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A transcriptomic perspective on the effect of UV irradiation on vitamin C content in pea sprouts

Ying-hao XU[1], Xin FU[2], Ming LU[2], Bao-dong WEI[3]*

Abstract

Pea sprouts are derived from sprouted peas and are popular for their high vitamin C and flavonoid content, as well as their rapid growth and ease of production. Enhancing its nutritional value with simple treatments is worth exploring, In the present study, we applied supplemental UV irradiation to pea sprouts and examined various components such as vitamin C and flavonoid substances in the treated pea sprouts. Meanwhile, to investigate the mechanism of action of UV irradiation on pea sprouts, we performed high-throughput RNA sequencing (RNA-Seq) analysis to probe the genome-wide expression profile of pea sprouts under UV irradiation, generating 41.90 Gb of Clean Data, the results were also validated by reverse transcription-polymerase chain reaction (RT-qPCR), and the raw data of sequencing were submitted to the SRA repository. Further transcriptome analysis showed that UV irradiation significantly affected the expression of genes in metabolic pathways, especially those of some key enzymes that affect substance metabolism.

Keywords: UV irradiation; pea sprouts; RNA-seq; differentially expressed genes.

Practical Application: To explore ways to enhance the nutritional value of pea shoots with treatments and to investigate the mechanisms of plant response to UV irradiation.

1 Introduction

Pea (*Pisum sativum* L.) sprouts are popular because they are nutritious and easy to prepare. At the same time, UV radiation is a common and simple means of treatment. As the most common form of radiation in nature, UV irradiation can often have a complex effect on plants and has the potential to be a method of treating sprouts to improve their nutritional value.

UVB includes A, B, and C, with UV-B having the most biological effects (Feister et al., 2011). UV-B causes physiological and ecological changes in plants (Mewis et al., 2012), and in the face of UV irradiation, the content of UV-absorbing substances in plant leaves increases (Tevini et al., 1991), and the transcript levels of related protective enzymes increase in the short term (Rao et al., 1996), which can reduce the damage to tissues from UV irradiation (Cen & Bornman, 1993).

Plants appear to react similarly in the face of UV irradiation; in general, plants show a decrease in yield and a decrease in photosynthetic pigment content (Zhang et al., 2010), while the content of phenolics (Ambasht & Agrawal, 1998), flavonoids (Wen et al., 2015), and anthocyanins (Yan et al., 2014) increases, enzyme activity increases (Ye-Fei et al., 2008), and antioxidant capacity increases (Erkan et al., 2008), and such changes generally occur immediately after irradiation (Wang et al., 2009). It has been shown that in the face of UV stress, UV-absorbing compounds are the main target products of plants acting under the mechanism of resistance to adversity damage, mainly including secondary metabolites of various plant physiological processes, such as flavonoids and phenolics (Zhao et al., 2014).

When plants were exposed to UV light, the activity of flavonoid synthase was increased (Strid et al., 1994), and there was also an increase in vitamin content (Liu et al., 2019).

In this study, physiological experiments were conducted on pea sprouts irradiated with different intensities of supplemental UV light and transcriptome analysis was performed to determine the effect of UV light irradiation on the content and regulatory mechanisms of vitamin C and other substances in plants.

2 Materials and methods

2.1 Handling of materials

The variety of peas (*Pisum sativum* L.) used is the more widely distributed variety of vegetable peas in the world, white peas. Peas of moderate size, uniform color, no scar, fullness and no mold were selected and evenly divided into two groups, CK and UV groups, and germinated in BD-ZGX-400G-4P plant growth incubator (Nanjing Beidi Experimental Instrument Co., China) at a constant temperature of 25 °C after decontamination. After germination, they were subjected to UV irradiation for 0 and 10 min at a wavelength of 253.7 nm and a distance of 40 cm by SW-CJ-1FD type clean bench (Shanghai Hujing Medical Equipment Co., Ltd., China), respectively. Samples were snap

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[1]*Department of Agricultural Products Processing and Storage Engineering, Shenyang Agricultural University, Shenyang, P.R. China*
[2]*Institute of Food and Processing, Liaoning Academy of Agricultural Sciences, Shenyang, P.R. China*
[3]*Department of Food Science, Shenyang Agricultural University, Shenyang, P.R. China*
*Corresponding author: bdweisyau@163.com

frozen in liquid nitrogen immediately after sampling and stored in an ultra-low temperature refrigerator at -80 °C. The experiments were performed immediately after the samples were thawed.

2.2 Measurement of physiological indicators

The vitamin C content in pea sprouts was determined using an ascorbic acid assay kit (Suzhou Grace Biotechnology Co., Ltd., China); the flavonoid and total phenolic content was determined by referring to the method in Cao et al. (2007).

2.3 Sequencing and analysis of the transcriptome

Whole transcriptome sequencing was based on the Illumina Novaseq 6000 sequencing platform (Illumina, USA), and experiments were performed using the Illumina TruseqTM RNA sample prep Kit (Illumina, USA) method for library construction. Analysis and statistics of gene expression were performed using RSEM software; analysis of variance was performed using DESeq2 software with the difference criteria of p-adjust < 0.05 & |log2FC| >= 1.

2.4 Validation of differential genes by RT-qPCR

The purpose of the experiments was to assess the specific process by which differences in the genes of interest occur and to validate the correctness of the RNA-Seq data. RNA samples were reverse transcribed using ChamQ SYBR Color qPCR Master Mix (2X) reagent (Nanjing Novozymes Biotechnology Co., Ltd., China) and detected using an ABI 7500 fluorescent quantitative PCR instrument (Applied Biosystems, USA). EF-1a was chosen as the internal reference gene.

2.5 Statistical analysis

All experimental results were expressed using standard deviations. Also each experiment had three biological replicates and was analyzed for significance.

3 Results

3.1 Effect of UV irradiation on physiological indicators of pea sprouts

The changes of physiological indicators of pea sprouts after UV irradiation are shown in Figure 1.

3.2 Comprehensive analysis of the transcriptome

A total of 41.90 Gb of Clean Data was obtained from this sequencing, and each sample had more than 6.27 Gb of Clean Data, with the percentage of Q30 bases above 94.42%. The matches between the tested samples and the reference genome were all in the range of 93.6-94.29%, meeting the requirements of the analysis. To explore the effect of UV irradiation on pea sprouts as a whole, the genes that produced significant differential

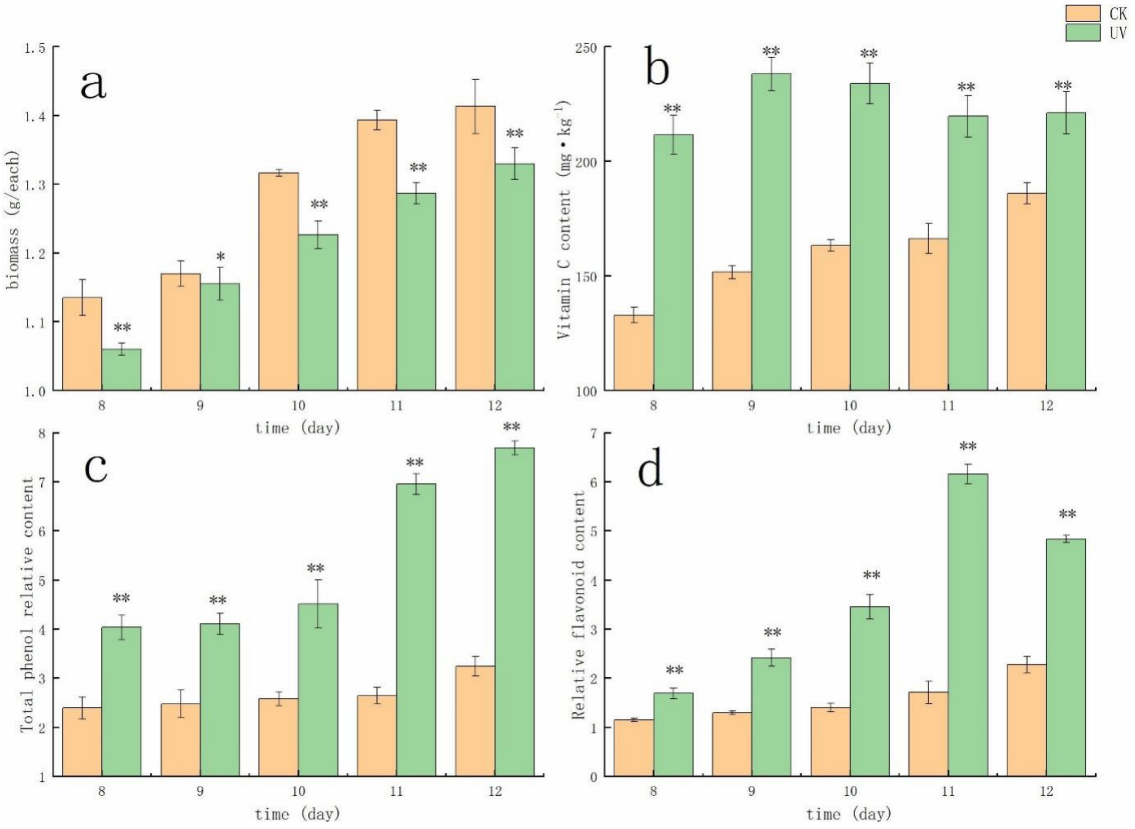

Figure 1. Changes in biomass (a), vitamin C content (b), total phenol relative content (c) and relative flavonoid content (d) of pea sprouts under UV irradiation.* indicates significant difference (P<0.05) and ** indicates highly significant difference (P<0.01).

expression in the UV group relative to the CK group were counted as shown in Figure 2, and a large number of genes were differentially expressed under UV irradiation.

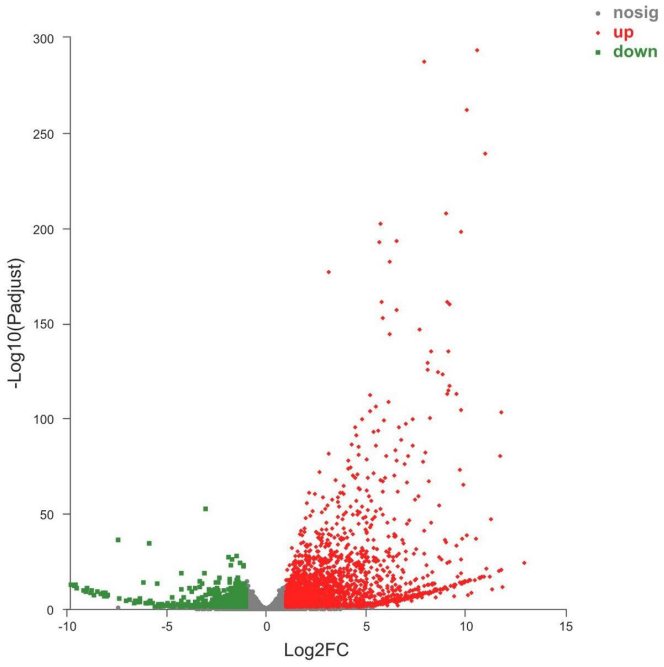

Figure 2. Differential genes in the group CK and UV10.

3.3 GO annotation analysis

The GO analysis database GO is a comprehensive database that categorizes and summarizes the results of all gene-related studies worldwide. The database standardizes the biological terminology of genes and gene products in different databases and provides a uniform qualification and description of gene and protein functions. The GO annotation situation is shown in Figure 3. Compared with the CK group, 15248 genes were annotated in the UV group and classified into three secondary categories of biological processes, cellular components and molecular functions, containing a total of 54 functional groups.

The top five GO terms in molecular function were binding(1946),catalytic activity(1854),transporter activity(245),transcription regulator activity(171),molecular transducer activity(99).the top five GO terms in biological processes were metabolic process(1665), cellular process(1611), biological regulation(541), response to stimulus(401) and localization(370). The top five GO terms in cellular component were cell part(1501), membrane part(1246), cell(855), organelle(649) and membrane(620).

3.4 KEGG enrichment analysis

To gain a clearer understanding of the function of differentially expressed genes in response to UV irradiation, enrichment analysis was performed using the KEGG database and the results are shown in Figure 4. A total of 1113 genes were enriched in

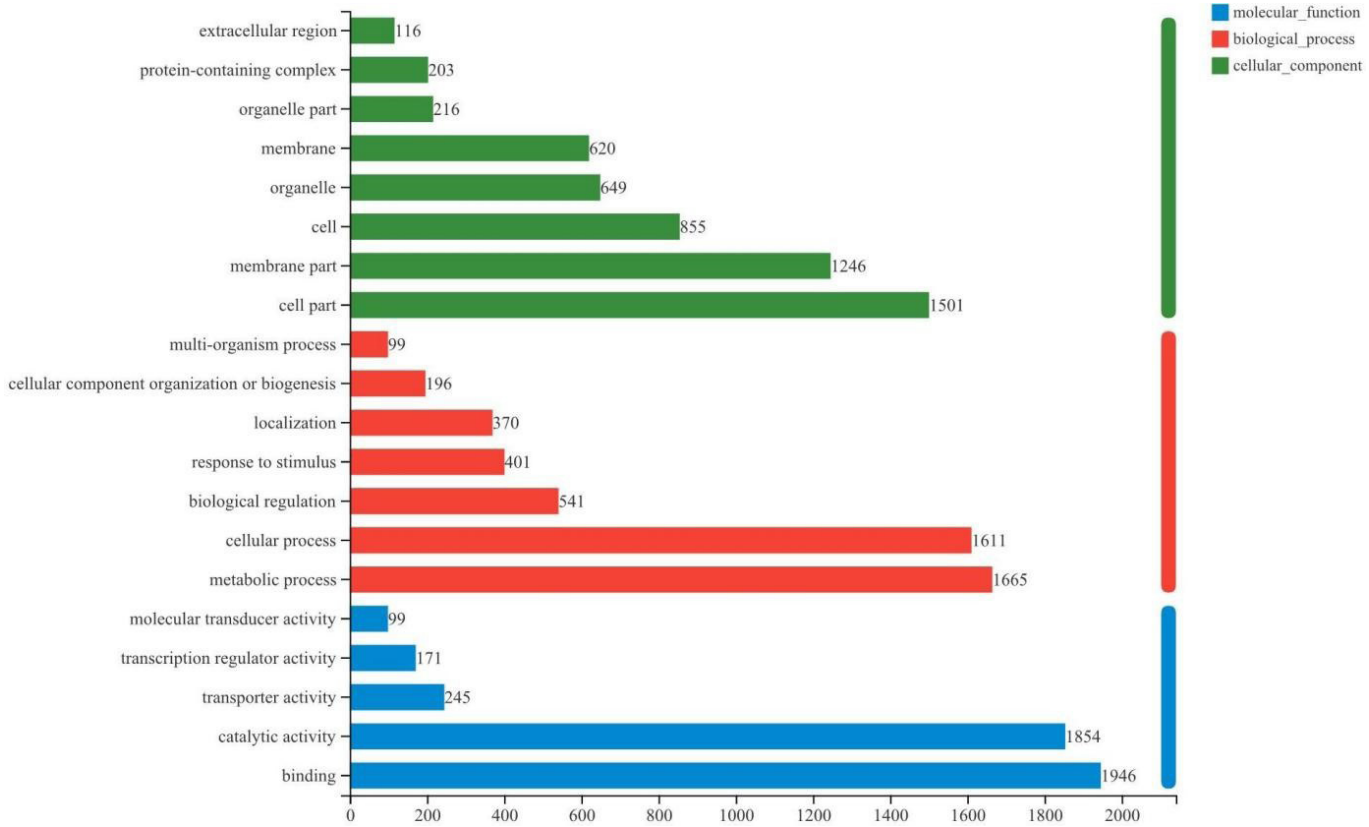

Figure 3. GO annotation analysis.

121 pathways in the UV group compared with the CK group. Among these pathways, the five most significantly enriched were phenylpropanoid biosynthesis, plant-pathogen interaction, MAPK signaling pathway - plant, glutathione metabolism and isoflavonoid biosynthesis. In addition, pathways associated with the metabolism of bioactive substances, such as flavonoid biosynthesis, biosynthesis of various secondary metabolites - part 2, plant hormone signal transduction, ascorbate and aldarate metabolism and betalain biosynthesis.

3.5 Analysis of differentially expressed genes

Differential genes involved in vitamin C metabolism

Vitamin C is a small molecule antioxidant in plants and plays an important role in the plant antioxidant system. The differential genes in the vitamin C metabolic pathway of pea sprouts after UV irradiation are shown in Table 1, and a total of 16 related genes were differentially expressed.

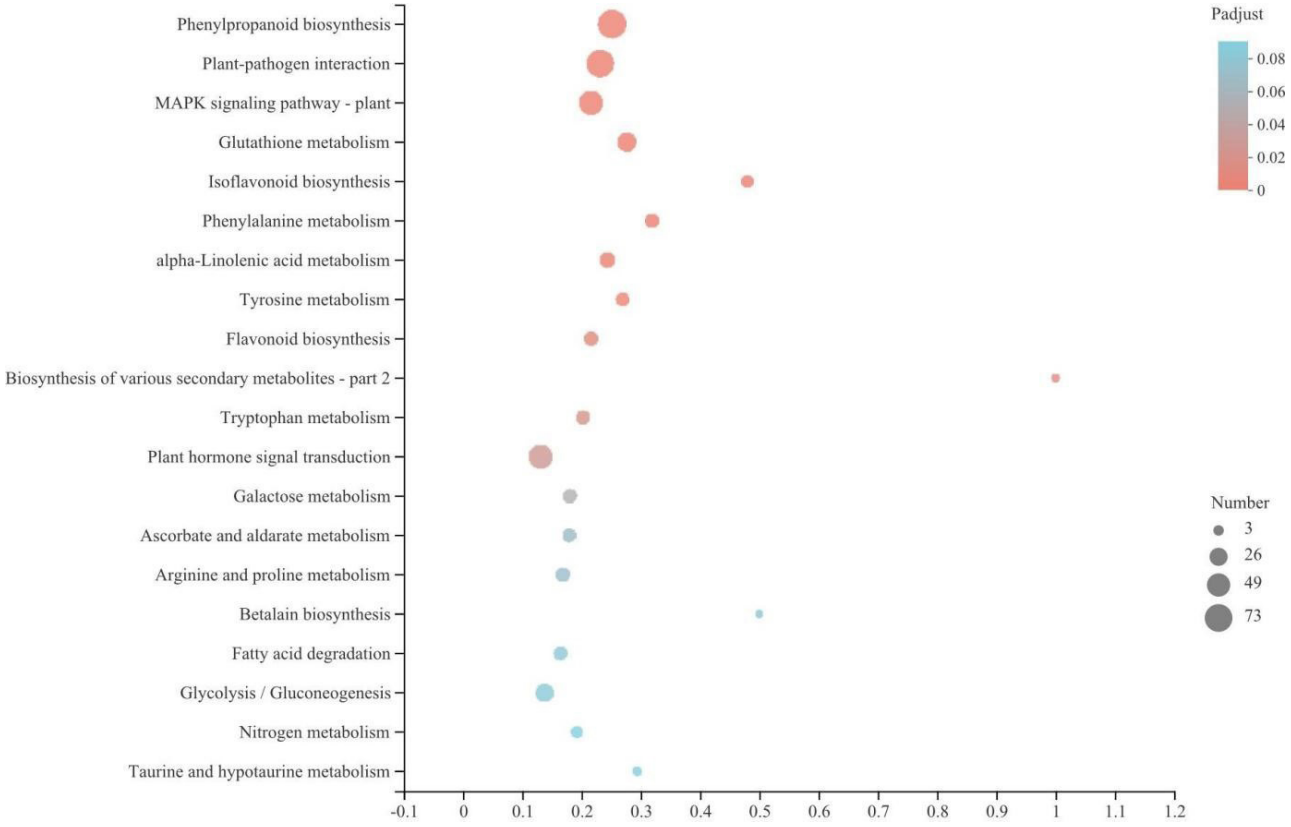

Figure 4. KEGG enrichment analysis.

Table 1. Differential genes involved in vitamin C metabolism.

Gene ID	log2FC	regulated	Gene Description
Psat0s1315g0480	-1.23495281	down	D-arabinono-1,4-lactone oxidase activity
Psat0s1315g0440	-1.339306525	down	L-gulonolactone oxidase activity
Psat2g069720	-1.07724861	down	L-ascorbate peroxidase activity
Psat0s1315g0400	-1.050108136	down	L-gulonolactone oxidase 3
Psat5g101760	-3.237187628	down	L-ascorbate oxidase activity
Psat5g191720	-2.49942334	down	UDP-glucose 6-dehydrogenase activity
Psat3g101720	2.667542861	up	glutathione dehydrogenase
Psat0ss3069g0160	2.938406902	up	aldehyde dehydrogenase family 3 member H1
Psat4g196000	1.663185033	up	Myo-inositol oxygenase 1
Psat0ss3069g0120	4.063037558	up	Aldehyde dehydrogenase family 3 member H1
Psat4g126400	3.349274957	up	Aldehyde dehydrogenase family 2 member B7
Psat7g019040	3.24246245	up	Myo-inositol oxygenase
Psat1g053480	1.07442004	up	glucuronokinase 1
Psat5g086480	3.170161696	up	Aldehyde dehydrogenase family 3 member H1
Psat0s744g0080	7.877840601	up	monodehydroascorbate reductase
Psat7g122160	1.575156504	up	monodehydroascorbate reductase

Differential genes involved in flavonoid synthesis

The differential genes involved in flavonoid synthesis in pea sprouts after UV irradiation are shown in Table 2, and a total of 16 related genes were differentially expressed.

3.6 RT-qPCR to verify the expression of key enzyme genes

Genes of key enzymes were selected for RT-qPCR. These genes were genes for key enzymes of vitamin C metabolism in differential genes, Psat5g101760 encoding ascorbate peroxidase (AO), Psat0s744g0080 encoding monodehydroascorbate reductase (MDHAR), and Psat3g101720 encoding dehydroascorbate reductase (DHAR).The experiments examined the gene expression changes in the CK and UV groups after receiving UV irradiation for 5h, 25h, 72h and 120h, and the results are shown in Figure 5.

4 Discussion

Pea sprouts are an easily available sprouting food rich in nutrients and widely distributed around the world. In this experiment, we treated pea sprouts with UV irradiation and

Table 2. Differential genes involved in flavonoid metabolism.

Gene ID	log2FC	regulated	Gene Description
Psat7g077640	9.750173918	up	O-methyltransferase
Psat5g069280	1.077122106	up	Chalcone-flavanone isomerase
Psat3g159880	4.954094429	up	Cytochrome P450
Psat2g003880	7.324688599	up	Aldo-keto reductase signature
Psat2g003920	8.137509965	up	Aldo-keto reductase signature
Psat4g099800	5.727782611	up	2OG-Fe(II) oxygenase superfamily
Psat7g095800	2.29782756	up	O-methyltransferase
Psat6g237840	1.453135146	up	Chalcone-flavanone isomerase
Psat7g080040	4.471285515	up	Transferase family
Psat5g201640	5.064425348	up	Cytochrome P450
Psat1g049080	8.051011792	up	O-methyltransferase
Psat0s2452g0040	1.72549776	up	O-methyltransferase
Psat4g191760	9.622135739	up	NAD dependent epimerase/dehydratase family
Psat0s2452g0080	1.799415371	up	O-methyltransferase
Psat3g088280	8.303965347	up	Transferase family
Psat6g238360	5.248072022	up	Chalcone-flavanone isomerase

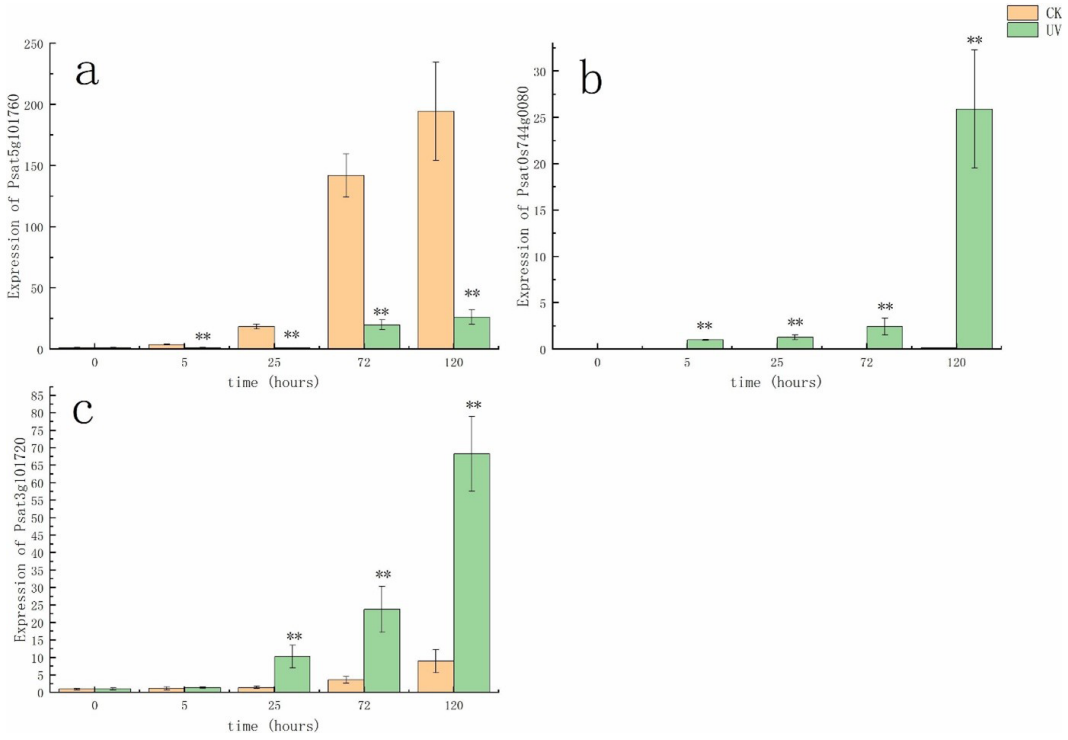

Figure 5. Expression of the gene Psat5g101760 (a), which regulates AO, Psat0s744g0080 (b), which regulates MDHAR, and Psat3g101720 (c), which regulates DHAR, with irradiation time. * indicates significant difference (P<0.05) and ** indicates highly significant difference (P<0.01).

investigated the effects of UV irradiation on relevant physiological indicators of pea sprouts, mainly vitamin C and flavonoids, and explored the changes in expression of relevant genes by transcriptome sequencing.

Vitamin C is a small-molecule antioxidant in plants that plays an important role in the plant antioxidant system (Du & Jin, 2000), while flavonoids are secondary metabolites in plant leaves that are actively and universally involved in various plant life activities and play a crucial role in protecting plants from UV damage (Harborne & Williams, 2000). In our study, we found a significant differential expression of genes for key enzymes of vitamin C catabolism. The expression of genes encoding APX and AO, key enzymes that contribute to vitamin C catabolism, was significantly reduced. The expression of the gene encoding DHAR was significantly increased, while it could revert the already oxidized monodehydroascorbic acid (MDHA) and dehydroascorbic acid (DHA).And to explore the specific process of gene generation changes, we chose different time points after UV irradiation treatment and did RT-qPCR on key genes, and the results validated our RNA-Seq results. We found that the overall expression of the relevant genes increased with the growth of pea sprouts, but a gap appeared between the two groups after UV irradiation treatment. The difference in expression of the relevant genes between the treated and control groups was not significant after only 5 hours of UV irradiation, but with increasing time, but already after 25 hours of treatment, extremely significant differences started to appear. Among them, the expression of the gene Psat5g101760, which regulates AO, was 7.4-fold higher in the treatment group than in the control group; the expression of the gene Psat0s744g0080, which regulates MDHAR, was 26-fold higher, while it was almost zero in the control group; and the expression of the gene Psat3g101720, which regulates DHAR, was 7.6-fold higher in the treatment group than in the control group. In addition, the metabolic mechanism of flavonoid-related substances is still unclear, but it was still found that the expression of a large number of genes in the synthetic pathway of flavonoids underwent a significant increase under UV irradiation.

5 Conclusion

In this study, UV irradiation was found to reduce the biomass of pea sprouts and significantly increase the content of vitamin C and flavonoids in pea sprouts. The differential genes in the vitamin C metabolic pathway in UV-irradiated pea sprouts were concentrated in the catabolic pathway, with a significant decrease in the expression of genes promoting vitamin C catabolism and a significant increase in the expression of genes aiding reduction; also a significant increase in the expression of genes related to flavonoid synthesis was observed.

Availability of data and material

The datasets generated during the current study are available in the SRA repository, PRJNA792203 (https://www.ncbi.nlm.nih.gov/sra/PRJNA792203).

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