Variations of Metabolites and Proteome in *Lonicera japonica* Thunb. Buds and Flowers under
UV Radiation

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Abbreviations

ACO, 1-aminocyclopropane-1-carboxylate oxidase; DPPH, 1,1-diphenyl-2-picrylhydrazyl; 2D-PAGE, two-dimensional polyacrylamide gel electrophoresis; GSH, glutathione; HSP, heat shock proteins; IPG, immobilized pH gradient; LC-MS/MS, liquid chromatography-mass spectrometry/ mass spectrometry; MALDI-TOF/TOF, matrix assisted laser desorption ionization-time-of-flight/ time-of-flight; PPP, pentose phosphate pathway; TCA, tricarboxylic acid cycle; TCM, traditional Chinese medicine; TIC, total ion chromatogram; UV, ultraviolet.

ABSTRACT

Lonicera japonica Thunb., also known as Jin Yin Hua and Japanese honeysuckle, is used as a herbal medicine in Asian countries. Its flowers have been used in folk medicine in the clinic and in making food or healthy beverages for over 1500 years in China. To investigate the molecular processes involved in *L. japonica* development from buds to flowers exposed to UV radiation, a comparative proteomics analysis was performed. Fifty-four proteins were identified as differentially expressed, including 42 that had increased expression and 12 that had decreased expression. The levels of the proteins related to glycolysis, TCA/organic acid transformation, major carbohydrate metabolism, oxidative pentose phosphate, stress, secondary metabolism, hormone, and mitochondrial electron transport were increased during flower opening process after exposure to UV radiation. Six metabolites in *L. japonica* buds and flowers were identified and relatively quantified using LC-MS/MS. The antioxidant activity was performed using a 1,1-diphenyl-2-picrylhydrazyl assay, which revealed that *L. japonica* buds had more activity than the UV irradiated flowers. This suggests that UV-B radiation induces production of endogenous ethylene in *L. japonica* buds, thus facilitating blossoming of the buds and activating the antioxidant system. Additionally, the higher metabolite contents and antioxidant properties of *L. japonica* buds indicate that the *L. japonica* bud stage may be a more optimal time to harvest than the flower stage when using for medicinal properties.

Keywords: *Lonicera japonica* Thunb., proteomics, LC-MS/MS, flower processing, UV radiation

1. Introduction

Lonicera japonica Thunb., also known as *Jin Yin Hua* and *Japanese honeysuckle*, is used as an herbal medicine in Asian countries. It has been used in folk medicine in the clinic and in making food and healthy beverages for 1500 years in China [1]. Modern pharmacological studies of *L. japonica* have confirmed its high medicinal value. *L. japonica* extracts had inhibitory effect on lipid peroxidation, superoxide radical scavenging activity, and tyrosinase activity *in vitro* [2]. Both extracts of *L. japonica* and *Scutellaria baicalensis* exhibited significant antiviral properties and have been used in the clinic to treat influenza, tonsillitis, pharyngitis, and upper respiratory infections [3]. Ethanol extracts of *L. japonica* leaves were potentially antibacterial against food-borne pathogens [4]. Tannins derived from *L. japonica* selectively inhibited human immunodeficiency virus [5]. The chemical composition of *L. japonica* consists of essential oils, organic acids, flavones, saponins, iridoids and inorganic elements, where caffeoylquinic acid and iridoid glycosides are the main active ingredients [6].

Systems biology has been increasingly used to study plant evolution, stress defense and species separation. Proteomics is one of the major techniques utilized in systems biology, and has been widely used in the study of model plants and crops. However, few proteomic studies have focused on traditional Chinese medicine (TCM). Strategies utilized in proteomics are valuable tools in quality control, toxicity studies, and standardization of preparations and decoctions in TCM [7], as well as single bioactive compound discovery, and multi-target effect characterizations of complex herbal preparations [8]. Ginseng is one of the best studied TCM, and has been evaluated by comparative proteomics. Two-dimensional electrophoresis (2 DE) maps of different parts of the Oriental ginseng, *Panax ginseng* C. A. Meyer, including

the main root, lateral roots, rhizome head and ginseng skin were generated, and the main roots of *American ginseng* (*Panax quinquefolius*) and Oriental ginseng were compared [9]. The 2 DE patterns of the main root and leaves of *Panax ginseng* was different from that of the cultured hairy root [10]. The 2 DE patterns of the Korean Ginseng (*Panax ginseng* C. A. Meyer) and Indian Ginseng (*Withania somnifera*) were also distinct [11]. The proteome of *Gynura procumbens* (Lour.) Merr. leaves was characterized, and it was found most of the proteins identified were related to photosynthesis and energy metabolism [12]. These studies suggested that proteomic analysis can be used to distinguish between common and unique protein spots, allowing easy discrimination between different species and tissues. The use of “marker proteins” would undoubtedly aid in understanding protein expression and functions in the physiology of medicinal plants.

Ultraviolet (UV) is one of the most important environmental factors affecting plant growth and development, and may cause alterations in plant physiology and biochemistry, including the accumulation of secondary metabolites [13]. Previously, Ning et al. [14] found that the content of active metabolites in *L. japonica* flower buds was increased significantly under UV radiation. A comparative proteomics study of UV irradiated *L. japonica* flower buds revealed an increase in two bioactive components after UV radiation and generated a profile of UV-response proteins in *L. japonica* flower buds [15]. In the present study, *L. japonica* flower buds bloomed after treatment with UV radiation and dark incubation. The main active components in flower buds, and flowers treated with or without UV radiation were analyzed by LC-MS/MS. The antioxidant activity of *L. japonica* flowers treated with UV-B radiation was higher than in the other cohorts. Comparative proteomics was employed

to uncover the mechanism of action of UV radiation on *L. japonica* during flower opening. The changes in the active components of *L. japonica* exposed to UV radiation, as well as different flower stages, affected the quality and quantity of the resulting medicinal materials. These results will facilitate our understanding of the molecular physiology and determining the optimal harvest period for *L. japonica*.

2. Materials and methods

2.1 *Plant materials and treatments*

L. japonica flower buds were obtained from Pingyi cultivation base (Shandong, China). More than 100 plants were grown in the same environment, collected in late May and used for the experiments in this study. The postharvest flower buds were randomly divided into three groups (control buds, control flowers, and UV-B flowers) for the following experiments. The UV-B flowers were exposed to UV-B light (306 nm, $89.56 \mu\text{W}\cdot\text{cm}^{-2}$) for 4 h [15], and then cultured to blossom for 36 h at 28°C and 80% humidity in the dark. The control buds and flowers were cultured under the same conditions with the exception of not being exposed to UV-B irradiation. For each group, three independent experiments were performed as biological replicates. For analysis using liquid chromatography-tandem mass spectrometry (LC-MS/MS), all samples were dried and then ground into coarse powder. For proteomics analysis, samples were frozen in liquid nitrogen and stored at -80 °C.

2.2 *Protein extraction and 2-DE*

Total proteins were extracted by the modified phenol (Phe)-based method protocol [16], and then the protein concentrations were measured using the Bradford [17] method with bovine serum albumin as the standard. Protein extract (1 mg) was loaded onto an IPG strip

holder with 24 cm, pH 4.0-7.0 linear gradient IPG strips (Bio-Rad, Hercules, CA, USA), and were rehydrated for 24 h at room temperature. The IEF program was performed using the process described by Zhang et al. [15]. Each separation was performed at least three times. The gels were stained using an improved CBB G-250 method [18], and were scanned with ImageScanner III (GE Healthcare).

Image analysis was performed using ImageMaster 2D Platinum Software (version 5.0, GE Healthcare). The optimized parameters (smooth, 6; min area, 70; saliency, 110) were used for automated detection and matching, then manual editing was conducted. The mean value of spot volumes from three biological replicates was calculated as the spot intensity. Volumes of the detected spots were normalized by dividing the sum of spot volumes for all detected spots, and then multiplied by 100 to obtain spot percentage (normalized spot volume, vol %). The protein spots with significant differences ($P < 0.05$) in buds and UV-B treated flowers were excised from the gel for further identification.

2.3 Identification of protein spots

The excised gel spots were digested and the resulting samples of extracted peptides were prepared for mass spectrum analysis using a previously described method [16]. After washing with ultrapure water, the gel spots were incubated in 50 μ L of destaining solution (50% acetonitrile, 25 mM ammonium bicarbonate) at 37 °C and dissolved in 10 μ L of alpha cyano-4-hydroxy-cinnamic acid saturated solution (alpha cyano-4-hydroxy-cinnamic acid in 0.1% trifluoroacetic acid and 50% acetonitrile) before being spotted onto the target plate. The obtained samples were analyzed using an ABI 4800 matrix-assisted laser desorption/ionization time-of-flight/time-of-flight (MALDI-TOF/TOF) plus mass

spectrometer (AB Sciex, Foster City, CA, USA). The UV laser was operated at a 200-Hz repetition rate at a wavelength of 355 nm. The accelerated voltage was 20 kV. Myoglobin digested by trypsin was used to calibrate the mass instrument with the internal calibration mode. Parent mass peaks range was 800-3500 Da and minimum S/N 20 were picked out for tandem TOF/TOF analysis. MS and MS/MS spectra were combined and submitted to Mascot (version 2.1, Matrix Science) by GPS 3.6 Explorer software (Applied Biosystems). The search parameters were described as follows: NCBI nr 20151214 (78002046 sequences; 28422168805 residues), taxonomy of Viridiplantae (Green Plants) (3406155 sequences), trypsin digest with one missing cleavage, fixed modifications of carbamidomethyl, none fixed modifications of oxidation, and MS tolerance of 0.15 Da, MS/MS tolerance of 0.25 Da. Known contaminant ions (keratin) were excluded. Mascot protein scores greater than 42 were considered well matched and the confidence interval of 95% ($p < 0.05$) was set as the significance threshold for Mascot protein scores.

2.4 Classification of proteins and protein-protein interactions

The protein ID was chosen by the highest score from the results of the database search, and the presence of at least two peptides were as one of the requirement. The Arabidopsis Information Resource (TAIR) (<http://arabidopsis.org>) was used to search for protein AGI codes [19]. These proteins were divided into several functional groups by MapMan software (version 3.5.1) (<http://mapman.gabipd.org/web/guest/mapman>) in conjunction with BLAST alignments. The functional interactions of the identified proteins were examined by the Search Tool for the Retrieval of Interacting Genes (STRING, version 9.1, <http://string-db.org/>) [20]. The required confidence (score) was set at medium confidence (0.04). The disconnect

nodes were hidden. The biological pathways of the identified proteins were deduced from the Kyoto Encyclopedia of Genes and Genomes (KEGG, <http://www.genome.jp/kegg/>).

2.5 *Extraction of metabolites*

Samples powder, 1g was extracted 3 times by resuspending in 100 mL of methanol for 60 min at 30 °C and ultrasonication. The resulting extracts were concentrated and dissolved in 10 mL of methanol. The liquid was filtered by 0.45 µm Millipore filters prior to LC-MS/MS analysis.

2.6 *LC-MS/MS apparatus and conditions*

LC analysis was performed using the Agilent 1200 series system (Agilent Corporation, CA, USA) equipped with a G1312B bin pump, a G1367C Hip-ALS auto sampler, and a G1316B TCC. Samples were separated on a reversed phase column (Agilent SB-Aq, 4.6 × 250mm, 5µm) with a set temperature of 40 °C and flow rate of 1 mL·min⁻¹. The mobile phase consisted of 0.4% formic acid in water (solvent A), and acetonitrile (solvent B): 0-25 min (10-20% B), 25-40 min (20-30% B), 40-40.01 min (30-95% B), 40.01-48 min (95% B), 48-48.01 min (95-10% B), and 48.01-55 min (10% B). The injection volume was 5 µL and detection wavelength was 254nm and 190-400 nm Scan spectrum by photodiode array detector (DAD) was used. The equipment used for mass spectrometry in the positive and negative ion mode was an Agilent 6410 triple quadrupole mass spectrometer (Agilent, Santa Clara, CA, USA). The post-column splitting to mass (1:1) was chosen to allow for the necessary lower flow-rate. The MS/MS conditions were listed as follows: electrospray ionization (ESI) interface, 35 psi nebulizer pressure, 350 °C dry gas temperature, 11 L·min⁻¹ gas flow rate, 4000 V capillary voltage, and scans between m/z 100 and 1200 with 500ms

scan time and 135 fragmentor.

Based on the comparison with the UV spectra, retention time, and m/z values of the standards, six metabolites were identified. And the total ion chromatogram (TIC) peak areas of these metabolites were measured for the relative quantification. Statistical significance was evaluated by the one-way ANOVA test. A p -value less than 0.05 was considered statistically significant.

2.7 1,1-diphenyl-2-picrylhydrazyl assay

To evaluate the free radical scavenging activity of extraction, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was performed. The volume of 0.4 mL of 1 $\text{mg} \cdot \text{mL}^{-1}$ diluted extract and 4.0 mL of $1.02 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$ DPPH methanol solution was incubated in the dark for 30 min at room temperature. The sample absorbance (A_i) in 517 nm was measured by spectrophotometer (Spectrum 752pc, Shanghai, China). The percent capability to scavenge the DPPH radical was calculated as $(A_c - A_i)/A_c \times 100\%$. A_c represents the absorbance of the DPPH solution itself. The samples from three biological replicates were analyzed.

2.8 Statistical analysis

The thresholds of identified protein spots were 1.5-fold changed abundance ratio and $p < 0.05$ with at least two peptide matches. Statistical analysis was performed by Statistical Product and Service Solutions software (v16.0; SPSS, Chicago, IL, USA). Statistical significance was evaluated by the Student's t -test when only two groups were compared and with the one-way ANOVA test when multiple groups were compared. All results are presented as mean $\pm$ SD from three independent biological replications. A p -value less than 0.05 was

considered statistically significant.

3. Results

3.1 L. japonica flowers and buds have differences in protein expression

To characterize the molecular processes occurring in flowers exposed to UV radiation, a comparative proteomic study on control buds, flowers, and UV-B exposed flowers of *L. japonica* was performed. Using a gel-based proteomic technique, more than 1000 CBB-stained flower protein spots were reproducibly detected, appearing on all of the 2D gels (Fig. S1 and Fig. S2). The 65 protein spots that displayed a change in intensity of over 1.5-fold in all replicates were then analyzed using MS. Fifty-four of these 65 proteins were positively identified using MALDI-TOF/TOF (Fig. 1, Table 1 and Table S1), where 42 and 12 proteins had increased and decreased expression, respectively. To determine the biological processes involved in flowering under UV radiation, the identified proteins were functionally classified into 19 categories using MapMan bin codes (Fig. 2). The primary functional categories were protein synthesis, postranslational modification, folding, degradation and activation (29.6%), photosynthesis (11.1%), stress (7.4%), amino acid metabolism (7.4%), oxidative pentose phosphate (5.6%), and secondary metabolism (5.6%). The abundance of the proteins related to major CHO metabolism, glycolysis, oxidative pentose phosphate, tricarboxylic acid cycle (TCA)/organic acid transformation, mitochondrial electron transport, secondary metabolism, hormone metabolism, stress, and cell division proteins were increased in flowers, while the abundance of proteins related to minor CHO metabolism, cell wall, redox, and RNA were decreased.

3.2 Protein-protein interactions and KEGG pathway assignments

To further analyze the differentially expressed proteins, predictions of protein-protein interactions and pathway were performed. The STRING database was used to calculate all potential direct interactions among 48 identified proteins. These interactions fell into eight closely connected categories, including photosynthesis, amino acid metabolism, TCA/organic acid transformation, oxidative pentose phosphate, stress, secondary metabolism, mitochondrial electron transport, and protein synthesis, degradation and modification (Fig. 3). The carbohydrate and energy metabolism related proteins are presented in a KEGG biosynthesis pathway (Fig. 4).

3.3 L. japonica flowers and buds have differences in metabolite

To elucidate the changes of metabolites between control buds and flowers and UV-radiated flowers, LC-MS/MS was employed for qualitative and relative quantification of metabolites. Positive and negative total ion chromatograms (TIC) were generated from methanol crude extracts of *L. japonica*, and 6 peaks with major changes were noted in the negative TIC (Fig. S3). Based on UV absorption (Fig. S4), the molecular formula, positive and negative mass spectrum and tandem mass spectrum fragments (Fig. S5 and Fig. S6), six compounds were identified as secologanic acid (peak 1), chlorogenic acid (peak 2), secoxyloganin (peak 3), secologanin (peak 4), aldosecologanin (peak 5), and isochlorogenic acid (peak 6) (Fig. S7 and Table S2).

The areas of the metabolite peak were measured to determine the relative quantity of metabolites and make comparisons between control buds and flowers, and UV-irradiated flowers (Fig. 5). The content of secologanic acid was significantly increased during flowering and UV radiation treatment ($P < 0.05$). By contrast, the content of aldosecologanin was

significantly decreased during flowering and UV radiation treatment ($P < 0.01$). The contents of chlorogenic acid, secologanin, and isochlorogenic acid were decreased after flowering ($P < 0.01$), while secoxyloganin displayed no significant changes. In addition, the content of isochlorogenic acid was increased after exposure to UV radiation compared to the control flowers, which had a level similar to buds. Secologanic acid, secoxyloganin, and isochlorogenic acid all increased after exposure to UV-B radiation compared to the control flowers, while, secologanin and aldosecologanin decreased and chlorogenic acid did not significantly change (Fig. 5).

3.4 Antioxidant activity of *L. japonica* buds and flowers extracts

To determine the antioxidant activity of *L. japonica* buds, control flowers, and UV-irradiated flowers, DPPH assays were performed. The scavenge capacity of control buds, control flowers, and UV-B flowers were 43.2%, 32.9%, and 55.3%, respectively. The antioxidant activity of the control buds and UV-B treated flowers was higher than the control flowers. Compared to the control flowers, UV radiation improved the antioxidant activity of *L. japonica* flowers (Fig. 6).

4. Discussion

4.1 Protein changes during flower opening under UV radiation

4.1.1 Carbohydrate metabolism and energy supply enhanced during flower opening with exposure to UV radiation

In most plants, carbohydrates, such as starch and sucrose, are rapidly hydrolyzed into glucose and fructose following flower opening [21, 22]. In non-green flowers, the carbon cost

of building the plant structure is higher than in green flowers, and photosynthesis in the floral crop plants may be enhanced [23]. In this present study, photosynthetic protein abundance increased, with the exception of the oxygen-evolving enhancer protein in *L. japonica* flowers compared to buds, which is consistent with previously published research. There are two anabolic and catabolic carbohydrate pathways, glycolysis and the pentose phosphate pathway (PPP), which take place primarily in the cytoplasm and plastids, respectively [24]. The ATP generated from glycolysis provides energy for physiological function, as well as serves as a primary precursor for further metabolism [25], while, PPP is an alternative pathway for generating NADPH and pentoses. In the present study, pfkB-type carbohydrate kinase was 2.73 times more abundant in UV-B treated flower than in buds, implying there is improved utilization of fructose during *L. japonica* flower opening. The TCA cycle is tightly related to glycolysis, provides energy, and is considered the hinge in carbohydrate, lipid, and proteins metabolism in higher plants [26]. The proteins identified in this study related to glycolysis, PPP, TCA cycle, and mitochondrial electron transport were 1.5-times more abundant in UV-B treated flowers than in buds, indicating that both glycolysis and the PPP were induced to provide energy and precursors for flowering.

4.1.2 Stress and protein synthesis, degradation and modification enzymes were activated

Heat shock proteins (HSPs) are typically considered proteins involved in responding to stresses, such as extreme temperature, drought, flooding, and UV radiation in plants [15]. HSP70 is a heat shock protein found in *Arabidopsis* that has been reported to influence seed maturation and germination [27]. In this present study, the abundance of HSP70 was higher in *L. japonica* flowers after UV radiation and, thus, may participate *in vivo* to protect and repair

photosystem II [28]. The abundance of ubiquitin and filamentation temperature-sensitive H (FtsH) in flowers exposed to UV radiation was higher than in control buds. Ubiquitin is involved in different developmental responses, including photoperiodic control of floral induction in *Arabidopsis* [29, 30]. In addition, the ubiquitin/proteasome system plays a central role in plant hormone signaling pathways, such as those involved in flowering [31]. In the present study, the abundance of ubiquitin proteins in flowers after exposure to UV radiation was twice that of buds, indicating that UV radiation may advance flowering. FtsH is induced by excessive illumination, accumulate in the grana and degrades the damaged reaction center-binding proteins of photosystem II [32]. Proteomic analysis showed that the abundance of FtsH in UV-B flower increased 1.83-times, thus helping protect the flowers from UV-B radiation.

4.1.3 Changes in cell structure related proteins

In flowers, the cell wall structure changes and loosens to allow for cell expansion during flower growth and opening [33]. The abundance of alpha-1,4-glucan-protein synthase, as a cell wall related protein, was decreased almost three times during flower opening in a manner similar to pear fruit maturation [34]. This might be due to the changing and loosening of the cell wall during *L. japonica* flower opening. However, the abundance of cell division control protein increased by more than seven times, which might be related to the pollen cell division and development during flowering.

4.1.4 Endogenous ethylene affected L. japonica flower opening

Plant hormones regulate every aspect of plant life and ethylene is an important plant hormone associated with plant growth, development, and senescence [35]. In the present study, the abundance of 1-aminocyclopropane-1-carboxylate oxidase (ACO), one of the rate

limiting enzymes in the ethylene synthetic pathway, increased by more than 4-fold in *L. japonica* flowers exposed to UV radiation compared to buds. Glutathione (GSH) modulates ACO to induce ethylene biosynthesis in *Arabidopsis* [36], and production of alanine aminotransferase was increased to produce more glutamate for GSH biosynthesis. In some plant species, ethylene affects flower opening. For example, flower opening of roses and cut carnation are influenced by endogenous ethylene, while, wintersweet flower opening and senescence is not [37]. Results from this study imply that *L. japonica* flower opening after exposure to UV radiation may be dependent upon the ethylene signal transduction pathway.

4.2 The *L. japonica* bud stage is optimal for harvesting

Proteomics results indicate that the abundance of 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (spot no. 856), a key enzyme in the shikimate pathway, displayed a 4-fold increase in *L. japonica* flower exposure to UV-B radiation. Secologanic acid and chlorogenic acid biosynthesis are both fed by shikimate pathway. During flower opening, the content of secologanic acid was increased, while, the content of chlorogenic acid was decreased, indicating a bias in monoterpenoid biosynthesis. However, chlorogenic acid is one of the major active constituent metabolites in *L. japonica*, and its content is different between flower buds and flowers [38]. In the present study, the contents of chlorogenic acid, secologanin, aldosecologanin, and isochlorogenic acid were decreased in opening flowers, and the antioxidant activity in flowers was lower than that of flower buds. This indicates that following flower opening, the medicinal value of *L. japonica* declines due to a decrease in active compounds. These results have significance by determining that the optimal harvest time is from flower bud blossoming through flowers

maturation. In addition, Yao et al. [39] suggested that UV-B radiation improves the active ingredients in chrysanthemum flowers. Compared to control flowers, the contents of secologanic acid, secoxyloganin, and isochlorogenic acid were significantly increased in UV-B treated *L. japonica* flower. This suggests that UV-B radiation improves the secondary metabolism in *L. japonica*.

4.3 The high antioxidant activity in L. japonica flowers exposed to UV radiation is related to metabolites and the ethylene signaling pathway

It has been shown that the antioxidant properties in *L. japonica* flowers in different stages are due to the different amounts of certain compounds [40]. In the present study, the antioxidant activity of the control flowers was 23.8% lower than the control buds, perhaps because the average chlorogenic acid and isochlorogenic acid content was lower in control flowers than control buds. However, the antioxidant activity of *L. japonica* flowers after exposure to UV-B radiation was 23.4% higher than in the control buds. As a signaling molecule, ethylene may affect the antioxidant system and photosystem II in plants in response to stresses, such as cold and heavy metals [41, 42]. The increased abundance of ethylene related proteins indicates that ethylene participates in the activation of antioxidant systems in *L. japonica* flowers exposed to UV-B radiation to enhance antioxidant activity.

5. Conclusion

L. japonica is a medicinal herb with widespread uses in folk medicine, and preparation of food and healthy beverages in Asian countries. To investigate the molecular mechanism

involved in flower opening under UV-B radiation, proteomic analyses of *L. japonica* buds and flowers exposed to UV-B radiation were performed. This study revealed an increased biosynthesis of endogenous ethylene, which is a hormone that could serve as a signal and mediate *L. japonica* flower opening under UV-B radiation. It may also activate the antioxidant system, resulting in higher antioxidant activity in flowers exposed to UV-B radiation. Delineating changes in the proteome and metabolome increases our understanding of the molecular mechanisms involved in flower opening under UV-B radiation, as well as reveals the optimal time to harvest *L. japonica*.

Accession Codes

The mass spectrometry proteomics data have been deposited with the ProteomeXchange Consortium ([http:// proteomecentral.Proteomexchange.org](http://proteomecentral.proteomexchange.org)) via the PRIDE partner repository [43] with the data set identifier PXD005324

Acknowledgments

This work was supported by the National Science Foundation of China (Grant No. 81473182, 81273394), the Research Project of the Chinese Ministry of Education (No.113037A). The Zhejiang Provincial Natural Science Foundation of China (Grant No. LZ14H280001).

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Table 1 Proteins with differential expression in UV-B treated *Lonicera japonica* flowers and control buds based on 2-DE proteomics

Spot No. ^a	Protein description	AGI code	M.P. ^b	Total Score ^c	Theor. Mr (Da)/pI ^d	Exper. Mr (Da)/pI ^e	NCBI accession no.	Fold change ^f	Function category ^g
Increase									
276	transketolase	AT3G60750	2	104	88.9/6.29	66.5/5.85	gi 587887488	3.42	photosynthesis
284	transketolase	AT3G60750	2	71	67.8/5.67	67.2/5.78	gi 357445031	3.20	photosynthesis
909	chloroplast sedoheptulose-1,7-bisphosphatase	AT3G55800	3	102	42.8/6.06	34.8/5.18	gi 118175929	2.37	photosynthesis
393	RuBisCO large subunit-binding protein subunit alpha, chloroplastic	AT2G28000	2	125	61.7/5.14	57.6/5.02	gi 464727	1.66	photosynthesis
422	RuBisCO large subunit-binding protein subunit beta, chloroplastic	AT1G55490	6	644	63.3/5.85	56.6/5.23	gi 2506277	3.24	photosynthesis
1046	pfkB-type carbohydrate kinase family protein	AT3G59480	3	147	35.5/4.94	32.7/4.99	gi 566211618	2.73	major CHO metabolism
413	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	AT1G09780	5	312	61.3/5.39	58.4/5.65	gi 587948991	1.75	glycolysis
674	phosphogluconate dehydrogenase family protein	AT3G02360	6	269	54.1/6.51	42.6/6.64	gi 566213382	1.90	OPP
1018	transaldolase	AT1G12230	3	166	40.1/7.01	32.5/5.34	gi 295687233	2.26	OPP
797	transaldolase family protein	AT5G13420	2	94	48.1/5.50	37.1/5.23	gi 566148667	2.14	OPP
880	succinyl-CoA ligase beta subunit	AT2G20420	3	204	45.5/6.41	35.1/5.42	gi 146215968	2.05	TCA / organic acid transformation
1012	isocitrate dehydrogenase V isoform 1	AT5G03290	4	329	40.7/6.38	32.3/5.93	gi 590630714	1.55	TCA / organic acid transformation
614	ATP synthase beta subunit	AT5G08680	7	717	59.3/5.56	44.4/5.48	gi 525291	1.51	mitochondrial electron transport
590	ATP synthase beta subunit 1	AT5G08680	6	403	60.0/5.90	44.5/5.32	gi 242129046	1.63	mitochondrial electron transport
514	Phospholipase C 3	AT3G03520	2	153	60.4/5.46	48.7/5.17	gi 587881725	3.24	lipid metabolism
575	alanine aminotransferase	AT1G17290	2	105	60.4/5.91	44.7/5.80	gi 9082270	1.87	amino acid metabolism
743	5-enol-pyruvylshikimate-phosphat	AT2G45300	3	154	48.0/5.19	40.2/5.41	gi 63334331	2.83	amino acid

	e synthase								metabolism
594	S-adenosyl-L-homocysteine hydrolase	AT4G13940	4	176	52.1/5.93	42.5/6.01	gi 3242075	8.45	amino acid metabolism
1247	1-aminocyclopropane-1-carboxylate oxidase	AT1G05010	3	94	36.5/5.26	29.2/5.68	gi 20152199	4.36	hormone metabolism
770	1-deoxy-D-xylulose 5-phosphate reductoisomerase	AT5G62790	2	125	51.4/6.04	39.1/5.36	gi 91241600	1.77	secondary metabolism
753	1-deoxy-D-xylulose 5-phosphate reductoisomerase	AT5G62790	6	331	51.1/5.64	38.4/5.64	gi 133753347	2.64	secondary metabolism
856	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase, chloroplastic	AT2G26930	2	62	37.9/5.04	36.3/5.43	gi 7227898	4.65	secondary metabolism
262	heat shock protein 70-2	AT5G49910	5	366	77.1/5.17	67.8/4.91	gi 15240578	1.90	stress
271	hsp70	AT5G02500	4	205	71.1/5.07	67.0/5.29	gi 20559	1.62	stress
247	heat shock protein 70	AT4G24280	4	321	75.5/5.15	67.8/4.93	gi 1143427	1.76	stress
244	heat shock protein 70	AT4G24280	3	245	74.4/5.41	67.8/4.96	gi 158635118	1.83	stress
1026	adenosine kinase isoform 2S	AT5G03300	3	191	37.9/5.17	32.5/5.47	gi 51949802	1.76	nucleotide metabolism
269	translation initiation factor eIF-3 subunit 7	AT4G20980	3	150	66.9/5.54	67.1/5.12	gi 15233469	3.73	protein synthesis
764	Helicase, C-terminal	AT3G13920	3	216	47.0/5.39	41.6/5.53	gi 124361137	3.10	protein synthesis
481	mitochondrial processing peptidase beta subunit	AT3G02090	2	59	58.6/6.42	49.3/6.22	gi 12802327	1.82	protein targeting
218	Acyl-peptide hydrolase-like protein	AT5G36210	3	90	75.9/5.34	69.5/5.09	gi 357459569	3.02	protein degradation
192	ATP-dependent Clp protease ATP-binding subunit ClpC	AT5G50920	7	517	103.6/6.36	76.1/5.54	gi 18423214	3.01	protein degradation
190	ATP-dependent Clp protease ATP-binding subunit clpC homolog, chloroplastic	AT5G50920	7	629	102.8/6.55	76.1/5.58	gi 461753	2.83	protein degradation
136	ATP-dependent Clp protease ATP-binding subunit ClpC	AT5G50920	5	275	103.6/6.36	80.3/5.58	gi 18423214	3.09	protein degradation
370	filamentation temperature-sensitive H 2A	AT2G30950	4	257	72.6/5.68	56.6/5.18	gi 413955061	1.83	protein degradation

	isoform 1								
447	Ubiquitin family protein	AT2G17200	2	69	50.5/4.87	53.6/4.87	gi 78708969	1.78	protein degradation
496	ubiquitin-like protein	AT2G17190	2	77	56.2/5.05	46.8/4.95	gi 224551496	1.89	protein degradation
1651	20S proteasome beta subunit C2	AT1G21720	3	283	31.5/6.27	19.4/5.23	gi 313585326	9.57	protein degradation
1238	20S proteasome alpha 6 subunit	AT5G42790	2	128	30.1/5.07	30.1/5.24	gi 22947842	4.08	protein degradation
482	Heat shock protein 60 isoform 1	AT3G23990	5	367	61.7/5.85	50.0/5.39	gi 590657227	2.48	protein folding
76	cell division control protein 48-A	AT3G09840	6	377	90.1/5.13	89.3/5.31	gi 15232776	7.57	cell
549	V-type proton ATPase subunit B2	AT4G38510	7	479	54.4/5.03	46.8/5.10	gi 15233891	2.73	transport
Decrease									
1381	oxygen-evolving enhancer protein 1-2	AT3G50820	3	122	35.2/5.92	26.9/5.44	gi 15230324	-8.58	photosynthesis
534	myo-inositol-1-phosphate synthase	AT2G22240	5	494	56.3/5.45	48.7/5.78	gi 18181983	-1.65	minor CHO metabolism
997	alpha-1,4-glucan-protein synthase	AT5G15650	3	164	41.7/5.75	32.5/6.03	gi 162463414	-2.83	cell wall
1602	flavodoxin-like quinone reductase 1 family protein	AT4G27270	3	87	17.6/5.59	20.0/6.25	gi 566154710	-2.49	lipid metabolism
1226	urease accessory protein G	AT2G34470	3	131	30.8/6.03	28.7/6.05	gi 13161908	-1.90	amino acid metabolism
2191	thioredoxin h	AT3G51030	2	104	12.9/5.29	12.3/5.31	gi 71534922	-2.83	redox
1594	inorganic pyrophosphatase	AT3G53620	2	113	24.4/5.56	23.1/5.46	gi 3885882	-2.12	nucleotide metabolism
2080	RNA-binding glycine-rich protein-1a	AT2G21660	3	238	15.8/5.31	14.1/5.17	gi 469070	-2.34	RNA
2075	EF hand family protein	AT1G12310	3	114	16.6/4.89	13.2/4.90	gi 48209896	-1.82	protein posttranslational modification
1451	20S proteasome alpha subunit E2 isoform 2	AT3G14290	6	481	26.1/4.70	24.3/4.78	gi 590621356	-1.73	protein degradation
1673	N-terminal nucleophile aminohydrolases (Ntn hydrolases) superfamily protein isoform 1	AT4G31300	2	196	25.2/5.18	19.4/60.4	gi 590571151	-4.80	protein degradation
1802	temperature-induced lipocalin	AT5G58070	3	112	21.7/6.10	17.7/6.35	gi 192911934	-1.88	transport

^a Spot ID was created by the Image Master 5.0 software and is labeled in Figure 1.

^b M.P., number of matched peptides.

^c The MASCOT score based on NCBI nr database search. Protein scores were derived from ion scores as a non-probabilistic basis by which to rank protein hits from the Mascot Search Results, individual ion scores > 43 indicate identity or extensive homology ($P < 0.05$).

^d Theoretical mass (kDa) and pI of identified proteins were retrieved from the protein database.

^e Experimental mass (kDa) and pI of identified proteins. Experimental values were calculated using Image Master 2D Platinum Software and standard molecular mass markers.

^f Ratio of protein levels (UV-B-treated flowers/control buds).

^g Protein function categorization using MapMan bin codes. Abbreviations: OPP, oxidative pentose phosphate; RNA, RNA processing/regulation of transcription.

Figure Legends

Fig. 1. *Lonicera japonica* flower protein differentiations in CBB G-250 stained gel images (pH range 4-7). Differentiated proteins are indicated using arrows and are numbered as displayed in Table 1.

Fig. 2. Functional categorization of differential proteins in *Lonicera japonica* flowers after exposure to UV-B radiation compared to buds. MapMan bin codes were applied for the categorization. Numbers of categorized proteins were shown in the graph. Black bars and white bars indicated the increased and decreased levels of proteins, respectively.

Abbreviations are as follows: TCA, tricarboxylic acid cycle; protein, protein synthesis, postranslational modification, folding, degradation and activation; cell, cell organization and vesicle transport.

Fig. 3. String visualization and protein-protein interaction network visualization of *Lonicera japonica*. Neighborhood, gene fusion, co-occurrence, co-expression, experiment, databases, and textmining were chosen as the active prediction methods.

Fig. 4. Protein changes in KEGG pathway during flower opening process under UV radiation associated with photosynthesis, carbohydrate metabolism, glycolysis, oxidative pentose phosphate, amino acid metabolism, TCA cycle, and secondary metabolism. Red color indicated increased proteins and blue color present decreased proteins. The abbreviations are as follows: ALT, alanine transaminase; CDME, 4-(cytidine

5'-diphospho)-2-C-methyl-D-erythritol; CDP-MEK,
 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase; DXP, 1-deoxy-D-xylulose 5-phosphate;
 DXR, 1-deoxy-D-xylulose-5-phosphate reductoisomerase; EPS,
 5-O-(1-carboxyvinyl)-3-phosphoshikimate; EPSPS, 5-enol-pyruvylshikimate-phosphate
 synthase; Erythrose-4P, erythrose 4-phosphate; Fructose-1,6P₂, fructose 1,6-bisphosphate;
 Fructose-6P, fructose 6-phosphate; Gluconate-6P, gluconate-6-phosphate; Glucose-6P,
 glucose 6-phosphate; Glyceraldehyde-3P, glyceraldehyde-3-phosphate; Glycerate-1,3P₂,
 3-phospho-D-glyceroyl phosphate; Glycerate-2P, 2-phospho-D-glycerate; Glycerate-3P,
 3-phospho-D-glycerate; IDH, isocitrate dehydrogenase; MEP, 2-C-methyl-D-erythritol
 4-phosphate; MIPS, myo-inositol-1-phosphate synthase; myo-Inositol-1P, myo-inositol
 3-phosphate; PCDME, 2-phospho-4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol; PEP,
 Phosphoenolpyruvate; PFK, pfkB-type carbohydrate kinase; PGAM,
 2,3-bisphosphoglycerate-independent phosphoglycerate mutase; PGD, phosphogluconate
 dehydrogenase; Ribulose-5P, ribulose 5-phosphate; S3P, 3-phosphoshikimate; SBPase,
 sedoheptulose-1,7-bisphosphatase; SUCLA, succinyl-CoA ligase; TAL, transaldolase; TKTL,
 transketolase; Xylulose-5P, xylulose 5-phosphate.

Fig. 5. Relative quantification of metabolites in control buds, control flowers, and UV-B flowers. Asterisk (*) indicates significantly different from control buds (* $P < 0.05$, ** $P < 0.01$). The symbol (#) indicates significantly different from control flowers (# $P < 0.05$, ## $P < 0.01$).

Fig. 6. Antioxidant activity of control buds and flowers, and UV-B exposed flowers

extracts. Asterisk (*) indicates significantly different from control buds (* $P < 0.05$, ** $P < 0.01$). The symbol (#) indicates significantly different from control flowers (# $P < 0.05$, ## $P < 0.01$).

- Oxidative pentose phosphate
- Amino acid metabolism
- Tricarboxylic acid cycle
- Protein
- Photosynthesis
- Stress
- Secondary metabolism
- Mitochondrial electron transport