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Transcriptome-sequencing analyses reveal flower color formation in *Strelitzia reginae*

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Abstract

Strelitzia reginae is a popular cut flower that has blue petals and orange sepals. Flower color is an important plant trait; however, little is known about its molecular mechanisms in *S. reginae*. In this study, cDNA libraries were constructed for blue petals and orange sepals of *S. reginae*. A total of 75 487 unigenes were obtained from transcriptome sequencing and *de novo* assembly, of which 41.86 % were annotated by public databases. Ultra-high performance liquid chromatography analysis revealed that anthocyanins were the main pigment in blue petals, and that carotenoids controled pigment formation in the orange sepals. Using a system analysis-based approach, 73 and 29 candidate genes related to anthocyanin and carotenoid biosyntheses were identified, respectively. Among these, *chalcone synthase 2*, *chalcone isomerase 1*, *flavanone 3-hydroxylase 1*, *flavonoid 3',5'-hydroxylase 1*, *dihydroflavonol 4-reductase 1*, *anthocyanidin synthase 1*, and *anthocyanidin 3-O-glucosyltransferase 1* were considered to be important in regulating the formation of blue petals, and *phytoene synthase 1*, *phytoene desaturaser 1*, *ζ-carotene desaturase 1*, *lycopene β-cyclase 3*, and *β-carotene hydroxylase 2* might play important roles in orange sepal formation. This study improves our understanding of flower color and provides evidence for future molecular breeding of ornamental plants based on flower color modifications.

Additional key words: anthocyanins, carotenoids, transcriptome sequencing.

Introduction

Flower color is one of the important characters that have been used in measuring the quality of ornamental plants. The quality of flowers is directly related to their ornamental and commercial values. In addition, flower color can attract insects to help pollination. Plant colors are mainly controlled by two pathways, namely, the anthocyanin pathway and the carotenoid pathway (Weiss 1995, Tanaka *et al*. 2008). Anthocyanins are the main pigments responsible for yellow, orange, red, purple, and blue colors (Tanaka *et al*. 1998, Iwashina 2000). Among these, blue is considered a rare color because it is difficult to obtain real blue flowers in many floricultural plants, such as carnation, chrysanthemum, rose, and lotus (Chandler and Tanaka 2007, Yang *et al*. 2009). Traditional breeding methods are difficult in direct breeding blue flowers, and genetic engineering shows some advantages (Sasaki and Nakayama 2015). Carotenoids comprise 40 carbon isoprenoids that are usually involved in yellow-orange coloration (Hirschberg 2001). To date, almost all of the genes related to carotenoid biosynthesis have been characterized (Pandurangaiah *et al*. 2016, Rezaei *et al*. 2016, Yang *et al*. 2016). However, there are few genetic data involved in carotenoid pathways of *Strelitzia reginae*.

Strelitzia reginae, also known as the bird of paradise, belongs to family *Strelitziaceae* and is native to southern Africa. It has a significant commercial value as cut flowers, which has blue petals and orange sepals (Paiva *et al*. 2004). However, pigment composition and genomic analyses in relation to flower color have not been conducted to date. The mechanisms of flower color formation in *S. reginae*, thus, remain unclear. Research studies on the regulatory mechanisms of flower color in *S. reginae* are therefore necessary.

Ribonucleic acid sequencing (RNA-seq) has become a

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Abbreviations: ANS - anthocyanidin synthase; CHI - chalcone isomerase; CHS - chalcone synthase; CHYB - β-carotene hydroxylase; DFR - dihydroflavonol 4-reductase; F3H - flavanone 3-hydroxylase; F3′5′H - flavonoid 3′,5′-hydroxylase; 3GTs - 3-glycosyltransferases; LCYB - lycopene β-cyclase; PSY - phytoene synthase; PDS - phytoene desaturase; UA3GT - anthocyanidin 3-O-glucosyltransferase; UPLC - ultra-high performance liquid chromatography; UPLC-QTOFMS - ultra performance liquid chromatography-quadrupole-time of flight-mass spectrometry; ZDS - ζ-carotene desaturase.
The full names of all enzymes are listed in Table 2 Suppl.

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particularly effective method for exploring the molecular mechanisms of certain characters, especially in non-model plants that have been used as reference genomes (Lou *et al*. 2014, Zhao *et al*. 2014, Zhang *et al*. 2015). In this study, we first sequenced the transcriptomes of orange sepals and blue petals of *S. reginae* on an *Illumina HiSeq™ 4000* platform. Qualitative and quantitative analyses of anthocyanins and carotenoids were performed with ultra performance liquid chromatography quadrupole time of flight mass spectrometry (UPLC-QTOFMS) and UPLC technologies. We analyzed the biochemical and molecular mechanisms of orange and blue color formation in *S. reginae* with the aim to improve understanding of flower color formation for possible future molecular breeding of ornamental plants based on flower color modifications.

Materials and methods

Plants and cultivation: *Strelitzia reginae* Aiton was open-air cultivated at the Fujian Academy of Agricultural Sciences in Fuzhou City, China. The blue petals and orange sepals of *S. reginae* were collected in October 2017. A total of 12 blue petals and 12 orange sepals from each plant were pooled. The samples were frozen in liquid nitrogen and then stored at -80 °C for transcriptomic analyses.

Library construction and sequencing: Total RNA was extracted from the blue petals and orange sepals using a universal RNA extraction kit (*Bioteke Corporation*, Beijing, China). The RNA integrity was examined with a *NanoDrop 2000* (*Thermo Scientific*, Waltham, MA, USA). Library constructions and transcriptome sequencing (*NCBI SRA* accession number PRJNA513215) were performed by *Biomarker Biotechnology Corporation* (Beijing, China) using an *Illumina HiSeq™ 4000*.

Assembly and functional annotation: High-quality clean reads were obtained from the *Illumina*-generated raw reads by removing low-quality reads. *De novo* assembly of the reads into contigs was performed using *Trinity* (Grabherr *et al*. 2011) and further assembled into transcripts using paired-end reads. Finally, the transcripts were screened by identifying unigenes.

Based on sequence similarity, *BLASTx* analysis against public databases was performed using an E-value threshold of 10^{-5}, which included *clusters of orthologous groups* (*COG*) of proteins, *gene ontology* (*GO*), *Kyoto encyclopedia of genes and genomes* (*KEGG*), *eukaryotic clusters of orthologous groups* (*KOG*), *protein family* (*Pfam*), *Swiss-Prot* protein database, evolutionary genealogy of genes: *non-supervised orthologous groups* (*eggNOG*), and *NCBI non-redundant protein* (Nr). A *Pfam* search was performed using the *HMMER 3.0* program with an E-value cut-off of 10^{-10} (Finn *et al.* 2011). Classification of *GO* terms was conducted using *Blast2GO* (Gotz *et al*. 2008).

Analysis of differentially expressed genes: The gene expression was estimated by RNA-Seq by expectation maximization (*RSEM*) (Li and Dewey 2011). The abundance of each gene was calculated using fragments per kilobase of transcript per million mapped reads (FPKM) (Trapnell *et al*. 2010). Differentially expressed genes (DEGs) among four different libraries were evaluated using the *DESeq* package (Anders and Huber 2010). A false discovery rate (FDR) < 0.01 and a fold-change value ≥ 2 were used as thresholds to confirm DEGs between two samples.

Quantitative real-time PCR analysis was performed using an *ABI 7500* real-time PCR system (*Applied Biosystems*, Waltham, MA, USA). Primers were designed for the determination of relative expressions of the candidate genes using *Primer Premier 5.0*. Melting curve analysis was conducted to confirm PCR specificity. Three independent replicates of each sample were analyzed. The *β-actin* gene was used as an internal control. A relative quantitative computing method ($2^{-\Delta\Delta Ct}$ method) was used to calculate changes in unigene expression.

Measurement of anthocyanin content: The frozen samples (100 mg) were grounded using a *Tissue Lyser JX-24* (*Jingxin*, Shanghai, China) with beads at 40 Hz for 4 min and extracted with 1 cm^3 of 60 % (v/v) ethanol containing 0.1 % (m/v) hydrochloric acid at 35 °C for 2 h. After centrifugation at 4 °C and 12 000 *g* for 15 min, the supernatant was collected and evaporated to remove ethanol. The concentrated extracts were then loaded into an *AB-8* resin column (200 × 20 mm). The anthocyanins absorbed onto the resin were washed with distilled water with 0.1 % hydrochloric acid and subsequently eluted with 60 % ethanol containing 0.1 % hydrochloric acid until colorless. The eluent was lyophilized and reconstituted in 1.5 cm^3 of 50 % (m/v) acetonitrile containing 0.5 % (v/v) formic acid. Following centrifugation at 12 000 *g* for 15 min, 0.09 cm^3 of the supernatant were combined with 0.01 cm^3 of 25 μg cm^{-3} lidocaine (internal standard), and the mixture was analyzed by *UPLC-QTOFMS*.

Chromatographic separation was performed on an *ACQUITY UPLC I-Class* system (*Waters Corporation*, Milford, MA, USA) with a *Waters Acquity UPLC HSS T3* column (2.1 × 100 mm, 1.7 μm) maintained at 45 °C. Chromatograms of anthocyanins were acquired at a wavelength of 525 nm. The injection volume was 3 mm^3. The mobile phases consisted of water (phase A) and acetonitrile (phase B), both with 0.5 % formic acid (v/v). A linear gradient elution was performed with the following program: 0 - 2 min, 1 % B; 3 min, 5 % B; 9 min, 20 % B; 12 min, 50 % B; 15 min, 100 % B; 17 min, 100 %B; and 17.1 min, 1 % B; and held to 20 min.

The eluents were analyzed on a *Vion IMS QTOF* mass spectrometer (*Waters Corporation*) on an ESI+ mode. The capillary voltage was set to 2 kV. The sampling cone voltage and cone gas flow were 40 V and 50 dm^3 h^{-1}, respectively. The desolvation gas was maintained at a flow rate of 900 dm^3 h^{-1} and a temperature of 450 °C, respectively. The ion source temperature was 115 °C. The time of flight mass spectrometry scan was operated at a high-resolution with 0.2 s survey scan time and a range of 50 - 1 000 m/z in the continuum mode both for function 1 and 2. To improve the

identification of unknown metabolites, the MS^E function was also performed to obtain fragment ion information with a ramp collision energy from 20 to 45 eV. The mass accuracy calibration was performed with the lock mass, leucine-enkephalin at 250 ng cm^{-3} and 5 mm^3 min^{-1}, with data acquisition frequency set at 30 s. The *UNIFI 1.8.1* (*Waters Corporation*) software was used to control the instrument and collecting data. Delphinidin 3-glucoside was used as quantification standard. Three biological replicates were employed.

Measurement of carotenoid content: Frozen flower samples (1 g) were extracted with acetone:petroleum ether (1:1) at 50 °C for 3 h, until the residue was whitish. The extract was evaporated to dryness at 40 °C using a rotary evaporator, dissolved in 20 cm^3 of petroleum ether containing 0.1 % (m/v) butylated hydroxytoluene, then 20 cm^3 of 10 % (m/v) KOH-methanol solution was added. When emulsification occurred, 10 % (m/v) NaCl was added to emulsification. The upper petroleum ether phase was collected, transferred to a separatory funnel, and the petroleum ether phase was washed repeatedly with double distilled water until the aqueous phase was neutral. Approximately 10 g of anhydrous sodium sulfate were added, filtered, and evaporated to dryness using a rotary evaporator at 40 °C. The solution was then dissolved in 2 cm^3 of acetone (containing 0.1 % BHT). The carotenoids were separated and identified using an *ACQUITY UPLC BEH* C18 column (2.1 × 50 mm, 1.7 µm) (*Waters Corporation*) with a mobile phase of acetonitrile:methanol (9:1) at a wavelength of 450 nm. Three biological replicates were performed.

Results

Using UPLC-QTOFMS, two anthocyanins (delphinidin 3-glucoside and hydroxycyanidin 3-glucoside) were detected in the blue petals. No anthocyanins were detected in the orange sepals (Fig. 1). The delphinidin 3-glucoside was the predominant anthocyanin, and a peak content of 54.82 $\mu g \cdot g^{-1}$(f.m.) was detected at the blooming stage (Table 1 Suppl.).

Using UPLC, three carotenoids (lutein, β-cryptoxanthin, and β-carotene) were detected in the orange sepals (Fig. 1). No carotenoids were detected in the blue petals. The β-carotene and β-cryptoxanthin were the predominant carotenoids, with peak content of 825 and 322 µg g^{-1}(f.m.) detected at the blooming stage (Table 1 Suppl.).

Due to the absence of available genomic information on *S. reginae*, *Illumina* sequencing and *de novo* assembly were conducted using the blue petals and orange sepals in this study. A total of 26.16 Gb high-quality clean reads were generated. *De novo* assembly of these reads respectively resulted in 127 803 transcripts and 75 487 unigenes, with N50 values of 1 981 and 1 565 bp, reflecting a good assembly. Of the obtained unigenes, 22 376 were longer than 1 000 bp, accounting for 31.8 % of all of the unigenes (Table 1).

To obtain further information about the unigenes, all

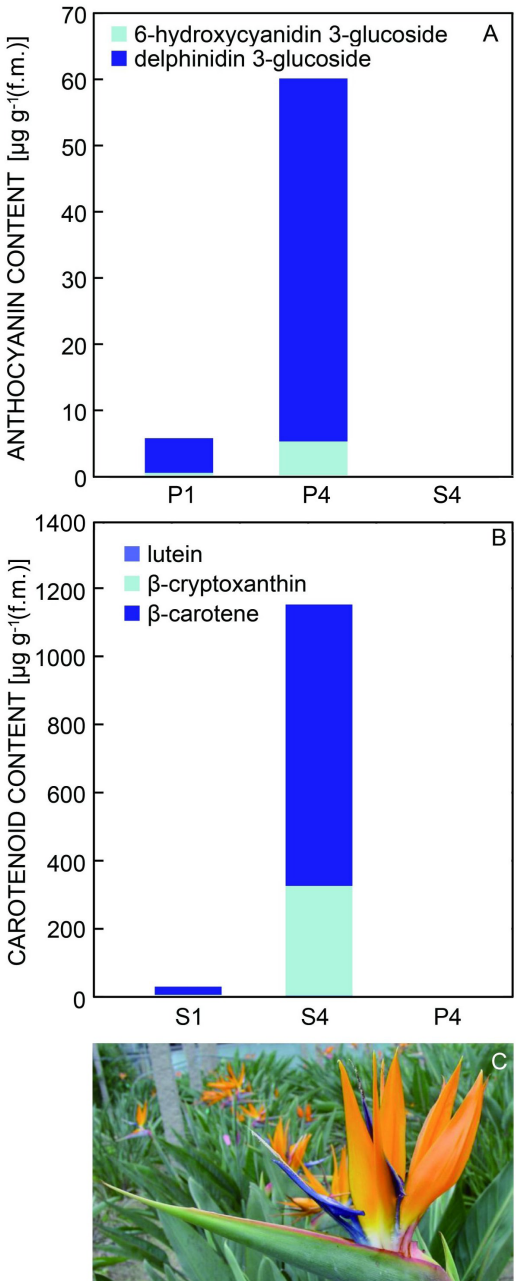

Fig. 1. Anthocyanins (*A*) and carotenoids (*B*) in blue petals and orange sepals (*C*). P1- bud stage of blue petals, P4 - blooming stage of blue petals, S1- bud stage of orange sepals, S4 - fully bloomed stage of orange sepals.

of the unigene sequences were annotated using *BLAST* analysis against eight public databases. Of the 75 487 unigenes, 29 505 assembled sequences were annotated, accounting for approximately 41.86 %, using an E-value ≤ 1e-5. A total of 15 936 unigenes (21.11 % of all unigenes) were functionally annotated into three major categories and 52 *GO* classes. In the molecular function categories, most unigenes were related to catalytic activity, binding, and transporter activity. However, a large proportion of assembled unigenes remained unannotated, which involved non-coding RNAs, untranslated regions,

Table 1. Quality parameters of the *Strelitzia reginae* transcriptome.

Length [bp]	Transcript number	Percent	Unigene number	Percent
200 - 300	18100	14.16	16478	21.83
300 - 500	21667	16.95	17197	22.78
500 - 1,000	30329	23.73	19436	25.75
1000 - 2000	32185	25.18	13376	17.72
2000+	25522	19.97	9000	11.92
Total number	127803		75487	
Total length	161149389		71961475	
N50 length	1981		1565	
Mean length	126092		95330	

or may be unique to *S. reginae*, which can be a good resource for studying novel genes (Chung *et al*. 2016, Kwenda *et al*. 2016).

KEGG is an important database that can be used to systematically analyze metabolic pathways and products and functions of related genes. Using the *KEGG* pathway, the DEGs in *S. reginae* were analyzed. Among them, 1 497 were grouped into 117 *KEGG* pathways. Four functional categories (phenylpropanoid biosynthesis, phenylalanine metabolism, flavonoid biosynthesis, and carotenoid biosynthesis) involved in flower color revealed significant enrichment. The enrichment of pathways involved in the anthocyanin biosynthesis and carotenoid biosynthesis were consistent with the observed high anthocyanin content in the blue petals and carotenoid content in the orange sepals. These provided a valuable resource for gene discovery and functional analysis of *S. reginae*.

To explore the molecular mechanism of formation of blue petals and orange sepals in *S. reginae*, a comparison of gene expression was conducted between these two plant parts. A total of 5 233 unigenes were differentially expressed between blue petals and orange sepals. Among them, 2 695 unigenes were upregulated, whereas 980 were downregulated in the blue petals. Transcript abundance was revealed by hierarchical cluster analysis. The expression of the blue petals and orange sepals showed pronounced differences.

The unigenes involved in anthocyanin biosynthesis were analyzed in *S. reginae*. A total of 73 unigenes were identified (Table 2 Suppl.). To obtain the key genes related to the metabolism of blue color, transcriptional profiles of genes involved in anthocyanin biosynthesis between blue petals and orange sepals were analyzed. The expressions of 21 key unigenes showed significant differences, which included 16 upregulated and 5 downregulated unigenes (Table 2 Suppl.). These DEGs included

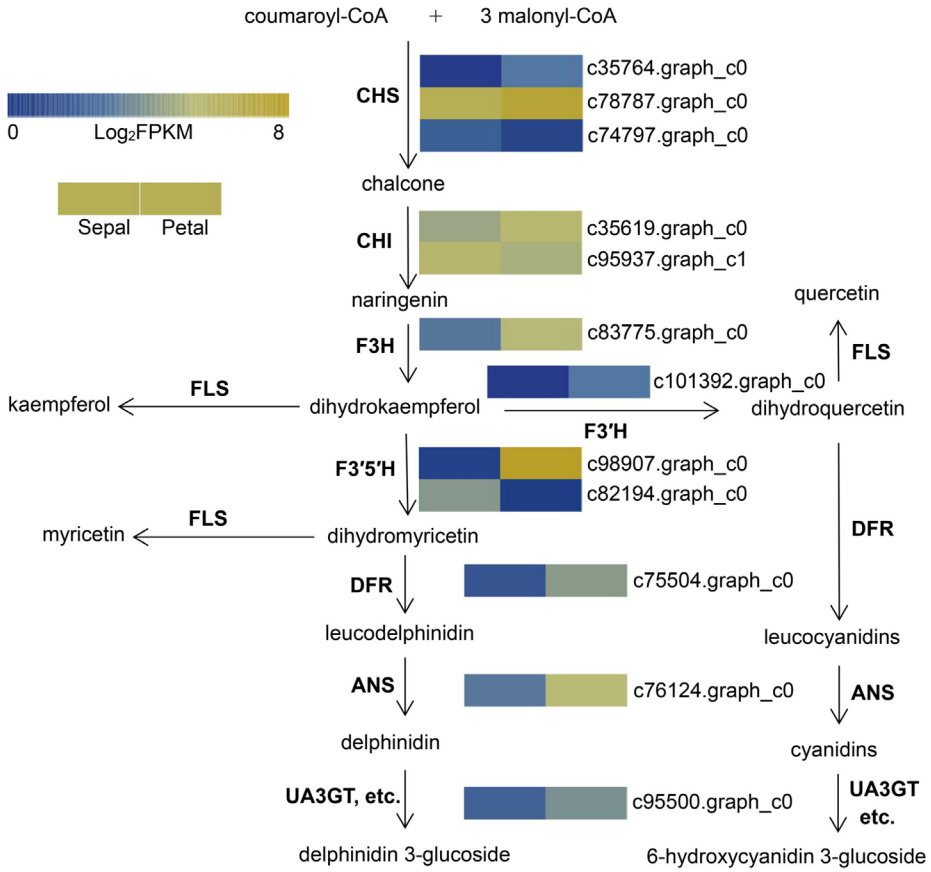

Fig. 2. Expression patterns of genes involved in anthocyanin biosynthesis. The abbreviated name of enzyme in each catalytic step is shown in *bold*. Gene expression - $\log_2$ of fragments per kilobase of transcript per million mapped reads (FPKM) in sepals and petals are represented by *color gradation*. The full names of the enzymes are listed in Table 2 Suppl.

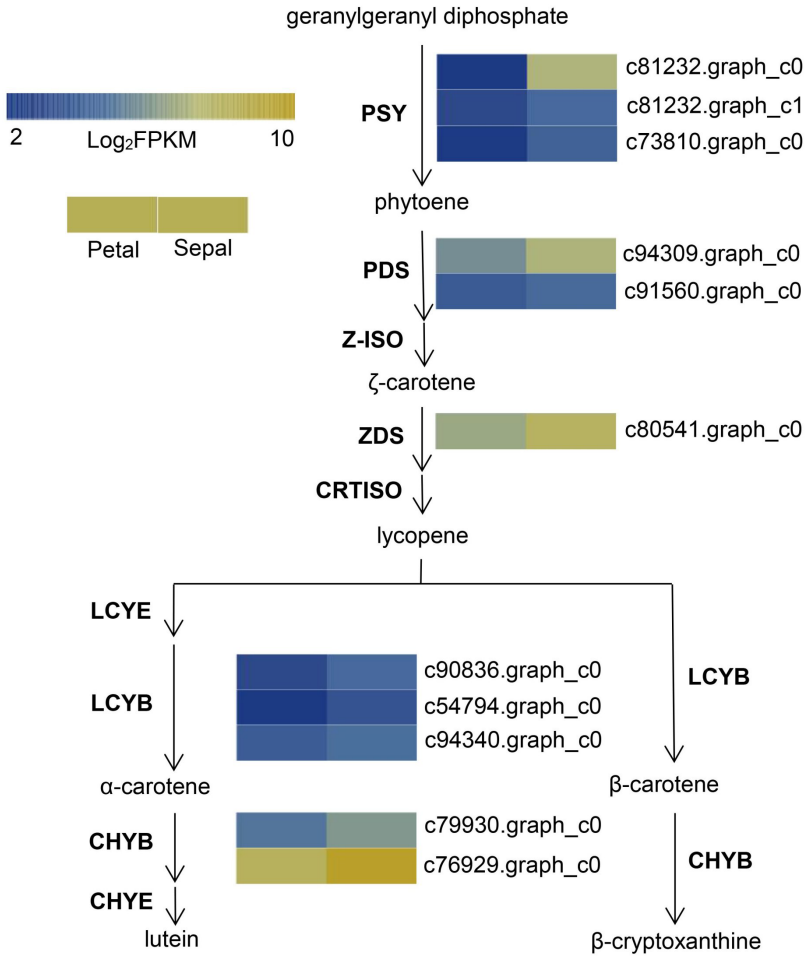

Fig. 3. Expression patterns of genes encoding enzymes involved in carotenoid biosynthesis. The abbreviated name of enzyme in each catalytic step is showed in *bold*. Gene expression - $\log_2$ of fragments per kilobase of transcript per million mapped reads (FPKM) in sepals and petals are represented by *color gradation*. The full names of enzymes are listed in Table 2 Suppl.

both upstream genes [*e.g.*, *chalcone synthase* (*CHS*), *chalcone isomerase* (*CHI*), and *flavanone 3-hydroxylase* (*F3H*)] and downstream genes [*e.g.*, *anthocyanidin 3-O-glucosyltransferase* (*UA3GT*)] (Fig. 2). Among these, seven structural unigenes *SrCHS2* (c78787.graph_c0), *SrCHI1* (c35619.graph_c0), *SrF3H1* (c83775.graph_c0), *flavonoid 3',5'-hydroxylase 1* (*SrF3'5'H1* c98907.graph_c0), *dihydroflavonol 4-reductase 1* (*SrDFR1* c75504.graph_c0), *anthocyanidin synthase 1* (*SrANS1* c76124.graph_c0), and *UA3GT1* (c95500.graph_c0) were upregulated in blue petals. And real-time quantitative PCR analysis showed that the expression of these seven genes gradually increased during flowering and peaked at the blooming stage, suggesting that these play important roles in the accumulation of "blue" pigments (Fig. 4). The formation and stability of the blue color was mainly dependent on glycosylation and hydroxylation rather than acylation and methoxylation because there were not observed acylated or methylated anthocyanin, and acylation or methoxylation genes in DEGs exhibited low expressions.

To identify the key genes involved in the formation of orange pigment, 28 unigenes related to carotenoid biosynthesis were obtained (Table 2 Suppl.). Among these, 11 genes revealed significantly different expressions, and all of the DEGs were upregulated in the orange sepals (Fig. 3). Five genes, *phytoene synthase 1* (*SrPSY1*, c81232.graph_c0), *phytoene desaturase 1* (*SrPDS1*, c94309.graph_c0), *ζ-carotene desaturase 1* (*SrZDS1*, c80541.graph_c0), *lycopene β-cyclase 3* (*SrLCYB3*, c94340.graph_c0), and *β-carotene hydroxylase 2* (*SrCHYB2*, c76929.graph_c0)] showed high transcript abundances. Real-time quantitative PCR analysis revealed that the expression patterns of five genes were relatively low at the bud stage and high at the blooming stage, reflecting that these play a crucial role in the formation of orange pigments (Fig. 4).

To verify the reliability of our transcriptome data, real-time quantitative PCR analyses were conducted based on the sequences of 24 core unigenes using the designed primers. The results revealed a positive correlation between quantitative PCR and the transcriptome sequence data ($R^2 = 0.8983$) (Fig. 1 Suppl.).

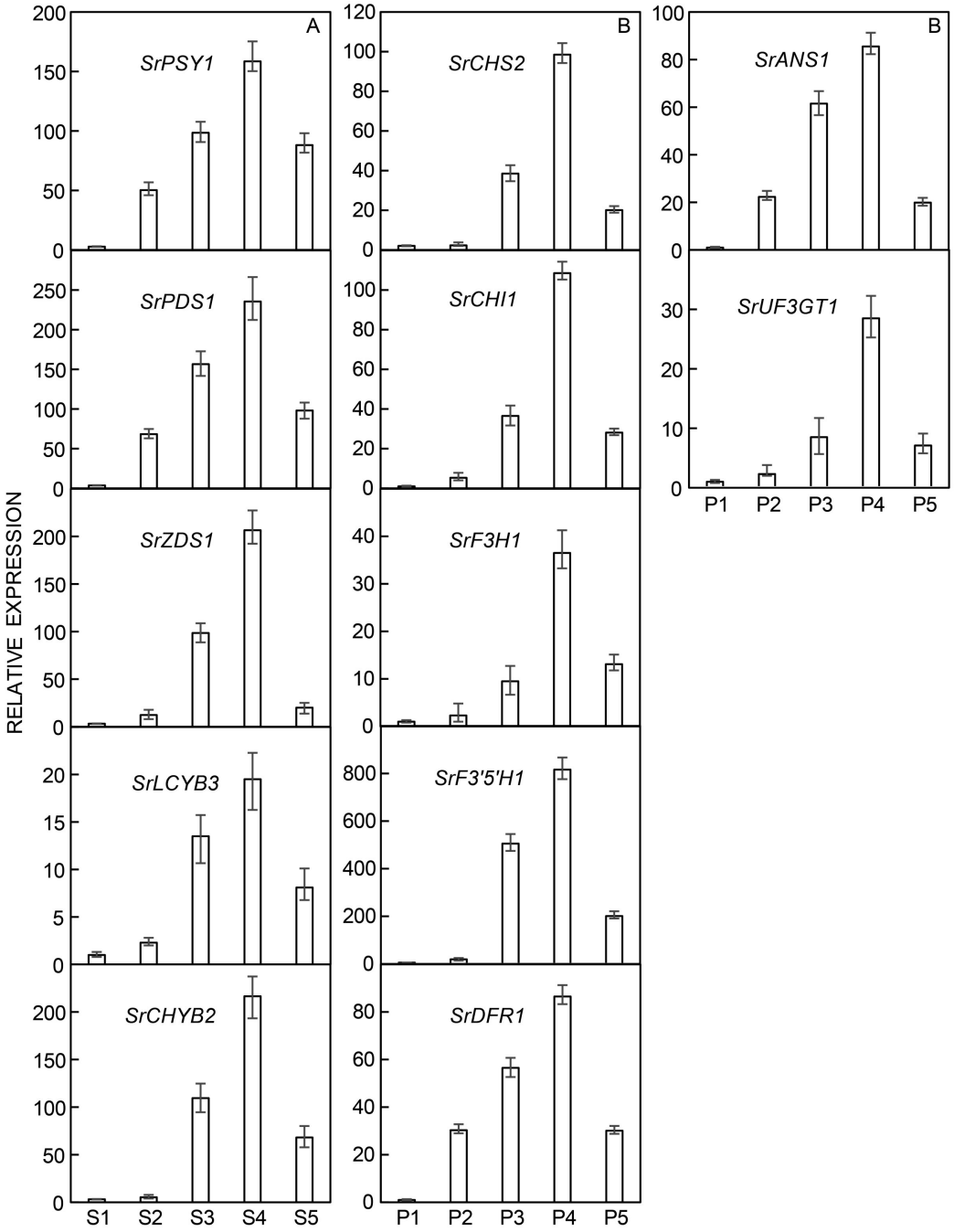

Fig. 4. Expression analyses of anthocyanin and carotenoid biosynthetic genes during flower development. P1 - bud stage of blue petals, P2 - mid-bud stage of blue petals, P3 - early flowering stage of blue petals, P4 - fully bloomed stage of blue petals, P5 - fade stage of blue petals, S1- early bud stage of orange sepals, S2 - mid-bud stage of orange sepals, S3- early flowering stage of orange sepals, S4 - fully bloomed stage of orange sepals, S5 - fade stage of orange sepals. The expressions were normalized to the expression of *β-actin*, and expressions P1 and S1 were set to 1.0. Means ± SDs, $n = 9$ (three biological replicates and three technical replicates).

Discussion

S. reginae is a popular ornamental plant that has blue petals and orange sepals. Blue flowers are considered rare, and many other important ornamental plants lack blue flowers, such as *Tulipa* and *Phalaenopsis.* To breed plants with blue-colored flowers, the mechanism of blue coloration should be elucidated. In addition, the formation of orange/yellow flowers is controlled by two pathways, namely the carotenoid and anthocyanin pathways. Investigating orange sepal formation in *S. reginae* may thus facilitate the identification of major pigmentation pathways in specific plant parts.

As the genome sequence of *S. reginae* has not been reported to date, it is difficult to determine the mechanism underlying blue and orange color formation. In this study,

transcriptome sequencing of blue and orange sepals was conducted. Approximately 75 487 unigenes were obtained from four cDNA libraries. A total of two anthocyanins were detected in blue petals, in which delphinidin-3-glucoside showed the highest content. Among DEGs related to anthocyanin biosynthesis, *SrCHS2*, *SrCHI1*, *SrF3H1*, *SrF3′5′H1*, *SrDFR1*, *SrANS1*, and *SrUA3GT1* were upregulated in blue petals and are involved in delphinidin-3-glucoside biosynthesis. The formation of blue petals is regulated by multiple genes. Three carotenoids were detected in the orange sepals, in which β-carotene and β-cryptoxanthin had high content. Among the DEGs involved in carotenoid biosynthesis, *SrPSY1*, *SrPDS1*, *SrZDS1*, *SrLCYB3*, and *SrCHYB2* were upregulated in the orange sepals. These unigenes were upstream of the carotenoid pathway, mainly regulating β-carotene and β-cryptoxanthin biosynthesis.

Flavonoid 3′-hydroxylase and F3′5′H determine the hydroxylation type of the B-ring of anthocyanins and are the key enzymes for cyanidin and delphinidin production, respectively. Thus, flavonoid 3′-hydroxylase and F3′5′H are essential for the structural organization of anthocyanins and are considered as the "red gene" and "blue gene" (Tanaka 2006). Flowers of numerous ornamental plants lack blue-color because these do not possess the *F3′5′H* gene. The heterologous overexpression of the *F3′5′H* gene in carnations (Chandler and Brugliera 2011), roses (Katsumoto et al. 2007), and chrysanthemums (Brugliera *et al*. 2013, Noda *et al*. 2013) results in a violet/blue color of their flowers. A total of eight *F3′5′H* genes were identified in *S. reginae*. One F3′5′H gene is upregulated in blue petals ($log_2FC = 11.23$) and upregulated during flower development, and peaked during the early flowering stage (Fig. 4). Delphinidin-3-glucoside is the predominant anthocyanin, which was in a relatively low content during the bud period and peaked during the early flowering stage (Fig. 1). This indicated that *F3′5′H* is a key gene that is related to delphinidin-3-glucoside content.

Dihydroflavonol 4-reductase uses one or three of dihydroflavonols (dihydromyricetin, dihydrokaempferol, or dihydroquercetin) to create leucoanthocyanidin, which is a key step to anthocyanin synthesis. The structure of the three substrates is similar and only differs in terms of the B phenyl ring, particularly in terms of the number of hydroxyl groups, in which there is no enzymatic action (Wu *et al*. 2016). Thus, DFRs can catalyze all three substrates in various plant species. However, in some species, such as *Petunia hybrida* and *Cymbidium hybrida*, DFR has strict substrate specificity (Johnson *et al*. 2001). In this study, only delphinidin and cyanidin-based anthocyanins were detected, and no pelargonidin-based anthocyanins were found. One possible explanation for this observation is that DFRs in *S. reginae* cannot utilize dihydrokaempferol or DFRs can utilize three dihydroflavonols, but flavonol synthase preferentially utilize dihydrokaempferol. In this study, DFR had a higher expression than flavonol synthase and, therefore, DFR had a greater priority. It is possible that DFR has substrate specificity and cannot utilize dihydrokaempferol in *S. reginae*.

Anthocyanins are modified by glycosylation, acylation, or methylation. These modifications keep the anthocyanins stable and soluble and facilitate the generation of different colors (Grotewold 2006). We hypothesize that acylation and methylation may not be as important as glycosylation during the formation of blue petals in *S. reginae* because acylated and methylated anthocyanins were not detected, and the genes involved in acylated and methylated modifications were downregulated. Delphinidin 3-glucoside and hydroxycyanidin 3-glucoside are major anthocyanins, reflecting that the modification of the 3-position is essential to blue petal color formation. Therefore, 3-glycosyltransferases (3GTs) might play a crucial role in the formation of blue petals in *S. reginae*. One 3GT has been identified, namely, UA3GT. Eleven UA3GT homologous unigenes were found in *S. reginae*. The expression of SrUA3GT1 is significantly upregulated from bud stage to blooming stage, suggesting that SrUA3GT1 could be the most important enzyme in delphinidin 3-glucoside accumulation. This study has improved our understanding of the molecular mechanism underlying blue flower pigmentation and provides a resource for the molecular breeding of color-modified ornamental plants.

In *Camellia nitidissima*, carotenoid synthesis serves as the main pathway for color formation in petals (Miyajima *et al*. 1985, Zhou 2012), which is basically controlled at the transcriptional level (Ohmiya 2013). In chrysanthemums, the expression of most genes in the carotenoid pathway, including *PSY*, *PDS*, *ZDS*, *carotenoid isomerase*, *LCYB*, *lycopene cyclase*, and *CHYB,* are upregulated during flower development (Ohmiya *et al*. 2006). The expression patterns of *PSY*, *ZDS*, *carotenoid isomerase*, and *CHYB* were similar in the Asiatic hybrid lily (Yamagishi *et al*. 2010). In this study, the content of β-carotene and β-cryptoxanthin, as detected by UPLC-QTOFMS, increased from the bud to the blooming stages, as well as those of *SrPSY1*, *SrPDS1*, *SrZDS1*, *SrLCYB3*, and *SrCHYB2.* These results indicate that the expression of these genes is crucial for carotenoid accumulation in sepal formation in *S. reginae,* and that the carotenoid pathway is mainly regulated at the transcriptional level.

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