isoforms from Single-Molecule Long-Read Sequences without a Reference Genome. Mol Ecol Resour 2017, 17(6). 28. Alessio C, Simonetta P, Marco F, Vania M, Angelo B, Mariangela S, Claudio P: A deficiency at the gene coding for zeta-carotene desaturase characterizes the sunflower non dormant-1 mutant. Plant & Cell Physiology 2004, 45(4):445-455. 29. Li NN, Yue C, Cao HL, Qian WJ, Hao XY, Wang YC, Wang L, Ding CQ, Wang XC, Yang YJ: Transcriptome sequencing dissection of the mechanisms underlying differential cold sensitivity in young and mature leaves of the tea plant (Camellia sinensis). J Plant Physiol 2018, 224-225:144-155. 30. Feng Y, Shan H, Gao R, Liu W, Yong T, Wang X, Wu X, Yang W: Growth of soybean seedlings in relay strip intercropping systems in relation to light quantity and red:far-red ratio. Field Crop Res 2014, 155(155):245-253. 31. Raik W, Lars D, Katharina BU, Wolfgang F, Thomas P: The long-term response to fluctuating light quality is an important and distinct light acclimation mechanism that supports survival of Arabidopsis thaliana under low light conditions. Planta 2008, 228(4):573-587. 32. Pu C, Gui XY, Yang Q, Li NZ, Cheng Z, Feng QZ, Rong ZG: iTRAQ-based quantitative proteomics analysis of Brassica napus leaves reveals pathways associated with chlorophyll deficiency. J Proteomics 2015, 113:244-259. 33. Floris, Maina, Robaglia, Christophe, Elodie, Bassi, Roberto, Alboresi: Post-transcriptional control of light-harvesting genes expression under;light stress. Plant Mol Biol 2013, 82(1-2):147-154. 34. Chen H, Zhang D, Guo J, Wu H, Jin M, Lu Q, Lu C, Zhang L: A Psb27 homologue in Arabidopsis thaliana is required for efficient repair of photodamaged photosystem II. Plant Mol Biol 2006, 61(4-5):567-575. 35. Dong L, Tu W, Liu K, Sun R, Liu C, Wang K, Yang C: The PsbS protein plays important roles in photosystem II supercomplex remodeling under elevated light conditions. J Plant Physiol 2015, 172:33-41. 36. Palacio S, Maestro MMG: Relationship between shoot-rooting and root-sprouting abilities and the carbohydrate and nitrogen reserves of mediterranean dwarf shrubs. Ann Bot-London 2007, 100(4):865-874. 37. Wang ZY, Bai MY, Oh E, Zhu JY: Brassinosteroid Signaling Network and Regulation of Photomorphogenesis. Annu Rev Genet 2012, 46(1):701. 38. Ning Z, Kallis RP, Ewy RG, Portis AR: Light modulation of Rubisco in Arabidopsis requires a capacity for redox regulation of the larger Rubisco activase isoform. Proc Natl Acad Sci U S A 2002, 99(5):3330-3334. 39. Tibor S, Boris H, Jiří `, Petr I, Bernhard G, Andrej P: Transcriptional and post-translational control of chlorophyll biosynthesis by dark-operative protochlorophyllide oxidoreductase in Norway spruce. Photosynth Res 2017, 132(2):1-15. 40. Valladares F, Niinemets U: Shade Tolerance, a Key Plant Feature of Complex Nature and Consequences. Annual Review of Ecology Evolution & Systematics 2008, 39(1):237-257. 41. Feng Y, Feng L, Liu Q, Wu X, Fan Y, Raza MA, Cheng Y, Chen J, Wang X, Yong T: Effect of interactions between light intensity and red-to- far-red ratio on the photosynthesis of soybean leaves under shade condition. Environmental & Experimental Botany 2018, 150. 42. Liu QH, Xiu WU, Chen

BC, Jia-Qing MA, Jie G: Effects of Low Light on Agronomic and Physiological Characteristics of Rice Including Grain Yield and Quality. Rice Sci 2014, 21(5):243-251. 43. Osanai T, Imashimizu M, Seki A, Sato S, Tabata S, Imamura S, Asayama M, Ikeuchi M, Tanaka K: ChlH, the H subunit of the Mg-chelatase, is an anti-sigma factor for SigE in Synechocystis sp. PCC 6803. P Natl Acad Sci Usa 2009, 106(16):6860-6865. 44. Khanal N, Bray GE, Grisnich A, Moffatt BA, Gray GR: Differential Mechanisms of Photosynthetic Acclimation to Light and Low Temperature in Arabidopsis and the Extremophile Eutrema salsugineum. PLANTS-BASEL 2017, 6(3). 45. Yan J, Yu L, Xuan J, Lu Y, Lu S, Zhu W: De novo transcriptome sequencing and gene expression profiling of spinach (Spinacia oleracea L.) leaves under heat stress. Sci Rep-Uk 2016, 6(4):19473. 46. Stefano C, Zhirong L, Niyogi KK, Roberto B, Luca D: The Arabidopsis szl1 mutant reveals a critical role of β-carotene in photosystem I photoprotection. Plant Physiol 2012, 159(4):1745-1758. 47. Jahns P, Holzwarth AR: The role of the xanthophyll cycle and of lutein in photoprotection of photosystem II □. Biochimica Et Biophysica Acta 2012, 1817(1):182-193. 48. Luca D, Holt NE, Shanti K, Marcel F, Stefano C, Donatella C, Frank HA, Jean A, Roberto B: Zeaxanthin protects plant photosynthesis by modulating chlorophyll triplet yield in specific light-harvesting antenna subunits. J Biol Chem 2012, 287(50):41820-41834. 49. Montserrat M, Albert M, Anna C, Carmen GR, Fernández-Checa JC: Mitochondrial glutathione, a key survival antioxidant. Antioxid Redox Signal 2009, 11(11):2685-2700. 50. N A, El-Baz F, Salama DZ, Adb El Baky H, Aly H, A G: Enhancement of phenolics, flavonoids and glucosinolates as antioxidants of broccoli in response to organic and bioorganic fertilizers. J Saudi Chem Soc 2012:135-142. 51. Kennedy JA, Jones GP: Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. Journal of Agricultural & Food Chemistry 2001, 49(4):1740-1746. 52. Zhang Y, Hu W, Peng X, Sun B, Wang X, Tang H: Characterization of anthocyanin and proanthocyanidin biosynthesis in two strawberry genotypes during fruit development in response to different light qualities. J Photoch Photobio B 2018, 186:225-231. 53. Agarwal A, Gupta SD: Impact of Light-Emitting Diodes (LEDs) and its Potential on Plant Growth and Development in Controlled-Environment Plant Production System. Current Biotechnology 2016, 5(1):-. 54. Jaakola L: New insights into the regulation of anthocyanin biosynthesis in fruits. Trends Plant Sci 2013, 18(9):477-483. 55. Miao L, Zhang Y, Yang X, Xiao J, Zhang H, Zhang Z, Wang Y, Jiang G: Colored light-quality selective plastic films affect anthocyanin content, enzyme activities, and the expression of flavonoid genes in strawberry (Fragaria × ananassa) fruit. Food Chem 2016, 207:S2122214610. 56. Pelletier MK, Murrell JR, Shirley BW: Characterization of flavonol synthase and leucoanthocyanidin dioxygenase genes in Arabidopsis. Further evidence for differential regulation of "early" and "late" genes. Plant Physiol 1997, 113(4):1437. 57. Katia P, Chiara T: Recent advances on the regulation of anthocyanin synthesis in reproductive organs. Plant Science An International Journal of Experimental Plant Biology 2011, 181(3):219-229. 58. Liu Y, Shen X, Zhao K, Ben Y, Guo X, Zhang X, Li T: Expression Analysis of Anthocyanin Biosynthetic Genes in Different Colored Sweet Cherries (Prunus avium L.) During Fruit Development. J Plant Growth Regul 2013, 32(4):901-907. 59. Wei H, Chen X, Zong X, Shu H, Gao D, Liu Q: Comparative Transcriptome Analysis of Genes Involved in Anthocyanin Biosynthesis in the Red and Yellow Fruits of Sweet Cherry (Prunus avium L.). Plos One 2015, 10(3). 60. Jiang-Fei M, Peng-Fei N, Teng-Fei X, Zhen-Wen Z: Effect of rain-shelter cultivation of Vitis vinifera cv. Cabernet Gernischet on the phenolic profile of berry skins and the incidence of grape diseases. Molecules 2012, 18(1):381-397. 61. Koyama K, Goto-Yamamoto N: Bunch Shading

During Different Developmental Stages Affects the Phenolic Biosynthesis in Berry Skins of 'Cabernet Sauvignon' Grapes. J Am Soc Hortic Sci 2008, 133(6):743-753. 62. Duan B, Song C, Zhao Y, Jiang Y, Shi P, Meng J, Zhang Z: Dynamic changes in anthocyanin biosynthesis regulation of Cabernet Sauvignon (Vitis vinifera L.) grown during the rainy season under rain-shelter cultivation. Food Chem 2019, 283:404-413.

Figures

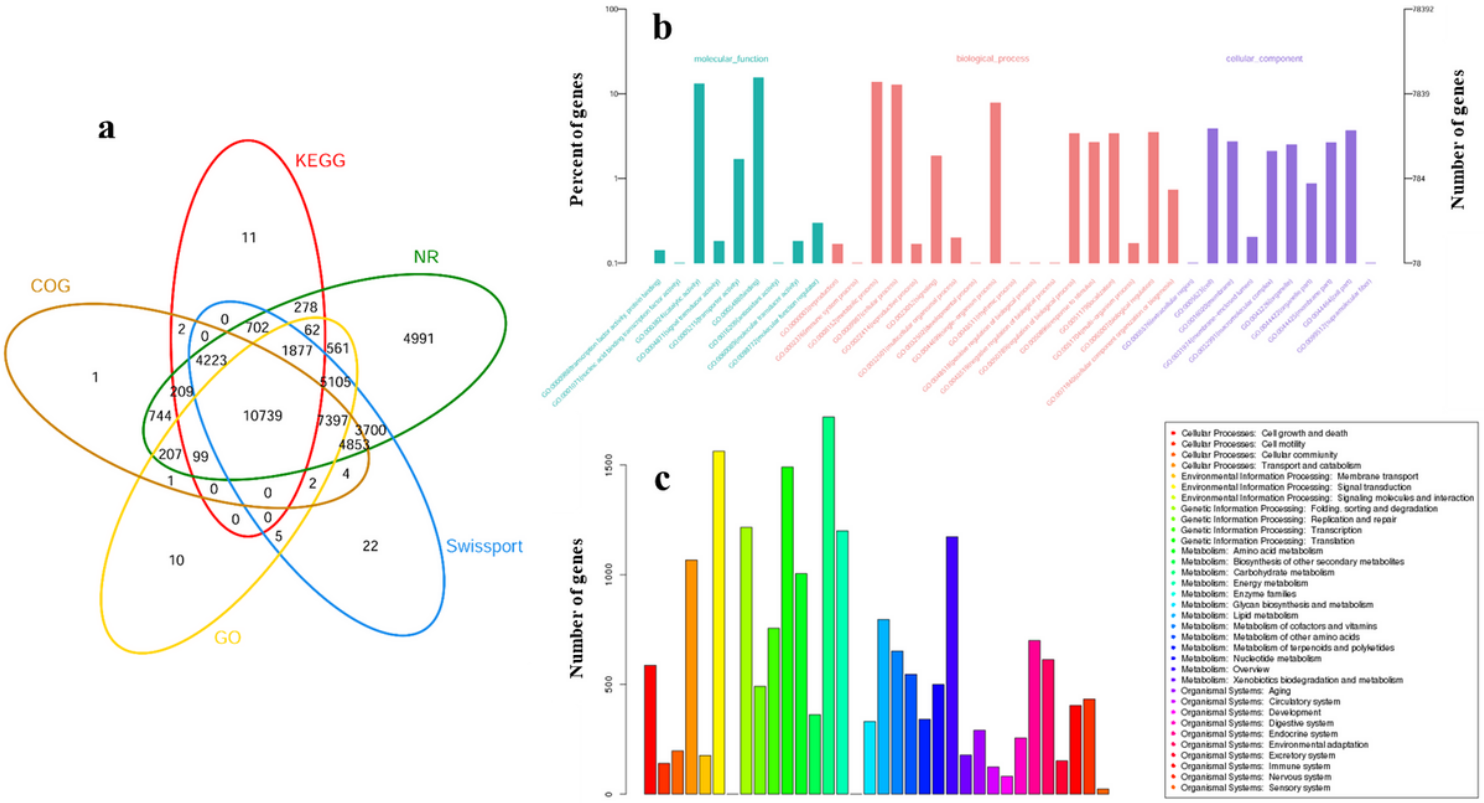

Figure 1

Annotation and functional classification of unigenes. (a) Venn diagram of unigenes functionally annotated for leaves and fruits. (b) Annotation of the GO database. The abscissa is the GO classification of gene function, the ordinate (left) is the proportion of genes in the annotation, and the ordinate (right) is the number of genes in the annotation. (c) Classification of KEGG annotation. The abscissa is the classification of gene function and the ordinate is the number of genes in the interpretation.

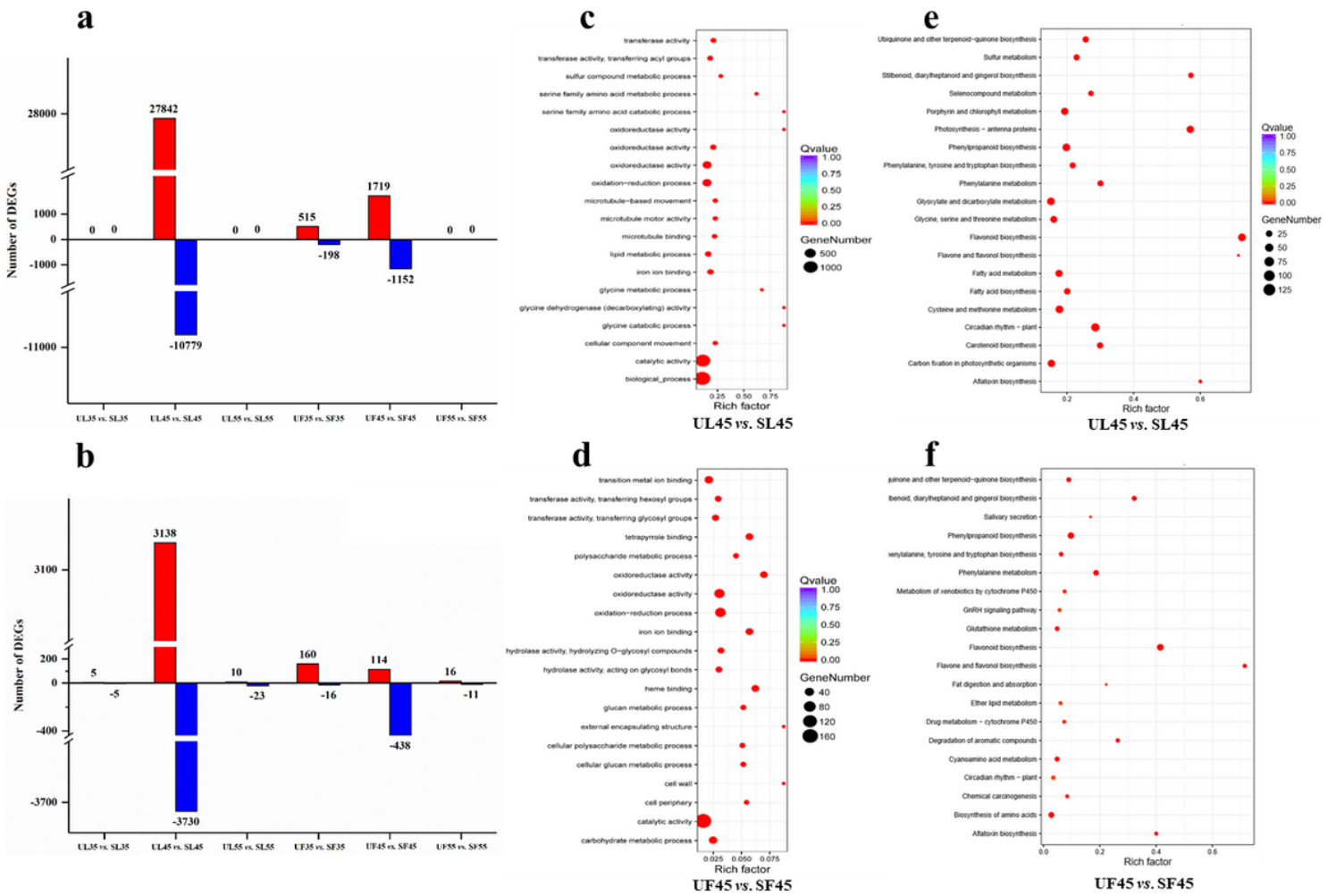

Figure 2

Expression patterns based on DEGs of leaves and fruits among three developmental stages. (a) DEG numbers from the GO database. (b) DEG numbers from the KEGG database. Scatter plots of GO and KEGG pathway-enrichment statistics of leaves and fruits at DAF45. (c) and (d), The top 20 upregulated enriched GO pathways of leaves and fruits, respectively. (e) and (f), The top 20 upregulated enriched KEGG pathways of leaves and fruits, respectively.

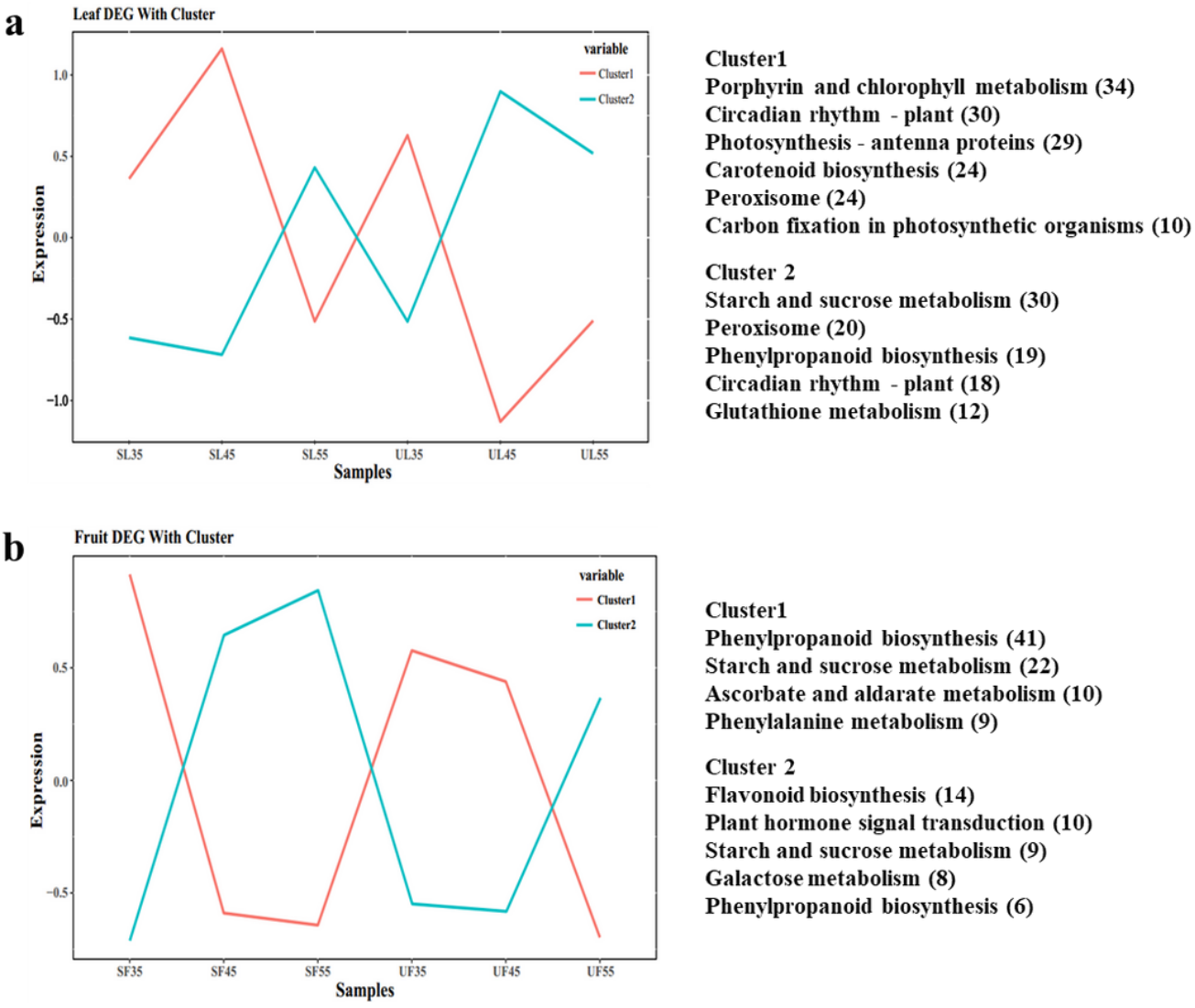

Figure 3

Cluster analysis of DEGs of leaves and fruits. The cluster analysis of DEGs showing significant changes in expression profiles of leaves (c) and fruits (d). Enriched KEGG pathways are listed in each cluster. Numbers in brackets indicate the quantity of DEGs enriched.

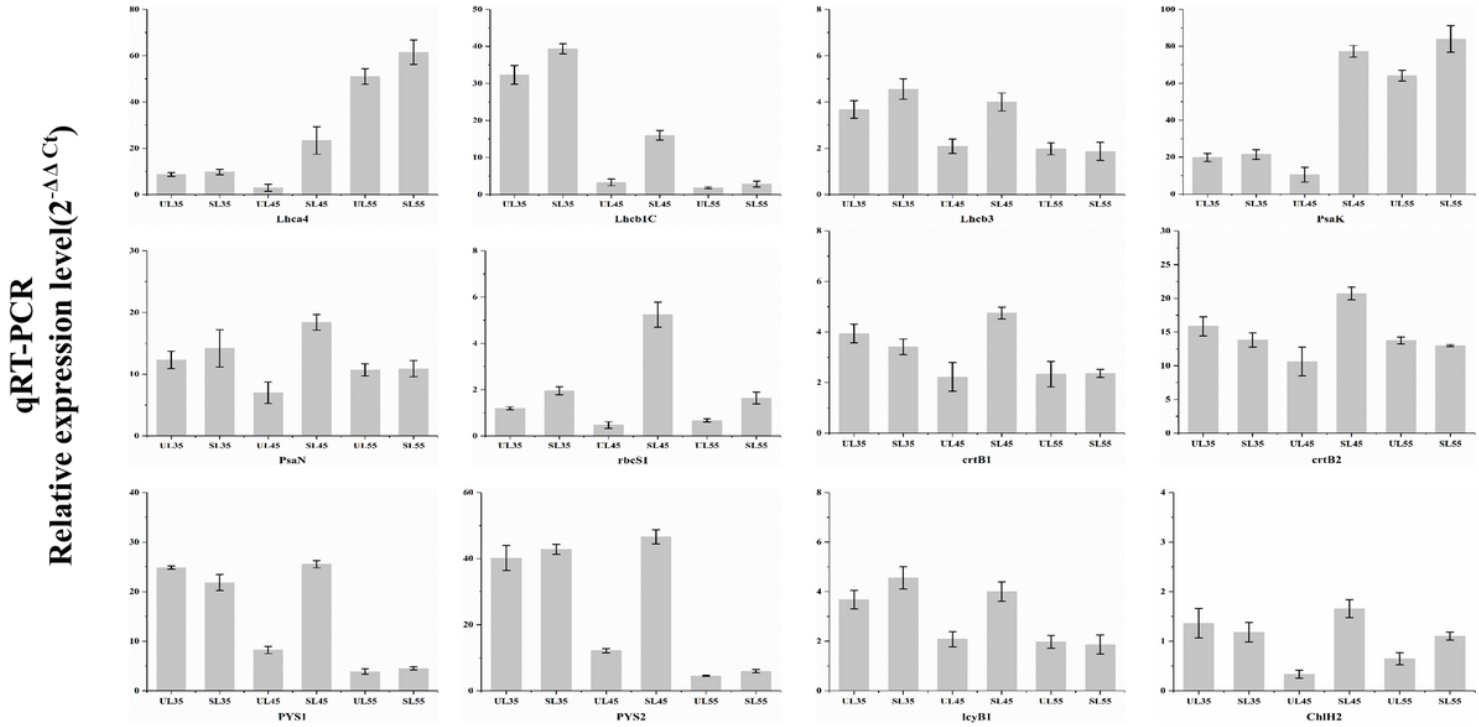

Figure 4

Validation of the RNA-Seq results using qRT-PCR analysis. The y-axis represents relative expression and the x-axis represents three stages of leaf development and different cultivation facilities. The standard error of the mean for three biological replicates (nested with three technical replicates) is represented by the error bars. Different letters on each symbol indicate statistically significant differences ($P < 0.05$) between two values according to ANOVA and Duncan's new multiple range tests.

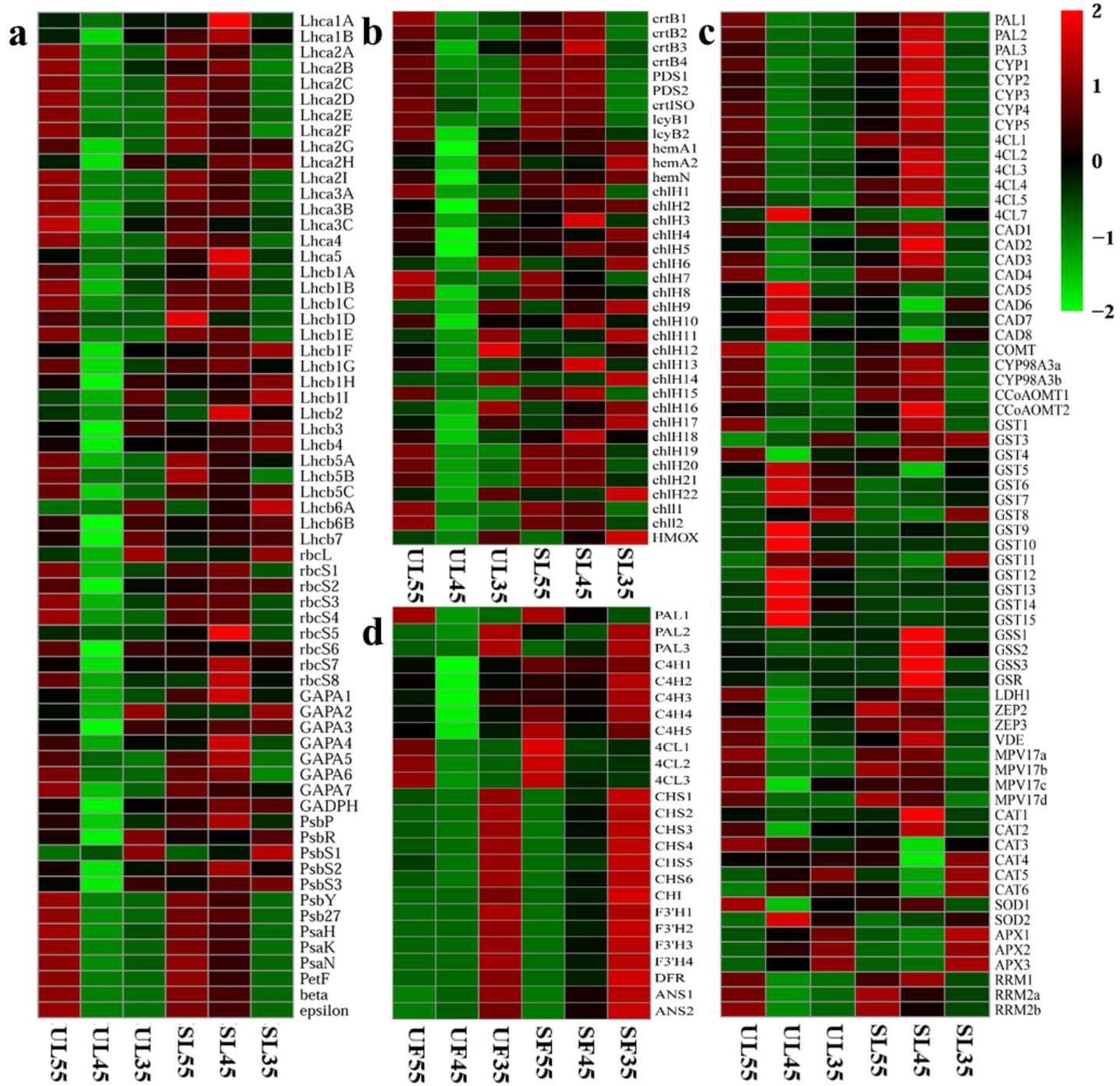

Figure 5

Heat maps representing the expression patterns of genes in leaves and fruits at DAF35, 45, and 55 under shelter covering and shelter-free conditions. (a) Genes involved in photosynthetic systems. (b) Genes related to chlorophyl and carotenoid synthesis. (c) Genes involved in antioxidant system. (d) Genes related to anthocyanin biosynthesis. The log2FoldChange (FPKM) values were used to generate the heat maps. The abscissa indicates the sampling time, while the ordinate indicates key differentially expressed genes. The green-black-red schemes are labeled above the heat maps. Red and green represent higher and lower expression levels, respectively, than those shown in black.

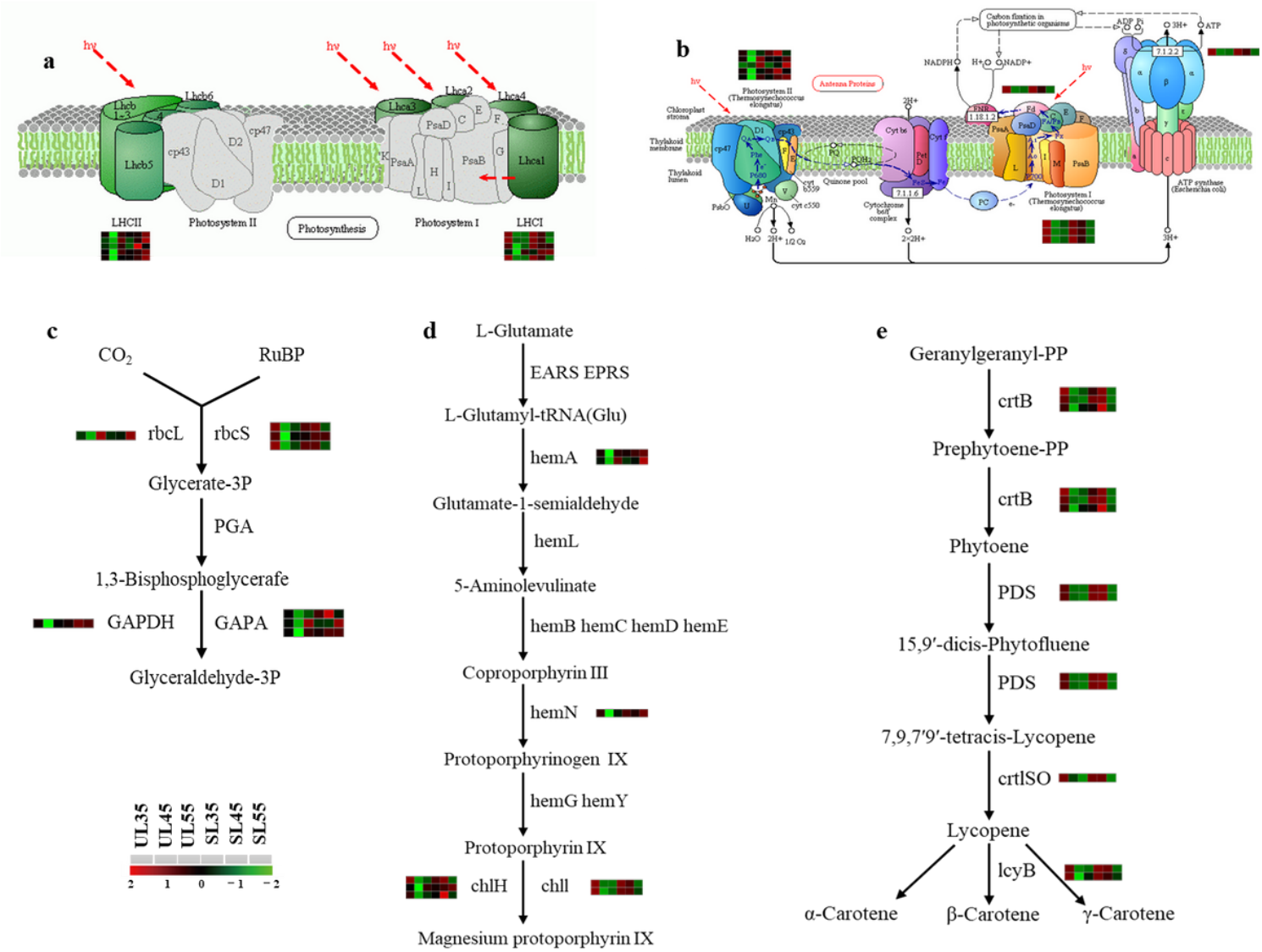

Figure 6

Photosynthesis and light and pigment synthesis pathways of cherry leaves under sheltered covering. (a) DEGs predicted to be involved in the photosynthesis-annotated proteins, (b) photosynthesis, (c) carbon fixation in photosynthetic organisms, (d) prophyrin and chlorophyl metabolism, and (e) carotenoid biosynthesis. Responsive progression of gene expression under sheltered covering C compared with the unsheltered conditions, indicated in six-box strings (including UL35, 45, 55, LR35, 45, and 55). Heat maps were drawn using log2-transformed FPKM values.

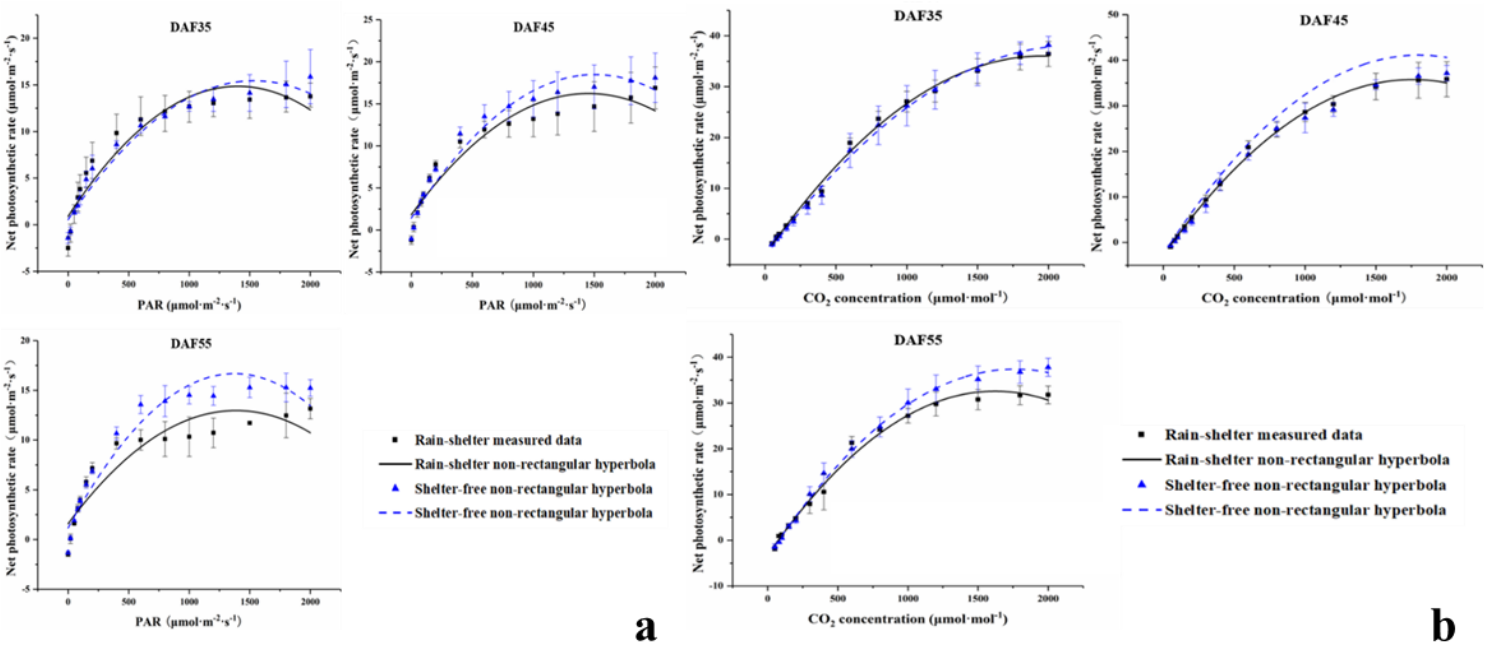

Figure 7

(a) PAR-Pn response curve of the leaves subjected to rain-shelter covering. (b) CO2-Pn response curve of the leaves subjected to sheltered covering.

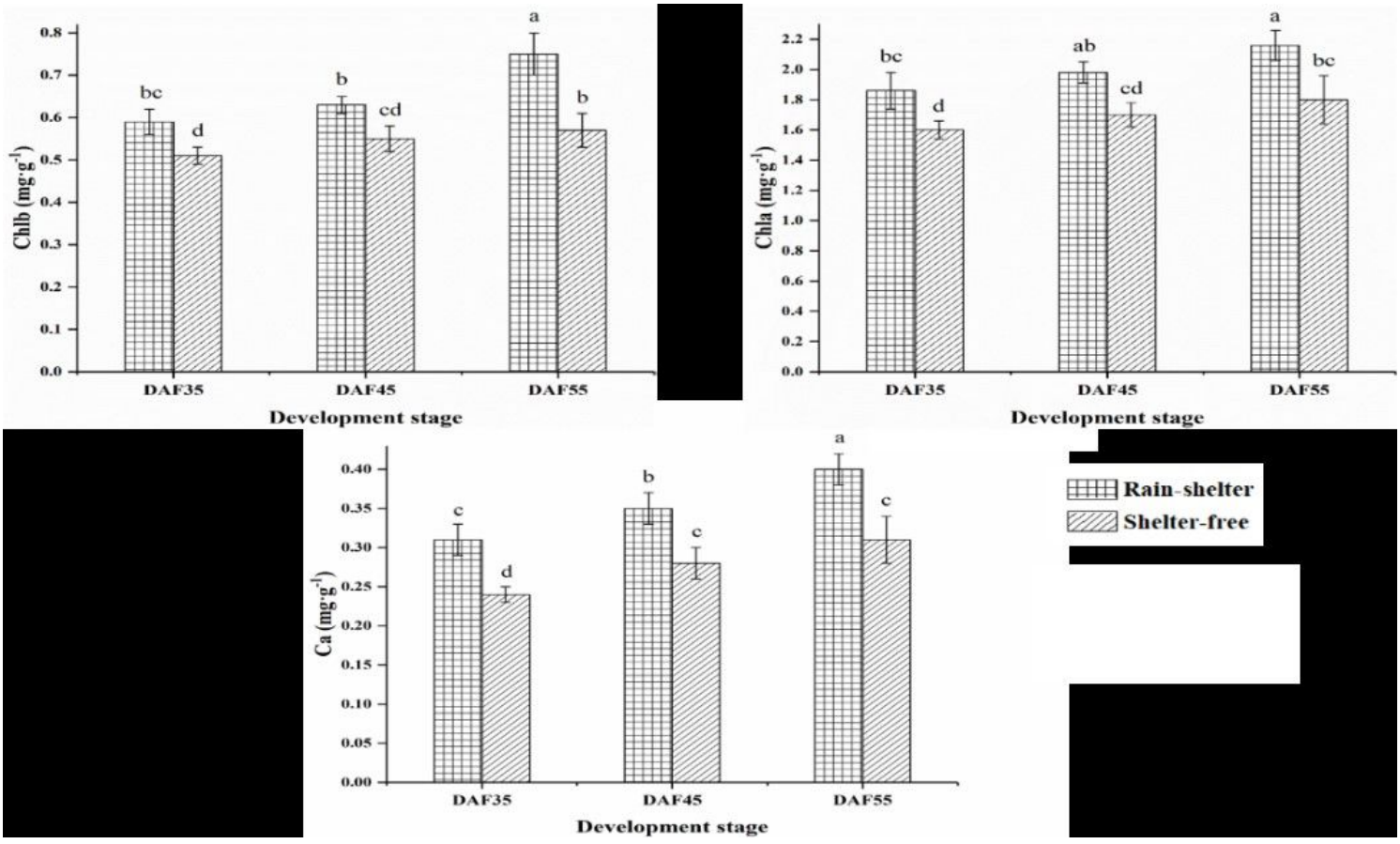

Figure 8

The contents of Chl a, Chl b, and carotenoid of leaves under sheltered covering. The vertical bars represent means of three trees (30 leaves·tree−1). The values marked with different letters are significantly different at P < 0.05.

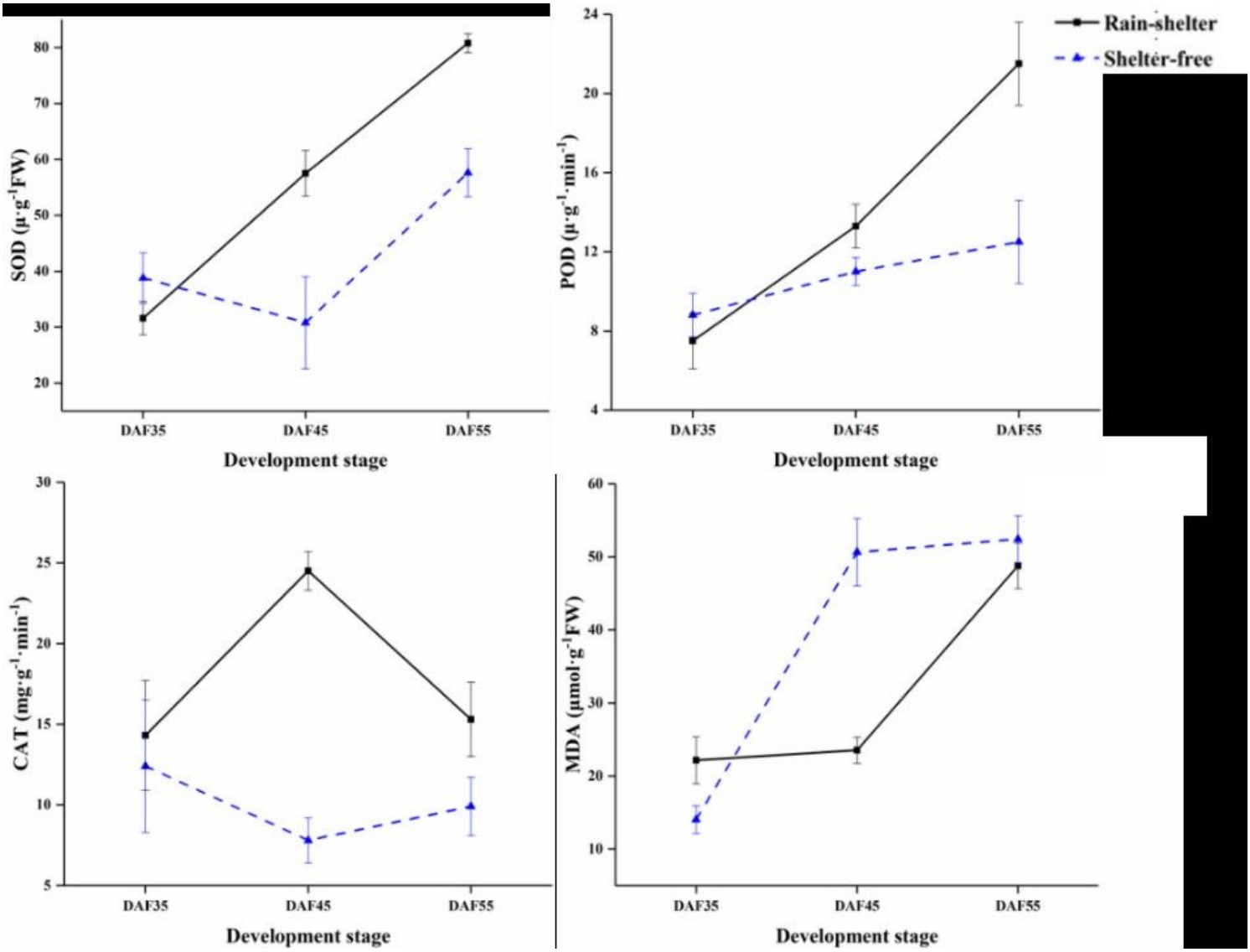

Figure 9

Effect of rain-shelter conditions on antioxidant enzyme activities and MDA contents in cherry leaves. The values represent mean ± SD of three trees (30 leaves·tree−1).

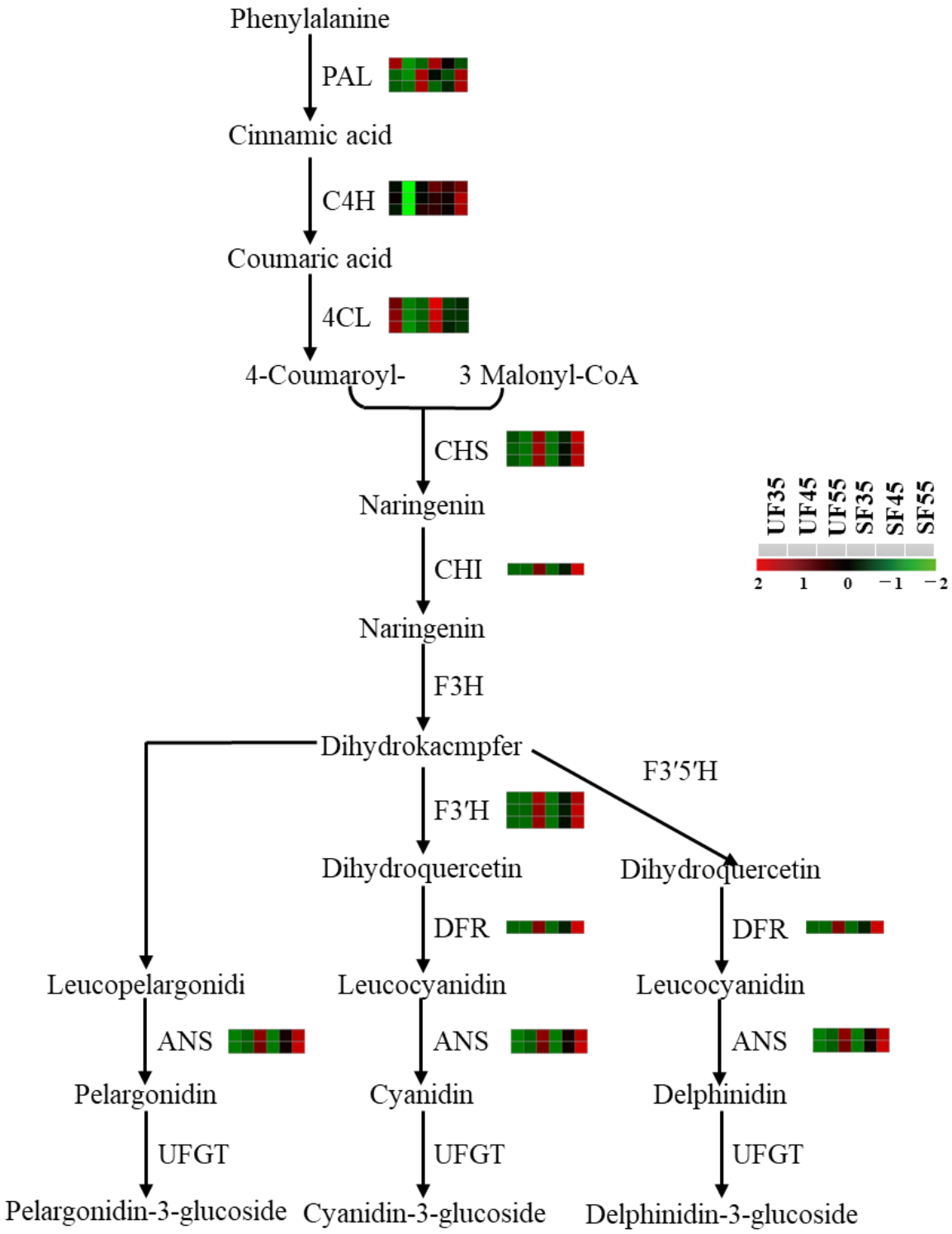

Figure 10

Biosynthetic pathway of anthocyanidins of cherry fruits under rain-shelter covering. Responsive progression of gene expression under sheltered conditions compared with that under unsheltered conditions, indicated in six-box strings (including UF35, 45, 55, SF35, 45, and 55). Heat maps were drawn using log2-transformed FPKM values.

Supplementary Files

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

- TableS4.docx
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- TableS1.docx
- TableS2.docx
- TableS3.docx
- FigureS1.docx