

# Transcriptome profiling reveals the regulatory mechanisms of ascorbic acid and flavonoid synthesis and metabolic processes in fruit development of *Ribes nigrum*

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## Research Article

**Keywords:** *Ribes nigrum* L., Transcriptional analysis, AsA, Flavonoids, Plant hormone, qRT-PCR, Expression analysis

**Posted Date:** October 6th, 2023

**DOI:** <https://doi.org/10.21203/rs.3.rs-3330314/v1>

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

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# Abstract

## Background

The blackcurrant (*Ribes nigrum* L.) is rich in nutritional value. It is rich in ascorbic acid and flavonoids with excellent antioxidant properties. Fruit quality is an important factor affecting subsequent processing and production, yet the molecular mechanisms underlying the transcriptional regulation of blackcurrant fruit quality are largely unknown.

## Results

In the current study, comparative transcriptome analysis investigated the similarities and differences between two blackcurrant varieties: 'Adelinia' and 'Heifeng' at four fruit developmental stages (young, expansion, veraison, and ripe fruit). Additionally, we observed variations in a number of physiological indicators during the growth of blackcurrant fruits. The results indicate that, during the development of blackcurrant fruits, ascorbic acid content continued to decrease, whereas flavonoid content was lowest in the veraison period. The fruit size of 'Adelinia' variety was larger than that of 'Heifeng' variety throughout the developmental. Based on the results of the transcriptome sequencing data, a total of 4295 up-regulated genes and 3529 down-regulated genes were obtained between 'Adelinia' and 'Heifeng' varieties; A total of 6,349 up-regulated and 5,770 down-regulated genes were identified between the four developmental periods, respectively. The expression of 4 genes, *PMM*, *APX*, *GME*, and *PGI* has a strong correlation with AsA content. while two *APX*, two *AO*, and one *MDHAR* genes showed a strong negative correlation with AsA content. A total of 19 genes related to flavonoid biosynthesis were screened. The KEGG pathway enrichment analysis showed that many DEGs were enriched in the plant hormone signal transduction (ko04075) pathway and the photosynthesis-antenna proteins (ko00196) pathway.

## Conclusions

We predicted that the *PMM*, *APX*, *GME*, and *PGI* genes, which are positively correlated with ascorbic acid, may play an important role in the biosynthesis of AsA; while the negatively correlated *APX*, *AO*, and *MDHAR* genes may be critical for AsA in the recycling pathway. *DFR*, *ANR*, *CHI*, *CHS*, *4CL*, and *LAR* genes are important regulators of flavonoid synthesis. Also predicted the regulatory influences of various plant hormones on the fruit size of blackcurrants. We provide genetic resources for improving fruit quality and size as well as for rationalizing the use of plant growth regulators to improve fruit quality.

## 1. Background

Blackcurrant (*Ribes nigrum* L.) is a perennial shrub that produces sweet and sour fruit packed with a wide range of nutrients. Biochemical analysis of blackcurrants has revealed the presence of numerous components, including flavonoids, polyunsaturated fatty acids, structural and non-structural

carbohydrates, non-volatile organic acids, tannins, and astragals[1]. Ascorbic acid (AsA), which can enhance antioxidant capacity and health potential[2], is also found in blackcurrants at higher levels than in raspberries and blueberries[3]. The high nutritional, medical, health, and cosmetic value of blackcurrant fruits is due to their bioactive components, which are effective in reducing thrombosis, inhibiting tumor growth[4], lowering cholesterol[5], inhibiting salmonella enteritidis[6], and preventing major diseases such as asthma, cardiovascular disease, heart disease, and hypertension[7]. In addition, blackcurrant is an effective anti-microbial agent[8] and has the potential to be used as a disinfectant and antiseptic[9].

The antioxidant properties of blackcurrants are largely due to their phenolic compounds, such as ANC, which can act as hydrogen donors or electron transfer agents depending on their chemical structure. The antioxidant activity of ANC is directly related to its chemical structure, and the differences in the antioxidant activity of flavonoids are due to differences in the type, position, and number of methyl and hydroxyl groups[10]. Blackcurrant fruits have significant value in nutrition, medicine, health, and beauty. Their bioactive components can effectively reduce blood clot formation, inhibit tumor growth, lower cholesterol levels[5], suppress Salmonella enteritidis infection[6], and prevent/treat major diseases such as asthma, cardiovascular disease, heart disease, and hypertension[7]. Additionally, blackcurrants are effective antimicrobial agents[8] and hold potential in the production of disinfectants and preservatives[9].

The rapid development of high-throughput sequencing technology has greatly improved the efficiency of gene discovery[11, 12]. The advancement of sequencing technology has provided powerful tools for scientists to study plant transcriptomes. Compared to woody plants, fruit trees have relatively less genomic information and have therefore been less extensively studied. Transcriptomes play a vital role in overcoming the bottleneck that hinders the development of fruit tree genomes. Currently, RNA-Seq has been widely applied to most fruit trees, such as sweet orange[13, 14], apple[15, 16], blackberry[17, 18], kiwi[19], blueberry[20], lychee[21], Chinese cherry[22], dragon fruit[23], pear[24], strawberry[25], raspberry[26], plum[24], and others. It has been used to investigate the synthesis of carotenoids in yellow-fleshed kiwi[27], gene expression changes during the maturation and development of blueberry[20], plum[24], and kiwi fruits[19], genetic differences among different varieties of dragon fruit[23] and strawberry fruits[25], changes in the cuticle layer during different stages of navel orange fruit[13], and the production of differentially expressed genes (DEGs) under Huanglongbing bacteria and decline viruses in citrus[14]. However, the fruit tree genome still faces several challenges, including imperfect bioinformatics studies and the difficulty of identifying key genes responsible for physiological changes. The quality of blackcurrant fruit not only determines its appearance, storage potential, and transportability but also affects subsequent product development and quality. The Northeast region of China has abundant blackcurrant germplasm resources and multiple varieties but lacks comprehensive blackcurrant varieties with superior economic traits. To explore the genetic basis of blackcurrant fruit quality, this study utilized RNA-Seq combined with de novo transcriptome assembly to analyze the differences in fruit quality during ripening between two varieties, 'Adelinia' and 'Heifeng'. The key genes involved in AsA biosynthesis and recycling, flavonoid biosynthesis, and plant hormone signal transduction were identified by integrating physiological experimental results. This study aims to investigate the major candidate genes influencing

fruit quality, laying the foundation for future research on gene function and high-quality blackcurrant fruit cultivation.

## **2. Results**

### **2.1. Changes in fruit weight, transverse and longitudinal diameter during the development of 'Adelinia' and 'Heifeng'**

During fruit growth and development, the variation in fruit weight and longitudinal and cross-sectional diameter between 'Adelinia' and 'Heifeng' was investigated (Fig. 1). The single fruit weight of 'Adelinia' was consistently greater than that of 'Heifeng', and the weight of the ripe fruit of 'Adelinia' was three times of 'Heifeng'. The most significant changes in fruit weight and transverse and longitudinal diameters occurred during the period from expansion to veraison, demonstrating an S-shaped trend of "slow-fast-slow". During fruit development, the longitudinal diameter developed faster, resulting in an oval fruit shape during the young fruit stage. After veraison, the transverse diameter gradually became larger than the longitudinal diameter, resulting in a spherical fruit shape.

### **2.2. Changes in fruit quality during the development of 'Adelinia' and 'Heifeng'**

The contents of soluble solids and titratable acids in blackcurrant fruits increased with fruit development (Table 1). Soluble solids increased the slowest from the young fruit stage to the expansion stage and the fastest from the veraison stage to the ripe stage. The contents of soluble solids increased by 4.6% and 3.0% from the veraison stage to the ripe stage in 'Heifeng' and 'Adelinia' respectively. The contents of soluble solids in 'Heifeng' were consistently higher than that in 'Adelinia' throughout the fruit development, reaching 1.4 times higher than that of 'Adelinia' at the ripe fruit stage.

Table 1  
Changes in quality parameters during fruit development of different cultivars of blackcurrant

| Varieties                                                                                                                                                                          | Developmental stage | The soluble solids (%) | Titrateable acid (%) | Ascorbic acid (mg/g) | Total phenol (mg/100g) | Flavonoid (mg/100g) |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|------------------------|----------------------|----------------------|------------------------|---------------------|
| Heifeng                                                                                                                                                                            | young fruit         | 7.6 ± 0.52cd           | 1.34 ± 0.02d         | 16.32 ± 0.05a        | 17.52 ± 0.04b          | 11.29 ± 0.93b       |
|                                                                                                                                                                                    | expansion fruit     | 8.2 ± 0.64c            | 1.97 ± 0.11b         | 15.56 ± 0.32b        | 13.68 ± 0.07d          | 3.24 ± 0.69c        |
|                                                                                                                                                                                    | veraison fruit      | 11.3 ± 0.97b           | 2.36 ± 0.21ab        | 11.33 ± 0.10c        | 0.13 ± 0.05h           | 0.26 ± 0.07e        |
|                                                                                                                                                                                    | ripe fruit          | 15.9 ± 0.72a           | 2.55 ± 0.45ab        | 7.62 ± 0.08f         | 0.55 ± 0.03f           | 2.18 ± 0.21d        |
| Adelinia                                                                                                                                                                           | young fruit         | 5.3 ± 0.44d            | 1.63 ± 0.05c         | 16.61 ± 0.91a        | 18.36 ± 0.06a          | 12.23 ± 0.27a       |
|                                                                                                                                                                                    | expansion fruit     | 6.4 ± 0.37d            | 2.45 ± 0.12ab        | 15.87 ± 0.99b        | 15.39 ± 0.07c          | 3.58 ± 0.34c        |
|                                                                                                                                                                                    | veraison fruit      | 8.5 ± 0.62c            | 3.07 ± 1.02a         | 10.18 ± 0.07d        | 0.16 ± 0.01g           | 0.32 ± 0.06e        |
|                                                                                                                                                                                    | ripe fruit          | 11.5 ± 0.43b           | 3.36 ± 0.58a         | 8.85 ± 0.03c         | 1.05 ± 0.01e           | 2.24 ± 0.28d        |
| Note: Data are expressed as mean ± standard deviation. Different letters within the same column indicate significant differences (p < 0.05) based on Duncan's multiple range test. |                     |                        |                      |                      |                        |                     |

The contents of titrateable acid in 'Adelinia' and 'Heifeng' increased most rapidly from the young fruit stage to the expansion stage, with the content increasing by 0.63% and 0.82%, respectively. The rate of increase in titrateable acid gradually slowed down from the expansion stage to the ripe stage. The content of titrateable acid in 'Adelinia' was consistently higher than that of 'Heifeng' throughout fruit development, reaching 1.3 times higher than that of 'Heifeng'.

The AsA content in the fruit showed a continuous decreasing trend during growth and development, with the highest content in the young fruit stage and the lowest in the ripe fruit. The AsA content decreased most from the expansion stage to the veraison stage, with 'Heifeng' decreasing by 4.23 mg/g and 'Adelinia' decreasing by 5.69 mg/g. The AsA content in the young, expansion, and ripe fruit of 'Adelinia' was higher than that of 'Heifeng'.

The changing trend of total phenols and total flavonoids was similar during fruit growth and development, with the maximum value observed in the young fruit stage and the minimum value in veraison fruit, which slightly increased in ripe fruit.

The contents of total phenols and flavonoids from the expansion stage to the veraison stage decreased the most in 'Heifeng' and 'Adelinia'. The content of total phenols decreased by 13.6 mg/100g and 15.23 mg/100g in 'Heifeng' and 'Adelinia', respectively. The content of total flavonoids decreased by 5.11 mg/100g and 7.95 mg/100g in 'Heifeng' and 'Adelinia', respectively. The contents of total phenols and flavonoids in 'Adelinia' fruit were consistently higher than those in 'Heifeng' fruit throughout the fruit development. The highest total phenolic content was 1.9 times that of 'Heifeng', and the highest total flavonoid content was 1.5 times that of 'Heifeng'.

## **2.3. Transcriptome sequencing quality of blackcurrant fruit and denovo-assemble of unigenes**

In order to explore the difference in gene expression in the two varieties during fruit ripening, transcriptome sequencing was performed on young, expansion, veraison, and ripe fruit stages of 'Adelinia' and 'Heifeng'. After removing low-quality reads, a total of 186.71 Gb reads were obtained from 24 samples, with an average of 6.74 Gb per sample (Table S2). The Q30 base percentage of all samples was greater than 94.08%, and GC content ranged from 44%-47%, indicating good sequencing quality and credible results.

Then the clean reads of all the samples were used for de novo assembly, and a total of 220,757 transcripts and 116,793 unigene were obtained. The average length was 1,129 bp and 729 bp respectively. The N50 was 1,998 and 1,342 respectively (Table S3), indicating that the assembly results were credible. Unigene length ranged from 150 to 16,047 bp, with most unigenes being 300 to 2,000 bp in length.

## **2.4. Gene function annotation, GO, and KOG functional classification**

All assembled unigenes were annotated with databases. A total of 23,788, 41,786, 22,044, 35,830, 41,872, 29,652, 64,522, and 66,902 unigenes were matched with COG, GO, KEGG, KOG, Pfam, Swissprot, eggNOG and NR databases, respectively. A total of 70,224 unigene were identified. There were 8,257, 13, 7,701, 12,554, 16,740, 13,041, 19,607, and 19,977 unigene genes with length  $\geq 1,000$  bp (Table S4).

GO annotation was performed on 41,786 unigenes, which are included in 54 branches. Twenty branches of biological processes involve 110,234 annotated information, accounting for 45.82% of all annotated information. The branches with the most unigenes are the "metabolic process" with 30,074 annotated information and the "cellular process" with 24,290 annotated information. In the cellular component category, 17 branches involve 78,115 annotated information, accounting for 32.47% of all annotated information. The branches with the most annotated information are "cell" and "cell part," both with 17,483 annotated information. In the molecular function category, 17 branches involve 52,216 unigenes, accounting for 21.71% of all annotated information. The branches with the most molecular function are "catalytic activity" with 23,645 unigenes and "binding" with 20,421 unigenes (Fig. 2).

The KOG database has a wide range of functions related to life activities, with a total of 35,830 genes annotated and divided into 26 categories represented by letters A to Z. The "General function prediction only" accounts for 20.56% of all gene sequences, with 7,368 genes in this category. The next most abundant category is "Posttranslational modification, protein turnover, chaperones", which accounts for 10.48% with 3,755 genes. Additionally, there are 3,202 genes involved in "Translation, ribosome structure, and biogenesis", accounting for 8.94% of the total sequence. Furthermore, there are 2,762 genes involved in "Signal transduction mechanisms", 2,488 genes involved in "Amino acid transport and metabolism", and 2,102 genes involved in "Carbohydrate transport and metabolism". The category with the fewest number of genes is "cell motility", with only 21 genes, followed by "extracellular structures" and "Nuclear structure" with 123 and 181 genes, respectively. The number of genes in each category is shown in Fig. 3.

## 2.5. DEGs analysis of ‘Adelinia’ and ‘Heifeng’ fruits at the same stage

The expression levels of unigenes in the fruits of ‘Adelinia’ and ‘Heifeng’ were compared at four developmental stages, namely, young fruit, expansion fruit, veraison fruit, and ripe fruit stages. A total of 483 DEGs were detected in the young fruit stage, including 241 up-regulated genes and 242 down-regulated genes. In the expansion stage, 1,909 DEGs were detected, including 1,020 up-regulated genes and 889 down-regulated genes. In the veraison fruit stage, 1,548 genes were differentially expressed, including 752 up-regulated genes and 796 down-regulated genes. In the ripe fruit stage, 2,822 genes were differentially expressed, including 1,602 up-regulated genes and 1,220 down-regulated genes (Fig. 4, A).

The KEGG enrichment analysis of the DEGs in the fruits of ‘Adelinia’ and ‘Heifeng’ at the same stage is shown in Fig. 4, B. Specifically, DEGs in the young fruits of ‘Adelinia’ and ‘Heifeng’ were significantly enriched in monoterpenoid biosynthesis (ko00902). DEGs in the expansion fruits of ‘Adelinia’ and ‘Heifeng’ were significantly enriched in plant hormone signal transduction (ko04075), DNA replication (ko03030), plant-pathogen interaction (ko04626), and mismatch repair (ko03430) pathway. DEGs in the veraison fruits of ‘Adelinia’ and ‘Heifeng’ were significantly enriched in photosynthesis-antenna proteins (ko00196), flavonoid biosynthesis (ko00941), and plant-pathogen interaction (ko04626) pathway. DEGs in the ripe fruits of ‘Adelinia’ and ‘Heifeng’ were significantly enriched in glyoxylate and dicarboxylate metabolism (ko00630), carbon metabolism (ko01200), citrate cycle (TCA cycle, ko00020), flavonoid biosynthesis (ko00941), photosynthesis-antenna proteins (ko00196), alanine, aspartate and glutamate metabolism alanine (ko00250), cutin, suberine and wax biosynthesis (ko00073), pyruvate metabolism (ko00620) pathway.

## 2.6. DEGs analysis of ‘Adelinia’ and ‘Heifeng’ fruits during ripening

To investigate the DEGs during fruit ripening, we compared young fruit with expansion fruit, expansion fruit with veraison fruit, and veraison fruit with ripe fruit in both ‘Adelinia’ and ‘Heifeng’. The number of DEGs was 1499 (810 up-regulated DEGs and 689 down-regulated DEGs) and 2311 (1163 up-regulated

DEGs and 1148 down-regulated DEGs) in the comparison group of young fruit with expansion fruit in ‘Adelinia’ and ‘Heifeng’, respectively. The number of DEGs was 2250 (850 up-regulated DEGs and 1400 down-regulated DEGs) and 3746 (1767 up-regulated DEGs and 1979 down-regulated DEGs) in the comparison group of expansion fruit with veraison fruit in ‘Adelinia’ and ‘Heifeng’, respectively. The number of DEGs was 1356 (1258 up-regulated DEGs and 98 down-regulated DEGs) and 960 (501 up-regulated DEGs and 456 down-regulated DEGs) in the comparison group of veraison fruit with ripe fruit in ‘Adelinia’ and ‘Heifeng’, respectively (Table 2).

Table 2  
Up-regulated and down-regulated DEGs in the young fruit, expansion fruit, veraison fruit, and ripe fruit stage

| Compared group | Total DEGs | Up-regulated | Down-regulated |
|----------------|------------|--------------|----------------|
| Y1 vs. Y2      | 1499       | 810          | 689            |
| Y2 vs. Y3      | 2250       | 850          | 1400           |
| Y3 vs. Y4      | 1356       | 1258         | 98             |
| H1 vs. H2      | 2311       | 1163         | 1148           |
| H2 vs. H3      | 3746       | 1767         | 1979           |
| H3 vs. H4      | 960        | 501          | 456            |

All DEGs in the comparison groups were mapped to KEGG pathway enrichment to analyze the enriched pathways. The DEGs in the comparison groups of Y1 vs Y2 and H1 vs H2 were both significantly enriched in plant hormone signal transduction (ko04075), Phenylpropanoid biosynthesis (ko00940), flavonoid biosynthesis (ko00941), Stilbenoid, diarylheptanoid and gingerol biosynthesis (ko00945), Starch and sucrose metabolism (ko00500), Phenylalanine metabolism (ko00360), Cutin, suberine, and wax biosynthesis (ko00073) pathway. The DEGs in the comparison groups of Y2 vs Y3 and H2 vs H3 were both significantly enriched in plant hormone signal transduction (ko04075), starch and sucrose metabolism (ko00500), phenylpropanoid biosynthesis (ko00360), homologous recombination (ko03440), phenylalanine metabolism (ko00360), flavonoid biosynthesis (ko00941), DNA replication (ko03030), Cutin, suberine, and wax biosynthesis (ko00073) pathway. While the DEGs in the comparison groups of Y3 vs Y4 and H3 vs H4 did not share the same significantly enriched pathway (Fig. 5).

## 2.7. Identification of genes involved in AsA biosynthesis and recycling pathway during fruit ripening

According to the gene expression level of transcriptome sequencing, crucial genes involved in AsA biosynthesis and recycling pathway were identified. Including 3 genes of glucose-6-phosphate isomerase (*PGI*), 1 gene of mannose-6-phosphate isomerase (*PMM*), 1 gene of GDP-mannose-3',5'-epimerase (*GME*), 2 genes of GDP-L-galactose phosphorylase (*GGP*), 1 gene of Myo-inositol oxygenase (*MIOX*) and 3 genes of L-galactono-1,4-lactone dehydrogenase (*GalLDH*) associated with AsA biosynthesis. And 6 genes of

ascorbate peroxidase (*APX*), 6 genes of ascorbate oxidase (*AO*), 3 genes of monodehydroascorbate reductase (*MDHAR*) and 1 gene of glutathione reductase (*GR*) associated with AsA recycling. As shown in Fig. 6, most of these genes were highly expressed in the young fruits of both 'Adelinia' and 'Heifeng'. Such as the expression of the *PGI* gene (c179793.graph\_c1 and c181113.graph\_c0) and *MDHAR* (c160843.graph\_c0) were highest in the young fruit and lowest in the veraison fruit in both 'Adelinia' and 'Heifeng'. The expression of the *PMM* gene (c184228.graph\_c0), *GME* gene (c181107.graph\_c0), *Ga/LDH* gene (c178565.graph\_c2), and *AO* gene (c185688.graph\_c1) was the highest in the young fruits and lowest in the ripe fruit.

In addition, we calculate the correlation coefficient ( $r^2$ ) of all the genes identified in AsA biosynthesis and recycling pathway with AsA content in the four fruit developmental stages of 'Adelinia' and 'Heifeng'. The expression of a *PMM* gene (c184228.graph\_c0) has a strong correlation ( $r^2 = 0.84$ ) with AsA content. Similarly, the expression of an *APX* (c176242.graph\_c0), a *GME* (c181107.graph\_c0), and a *PGI* (c181113.graph\_c0) gene were strongly correlated with AsA content, with  $r^2$  of 0.74, 0.71 and 0.71, respectively. These results revealed that the above genes may be crucial for AsA biosynthesis in blackcurrant. The expression of 2 *APX* (c176953.graph\_c0, c172886.graph\_c0) gene showed a strong negative correlation with AsA content, with  $-0.93$  and  $-0.77$ , respectively. The expression of 2 *AO* (c184540.graph\_c1, c185556.graph\_c0) genes has a strong negative correlation with AsA content, with  $r^2$  of  $-0.83$  and  $-0.74$ , respectively. An *MDHAR* (c172058.graph\_c0) gene also showed a strong negative correlation ( $r^2 = -0.72$ ) with AsA content. These genes may be crucial for the AsA recycling pathway in blackcurrant.

## 2.8. Identification of genes involved in flavonoid biosynthesis pathway during fruit ripening

As the flavonoid contents were variational during fruit ripening, we then identified the key genes involved in the flavonoid biosynthesis pathway. A total of 6 genes of 4-coumarate-CoA (*4CL*), 3 genes of chalcone synthase (*CHS*), 2 genes of chalcone isomerase (*CHI*), 1 gene of Flavanone-3-hydroxylase (*F3H*), 2 genes of dihydro flavonol reductase (*DFR*), and 1 gene of leucoanthocyanidin dioxygenase (*LDOX*), anthocyanin synthase (*ANS*), anthocyanidin reductase (*ANR*), flavonoid 3-O-glucosyl transferase (*UGT*) and leucoanthocyanidin reductase (*LAR*) were identified that associated with flavonoid biosynthesis. Most of these genes were expressed at their highest levels in the young fruits, by the flavonoid contents during fruit ripening. The expressions of the *4CL* gene (c183421.graph\_c1), *CHS* genes (c115942.graph\_c0, c183294.graph\_c1), *CHI* genes (c157264.graph\_c0, c163054.graph\_c0), *DFR* gene (c183820.graph\_c4), *ANS* gene (c187123.graph\_c2), *ANR* gene (c183720.graph\_c1) and *LAR* (c162573.graph\_c1) were highest in the young fruit of both 'Adelinia' and 'Heifeng', and were significantly down-regulated in the expansion fruits.

Similarly, the expression of a *DFR* (c183820.graph\_c4), an *ANR* gene showed a strong correlation (both  $r^2 = 0.88$ ) with flavonoid content. The expression of 2 *CHI* (c157264.graph\_c0, c163054.graph\_c0) with  $r^2$  of 0.87 and 0.83, and 2 *CHS* (c115942.graph\_c0, c183294.graph\_c1) genes with  $r^2$  of 0.82 and 0.80 showed

strong correlation with flavonoid content. A *4CL* and a *LAR* gene were also strongly correlated with flavonoid content, with  $r^2$  of 0.73 and 0.71, respectively. These results indicated that the above genes may be crucial for flavonoid biosynthesis in blackcurrant (Fig. 7).

## 2.9. Identification of DEGs involved in plant hormone signal transduction pathway

To explore changes in gene expression related to plant hormone signal transduction, we analyzed the DEGs involved in auxin, cytokinin, gibberellin, ethylene, abscisic acid, brassinosteroid, jasmonic acid, and salicylic acid signal transduction pathways. A total of 31 DEGs were detected involved in the auxin signal transduction pathway, and 18 genes were expressed highest in the young fruit stage of both 'Adelinia' and 'Heifeng' (Fig. 8). These genes included 2 *ARG7* genes (c171898.graph\_c0, c167577.graph\_c0), 4 *AUX10A* genes (c177750.graph\_c0, c179267.graph\_c0, c173102.graph\_c0, c178278.graph\_c0), *AUX28* (c182867.graph\_c3), *AUX6B* (c163920.graph\_c0), 2 *GH3.1* genes (c179273.graph\_c0, c187153.graph\_c1), *GH3.5* (c175332.graph\_c0), *GH3.9* (c175298.graph\_c0), *IAA7* (c159328.graph\_c0), *IAA9* (c177889.graph\_c1), *IAA14* (c184998.graph\_c1), 2 *IAA26* genes (c176546.graph\_c1, c176147.graph\_c3), *IAA27* gene (c179588.graph\_c0). This indicates that the auxin signal transduction pathway plays an important role in the young fruit stage. We also found that the expression of *ARG7* (c174082.graph\_c0), *IAA7* (c159328.graph\_c0), and *IAA11* (c164719.graph\_c0) was significantly higher in 'Adelinia' than 'Heifeng' in the expansion fruit stage. The expression of *AGR7* (c167451.graph\_c0) was significantly higher in 'Adelinia' than 'Heifeng' in the expansion and veraison fruit stages. As the fruit grows rapidly in the expansion stage, these genes may be responsible for the difference in volume and weight between 'Adelinia' and 'Heifeng' fruits. A total of 10 DEGs were detected as involved in the cytokinin signal transduction pathway. Three genes including *ARR3* (c178666.graph\_c0), *ARR9* (c173285.graph\_c1), and *AHP* gene (c181299.graph\_c0) were expressed at their highest level in the young fruit stage of both 'Adelinia' and 'Heifeng'. Two genes including *ARR1* (c179842.graph\_c0) and *ARR12* (c183630.graph\_c1) were expressed highest in the expansion fruit stage of both 'Adelinia' and 'Heifeng'. The *HK2* (c185911.graph\_c0) gene was expressed at its highest level in the ripe fruit stage of both 'Adelinia' and 'Heifeng'. A total of 4 DEGs were detected involved in the gibberellin signal transduction pathway. The expression of the *PIF3* (c182315.graph\_c1) and *GAI* (c151333.graph\_c0) gene was higher in the young and expansion fruit than veraison and ripe fruit of both 'Adelinia' and 'Heifeng'. While the expression of the *GID1B* (c184501.graph\_c3) gene was expressed higher in the veraison and ripe fruit than in young and expansion fruit of both 'Adelinia' and 'Heifeng'. A total of 5 DEGs were detected involved in the jasmonic acid signal transduction pathway. Four genes including *TIFY3B* (c179099.graph\_c0), *TIFY11B* (c183995.graph\_c4), *MYC2* (c182574.graph\_c0), and *bHLH14* (c167287.graph\_c0) were expressed highest in the young fruit of both 'Adelinia' and 'Heifeng'.

A total of 4 genes were detected involved in the ethylene signal transduction pathway. Two genes of *EBF1* (c168698.graph\_c0, c168698.graph\_c1) were expressed higher in the young and ripe fruit stage than expansion and veraison fruit stage of both 'Adelinia' and 'Heifeng'. In addition, the expression of

*EBF1* (c168698.graph\_c0, c168698.graph\_c1) and  *EIN3* (c180971.graph\_c4) genes was higher in the expansion fruit of 'Heifeng' than 'Adelinia'. A total of 11 DEGs were detected involved in abscisic acid signal transduction. The *PYL8* (c169965.graph\_c0) gene was expressed highest in the young fruit stage. One of the *ABI5* (c186178.graph\_c0) genes was expressed highest in the expansion fruit stage, while another *ABI5* (c172267.graph\_c0) gene was expressed highest in the veraison fruit stage. The *PP2C* (c177710.graph\_c0) gene was expressed highest in the ripe fruit stage. In addition, the expression of *PP2C* (c176253.graph\_c0) was higher in the expansion fruit of 'Adelinia' than 'Heifeng'. The expression of another *PP2C* (c177479.graph\_c0) and *PYR1* (c141701.graph\_c0) gene was higher in the ripe fruit of 'Heifeng' than in 'Adelinia'. A total of 8 DEGs were detected involved in the brassinosteroid signal transduction pathway. Three genes including *SIK* (c169178.graph\_c0, c186784.graph\_c1) and *THX* (c156073.graph\_c0) genes were expressed higher in the veraison and ripe fruit stages than in young and expansion fruit stages of both 'Adelinia' and 'Heifeng'. In addition, *CYCD3* (c173925.graph\_c0, c178067.graph\_c0) gene was expressed highest in the young fruit stage of both 'Adelinia' and 'Heifeng'. Three DEGs were detected as involved in the salicylic acid signal transduction pathway. *PR1* (c181784.graph\_c1) gene was expressed higher in the ripe fruit, while *TGA3* (c174510.graph\_c1) gene was expressed higher in the expansion fruit stage of both 'Adelinia' and 'Heifeng'. The result indicated that these DEGs involved in the plant hormone signal transduction pathway may play an important role in the fruit growth and development of blackcurrant.

## 2.10. Validation of DEGs by qRT-PCR

To confirm the accuracy and reliability of the transcriptome analysis results, three ascorbic acid biosynthesis and recycling-related genes, five flavonoid biosynthesis genes, and one hormone signal transduction-related gene were selected. The gene expression levels at different developmental stages were further examined by qRT-PCR, as shown in Fig. 9. For all nine genes, the qRT-PCR analysis results had the same expression trend as the FPKM data from RNA-seq.

## 3. Discussion

### 3.1. AsA biosynthesis and recycling in 'Adelinia' and 'Heifeng' during fruit ripening

Several factors can influence the content of ascorbic acid (AsA) in plants, including variety, climate, growing conditions, cultivation practices, maturity at harvest, and storage conditions[28]. For example, blackcurrant grown in high-latitude regions of Finland tend to have higher AsA content compared to those grown in lower latitudes[28]. The AsA content in blackcurrant fruits also varies under different light intensities, with shading leading to a decrease in AsA content[29]. Additionally, the AsA content changes during the ripening process of blackcurrant fruits. Under natural growth conditions, the accumulation of AsA in blackcurrant begins in the early stages of berry growth and reaches a stable period at fruit maturity[29]. This study also found that the AsA levels were highest during the early fruiting stage in the two varieties, 'Adelinia' and 'Heifeng', and gradually decreased as the fruits matured, with the lowest

content observed at the ripe stage. In addition, the synthesis of AsA in blackcurrant occurs through the L-galactose pathway[30], which is consistent with apple (*Malus domestica*), kiwifruit (*Actinidia*), tomato (*Solanum lycopersicum*), and citrus fruits[31]. In the AsA recycling pathway of blackcurrant, ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), and L-galactono-1,4-lactone dehydrogenase (GalLDH) collectively regulate the total AsA content in blackcurrant fruits[29]. In this study, a total of 26 genes were identified from the AsA biosynthesis and recycling pathway based on transcriptome sequencing results. Among them, the expression of a *PMM* (c184228.graph\_c0), an *APX* (c176242.graph\_c0), a *GME* (c181107.graph\_c0), and a *PGI* (c181113.graph\_c0) gene have a strong correlation with AsA content. These genes may be crucial for the AsA biosynthesis pathway in blackcurrant. Similarly, the suppressed expression of the *PMM* gene caused a substantial decrease in AsA content in tobacco[32]. In tomato, the overexpression of the *SIGMEs* gene enhanced AsA accumulation[33]. However, the expression of 2 *APX* (c176953.graph\_c0, c172886.graph\_c0) gene, 2 *AO* (c184540.graph\_c1, c185556.graph\_c0) gene, and an *MDHAR* (c172058.graph\_c0) gene showed strong negative correlation with AsA content. These genes may be crucial for the AsA recycling pathway in blackcurrant. Consist with our result, Shu et al. identified an *AcAO1* and an *AcAPX2* gene from comprehensive metabolic analysis of kiwifruit during ripening, and the overexpression of the two genes led to reduced L-AsA and total AsA content in tobacco[34]. The study on AsA indicated that the overexpression of chloroplastic monodehydroascorbate reductase (*MDHAR*) led to an increase in AsA content, a decrease in DHA content, and an approximately twofold increase in the AsA/DHA ratio in tomato[35]. The DEGs identified in this work will be a valuable gene resource for exploring the molecular mechanism of AsA biosynthesis and breeding of blackcurrant with high AsA levels.

## 3.2. Flavonoids biosynthesis in ‘Adelinia’ and ‘Heifeng’ during fruit ripening

Flavonoid compounds, as a type of phenolic substance, possess powerful antioxidant and free radical scavenging activities. There is a wide variety of flavonoids, including anthocyanins, flavanols, flavones, flavanones, and flavonols[36]. Flavonoids play important roles in plant defense against microbial stress[37] as well as in promoting human health[38]. Among all the components of flavonoids, myricetin has the highest content in blackcurrants, followed by quercetin[39]. The content of flavonoids shows significant variations at different stages of fruit development. The flavonoid content in *Rubus* fruits[40] and navel oranges [41] has been observed to decline with fruit maturation. In this study, the peak flavonoid content was observed in young fruit, while the minimum was observed in fruits during the coloration stage. Similarly, most of the genes involved in flavonoid biosynthesis showed their maximum expression levels in young fruit, consistent with the changes in flavonoid contents during fruit ripening. The DEGs in the comparison groups Y1 vs Y2, H1 vs H2, Y2 vs Y3, and H2 vs H3 were significantly enriched in both phenylpropanoid biosynthesis and flavonoid biosynthesis pathway. These findings may account for the pronounced variations in flavonoid synthesis and accumulation at different stages of fruit development. The molecular mechanisms regulating flavonoid synthesis have been elucidated in many plants. In recent years, significant progress has also been made in research on flavonoid synthesis

in fruit trees, including grapes, blueberries, apricots[42], blackberries[42], and apples. Flavonoid synthesis begins with phenylalanine. The health benefits of blackcurrants are partly attributed to their high flavonoid content, so it is important to elucidate the molecular mechanism of flavonoid accumulation in blackcurrants. In melon, flavonoid accumulation is regulated by the up-regulation of *4CL*, *F3H*, *F3'H*, *IFS*, *FNS*, and *FLS* genes and the down-regulation of *ANS*, and *UFGT* genes[43]. A study on apricot fruits has demonstrated the *4CL* gene transcript level has a strong positive correlation with flavonoid contents[42]. *CHS* was identified as the backbone gene for flavonoid biosynthesis in sweet orange[44]. Here, we identified 19 candidate genes associated with flavonoid biosynthesis. Among them, the expressions of *4CL* gene (c183421.graph\_c1), *CHS* genes (c115942.graph\_c0, c183294.graph\_c1), *CHI* genes (c157264.graph\_c0, c163054.graph\_c0), *DFR* gene (c183820.graph\_c4), *ANS* gene (c187123.graph\_c2), *ANR* gene (c183720.graph\_c1) and *LAR* gene (c162573.graph\_c1) followed the same trend as that of flavonoid content in fruits. These genes may be crucial for flavonoid biosynthesis in blackcurrants. Consistent with our results, Zhou et al. reported that the expression of *CHS*, *DFR*, and *ANS* genes peaked at the early stages, decreased when fruits turned white from green, and then increased during anthocyanin biosynthesis in strawberry[45]. Our results provide valuable information on the dynamic changes in flavonoid content and the molecular mechanism of flavonoid accumulation during the maturation of blackcurrants in the future.

### 3.3. The difference between 'Adelinia' and 'Heifeng' in fruit weight

Fruit size is one of the most important indicators of fruit appearance and quality, and it is one of the breeding objectives that breeders cannot ignore. Fruit size is affected by a variety of factors such as varietal characteristics, genetic factors, plant hormones, cultivation, and management levels, and is a quantitative trait with multi-gene control. The fruit weight of different varieties of blackcurrant varies greatly[46], and our data also show that 'Adelinia' fruit weight at maturity can be up to three times that of 'Heifeng'. The influence of genetic factors on fruit size is mainly determined by cell division and expansion, and are the most fundamental factors in determining fruit size. Plant hormones play an important role in fruit development and fruit size. For example, BRASSINOSTEROID INSENSITIVE1 (*BR1*) Enlarges Cell Size by Regulating the Cooperation of BR-GA Signaling in Tomato[47]. Hence, the genes involved in the regulation of cell development and plant hormone signaling are important regulators of fruit size. When studying the influencing factors of fruit size in apricot, Huang et al. identified a series of DEGs involved in plant hormone signal transduction, biosynthesis, and metabolism based on the KEGG enrichment results[48]. Genes associated with fruit size in the early stage of fruit development in *Pyrus pyrifolia* significant enrichment in the plant hormone signal transduction pathway of zeatin and gibberellin[49]. The main purpose of plant photosynthesis is to produce carbohydrates, an important energy material for plants to carry out various life activities. An adequate supply of carbohydrates plays an important role in plant growth[50]. Autotetraploid and diploid mulberry trees, with large fruit size differences, DEGs significant enrichment in the plant hormone signal transduction pathways, and many DEGs were involved in photosynthesis[51]. In our study, the difference in fruit size and weight between the two varieties increased significantly after the expansion stage, at the same time, KEGG pathway

enrichment analysis revealed that DEGs were significantly enriched in the plant hormone signal transduction (ko04075) pathway in the expansion fruit stage of 'Adelinia' and 'Heifeng', in photosynthesis-antenna proteins (ko00196) pathway in the veraison fruit stage and ripe fruit stage. Combined with the above results, we believe that the fruit size of blackcurrant is also affected by the regulation of hormone signals and photosynthesis. To clarify the specific regulation mechanism of different kinds of hormones on fruit size, we analyzed the DEGs involved in auxin, cytokinin, gibberellin, ethylene, abscisic acid, brassinosteroid, jasmonic acid, and salicylic acid signal transduction pathway. Among them, a total of 9 genes had different gene expression levels between the 'Adelinia' and 'Heifeng' varieties after the expansion stage. Such as *ARG7* (c174082.graph\_c0), *IAA7* (c159328.graph\_c0), and *IAA11* (c164719.graph\_c0) in the expansion fruit stage. *AGR7* (c167451.graph\_c0) in the expansion and veraison fruit stage. *EBF1* (c168698.graph\_c0, c168698.graph\_c1) and  *EIN3* (c180971.graph\_c4) gene in the expansion fruit. *PP2C* (c176253.graph\_c0) in the expansion fruit. *PP2C* (c177479.graph\_c0) and *PYR1* (c141701.graph\_c0) genes in the ripe fruit. *SIARF9* affects cell division in early tomato fruit development, *SIARF9-OE* fruit cells were on average bigger than in wild-type fruits, inversely the *SIARF9*-RNAi lines, cells were smaller[52]. Silencing of *Sl-IAA17* resulted in the production of larger fruit than the wild type[53]. *MdKNOX19* binds directly to the *MdABI5* promoter to activate expression-enhanced ABA sensitivity-affected organ development and fruit size[54]. genome-edited mutants of tomato ETHYLENE INSENSITIVE 2(*SIEIN2*) decreased auxin biosynthesis to reduce fruit size[55]. In brief, the above genes may be important influencing factors in cell enlargement and fruit enlargement, and they regulate plant growth and development by regulating the synthesis and metabolism of various hormones. The results provide new insights into the molecular mechanisms underlying fruit development and may be useful for improving fruit yield and quality.

## 4. Conclusions

In this study, we generated a high-quality, functionally annotated reference transcriptome for blackcurrant. Based on the transcriptome, a comparison of different developmental stages and different varieties was performed and DEGs were detected. Detection of differentially expressed genes led to the identification of regulatory genes associated with the AsA biosynthesis and recycling, flavonoids biosynthesis pathways. A large number of differentially expressed genes were also found to be involved in plant hormone signal transduction. In addition, the accuracy of the RNA-Seq results was further verified by qRT-PCR. Our study provides insights into AsA biosynthesis and recycling, flavonoid biosynthesis, and fruit size regulation in blackcurrant.

## 5. Materials and methods

### 5.1. Plant materials and growth condition

Two varieties of blackcurrants, 'Adelinia' and 'Heifeng', were used as samples in this study. These plants were grown in the germplasm nursery of Northeast Agricultural University, Harbin, China, at coordinates 44°04' N, 125°42' E, under natural conditions. Adelinia is an early-maturing variety, while Heifeng is a late-

maturing variety. Four stages of fruit development were collected, including young fruit, expansion fruit, veraison fruit, and ripe fruit. Samples were immediately frozen in liquid nitrogen and stored at -80°C for RNA extraction.

## **5.2. Determination of Soluble solids, titratable acid, Total phenol, flavonoids, and ascorbic acid**

The soluble solids were measured using a refractometer, according to the GB/T 12295 – 1990 method. Titratable acid content was measured using acid-base titration, according to the GB/T 12295 – 1990 method. Total phenol was measured using the method reported by John[56] with some modifications. Flavonoid content was determined using the method reported by Zou[57]. According to the manufacturer's instructions, AsA content was determined using a Solarbio detection kit. Three fruits from different trees were used for each measurement.

## **5.3. Library construction, and sequencing**

Total RNAs were extracted from the collected fruit samples using the CTAB method. The RNA quality was assessed using agarose gel electrophoresis and RNA quantity was determined by an Agilent 2100 Bioanalyzer (Palo Alto, USA). Strand-specific RNA-Seq libraries were constructed and sequenced on the Illumina HiSeq 2000 platform by Biomarker Technologies (Beijing, China). Three biological replicates of each fruit sample were used.

## **5.4. RNA-Seq data processing, and transcriptome assembly**

Raw RNA-Seq reads were processed using in-house Perl scripts to trim adapters and low-quality sequences. The transcriptome was assembled based on the left.fq and right.fq using Trinity[58] with the minimum Kmer coverage set to 2. Gene function was annotated based on NR (NCBI non-redundant protein sequences), Pfam (Protein family), KOG/COG/eggNOG (Clusters of Orthologous Groups of proteins), Swiss-Prot (A manually annotated and reviewed protein sequence database), KEGG (Kyoto Encyclopedia of Genes and Genomes) and GO (Gene Ontology) databases. GO functional enrichment analysis of genes in major KEGG enriched pathways was performed using BMKCloud ([www.biocloud.net](http://www.biocloud.net)). For convenience, the young fruits of 'Adelinia' and 'Heifeng' were labeled as Y1 and H1. The expansion fruits of 'Adelinia' and 'Heifeng' were labeled as Y2 and H2, the veraison fruits of 'Adelinia' and 'Heifeng' were labeled as Y3 and H3, and the ripe fruits of 'Adelinia' and 'Heifeng' were labeled as Y4 and H4, respectively.

## **5.5. Identification of differentially expressed genes (DEGs)**

Differential expression analysis was performed using the DESeq R package to identify DEGs between the two groups of samples collected at different stages of fruit development, including young fruit, expansion fruit, veraison fruit, and ripe fruit, for Adelinia and Heifeng varieties. For identifying differentially expressed genes (DEGs), a fold change (FC)  $\geq 2$  and false discovery rate (FDR)  $< 0.01$  were used as the screening criteria. FC represents the ratio of gene expression values in two groups. Genes with an adjusted P-value  $< 0.05$  determined by DESeq were assigned as differentially expressed.

## 5.6. Quantitative Reverse Transcription PCR (qRT-PCR)

Gene expression analysis was performed for each sample using RT-qPCR. Total RNA from ‘Adelinia’ and ‘Heifeng’ fruit was extracted using the Biomarker Plant Total RNA Isolation Kit (Polysaccharides & Polyphenolics–rich) according to the manufacturer’s instructions. The RNA concentration and quality were measured using an ultraviolet spectrophotometer (NanoPhotometer NP80, IMPLEN) and 1% agarose gel electrophoresis. cDNA was synthesized from 500 ng of the total RNA using ReverTra Ace qPCR RT Master Mix (TOYOBO) according to the manufacturer’s instructions. Specific primers for qRT-PCR are listed in Table S1, which were designed using Premier 5.0.

The qRT-PCR reaction was performed in a 20 µL reaction volume containing 10 µL of SYBR®Green Realtime PCR Master Mix (TOYOBO), 2 µL of cDNA, 0.8µL of each forward and reverse primer, and 6.4 µL ddH<sub>2</sub>O. The analysis was carried out on three biological replicates on a qTOWER3 fluorescence quantitative PCR instrument (Analytik Jena). qRT-PCR was carried out under the following conditions: denaturation at 95 °C for 3 min prior to 45 amplification cycles (95°C for 5 s, 60°C for 30 s), followed by melt-curve analysis (60–95°C, 1°C/increment for 15 s per step) to test the amplicon specificity. Relative expression was assessed by the  $2^{-\Delta\Delta CT}$  method using *Actin* (c178043.graph\_c0) as the internal control gene[59].

## Abbreviations

DEGs

Differentially expressed genes

qRT-PCR

Quantitative real-time PCR

GO

Gene ontology

KOG

Clusters of orthologous groups

KEGG

Kyoto encyclopedia of genes and genomes

AO

Ascorbate oxidase

APX

Ascorbate peroxidase

GalDH

L-galactose dehydrogenase

GGP

GDP-L-galactose-phosphorylase

GalLDH

L-galactono-1,4-lactone dehydrogenase  
GME  
GDP-D-mannose 3', 5'-epimerase  
GMP  
GDP-mannose pyrophosphorylase  
GPP  
L-galactose-1-phosphate phosphatase  
GSH  
Glutathione  
GSSH  
Oxidized glutathione  
MDHAR  
Monodehydroascorbate reductase  
MIOX  
Myo-inositol oxygenase  
PGI  
Phosphoglucose isomerase  
PMI  
Phosphomannose isomerase  
PMM  
Phosphomannomutase  
GR  
Glutathione reductase  
4CL  
4-coumarate-CoA  
CHS  
Chalcone synthase  
CHI  
Chalcone isomerase  
F3H  
Flavanone-3-hydroxylase  
DFR  
Dihydro flavonol reductase  
LDOX  
Leucoanthocyanidin dioxygenase  
ANS  
Anthocyanin synthase  
ANR  
Anthocyanidin reductase  
UFGT

Flavonoid 3-O-glucosyl transferase

LAR

Leucoanthocyanidin reductase

ARG

Argentina

AUX

Auxin

IAA

Indole acetic acid.

## Declarations

### Acknowledgments

We thank all contributors for their work and would like to thank the editor and reviewers for their valuable comments and suggestions.

### Authors' contribution

DQ and JWH designed and managed the experiments. HXG and XLZ drafted and revised the manuscript. HXG and DNZ conducted the sequence data analysis. XLZ performed the experiments. The authors have carefully read and approved the final manuscript.

### Funding

This research was supported by the following fundings: National Key R&D Program of China (2022YFD1600500), Academic Backbone Project of Northeast Agricultural University (20XG04), China Postdoctoral Science Foundation (2022MD713724) and Central Government Supports the Reform and Development Fund Talent Training Project of Local Colleges and Universities, China.

### Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files. The raw RNA-Seq data in this manuscript are available for downloading from the NCBI Sequence Read Archive (BioProject ID: PRJNA1021373).

<https://www.ncbi.nlm.nih.gov/sra/PRJNA1021373>

### Ethics approval and consent to participate

The fruits of blackcurrant in this study was collected from the germplasm nursery of Northeast Agricultural University, Harbin, China. No specific permits are required for sample collection. The use of plant parts in present study complies with international, national and/or institutional guidelines.

### Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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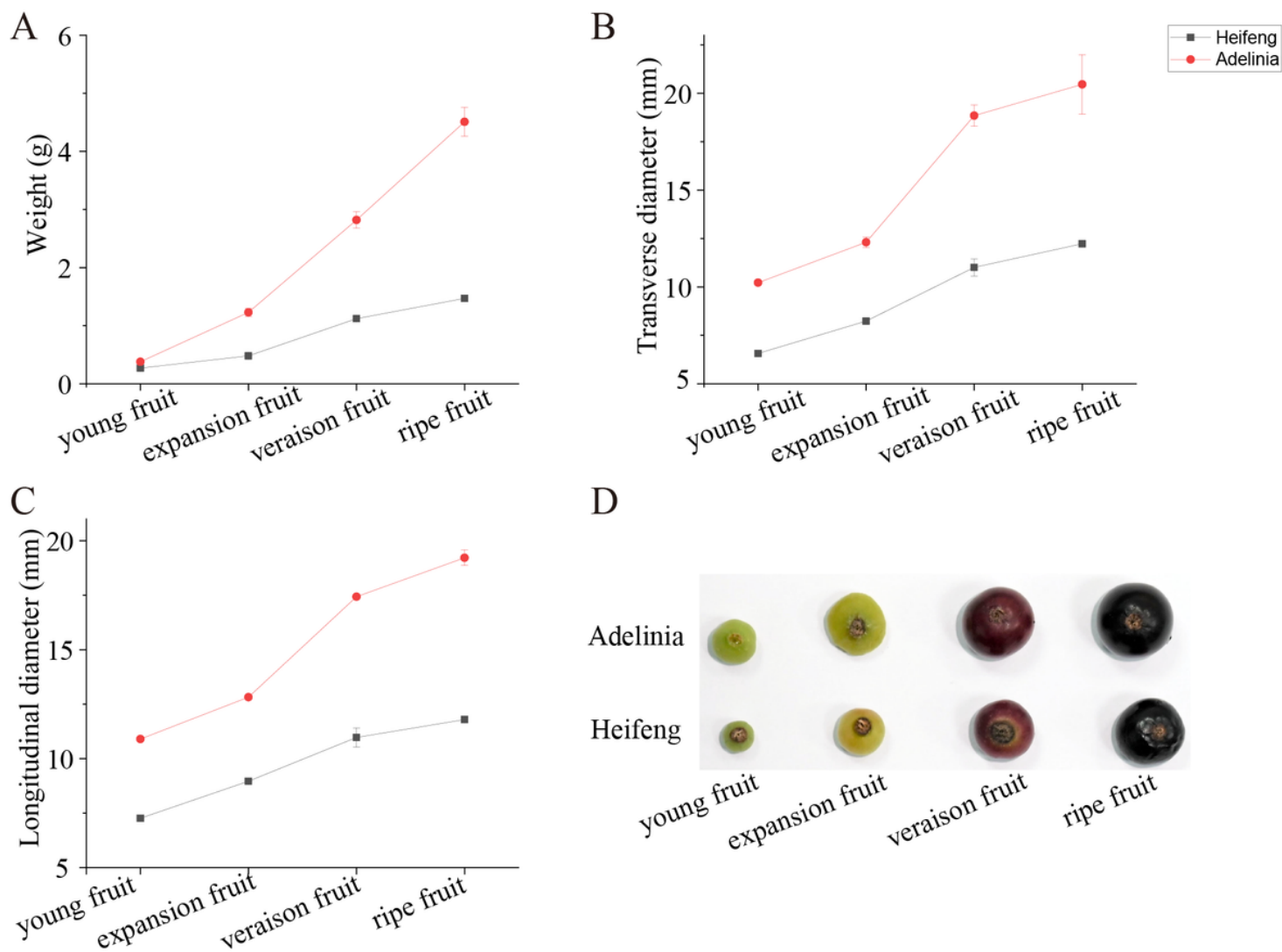
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## Figures



**Figure 1**

Characteristics of fruit development and ripening in the two varieties of ‘Heifeng’ and ‘Adelinia’. (A) Fruit weight, (B) transverse, and (C) longitudinal diameters during fruit development and ripening. (D) Phenotypes at four stages in ‘Heifeng’ and ‘Adelinia’.

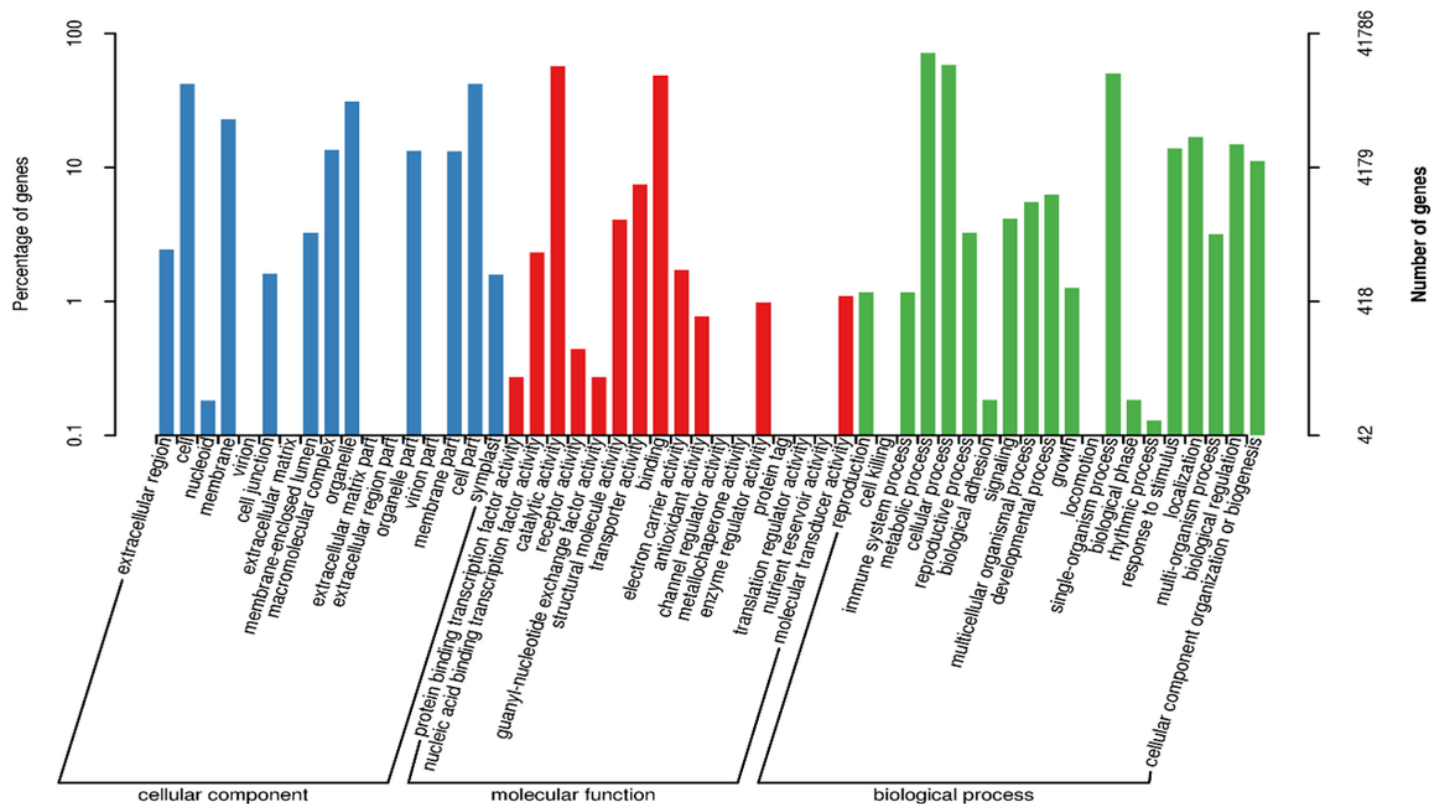
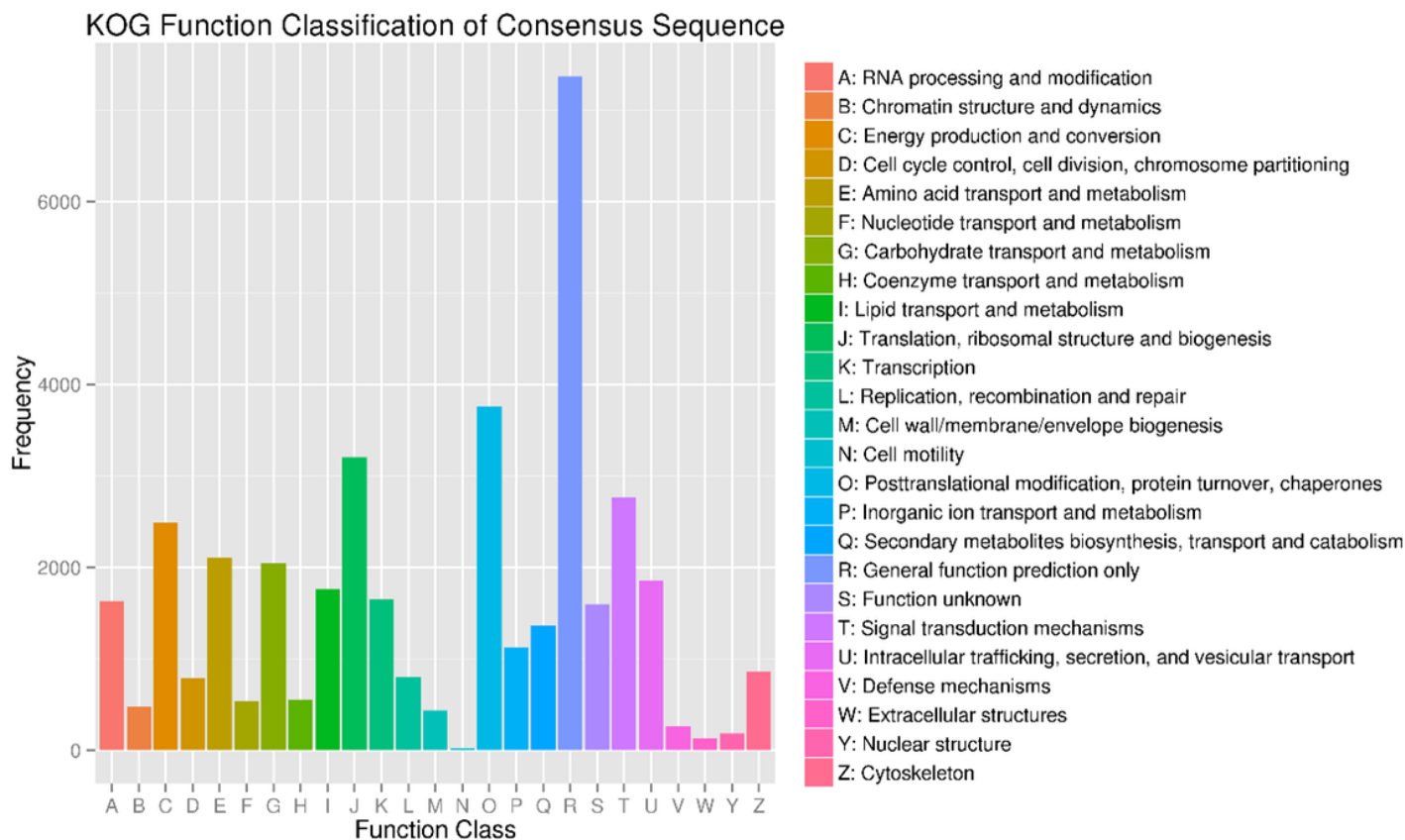


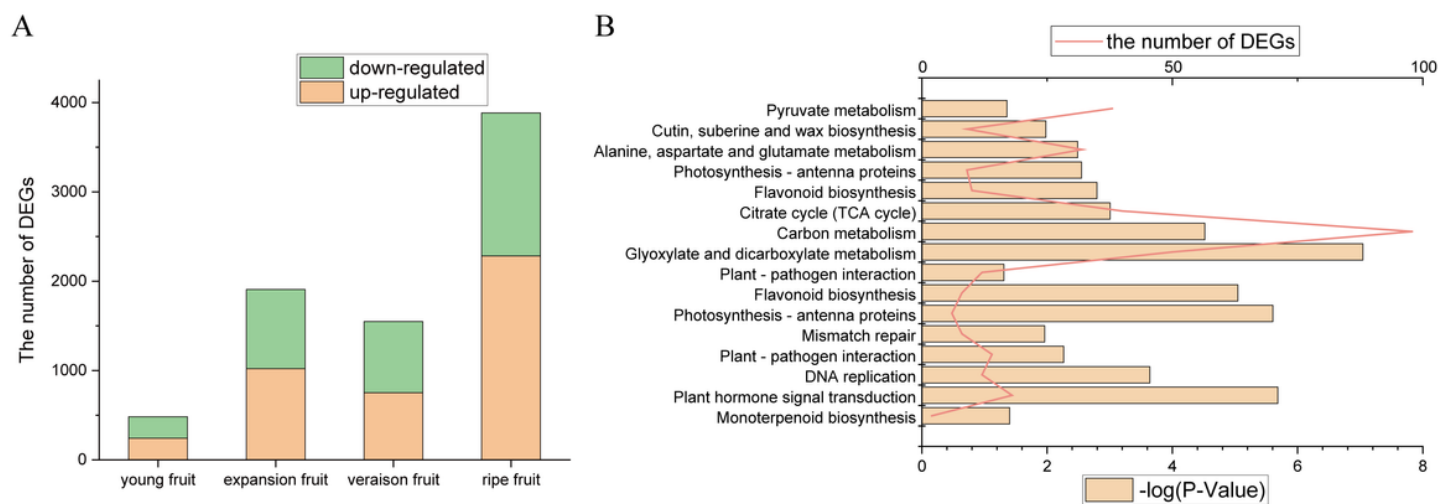
Figure 2

The alignment results against the gene ontology (GO) database.



**Figure 3**

KOG function classification of consensus sequence

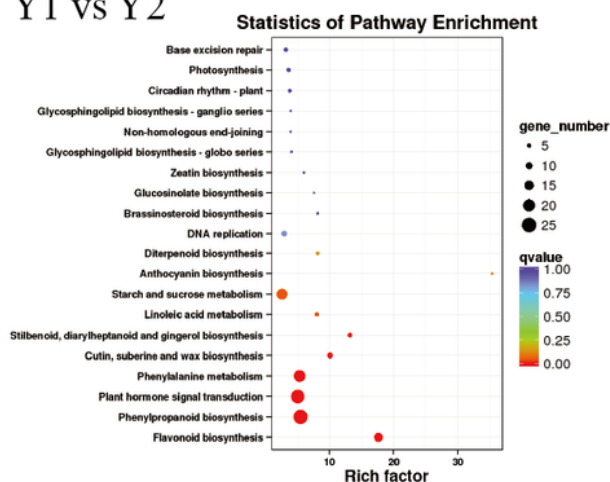


**Figure 4**

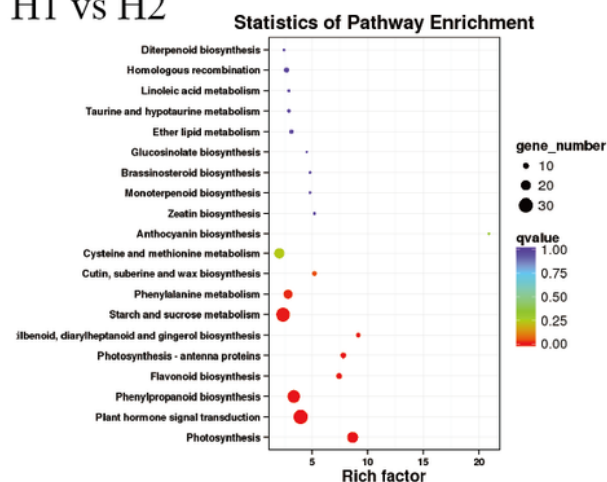
Analysis of expression differences between 'Adelinia' and 'Heifeng' fruits at the same stage.

(A) Up-regulated and down-regulated DEGs in 'Adelinia' and 'Heifeng'. (B) Statistical results of significant enrichment of KEGG.

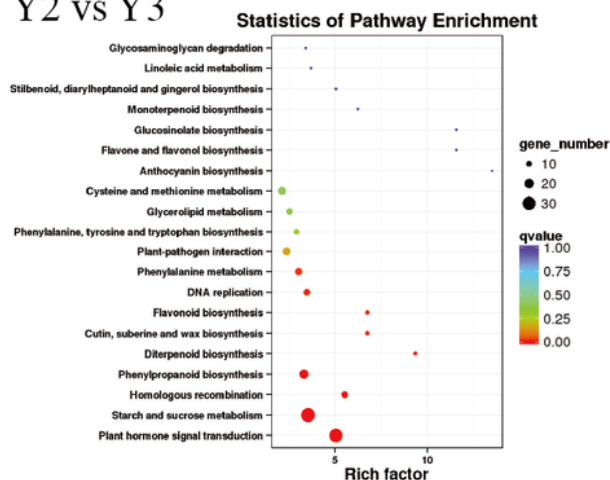
Y1 vs Y2



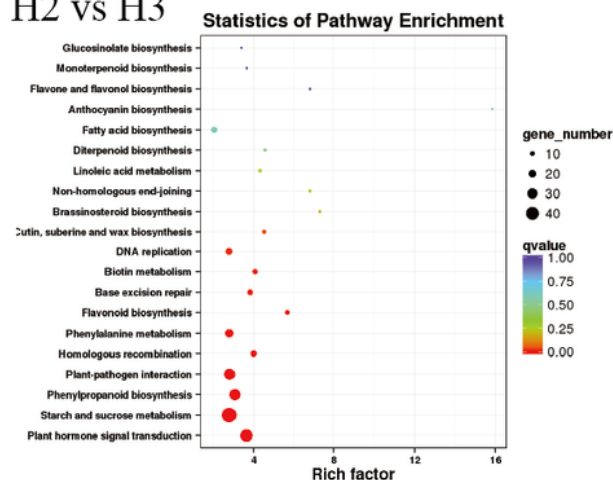
H1 vs H2



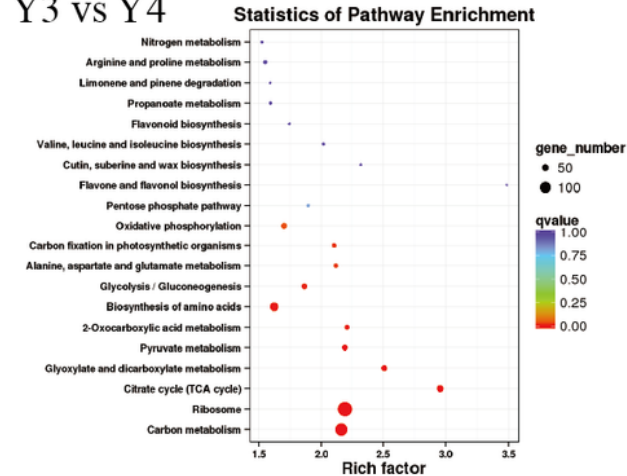
Y2 vs Y3



H2 vs H3



Y3 vs Y4



H3 vs Y4

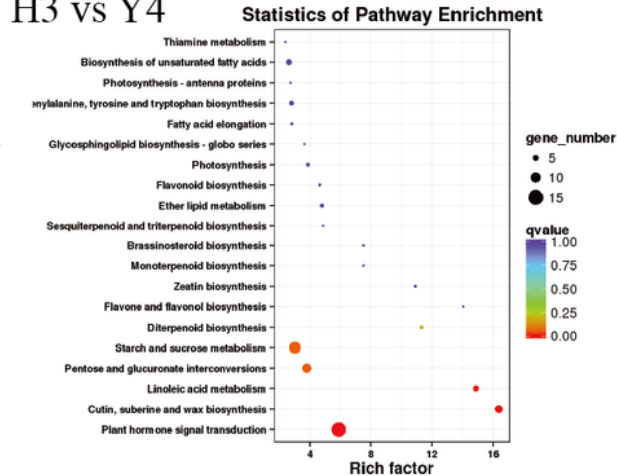
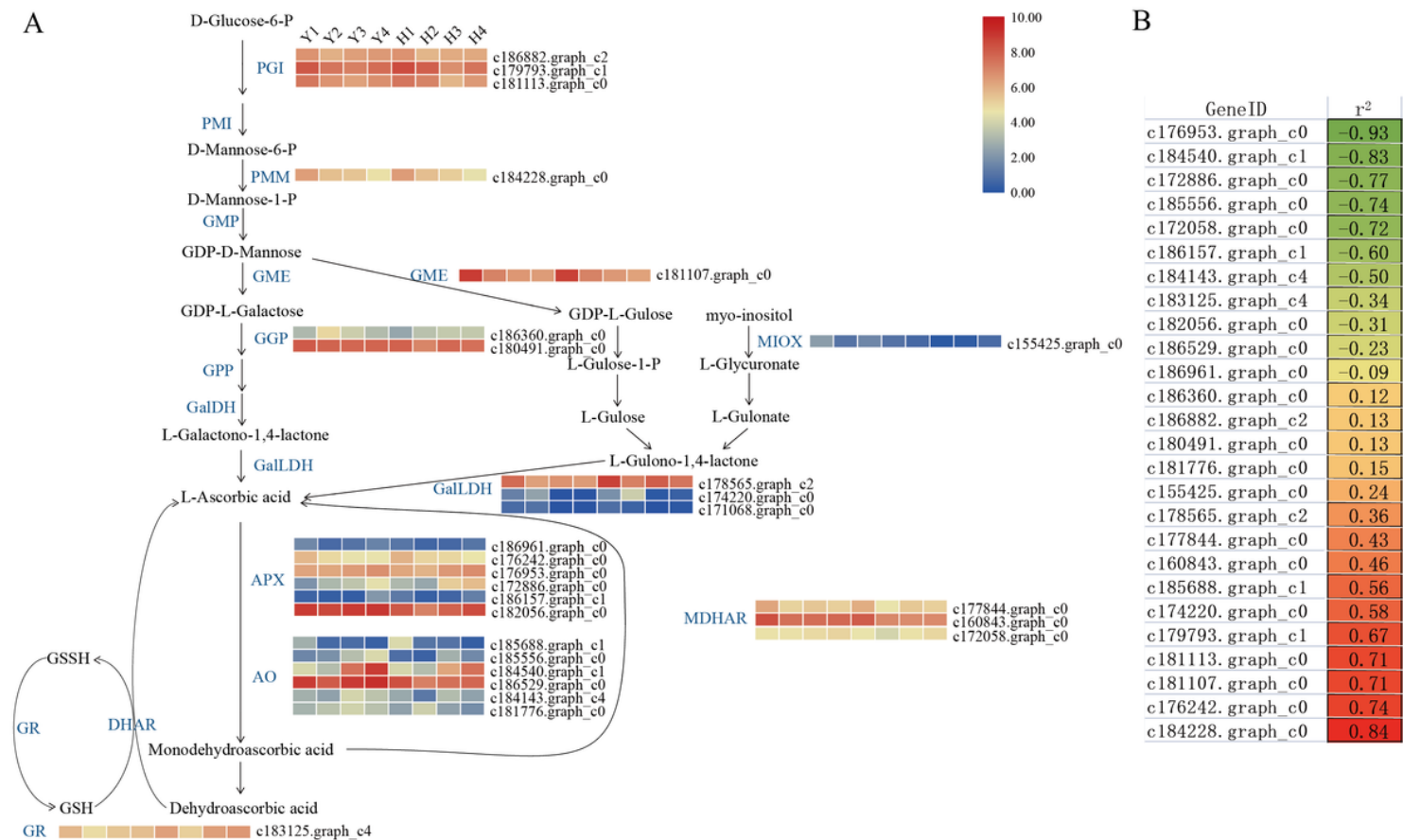


Figure 5

The top 20 enriched KEGG pathways of DEGs.

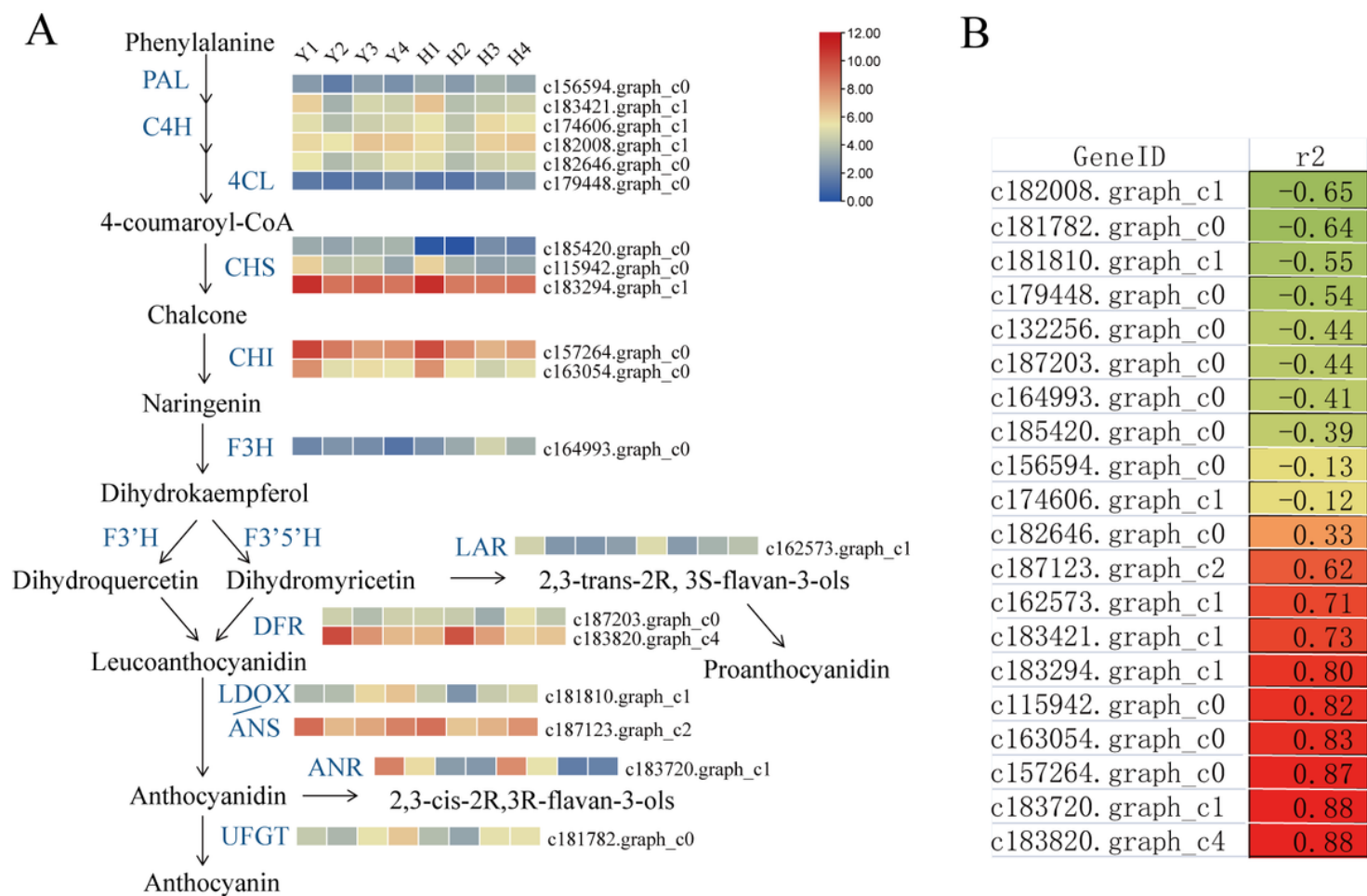
Note: Y1–Y4 indicate the young fruit, expansion fruit, veraison fruit, and ripe fruit of ‘Adelinia’. H1-H4 indicates the young fruit, expansion fruit, veraison fruit, and ripe fruit of ‘Heifeng’.



**Figure 6**

Genes expression in AsA biosynthesis and recycling pathway during fruit ripening.

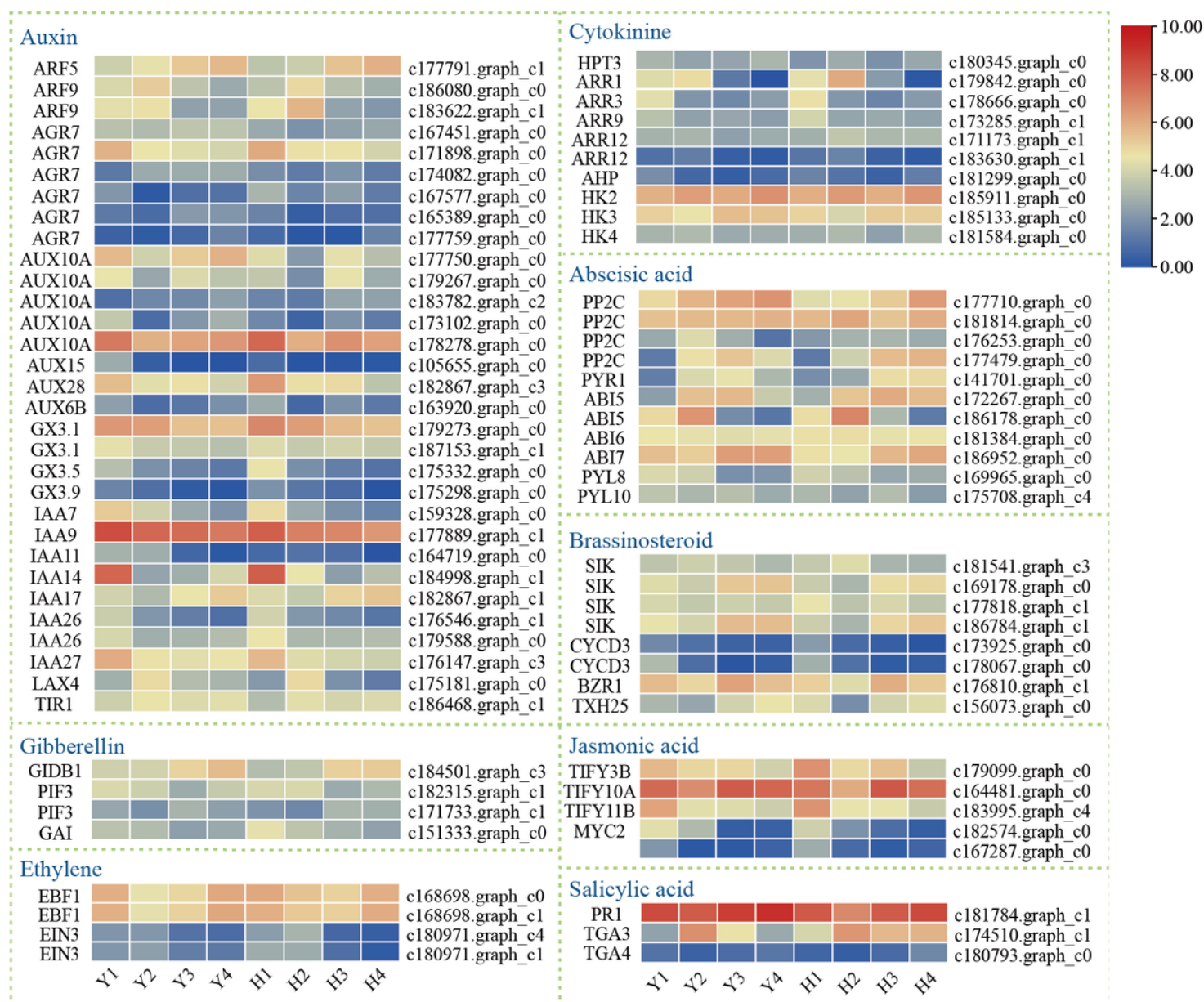
(A) AsA biosynthesis and recycling pathway in the blackcurrant fruits. (B) The correlation coefficient ( $r^2$ ) of all the genes identified in AsA biosynthesis and recycling pathway with AsA content in the four fruit developmental stages of 'Adelinia' and 'Heifeng'.



**Figure 7**

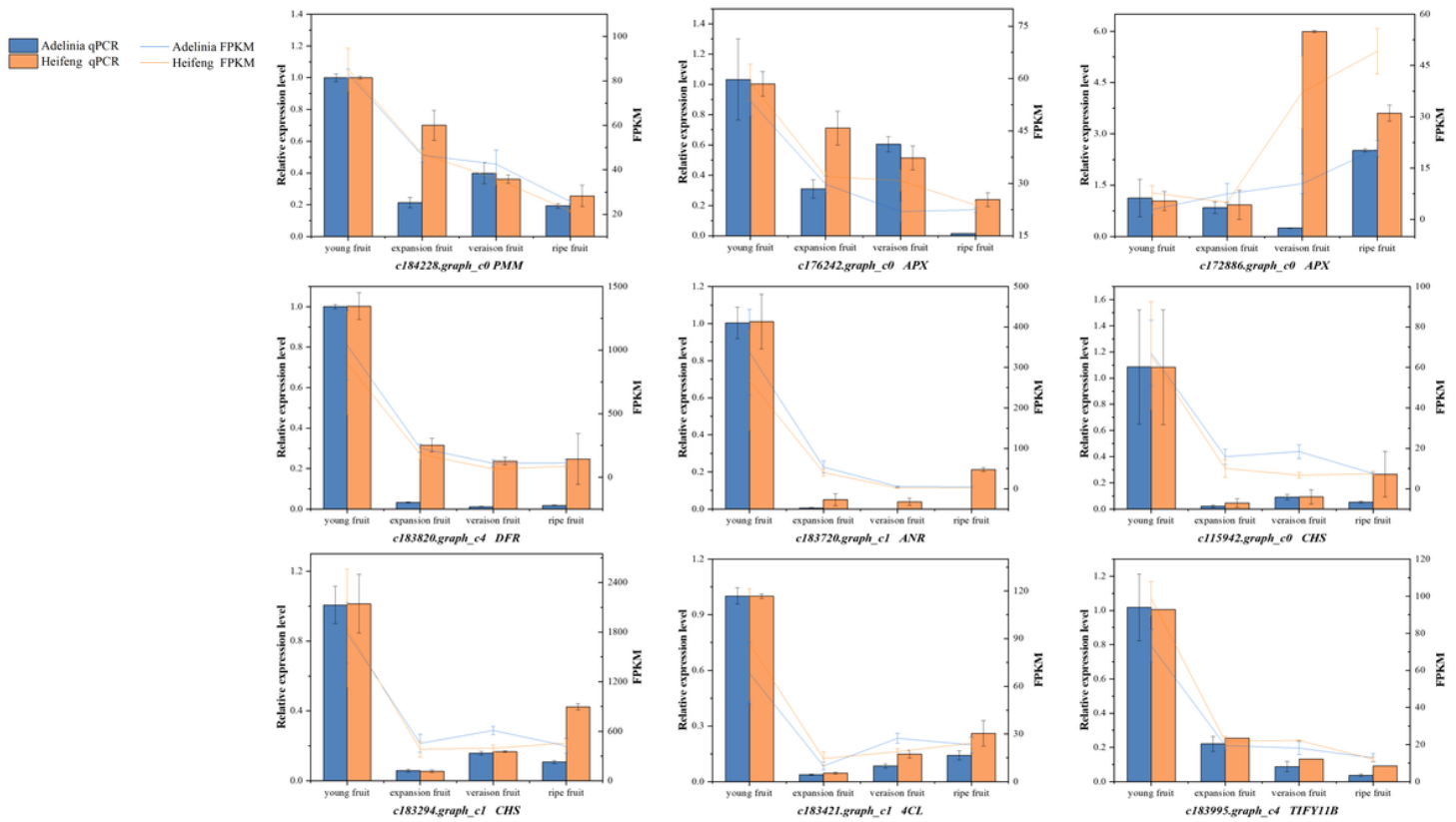
Genes expression in flavonoids biosynthesis pathway during fruit ripening.

(A) Flavonoid biosynthesis pathway in the blackcurrant fruits. (B) The correlation coefficient ( $r^2$ ) of all the genes identified in flavonoid biosynthesis pathway with flavonoid content in the four fruit developmental stages of 'Adelinia' and 'Heifeng'.



**Figure 8**

Genes expression in plant hormone signal transduction pathway during fruit ripening



**Figure 9**

The expression of nine genes in the fruit of 'Adelinia' and 'Heifeng'.

Left y-axis: Quantitative real-time PCR validation of DEGs at four stages. Right y-axis: Expression profiling of genes at four stages from RNA-seq data. Error bars represent  $\pm$  SE of biological replicates.

## Supplementary Files

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

- [SupplementalTables.doc](#)