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Effect of UV-B Irradiation on Bioactive Compounds of Red Perilla (*Perilla frutescens* (L.) Britton) Cultivated in a Plant Factory with Artificial Light

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Abstract: In this study, we investigated the effect of UV-B irradiation 3 days prior to harvest, on the accumulation of rosmarinic acid (RA) and anthocyanin, and the expression of genes related to phenylpropanoid and flavonoid biosynthetic pathways, in red perilla (*Perilla frutescens* L.). In experiment 1, seedlings at 60 days after sowing (DAS) were subjected to UV-B irradiation at 0 (control), 6, and 10 W m^{-2} under a 16 h light period; while in experiment 2, seedlings at 45 DAS were subjected to UV-B irradiation at 0 (control), 4 W m^{-2} at continuous irradiation, and 6 W m^{-2} at 16 h irradiation. UV irradiation of 10 W m^{-2} for 16 h negatively affected leaf color, while irradiation at 6 W m^{-2} enhanced RA biosynthesis and antioxidant capacity. Continuous UV-B irradiation of 4 W m^{-2} increased the RA concentration by 92% compared to the control; however, this effect was smaller than that of UV-B irradiation at 6 W m^{-2} for 16 h, 141% higher than that of the control and had a lower antioxidant capacity against UV-mediated ROS overproduction during the dark period. Results demonstrate that 6 W m^{-2} of UV-B irradiation for 16 h is suitable for enhancing the RA concentration and content of red perilla.

Keywords: anthocyanin; antioxidant capacity; flavonoid; gene expression; phenylpropanoid; rosmarinic acid

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1. Introduction

Red perilla (*Perilla frutescens* (L.) Britton) belonging to the Lamiaceae family is an aromatic herb used as a food, flavoring, coloring agent, and traditional medicine [1,2]. Furthermore, this species contains various bioactive compounds [3], which are beneficial to human health [2].

Perillaldehyde (PA), the main compound in red perilla leaves, has antibacterial properties, whereas rosmarinic acid (RA) and anthocyanins (ANT), which are used as food ingredients, have antioxidant properties. RA and ANT are produced via the phenylpropanoid and flavonoid biosynthetic pathways, respectively. The demand for functional foods containing a variety of young leaves has been increasing in the food market. Thus, young leaves of red perilla containing high concentrations of RA and ANT are highly valuable.

Environmental stresses, including high light intensity and low or high temperatures, have been utilized to increase the accumulation of bioactive compounds in plants. Abiotic stress, such as ultraviolet (UV) irradiation, can be a powerful environmental stressor [4,5]; UV-A (315–400 nm) and UV-B (280–315 nm) exposure elevates bioactive compound accumulation in plants (including the polyphenolic content) which subsequently enhances its antioxidant capacity in defense against the reactive oxidative species (ROS) generated by UV radiation [6].

Currently, red perilla is cultivated mostly in open fields; however, it is difficult to achieve stable yield quality in fields with variable cultivation conditions. In contrast, cultivation conditions can be completely controlled in a plant factory with artificial light

(PFAL) and a vertical farm [7]. Indeed, it is possible to create environments with suitable light intensity, air temperature, humidity, and gas concentrations, to promote plant growth and increase bioactive compound concentrations [8,9].

Moreover, the application of a UV light source can be easily realized in a PFAL by the addition of UV lamps to cultivation lamps. Therefore, the effects of UV-A and UV-B radiation under controlled environments have been studied extensively for vegetable leaves and sprouts to increase the concentration of bioactive compounds.

For example, Ebisawa et al. [10] determined that UV-B supplemented with blue light during the night increases the quercetin content and expression of genes related to flavonol synthase in lettuce. Furthermore, Nishimura et al. [11] reported that UV-B radiation suppresses the growth and accumulation of anthocyanin in perilla. Lee et al. [12] reported that in red leaf lettuce the expression of genes related to ANT biosynthesis (phenylalanine ammonia lyase, PAL) increased after UV irradiation treatment for 4 d. Additionally, Goto et al. [13] reported that UV-B radiation enhanced the biosynthesis of antioxidant polyphenols in red leaf lettuce and concluded that UV-B irradiation 1 to 3 d prior to harvest effectively increases the concentration of functional phytochemicals in lettuce. Similarly, the total phenolic and flavonoid concentrations in broccoli sprouts under UV-B irradiation were considerably high compared to those under UV-A irradiation at the same intensity and duration [14].

Previous research reported that UV-A radiation enhances the concentrations of functional phytochemicals in lettuce [15–17]. Studies on the members of Lamiaceae further demonstrate that the concentration of certain phytochemicals of the plants increased under UV-B irradiation [18–22]. Although irradiation with both UV-A and UV-B is effective in the accumulation of bioactive compounds in leaves, a previous study concluded that compared to UV-A at the same light intensity, UV-B radiation has a greater effect on the accumulation of bioactive compounds [14]. Moreover, some reports indicated that short-term (several days) UV-B irradiation might be a useful pre-harvest treatment to yield antioxidant-rich plant products [23,24].

We previously reported that UV-B irradiation activates the phenylpropanoid pathway for the biosynthesis of RA and increases the antioxidant capacity of red perilla [25]; however, we neither altered the UV-B irradiation intensity nor discussed the timing and duration of irradiation. We hypothesized that optimal pre-harvest conditions of UV-B irradiation exist to promote the accumulation of bioactive compounds. Therefore, in this study, we elucidate the effect of UV-B irradiation doses on the accumulation of RA and ANT and the expression of genes related to these biosynthetic pathways in red perilla after short-term irradiation prior to harvest.

2. Materials and Methods

2.1. Plant Material and Cultivation Conditions

The experiments were conducted at Chiba University, Japan, in a PFAL with multilayer cultivation shelves. Red perilla (*Perilla frutescens* var. crispa 'Houkouakashiso' Nakahara Seed Product Co., Ltd., Fukuoka, Japan) was used as plant material. Seeds were sown in water-saturated urethane sponges in a tray put on the cultivation shelf (Figure S1A). Ninety-six germinated seeds were grown at the following conditions: 25/20 °C (light/dark period) air temperature, 70% relative humidity, 16/8 h light/dark period, 150 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) at the canopy level using white fluorescent lamps, and 1000 ppm of CO_2 concentration.

Plants were transplanted into hydroponic containers when the first true leaf appeared and cultivated until 60–65 days after sowing (DAS) (Figure S1B). The PPFD was set to 200 μmol m^{-2} s^{-1} at the canopy level. Quarter-strength OAT house A prescription (OAT Agrio Co., Ltd., Tokyo, Japan) was used as the nutrient solution for all the experiments. The pH and electrical conductivity of the solution were set to 6.4–6.5 and 1.0–1.1 dS m^{-1}, respectively.

When the leaves at the 5th node (from the plant base) were just unfolded, the UV-B treatment started. At this point, the leaves at the 1st–3rd nodes had fully expanded while the leaves at the 4th node did not fully expand. Seventy-two seedlings were selected, divided into three groups, and moved to three hydroponic containers (Figure S2).

2.2. UV-B Treatment

UV-B fluorescent lamps (TL20W/01 RS; Philips, Hamburg, Germany) with a peak wavelength of 310 nm were used as the UV light sources (Figure 1). The UV-B lamp spectrum was determined using a spectroradiometer and the UV-B irradiation intensity was adjusted using a UV radiometer (UV203; Irradian Ltd., Scotland, UK). Table S1 shows the spectral characteristics of these lamps. The biologically effective UV-B radiation ($UV\text{-}B_{BE}$) was determined according to the method described by Caldwell [26] and Nouchi [27]. Red LED lamps with a 660 nm peak wavelength (CIVILIGHT, DPT2RB120Q33 40 type, Showa Denko K.K., Tokyo, Japan) were used as the cultivation light source (Figure S2) to exclude the effect of blue light on gene expression from that of UV light. Goto et al. [28] reported that red perilla can be grown under 660 nm red LEDs and its net photosynthetic rate is higher than that of plants grown under blue-rich white lamps because the ANT pigments that accumulate in the leaves absorb large amounts of blue light in the epidermis, thereby reducing photosynthetic rate under blue-light-rich conditions.

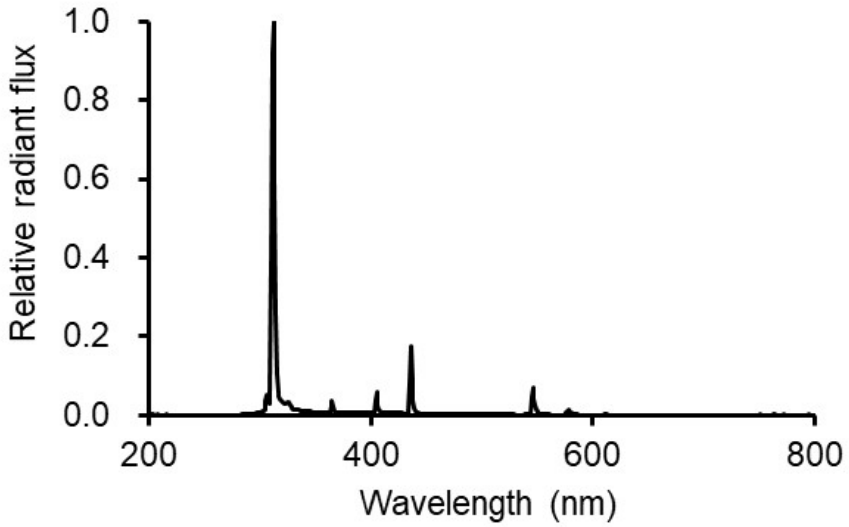

Figure 1. Spectral radiant flux of the UV-B lamps used in this study (TL20W/01 RS; Philips, Hamburg, Germany).

In experiment 1, seedlings at 60–65 DAS were subjected to UV-B irradiation at 3 intensities of 0 W m^{-2} (control), 6 W m^{-2}, and 10 W m^{-2} under a 16 h light period for 3 d (Figure 2 and Table S2).

In experiment 2, seedlings at 60–65 DAS were subjected to UV-B irradiation at 4 W m^{-2} with continuous irradiation (4 W 24 h) and 6 W m^{-2} with 16 h irradiation (6 W 16 h) for 3 d (Figure 3 and Table S3). For the control treatment seedlings were cultivated without UV-B irradiation under a 16 h light period.

Figures 2 and 3 show the time points of leaf sampling for growth measurement, bioactive compound analysis, and gene expression analysis. The experiments were performed once. The expanding leaves at the 4th node, 5 cm or longer in length were used for the analysis of bioactive compounds and gene expression because RA and ANT concentrations are constant from this size to full expansion size under normal cultivation conditions (Figure S3).

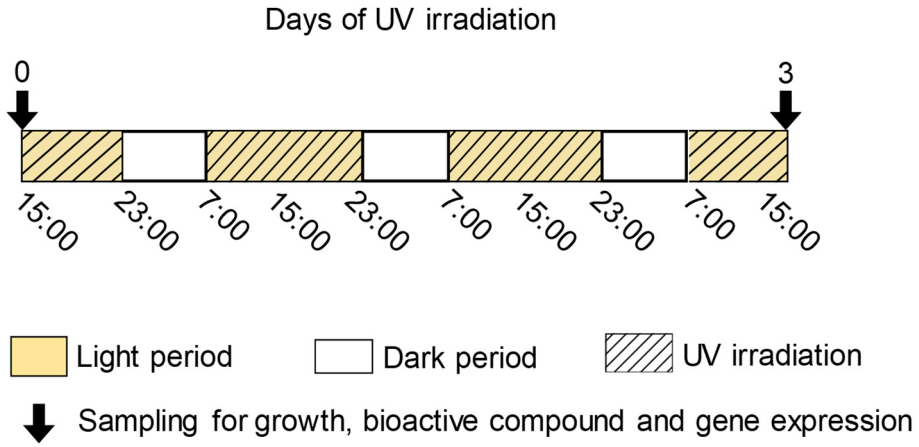

Figure 2. A schematic representation of UV-B sampling and exposure times (experiment 1). UV-B irradiation intensity was set to 0, 6, and 10 W m^{-2}. Sampling was conducted at 0, 24, 48, and 72 h for the analysis of time-dependent responses to UV-B irradiation.

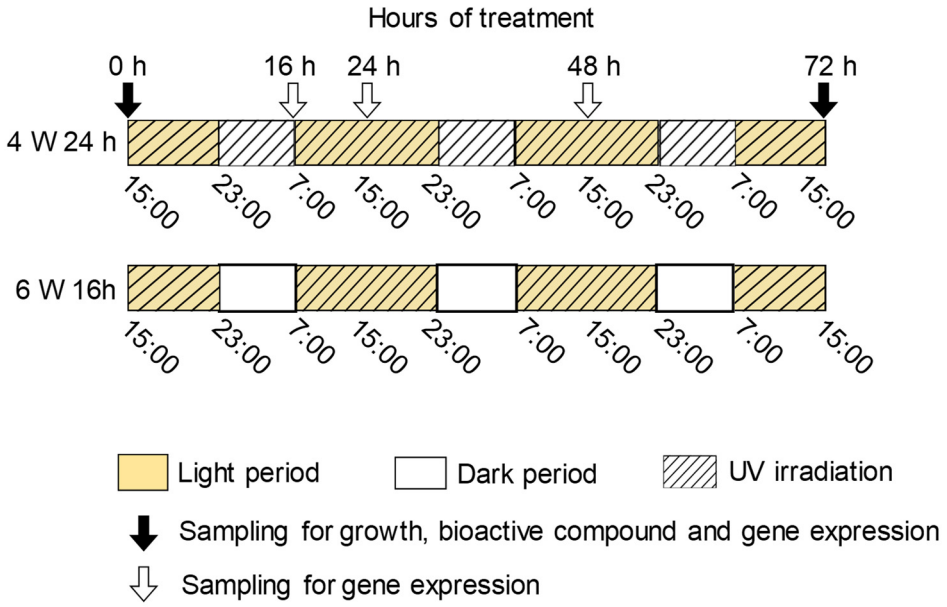

Figure 3. A schematic representation of UV-B sampling and exposure times (experiment 2. The plants were subjected to UV-B irradiation at 4 W m^{-2} with continuous irradiation (4 W 24 h) and 6 W m^{-2} with 16 h irradiation (6 W 16 h). Sampling was conducted at 0, 16, 24, 48, and 72 h for the analysis of time-dependent responses to UV-B irradiation.

2.3. Measurement of RA, PA, and ANT Concentrations

Leaves were harvested from four uniform plants. The phyllotaxis of perilla plants is decussate, with each node having two leaves at the same growth stage. The fresh weights of both leaves were measured using a digital balance immediately after harvesting. One leaf at the 4th node was immediately stored at −80 °C for subsequent biochemical and gene expression analyses. Another leaf at the 4th node was first oven-dried at 80 °C for 72 h, weighed for dry weight measurement using a digital balance, and further used for calculation of percent dry matter. The percent dry matter was calculated by dividing leaf dry weight by the leaf fresh weight, multiplied by 100.

Leaves at the 4th node with 0.05–0.10 g of fresh weight ground in liquid nitrogen were used for measurement of RA and PA concentrations. The concentrations were analyzed according to the method described by Ogawa et al. [29]. Briefly, 1.0 mL of methanol was

added to a 2.0 mL microtube containing the leaf tissue; the samples were sonicated for 30 min at 10–15 °C and centrifuged at 20,380× *g* for 10 min at 4 °C, and the supernatants were collected. Methanol (0.5 mL) was added to the residue and the samples were sonicated and centrifuged as described in the first step. The supernatants were combined and centrifuged and filtered using disposable syringe filter units (13HP020AN; pore size: 0.20 µm; Advantec Toyo Kaisha, Ltd., Tokyo, Japan). RA and PA concentrations were determined using a UHPLC (Nexera, Shimadzu Corporation, Kyoto, Japan) equipped with a UPLC BEH C18 column (φ 1.7 µm, 2.1 × 50 mm, Waters Corporation, Milford, Massachusetts, USA). The temperature of the column oven was maintained at 50 °C. The retention times of RA and PA were 6.0 and 8.7 min at 326 and 327 nm of detection wavelengths, respectively. Quantitation was conducted using the absolute calibration curve method, and standard curves were prepared with diluted solutions of RA standard reagent (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and PA standard reagent (Wako Pure Chemical Industries, Ltd., Osaka, Japan), respectively.

Leaves at the 4th node with 0.02–0.05 g of fresh weight ground in liquid nitrogen were used for measurement of ANT concentration. ANT concentration was analyzed according to the method described by Gong et al. [30] and partially modified by Ogawa et al. [28]. First, 1.5 mL of 1% HCl-methanol was added to 2.0 mL microtubes containing the leaf tissue. The samples were refrigerated at 4 °C for 48 h and, subsequently mixed and incubated for 30 min; the supernatant was diluted 10-fold with 1% HCl-methanol. The absorbance of the samples was then measured at 517 nm using a spectrophotometer. Quantitation was conducted using the absolute calibration curve method with a standard curve prepared using a diluted solution of cyanidin 3-glucoside chloride standard reagent (Funakoshi Ltd., Tokyo, Japan). The concentration of ANT was calculated as the equivalent of cyanidin 3-glucoside (C3G).

2.4. Measurement of Total Oxygen Radical Absorbance Capacity (ORAC)

Leaves at the 4th node with 0.05 g of fresh weight ground in liquid nitrogen were used for ORAC measurement. ORAC values were measured according to the protocol described by Zhao et al. [31]. Treated solutions were measured using a microplate reader (SH-9000Lab, Hitachi High-Technologies Corp., Tokyo, Japan). The ORAC values were expressed as Trolox equivalents.

2.5. Gene Expression Analysis

Leaves at the 4th node with 0.03–0.04 g of fresh weight ground in liquid nitrogen were used for gene expression analysis. The analysis was carried out according to the method described by Goto et al. [25]. The primers used in the experiments were designed according to information obtained from the GenBank database (Table S4).

The following mRNAs related to phenylpropanoid and flavonoid biosynthetic pathways (Figure S4) were amplified: phenylalanine ammonia lyase (PAL), tyrosine aminotransferase (TAT), rosmarinic acid synthase (RAS), chalcone synthase (CHS), flavanone 3-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR), and anthocyanidin synthase (ANS). The mRNA expression levels were normalized using actin (ACT) as the reference gene, and relative gene expression was calculated using the log2 treatment to control ratio.

2.6. Statistical Analysis

All data were analyzed using SPSS for Windows (Version 24.0; SPSS Inc., Chicago, IL, USA). The means were compared using the Tukey-Kramer test, with a p-value of <0.05.

3. Results

3.1. Experiment 1

Fresh weights of above-ground and 4th leaf samples of plants exposed to UV-B irradiation of 6 and 10 W m^{-2} were significantly lower compared to those of the control samples (Table 1). The dry weights of the above-ground and 4th leaf samples of the plants exposed

to UV-B irradiation decreased as the UV irradiation intensity increased. The percent dry weights of the 4th leaf samples of the plants grown at 6 and 10 W m^{-2} were significantly higher than that of the control. The color of the 3rd–5th leaves of the plants grown at 10 W m^{-2} turned relatively green after 3 d of irradiation (Figure 4).

Table 1. Effect of UV irradiation on the growth of the above-ground parts and 4th leaf of red perilla after 3 d of UV-B treatment (experiment 1). Plants were exposed to UV-B irradiation at 0 (control), 6 (6 W), and 10 W m^{-2} (10 W).

Days of Treatment	Treatment	Fresh Weight		Dry Weight		Percent Dry Weight
		Above-Ground Part (g)	4th Leaf (g)	Above-Ground Part (g)	4th Leaf (g)	4th Leaf (%)
0		2.46	0.66	0.39	0.14	20.6
3	Control	4.06 a	1.24 a	0.78 a	0.27 a	22.3 b
3	6 W	3.41 b	0.88 b	0.72 ab	0.23 ab	25.7 a
3	10 W	3.02 b	0.76 b	0.63 b	0.19 b	25.4 a

Different letters indicate a significant difference among treatments by the Tukey-Kramer test at $p < 0.05$ ($n = 6$).

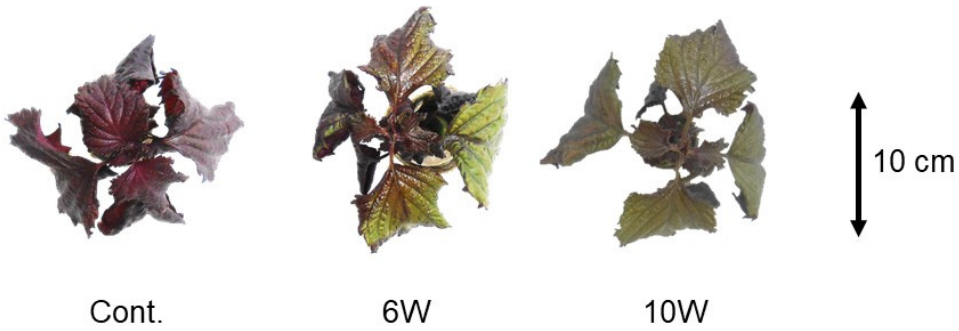

Figure 4. Red perilla was grown for 3 d under different UV irradiation intensities (experiment 1). Plants were exposed to UV-B irradiation at 0 (control), 6 (6 W), and 10 W m^{-2} (10 W).

The RA concentration was approximately 40% and 120% higher at 6 and 10 W m^{-2}, respectively, than that of the control (Figure 5), whereas the PA concentration did not differ among the treatments. The ANT concentrations at 6 and 10 W m^{-2} were significantly lower than that of the control, and the ORAC values at both 6 and 10 W m^{-2} were significantly higher than those of the control.

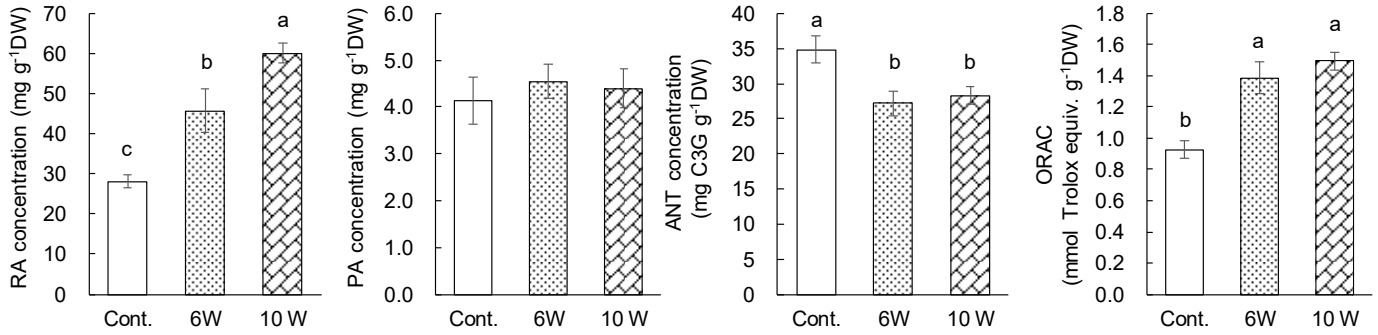

Figure 5. Effect of UV irradiation on rosmarinic acid (RA), perillaldehyde (PA), and anthocyanin (ANT) concentrations and an ORAC value of the 4th leaf of red perilla after 3 d of UV-B treatment (experiment 1). The plants were exposed to UV-B irradiation at 0 (control), 6 (6 W), and 10 W m^{-2} (10 W). The vertical bars indicate SE ($n = 4$). Different letters indicate significant differences among treatments by the Tukey-Kramer test at $p < 0.05$.

3.2. Experiment 2

Growth analysis after 3 d of UV-B treatment indicated that there were no differences in the fresh and dry weights of the above-ground and 4th leaf samples of the 4 W 24 h and 6 W 16 h plants compared with those of the control (Table 2). The percent dry weights of the 4th leaf of the plants exposed to UV-B irradiation were significantly higher than those of the control. The color of the leaves of the 4 W 24 h and 6 W 16 h plants turned slightly green after 3 d of irradiation (Figure 6).

Table 2. Effect of UV irradiation on growth of the above-ground parts and 4th leaf of red perilla after 3 d of UV-B treatment [experiment 2]. The plants were exposed to UV-B irradiation at 0 W m^{-2} (control), 4 W m^{-2} at continuous irradiation (4 W 24 h), and 6 W m^{-2} for a 16 h light period (6 W 16 h).

Days of Treatment	Treatment	Fresh Weight		Dry Weight		Percent Dry Weight
		Above Ground Part (g)	4th Leaf (g)	Above Ground Part (g)	4th Leaf (g)	4th Leaf (%)
0		1.24 ± 0.15	0.50 ± 0.03	0.22 ± 0.03	0.10 ± 0.01	20.3 ± 1.43
3	Control	2.14 ± 0.19	0.90 ± 0.10	0.36 ± 0.02	0.18 ± 0.01	18.8 ± 0.71 b
3	4 W 24 h	1.95 ± 0.18	0.73 ± 0.07	0.32 ± 0.03	0.19 ± 0.01	26.1 ± 0.88 a
3	6 W 16 h	2.25 ± 0.31	0.73 ± 0.12	0.37 ± 0.04	0.20 ± 0.03	27.5 ± 1.85 a

Data are presented as mean ± SE (n = 4). Different letters indicate a significant difference among treatments by the Tukey-Kramer test at $p < 0.05$.

Figure 6. The 4th leaves of red perilla were grown for 3 d under different UV irradiation intensities (experiment 2). Plants were exposed to UV-B irradiation at 0 W m^{-2} (control), 4 W m^{-2} at continuous irradiation (4 W 24 h), and 6 W m^{-2} for a 16 h light period (6 W 16 h).

The RA concentrations in 4 W 24 h and 6 W 16 h treatments were 92% and 141% higher than that of the control at 64.4 and 80.4 mg g^{-1} leaf DW, respectively (Table 3). Furthermore, the RA contents in 4 W 24 h and 6 W 16 h treatments were 12.2 and 16.1 mg per leaf, respectively, which was 103% and 168% higher than that of the control (Table 4). In contrast, ANT concentration and content in 4 W 24 h and 6 W 16 h treatments were significantly lower than those in the control.

The expression of *PAL*, *TAT*, and *RAS* genes related to the RA biosynthetic pathway increased significantly in 4 W 24 h and 6 W 16 h treatments after 48 h and 72 h, respectively (Figure 7A–C). The expression of *PAL*, *TAT*, and *RAS* genes at 48 h was approximately 2, 3.2, and 1.5 times higher than that of the control, respectively, and after 16 h of treatment, the expression of the three genes in 4 W 24 h treatments was relatively low compared to that in the control, whereas the expression in 6 W 16 h treatments was similar to that in the control.

Table 3. Effect of UV irradiation on rosmarinic acid (RA) and anthocyanin (ANT) concentrations in the 4th leaf of red perilla after 3 d of UV-B treatment (experiment 2). The plants were exposed to UV-B irradiation at 0 W m^{-2} (control), 4 W m^{-2} at continuous irradiation (4 W 24 h), and 6 W m^{-2} for a 16 h light period (6 W 16 h).

Days of Treatment	Treatment	RA Conc. (mg g^{-1} DW)	ANT Conc. (mg C3G g^{-1} DW)
0		35.4 ± 5.4	27.4 ± 2.8
3	Control	33.4 ± 3.3 c	37.2 ± 5.2 a
3	4 W 24 h	64.4 ± 2.9 b	25.5 ± 0.6 b
3	6 W 16 h	80.4 ± 4.1 a	26.3 ± 2.9 b

Data are presented as mean ± SE (n = 4). Different letters indicate significant differences among treatments by the Tukey-Kramer test at $p < 0.05$.

Table 4. Effect of UV irradiation on rosmarinic acid (RA) and anthocyanin (ANT) contents in the 4th leaf of red perilla after 3 d of UV-B treatment (experiment 2). The plants were exposed to UV-B irradiation at 0 W m^{-2} (control), 4 W m^{-2} at continuous irradiation (4 W 24 h), and 6 W m^{-2} for a 16 h light period (6 W 16 h).

Days of Treatment	Treatment	RA Content (mg/leaf)	ANT Content (mg/leaf)
0		3.5	2.7
3	Control	6.0 c	6.7 a
3	4 W 24 h	12.2 b	4.8 b
3	6 W 16 h	16.1 a	5.3 b

The RA and ANT content was calculated by multiplying their concentration in the 4th leaf by the dry weight of the 4th leaf. Different letters indicate significant differences among treatments by the Tukey-Kramer test at $p < 0.05$ (n = 4).

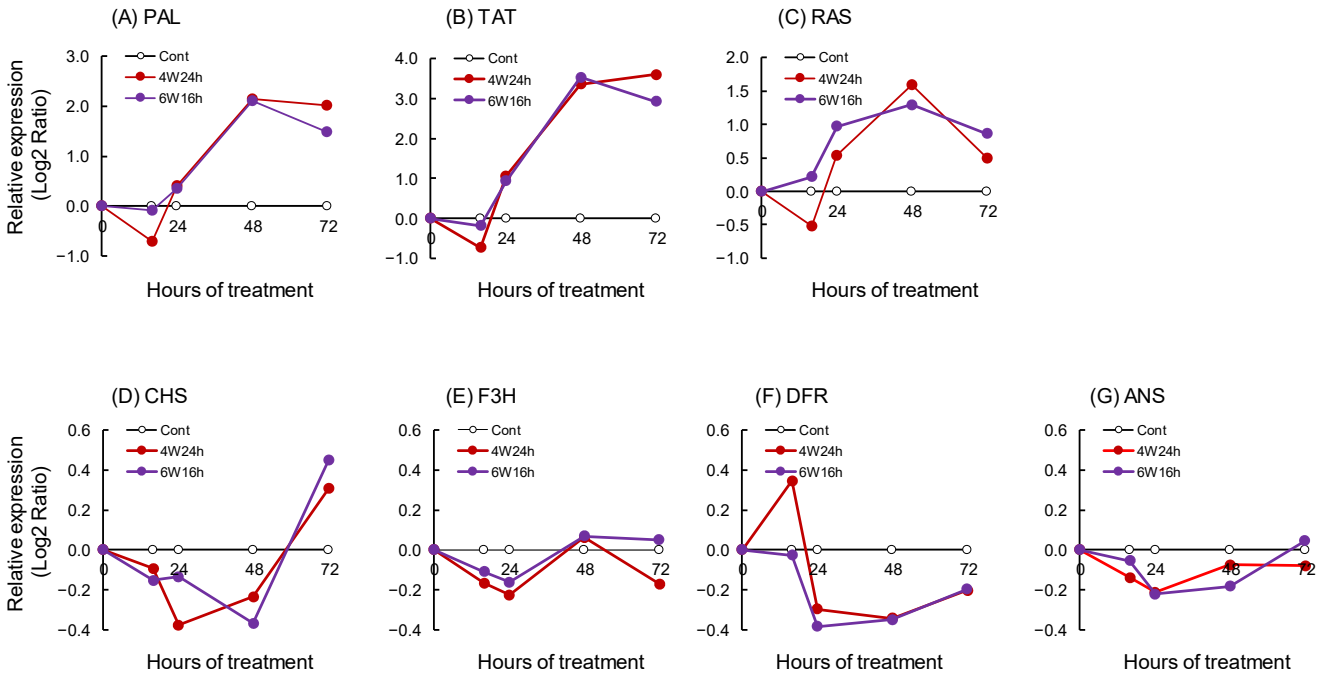

Figure 7. Time courses of *PAL* (**A**), *TAT* (**B**), *RAS* (**C**), *CHS* (**D**), *F3H* (**E**), *DFR* (**F**), and *ANS* (**G**) mRNA expression in the 4th leaf of red perilla (n = 4). Red perilla plants with 5 leaves were subjected to the following UV-B irradiation intensities: 0 W m^{-2} (control), 4 W m^{-2} at continuous irradiation (4 W 24 h), and 6 W m^{-2} for 16 h light period (6 W 16 h). Line graphs indicate $\log_2$ fold changes (treatment:control) in gene expression levels.

The expression of *CHS*, *F3H*, *ANS*, and *DFR* genes related to the ANT biosynthesis pathway decreased considerably under UV-B irradiation during 16–48 h (Figure 7D–G), while expression of the *CHS* gene under UV-B irradiation increased slightly at 72 h.

4. Discussion

4.1. Variations in RA, ANT, and PA Concentrations and ORAC Values under UV-B Irradiation

4.1.1. Variations in PA Concentrations

Lu et al. [32] cultivated green and red perilla under different PPFDs and electrical conductivities of nutrient solutions. They found that the PA in red perilla was unaffected, whereas that in green perilla was largely affected at PPFDs of 100, 200, and 300 μmol m^{-2} s^{-1}. In experiment 1, the PA concentration did not differ among the treatments (Figure 6), suggesting that PA biosynthesis is unaffected by environmental factors. Considering that PA is produced via the terpenoid biosynthetic pathway and does not exhibit high antioxidant capacity, it may not respond to the ROS generated by UV irradiation.

4.1.2. Variations in RA Concentrations and ORAC Values

The ORAC values increased significantly under UV treatment compared to that in the control, although the ANT concentration did not increase. RA and ANT are the major bioactive compounds that respond to the ROS generated by UV irradiation. Iwai et al. [33] reported that the RA concentration was significantly higher in red perilla plants grown under artificial light than those grown in a greenhouse. Furthermore, Nguyen and Oh [34] reported that UV-A irradiation increased the anthocyanin and total phenolic contents, antioxidant capacity, and RA accumulation in red perilla.

4.1.3. Variations in ANT Concentrations and Leaf Growth

The leaves of the plants grown at 10 W m^{-2} turned relatively green after 3 d of UV-B treatment (Figure 5). The dry weights of the above-ground parts and the 4th leaf of the plants exposed to UV-B irradiation decreased as UV irradiation intensity increased (Table 1). Lee et al. [35] reported that short-term (5 d) UV-A treatment substantially increased the bioactive compound content and biomass of kale without altering its appearance. However, in experiment 1, UV-B radiation appeared to have a greater impact on growth and appearance than UV-A radiation, owing to its relatively higher energy. Based on the results of previous studies, we hypothesized that ANT would increase under UV-B irradiation, yet this was not the case. This result indicates that a fraction of ANT accumulated on the leaf surface may be decomposed by UV-B radiation. Although the leaf color of the plants grown at 6 W m^{-2} did not change, they did turn green if the treatment period was extended over 3 d. Additionally, ANT accumulation may vary depending on the strength and dose of UV-B irradiation.

4.1.4. RA and ANT for Improvement of Red Perilla Quality

Our results suggest that in the plants that responded to the ROS generated by UV-B irradiation, RA is a major compound affecting changes in the ORAC values of plants under UV-B irradiation. Based on these results, in experiment 2, we focused on RA and ANT accumulation and gene expression associated with RA and ANT biosynthetic pathways.

4.2. Comparison of RA and ANT Accumulations under UV Treatments in Light and Dark Periods

4.2.1. Intensity and Dosage of UV-B Irradiation

Previous studies have investigated the impacts of UV-B irradiation intensity and dosage on morphology, growth, and the synthesis of bioactive compounds [25,35]. These studies have demonstrated that the accumulation of bioactive compounds is influenced by cumulative UV-B irradiation [17].

We hypothesized that at a given dose and period, UV-B irradiation, with or without a dark period, would have the same effect on RA and ANT accumulation. If a low UV-B irradiation intensity has the same effect as a high irradiation intensity, the initial cost of

building a PFAL would reduce, requiring fewer UV-B lamps. Therefore, in experiment 2, we focused on the effects of UV irradiation with or without a dark period.

UV irradiation for the 4 W 24 h treatment continued during the dark period, whereas the 6 W 16 h treatment was applied only during 16 h of the light period. The dose was the same for all the treatments. On the first day of the UV-B treatment, plants were subjected to 16 h of the light period—8 h before and after the start of UV irradiation (Figure 3), and gene expression was analyzed after 16 h of treatment. Thereafter, samples were obtained from these plants after 8 h of the dark period.

4.2.2. Time Courses of Expression of Genes Related to RA under UV-B Irradiation with or without a Dark Period

The expression of *PAL*, *TAT*, and *RAS* genes in the 4 W 24 h treatment was low compared to that in the control, whereas the gene expression in the 6 W 16 h treatment was similar to that in the control (Figure 7A–C). The results indicated that continuous UV irradiation during light and dark periods negatively affected gene expression, although the UV irradiation intensity in 4 W 24 h treatment was 4 W m^{-2}, which was two-thirds the intensity in 6 W 16 h treatment. A possible explanation for these results is that during the dark period, new photoassimilates are not produced by photosynthesis, thus limiting the resources required for the synthesis of antioxidant enzymes and/or bioactive compounds required to combat the UV-mediated ROS overproduction [36]. As a result, the RA concentration under 4 W 24 h treatment could be lower than that under 6 W 16 h treatment.

The expression of *PAL*, *TAT*, and *RAS* genes increased gradually after 24 h and showed the highest values at 48 h compared to those of the control (Figure 7A–C) and continued to increase until 72 h. The RA concentrations and contents in 4 W 24 h and 6 W 16 h treatments after 3 d of UV treatment were 92% and 141% higher and 103% and 168% higher, respectively, than those in the control, respectively (Tables 3 and 4).

In a previous study, we demonstrated a temporal difference between the induction of genes related to the synthesis and accumulation of antioxidant compounds in the phenylpropanoid and flavonoid biosynthetic pathways in UV-B-treated canola plants [37]. Additionally, previous research has shown that upregulation of the expression of genes related to the synthesis of bioactive secondary metabolite triggered by UV-B exposure may vary over time [24,38].

Our results are in agreement with those of previous studies and demonstrated that gene expression increased gradually after 24 h and reached the highest values after 48 h. The RA concentration reached high values after 3 d of UV-B irradiation, indicating that 3 d of UV-B irradiation augmented RA biosynthesis, accompanied by the activation of gene expression.

4.2.3. Changes in ANT and Leaf Growth

ANT concentrations and contents under 4 W 24 h and 6 W 16 h treatments were significantly lower than those in the control (Tables 3 and 4). The two UV irradiation treatments did not show any difference in the expression of genes related to ANT biosynthetic pathways and accumulation. Our previous study using canola plants demonstrated that the expression of genes related to ANT biosynthetic pathways and accumulation significantly increased during 3 d of UV-B irradiation at 3, 6, and 9 W m^{-2} for 16 h [38]. In contrast, ANT accumulation in the red perilla was restricted by UV irradiation. In experiment 2, the color of the leaves of the 4 W 24 h and 6 W 16 h plants turned slightly green after 3 d of irradiation probably because a fraction of ANT accumulated at the leaf surface may have been decomposed by UV-B radiation. However, the marketability of both UV-B-treated plants was not changed. This demonstrates that the effect of UV irradiation on ANT accumulation may vary depending on plant species.

5. Conclusions

A comparison between UV irradiation intensities during a 16 h light period showed that irradiation at 10 W m^{-2} for 16 h had a negative effect on leaf color, whereas irradiation at 6 W m^{-2} for 16 h was suitable for the enhancement of RA biosynthesis and ORAC value in red perilla. Furthermore, 4 W m^{-2} of continuous UV-B irradiation increased the RA concentration in red perilla, but this positive impact was smaller than that of UV-B irradiation at 6 W m^{-2} for 16 h. Moreover, the 4 W m^{-2} of continuous UV-B irradiation resulted in a lower antioxidant capacity against UV-mediated ROS overproduction during the dark period. Therefore, we conclude that 6 W m^{-2} of UV-B irradiation for a 16 h light period is suitable for enhancing RA concentration and content of red perilla leaves.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8080725/s1, Table S1: Spectral characteristics of the UV lamp used in the experiments; Table S2: UV irradiation conditions during experiment 1; Table S3: UV irradiation conditions during experiment 2; Table S4: Primers used for the real-time PCR of phenylpropanoid and flavonoid pathway genes and the internal reference gene, actin. Figure S1: Red perilla cultivation procedure. Figure S2: The illustration of the UV lamp installation above the plants. Figure S3: Changes in dry weight, rosmarinic acid, and anthocyanin concentrations in the 4th leaves of red perilla plants. Figure S4. List of analyzed mRNAs related to phenylpropanoid and flavonoid biosynthetic pathways.

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