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Biochemical traits and proteomic changes in postharvest flowers of medicinal chrysanthemum exposed to enhanced UV-B radiation

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ABSTRACT

The article studied UV-B effects on biochemical traits and proteomic changes in postharvest flowers of medicinal chrysanthemum. The experiment about UV-B effects on biochemical traits in flowers included six levels of UV-B treatments (0 (UV0), 50 (UV50), 200 (UV200), 400 (UV400), 600 (UV600) and 800 (UV800) $\mu W\ cm^{-2}$). UV400, UV600 and UV800 treatments significantly increased the contents of hydrogen peroxide, malondialdehyde and UV-B absorbing compounds, and the activity of phenylalanine ammonia lyase enzyme over the control. The contents of chlorogenic acid and flavone in flowers were significantly increased by UV-B treatments (except for UV50 and UV800). Two-dimensional gel electrophoresis was utilized to analyze proteomic changes in flowers with or without UV-B radiation. Results indicated that 43 protein spots (>1.5-fold difference in volume) were detected, including 19 spots with a decreasing trend and 24 spots with an increasing trend, and 19 differentially expressed protein spots were successfully indentified by MALDI-TOF MS. The indentified proteins were classified based on functions, the most of which were involved in photosynthesis, respiration, protein biosynthesis and degradation and defence. An overall assessment using biochemical and differential proteomic data revealed that UV-B radiation could affect biochemical reaction and promote secondary metabolism processes in postharvest flowers.

1. Introduction

The ozone layer thinning resulted in the increase of ultraviolet-B (UV-B, 280–320 nm) radiation on the Earth's surface, which has been recognized as one of the serious global environmental problems, and surface UV-B radiation will still continuously increase in the next few decades [1]. Because the DNA and proteins in the plants can absorb UV spectrum, they are easily damaged under high level of UV-B radiation. Many studies have indicated the deleterious effects of enhanced UV radiation on plants, such as reduced photosynthesis and growth, oxidative damages, and damage to DNA and proteins [2–4].

However, some interesting results about UV-B-beneficial effects on secondary metabolism processes in medicinal plants have been found in recent years [5–12]. Most of medically active ingredients in medicinal plants are secondary metabolites. In the experiment performed by Kumari and Agrawal, a higher dose of UV-B radiation (elevated 3.6 $kJ\ m^{-2}\ d^{-1}$ above ambient) produced a 17.6% reduction in biomass and increased phenolic compounds concentrations [5]. Germ et al. reported that enhanced UV-B radiation increased flavonoid and tannin content in the leaves of St. John's wort (*Hypericum perforatum* L.) [7]. Manukyan found that low dose of UV-B radiation increased polyphenol content of lemon catmint (*Nepeta cataria* L. f. citriodora), lemon balm (*Melissa officinalis* L.) and sage (*Salvia officinalis* L.) [8]. From previous studies to know, UV-B radiation could induce secondary metabolism processes, and increase active ingredients content in medicinal plants. However, enhanced UV-B radiation in these studies was applied during growth, which was difficult to operate (especially for regions with no reliable electricity source) and required a large investment in production. Therefore, further studies to explore the method for improving active ingredients content in medicinal plants by UV-B radiation were deemed necessary. In addition, the content of previous studies mainly focused on growth, development, and physiological traits about UV-B effects on medicinal plants. We still do not have a complete understanding of the molecular bases of these responses. The proteomic analysis becomes increasingly popular as it can reveal novel mechanisms of plant adaptations to stressful conditions, and have been successfully applied to examine the proteome responses to abiotic stresses, such as drought, chilling stress and salt stress [13–15]. However, there has been only limited research on UV-B effects on proteins in medicinal plants.

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Medicinal chrysanthemum (*Chrysanthemum morifolium* Ramat) is one of important export medicines in China. Flowers of medicinal chrysanthemum are used in traditional medicine where they play a role in improving liver function, decreasing inflammation, improving eyesight and serving other anti-inflammatory detoxification roles. In this experiment we studied the changes in biochemical traits and in proteome profile of medicinal chrysanthemum postharvest flowers exposed to enhanced UV-B radiation, in order to better understanding the responses of postharvest flowers to enhanced UV-B radiation.

2. Materials and methods

2.1. Plant material and experimental design

The research was conducted at Hebei University, Baoding, China. The seedlings of Huai chrysanthemum were obtained from Jiaozuo Chinese herbal medicine planting base, Henan province, China. The seedlings of same size were selected based on plant height (10.26 ± 0.64 cm) and planted into the farmland. Routine field managements were conducted during growth (from 4/15 to 11/15). Fresh flowers were collected when 2/3 of the tubular flowers in the flower head were in bloom.

2.2. Experiment 1

The harvested fresh flowers were immediately treated with UV-B radiation for 120 min. The experiment included six levels of UV-B radiation intensity: (1) 0 $\mu W\ cm^{-2}$ (UV0); (2) 50 $\mu W\ cm^{-2}$ (UV50); (3) 200 $\mu W\ cm^{-2}$ (UV200); (4) 400 $\mu W\ cm^{-2}$ (UV400); (5) 600 $\mu W\ cm^{-2}$ (UV600) and (6) 800 $\mu W\ cm^{-2}$ (UV800). After UV-B radiation, the samples were put into the incubator (25 °C, 80% humidity) for 12 h. Each treatment had five replications. The experiment mainly investigated the effects of UV-B radiation intensities on biochemical traits in postharvest flowers of chrysanthemum.

2.3. Experiment 2

The harvested fresh flowers were immediately treated with UV-B radiation for 120 min. The experiment included two levels of UV-B radiation intensity: (1) 0 $\mu W\ cm^{-2}$ (UV0); (2) 600 $\mu W\ cm^{-2}$ (UV600). After UV-B radiation, the samples were put into the incubator (25 °C, 80% humidity) for 12 h. Each treatment had five replications. The experiment mainly investigated proteomic changes in postharvest flowers treated with or without UV-B radiation.

2.4. UV-B treatments

Enhanced UV-B was produced by UV-B fluorescent lamps (40 W, 305 nm, Beijing Electronic Resource Institute, Beijing, China) mounted in metal frames. In the control, UV-B from the lamps was excluded by wrapping the tubes with 0.125 mm polyester film (Chenguang Research Institute of Chemical Industry, China), which transmits UV-A.

2.5. Determination of hydrogen peroxide and malondialdehyde

Hydrogen peroxide (H_2O_2) concentration was determined as described by Prochazkova et al. [16]. The 0.5 g sample was ground with 5 mL cooled acetone in a cold room (10 °C). Mixture was filtered with filter paper followed by the addition of 2 mL 5% titanium sulfate and 5 mL ammonium solution to precipitate the titanium–hydrogen peroxide complex. The reaction mixture was centrifuged at 10000*g* for 10 min. The precipitate was dissolved in 5 mL 2 mol L^{-1} H_2SO_4 and then recentrifuged. The absorbance of supernatant was measured at 415 nm by spectrophotometer.

The degree of lipid peroxidation in flower tissue was assessed by malondialdehyde (MDA) concentration. MDA concentration was measured as described by Feng et al. [17] with minor modification. The 0.5 g sample was extracted with 5 mL of 20% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 3500*g* for 20 min. 2 mL supernatant was added to 2 mL of 20% TCA containing 0.5% (w/v) thiobarbituric acid (TBA). The mixture was kept in boiling water bath for 30 min and then quickly cooled on ice. After refrigeration, the solution was centrifuged at 10000*g* for 15 min. The absorbance of supernatant was measured at 532 nm and 600 nm by spectrophotometer. The value for non-specific absorption at 600 nm was subtracted from the value at 532 nm. MDA concentration was calculated using MDA's extinction coefficient of 155 $mmol^{-1}\ cm^{-1}$. Results were expressed as nmol g^{-1} fresh weight (FW).

2.6. Determination of phenylalanine ammonia lyase activity

Phenylalanine ammonia lyase enzyme (PAL, EC 4.3.1.5) activity was expressed as the content of trans-cinnamic acid generated from L-phenylalanine by PAL. PAL activity was measured as described by Liu et al. with minor modification [18]. The 0.5 g sample was ground with 5 mL of borate buffer (pH 8.7) containing 5 mmol L^{-1} mercaptoethanol and 0.1% polyvinylpolypyrrolidone. Extracts were centrifuged at 10000*g* for 15 min at 4 °C. The supernatant was used for the analysis of PAL activity. The reaction mixture contained 0.2 mL of the supernatant, 1 mL of 0.02 mol L^{-1} L-phenylalanine and borate buffer (0.05 mol L^{-1}, pH 8.7) in a total volume of 3.0 mL, and it was incubated at 30 °C for 30 min. The reaction was stopped by the addition of 0.5 mL HCl (6 mol L^{-1}). The absorbance of solution was measured spectrophotometrically at 290 nm. The PAL activity was expressed as A h^{-1} g^{-1} FW.

2.7. Determination of UV-B absorbing compounds

UV-B absorbing compounds concentration was determined according to the method described by Nogués and Baker [19]. The 0.5 g sample was extracted with 5 mL of acidified methanol (methanol:HCl = 99:1, v:v) in the dark for 3 days. UV-B absorbing compounds concentration was estimated from absorbance at 285 nm. The results were expressed as A g^{-1} FW.

2.8. Determination of total flavone and chlorogenic acid

Total flavone concentration was determined according to the method described by He and Liu [20] with minor modifications. The 0.5 g dried sample was extracted with 20 mL of 50% ethanol solution in ultrasonic bath for 30 min. The supernatant was filtered to a 50 mL volumetric flask and the volume was made up with 50% ethanol. 5 mL extracted solution was mixed with 8 mL of 1.5% $AlCl_3$ and 4 mL acetic acid–sodium acetate (pH 5.5), and the mixture volume was made up with 50% ethanol to 25 mL. After 30 min, the absorbance of solution was measured at 420 nm using spectrophotometer. The flavone content was expressed as g rutin 100 g^{-1} dried weight (DW).

Chlorogenic acid concentration was measured according to the method described by Zhang et al. [21]. The 0.5 g dried sample was extracted with 20 mL of 50% methanol solution in ultrasonic bath for 30 min. The supernatant was filtered to 50 mL volumetric flask. The filtered residue was again extracted by the same method, and the volume was made up with 50% methanol to 50 mL. 5 mL extracted solution was mixed with 0.5 mL of 0.02 mol L^{-1} $FeCl_3$.