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Responses of the flavonoid pathway to UV–B radiation stress and the correlation with the lipid antioxidant characteristics in the desert plant *Caryopteris mongolica*

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ABSTRACT

Caryopteris mongolica is a dwarf shrub mainly found in grassland and desert areas of north-west China, and which can survive severe environmental stress. This study aimed to assess the responses of the flavonoid pathway to UV-B radiation treatments and its correlation to the lipid peroxide and antioxidant systems in *C. mongolica*. In UV-B radiation experiments, plants were exposed to UV-B radiation treatments with a intensity of 30 J/s for 1, 4 and 24 h, respectively. A control group without UV-B radiation treatment was also used. The chlorophyll fluorescence parameters, contents of chlorophyll and carotenoid, levels of lipid peroxidation, activities of antioxidant system enzymes, accumulations of total flavonoids and anthocyanins, and activities of phenylalanine ammonialyase (PAL) and chalcone isomerase (CHI) under different UV-B radiation treatments were investigated. The correlations between products and key enzymes in the flavonoid pathway and the lipid peroxide and antioxidant systems were also analyzed. The results showed that chlorophyll fluorescence parameters decreased within 24 h of treatment. The chlorophyll contents decreased within 4 h and remained stable after 24 h. Carotenoid content significantly increased. The level of MDA, the activities of superoxide dismutase (SOD), ascorbate peroxidase (APX) and peroxidase (POD) and the contents of total flavonoids and anthocyanidins increased, while catalase (CAT) activity decreased under UV-B stress. The activities of PAL and CHI also increased with the increased content of total flavonoids. The flavonoid products anthocyanidins had a significant positive correlation with MDA level, as well as the activities of antioxidant enzyme SOD. In conclusion, UV-B radiation induced the degradation of photosynthetic pigments and decreased photochemical efficiency of Photosystem II; increased the contents of MDA, total flavonoids and anthocyanidins; and also enhanced activities of antioxidant enzymes (SOD, APX and POD) and key enzymes (PAL and CHI) in the flavonoid pathway in *C. mongolica*. Thus, we speculate that the flavonoid pathway were involved in the regulation of stress resistance in *C. mongolica*.

1. Introduction

Due to the depletion of ozone layer, elevated UV-B radiation is becoming one of the most important components of terrestrial radiation that plants are exposed to at the Earth's surface. Increased UV-B radiation significantly affects growth, development, morphology, self-protection and other physiological and biochemical characteristics of plants [1]. It is important to consider the potential effects of the increased UV-B radiation on photosynthetic systems, activity of enzymes, membrane systems and secondary metabolism in plants. Studies have indicated that UV-B radiation can lead to lipid peroxidation and accumulation of MDA [2]. Several studies have demonstrated that UV-B radiation decrease the growth, chlorophyll content and expression of genes involves in photosynthesis [3]. Plants adapt by using different strategies which play a protective role against potential damage by UV-B irradiation during their life cycle. UV-B radiation increase the content of antioxidants and activities of antioxidant enzymes [4,5]. The leaf thickness and the concentration of phenolic compounds in leaf were found to increase under enhanced UV-B radiation [6,7]. Enhanced UV-B radiation can induce the expression of flavonoid pathway genes and accumulation of UV-absorbing flavonoids [8]. In addition to acting as pigments in plants, flavonoids play a vast array of other biological functions, especially in stress resistance [9].

Caryopteris mongolica, a native of Mongolia and western China, is a type of sub-shrub belonging to Verbenaceae, Caryopteris. *C.*

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mongolica can survive in severe extreme environment, show tolerance to drought, salinity, extreme temperature and UV radiation [10]. Consequently, there is an interest in studying stress resistance in shrubs. *C. mongolica* is one of the most promising plants for such studies. In present paper, we analyzed the correlations between products and key enzymes in the flavonoid pathway and the lipid peroxide and antioxidant systems under UV-B radiation of 24 h. According to these studies, we hope to explain the protective role of flavonoids after UV-B radiation in *C. mongolica*, to explore the regulation mechanism of flavonoids involve in the stress resistance further.

2. Materials and methods

2.1. Materials and treatments

Seeds of *C. mongolica* were obtained from the Baita foothills of Lanzhou City, Gansu, China. The seeds were treated with 1% hypochlorite potassium for 10 min, washed with tap water, soaked in water for 12 h, and then planted in individual 9-L plastic pots containing soil. The pots were placed in a greenhouse (16/8 h photoperiod; 25 °C/12 °C day/night; PAR 150 mol/m^2/s, relative humidity 30%). In the UV-B radiation experiments, plants were exposed to UV-B radiation treatments with a intensity of 30 J/s for 1, 4 and 24 h, respectively. A control group without UV-B radiation treatment was also used. The UV-B lamps were made in the Wuxi Jinhua test equipment company, China, and the irradiation dose was measured with a UV-B radiation detector produced by Beijing Normal University. Leaves from plants for each treatment were immediately placed in liquid nitrogen and stored at −80 °C prior to extraction.

2.2. Methods

The level of lipid peroxidation in leaf samples was determined in terms of MDA content according to the method of Zhang [11]. The activities of antioxidant system enzymes were measured by the methods of Liu [12]. The relative contents of total flavonoids and anthocyanidins were measured according to the methods of Liu [13]. CHI activity was measured with the method of Li [14]. PAL activity was measured and calculated by a modified method of Zhang [11]. The chlorophyll content and chlorophyll fluorescence parameters were measured according to the method of Liu [15]. A PAM-2000 chlorophyll fluorometer was used. Analysis of F_0 and F_m were conducted after 30 min of dark-adaption.

All data were presented as means ± standard deviations of three determinations. Statistical analyses were performed using the Student's *t*-test and one-way analysis of variance. Multiple comparisons of means were done by the LSD (least significant difference) test. Statistical assessments of differences with the same letter between mean values were performed by Duncan's multiple range test at $p \leqslant 0.05$.

3. Results

3.1. Effect on chlorophyll fluorescence parameters and photosynthesis pigments

Chlorophyll fluorescence parameters and photosynthetic pigment content is shown in Fig. 1. Under UV-B radiation treatments, little changes in the value of F_0, and variations between different treatments were not significant (Fig. 1A). The values of F_m decreased as the UV-B treatment time increased (Fig. 1B). The values of F_v/F_m (photochemical efficiency of Photosystem II) displayed decreases, and the variation were significant after 24 h (Fig. 1C).

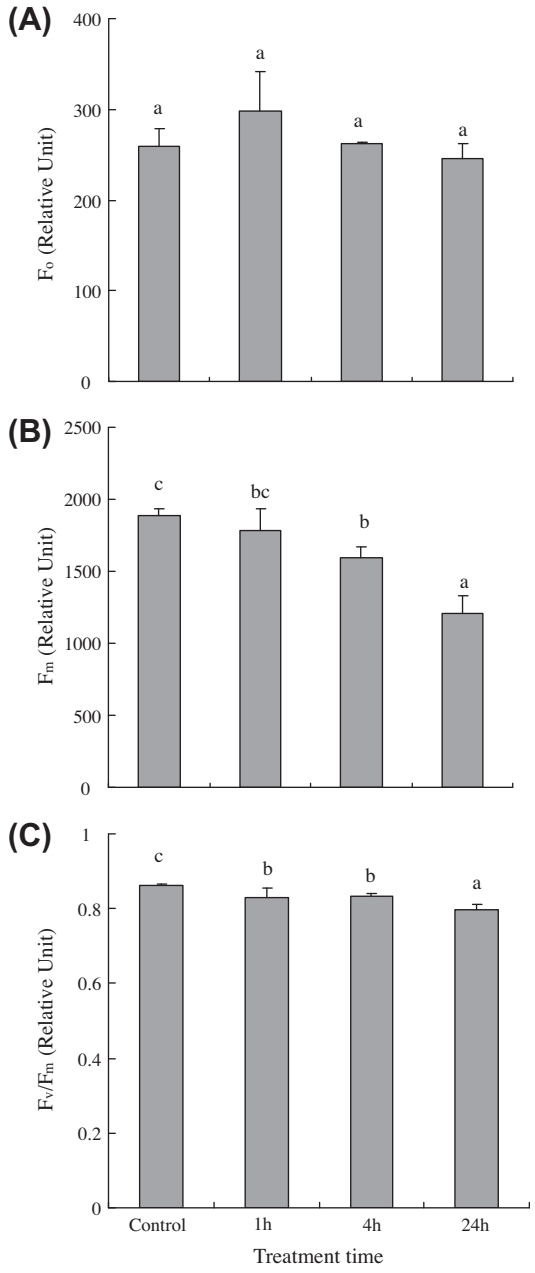

Fig. 1. Effects of UV–B radiation treatment (1 h, 4 h, 24 h) on chlorophyll fluorescence parameters in *Caryopteris mongolica* Bunge. Note: (A) F_o; (B) F_m; (C) F_v/F_m. Little letters indicate significant differences ($p < 0.05$) among the different treatment time under UV–B expose.

The content of chlorophyll reflects the photosynthesis capacity of plants. The content of chlorophyll decreased under UV-B stress, and had a negative correlation with treatment time (Fig. 2A). Chla, Chlb contents, and the ratio of Chla/Chlb displayed significant decreases within 4 h (Fig. 2B). The content of chlorophyll showed a decline trend within 4 h, and kept stable after 4 h. The caretonoid content exhibited an increase trend during the experiment (Fig. 2A). In the leaves of *C. mongolica*, the ratio of caretonoids/Chl revealed a significant increase trend (Fig. 2B).

3.2. Effect on MDA and antioxidant enzymes

MDA content is usually used to measure the extent of lipid peroxidation. The content of MDA were not changed within 1 h of UV-B treatment in *C. mongolica*. However, the content of MDA