Transcriptomic analysis of berry development and a corresponding analysis of anthocyanin biosynthesis in teinturier grape

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1. Introduction

Grape (V. vinifera L.) is one of the most economically important and widely cultivated fruit crops globally. The estimate of commercial grape production globally in 2016 was more than 77 million tons (FAOSTAT, http://faostat.fao.org/, December 28, 2017). Since there has been an increasing demand for high fruit quality and appearance, many studies have been conducted on fruit development.

Anthocyanins are widely present throughout the plant kingdom and contribute to the coloration of plant organs, especially fruit. In grape berries, anthocyanins are generally confined to skin tissues; however, in some grape cultivars, referred to as teinturier (Guan et al. 2012), anthocyanins can accumulate in both skin and pulp tissues. Teinturier grape berries are noted for their nutritional and pharmacological properties due to their high concentration of anthocyanins (Monrad et al. 2010). Teinturier grapes also have a significant effect on wine quality due to their multiple color pigments and a high level of tannins, the latter of which are structurally related to anthocyanins (Richardson et al. 2008). Different cultivars of teinturier grape accumulate anthocyanins in different plant tissues such as fruit, stems, leaves, shoots, and seeds (Falginella et al. 2012; Guan et al. 2012). Since the majority of the anthocyanins accumulate in grape berries, most studies conducted on teinturier cultivars have focused on the genetic basis of anthocyanin accumulation in berries (Castillomunoz et al. 2009; Santiago et al. 2010).

Anthocyanins are responsible for the red or purple color in red grapes, especially teinturier cultivars. Due their small berry size, low sugar content, and darker color, teinturier grapes are typically used to increase the color intensity and modify the quality of red wine produced from traditional cultivars of wine grapes. Previous studies have focused on the differences in anthocyanin composition between the skin and pulp of teinturier grapes. For example, in ‘Yan73,’ a teinturier grape variety in China, the skin contained the highest ratio of delphinidin, petunidin, and malvidin and their acylated derivatives cyanidin, peonidin, relative to pulp tissues (He et al. 2010). Xie et al. (2015) reported that tissue-specific expression of OMT (O-methyltransferase), F3’5’H (flavanoid 3’5’-hydroxylase), and LDOX (leucoantho-cyanidin dioxygenase) genes in ‘Yan73’ pulp cells was the potential basis for the specific localization of anthocyanins. Limited studies have been conducted, however, on the molecular mechanisms responsible for the flesh color in teinturier grapes. Therefore, the present study was designed to provide the first temporal transcriptomic atlas of teinturier grape berries.

Anthocyanins are a class of secondary metabolites in plants, referred to as flavonoids, and are generally present in the form of glycosides within plant vacuoles. Anthocyanin biosynthesis is genetically regulated and controlled through the expression of structural and regulatory genes, as well as enzyme complexes formed by a collection of enzymes that catalyze sequential biosynthetic reactions (Zhao and Dixon 2010). Phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), flavonoid 3’-hydroxylase (F3’5’H), flavonoid 3’5’-hydroxylase (F3’5’H), dihydroflavonol 4-reductase (DFR), leuкоantho-cyanidin dioxygenase (LDOX), UDP-
glucose-flavonoid 3-O-glucosyltransferase (UGFT), and O-methyltransferase (OMT) are all enzymes involved in the anthocyanin biosynthesis pathway (Zhao et al. 2016). Among these components, the correlation between PAL gene expression and anthocyanin accumulation was poorly understood since anthocyanins only represent a small fraction of the total flavonoids produced by plants (Lister et al. 1996). In contrast, the expression of CHS3 clearly exhibits a positive correlation with anthocyanin production (Zhao et al. 2016). Additionally, the relative expression of F3′5′H has also been significantly correlated with anthocyanin levels in pomegranate pulp (Guan et al. 2016) and complementation of an F3′Hs transgene in an anthocyanin-reduced potato mutant increased anthocyanin levels to wild-type levels (Maloney et al. 2014). Recent studies have revealed that R2R3-MYB transcripts, basic helix–loop–helix transcripts (bHLH), and WD40 protein domains regulate the expression of genes known to be involved in red-flesh development in fruits. The R2R3-MYB gene family has been reported to regulate red pigment synthesis in apple (Chagné et al. 2007; Umemura et al. 2013), Chinese bayberry (Niu et al. 2010), pear (Li et al. 2012), and grape (Cardoso et al. 2012). In grapes, a retrotransposon insertion in VvmybA1 produces white grape berries (Kobayashi et al. 2004). In the white-skinned cultivar, `Italia,’ there is a retrotransposon, Gret1, inserted in VvmybA1; while the other heterozygous allele VvmybA1b has no insertion. No expression of VvmybA1 is detected in `Italia.’ In red-skinned grape cultivars, however, VvmybA1 has been detected; suggesting that the retrotransposon insertion may prevent the transcription of VvmybA1.

Several environmental factors induce anthocyanin biosynthesis and sunlight is known to play a key role in color formation (Jackson and Lombard 1993). Previous studies have demonstrated a relationship between sunlight and the accumulation of anthocyanins in teinturier grapes. Anthocyanin biosynthesis in `Yan73’ is reduced and delayed in berry skin and pulp when exposure to sunlight is excluded (Guan et al. 2014). Different wavelengths of light, such as UV-light, blue light, and red light also affect anthocyanin biosynthesis (Peng et al. 2013; Liu et al. 2018). In particular, blue light is considered to be a wavelength that has a major impact on regulating the biosynthesis of anthocyanins in fruits. Anthocyanin production in response to blue light is mediated by cryptochrome (CRY) and phototropin (PHOT). Responses to red and far-red light are mediated by phytochrome (PHY). Downstream genes, such as CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1), and LONG HYPOCOTYL 5 (HY5) mediate photoreceptors to regulate anthocyanin biosynthesis (Liu et al. 2018).

RNA-seq is an efficient, powerful tool for transcriptome analysis that utilizes high-throughput sequencing technologies to generate millions of short cDNA reads. RNA-seq analysis was used in studies of color-mutant fruits to elucidate the molecular mechanisms underlying the shortage of anthocyanin in the yellow-skin apple mutant `Blondee’ and its red-skin parent `Gala.’ Results indicated that the expression of 34 genes was highly correlated with anthocyanin levels (Elsharkawy et al. 2015). The expression of Mdmyb10 and MdGST was found to be the most inhibited in `Blondee’ and further analysis indicated that Mdmyb10 and MdGST were methylated in fruit skin cells and that two regions of the Mdmyb10 promoter exhibited sequence differences between `Blondee’ and `Gala.’

In the current study, a newly identified teinturier germplasm, ZhongShan-HongYu (ZS-HY), was used to investigate the molecular mechanism regulating anthocyanin accumulation in teinturier grape berries. Unfortunately, the genetic background of ZS-HY is still unknown and needs to be determined. RNA-seq technology was used to identify the molecular basis of the regulation of the coloring of the pulp of grape berries at the transcriptional level. The composition and level of the different anthocyanins present in the pulp of the teinturier grape berries were also determined to better understand the relationship between changes in genes expression and anthocyanin development. The results of the study provide a strong foundation for understanding anthocyanin biosynthesis in teinturier grape and generated information that will be useful for the breeding of red-flesh grapes.

2. Material and methods

2.1. Plant material

`ZS-HY,’ a newly identified source of teinturier germplasm, was used in the study. A genetic analysis identified highly specific simple sequence repeats (SSRs) among ZS-HY, `Yan73’ (Muscat Hamburg × Alicante), and `Kolor’ of teinturier grape (Supplementary file 1; Supplementary file 2). In addition, leaf morphology was also distinctly different among these three genotypes (Supplementary file 3). The change in color, or veraison, of the pulp of ZS-HY occurred approximately 6 days after anthesis (DAA), while the skin veraison occurred about 35 DAA and the fruit maturity occurred at about 77 DAA. In other teinturier cultivars, such as `Alicante Bouschet,’ pulp veraison occurs about 56 DAA and skin veraison occurs about 67 DAA (Falginella et al. 2012). The average mature berry weight in ZS-HY was about 5.65 g and soluble solid content was approximately 15.7 °Brix.

Ripening berries of two-year-old `ZS-HY’ grape plants (8 m between rows and 3 m between plants) growing under a rain shelter were collected from the Nanjing Agricultural University vineyard located in the Tangshan Valley, Nanjing, Jiangsu Province. The berries were sampled at 3 days after anthesis (3 DAA, green-pulp, May 18th), 6 days after anthesis (6 DAA, pulp-veraison, May 21st), and 22 days after anthesis (22 DAA, pulp-half-colored, June 6th) in 2017 (Figure 1). Mature berries (77 DAA, July 31st) were also sampled and immediately separated into skin and pulp. All samples were frozen in liquid nitrogen and maintained at −80°C until further processing. The longitudinal and cross-sectional diameters of sampled berries at 3 DAA and 6 DAA were about 2.0–4.5 mm and were too small to separate into skin and pulp. The pulp in the grape berries began to develop a red color at 3 DAA, 6 DAA and 22 DAA, while the skin of the berries remained green (Figure 1). Thus, whole berries sampled at 3 DAA, 6 DAA, and 22 DAA were used in the transcriptome and anthocyanin analyses.

2.2. RNA extraction, library preparation, and sequencing

Samples collected at 3 DAA, 6 DAA and 22 DAA were used to construct transcriptome libraries. Total RNA was extracted from frozen samples using a Foregene RNA isolation kit (Foregene Co. Ltd, China) according to the
manufacturer’s instructions. The RNA quality was assessed using a 2200 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA). Two biological replicates were used for each developmental stage to construct two independent RNA-seq libraries for each developmental stage. A total of 3 µg RNA per sample was used as input material for subsequent construction of RNA-seq libraries. Sequencing libraries were constructed using an NEB Next Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA). The clustering of the index-coded samples was performed on a cBot Cluster Generation System using TruSeq PE Cluster Kit v3-cBot-HS (Illumina) software according to the manufacturer’s instructions. The libraries were sequenced after cluster generation on an Illumina Hiseq platform that generated 150 bp paired-end reads.

2.3. Mapping reads to the reference genome and gene annotation

The V. vinifera reference genome and gene model annotation files were downloaded from the grape genome website. An index of the reference genome was built and paired-end, clean reads were aligned to the grape reference genome using HISAT2. HISAT2 was selected as the mapping tool to generate a database of splice junctions based on the gene model annotation file. Gene function was annotated utilizing the six following databases: Nr (NCBI non-redundant protein sequences), Nt (NCBI non-redundant nucleotide sequences), Pfam (Protein family), KOG (Clusters of Orthologous Groups of proteins), Swiss-Prot (A manually annotated and reviewed protein sequence database), KO (KEGG Ortholog database), and GO (Gene Ontology). Generally, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) based enrichment analysis are the most commonly used gene function classification system used in transcriptome studies.

2.4. Differential expression analysis, GO and KEGG pathway enrichment analysis

Differential expression analysis of two samples was performed using the DESeq R package (1.10.1). DESeq provides the ability to determine statistical significance in digital gene expression data using a model based on a negative binomial distribution. The resulting p values were adjusted using the Benjamini and Hochberg’s approach for controlling the false discovery rate. Genes with an adjusted p-value <0.05 found by DESeq were determined to be differentially

Figure 1. Observation of berry appearance under stereomicroscope. There are three developmental stages of our material, green-pulp (May 18th, 3 DAA), pulp-veraison (May 21st, 6 DAA) and pulp-half-colored (June 6th, 22 DAA).
expressed. The p-value was also adjusted by using a q value. FDR < 0.01 and \(|\log_{2}\) (fold change) \(\geq 1\) was set as the threshold for significant differential expression.

GO and KEGG enrichment analysis of differentially expressed genes (DEGs) was used to identify candidate genes related to anthocyanin biosynthesis. Gene Ontology (GO) enrichment analysis of the DEGs was carried out using GO seq R packages based on a Wallenius non-central hyper-geometric distribution (Young et al. 2010). KOBAS (Mao et al. 2005) software was used to test the statistical significance of the enrichment of DEGs in the KEGG pathways.

### 2.5. RT-qPCR analysis

Nine candidate genes were selected for validation by Reverse Transcription – quantitative PCR (RT-qPCR). Gene-specific primer pairs were designed using Beacon Designer software and are listed in Supplementary File 4. VvACTIN was used as a house-keeping gene for normalization. RNA extracted from 3 DAA, 6 DAA and 22 DAA berries was used to synthesize cDNA using a Takara reverse transcription kit. RT-qPCR was performed on an ABI 7300 system and the amplification program was as follows: 30 s at 95°C, followed by 40 cycles of 5 s at 95°C and 30 s at 60°C. Three biological and technical replicates were used for the RT-qPCR analysis of each gene and sample.

### 2.6. Extraction, compositional and quantitative analysis of anthocyanins

A 1.00 g sample of dried grape powder, obtained by grinding grape berry samples in liquid nitrogen, was immersed in 10 ml methanol containing 1% formic acid. The extraction was performed with the aid of ultrasonic irradiation for 40 min at 40°C. Samples were then centrifuged at 8000 x g for 20 min and the supernatant was collected. The extraction process was repeated three times for each sample after which the supernatants were combined.

Total anthocyanin content was determined by measuring absorbance of the supernatant at 525 and 600 nm. The OD525 nm – OD600 nm represented the relative anthocyanin content per 0.01 and was defined as one unit of anthocyanin content.

The identity and quantification of individual anthocyanins was determined using an AB SCIEX Triple TOF 5600+ liquid mass spectrometer and was performed according to the previously reported protocol (Zhang et al. 2016). The mobile phase of aqueous 0.1% formic acid (solvent A) and acetonitrile (solvent B) were employed for high-performance liquid chromatography (HPLC) analysis at a flow rate of 0.2 mL·min\(^{-1}\). The following linear gradient was used (proportion of solvent B): 0–5 min, 10%; 5–40 min, 5–95%; 40–50 min, 95%; 50–52 min, 95–10%; and 52–60 min, 10%. A 5 μL shot volume was used each time and 525 nm was set as the wavelength for detection. The positive ion was carried out on mass spectrometry (MS) pattern and the instrument parameters using an electrospray ionization (ESI) interface; 35 psi nebulizer pressure; using N2, 10 L·min\(^{-1}\) drying gas and using 300 °C to dry the gas; and a scan range, 150–1200 m/z. Three biological replicates were utilized and obtained from the HPLC-MS of each sample.

### 2.7. Statistical analysis

The main anthocyanins were identified by comparing the chemical formula and relative molecular weight with detected data using Masterview software. No sample standards were used in this study and no standard curves were generated. Peak intensity and peak area square were used to approximate the relative content of anthocyanin. All data were analyzed by SPSS.

### 3. Results

#### 3.1. Anthocyanin composition and content

Ten different anthocyanins were identified collectively by LC/MS analysis in the different developmental stages of teinturier grape berries, i.e. green-pulp (3 DAA, May 18th), pulp-veraison (6 DAA, May 21st), pulp-half-colored (22 DAA, June 6th) and mature (77 DAA, July 31st). The different anthocyanins included 4 non-acylated and 6 acylated glucosides (Table 1). All of the detected anthocyanins were monoglycosides. Absorption peak areas were used to estimate the relative content of the different anthocyanins. The relative content of malvidin derivatives increased significantly during the first three stages of fruit development, while the relative content of Delphinidin-3-O-monoglucoside decreased at the same time. The other anthocyanins, including Peonidin-3-O-monoglucoside, Pelargonidin-3-O-monoglucoside, and Cyanidin-3-O-acetylglucoside, gradually increased in content or remained stable. The relative content of anthocyanins in pulp tissues in mature berries was lower than in skin tissues. A greater number of malvidin derivatives were detected in skin samples than in pulp samples, while peonidin derivatives exhibited the opposite pattern (Table 1). Overall, the total content of anthocyanins continually increased over the course of grape berry development (Supplementary file 5).

### Table 1. Anthocyanins composition and relative content in three stages.

| Peak | Compound                      | Absorption peak area* | 3 DAA | 6 DAA | 22 DAA | Ripen (skin) | Ripen (pulp) | Retention time | M* & MS2(m/z) |
|------|-------------------------------|-----------------------|-------|-------|--------|-------------|--------------|----------------|--------------|
| 1    | Cyanidin-3-O-acetlyglucoside  | 1.09                  | 1.77  | 1.43  | 6.56   | 2.77        | 2.61         | 491, 287, 449 | 493, 331     |
| 2    | Malvidin-3-O-monoglucoside    | 5.32                  | 11.78 | 8.09  | 4889.11| 406.25      | 12.19        | 493, 331      |              |
| 3    | Pelargonidin-3-O-monoglucoside| 0.32                  | 0.71  | 1.37  | 7.15   | 10.94       | 12.45        | 433, 271      |              |
| 4    | Petunidin-3-O-acetlyglucoside | 0.69                  | 1.00  | 2.97  | 14.80  | 2.35        | 11.42        | 521, 317, 479 |              |
| 5    | Delphinidin-3-O-monoglucoside | 17.13                 | 10.09 | 1.29  | 23.73  | 30.43       | 13.79        | 465, 303      |              |
| 6    | Peonidin-3-O-monoglucoside    | 5.70                  | 9.25  | 11.80 | 527.33 | 750.27      | 1.13         | 463, 301      |              |
| 7    | Peonidin-3-O-acetylglucoside  | 0.28                  | 1.14  | 0.81  | 27.23  | 10.33       | 13.98        | 505, 301, 463 |              |
| 8    | Malvidin-3-O-cafeoylglucoside | 2.16                  | 2.91  | 2.63  | 83.82  | 28.28       | 1.12         | 625, 301, 463 |              |
| 9    | Malvidin-3-O-cafeoylglucoside | 0.98                  | 2.84  | 5.90  | 9.48   | 10.58       | 15.2         | 655, 331, 493 |              |
| 10   | Malvidin-3-O-coumarylglucoside| 0.60                  | 4.44  | 11.26 | 7306.54| 53.49       | 2.01         | 639, 331, 493 |              |

*Absorption peak area was used to refer to the relative content in three stages.
Table 2. Summary of RNA-seq data.

| Sample      | Clean reads | Reads aligned | Q20 (%) | Q20 (%) | GC content (%) |
|-------------|-------------|---------------|---------|---------|----------------|
| 3 DAA-rep1  | 21372814    | 92.36         | 93.24   | 45.17   |
| 3 DAA-rep2  | 25917780    | 92.60         | 93.75   | 45.81   |
| 6 DAA-rep1  | 20084229    | 93.50         | 94.85   | 45.77   |
| 6 DAA-rep2  | 26164988    | 94.03         | 95.47   | 45.96   |
| 22 DAA-rep1 | 21150550    | 94.03         | 95.66   | 45.58   |
| 22 DAA-rep2 | 25921780    | 92.36         | 95.77   | 45.53   |

Sample: a-rep1 and -rep2: two biological replicates.
Reads aligned: the number of reads after controlling the sequencing raw data quality.
Q20 and Q30: Phred score, refer to the accuracy of sequenced bases were 99 and 99.9%.
GC content: the percentage of G and C in total bases.

3.2. RNA-seq analysis

A total of six cDNA libraries were constructed from the three stages of berry development (two biological replicates for each stage). After removal of the adaptor sequences (reads containing poly-N and low-quality reads), a range of 20–26 million paired-end clean reads were obtained from each sample (Table 2), and used in the subsequent analyses. The Pearson’s Correlation Coefficient for the two replicates of each sample was greater than 0.97 (Additional files 6), indicating that the correlation between two replicates was strong and that the sequencing results obtained from each of the two replicates were similar.

After quality control, the clean data were mapped to the grape reference genome (ftp://ftp.ncbi.nlm.nih.gov/genomes/Vitis_vinifera/) using HISAT2. A total of 92.36–94.03% of the genes obtained from each sample could be mapped to the grape genome. Approximately 70% of the aligned reads from each sample mapped to exonic regions of the reference genome, 12–14% mapped to intergenic regions, and 14–16% mapped to intronic regions (Supplementary file 7).

3.3. Identification of differentially expressed genes (DEGs).

Thousands of DEGs were identified among three different stages of grape berry development based on the RNA-seq analysis. Three pairwise comparisons, 6 DAA vs. 3, 22 DAA vs. 6 DAA, and 22 DAA vs. 3 DAA, were conducted; resulting in the identification of 3727, 3866, and 5936 DEGs, respectively. DEGs were designated as significant using the following parameter; fold change ≥1 or ≤−1, and padj (p-value after multiple calibration corrected) ≤0.05. The maximum number of DEGs was identified in the 22 DAA vs. 3 DAA comparison with 2331 up-regulated genes and 3605 down-regulated genes. The 6 DAA vs. 3 DAA and the 22 DAA vs. 6 DAA comparisons resulted in the identification of 1373 and 1778 up-regulated genes, and 2354 and 2088 down-regulated genes, respectively. A total of 1131 differentially expressed genes were identified in the 6 DAA vs. 3 DAA and the 22 DAA vs. 6 DAA comparison; while only 462 DEGs were identified in the 6 DAA vs. 3 DAA and 22 DAA vs. 3 DAA comparisons. A total of 182 significantly up-regulated and 279 down-regulated genes were also identified in all three comparisons (Figure 2).

3.4. Functional annotation and enrichment analysis of DEGs.

A series of complementary approaches were used to annotate the DEGs and six kinds of functional annotations (NR, Pfam, Swiss-Port, KOG, GO, and KEGG) of DEGs are shown in Table 3. They include 3447 (3593; 5498), 2715 (2816; 4249), 2685 (2793; 4223), 1599 (1788; 2642), 2534 (2704; 4044) and 581 (657; 936) DEGs in the 6 DAA vs. 3 DAA, 22 DAA vs. 6 DAA, and 22 DAA vs. 3 DAA comparisons that were annotated in the NR, Pfam, Swiss-Port, KOG, GO, and KEGG databases, respectively.

GO enrichment analysis was used to predict the biological function of the DEGs within three main categories: biological process, molecular function, and cellular component. Biological process contained the largest proportion of DEGs, totaling 46.63% (24,985 of 53,578), followed by cellular component (31.05%; 16,636), and molecular function (22.32%; 11,957). In the 6 DAA vs. 3, 22 DAA vs. 6 DAA, and 22 DAA vs. 3 DAA comparisons, metabolic process (24.90%; 1703, 25.65%; 1895, 24.97%; 2687), cellular process (18.65%; 1275, 19.00%; 1404, 19.22%; 2068) and single-organism process (18.06%; 1235, 18.39%; 1359, 18.38%; 1977) were identified as the most enriched subclasses of biological process, respectively. Cell part (23.37%; 975, 22.11%; 1116, 22.68%; 1682), cell (23.27%; 971, 22.03%; 1112, 22.63%; 1678), and organelle (16.56%; 691, 15.91%; 803, 16.63%; 803) were designated as significantly up-regulated and 279 down-regulated common genes among three stages.

Figure 2. Venn diagram of up-regulated (A) and down-regulated (B) DEGs in three stages during fruit development. And there are 76 up-regulated and 183 down-regulated common genes among three stages.
16.40%; 1216) were identified as the most enriched sub-classes of cellular component, respectively. Catalytic activity (42.72%; 1422), binding (41.63%; 1386), and nucleic acid binding transcription factor activity (4.80%; 160) were the top three enriched subclasses of molecular function in the 6 DAA vs. 3 DAA comparison, while catalytic activity (44.78%; 1550), binding (39.38%; 1363), and transporter activity (6.04%; 209, 5.57%; 2236), binding (39.38%; 1363, 41.15%; 2126), and transporter activity (6.04%; 209, 5.57%; 2236) were the most enriched subclasses in the 22 DAA vs. 6 DAA and 22 DAA vs. 3 DAA comparisons, respectively. Some GO pathways related to light response were also identified which helped to clarify the relationship between pulp veraison and other regulatory factors. A total of 97 DEGs associated with light were identified from 23 GO pathways in the 6 DAA vs. 3 DAA comparison. Among these DEGs, 71 were up-regulated and 26 were down-regulated. A total of 118 DEGs associated with light were identified from 20 GO pathways in the 22 DAA vs. 6 DAA comparison. Among these DEGs, 21 were up-regulated and 97 DEGs were down-regulated. A total of 104 DEGs associated with light were identified from 22 GO pathways in the 22 DAA vs. 3 DAA comparison. Among these DEGs, 33 were up-regulated and 71 were down-regulated (Supplementary file 8).

KEGG pathway enrichment analysis was conducted for the 6 DAA vs. 3 DAA, 22 DAA vs. 6 DAA, and 22 DAA vs. 3 DAA comparisons. Results indicated that carbohydrate metabolism was the most enriched pathway in the 6 DAA vs. 3 DAA, and 22 DAA vs. 3 DAA comparisons, containing 77 and 115 genes, respectively. This was followed by energy metabolism, containing 64 and 112 genes, respectively, and amino acid metabolism, containing 60 and 111 genes, respectively. In the 22 DAA vs. 6 DAA comparison, energy metabolism was the most enriched pathway, containing 98 genes, followed by carbohydrate metabolism (71 genes), and amino acid metabolism (67 genes). Light has been previously reported to promote anthocyanin biosynthesis (Guan et al. 2014). In our results, the photosynthesis – antenna proteins pathway was found to be enriched in all three comparisons; while the photosynthesis pathway was found to be enriched in the 22 DAA vs. 6 DAA and 22 DAA vs. 3 DAA comparisons (Supplementary file 9). The photosynthesis – antenna proteins and photosynthesis pathways contained 11 and 9 up-regulated DEGs, respectively, in the 22 DAA vs. 3 DAA comparison. In the 22 DAA vs. 6 DAA comparison, the photosynthesis – antenna proteins and photosynthesis pathways contained 17 and 39 down-regulated DEGs, respectively. The photosynthesis – antenna proteins and photosynthesis pathways in the 22 DAA vs. 3 DAA comparison contained 11 and 35 down-regulated DEGs, respectively. The DEGs in the photosynthesis – antenna proteins and photosynthesis pathways appear to be associated with the upstream regulation of anthocyanin biosynthesis.

### Table 3. The number of DEGs through different functional annotation.

| DEG Set* | Swiss-Prot* | GO* | KEGG* | KOG* | Pfam* | NR* |
|----------|-------------|-----|-------|------|-------|-----|
| 6 DAA vs 3 DAA | 2685 | 2534 | 581 | 1599 | 2715 | 3447 |
| 22 DAA vs 3 DAA | 2423 | 2044 | 936 | 2642 | 4249 | 5498 |
| 23 DAA vs 22 DAA | 2793 | 2704 | 657 | 1788 | 2816 | 3593 |

*DEG Set: three pairwise comparisons. Swiss-Prot, GO, KEGG, KOG, Pfam and NR: the number of DEGs annotate from different database.

3.5. Selection of candidate genes

#### 3.5.1. Candidate genes associated with the anthocyanin biosynthesis pathway

In the present study, 56 DEGs that putatively participate in anthocyanin biosynthesis were identified; including 30 structural genes and 26 regulatory genes (Tables 4 and 5). These candidate genes were primarily identified based on their assignment in the category of metabolism in the KEGG pathway analysis and metabolic processes in the GO analysis. Seven structural genes (Table 4) and 6 regulatory genes (Table 5) were up-regulated in the 6 DAA vs. 3 DAA comparison, representing the stage at which the color of the pulp began to turn red; and were subsequently down-regulated in the 22 DAA vs. 6 DAA comparison. Five structural genes and 1 regulatory gene exhibited a decrease in expression during the period from 3 DAA to 22 DAA. Additionally, 8 structural genes and 16 regulatory genes exhibited a down-regulation, while 10 structural genes and 3 regulatory genes exhibited up-regulation during the time course of the experiment. The structural genes, F3’5’H, F3H, UFGT and OMT, exhibited a higher level of expression than other candidate structural genes. A total of 26 regulatory genes (Table 5), including eighteen Myb transcripts, six bHLH transcripts, and two WD40 transcripts were identified. The four Myb transcripts and two WD40 transcripts exhibited an up and then down trend of expression over the course of the experiment. Three Myb transcripts and one bHLH transcript exhibited an upward trend of expression over the course of grape berry development. Some of the up-regulated genes had significantly higher expression levels than others, such as GSVIVT01009424001 (Myb44), GSVIVT01020205001 (bHLH155), and GSVIVT01009000501 (putative WD40).

#### 3.5.2. Candidate genes that putatively participate in light-responsive reactions and their association with anthocyanin biosynthesis

In our study, 20–23 GO pathways were identified in the three comparisons that are associated with light. Two KEGG pathways, photosynthesis – antenna proteins and photosynthesis, were also significantly enriched in the three comparisons. Based on our analyses, a total of seven candidate genes were identified that are associated with light absorption and response and that might also regulate downstream genes participating in anthocyanin biosynthesis (Table 6). Three genes associated with light reaction were up-regulated at the pulp-veraison stage, including one cryptochrome-related gene (GSVIVT01009033001), one phototropin-related gene (GSVIVT01023824001), one gene annotated as a bZIP family HY5, and one CON- STANS-like (COL) B-box gene.

3.6. RT-qPCR analysis

Nine candidate genes were randomly selected to validate the accuracy and reproducibility of the RNA-seq data. In each case, the results of the RT-qPCR analysis were similar to the results obtained by the RNA-seq analysis (Figure 3).
Table 4. Candidate structural genes related to the anthocyanins biosynthesis.

| Gene ID                  | Log-Fold | 3 DAA   | 6 DAA   | 22 DAA  | Description                                       |
|-------------------------|----------|---------|---------|---------|---------------------------------------------------|
| GSVVIT01000219001       | 2.97     | 1.83    | 5.54    | 12.45   | MYB60                                             |
| GSVVIT01004573001       | 1.05     | 28.12   | 47.14   | 140.08  | Hypothetical protein LOC100247039                 |
| GSVVIT01028129001       | 1.20     | 3.14    | 5.97    | 1.51    | Anthocyanidin 3-O-glucosyltransferase 7           |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |
| GSVVIT01016409001       | 1.60     | 2.22    | 0.65    | 6.27    | Anthocyanidin 3-O-glucosyltransferase 6           |
| GSVVIT01016427001       | 3.59     | 18.46   | 251.61  | 0.87    | UDP-glycosyltransferase 76C3                      |
| GSVVIT01034131001       | 1.52     | 17.74   | 3.44    | 2.66    | Flavonol synthase/flavanone 3-hydroxylase          |
| GSVVIT01023904001       | 2.97     | 1.83    | 5.54    | 12.45   | MYB60                                             |
| GSVVIT01028235001       | 2.59     | 3.60    | 8.01    | 20.24   | Hypothetical protein LOC100247039                 |
| GSVVIT01028961001       | 1.40     | 1.98    | 4.48    | 1.83    | /-deoxyloganetic acid glucosyltransferase         |
| GSVVIT01028929001       | 2.48     | 1.57    | 0.34    | 0.17    | Anthocyanidin 3-O-glucosyltransferase 2           |
| GSVVIT01029126001       | 1.20     | 3.14    | 5.97    | 1.51    | Anthocyanidin 3-O-glucosyltransferase 7           |
| GSVVIT01029758001       | 1.12     | 16.27   | 32.44   | 9.47    | Transcription factor RAE1                         |

Table 5. Candidate regulatory genes related to the anthocyanins biosynthesis.

| Gene ID                  | Log-Fold | 3 DAA   | 6 DAA   | 22 DAA  | Description                                       |
|-------------------------|----------|---------|---------|---------|---------------------------------------------------|
| GSVVIT01012725001       | 1.45     | 11.41   | 27.91   | 5.46    | MYB-like transcription factor ETC3 isoform X2     |
| GSVVIT01012725001       | 1.45     | 11.41   | 27.91   | 5.46    | MYB-like transcription factor ETC3 isoform X2     |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |
| GSVVIT01028961001       | 1.45     | 1.43    | 1.77    | 3.70    | Anthocyanidin 5,3-O-glucosyltransferase 6          |

4. Discussion

4.1. Anthocyanin composition and content of teinturier grape berries during development

Anthocyanins, which are synthesized through the flavonoid pathway, are primarily responsible for the red/purple color of grape berries. Generally, anthocyanins only accumulate in grape skin, but we investigated a teinturier grape genotype of grape berries. Generally, anthocyanins only accumulate in grape skin, but we investigated a teinturier grape genotype in which anthocyanins are also produced in the pulp tissue at an earlier stage than in skin tissues. Six main types of anthocyanins were identified (Table 1); including Pelargonidin (Pg), Delphinidin (Dp), Malvidin (Mv), Cyanidin (Cy), Peonidin (Pn), and Petunidin (Pt), in both their hydroxylated and methylated forms. Collectively, a total of ten types of anthocyanins were identified, which were all monoglucosides.

Our results are in accordance with a previous study that reported that the predominant anthocyanins in V. vinifera grape berries were monoglucoside derivatives (Cruz et al.)
In contrast, diglucoside derivatives are typically detected in Chinese wild grapes (Liang et al. 2013). By comparing the absorption peak area of each anthocyanin, Malvidin derivatives were found to be the most abundant at the beginning of veraison and their relative content increased more rapidly than other anthocyanins. Jeong et al. (2006) and Castellarin et al. (2006) reported that the expression ratio of flavonoid 3'-hydroxylase (F3'H) to flavonoid 3'5'-hydroxylase (F3'5'H) reflected the ratio of di-substituted (cyanidin- and peonidin-) anthocyanins to tri-substituted anthocyanins.

### Table 6. Candidate regulatory genes related to the light absorption and response.

| Gene ID          | Log2Fold | 3 DAA | 6 DAA | 22 DAA | FPKM* |
|------------------|----------|-------|-------|--------|-------|
| GSVVIT01009033001 | 1.11     | 14.34 | 27.74 | 21.97  |       |
| GSVVIT01028420001 | 2.34     | 0.23  | 1.10  | 1.25   |       |
| GSVVIT01029894001 | −1.76    | 0.84  | 0.08  | 0.02   |       |
| GSVVIT01030081001 | 1.39     | 3.44  | 7.09  | 3.67   |       |
| GSVVIT01021381001 | 1.22     | 7.55  | 15.67 | 13.52  |       |
| GSVVIT01032821001 | −2.78    | 16.77 | 4.50  | 7.39   |       |
| GSVVIT01036499001 | 3.06     | 6.26  | 46.81 | 15.70  |       |

*Expression level is shown in RPKM for each sample.

![Figure 3.](image)
(delphinidin, petunidin and malvidin) anthocyanins. In this regard, the present study indicated that the expression level of F3’5’H was 10-fold higher than F3’H (Table 4), which was consistent with the observed ratio of tri-substituted anthocyanins to di-substituted anthocyanins. Peonidin derivatives became the predominant anthocyanins in pulp tissue at the mature berry stage, while malvidin derivatives were the predominant components in skin tissue. Interestingly, Pelargonidin-3-O-monoglucoside is rarely produced in most varieties of V. vinifera (Boss et al. 2010; Niu et al. 2016) but detected in both pulp and skin tissues of ZS-HY. This suggests that the different genes may regulate and be responsible for anthocyanin biosynthesis in ZS-HY.

A trace amount of anthocyanin was detected in 3 DAA samples, at which time grape berries had not begun to develop red color. A recent study demonstrated that the skin of some white grape cultivars also contains measurable traces of anthocyanins (Arapitsas et al. 2015), most likely due to some putative UDP-glycosyltransferases (UGTs). In our study, ten candidate UGTs were identified that may putatively participate in anthocyanin biosynthesis prior to veraison (Figure 4). The expression of these genes may be related to the accumulation of anthocyanins in ZS-HY.

4.2. Biosynthesis and modification of anthocyanins

Several researchers have reported on the biosynthetic pathway leading to anthocyanins in many species and key structural genes have been examined to determine the mechanism responsible for controlling the anthocyanin pathway (Sparvoli et al. 1994; Jaakola et al. 2002; Han et al. 2012). In V. vinifera, the anthocyanin biosynthetic pathway has also been thoroughly studied in grape berry skin, but the molecular mechanism responsible for pulp veraison in teinturier grapes has not been completely elucidated. Phenylalanine ammonia-lyase (PAL), cinnamate-4-hydroxylase (C4H) and 4-coumaroyl-CoA synthase (4CL) play a functional role in the general phenylpropanoid pathway going from Phenylalanine (PAL) to flavonoid, while the expression of other F3Hs (F3H1 and F3H2) coincides with the biosynthesis of flavonol, while the expression of only F3H2 coincides with anthocyanin biosynthesis. F3Hs expression leads to the formation of dihydroflavonol and F3H2 has been reported to exhibit higher expression levels in berry skin at the ripening stage, which is also called the veraison stage, when the grape skin is turning red (Jeong et al. 2008). Two F3Hs (GSVIVT01011165001 and GSVIVT01008907001) were identified in the current study. The expression level of GSVIVT01008907001 was only up-regulated in the 22 DAA vs. 6 DAA comparison, while the other F3H (GSVIVT01011165001) was down-regulated. These results indicate that F3Hs may play an important role in anthocyanin production after pulp veraison and are not the key genes that influence the pulp red coloring in

Figure 4. Heatmap of differential expressed UGTs. Each row represents a gene and each column represents a stage of sample. Red color means up-regulated and green color means down-regulated. Five of UGTs were up-regulated at veraison.
teinturier genotypes. Flavonoid 3’-hydroxylase (F3’H) and flavonoid 3’-hydroxylase (F3’5’H) in *V. vinifera* belong to the cytochrome P450 super family and determine the ratio of cyanidin-based anthocyanins to delphinidin-based anthocyanins by catalyzing the addition of a hydroxyl group at the 3’ or 3’5’ position of the B-ring of flavonoids. Thus, F3’H and F3’5’H could divide and separate the flavonoid pathway into two branches that lead to the accumulation of cyanidin-; peonidin-glycosides (red to purple) and delphinidin-; malvidin-; petunidin-glycosides (blue to purple), respectively. A previous study (Mu et al. 2014) on a teinturier cultivar, ’Yan-73,’ demonstrated that the transcript level of F3’H was much higher than F3’5’H in pulp tissue. In our study, however, the expression level of F3’5’H was significantly higher than F3’H. Following an optimization of gene structures, several new DEGS were annotated. Specifically, two were annotated as F3’5’H (MSTRG.20641 and MSTRG.20648) and one was annotated as F3’H (MSTRG.20646). The expression level of both F3’H and F3’5’H were up-regulated in the 6 DAA vs. 3 DAA comparison and the expression level of F3’5’H (MSTRG.20641) was 10-fold higher than the expression level of F3’H (MSTRG.20646). This observation is consistent with the ratio of tri-substituted anthocyanins to di-substituted anthocyanins. As a result, the F3’5’Hs are probably the predominant genes that influence the formation of different anthocyanins in the pulp samples of our material at the initial stage of veraison. Additionally, dihydroflavonols are converted to anthocyanins by dihydroflavonol 4-reductase (DFR) and leucoantho-cyanidin dioxygenase (LDOX) and we identified DEGs annotated as DFR (GSVIVT01014573001) and LDOX (GSVIVT01034131001). These results are also consistent with the findings of previous studies (Gutha et al. 2010; Zhao and Dixon 2010; Pastore 2011).

After a series of biosynthetic processes, anthocyanins are further modified by glycosylation and methylation, which is proposed to stabilize them. UDP-glycosyltransferases (UGTs) in *V. vinifera* catalyze glycosylation at the C3 position, whereas UGTs also catalyze glycosylation at the C5 position in some species. Previous studies have shown that *VvUGGT* in grapes is the major gene that controls grape berry veraison (Kobayashi et al. 2001; Ageorges et al. 2006). Only one gene (GSVIVT01029680001) in the current study was annotated as UDP-glucose-3-epi-flavanone-3-O-glucosyltransferase (UGFT) and its expression was modestly up-regulated during the stage of pulp veraison. These data indicate that UGFT may not be the key gene regulating pulp veraison in ZS-HY. A total of 181 putative regulatory genes were identified during the initial stage of anthocyanin accumulation. This may lead to the up-regulation of anthocyanin accumulation in ZS-HY grape berries. The role of *VvMYBPA1* and *VvMYBPA2* in the synthesis of proanthocyanidins in grapevine have been studied and found to be highly expressed during early grape berry development and in seeds. The same transcripts are then subsequently down-regulated during the accumulation of anthocyanins in grape berries (Bogs et al. 2007; Terrier et al. 2009). *VvMYBA1* (GSVIVT01022659001) or *VvMYBA2* (GSVIVT01022664001) and one of them was annotated as *VvMYB2* (GSVIVT01022656001). These three genes were up-regulated in 6 the DAA vs. 3 DAA comparison but down-regulated in the 22 DAA vs. 6 DAA comparison. This pattern of expression is consistent with the expression level of the gene annotated as anthocyanidin 3-O-glucosyltransferase (GSVIVT01022659001) (Table 4). Thus, we suggest that *VvMYBA1*, *VvMYBA2*, and *VvMYBA3* may play a major role in the regulation of anthocyanin accumulation in ZS-HY grape berries. The role of *VvMYBPA1* and *VvMYBPA2* in the synthesis of proanthocyanidins in grapevine has been studied and found to be highly expressed during early grape berry development and in seeds. The same transcripts are then subsequently down-regulated during the accumulation of anthocyanins in grape berries (Bogs et al. 2007; Kuhn et al. 2014). In the current study, *VvMYBPA1* was highly up-regulated in grape berry samples collected on May 18th and then down-regulated at the beginning of anthocyanin accumulation. This may lead to the up-regulation of anthocyanin-related structural genes, such as *F3’5’H*. In contrast, *VvMYBPA2* was up-regulated at the initial stage of anthocyanin synthesis and was subsequently down-regulated.

MYB transcripts are thought to be the key component regulating the expression of structural genes in the flavonoid pathway, while bHLH transcripts and WD40 proteins may also have similar, overlapping regulatory targets (Jaakola 2013). *VvMYC1* in *V. vinifera* has been reported to take part in the synthesis of anthocyanins and proanthocyanidins and interact with different MYB proteins, such as *VvMYBSa*, *VvMYBSb*, and *VvMYBA1/A2* (Hichri et al. 2010). WD40 genes are probably the predominant genes that influence the formation of different anthocyanins in the pulp samples of our material at the initial stage of veraison. Additionally, dihydroflavonols are converted to anthocyanins by dihydroflavonol 4-reductase (DFR) and leucoantho-cyanidin dioxygenase (LDOX) and we identified DEGs annotated as DFR (GSVIVT01014573001) and LDOX (GSVIVT01034131001). These results are also consistent with the findings of previous studies (Gutha et al. 2010; Zhao and Dixon 2010; Pastore 2011).
proteins interact with bHLH and are then thought to be mobilized to the nucleus where they physically interact with MYBs (Matus et al. 2010). Six DEGs belonging to the bHLH family and two DEGs belonging to the WD40 (GSVIVT01009005001 and GSVIVT01029758001) were identified in the RNA-seq data in the present study (Table 5). Among these DEGs, one of the bHLHs (GSVIVT01020205001) was up-regulated during berry development and both of the WD40s (GSVIVT01009005001 and GSVIVT01029758001) were up-regulated in the 6 DAA vs. 3 DAA comparison and down-regulated in the 22 DAA vs. 6 DAA comparison. One of the candidate bHLHs (GSVIVT01013156001) was also found to be functionally similar to the MYC transcription factor by KEGG analysis and the expression pattern of this gene (GSVIVT01013156001) was similar to VvMYBPA1 (GSVIVT01027182001). It is possible that GSVIVT01013156001 may interact with VvMYBPA1 and function in the synthesis of proanthocyanidins. We suggest that GSVIVT01020205001, GSVIVT01009005001, and GSVIVT01029758001 may play an important role in regulating the genes associated with anthocyanin biosynthesis and probably interact with R2R3-MYBs.

4.4. Light response related genes and their association with the biosynthesis of anthocyanins

The accumulation of anthocyanins was reported to be enhanced by ABA treatment and suppressed by light exclusion in grape berry skins (Jeong et al. 2004). Several studies have demonstrated that light has a major impact on the biosynthesis of anthocyanins (Guan et al. 2014). The accumulation of anthocyanins is also regulated by the quality of light. Previous studies demonstrated that blue light had a greater effect on anthocyanin accumulation than red and far-red light (Meng et al. 2004; Liu et al. 2018). Blue light exposure and overexpression of the CRY1a gene has been reported to be associated with increased accumulation of anthocyanin in tomato (Liu et al. 2018) and suggested that SlHY5 may mediate CRY1a effects on anthocyanin biosynthesis. One gene annotated as CRY1 was identified to be blue light photoreceptor associated with downstream gene expression. One bZIP transcription factor, HY5 (GSVIVT01035829001), and one B-box gene, COL2 (GSVIVT01036499001), identified in our current study are both light-responsive genes and may regulate the downstream transcription of several anthocyanin-related structural genes (CHS, CHI, F3’5’H) (Zhang et al. 2011). Phytochrome may regulate plant responses to red and far-red light in Arabidopsis thaliana that may be related to anthocyanin biosynthesis (Steinitz et al. 1979). Kadomura-Ishikawa et al. (2013) demonstrated that FaPHOT2 expression increases anthocyanin content in Fragaria x ananassa. In the current study, one gene annotated as Phototropin-2 (PHOT2), may act as a blue light receptor, and exhibited a high level of expression during pulp veraison.

A transcriptional regulatory model illustrating light-responsive reactions in ‘ZhongShan-HongYu’ teinturier grape inferred from the RNA-seq analysis is presented in Figure 5. After grapes flower, photoreceptors, such as CRY (GSVIVT01009005001) and PHOT (GSVIVT01023842001), receive light and activate the transcription of HY5 (GSVIVT01035829001) and COL2 (GSVIVT01036499001); thereby inducing the transcription of downstream transcription factors (TFs), such as MYBA1 (GSVIVT01022659001), MYBA2 (GSVIVT010226560001), and structural genes, CHS (MSTRG.9521), CHI (GSVIVT01032392001), and F3’5’H (MSTRG.20641). This chain of events may result in the induction of pulp veraison in teinturier grapes.

5. Conclusion

In the present study, new information was obtained pertaining to the content and composition of anthocyanin in teinturier grape berries. RNA-seq was used to identify DEGs during grape berry development and identify potential candidate genes responsible for the synthesis and accumulation of anthocyanins in pulp and skin tissues of developing grape berries. New candidate genes related to anthocyanin biosynthesis and genes that were highly expressed during pulp veraison were identified and warrant further study. The potential mechanism of anthocyanin biosynthesis in teinturier grape berries was elucidated and a putative model for light-induced anthocyanin biosynthesis in teinturier grapes was postulated. The present study provides a scientific foundation for further studies on the breeding of high-quality and economically viable teinturier grape cultivars.

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QLW and JMT designed the research and wrote the manuscript. QLW, SMG and HL performed the experiments. QLW analyzed the data. HZ reviewed and revised the manuscript. All authors read and approved the final manuscript.

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