

# Integrated biochemical, metabolomic, transcriptomic, and phytohormone analyses reveal cultivar-specific metabolic regulation in *Chaenomeles speciosa* fruit during harvest

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## Research Article

**Keywords:** *Chaenomeles speciosa*, harvest, cultivar-specific, metabolome, transcriptome, phytohormones

**Posted Date:** October 19th, 2026

**DOI:** <https://doi.org/10.21203/rs.3.rs-10802346/v1>

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**Additional Declarations:** No competing interests reported.

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# Abstract

## Background *Chaenomeles speciosa*

is a dual-purpose fruit with high value for both medicinal and food applications. Its fruit quality varies substantially among cultivars and harvest stages, yet the molecular basis underlying metabolic divergence between cultivars during the harvest window remains poorly understood.

## Results

Here, we analyzed cultivars 'P' and 'L' at 105 and 120 days after full bloom in terms of morphology and chemical composition, integrating transcriptomics, metabolomics, and hormone profiling to investigate the quality variation. A total of 896 metabolites were identified, with significant variety among the cultivars in carbohydrates (stachyose, D-glucose), phenolic acids (caffeic acid and chlorogenic acid) and flavonoids. Phytohormone profiling revealed that during harvesting, auxin, salicylic acid (SA), jasmonic acid (JA), ethylene, abscisic acid, and cytokinin declined, whereas gibberellin (GA) exhibited the opposite trend. Through integrated correlation analysis of differentially accumulated metabolites, differentially expressed genes, and phytohormones, we predicted a potential GA- JA- SA crosstalk network that may coordinate the balance between fruit growth and defense during the harvest period. Notably, the persistent-calyx 'L' variety exhibited greater metabolic stability and higher accumulation of functional saccharides, while the deciduous-calyx 'P' variety showed higher peak phenolic content but rapid decline in the later stage of the harvest window.

## Conclusions

Our study provides the first integrated multi-omics and phytohormone profiling of cultivar-specific metabolism in *Ch. speciosa* fruit during harvest and establish a regulatory framework linking hormone signaling to variety-specific metabolic divergence. The findings offer new insights into the molecular mechanism of cultivar-specific fruit development in multi-purpose medicinal plants.

## 1. Background

*Chaenomeles speciosa*, a member of the Rosaceae family (*Pomoideae* subfamily), is a horticulturally valuable species native to East Asia and now widely cultivated across the Eurasian continent. Beyond its ornamental appeal, the fruit of *Ch. speciosa* has been used for centuries as both a traditional Chinese medicine (State Pharmacopoeia Commission, 2025). Modern phytochemical studies have revealed that the fruit is rich in phenolics, flavonoids, amino acids, and triterpenoids, which contribute to a range of beneficial effects, including antihyperuricemic (Xu et al., 2024), antimicrobial, gastroprotective (He et al., 2020), and anti-rheumatic activities (Yeo et al., 2022). Recent comprehensive reviews have systematically summarized the phytochemical diversity and bioactivities of *Chaenomeles* species,

underscoring their potential as a valuable source of functional ingredients, including the production of preserved fruits, beverages, and related products (Turkiewicz et al., 2020; Tian et al., 2025). In China, *Ch. speciosa* is extensively cultivated, with Xuancheng City in Anhui Province recognized as the genuine production region. Centuries of local cultivation have given rise to two primary agricultural cultivars: cv. 'Luohanqi' (hereafter 'L') and cv. 'Pingguoxing' (hereafter 'P'). Our previous studies showed that these two cultivars distinct morphological and phytochemical profiles (Zheng et al., 2025), yet the molecular basis underlying metabolic divergence between cultivars during the harvest window remains poorly understood, leading to inconsistent fruit quality and limiting their optimized utilization for medicinal versus food purposes.

Harvest timing is a critical determinant of fruit quality, as it governs the accumulation and transformation of metabolites (Kader, 1999). For *Ch. speciosa*, the fruit is traditionally harvested at near-maturity, a stage characterized by firm texture and subtle external color changes, which makes it difficult to assess internal biochemical processes based solely on visual appearance. Our previous studies have systematically characterized the dynamic changes in agronomic traits, chemical composition, and antioxidant activity during fruit development in a single *Ch. speciosa* variety (Fang et al., 2021; Fang et al., 2023). We demonstrated that at approximately 105 days after full bloom (DAFB), the accumulation of bioactive pharmaceutical ingredients peaks, making this time point optimal for medicinal harvest. In contrast, at approximately 120 DAFB, carbohydrate metabolism is markedly enhanced while phenolic metabolism declines, rendering the fruit more suitable for edible product processing. However, these earlier investigations focused on a single variety and did not address the molecular basis of cultivar-specific metabolic divergence during the harvest window, which limits our understanding of the developmental programs governing fruit quality formation.

Plant phytohormones are key regulators of fruit development, ripening, and postharvest physiology (Kou et al., 2021; Gu et al., 2019). Cytokinins (CK), auxins (IAA), gibberellins (GA), abscisic acid (ABA), ethylene (ETH), jasmonic acid (JA), and salicylic acid (SA) have all been implicated in coordinating the expression of genes involved in primary and secondary metabolism (Upadhyay et al., 2024; Zhao et al., 2024). Notably, different cultivars of the same fruit species may employ distinct hormonal regulatory mechanisms. For example, in pear, the variety 'Blanquilla' exhibits increased ABA content during late development, which enhances ethylene production and promotes postharvest ripening, whereas in the 'Conference' variety, GA, SA, and JA act synergistically to suppress ethylene production after harvest (Lindo-García et al., 2020). Such variety-specific hormonal regulation has also been linked to metabolic shifts in both primary (Li et al., 2019) and secondary metabolism (Dou et al., 2022). For instance, MYC2, a master regulator of JA signaling, has been shown to regulate genes involved in carbohydrate metabolism, phenylpropanoid biosynthesis, and carotenoid accumulation during tomato fruit development and ripening (Zhao et al., 2024). Till now, the hormonal regulation of metabolites during the harvest of *Ch. speciosa* fruit remains largely unexplored, particularly regarding cultivar-specific differences and the potential crosstalk among signaling pathways.

In this study, we performed integrated metabolomic, transcriptomic, and phytohormone analyses on the two major *Ch. speciosa* cultivars, cv. 'L' and cv. 'P', harvested at two distinct stages (DAFB105 and DAFB120), corresponding to their traditional harvest window. Our objectives were to: (i) characterize the cultivar-specific metabolic profiles of *Ch. speciosa* fruit at harvest; (ii) identify key phytohormones associated with metabolic divergence between cultivars; and (iii) construct co-expression networks linking phytohormones, regulatory genes, and quality-associated metabolites. By elucidating the hormone, metabolism, and molecular networks of different cultivars of *Ch. speciosa* fruits during harvest, this study aims to provide basic research for understanding the development mechanism of fruit quality formation.

## 2. Materials and methods

### 2.1 Plant materials

All samples were collected from a certified commercial orchard in Xuancheng, Anhui Province, China (118°72.2470' E, 30°88.3826' N), a region historically recognized as the genuine production area ("Daodi") for *Ch. speciosa*. Based on our previous studies of *Ch. speciosa* fruit maturation, two harvest time points with clear functional relevance were selected: 105 DAFB, representing the optimal window for medicinal component accumulation, and 120 DAFB, representing the transition toward edible-use quality traits. Two primary agricultural cultivars were selected: cv. 'Luohanqi' (hereafter 'L') and cv. 'Pingguo' (hereafter 'P'). All trees were managed under standardized agronomic protocols with integrated pest management and no application of plant growth regulators. Under the principle of representativeness, healthy adult *Ch. speciosa* of similar age and ecological conditions were selected. At each harvest time point, approximately 8–12 fruits were collected from each tree, immediately sliced into 1 cm thick sections, and pooled by tree to form one biological replicate. A total of three independent biological replicates were prepared for each variety × time point combination, resulting in 12 samples. All samples were transported on dry ice within 2 h of harvest, frozen in liquid nitrogen, pulverized, and stored at -80°C until analysis.

### 2.2 Determination of total phenolic acids, flavonoids, and amino acids

Total flavonoid and phenolic acid contents were determined using a commercial test kit (Suzhou Grace Biotechnology Co.,Ltd., Suzhou, China) and a microplate reader. Amino acids were quantified by ninhydrin colorimetry according to previously described method (Fang et al., 2022). All experiments were performed in triplicate, and results were expressed as mean ± standard deviation. Statistical analyses were conducted using GraphPad Prism software (version 10.0).

### 2.3 RNA extraction, cDNA library construction, and Illumina sequencing

Total RNA was extracted from fruits using the RNA prePure Plant Plus kit (DP441; Tiangen, Beijing, China) following the manufacturer's protocol. RNA concentration and purity were determined with a Nanodrop 2000 spectrophotometer, and integrity was assessed using an Agilent 2100 system. High-quality RNA from three biological replicates was reverse transcribed into cDNA. Libraries were sequenced on an Illumina platform by Metware Biotechnology Co., Ltd. (Wuhan, China).

## **2.4 Transcriptome data analysis and differentially expressed genes**

Raw data were filtered using Fastp to remove adapter-containing reads. All subsequent analyses were based on clean reads. Raw data quality control: (1) adapter contamination removal using Fastp (v0.20.0); (2) per-base sequence quality assessment using FastQC (v0.11.9), (3) Q20, Q30, and GC content calculated for each sample. Approximately 116.74 GB of clean data were generated, with all samples achieving  $Q30 \geq 94.2\%$  and  $Q20 \geq 98.1\%$ . Transcriptome assembly was performed with Trinity, and gene functions were annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Gene expression levels were estimated using RSEM, and fragments per kilobase of transcript per million mapped reads (FPKM, fragments per kilobase of transcript per million mapped reads) were calculated. Analysis of the differential expression between the two groups was conducted using DESeq2, and pathway enrichment was assessed using a hypergeometric test with KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway units.

## **2.5 Metabolomic analysis and differential metabolites**

Biological samples were freeze-dried, dissolved, and extracted using MetWare Biotechnology Co., Ltd. ([www.metware.cn](http://www.metware.cn)). A triple quadrupole linear ion trap mass spectrometer (QTRAP; AB4500 QTRAP UPLC/MS/MS System) was used to acquire the LIT and triple quadrupole scans. The ESI source operation parameters were as follows: ion source, turbo spray; source temperature 550°C, ion spray voltage (IS) 5500 V (positive ion mode)/-4500 V (negative ion mode); curtain gas (CUR) was set at 50, 60, and 25.0 psi. High-collision-activated dissociation was applied. Multiple reaction monitoring (MRM) experiments were conducted with nitrogen as the collision gas. Declustering potential (DP) and collision energy (CE) for each MRM transition were optimized, and a specific set of transitions was monitored according to metabolite elution periods. Data were unit variance-scaled and normalized following standard procedures (Chen et al., 2013). Six consecutive injections of the pooled QC sample at run initiation to equilibrate the system and verify retention time stability ( $RSD < 2.0\%$ ). An extraction blank (solvent only) was injected every 10 samples to monitor background contamination. Pooled QC (equal aliquots from all 12 samples) was injected every 8–10 analytical runs throughout the sequence to assess system stability. All metabolite intensities were normalized to respective internal standard responses. Metabolites with  $CV > 30\%$  in pooled QC samples were excluded from downstream differential analysis. Unsupervised Principal component analysis (PCA) was performed using the prcomp function in R ([www.r-project.org](http://www.r-project.org)). Identified metabolites were annotated using the KEGG Compound database (<http://www.kegg.jp/kegg/compound/>), and annotated metabolites were mapped to the KEGG Pathway database (<http://www.kegg.jp/kegg/pathway.html>). Pathways with significantly

regulated metabolites were then subjected to metabolite set enrichment analysis (MSEA). Metabolites with fold change (FC)  $\geq 2$  or  $\leq 0.5$  and a P-value of  $\leq 0.05$  were considered differential metabolites (DAMs).

## 2.6 Phytohormone metabolome determination

Fresh plant samples were immediately frozen in liquid nitrogen. Plant samples (50 mg) were dissolved in 1 mL of methanol/water/formic acid (15:4:1, v/v/v). Stable isotopically labelled internal standards ( $[^2\text{H}_6]$ - $(\pm)$ -JA,  $[^2\text{H}_2]$ -(-)-JA-Ile,  $[^2\text{H}_5]$ -OPDA,  $[^2\text{H}_5]$ -IAA,  $[^2\text{H}_4]$ -SA,  $[^2\text{H}_6]$ -(+)-cis, trans-ABA,  $[^2\text{H}_2]$ -GA1,  $[^2\text{H}_2]$ -GA3,  $[^2\text{H}_5]$ -Tz) were added to each sample at 10  $\mu\text{L}$  with a concentration of 100 ng/mL.

Extracts were vortexed, dried, redissolved in 100  $\mu\text{L}$  of 80% methanol, and filtered through a 0.22  $\mu\text{m}$  membrane for LC-MS/MS analysis as described previously (Li et al., 2016). Phytohormones were quantified using the AB Sciex QTRAP 6500 LC-MS/MS platform (Metware, <http://www.metware.cn/>). Data acquisition was performed using Analyst 1.6.3 software (Sciex). Multiquant 3.0.3 software (Sciex) was used to quantify all metabolites.

## 2.7 Integrated analyses of metabolome, transcriptome, and phytohormones

To investigate regulatory mechanisms of hormones and metabolites during harvesting, integrated analyses of transcriptome, metabolome, and phytohormones were conducted. Key pathways were identified through transcriptome–metabolome co-enrichment, followed by Pearson correlation analysis of differentially expressed genes (DEGs) and differentially accumulated metabolites (DAMs) related to phytohormones. Visualization of the regulatory networks was performed using Cytoscape, with a Pearson correlation coefficient threshold of  $> 0.8$ .

## 2.8 Validation of DEGs using qRT-PCR

Quantitative real-time polymerase chain reaction (qRT-PCR) was used to validate gene expression using combined sequencing platforms. Twelve DEGs involved in flavonoid and hormone biosynthesis were selected. *TUB* and *18S* rRNA served as internal reference genes. qRT-PCR was performed using the 2 $\times$  Realab Green PCR Fast mixture (LABLEAD, Beijing) on an Agilent Stratagene Mx3000P system. All reactions were conducted in triplicate to confirm the reliability of RNA-seq results.

## 3. Results

### 3.1 Morphological characteristics and major compound changes in two *Ch. speciosa* fruit cultivars during harvesting time

The two cultivars of *Ch. speciosa* fruit, shown in **Fig. 1A**, exhibited significant differences in morphological characteristics. The 'L' variety had an elongated fruit shape, with the calyx contributing to

development, whereas the 'P' variety displayed a rounder fruit shape, with the calyx either shedding or failing to expand. To further compare the chemical profiles of the cultivars during the harvest window, we quantified the contents of phenolic acids, flavonoids, and amino acids (Fig. 1B–D). Both cultivars showed a decline in phenolic acid and flavonoid contents to varying degrees. The 'P' variety showed a sharp decrease in these compounds over time, whereas the 'L' variety exhibited relatively stable levels with only minor fluctuations. At the same harvest time point, the two cultivars also differed in their major compound profiles: the 'L' variety contained higher levels of amino acids, whereas its phenolic acid and flavonoid contents were slightly lower than those of the 'P' variety.

**Figure\_1.** Morphological characteristics and major phytochemical contents of *Ch. speciosa* fruit during harvest. (A) Appearance of fruit cultivars during harvesting time (near-maturation and maturation stages). (B–D) Total phenolic acids, flavonoids, and amino acid contents of four samples. 'L' represents cv. 'Luohanqi'; 'P' represents cv. 'Pingguo'; '105' represents '105 days after full bloom' for early harvest; and '120' represents '120 days after full bloom' for late harvest. Data bars represent means  $\pm$  standard errors (n = 3). \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ .

## 3.2 Metabolome profiling in two *Ch. speciosa* fruit cultivars during harvesting time

To gain a clearer understanding of metabolic profiles, primary and secondary metabolites were identified in the cv. 'P' and cv. 'L' at two harvest stages using a widely targeted metabolomics approach. A total of 1727 metabolites (**Supplementary Table 1**), classified into more than 12 categories, were detected in the P-105, P-120, L-105, and L-120 samples. These included phenolic acids (16.79%), flavonoids (18.82%), amino acids and their derivatives (10.6%), terpenoids (7.87%), alkaloids (8.8%), organic acids (5.79%), nucleotides and their derivatives (4.34%), and others (13.03%) (Fig. 2A). PCA (**Supplementary Fig. 1**) was performed to separate the 12 samples into four groups, with PC1 and PC2 accounting for 33.99% and 10.58% of the variance, respectively. The four groups, corresponding to cultivars and harvest periods, were clearly distinguished (Fig. 2B). Pairwise comparisons were then performed to elucidate metabolic differences among L-105, L-120, P-105, and P-120. The clustering analysis (Fig. 2C) illustrated their overall changes. These results indicated that while the four fruit samples shared similar metabolite categories, they exhibited distinct expression patterns. Furthermore, the differences between harvest times were greater than those between cultivars.

**Figure\_2.** Metabolomic profiling and dynamic metabolic changes in *Ch. speciosa* fruit cultivars during the harvesting stage. (A) Hierarchical pie chart depicting the relative abundance of all metabolites. (B) PCA score plot of metabolome data for the four comparisons. (C) Heatmap showing the expression patterns of metabolites. (D) Number of metabolites differing in relative abundance among comparison groups across harvest times and cultivars. (E-1) KEGG enrichment analysis of DAMs between harvest times ('L-105 vs. L-120', 'P-105 vs. P-120'). (E-2) KEGG enrichment analysis of DAMs between cultivars ('L-105 vs. P-105', 'L-120 vs. P-120'). Star markings represent repeated pathways across comparison groups.

### 3.3 Dynamic metabolic changes of different *Ch. speciosa* fruit cultivars during the harvest period

To investigate changes in the patterns of metabolite expression in the four samples, we analyzed DAMs using a fold-change threshold of  $> 2$  or  $< 0.5$  and a  $P$ -value  $\leq 0.05$ . Pairwise comparisons were conducted to clarify metabolic differences. Specifically, 'L-105 vs. P-105' and 'L-120 vs. P-120' reflected varietal differences, whereas 'L-105 vs. L-120' and 'P-105 vs. P-120' represented harvest-stage differences. The number of DAMs was quantified, and the bar chart illustrates the overall trends (Fig. 2D). The group with the largest number of DAMs was 'L-105 vs. L-120,' with 516 DAMs (231 upregulated and 285 downregulated). Comparisons between two groups of 'P-105 vs. L-105', 'P-120 vs. L-120' showed that the closer it was to the early harvest period, the greater the difference between the two cultivars.

KEGG pathway enrichment analysis was performed (Fig. 2E). For harvest-stage comparisons, DAMs were enriched in pathways such as 'Aminoacyl-tRNA biosynthesis', 'D-Amino acid metabolism', 'Flavonoid biosynthesis', 'Folate biosynthesis', 'Galactose metabolism', 'Glucosinolate biosynthesis', 'Glycolysis /Gluconeogenesis', 'Tropane, piperidine and pyridine alkaloid biosynthesis'. Additionally, in the comparison between different cultivars, DAMs were enriched in pathways including 'the biosynthesis of secondary metabolites', 'biosynthesis of various plant secondary metabolites', 'arginine and proline metabolism', and 'ABC transporters'.

We focused on DAMs related to phenolic acids, flavonoids, and carbohydrates. For harvest-stage comparisons (**Supplementary Table 2a**), several key flavonoids and phenolic acids in *Ch. speciosa* fruit, such as dihydroquercetin, cinnamic acid, chlorogenic acid, naringenin chalcone, caffeic acid, kaempferol, and catechin, showed a decreasing trend with prolonged harvesting (in both 'L-105 vs. L-120' and 'P-105 vs. P-120'). Notably, some flavonols, myricetin-3-O-galactoside, stachyose (sugar), and amygdalin (alkaloid) were upregulated at the end of the harvesting stage. We have also summarized the results in two comparison groups of cultivars (**Supplementary Table 2b**). The results showed that some flavonoids and triterpenoids, such as quercetin, oleanolic acid, myricetin-3-O-galactoside, betulinic acid, soyasapogenol E, were significantly accumulated in the 'P' variety. Conversely, D-maltotetraose, stachyose, D-glucose-6-phosphate, and trehalose-6-phosphate were significantly higher in the 'L' variety.

### 3.4 Analysis of the transcriptome of different *Ch. speciosa* fruit cultivars during the harvest period

To further investigate alterations in gene expression profiles, transcriptome sequencing was conducted on 12 samples from the two cultivars at different harvest stages using the Illumina high-throughput sequencing platform. After data filtering, approximately 116.74 GB of clean data were obtained. All samples exhibited Q30 and Q20 base percentages exceeding 98% and 94%, respectively, with GC contents ranging from 47.42% to 47.8%. A total of 86,485 genes were identified with an average length of 1,496 bp (**Table S3**). PCA was performed for the four comparison groups, with the cumulative variance contributions of PC1 and PC2 reaching 85% or higher (Fig. 3A). These results indicate significant

differences between *Chaenomeles* cultivars and developmental stages, warranting further investigation. The numbers of DEGs in each comparison group were shown in **Fig. 3B**. DEG counts associated with harvest stage were considerably higher than those linked to cultivar differences, consistent with the metabolome findings. Comparisons of 'L-105 vs. L-120' and 'P-105 vs. P-120,' representing harvest stage differences, identified 6,423 and 6,864 DEGs, respectively. In contrast, comparisons of 'L-120 vs. P-120' and 'L-105 vs. P-105,' representing genetic differences between cultivars, identified 1,860 and 2,194 DEGs, respectively.

KEGG pathway analysis of the four comparison groups revealed that 49,172 of the 86,486 genes (56.86%) were successfully annotated. The top 10 significantly enriched KEGG pathways for each group were shown in **Fig. 3C**, with the most enriched common pathways across comparisons being 'Metabolic pathways', 'Biosynthesis of secondary metabolites', and 'Phenylpropanoid biosynthesis'. In the harvest stage comparisons, both 'L-105 vs. L-120' and 'P-105 vs. P-120' showed enrichment of DEGs in pathways related to 'flavonoid biosynthesis', 'flavone and flavonol biosynthesis', 'isoflavonoid biosynthesis', and 'plant hormone signal transduction'. Differences between cultivars were more significant in 'isoflavonoid' and 'flavone biosynthesis', particularly at the early harvest stage. The 'P' variety exhibited greater metabolic activity in flavonoid and phenolic acid biosynthesis during the early harvest stage compared with the 'L' variety, whereas such differences diminished at the late harvest stage.

Notably, the 'Plant hormone signal transduction' pathway (ko04075) was significantly enriched in both comparisons involving the harvest stage. Although this pathway was not enriched in cultivar comparisons, we observed enrichment of pathways related to precursors of endogenous hormone synthesis, such as  $\alpha$ -linolenic acid metabolism and tyrosine and tryptophan biosynthesis. These findings suggest that during the harvest stage, *Chaenomeles* fruits undergo extensive physiological and biochemical changes, which are tightly regulated by hormonal crosstalk and transcriptional networks.

**Figure\_3.** Dynamic transcriptomic changes of different *Ch. speciosa* cultivars during the harvest period. (A) PCA score plot of metabolome data in the four comparisons. (B) The number of metabolites with differences in relative abundance between the comparison groups in harvesting time and in cultivars. (C-1) KEGG enrichment analysis of DAMs in the harvesting time ('L-105 vs. L-120', 'P-105 vs. P-120'). (C-2) KEGG enrichment analysis of DAMs in the cultivars ('L-105 vs. P-105', 'L-120 vs. P-120'). The star markings represent repeated pathways in comparison groups. (D) The biosynthesis pathways of saccharides, phenolic acids, and flavonoids in *Ch. speciosa* fruit. The heatmap represents the expression of corresponding structural DEGs in different maturation stages; blue to yellow indicates the FPKM values of DEGs ranging from low to high. The histogram represents DEMs content, the abscissa presents the samples (from left to right show L-105, P-105, L-120, and P-120), and the ordinate represents the abundance value.

### 3.5 Hormone content analysis and key differential hormones screening of different *Ch. speciosa* fruit cultivars during the harvest period

The aforementioned transcriptomic analysis revealed that fruit development stimulated the expression of genes involved in plant hormone signal transduction in *Chaenomeles* fruit. To further clarify the relationship between fruit metabolites and phytohormones, we examined the hormone metabolomes of different fruit cultivars during the harvest period. PCA revealed significant differences in hormone levels between different periods and cultivars (Fig. 4A). The two principal components (PC1 and PC2) effectively distinguished the comparisons, with total contributions of 58.84% in 'P-105 vs. L-105', 57.63% in 'P-120 vs. L-120', 70.37% in 'P-105 vs. P-120', and 64.73% in 'L-105 vs. L-120'. These results indicate significant differences in endogenous hormone levels among the different *Ch. speciosa* fruit cultivars and harvest periods.

A total of 64 hormones were detected in the samples, classified into eight categories: CK, ETH, ABA, auxin, JA, SA, GA, and SL. The total content of each hormone type was then summed (Fig. 4B). Among the eight hormones, auxin and ABA were the most abundant, with average values exceeding 200 ng/g. The levels of six hormones, namely, auxin, ETH, ABA, SA, JA, and CK, were higher during the '105 DAFB' period and decreased at '120 DAFB' in both *Ch. speciosa* cultivars. In contrast, GA and SL, particularly GA, exhibited the opposite trend. GA, ABA, and JA were significantly upregulated in the 'P' variety, while SL, SA, and CK were significantly upregulated in the 'L' variety. At the early harvest stage, CK content was specifically upregulated in the 'L' variety. Overall, 51 differential hormones exhibited significant differences in the comparisons of 'L-105 vs. L-120' and 'P-105 vs. P-120', while 46 differential hormones were identified in the comparisons of 'L-105 vs. P-105' and 'L-120 vs. P-120'. All hormone measurements are presented in a heatmap (Fig. 4C). As hormones play critical roles in plant growth and development, significant differences in the levels of several individual hormones among the four samples suggest that specific hormones may affect fruit quality in different *Ch. speciosa* cultivars and at different harvest stages.

Based on these analyses, we employed OPLS-DA (orthogonal projections to latent structures discriminant analysis) to distinguish the groups based on hormone content profiles. The permutation test of the OPLS-DA model was performed using hormone content data from 'L-105 vs. L-120', 'P-105 vs. P-120', 'L-105 vs. P-105', and 'L-120 vs. P-120'. The permutation test showed that the model achieved significant goodness of fit ( $R^2Y$ ) and predictability ( $Q^2$ ), as shown in **Supplementary Fig. 2**. Differential hormones with  $|\log_2(\text{fold change})| \geq 1.5$  and  $VIP > 1$  (Variable importance in projection, VIP) were marked in the comparison groups to identify key differential hormones (Fig. 4D).  $GA_3$ ,  $GA_5$ ,  $GA_6$ ,  $GA_{19}$ , and IAA-Gly were significantly increased in both *Ch. speciosa* cultivars during the harvest stage, whereas JA and SAG were the key differential hormones that significantly decreased. In the comparison of cultivars,  $GA_{19}$ ,  $GA_8$ , ABA-GE, iP9G, and IP showed higher levels in the 'L' variety, while SA, cZROG, and OxIAA were more abundant in the 'P' variety.

**Figure\_4.** Hormone content analysis of *Ch. speciosa* cultivars during the harvest period and screening analysis for key differential hormones. (A) Principal component analysis of hormone content in different cultivars during the harvest period; (B) Analysis of different categories of hormone content; (C) Heatmap analysis of hormone content and categories. Numbers between groups indicate significant differences

( $p < 0.05$ ). Red dots indicate differential hormones with FC greater than 1.5 or less than 1/1.5 in the control groups; (D) Screening analysis by VIP-FC value lollipop plot of key differential hormones. The circles represent the VIP value. The larger the circle, the higher the VIP level. Among them, the hormones with a VIP value greater than 1 and ranking in the top 20 in each comparison group are displayed in the VIP-FC lollipop chart.

### 3.6 Expression of genes involved in carbohydrates, flavonoids, and phenolic acids metabolism pathways, and plant phytohormone signal transduction

According to KEGG enrichment analysis, the genes were associated with carbohydrate, flavonoid, and phenolic acid metabolism pathways as well as plant phytohormone signal transduction. Expression levels (FPKM values) varied among the four samples (Fig. 5 and **Supplementary Table 4**). Fragment sequences were removed, genes with FPKM > 1 were screened, and BLAST analysis was performed using NCBI. A total of 124 genes belonging to 42 gene families were enriched in the sucrose and phenylalanine metabolic pathways, including those related to carbohydrates, flavonoids, and phenolic acids (Fig. 5A). In sucrose metabolism, two *GLA*, one *GULO*, and one *SUS* gene were upregulated in the late harvest stages of both cultivars. Notably, the *SUS* gene (Cluster-21072.16) and *GLA* genes (Cluster-32063.0 and Cluster-36258.1) showed significantly high expression in the 'L' variety, consistent with the metabolome results. In addition, genes encoding enzymes in phenylalanine and flavonoid biosynthetic pathways, including two *PAL*, four *4CL*, five *CHS*, two *CHI*, two *CYP73A*, two *F3H*, *DFR* and *ANS*, two *ANR*, two *CYP75B1*, and *LAR*, exhibited significantly high expression near maturity but were downregulated at full maturity. These results indicate that these genes are strongly associated with metabolite changes during the harvesting period.

Plant hormones such as GA, IAA, CK, ABA, SA, and JA regulate fruit maturation in both cultivars. From the four comparisons, a large number of DEGs involved in phytohormone synthesis and signal transduction pathways were filtered to analyze *Ch. speciosa* fruit cultivars during harvest. After filtration and screening, 61 enriched DEGs were identified (Fig. 5B and **Supplementary Table 4**), which were classified into six sub-pathways. In the GA signaling pathway, all DEGs were downregulated during maturation in both cultivars, including *GID1*, *DELLA*, *GID2*, and *PIF4*. Similarly, *TGA* and *PR-1* for salicylic acid; *JAR1*, *JAZ*, and *MYC2* for JA; and *AUX1*, *AUX*, *ARF*, and *IAA* for auxins were downregulated during fruit maturation. In contrast, genes involved in other hormone signal transduction pathways were primarily upregulated at the '120 DAFB' stage, including *COI1* for JA; two *TIR1*, *ARF*, and *SAUR* for auxin; *CRE1* and *A-APP* for CK; and *PP2C* and *ABF* for ABA, which showed high expression levels in the 'P' variety. In summary, a combined transcriptomic and metabolomic analysis of hormone regulatory networks was performed to explore the mechanisms underlying metabolite changes in *Ch. speciosa* fruit during harvesting.

**Figure\_5.** Expression of genes involved in carbohydrate, flavonoid, and phenolic acid metabolism pathways, and plant phytohormone signal transduction. (A) Expression of DEGs related to carbohydrate, phenolic acid, and flavonoid metabolism pathways in *Ch. speciosa* fruit. (B) Heatmap of DEGs related to

plant hormone signal transduction pathways in different cultivars of *Ch. speciosa* fruit during harvesting. Purple to green indicates FPKM values of DEGs from low to high. The histogram represents DEM content, with the abscissa showing samples (from left to right: L-105, P-105, L-120, P-120) and the ordinate representing abundance values.

### 3.7 Co-expression networks reveal the relationships between DAMs, DEGs, and key differential hormones

We analyzed the interactions among metabolites, transcriptome data, and key differentially expressed hormones. Pathways were identified through transcriptome and metabolome co-enrichment, followed by Pearson's correlation analysis of DEGs and DAMs with respect to the expression of key differential hormones. Several metabolites were strongly correlated with hormones and DEGs (Fig. 6 and **Supplementary Table 5**). Among the physiological traits, catechin exhibited a strong positive correlation with SA, JA, and isochlorogenic acid A, whereas pinobanksin showed a negative correlation with GA<sub>3</sub>. Further analysis revealed that SA, GA, IAA, and other key hormones were strongly correlated with DEGs. Salicylic acid was highly positively correlated with key enzyme genes in various flavonoid pathways, including *LAR-2* (Cluster-29009.0), *4CL-3* (Cluster-34137.0), *DFR* (Cluster-21907.0), and *CHS* (Cluster-34728.7, Cluster-34728.5, Cluster-34728.0, etc.), with correlation coefficients greater than 0.89. These genes encode key enzymes in catechin biosynthesis. The salicylic acid response gene PR-1 also showed a strong positive correlation with catechin.

**Figure\_6.** Connection network between DEMs, DEGs, and key differential hormones in metabolic pathways. Key enzyme genes in saccharide, phenolic acid, and flavonoid pathways are marked with yellow circles; hormone-related DEGs with blue circles; DAMs (sugars, phenolic acids, and flavonoids) with red diamonds; and DAMs (hormones) with green inverted triangles. Red and blue lines indicate positive and negative correlations, respectively. Larger circles represent higher connectivity, and thicker lines indicate stronger correlation coefficients.

### 3.8 Validation of DEGs using qRT-PCR

A total of 12 key genes related to phenolic acid, flavonoid, and hormone signaling pathways were selected to validate the accuracy and reliability of the transcriptomic data by qRT-PCR. These genes included *PAL* (Cluster-22888.0), *CHS* (Cluster-34728.5, Cluster-34728.7, Cluster-35427.1), *F3H* (Cluster-29319.0), *CYP73A* (Cluster-6631.9, Cluster-6631.3), *CYP75B1-1* (Cluster-21670.1), *MYC2* (Cluster-4013.7), *GID2-2* (Cluster-33873.4), and *PR-1-3* (Cluster-17736.0). Gene-specific primers are listed in **Supplementary Table 6**. Overall, the expression levels of the genes detected by qRT-PCR were consistent with FPKM values, validating the accuracy and reliability of the RNA-seq data (Fig. 7).

**Figure\_7.** qRT-PCR and FPKM value analyses of twelve isoforms encoding enzymes involved in flavonoid biosynthesis in *Ch. speciosa* fruit at harvesting time. Data bars represent means  $\pm$  standard errors (n = 3).

## 4. Discussion

## 4.1 Variety-specific morphological and metabolic divergence at harvest

The two varieties of *Ch. speciosa* exhibited pronounced morphological divergence at harvest, primarily in fruit shape and calyx persistence. As a pome fruit, *Ch. speciosa* fruit flesh derives mainly from the fused hypanthium and sepal base (Liu et al., 2020). The persistence or abscission of the calyx, a trait distinguishing the cv. 'L' (persistent) and 'P' (deciduous), has been linked to internal quality in other Rosaceae fruits (Fan, Mattheis, & Fellman, 1998; Jin et al., 2022; Konarska, 2013). Notably, pear fruits with a persistent calyx typically exhibit higher acid content, firmer texture, and delayed ripening compared to calyx-shedding types (Xu et al., 2024; Wen et al., 2024). Our hormonal data support a similar interpretation: the persistent-calyx 'L' variety maintained significantly higher levels of GA<sub>8</sub>, GA<sub>19</sub>, ABA-GE, and specific cytokinins (iP9G, IP) at harvest, which are associated with cell division and early development. This hormonal signature suggests that 'L' fruits retain juvenile physiological characteristics for a longer duration, consistent with their slower post-maturation decline in phenolic and flavonoid content (Fig. 1B–C). In contrast, the deciduous-calyx 'P' variety exhibited elevated SA, cZROG, and OxIAA levels, along with rapid phenolic decline and accelerated sugar accumulation, indicative of more advanced developmental progression.

Metabolite profiling further revealed that the 'L' variety accumulated significantly higher levels of stachyose, trehalose-6-phosphate, and D-maltotetraose, which played roles in carbon status signaling and stress adaptation (Kerbl et al., 2023; Mollavali & Börnke, 2022). Notably, this is the first report of stachyose in *Ch. speciosa*, a functional oligosaccharide with low sweetness and low caloric content, offering potential for functional food development (Ta et al., 2024; Li et al., 2019b). Conversely, the cv. 'P' contained higher levels of quercetin glycosides, oleanolic acid, and betulinic acid, which have antifungal and anti-herbivore activities. These findings indicate that the two cultivars operate under fundamentally different developmental programs, with the 'L' variety favoring carbon accumulation and metabolic stability, and the 'P' variety investing more heavily in constitutive chemical defenses. From a practical perspective, the 'L' variety appears better suited for food applications due to its stable sugar profile, whereas the 'P' variety, with its higher peak phenolic content at early harvest, is more appropriate for medicinal use when harvested at 105 DAFB.

## 4.2 GA<sub>3</sub>, JA, and SA exert a crucial influence on the growth of *Ch. speciosa* fruits and the regulation of metabolites during the harvest window

Consistent with previous reports on Rosaceae fleshy fruits (Sánchez-Gómez et al., 2022; Aslam et al., 2019), both *Ch. speciosa* cultivars exhibited a decline in phenolic acids and flavonoids during the harvest period, accompanied by an increase in carbohydrate levels. The identified bioactive compound, including chlorogenic acid, caffeic acid, catechin, epicatechin, and various flavonoids which align with known bioactive components of *Chaenomeles* (Li et al., 2024; Gao et al., 2020). Our integrated multi-omics analysis identified GA, SA, and JA as core regulatory hormones driving these metabolic shifts.

Gibberellin (GA), specifically GA<sub>3</sub>, emerged as a key regulator. Both cultivars showed a marked increase in GA<sub>3</sub> and GA<sub>5</sub> (an inhibitor of GA<sub>3</sub>-oxidase) during late harvest, whereas GA<sub>4</sub> decreased and GA<sub>1</sub> was not detected. This pattern suggests that GA<sub>3</sub> is the predominant bioactive GA form during the *Ch. speciosa* harvest window. Exogenous GA<sub>3</sub> is known to increase firmness and delay ripening in both climacteric and non-climacteric fruits (Kuhn et al., 2021; Li et al., 2019c; Wu et al., 2024; Feng et al., 2025), primarily by antagonizing ripening-promoting hormones. In our study, elevated GA<sub>3</sub> and GA<sub>5</sub> levels coincided with sharp declines in SA and JA contents, as well as downregulation of *MYC2* transcripts and downstream flavonoid biosynthetic genes. Notably, while total jasmonates (excluding methyl jasmonate) decreased during harvest, methyl jasmonate (MeJA) accumulated at 120 DAFB, which may reflect a specific role in volatile-mediated inter-organ communication.

JA and SA are defense hormones that also influence fruit quality. JA has been shown to enhance anthocyanin and phenolic accumulation in apples, blueberries, grapes, and strawberries (Shafiq et al., 2013; Ruiz-Garcia et al., 2012; Garrido-Bigotes et al., 2018). In apples, JA peaks during early development and declines sharply at full ripeness (Fan et al., 1998), this pattern is coordinated with our *Ch. speciosa* data. The antagonistic interaction between GA and JA signaling, mechanistically elucidated in model plants, involves DELLA proteins (negative GA regulators) interacting with JAZ proteins to modulate *MYC2* activity (Hou et al., 2010; Wang et al., 2023; Mu et al., 2025). GA promotes DELLA degradation, releasing JAZ to bind and suppress *MYC2*, thereby attenuating JA-responsive gene expression. Our correlation analyses in *Ch. speciosa* revealed expression patterns consistent with this model: *DELLA* genes were positively correlated with *JAZs* and *MYC2s*, while GA content increased coincident with decreased JA levels and downregulation of JA-responsive genes. SA biosynthesis can occur via the ICS or PAL pathway (Wang et al., 2025; Lefevere et al., 2020). In our study, the 'P' variety—which exhibited a more pronounced decline in phenolics—also showed higher SA levels, suggesting a potential link between SA signaling and the rapid turnover of phenolic compounds. Collectively, our data support a possible model in which GA<sub>3</sub> acts antagonistically with SA and JA to regulate the transition from active secondary metabolism (high phenolics/flavonoids) to carbohydrate accumulation during the *Ch. speciosa* harvest window, with variety-specific hormonal balances dictating the extent of these metabolic shifts.

**Figure\_8.** Proposed model from correlation analysis for the transcriptional cascade regulating metabolite transformation patterns during the harvesting process of *Ch. speciosa*.

## 4.3 Practical implications for variety-specific harvest optimization

The integrated multi-omics dataset presented here, combined with our previously established metabolic trajectory of *Ch. speciosa* maturation (Fang et al., 2023), enables evidence-based refinement of harvest strategies for the two widely cultivated varieties. For medicinal applications—targeting phenolic acids and flavonoids, the 'P' variety harvested at 105 DAFB provides optimal material. At this time point, total phenolic content in 'P' fruits is approximately 35% higher than in 'L' fruits. However, cv. 'P' undergo rapid

post-105 DAFB decline in these components; therefore, the harvest window for medicinal-grade 'P' fruit is relatively narrow. In contrast, the cv. 'L' maintains more stable phenolic content across the harvest period, offering greater operational flexibility for large-scale medicinal material production despite slightly lower peak values. For edible/processing applications, the 'L' variety harvested at 120 DAFB presents distinct advantages. The accumulation of stachyose, D-maltotetraose, and sugar phosphates in 'L' fruits enhances nutritional value and processing characteristics. Trehalose-6-phosphate, beyond its role as a metabolic intermediate, functions as a central signaling metabolite integrating carbon status with stress tolerance (Kerbler et al., 2023); its accumulation suggests improved postharvest resilience in 'L' fruits.

## 5. Conclusion

This study provides the first integrated biochemical, metabolomic, transcriptomic, and phytohormone profiling of two *Ch. speciosa* cultivars at harvest. We demonstrate that there were significant differences between cultivars during the harvest period. The metabolic divergence is underpinned by distinct hormonal signatures, particularly GA<sub>3</sub>, JA, and SA, which orchestrate the transition from phenolic/flavonoid accumulation to carbohydrate metabolism in the harvest window. The persistent-calyx 'L' variety exhibits greater metabolic stability and accumulates higher levels of stachyose and trehalose-6-phosphate, whereas the deciduous-calyx 'P' variety shows higher peak phenolic content but rapid post-harvest decline. Through correlation analysis of differentially expressed genes, differentially accumulated metabolites, and phytohormones, we constructed a regulatory network linking hormone signaling to the biosynthesis of sugars, flavonoids, and phenolic acids. These findings establish a scientific basis for cultivar-differentiated harvest strategies and provide a multi-omics framework applicable to harvest optimization in *Ch. speciosa* fruits. Our study also offers a foundational resource for future functional validation of hormone-responsive genes and for the development of value-added products from *Ch. speciosa* genetic resources.

## Abbreviations

cv 'P', cultivar 'Luohanqi'

cv 'L', cultivar 'Pingguo'

DAFB, days after full bloom

DEGs, differentially expressed genes

DAMs, differentially accumulated metabolites;

SUS, sucrose synthase;

UGDH, UDP-glucose 6-dehydrogenase;

INV, beta-fructofuranosidase;

GLA, alpha-galactosidase;

GULO, L-gulonolactone oxidase;

PAL, phenylalanine ammonia-lyase;

4CL, 4-coumarate-CoA ligase;

CYP73A, trans-cinnamate 4-monooxygenase;

1L4I1, L-amino-acid oxidase;

CHS, chalcone synthase;

F3H, flavanone 3-dioxygenase;

CHI, chalcone isomerase;

DFR, dihydroflavonol 4-reductase;

CYP75B1, flavonoid 3'-monooxygenase;

ANS, anthocyanidin synthase;

ANR, anthocyanidin reductase;

LAR, leucoanthocyanidin reductase;

CRE1, arabidopsis histidine kinase 2/3/4;

AHP, histidine-containing phosphotransfer protein;

B-ARR, two-component response regulator ARR-B family;

A-ARR, two-component response regulator ARR-A family;

GID1, gibberellin receptor GID1;

GID2, F-box protein GID2;

DELLA, DELLA protein;

PIF4, phytochrome-interacting factor 4;

NPR1, regulatory protein NPR1;

TGA, transcription factor TGA;

PR-1, pathogenesis-related protein 1;

PYL, abscisic acid receptor PYR/PYL family;

PP2C, protein phosphatase 2C;

SnRK2, serine/threonine-protein kinase SRK2;

ABF, responsive element binding factor;

AUX1, auxin influx carrier;

TIR1, transport inhibitor response 1;

AUX/IAA, auxin-responsive protein IAA;

ARF, auxin response factor;

GH3, auxin responsive GH3 gene family;

SAUR, SAUR family protein;

JAR1, jasmonic acid-amino synthetase;

COI1, coronatine-insensitive protein 1;

JAZ, jasmonate ZIM domain-containing protein;

MYC2, transcription factor MYC2;

FPKM, fragments per kilobase of transcript per million mapped reads;

KEGG, Kyoto Encyclopedia of Genes and Genomes;

VIP, Variable importance in projection;

OPLS-DA, orthogonal projections to latent structures discriminant analysis;

## Declarations

### Ethics approval and consent to participate

This study does not involve human participants or animals, and therefore ethics approval and consent to participate are not applicable.

## Consent for publication

All authors have read and approved the final version of the manuscript. They consent to the submission of this manuscript to the journal for peer review and potential publication.

## Availability of data and material

All original data supporting the findings of this study are provided within the article and the supplementary materials. Any additional data or materials not included in the manuscript can be obtained from the corresponding author upon reasonable request. The sequenced raw reads produced in this research have been submitted to the National Center for Biotechnology Information (NCBI) with BioProject ID: PRJNA1516665.

## Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Funding

This work was supported by Key project at central government level: The ability establishment of sustainable use for valuable Chinese medicine resources (2060302); MOE-Anhui Joint Collaborative Innovation Center For Quality Improvement of Anhui Genuine Chinese Medicinal Materials (xtcx202403); The University Synergy Innovation Program of Anhui Province (GXXT-2023-072); and Anhui Province Higher Education Scientific Research Project (2023AH050729).

## Author contributions

Qingying Fang contributed to writing – original draft, methodology, and formal analysis; Yan Fang and Yubin Fang analyzed the omics data and drafted the manuscript; Letian Zheng, Yujie Wu, and Guangqi Xu assisted with sample collection and biochemicals determination; Yong Yao provided *Chaenomeles speciosa* production management and sample resources; Liangping Zha and Daiying Peng acquired the research funding. All authors reviewed the manuscript.

## Acknowledgements

The authors wish to acknowledge the contributions of all individuals who supported this work.

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## Figures

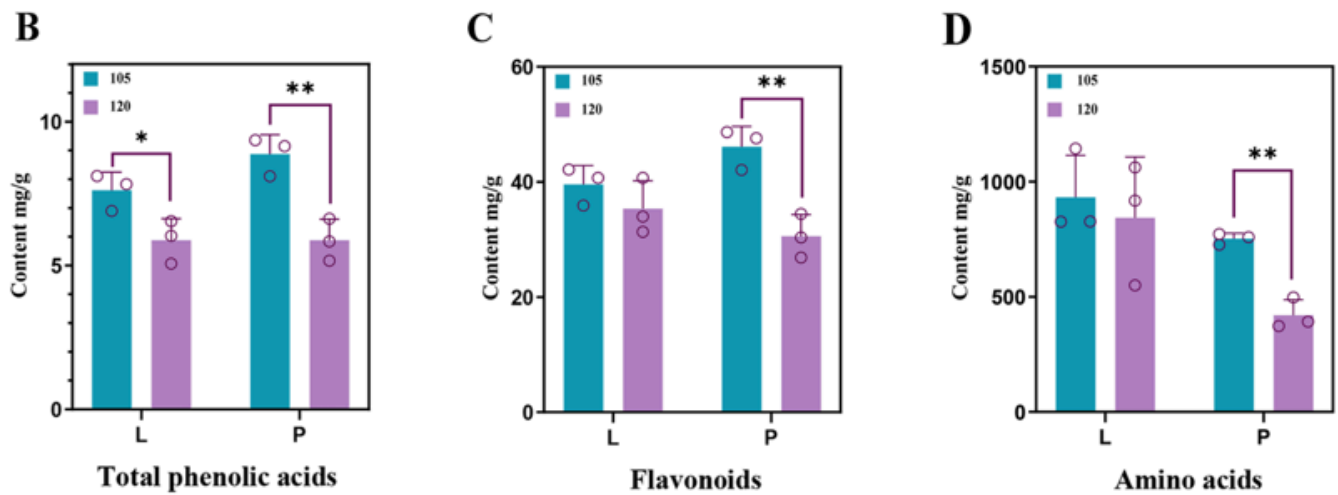


**Figure 1**

Morphological characteristics and major phytochemical contents of *Ch. speciosa* fruit during harvest. (A) Appearance of fruit cultivars during harvesting time (near-maturation and maturation stages). (B–D) Total phenolic acids, flavonoids, and amino acid contents of four samples. ‘L’ represents cv. ‘Luohanqi’; ‘P’ represents cv. ‘Pingguo’; ‘105’ represents ‘105 days after full bloom’ for early harvest; and ‘120’ represents ‘120 days after full bloom’ for late harvest. Data bars represent means  $\pm$  standard errors (n = 3). \*, p < 0.05; \*\*, p < 0.01.

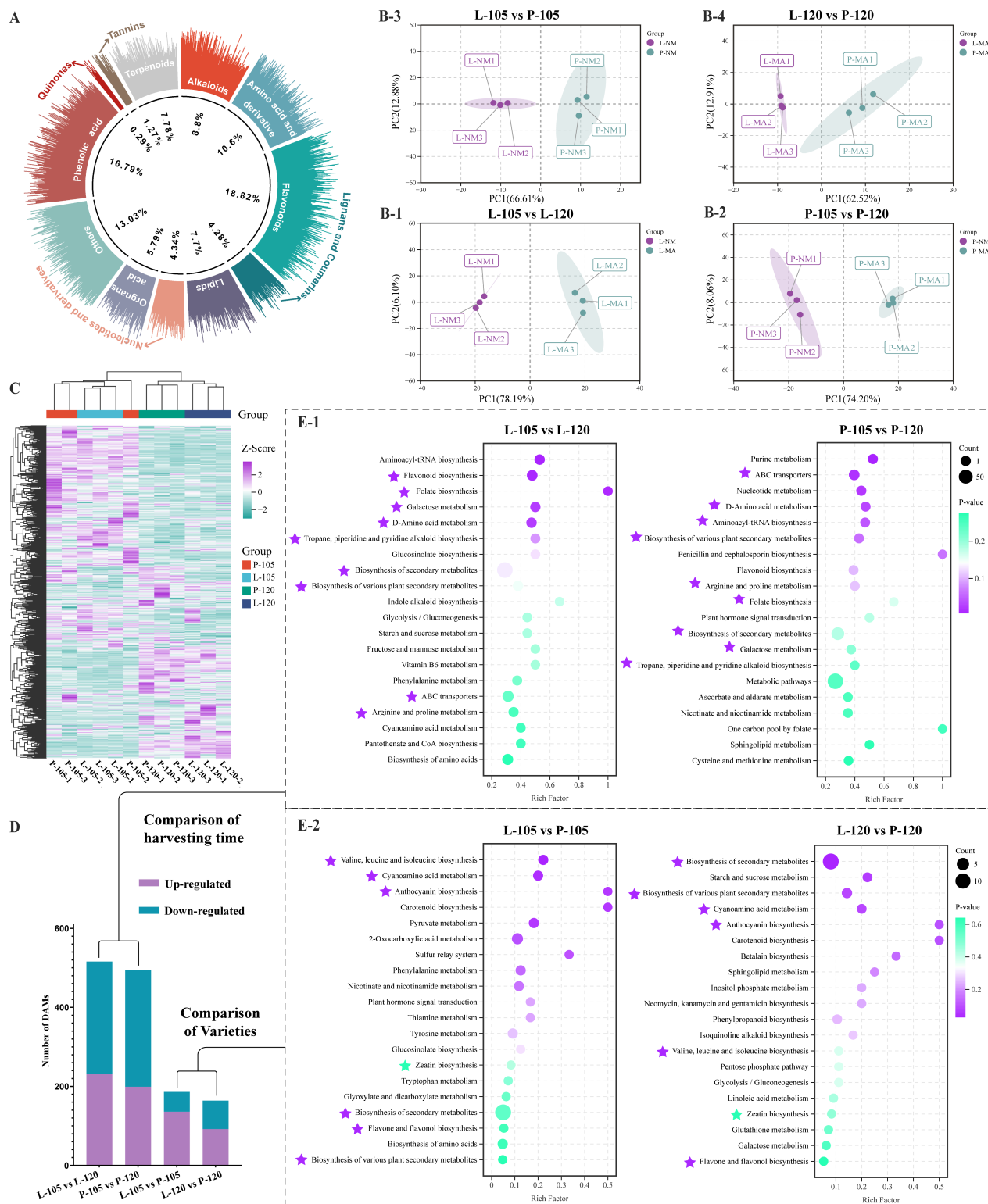


**Figure 2**

Metabolomic profiling and dynamic metabolic changes in *Ch. speciosa* fruit cultivars during the harvesting stage. (A) Hierarchical pie chart depicting the relative abundance of all metabolites. (B) PCA score plot of metabolome data for the four comparisons. (C) Heatmap showing the expression patterns of metabolites. (D) Number of metabolites differing in relative abundance among comparison groups across harvest times and cultivars. (E-1) KEGG enrichment analysis of DAMs between harvest times ('L-

105 vs. L-120', 'P-105 vs. P-120'). (E-2) KEGG enrichment analysis of DAMs between cultivars ('L-105 vs. P-105', 'L-120 vs. P-120'). Star markings represent repeated pathways across comparison groups.

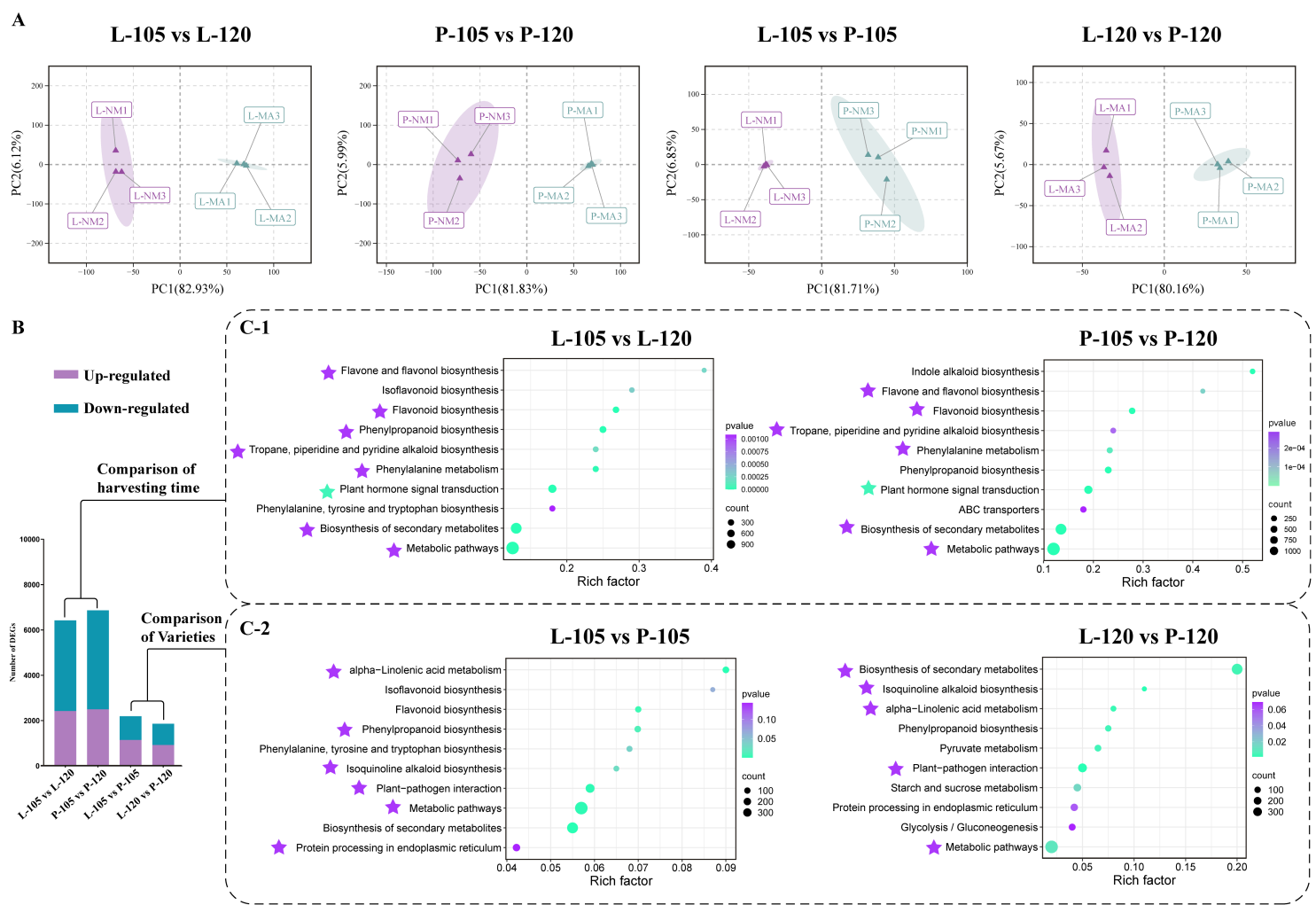


**Figure 3**

Dynamic transcriptomic changes of different *Ch. speciosa* cultivars during the harvest period. (A) PCA score plot of metabolome data in the four comparisons. (B) The number of metabolites with differences in relative abundance between the comparison groups in harvesting time and in cultivars. (C-1) KEGG enrichment analysis of DAMs in the harvesting time ('L-105 vs. L-120', 'P-105 vs. P-120'). (C-2) KEGG enrichment analysis of DAMs in the cultivars ('L-105 vs. P-105', 'L-120 vs. P-120'). The star markings represent repeated pathways in comparison groups. (D) The biosynthesis pathways of saccharides, phenolic acids, and flavonoids in *Ch. speciosa* fruit. The heatmap represents the expression of corresponding structural DEGs in different maturation stages; blue to yellow indicates the FPKM values of DEGs ranging from low to high. The histogram represents DEMs content, the abscissa presents the samples (from left to right show L-105, P-105, L-120, and P-120), and the ordinate represents the abundance value.

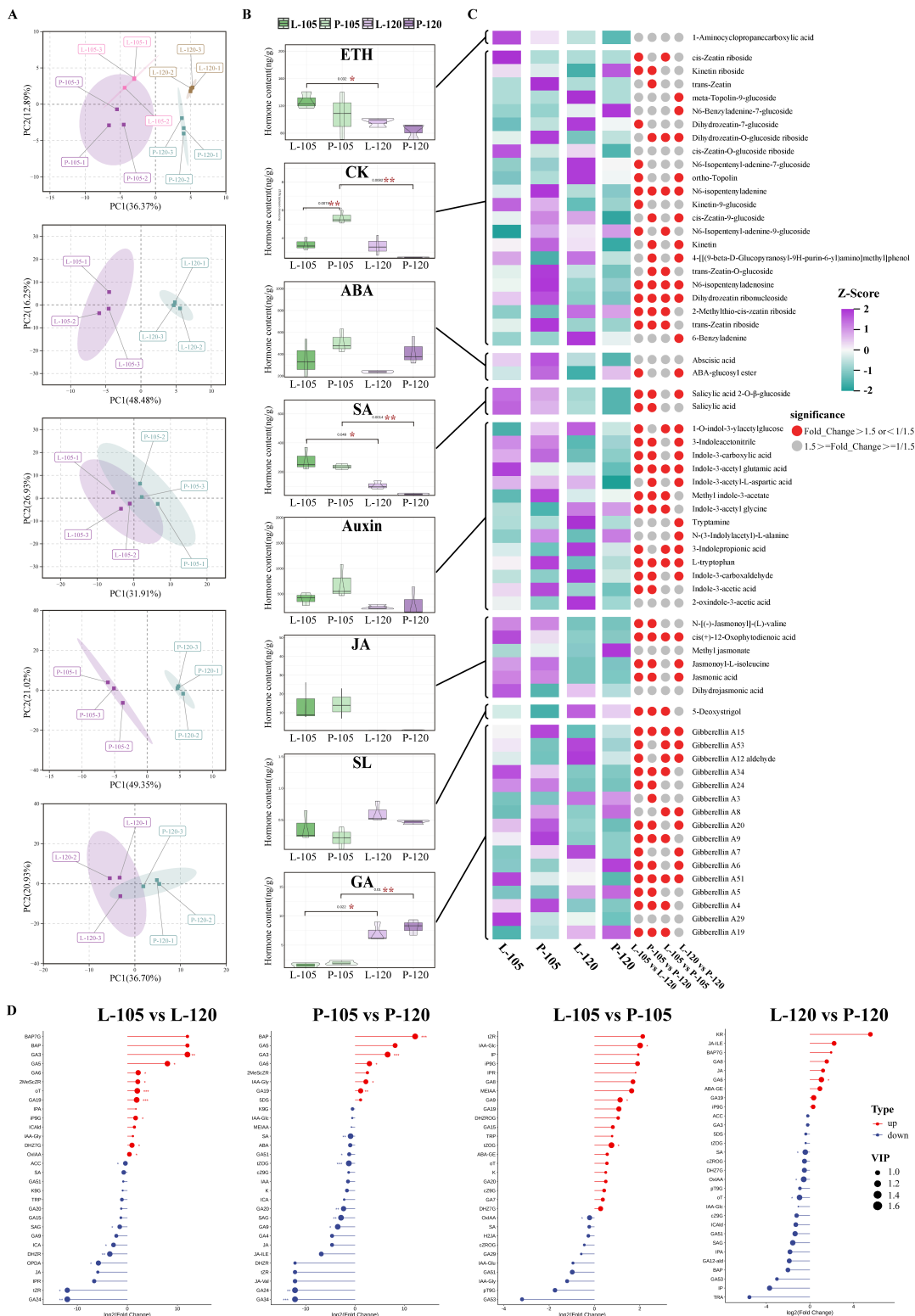
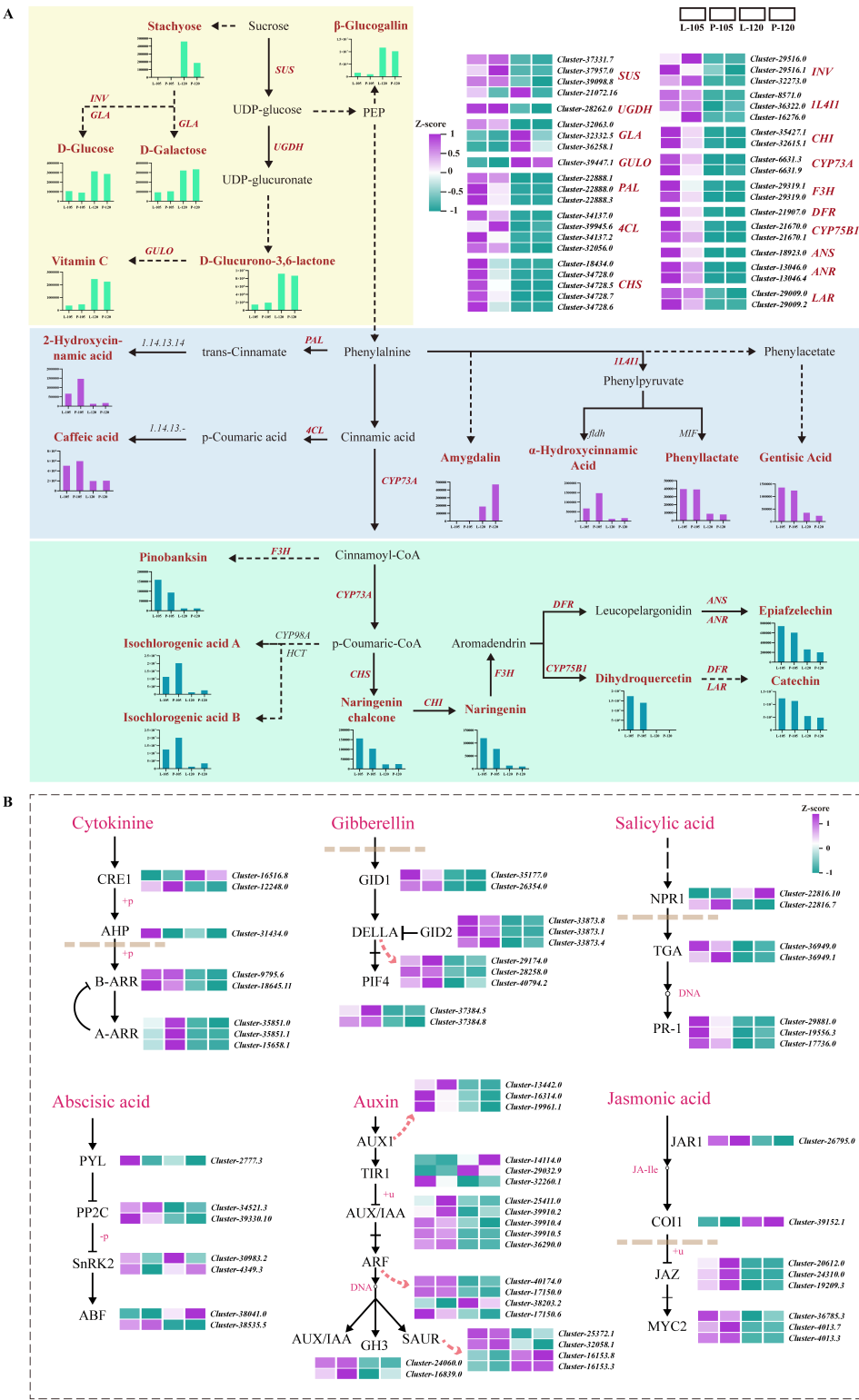


**Figure 4**

Hormone content analysis of *Ch. speciosa* cultivars during the harvest period and screening analysis for key differential hormones. (A) Principal component analysis of hormone content in different cultivars during the harvest period; (B) Analysis of different categories of hormone content; (C) Heatmap analysis of hormone content and categories. Numbers between groups indicate significant differences ( $p < 0.05$ ). Red dots indicate differential hormones with FC greater than 1.5 or less than 1/1.5 in the control groups;

(D) Screening analysis by VIP-FC value lollipop plot of key differential hormones. The circles represent the VIP value. The larger the circle, the higher the VIP level. Among them, the hormones with a VIP value greater than 1 and ranking in the top 20 in each comparison group are displayed in the VIP-FC lollipop chart.



Expression of genes involved in carbohydrate, flavonoid, and phenolic acid metabolism pathways, and plant phytohormone signal transduction. (A) Expression of DEGs related to carbohydrate, phenolic acid, and flavonoid metabolism pathways in *Ch. speciosa* fruit. (B) Heatmap of DEGs related to plant hormone signal transduction pathways in different cultivars of *Ch. speciosa* fruit during harvesting. Purple to green indicates FPKM values of DEGs from low to high. The histogram represents DEM content, with the abscissa showing samples (from left to right: L-105, P-105, L-120, P-120) and the ordinate representing abundance values.

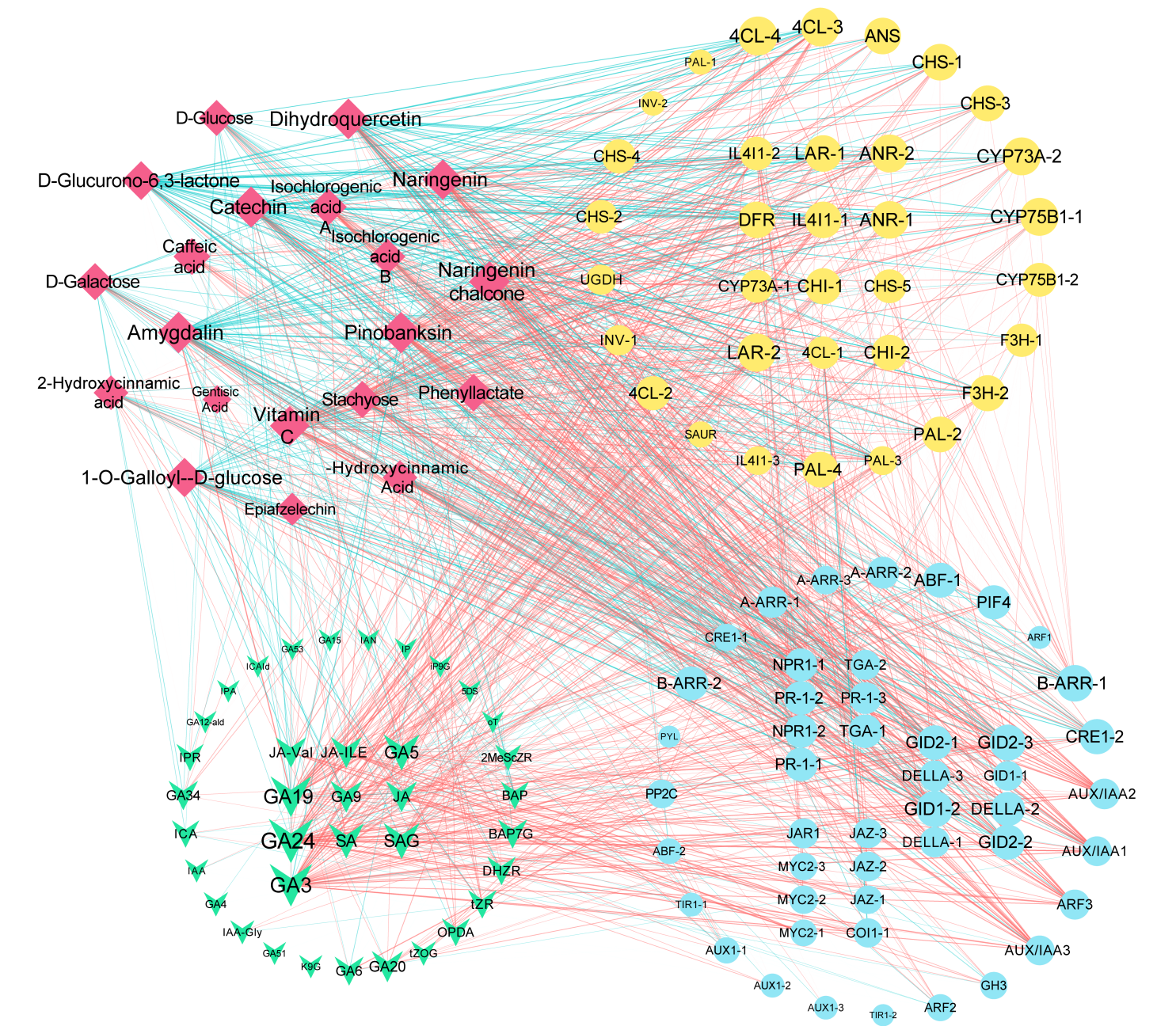


Figure 6

Connection network between DEMs, DEGs, and key differential hormones in metabolic pathways. Key enzyme genes in saccharide, phenolic acid, and flavonoid pathways are marked with yellow circles; hormone-related DEGs with blue circles; DAMs (sugars, phenolic acids, and flavonoids) with red diamonds; and DAMs (hormones) with green inverted triangles. Red and blue lines indicate positive and negative correlations, respectively. Larger circles represent higher connectivity, and thicker lines indicate stronger correlation coefficients.

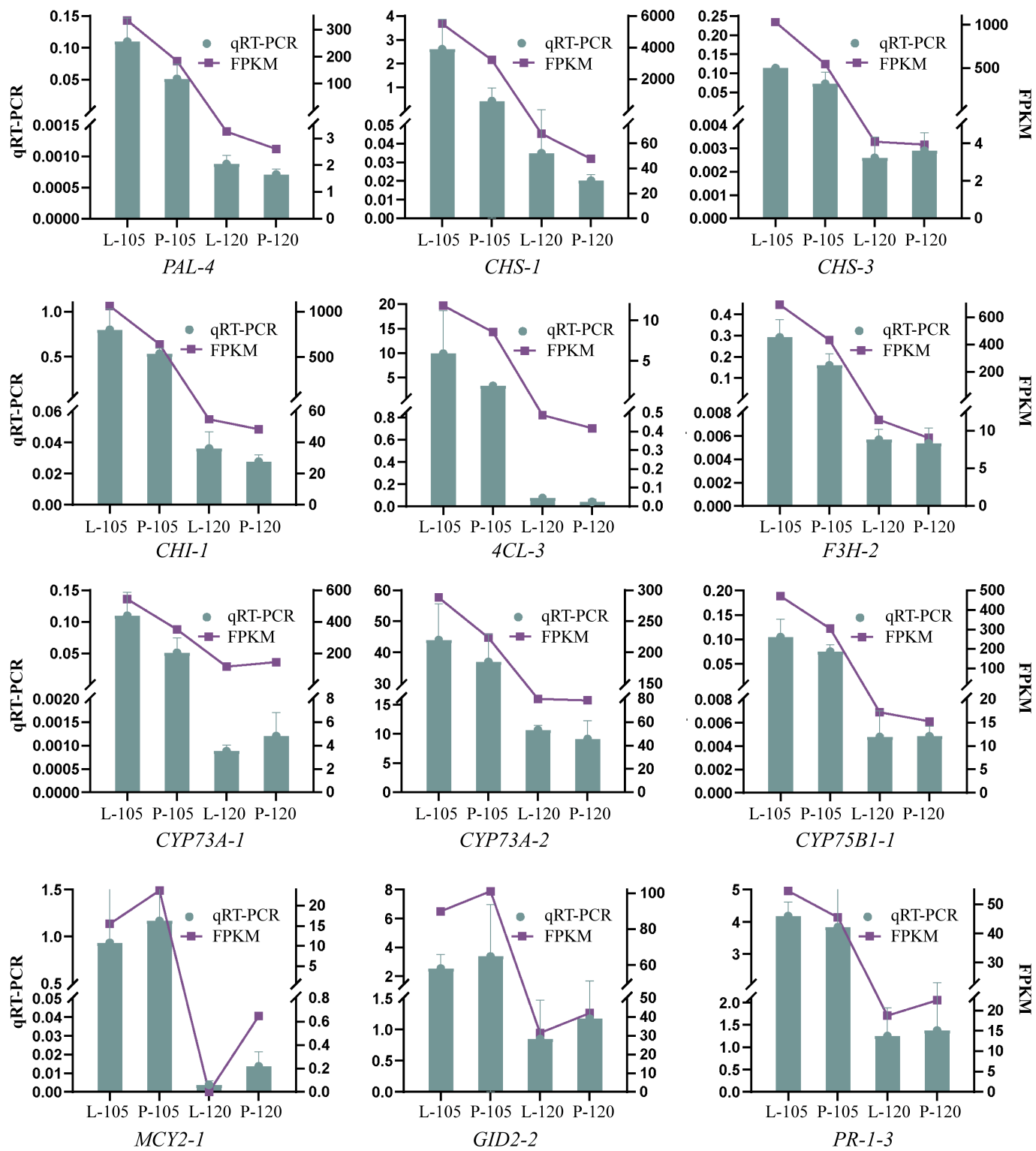


Figure 7

qRT-PCR and FPKM value analyses of twelve isoforms encoding enzymes involved in flavonoid biosynthesis in *Ch. speciosa* fruit at harvesting time. Data bars represent means  $\pm$  standard errors (n= 3).

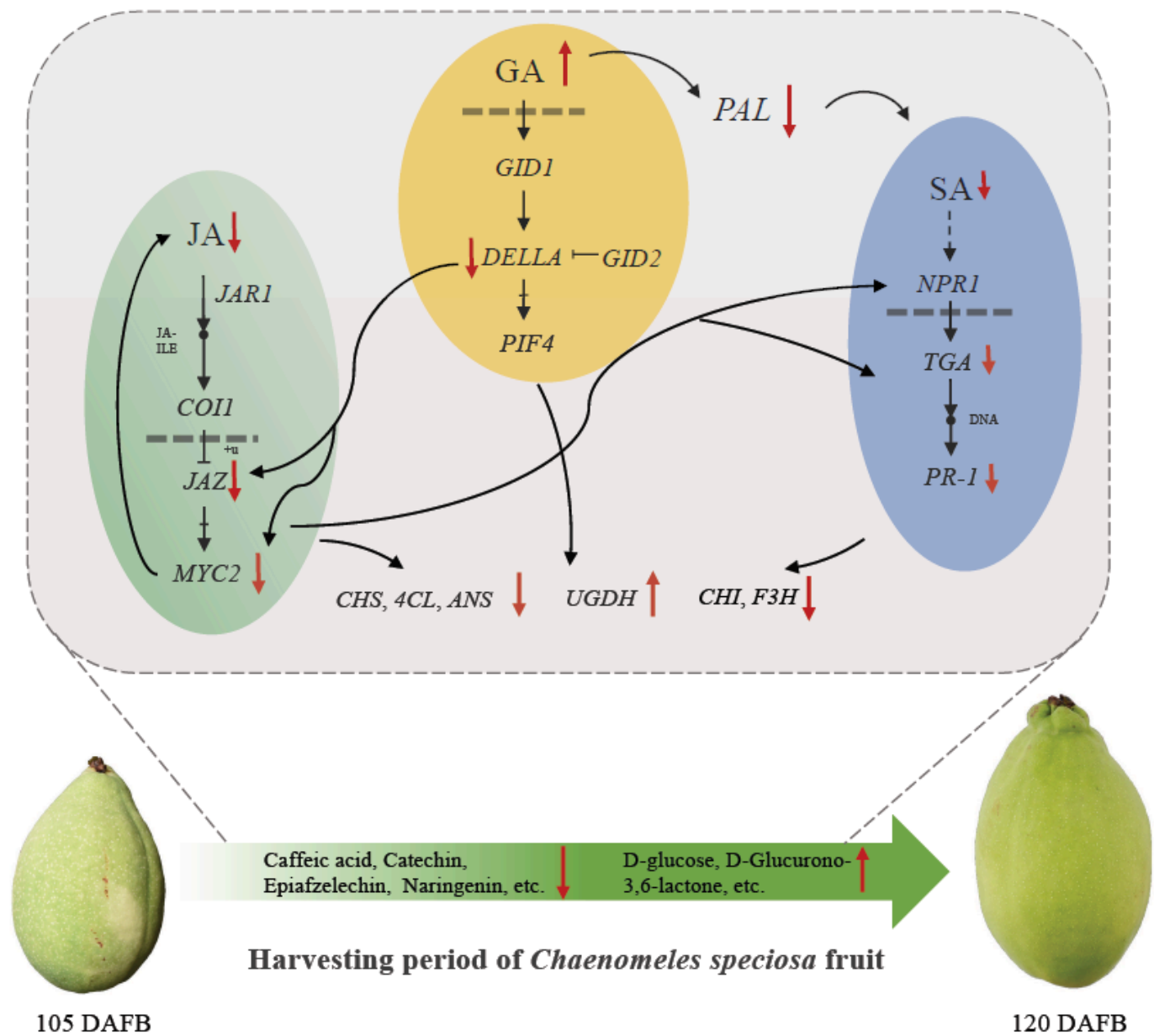


Figure 8

Proposed model from correlation analysis for the transcriptional cascade regulating metabolite transformation patterns during the harvesting process of *Ch. speciosa*.

## Supplementary Files

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

- [SupplementaryTable16.xlsx](#)
- [FigureS1PCA20250815.png](#)
- [FigureS2OPLSDA20250815.png](#)