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UV-B irradiation promotes anthocyanin biosynthesis in the leaves of *Lycium ruthenicum Murray*

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Anthocyanins are the most valuable nutrients in *Lycium ruthenicum Murray* (*L. ruthenicum Murr.*). Although ultraviolet-B (UV-B) irradiation is a key environmental factor influencing anthocyanin biosynthesis in *L. ruthenicum Murr.*, the deep molecular mechanism remains unclear. Herein, we examined the changes in anthocyanin content and transcriptomic characteristics of *L. ruthenicum Murr.* leaves following UV-B irradiation treatment. The results showed a twofold increase in anthocyanin content in the leaves of *L. ruthenicum Murr.* after the treatment. The transcriptome analysis showed that the expression of 24 structural genes identified in the anthocyanin synthesis pathway was up-regulated. In particular, F3'H (Unigene0009145) and C4H (Unigene0046607) exhibit notable up-regulation, suggesting their potential roles in anthocyanin synthesis. Protein interaction network results revealed that MYB1 (Unigene004770600) had the highest connectivity, followed by bHLH (Unigene0014085). Additionally, UVR8 (Unigene0067978) and COP1 (Unigene0008780), were found to be highly involved in the transduction of UV-B signals. These findings provide fundamental data for future molecular biology research on promoting anthocyanin accumulation in *L. ruthenicum Murr.* under UV-B irradiation.

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Abstract

Anthocyanins are the most valuable nutrients in *Lycium ruthenicum Murray* (*L. ruthenicum Murr.*). Although ultraviolet-B (UV-B) irradiation is a key environmental factor influencing anthocyanin biosynthesis in *L. ruthenicum Murr.*, the deep molecular mechanism remains unclear. Herein, we examined the changes in anthocyanin content and transcriptomic characteristics of *L. ruthenicum Murr.* leaves following UV-B irradiation treatment. The results showed a twofold increase in anthocyanin content in the leaves of *L. ruthenicum Murr.* after the treatment. The transcriptome analysis showed that the expression of 24 structural genes identified in the anthocyanin synthesis pathway was up-regulated. In particular, F3'H (Unigene0009145) and C4H (Unigene0046607) exhibit notable up-regulation, suggesting their potential roles in anthocyanin synthesis. Protein interaction network results revealed that MYB1 (Unigene0047706) had the highest connectivity, followed by bHLH (Unigene0014085). Additionally, UVR8 (Unigene0067978) and COP1 (Unigene0008780), were found to be highly involved in the transduction of UV-B signals. These findings provide fundamental data for future molecular biology research on promoting anthocyanin accumulation in *L. ruthenicum Murr.* under UV-B irradiation.

Keywords: *Lycium ruthenicum Murray*; UV-B irradiation; Anthocyanins; Transcriptome analysis; Gene expression

Introduction

L. ruthenicum Murr. is an important ecological and economic species in the Qinghai-Tibet Plateau. The leaves of *L. ruthenicum Murr.* are a byproduct of the planting industry and contain functional components similar to its fruits (Kumar et al., 2022). Therefore, the leaves are commonly used for both medicinal and culinary purposes due to their nutritional value and cost-effectiveness (Sharma et al., 2022). The utilization of the leaves has also increased the value of the *L. ruthenicum Murr.*. Anthocyanins are valuable functional components found in the leaves of *L. ruthenicum Murr.*. These active substances have various physiological benefits, such as antioxidant, anti-cancer, and anti-diabetic properties (Liu et al., 2020; Zheng et al., 2011; Cappellini et al., 2021).

UV-B irradiation has various physiological and biochemical effects on plants, impacting their growth, development, and metabolic processes (Jenkins, 2009). Plants detect UV-B radiation through photoreceptors and transmit signals to the nucleus, leading to the expression of genes related to anthocyanin synthesis. This, in turn, regulates anthocyanin synthesis in plants (Takshak & Agrawal, 2019; Li et al., 2023). Specifically, UV-B irradiation can induce the expression of transcription factors for anthocyanin synthesis, such as SIBBX20/SIBBX21, MYB75/PAP1, HY5, COP1, and WRKY11 (Yang et al., 2022; Teng et al., 2005; Bursch et al., 2020; Liu et al., 2019). These genes directly or indirectly regulate downstream structural genes, promoting transcription of genes such as PAL, C4H, 4CL, CHS, CHI, F3H, FLS, DFR, ANS, and UFGT. These genes encode enzymes involved in the anthocyanin synthesis pathway. The increased expression of the enzymes leads to a subsequent increase in anthocyanin content (Chaves-Silva et al., 2018; Liu et al., 2018).

Research has shown that MdBBX20 interacts with MdHY5 under UV-B irradiation and can bind to the promoters of the biosynthesis genes MdDFR and MdANS, regulating their expression and promoting anthocyanin synthesis in *apples* (Fang et al., 2019). Additionally, UV-B induces the expression of MdWRKY71-L, which directly regulates anthocyanin biosynthesis by forming a transcriptional complex with MdHY5-MdMYB1 and interacting with the MdUFGT promoter (Su et al., 2022). Besides, research on *Arabidopsis thaliana* has shown that MYB13 plays a positive role in UV-B-induced cotyledon expansion in a UVR8-dependent manner. The MYB13 binds directly to the promoters of CHS, CHI, and FLS, positively regulating their expression for flavonoid accumulation and UV-B tolerance (Qian et al., 2020). Similarly, studies have found

that under long-term UV-B irradiation, the expression of transcription factors such as UVR8, COP1, HY5, and MYB, as well as structural genes like FLS and F3'H, increased in *Ginkgo biloba* leaves, leading to an increase in flavonol content (Zhao et al., 2020).

L. ruthenicum Murr. also exhibits a significant response to UV-B irradiation. Transcriptome analysis revealed a significant increase in the expression of the UDP-glucose flavonoid 3-O-glucosyltransferase (UFGalT) gene, which is related to anthocyanin synthesis, in the leaves of *L. ruthenicum Murr.* after UV-B irradiation(Chen et al., 2015). However, there is currently a lack of systematic research on genes related to anthocyanin synthesis in the leaves of *L. ruthenicum Murr.* under UV-B irradiation. The molecular mechanism by which UV-B promotes anthocyanin accumulation has not been well understood.

In this paper, we studied the effects of UV-B irradiation treatment on the anthocyanin biosynthesis in *L. ruthenicum Murr.* leaves using transcriptome analysis. The study investigated the changes in anthocyanin content caused by UV-B irradiation and identified key regulatory genes that promote anthocyanin accumulation in the leaves. The findings provide fundamental data for future molecular biology research on promoting anthocyanin accumulation in *L. ruthenicum Murr.* under UV-B irradiation. Additionally, the study offers insights for optimizing UV-B radiation dosage to produce *L. ruthenicum Murr.* tea with increased anthocyanin content.

Materials and methods

Plant materials and UV-B irradiation treatment

Seeds used in this experiment of *L. ruthenicum Murr.* were collected from the desert area of Dulan County, Qinghai Province, China. The seeds with intact particles were selected and germinated in a petri dish for 15 days before being transplanted into organic soil. The seedlings were then grown for 80 days in an environment with a light/dark cycle (16 h/8 h, 25/20°C) and a humidity of 60%. Twelve plants with comparable growth vigor and size were selected, and each of the four plants was divided into a separate group. Each group represents a biological replication, resulting in a total of three biological replications. All data were obtained based on the three biological repetitions. The samples that were not subjected to UV-B irradiation served as the control group (CK), while the samples that were irradiated with UV-B constituted the experimental group.

UV-B irradiation was produced by a UV-B lamp with a main spectral line of 253.7 nm and a real-time power density of $10\mu w/cm^2$. During each light/dark cycle, the plants in the experimental group were intermittently exposed to the UV-B irradiation for 12 periods during the light cycle in each day. The irradiation duration of each period was 10 minutes (i.e. 8:00-8:10, 9:00-9:10..., 19:00-19:10). After being irradiated for 0.5 days (6 periods), 1 day (12 periods), 1.5 days ((12+6) periods), 2 days ((12+12) periods), and 4 days ((12+12+12+12) periods), the leaves

were collected from the same batch of plants for physiological indicators and RNA-Seq analysis.

Determination of anthocyanin content

Anthocyanin content was determined using the methods described in Ai (2016) (Ai et al., 2016). Fresh leaves of *L. ruthenicum Murr.* (0.1 g) were ground into fine pieces and extracted with 65% methanol/water solution (hydrochloric acid volume ratio: 1%) using ultrasonication with three replicates. The optical density of 530 nm (A_{530}) and 657 nm (A_{657}) were measured using a microplate reader, then the anthocyanin content was calculated as (A_{530}-0.25*A_{657})/M.

Detection of reactive oxygen species metabolism

Reactive oxygen species metabolism content was detected using peroxidase (POD), hydrogen peroxide (H_2O_2), catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA) detection kits.

RNA-Seq analysis

RNA-Seq analysis was performed using the Illumina HiseqTM 4000 platform (Chidio, China). There were three biological replications in each sample. Differential expression genes（DEG） were screened using the FDR value and the difference fold log2FC after comparing the sequencing results with the annotated genes in the database. The threshold value was set as FDR<0.05, |log2FC|≥1.

qPCR analysis

RNA was extracted using the Polysaccharide Polyphenol Plant Total RNA Extraction Kit (Tiangen, China, DP441). cDNA synthesis was performed using the Rapid Reverse Transcription Kit (Takara, RR092A). qPCR analysis was performed using a quantitative PCR kit (Takara, RR820A) with three replicates. The primer sequences are shown in Table 1. Actin from *L. ruthenicum Murr.* was used as the internal reference gene. The expression of genes was calculated using the $2^{-\Delta\Delta Ct}$ method.

Interaction network analysis of structural genes and transcription factors for anthocyanin synthesis

Using the STRING protein interaction database (http://string-db.org), the Omicsmart online platform of Guangzhou Kidio Company predicted the transcription factors that interact with 24 structural genes involved in anthocyanin synthesis. Out of the 12,720 genes that were differentially expressed after UV irradiation treatment, 424 were identified as transcription factors. An interaction network was created by using the 24 structural genes as a target gene set and the 424 transcription factors as an associated gene set. Only prediction results with a combined score > 400 were retained.

Results

UV-B induced anthocyanin accumulation and changes in antioxidant enzymes

After being exposed to UV-B irradiation for 0.5 days, 1 day, 1.5 days, 2 days, and 4 days, the color of the anthocyanin extraction solution obtained with the leaves of *L. ruthenicum Murr.* changed significantly, as shown in Fig. 1. The color of the anthocyanin extraction solution became darker after UV-B treatment compared to the control group (CK).

Figure 2A displays the anthocyanin content measured by spectrophotometry for varying durations of UV-B irradiation. The highest level of anthocyanin content was observed after 1 day of exposure to UV-B irradiation, with a relative content of 5.43 (A_{530}-0.25*A_{657}) g^{-1}, which is approximately 2.8 times higher than that of the control group. However, as the duration of UV-B irradiation increased, the anthocyanin content gradually decreased. Furthermore, Figs. 2B~2F displays the changes in the content of five antioxidant enzymes, i.e., H_2O_2, MDA, SOD, POD, and CAT, in the leaves of *L. ruthenicum Murr.* after UV-B irradiation treatment. The content of antioxidant enzymes (excluding MDA) increased after UV-B irradiation treatment, indicating that increasing antioxidant enzyme activity is a physiological response for plants to adapt to UV-B irradiation stress.

Differential expression genes after UV-B irradiation treatment

The anthocyanin content in the leaves of *L. ruthenicum Murr.* changed significantly after 1 day of UV-B irradiation treatment. Therefore, the leaves that were exposed to UV-B irradiation for 1 day were selected for transcriptome analysis. Six transcriptome libraries of *L. ruthenicum Murr.* leaves were constructed, which were then divided into a control group (CK) and a treatment group. The control group consisted of Lr-CK-1, Lr-CK-2, and Lr-CK-3, while the treatment group consisted of Lr-UV-B-1, Lr-UV-B-2, and Lr-UV-B-3.

A total of 63698 Unigenes were assembled from clean reads and subsequently annotated using BLASTx (E-value $<1\times10^{-5}$) searches of four public databases: NCBI nr database, Swiss-Prot protein database, KEGG database, and COG database. The annotation results are presented in Table 2. A total of 63,698 unigenes were processed and low expressed genes were removed, resulting in a total of 43,989 unigenes. The statistical power of this experimental design, calculated in *RnaSeqSampleSize* is 0.82.

To identify differential expression genes after UV-B irradiation treatment, we used DESeq2 software to generate histograms and volcano plots of differential genes, as shown in Fig. 3. The thresholds used to identify differential expression genes between each group were DFR<0.05 and log2 FC>>log2(2). As a result, we identified a total of 12,720 differential expression genes, including 6,130 genes with increased expression and 6,617 genes with decreased expression.

To study the functions of differential expression genes, we performed KEGG functional enrichment analysis. The Unigenes were divided into 140 pathways in the KEGG functional classification, with the top 20 pathways shown in Fig. 4. The metabolic pathway was the most

significantly enriched with 1204 Unigenes, followed by the biosynthesis of secondary metabolites pathway with 705 Unigenes. The pathways that have been significantly enriched include phenylpropanoid metabolism, flavonoid metabolism, and hormone signal transduction. Other enriched pathways include phenylpropanoid metabolism, flavonoid metabolism, and hormone signal transduction.

Analysis of genes related to UV-B signal transduction

The differential expression genes in the UV-B signaling pathway were identified. The expression of seven genes was observed to change: a COP1 gene (Unigene0008780), a HY5 gene (Unigene0072293), and five UVR8 genes (Unigene0015353, Unigene0003921, Unigene0067978, Unigene0020095 and Unigene0078284). Specifically, following exposure to UV-B irradiation, the majority of UVR8 genes exhibited a decrease (Fig. 5A), while the Unigene0067978 demonstrated a significant increase. In contrast, the COP1 genes exhibited a decrease, while the expression of HY5 increased.

The results of the qPCR analysis indicated a significant increase in the expression of UVR8 (Unigene0067978) following one day of UV-B treatment, which then decreased (Fig. 5B). The expression of COP1 initially decreased and then increased significantly with the duration of treatment (Fig. 5C). The expression of HY5 increased continually with the treatment duration (Fig. 5D).

Analysis of genes related to anthocyanin synthesis

We analyzed the Unigenes involved in the synthesis pathways of flavonoids (Fig. 6A) and anthocyanins under UV-B irradiation. A total of 24 enzyme-encoding genes were identified and their expression was found to be up-regulated, as shown in Fig. 6B. Among the identified genes, the two genes with the most significant increase in expression were F3’H (Unigene0009145, FPKM is 891 times that of CK), and C4H (Unigene0046607, FPKM is 18 times that of CK).

To ensure the reliability of the RNA-seq data, we selected CHS (Unigene0010556) and UFGT (Unigene0001601), which are enriched in the anthocyanin biosynthesis pathway (ko00942), for qPCR analysis. The results showed a significant increase in the expression of CHS (Unigene0010556) following one day of UV-B treatment (Fig. 6C). However, the expression decreased as the duration of UV-B irradiation treatment increased. The expression of UFGT (Unigene0001601) initially increased and then decreased with UV-B treatment time, as shown in Fig. 6D. The results of the qPCR analysis were consistent with the transcriptome analysis results.

UV-B signal transduction and anthocyanins synthesis transcription factors

A total of 424 transcription factors, belonging to 36 gene families, were identified among the differential expression genes. The largest family group was MYB transcription factors (15%), followed by ERF (14%), bHLH (10%), and WRKY (10%). The changes in expression of MYB, bHLH, and WRKY transcription factors were examined. The expression of most transcription

factors was found to decrease in response to UV-B irradiation, as shown in Fig. 7. However, some transcription factors showed a significant increase in expression, such as WRKY1 (Unigene0066868, FPKM value 100 times higher than that of CK), WRKY2 (Unigene0013893, FPKM value 63 times higher than that of CK), WRKY3 (Unigene0039337, FPKM value 47 times higher than that of CK), and WRKY4 (Unigene0078996, FPKM value 14 times higher than that of CK).

The qPCR analysis was conducted with the following transcription factors: MYB (Unigene0070868), bHLH (Unigene0025729), and WRKY (Unigene0066868, Unigene0013893, Unigene0039337, Unigene0078996). The results are presented in Fig. 8, which demonstrates that the change in the expression of the six transcription factors, with the exception of MYB, is consistent with the transcriptome analysis results. Furthermore, the expression of WRKY transcription factors significantly increased following exposure to UV-B irradiation. This indicates that WRKY family genes may play a crucial role in responding to UV-B irradiation.

Network analysis of interactions between structural genes and transcription factors in anthocyanin biosynthesis

To study the regulatory role of transcription factors in anthocyanin biosynthesis, we analyzed the interactions between 24 structural genes involved in anthocyanin synthesis and transcription factors. A network graph was constructed based on protein-protein interactions with a combined score above 400. As shown in Fig. 9 A, a total of 12 structural genes involved in anthocyanin synthesis formed 40 pairs of interactions with 11 transcription factors, all of which exceeded the score threshold. Notably, MYB1 (Unigene004770600) showed the highest connectivity, interacting with all 11 structural genes. Following MYB1, bHLH (Unigene0014085) and MYB308 (Unigene0053002) interacted with 9 and 5 structural genes, respectively. Among the structural genes, the CHS genes (Unigene0004160, Unigene0010556) showed the highest connectivity, interacting with 6 transcription factors, followed by ANT17 (Unigene0004160). As shown in Fig. 9B, most of the interacting transcription factors showed up-regulated expression, with the exception of JAF13, MYB4, and TAF1.

Discussion

Anthocyanins provide protection by acting as UV-B filters and/or scavenging reactive oxygen under UV-B stress(Neill & Gould, 2003; Landi et al., 2015). Our findings indicate that the leaves of *L. ruthenicum Murr.* are abundant in anthocyanins, with a significant increase in content observed following exposure to UV-B irradiation. This suggests that anthocyanin content may be a direct response of plants to UV-B irradiation (Del et al., 2015). Studies on many other plants have also shown an increase in anthocyanin content following UV-B irradiation. These include *Populus alba* and *Populus russkii*(Ma et al., 2016), *Artemisia annua*(Ma et al., 2016), and

Indigofera tinctoria(Ravindran et al., 2010).

The molecular mechanism of anthocyanin biosynthesis in the leaves of *L. ruthenicum Murr.* following UV-B irradiation was investigated by transcriptome testing. The analysis revealed that the 24 identified structural genes in the anthocyanin synthesis pathway were up-regulated, resulting in an increase in anthocyanin content. Furthermore, the study analyzed the genes expressed on the UV-B signaling pathway. The results demonstrated that following UV-B irradiation, UVR8 (Unigene0067978), COP1, and HY5 were up-regulated. This result is consistent with the expression patterns of UVR8, COP1 and HY5 in *Arabidopsis thaliana* and *Ginkgo biloba* leaves (Yang et al., 2020; Favory et al., 2009).

The expression of WRKY2 increased significantly and reached its highest level after 1 day of UV-B irradiation treatment. As previously stated, the anthocyanin content in the leaves of *L. ruthenicum Murr.* also reached its highest level after 1 day of UV-B irradiation treatment. Therefore, the trend of anthocyanin content changing with UV-B irradiation treatment duration was consistent with the changing trend of WRKY2 expression. This suggests that the WRKY2 transcription factor positively regulates anthocyanin synthesis in *L. ruthenicum Murr.* leaves in response to UV-B irradiation. Similar results have also been obtained in *apples* (Liu et al., 2019)(Hu et al., 2020).

The prediction of transcription factors that interact with structural genes revealed that MYB1 had the highest connectivity and interacted with 11 structural genes. MYB1 was identified as an AN2-like protein (Li et al., 2020), a transcription factor that plays a crucial role in regulating anthocyanin synthesis in *L. ruthenicum Murr.*. Therefore, MYB1 may be a key transcription factor in regulating anthocyanin synthesis in *L. ruthenicum Murr.* leaves following UV-B radiation. Additionally, we found that HY5 interacts with two CHS genes. Previous research has demonstrated that under UV-B irradiation, HY5 can bind to the promoter of CHS and activate its transcription (Ang et al., 1998). Furthermore, studies have shown that HY5 protein can bind to the CHS promoter sequence both in vitro and in vivo (Lee et al., 2007). Therefore, HY5 and CHS are also considered key genes for anthocyanin synthesis.

Conclusions

In this study, we investigated the impact of UV-B irradiation on anthocyanin content and identified key regulatory genes that promote anthocyanin accumulation in the leaves of *L. ruthenicum Murr.* Our findings can be summarized as follows:

(1) A twofold increase in anthocyanin content was observed in the leaves of *L. ruthenicum Murr.* following a one-day UV-B irradiation treatment.

(2) The expression of 24 structural genes identified in the anthocyanin synthesis pathway was up-regulated. In particular, F3'H (Unigene0009145) and C4H (Unigene0046607) exhibited

notable up-regulation, suggesting their potential roles in anthocyanin synthesis.

(3) The transcription factor MYB1 (Unigene004770600) exhibited the highest connectivity, followed by bHLH (Unigene0014085), suggesting their potential roles in regulating anthocyanin synthesis.

(4) Two genes, UVR8 (Unigene0067978) and COP1 (Unigene0008780), were found to be highly involved in the transduction of UV-B signals.

These findings provide fundamental data for future molecular biology research on promoting anthocyanin accumulation in *L. ruthenicum Murr.* under UV-B irradiation.

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Table 1(on next page)

Primers used for RT-qPCR analysis.

1

Unigene	Genename	ForwardprimerSequence(5'-3')	ReverseprimerSequence(5'-3')
Unigene0067978	UVR8	CTTGGGTTTCACCTACAGAGAG	CATCACTTTCCGGCACATTTAC
Unigene0008780	COP1	TGTACGCAGATCATAAGGGATG	GAGTTGGCTGGTAGTGAGATAA
Unigene0072293	HY5	GTATGGAGAGTGATGATGAGA	TTGCTTGCTGTGCTGATA
Unigene0010556	CHS	GTGACACTCACTTGGATAGTATGG	GGCCTTTCAACCTCTGGTAAT
Unigene0001601	UFGT	TCGGACATGTAGGGAACTAGAA	GATGATGGTTCGGGCAGTATAG
Unigene0070868	MYB	CCCTAAAGAACCCGTGAATGT	GAAAGCGGAGAAGGCTCAATA
Unigene0025729	bHLH	CCGGTACTGAAGGATTTGGTTAT	GTGGGCAGCTTGTAGTTCTT
Unigene0066868	WRKY1	GCCATCTCATTCCCACTCTT	GGCCAATTGCCTTATCCTTTG
Unigene0013893	WRKY2	ACAGGAAACCAAAGAGGATGT	AGACGATATAACATCTGGCGATAA
Unigene0039337	WRKY3	GAAGAGGACGCTACAAGAGAAG	CCTTGGATATTTGGCATTGAGG
Unigene0078996	WRKY4	GCTGATGCAGCTGTTACAAAG	CAGGATGAGGAGAAGCAATAGG
Reference gene	Actin	GAAGGGTGTCCCTCAGATCA	CCGTCCATGTCGTCTCTTTT

2

Table 2(on next page)

Annotation statistics for the Unigenes.

Total Unigenes	Nr	KEGG_ with_KO	KEGG_with Pathway	KOG	SwissProt	Annotation genes	Without annotation gene
63698	32130	11450	6064	16060	19711	32535	31163

1

Figure 1

The color of the anthocyanin extraction solution with different UV-B irradiation duration.

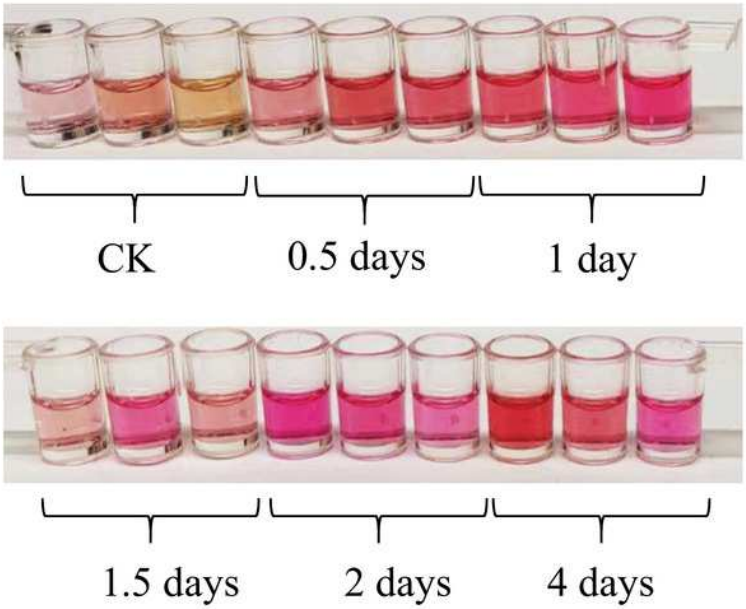
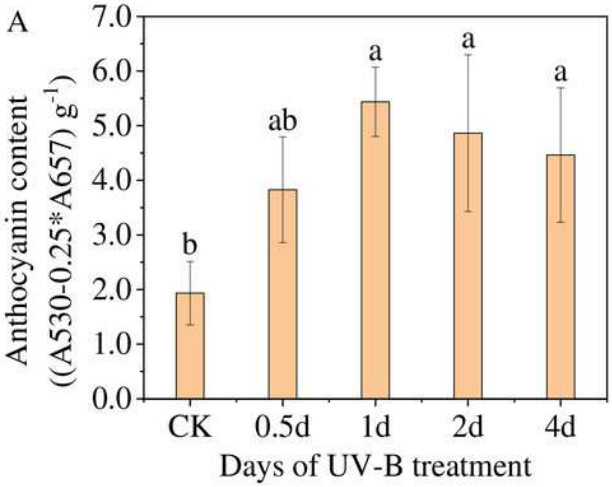
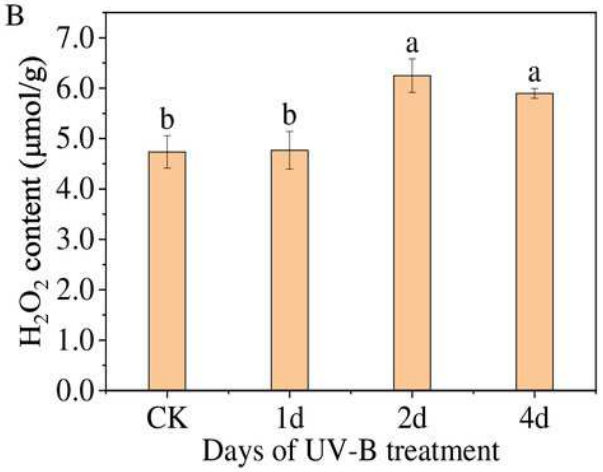
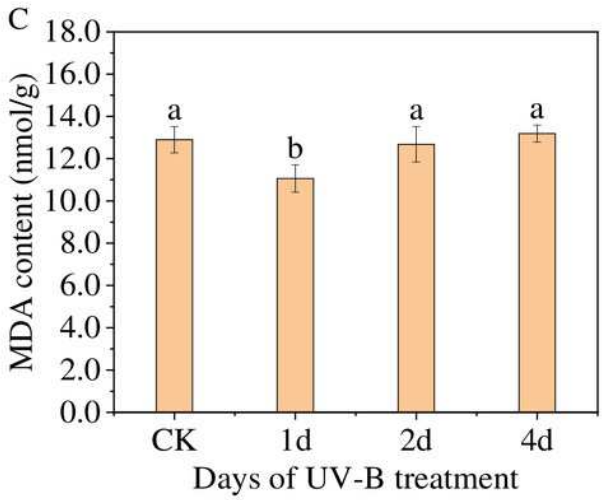


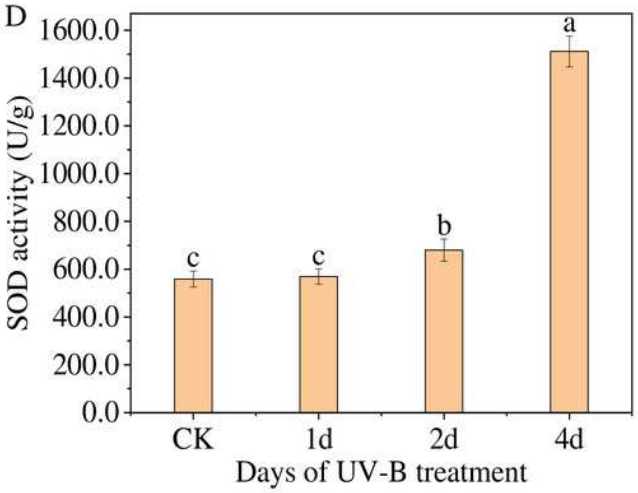
Figure 2

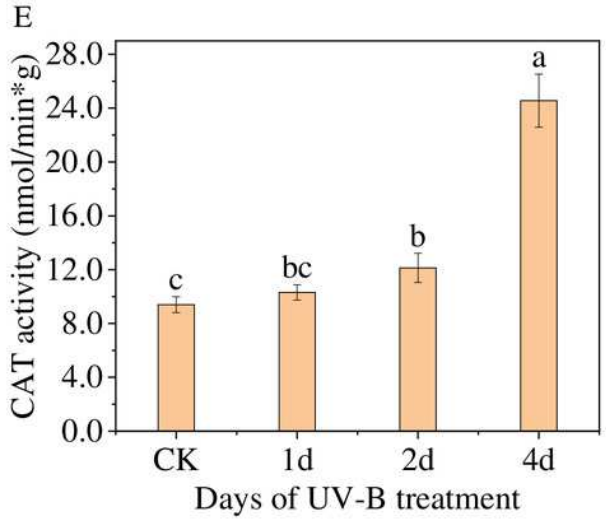
Total Content of anthocyanins and antioxidant enzymes in the leaves of *L. ruthenicum Murr.* after 0.5, 1, 2, 4 days of UV-B irradiation.


A
Anthocyanin content ((A530-0.25*A657) g-1)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
b
ab
a
a
a
CK
0.5d
1d
2d
4d
Days of UV-B treatment


B
H2O2 content (μmol/g)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
b
b
a
a
CK
1d
2d
4d
Days of UV-B treatment


C
MDA content (nmol/g)
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
a
b
a
a
CK
1d
2d
4d
Days of UV-B treatment


D
SOD activity (U/g)
1600.0
1400.0
1200.0
1000.0
800.0
600.0
400.0
200.0
0.0
c
c
b
a
CK
1d
2d
4d
Days of UV-B treatment


E
CAT activity (nmol/min*g)
28.0
24.0
20.0
16.0
12.0
8.0
4.0
0.0
c
bc
b
a
CK
1d
2d
4d
Days of UV-B treatment

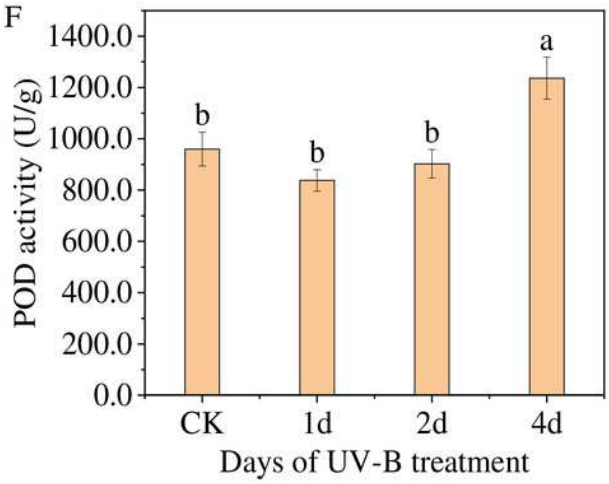

F
POD activity (U/g)
1400.0
1200.0
1000.0
800.0
600.0
400.0
200.0
0.0
b
b
b
a
CK
1d
2d
4d
Days of UV-B treatment

Figure 3

Statistics of differential expression genes.

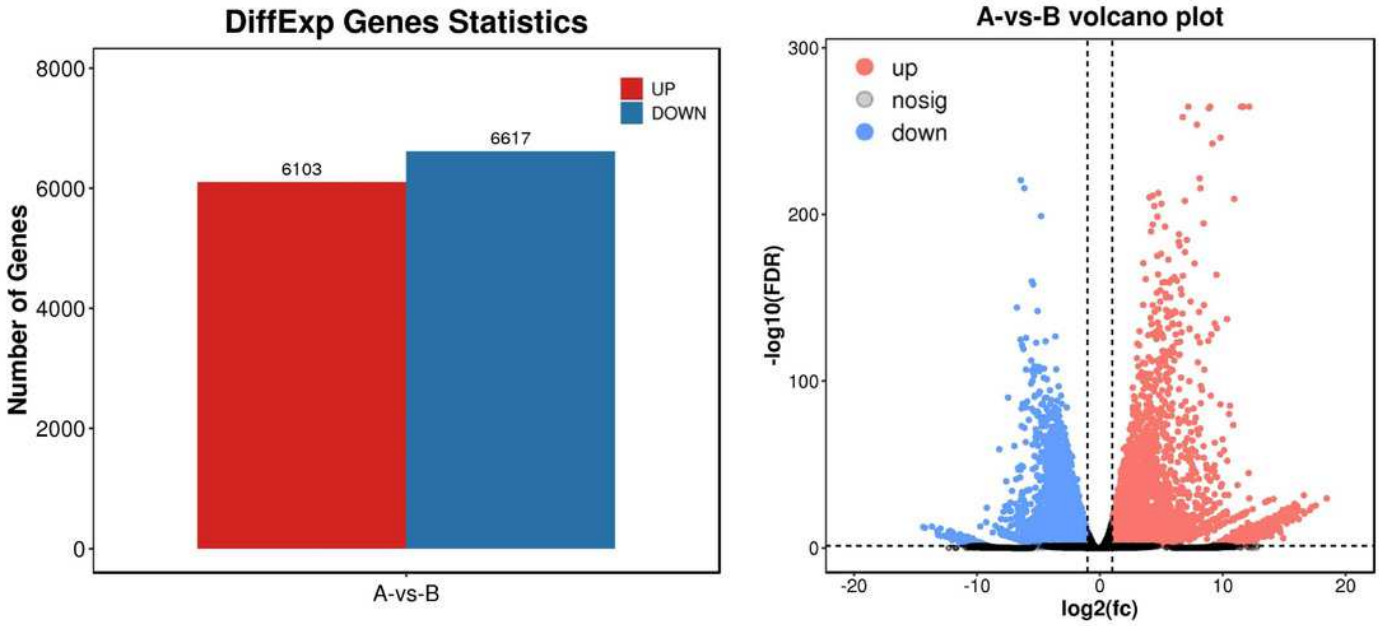


Figure 4

Top 20 enriched KEGG pathways of DEGs.

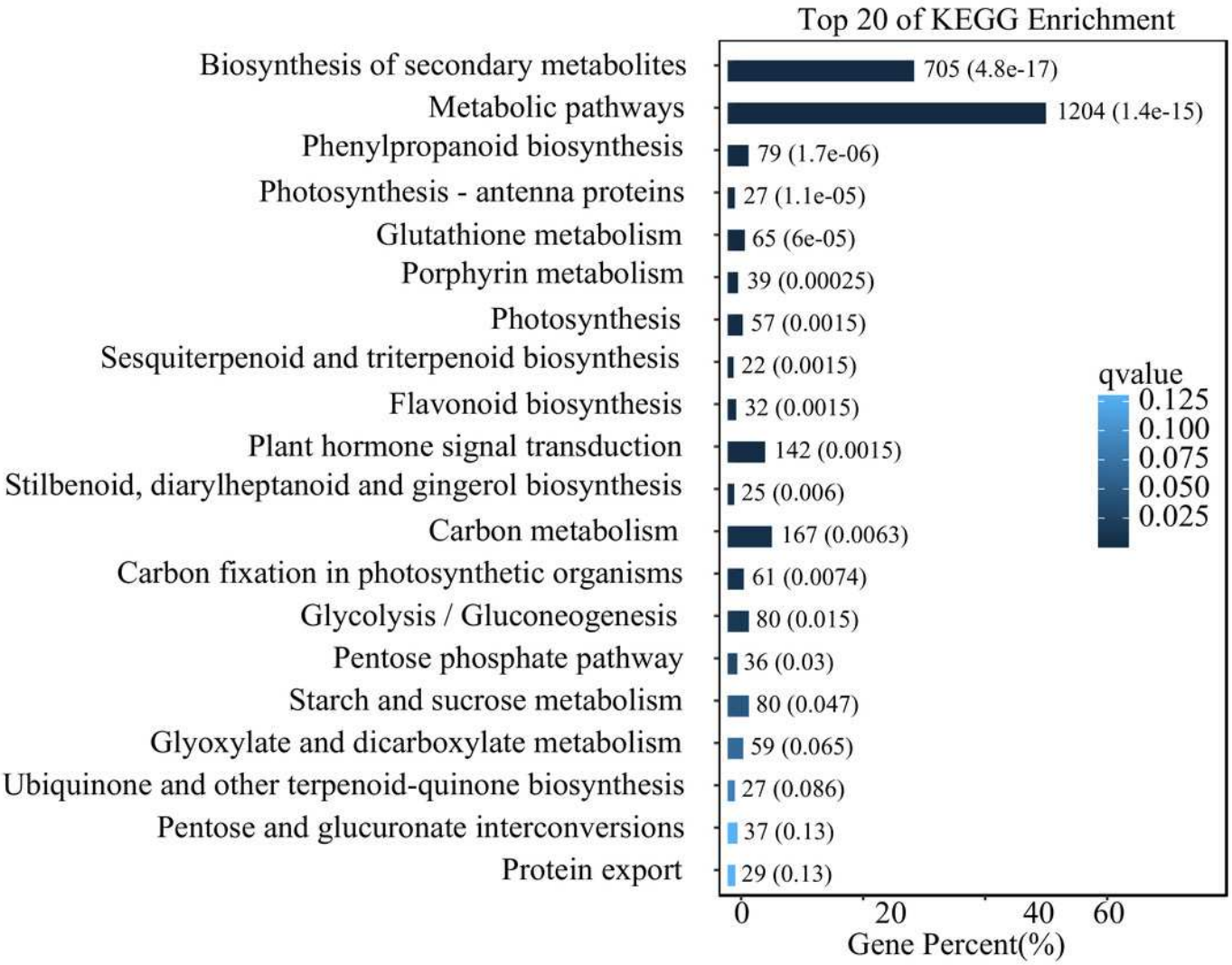


Figure 5

Expression heat map in the UV-B signaling pathway after UV-B irradiation and RT-qPCR verification of the differential expression genes. (A) Expression heat map. (B)-(D) The RT-qPCR results of UVR8, COP1 and HY5.

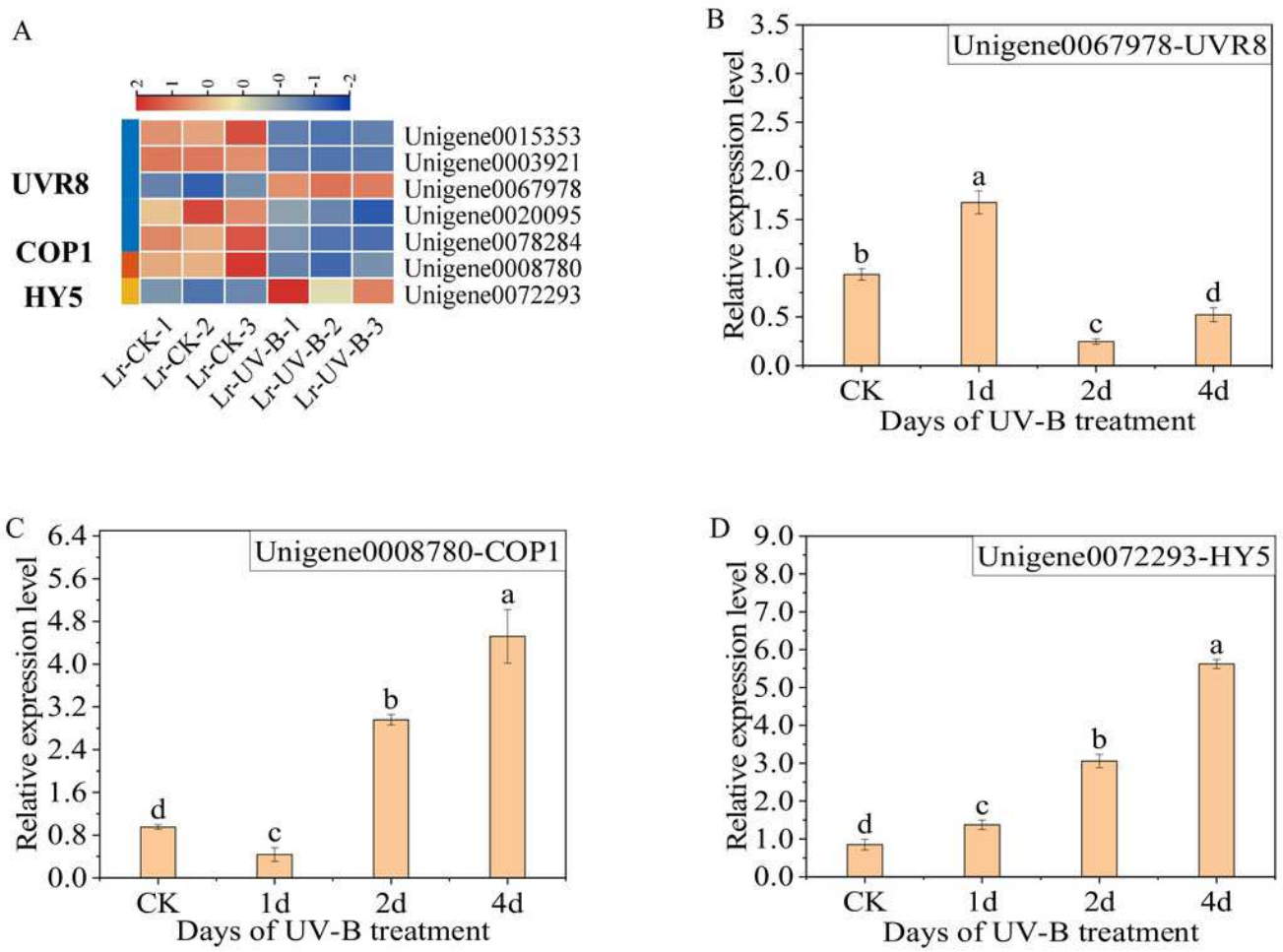


Figure 6

Expression heat map and RT-qPCR verification of the DEGs in the anthocyanin biosynthesis. (A) Simplified scheme. (B) Expression heat map. (C)(D) The RT-qPCR results of CHS and UFGT.

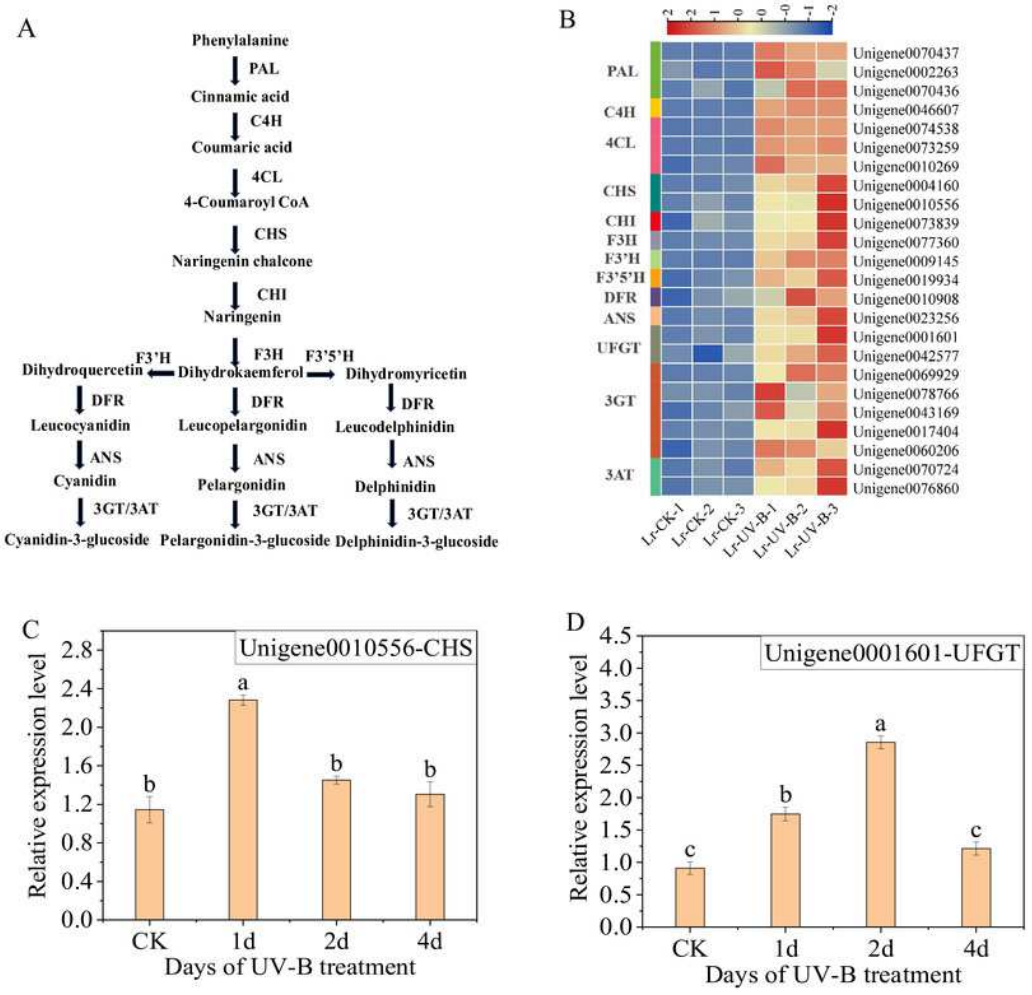


Figure 7

Classification and proportion of differential expression transcription factors and expression heat map.

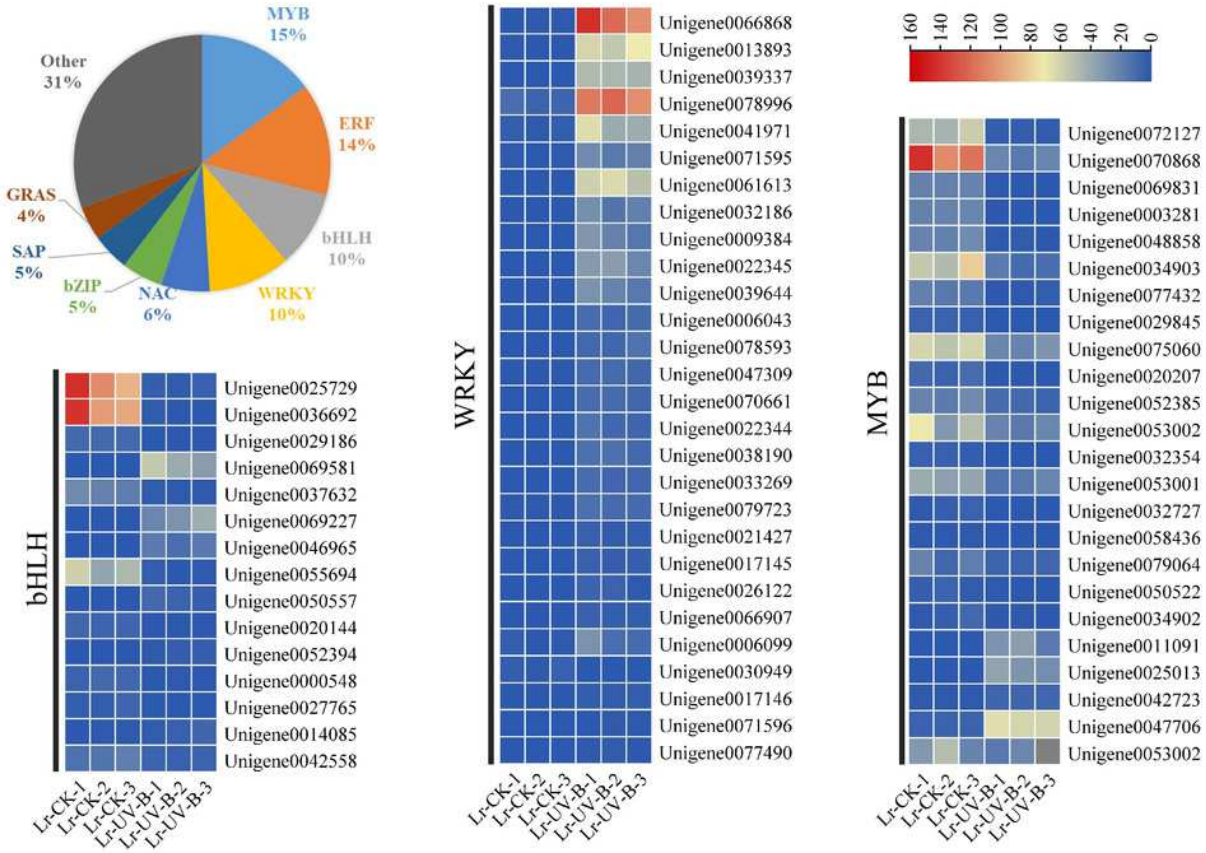
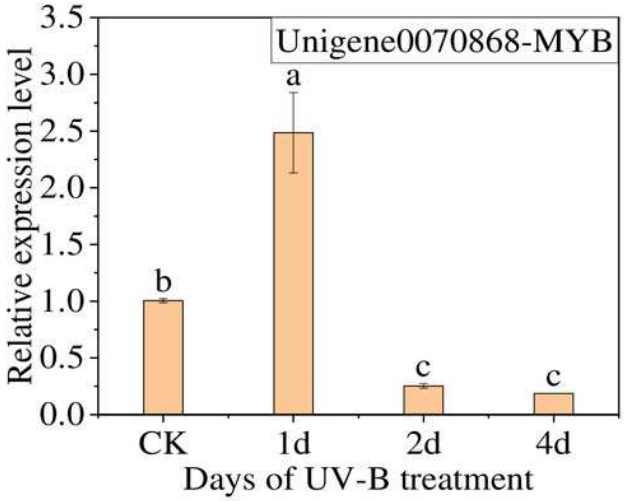
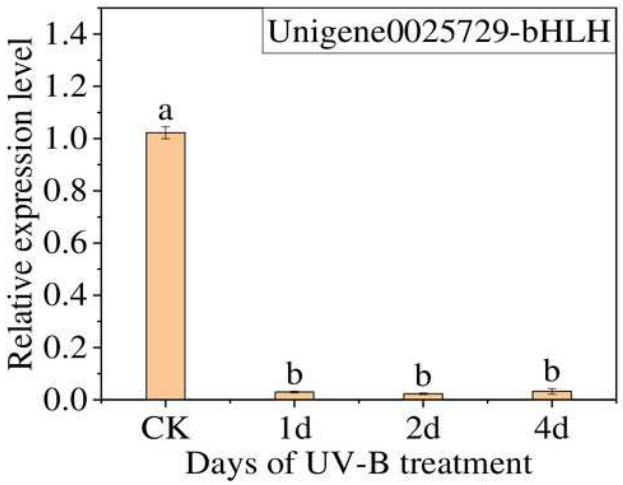
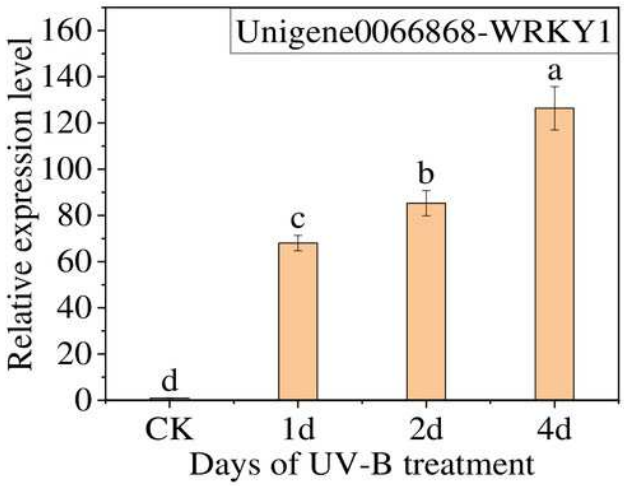


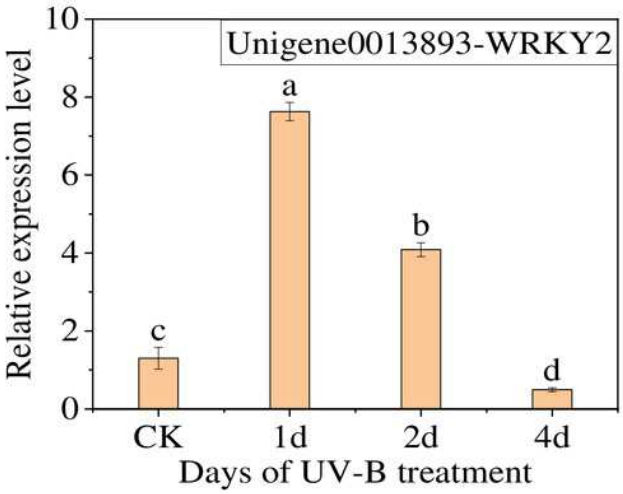
Figure 8

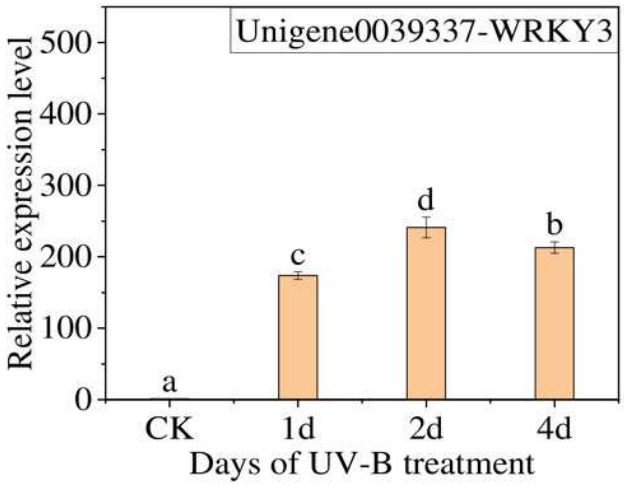
The results of RT-qPCR for the transcription factors.


Unigene0070868-MYB
Relative expression level
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
a
b
c
c
CK
1d
2d
4d
Days of UV-B treatment


Unigene0025729-bHLH
Relative expression level
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
a
b
b
b
CK
1d
2d
4d
Days of UV-B treatment


Unigene0066868-WRKY1
Relative expression level
160
140
120
100
80
60
40
20
0
d
c
b
a
CK
1d
2d
4d
Days of UV-B treatment


Unigene0013893-WRKY2
Relative expression level
10
8
6
4
2
0
c
a
b
d
CK
1d
2d
4d
Days of UV-B treatment


Unigene0039337-WRKY3
Relative expression level
500
400
300
200
100
0
a
c
d
b
CK
1d
2d
4d
Days of UV-B treatment

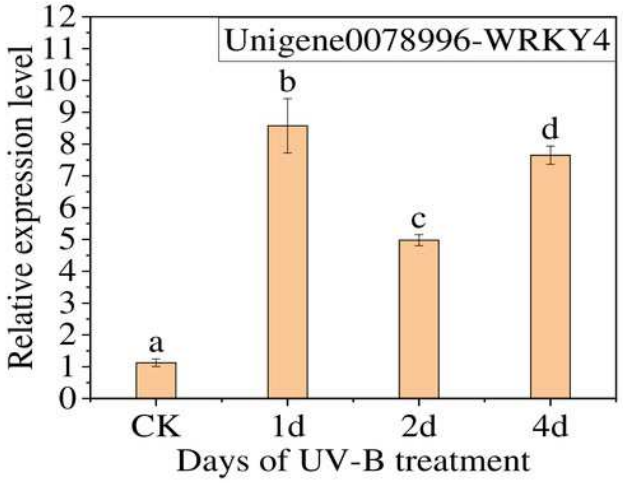

Unigene0078996-WRKY4
Relative expression level
12
11
10
9
8
7
6
5
4
3
2
1
0
a
b
c
d
CK
1d
2d
4d
Days of UV-B treatment

Figure 9

Protein-protein interaction networks and heat map of their expressions. (A) Interaction network between structural genes of anthocyanin synthesis and transcription factors. (B) Expression heat map of genes.