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Transcriptomic analyses of *Chrysanthemum morifolium Ramat* under UV-B radiation treatment reveal variations in the metabolisms associated with bioactive components

Yanjun Yang[a,b], Zhifang Jiang[a,b], Jiena Guo[a,b], Xiuli Yang[a,b], Ning Xu[c], Zhehao Chen[d], Juan Hao[a,b], Jiaxun Li[d], Jiliang Pang[d], Chenjia Shen[a,b,*], Maojun Xu[a,b,*]

[a] Zhejiang Provincial Key Laboratory for Genetic Improvement and Quality Control of Medicinal Plants, Hangzhou Normal University, Hangzhou, China
[b] Key Laboratory of Hangzhou City for Quality and Safety of Agricultural Products, College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou, China
[c] Institute of Microbiology, Chinese Academy of Sciences, Beijing, China
[d] College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou, China

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ABSTRACT

Ultraviolet-B is an effective elicitor that enhances the production of bioactive ingredient in various plants. However, the mechanism underlying ultraviolet-B responses in the genus *Chrysanthemum* is largely unknown. *Chrysanthemum morifolium Ramat* is used as herbal medicine, beverage material, and seasoning in many Asian regions. A massive amount of bioactive ingredients, such as caffeoylquinic acids and flavonoids, are contained in *C. morifolium* flowers and leaves, leading to an extensive number of applications for their extracts. In this study, complementary DNA libraries from *Chrysanthemum morifolium Ramat* leaves under ultraviolet-B radiation at various time points (0 h, 3 h, 6 h and 12 h) were separately sequenced. A large number of differential expressed genes were screened during the ultraviolet-B radiation process. The expression of most glycosylsis-, glycosyltransferase-related genes, flavonoid, fatty acid and caffeoylquinic acid biosynthesis related genes were largely induced by ultraviolet-B radiation. Furthermore, the contents of flavonoids, caffeoylquinic acids and fatty acids were significantly induced by ultraviolet-B radiation, suggesting a close relationship between the contents of flavonoids, caffeoylquinic acids and fatty acids and the resistance of plant to ultraviolet-B-induced stress. Our data provides a comprehensive resource for identifying genes associated with ultraviolet-B-induced variations in the bioactive ingredients of *C. morifolium*.

1. Introduction

Ultraviolet-B (UV-B) radiation, an important component of solar radiation, is absorbed by the stratospheric ozone layer and little of it reaches the surface of the Earth (McKenzie et al., 2007). Nonetheless, UV-B exposure can cause deleterious changes including DNA damage, membrane composition alteration and reactive oxygen species accumulation, and leads to a series of negative effects on the growth and development of plants (Gill et al., 2015). To deal with this threat, plants have adapted and developed the ability to respond to changes environmental conditions and UV-B exposure (Dotto and Casati, 2017). Conversely, low-dose UV-B is also involved in a variety of processes, including pathogen defense, regulation of growth, signal transduction stimulation, and increasing secondary metabolites accumulation, in different plant species (Brosché and Strid, 2003; Escobar-Bravo et al., 2017; Pan et al., 2014).

For years, UV-B has been applied as an effective elicitor to enhance the biosynthesis of various secondary metabolites in plants (Pandey and Pandey-Rai, 2014). Various active ingredients, such as catharanthine in *Catharanthus roseus*, glycyrrhizin in *Glycyrrhiza uralensis*, and taxol in *Taxus mairei*, have been over accumulated under UV-B treatments (Afreen et al., 2005; Ramani and Chelliah, 2007; Zu et al., 2010). Considerable research has been done to understand the molecular and physiological responses of plants to UV-B exposure (Hectors et al., 2007; Pontin et al., 2010). However, the mechanism underlying UV-B responses in the genus *Chrysanthemum* is currently largely unknown.

Chrysanthemum morifolium Ramat (*C. morifolium*) is a traditional medicinal plants in China. Research shows that *C. morifolium* possesses various biological activities, including antibacterial, antioxidant, anti-inflammatory, antitumoral, cardiovascular protection, and

* Corresponding authors at: Hangzhou Normal University, College of Life and Environmental Sciences, 16 Xuelin Street, Hangzhou, 310036, China.
E-mail addresses: shencj@hznu.edu.cn (C. Shen), xumaojunhz@163.com (M. Xu).

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hepatoprotective (Duh, 1999; Gao et al., 2016; Jiang et al., 2005; Akihisa et al., 1996; Ukiya et al., 2002). The flower head of *C. morifolium* has also been used as herbal tea, beverage material, and seasoning due to its unique flavor and health benefits. To meet the demands of an increasing consumer market, *C. morifolium* has been widely cultivated in East Asia, and studies have been done regarding molecular breeding (Shibata, 2008; Wang et al., 2014). A massive amount of bioactive ingredients, such as caffeoylquinic acids and flavonoids, are contained in *C. morifolium* flowers and leaves, leading to extensive applications of their extracts (Beninger et al., 2004; Beninger and Hall, 2005; Chen et al., 2015). Therefore, it is important to evaluate variations in the metabolisms associated with the bioactive ingredients under UV-B radiation in *C. morifolium*. An improved understanding of underlying processes will facilitate effective manipulation to improve application of the bioactive substances in the plants.

The development of high-throughput sequencing now allows for a global comparison of gene expression changes in non-model species under various environmental stresses (Ozsolak and Milos, 2011). Chrysanthemum is an allohexaploid (2n = 6× = 54) with a complex genome, and thus, the whole genome of *C. morifolium* is currently unavailable (Sasaki et al., 2017; Shibata, 2008). Recently, RNA sequencing has been used to uncover various biological aspects of *C. morifolium*. In 2014, the first transcriptome of *C. morifolium* was published using an aphid-attacked leaf (Xia et al., 2014). High-throughput sequencing has also identified differentially expressed genes of floral organs, several transcription factor families encoding genes, floral transition differentially expressed genes, and petal elongation related genes were identified by high-throughput sequencing (Liu et al., 2015, 2016; Ren et al., 2016; Sasaki et al., 2017; Song et al., 2016; Wang et al., 2017a). Comparative transcriptome analysis reveals large variations in gene selection patterns between cultivated and wild *Chrysanthemum* species (Won et al., 2017). A deep transcriptome analysis assumed that a low chlorophyll (Chl) biosynthesis rate and a high Chl degradation rate resulted in the absence of Chls in white chrysanthemum petals (Ohmiya et al., 2017). Currently, however, there is no information on transcriptomic responses to UV-B radiation in *C. morifolium*.

To understand the complexity of the responses to UV-B in *C. morifolium* and move forward on the elucidation of bioactive ingredients and their biosynthesis, transcriptomic analyses were performed to investigate the changes in gene expressions under UV-B radiation. Differences in the expression of the candidate genes involved in various metabolic pathways may give us an opportunity to explain the roles of UV-B in the biosynthesis of bioactive ingredients.

2. Materials and methods

2.1. Plant materials and UV-B treatments

The chrysanthemum (*Chrysanthemum morifolium Ramat*) cultivar '*Xiaoyangju*' was planted in a growth chamber of Hangzhou Normal University, Hangzhou, China, at a temperature of 25 ± 2 °C with a light/dark cycle of 12/12 h and 60%–70% relative humidity. The cultivated seedlings were randomly grouped into four groups with 20 pots each. One group of two month-old seedlings was grown in normal condition and treated as the control, and the three other groups were exposed to UV-B radiation for 3 h, 6 h and 12 h, respectively. UV-B was artificially produced by a UV-B fluorescent lamp (Q-Lab narrowband UV-B tubes). To filter out the UV-C (lower than 280 nm) radiation, the UV-B lamps were wrapped with 3-mm transmission cutoff filters (Schott, Mainz, Germany). UV-B irradiance at the leaves of the seedlings was controlled at 10 μmol m^{-2} s^{-1}. UV-B irradiance was determined by an ultraviolet intensity meter (Apogee Instruments). Fresh leaves samples were collected after the treatments, put in liquid N_2 and kept at −80 °C for further study.

2.2. RNA isolation and cDNA library preparation

Total RNAs were isolated from three independent samples for each group using a TRIzol reagent (Invitrogen, Shanghai, China) according to its protocol. The quantity and quality of RNAs were determined using a NanoDrop ND-430 1000 spectrophotometer (Agilent, Santa Clara, CA, USA). RNA samples with a 260/280 ratio within 1.8–2.1, a 260/230 ratio within 2.0–2.5 and an integrity number > 7.0 were used for library construction. A mount of 10 μg RNA from each sample (4 × 3 biological replicates) was subjected to oligo-dT binged magnetic beads (Thermo Fisher Scientific, Shanghai, China) and broken into short fragments by divalent cations at a relatively high temperature. Fragmentary RNAs were used as template for first strand cDNA synthesis by an cDNA preparation kit (Illumina, San Diego, CA, USA). Second strand cDNA synthesis was performed by a reaction with DNA polymerase I (Takara, Dalian, China). After purification with a QiaQuick PCR purification kit (Qiagen, Valencia, CA, USA), the resulting DNA fragments were ligated to sequencing adapters. Paired-end sequencing was performed on an Illumina Hiseq2500 platform according to the protocol, and a large number of small paired/single-end reads were generated.

2.3. Quality control, de novo assembly and gene annotation

Several parameters, including GC-content, Q20 and Q30 values, and duplication levels, were calculated using in-house Perl scripts (provided by LC-Bio, Hangzhou, China) to evaluate the quality of the Raw reads. Low quality reads were discarded to generate clean reads. After removing the low quality reads, a *de novo* strategy was used to assemble the clean reads into distinct contigs using Trinity assembly software (Grabherr et al., 2011). All of the contigs were clustered, and the contigs with the longest sequences were defined as unigenes. For functional annotation, the unigenes, through a BLASTall algorithm-based program with a threshold of *E*-value < 10^{-10}, were searched against different databases, including Nr (https://www.ncbi.nlm.nih.gov/refseq/about/nonredundantproteins/), Swiss-Port (https://www.ebi.ac.uk/uniprot), KEGG (https://www.genome.jp/kegg/), and KOG (https://genome.jgi.doe.gov/help/kogbrowser.jsf/), and the directions of unigenes were determined based on perfect alignment results. GO classification were performed using a Blast2GO software (Conesa et al., 2005).

2.4. Identification of differentially expressed genes (DEGs)

The *C. morifolium* transcriptome from all samples was applied as a reference sequence for DEGs screening. All transcripts were mapped onto the assembled reference transcriptome using Bowtie ver. 0.12.7 alignment software. The expression level of each unigene was normalized by the reads per kilobase per million mapped reads (RPKM) method (Mortazavi et al., 2008). The false discovery rate (FDR) method was applied to correct the threshold of the *P* values in multiple tests for identifying the differences between two groups. A FDR < 0.001 and an absolute value of log2 (ratio) > 2 were applied as thresholds to identify significant differences between two groups (Mortazavi et al., 2008).

2.5. Functional enrichment analysis

Within the DEGs, a two-tailed Fisher's exact test was used to reveal GO and KEGG functional enrichments. A standard FDR control method (FDR < 0.001) was applied to correct the multiple hypothesis test. GO and KEGG terms with a corrected *P* value lower than 0.05 were considered significant. K-means clustering was analyzed using MeV software. To analyze GO-base and KEGG-base enrichments, all the sequences in the databases were set as the backgrounds.