Transcriptomic and proteomic analyses of **leaves** from *Clematis terniflora* DC. under high level **of** ultraviolet-B irradiation followed by dark treatment

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Running title: *Clematis* Transcriptomics under Binary Stress

Abbreviations: ACL, ATP-citrate lyase; AH, aconitate hydratase; CCD, carotenoid cleavage dioxygenase; CCR, cinnamoyl-CoA reductase; CHS, chalcone synthase; COMT, caffeic acid 3-*O*-methyltransferase; CS, citrate synthase; CMS, chorismate synthase; C4H, *trans*-cinnamate 4-monooxygenase; DAPs, differentially abundant proteins; DEGs, differentially expressed genes; DLAT, dihydrolipoyllysine-residue

26 acetyltransferase; DLD, dihydrolipoyl dehydrogenase; DLST,
 27 dihydrolipoyllysine-residue succinyltransferase; FH, fumarate hydratase; F3'5'H,
 28 flavonoid 3',5'-hydroxylase; GPS, geranylgeranyl pyrophosphate synthase; HBP,
 29 heme-binding protein 2; HMDS, 4-hydroxy-3-methylbut-2-en-1-yl diphosphate
 30 synthase; HPPD, 4-hydroxyphenylpyruvate dioxygenase; HPT, homogentisate
 31 phytyltransferase; HUV-B+D, high level of UV-B irradiation followed by dark
 32 treatment; IDI, isopentenyl-diphosphate delta-isomerase; IRH, isoflavone reductase
 33 homolog; LC, liquid chromatography; MDH, NADP-malate dehydrogenase; ME,
 34 NADP-dependent malic enzyme; MECS, 2-*C*-methyl-*D*-erythritol
 35 2,4-cyclodiphosphate synthase; MGL, methionine gamma-lyase; MPMEC,
 36 magnesium-protoporphyrin IX monomethyl ester cyclase; MS, mass spectrometry;
 37 OGDH, 2-oxoglutarate dehydrogenase; PAL, phenylalanine ammonia-lyase; PDH,
 38 pyruvate dehydrogenase; PS, phytoene synthase; RPKM, reads per kilobase million;
 39 SCL, succinyl-CoA ligase; SDS, solanesyl-diphosphate synthase; SP, starting point;
 40 UV-B, ultraviolet-B; SSDH, succinate-semialdehyde dehydrogenase; SUCL,
 41 succinyl-CoA ligase; 4CL, 4-coumarate: CoA ligase.

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Abstract

High level of UV-B irradiation followed by dark treatment (HUV-B+D) causes accumulation of secondary metabolites in *Clematis terniflora* DC. To investigate the response mechanism under HUV-B+D, transcriptomic and proteomic analyses were performed in leaves of *C. terniflora*. The number of genes related to tetrapyrrole synthesis, amino acid metabolism, tricarboxylic acid cycle, and mitochondrial electron transport chains was hierarchically changed in leaves of *C. terniflora* under HUV-B+D. Data from RNA-sequencing transcriptomics and gel-free/label-free proteomics were integrated. The genes related to biosynthesis of lignins and flavonoids/isoflavonoids were significantly upregulated. Luteolin 7-O- β -D-glucosiduronic acid, rutin, and kaempferol 3-O-rutinoside were accumulated. The number of differentially expressed genes (DEGs) and differentially abundant proteins (DAPs) related to protein metabolism were largely changed in posttranslational modification, ubiquitin proteasome, and ribosomal protein. The expression of NADP-dependent malic enzyme and the abundance of NADP-malate dehydrogenase were upregulated and increased, respectively. The activities of these two enzymes were also enhanced. These results suggest that the secondary metabolism pathway and tricarboxylic acid cycle might be activated in leaves of *C. terniflora* in response to HUV-B+D.

Keywords: transcriptomics, proteomics, *Clematis terniflora* DC., ultraviolet-B irradiation, dark treatment

1. Introduction

Ultraviolet-B (UV-B) regulates plant growth by antagonizing auxin signaling/shade avoidance [1] and promotes the synthesis of UV-B protective secondary metabolites [2]. Hierarchical doses of UV-B irradiation defined as high level (HUV-B; more than $15 \text{ kJ m}^{-2} \text{ d}^{-1}$), moderate level ($1\text{-}3 \text{ kJ m}^{-2} \text{ d}^{-1}$), and low level (less than $1 \text{ kJ m}^{-2} \text{ d}^{-1}$) have different effects on plants [3, 4]. Low level of UV-B irradiation affects the acclimation of plant to drought or cadmium in wheat [5]. In *Arabidopsis* Col-0 cell suspension cultures, the crosstalk between low level of UV-B irradiation and bacterial elicitor involved antagonistic regulation of opposing transcription factors through metabolites [6]. HUV-B increased the essential oil compositions and total phenolics content in *Acorus calamus* [7]. HUV-B followed by dark treatment (HUV-B+D) causes the accumulation of Diels-Alder adducts in *Morus alba* L. [8] and alkaloids in *Catharanthus roseus* [9]. The molecular mechanisms in the plant under HUV-B+D are not clarified.

Transcriptomic analysis indicated that genes related to stress and cell cycle/protein degradation were upregulated and downregulated, respectively, in leaves of *Vitis vinifera* under HUV-B [10]. Transcriptomic study indicated that genes related to antioxidant and defense were downregulated in *Lycium ruthenicum* under HUV-B [11]. On the other hand, proteomic analysis indicated that proteins related to amino acid metabolism, energy metabolism, and cofactor biosynthesis were increased in three *Anabaena* species under HUV-B [12]. Proteomic study indicated that proteins related to synthesis of secondary metabolites such as caffeoylquinic acids and iridoids were increased in leaves of *Lonicera japonica* Thunb. under HUV-B [13]. Because of loose correlation between mRNA and protein, the integration of transcriptomics and proteomics is useful for functional analysis of plant under stress.

Many kinds of secondary metabolites have significant pharmaceutical activities such as anti-tumor [14] and anti-inflammatory [15]. *Clematis terniflora* DC. is one of *Clematis* species which has important pharmaceutical value such as antinociceptive, antipyretic [16], and anti-inflammatory [17-19]. *C. terniflora* widely spreads in Asia, Europe, and Africa [20] and serves as the folk medicine for the treatment of urinary inflammation [21]. Many kinds of metabolites with pharmaceutical effects such as alkaloids [22], saponins [23], and flavonoids [24] have been isolated from *C. terniflora*. Furthermore, recent studies have proved that the extracts from leaves and stem of *C. terniflora* have anti-tumor [21], anti-inflammatory, and antinociceptive [25, 26] effects. However, the biosynthetic mechanism of secondary metabolites in this plant is not clarified. HUV-B+D has a prominent effect on the biosynthesis of secondary metabolites in plant [8, 9]. In this study, *C. terniflora* was treated with $104.4 \text{ kJ m}^{-2} \text{ d}^{-1}$ (7 times than $15 \text{ kJ m}^{-2} \text{ d}^{-1}$) of HUV-B+D, because this intensity of UV-B resulted in a furthest stress response in the leaves of *C. terniflora* (A lethal dose of HUV-B+D was proved to be $150 \text{ kJ m}^{-2} \text{ d}^{-1}$ (10 times than $15 \text{ kJ m}^{-2} \text{ d}^{-1}$)) (see Figure 1 in Ref [27]). Transcriptomic and proteomic technologies were used to reveal the response mechanisms in leaves of *C. terniflora* under the treatment. Bioinformatic, phytochemical, and enzymatic techniques were used for confirmation of results from proteomics and transcriptomics.

2. Materials and Methods

2.1. Plant material and treatments

Seeds of *Clematis terniflora* DC. were incubated in water and the germinated seeds were sown into seedbeds. The seedlings were transplanted into potted containers and placed in greenhouse, which was controlled at 28-30°C, 70-80% relative humidity,

and $160 \mu\text{mol m}^{-2} \text{sec}^{-1}$ of white light irradiance. After 6 weeks, plants were treated with $104.4 \text{ kJ m}^{-2} \text{d}^{-1}$ of UV-B irradiation in a cabinet, which was controlled at 25-30°C and 80% relative humidity. Before being irradiated, plants were considered to be at starting point (SP). Intensity of UV-B irradiation was measured by a UV Light Meter (Beijing Normal University, Beijing, China). After HUV-B for 5 h, plants were treated with dark (HUV-B+D) for 48 h. Leaves in the area from the basal 10 to 60 cm in each plant at SP, HUV-B for 5h, and HUV-B+D for 48h were collected for transcriptomic, proteomic, phytochemical, and enzymatic analyses (see Figure 2 in Ref [27]). Five leaves were collected for one replication and 3 independent biological replicates were performed. Independent biological replicates mean that plants were treated with different date.

2.2. Protein extraction, enrichment, and digestion for proteomics

A portion (50 mg) of each sample was ground to powder in liquid nitrogen using a mortar and pestle. The powder was added to an acetone solution containing 10% trichloroacetic acid and 0.07% 2-mercaptoethanol [28]. The resulting mixture was vortexed and sonicated for 10 min. The suspension was incubated for 1 h at -20°C with vortexing every 15 min and centrifuged at $9,000 \times g$ for 20 min at 4°C. The pellet was washed twice with 0.07% 2-mercaptoethanol in acetone, dried using a Speed-Vac concentrator (Savant Instruments, Hickville, NY, USA), and resuspended in lysis buffer consisting of 7 M urea, 2 M thiourea, 5% CHAPS, and 2 mM tributylphosphine by vortexing for 1 h at 25°C. The resulting suspension was centrifuged at $20,000 \times g$ for 20 min at 25°C, and the supernatant was collected as crude extract. Protein concentrations were determined using the Bradford assay [29] with bovine serum albumin as the standard.

Proteins (100 μg) were cleaned up with methanol and chloroform to remove any

detergent from the sample solutions [30]. Briefly, 400 μ L of methanol was added and mixed before the further addition of 100 μ L of chloroform and 300 μ L of water. After mixing, the samples were centrifuged at 20,000 x g for 10 min to achieve phase separation. The upper aqueous phase was discarded and 300 μ L of methanol was added slowly to lower phase. The samples were centrifuged at 20,000 x g for 10 min and the pellets were dried. The dried pellets were resuspended in 50 mM NH_4HCO_3 , reduced with 50 mM dithiothreitol for 30 min at 56°C, and alkylated with 50 mM iodoacetamide for 30 min at 37°C in the dark. Alkylated proteins were digested with trypsin and lysyl endopeptidase (Wako, Osaka, Japan) at 1:100 enzyme/protein concentrations at 37°C for 16 h. The resulting tryptic peptides were acidified by formic acid (pH less than 3), and centrifuged at 20,000 x g for 10 min. The obtained supernatant was collected for the following analysis.

2.3. Nanoliquid chromatography-tandem mass spectrometry analysis

Peptides in 0.1% formic acid were loaded onto an Ultimate 3,000 nanoLC system (Dionex, Germering, Germany) equipped with a C18 PepMap trap column (300 μ m ID x 5 mm; Dionex). Peptides were eluted from the trap column and separated using 0.1% formic acid in acetonitrile at a flow rate of 200 nL/min on a C18 Tip column (75 μ m ID x 120 mm; Nikkyo Technos, Tokyo, Japan) with a spray voltage of 1.8 kV. Peptide ions were analyzed on a nanospray LTQ Orbitrap MS (Thermo Fisher Scientific, San Jose, CA, USA) operated in data-dependent acquisition mode with the installed Xcalibur software (version 2.1; Thermo Fisher Scientific). Full-scan mass spectra were acquired in the LTQ Orbitrap MS over 400-1,500 m/z with a resolution of 30,000. A lock mass function was used to obtain high mass accuracy [31]. As the lock mass, the ions $\text{C}_{24}\text{H}_{39}\text{O}_4^+$ (m/z 391.28429), $\text{C}_{14}\text{H}_{46}\text{NO}_7\text{Si}_7^+$ (m/z 536.16536), and

$C_{16}H_{52}NO_8Si_8^+$ (m/z 610.18416) were used. Values for ion isolation window were set as follows: activation type was collision-induced dissociation, default charge state was 2, isolation width was 2.0 m/z , normalized collision energy was 35%, and activation time was 30 ms. Values for dynamic exclusion were determined as follows: repeat count was 2, repeat duration was 30 sec, exclusion list size was 500, exclusion duration was 90 sec, and exclusion mass width was ± 1.5 Da. The ten most intense precursor ions above a threshold value of 500 were selected for collision-induced fragmentation. The acquired MS spectra were used for protein identification.

2.4. Protein identification from the mass spectrometry data

Proteins were identified using the Mascot search engine (version 2.5.1; Matrix Science, London, UK) with UniProtKB/Swiss-Prot database (Release 2015_11, 37,036 sequences, <http://www.uniprot.org/uniprot/>). DTA files were generated from acquired raw data files and then converted to Mascot generic files using Proteome Discoverer software (version 1.4.0.288; Thermo Fisher Scientific). The parameters used in the Mascot searches were as follows: carbamidomethylation of cysteine was set as a fixed modification and oxidation of methionine was set as a variable modification. Trypsin was specified as the proteolytic enzyme and one missed cleavage was allowed. Peptide mass tolerance was set at 10 ppm, fragment mass tolerance was set at 0.8 Da, and peptide charges were set at +2, +3, and +4. An automatic decoy database search was performed as part of the search. Mascot results were filtered with the Mascot percolator to improve the accuracy and sensitivity of peptide identification [32]. False discovery rates for peptide identification of all searches were less than 1.0%. Peptides with a percolator ion score of more than 13 and a p -value less than 0.05 were used for protein identification.

2.5. Analysis of differentially abundant proteins using acquired mass spectrometry data

The Mascot search results were exported in msf format for SIEVE analysis (version 2.1.377; Thermo Fisher Scientific). The relative abundances of peptides and proteins were compared between samples. For the analysis, the chromatographic peaks detected by MS were aligned and the peptide peaks were detected as a frame using the following settings: frame time width was 5 min, frame m/z width was 10 ppm, and produced frames on all parent ions were scanned by MS/MS. Chromatographic peak areas of each sample within a single frame were compared and the ratios between samples in each frame were determined. The frames detected in the MS/MS scan were matched to the imported Mascot search results. The ratio of peptides between samples was determined from the relative variance-weighted average of the ratios in frames, which matched the peptides in the MS/MS spectrum. The ratios of peptides were further integrated to determine the ratio of the corresponding protein. In the differential analysis of protein abundance, total ion current was used for normalization. The minimum requirement for the identification of a protein was a minimum of 2 matched peptides and a p -value less than 0.05.

2.6. RNA extraction for transcriptomics

A portion (100 mg) of each sample was ground to powder in liquid nitrogen using a mortar and pestle. Total RNA was isolated using RNeasy Plant Mini kit (Qiagen, Hilden, Germany). The quantity and quality were determined using Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). The poly (A) mRNA was isolated from total RNA samples with Magnetic Oligo (dT) Beads (Illumina, San

Diego, CA, USA) and used for mRNA-Sequencing library construction. cDNA was synthesized using the fragmented mRNA and end-repaired followed by a single 'A' base addition. mRNA-Sequencing Sample Preparation Kit (Illumina) was used to prepare the DNA fragments for ligation to the adapters. After purification, the cDNA fragments (200 ± 25 bp) were excised and retrieved. Polymerase chain reaction (PCR) was performed to selectively enrich and amplify the cDNA fragments. Libraries were prepared from a 150–200 bp size-selected fraction following adapter ligation and agarose gel separation.

2.7. Transcriptomic sequencing, data processing, and transcriptomic analysis

The quality control analysis of the sample libraries was performed. After validation with a Mastercycler ep realplex Real-time PCR System (Eppendorf, Hamburg, Germany), the individual libraries in the same group were mixed using the same amount of each library and resulting in 3 library pools. The mRNA-sequencing libraries were sequenced using a paired end read protocol with 100 base pairs of data collected per run on the Hiseq 2000 sequencing platform (Illumina). *De novo* assembly of the transcripts was carried out using the short read assembly program 'Trinity' [33, 34]. Unigene sequences were aligned by BLASTX to UniProtKB/Swiss-Prot database using an E-value cut-off of 10^{-5} . Coding sequence regions were determined for the highest-ranked proteins using BLAST. Unigenes that could not be aligned to database were scanned by ESTScan [35] to determine the nucleotide (5'–3') and amino acid sequences of the coding regions. The value of reads per kilobase million (RPKM) was used to represent the expression level of genes. For gene identification analysis, the alternatively spliced genes were filed according to the criterion which is more than 100 by the RPKM value of transcripts [36]. For gene expression analysis, all the genes with

RPKM value were used. A significant change of gene expression between samples is identified as 10-fold change of RPKM value.

2.8. Functional annotation

Protein and gene functions were categorized using MapMan bin codes (<http://mapman.gabipd.org/>) [37]. The predication of identified proteins and genes derived from *C. terniflora* was performed by transferring annotations to the *Arabidopsis* genome and consideration of orthologous genes. Pathway mapping of identified proteins and genes was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (<http://www.genome.jp/kegg/>) [38].

2.9. QRT-PCR analysis

Leaves of *C. terniflora* were collected at SP, HUV-B for 2 h/5 h, and HUV-B+D for 12 h/24 h/48 h. A portion (100 mg) of each sample was ground to powder in liquid nitrogen using a mortar and pestle. Total RNA was isolated using the method described above. RNA was reverse-transcribed using a cDNA synthesis kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. The qRT-PCR was performed using an EvaGreen qPCR MasterMix (Applied Biological Materials, Richmond, BC, Canada) on a MyiQ2 two-color real-time PCR detection system (Bio-Rad, Hercules, CA, USA). The reaction conditions were as follows: 95°C for 600 sec, followed by 35 cycles of 95°C for 15 sec and 60°C for 60 sec. Gene expression was normalized using *GAPDH* as an internal control. Three biological replicates were used for each gene in each treatment and each biological replicate was technically duplicated to reduce the error rate. The primers (see Table 1 in Ref [27]) were designed using the Primer Premier 5.0 software (Premier Biosoft International, Silicon Valley, CA, USA). The

relative quantification method ($2^{-\Delta\Delta C_t}$) was used to evaluate quantitative variation between different treatments [39].

2.10. Secondary metabolites analyses

Secondary metabolites were analyzed according to the method of Zhu et al. [40] with minor modifications. A portion (300 mg) of each freeze-dried leaf sample was added to 3 mL methanol and the resulting mixture was sonicated for 1 h at 4°C. The suspension was centrifuged at 10,000 x g for 10 min at 4°C and the obtained supernatant was filtered through a syringe-driven filter (0.45 µm). The filtrate was dried in a vacuum concentrator, re-dissolved in 100 µL methanol, and used as metabolite extract. Three biological replicates were performed and each biological replicate was technically duplicated to reduce the error rate.

The metabolite samples were analyzed on a Triple-TOF 5600 System (AB Sciex, Framingham, MA, USA) fitted with a DuoSpray ion source (AB Sciex), which was connected to an LC system. The separation of all samples was performed on a C18 column (2.1 mm ID x 100 mm, Waters, Dublin, Ireland) with a column temperature at 40°C. The flow rate was 1 mL/min, and the mobile phase consisted of 0.1% formic acid aqueous and acetonitrile: 0-10 min (8% acetonitrile), 11-20 min (13.6% acetonitrile), 21-30 min (15.2% acetonitrile), 31-60 min (19.2% acetonitrile), 61-80 min (47.2% acetonitrile), and 81-90 min (95% acetonitrile). The sample injection volume was 2 µL. The source voltage and temperature were set to 4,500 V and 550°C for negative ionization mode. The de-clustering potential was 100 V and the collision energy was 35±10 eV. The curtain gas flow was set to 30 arbitrary units and the ion source gas pressure was 50 psi. The mass range was 50-1,000 m/z. The accumulation time was set at 0.15 sec for MS analysis and 0.08 sec for MS/MS analysis. Acquisition

of the MS/MS spectra was controlled by the information-dependent acquisition (IDA) function of Analyst TF software (version 1.5.1; AB Sciex). Mass accuracy was maintained by use of an automated calibrant delivery system (AB Sciex) interfaced to the second inlet of the DuoSpray source. The calibrant delivery system was set to be an external calibration for each sample. Total sum area module was used for total ion chromatogram normalization and the normalized intensities were integrated for each sample. The data processing was performed using PeakView software (version 1.1, AB Sciex) and MS/MS libraries (Chemspider, <http://www.chemspider.com>; Metlin, <http://metlin.scripps.edu/xcms/>; HMDB, <http://www.hmdb.ca/>).

2.11. Enzyme activity analyses

Enzyme extraction: The activity of NADP-dependent malic enzyme (ME) and NADP-malate dehydrogenase (MDH) was measured according to the method of Tsuchida et al. [41] with minor modifications. A portion (20 mg) of freeze-dried sample was homogenized on ice using a mortar and pestle with 1 mL of extraction buffer containing 90 mM HEPES-KOH (pH 8.0), 1 mM EDTA, 5 mM dithiothreitol, and 10% glycerol. The homogenate was centrifuged at 15,000 x g for 15 min at 4°C.

For ME assay: Twenty-five µL of crude enzyme was mixed with 975 µL of reaction buffer containing 90 mM HEPES-KOH (pH 8.0), 1 mM EDTA, 2 mM MgCl₂, 0.5 mM NADP⁺, and 5 mM malate. The reaction was started by adding the malate-NaOH (pH 7.0).

For MDH assay: Fifty µL of crude enzyme was mixed with 950 µL of reaction buffer containing 90 mM HEPES-KOH (pH 8.0), 1 mM EDTA, 0.2 mM NADPH, 70 mM KCl, 1 mM dithiothreitol, and 2 mM oxaloacetate. The reaction was started by adding the oxaloacetate.

Activity assay: The reactions were monitored by absorbance at 340 nm. One unit of enzyme activity is defined as the amount of enzyme resulting in the reduction of 1 nmol NADPH per min ($\text{nmol min}^{-1} \text{mg protein}^{-1}$).

2.12. Malondialdehyde content analysis

The malondialdehyde content was measured according to the method of Qin et al. [42] with minor modifications. A portion (30 mg) of each fresh leaf sample was homogenized on ice using a mortar and pestle with 5 mL of 10% trichloroacetic acid solution. The homogenate was centrifuged at 5,000 x g for 10 min at 4°C. Two mL of the supernatant was mixed with 2 mL of 0.6% thiobarbituric acid solution (thiobarbituric acid was dissolved in 10% trichloroacetic acid solution). After heating at 100°C for 15 min in a water bath, the reacted mixture was quickly cooled in an ice bath and centrifuged at 5,000 x g for 10 min at 4°C. The absorbance was measured at 450 nm, 532 nm, and 600 nm using a spectrophotometer. Three independent biological replicates were performed. The malondialdehyde content was calculated using the following equation: malondialdehyde content ($\mu\text{mol g}^{-1}$) = $(6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}) \times 5 \times W^{-1}$ (A_{450} , A_{532} , and A_{600} represent the absorbances of the reacted mixture; W represents the fresh weight of leaves).

2.13. Statistical analysis

The SPSS statistical software (version 22.0; IBM, Armonk, NY, USA) was used for statistical evaluation. Statistical significance was evaluated by the Student's *t*-test when only two groups were compared or one-way ANOVA followed by Tukey's test when multiple groups were compared. A *p*-value less than 0.05 was considered as the statistical significance.

3. Results

3.1. Effects of HUV-B+D on genes in leaves of *C. terniflora*

To investigate the effects of HUV-B+D in leaves of *C. terniflora*, transcriptomic technique was used. A total of 467, 546, and 463 genes were identified in leaves at SP (see Table 2 in Ref [27]), HUV-B (see Table 3 in Ref [27]), and HUV-B+D (see Table 4 in Ref [27]), respectively. Among these genes, 112, 89, and 130 genes were specifically expressed at SP, HUV-B, and HUV-B+D, respectively (Figure 1). Based on the functional analysis, the number of genes related to protein degradation, amino acid metabolism, tricarboxylic acid cycle, mitochondrial electron transport chains, miscellaneous, and others (a) was hierarchically increased in response to HUV-B+D. However, the number of genes related to protein synthesis, photosynthesis, redox, cell, tetrapyrrole synthesis, and others (b) was hierarchically decreased in response to HUV-B+D. The number of genes included in others and miscellaneous was not hierarchically changed under the treatment. Furthermore, the number of genes related to photosynthesis, tetrapyrrole synthesis, stress, amino acid metabolism, tricarboxylic acid cycle, and mitochondrial electron transport chains was changed by 2 times in response to HUV-B+D compared with SP (Figure 1). The number of genes related to stress and secondary metabolism was increased by more than 2 times in response to HUV-B and HUV-B+D compared with SP (Figure 1). Genes related to tetrapyrrole synthesis, amino acid metabolism, and mitochondrial electron transport chains were listed (Table 1).

In terms of secondary metabolism, the genes were mapped onto pathway of secondary metabolism using KEGG database. *Solanesyl-diphosphate synthase (SDS)* and *phytoene synthase (PS)/carotenoid cleavage dioxygenase (CCD)*, which are

mainly related to biosynthesis of plastoquinol-9 and carotenoid, respectively, were specifically identified at SP compared with HUV-B and HUV-B+D (Figure 2). *2-C-Methyl-D-erythritol 2,4-cyclodiphosphate synthase (MECS)*, *4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase (HMDS)*, *isopentenyl-diphosphate delta-isomerase (IDI)*, *geranylgeranyl pyrophosphate synthase (GPS)*, *homogentisate phytyltransferase (HPT)*, and *4-hydroxyphenylpyruvate dioxygenase (HPPD)*, which are mainly related to biosynthesis of vitamin E, were specifically identified at HUV-B and HUV-B+D compared with SP (Figure 2). *Phenylalanine ammonia-lyase (PAL)*, *trans-cinnamate 4-monooxygenase (C4H)*, and *flavonoid 3',5'-hydroxylase (F3'5'H)*, which are related to biosynthesis of flavonoids, as well as *caffeic acid 3-O-methyltransferase (COMT)*, which is mainly related to biosynthesis of lignins, were specifically identified at HUV-B and HUV-B+D compared with SP (Figure 2). *4-Coumarate: CoA ligase (4CL)*, *chalcone synthase (CHS)*, and *isoflavone reductase homolog (IRH)*, which are mainly related to biosynthesis of flavonoids/isoflavonoids, as well as *cinnamoyl-CoA reductase (CCR)*, which is mainly related to biosynthesis of lignins, were specifically identified at HUV-B+D compared with SP and HUV-B (Figure 2).

3.2. Functional distribution of differentially expressed genes (DEGs) in leaves of *C. terniflora* under HUV-B+D

To determine the mechanism of effects of HUV-B+D in leaves of *C. terniflora*, the expression level of each gene was compared. Genes at HUV-B and HUV-B+D were compared with those at SP. DEGs were selected according to the criterion, which is more than 10-fold change in RPKM values. A total of 685 (see Table 5 in Ref [27]) and 3360 (see Table 6 in Ref [27]) genes were differentially expressed in response to

HUV-B and HUV-B+D, respectively. A total of 198 and 2873 genes were unique in response to HUV-B and HUV-B+D, respectively (Figure 3). Based on the functional analysis, the DEGs in response to HUV-B were mainly related to RNA, protein metabolism, signaling, stress, and transport. The DEGs in response to HUV-B+D were mainly related to protein metabolism, RNA, signaling, transport, and cell (Figure 3). Furthermore, the percentage of DEGs related to protein metabolism, cell, and tricarboxylic acid cycle was increased in response to HUV-B+D compared with HUV-B (Figure 3).

3.3. Functional distribution of differentially abundant proteins (DAPs) in leaves of *C. terniflora* under HUV-B+D

To determine the mechanism of effects of HUV-B+D in leaves of *C. terniflora*, a gel-free/label-free proteomic technique was used. Proteins at HUV-B and HUV-B+D were compared with those at SP. SIEVE software was used for comparing relative abundance of peptides and proteins between samples. A total of 210 (see Table 7 in Ref [27]) and 293 (see Table 8 in Ref [27]) proteins were differentially changed in response to HUV-B and HUV-B+D, respectively. A total of 105 and 188 proteins were unique in response to HUV-B and HUV-B+D, respectively (Figure 4).

Based on the functional analysis, the DAPs in response to HUV-B were mainly related to photosynthesis, protein metabolism, cell, and stress. The DAPs in response to HUV-B+D were mainly related to protein metabolism, photosynthesis, amino acid metabolism, and tricarboxylic acid cycle, and others (a) (Figure 4). Furthermore, the percentage of DAPs related to amino acid metabolism, glycolysis, and tricarboxylic acid cycle, and others (a) was increased in response to HUV-B+D compared with HUV-B (Figure 4).

3.4. Effects of HUV-B+D on protein metabolism in leaves of *C. terniflora*

The DEGs and DAPs related to protein metabolism were leading in number (more than 10% of DEGs and 20% of DAPs, respectively) in leaves of *C. terniflora* under HUV-B and HUV-B+D (Figures 3 and 4). To further determine the effects of HUV-B+D on protein metabolism, DEGs and DAPs related to protein metabolism were subclassified using MapMan bin codes. DEGs related to ribosomal protein/ubiquitin-conjugating enzyme and DAPs related to ribosomal protein/ubiquitin proteasome were listed (Tables 2 and 3). Based on the functional analysis, proteins and genes categorized in postranslational modification were increased in quantity and upregulated, respectively, in response to HUV-B compared with SP, but the number of upregulated and downregulated genes was increased in response to HUV-B+D compared with HUV-B (Figure 5). Proteins and genes categorized in ubiquitin proteasome were increased/decreased in quantity and upregulated/downregulated, respectively, in response to HUV-B, and the protein were increased in quantity in response to HUV-B+D compared with SP; the number of proteins increased in quantity and upregulated/downregulated genes was increased in response to HUV-B+D compared with HUV-B (Figure 5). Proteins and genes categorized in ribosomal protein were increased/decreased in quantity and upregulated/downregulated in response to HUV-B compared with SP; the number of proteins increased in quantity and upregulated/downregulated genes was increased in response to HUV-B+D, but the number of proteins decreased in quantity was decreased in response HUV-B+D compared to HUV-B (Figure 5).

3.5. Effects of HUV-B+D on tricarboxylic acid cycle in leaves of *C. terniflora*

To investigate the effects of HUV-B+D on tricarboxylic acid cycle in leaves of *C. terniflora*, the DEGs and DAPs related to tricarboxylic acid cycle were mapped onto pathway of tricarboxylic acid cycle using KEGG database. The number of upregulated ME and increased MDH was hierarchically increased in response to HUV-B+D (Figure 6). The number of upregulated dihydrolipoyl dehydrogenase (DLD), MDH, aconitate hydratase (AH), succinyl-CoA ligase (SUCL), dihydrolipoyllysine-residue acetyltransferase (DLAT), fumarate hydratase (FH), succinate-semialdehyde dehydrogenase (SSDH), dihydrolipoyllysine-residue succinyltransferase (DLST), and 2-oxoglutarate dehydrogenase (OGDH) was increased in response to HUV-B+D (Figure 6). The numbers of upregulated genes and increased proteins of pyruvate dehydrogenase (PDH), succinate dehydrogenase, isocitrate dehydrogenase (NADP+), isocitrate dehydrogenase (NAD+), and citrate synthase were increased in response to HUV-B+D (Figure 6). The gene expression and protein quantity of ATP-citrate lyase (ACL) were upregulated and decreased, respectively, in response to HUV-B+D (Figure 6).

To determine the effects of HUV-B+D on the function of tricarboxylic acid cycle, the activities of ME and MDH were measured. The activity of ME was increased with 46% and 57% in response to HUV-B and HUV-B+D, respectively, compared with SP (Figure 7A). The activity of MDH did not change in response to HUV-B; however, it was increased with 11% in response to HUV-B+D compared with SP (Figure 7B).

3.6. QRT-PCR validation of genes identified in leaves of *C. terniflora* under HUV-B+D

The number of genes related to tetrapyrrole synthesis, amino acid metabolism, and tricarboxylic acid cycle was largely changed in response to HUV-B+D compared

with SP (Figure 1). The mRNA expression levels of *methionine gamma-lyase* (*MGL*) and *chorismate synthase* (*CMS*) related to amino acid metabolism, *succinyl-CoA ligase* (*SCL*) and *citrate synthase* (*CS*) related to tricarboxylic acid cycle, and *heme-binding protein 2* (*HBP*) and *magnesium-protoporphyrin IX monomethyl ester cyclase* (*MPMEC*) related to tetrapyrrole synthesis were analyzed by qRT-PCR (Figure 8). The qRT-PCR results showed that the mRNA expression levels of *MGL* and *CMS* were slightly upregulated under HUV-B+D. Furthermore, they were 150 and 9-fold upregulated, respectively, at HUV-B+D compared with SP (Figure 8A-B). The variation trends of mRNA expression levels of *SCL* and *CS* were the same under HUV-B+D. The mRNA expression levels of the two genes were significantly upregulated under HUV-B followed by dark treatment for 24 h and 48 h compared with SP (Figure 8C-D). The mRNA expression levels of *HBP* and *MPMEC* were commonly downregulated under HUV-B. Furthermore, the mRNA expression levels of the two genes were 16 and 100-fold downregulated under HUV-B+D compared with SP (Figure 8E-F).

3.7. Effects of HUV-B+D on flavonoids in leaves of *C. terniflora*

Transcriptomic analysis indicated that *PAL*, *C4H*, and *F3'5'H* were specifically identified at HUV-B/HUV-B+D compared with SP and *4CL*, *CHS*, and *IRH* were specially identified at HUV-B+D compared with SP/HUV-B (Figure 2), implying the activation of metabolic pathway of flavonoids in leaves of *C. terniflora* under HUV-B+D. To determine the effects of HUV-B+D on flavonoids, luteolin 7-O- β -D-glucosiduronic acid, rutin, and kaempferol 3-O-rutinoside were analyzed as the targeted metabolites. The intensity of luteolin 7-O- β -D-glucosiduronic acid was significantly increased under HUV-B+D compared with HUV-B (Figure 9A). The

intensities of rutin and kaempferol 3-O-rutinoside were 2 and 1-fold increased, respectively, under HUV-B+D compared with SP (Figure 9B-C).

4. Discussion

4.1. Genes related to tetrapyrrole synthesis, amino acid metabolism, and mitochondrial function are hierarchically changed in leaves of *C. terniflora* in response to HUV-B+D

Transcriptomic analysis indicated that the number of genes related to tetrapyrrole synthesis, amino acid metabolism, and mitochondrial electron transport chains was hierarchically changed. Tetrapyrroles play a vital role in primary metabolic processes of plant [43]. Glutamyl-tRNA-reductase regulates the synthesis of aminolevulinic acid [44], which is the universal precursor for all the tetrapyrroles [45] including chlorophyll. Magnesium-chelatase [46] and MPMEC [47] are involved in the biosynthesis of chlorophyll in the downstream of biosynthesis of tetrapyrroles. HUV-B caused an inhibition in photosynthesis in symbiotic dinoflagellates [48]. In this study, the inhibition of glutamyl-tRNA reductase, magnesium-chelatase, and MPMEC (Table 1) implies that the photosynthetic capacity is also inhibited through the decreased biosynthesis of chlorophyll in leaves of *C. terniflora* in response to HUV-B+D.

Shikimate kinase and CMS are involved in shikimate pathway and forms chorismate [49]. Chorismate is a precursor for biosynthesis of aromatic amino acids, salicylate [50], and phylloquinone [51]. Indole-3-glycerol phosphate lyase catalyzes indole-3-glycerol phosphate into free indole [52]. Tryptophan synthase beta chain 2 functioned in the biosynthesis of tryptophan from indole [53]. Indole-3-glycerol phosphate lyase was expressed by stress signals, such as herbivory, wounding, and jasmonates [52, 54]; especially, it was upregulated in response to HUV-B [55].

Aromatic amino acids serve as precursors for biosynthesis of a series of secondary metabolites with roles in stress-protection in plant [56]. In this study, the induction of shikimate kinase, CMS, indole-3-glycerol phosphate lyase, and tryptophan synthase beta chain 2 (Table 1) implies that the HUV-B protective mechanism might be induced by the formation of aromatic amino acids through the activation of their biosynthesis in leaves of *C. terniflora* in response to HUV-B+D.

Mitochondrial electron transport chains function in adjusting whole-cell redox homeostasis and influencing metabolic pathways in leaves of tobacco [57]. Nitric oxide induced alternative oxidase through the accumulation of citrate [58]. The alternative oxidase played a role in lowering the accumulation of mitochondrial reactive oxygen species in tobacco cells [59]. Furthermore, nitric oxide reduced oxygen by modifying the redox state of cytochrome *c* oxidase [60]. HUV-B systemically induced the nitric oxide in leaves of maize [61]. A loss of cytochrome *c* oxidase in mitochondria of maize under cold stress had a constraint on the electron transport chains, which enhanced the production of reactive oxygen species [62]. In this study, alternative oxidase and cytochrome *c* oxidase were induced by HUV-B+D (Table 1), implying that the defense response against HUV-B+D in leaves of *C. terniflora* under HUV-B+D might be activated through regulating the reactive oxygen species.

4.2. Pathway of secondary metabolism is enhanced in leaves of *C. terniflora* in response to HUV-B+D

Genes related to secondary metabolism had a significant increase in number under HUV-B and HUV-B+D (Figure 1). A *PAL* mutant of *Arabidopsis* was sensitive to HUV-B [63]. The tendency of expressions of *PAL* and *C4H* was positively

correlated to that of catechin content in tea [64]. Furthermore, the expression of *C4H* was also responsible for the accumulation of lignins [65]. The *4CL*, which was activated by UV-B irradiation [66], converted its substrates into corresponding CoA esters involved in biosynthesis of flavonoids and lignins. In this study, *PAL*, *C4H*, and *4CL* were induced in response to HUV-B+D (Figure 2), implying that the upstream of lignins and flavonoids biosynthesis is also activated in leaves of *C. terniflora* under HUV-B+D.

The *CCR* and *COMT* catalyzed the key steps in biosynthesis of lignins in *Lolium perenne* [67]. The increased lignins serve as protective response to cellular damage in root suspension cultures of *Panax ginseng* under copper stress [68]. Furthermore, lignins were impregnated in outer layer cell walls in leaves of *Kalanchoe pinnata* exposed to HUV-B [69]. The *CCR* and *COMT* were induced in response to HUV-B+D in the downstream of *PAL*, *C4H*, and *4CL* (Figure 2). These results with previous findings imply that a defense mechanism against HUV-B+D might be activated by the accumulation of lignins through the expression of relative genes in leaves of *C. terniflora*.

The *CHS* catalyzes the first step in biosynthesis of flavonoids [70]. The *F3'5'H* and *IRH* were involved in the biosynthesis of flavonoids [71] and isoflavones [72]. Increase of flavonoids and upregulation of *CHS* in *Arabidopsis* contributed a high tolerance to extremely HUV-B [73]. In this study, the induction of *CHS*, *F3'5'H*, and *IRH* indicates the activation of the metabolic pathway of flavonoids (Figure 2), which were proved by the accumulation of luteolin 7-O- β -D-glucosiduronic acid, rutin, and kaempferol 3-O-rutinoside (Figure 9). It was reported that kaempferol 3-O-rutinoside had the free radical scavenging activity [74] and rutin enhanced the defense system in buckwheat under HUV-B [75]. These results with previous findings imply that the

defense system against HUV-B+D might be enhanced through the activation of metabolic pathway of flavonoids.

4.3. Protein metabolism is enhanced in leaves of *C. terniflora* in response to HUV-B+D

Ubiquitin proteasome system is an essential process in degrading intracellular proteins [76]. The ubiquitin-conjugating enzyme was specifically involved in the proteolytic degradation of abnormal proteins in tomato under metal stress [77]. Additionally, the ubiquitin proteasome system played an important role in plant tolerance against abiotic stresses, such as cold [78] and drought [79]. The overexpression of *ubiquitin-conjugating enzyme* in *Arabidopsis* enhanced the stress tolerance against drought and salt stress [80]. In rice, UV-B irradiation highly induced the gene of ubiquitin-conjugating enzyme, which conferred the plant protection by binding the *PAL* promoter *cis*-element [81]. In this study, the upregulation and increase of gene and protein of ubiquitin-conjugating enzyme (Tables 2 and 3) imply that the UV-B-protective mechanism might occur to enhance the stress tolerance against HUV-B+D through the induction of *PAL* by ubiquitin-conjugating enzyme in leaves of *C. terniflora*.

During the stress period, the increased protein synthesis and protein turnover in salinity-tolerant rice occurred followed by the induction of stress-responsive and defense-related transcripts [82]. UV-B irradiation damaged the ribosome and resulted in a progressive decrease in the production of new protein [83]. On the other hand, HUV-B specifically induced the *de novo* synthesis of ribosomal proteins in maize [83]. Ribosomal protein S6 was phosphorylated and leaves damage were recovered [83]. Ribosomal proteins S2, S4, and L20 were essential for cell survival and ribosomal L33

increased the stress tolerance by sustaining sufficient plastid translation capacity in tobacco under cold stress [84]. A number of genes of ribosomal protein were upregulated in response to UV-B irradiation in maize [85]. In this study, the upregulation of genes of ribosomal protein S6, S2, S4, L20, and L33 (Table 2) and the increase of protein ribosomal protein S6 (Table 3) imply that the stress tolerance might occur through the synthesis of cell survival- and defense-related ribosomal proteins in leaves of *C. terniflora* in response to HUV-B+D.

4.4. Tricarboxylic acid cycle is activated in leaves of *C. terniflora* in response to HUV-B+D

Tricarboxylic acid cycle in plant mitochondrion plays a central action not only generating adenosine triphosphate [86], but also providing carbon skeletons for a range of metabolic processes [87]. ME functioned in enhancement of stress tolerance in plant [88]. The activity and transcriptional level of ME were increased in maize leaves under UV-B irradiation after dark period [89]. A further study proved that the ME was induced by reactive oxygen species under HUV-B [90]. The increase of ME activity implies that stress tolerance might be enhanced by the increased reactive oxygen species in leaves of *C. terniflora* under HUV-B+D.

The MDH is one of sensitive targets for flux regulation of tricarboxylic acid cycle [91]. It was accumulated in the mitochondrion of pea in response to oxidative stress [92]. Furthermore, Yoshida and Hisabori [93] reported that the MDH was not a redox-regulated enzyme and its activity was mainly inhibited by adenosine triphosphate. The overexpression of MDH in *Arabidopsis* lowered the leaf respiration, ameliorated the photorespiration, and increased the plant growth [94]. In this study, the increase of MDH activity implies that the stress tolerance against HUV-B+D might be

enhanced through the inhibition of respiration and the amelioration of photorespiration in leaves of *C. terniflora*.

MDH reduces oxaloacetate to malate by using NADH [94]. **ME** functions in the catalyzation from malate to pyruvate [95]. **FH** catalyzes the hydration of fumarate to malate [96]. The expression of **FH** was inhibited by light [97]. As acetyl-CoA is the precursor for biosynthesis of fatty acids, **PDH** and **DLD** are involved in mitochondrial **PDH** complex, which links the processes in glycolysis, tricarboxylic acid cycle, and biosynthesis of fatty acids, by catalyzing the oxidative decarboxylation of pyruvate to acetyl-CoA [98]. The activity of mitochondrial **PDH** is stimulated in the presence of malate [99]. In this study, the abundance of **MDH** and expression of **ME** were hierarchically increased and upregulated, respectively (Figure 6), implying that the function of tricarboxylic acid cycle might be hierarchically enhanced in leaves of *C. terniflora* under HUV-B+D. Furthermore, the **FH** and **PDH** were induced in the additional dark treatment (Figure 6). These results suggest that the metabolic flux from tricarboxylic acid cycle to fatty acid biosynthesis might be enhanced through the upregulated expression of relative genes in leaves of *C. terniflora* in response to HUV-B+D.

5. Conclusion

Transcriptomic and proteomic analyses were performed to reveal the response mechanism in leaves of *C. terniflora* under HUV-B+D. The main findings are as follows: (i) the number of genes related to tetrapyrrole synthesis, amino acid metabolism, tricarboxylic acid cycle, and mitochondrial electron transport chains was hierarchically changed; (ii) the genes related to biosynthesis of lignins and flavonoids/isoflavonoids were significantly upregulated; (iii) the number of DEGs and

DAPs related to protein metabolism were largely changed in postranslational modification, ubiquitin proteasome, and ribosomal protein; (iv) ME and MDH were activated in tricarboxylic acid cycle; luteolin 7-O- β -D-glucosiduronic acid, rutin, and kaempferol 3-O-rutinoside were accumulated in secondary metabolism. Taken together, these results suggest that the stress tolerance against HUV-B+D might be increased through the secondary metabolism pathway related to lignins and flavonoids/isoflavonoids in leaves of *C. terniflora*. Furthermore, the metabolic flux of tricarboxylic acid cycle might be enhanced through the activation of ME and MDH in leaves of *C. terniflora* in response to HUV-B+D.

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Figure legends

Figure 1. Functional distribution of identified genes in leaves of *C. terniflora* under high level of UV-B irradiation followed by dark treatment (HUV-B+D). Leaves of *C. terniflora* were collected at starting point (SP), HUV-B, and HUV-B+D. RNAs were extracted, sequenced, and analyzed. *De novo* assembly of the transcripts was carried out and the criterion is more than 100 by the RPKM values of transcripts. Gene functions were predicated and categorized using MapMan bin codes. Abbreviations are as follows: cell, cell organization/vesicle transport/division/cycle; TCA, tricarboxylic acid; ETC, electron transport chains; CHO, carbohydrate. ^aOthers include DNA, co-factor/vitamin metabolism, C1-metabolism, and biodegradation of xenobiotics. ^bOthers include folding, targeting, posttranslational modification, and assembly/co-factor ligation.

Figure 2. Pathways of secondary metabolism based on genes identified in leaves of *C. terniflora* under HUV-B+D. Pathways of secondary metabolism in leaves of *C. terniflora* at SP, HUV-B, and HUV-B+D were mapped using KEGG database. Red symbols shows identified genes. Abbreviations: C9'10'CD, carotenoid 9,10 (9',10')-cleavage dioxygenase; DXPS, 1-deoxy-D-xylulose-5-phosphate synthase; DXPR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; GDR, geranylgeranyl diphosphate reductase; HMDR, 4-hydroxy-3-methylbut-2-enyl diphosphate reductase; IEM, 37 kDa inner envelope membrane protein.

Figure 3. Functional distribution of differentially expressed genes (DEGs) in leaves of *C. terniflora* under HUV-B+D. Genes in leaves at HUV-B and HUV-B+D were compared with those at SP. DEGs were selected according to the criterion which is

more than 10-fold change in RPKM values. Gene functions were predicated and categorized using MapMan bin codes. Abbreviations are as follows: cell, cell organization/vesicle transport/division/cycle; ETC, electron transport chains; CHO, carbohydrate; OPP, oxidative pentose phosphate; TCA, tricarboxylic acid. ^aOthers include tetrapyrrole synthesis, metal handling, fermentation, and polyamine metabolism. ^bOthers include synthesis, amino acid activation, assembly/co-factor ligation, and glycosylation.

Figure 4. Functional distribution of differentially abundant proteins (DAPs) in leaves of *C. terniflora* under HUV-B+D. Leaves of *C. terniflora* were collected at SP, HUV-B, and HUV-B+D. Proteins were extracted, reduced, alkylated, digested, and analyzed by nanoLC-MS/MS. Proteins in leaves at HUV-B and HUV-B+D were compared with those at SP. SIEVE software was used for comparing relative abundance of peptides and proteins between samples. Protein functions were predicated and categorized using MapMan bin codes. Abbreviations are as follows: cell, cell organization/vesicle transport/division; TCA, tricarboxylic acid; CHO, carbohydrate; ETC, electron transport chains. ^aOthers include signaling, oxidative pentose phosphate, lipid metabolism, and C1-metabolism. ^bOthers include targeting, folding, amino acid activation, and posttranslational modification.

Figure 5. Functional distribution of DEGs and DAPs identified in leaves of *C. terniflora* under HUV-B (A) and HUV-B+D (B). Genes and proteins related to protein metabolism selected from Figures 3 and 4, respectively, were predicated and categorized using MapMan bin codes. Red and blue colors indicate increase and decrease of proteins in quantity, respectively. Orange and green colors indicate

upregulation and downregulation of genes, respectively.

Figure 6. Integrated pathway of tricarboxylic acid cycle based on DEGs and DAPs identified in leaves of *C. terniflora* under HUV-B (A) and HUV-B+D (B) as compared with those at SP. Genes and proteins were mapped on pathway of tricarboxylic acid cycle using KEGG database. Red square, blue square, and orange circle indicate increased protein, decreased protein, and upregulated gene, respectively. Abbreviations are as follows: IDH, isocitrate dehydrogenase; SDH, succinate dehydrogenase;

Figure 7. Enzyme activities of NADP-dependent malic enzyme (ME) and NADP-malate dehydrogenase (MDH) in leaves of *C. terniflora* under HUV-B+D. Leaves of *C. terniflora* were collected at SP, HUV-B, and HUV-B+D. Enzyme activities of ME and MDH were measured in extracts of leaves. All data are shown as the mean $\pm$ SD from three independent biological replicates. Asterisks were used to indicate significant changes measured by Student's *t*-test (* p <0.05; ** p <0.01; *** p <0.001).

Figure 8. Expression level of selected genes in leaves of *C. terniflora* under HUV-B+D. Leaves of *C. terniflora* were collected at SP, HUV-B for 2 h and 5 h, HUV-B+D for 12 h, 24 h, and 48 h. RNAs were extracted from leaves and the expression of genes was measured by qRT-PCR. Relative mRNA abundances of methionine gamma-lyase (MGL), chorismate synthase (CMS), succinyl-CoA ligase (SCL), citrate synthase (CS), heme-binding protein 2 (HBP), and magnesium-protoporphyrin IX monomethyl ester cyclase (MPMEC) were normalized against GAPDH abundance. The data are presented as the mean $\pm$ S.D. from three

independent biological replicates. Means with the same letter were not significantly different among the 5 examined time points based on one-way ANOVA ($p<0.05$).

Figure 9. Secondary metabolites in leaves of *C. terniflora* under HUV-B+D. Leaves of *C. terniflora* were collected at SP, HUV-B, and HUV-B+D. Secondary metabolites were extracted from leaves and peak intensity of metabolites was measured by LC Time-of-Flight MS/MS. Total sum area module was used for total ion chromatogram normalization and the normalized intensities were integrated for each sample. “(-)” means no intensity was detected. All data are shown as the mean $\pm$ SD from three independent biological replicates. Asterisks were used to indicate significant changes measured by Student’s *t*-test (* $p<0.05$; ** $p<0.01$).

Table 1

Table 1. List of genes related to tetrapyrrole synthesis, amino acid metabolism, and mitochondrial ETC in leaves of *Clematis terniflora* DC. exposed to HUV-B+D.

no.	gene ID	description	species	E-value	identify	RPKM			function
						SP	HUV-B	HUV-B+D	
2	P31593	Glutamate-1-semialdehyde 2,1-aminomutase	<i>Nicotiana tabacum</i>	3.00E-97	66.67	673.44	(-)	(-)	tetrapyrrole synthesis
3	Q9Y5Z4	Heme-binding protein 2	<i>Homo sapiens</i>	4.00E-24	35.56	495.29	121.33	(-)	tetrapyrrole synthesis
4	Q6SJV8	Magnesium-protoporphyrin IX monomethyl ester cyclase	<i>Gossypium hirsutum</i>	0	86.1	477.36	(-)	(-)	tetrapyrrole synthesis
5	P45621	Glutamate-1-semialdehyde 2,1-aminomutase	<i>Glycine max</i>	0	82.53	387.33	202.74	231.44	tetrapyrrole synthesis
6	Q41249	Protochlorophyllide reductase	<i>Cucumis sativus</i>	0	79.5	345.54	(-)	(-)	tetrapyrrole synthesis
7	O50314	Magnesium-chelatase subunit H	<i>C. parvum</i>	0	33.72	240.39	(-)	(-)	tetrapyrrole synthesis
8	Q43082	Porphobilinogen deaminase	<i>Pisum sativum</i>	2.00E-177	83.49	159.66	(-)	(-)	tetrapyrrole synthesis
9	P93111	Glutamyl-tRNA reductase 1	<i>Cucumis sativus</i>	0	78.53	153.2	(-)	(-)	tetrapyrrole synthesis
10	P93162	Magnesium-chelatase subunit chlI	<i>Glycine max</i>	0	88.43	140.87	(-)	(-)	tetrapyrrole synthesis
11	P35055	Coproporphyrinogen-III oxidase	<i>Glycine max</i>	0	83.78	122.1	(-)	(-)	tetrapyrrole synthesis
12	Q93ZB6	Uroporphyrinogen decarboxylase 1	<i>Arabidopsis thaliana</i>	0	82.37	111.81	(-)	(-)	tetrapyrrole synthesis
13	P31752	Asparagine synthetase	<i>Asparagus officinalis</i>	0	84.86	(-)	(-)	532.55	amino acid metabolism
14	Q56WD9	3-ketoacyl-CoA thiolase 2 peroxisomal	<i>Arabidopsis thaliana</i>	0	79.08	79.08	519.28	516.22	amino acid metabolism
15	Q43317	Cysteine synthase	<i>Citrullus lanatus</i>	0	89.23	89.23	140.32	303.15	amino acid metabolism
16	P27608	Phospho-2-dehydro-3-deoxyheptonate aldolase	<i>Nicotiana tabacum</i>	0	84.68	(-)	336.11	271.91	amino acid metabolism
17	P13254	Methionine gamma-lyase	<i>Pseudomonas putida</i>	2.00E-48	37.87	(-)	(-)	262.65	amino acid metabolism
18	P55217	Cystathionine gamma-synthase	<i>Arabidopsis thaliana</i>	0	68.21	68.21	371.86	244.55	amino acid metabolism
19	Q9S7E9	Glutamate--glyoxylate aminotransferase 2	<i>Arabidopsis thaliana</i>	0	87.03	(-)	(-)	243.15	amino acid metabolism
20	Q7XA67	5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase 2	<i>Arabidopsis thaliana</i>	4.00E-110	68.92	(-)		235.63	amino acid metabolism
21	P27793	Chorismate synthase	<i>C. sempervirens</i>	0	79.63	(-)	141.29	232.37	amino acid metabolism
22	A7NVX9	S-adenosylmethionine synthase 2	<i>Vitis vinifera</i>	0	95.67	(-)	355.37	221.48	amino acid metabolism
23	Q9LDV4	Alanine aminotransferase 2	<i>Arabidopsis thaliana</i>	0	83.16	(-)	206.63	202.17	amino acid metabolism
24	P42390	Indole-3-glycerol phosphate lyase	<i>Zea mays</i>	4.00E-101	59	(-)	(-)	174.17	amino acid metabolism
25	Q42699	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	<i>Catharanthus roseus</i>	0	90.98	90.98	251.22	142.2	amino acid metabolism
26	O50046	Tryptophan synthase beta chain 2	<i>C. acuminata</i>	0	88.31	(-)	(-)	121.78	amino acid metabolism
27	P32069	Anthranilate synthase component I-2	<i>Arabidopsis thaliana</i>	0	75.23	(-)	(-)	121.38	amino acid metabolism
28	Q00497	Shikimate kinase	<i>S. lycopersicum</i>	9.00E-90	53.06	(-)	160.91	115.25	amino acid metabolism
29	Q9ZUY3	Arogenate dehydratase 3	<i>Arabidopsis thaliana</i>	0	78.45	(-)	124.74	109.17	amino acid metabolism
30	Q9ZPF5	Probable arginase	<i>Arabidopsis thaliana</i>	2.00E-177	81.21	81.21	(-)	(-)	amino acid metabolism
31	O81155	Cysteine synthase	<i>Solanum tuberosum</i>	2.00E-155	78.18	78.18	165.19	(-)	amino acid metabolism
32	Q96255	Phosphoserine aminotransferase	<i>Arabidopsis thaliana</i>	0	78.5	(-)	138.99	(-)	amino acid metabolism
33	P50246	Adenosylhomocysteinase	<i>Medicago sativa</i>	0	91.55	(-)	120.26	(-)	amino acid metabolism
34	Q8GXX1	Probable isoaspartyl peptidase/L-asparaginase 2	<i>Arabidopsis thaliana</i>	0	84.83	(-)	107.1	(-)	amino acid metabolism

35	Q9T070	Cytochrome c oxidase subunit 6a	<i>Arabidopsis thaliana</i>	2.00E-45	72.73	121.81	115.12	226	mitochondrial ETC
36	Q96253	ATP synthase subunit epsilon	<i>Arabidopsis thaliana</i>	3.00E-33	77.27	146.27	108.25	221.68	mitochondrial ETC
37	Q0DI31	Cytochrome c	<i>Oryza sativa</i>	2.00E-70	91.07	(-)	394.02	194.49	mitochondrial ETC
38	Q40578	Alternative oxidase 2	<i>Nicotiana tabacum</i>	3.00E-157	81.75	(-)	487.72	181.61	mitochondrial ETC
39	P25076	Cytochrome c1-1 heme protein	<i>Solanum tuberosum</i>	1.00E-165	86.64	134.3	145.65	167.17	mitochondrial ETC
40	P26360	ATP synthase subunit gamma	<i>Ipomoea batatas</i>	0	82.66	105.26	105.03	141.5	mitochondrial ETC
41	Q41266	Alternative oxidase 2	<i>Glycine max</i>	2.00E-160	76.61	(-)	375.22	139.77	mitochondrial ETC
42	Q9SJ12	Probable ATP synthase 24 kDa subunit	<i>Arabidopsis thaliana</i>	3.00E-114	71.37	102.03	(-)	135.17	mitochondrial ETC
43	P48502	Cytochrome b-c1 complex subunit 7	<i>Solanum tuberosum</i>	1.00E-53	74.14	(-)	(-)	130.89	mitochondrial ETC
44	Q9S7L9	Cytochrome c oxidase subunit 6b-1	<i>Arabidopsis thaliana</i>	8.00E-41	90	(-)	(-)	129.08	mitochondrial ETC
45	Q54PY7	Probable mitochondrial 2-oxoglutarate/malate carrier protein	<i>D. discoideum</i>	1.00E-61	43.16	(-)	242.36	106.52	mitochondrial ETC
46	P19023	ATP synthase subunit beta	<i>Zea mays</i>	0	96.15	(-)	100.96	(-)	mitochondrial ETC

Gene ID, according to UniProtKB/Swiss-Prot database. E-value, expected value for putative annotation in UniProtKB/Swiss-Prot database. Abbreviations: RPKM, reads per kilobase million. ETC, electron transport chains. (-), no expression or RPKM value < 100.

Table 2

Table 2. List of DEGs related to ubiquitin-conjugating enzyme and ribosomal protein in leaves of *Clematis terniflora* DC. exposed to HUV-B+D.

no.	uniprot ID	description	species	E-value	identity	RPKM		function
						SP_HUV-B	SP_HUV-B+D	
1	P25867	Ubiquitin-conjugating enzyme E2-17 kDa	<i>D. melanogaster</i>	2.00E-106	100	(-)	693	protein. ubiquitin proteasome
2	P68037	Ubiquitin-conjugating enzyme E2 L3	<i>Mus musculus</i>	3.00E-90	81.82	(-)	306	protein. ubiquitin proteasome
3	P62255	Ubiquitin-conjugating enzyme E2 G1	<i>Rattus norvegicus</i>	6.00E-101	83.64	(-)	299	protein. ubiquitin proteasome
4	Q5R6C9	Ubiquitin-conjugating enzyme E2 variant 2	<i>Pongo abelii</i>	2.00E-72	71.83	(-)	245	protein. ubiquitin proteasome
5	P35128	Ubiquitin-conjugating enzyme E2 N	<i>D. melanogaster</i>	3.00E-65	90.4	(-)	202	protein. ubiquitin proteasome
6	P52486	Ubiquitin-conjugating enzyme E2-22 kDa	<i>D. melanogaster</i>	4.00E-81	77.12	(-)	166	protein. ubiquitin proteasome
7	P25153	Ubiquitin-conjugating enzyme E2-17 kDa	<i>D. melanogaster</i>	4.00E-106	98.01	(-)	144	protein. ubiquitin proteasome
8	P35134	Ubiquitin-conjugating enzyme E2 11	<i>Arabidopsis thaliana</i>	8.00E-80	84.35	65	110	protein. ubiquitin proteasome
9	Q7TSS2	Ubiquitin-conjugating enzyme E2 Q1	<i>Mus musculus</i>	1.00E-27	65.28	(-)	97	protein. ubiquitin proteasome
10	B5DEI4	Ubiquitin-conjugating enzyme E2 W	<i>Rattus norvegicus</i>	6.00E-38	89.23	(-)	88	protein. ubiquitin proteasome
11	Q9JJZ4	Ubiquitin-conjugating enzyme E2 J1	<i>Mus musculus</i>	1.00E-43	74.44	(-)	87	protein. ubiquitin proteasome
12	Q95044	Ubiquitin-conjugating enzyme E2 C	<i>Spisula solidissima</i>	3.00E-40	58.26	(-)	80	protein. ubiquitin proteasome
13	P62840	Ubiquitin-conjugating enzyme E2 D2	<i>Xenopus laevis</i>	5.00E-15	61.82	(-)	68	protein. ubiquitin proteasome
14	P42747	Ubiquitin-conjugating enzyme E2 14	<i>Arabidopsis thaliana</i>	2.00E-71	82.03	12	12	protein. ubiquitin proteasome
15	Q95V32	40S ribosomal protein S6	<i>S. frugiperda</i>	8.00E-100	77.29	(-)	5187	protein. ribosomal protein
16	A2I3Y6	60S ribosomal protein L17	<i>M. hirsutus</i>	4.00E-101	83.78	(-)	4923	protein. ribosomal protein
17	Q962T1	60S ribosomal protein L32	<i>S. frugiperda</i>	1.00E-76	82.58	(-)	4780	protein. ribosomal protein
18	Q56K10	40S ribosomal protein S15	<i>Bos taurus</i>	1.00E-78	79.31	(-)	4755	protein. ribosomal protein
19	P08570	60S acidic ribosomal protein P1	<i>D. melanogaster</i>	9.00E-26	84.13	(-)	4722	protein. ribosomal protein
20	Q56K03	60S ribosomal protein L27a	<i>Bos taurus</i>	2.00E-53	74.81	(-)	4612	protein. ribosomal protein
21	Q962R2	40S ribosomal protein S17	<i>S. frugiperda</i>	2.00E-70	87.2	(-)	4395	protein. ribosomal protein
22	P61255	60S ribosomal protein L26	<i>Mus musculus</i>	3.00E-54	79.61	(-)	4335	protein. ribosomal protein
23	Q6XIM8	40S ribosomal protein S15a	<i>Drosophila yakuba</i>	1.00E-79	91.34	(-)	4318	protein. ribosomal protein
24	Q962U1	60S ribosomal protein L13	<i>S. frugiperda</i>	5.00E-81	72.02	(-)	4293	protein. ribosomal protein
25	O76756	40S ribosomal protein S8	<i>Apis mellifera</i>	6.00E-103	82	(-)	4266	protein. ribosomal protein
26	Q962Q7	40S ribosomal protein S23	<i>S. frugiperda</i>	1.00E-97	96.5	(-)	4249	protein. ribosomal protein
27	Q0PXY6	60S ribosomal protein L18	<i>Diaphorina citri</i>	1.00E-71	70.41	(-)	4245	protein. ribosomal protein
28	P45842	60S ribosomal protein L34	<i>Aedes albopictus</i>	9.00E-47	68.52	(-)	4235	protein. ribosomal protein
29	Q9VMU4	60S ribosomal protein L37a	<i>D. melanogaster</i>	5.00E-39	87.91	(-)	4113	protein. ribosomal protein
30	Q5R8K6	60S ribosomal protein L35a	<i>Pongo abelii</i>	3.00E-42	60.91	(-)	3990	protein. ribosomal protein
31	P48588	40S ribosomal protein S25	<i>D. melanogaster</i>	8.00E-35	81.08	(-)	3957	protein. ribosomal protein
32	Q69CJ9	60S ribosomal protein L35	<i>Ophiophagus hannah</i>	6.00E-35	64.23	(-)	3955	protein. ribosomal protein
33	Q962R1	40S ribosomal protein S18	<i>S. frugiperda</i>	8.00E-86	88.89	(-)	3939	protein. ribosomal protein
34	P97461	40S ribosomal protein S5	<i>Mus musculus</i>	1.00E-124	89.45	(-)	3814	protein. ribosomal protein

35	Q9NB33	60S ribosomal protein L44	<i>Aedes triseriatus</i>	1.00E-56	94.23	(-)	3814	protein. ribosomal protein
36	Q6F473	40S ribosomal protein S29	<i>Plutella xylostella</i>	2.00E-33	85.71	(-)	3797	protein. ribosomal protein
37	P36241	60S ribosomal protein L19	<i>D. melanogaster</i>	6.00E-72	87.34	(-)	3774	protein. ribosomal protein
38	P31009	40S ribosomal protein S2	<i>D. melanogaster</i>	3.00E-130	90.99	(-)	3754	protein. ribosomal protein
39	Q8ITC3	40S ribosomal protein S19	<i>A. irradians</i>	5.00E-58	64.39	(-)	3699	protein. ribosomal protein
40	P58375	60S ribosomal protein L30	<i>S. frugiperda</i>	2.00E-52	75.89	(-)	3608	protein. ribosomal protein
41	P46222	60S ribosomal protein L11	<i>D. melanogaster</i>	1.00E-106	87.93	(-)	3552	protein. ribosomal protein
42	O76732	60S ribosomal protein L7a	<i>Anopheles gambiae</i>	2.00E-113	68.53	(-)	3524	protein. ribosomal protein
43	Q963B6	60S ribosomal protein L10a	<i>S. frugiperda</i>	6.00E-114	84.79	(-)	3512	protein. ribosomal protein
44	P62282	40S ribosomal protein S11	<i>Rattus norvegicus</i>	1.00E-54	93.18	(-)	3485	protein. ribosomal protein
45	Q962R9	40S ribosomal protein S10	<i>S. frugiperda</i>	6.00E-58	66.44	(-)	3433	protein. ribosomal protein
46	Q962T5	60S ribosomal protein L24	<i>S. frugiperda</i>	5.00E-47	87.5	(-)	3343	protein. ribosomal protein
47	Q5XUC7	60S ribosomal protein L5	<i>Toxoptera citricida</i>	0	98.33	(-)	3332	protein. ribosomal protein
48	P55841	60S ribosomal protein L14	<i>D. melanogaster</i>	4.00E-44	52.99	(-)	3297	protein. ribosomal protein
49	P48153	40S ribosomal protein S3	<i>Manduca sexta</i>	9.00E-129	79.48	(-)	3181	protein. ribosomal protein
50	P32100	60S ribosomal protein L7	<i>D. melanogaster</i>	2.00E-102	62.65	(-)	3074	protein. ribosomal protein
51	P19889	60S acidic ribosomal protein P0	<i>D. melanogaster</i>	7.00E-141	78.55	(-)	2944	protein. ribosomal protein
52	Q8WQI7	60S ribosomal protein L18a	<i>S. frugiperda</i>	9.00E-87	75.28	(-)	2923	protein. ribosomal protein
53	A2I469	60S ribosomal protein L38	<i>M. hirsutus</i>	1.00E-40	89.47	(-)	2898	protein. ribosomal protein
54	Q0PXX8	40S ribosomal protein SA	<i>Diaphorina citri</i>	2.00E-127	71.76	(-)	2890	protein. ribosomal protein
55	Q962T2	60S ribosomal protein L28	<i>S. frugiperda</i>	2.00E-45	60.48	(-)	2859	protein. ribosomal protein
56	Q963B7	60S ribosomal protein L9	<i>S. frugiperda</i>	1.00E-101	79.26	(-)	2846	protein. ribosomal protein
57	P14130	40S ribosomal protein S14	<i>D. melanogaster</i>	3.00E-66	92.14	(-)	2832	protein. ribosomal protein
58	P29504	Ubiquitin-40S ribosomal protein S27a	<i>Manduca sexta</i>	7.00E-63	87.84	(-)	2765	protein. ribosomal protein
59	P47904	40S ribosomal protein S27	<i>Xenopus laevis</i>	2.00E-48	83.33	(-)	2762	protein. ribosomal protein
60	Q9GNE2	60S ribosomal protein L23	<i>Aedes aegypti</i>	3.00E-84	94.66	(-)	2706	protein. ribosomal protein
61	P08429	60S ribosomal protein L4-A	<i>Xenopus laevis</i>	1.00E-174	64.67	(-)	2654	protein. ribosomal protein
62	Q9GT45	40S ribosomal protein S26	<i>Anopheles gambiae</i>	7.00E-64	84.07	(-)	2640	protein. ribosomal protein
63	Q24154	60S ribosomal protein L29	<i>D. melanogaster</i>	6.00E-19	63.49	(-)	2621	protein. ribosomal protein
64	Q962S4	60S ribosomal protein L39	<i>S. frugiperda</i>	1.00E-16	96.97	(-)	2610	protein. ribosomal protein
65	P47772	40S ribosomal protein S13	<i>Ictalurus punctatus</i>	2.00E-87	84.77	(-)	2588	protein. ribosomal protein
66	P23403	40S ribosomal protein S20	<i>Xenopus laevis</i>	2.00E-50	71.3	(-)	2515	protein. ribosomal protein
67	Q4GXP3	40S ribosomal protein S21	<i>Agriotes lineatus</i>	4.00E-37	73.49	(-)	2515	protein. ribosomal protein
68	Q2YGT9	60S ribosomal protein L6	<i>Sus scrofa</i>	1.00E-38	41.89	(-)	2417	protein. ribosomal protein
69	Q5G5C4	40S ribosomal protein S3a	<i>P. americana</i>	1.00E-133	80.08	(-)	2314	protein. ribosomal protein
70	P62864	40S ribosomal protein S30	<i>Rattus norvegicus</i>	6.00E-19	83.33	(-)	2236	protein. ribosomal protein

71	O16797	60S ribosomal protein L3	<i>D. melanogaster</i>	0	79.15	(-)	2221	protein. ribosomal protein
72	Q95V31	40S ribosomal protein S16	<i>S. frugiperda</i>	2.00E-83	85.11	(-)	2143	protein. ribosomal protein
73	P48155	40S ribosomal protein S7	<i>Manduca sexta</i>	8.00E-102	80.75	(-)	2137	protein. ribosomal protein
74	Q962S0	40S ribosomal protein S7	<i>S. frugiperda</i>	1.00E-104	83.42	(-)	2032	protein. ribosomal protein
75	P05389	60S acidic ribosomal protein P2	<i>D. melanogaster</i>	3.00E-11	51.92	(-)	1980	protein. ribosomal protein
76	Q9W334	40S ribosomal protein S28	<i>D. melanogaster</i>	6.00E-26	92.16	(-)	1772	protein. ribosomal protein
77	P55935	40S ribosomal protein S9	<i>D. melanogaster</i>	1.00E-84	95.62	(-)	1755	protein. ribosomal protein
78	P52865	60S ribosomal protein L22	<i>Gadus morhua</i>	2.00E-25	74.74	(-)	1573	protein. ribosomal protein
79	Q7KF90	60S ribosomal protein L31	<i>S. frugiperda</i>	2.00E-59	74.19	(-)	1556	protein. ribosomal protein
80	Q95V39	60S ribosomal protein L8	<i>S. frugiperda</i>	1.00E-156	87.84	(-)	1553	protein. ribosomal protein
81	P35979	60S ribosomal protein L12	<i>Mus musculus</i>	3.00E-72	80.54	(-)	1515	protein. ribosomal protein
82	Q5XH16	NHP2-like protein 1	<i>Xenopus laevis</i>	7.00E-58	83.87	(-)	870	protein. ribosomal protein
83	Q4GXU6	40S ribosomal protein S4	<i>Carabus granulatus</i>	2.00E-164	86.54	(-)	613	protein. ribosomal protein
84	P52818	60S ribosomal protein L15	<i>Anopheles gambiae</i>	1.00E-93	80.88	(-)	551	protein. ribosomal protein
85	Q9VGN9	Ribosome biogenesis protein RLP24	<i>D. melanogaster</i>	2.00E-80	76.71	(-)	471	protein. ribosomal protein
86	O96647	60S ribosomal protein L10	<i>Bombyx mandarina</i>	2.00E-145	89.04	(-)	464	protein. ribosomal protein
87	Q9VNE9	60S ribosomal protein L13a	<i>D. melanogaster</i>	6.00E-72	53.69	(-)	464	protein. ribosomal protein
88	P23209	Ribosomal protein S13	<i>Daucus carota</i>	4.00E-74	93.97	54	398	protein. ribosomal protein
89	P62752	60S ribosomal protein L23a	<i>Rattus norvegicus</i>	1.00E-52	74.07	(-)	389	protein. ribosomal protein
90	Q962Q6	40S ribosomal protein S24	<i>S. frugiperda</i>	3.00E-49	84.04	(-)	372	protein. ribosomal protein
91	P49630	60S ribosomal protein L36	<i>D. melanogaster</i>	1.00E-46	70	(-)	331	protein. ribosomal protein
92	Q6B860	28S ribosomal protein S14	<i>Bos taurus</i>	1.00E-33	54.29	(-)	302	protein. ribosomal protein
93	Q0E959	39S ribosomal protein L34	<i>D. melanogaster</i>	7.00E-14	60	(-)	216	protein. ribosomal protein
94	Q9V3U2	H/ACA ribonucleoprotein complex subunit 2-like protein	<i>D. melanogaster</i>	7.00E-44	58.33	(-)	198	protein. ribosomal protein
95	Q9DB15	39S ribosomal protein L12	<i>Mus musculus</i>	3.00E-28	47.69	(-)	176	protein. ribosomal protein
96	P82911	28S ribosomal protein S11	<i>Bos taurus</i>	3.00E-36	52.03	(-)	169	protein. ribosomal protein
97	Q9CQL4	39S ribosomal protein L20	<i>Mus musculus</i>	3.00E-16	45.68	(-)	166	protein. ribosomal protein
98	Q9DCU6	39S ribosomal protein L4	<i>Mus musculus</i>	2.00E-25	52.69	(-)	163	protein. ribosomal protein
99	Q9VHN6	39S ribosomal protein L19	<i>D. melanogaster</i>	2.00E-79	66.35	(-)	159	protein. ribosomal protein
100	Q58DQ5	28S ribosomal protein S9	<i>Bos taurus</i>	7.00E-31	65.33	(-)	159	protein. ribosomal protein
101	Q9UKD2	mRNA turnover protein 4 homolog	<i>Homo sapiens</i>	1.00E-62	56.1	(-)	157	protein. ribosomal protein
102	Q9VFJ2	39S ribosomal protein L11	<i>D. melanogaster</i>	3.00E-29	56.25	(-)	153	protein. ribosomal protein
103	Q9VJ38	39S ribosomal protein L13	<i>D. melanogaster</i>	2.00E-65	59.03	(-)	150	protein. ribosomal protein
104	O94690	54S ribosomal protein c83.06c	<i>S. pombe</i>	7.00E-06	57.58	(-)	140	protein. ribosomal protein
105	Q9VZD5	Probable 28S ribosomal protein S6	<i>D. melanogaster</i>	6.00E-27	45.63	(-)	139	protein. ribosomal protein

106	Q9HD33	39S ribosomal protein L47	<i>Homo sapiens</i>	2.00E-44	42.86	(-)	137	protein. ribosomal protein
107	Q291A0	39S ribosomal protein L41	<i>D. pseudoobscura</i>	6.00E-21	62.96	(-)	133	protein. ribosomal protein
108	Q99N92	39S ribosomal protein L27	<i>Mus musculus</i>	4.00E-23	46.3	(-)	133	protein. ribosomal protein
109	Q29IK4	39S ribosomal protein L22	<i>D. pseudoobscura</i>	9.00E-23	59.15	(-)	116	protein. ribosomal protein
110	Q1JQ99	39S ribosomal protein L14	<i>Bos taurus</i>	2.00E-13	51.61	(-)	116	protein. ribosomal protein
111	Q9VY28	Probable 28S ribosomal protein S25	<i>D. melanogaster</i>	1.00E-47	62.73	(-)	115	protein. ribosomal protein
112	Q498T4	39S ribosomal protein L2	<i>Rattus norvegicus</i>	9.00E-38	50.35	(-)	112	protein. ribosomal protein
113	Q8MS27	39S ribosomal protein L35	<i>D. melanogaster</i>	3.00E-40	67.37	(-)	107	protein. ribosomal protein
114	Q9VMY1	Probable 39S ribosomal protein L24	<i>D. melanogaster</i>	7.00E-83	53.15	(-)	105	protein. ribosomal protein
115	P82917	28S ribosomal protein S18c	<i>Bos taurus</i>	4.00E-23	48.94	(-)	105	protein. ribosomal protein
116	Q6PDW6	39S ribosomal protein L17	<i>Rattus norvegicus</i>	4.00E-17	38.05	(-)	95	protein. ribosomal protein
117	Q6UZF7	60S ribosomal protein L35	<i>Hippocampus comes</i>	1.00E-53	96.58	15	94	protein. ribosomal protein
118	Q8WTC1	28S ribosomal protein S15	<i>D. melanogaster</i>	1.00E-16	39.25	(-)	94	protein. ribosomal protein
119	P51398	28S ribosomal protein S29	<i>Homo sapiens</i>	2.00E-21	40.54	(-)	91	protein. ribosomal protein
120	Q90YQ0	40S ribosomal protein S24	<i>Ictalurus punctatus</i>	2.00E-20	83.82	(-)	88	protein. ribosomal protein
121	Q2TBS2	39S ribosomal protein L21	<i>Bos taurus</i>	8.00E-15	49.33	(-)	87	protein. ribosomal protein
122	Q9VMX0	39S ribosomal protein L28	<i>D. melanogaster</i>	2.00E-21	54.29	(-)	84	protein. ribosomal protein
123	P80455	40S ribosomal protein S12	<i>D. melanogaster</i>	4.00E-30	76.12	(-)	75	protein. ribosomal protein
124	Q56FH2	40S ribosomal protein S4	<i>L. testaceipes</i>	8.00E-31	74.63	(-)	74	protein. ribosomal protein
125	Q9CR47	Ribosome biogenesis protein NSA2	<i>Mus musculus</i>	4.00E-41	97.06	(-)	73	protein. ribosomal protein
126	O42699	60S ribosomal protein L19-B	<i>S. pombe</i>	7.00E-19	73.49	23	73	protein. ribosomal protein
127	P47955	60S acidic ribosomal protein P1	<i>Mus musculus</i>	2.00E-16	72.73	(-)	72	protein. ribosomal protein
128	Q5ZKT8	39S ribosomal protein L15	<i>Gallus gallus</i>	1.00E-29	57.61	(-)	66	protein. ribosomal protein
129	P62919	60S ribosomal protein L8	<i>Rattus norvegicus</i>	1.00E-38	78.21	(-)	65	protein. ribosomal protein
130	Q9V6Y3	Probable 28S ribosomal protein S16	<i>D. melanogaster</i>	7.00E-22	58.82	(-)	64	protein. ribosomal protein
131	P82675	28S ribosomal protein S5	<i>Homo sapiens</i>	1.00E-19	54.79	(-)	60	protein. ribosomal protein
132	P41056	60S ribosomal protein L33-B	<i>S. cerevisiae</i>	2.00E-24	61.43	(-)	57	protein. ribosomal protein
133	P05750	40S ribosomal protein S3	<i>S. cerevisiae</i>	1.00E-17	71.19	36	45	protein. ribosomal protein
134	P46778	60S ribosomal protein L21	<i>Homo sapiens</i>	4.00E-100	85.62	(-)	33	protein. ribosomal protein
135	Q8L805	60S ribosomal protein L35	<i>Triticum aestivum</i>	4.00E-29	77.94	28	29	protein. ribosomal protein
136	Q9GCB9	Ribosomal protein S2	<i>Arabidopsis thaliana</i>	2.00E-70	59.51	(-)	21	protein. ribosomal protein
137	P42793	60S ribosomal protein L5	<i>Arabidopsis thaliana</i>	4.00E-106	88.65	(-)	17	protein. ribosomal protein
138	P93311	60S ribosomal protein L2	<i>Arabidopsis thaliana</i>	5.00E-108	83.17	(-)	16	protein. ribosomal protein
139	P29757	Cytochrome b	<i>Solanum tuberosum</i>	0	98.71	(-)	15	protein. ribosomal protein
140	P92557	Ribosomal protein S7	<i>Arabidopsis thaliana</i>	4.00E-90	91.89	(-)	13	protein. ribosomal protein
141	Q08745	40S ribosomal protein S10-A	<i>S. cerevisiae</i>	2.00E-47	76.09	(-)	12	protein. ribosomal protein

142	Q31708	Ribosomal protein S4	<i>Arabidopsis thaliana</i>	4.00E-58	79.08	(-)	11	protein. ribosomal protein
143	Q9ASV6	30S ribosomal protein S20	<i>Arabidopsis thaliana</i>	5.00E-45	54.45	(-)	0.10	protein. ribosomal protein
144	Q9FJP3	50S ribosomal protein L29	<i>Arabidopsis thaliana</i>	1.00E-49	65.41	(-)	0.10	protein. ribosomal protein
145	P51412	50S ribosomal protein L21	<i>Arabidopsis thaliana</i>	3.00E-46	77.34	(-)	0.09	protein. ribosomal protein
146	P82412	30S ribosomal protein 3	<i>Spinacia oleracea</i>	2.00E-60	62.29	(-)	0.08	protein. ribosomal protein
147	P93014	30S ribosomal protein S5	<i>Arabidopsis thaliana</i>	3.00E-107	73.64	(-)	0.08	protein. ribosomal protein
148	O80361	50S ribosomal protein L4	<i>Nicotiana tabacum</i>	2.00E-105	81.69	(-)	0.07	protein. ribosomal protein
149	Q5FFA8	30S ribosomal protein S16	<i>E. ruminantium</i>	5.00E-21	54.79	(-)	0.07	protein. ribosomal protein
150	O80362	50S ribosomal protein L10	<i>Nicotiana tabacum</i>	6.00E-91	68.42	(-)	0.07	protein. ribosomal protein
151	Q9SKX4	50S ribosomal protein L3-1	<i>Arabidopsis thaliana</i>	4.00E-117	83.64	(-)	0.07	protein. ribosomal protein
152	Q9SX68	50S ribosomal protein L18	<i>Arabidopsis thaliana</i>	4.00E-67	80.88	(-)	0.07	protein. ribosomal protein
153	P82244	50S ribosomal protein L34	<i>Spinacia oleracea</i>	2.00E-17	55.22	(-)	0.06	protein. ribosomal protein
154	O23049	50S ribosomal protein L6	<i>Arabidopsis thaliana</i>	2.00E-101	71.03	(-)	0.06	protein. ribosomal protein
155	Q6JJ61	50S ribosomal protein L9	<i>Ipomoea trifida</i>	1.00E-57	81.25	(-)	0.06	protein. ribosomal protein
156	O80439	30S ribosomal protein S31	<i>Arabidopsis thaliana</i>	7.00E-20	58.04	(-)	0.05	protein. ribosomal protein
157	Q9FKP0	50S ribosomal protein 6	<i>Arabidopsis thaliana</i>	9.00E-23	67.47	(-)	0.05	protein. ribosomal protein
158	P25873	50S ribosomal protein L15	<i>Arabidopsis thaliana</i>	1.00E-88	85.63	(-)	0.05	protein. ribosomal protein
159	P23326	50S ribosomal protein L35	<i>Spinacia oleracea</i>	3.00E-42	84.95	(-)	0.05	protein. ribosomal protein
160	P36688	50S ribosomal protein L12	<i>Nicotiana sylvestris</i>	6.00E-29	68.5	(-)	0.04	protein. ribosomal protein
161	P0CX83	60S ribosomal protein L19-B	<i>S. cerevisiae</i>	4.00E-19	78.26	27	(-)	protein. ribosomal protein
162	P46777	60S ribosomal protein L5	<i>Homo sapiens</i>	0	89.08	0.08	(-)	protein. ribosomal protein
163	P60868	40S ribosomal protein S20	<i>Rattus norvegicus</i>	2E-77	98.32	0.06	(-)	protein. ribosomal protein

Uniprot ID, according to UniProtKB/Swiss-Prot database. E-value, expected value for putative annotation in UniProtKB/Swiss-Prot database.
Abbreviations: (-), 0.1 < fold change < 10.

Table 3

Table 3. List of DAPs related to ribosomal protein and ubiquitin proteasome in leaves of *Clematis terniflora* DC. exposed to HUV-B+D.

no.	uniprot ID	description	species	M.P.	Ratio		p-value		function	
					SP	HUV-B	HUV-B+D	HUV-B		HUV-B+D
1	Q9M385	50S ribosomal protein L17	<i>Arabidopsis thaliana</i>	2	1	0.72	0.82	0.04	0.01	protein. ribosomal protein
2	Q9FJA6	40S ribosomal protein S3_3	<i>Arabidopsis thaliana</i>	3	1	0.76	0.74	0.03	0.00	protein. ribosomal protein
3	Q943F3	60S ribosomal protein L18a	<i>Oryza sativa</i>	4	1	0.33	0.68	0.00	0.00	protein. ribosomal protein
4	Q8LD46	60S ribosomal protein L23a_1	<i>Arabidopsis thaliana</i>	3	1	0.94	0.91	0.04	0.01	protein. ribosomal protein
5	Q8L7K0	60S ribosomal protein L18a_1	<i>Arabidopsis thaliana</i>	2	1	0.27	0.61	0.01	0.03	protein. ribosomal protein
6	P50346	60S acidic ribosomal protein P0	<i>Glycine max</i>	2	1	3.77	5.74	0.01	0.00	protein. ribosomal protein
7	O80360	50S ribosomal protein L3	<i>Nicotiana tabacum</i>	2	1	0.47	1.34	0.01	0.05	protein. ribosomal protein
8	O50003	60S ribosomal protein L12	<i>Prunus armeniaca</i>	3	1	1.53	1.56	0.00	0.00	protein. ribosomal protein
9	O04204	60S acidic ribosomal protein P0_1	<i>Arabidopsis thaliana</i>	3	1	1.19	1.82	0.02	0.00	protein. ribosomal protein
10	A4QK56	30S ribosomal protein S3	<i>Arabis hirsuta</i>	3	1	1.00	0.90	0.02	0.03	protein. ribosomal protein
11	P59223	40S ribosomal protein S13_1	<i>Arabidopsis thaliana</i>	4	1	(-)	1.41	(-)	0.00	protein. ribosomal protein
12	P49201	40S ribosomal protein S23_2	<i>Arabidopsis thaliana</i>	2	1	(-)	4.56	(-)	0.00	protein. ribosomal protein
13	P29345	40S ribosomal protein S6	<i>Nicotiana tabacum</i>	2	1	(-)	3.94	(-)	0.03	protein. ribosomal protein
14	P49199	40S ribosomal protein S8	<i>Oryza sativa</i>	2	1	(-)	3.43	(-)	0.00	protein. ribosomal protein
15	Q9LXG1	40S ribosomal protein S9_1	<i>Arabidopsis thaliana</i>	3	1	(-)	1.67	(-)	0.01	protein. ribosomal protein
16	Q9FF90	60S ribosomal protein L13_3	<i>Arabidopsis thaliana</i>	2	1	(-)	2.64	(-)	0.04	protein. ribosomal protein
17	P49627	60S ribosomal protein L13	<i>Nicotiana tabacum</i>	3	1	(-)	2.38	(-)	0.01	protein. ribosomal protein
18	Q9FKC0	60S ribosomal protein L13a_4	<i>Arabidopsis thaliana</i>	2	1	(-)	3.17	(-)	0.00	protein. ribosomal protein
19	Q07761	60S ribosomal protein L23a	<i>Nicotiana tabacum</i>	2	1	(-)	1.29	(-)	0.04	protein. ribosomal protein
20	Q9FUL4	60S ribosomal protein L24	<i>Prunus avium</i>	3	1	(-)	4.24	(-)	0.00	protein. ribosomal protein
21	P35684	60S ribosomal protein L3	<i>Oryza sativa</i>	3	1	(-)	7.56	(-)	0.00	protein. ribosomal protein
22	Q8LBI1	60S ribosomal protein L5_1	<i>Arabidopsis thaliana</i>	2	1	(-)	1.48	(-)	0.04	protein. ribosomal protein
23	P34091	60S ribosomal protein L6	<i>M. crystallinum</i>	2	1	(-)	1.71	(-)	0.03	protein. ribosomal protein
24	P49210	60S ribosomal protein L9	<i>Oryza sativa</i>	2	1	(-)	1.35	(-)	0.00	protein. ribosomal protein
25	O19990	30S ribosomal protein S4	<i>Iris domestica</i>	2	1	(-)	1.77	(-)	0.00	protein. ribosomal protein
26	A6MM72	30S ribosomal protein S8	<i>Buxus microphylla</i>	2	1	(-)	1.47	(-)	0.00	protein. ribosomal protein
27	P35266	60S ribosomal protein L17_1	<i>Hordeum vulgare</i>	2	1	(-)	0.79	(-)	0.02	protein. ribosomal protein
28	O22644	60S ribosomal protein L23A	<i>Fritillaria agrestis</i>	2	1	(-)	0.99	(-)	0.01	protein. ribosomal protein
29	P36212	50S ribosomal protein L12_3	<i>Arabidopsis thaliana</i>	2	1	(-)	0.64	(-)	0.05	protein. ribosomal protein
30	A4QJF1	50S ribosomal protein L14	<i>A. cordifolium</i>	2	1	(-)	0.27	(-)	0.00	protein. ribosomal protein
31	O65731	40S ribosomal protein S5	<i>Cicer arietinum</i>	3	1	1.02	(-)	0.04	(-)	protein. ribosomal protein
32	Q2MII5	30S ribosomal protein S4	<i>S. bulbocastanum</i>	3	1	1.32	(-)	0.02	(-)	protein. ribosomal protein
33	P42036	40S ribosomal protein S14_3	<i>Arabidopsis thaliana</i>	2	1	0.29	(-)	0.00	(-)	protein. ribosomal protein
34	Q9LZ17	40S ribosomal protein S17_4	<i>Arabidopsis thaliana</i>	3	1	0.51	(-)	0.02	(-)	protein. ribosomal protein

35	P34788	40S ribosomal protein S18	<i>Arabidopsis thaliana</i>	2	1	0.33	(-)	0.02	(-)	protein. ribosomal protein
36	Q9LHP1	60S ribosomal protein L7_4	<i>Arabidopsis thaliana</i>	3	1	0.62	(-)	0.00	(-)	protein. ribosomal protein
37	P60039	60S ribosomal protein L7_3	<i>Arabidopsis thaliana</i>	4	1	0.52	(-)	0.00	(-)	protein. ribosomal protein
38	A8SED6	30S ribosomal protein S11	<i>C. demersum</i>	2	1	0.68	(-)	0.01	(-)	protein. ribosomal protein
39	A6MM38	30S ribosomal protein S4	<i>Buxus microphylla</i>	3	1	0.62	(-)	0.05	(-)	protein. ribosomal protein
40	P31165	50S ribosomal protein L15	<i>Pisum sativum</i>	2	1	0.66	(-)	0.01	(-)	protein. ribosomal protein
41	Q94A97	Ubiquitin_conjugating enzyme E2 35	<i>Arabidopsis thaliana</i>	2	1	(-)	1.97	(-)	0.00	Protein. ubiquitin proteasome
42	Q9FZ48	Ubiquitin_conjugating enzyme E2 36	<i>Arabidopsis thaliana</i>	3	1	1.48	1.92	0.04	0.00	protein. ubiquitin proteasome
		26S proteasome non_ATPase regulatory subunit 2 homolog A								
43	Q9SIV2	subunit 2 homolog A	<i>Arabidopsis thaliana</i>	2	1	1.82	2.45	0.04	0.00	protein. ubiquitin proteasome
44	Q9SL67	26S proteasome regulatory subunit 4 homolog	<i>Arabidopsis thaliana</i>	3	1	(-)	2.07	(-)	0.00	protein. ubiquitin proteasome
45	P54776	26S protease regulatory subunit 6A homolog	<i>Solanum lycopersicum</i>	4	1	(-)	1.90	(-)	0.00	protein. ubiquitin proteasome
46	P46466	26S protease regulatory subunit 4 homolog	<i>Oryza sativa</i>	3	1	0.79	1.82	0.00	0.00	protein. ubiquitin proteasome
47	O04019	26S protease regulatory subunit 6A homolog B	<i>Arabidopsis thaliana</i>	5	1	(-)	1.81	(-)	0.00	protein. ubiquitin proteasome
48	O23715	Proteasome subunit alpha type_3	<i>Arabidopsis thaliana</i>	2	1	(-)	1.66	(-)	0.00	protein. ubiquitin proteasome
49	Q9SEI4	26S protease regulatory subunit 6B homolog	<i>Arabidopsis thaliana</i>	4	1	(-)	1.29	(-)	0.03	protein. ubiquitin proteasome
50	Q9MAK9	26S protease regulatory subunit S10B homolog	<i>Arabidopsis thaliana</i>	2	1	(-)	1.28	(-)	0.01	protein. ubiquitin proteasome
51	P0CG86	Ubiquitin_40S ribosomal protein S27a	<i>Hordeum vulgare</i>	3	1	1.18	(-)	0.03	(-)	protein. ubiquitin proteasome
52	P52428	Proteasome subunit alpha type_1	<i>Oryza sativa</i>	2	1	0.81	(-)	0.04	(-)	protein. ubiquitin proteasome
53	Q9SEI3	26S protease regulatory subunit 10B homolog	<i>Arabidopsis thaliana</i>	2	1	0.65	(-)	0.01	(-)	protein. ubiquitin proteasome

Protein ID, according to UniProtKB/Swiss-Prot database. M.P., number of more than 2 matched peptides; function, protein function categorized using MapMan bin codes.

Abbreviations: (-), protein was not identified.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 7

Figure 8

Figure 9

Biological significance

This study reported response mechanism in [leaves](#) of *Clematis terniflora* DC. under high level [of](#) UV-B irradiation followed by dark treatment using transcriptomic and proteomic techniques. Transcriptomic analysis [revealed](#) that the number of genes related to secondary metabolism was increased by more than 2 times. *Phenylalanine ammonia-lyase*, *4-Coumarate: CoA ligase*, *chalcone synthase*, *isoflavone reductase homolog*, and *cinnamoyl-CoA reductase* were significantly upregulated, which suggests that the secondary metabolism pathway related to lignins and flavonoids/isoflavonoids might be activated. Genes and proteins related to protein metabolism were largely changed in postranslational modification, ubiquitin proteasome, and ribosomal protein. NADP-dependent malic enzyme and NADP-malate dehydrogenase were activated in tricarboxylic acid cycle, which suggests that tricarboxylic acid cycle might be enhanced in leaf of *C. terniflora* in response to [HUV-B+D](#).

Clematis terniflora DC.