Vegetable Research

ARTICLE

Open Access

https://doi.org/10.48130/VR-2022-0021
Vegetable Research **2022**, 2:21

Integrated metabolome and transcriptome analysis revealed carotenoid metabolism difference in pepper (*Capsicum annuum* L.) yellowing mutants under different light quality

Zhoubin Liu[1#], Yunhua Dai[1#], Lianzhen Mao[1], Sha Yang[1], Bozhi Yang[1], Ying Sun[1], Yao Zhou[1], Shaoxiang Wang[2], Xuexiao Zou[1*], and Lijun Ou[1*]

[1] *Engineering Research Center of Education Ministry for Germplasm Innovation and Breeding New Varieties of Horticultural Crops, Key Laboratory of Vegetable Biology of Hunan Province, College of Horticulture, Hunan Agricultural University, Changsha 410128, Hunan, China*
[2] *Wenshan Academy of Agricultural Sciences, Wenshan 663000, Yunnan, China*
[#] These authors contributed equally: Zhoubin Liu, Yunhua Dai
[*] Corresponding authors, E-mail: zouxuexiao428@163.com; ou9572@hunau.edu.cn

Abstract

Light is an important environmental factor for plant growth and development, different light qualities have different regulatory effects on plants. To investigate the effect of light quality on plants, we determined the physiological characteristics, transcriptome and metabolome analysis of pepper yellowing mutants *yl1* treated with blue, red, green, and purple light. Results showed that the leaf of *yl1* was obviously yellowing, and the contents of chlorophyll, carotenoid and net photosynthetic rate in *yl1* were significantly decreased under purple light. A total of 31,853 genes were quantified under blue, red, green and purple light. The genes related to carotenoid metabolism pathway such as *PSY*, *LUT5* and *VDE* were significantly increased, while the expression levels of chlorophyll synthesis related genes *POR* and *CAO* were significantly decreased under purple light. At the same time, 21 carotenoid pathway metabolites were detected under the four light different lights, and 10 metabolites were more abundant in pepper leaves. α-carotene, β-carotene, lutein, neoxanthin, α-cryptoxanthin and β-cryptoxanthin were significantly accumulated under blue, red and green light. However, zeaxanthin and antheraxanthin were accumulated in large quantities under purple light. After silencing the *CaVDE* gene under purple light, leaf etiolation degree was significantly weakened, chlorophyll, carotenoids and net photosynthetic rate were significantly increased, and the accumulation of zeaxanthin and antheraxanthin was significantly decreased. These results provide a reference for analyzing the changes of carotenoid components induced by *VDE* in purple light and provide new insights into the mechanism of leaf color change in plants.

Citation: Liu Z, Dai Y, Mao L, Yang S, Yang B, et al. 2022. Integrated metabolome and transcriptome analysis revealed carotenoid metabolism difference in pepper (*Capsicum annuum* L.) yellowing mutants under different light quality. *Vegetable Research* 2:21 https://doi.org/10.48130/VR-2022-0021

INTRODUCTION

Plant leaf color mutation is a character with high frequency and easy identification. Leaf etiolation is widely found in many plants such as Arabidopsis[1], rice[2] and cucumber[3]. In recent years, leaf color mutants have been widely used in basic studies of genetic patterns, pigment synthesis, photosynthesis, cell structure and development, and have become one of the hot spots in molecular and developmental biology[4–6]. A large number of studies have divided leaf color mutants into total chlorophyll increased or decreased type, chlorophyll b deleted type and carotenoid increased or decreased type. In higher plants, most of the photosynthetic pigments are synthesized in the chloroplast. If the genes related to chloroplast development are mutated, the synthesis of photosynthetic pigments will be blocked, which will eventually lead to the change of leaf color. Therefore, a lot of research has focused on the influence of chloroplast and chlorophyll development metabolism on leaf color change.

Chlorophyll organisms begin with δ-aminolevulinic acid, which is processed by 15 enzymes such as aminolevulinic acid synthetase to form aminolevulinic acid, protoporphyrin ix and other metabolites, and finally converted into chlorophyll a and b[7]. Changes in the expression of any gene in this pathway will affect the accumulation of chlorophyll and reduce the photosynthetic capacity[8]. In rice, at least 50 related genes have been cloned and located through the study of a large number of leaf color mutants, some of which are directly involved in the metabolic pathways of photosynthetic pigments, such as *OsCHLH*, *OsCAO*, *YGL1* and *OsDVR*[6]. In other crops, such as the yellow leaf mutant of Populus deltoides W. Bartram ex Marshall, the photosynthetic pigment content was significantly reduced, and the expression of chlorophyllase (*CLH*) related to chlorophyll degradation was up-regulated[9]. Under low temperature conditions, the expression of *POR* in the kale leaf color mutant showed a significant down-regulation trend[10]. In the yellow cabbage mutant, the expression of *CHLG* gene encoding chlorophyll synthase was significantly reduced[4].

However, in addition to chlorophyll, photosynthetic pigments also include carotenoids. Although chlorophyll accounts for a higher proportion of photosynthetic pigments, the proportion of carotenoids still reaches about 20%[11]. In plants, carotenoids are also one of the factors that determine the color (yellow, orange, and red) of the plant[12]. Li et al.[13] found that

PPO and *NYC/NOL* inhibited the synthesis of chlorophyll in ginkgo biloba leaves, while *Z-ISO*, *ZDS* and *LCYE* promoted the accumulation of carotenoids, which confirmed that the change of leaf color was accompanied by the change of carotenoid content.

Light intensity and light quality play an important role in the regulation of plant carotenoid metabolism and are the most important signals for regulating the expression of carotenoid structural genes in plant photosynthetic tissues[14,15]. Light quality refers to the spectral composition of the light that plants receive, and each spectral band has different effects on plants. Plants sense light in different wavelength ranges such as red, blue, and green through various pigment systems in the cell, thereby regulating plant leaf elongation and stem elongation, photoprotection mechanisms, and assimilation and transport processes[16,17]. Studies have found that the difference in light quality not only affects the total chlorophyll content of plants[18,19], but also has different regulatory mechanisms on plant carotenoids[20]. The concentration of β-carotene and lutein in spinach (*Spinacia oleracea* L.) can be significantly increased by using blue LED light at a photosynthetic photon flux of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$[21]. The lutein content of purple cabbage (*Brassica oleracea* L. Winterbor) reaches the maximum at a wavelength of 640 nm, and the concentration of β-carotene reaches the maximum at 440 nm[22]. In the study of broccoli, short-time blue light could significantly increase the content of β-carotene and xanthophyll cycle pigments compared with red-blue mixed LED treatment[23]. The lutein and neoxanthin of broccoli sprouts under UV-A irradiation increased by 22.4% and 33.5%, respectively. While UV-B irradiation lutein and neoxanthin increased significantly by 16.9% and 36.7%[24]. However, the existing studies have paid less attention to carotenoids in leaf color mutants, and the research on effects of light quality differences on the changes of carotenoid components in leaf color mutants is even less.

Pepper (*Capsicum annuum* L.), belonging to the genus Capsicum in the Solanaceae family, is the largest seasoning crop and the third largest vegetable crop in the world. Although some studies have been carried out on the color of pepper[25,26] the study of carotenoid metabolism in pepper is still not in-depth, and mainly focuses on capsanthin or capsorubin. There are few studies on other substances in the carotenoid pathway, and the regulatory mechanism is still unclear. Based on the above reasons, this study intends to comprehensively study the dynamic changes of carotenoid components and gene expression in leaf yellowing mutants of pepper under different light quality by transcriptomics and metabolomics, and reveal the changes of key pigments and key regulatory genes related to leaf color transformation. This study will provide a reference for further understanding of the molecular mechanism of light quality in regulating carotenoid metabolism and inducing leaf color change.

RESULTS

Phenotypic identification and pigment content analysis

The leaves of the mutants under different light quality showed obvious differences, among which the leaves under purple light were significantly yellowing, the leaves under blue light were pale green, and the leaves under red and green light were still green (Fig. 1a). The contents of chlorophyll a, chlorophyll b, chlorophyll (a+b) and carotenoids were the highest in the red and green light treatments, and the contents of these photosynthetic pigments were the lowest in the purple light treatments, which decreased significantly by 64.41%, 40.75%, 57.95% and 58.12% compared with the blue light treatments, respectively (Fig. 1b). By measuring the color parameters of mutant leaves under different light quality, it was found that the red value (Δa), color luminance value (ΔL), yellow value (Δb) and total chromaticity value (ΔE) of leaves under purple light treatment were significantly higher than blue, red and green light treatment (Fig. 1c). And it was also found that the Pn and Fv/Fm of yellowed leaves under purple light were significantly lower than those under other light quality, the qP value was significantly higher than that under other light quality, and the NPQ value was significantly higher than that under red and green light (Fig. 1d).

Transcriptome sequencing analysis

A total of 77,910,471,234 raw reads were obtained from 12 pepper samples by Illumina-nova 6000 sequencing. After removing adaptor sequences, low-quality sequences and rRNA, a total of 261,581,124 clean high-quality reads were obtained. A total of 43,751 genes were identified after mapping to the *C. annuum* L_Zunla-1 Database[27], among which 31,853 genes were quantified, of which 27,085 genes (85.03%) were quantified in all samples (Fig. 2a, Supplemental Table S1). At the same time, it was also found that some genes were only quantified under certain light quality treatments. Among them, 666, 401, 369 and 228 genes were unique to purple, green, red and blue light treatment, respectively.

Analysis of differentially expressed genes (DEGs)

A total of 7,175 DEGs were identified by comparing gene expression between different light quality (Supplemental Table S2). Among these comparison groups, the R_*vs*_P group showed the largest difference in gene expression, with 5,403 DEGs, while there were only 713 DEGs in R_*vs*_G group (Fig. 2b). By comparing green leaves under blue, red and green light with yellowing leaves under purple light, 1,239 genes were found to be significantly different in all three groups (Fig. 2c). However, among the green leaves treated with blue, red and green light, only 58 genes had significant changes in expression between the comparison groups (Fig. 2d). This indicates that the differential expression of these 1,239 genes may be closely related to leaf etiolation.

Further analysis showed that genes involved in chlorophyll synthesis and metabolism were differentially expressed under different light quality. Among them, the expression levels of *POR* (Capana10g000065) under red and green light were at higher levels, which were 2.15 and 1.63-fold higher than those under purple light, respectively. The *CAO* (Capana06g001723) gene also maintained a high level of expression under red and green light, and the green light was 1.71-fold higher than the purple light treatment. The expression trend of *CHLG* (Capana09g001431) was different from that of the above genes, which significantly increased under purple light and was 1.45-fold higher than that under red light. The differential expression of these genes related to chlorophyll biosynthesis pathway under different light quality may be closely related to the changes of leaf color under different light quality.

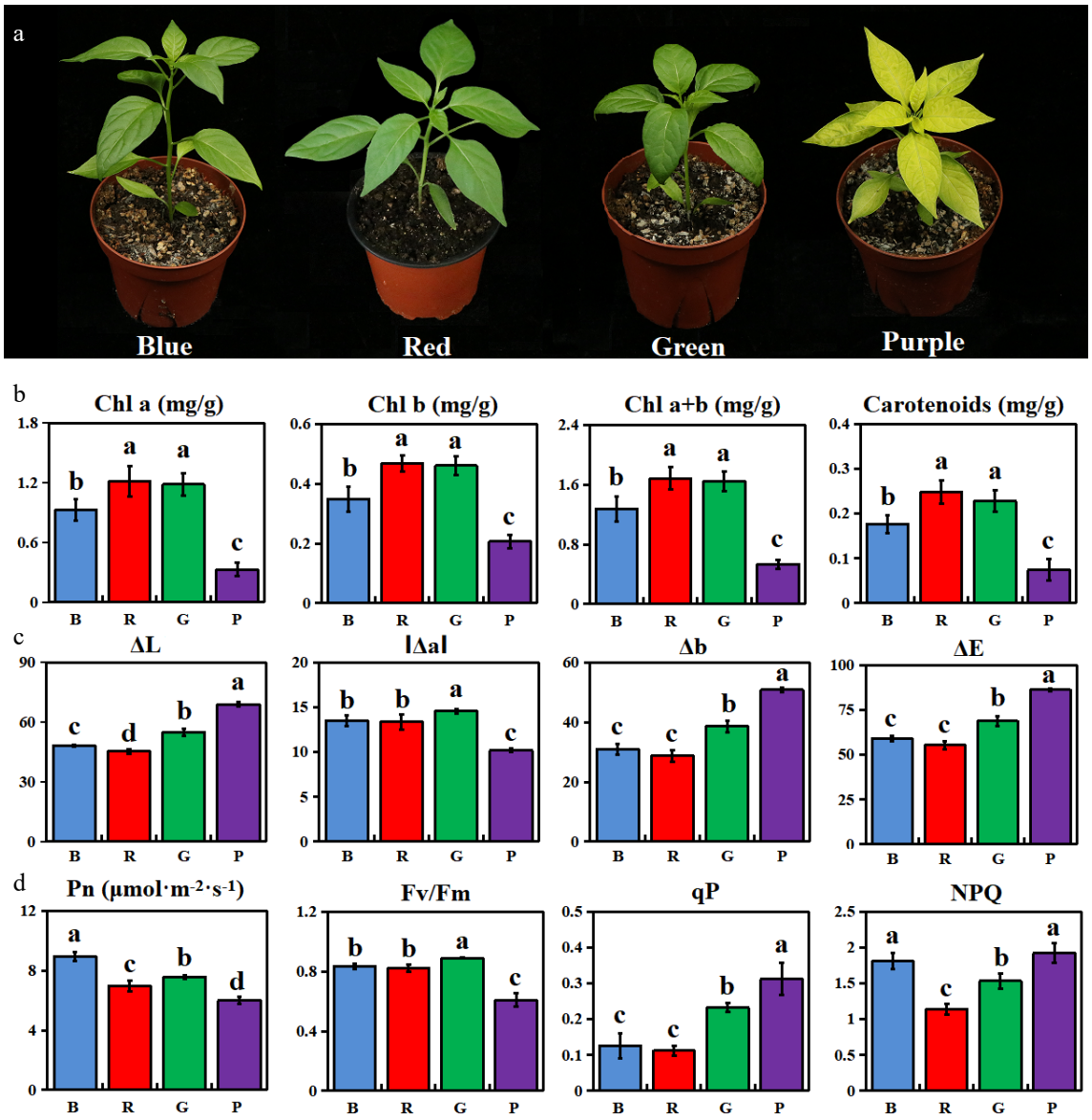

Fig. 1 Analysis of phenotypes and physiological characteristics of mutants under different light qualities. (a) Phenotypic identification of pepper leaves; (b) Photosynthetic pigment content; (c) Color chromaticity value; (d) Characteristics of photosynthesis and chlorophyll fluorescence.

Functional enrichment analysis of DEGs

The quantified DEGs were classified using the gene ontology (GO) database, and it was found that these DEGs cover a wide range of molecular function, biological process and cellular component, which can be classified into 21, 15 and 18 terms, respectively (Fig. 3a). The top 10 GO terms in each category were further analysed and results showed that catalytic activity and binding processes were predominant in the category of molecular function. In terms of biological process, the largest group is metabolic process, followed by cell process and single-organism process. Then the cellular components of these DEGs mainly enriched in membrane, membrane part, cell, cell part, and organelle. Among the light quality comparison groups, R_vs_P and G_vs_P had the most enriched DEGs in each GO terms, while R_vs_G had the least enriched DEGs.

KEGG pathway enrichment analysis was also performed on the top 20 DEGs in each comparison group (Fig. 3b). Results showed that DEGs were mainly significantly enriched in MAPK signaling pathway-plant, plant hormone signal transduction, carbon metabolism, biosynthesis of amino acids, phenylpropanoid biosynthesis starch and metabolism, in all light quality comparison groups. The number of DEGs enriched in R_*vs*_P was also the largest in all comparison groups, which DEGs enriched in MPAK signaling pathway-plant and plant hormone signal transduction were both 116 more than those in R_*vs*_G groups.

Gene expression patterns of the carotenoid pathway

Through the expression analysis of genes involved in the carotenoid metabolism pathway (Fig. 4a), it was found that there were significant differences in gene expression under different light quality. Among different light quality treatments, the expression levels of *PSY* (Capana04g002519) and *LUT5* (Capana12g001743) under purple light were the highest, which were significantly higher than other lights. *CrtISO* (Capana 00g004805, Capana11g002179), *LCYB* (Capana05g000023),

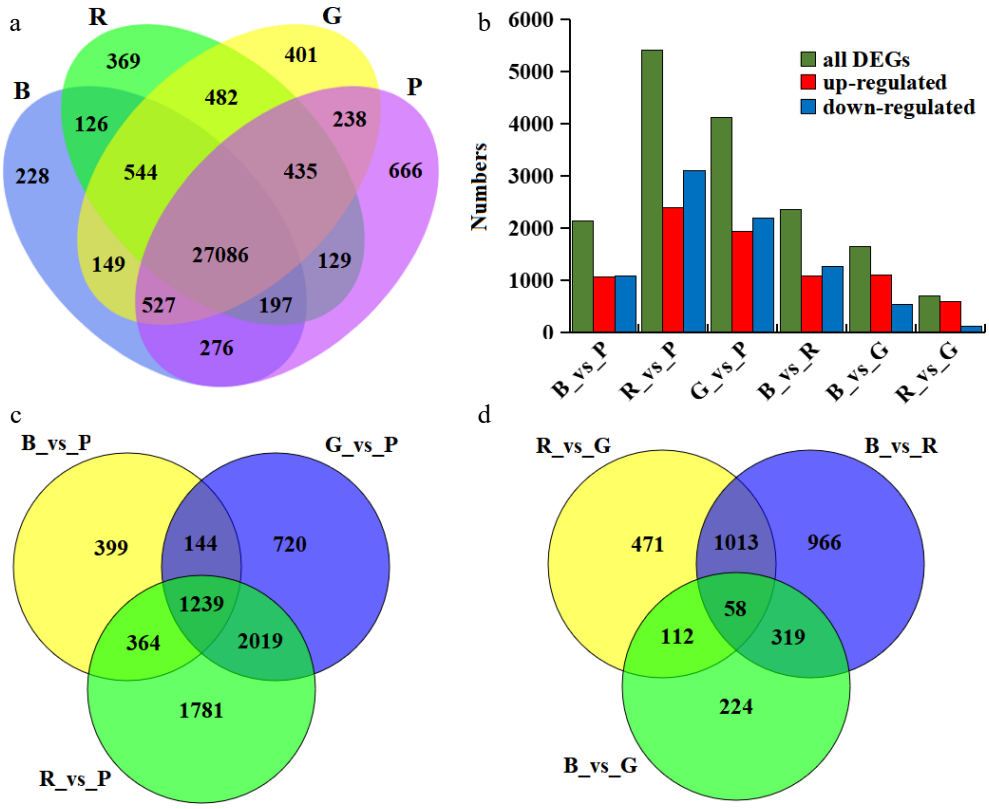

Fig. 2 Statistical analysis of the genes identified by transcriptome analysis. (a) Quantified genes under different light qualities; (b) Differentially expressed genes under different light qualities; (c) Venn diagram of differentially expressed genes between purple light compared to red, blue, and green light; (d) Venn diagram of differential genes between red, blue, and green light.

LUT1 (Capana10g001912), *PDS* (Capana03g000054), *VDE* (Capana12g001449) and *Z-ISO* (Capana12g000229) gene expression levels were significantly higher than those of red and green light, while slightly higher than those of blue light. The expression trends of *CrtZ-2* (Capana06g002492) and *ZEP* (Capana02g003105) were different from those of the above genes, among which *CrtZ-2* was highly expressed under red and green light, and significantly decreased under purple light. While *ZEP* was the highest under blue light, it was significantly higher than that of red and green light, but had no significant difference with purple light. Pearson correlation matrix analysis showed that all genes were positively correlated with each other except *LCYE* (Capana00g002014) and *CRTZ-2* (Capana 06g002492), which were negatively correlated with other genes in the carotenoid pathway.

Content of metabolites in the carotenoid pathway

In order to study the pattern of carotenoid accumulation in leaves, the distribution of carotenoid metabolites under different light quality treatments was analyzed by liquid chromatography-mass spectrometry/mass spectrometry. A total of 21 carotenoids were detected from the four light quality treatments (Fig. 4c, Supplemental Table S3). Ten carotenoids including Phytoene, α-Carotene, β-Carotene, Antheraxanthin, Lutein, Neoxanthin, Violaxanthin, Zeaxanthin, β-Cryptoxanthin and α-Cryptoxanthin were abundant in pepper leaves, and the contents of these carotenoid metabolites were significantly different under different light quality treatments. Among them, α-Carotene, β-Carotene, Lutein, Neoxanthin, α-cryptoxanthin and β-Cryptoxanthin were accumulated in a large amount under blue, red and green light, but were less accumulated in purple light. However, zeaxanthin and antheraxanthin, two important metabolites of the xanthophyll cycle, accumulated in large quantities under purple light, and their contents were lower under blue, red and green light, resulting in their significantly higher contents under purple light than under blue, red and green light. In addition, pearson correlation coefficient matrix analysis showed that zeaxanthin and antheraxanthin were mainly negatively correlated with other metabolites in different degrees (Fig. 4d).

Real Time PCR validation

qRT-PCR experiments were performed on carotenoid pathway genes including *PSY, PDS, Z-ISO, CrtISO, VDE, LCYE, CrtZ-2, LUT5, ZEP, LCYB, LUT1* and *CAO, CHLG, POR* genes related to chlorophyll metabolism using gene-specific primers. By comparing the transcript abundance (FPKM) obtained by transcriptome sequencing, it was found that the qRT-PCR results of related genes under different light were basically consistent with the variation trend of FPKM values, indicating that the transcriptome sequencing data was reliable in this study (Fig. 4e).

Phenotypic identification and expression analysis of *CaVDE* silenced lines

Since the mutant leaves only turn yellow under purple light, we found that the expression level of *VDE* gene in leaves under purple light was significantly increased by RNA-Seq and qRT-PCR. Meanwhile, the contents of zeaxanthin and antheraxanthin were significantly increased under purple light. Therefore, we speculated that the increased expression of VDE, a key gene

a

GO terms	B_vs_P	R_vs_P	G_vs_P	B_vs_R	B_vs_G	R_vs_G	
catalytic activity	830	2048	1544	987	676	290	Molecular Function
binding	775	2008	1544	827	557	285	
transporter activity	100	223	167	113	69	37	
nucleic acid binding transcription factor activity	41	101	86	42	34	16	
molecular function regulator	30	62	44	35	23	13	
electron carrier activity	29	57	44	22	16	10	
structural molecule activity	24	58	44	11	9	2	
antioxidant activity	21	32	27	17	14	5	
signal transducer activity	13	21	12	10	7	3	
molecular transducer activity	12	20	16	9	7	1	
metabolic process	736	1891	1455	850	604	231	Biological Process
cellular process	575	1620	1223	639	475	188	
single-organism process	491	1158	866	559	391	188	
biological regulation	210	517	415	215	174	73	
response to stimulus	170	434	319	195	143	72	
localization	154	336	247	161	102	51	
cellular component organization or biogenesis	90	261	191	89	79	42	
signaling	49	117	93	54	45	15	
multicellular organismal process	47	134	101	49	40	19	
developmental process	41	112	82	43	36	12	
membrane	494	1288	966	640	413	167	Cellular Component
membrane part	443	1139	864	567	370	149	
cell	395	1126	839	386	320	153	
cell part	395	1126	839	386	320	153	
organelle	319	926	696	296	241	132	
organelle part	116	369	246	109	100	59	
macromolecular complex	93	255	166	53	65	44	
extracellular region	42	77	66	55	41	16	
other organism	14	37	15	7	9	20	
other organism part	14	37	15	7	9	20	

b

KEGG-pathway	B_vs_P	R_vs_P	G_vs_P	B_vs_R	B_vs_G	R_vs_G
MAPK signaling pathway - plant	56	32	72	142	101	26
Plant hormone signal transduction	57	61	81	134	111	18
Carbon metabolism	24	27	56	92	61	7
Biosynthesis of amino acids	25	16	30	71	51	7
Phenylpropanoid biosynthesis	47	32	37	68	57	17
Starch and sucrose metabolism	32	36	35	67	56	8
Amino sugar and nucleotide sugar metabolism	21	16	22	62	46	9
Pentose and glucuronate interconversions	20	23	29	60	42	8
Ribosome	23	3	5	57	41	1
Glycolysis / Gluconeogenesis	15	14	30	48	29	5
Glyoxylate and dicarboxylate metabolism	16	12	22	47	35	5
Photosynthesis	18	7	7	46	33	1
Endocytosis	12	12	18	43	30	6
Glycerophospholipid metabolism	20	12	15	41	31	2
Protein processing in endoplasmic reticulum	20	16	22	40	40	2
Galactose metabolism	23	13	17	40	27	7
Cysteine and methionine metabolism	20	11	25	39	25	7
ABC transporters	23	10	18	38	19	7
Spliceosome	13	9	15	37	32	5
Glutathione metabolism	9	8	15	37	19	6

Fig. 3 Functional enrichment analysis of DEGs. (a) GO enrichment analysis; (b) KEGG enrichment analysis.

in the lutein cycling pathway, may lead to the accumulation of zeaxanthin and antheraxanthin and eventually lead to leaf yellowing. To verify this hypothesis, we constructed a silenced expression vector using the specific fragment of *CaVDE* gene, and introduced the vector into leaf color mutants and incubated it under purple light environment. The results showed that the leaves of the empty vector control (EV) line turned yellowing under purple light, while the leaves of the pTRV2-*CaVDE* silencing line turned green, and the degree of yellowing was obviously weakened (Fig. 5a). Further analysis showed that the contents of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids in *CaVDE*-silenced lines were significantly higher than those in EV line (Fig. 5b). Color parameters analysis also showed that the color luminance value (ΔL), yellow value

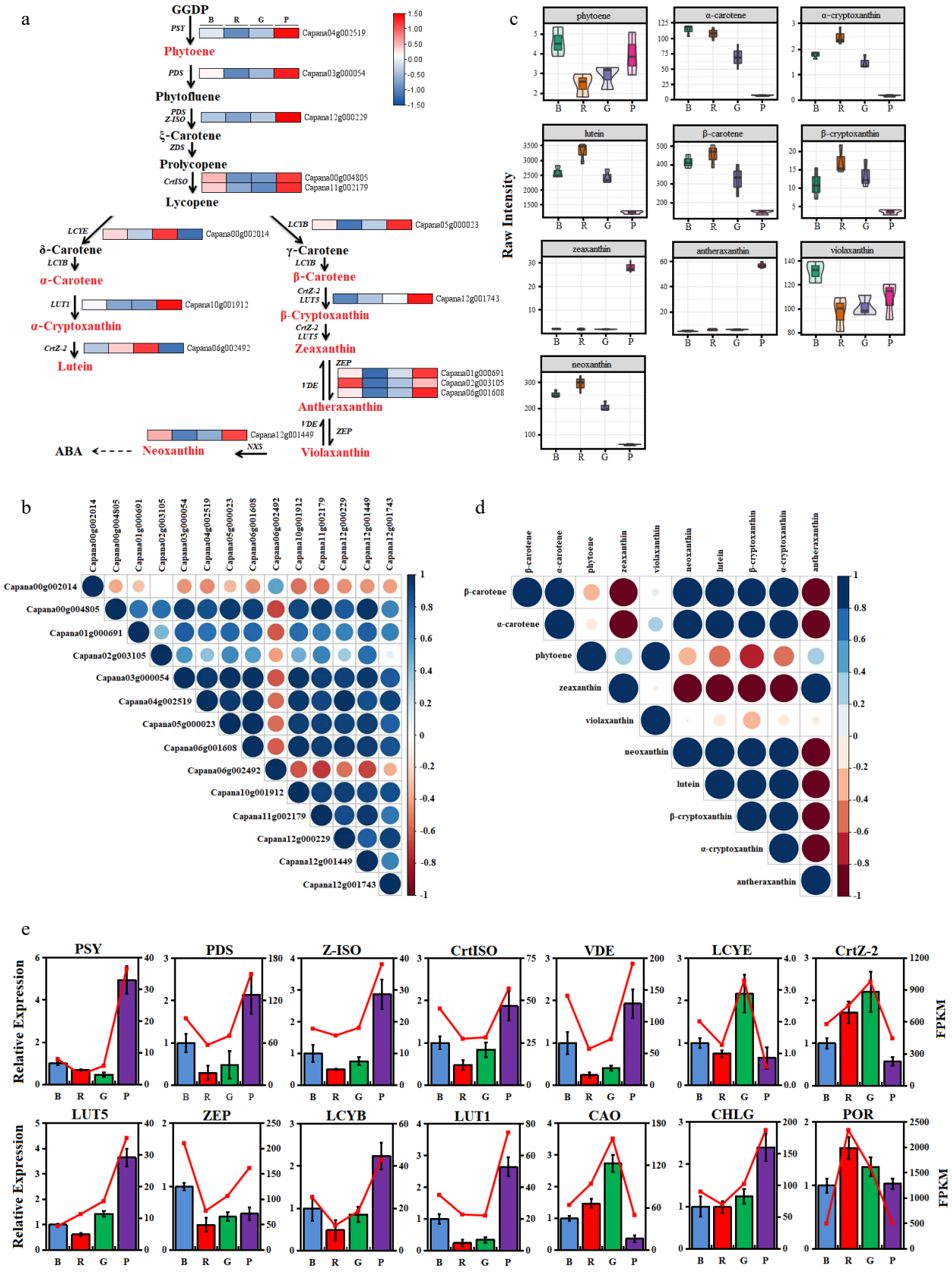

Fig. 4 Expression of carotenoid pathway genes and metabolites. (a) Analysis of gene expression; (b) Gene correlation analysis; (c) Analysis of metabolites synthesis; (d) Metabolite correlation analysis; (e) Quantitative verification of carotenoid pathway genes and chlorophyll metabolism related genes.

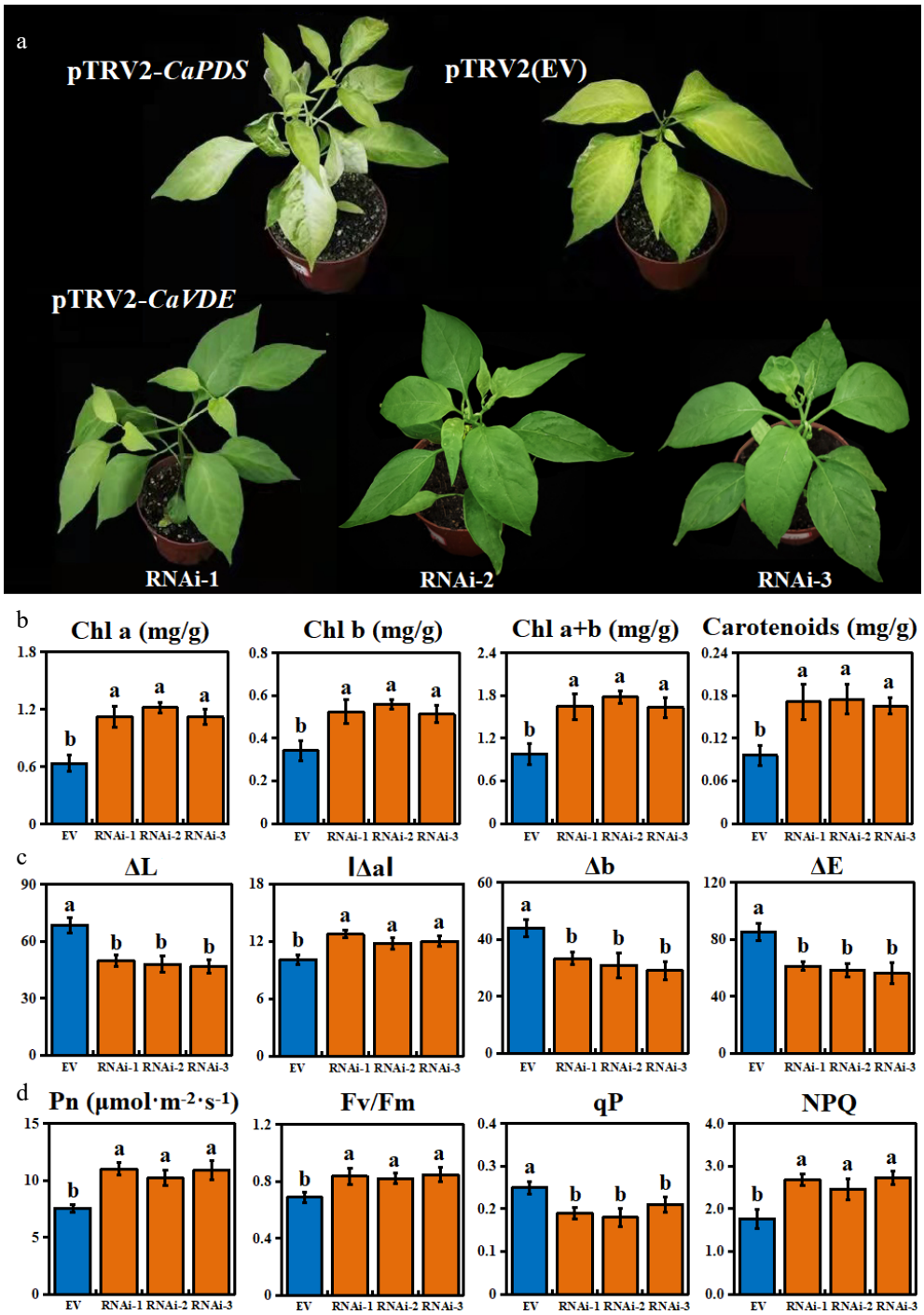

Fig. 5 Phenotype and physiological characteristics of *CaVDE* silenced lines. (a) Phenotypic identification of *CaVDE* silenced and EV line; (b) Photosynthetic pigment content of *CaVDE* silenced and EV line; (c) Color chromaticity value of *CaVDE* silenced and EV line; (d) Characteristics of photosynthesis and chlorophyll fluorescence of *CaVDE* silenced and EV line.

(Δb) and total chromaticity value (ΔE) of *CaVDE*-silenced lines were significantly lower than those of the EV line (Fig. 5c). These results indicated that silencing *CaVDE* gene could weaken leaf etiolation and deepen green color of mutant seedlings under purple light. At the same time, the Pn, Fv/Fm and NPQ values of the silent lines were found significantly higher than those of the EV line, and the qP values were significantly lower, which indicated that the photosynthetic characteristics of the *CaVDE*-silenced lines restored to green were enhanced (Fig. 5d).

Changes of carotenoid pathway gene expressions and contents in *CaVDE* silenced lines

In order to confirm the changes in the content of carotenoid metabolites in leaf yellowing, we further measured the content of carotenoid metabolites in empty vector control and *CaVDE*-silenced lines (Fig. 6a). The results showed that α-Carotene, α-

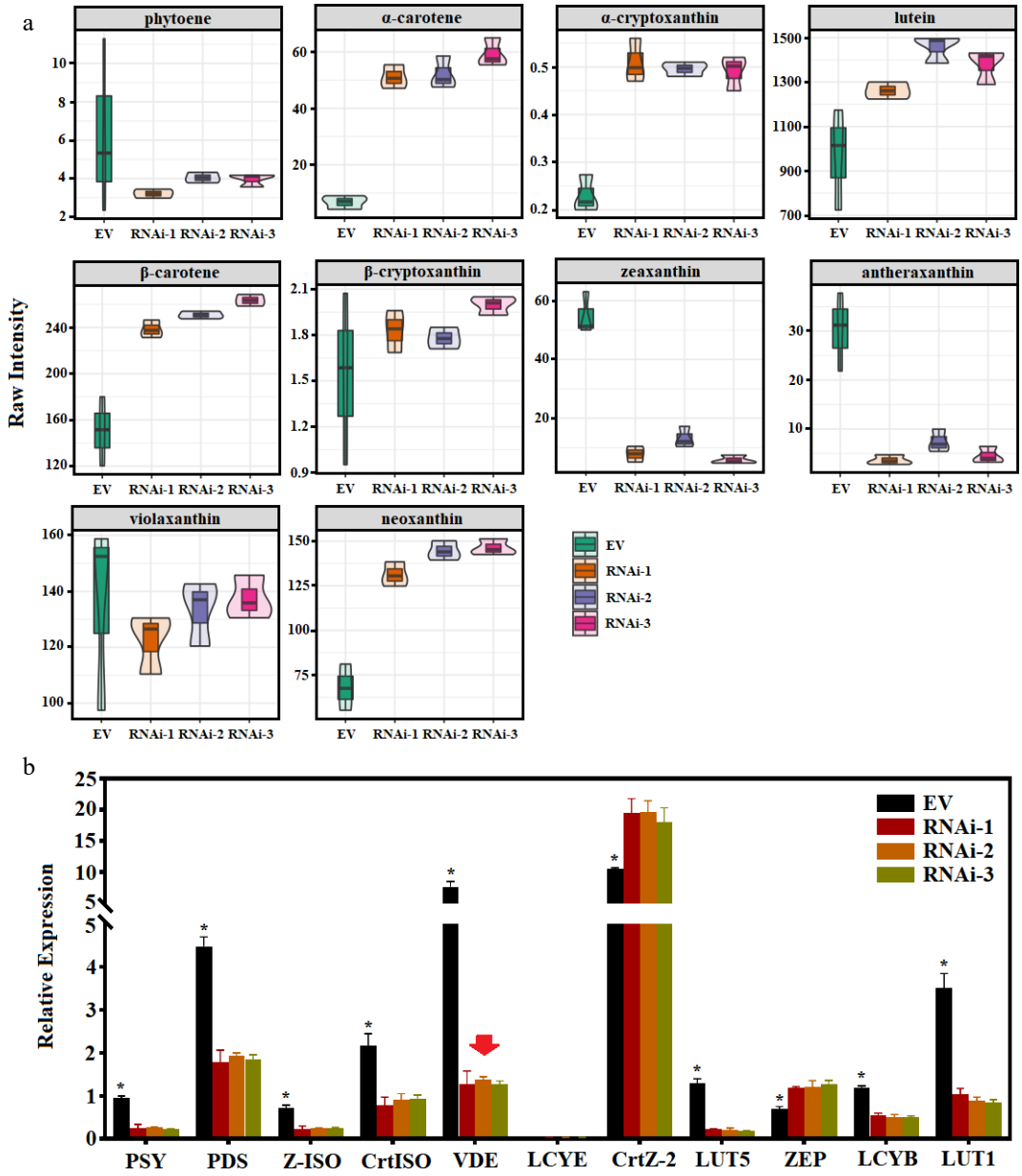

Fig. 6 Carotenoid content and gene expression of *CaVDE* silenced lines. (a) Volin plot of raw values of *CaVDE* silenced and EV line; (b) Quantitative verification of carotenoid pathway genes of *CaVDE* silenced and EV line.

cryptoxanthin, Lutein, *β*-Carotene and Neoxanthin of *CaVDE*-silenced lines were significantly increased by 689.52%, 116.58%, 41.98%, 63.56% and 101.97% compared with empty vector control line, respectively. However, the accumulation of zeaxanthin and antheraxanthin was less, which decreased significantly by 83.61% and 79.42% compared with empty vector control lines. In addition, we also performed qRT-PCR analysis of carotenoid pathway genes in pTRV2 and PTRV2-*CavDE* lines(Fig. 6b). The results showed that the expression levels of *PSY*, *PDS*, *Z-ISO*, *CrtISO*, *LUT5*, *LCYB*, *LUT1* genes were significantly down-regulated, while the expression levels of *CrtZ-2* and *ZEP* genes were significantly up-regulated in *CaVDE*-silenced lines. This indicates that the silencing of *VDE* in pTRV2-*CaVDE* lines inhibits the process of catalyzing the production of zeaxanthin and antheraxanthin from violaxanthin in the xanthophyll cycle, resulting in a decrease in the accumulation of zeaxanthin and antheraxanthin, and further promoting the greening of leaves.

DISCUSSION

The photosynthetic pigments of higher plants are mainly composed of chlorophyll and carotenoids. Leaf etiolation is mainly manifested in the inhibition of chlorophyll and carotenoid biosynthesis, resulting in the reduction of chlorophyll and carotenoid content, and finally leading to the generation of etiolated varieties[28]. Light quality plays an important role in regulating the content of photosynthetic pigments in plants[29], and different light qualities have different biological effects. Plants perceive different light qualities through photoreceptors, thus causing changes in photosynthetic pigments. Studies have found that the chlorophyll content of eggplant[30] and cucumber[31] plants under red light was significantly higher

than that under blue light. Other studies have shown that green light could promote chlorophyll production by *Chlorella vulgaris*[32]. In plants, *POR* is a key enzyme in chlorophyll synthesis. It depends on light and catalyzes the conversion of DV-Pchlide to DV-Chlide a or the conversion of Pchlide a to Chlide a[33]. Chlorophyll A oxygenase (*CAO*) could convert Chlide a into Chlide b[34]. Chlorophyll synthetase (*CHLG*) can catalyze the synthesis of chlorophyll A and B in higher plants, completing the final step of chlorophyll synthesis[35]. In this study, *POR* (Capana10g000065) and *CAO* (Capana06g001723) were highly expressed under red and green light, while their expression levels were significantly decreased under blue and purple light, especially purple light. The high expression of *POR* and *CAO* genes further led to the synthesis of chlorophyll under the red and green light treatments. At the same time, although the expression of *CHLG* (Capana09g001431) was significantly increased under purple light, the low expression of *POR* and *CAO* under purple light resulted in less accumulation of chlorophyll synthesis precursor products, which ultimately resulted in the lowest chlorophyll content under purple light.

As part of the photosystem, carotenoids are essential for their assembly, light harvesting and light protection. The most important factor affecting the change of carotenoid content is light quality and light intensity, in which different wavelengths of light affect the composition and structure of the photosystem, thus providing the possibility to influence the concentration and composition of carotenoids in the photosystem by specific narrowband spectral illumination[36,37]. However, the effect of different light qualities on carotenoid concentrations appears to be species dependent. The study found that blue light can increase the concentration of carotenoids in lettuce[18,38] and spinach[39], and red light supplementation can promote the accumulation of carotenoids in tomatoes[40,41], while komatsuna has the highest carotenoid content under white fluorescent light[39]. In this study, the highest carotenoid concentration was observed under red and green light treatment, while the lowest was observed under purple light treatment. This suggests that light quality has an important effect on carotenoid concentration in leaf yellowing mutants. At the same time, previous studies found that carotenoid content of Sinapis alba, Arabidopsis thaliana and tobacco increased during photoinduced de-etiolation[42,43]. However, in this study, the leaf color of the mutant was significantly yellowed under purple light, indicating that the etiolation process was not only accompanied by the decrease of chlorophyll content, but also ccompanied with carotenoid content decreased.

The change of light quality not only affects the concentration of carotenoids, but also the composition of carotenoids. Previous studies have shown that changes in light quality can affect the contents and proportions of β-cryptoxanthin, zeaxanthin, β-carotene and lutein in citrus[44], Tartary buckwheat[45], and tomato[46]. In this study, contents of lutein, neoxanthin, α-cryptoxanthin, β-cryptoxanthin and β-carotene were higher under red light than those with other light conditions. The content of above metabolites were very low under purple light treatment, while zeaxanthin and antheraxanthin had a large amount of accumulation and were significantly higher than other light quality treatments. We believe that the difference in carotenoid component content may be caused by the different expression of genes related to carotenoid metabolism pathway induced by different light quality treatments.

Carotenoid biosynthesis in plants is mainly regulated by a series of enzymes or genes. *PSY* is considered to be the rate-limiting enzyme in the carotenoid synthesis pathway, its expression level and activity can affect the metabolic flux of the synthetic pathway[47]. The overexpression of *PSY* in some plants can significantly promote the accumulation of phytoene in leaves and greatly increase the synthesis of β-carotene[48,49]. It has been found that a large number of genes are activated during plant etiolation[50], among which the transcript abundance of genes controlling metabolic fluxes (such as *PSY* and *DXS*) is greatly enhanced[47,51]. In this study, the expression of *PSY* (Capana04g002519) gene under purple light was significantly increased compared with other light quality, and the phenomenon of leaf etiolation was also observed, suggesting that the increase of *PSY* gene expression promoted leaf etiolation. However, it has also been found that *PSY* transcripts are similarly increased during de-etiolation in Sinapis alba, Arabidopsis thaliana and tobacco seedlings[42,43]. These results indicated that leaf etiolation was not only determined by *PSY* expression level, but also regulated by downstream related genes.

There are many carotenoid synthetases, which can form multi-enzyme complexes to play a role and accelerate the metabolic intensity of carotenoid biosynthesis[52]. At present, there are four putative models of carotenoid metabolic enzyme complexes, including *PDS*, *Z-ISO*, *ZDS*, *CrtISO* and *LCYB* complexes, which are capable of synthesizing β-carotene from phytoene[53]. In this study, the expression of *PDS (*Capana03g 000054), *CRTISO* (Capana00g00480、Capana11g002179) and *Z-ISO* (Capana12g000229) under purple light were significantly higher than those under blue, red and green light, which was conducive to the massive synthesis of lycopene under purple light. However, the content of related metabolites was not identified, which may be that the intermediate lycopene was further catalyzed by the downstream enzymes to synthesize other substances. *LCYB* and *LCYE* are important nodes that control the diversion of lycopene to downstream branches, its relative expression levels determine the contents of β-carotene and α-carotene in the downstream of the branches, and the proportion of zeaxanthin, violaxanthin, lutein, neoxanthin, etc, in the total carotenoids[54–56]. In this study, *LCYB* (Capana05g 000023) gene was highly expressed under purple light, while the expression trend of *LYCE* (Capana00g002014) was opposite to that of *LCYB*. This made a large number of upstream metabolites that were involved in β-carotene metabolic branch and less metabolite that was involved in α-carotene metabolic branch under purple light. While higher amounts of upstream metabolites were involved in α-carotene metabolic branch rather than β-carotene metabolic branch under red and green light. Meanwhile, higher expression of *CRTZ-2* (Capana06g 002492) under blue, red and green light resulted in further catalytic production of lutein by metabolites that are involved in the α-carotene metabolic branch. The low expression of *LUT5* (Capana12g001743) results in metabolites involved in the β-carotene metabolic branch that cannot be further catalyzed in large quantities, resulting in accumulation of β-carotene and β-crpytoxanthin. However, the higher expression of *LUT5* under purple light made β-carotene and β-crpytoxanthin could be further catalyzed to synthesize downstream metabolites, which significantly reduced the accumulation of β-carotene and β-

crpytoxanthin.

The Xanthophyll cycle is located downstream of the carotenoid pathway[50] and is considered to be the main process of plant defense against stress damage such as strong light[55]. In chloroplasts, zeaxanthin epoxidase (*ZEP*) and violaxanthin de-epoxidase (*VDE*)[57,58] are key enzymes in the regulation of the Xanthophyll cycle[50], which catalyze the interconversion of antherxanthin, violaxanthin and zeaxanthin components. Light intensity and redox status of chloroplasts can regulate the composition of xanthophyll[59]. When light energy is excessive in plants, a low pH environment will be formed in chloroplasts, which promotes the activation of *VDE*, thereby producing zeaxanthin[53]. Zeaxanthin can dissipate the excess light energy in the light-trapping complex (LHC), which can protect the photosynthetic organs from bright light damage[60]. However, when light energy is no longer excess, the direction of transformation is reversed, and zeaxanthin is regenerated into violaxanthin through antheraxanthin under the catalysis of *ZEP*[61]. In potato tubers, reduced expression of the *ZEP* gene promotes a large accumulation of zeaxanthin, resulting in an orange phenotype[62]. In this study, the expression of *ZEP* (Capana02g003105) and *VDE* (Capana12g001449) was at a high level under both blue and purple light, but the antheraxanthin and zeaxanthin accumulated a lot under purple light, but less accumulated under blue light. This is because the expression of *VDE* under purple light treatment was 36.01% higher than that under blue light, while the expression of *ZEP* was 23.24% lower than that under blue light. Due to such expression differences, the process of *VDE* catalyzed deepoxidation of violaxanthin to form antheraxanthin and zeaxanthin under purple light was enhanced, while the catalytic process of zeaxanthin to violaxanthin under the catalysis of *ZEP* is weakened. As a result, the blue light treatment catalyzed the synthesis of more violaxanthin, while the purple light treatment catalyzed the synthesis of antheraxanthin and zeaxanthin in large quantities, thus making the leaves under purple light treatment yellow.

CONCLUSIONS

In this study, a total of 31,853 genes were quantified and 1,239 genes were differentially expressed in blue, red, green and purple light. The genes related to carotenoid metabolism pathway such as *PSY*, *LUT5* and *VDE* were significantly increased, while the expression levels of chlorophyll synthesis related genes *POR* and *CAO* were significantly decreased under purple lught. At the same time, 10 kinds of metabolites were detected more abundant in pepper leaves. Among them, zeaxanthin and antheraxanthin were accumulated in large quantities under purple light. After silencing *CaVDE* gene under purple light, leaf etiolation degree was significantly weakened, chlorophyll, carotenoids and net photosynthetic rate were significantly increased, and the accumulation of zeaxanthin and antheraxanthin was significantly decreased.

MATERIAL AND METHODS

Plant growth and sampling

Pepper leaf yellowing mutant material *yl1* was provided by the College of Horticulture, Hunan Agricultural University. Seeds were sown in 50 hole trays with the nutrient substrate (organic matter ≥ 20%) and grown in a greenhouse (the photoperiod was set to 16/8 h day/night, the temperature was 30 ± 2 °C / 20 ± 2 °C). After growing to two-leaf stage, the seedlings were transplanted to 6 cm × 10 cm pots and then placed under four different light qualities of blue (B), red (R), green (G) and purple light (P) for 15 d. The light intensity was set to 60 ± 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Supplemental Fig. S1), the photoperiod was set to 16/8 h day/night, the temperature was 28/20 °C, and the humidity was controlled at 65% ± 5%. Fifteen seedlings were planted in each light treatment and each treatment was repeated three times. After 15 d of normal fertilizer and water management of each light treatment, the color index, photosynthetic rate and chlorophyll fluorescence parameters of seedlings were measured at 10:00 to 11:00 in the morning. Five pepper seedlings with the same growth were selected from each repetition, and the 3rd to 8th true leaves (six leaves in total) from top to bottom of the seedlings were sampled, mixed and divided into three parts, and quickly frozen into liquid nitrogen and stored at −80 °C for physiological measurement, transcriptome, and metabolome analysis.

Determination of leaf color index, photosynthesis and chlorophyll fluorescence parameters

The ΔL, Δa and Δb values of pepper leaves under different light quality treatments were measured using a spectrophotometer (Ts7600, Shenzhen 3nh Technology Co., Ltd., Shenzhen, China). At 9:00−11:00 in the daytime, the photosynthetic parameters of pepper seedling functional leaves (3rd to 4th leaves from the top) were measured by LI-6400 photosynthetic apparatus. The photosynthetic apparatus was set as red and blue light source, the light intensity was 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and the CO_2 concentration was 400 $\mu mol\ L^{-1}$. After the photosynthetic parameters were determined, the functional leaves of pepper under various light quality treatments were fully dark adapted, and the chlorophyll fluorescence parameters were determined by Fluorpen (FP110/D) handheld chlorophyll fluorescence instrument. The maximum photochemical efficiency Fv/Fm, photochemical quenching coefficient qP and non-photochemical quenching coefficient NPQ of light system II were calculated according to the method of Maxwell & Johnson[63]. Three plants were selected for each treatment and repeated three times.

Determination of chlorophyll content

Referring to the method reported by Arnon[64], the materials were extracted with 80% acetone and then whitened. The absorbance values at wavelengths of A663, A645 and A470 were measured by multi-functional microplate reader (TECAN/SPARK), and the contents of chlorophyll a, chlorophyll b and carotenoids were calculated.

Construction of gene fragment and VIGS system

The RNA of pepper leaves was extracted by the TRIzol[65] method, and the extracted RNA was reverse transcribed into cDNA using Vazyme reverse transcriptase kit (Jiangsu, China). The NCBI database (www.NCBI.nlm.nih.gov) was searched for the full-length CDS sequence of VDE. The most effective silent area on site https://vigs.solgenomics.net/. was found according to the principle of primer specific design, the primers for silencing *VDE* gene sequence (F: CGCTTGTCCCTCAGTCAAAC, R: TCTTCCGCACCATCAAAGCT) were designed by Primer 5.0 software. The target fragment was obtained by PCR, and the selected endonuclease EcoRI and ExnaseII, recombine the purified product of the target fragment with TRV2 vector, and

transfer the recombinant product into *E. coli* DH5 α. Monoclonal cells were selected for PCR identification and sequencing verification. Finally, the right monoclonal solution was selected to extract the plasmid, and then transferred into Agrobacterium tumefaciens GV3101. After PCR identification of Agrobacterium tumefaciens, the infection solution was prepared for use in the infection of pepper leaves by Agrobacterium tumefaciens.

Method for detecting efficiency of gene silencing plants

Three weeks after the injection of Agrobacterium tumefaciens into pepper plants, the leaves of some plants inoculated with TRV1-PDS showed albinism, and the inoculation was confirmed to be successful. The TRV1-*PDS* plants, TRV1-*TRV2* plants and TRV1-*VDE* silenced plants were placed under purple light (light intensity 60 ± 5 μmol·m^{-2}·s^{-1}, photoperiod 16/8 h day/night, temperature 28/20 °C, humidity 65% ± 5%), 15 d after treatment. The color value, photosynthetic rate and chlorophyll fluorescence parameters were measured. At the same time, the leaves of TRV1-*TRV2* no-load plants and TRV1-*VDE* silencing plants were collected and stored at −80 °C for qPCR determination. The primers were listed in Supplemental Table S4. The relative expression levels were measured and calculated by Roche Lightcycler® 96 instrument.

RNA Extraction, library preparation and sequencing

According to the manufacturer's instructions, TRI reagent (Sigma Life Science, USA) was used to extract total RNA instructions from tissues. RNA was examined by non-ribonuclease agarose gel electrophoresis to avoid possible degradation and pollution, and then verified by Agilent 2100 biological analyzer (Agilent Technology Company, Santa Clara, California, USA). Next, oligo-dT beads (Qiagen, Germany) were used to isolate Poly (A) mRNA, and then the mRNA was randomly disrupted into 200–300 bp short segments by adding Fragmentation Buffer. The first cDNA chain was synthesized by using mRNA as the template with random hexamers, and then the second cDNA chain was synthesized by adding buffer, dNTPs, RNaseH and DNA polymerase I. The cDNA was purified by AMPure XP beads. After purification, the double-stranded cDNA was subjected to terminal repair. A tail was added, and the sequencing joint was ligated, and then AMPure XP beads were used for fragment size selection. Finally, cDNA library was obtained by PCR enrichment. Then Illumina sequencing platform (Illumina-nova6000) using paired-end sequencing technology. Each strain was subjected to three biological replicates, and finally 12 DGE libraries were generated and sequenced.

Transcriptome analysis

The original image data generated by the sequencer is converted into a sequence, which is defined as 'original read'. Low-quality sequences were removed (more than 50% of the base mass of a sequence is less than 20, 5% of the unknown base (N) readings and reads containing adapter sequences) to obtain high-quality clean data. All downstream analysis in this article is based on high-quality clean data[66].

TopHat2 were used to map clean readings to the pepper reference genome, allowing up to one mismatch. All successfully mapped transcripts were identified and analyzed using R packaging edgeR, and the expression levels of each gene were calculated and normalized to FPKM. Log_2fold change (log_2FC) value > 2 (up-regulated) or < −2 (down-regulated). FDR was used to determine the threshold of P value in multiple experiments. The transcripts with FDR < 0.05 were considered to be meaningful and used as the significant indigenous boundary for gene expression differences. Differential genes were used for GO and KEGG enrichment analysis. GO term with P-value < 0.05 and KEGG pathway with P-value < 0.05 were considered as significant enrichment of differentially expressed genes.

Metabolite extraction

The pepper leaves were freeze-dried and ground into powder on a grinding machine, then the tissue samples (50 mg) were weighed and dissolved in 500 μL extract (50% methanol aqueous solution containing 0.1% hydrochloric acid). The samples were subjected to the following procedures: vortex 5 min, ultrasonic 5 min, centrifuge 3 min (12,000 r/min, 4 °C), extract the supernatant, repeat the operation once; the supernatant was combined twice, and the samples were filtered from the microporous membrane (0.22 μm pore size) and collected into a bottle for LC-MS/MS analysis. Supernatant mixtures of biological samples of the same volume were used as quality control (QC) samples to evaluate the stability of the system. In addition, the blank control was established to run with QC samples to remove background pollution[67] .

HPLC-MS/MS analysis

ExionLC was used for LC-MS/MS analysis of the AD system (SCIEX) of TM New Gene Co., Ltd. (Beijing, China) coupled with QTRAP® 6500+mass spectrometer (SCIEX). The samples were subjected to chromatographic column (ACQUITY BEH C18 1.7 μm, 2.1 mm × 100 nm), flow rate 0.35 mL·min^{-1}, column temperature 40 °C, and positive/negative polarity mode. The mobile phase was divided into phase A and phase B, with liquid phase A containing ultrapure water (0.1% formic acid) and liquid phase B containing methanol (0.1% formic acid). Solvent gradient program is set as follows: 0.00 min, 5% B; 50% B, 6.00 min; 95% B, 12.0 min; 2 min, 5% B, 14; 2% B, balance 2 min. Mass spectrometry conditions mainly include electrospray ionization (ESI) QTRAP® 6500+mass spectrometer operating in positive ion mode, temperature parameter setting is 550 °C, ion spraying voltage is set to 5,500 V, Curtain Gas (CUR) is set to 35 psi, medium collision gas, ion source gas 1:60, 2:60 ion source gas. QTRAP® 6500+mass spectrometer works in negative mode, voltage-4500V, temperature 550 °C, medium collision gas, ion source gas 1:60, ion source gas 2:60.

Metabolic data analysis

The gene sequences were obtained from the NCBI (ftp://ftp.ncbi.nih.gov/blast/db/) database, and the metabolites were annotated using the KEGG database (www.genome.jp/kegg), HMDB database (www.hmdb.ca) and Lipidmaps database (www.lipidmaps.org) principal component analysis (PCA)) and partial least squares discriminant analysis (PLS-DA)) in metaX (a flexible and comprehensive processing software). The statistical significance (P value) was calculated by single factor analysis (t test). The metabolites with VIP > 1, P value < 0.05, fold change ≥ 2 or FC ≤ 0.5 were differential metabolites. Volcanic maps are used to screen interested metabolites according to the metabolites of Log_2 (FC) and −log10 (P value) ggplot2 in R language. For the clustering heat map, the z scores of different metabolite intensity regions are used to normalize the data, and the Pheatmap package in R language is used to

draw the data. The correlation between different metabolites was analyzed by R language cor, and the correlation between different metabolites was corrected by R language. When the metabolite metabolic pathway P-value < 0.05, statistically significant enrichment was considered, and the correlation mapping language was used in the Corrplot software package to study the function and metabolic pathways of these metabolites using the KEGG database when x/n > y/n.

Quantitative real-time PCR

The qRT-PCR method was performed according to Taylor et al.[68]. Using cDNA as template, the Vazyme fluorescent quantitative kit (ChamQTM SYBR® qPCR Master Mix, Jiangsu, China) was used for qRT-PCR validation. Gene-specific primers for qPCR were designed based on selected sequences from RNA-seq (Supplemental Table S4). The relative expression level of genes was standardized by $2^{-\Delta\Delta C_T}$ method[69] .

Data analysis

The experimental results were expressed in mean ± standard error and analyzed in Excel 2010 and SPSS 23.0. Duncan's test at a significance level of $P < 0.05$ was used to analyze the significance of the difference between different data sets.

Registration mark

The RNA-Seq data generated in this study are available from the SRA archive (www.ncbi.nlm.nih.gov/sra) registration number: PRJNA868108.

ACKNOWLEDGMENTS

This research was Supported by the Project of Education Department of Hunan Province (Grant No. 22B0229), the China Agriculture Research System of MOF and MARA (Grant No. CARS-24-A05), and the Special Project of Biological Seed Industry and Fine and Deep Processing of Agricultural Products (Grant No. 202202AE090031)

Conflict of interest

The authors declare that they have no conflict of interest.

Supplementary Information accompanies this paper at (https://www.maxapress.com/article/doi/10.48130/VR-2022-0021)

Dates

Received 8 October 2022; Accepted 22 November 2022; Published online 19 December 2022

REFERENCES

1. Guan Z, Wang W, Yu X, Lin W, Miao Y. 2018. Comparative proteomic analysis of coregulation of CIPK14 and WHIRLY1/3 mediated pale yellowing of leaves in Arabidopsis. *International Journal of Molecular Sciences* 19:2231
2. Yamatani H, Kohzuma K, Nakano M, Takami T, Kato Y, et al. 2018. Impairment of Lhca4, a subunit of LHCI, causes high accumulation of chlorophyll and the stay-green phenotype in rice. *Journal of Experimental Botany* 69:1027–35
3. Hsieh EJ, Waters BM. 2016. Alkaline stress and iron deficiency regulate iron uptake and riboflavin synthesis gene expression differently in root and leaf tissue: implications for iron deficiency chlorosis. *Journal of Experimental Botany* 67:5671–85
4. Zhang K, Liu Z, Shan X, Li C, Tang X, et al. 2017. Physiological properties and chlorophyll biosynthesis in a Pak-choi (*Brassica rapa* L. ssp. *chinensis*) yellow leaf mutant, pylm. *Acta Physiologiae Plantarum* 39:1–10
5. Liu X, Lan J, Huang Y, Cao P, Zhou C, et al. 2018. WSL5, a pentatricopeptide repeat protein, is essential for chloroplast biogenesis in rice under cold stress. *Journal of Experimental Botany* 69:3949–61
6. Ma X, Sun X, Li C, Huan R, Sun C, et al. 2017. Map-based cloning and characterization of the novel yellow-green leaf gene *ys83* in rice (*Oryza sativa*). *Plant Physiology and Biochemistry* 111:1–9
7. Nagata N, Tanaka R, Satoh S, Tanaka A. 2005. Identification of a vinyl reductase gene for chlorophyll synthesis in *Arabidopsis thaliana* and implications for the evolution of Prochlorococcus species. *The Plant Cell* 17:233–40
8. Wu Z, Zhang X, He B, Diao L, Sheng S, et al. 2007. A chlorophyll-deficient rice mutant with impaired chlorophyllide esterification in chlorophyll biosynthesis. *Plant Physiology* 145:29–40
9. Zhang S, Wu X, Cui J, Zhang F, Wan X, et al. 2019. Physiological and transcriptomic analysis of yellow leaf coloration in *Populus deltoides* Marsh. *PLoS One* 14:e0216879
10. Zhou Y, Gong Z, Yang Z, Yuan Y, Zhu J, et al. 2013. Mutation of the *light-induced yellow leaf 1* gene, which encodes a geranylgeranyl reductase, affects chlorophyll biosynthesis and light sensitivity in rice. *PLoS One* 8:e75299
11. Sakai A, Takano H, Kuroiwa T. 2004. Organelle nuclei in higher plants: structure, composition, function, and evolution. *International Review of Cytology* 238:59–118
12. Keyhaninejad N, Richins RD, O'Connell MA. 2012. Carotenoid content in field-grown versus greenhouse-grown peppers: Different responses in leaf and fruit. *HortScience* 47:852–55
13. Li W, Yang S, Lu Z, He Z, Ye Y, et al. 2018. Cytological, physiological, and transcriptomic analyses of golden leaf coloration in *Ginkgo biloba* L. *Horticulture Research* 5:12
14. Llorente B, Martinez-Garcia JF, Stange C, Rodriguez-Concepcion M. 2017. Illuminating colors: regulation of carotenoid biosynthesis and accumulation by light. *Current Opinion in Plant Biology* 37:49–55
15. Quian-Ulloa R, Stange C. 2021. Carotenoid biosynthesis and plastid development in plants: the role of light. *International Journal of Molecular Sciences* 22:1184
16. Gruszecki WI, Luchowski R, Zubik M, Grudzinski W, Janik E, et al. 2010. Blue-light-controlled photoprotection in plants at the level of the photosynthetic antenna complex LHCII. *Journal of Plant Physiology* 167:69–73
17. Johkan M, Shoji K, Goto F, Hahida SN, Yoshihara T. 2012. Effect of green light wavelength and intensity on photomorphogenesis and photosynthesis in *Lactuca sativa*. *Environmental and Experimental Botany* 75:128–33
18. Johkan M, Shoji K, Goto F, Hashida SN, Yoshihara T. 2010. Blue light-emitting diode light irradiation of seedlings improves seedling quality and growth after transplanting in red leaf lettuce. *HortScience* 45:1809–14
19. Mouget JL, Rosa P, Tremblin G. 2004. Acclimation of Haslea ostrearia to light of different spectral qualities–confirmation ofchromatic adaptation'in diatoms. *Journal of Photochemistry and Photobiology B:Biology* 75:1–11
20. Joubert C, Young PR, Eyéghé Bickong HA, Vivier MA. 2016. Field-grown grapevine berries use carotenoids and the associated xanthophyll cycles to acclimate to UV exposure differentially in high and low light (shade) conditions. *Frontiers in Plant Science* 7:786
21. Li J, Hikosaka S, Goto E. 2009. Effects of light quality and photosynthetic photon flux on growth and carotenoid pigments in spinach (Spinacia oleracea L.). *Acta Horticulturae* 907:105–10
22. Lefsrud MG, Kopsell DA, Sams CE. 2008. Irradiance from distinct wavelength light-emitting diodes affect secondary metabolites in kale. *HortScience* 43:2243–44

23. Kopsell DA, Sams CE. 2013. Increases in shoot tissue pigments, glucosinolates, and mineral elements in sprouting broccoli after exposure to short-duration blue light from light emitting diodes. *Journal of the American Society for Horticultural Science* 138:31−37
24. Moreira-Rodríguez M, Nair V, Benavides J, Cisneros-Zevallos L, Jacobo-Velázquez DA. 2017. UVA, UVB light, and methyl jasmonate, alone or combined, redirect the biosynthesis of glucosinolates, phenolics, carotenoids, and chlorophylls in broccoli sprouts. *International Journal of Molecular Sciences* 18:2330
25. Matsufuji H, Ishikawa K, Nunomura O, Chino M, Takeda M. 2007. Anti-oxidant content of different coloured sweet peppers, white, green, yellow, orange and red (*Capsicum annuum* L.). *International Journal of Food Science & Technology* 42:1482−88
26. Huh JH, Kang BC, Nahm SH, Kim S, Ha KS, et al. 2001. A candidate gene approach identified phytoene synthase as the locus for mature fruit color in red pepper (*Capsicum* spp.). *Theoretical and Applied Genetics* 102:524−30
27. Qin C, Yu C, Shen Y, Fang X, Chen L, et al. 2014. Whole-genome sequencing of cultivated and wild peppers provides insights into *Capsicum* domestication and specialization. *Proceedings of the National Academy of Sciences* 111:5135−40
28. Marles MS, Gruber MY, Scoles GJ, Muir AD. 2003. Pigmentation in the developing seed coat and seedling leaves of *Brassica carinata* is controlled at the dihydroflavonol reductase locus. *Phytochemistry* 62:663−72
29. Peng J, Feng Y, Wang X, Li J, Xu G, et al. 2021. Effects of nitrogen application rate on the photosynthetic pigment, leaf fluorescence characteristics, and yield of indica hybrid rice and their interrelations. *Scientific Reports* 11:7485
30. Di Q, Li J, Du Y, Wei M, Shi Q, et al. 2021. Combination of red and blue lights improved the growth and development of eggplant (*Solanum melongena* L.) seedlings by regulating photosynthesis. *Journal of Plant Growth Regulation* 40:1477−92
31. Su N, Wu Q, Shen Z, Xia K, Cui J. 2014. Effects of light quality on the chloroplastic ultrastructure and photosynthetic characteristics of cucumber seedlings. *Plant Growth Regulation* 73:227−35
32. Mohsenpour SF, Willoughby N. 2013. Luminescent photobioreactor design for improved algal growth and photosynthetic pigment production through spectral conversion of light. *Bioresource Technology* 142:147−53
33. Yuan M, Zhang D, Zhang Z, Chen Y, Yuan S, et al. 2012. Assembly of NADPH: protochlorophyllide oxidoreductase complex is needed for effective greening of barley seedlings. *Journal of Plant Physiology* 169:1311−16
34. Liu M, Lu Y, Wang S, Wu F, Li J, et al. 2018. Characterization of the leaf color mutant hy and identification of the mutated gene in Chinese cabbage. *Journal of the American Society for Horticultural Science* 143:363−69
35. Shalygo N, Czarnecki O, Peter E, Grimm B. 2009. Expression of chlorophyll synthase is also involved in feedback-control of chlorophyll biosynthesis. *Plant molecular biology* 71:425
36. Frede K, Schreiner M, Baldermann S. 2019. Light quality-induced changes of carotenoid composition in pak choi *Brassica rapa* ssp. chinensis. *Journal of Photochemistry and Photobiology B: Biology* 193:18−30
37. Demotes-Mainard S, Péron T, Corot A, Bertheloot J, Le Gourrierec J, et al. 2016. Plant responses to red and far-red lights, applications in horticulture. *Environmental and Experimental Botany* 121:4−21
38. Li Q, Kubota C. 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. *Environmental and Experimental Botany* 67:59−64
39. Ohashi Kaneko K, Takase M, Kon N, Fujiwara K, Kurata K. 2007. Effect of light quality on growth and vegetable quality in leaf lettuce, spinach and komatsuna. *Environmental Control in Biology* 45:189−98
40. Xie B, Wei J, Zhang Y, Song S, Su W, et al. 2019. Supplemental blue and red light promote lycopene synthesis in tomato fruits. *Journal of Integrative Agriculture* 18:590−98
41. Li Y, Chen Q, Xie X, Cai Y, Li J, et al. 2020. Integrated metabolomics and transcriptomics analyses reveal the molecular mechanisms underlying the accumulation of anthocyanins and other flavonoids in Cowpea Pod (*Vigna unguiculata* L.). *Journal of Agricultural and Food Chemistry* 68:9260−75
42. Von Lintig J, Welsch R, Bonk M, Giuliano G, Batschauer A, et al. 1997. Light-dependent regulation of carotenoid biosynthesis occurs at the level of phytoene synthase expression and is mediated by phytochrome in *Sinapis alba* and *Arabidopsis thaliana* seedlings. *The Plant Journal* 12:625−34
43. Woitsch S, Römer S. 2003. Expression of xanthophyll biosynthetic genes during light-dependent chloroplast differentiation. *Plant Physiology* 132:1508−17
44. Zhang L, Ma G, Kato M, Yamawaki K, Takagi T, et al. 2012. Regulation of carotenoid accumulation and the expression of carotenoid metabolic genes in citrus juice sacs in vitro. *Journal of Experimental Botany* 63:871−86
45. Tuan PA, Thwe AA, Kim YB, Kim JK, Kim SJ, et al. 2013. Effects of white, blue, and red light-emitting diodes on carotenoid biosynthetic gene expression levels and carotenoid accumulation in sprouts of tartary buckwheat (*Fagopyrum tataricum* Gaertn.). *Journal of Agricultural & Food Chemistry* 61:12356−61
46. Li Y, Liu C, Shi Q, Yang F, Wei M. 2021. Mixed red and blue light promotes ripening and improves quality of tomato fruit by influencing melatonin content. *Environmental and Experimental Botany* 185:104407
47. Meier S, Tzfadia O, Vallabhaneni R, Gehring C, Wurtzel ET. 2011. A transcriptional analysis of carotenoid, chlorophyll and plastidial isoprenoid biosynthesis genes during development and osmotic stress responses in *Arabidopsis thaliana*. *BMC Systems Biology* 5:77
48. Welsch R, Arango J, Bär C, Salazar B, Al Babili S, et al. 2010. Provitamin a accumulation in cassava (*Manihot esculenta*) roots driven by a single nucleotide polymorphism in a phytoene synthase gene. *The Plant Cell* 22:3348−56
49. Maass D, Arango J, Wüst F, Beyer P, Welsch R, et al. 2009. Carotenoid Crystal Formation in Arabidopsis and Carrot Roots Caused by Increased Phytoene Synthase Protein Levels. *PLoS One* 4:e6373
50. Cazzonelli CI, Pogson BJ. 2010. Source to sink: regulation of carotenoid biosynthesis in plants. *Trends in Plant Science* 15:266−74
51. Welsch R, Beyer P, Hugueney P, Kleinig H, von Lintig J. 2000. Regulation and activation of phytoene synthase, a key enzyme in carotenoid biosynthesis, during photomorphogenesis. *Planta* 211:846−54
52. Ruiz Sola MÁ, Coman D, Beck G, Barja MV, Colinas M, et al. 2016. *Arabidopsis* GERANYLGERANYL DIPHOSPHATE SYNTHASE 11 is a hub isozyme required for the production of most photosynthesis-related isoprenoids. *New Phytologist* 209:252−64
53. Nisar N, Li L, Lu S, Khin NC, Pogson BJ. 2015. Carotenoid metabolism in plants. *Molecular Plant* 8:68−82
54. Giorio G, Yildirim A, Stigliani AL, D'Ambrosio C. 2013. Elevation of lutein content in tomato: A biochemical tug-of-war between lycopene cyclases. *Metabolic Engineering* 20:167−76
55. Liang M, Zhu J, Jiang J. 2018. Carotenoids biosynthesis and cleavage related genes from bacteria to plants. *Critical Reviews in Food Science and Nutrition* 58:2314−33
56. Colasuonno P, Lozito ML, Marcotuli I, Nigro D, Giancaspro A, et al. 2017. The carotenoid biosynthetic and catabolic genes in wheat and their association with yellow pigments. *BMC Genomics* 18:122
57. Joyard J, Ferro M, Masselon C, Seigneurin-Berny D, Salvi D, et al. 2009. Chloroplast proteomics and the compartmentation of plastidial isoprenoid biosynthetic pathways. *Molecular Plant* 2:1154−80
58. Bruley C, Dupierris V, Salvi D, Rolland N, Ferro M. 2012. AT_CHLORO: A Chloroplast Protein Database Dedicated to Sub-Plastidial Localization. *Frontiers in Plant Science* 3:205

59. Jahns P, Holzwarth AR. 2012. The role of the xanthophyll cycle and of lutein in photoprotection of photosystem II. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* 1817:182–93
60. Pinnola A, Dall'Osto L, Gerotto C, Morosinotto T, Bassi R, et al. 2013. Zeaxanthin binds to light-harvesting complex stress-related protein to enhance nonphotochemical quenching in *Physcomitrella patens*. *The Plant Cell* 25:3519–34
61. Chen C, Li H, Zhang D, Li P, Ma F. 2013. The role of anthocyanin in photoprotection and its relationship with the xanthophyll cycle and the antioxidant system in apple peel depends on the light conditions. *Physiologia Plantarum* 149:354–66
62. Wolters AMA, Uitdewilligen JGAML, Kloosterman BA, Hutten RCB, Visser RGF, et al. 2010. Identification of alleles of carotenoid pathway genes important for zeaxanthin accumulation in potato tubers. *Plant Molecular Biology* 73:659–71
63. Maxwell K, Johnson GN. 2000. Chlorophyll fluorescence—a practical guide. *Journal of Experimental Botany* 51:659–68
64. Arnon DI. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology* 24:1
65. Rio DC, Ares M, Hannon GJ, Nilsen TW. 2010. Purification of RNA using TRIzol (TRI reagent). *Cold Spring Harbor Protocols* 2010:pdb.prot5439
66. Davidson NM, Oshlack A. 2014. Corset: enabling differential gene expression analysis for *de novo* assembled transcriptomes. *Genome Biology* 15:410
67. Hao Z, Liu S, Hu L, Shi J, Chen J. 2020. Transcriptome analysis and metabolic profiling reveal the key role of carotenoids in the petal coloration of Liriodendron tulipifera. *Horticulture Research* 7:70
68. Taylor SC, Nadeau K, Abbasi M, Lachance C, Nguyen M, et al. 2019. The ultimate qPCR experiment: producing publication quality, reproducible data the first time. *Trends in Biotechnology* 37:761–74
69. Mestdagh P, Van Vlierberghe P, De Weer A, Muth D, Westermann F, et al. 2009. A novel and universal method for microRNA RT-qPCR data normalization. *Genome Biology* 10:R64