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Original Article

Physiological Responses of *Melissa officinalis* Seedling to Different Bands of Ultraviolet (UV) Radiation

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ABSTRACT

Ultraviolet (UV) rays are part of solar radiation, which induces physiological processes mediated by photoreceptors. This research investigated the effect of short-term exposure to different bands of UV rays (UV-A, UV-B, and UV-C) with the wavelengths of 365, 312, and 254 nm on *Melissa officinalis* L. seedlings, respectively. The amount of Chl. *a*, *b*, total, carotenoids, anthocyanin, UV absorbing compounds, and proteins in the leaves were measured. The control group was not treated with any UV rays. The total chlorophyll content decreased under UV-A (19%), UV-B (23%), and UV-C (49%) treatments. The lowest amount of Chl. *a*, *b*, and total chlorophyll belonged to UV-C with about 50, 46, and 49%, respectively. The carotenoid contents significantly decreased under UV-A and UV-C treatments. The ratio of carotenoids to total chlorophyll increased under UV-B (17%), and UV-C (45%) treatments compared with the control. The reduction of carotenoids content under UV treatment was less than that of the chlorophyll. The amount of anthocyanin and UV-absorbing compounds increased under different bands of UV radiation. The UV-A and UV-B increased protein contents, while UV-C treatment decreased protein content. This research indicated that the UV-B ray stimulate plant antioxidant system helping to plant survival under UV stress.

INTRODUCTION

Plants can perceive the quality and quantity of light through different photoreceptors. Light controls plant growth and developmental processes, such as seed germination, plant etiolation, plant architecture, stomatal movement, and growth and flowering via creating a complex network of transcriptomes [1]. Ultraviolet (UV) rays are part of the light rays that are absorbed by plants. Ultraviolet rays are classified based on their wavelengths into UV-C (200-280 nm), UV-B (280-320 nm), and UV-A (320-390 nm) [2, 3]. Ultraviolet rays cause various changes in plants. It was reported that moderate UV-B stress induced photo-morphogenesis responses in plants. While intense UV-B or UV-C stress resulted in the inhibition of the cell cycle, disruption of mitochondrial and chloroplast function, damaging the lipids and DNA, and interfering with some important physiological processes, such as photosynthesis and programmed cell death (PCD) [1]. The physiological changes are induced by UV-C and UV-B rays via UVR8 (UV RESISTANCE LOCUS8) protein that acts as a UV-B photoreceptor in plant cells [4, 5]. It was reported that UVR8 induced UV-absorbing compounds, scavenged ROS, and activated defense responses against UV radiation [1, 4-6]. The plants' response to UV rays depended on the plant species' sensitivity threshold, radiation intensity, wavelength, and exposure duration. Broad-leaved plants are more sensitive than coniferous due to their higher exposure surfaces, which results in receiving higher portions of UV rays [7, 8]. In most plants, the amount of total protein decreases under UV radiation treatment. While a novel 34 kDa polypeptide was increased in UV-treated potato plants. This stress-induced polypeptide led to thylakoid stability and prevented oxidative stress [9]. Plants, cells, and intracellular structures are protected against UV stress by antioxidant enzymes, such as catalase, ascorbate peroxidase, superoxide dismutase, and glutathione reductase. In addition to

antioxidant enzymes, many phenylpropanoid compounds including hydroxycinnamic acid derivatives, glutathione, ascorbic acid, carotenoids, phenolic compounds, flavonoids, and anthocyanins protect the cell membrane and intracellular structures against oxidative stress by scavenging oxygen free radicals [2, 3, 6, 10].

The lemon balm plant (*Melissa officinalis*) is a medicinal plant from the Lamiaceae family. The plant contains bio-compounds that are responsible for strengthening memory and nerve system, and act as tranquilizer and anti-bloating [11].

MATERIALS AND METHODS

The ultraviolet treatments were applied in a rectangular Plexiglas chamber (50×40×90 cm) equipped with 8 watts UV-A (365 nm), UV-B (312 nm), and UV-C (254 nm) lamps (Philips company, Actinic BL model, Poland). The treatments were applied two times for each replicate.

The experiments were conducted at plant research facility at Payame Noor University in 2022. *M. officinalis* plant seeds were obtained from Pakan Bazr Company, Isfahan, Iran. The seeds were washed and planted in containers containing coarse, medium, and fine perlite (1:1:2). After germination, the plantlets were watered once a week with one-fifth of Hoagland nutrient solution until the appearance of the third leaf [12]. After the second leaves were fully expanded, the plants were transferred to 700 ml pots containing washed perlite. The plants were placed in a growth chamber with a photoperiod of 16 h light at 25±2 °C and 8 h darkness at 17±2 °C. The plantlets let grow to reach the seven-leaf stage. The lemon balm plants were exposed to ultraviolet rays as follows: UV-A and UV-B for 20 minutes and UV-C for 3 minutes at a distance of 30 cm from UV lamps for one week. The control plants were exposed to visible (full spectrum) light under the same conditions. At the end of the treatments, photosynthetic pigments (Chl. *a*, *b*, total, and carotenoids), anthocyanins, UV-absorbing compounds, and the proteins contents were assayed.

Photosynthetic Pigments

The photosynthetic pigments, including Chl. *a*, *b*, total Chl., and carotenoids (Car) were measured as described by Lichtenthaler, H.K.C. Buschmann [13]. For Chl. assays the pigments extract was measured at 665.2 and 652.4 nm wavelengths. The 470 nm wavelength was used for Car assays using a spectrophotometer (UV-1601, Rayleigh, China). Pure methanol was used as the blank.

Anthocyanins

The anthocyanins content was assayed with the acidified methanol method reported by Wagner, G.J. [14].

UV Absorbing Compounds

The amount of UV-absorbing compounds was measured by the method of Day, T., G. MartinT. Vogelmann [7]. The leaves were ground in acidic methanol (pure methanol:hydrochloric acid:water with a ratio of 1:9:90) and then heated for 10 min at 80 °C. The absorption of the supernatant extract was read at the wavelength of 300 nm.

Total Protein Content

The Bradford, M.M. [15] method by Coomassie Blue G-250 protein assay reagent was applied to determine total protein content. Bovine serum albumin was used as standard.

Statistical Analysis

Three pots each containing four plants were considered for each treatment. All experiments were performed in a completely randomized design (CRD) with three replications (n=3). The analysis of variance (ANOVA) and SPSS software were used for the statistical analysis of data and correlation between parameters. Mean data were compared by Duncan's multiple range test ($p \leq 0.05$).

RISULT AND DISCUSSION

Chlorophyll and Carotenoids

The ANOVA showed that the amount of Chl. *a*, *b*, and total Chl. in all of the plants treated with UV was significantly decreased compared to the control (Table 1). The reduction in Chl. *a* under the influence of UV-A, UV-B, and UV-C was 18, 19 and 50%, respectively. While the amount of Chl. *b* under the influence of UV-A, UV-B, and UV-C decreased by 20, 15 and 46%, respectively. The highest amount of decline in total chlorophyll content belonged to UV-C with about 48.8% compared to the control.

These results are consistent with earlier studies on *Capsicum annuum* [16], *Arabidopsis thaliana* [17], and *Caryopteris mongolica* [18]. It has been reported that UV-B caused a 39.49% decline in photosynthetic pigments' content in bell pepper

leaves compared to control plants [16]. The severity of the reduction in chlorophyll content under UV-B radiation was correlated with the duration of treatment in *C. mongolica* [18]. The effects of ultraviolet rays on chlorophyll are different depending on the plant species. The UV rays led to photo-oxidation of chlorophyll, increasing the amount of chlorophyllase enzyme activity and preventing the biosynthesis of these pigments in plants [10, 19, 20]. The carotenoids content in seedlings treated with UV-A and UV-C decreased by 15 and 28%compared to the control, respectively. While in the plants treated with UV-B, no significant difference was observed in carotenoids content compared to the control plants. There was a positive correlation between carotenoids content and Chl. *a*, *b*, and total contents in the treated plants (R^2: 0.80, 0.92, and 0.86), respectively (Table 2). The reduction of carotenoids content in UV treatments was less than that of the chlorophyll. The decrease in the carotenoids to total chlorophyll ratio under the influence of UV-B and UV-C was 17 and 45%, respectively.

Carotenoids contribute to the plants' survival against oxidative stress by protecting against chlorophyll oxidation in photosystems and light-harvesting complexes by ROS generation [20]. There are conflicting reports about the effect of UV rays on carotenoids content. It was reported that the carotenoids content in soybean plants [21] and *Caryopteris mongolica* [18] decreased under UV-B and UV-C treatment. While León-Chan, R.G., M. López-Meyer, T. Osuna-Enciso, J.A. Sañudo-Barajas, J.B. HerediaJ. León-Félix [16] for bell pepper plant and Badmus, U.O., G. Crestani, N. Cunningham, M. Havaux, O. UrbanM.A. Jansen [17] for *Arabidopsis thaliana* plants reported that one of the adaptive responses to ultraviolet rays was an increase in carotenoids content. Carotenoids can perceive high energy and short wavelengths of light spectrum and convert singlet oxygen into triplet oxygen, protecting plants from damages caused by oxidative stress [2, 10]. Moreover, carotenoids cause oxygen consumption and protection of chlorophyll against photo-oxidation through the xanthophyll cycle with epoxidation and de-oxidation reactions. Carotenoids play an important role in protecting chlorophyll from photooxidation and absorbing and transferring light energy to Chl. *a* [18, 22]. Liu, M., B. Cao, S. ZhouY. Liu [18] reported that the changes in carotenoids/Chl. ratio under UV-B treatment was an indication of the photosynthetic capacity decline, while the resistance potential was induced. The decrease in the carotenoids content in UV-A and UV-B is due to the conversion of carotenoids into abscisic acid, which occurs during many environmental stresses [17].

Table 1 Impacts of different bands of ultraviolet rays on the amount of chlorophyll *a* (Chl. *a*), chlorophyll *b* (Chl. *b*), total chlorophyll (T. Chl.), carotenoid (Car) and ratio of carotenoids to total chlorophyll of *M. officinalis* seedlings. The data are mean of three replicates ±StD. Different letters indicate significant differences using Duncan's test.

Treatment	Chl. *A* (mg/g FW)	Chl. *B* (mg/g FW)	T. Chl. (mg/g FW)	Car. (mg/g FW)	Car. / T. Chl.
Control	6.18±0.39 a	4.66±0.19 a	10.84±0.41 a	5.48±0.53 a	0.51±0.05 c
UV-A	5.04±0.44 b	3.73±0.28 b	8.77±0.72 b	4.67±0.28 bc	0.53±0.02 bc
UV-B	4.40±0.26 b	3.98±0.12 b	8.38±0.31 b	5.04±0.25 ab	0.59±0.01 b
UV-C	3.06±0.25 c	2.50±0.17 c	5.55±0.40 c	3.93±0.45 c	0.73±0.04 a

Table 2 Correlation matrices showing relationships between measured parameters of *M. officinalis* seedlings in response to different bands of ultraviolet rays.

	Chl. *a*	Chl. *b*	T. Chl.	Car.	Car/ T. Chl.	Antho	UVAC
Chlorophyll *a* (Chl. *a*)	1	-	-	-	-	-	-
Chlorophyll *b* (Chl. *b*)	0.922 **	1	-	-	-	-	-
Total Chl. (T. Chl.)	0.987 **	0.972 **	1	-	-	-	-
Carotenoid (Car.)	0.798 **	0.916 **	0.863 **	1	-	-	-
Ratio Car. to T. Chl.	-0.928 **	-0.869 **	-0.922 **	-0.649 *	1	-	-
Anthocyanin (Antho)	-0.689 *	-0.45	-0.603 *	-0.379	0.487	1	-
UV absorbing compound (UVAC)	-0.902 **	-0.923 **	-0.928 **	-0.836 **	0.846 **	0.518	1
protein	0.450	0.577 *	0.512	0.514	-0.610 *	0.191	-0.517

**and * Correlation is significant at the 0.01 and 0.05 levels.

Anthocyanins and UV-absorbing Compounds

The anthocyanins content showed an increase of 32, 67, and 43% in the seedlings treated with UV-A, UV-B, and UV-C compared to the control, respectively (Figure 1). These results are consistent with other studies on tomato plant [23], three varieties of wheat [24], and lettuce [25].

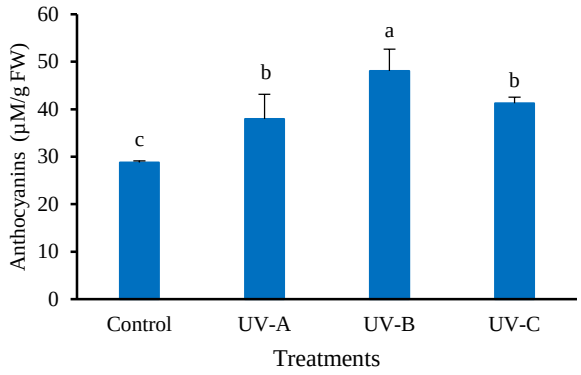

Fig. 1 Impacts of different bands of ultraviolet rays on anthocyanin content of *M. officinalis* seedlings. The data are mean of three replicates ±StD. Different letters indicate significant differences using Duncan's test.

In this study, the content of UV-absorbing compounds in plants treated with UV-A, UV-B, and UV-C increased by about 27, 35 and 69% compared to the control plants, respectively (Figure 2). The amounts of UV-absorbing compounds showed negative correlation (R^2=0.9) with photosynthetic pigments, but positive correlation (R^2=0.86) with anthocyanin (Table 2).

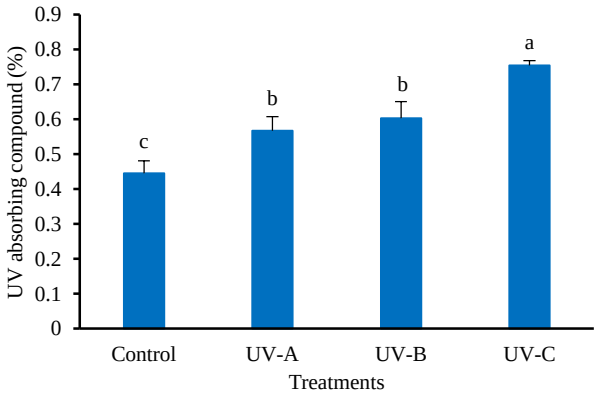

Fig. 2 Impacts of different bands of ultraviolet rays on UV absorbing compound of *M. officinalis* seedlings. The data are mean of three replicates ±StD. Different letters indicate significant differences using Duncan's test.

The UV rays damage PSII proteins, such as D_1 protein, and prevent oxygen from entering the center and turning it into $^1O_2$. Therefore, it is possible that the increase of antioxidants by UV resulted in the elimination of ROS [16]. Plants activate defense mechanisms to deal with the harmful effects of UV rays. One of these mechanisms is the increase and accumulation of light-absorbing pigments, such as phenolic compounds, flavonoids, anthocyanins, and other UV-absorbing compounds, etc. It has been reported that these compounds accumulated due to the plant exposure to UV-B and UV-C rays in the vacuoles of the upper and lower epidermis cells of mature leaves in most plants [10, 18, 20]. Anthocyanins are structurally similar to flavonoids and are a group of phenolic compounds in plants. By changing the quality and quantity of the absorbed light, and scavenging free radicals, anthocyanins can filter UV rays and reduce the adverse effects of these rays in plants [20]. Phenolic compounds and anthocyanins are synthesized from the phenylpropanoid pathway. A large number of key enzymes of this pathway are activated under the influence of UV rays [3, 6, 26]. Studies showed that ultraviolet rays increased PAL enzyme activity and cinnamic acid production and activated anthocyanins biosynthesis pathway. It has been reported that the amount of anthocyanins increases depending on the intensity and duration of UV treatment in strawberries [10, 19, 20]. Despite the increase of these compounds and their protective role in supporting the photosynthetic apparatus, the content of photosynthetic pigments decreased rapidly in response to ultraviolet radiation, especially UV-B and UV-C rays. Therefore, it seemed that the increase of these organic substances in plants is induced by UV rays through affecting the genes encoding the enzymes of this pathway.

Proteins

As shown in Figure 3, the proteins content in UV-A and UV-B treated seedlings increased about 20.2% and 20.5% compared to the control, respectively. But UV-C led to a 30% reduction in proteins content in treated plants.

The reduction in the amount of protein in pepper plants treated with UV rays has been reported by Mahdavian *et al*. (2008). Protein metabolism depends on the tissue and the age of the plant. Stress causes the reduction in cell polysomes and ultimately decreases the production of proteins in several plant species. Moreover, stress results in the activation of antioxidant protein genes in plants.

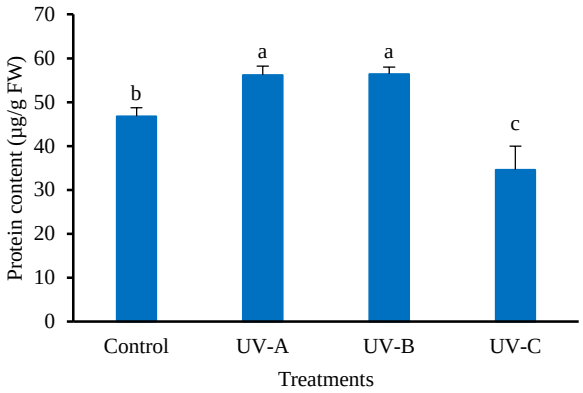

Fig. 3 Impacts of different bands of ultraviolet rays on protein content of *M. officinalis* seedlings. The data are mean of three replicates ±StD. Different letters indicate significant differences using Duncan's test.

The proteins produced under stress conditions protect the enzymes and proteins in the cell structure. Therefore, they prevent the accumulation of abnormal and denatured proteins and the condensation of proteins [6, 10, 27]. Ultraviolet rays destroy ATPase, Rubisco, and protein subunits of photosystems I and II that lead to lower photosynthesis and protein production in plants [20]. Moreover, cyclic amino acids including tryptophan, tyrosine, and phenylalanine have a high capacity to absorb UV rays and therefore will be damaged by UV radiation quickly. The chlorophyll content decreases under the influence of ROS produced during light stress. Stresses lead to the breakdown of chlorophylls in chloroplasts and the disappearance of thylakoid structures, causing a reduction in the photosynthesis rate [10]. The decrease in the protein contents caused by UV-C in this research may be caused by the destruction or reduction in the biosynthesis of these compounds. The increase in the amount of protein in the UV-B and UV-A treatments is due to the increase in antioxidant enzymes in the lemon balm plant. Despite the increase in the amount of antioxidants under the UV-B treatment, stress conditions were still observed.

CONCLUSION

This study investigated some physiological effects of 3 different UV wavelengths on *M. officinalis* seedlings. The results showed that increased carotenoids, UV absorbing compounds, and anthocyanins contents were the main defense mechanisms of the plants against ultraviolet radiations. However, due to the adverse effect of UV spectrums on plant subcellular and membrane structures, a decline in the pigments and proteins content due to ultraviolet radiation was observed. Higher antioxidants contents allowed the plants to survive under oxidative stress induced by UV treatments. Since living organism are overwhelmingly exposed to various abiotic stresses caused by anthropogenic activities, obtaining more empirical data about the effect of such elicitors on living cells including plant cells is important. The results of this study can contribute to our understanding of the overall effects of various light spectrums on plants.

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