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RESEARCH ARTICLE

Comparative Transcriptome Analysis of Flower Senescence of *Camellia lutchuensis*

Weixin Liu[1,2], Hengfu Yin[1,2], Yi Feng[1,2], Suhang Yu[1,2], Zhengqi Fan[1,2], Xinlei Li[1,2] and Jiyuan Li[1,2,*]

[1]*State Key Laboratory of Tree Genetics and Breeding, Research Institute of Subtropical Forestry, Chinese Academy of Forestry, Hangzhou, Zhejiang 311400, China;* [2]*Key Laboratory of Forest Genetics and Breeding, Research Institute of Subtropical Forestry, Chinese Academy of Forestry, Hangzhou, Zhejiang 311400, China*

Abstract: ***Background*:** Flower senescence is the last stage of flower development and affects the ornamental and economic value of flower plants. There is still less known on flower senescence of the ornamental plant *Camellia lutchuensis*, a precious species of *Camellia* with significant commercial application value.

***Methods*:** Transcriptome sequencing was used to investigate the flower senescence in five developmental stages of *C. lutchuensis*.

***Results*:** By Illumina HiSeq sequencing, we generated approximately 101.16 Gb clean data and 46649 differentially expressed unigenes. Based on the different expression pattern, differentially expressed unigenes were classified into 10 Sub Class. And Sub Class 9 including 8252 unigenes, was highly expressed in the flower senescent stage, suggesting it had a potential regulatory relationship of flower senescence. First, we found that ethylene biosynthesis genes *ACSs*, *ACOs*, receptor *ETR* genes and signaling genes *EINs*, *ERFs* all upregulated during flower senescence, suggesting ethylene might play a key role in the flower senescence of *C. lutchuensis*. Furthermore, reactive oxygen species (ROS) production related genes *peroxidase* (*POD*), *lipase* (*LIP*), *polyphenoloxidase* (*PPO*), and ROS scavenging related genes *glutathione S-transferase* (*GST*), *glutathione reductase* (*GR*) and *superoxide dismutase* (*SOD*) were induced in senescent stage, suggesting ROS might be involved in the flower senescence. Besides, the expression of monoterpenoid and isoflavonoid biosynthesis genes, transcription factors (*WRKY*, *NAC*, *MYB* and C_2H_2), *senescence-associated gene SAG20* also were increased during flower senescence.

***Conclusion*:** In *C. lutchuensis*, ethylene pathway might be the key to regulate flower senescence, and ROS signal might play a role in the flower senescence.

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1. INTRODUCTION

Flower longevity is an important basis of flower quality and economic value [1] and flower senescence is the last stage of flower development and the crucial factor of flower longevity [2]. Flower senescence first manifested as petal rolling, dehydration, color change, black spots and so on, which seriously affected the ornamental and economic value [3]. A series of studies have shown that key factors are involved in the regulation of flower senescence, such as ethylene regulation [4, 5], Reactive Oxygen Species (ROS) and enzyme metabolism [6, 7], sugar metabolism [8, 9], DNA and nuclear degeneration [10, 11] and programmed cell death (PCD) [12], senescence related genes regulation networks [13, 14] and so on.

The onset of flower senescence requires the coordinated regulation of a series of genes which is triggered by internal and external factors [15]. The plant hormones ethylene has been regarded as an internal regulator of senescence regulation network in many plants, such as *Oncidium* [1] and *Rosa hybrida* [4]. In plant senescent organs, such as flower, leaf and fruit, ethylene were induced and ethylene-related genes were involved in organ senescence [16]. In cut roses, ethylene was the main factor affecting the flower longevity, *RhACO1* expression level was strongly related to flower longevity and petal senescence [17]. ROS concentrations rise at flower opening and then again senescence symptoms become evident. Enzymatic scavenging of ROS, such as superoxide dismutase activity, remained high in carnation peroxisomes during senescence [6]. Sucrose and trehalose treat-

*Address correspondence to this author at the State Key Laboratory of Tree Genetics and Breeding, Research Institute of Subtropical Forestry, Chinese Academy of Forestry, Hangzhou, Zhejiang 311400, China;
Tel: +86-571-63346372; E-mail: jiyuan_li@126.com

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ments delayed flower senescence in cut astilbe [18, 19] and fructose and glucose content decreased during petals senescent stage [20]. In addition, nitric oxide [21] and ubiquitination [22] as endogenous signal are involved in flower senescence. And the antioxidants, such as nano-silver [3, 23], lanthanum [24] and 8-hydroxychinoline [3, 25] delay petal senescence.

The expression of senescence related genes is closely related to plant senescence. In petunia, *PaACL* (*ATP-citrate lyase*) silencing reduced anthocyanin contents, increased ethylene production and accelerated flower senescence [13]. The expression of *PhPR10.1* induced ethylene and flower senescence, but silenced *PhPR10.1* by VIGS accelerated flower senescence, indicating that *PhPR10.1* played an antagonistic role in the petal senescence in rose [4]. RhABF2 regulates RhFer1 for maintaining iron levels and enhances dehydration tolerance during senescence in rose flowers [26].

Camellia is one of the top ten traditional flowers in China and the precious woody flowers in the world possessing high ornamental and great economic value [27]. *Camellia lutchuensis* is a precious species of *Camellia* and is famous for being fragrant in *Camellia* plants [28]. However, the blooming period of *C. lutchuensis* is short and the flowers are easy to wither, which restricts its ornamental value. Therefore, the study on the flower senescence mechanism of *C. lutchuensis*, can lay a foundation for inhibiting the senescence process through molecular biological means and prolong the full bloom period, which can greatly improve the ornamental and economic value.

To test the mechanism of flower senescence of *C. lutchuensis*, we initiated a study to investigate the flower senescence at the transcriptome level within five developmental stages. Based on the results of transcriptome data, we analyzed the possible regulation pathway of flower senescence of *C. lutchuensis*.

2. MATERIALS AND METHODS

2.1. Plant Materials and Sample Preparation

We took *C. lutchuensis* as materials, and according to the progress of flower development, it can be divided into early bud stage, late bud stage, half opening stage, complete opening stage and senescent stage (Supplementary Fig. **1**). Fresh plant materials were harvested, immediately frozen in liquid nitrogen, and stored at -80°C until needed.

2.2. RNA Extraction, Library Preparation and RNAseq

Samples of five stage were ground to a powder in liquid nitrogen for RNA extraction. Total RNA was extracted using TRIzol reagent according to the manufacturer's protocol (Takara Bio Inc., Otsu, Japan). The double stranded cDNA was purified by AMPure XP beads. The purified double stranded cDNA was repaired at the end, added A tail and connected to the sequencing adaptor. Finally, the final cDNA library was obtained by PCR enrichment, and the RNAseq libraries were prepared. RNAseq was performed using Illumina HiSeq™ 2000 (Illumina, San Diego, CA, USA).

2.3. Data Filtering and *de novo* Assembly

After RNAseq, the raw data was raw reads, which containing low-quality, adaptor-polluted and high content of unknown base (N) reads, should be processed to remove these reads before downstream analyses. After reads filtering, clean reads were obtained. We used Trinity [29] to perform de novo assembly with clean reads, and use Tgicl [30] to cluster transcripts to Unigenes.

2.4. Unigene Functional Annotation and Expression Analyses

After assembly, BLAST software was used to compare sequences of Unigene with KEGG (Kyoto Encyclopedia of Genes and Genomes), NR (NCBI non-redundant protein sequences), Swiss-Prot, GO (Gene Ontology), COG/KOG (COG: Clusters of Orthologous Groups of proteins; KOG: euKaryotic Ortholog Groups) and Trembl databases. After predicting the amino acid sequences of Unigene, HMMER software was used to compare with Pfam database to obtain annotation information of Unigene. To predict unigene expression levels in different samples, we calculated unigene expression using the fragments per kb per million fragments (FPKM) method. This method can eliminate the influence of different gene lengths and sequencing levels, allowing the calculated unigene expression levels to be directly used to compare unigene expression differences between samples. The DESeq2 method [31] was used to analyze the differentially expressed genes (DEGs) between the two groups. Finally, according to Unigene annotation results and official classification, GO function and KEGG biological pathway of DEGs were analyzed.

2.5. Quantitative Real-time PCR (qRT-PCR) Analyses

To validate the reproducibility of the transcriptome sequencing results, six differentially expressed genes were randomly selected to conduct qRT-PCR. *Glyceraldehyde 3-phosphate dehydrogenase* (*GAPDH*) was used as an internal reference gene for relative quantification [27]. Gene-specific primers were designed using Primer Premier 5.0 (Premier Biosoft International, Palo Alto, CA, USA) and given in Supplementary Table **1**. qRT-PCR was performed with SYBR® Premix Ex TaqTM II (TaKaRa) on a Mastercycler® ep realplex Real-Time PCR System (Eppendorf, Germany). Each reaction was composed of 10 μL of SYBR Green PCR master mix, 0.5 μL of each primer (10 μM), 8 μL of H_2O, and 1 μL of cDNA. The PCR cycling regime comprised an initial denaturation at 95°C for 30 s, followed by 40 cycles at 95°C for 5 s and 60°C for 30 s. Normalized transcript abundances were calculated by applying the $2^{-\Delta\Delta CT}$ method [32]. Three biological replicates were performed per sample.

3. RESULTS

3.1. Gene Expression Profiling of Flower Development Stages of *C. lutchuensis*

To understand the gene expression of different flower development stages of *C. lutchuensis*, fifteen libraries, five de-

velopment stages (Supplementary Fig. **1**) with three replicates, were generated for transcriptome sequencing. In total, we generated approximately 101.16 Gb clean data by Illumina HiSeq sequencing. The clean data of all samples reached 6 GB, and the percentage of Q30 base was 92% or more (Supplementary Table **2**). After sequence assembly, we finally obtained 386908 unigenes in the 7 functional databases (Supplementary Table **3**) with 46649 differentially expressed unigenes. To detect the correlation of biological duplication of samples, we used Pearson's correlation coefficient (R) as the evaluation index. The closer R^2 was to 1, the stronger the correlation between the two duplicate samples was. In this project, the R^2 of biological repeated samples was greater than 0.8 (Supplementary Fig. **2**).

To validate the reproducibility and reliability of the RNA-seq data, we randomly selected six genes for further qRT-PCR investigations. It included *xyloglucan endotransglucosylase 16* (Cluster-40156.173030), *Wound-induced protein 1* (Cluster-40156.160567), *senescence/dehydration-associated protein* (Cluster-40156.132305), *WRKY transcription factor 31* (Cluster-40156.157861), *MYB116* (Cluster-40156.156465) and *NAC transcription factor 2* (Cluster-40156.161640). The results showed that the expression trend of qRT-PCR was consistent with the RNAseq data (Fig. **1**).

3.2. Screening of Differentially Expressed Unigenes

In order to study the gene expression pattern in the flower senescence process of *C. lutchuensis*, the FPKM of gene was first centralized and standardized, and then K-means clustering analysis was performed. The same kind of genes have similar changing trends under different development stages and may have similar functions. The results showed that 46649 differentially expressed unigenes were classified into 10 Sub Class, which had different expression patterns (Fig. **2**). And only Sub Class 9, included 8252 unigenes, was specifically expressed in the senescent stage. It suggested that the expression pattern of Sub Class 9 genes was the most relevant to the flower senescence of *C. lutchuensis*. So, in the following analysis, we focused on inspection of Sub Class 9 genes.

3.3. Ethylene is involved in Flower Senescence

To investigate the possible regulation pathway of flower senescence in *C. lutchuensis*, we compared the differentially expressed genes of Sub Class 9, and many ethylene-related genes were identified and exhibited changes in expression, including ethylene synthesis and ethylene signaling genes. Ethylene biosynthesis involved two key enzymatic steps, *S*-adenosylmethionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS) and ACC to ethylene by ACC oxidase (ACO). As shown in Fig. (**3**), the expression of ethylene related genes is low in early bud stage (S1) to complete opening stage (S4), but highly expressed in senescent stage (S5). First, we revealed that the expression of the ethylene synthesis genes *ACSs* (5 uningenes) and *ACOs* (11 uningenes) was induced in S5 (Fig. **3**). Second, the expression of ethylene receptor *ETR* genes was increased. Last, the expression of ethylene signaling genes *EINs* and *ERFs* also was upregulated. The results revealed that ethylene pathway was positively correlated with flower senescence of *C. lutchuensis*.

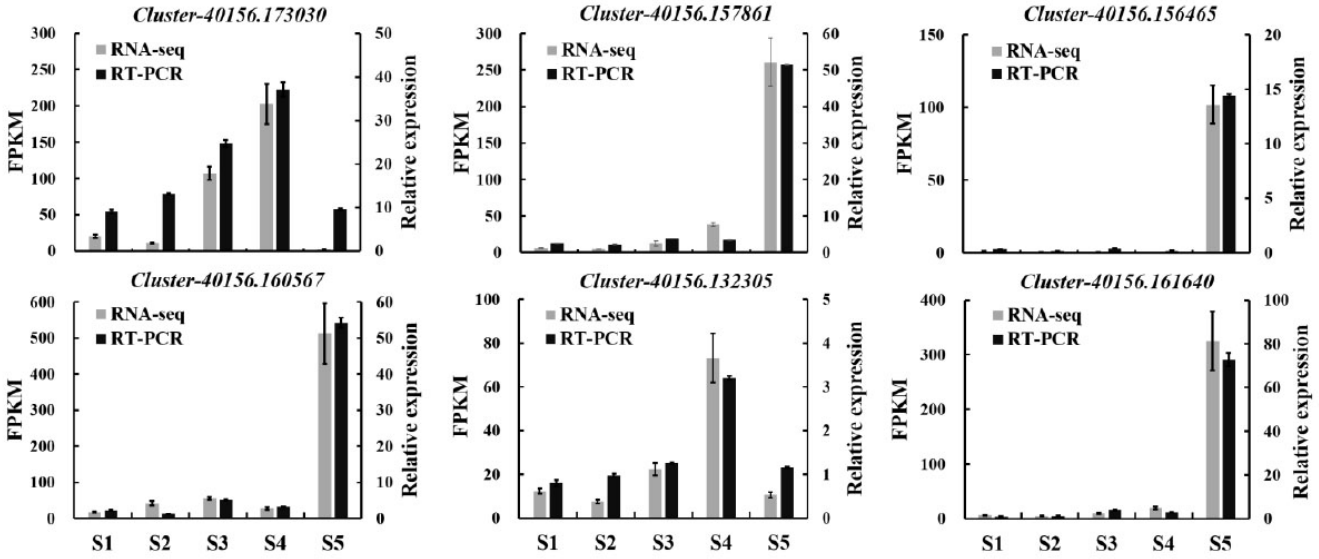

Fig. (1). Validation of the RNAseq results by qRT-PCR. FPKM, fragments per kb per million fragments, is the abundance of genes determined from the transcriptome library sequencing data. S1 represents early bud stage, S2 represents late bud stage, S3 represents half opening stage, S4 represents complete opening stage, S5 represents senescent stage.

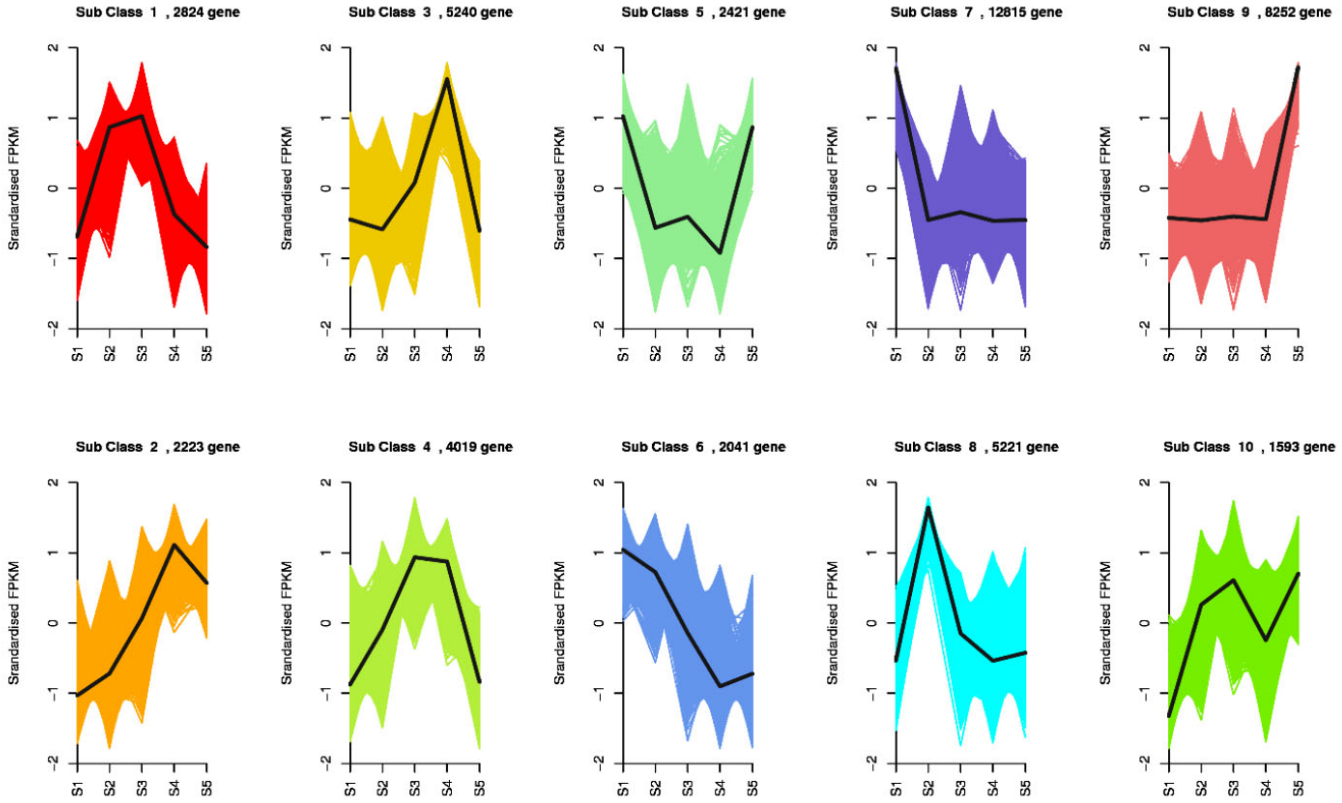

Fig. (2). K-means clustering analysis. The abscissa represents the samples of different development stages, and the ordinate represents the centralized and standardized expression. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

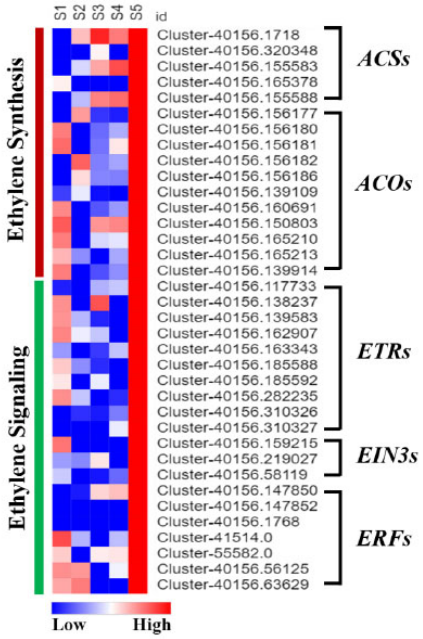

Fig. (3). Heat map of ethylene-related genes from RNA-seq data. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

3.4. Related Genes of Reactive Oxygen Species Production and Scavenging

To test whether ROS signal was involved in the flower senescence of *C. lutchuensis*, we analyzed the expression of genes related to ROS production and scavenging. We found that ROS production related genes, such as *peroxidase* (*POD*), *lipase* (*LIP*), *polyphenoloxidase* (*PPO*), and ROS scavenging related genes, such as *glutathione S-transferase* (*GST*), *glutathione reductase* (*GR*) and superoxide dismutase (SOD), changed significantly. As shown in Table **1**, the expression levels of *POD*s, *PPOs*, *LIPs*, *GSTs*, *GRs* and *SOD* remained low in the first four developmental stages (S1 to S4), but the expression of these genes drastically increased after S5, and the increase was all more than 10 times. The difference of gene expression indicates that the ROS may be involved in flower senescence of *C. lutchuensis*.

3.5. Biosynthesis of Secondary Metabolites: Monoterpenoid and Isoflavonoid

Secondary metabolism plays critical roles in the regulation of plant growth and development. In our study, the abundance of secondary metabolism related transcripts was determined from the RNA-seq data, such as biosynthesis genes of monoterpenoid and isoflavonoid.

Monoterpenoid is a class of secondary metabolite with important biological activity and function. In the RNA-seq

Table 1. Summarized results from transcriptome analysis.

Type	Genes ID	FPKM				
		S1	S2	S3	S4	S5
Peroxidase	Cluster-40156.113681	0.543	0.153	0.437	0	257.89
	Cluster-40156.124064	0.617	0	0	0	339.537
	Cluster-40156.153738	0.303	0.033	0.33	0.587	255.81
	Cluster-40156.156716	1.52	5.62	7.537	17.847	186.153
	Cluster-40156.160502	2.213	0.023	0.193	0.39	149.57
	Cluster-40156.158603	0.017	0.03	0.077	0.207	330.547
	Cluster-40156.160784	8.827	0.077	0	0.467	187.583
Lipase	Cluster-40156.165543	0	0.433	1.75	0.223	116.273
	Cluster-40156.165596	0.1	0.42	1.033	0.097	101.133
	Cluster-40156.165602	0.11	0	0	0	159.99
	Cluster-40156.154849	0	0.653	2.987	0.9	191.307
	Cluster-40156.164235	0	0	0	0	238.023
	Cluster-40156.154855	0.16	0	0.293	0.083	139.707
	Cluster-40156.161244	0.713	0	0	0.06	212.66
Polyphenoloxidase	Cluster-40156.160989	0.513	4.07	6.43	4.56	1229.253
	Cluster-40156.161094	4.593	5.42	11.517	21.493	2469.673
	Cluster-40156.160991	3.153	1.413	4.773	10.187	917.257
GST	Cluster-40156.101531	0	0	0	0	107.14
	Cluster-40156.152610	6.39	8.897	8.03	9.613	179.127
	Cluster-40156.152729	0.21	0.267	0.603	1.95	305.023
	Cluster-40156.152730	0	0	0.217	0.153	102.28
	Cluster-40156.156371	0.847	0	0	0.41	222.833
	Cluster-40156.156398	0	0	0	0.257	101.59
	Cluster-40156.160506	13.55	1.58	1.217	5.017	179.307
	Cluster-40156.162415	3.177	2.06	2.68	2.313	260.583
	Cluster-40156.162571	35.163	18.08	11.847	3.97	763.66
	Cluster-40156.162763	30.26	10.37	8.527	5.527	603.903
	Cluster-40156.163283	4.77	0.967	1.6	1.573	298.063
	Cluster-40156.19373	1.167	0.413	1.89	1.133	104.55
	Cluster-40156.205640	15.217	1.647	2.677	4.767	500.417
	Cluster-40156.178385	0.187	0	0	0.74	529.313
GR	Cluster-40156.170214	9.913	26.697	22.897	34.813	310.447
	Cluster-40156.159982	19.95	4.71	9.093	21.75	236.063
SOD	Cluster-40156.160081	4.81	0.64	0.897	0.713	274.297

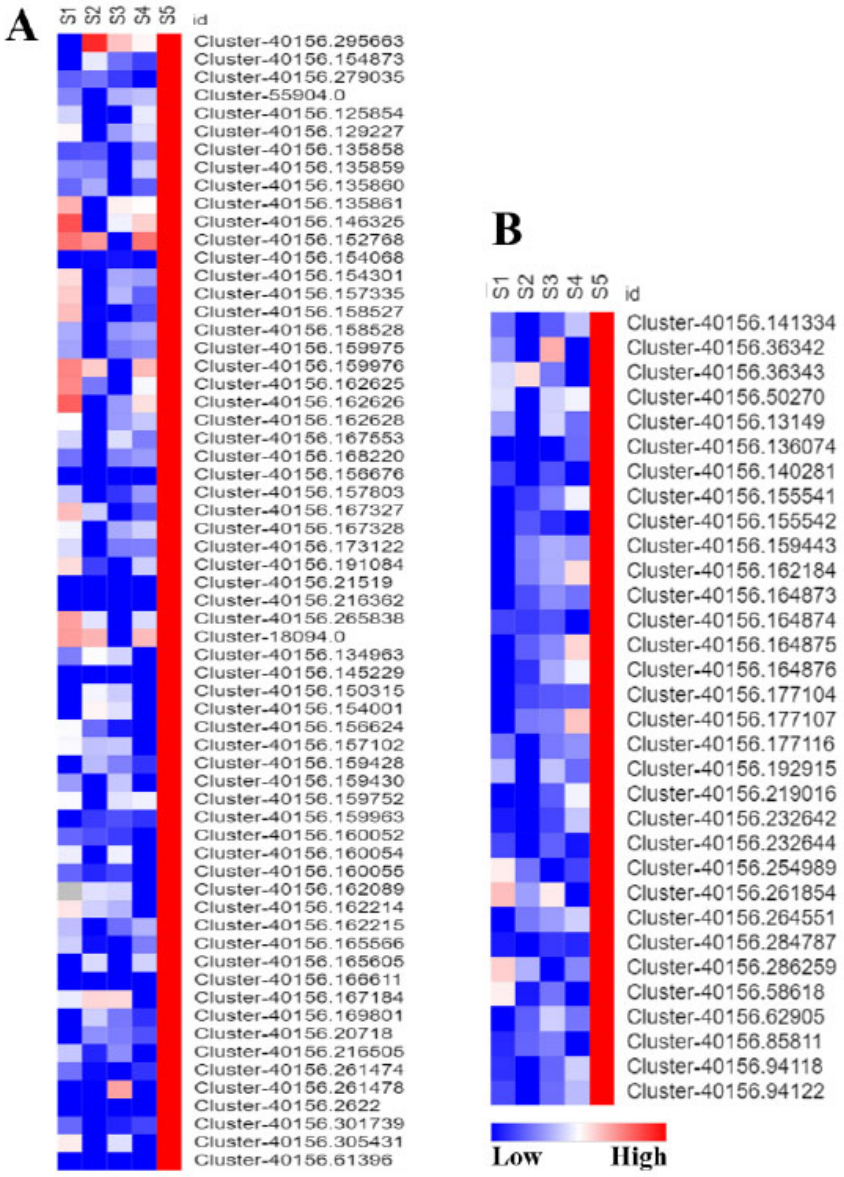

Fig. (4). Heat map of biosynthesis related genes of secondary metabolites from RNA-seq data. (**A**) biosynthesis related genes of monoterpenoid, (**B**) biosynthesis related genes of isoflavonoid. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

data, monoterpenoid biosynthesis genes, such as neomenthol dehydrogenase (Cluster-40156.129227), hydroxygeraniol dehydrogenase (Cluster-40156.216362), deoxyloganetic acid glucosyltransferase (Cluster-40156.279035), deoxyloganetin glucosyltransferase (Cluster-40156.159752), terpineol synthase (TPS, Cluster-40156.295663) were specifically expressed in S5 stage (Fig. **4A**).

Isoflavonoid, a branch of flavonoid metabolism, was produced by naringenin through a series of enzymes, such as malonyltransferase and hydroxylase. Isoflavone 7-O-glucoside-6”-O-malonyltransferase and isoflavone/4'-methoxyisoflavone 2'-hydroxylase catalyzed genistein to produce genistein 7-O-glucoside-6”-O-malonate and 2'-hydroxybiochanin A, respectively. In our study, the genes encoding isoflavone 7-O-glucoside-6”-O-malonyltransferase (Cluster-40156.141334) and isoflavone/4'-methoxyisoflavone 2'-hydroxylase (Cluster-40156.164875) were highly expressed at S5 stage (Fig. **4B**).

3.6. Other Senescence Related Genes

In addition to the genes in the above pathway, there are a large number of genes specifically expressed in S5 stage in transcriptome data of *C. lutchuensis* (Supplementary Table **4**). First, the expression level of senescence-associated gene

SAG20 (Cluster-40156.160567) sharply increased during flower senescence. Second, many transcription factors (TFs) drastically altered the flower senescence of *C. lutchuensis*. There were 9 *WRKYs*, 12 *NACs*, 3 *MYBs* and 4 C_2H_2s genes high expression in S5 (Supplementary Table **4**). Besides, we found that lots of genes encoding chitinase, oxidosqualene-lanosterol cyclase and cytochrome P450 were upregulated in the senescence stage of *C. lutchuensis*. The results implied that these genes might be associated with senescence regulation of flowers in *C. lutchuensis*.

4. DISCUSSION

4.1. Ethylene may Play a key Role in Flower Senescence of *C. lutchuensis*

The phytohormone ethylene plays a key role in plant growth, development and senescence [33]. *ACS* and *ACO* are the key genes of ethylene synthesis, encoding enzymes that catalyze the synthesis of ethylene from methionine [34, 35]. The accelerated induction of *DcACS1* and *DcACO1* genes during petal senescence in cut carnation flowers [20, 36]. In rosa, silencing both *ACS1* and *ACS2* obviously suppressed petal and gynoecia dehydration induced by ethylene [37]. Similarly, when the flowers of *C. lutchuensis* entered the senescence stage, the expression of *ACSs* and *ACOs* rapidly increased (Fig. **3**). The expression of synthesis genes was induced, which indicated that ethylene synthesis increased to some extent. The results suggested that ethylene might be involved in the regulation of flower senescence of *C. lutchuensis*. After ethylene synthesis, it bonded with receptor protein and removed the inhibition of downstream ethylene signaling pathway [38, 39]. The mutant *ethylene-insensitive 1-1* (*etr1-1*), an ethylene receptor gene, was known to have longer flower longevity [40]. In *Dendrobium* orchid, high expression of ethylene receptor *ERS1* (*ethylene response sensor 1*) was found at the early phase of flower senescence [41]. In our study, the expression of ethylene receptor *ETR* genes also increased in the senescent stage (Fig. **3**), which indicated again that ethylene might be involved in flower senescence. EIN3, a key regulator of ethylene signaling pathway, is bound directly to the conserved element *EBS* (EIN3 binding site) in the promoter region of downstream gene by forming a dimer, thus activating or inhibiting the expression of downstream gene, such as *ERFs* and *PMP1* [42, 43]. In cut carnation, silver nanoparticles treatment delayed petal senescence, decreased ethylene synthesis, and the expression levels of *EIL1/2,* a homolog of *EIN3*, were significantly downregulated in the silver nanoparticles treatment compared with control flowers [44]. The expression of *PhEIL1/2* increased during corolla senescence and VIGS mediated *PhEIL1/2* silencing delayed flower senescence and reduced ethylene production [45]. During flower senescence of *C. lutchuensis*, *EIN3* genes upregulated (Fig. **3**), which demonstrated again that ethylene signaling was positively correlated with flower senescence in the *C. lutchuensis*. And ERF, ethylene responsive transcription factor, as the direct downstream of *EIN3* gene, participated in ethylene signaling [46]. In petunia, *PhERF2/3/4/5* genes, belonging to group VII ERFs were isolated and showed a strong association with flower senescence [47]. In *Arabidopsis thaliana*, the expression of *EDF1/2/3/4*, a subfamily of *ERFs*, caused the activation of the senescence-associated genes, and promoted flower senescence [48]. Same as *EIN3*, the expression of *ERFs* obviously was upregulated in *C. lutchuensis* (Fig. **3**). Based on the response to ethylene, flowers are classified ethylene sensitive or ethylene insensitive [49]. In most ethylene sensitive flowers, ethylene pathway is a major signaling that regulates flower senescence [16]. In *C. lutchuensis*, ethylene synthesis genes (*ACSs, ACOs*) and ethylene signaling genes (*ETRs*, *EIN3*, *ERFs*) all upregulated during flower senescent stage. The results implied that *C. lutchuensis* might be ethylene sensitive flowers. And ethylene pathway might be the key to regulate flower senescence in *C. lutchuensis*.

4.2. ROS may be involved in the Flower Senescence of *C. lutchuensis*

In plants, petal senescence was often accompanied by a rise in ROS and a change in redox balance [50]. ROS metabolism included ROS production and scavenging. During plant senescence, ROS, such as singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), superoxide (O_2^-), and hydroxyl (OH^-), was accumulated, in which a variety of enzymes were involved [51]. ROS accumulation caused oxidation and destroyed proteins, lipids, and damaged cell structure [52]. Lipases catalyzed the hydrolysis of lipids. In Iris, the enzymes involved in the degradation of lipids were increased activity during the process leading to cellular death [12]. Genes related to lipases were generally upregulated during petal senescence [50]. And in cut carnation, the gene (*Dc428*), encoded lipase enzyme, was significantly up-regulated during petal senescence [53]. In the flower senescence of *C. lutchuensis*, 7 lipase genes had a sharp increase at the transcriptional level (Table **1**). The results showed that the lipids might be degraded in the senescent stage in *C. lutchuensis*. ROS accumulation was accompanied by the increase of oxidase activities. H2O2, one type of ROS, was generated by the catalysis of oxidases, such as peroxidases [50]. Several peroxidase genes upregulated dramatically in the carnation petal senescence [53]. Peroxidase and polyphenol oxidase activities increased during rice leaf senescence [54]. In *C. lutchuensis*, 7 peroxidase genes and 3 polyphenol oxidase genes were included during flower senescence. In our study, the high expression of lipase, peroxidase and polyphenol oxidase genes indicated that ROS production increased. ROS production was strongly associated with petal senescence [50].

In plant, there was a redox balance mechanism in ROS metabolism. ROS production might be counteracted by the rise in ROS-scavenging enzymes and antioxidants [6, 55]. Glutathione (GSH), an important antioxidant, plays a central role in redox balance. GSH could scavenge and relieve the damage caused by ROS [56]. GSH converted to the oxidized form (GSSG) by dehydroascorbate reductase (DHAR) during senescence, while glutathione reductase (GR), as a ROS-scavenging enzyme, catalyzed the conversion of GSSH to GSH [57]. GR activity peaked in the middle of senescence,

was followed by a sharp decline in the petal development process of cut Gladiolus [58]. Besides, GST could conjugate GSH to electrophilic substrates, which played a key role in the removal of ROS. In most plants, GSTs were induced during senescence [50]. The transcript levels of *GSTs* were increased during petal senescence of cut carnation [53]. There were 14 *GST* ang 2 *GR* genes upregulated in the flower senescent stage of *C. lutchuensis* (Table **1**). SOD, a ROS-scavenging enzyme, catalyzed the dismutation of superoxide to hydrogen peroxide, which played a role in maintenance of low superoxide concentrations [59]. In day lily, high accumulation of hydrogen peroxide induced *SOD* gene action [55]. In *C. lutchuensis*, *SOD* gene (Cluster-40156.160081) had a sharp increase in transcript level during flower senescence (Table **1**). In our study, genes related to ROS production and scavenging were transcriptionally regulated during flower senescence. The results suggested that ROS signaling might be involved in the flower senescence of *C. lutchuensis*.

4.3. Monoterpenoid may affect Flower Senescence

Terpenoid compounds, a class of secondary metabolites, were widely distributed in plants and participated in the regulation of plant growth and development and various stress, such as floral scent [60] and defense against biological enemies [61]. Terpenoids are also involved in the regulation of plant or cell senescence. In barley, terpenoid treatment promoted leaf senescence [62]. Compared with healthy callus tissues, terpenoid biosynthesis mechanism was found more active in the senescing callus [63]. The apple a-farnesene synthase gene *AFS* was ectopically expressed in tobacco and transgenic plants showed an early senescence characteristic [64]. Oridonin, a tetracycline terpenoid compound, induced senescence in colorectal cancer cells [65]. In *C. lutchuensis*, monoterpenoid biosynthesis genes *neomenthol dehydrogenase*, *terpineol synthase*, *hydroxygeraniol dehydrogenase*, *deoxyloganetic acid glucosyltransferase*, *deoxyloganetin glucosyltransferase* were induced in flower senescent stage. The results demonstrated monoterpenoid might be involved in the flower senescence of *C. lutchuensis*.

4.4. Isoflavonoid may play a Role in the Flower Senescence

Isoflavone is one of the flavonoid compounds, which is synthesized through phenylpropanoid metabolism pathway [66]. Studies have shown that isoflavones and other flavonoids are involved in the regulation of many aspects of plant growth and development [67, 68]. In autumn, senescence of deciduous trees was due to flavonoid synthesis and chlorophyll degradation [69]. Flavonoid biosynthesis genes upregulated in the sugar induced senescence in *A. thaliana* [70]. Now, there are few reports about isoflavones involved in the regulation of plant senescence. However, the expression of *isoflavone 7-O-glucoside-6"-O-malonyltransferase* and *isoflavone/4'-methoxyisoflavone 2'-hydroxylase*, isoflavone synthesis genes, was highly up-regulated during flower senescent stage in *C. lutchuensis*. In the field of Medicine, isoflavone was proven to have good pharmacological activities, such as anti-aging, antibacterial, antioxidant and so on [67]. Therefore, isoflavone metabolism may also be a regulatory pathway of plant senescence regulatory network of *C. lutchuensis*.

4.5. Other related Genes under the Flower Senescence

Senescence was a complex regulatory network which was regulated by multiple genes. TFs play a key role in the regulation of senescence. NAP2, a NAC TF, had a central role in controlling senescence by controlling the expression of senescence-related genes *SAG113*, *SGR1* and ABA biosynthesis genes [71]. WRKY6, a GA-repressible TF, activated *SAG12*, *SGR1*, *NYC1* and regulated the GA-suppressed senescence process [72]. The transcription factors *NAC*, *WRKY*, *MYB*, C_2H_2 and *ERF* were differentially expressed in the petal senescence [49, 73, 74]. *SAG* is marker gene of senescence [75]. The expression of *SAGs* was positively correlated with flower senescence [18, 76]. During post-harvest senescence of *Alstroemeria* petals, *chitinase* genes and *cytochrome P450* genes were upregulated [77]. In the flower senescence of *C. lutchuensis*, the expression of *TFs* (*NACs*, *WRKYs*, *MYBs*, C_2H_2s), *SAGs*, *chitinase* genes and *cytochrome P450* genes was increased. Besides, oxidosqualene-lanosterol cyclase genes also specifically induced in the flower senescent stage of *C. lutchuensis*. The differential expression of these genes demonstrated that they might participate in flower senescence regulation.

CONCLUSION

Transcriptome analysis of *C. lutchuensis* resulted in the identification and functional classification of differentially expressed unigenes during flower senescence. We found that 8252 unigenes (Sub Class 9) were specifically highly expressed in flower senescent stage, revealing they might be related to flower senescence. And these unigenes were assigned to various regulatory pathways, including ethylene synthesis genes (*ACSs, ACOs*) and ethylene signaling genes (*ETRs*, *EIN3*, *ERFs*), ROS production and scavenging genes, monoterpenoid and isoflavonoid metabolites genes, *TFs* (*NACs*, *WRKYs*, *MYBs*, C_2H_2s), *SAGs*, *chitinase* genes, *cytochrome P450* genes and oxidosqualene-lanosterol cyclase genes. There are various regulatory pathways in the flower senescence of *C. lutchuensis*, suggesting *Camellia lutchuensis*being a potential good model plant for studying flower senescence in Camellia. And the mechanism of senescence regulation of these key unigenes needs to be further studied in order to delay petal senescence by molecular biological methods.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

RESEARCH INVOLVING PLANTS

The study was conducted in accordance with the international guidelines and all experimental research on plants complied with the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

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