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The changes in quality ingredients of Qi chrysanthemum flowers treated with elevated UV-B radiation at different growth stages

Xiaoqin Yao [a,*], Jianzhou Chu [a], Xueli He [a], Chunhui Ma [a], Chao Han [b], Haiyu Shen [b]

[a] The College of Life Sciences, Hebei University, Baoding 071002, China
[b] Department of Biological Science, Handan College, Handan 056005, China

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ABSTRACT

The paper mainly reported the changes in quality ingredients of Qi chrysanthemum flowers treated with elevated UV-B radiation at different growth stages. The experiment included two levels of UV-B radiation (ambient UV-B, a 10% increase in ambient UV-B). Elevated UV-B radiation was carried out for 10-days during seedling, vigorous growth, bud and flower stages of Qi chrysanthemum, respectively. Elevated UV-B treatments applied during four development stages did not significantly affect flower yield, the rate of superoxide radical production and malondialdehyde concentration in flowers, while increased free amino acid concentration. The amino acid concentration induced by elevated UV-B radiation applied during bud stage was higher than that during the other stages. Elevated UV-B radiation applied during vigorous growth (except for flavone), bud and flower stages of chrysanthemum significantly increased hydrogen peroxide concentration, phenylalanine ammonia lyase enzyme activity, vitamin C, chlorogenic acid and flavone concentrations in flowers. These results suggested that active and nutritional ingredients in flowers of chrysanthemum could be increased by elevated UV-B radiation applied during the later growth stages of chrysanthemum. The paper supplied a simple and environmental-friendly method to improve quality of medicinal plants.

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]. Elevated UV-B radiation can indeed negatively affect growth, physiology and productivity of plants [2–7], which have been primarily done on agricultural crops or key species in natural ecosystems.

At present, a few literatures have reported the effects of elevated UV-B radiation on medicinal plants. Nishimura et al. found that elevated UV-B radiation suppressed the growth and anthocyanins production of *Perilla* seedlings [8]. 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 [9]. Wen et al. reported that short-term elevated UV-B radiation induced a significant increase in berberine concentration in root of *Coptis chinensis* [10]. From previous researches to know, elevated UV-B radiation could affect secondary metabolism processes in medicinal plants. To our knowledge, there have been limited efforts to know the responses of medicinal plants to short-term elevated UV-B radiation applied during different growth stages. So, more works should be done for better evaluation of the application of UV-B radiation in medicinal plants.

It is widely believed that elevated UV-B radiation can induce the synthesis of UV-B absorbing compounds in plants (mainly phenolic and flavonoids compounds) [11–14], which can strongly absorb UV-B radiation of sunlight, and mitigate UV-B-induced damages in plants to some extent. UV-B absorbing compounds induced by elevated UV-B radiation are secondary metabolites produced in secondary metabolism processes. Most of active ingredients in medicinal plants are secondary metabolites. Modern pharmacological studies have shown that pharmacological effects of many medicinal plants are relevant with active ingredients content [15,16]. However, active ingredients concentrations in many medicinal plants are not high enough to have a health-promoting effect. Therefore, it will be an interesting study about improving active ingredients level in medicinal plants by elevated UV-B radiation technology which is a simple and environmental-friendly method.

Medicinal chrysanthemum (*Chrysanthemum morifolium* Ramat) is commonly used in traditional Chinese medicine, and is also one of important export medicines in China. Flowers of medicinal

* Corresponding author.
E-mail address: yaoxiao301@126.com (X. Yao).

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chrysanthemum with scattered wind heat, removing liver-heat, improving eyesight, and other anti-inflammatory role of detoxification are used as medicine [17]. Flavonoids and chlorogenic acid are main active ingredients in flowers of medicinal chrysanthemum. The paper mainly studied short-term elevated UV-B radiation effects applied during different growth stages on active and nutritional ingredients in flowers of Qi chrysanthemum, in order to better know the roles of short-term elevated UV-B radiation in medicinal plants. We suggested that (1) short-term UV-B radiation applied during different growth stages could not inhibit flower yield of Qi chrysanthemum, and (2) elevated UV-B radiation applied during the later growth stages of chrysanthemum could increase active and nutritional ingredients content. This will be helpful for further research about the application of UV-B radiation on medicinal plants.

2. Material and methods

2.1. Plant material and experimental design

A pot experiment was conducted at Hebei University, Baoding, China. The seedlings of Qi chrysanthemum were obtained from Anguo Chinese herbal medicine planting base, Hebei province, China. The seedlings of the same size were selected based on plant height. Seedlings were transplanted into the plastic pots (30-cm diameter and 40-cm depth, one seedling per pot) with an average 9 h photoperiod and a daily average 950 μmol m^{-2} s^{-1} photosynthetic photon flux density from May to October, 2012. The substrate used for growing the seedlings was sieved topsoil from farmland. The content of soil organic matter, soil available N, P, K, and soil pH was 15.06 g kg^{-1}, 60.29 mg kg^{-1}, 25.36 mg kg^{-1}, 110.56 mg kg^{-1}, and 8.0, respectively. N (0.4 g kg^{-1}), P (0.2 g P_2O_5 kg^{-1}) and K (0.3 g K_2O kg^{-1}) were added to the soil as base fertilizers before transplanting to meet the nutrient demand of chrysanthemum growth. Routine field management was conducted during growing stages.

Elevated UV-B treatments were carried out for 10-days during different growth stage of Qi chrysanthemum. After 10-days exposure, the chrysanthemum was grown under ambient UV-B radiation. The experiment included five treatments: (1) ambient UV-B radiation (CK); (2) elevated UV-B radiation during seedling stage (S, 24/5-2/6; temperature, 18 °C; rainfall, 90 mm; photoperiod, 9 h); (3) elevated UV-B radiation during vigorous growth stage (V, 10/7-19/7; temperature, 25 °C; rainfall, 170 mm; photoperiod, 10 h); (4) elevated UV-B radiation during bud stage (B, 20/9-29/9; temperature, 17.5 °C; rainfall, 120 mm; photoperiod, 9 h); and (5) elevated UV-B radiation during flower stage (F, 10/10-19/10; temperature, 15.5 °C; rainfall, 50 mm; photoperiod, 8 h). Each treatment had four blocks and each block had three pots.

2.2. UV-B treatments

Elevated UV-B radiation was produced by UV-B fluorescent lamps (40 W, 305 nm, Beijing Electronic Resource Institute, Beijing, China) mounted in metal frames with minimum shading. In ambient UV-B frames, 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. In elevated UV-B frames, lamps were wrapped with 0.10 mm cellulose diacetate film, which transmits both UV-B and UV-A. Guard rows were provided between the frames in order to prevent the UV-B radiation from reaching the control seedlings. The spectral irradiance from the lamps was determined with an Optronics Model 742 (Optronics Laboratory Inc., Orlando, FL) spectroradiometer, and was calibrated according to the generalized plant action spectrum and normalized at 300 nm to obtain effective radiation (UV-B_{BE}) [18]. The elevated UV-B_{BE} dose was 0.82 kJ m^{-2} day^{-1} (a 10% increase above ambient UV-B_{BE}) in addition to the effective 8.20 kJ m^{-2} day^{-1} UV-B_{BE} (ambient UV-B_{BE}). All pots also received natural solar radiation. Seedlings were irradiated for 8 h (from 9:00 to 17:00) daily centered on the solar noon.

2.3. Sample preparation

Flowers were taken and frozen to determine the physiological changes, when petals fully turned white, and about 50% flowers were in bloom. The other flowers were harvested and dried to constant mass. The dried samples were ground to determine active and nutritional ingredients in flowers.

2.4. The rate of superoxide radical production, hydrogen peroxide and malondialdehyde concentrations

The rate of superoxide radical production (O_2^-) was measured as described by Ke et al. by monitoring the nitrite formation from hydroxylamine in the presence of O_2^- [19]. 0.5 g fresh sample was homogenized with 1.5 mL of 65 mmol L^{-1} potassium phosphate (pH 7.8) and centrifuged at 5000*g* for 10 min. The incubation mixture contained 0.45 mL of 65 mmol L^{-1} phosphate buffer (pH 7.8), 0.5 mL of 10 mmol L^{-1} hydroxylamine hydrochloride, and 0.5 mL of the supernatant. After incubation at 25 °C for 20 min, 0.5 mL of 8.5 mmol L^{-1} sulfanilamide and 0.5 mL of 3.5 mmol L^{-1} α-naphthylamine were added to the incubation mixture. After reaction at 25 °C for 20 min, the absorbance of solution was measured by spectrophotometer at 530 nm. A standard curve with NO_2^- was used to calculate the production rate of O_2^- from the chemical reaction of O_2^- and hydroxylamine.

Hydrogen peroxide (H_2O_2) concentration was determined according to Prochazkova et al. [20]. 0.5 g fresh 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 of 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 according to Feng et al. [21] with minor modification. 0.5 g fresh 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. 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, respectively. 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 μmol g^{-1} fresh weight (FW).

2.5. Phenylalanine ammonia lyase enzyme

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 [22]. 0.5 g fresh 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