

Metabolome and transcriptome integration provide insights into petal color variation of *Chrysanthemum indicum*

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Abstract

Anthocyanins and carotenoids are essential for plant color formation and play significant roles in various physiological processes. This study explores the underlying molecular mechanisms of petal color variation in two *Chrysanthemum indicum* cultivars. Targeted metabolomics quantified 57 carotenoids, revealing higher total carotenoid content in yellow petals than in white petals. The yellow-petaled *C. indicum* exhibited higher levels of key carotenoids, including lutein and zeaxanthin dipalmitate, where total carotenoid accumulation was markedly reduced. In addition, flavonoid profiling identified 544 metabolites, Cyanidin-3-O-(6"-O-p-Coumaroyl) glucoside was the main color constituent in yellow-petaled *C. indicum*, while comparative analysis revealed significantly reduced accumulation levels of 10 anthocyanins in the white-petaled *C. indicum*. Transcriptome analysis revealed that the white-petaled *C. indicum* is primarily due to the upregulation of carotenoid degradation genes, particularly *CiCCD4*, which leads to a loss of carotenoids despite active biosynthesis. The yellow petal color is a result of the upregulation of carotenoid biosynthetic genes, such as *CiDXS2*, and the significant accumulation of specific anthocyanins. Further analysis identified critical structural genes, such as *CiDXS* and *CiPSY*, along with transcription factors that likely regulate carotenoid biosynthesis. Furthermore, the structural genes *Ci4CL1*, *Ci3GT*, and the transcription factor *CiDBB2* may play significant roles in regulating petal color. This study provides a theoretical basis for understanding the phenotypic differences between the petal colors and offers valuable insights into the cultivation and breeding of *C. indicum*.

Introduction

Chrysanthemum indicum (Asteraceae), a traditional Chinese medicinal herb, has been utilized for centuries to treat various ailments, including headache, dizziness, and malaria[1]. Recent studies have unveiled its potent pharmacological properties, such as anti-inflammatory [2], anti-oxidation, and neuroprotection activities [3], garnering significant interest for drug development targeting G protein-coupled receptors [4]. Notably, the total flavonoids in *C. indicum* exhibit substantial anti-inflammatory potential [5], with linarin serving as the sole quality control standard in the Chinese Pharmacopoeia 2015. These findings highlight the importance of systematically characterize the flavonoid in *C. indicum*.

Flavonoids are pivotal secondary metabolites that determine plant coloration, significantly influencing the quality and market value of ornamental plants [6, 7]. Anthocyanins underlie the pink, orange, purple, scarlet and blue tones of most angiosperms [8, 9]. Additionally, carotenoids, a distinct class of terpenoid pigments, contribute to yellow coloration in flowers and leaves through the accumulation of compounds such as lutein and zeaxanthin. For example, pelargonidin 3-O-glucoside drives the red coloration in Japanese tree peony cultivars 'Taiyoh' [10]. Metabolomic and transcriptomic analyses of *Camellia oleifera* have identified anthocyanins responsible for pink and red petals [11]. In *Osmanthus fragrans*, the differential accumulation of α -carotene and β -carotene serves as a key distinguishing factor among the orange-red, orange-yellow, and pale yellow-white flower variants[12]. Here, we observed two petal phenotypes in *C. indicum* under identical cultivation conditions. Elucidating the specific pigments and their regulatory mechanisms will enhance breeding efforts for targeted ornamental and medicinal traits.

Anthocyanin production is a well-characterized branch of the phenylpropanoid pathway [6, 8], involving key genes such as *PAL*, *C4H*, and *4CL*. The biosynthesis of anthocyanin from 4-coumarin CoA requires enzymes like CHS, CHI, F3H, F3'H, F3'5'H [13–15], as well as DFR, ANS, and UFGT [16]. Carotenoid biosynthesis, which originates from the plastidial MEP pathway via IPP and GGPP, is initiated by PSY-catalyzed formation of phytoene and proceeds through desaturation and cyclization to produce α - and β -carotene[17]. Transcript levels of these genes directly dictate petal color. For instance, overexpression of *F3'5'H* in rose and carnation plants results in violet flowers rich in delphinidin-based anthocyanins [18, 19]. In cotton, the phytoene-synthase gene *GbDYA* is highly expressed in yellow-pigmented anthers, thereby boosting carotenoid flux; when this gene is silenced by a retrotransposon insertion, both PSY transcript levels and carotenoid content drop, switching the anther color to light yellow[20]. Transcription factors including R2R3-MYB, bZIP, bHLH, ERF, and WRKY, orchestrate both anthocyanin and carotenoid production, with the MYB-bHLH-WD40 (MBW) complex activating structural genes [21–23].

In this study, we employed UPLC-MS/MS technology to comprehensively characterize the flavonoids compounds of white and yellow-petaled *C. indicum*. Integrated with RNA-seq analysis, we identified key structural and regulatory genes associated with pigment accumulation. These findings elucidate the anthocyanin and carotenoid biosynthetic networks in *C. indicum* petals, providing theoretical insights for breeding cultivars with desired flower colors and enhanced medicinal value.

Materials and Methods

Plant materials

Two *C. indicum* cultivars with contrasting white and yellow petal were selected for this study. Plant materials were obtained from the Chrysanthemum Germplasm Resource Base of China Resources Sanjiu Medical & Pharmaceutical Co., Ltd. (Yangxin County, Hubei Province, China). Flowers were harvested at the full-blooming stage and designated as FW (white-petaled *C. indicum*) and FY (yellow-petaled *C. indicum*). All collected samples were immediately frozen in liquid nitrogen and stored at -80°C until subsequent transcriptomic and metabolomic analyses.

Targeted metabolic profiling of carotenoids and flavonoids

UPLC (ExionLC™ AD; MS: AB SCIEX Triple Quad™ 6500) was employed for the analysis of carotenoids and flavonoids. Carotenoids were extracted from 50 mg of flower powder from white- and yellow-petaled *C. indicum* cultivars using 0.5 mL of a n-hexane-acetone-ethanol mixture (1:1:1, v/v/v). After vortex-extraction for 20 min, the supernatant was collected following centrifugation at 4°C and 12,000 r/min for 5 min. The extracts were concentrated under a nitrogen stream, reconstituted in 150 μ L of dichloromethane, and filtered through a 0.22 μ m microporous membrane prior to injection. Analytical conditions: YMC C30 column (3 μ m, 100 mm $\times$ 2.0 mm); mobile phase A consisted of methanol-acetonitrile (1:3, v/v) containing 0.01% BHT and 0.1% formic acid, while mobile phase B was methyl tert-

butyl ether with 0.01% BHT; gradient program: 0–3 min holding at 0% B, 3–5 min linearly increasing to 70% B, 5–9 min rising to 95% B, and 10–11 min returning to 0% B; flow rate 0.8 mL/min; column temperature 30°C; injection volume 2 µL. Detection was performed using APCI ion source in positive ion mode with ion source temperature at 350°C and curtain gas pressure at 25.0 psi. Scheduled multiple reaction monitoring (MRM) mode was used for data acquisition, and quantification was carried out with Multiquant 3.0.3.

Flavonoids were extracted from 50 mg of flower powder with 1.2 mL 70% methanol (vortex 30 min), centrifuged, filtered (0.22 µm) and analyzed by UPLC-MS/MS. Chromatography: Agilent SB-C18 (1.8 µm, 2.1 × 100 mm, 40°C); 0.35 mL min⁻¹. Eluents: A = 0.1% formic acid in water, B = 0.1% formic acid in acetonitrile. Gradient: 0–9 min 5–95% B, 9–10 min 95% B, 10–11 min 95–5% B, 11–14 min 5% B. ESI ± switching: +5.5 / – 4.5 kV, 550°C, curtain gas 25 psi, GS1 50 psi, GS2 60 psi; MRM acquisition, with data acquisition in MRM mode. Chromatographic peaks were integrated and corrected using MultiaQuant. Differential metabolites of two *C. indicum* were selected by OPLS-DA (VIP ≥ 1, FC ≥ 2 or ≤ 0.5) and > 8-fold changes visualized in a heatmap.

RNA sequencing

Total RNA was extracted from the flowers of two *C. indicum* using The Plant RNA Kit (R6827, Omega Bio-Tek). The quality, purity and concentration of RNA were assessed through agarose gel electrophoresis, NanoDrop One spectrophotometer (NanoDrop Technologies, Wilmington, DE) and Qubit 3.0 Fluorometer (Life Technologies, Carlsbad, CA, USA), respectively. For each sample, a cDNA library was constructed using the MGIEasy RNA Library Prep Kit for BGI®. After library validation, transcriptome sequencing was performed on the DNBSEQ-T7 platform using the High-throughput Sequencing Set V3.0. Each sample was analyzed in triplicate to ensure biological reproducibility.

Functional annotation of differential expression genes (DEGs)

Raw reads from two *C. indicum* cultivars were processed using fastq (v 0.21.0) to generate clean reads [24]. Transcripts were assembled using Trinity (v 0.21.0) parameters: –min_kmer_cov 2 [25], followed by clustering into unigenes using CD-Hit [26]. Assembly completeness was assessed using Benchmarking Universal Single-Copy Orthologs (BUSCO) [27]. Coding sequences were predicted with TransDecoder (v 5.5.0) under parameters: –m 50, –single_best_only. Transcripts and unigenes were annotated against seven databases: including Nr, Pfam [28], Uniprot, KEGG [29], GO [30], KOG [31], and PATHWAY. Transcription factors were identified and annotated using PlantTFDB v5.0 [32]. RSEM software was employed to calculate FPKM values for transcripts and unigenes across samples [33]. Differential expression analysis was performed using DESeq2 software (v 1.26.0) with thresholds set at p-adj < 0.05 and |log₂FoldChange| > 1 [34]. Finally, KEGG and GO enrichment analysis of differentially expressed genes were conducted using clusterProfiler (v 3.14.3) [35].

Identification of metabolic genes involved in carotenoid and anthocyanin biosynthesis

To identify genes associated with carotenoid and anthocyanin biosynthesis, functional protein sequences related to carotenoid and anthocyanin biosynthesis and transcriptional regulation were downloaded from NCBI (**Supplementary data 1**). Candidate genes in two *C. indicum* cultivars were identified using BLASTP with an e-value cutoff of $1e^{-10}$. Hits with $\geq 50\%$ coverage of seed protein sequences and $> 50\%$ protein sequence identity were selected as candidate anthocyanin and carotenoids-associated genes. Gene expression patterns for carotenoids metabolic and anthocyanin biosynthesis were visualized using the Tbtools [36].

qRT-PCR analysis

To validate the reliability of the transcriptome data, we conducted qRT-PCR experiments to quantify the expression levels of genes involved in anthocyanin biosynthesis and transcriptional regulation. Candidate genes were selected based on their high relevance to anthocyanin metabolism, including structural genes and transcription factors. Specific primers for these genes were designed using Primer5.0 and synthesized by Sangong Bioengineering (Shanghai). The relative expression levels of these genes were normalized with the $2^{-\Delta\Delta C_t}$ method [37], using *EF1a* serving as the internal control. qRT-PCR experiments were carried out on a QX Real-Time PCR System (Sichuan Jelamet Technology Co.) using the TB Green Premix Ex Taq II (Takara Bio, Beijing, China). Each qRT-PCR experiment was performed with three biological and technical replicates. The sequences of specific primers were listed in **Table S1**.

Results

Metabolic profiling of carotenoids in two *C. indicum* cultivars

To investigate the metabolic basis of petal color variation, we conducted targeted carotenoid profiling in the white- and yellow-petaled *C. indicum*. A total of 57 carotenoids (5 carotenes and 52 xanthophylls) were quantified via scheduled multiple reaction monitoring (MRM) (Fig. 2A and **Supplementary data 2**). The total carotenoid content in yellow-petaled *C. indicum* was significantly higher than white-petaled. Five predominant compounds (lutein, zeaxanthin dipalmitate, lutein palmitate, lutein dipalmitate, and lutein distearate) collectively represented approximately 60.4% and 63.2% of the total carotenoid pool in yellow and white flowers, respectively. Furthermore, nine carotenoids including lutein, zeaxanthin dipalmitate, lutein dipalmitate, and β -carotene, were substantially more abundant (content difference > 1.5) in yellow flowers. Among these, violaxanthin palmitate, violaxanthin myristate, and lutein dimyristate exhibited over 1.5-fold higher accumulation in yellow compared to white flowers (Fig. 2B). This distinct

pattern highlights the critical role of these differentially accumulated carotenoids in yellow color formation.

Metabolic profiling of flavonoids in two *C. indicum* cultivars

The targeted metabolomics analysis identified 544 metabolites, with the majority (536) belonging to the flavonoid class, including 241 flavones, 137 flavanols, 51 flavanones, 27 chalcones, 26 isoflavones, 10 anthocyanidins, 12 biflavones, 8 flavanols, 7 flavanonols, 3 auronones, and 14 categorized as other flavonoids (**Table S2** and Fig. 3A). 456 differentially accumulated metabolites in two *C. indicum*, including anthocyanin, flavonoid, flavone, and flavonol compounds. Using screening criteria with $|\text{Log}_2\text{FC}| \geq 8$ or ≤ 0.125 , we identified 52 significantly different metabolites (24 up-regulated and 28 down-regulated) in the FW vs FY (**Fig. S1**). Ten types of anthocyanins were identified in two *C. indicum*, including Pelargonidin (Pg), Malvidin (Mv), Cyanidin (Cy), Petunidin (Pt), and Peonidin (Pn) (Fig. 3B). Yellow-petaled *C. indicum* exhibited significantly higher anthocyanin accumulation compared to white-petaled cultivars (FW vs FY). Notably, Cyanidin-3-O-(6"-O-p-Coumaroyl) glucoside (Cy-3-ca-Glu), which accounted for over half of the anthocyanin metabolites, was 515.6-fold more abundant in yellow-petaled cultivars. In white-petaled cultivars, 10 differentially accumulated anthocyanins were detected in flowers (8 down-regulated and 2 up-regulated). Key metabolites such as Peonidin-3-O-(6"-O-p-Coumaroyl)glucoside)-5-O-Glucoside (Pn-3-coGlu-Glu), Cy-3-pC-Glu were significantly down-regulated in white flowers, with fold changes ranging from 0.002 to 0.456. Conversely, Petunidin-3-O-(6"-O-caffeoyl) glucoside (Pt-3-ca-Glu), and Malvidin-3-O-(6"-O-caffeoyl) glucoside (Mv-3-ca-Glu) were up-regulated by 2.297-, and 3.050-fold in white-petaled *C. indicum*, respectively. These findings suggest that the differential accumulation of anthocyanins is a key factor underlying the distinct petal colors in *C. indicum*.

Transcriptome sequencing and characterization of *C. indicum*

Transcriptome analysis of flowers from two *C. indicum* were generated 36.07 Gb clean data with a Q30 rate exceeding 97.0% (**Table S3**). A total of 558,451 transcripts and 200,968 unigenes were identified, of which 72,623 (36.14%) and 66,086 (32.88%) unigenes were functionally annotated using Nr and Uniprot database, respectively. Compared to white-flowered, 9,644 genes were up-regulated and 7,637 genes were down-regulated in yellow-petaled cultivars (Fig. 4A). KEGG enrichment analysis of these differentially expressed genes indicated that 67 unigenes were involved in Aminoacyl-tRNA biosynthesis, 61 unigenes were related in Phenylpropanoid biosynthesis, and 54 unigenes were annotated for Starch and sucrose metabolism (Fig. 4B). GO enrichment analysis revealed 90 unigenes related to DNA repair, 88 unigenes related to protein dimerization activity, 78 unigenes related to monooxygenase activity, and 70 unigenes related to UDP-glycosyltransferase activity (Fig. 4C).

Structural Genes Associated with Carotenoid Metabolism

To investigate the expression patterns of carotenoid metabolic genes in white and yellow-petaled *C. indicum*, we first conducted a blastp-based homology search using well-annotated proteins as

references to identify candidate genes. Our analysis identified 26 carotenoid metabolic genes, covering core biosynthetic enzymes (e.g., *CiDXS*, *CiPSY*, *CiLCYB*, *CiZEP*) and those involved in degradation/turnover (e.g., *CiNCED*, *CiCCDs*). Transcriptome data from the two *C. indicum* revealed that the expression level of *CiDXS2* in yellow flowers was approximately four-fold higher than that in white flowers (Fig. 5). In contrast, *CiCCD4* genes (*CiCCD4-1* and *CiCCD4-3*), which encode enzymes that catalyze carotenoid degradation, were markedly up-regulated in white flowers. Notably, *CiCCD4-1* exhibited an FPKM value of approximately 18.5 in white flowers, whereas it was nearly undetectable in yellow flowers. Despite the higher expression of several biosynthetic genes (e.g., *CiNXS*, *CiPSY*, *CiZEP1*) in white flowers, the concurrent strong up-regulation of degradation genes likely overwhelmed the biosynthetic capacity, leading to net carotenoid loss. The expression level of other carotenoid metabolic genes showed similar expression levels in flowers of two *C. indicum*. Therefore, we conclude that the reduced carotenoid accumulation in white-petaled *C. indicum* is primarily attributable to the elevated expression of *CCD* genes, which enhances carotenoid degradation.

Key DEGs related to anthocyanin biosynthesis pathway

A total of 26 genes involved in anthocyanin biosynthesis were identified in *C. indicum* using the BLASTp with functional gene protein sequences as queries. These genes included two *PAL*, one *C4H*, three *4CL*, eight *CHS*, one *F3H*, one *FLS*, one *F3'H*, one *F3'5'H*, one *DFR*, three *FNS*, one *UFGT*, one *FLS*, and two *CiF3G2Gt* (**Supplementary data 3**). The expression profiles of anthocyanin biosynthetic genes in flowers of two *C. indicum* were distinct, as visualized by a heatmap (Fig. 6). 7 (3 down-regulated and 4 up-regulated) DEGs involved in anthocyanin biosynthesis were identified in FW vs FY (**Supplementary data 4**). For instance, the expression levels of *Ci4CL3*, *CiCHS3*, *CiCHS6* in the flowers of yellow-petaled *C. indicum* were 4.2-, 147.6-, and 32.8-fold higher than those in flowers of white-petaled *C. indicum*, respectively. Notably, key anthocyanin biosynthetic genes such as *CiDFR*, *CiCHS5*, *CiCHS7*, and *CiF3'H* were down-regulated in yellow-petaled *C. indicum*, despite their higher anthocyanin accumulation. Specifically, *CiDFR* was expressed in the white flowers but either absent or minimally expressed in yellow flowers. *CiCHS5* and *CiCHS7* were 26.1- and 17.4-fold higher in white flowers, respectively. Additionally, we have identified key enzymatic genes responsible for the biosynthesis of linarin, a major bioactive compound in *C. indicum*. This includes three *CiCOMT10*, five *CiUGT11*, and two *CiF3G2Gt* genes. Notably, the expression of *CiCOMT10-2* and *CiF3G2Gt1* was 3-fold and 5.8-fold higher in yellow flowers than in white flowers, respectively, correlating with the elevated linarin content in yellow-petaled plants. These findings suggest that the expression levels of anthocyanin biosynthetic genes may not be the primary determinant of petal color variation in *C. indicum*.

Identification of transcription factors (TFs) involved in carotenoids and anthocyanin biosynthesis

Transcription factors play a critical role in carotenoids and anthocyanin metabolism, particularly the MYB, bHLH, WRKY and NAC TFs. In this study, 1,585 TFs were identified in white and yellow-petaled *C. indicum*, encompassing 56 families, with C2H2 (146), bHLH (129), ERF (121), C3H (93) being the most

abundant (**Supplementary data 5 and Fig. S2**). In the FW vs FY, the expression level of 119 TFs exhibited up-regulated while 134 TFs showed down-regulation. Among them, 113 differentially expressed TFs exhibited absolute $\text{Log}_2(\text{Fold Change})$ values greater than 2 in both the white and yellow flowers of two *C. indicum* cultivars, with the major families including 13 bHLH, 11 ERF, 11 MYB, 8 LBD, 7 Nin-like, 7 FAR1 and 6 NAC transcription factors (Fig. 7). After filtering out TFs with the FPKM value lower than 1 in white and yellow flowers of two *C. indicum* cultivars, 19 TFs of above eight families were identified. Two *CibHLHs* (Unigene176947 and Unigene62166), *CiMYBs* (Unigene14273 and Unigene34688), and *CiNin*-likes (Unigene52997 and Unigene121052) exhibited high expression and were significantly upregulated in the yellow-petaled *C. indicum* compared to the white-petaled. These TFs might play important roles in the metabolism of carotenoids and anthocyanin.

Verification of RNA-seq results by qRT-PCR

To validate the RNA-seq results, the expression levels of several anthocyanin related genes were quantified with qRT-PCR. These included three structural genes associated with anthocyanin biosynthesis (*CiPAL1*, *Ci4CL*, and *CiFLS*), one bZIP (*CibZIP5*), one Ninlike (*CiC3H3*), one DBB (*CiDBB2*), and one GRAS (*CiGRAS6*). The relative expression levels of these seven genes were normalized to *EF1a* expression (Fig. 8). Further analysis indicated that qRT-PCR results were generally consistent with and validated the reliability of the RNA-Seq data.

Discussion

Carotenoids are a class of yellow, orange, and red pigments found in nature that contribute to the vibrant colors of various plant parts[38]. The types and concentrations of carotenoids determine the final coloration of the plant [38]. For instance, lycopene imparts a red hue to tomatoes, while beta-carotene is responsible for the orange color of carrots[39]. In *Chrysanthemum*, carotenoids such as lutein and zeaxanthin are key contributors to yellow petal coloration, and the absence or degradation of these pigments can result in white petals[40]. Our targeted carotenoid profiling revealed that yellow-petaled *C. indicum* contained significantly higher total carotenoid levels than white-petaled *C. indicum*, with compounds like lutein and zeaxanthin dipalmitate playing crucial roles in determining flower color. This finding corroborates previous studies highlighting the importance of carotenoids in plant pigmentation.

Anthocyanins are composed of a flavonoid backbone with attached sugar molecules. Numerous studies have identified that anthocyanins contribute to certain color in plants [7], Which may depend on their structure [41], and their concentration [42]. For instance, rose mutants accumulate more peonidin 3-O-glucoside chloride, altering flower color [43]. Black peanut seed coats, which contain higher anthocyanin levels than pink varieties, display distinct pigmentation [44]. In *Chrysanthemum*, the presence of anthocyanins, particularly delphinidin, is crucial for petal coloration, with white petals lacking these pigments [45–47]. Research indicates that flavonoid content surpasses anthocyanins in normally colored petals, especially in white ones [48]. Previous studies links delphinidin-like anthocyanins to blue coloration and cyanidin-like anthocyanins to pink and purplish-red hues in *Chrysanthemums* [40]. In the investigation of the *Combretum indicum*, cyanidin 3-O-glucoside was identified as a key compound driving

the transition from white to vibrant red coloration [49]. Similarly, the color variation between purple and white flowers in *Salvia miltiorrhiza* has been linked to the content of cyanidin 3,5-O-diglucoside, cyanidin 3-O-galactoside, and malvidin 3,5-diglucoside [50]. This study detected 548 metabolites in the two *C. indicum*, including 14 anthocyanins classified into six categories. Notably, anthocyanins expression was significantly higher in yellow-petaled *C. indicum*. Specifically, Pn-3-coGlu-Glu accumulated 643, more in the yellow compared to white-petaled *C. indicum*. Our analysis identified a substantial increase in anthocyanin content in yellow flowers, with Cyanidin-3-O-(6"-O-p-Coumaroyl) glucoside being the most prominent. These findings suggest that these anthocyanins play a crucial role in the color differentiation between the two cultivars.

While the anthocyanin and carotenoid metabolic pathway were well characterized, variations in key structural genes or TFs within the pathway can determine their biosynthesis, thereby influencing the formation of plant color phenotypes [51]. For example, in the 'Dante Purple' *chrysanthemum*, the CmMYB308 gene inhibits the expression of regulatory genes in the anthocyanin synthesis pathway, leading to reduced anthocyanin production and altered flower color [52]. In *Freesia hybrida*, four TFs from the WRKY and AP2 families have been identified as having regulatory roles in flavonoid synthesis, impacting flower color formation [53]. Similarly, a single amino acid insertion mutation in the lycopene β -cyclase gene (LCYB2) has been shown to redirect carotenoid biosynthesis in *Daucus carota* (carrot), significantly reducing the accumulation of β -carotene while promoting the buildup of lycopene, which shifts the taproot color from orange to red. CsERF061 plays a pivotal role in regulating carotenoid biosynthesis in citrus. Induced by ethylene, it directly binds to the promoters of key carotenogenic genes to activate their expression, thereby enhancing the accumulation of β -carotene and lutein and deepening the orange coloration of citrus fruits. Our analysis revealed 33,001 DGEs in FY and FW, including 26 core anthocyanin-biosynthetic and 26 carotenoid-metabolic genes. In FW, carotenoid content is low because the *CiCCD4-1* and *CiCCD4-3* genes are strongly up-regulated. Conversely, FY accumulate carotenoids via a four-fold higher expression of the rate-limiting *CiDXS2* and *CiCCD4* activity. Among 1,585 TFs identified (56 families), 253 were differentially expressed. Notably, two bHLHs, two MYBs and two Nin-likes were highly expressed and significantly up-regulated in FY, implying that they may co-ordinate the elevated flux through both the MEP and carotenoid pathways. Thus, the combined differential expression of structural genes and their TF regulators we observed in *C. indicum* provides a clear molecular explanation for the white versus yellow petal phenotypes.

Conclusion

Anthocyanins and Carotenoids are important substances that regulate flower color and significantly contribute to its formation. Our results indicated that the anthocyanin contents of white and yellow-petaled *C. indicum* were different, with yellow-petaled *C. indicum* exhibiting higher levels. This study elucidates the molecular mechanisms of petal color variation in *C. indicum*, revealing that white petals result from active degradation of carotenoids by upregulated *CiCCD4* genes, while yellow petals are formed by the dual accumulation of carotenoids and specific anthocyanins, particularly Cyanidin-3-O-(6"-O-p-Coumaroyl) glucoside (Cy-3-ca-Glu). This complex process is orchestrated by a network of

differentially expressed structural genes and transcription factors, which can serve as a preliminary investigation into the two petal-color variants of *C. indicum*, providing valuable theoretical support for understanding the mechanisms underlying flower color variation.

Abbreviations

DEGs	Differentially expressed genes
PAL	phenylalanine ammonia lyase
C4H	cinnamate 4-hydroxylase
4CL	4-coumarate-CoA ligase
CHS	chalcone synthase
CHI	chalcone isomerase
F3H	flavonoid 3-hydroxylases
F3'H	flavonoid 3'-hydroxylases
F3'5'H	flavonoid 3'5'-hydroxylases
DFR	dihydroflavonol reductase
ANS	anthocyanin synthase
UFGT	flavonoid 3-O-glucosyltransferase
PCA	Principal component analysis

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Clinical trial

Not applicable

Availability of data and materials

The transcriptome data of two *C. indicum* cultivars has been submitted to the NCBI database (<https://www.ncbi.nlm.nih.gov/>) under the accession number PRJNA1159015.

Competing interests

The authors declare that the research was conducted devoid of any commercial or financial affiliations that may create a perception of potential conflicts of interest.

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Authors' contributions

The manuscript was conceived and designed by HTY and CW. XFL and CW were responsible for the analysis of data and the writing of the manuscript. MW, LJM, and HTY provided experimental samples. CW and HTY performed manuscript editing. Additionally, LL and CS conducted language editing and granted final approval for the manuscript. All authors made significant contributions to the article and have approved the submitted version.

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Figures

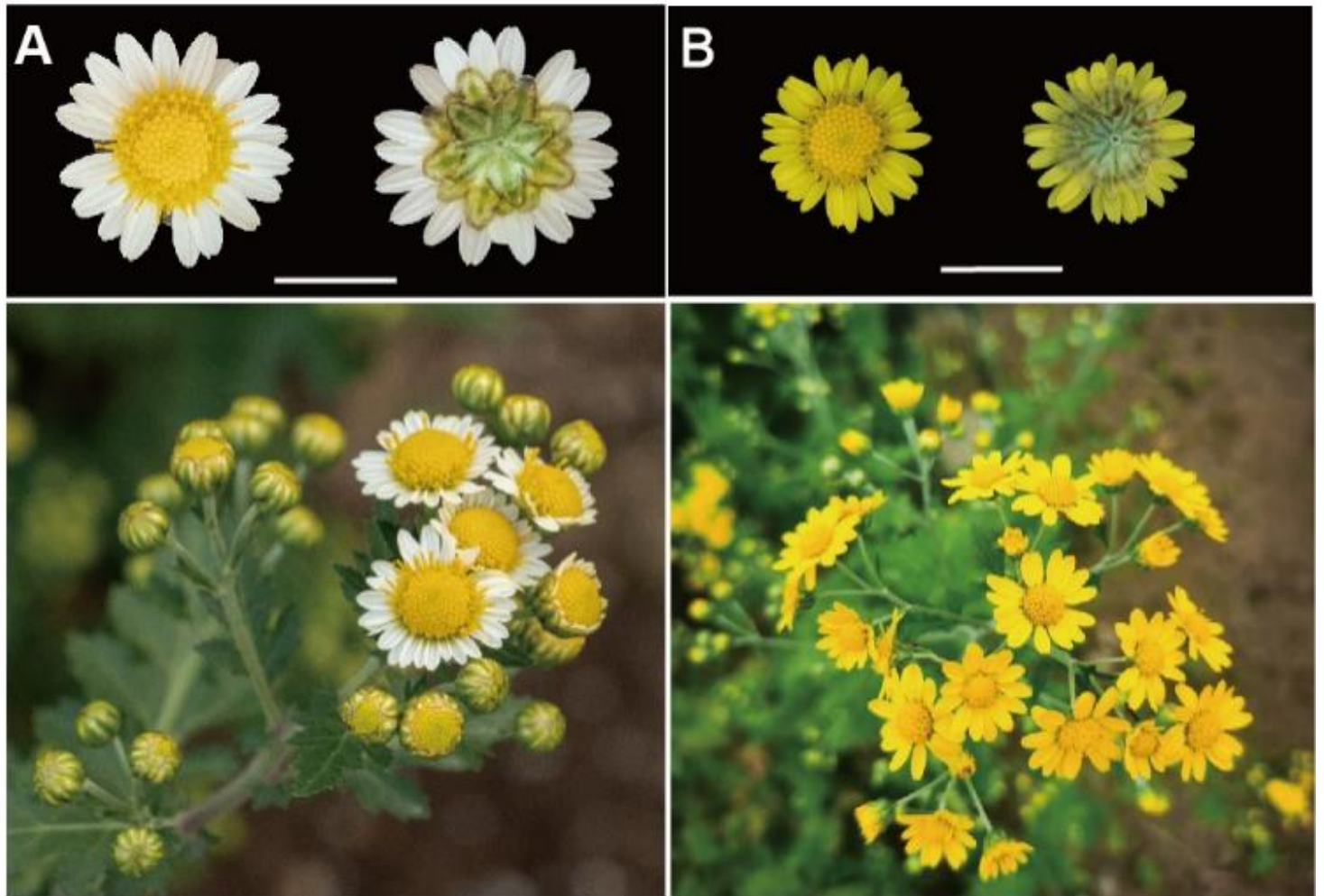


Figure 1

Phenotypic characteristics of flowers and plants in two *C. indicum* cultivars. (A). FW: white-petaled *C. indicum*. (B). FY: yellow-petaled *C. indicum*. Scale bar= 1 cm.

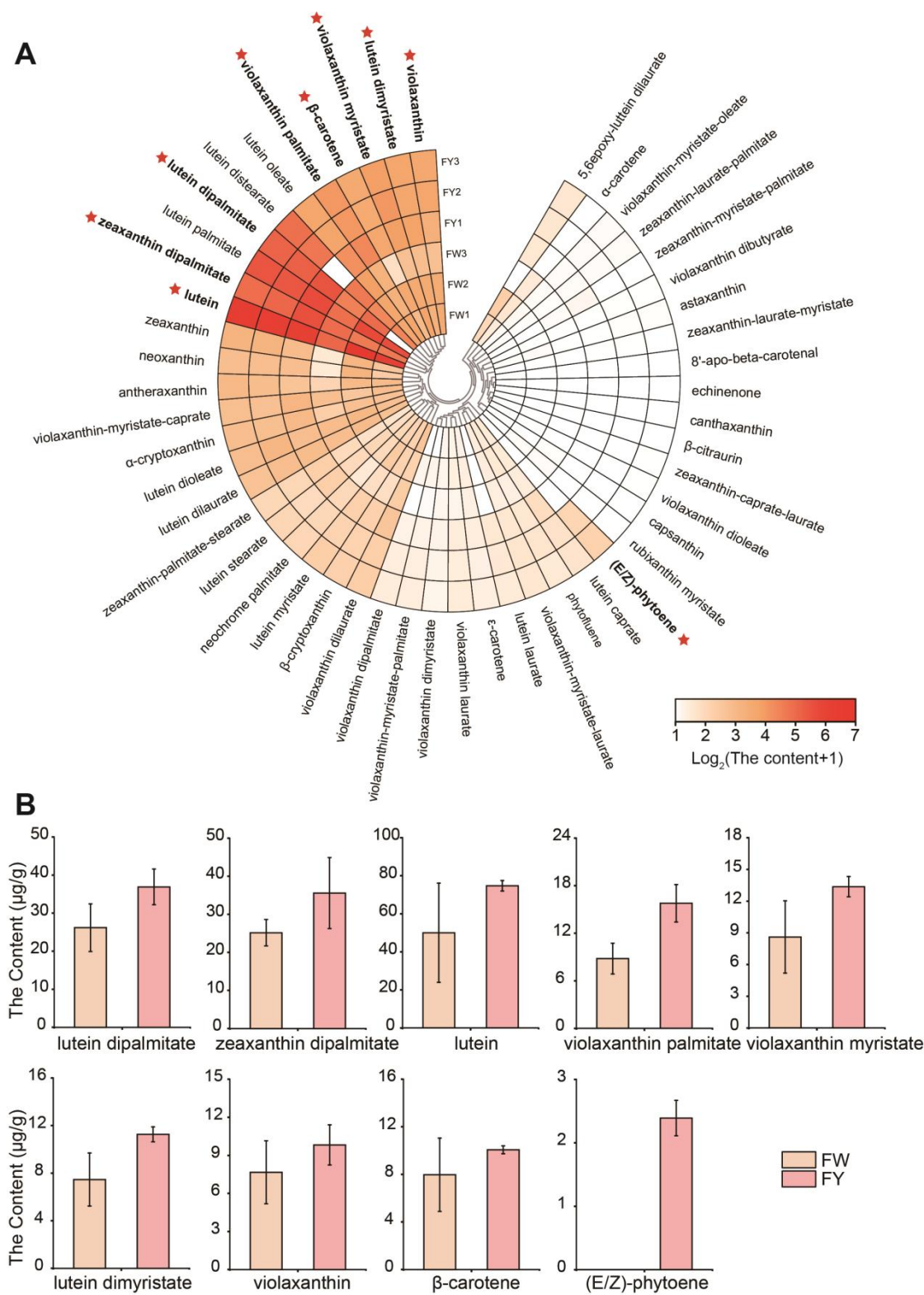


Figure 2

Comparative analysis of carotenoid profiles in white- and yellow-petaled *C. indicum*. (A) Heatmap depicting the contents of identified carotenoids in the two *C. indicum*. Red and white colors represent high and low abundance, respectively. (B) Contents of major differential carotenoids, with a content difference greater than $1.5 \mu\text{g/g}$ between the two cultivars.

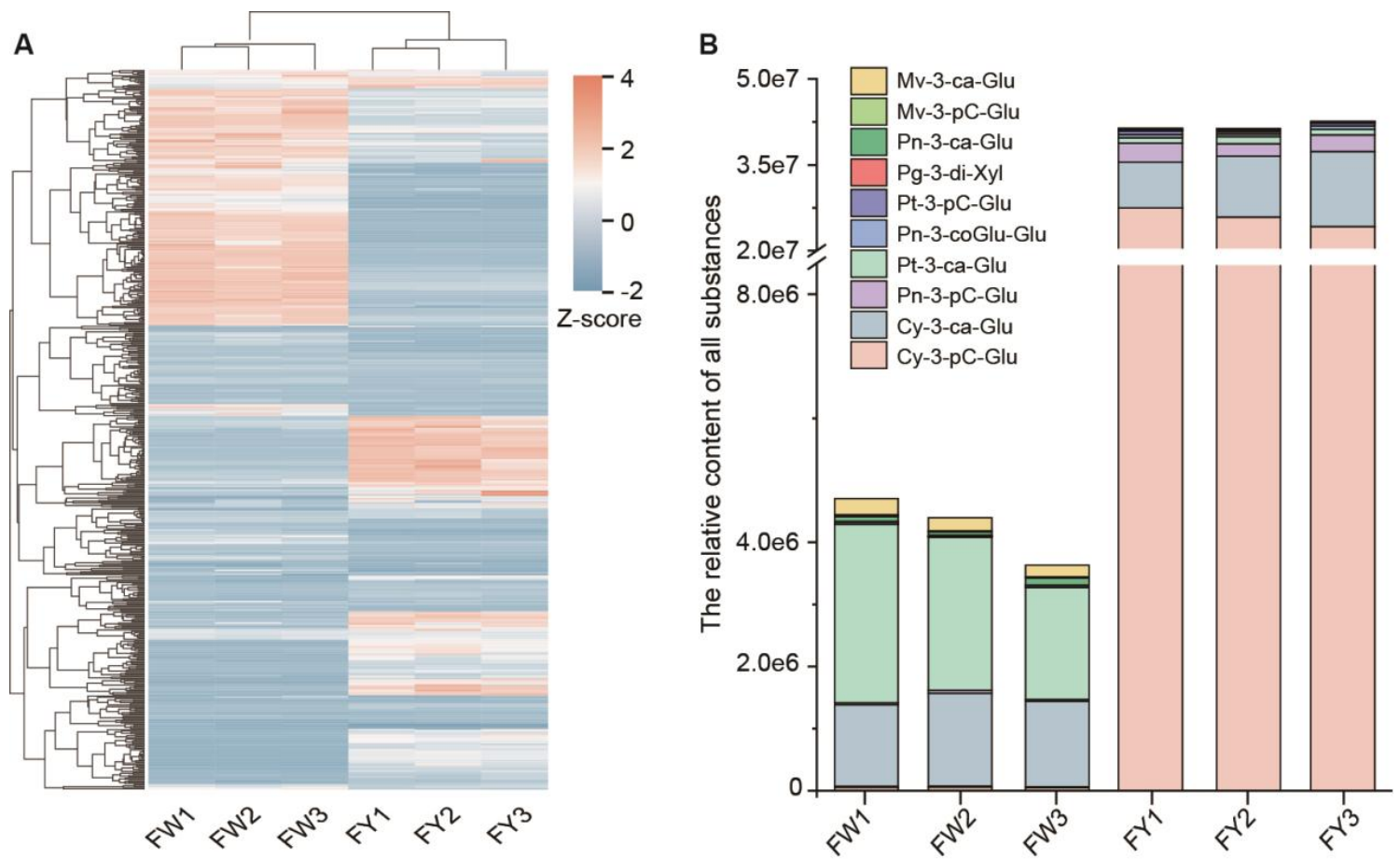


Figure 3

Metabolome profiles of flowers in two *C. indicum*. A. Hierarchical clustering analysis of significantly differential metabolites. B. The relative amount of ten anthocyanins in two *C. indicum*. Each bar represents the sum of the relative contents of the 10 anthocyanins in that sample.

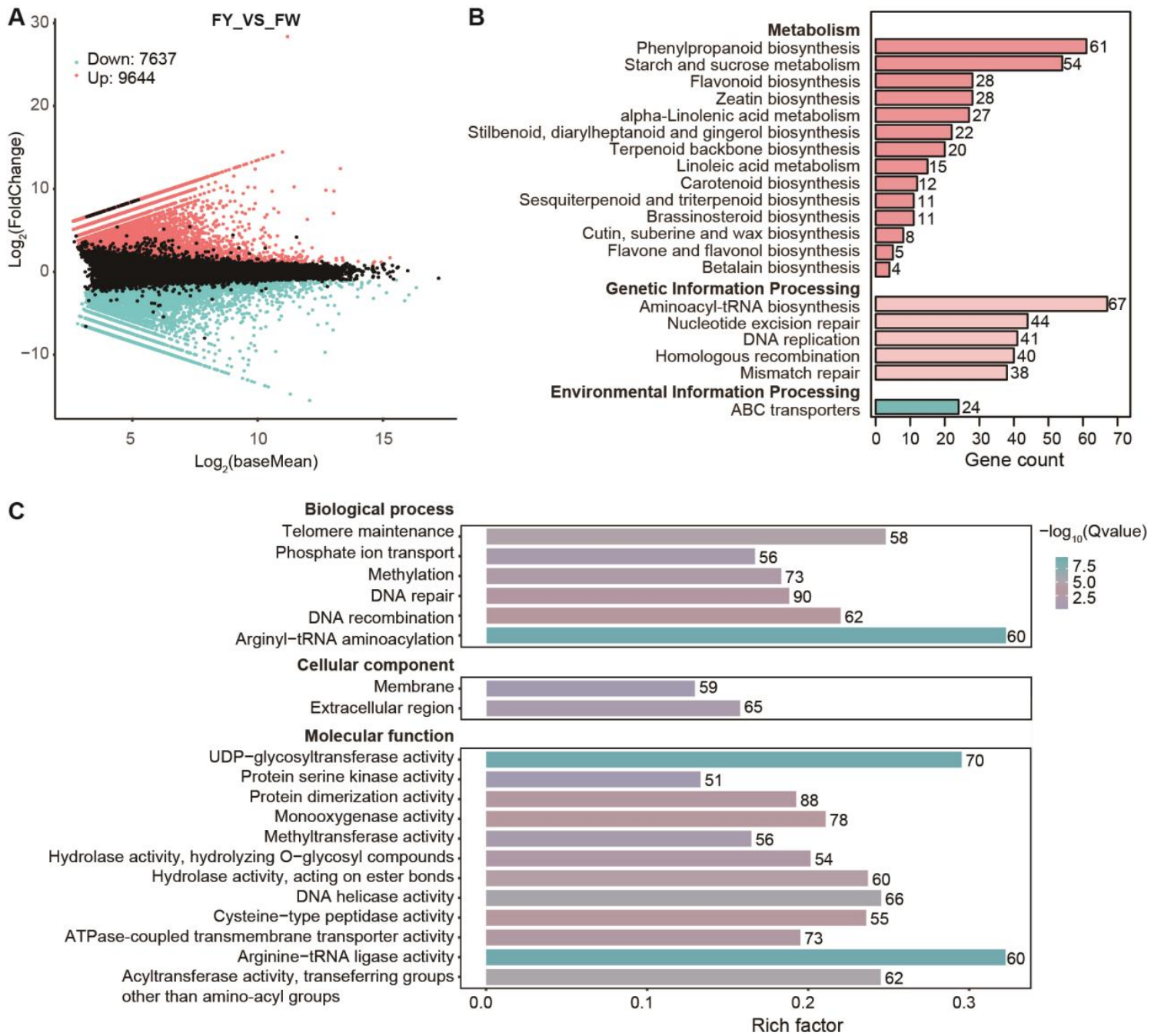


Figure 4

Differentially expressed genes (DEGs) in flowers of two *C. indicum*. A. DEGs between FY and FW. (B) KEGG enrichment analysis of DEGs in FW vs FY; (C) GO enrichment analysis of DEGs in FW vs FY. FW: white-petaled *C. indicum*. FY: yellow-petaled *C. indicum*.

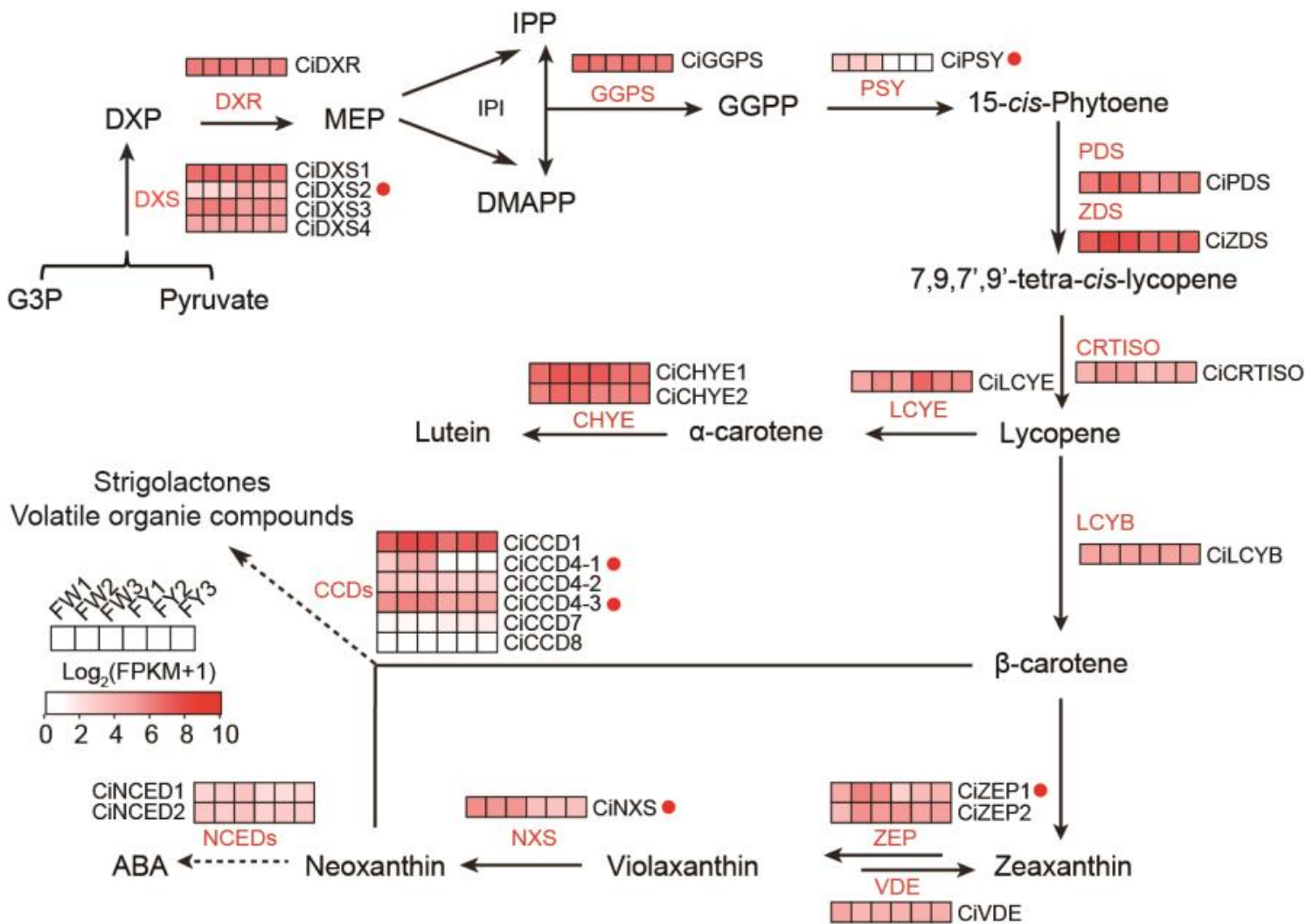


Figure 5

The expression patterns of carotenoid metabolic genes in flowers of two *C. indicum* cultivars

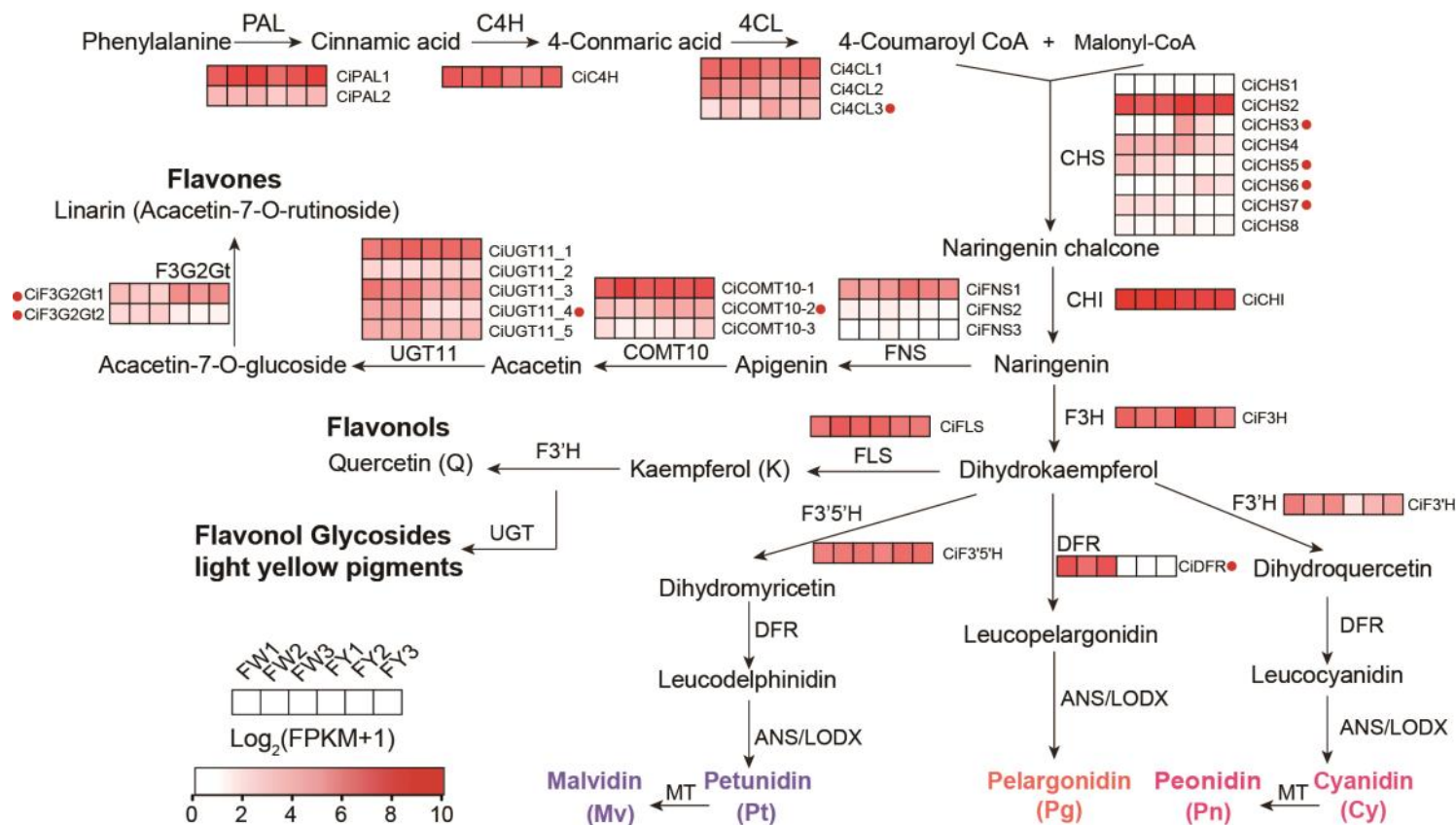


Figure 6

The expression patterns of anthocyanin biosynthetic genes in two *C. indicum*.

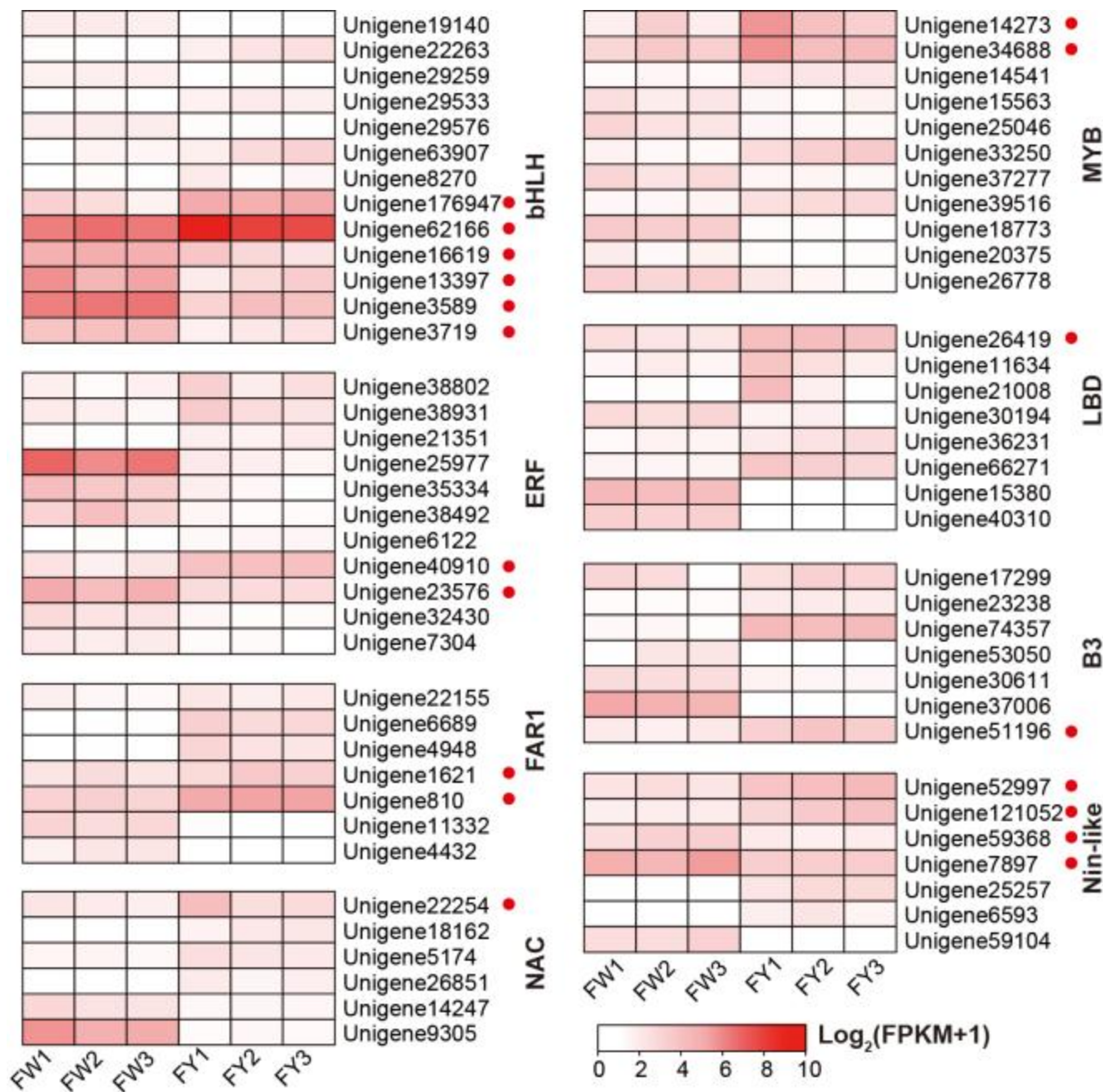


Figure 7

Expression profile of differentially expressed TFs.

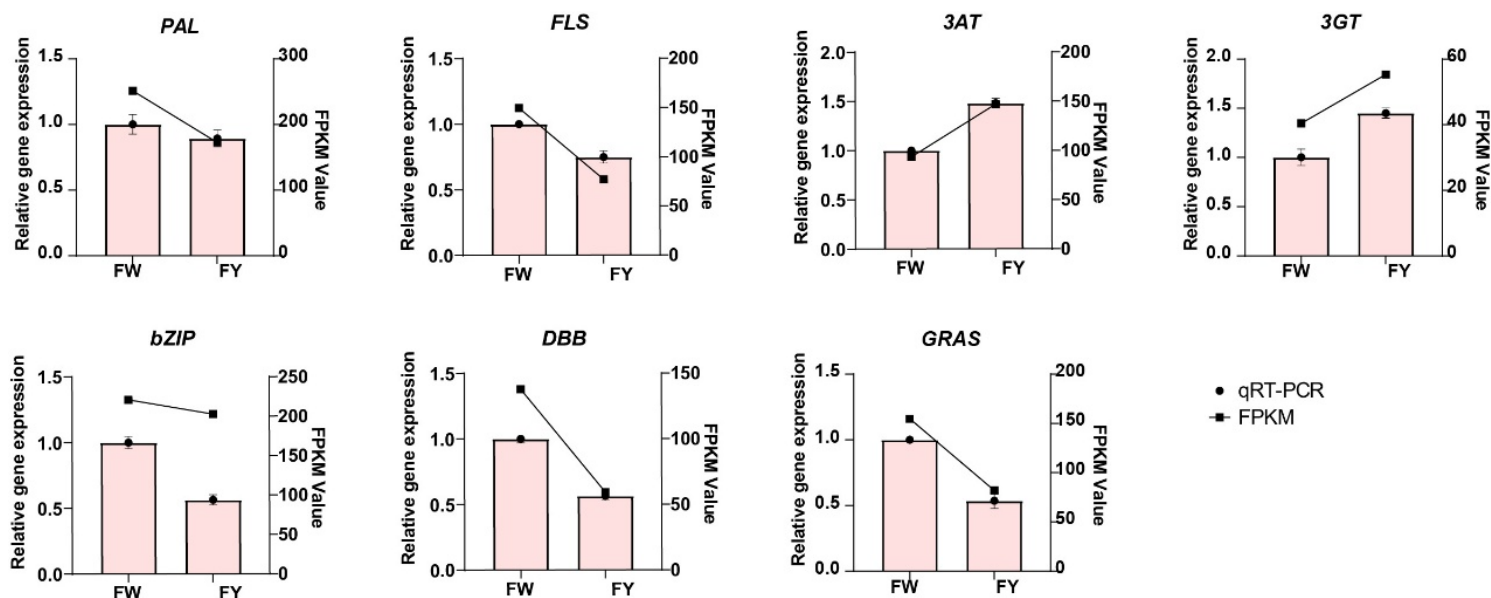


Figure 8

The relative expression levels of structural genes and transcription factors related to anthocyanin biosynthesis.

Supplementary Files

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