Transcriptome sequencing and anthocyanin metabolite analysis determines leaf color of *Acer pseudo-sieboldianum* in autumn

Donghui Zhao

Yanbian University

Yufu Gao

Yanbian University

Liping Rong (✉ rongliping2013@163.com)

Yanbian University

Research article

Keywords: *Acer pseudo-sieboldianum*, Transcriptome, Differentially expressed genes, Anthocyanin

Posted Date: December 6th, 2019

DOI: <https://doi.org/10.21203/rs.2.18312/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Transcriptome sequencing and anthocyanin metabolite analysis determines leaf color of *Acer pseudo-sieboldianum* in autumn

Donghui Zhao¹, Yufu Gao¹, Liping Rong^{1*}, Jiaqi Zhang¹ and Jiashuo Chen¹

Abstract

Background: *Acer pseudo-sieboldianum* is an often used color-leafed tree native to northeastern China, whose leaves are gorgeous in autumn and is used to great effect in landscaping. Leaf color is an important index affecting the ornamental traits of color-leafed plants. Thus, it is of great significance to study the mechanism in leaf coloring in *Acer pseudo-sieboldianum* as the development and application prospects of this research are broad, and further the utilization of local characteristics tree species with colored leaves.

Results: In this study, the leaves of *Acer pseudo-sieboldianum* from three stages in the autumn color-changing period were used as materials. In order to investigate the related differentially expressed genes in the process of color transformation, we determined the anthocyanins in leaves and used high-throughput sequencing to analyze the associated transcriptomes. The results demonstrated that five anthocyanins were detected in this experiment. It is worth mentioning that Peonidin O-hexoside, Cyanidin 3-O-glucoside (Kuromanin) and Cyanidin 3,5-O-diglucoside (Cyanin) have significant differences in their content change in two of the tested periods, meaning these may be the key substances determining the final color of *Acer pseudo-sieboldianum*. A total of 67.47 Gb of clean data were obtained from our sequencing results. Functional annotation of unigenes, including comparison with COG and GO databases, yielded 35,316 unigene annotations. 16521 differentially expressed genes were identified from a statistical analysis of differentially gene expression. We were able to cluster genes related to leaf

* Correspondence: rongliping2013@163.com

¹ Agriculture college, Yanbian University, Yanji 133002, China

Full list of author information is available at the end of the article

color formation in *Acer pseudo-sieboldianum*, including ANS, DFR, F3H, UFGT, and so on, based on the expression at specific times.

Conclusions: This study enriched the available transcriptome information for *Acer pseudo-sieboldianum* and identified a series of differentially expressed genes related to leaf color, which provides valuable information for further study on the genetic mechanism of leaf color expression in *Acer pseudo-sieboldianum*.

Key words: *Acer pseudo-sieboldianum*, Transcriptome, Differentially expressed genes, Anthocyanin

Background

Ornamental plants are widely used in landscape greening and green plant allocation. Leaf color and flower color are one of the most important characteristics of ornamental plants [1, 2]. Some scholars have systematically analyzed and determined the pigments and physiological indexes of the leaves of color-leaved plants [3–5]. Research shows that the change of leaf color is closely related to the synthesis and accumulation of flavonoids and anthocyanins in leaves[6]. Flavonoids are one of the important secondary metabolites of plants and they often have anti-cancer, anti-oxidation and anti-atherosclerosis properties[7]. Flavonoids can be classified into flavanone, isoflavone, flavone, flavonol, dihydroflavone and anthocyanin depending on their different structures[8]. In recent years, some scholars have identified PAL, CHS, CHI, DFR, ANS, F3H, F3'H, F3'5'H [9–12] and a few related regulatory genes such as MYB, bHLH and WDR, in color changing leafed plants[13, 14]. However, the process of anthocyanin synthesis and accumulation is relatively complex, and is regulated by multiple enzymes and transcription factors[15], as well as being influenced by external environmental factors such as light[16], water stress[17], temperature[18] and other factors. Thus, the mechanism of leaf color change in color-leaved plants needs to be further studied.

Acer pseudo-sieboldianum, belongs to the *Acer* genus of the family *Sapindaceae*. It is a small deciduous tree, and because of its beautiful shape and brilliant leaves, it is an often used autumn leaf ornamental tree species [19]. It is a type of nectar plant, whose woods can be used for making utensils and leaves can be used as dyes, giving it high

economic value [20]. Recently, some scholars have reported and studied the introduction, cultivation and breeding of *Acer pseudo-sieboldianum* [21, 22]. However, the key genes affecting leaf color change in *Acer pseudo-sieboldianum* have not been determined yet, and genome information on *Acer pseudo-sieboldianum* is relatively scarce. This fact means that the molecular regulatory mechanisms related to leaf color formation needs further study.

In recent years, transcriptome high-throughput sequencing technology has been widely used to study the mechanism of leaf color in various plants [23, 24]. In this study, we performed transcriptome sequencing, assembly, annotation and bioinformatic analysis on leaves from *Acer pseudo-sieboldianum* at different color-changing stages in autumn. We then combined this data with anthocyanin metabolism analysis data to identify some enzymes related to flavonoid and anthocyanin synthesis, and using real-time fluorescence quantitative qPCR, we analyzed and validated these DEGs in the leaves of *Acer pseudo-sieboldianum* at different transformation stages in autumn. We thus screened to identify the candidate genes affecting leaf color in *Acer pseudo-sieboldianum*, which provides a theoretical basis for studying the molecular mechanism of leaf color in *Acer pseudo-sieboldianum*.

Materials and methods

Plant materials and treatments

The materials tested were *Acer pseudo-sieboldianum* plants that were 5 years old from Tianchi Square, Yanji City, Jilin Province. Samples from the same *Acer pseudo-sieboldianum* plant were collected separately and storing in a refrigerator at -80°C for transcriptome sequencing and anthocyanin metabolite analysis from three periods on September 21, 2018 (B), September 30, 2018 (M) and October 11, 2018 (A). Harvest was repeated three times per treatment facility (Fig. 1).

Fig. 1 The color change of leaf transcriptome level of *Acer pseudo-sieboldianum* at different periods in autumn can be divided into three stages. Initial stage: at this early stage (B), the leaves were all green. Mid stage: at this time, the leaves were both red and green (M). The last stage: the leaves were all red.

Extraction, identification and data analysis of anthocyanin metabolites

Leaf tissue samples of *Acer pseudo-sieboldianum* were ground to a powder, and 100 mg of powder was dissolved in 1.0 ml extract solution (70% methanol aqueous solution). The dissolved sample was placed in a refrigerator overnight at 4°C, and then vortexed three times during the period to improve the extraction rate. After centrifugation, the supernatant was reserved, the sample was filtered with a microporous filter membrane, and stored in a sample bottle for LC-MS / MS analysis. We used multiple reaction monitoring (MRM) for qualitative and quantitative analysis of metabolites by mass spectrometry. Combining single variable statistical analysis and multivariate statistical analysis, we calculated the fold-change, and called a metabolite as a differential metabolite when its values was between a fold change ≥ 2 and a fold change ≤ 0.5 . The differential metabolites were then annotated using the KEGG database[25].

RNA isolation, library construction and RNA-Seq

Total RNA was extracted from leaf samples using an RNA extraction kit (Beijing Tiangen in China). Agarose electrophoresis and the Agilent 2100 Bioanalyzer were used to determine the concentration, purity and integrity of RNA samples. Then, PolyA mRNA was reverse transcribed into cDNA, and the construction and sequencing of the cDNA library was completed by the BMK Technology company in Beijing. Raw reads were obtained using an Illumina Hiseq 2500 sequencing platform, and after filtering, clean

reads were obtained. Contigs were assembled by overlapping information between sequences, transcripts were locally assembled, and unigenes were obtained by homologous clustering and splicing of transcripts with Tgicl and Phrap software, respectively[26].

De novo assembly and functional annotation

After obtaining high quality sequencing data, it was necessary to assemble the genomic sequence of *Acer pseudo-sieboldianum*. First, Trinity software parsed the sequencing reads into shorter fragments (K-mers), extends these fragments into longer fragments (Contig), and uses the overlap between these fragments to determine the fragment set (Component). Finally, using the dual methods of De Bruijn mapping and sequencing read information analysis, each transcript sequence was identified in each fragment set. The Unigene sequence was compared with the gene sequence in NR [27], Swiss-Prot [28], GO [29], COG[30], KOG [31], eggNOG4.5 [32], KEGG database by Blast software [33] ($e < 0.00001$). Using KOBAS 2.0 [34], the KEGG orthology result of unigenes from KEGG was obtained, and after predicting the amino acid sequence of each unigene, we used HMMER [35] software to compare with the Pfam [36] database, select unigenes whose BLAST parameter E-values were not greater than $1e^{-5}$ and whose HMMER parameter E-values were not more than $1e^{-10}$, and thus, finally obtained a unigene with annotation information.

Expression and differentially expressed unigene annotation

Bowtie[37] was used to compare the sequenced reads with a unigene library, and RSEM[38] was used to estimate the expression level. The expression abundance of each corresponding unigene was expressed by its FPKM [39] value. It is a common method for estimating gene expression level in transcriptome sequencing data analysis. The use of FPKM values can eliminate the influence of gene length and sequencing on calculations of gene expression. When detecting differentially expressed genes, DESeq2 was used to analyze the differentially expressed genes between the sample groups, and the differentially expressed gene sets between two different conditions were identified. In the process of differential expression analysis, the Benjamini – Hochberg method was

used to correct the significance p-value of the original hypothesis test, so as to reduce the false positives in independent statistical hypothesis testing for a large number of gene expression values. In the screening process, the criterion was that the FDR (False Discovery Rate) was less than 0.01 and the difference factor FC (Fold Change) was greater than or equal to 2. Between these two factors, the FC represented the ratio of expression between two samples (groups).

Gene validation and expression analysis

In order to validate our differential gene expression analysis, RT-qPCR was used to validate the differentially expressed genes related to flavonoid anthocyanin biosynthesis[40]. We used a fluorescence quantitative Kit ($2 \times$ SYBR® green premix) and an analytikjena-qTOWER2.2 fluorescence quantitative PCR instrument for quantitative analysis. The primer sequences can be found in File. S1. The reaction procedure was as follows: 95°C for 3 min, 95°C for 10 s, 58°C for 30 s, for a total of 39 cycles. Melt curve analysis (60 °C~ 95 °C, +1 °C/cycle, holding time 4 s), and carried out centrifugation on PCR plate centrifuge at 4°C 6000 rpm for 30s. Finally, we put it in quantitative PCR for amplification, using c110191.graph_c0 as an internal reference gene. The procedure was repeated three times for each sample, and we calculated the relative gene expression of each sample using the $2^{-\Delta\Delta Ct}$ method.

Results

Contents of flavonoid-anthocyanin biosynthesis in the leaves of *Acer pseudo-sieboldianum*

In order to further understand the mechanism of pigment formation in *Acer pseudo-sieboldianum* leaves, we carried out qualitative analysis of anthocyanin components in the middle and last stage of leaf color transformation. According to our UPLC–Q–TOF–MS data, five anthocyanins were identified (Table 1). These were Peonidin O-hexoside, Rosinidin O-hexoside, Cyanidin 3-O-glucoside (Kuromanin), Cyanidin 3,5-O-diglucoside (Cyanin) and Pelargonidin 3-O-beta-D-glucoside (Callistephin chloride).

The content changes of these five metabolites were different during the middle and last stages of color transformation. Rosinidin O-hexoside and Pelargonidin 3-O-beta-D-glucoside (Callistephin chloride) anthocyanin content showed little difference between these two periods. But Peonidin O-hexoside, Cyanidin 3-O-glucoside (Kuromanin) and Cyanidin 3,5-O-diglucoside (Cyanin) displayed significant differences in content change between these two periods, meaning they may be the key substances for the final color of *Acer pseudo-sieboldianum* (File. S2).

Table1 Anthocyanins detected in *Acer pseudo-sieboldianum*

Index	KEGG ID	Compounds	Average content in M stage	Average content in A stage
Pma1590	-	Peonidin O-hexoside	1425333	26682600
Pmb0545	-	Rosinidin O-hexoside	48403	27156
Pmb0550	C08604	Cyanidin3-O-glucoside	2420000	6970767
Pme1777	C08639	Cyanidin 3,5-O-diglucoside	14336000	70564000
Pme3392	-	Pelargonidin 3-O-beta-D-glucoside	42512	62136

RNA sequencing and de novo assembly of the *Acer pseudo-sieboldianum* genome

In order to understand the molecular mechanism of color change in *Acer pseudo-sieboldianum* leaves in autumn, nine libraries (early stage: B1, B2, and B3, medium stage: M1, M2, and M3, last stage: A1, A2, and A3) were constructed from leaves from the three different stages, with three replicate libraries being constructed per stage. Sequencing was performed using the Illumina Hiseq 2500, and a total of 67.47 Gb of clean data was obtained from these sequencing results. The clean data from all samples totally 6.14 Gb, and the percentage of Q30 bases was 93.10% or more. After assembly, 50,501 unigenes were identified. Among these, there were 20,706 unigenes over 1 kb in length, and the error rate of sequencing was less than 0.1%, which indicates that the quality of sequencing data was good and could be used for subsequent analysis (Table 2).

These recombinant sequence dataset yielded 115,413 transcripts and 50,501 unigenes, among which, the N50 (accounting for 50% of the maximum length nucleotide sequence of all single genes) was 2267 nt and 1979 nt, respectively. There were 17,366 (34.39%) unigenes between 300 and 500 nt, 23,580 (46.69%) unigenes between 500 and 2000 nt, and 9,555 (18.92%) unigenes longer than 2000 nt (Table 3).

Table 2 Statistics of sequencing data across the nine libraries in *Acer pseudo-sieboldianum*

Sample II	Clean number	Base number	GC contmer	%≥Q30	Mapped reads	Mapped ratio
B1	24,891,319	7,411,181,104	45.12%	94.12%	20,710,424	83.20%
B2	24,754,322	7,373,450,408	45.72%	94.25%	20,509,397	82.85%
B3	26,344,753	7,839,350,686	44.02%	93.10%	21,123,549	80.18%
M1	20,586,684	6,140,849,238	44.61%	94.04%	17,004,609	82.60%
M2	24,264,520	7,220,440,952	45.08%	94.33%	20,030,805	82.55%
M3	20,951,279	6,256,334,358	44.34%	93.99%	17,134,598	81.78%
A1	26,611,490	7,923,701,850	44.84%	94.03%	21,802,156	81.93%
A2	28,672,411	8,529,990,156	44.95%	93.73%	23,549,563	82.13%
A3	29,433,309	8,770,181,170	45.13%	93.44%	24,196,882	82.21%

Table 3 Length distributions of the transcripts and unigenes from *de novo* assembly

Length range	Transcript	Unigene
300-500	24,236(21.00%)	17,366(34.39%)
500-1000	26,476(22.94%)	12,429(24.61%)
1000-2000	33,114(28.69%)	11,151(22.08%)
2000+	31,587(27.37%)	9,555(18.92%)
Total Number	115,413	50,501
Total Length	179,159,431	62,348,493
N50 Length	2,267	1,979
Mean Length	1552.33	1234.60

Functional annotation and classification

Unigene sequence was then compared with gene sequences in the NR, Swiss-Prot, GO, COG, KOG, eggNOG4.5, and KEGG databases using BLAST software ($e < 0.00001$). 35,316 unigenes were identified, accounting for 70.01% of the 50,501 unigenes. 12,984 unigenes were annotated in the COG database, 25,375, 12,487 and 19,460 unigenes were annotated in the GO, KEGG and KOG databases, respectively. 25,226 unigenes were annotated in the Pfam database. 19,796 unigenes and 32,498 unigenes were also annotated in the Swanshot and eggNOG databases, respectively (Table 4). According to NCBI NR database and E-value distribution, the number of unigenes annotated in our dataset was 35,024, of which 71.53% of these unigenes ($E < 10^{-50}$) had strong homology and 47.87% of these unigenes ($E < 10^{-100}$) had very strong homology (Fig. 2a).

Ten popular-related species were also annotated based on the NCBI NR database (Fig. 2b). The highest homology to *Acer pseudo-sieboldianum* was *Citrus sinensis*, accounting for 12.25% homology, followed by *Citrus clementina*, which accounted for 9.74% homology.

The COG (Clusters of Orthologous Groups) database is a database for homologous classification of gene products. It is an early database used for the identification of directly homologous genes. It is based on a large number of comparisons of protein sequences from various organisms. 12,984 unigenes from our dataset were classified into 24 protein families, of which 1,867 unigenes (12.67%) were classified as general function predictions only, followed by carbohydrate transport and metabolism, with 1,380 unigenes (9.36%). The smallest number of identified unigenes was in extracellular structures, with only 15 unigenes (0.1%). Also, a large number of unigenes were assigned to translation, ribosomal structure and biogenesis (9.26%), posttranslational modification, protein turnover, chaperones (8.09%), lipid transport and metabolism (7.4%), energy production and conversion (6.68%), amino acid transport and metabolism (6.41%) and signal transduction mechanisms (6.26%) (Fig. 3).

GO (Gene Ontology) databases are divided into three categories: Biological Process, Cellular Component and Molecular Function, which are further divided into 42

functional subgroups. In Biological Process, which had the largest number of annotated unigenes, included metabolic process and cellular process with 13,141 (51.78%) unigenes and 11,546 (45.5%) unigenes, respectively. The Cellular Component class mainly included cell and cell part, with 11,886 (46.84%) unigenes and 11,806 (46.53%) unigenes, respectively. The Molecular Function category mainly included catalytic activity and binding, and there were 12,691 (50.01%) unigenes and 1,1049 (43.54%) unigenes in these two groups (File. S3).

These KEGG database annotated 12,487 unigenes assigned to 130 different KEGG pathways. Our results showed that the largest pathway group was Ribosome, which contained 832 (11.32%) unigenes, followed by Carbon metabolism and Biosynthesis of amino acids, which contained 661 (8.99%) and 487(6.63%) unigenes, respectively.

Metabolic pathways directly related to plant leaf color and leaf growth (number of unigenes annotated) were Flavonoid biosynthesis (ko00941 28 unigenes), Phenylalanine metabolism (ko00360 74 unigenes), Anthocyanin biosynthesis (ko00942 1 unigene), and Porphyrin and chlorophyll metabolism (ko00860 73 unigenes), etc. (File. S4).

Fig. 2 Characteristics of homology search of *Acer pseudo-sieboldianum* unigenes. (a) E-value distribution in the NR database for each unigene. (b) Species taxonomy based on the NR database.

Table 4 Statistics of comparisons with databases

Anno	Database	COG	GO	KEGG	KOG	Pfam	Swisspro	eggNOG	Nr	All
300<=length<1000		4534	11027	4861	7536	9281	6715	14214	16192	16431
length>=1000		8450	14348	7626	11924	15945	13081	18284	18832	18885
annotated number		12984	25375	12487	19460	25226	19796	32498	35024	35316

Fig.3 COG classifications of annotated unigenes

Differentially expressed genes (DEGs)

In order to explore the genes related to anthocyanin biosynthesis in *Acer pseudo-sieboldianum* at different color-changing stages, the differential expression of *Acer pseudo-sieboldianum* samples at different color-changing stages were then analyzed. The results showed that there were 16,521 DEGs in the three color-changing periods of *Acer pseudo-sieboldianum*. Comparing between the early stage (B) and the middle stage (M), there were 87 significant DEGs, with 52 up-regulated and 35 down-regulated. Between with the early stage (B) and the final stage (A), there were 14,855 DEGs, of which 7984 were up-regulated and 6871 were down-regulated. In a comparison of the middle stage (M) and the final stage (A), there were 12,402 DEGs, 5683 up-regulated and 6719 down-regulated, in *Acer pseudo-sieboldianum* (Figs. 4 and 5).

In order to further understand the function of these respective DEGs, we carried out KEGG pathway enrichment analysis in the three stages of *Acer pseudo-sieboldianum*. Our results showed that there were 16,521 differentially expressed genes in the three stages (B, M and A). The flavonoid biosynthesis pathways related to leaf tone control were significantly enriched in B vs M and B vs A up-regulated genes. Phenylalanine metabolic pathways were significantly enriched in B vs M and B vs A up-regulated genes. In addition, photosynthesis and photosynthesis-antenna protein pathways were significantly down-regulated in B vs A (File. S5-S7).

Fig. 4 Differentially expressed genes in the three stages

Fig. 5 Relationship of differentially expressed genes across development time.

Candidate genes involved in the flavonoid-anthocyanin biosynthesis

Pathway

Flavonoids are important secondary metabolites in plants. The pathway for flavonoid biosynthesis has been well verified. We identified 20 candidate genes that covered almost all known enzymes involved in flavonoid biosynthesis (Fig. 6). Four PAL genes were detected in the upstream phenylalanine pathway (c118011.graph_c0, c118229.graph_c0, c60818.graph_c0, c97964.graph_c0), one CHS gene was detected (c100615.graph_c0), one CHI gene (c108255.graph_c0), two F3H genes (c114916.graph_c0, c56266.graph_c0), one F3'5'H gene (c111360.graph_c0), and two F3'H genes (c110935.graph_c0, c108910.graph_c0) were also detected. Among the four PAL genes, the expression of a single gene, c118011.graph_c0, was significantly up-regulated in M vs A, but not in B vs M. The expression of the gene c118229.graph_c0 decreased in B vs M, but not in M vs A. Additionally, the expression of the genes c60818.graph_c0 and

c97964.graph_c0 were up-regulated in B vs A . The expression of the single gene c100615.graph_c0 was significantly down-regulated in M vs A. In contrast, the expression of the gene c108255.graph_c0 was down-regulated in M vs A, but not significantly in B vs M. The expression of the single gene c114916.graph_c0 increased in B vs M, but decreased significantly in M vs A. The expression of c111360.graph_c0 decreased significantly in M vs A, while the expression of c110935.graph_c0 increased significantly during the B vs M period.

One ANS gene (c60813.graph_c0) was found in the anthocyanin pathway branch, and its expression increased during B vs M. Two genes (c107656.graph_c0, c99050.graph_c0) encoding DFR were identified, and the expression of the gene c107656.graph_c0 increased significantly during B vs M. In the final modification stage of the anthocyanin pathway, six UFGT genes were identified, of which the expression of the single gene c103768.graph_c0 increased significantly during the B vs M period, while the expression of c120331.graph_c0 did not differ significantly during B vs M, while the expression of both genes decreased significantly during the M vs A period. The UFGT gene (c103768.graph_c0) increased significantly in the B vs M period, and decreased significantly in M vs A, which indicated that UFGT was first highly accumulated and then rapidly consumed. Combined with the detection and quantification of metabolites, paeoniflorin may be the most important anthocyanin species that caused the color to change to red in *Acer pseudo-sieboldianum* leaves.

Fig. 6 Thermographic analysis of gene pathways related to flavonoid synthesis in *Acer pseudo-sieboldianum* leaves at three stages. B, M and A are arranged horizontally at all stages, and single genes are listed vertically. The annotations are displayed next to the corresponding genes. All FPKM values of single genes are plotted logarithmically.

qRT-PCR confirmation of RNA-seq data

In order to verify the accuracy of our sequencing data, we selected eight genes involved in flavonoid anthocyanin biosynthesis, and analyzed the expression level in leaves of different color from these three different stages of *Acer pseudo-sieboldianum* by qRT-PCR (Fig. 7). The results showed that these gene expression levels were consistent with our RNA sequencing data, demonstrating the reliability of our data.

Fig. 7 Expression analysis of eight differentially expressed genes related to flavonoid and

anthocyanin biosynthesis in *Acer pseudo-sieboldianum*.

Discussion

Acer pseudo-sieboldianum is an important ornamental plant in China. Its leaves are attractive in autumn, beautiful in shape, shining like the morning sun and flowers. Both *Acer pseudo-sieboldianum* and *Acer palmatum* Thunb. belong to Sect. Palmata Pax and Ser. Palmata (Pax) Pojark. *Acer palmatum* Thunb. has many varieties, gorgeous leaves, fast growth and strong ecological adaptability [41]. However, the distribution of *Acer pseudo-sieboldianum* is limited, and its growth potential is relatively slow. Therefore, most *Acer pseudo-sieboldianum* are still in a wild state and have not been widely used in gardens. Some scholars have analyzed the color mechanism of the related species in *Acer rubrum* L. [42].

At present, transcriptome sequencing technology has been used to study leaf color formation [43], flower color mechanisms [44, 45], fruit development [46, 47], and so on. However, due to the lack of genomic reference sequences, the molecular mechanism of leaf color is difficult to decipher in *Acer pseudo-sieboldianum*. The change of anthocyanin content in plants was shown to be related to the differential expression of key genes encoding structural enzymes in the anthocyanin biosynthesis pathway [48]. Jiang HB [49] screened the different genes in the flavonoid biosynthesis pathway from the purple bud tea plant by transcriptome sequencing, and there were nine enzyme genes, including PAL, CHS, ANS, UFGT, FLS, C4H, 4CL, DFR, and ANR, that were identified. In this study, we used transcriptome sequencing technology to sequence and compare three different coloring stages of *Acer pseudo-sieboldianum* leaves in autumn. We detected four PAL, one CHS, one CHI, two F3H, two F3'H, one F3'5'H, two DFR, one ANS, and six UFGT genes in the flavonoid anthocyanin complex related to leaf color in *Acer pseudo-sieboldianum*. Three of the UFGT single genes were down regulated in the M vs A stage, which indicated that the change of leaves from green to red was controlled by multiple single genes.

Both F3'H and F3'5'H belong to the cytochrome P450 superfamily [50]. F3'H is an important intermediate in the synthesis of cyanidin. F3'5'H is a key enzyme in the

343 synthesis of blue flower anthocyanin. Masukawa T [51] found F3'H could make red
344 cyanidin accumulate in purple and red root radishes. Wu Q [52] found that F3'5'h
345 mainly accumulated in the blue waterlily. Many important flower crops can't produce
346 turquoise, meaning they cannot appear blue. In this study, we detected two F3'H and
347 one F3'5'H, but the content of F3'5'H in the M vs A period was very small and there
348 was no significant difference, so *Acer pseudo-sieboldianum* leaves did not appear blue
349 at this time.

350 DFR is the key enzyme that catalyzes the conversion of dihydroflavonol to
351 corresponding colorless geranium, delphinium and cyanidin [53]. The main function
352 of ANS is to oxidize colorless proanthocyanidins to produce colored anthocyanidins,
353 which are the first colored compound in the anthocyanin synthesis pathway [54]. ANS
354 was originally identified in a maize A2 mutant and cloned by the transposon tagging
355 technique [55]. UFGT is mainly responsible for transforming unstable anthocyanins
356 into stable anthocyanins. Studies have shown that the expression of UFGT is different
357 in different varieties [56]. For example, anthocyanin accumulation in apple was
358 positively correlated with UFGT activity. The change of UFGT activity in grape leads
359 to the change of their phenotype from white to red [57]. In this study, UFGT
360 (c103768.graph_c0) expression in *Acer pseudo-sieboldianum* leaves first accumulated
361 and then was consumed in the process of leaf color formation, which was consistent
362 with the conclusion that UFGT consumption was needed for paeoniae anthocyanin
363 synthesis.

364 It is worth mentioning that we found Rosinidin O-hexoside in five anthocyanin
365 components, although the content was very small. There was no Rosinidin
366 O-hexoside found in previous studies on anthocyanins of *Acer*. Schmitzer V [58]
367 found that the content of cyanidin 3-glucoside could be used as a quantitative index to
368 determine the position effect of *Acer palmatum atropurpureum*. Cai XY [59] found
369 that the reason leaf color changed from red to green in *Acer palmatum* Thunb. was the
370 result of the increase of the mass fraction of cyanidin galactoside and the decrease of
371 the mass fraction of chlorophyll. The distribution of Rosinidin O-hexoside in plants is
372 very limited and has only been reported in *Catharanthus roseus* [60] and *Primula* [61].

The contents of differential metabolites were very high in terms of Peonidin O-hexoside, Cyanidin 3-O-glucoside (Kuromanin) and Cyanidin 3,5-O-diglucoside (Cyanin). These results demonstrate a molecular mechanism of leaf color change in autumn in *Acer pseudo-sieboldianum* and provide the basis for molecular breeding theory for ornamental plant leaf color improvement.

Conclusions

In this study, we detected five anthocyanins in the leaves of *Acer pseudo-sieboldianum*, including Rosinidin O-hexoside, the distribution of which in *Acer* plants is very limited. We used the transcriptome data obtained using the high-throughput sequencing technology to provide an effective method for the identification of three important single genes in autumn leaves of *Acer pseudo-sieboldianum*. A total of 50,501 unigenes were produced in three stages of *Acer pseudo-sieboldianum* leaves, among which 16,521 DEGs and 64 unigenes were identified as color-related homologous genes. In this study, we identified anthocyanins related to the anthocyanin synthesis pathway and red leaf formation. We detected four PAL, one CHS, one CHI, two F3H, two F3'H, one F3'5'H, two DFR, one ANS, and six UFGT in the flavonoid anthocyanin complex related to leaf color in *Acer pseudo-sieboldianum*. Then, UFGT was selected as the most important candidate compound to participate in the anthocyanin metabolism pathway and induce red leaf formation. This study provides a theoretical basis for the formation of leaf color in *Acer pseudo-sieboldianum*.

Additional files

Additional files 1: Primer Information in *Acer pseudo-sieboldianum*. (DOCX 14 kb)
Additional files 2: Anthocyanins detected in *Acer pseudo-sieboldianum*. (XLSX 1565 kb)
Additional files 3: Histogram of GO classification of assembled unigenes. (DOCX 160 kb)
Additional files 4: KEGG pathway annotation in *Acer pseudo-sieboldianum* unigenes. (XLSX 15 kb)
Additional files 5: KEGG pathway enrichment analysis of DEGs between B and A. (XLSX 22 kb)
Additional files 6: KEGG pathway enrichment analysis of DEGs between B and M. (XLSX 13 kb)
Additional files 7: Analysis map of gene pathway for differential expression of

flavonoid biosynthesis pathway. Map of differentially expressed gene pathways in phenylalanine metabolism.(DOCX 96 kb)

Abbreviations

RNA-Seq: RNA sequencing; RT-qPCR: Real-time quantitative polymerase chain reaction; Q30 percentage: Percentage of bases with sequencing error rate lower than 1 %; NR: Non-redundant; Pfam: Protein family; DEGs: Differentially expressed genes; COG: Clusters of Orthologous Groups of proteins; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; KOG: Eukaryotic Orthologous Groups of proteins; ANS: Anthocyanidin synthase; CHI: Chalcone isomerase; CHS: Chalcone synthase; DFR: Dihydroflavonol 4-reductase; F3H: Flavanone 3-hydroxylase; F3'5'H: Flavonoid 3', 5'-hydroxylase; F3'H: Flavonoid 3'-hydroxylase; UA3GT: UDP-glycoside:Anthocyanin 3-O-glycosyltransferase; UFGT:UDP-glycoside: flavonoid 3-O-glucosyl-transferase.

Funding

The project was supported by Science and Technology Development Plan Project of Jilin Province (20180520218JH).

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional files.

Authors' contributions

DZ and YG contributed equally to this work. DZ and YG carried out sequence data analysis and drafted the manuscript. LR organized the manuscript and supervised the study. JZ and JC collected taproot samples. All authors have read, revised the manuscript critically for important intellectual content, and approved the final manuscript. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the manuscript are appropriately investigated and resolved.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹ Agriculture college, Yanbian University, Yanji 133002, China

References

- [1] Chalker SL. Environmental significance of anthocyanins in plant stress responses. Photochemistry and Photobiology. 1999;70:1-9. DOI:10.1111/j.1751-1097.1999.tb01944.x
- [2] Dai SL, Huang H, Fu JX, Hong Y. Advances in molecular breeding of ornamental plants. Chinese Bulletin of Botany. 2013;48:589-607. DOI:10.3724/SP.J.1259.2013.00589
- [3] Li XM. Physiological characters of *Pyracantha fortuneana* ‘Harlequin’ leaves during color-changing period in autumn and winter. Journal of Shanghai Jiaotong University(Agricultural Science). 2013;31:82-87. DOI:10.3969/J.
- [4] Ren J, Chen Z, Tang F, Xuan Y, Yang F, Lu XY, Fu SL. Study on leaf color related chemicals components based on comparing *Acer rubum* L. cv. ‘Yanhong’ and ‘Jinsequitian’. Journal of Anhui Agricultural University. 2019;46:420-425. DOI:10.13610/j.
- [5] Zhang M, Huang LB, Zhou P, Qian M, Dou QQ. Physiological and biochemical changes in *Zelkova serrata* leaves during leaf color transformation in autumn. Scientia Silvae Sinicae. 2015;51:44-51. DOI:10.11707/j.1001-7488.20150806
- [6] Li BB, Hou ZX, Yang JF, Chen L, Wan RM. Variations of flavonoids and soluble sugars in ‘Northland’ blueberry leaf during the color changing process. Journal of Agricultural Science and Technology. 2018;20:20-29. DOI:10.13304/j.nykjdb.2017.0210
- [7] Song XW, Wei XB, Di SK, Pang YZ. Recent advances in the regulation mechanism of transcription factors and metabolic engineering of anthocyanins. Chinese Bulletin of Botany. 2019;54:133-156. DOI:10.11983/CBB 18016
- [8] Yao YF, Zhang SP, Lian DM, Lai ZF, Huang HM, Hong JJ. Transcriptome sequencing and differential expression analysis of flavonoid metabolism in flowers and fruits of Okra. Acta Bot. Boreal. Occident. Sin. 2018;38:2000-2009. DOI:10.7606/j.
- [9] Zhang SP, Qiu SL, Zhang S, Hong JM, Lin BM, Zheng KB. Transcriptome analysis of anthocyanidins and their synthesis regulatory genes in *Gynura bicolor* leaves. Acta Bot. Boreal. Occident. Sin. 2019;39:808-816. DOI:10.7606/j.
- [10] Jin XH. The study of anthocyanidins biosynthesis pathway in *Senecio cruentus* based on the high-throughput RNA sequencing technique. Beijing Forestry University. 2013.

466 [11] Wang W, Zheng W, Xu XD, Chen J, Wang TX. Coloring mechanism analysis of mosaic le
 467 aves in *Camellia reticulata* Lindl. based on sequencing of transcriptome. *Acta Bot. Boreal. Occid*
 468 ent. Sin. 2017;37:1720-1727. DOI:10.7606/j.

469 [12] Jia ZD, Ma PY, Bian XF, Yang Q, Guo XD, Xie YZ. Biosynthesis metabolic pathway and
 470 molecular regulation of plants anthocyanin. *Acta Bot. Boreal. Occident. Sin.* 2014;34:1496-1506.
 471 DOI:10.7606/j.

472 [13] Liu XF, Li F, Yin XR, Xu CJ, Chen KS. Recent advances in the transcriptional regulation
 473 of anthocyanin biosynthesis. *Acta Horticulturae Sinica.* 2013;40:2295-2306. DOI:10.16420/j.

474 [14] Duan YJ, Zhang LG, He Q, Zhang MK, Shi JC. Expression of transcriptional factors
 475 and structural genes of anthocyanin biosynthesis in Purple-heading Chinese Cabbage. *Acta H*
 476 orticulturae Sinica. 2012;39:2159-2167. DOI:10.16420/j.

477 [15] Zhu JL. Physiological characteristics of leaf color establishment and key
 478 gene screening of *Eucommia ulmoides* 'Hongye'. Chinese Academy of Forestry. 2017.

479 [16] Grace SC, Logan BA, Adams WW. Seasonal differences in foliar content of chlorogenic a
 480 cid, A phenylpropanoid antioxidant, in mahonia repens. *Plant, Cell & Environment*, 1998;21:513-
 481 521. DOI:10.1046/j.1365-3040.1998.00282.x

482 [17] Zhang X, Xiao TT, Li J, Wang YT, Liu GL. Effects of water stress on the growth and leaf color in
 483 *Acer rubrum*. *Jiangsu Agricultural Sciences*, 2016;44:224-227. DOI:10.15889/j.

484 [18] Oberbauer SF, Starr G. The role of anthocyanins for photosynthesis of alaskan arctic everg
 485 reens during snowmelt. *Advances in Botanical Research.* 2002;37:129-145. DOI:10.1016/S0065-22
 486 96(02)37047-2

487 [19] Sun B, Zheng DC, Cui HM. Leaf color change in *Acer pseudo-sieboldianum* in autumn. *Journal of*
 488 Northeast Forestry University. 2009;37:14-15. DOI:CNKI:SUN:DBLY.0.2009-02-007

489 [20] Pang QY, Zhuo LH. Spatial difference in physiological indexes of autumn colored Maple leaves.
 490 *Journal of Northeast Forestry University.* 2007;35:16-17,20. DOI:10.13759/j.

491 [21] Xian Y, Dong X, Xie XM, Wu D, Han B, Wang Y. Effect of conservation conditions on restricting
 492 conservation of *Acer rubrum* cv. 'Somerset'. *Chinese Bulletin of Botany.* 2019;54:64-71.
 493 DOI:10.11983/CBB 18010

494 [22] Qu X, Sun B, Yang YH. Leaf color performance and its application of introduced *Acer*
 495 *pseudo-sieboldianum*. *Territory & Natural Resources Study.* 2012;;86-88. DOI:10.16202/j.

496 [23] Zhao LY, Cao SY, Long YS, Zhang YH, Xu JQ. Applications and research progresses of f
 497 ull-length transcriptome sequencing in plants. Journal of Plant Genetic Resources. 2019;20:1-12.
 498 DOI:10.13430/j.

499 [24] Wang YS, Wang H, Fan ZY, Chen Y, Jin YF, Gao ML. Identifying genes associated with leaf
 500 color in kale (*Brassica oleracea* L. var. *acephala* DC.) based on transcriptome analysis. Genomics
 501 and Applied Biology. 2019;38:1-9.
 502 <http://kns.cnki.net/kcms/detail/45.1369.Q.20190422.1420.006.html>

503 [25] Kanehisa M, Goto S, Kawashima S, Okuno Y, Hattori M. The KEGG resource for deciphering
 504 the genome. Nucleic Acids Research. 2004;32:277-280. DOI: 10.1093/nar/gkh063

505 [26] Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L,
 506 Raychowdhury R, Zeng QD, Chen ZH, Mauceli E, Hacohen N, Gnirke A, Rhind N, Dipalma
 507 F, Birren BW, Nusbaum C, Lindblad-Toh K, Friedman N, Regev A. Full length transcriptome assembly
 508 from RNA Seq data without a reference genome. Nature Biotechnology. 2011;29:644-65
 509 2. DOI:10.1038/nbt.1883

510 [27] Deng YY, Li JQ, Wu SF, Zhu YP, Chen YW, He FC. Integrated nr database in protein annotation
 511 system and its localization. Computer Engineering. 2006; 32:71-74. DOI:10.1109/INFOCOM.
 512 OM.2006.241

513 [28] Apweiler R, Bairoch A, Wu CH, Barker WC, Boeckmann B, Ferro S, Gasteiger E, Huang
 514 HZ, Lopez R, Magrane M, Martin MJ, Natale DA, O'Donovan C, Redaschi N, Yeh, LSL. UniProt: the
 515 Universal Protein knowledgebase. Nucleic Acids Research. 2004;32:115-9. DOI:
 516 10.1093/nar/gkh131

517 [29] Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K,
 518 Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC,
 519 Richardson JE, Ringwald M, Rubin GM, Sherlock G. Gene ontology: tool for the unification of
 520 biology. Nature Genetics. 2000;25:25-29. DOI:10.1038/75556

521 [30] Tatusov RL, Galperin MY, Natale DA, Koonin EV. The COG database: a tool for genome
 522 scale analysis of protein functions and evolution. Nucleic Acids Research. 2000;28:33-36. DOI:
 523 10.1093/nar/28.1.33

524 [31] Koonin EV, Fedorova ND, Jackson JD, Jacobs AR, Krylov DM, Makarova KS, Mazumber
 525 R, Mekhedov SL, Nikolskaya AN, Rao BS, Rogozin IB, Smirnov S, Sorokin AV, Sverdlov AV,

526 Vasudevan S, Wolf YI, Yin JJ, Natale DA. A comprehensive evolutionary classification of prot
 527 eins encoded in complete eukaryotic genomes. *Genome Biology*. 2004;5:R7. DOI:10.1186/gb-200
 528 4-5-2-r7
 529 [32] Huerta-Cepas J, Szklarczyk D, Forslund K, Cook H, Heller D, Walter MC, Ratteri T, Mende
 530 DR, Sunagawa S, Kuhn M, Jensen LJ, Mering CV, Bork P. eggNOG 4.5: a hierarchical orthology
 531 framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences.
 532 *Nucleic Acids Research*. 2016;44:286-293. DOI:10.1093/nar/gkv1248
 533 [33] Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped
 534 BLAST and PSI BLAST: A new generation of protein database search programs. *Nucleic Acids R*
 535 *esearch*. 1997;25: 3389-3402. PMID:9254694
 536 [34] Xie, C, Mao, XZ, Huang, JJ, Ding, Y, Wu, JM, Dong, S, Kong, L, Gao, G, Li CY, Wei,
 537 LP. KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseas
 538 es. *Nucleic Acids Research*. 2011;39:316-322. DOI:10.1093/nar/gkr483
 539 [35] Eddy SR. Profile hidden Markov models. *Bioinformatics*. 1998;14:755-763. DOI:10.1093/bio
 540 informatics/14.9.755
 541 [36] Finn RD, Bateman A, Clements J, Coghill P, Eberhardt RY, Eddy SR, Heger A, Hetheringt
 542 on K, Holm L, Mistry J, Sonnhammer ELL, Tate J, Punta M. Pfam: the protein families databa
 543 se. *Nucleic Acids Research*. 2014;42:222-230. DOI:10.1093/nar/gkt1223
 544 [37] Langmead B, Pop M. Ultrafast and memory-efficient alignment of short DNA sequences to
 545 the human genome. *Genome Biology*. 2009;10:R25. DOI:10.1186/gb-2009-10-3-r25
 546 [38] Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or wit
 547 hout a reference genome. *BMC Bioinformatics*. 2011;12:323 DOI:10.1186/1471-2105-12-323
 548 [39] Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, Salzberg SL,
 549 Wold BJ, Pachter L. Transcript assembly and quantification by RNA Seq reveals unannotated tra
 550 nscripts and isoform switching during cell differentiation. *Nature Biotechnology*. 2010;28:511-515.
 551 DOI:10.1038/nbt.1621
 552 [40] Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nuc*
 553 *leic Acids Research*. 2001;29:e45. DOI:10.1093/nar/29.9.e45
 554 [41] Rong LP, Li QZ, Li SS, Tang L, Wen J. De novo transcriptome sequencing of *Acer palma*
 555 *tum* and comprehensive analysis of differentially expressed genes under salt stress in two contra

556 sting genotypes. *Molecular Genetics and Genomics*. 2016;291:575-586. DOI:10.1007/s00438-015-1
 557 127-2
 558 [42] Chen Z, Lu XY, Xuan Y, Tang F, Wang JJ, Shi D, Fu SL, Ren J. Transcriptome analysis
 559 based on a combination of sequencing platforms provides insights into leaf pigmentation in *Acer*
 560 *rubrum*. *BMC Plant Biology*. 2019;19:240. DOI:10.1186/s12870-019-1850-7
 561 [43] Fang Z, Zhou DR, Ye XF, Jiang CCi, Pan SL. Identification of candidate anthocyanin-related genes
 562 by transcriptomic analysis of 'Furongli' Plum (*Prunus salicina* Lindl.) during fruit ripening using
 563 RNA-Seq. *Front Plant Science*. 2016;7:1338. DOI:10.3389/fpls.2016.01338
 564 [44] Qu Y, Ou Z, Yang FS, Wang S, Peng JS. The study of transcriptome sequencing for flower
 565 coloration in different anthesis stages of alpine ornamental herb (*Meconopsis* 'Lingholm'). *Gene*.
 566 2018;689:220-226. DOI:10.1016/j.gene.2018.12.017
 567 [45] Zhang HS, Tian H, Chen MX, Xiong JB, Cai H, Liu Y. Transcriptome analysis reveals potential
 568 genes involved in flower pigmentation in a red-flowered mutant of white clover (*Trifolium*
 569 *repens* L.). *Genomics*. 2018;110:191-200. DOI:10.1016/j.ygeno.2017.09.011
 570 [46] Kodama M, Brinch-Pedersen H, Sharma S, Holme IB, Joernsgaard B, Dzhanfezova T, Amby DB,
 571 Vieira FG, Liu SL, Gilbert MTP. Identification of transcription factor genes involved in anthocyanin
 572 biosynthesis in carrot (*Daucus carota* L.) using RNA-Seq. *BMC genomics*, 2018,19:811.
 573 DOI:10.1186/s12864-018-5135-6
 574 [47] Shi LY, Chen X, Chen W, Zheng YH, Yang ZF. Comparative transcriptomic analysis of white and
 575 red Chinese bayberry (*Myrica rubra*) fruits reveals flavonoid biosynthesis regulation. *Scientia*
 576 *Horticulturae*. 2018,235:9-20. DOI:10.1016/j.scienta.2018.02.076
 577 [48] Zhuang HM, Lou Q, Liu HF, Han HW, Wang Q, Tang ZH, Ma YM, Wang H. Differential
 578 regulation of anthocyanins in green and purple turnips revealed by combined de novo transcriptome
 579 and metabolome analysis. *International Journal of Molecular Sciences*. 2019;20:4387. DOI:
 580 10.3390/ijms20184387
 581 [49] Jiang HB, Xia LF, Tian YP, Dai WD, Sun YN, Chen LB. Transcriptome analysis of antho
 582 cyanin synthesis related genes in purple bud tea plant. *Journal of Plant Genetic Resources*. 2018;
 583 19:967-978. DOI:10.13430/j.

584 [50] Rosati C, Cadic A, Duron M, Ingouff M, Simoneau P. Molecular characterization of the an
585 thocyanidin synthase gene in *Forsythia ×intermedia* reveals organ-specific expression during flow
586 er development. Plant Science. 1999;149:73-79. DOI:10.1016/s0168-9452(99)00146-6

587 [51] Masukawa T, Cheon KS, Mizuta D, Kadowaki M, Nakatsuka A, Kobayashi N. Developmen
588 t of mutant RsF3'H allele-based marker for selection of purple and red root in radish (*Raphanu*
589 *s sativus* L.var.longipinnatus L.H.Bailey). Euphytica. 2019;215:1-9. DOI:10.1007/s10681-019-2442-
590 1

591 [52] Wu Q, Wu J, Li SS, Zhang HJ, Feng CY, Yin DD, Wu RY, Wang LS. Transcriptome
592 sequencing and metabolite analysis for revealing the blue flower formation in waterlily. BMC
593 Genomics. 2016;17:897. DOI:10.1186/s12864-016-3226-9

594 [53] Holton TA, Cornish EC. Genetics and biochemistry of anthocyanin biosynthesis. The Plant
595 Cell. 1995;7:1071-1083. DOI:10.2307/3870058

596 [54] Springob K, Nakajirna J, Yamazaki M, Saito K. Recent advances in the biosynthesis and a
597 ccumulation of anthocyanins. Natural Product Reports. 2003;20:288-303. DOI:10.1039/b109542k

598 [55] Menssen A, Hohmann S, Martin W, Schnable PS, Peterson PA, Saedler H, Gierl A. The E
599 n/Spm transposable element of *Zea mays* contains splice sites at the termini generating a novel i
600 ntron from a dSpam element in the A2 gene. The EMBO Journal. 1990;9:3051-3057. DOI:
601 10.1002/j.1460-2075.1990.tb07501.x

602 [56] Li JC, Li TH, Wang ZG, Li BJ. Cloning and expression of UFGT gene in skin of max re
603 d Bartlett. Acta Botanica Boreali-Occidentalia Sinica. 2010;30:30-34. DOI:10.3724/SP.J.1238.2010.
604 00512

605 [57] Kobayashi S, Ishimaru M, Ding CK, Yakushiji H, Goto N. Comparison of UDP-glucose:fla
606 vonoid 3-O-glucosyltransferase(UFGT) gene sequences between white grapes (*Vitis vinifera*) and
607 their sports with red skin. Plant Science. 2001;160:543-550. DOI:10.1016/s0168-9452(00)00425-8

608 [58] Schmitzer V, Stampar F, Veberic R, Osterc G. Phase change modifies anthocyanin synthesis
609 in *Acer palmatum* Thunb. (Japanese maple) cultivars. Acta Physiologiae Plantarum. 2009;31:415
610 -418. DOI:10.1007/s11738-008-0237-4

611 [59] Cai XY, Li HH, Li L, Yu H, Chen G, Bao NNDT. Pigment composition and leaf color ch
612 ange in *Acer palmatum*. Journal of Northeast Forestry University. 2015;43:63-67. DOI:10.13759/j.

613 [60] Toki K, Saito N, Irie Y, Tatsuzawa F, Shigihara A, Honda T. 7-O-Methylated anthocyanidin
614 glycosides from *Catharanthus roseus*. *Phytochemistry*. 2008;69:1215-1219. DOI:10.1016/j.phytoch
615 em.2007.11.005

616 [61] Valentina S, Maja MP, Franci S, Vlasta C. Phenolic accumulation in Hybrid Primrose and pigment
617 distribution in different flower segments. *Journal of the American society for horticultural science*.
618 2017;142:192-199. DOI:10.21273/JASHS04038-17

619

620

621

Supplementary Files

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

- [File.S5.xlsx](#)
- [File.S7.docx](#)
- [File.S2.xlsx](#)
- [File.S3.docx](#)
- [File.S6.xlsx](#)
- [File.S1.docx](#)
- [File.S4.xlsx](#)