Difference between Nighttime and Daytime UV-B Irradiation with Respect to the Extent of Damage to Perilla Leaves

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Although ultraviolet-B (UV-B) light (280–315 nm) irradiation effectively controls spider mites in horticultural crop production, it also causes plant damage, leading to growth suppression, changes in morphology, and leaf scorching. However, sensitivity to UV-B varies among plant species. Here, we assessed the effect of UV-B on growing perilla (Perilla frutescens var. crispa) plants. Three experiments were conducted. In the first experiment, plants of the variety ‘Akachirimenshiso’ were grown in a plastic house with natural light conditions and subjected to three treatments: natural UV (control), −UV, and natural UV plus artificial UV-B treatment (50 mW·m⁻² from 0:00 to 3:00). The length of leaf blades that received additional UV-B treatment was reduced by 16.5% and 32.3% in two trials compared with that of the respective controls. In this experiment, additional UV-B irradiation turned the leaf color greener. During UV-B treatment, the values of leaf a*, an indicator of leaf redness, were significantly lower than their respective controls in both trials: 21.5 vs. 31.6 in trial I and 20.2 vs. 30.7 in trial II. For most of the parameters measured in this experiment, no differences were observed between the control and −UV treatment groups. In the second experiment, plants were irradiated with UV-B for 3 weeks at nighttime (0:00–3:00) or daytime (12:00–15:00). In the cultivar ‘Houkouakashiso’, the length of leaf blades significantly decreased by 15.9% and 20.6% under nighttime UV-B irradiation at 80 and 120 mW·m⁻², respectively, compared with that of the non-irradiated controls. Irradiation at 80 mW·m⁻² also decreased the width of the leaf blades by 13.1% and that at 120 mW·m⁻² further decreased it by 25.0%. These results showed that UV-B irradiation at night decreased the size of perilla leaves. In addition, the value of a* became lower under UV-B irradiation in the nighttime. Thus, UV-B irradiation appeared to turn purple perilla leaves green. When plants were irradiated with UV-B in the daytime, there was no significant difference between irradiated and non-irradiated plants in the length or width of leaf blades, a*, or fresh weight of aerial parts and number of nodes on the main stem. In the third experiment, visible rays (VIS) emitted by fluorescent lamps were applied at 0:00–3:00 and 6:00–22:00. Plants were irradiated with 120 mW·m⁻² of UV-B at 0:00–3:00. UV-B + VIS treatment of ‘Akachirimenshiso’ with VIS from fluorescent lamps did not significantly affect the parameters measured in this experiment compared to −UV treatment. The results of this study suggest that UV-B damage to perilla leaves can be avoided by combined irradiation with visible light.

Key Words: anthocyanin, pest control, photoreactivation, visible light.

Introduction

Perilla (Perilla frutescens var. crispa) is a leaf vegetable commonly consumed in East Asian countries such as Japan, China, and Korea. The purple leaves of perilla are high in anthocyanins and are used as a red coloring of foods such as umeboshi (pickled Japanese plums). Additionally, the leaves of perilla are used as crude materials in Chinese herbal medicine. Herbivorous spider mites are one of the most economically important pests in the production of perilla leaves. Spider mites feed on perilla leaves, the edible part of perilla plants. Most acaricides are sprayed directly on leaves. Consumers tend to avoid crops cultivated using pesticides. To
ensure a safe food supply and integrated pest management (IPM), there is a need for alternative methods of mite control in perilla production.

In recent years, there have been attempts to control pests by irradiating plants with artificial ultraviolet-B (UV-B) light (280–315 nm). Artificial UV-B irradiation was reported to reduce the incidence of fungal diseases, such as strawberry powdery mildew (Kanto et al., 2009, 2014), rose powdery mildew (Kobayashi et al., 2013), and eggplant leaf mold caused by Mycovellulosiella nattrassii (Oka et al., 2011). Moreover, it was reported that UV-B irradiation hindered the survival and reproduction of the herbivorous spider mite Tetranychus urticae (Murata and Osakabe, 2013; Ohtsuka and Osakabe, 2009). The mortalities of adult females, larvae, and eggs of T. urticae were proportional to the cumulative UV-B irradiance to which they were exposed (Murata and Osakabe, 2013). Greenhouse experiments revealed an excellent controlling effect of UV-B irradiation on T. urticae populations on strawberries (Tanaka et al., 2016).

Although UV-B irradiation has beneficial effects in pest control, it possibly damages horticultural crops via DNA lesions and injury to the photosynthetic machinery and causes growth suppression, morphological changes, and leaf scorching. However, the vulnerability to UV-B radiation varies among plant species (Jansen et al., 1998; Teramura, 1983). Kanto et al. (2014) recommended 2 h of UV-B irradiation at daytime to control powdery mildew in strawberry cultures. On the other hand, Kobayashi et al. (2013) reported that irradiation for 2 h (0.5–1.0 kJ·m⁻²·day⁻¹) at nighttime had an effect equivalent to daytime irradiation for 6 h (1.5–3.0 kJ·m⁻²·day⁻¹) in preventing powdery mildew during rose production. From economic and practical viewpoints, irradiating at night for 2 h (0.32–1.0 kJ·m⁻²·day⁻¹) has been recommended for powdery mildew control in strawberries (Matsuura et al., 2012). To control spider mites, Tanaka et al. (2016) reported that 1.8 kJ·m⁻²·day⁻¹ UV-B irradiation at night effectively suppressed their presence in strawberry cultures. In addition, from the viewpoint of agricultural practice, nighttime irradiation is preferable because it is safer for farmers than daytime irradiation.

In this study, with the aim of reducing the use of agrochemicals in perilla production, we addressed the effect of UV-B irradiation on perilla plants. Specifically, we evaluated damage to perilla leaves when irradiated with UV-B at day or nighttime. Accordingly, we first comprehensively investigated the responses of perilla to UV-B irradiation in a plastic house and examined the factors affecting the extent of damage to perilla plants caused by UV-B. We then investigated the effect of irradiation time (day or night) on damage incidence in perilla plants.

Materials and Methods

Plant materials

A perilla ‘Akachirimenshiso’ was used in all experiments and ‘Houkouakashiso’ was also used in experiment 2 and 3. Seeds were sown in a horticultural soil mix (Nihon Hiryo Co. Ltd., Tokyo, Japan) and allowed to germinate in a growth chamber controlled at 25°C as the maximum temperature (minimum temperature was not controlled), and a 16-h photoperiod (6:00–22:00). Light was produced with white fluorescent lamps (FHF32EX-N-H; Panasonic Co., Osaka, Japan), illuminating at three wavelengths with peaks around 437, 545, and 610 nm and at an intensity of 190 μmol·m⁻²·s⁻¹. Each seedling was transplanted to a 6-cm diameter plastic pot filled with 90 mL of horticultural soil mix and pots were placed in a growth chamber until use in the experiments detailed below.

Exp. 1 Effects of UV-B on perilla plants grown in a plastic house

Three seedlings grown in the growth chamber until they had four expanded true leaves were transplanted to a plastic container filled with 5-L granular rock wool (Nippon Rockwool Co., Tokyo, Japan). Containers were placed in a plastic house at the experimental farm of the Department of Agriculture of Kyoto University, Kyoto, Japan. Three conditions of UV-B radiation were established: (i) control: growing perilla plants under natural conditions in a plastic house covered by ETFE film (F-CLEAN natural; AGC, Tokyo, Japan); (ii) −UV: growing perilla plants under UV cut film (Achilles Tooshimasen; Achilles Co., Tokyo, Japan) installed in the plastic house; and (iii) +UV-B: growing perilla plants under natural conditions in a plastic house and exposing them to 50 mW·m⁻² of UV-B radiation for 3 h (0:00–3:00) every day. UV-B radiation, with a peak at 310 nm and wavelength spectrum full width at half maximum (FWHM) 30 nm, was produced by UV-B fluorescent lamps (YGRKX21799; Panasonic Co.) located at the plant level. The intensity of UV-B was measured with a photodiode (HD2302.0; Delta OHM S. r. L.). Treatments were started 1 week after transplanting. Plants were irrigated and fertilized with 50% Enshi nutrient solution as needed. After 32 days of treatment, plants were harvested and the fresh weight of the aerial parts, number of nodes on the main stem, and length, width, a* (mentioned below), and anthocyanin concentration of the leaf blades at the second node from the top were measured. The experiments were conducted twice from late June to late July in 2015 (period I) and from early August to early September in 2015 (period II). Three and five containers were used for each treatment in periods I and II, respectively.
Exp. 2 Effects of UV-B irradiation time on perilla growth and appearance

Twelve seedlings with eight true leaves were placed on a plastic tray in the growth chamber described above for each treatment. Three grains of IB synthetic fertilizer (10N–10P–10K–1Mg, Mitsubishi Chemical Agri Inc., Tokyo, Japan) were placed on the soil surface in each pot. Tap water was supplied to the trays every day. Plants were irradiated with UV-B every day for 3 weeks in the nighttime (0:00–3:00) or daytime (12:00–15:00). The intensity of UV-B was adjusted to 0, 80, or 120 mW·m^{-2}. After 3 weeks of treatment, the plants were harvested and the fresh weight of the aerial parts, number of nodes on the main stem, and length, width, a*, and anthocyanin concentration of the leaf blades at the second node from the top were measured. The ‘Houkouakashiso’ plants that underwent nighttime irradiation were harvested on October 5 and those of the same cultivar that underwent daytime irradiation were harvested on January 1. For the ‘Akachirimenshiso’ plants that underwent nighttime irradiation, the harvest took place on October 9 and those that underwent daytime irradiation it were harvested on December 18. The temperature inside the chamber was set to a maximum of 25°C (daytime) and was not controlled at nighttime.

Exp. 3 Effects of irradiation with visible rays together with UV-B on perilla growth and appearance

Twelve seedlings of ‘Akachirimenshiso’ and nine seedlings of ‘Houkouakashiso’ with eight true leaves were placed on a plastic tray in the growth chamber described above for each treatment. They were grown under the same conditions as in experiment 2, except with different lighting conditions. Visible rays (VIS) were provided by fluorescent lamps (FFH32EX-N-H; Panasonic Co.) at 0:00–3:00 and 6:00–22:00. Plants were irradiated with 120 mW·m^{-2} of UV-B at 0:00–3:00 in the nighttime UV-B + VIS treatment (Fig. 1). Control plants were not irradiated with UV-B. After 3 weeks of treatment, plants were harvested and the fresh weight of the aerial parts, number of nodes on the main stem, and length, width, a*, and anthocyanin concentration of the leaf blades at the second node from the top were measured. Plants were grown in the same growth chamber at that used in Experiment 2 and harvested on January 29.

Color measurement of leaves

As an index of the extent of red and green color of the adaxial surface of leaves, a* indicates the color difference between red and green in the CIE 1976 (L*, a*, b*) color space, and negative values indicate green, whereas positive values indicate red, as measured using a hand spectrophotometer (NR-3000; Nippon Denshoku Industries Co., Ltd., Tokyo, Japan). The mean values of a* were calculated from five areas on the adaxial surface of leaves at the second node.

Quantification of anthocyanin concentration

For anthocyanin quantification, 100 mg of leaf at the second node was sampled. Samples were immediately frozen with liquid nitrogen and stored at −80°C until measurement of the anthocyanin concentration. The frozen samples were homogenized in liquid nitrogen, and 1 mL of extraction buffer (10% hydrochloric acid in 50% methanol) was added. The extracted samples were centrifuged at 4°C at 15000 rpm for 15 min and the supernatant was collected. The supernatant was diluted 50 times with the same solvent and the absorption at 530 nm was measured with a spectrophotometer (UV-1800; Shimadzu Co., Kyoto, Japan). The amount of anthocyanin per 100 mg of fresh leaves was calculated from the absorption value using a standard curve prepared using cyanidin chloride (Polyphenols Laboratories AS, Sandnes, Norway).

Transmission electron microscopy

For transmission electron microscopy (TEM), fully expanded leaves were collected from the plants irradiated with 120 mW·m^{-2} of UV-B in the nighttime or daytime after 3 weeks of treatment in experiment 2. Leaves from the plants grown without artificial UV-B irradiation were also collected as a negative control. Leaf segments (2 × 2 mm) were fixed with 2.5% (w/v)
UV-B lamps were examined. The fresh weight of aerial parts and the number of nodes on the main stem were reduced by UV-B irradiation, and in period II, that of the UV-B irradiated plants was 21.2% smaller than that of the control plants (5.2 cm to 6.6 cm of control plants) (Table 1). From these results, it was inferred that artificial UV-B irradiation of perilla at night decreased the leaf area. Artificial UV-B irradiation in this experiment turned the leaf color greener (Fig. 2). The value of $a^*$ significantly decreased from 31.6 (control) to 21.5 in period I and from 30.7 (control) to 20.2 in period II by UV-B treatment (Table 1). Moreover, the content of anthocyanins was significantly reduced by UV-B irradiation from 0.87 to 0.56, and from 1.00 to 0.67 mg/100 mg fresh leaf in periods I and II, respectively (Table 1).

Exp. 2 Difference between day and night treatments in the extent of damage to perilla leaves by UV-B irradiation

To investigate the effect of irradiation time, we compared the extent of damage to perilla plants treated in the nighttime and daytime. In 'Houkouakashiso', there was no significant difference in the fresh weight of the aerial parts and the number of nodes on the main stem between the non-irradiated and irradiated plants in the nighttime treatments (Table 2). However, the length of the leaf blades decreased significantly by 15.9% (10.7 cm to 9.0 cm) and 20.6% (10.7 cm to 8.5 cm) in 'Akachirimenshiso' grown in a plastic house.

Table 1. The effects of UV-B on the growth and appearance of ‘Akachirimenshiso’ grown in a plastic house.

<table>
<thead>
<tr>
<th>Periods</th>
<th>Treatments</th>
<th>Fresh weight of aerial parts (g)</th>
<th>Number of nodes on the main stem</th>
<th>Length of leaf blades (cm)</th>
<th>Width of leaf blades (cm)</th>
<th>Anthocyanin concentration (mg/100 mg fresh leaf)</th>
<th>$a^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>62.5 a</td>
<td>9.2 a</td>
<td>10.3 b</td>
<td>8.0 a</td>
<td>31.6 a</td>
<td>0.87 a</td>
</tr>
<tr>
<td></td>
<td>UV</td>
<td>56.1 a</td>
<td>9.3 a</td>
<td>12.6 a</td>
<td>8.9 a</td>
<td>30.8 a</td>
<td>0.91 a</td>
</tr>
<tr>
<td></td>
<td>+UV-B</td>
<td>65.0 a</td>
<td>9.7 a</td>
<td>8.6 c</td>
<td>7.7 a</td>
<td>21.5 b</td>
<td>0.56 b</td>
</tr>
<tr>
<td>II</td>
<td>Control</td>
<td>95.5 a</td>
<td>10.9 a</td>
<td>9.3 a</td>
<td>6.6 a</td>
<td>30.7 a</td>
<td>1.00 a</td>
</tr>
<tr>
<td></td>
<td>UV</td>
<td>87.8 a</td>
<td>11.4 a</td>
<td>9.0 a</td>
<td>6.5 a</td>
<td>30.0 a</td>
<td>1.02 a</td>
</tr>
<tr>
<td></td>
<td>+UV-B</td>
<td>77.9 a</td>
<td>12.1 a</td>
<td>6.3 b</td>
<td>5.2 b</td>
<td>20.2 b</td>
<td>0.67 b</td>
</tr>
</tbody>
</table>

7 Approximately 50 mW·m$^{-2}$ UV-B was supplied at night (0:00–3:00) in +UV-B treatment.
8 Anthocyanin concentration was measured at pH 7.4, followed by post-fixation with 2% (w/v) osmium tetroxide in phosphate buffer. Fixed samples were dehydrated through an ethanol series and embedded in epoxy resin. Ultrathin sections (ca. 90 nm thick) were cut with a diamond knife using an ultramicrotome (Ultracut N; Reichert Nissei, Tokyo, Japan) and collected on Formvar-coated copper grids. The grids were stained with 2% (w/v) aqueous uranyl acetate for 10 min and Reynolds' lead citrate (Reynolds, 1963) for 1 min and were examined with a TEM (JEM-1400; JEOL Ltd., Tokyo, Japan) at 100 kV.

Statistical analysis

All statistical analyses were performed using Excel statistics 2010 (Social Survey Research Information, Tokyo, Japan). Significant differences among the means were identified at $P < 0.05$ by the Tukey–Kramer test in experiments 1 and 2 and the $t$-test in the experiment 3.

Results

Exp. 1 Effects of UV-B on perilla plants grown in a plastic house

At first, we tested the effects of solar UV radiation by growing perilla plants under UV cut film on the fresh weight of aerial parts, number of nodes on a main stem, length and width of leaf blades, $a^*$, and anthocyanin concentration. In period I, there were no significant differences between the −UV and control plants except in the length of the leaf blades (Table 1). Also in period II, there was no significant difference between the −UV treatment and control plants in any parameter measured in this experiment (Table 1).

Second, the effects of artificial UV-B irradiation by UV-B lamps were examined. The fresh weight of aerial parts and the number of nodes on the main stem were not affected by UV-B irradiation in either period (Table 1). However, the lengths of leaf blades were reduced by 16.5% (10.3 cm to 8.6 cm) and 32.3% (9.3 cm to 6.3 cm) compared with that of control plants in periods I and II, respectively (Table 1). In period I, the width of the leaf blades was slightly but non-significantly reduced by UV-B irradiation, and in period II, that of the UV-B irradiated plants was 21.2% smaller than that of the control plants (5.2 cm to 6.6 cm of control plants) (Table 1). From these results, it was inferred that artificial UV-B irradiation of perilla at night decreased the leaf area. Artificial UV-B irradiation in this experiment turned the leaf color greener (Fig. 2). The value of $a^*$ significantly decreased from 31.6 (control) to 21.5 in period I and from 30.7 (control) to 20.2 in period II by UV-B treatment (Table 1). Moreover, the content of anthocyanins was significantly reduced by UV-B irradiation from 0.87 to 0.56, and from 1.00 to 0.67 mg/100 mg fresh leaf in periods I and II, respectively (Table 1).

Exp. 2 Difference between day and night treatments in the extent of damage to perilla leaves by UV-B irradiation

To investigate the effect of irradiation time, we compared the extent of damage to perilla plants treated in the nighttime and daytime. In 'Houkouakashiso', there was no significant difference in the fresh weight of the aerial parts and the number of nodes on the main stem between the non-irradiated and irradiated plants in the nighttime treatments (Table 2). However, the length of the leaf blades decreased significantly by 15.9% (10.7 cm to 9.0 cm) and 20.6% (10.7 cm to 8.5 cm) in 'Akachirimenshiso' grown in a plastic house.
under 80 and 120 mW·m$^{-2}$ of nighttime UV-B irradiation, respectively (Table 2). Irradiation with 80 mW·m$^{-2}$ of UV-B also decreased the width of the leaf blades by 13.1% (8.4 cm to 7.3 cm) and that with 120 mW·m$^{-2}$ further decreased it by 25.0% (8.4 cm to 6.3 cm) (Table 2). Thus, UV-B irradiation in the nighttime appeared to turn purple perilla leaves green. The values of $a^*$ of the leaves irradiated with 80 or 120 mW·m$^{-2}$ significantly decreased compared with that of the control plants (Table 2). When UV-B irradiation was applied in the daytime, there was no significant difference between the irradiated and non-irradiated plants in the length or width of leaf blades or $a^*$, or in the fresh weight of the aerial parts or the number of nodes on the main stem (Table 2).

Similar trends were found for ‘Akachirimenshiso’. UV-B irradiation did not affect the fresh weight of the aerial parts of the plant under nighttime or daytime treatment (Table 2). Under the nighttime treatment, the number of nodes on the main stem was increased by UV-B irradiation (Table 2). UV-B irradiation at night significantly decreased the length and width of the leaf blades (Table 2). The values of $a^*$ of the leaves irradiated with 120 mW·m$^{-2}$ UV-B were lower than those of the leaves irradiated with 80 mW·m$^{-2}$, although there was no significant difference between these groups and the control leaves (Table 2). Anthocyanin concentration was decreased by 120 mW·m$^{-2}$ UV-B, but without a significant difference (Table 2). Then, we examined the ultra-structures of adaxial epidermal cells using TEM. Results indicated that 120 mW·m$^{-2}$ UV-B irradiation in the daytime or nighttime did not lead to significant destruction of ultra-structures in these cells, even in leaves that turned green following nighttime exposure to UV-B (Fig. 3). In all treatments, no plasma membrane or tonoplast collapse was observed.

**Exp. 3 Alleviation effects of visible irradiation with fluorescent lamps together with UV-B on UV-B damage to perilla leaves**

The results of experiment 2 suggested that the sensitivity of perilla plants to UV-B differed with irradiation time. In other words, damage from UV-B tended to be more severe following nighttime rather than daytime irradiation. We accordingly investigated the effects of visible irradiation by fluorescent lamps together with UV-B on the extent of UV-B damage to perilla plants. In ‘Houkouakashiso’, UV-B irradiation at night with VIS provided by fluorescent lamps did not significantly affect the measured parameters except for the length of leaf blades (Table 3). Visible appearance was not different compared to control plants (data not shown). UV-B irradiation of ‘Akachirimenshiso’ with VIS provided by

![Fig. 3. Transmission electron microscopy showing the ultrastructure of perilla adaxial epidermal cells. (A) Control plant grown under natural conditions in a plastic house. (B) Plant irradiated with 120 mW·m$^{-2}$ of UV-B during daytime (12:00 to 15:00). (C) Plant irradiated with 120 mW·m$^{-2}$ of UV-B during nighttime (0:00 to 3:00). Black bars indicate 2 µm.](image-url)

**Table 2. Effects of UV-B irradiation in the nighttime or daytime on the growth and appearance of ‘Houkouakashiso’ and ‘Akachirimenshiso’.

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Irradiation time</th>
<th>UV-B intensity (mW·m$^{-2}$)</th>
<th>Fresh weight of aerial parts (g)</th>
<th>Number of nodes on the main stem</th>
<th>Length of leaf blades (cm)</th>
<th>Width of leaf blades (cm)</th>
<th>$a^*$</th>
<th>Anthocyanin concentration (mg/100 mg fresh leaf)</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Houkouakashiso’</td>
<td>Nighttime* (0:00-3:00)</td>
<td>0</td>
<td>12.2 a*</td>
<td>7.2</td>
<td>10.7 a</td>
<td>8.4 a</td>
<td>27.5 a</td>
<td>0.88 a</td>
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<td></td>
<td></td>
<td>80</td>
<td>13.1 a</td>
<td>7.3</td>
<td>9.0 b</td>
<td>7.3 b</td>
<td>23.1 b</td>
<td>0.81 a</td>
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<td></td>
<td></td>
<td>120</td>
<td>12.2 a</td>
<td>7.3</td>
<td>8.5 b</td>
<td>6.3 c</td>
<td>20.6 b</td>
<td>0.82 a</td>
</tr>
<tr>
<td></td>
<td>Daytime (12:00-15:00)</td>
<td>0</td>
<td>12.6 a</td>
<td>7.2</td>
<td>9.7 a</td>
<td>8.1 a</td>
<td>25.7 a</td>
<td>1.38 ab</td>
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<td></td>
<td></td>
<td>80</td>
<td>12.4 a</td>
<td>7.1 a</td>
<td>10.1 a</td>
<td>8.2 a</td>
<td>26.1 a</td>
<td>1.33 b</td>
</tr>
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<td></td>
<td></td>
<td>120</td>
<td>11.7 a</td>
<td>7.1 a</td>
<td>9.6 a</td>
<td>8.0 a</td>
<td>25.7 a</td>
<td>1.69 a</td>
</tr>
<tr>
<td>‘Akachirimenshiso’</td>
<td>Nighttime* (0:00-3:00)</td>
<td>0</td>
<td>14.3 a</td>
<td>6.8 b</td>
<td>10.7 a</td>
<td>9.1 a</td>
<td>25.4 ab</td>
<td>0.96 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>14.8 a</td>
<td>7.0 ab</td>
<td>9.2 b</td>
<td>7.8 b</td>
<td>26.1 a</td>
<td>0.96 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>14.3 a</td>
<td>7.3 a</td>
<td>8.4 b</td>
<td>6.9 b</td>
<td>22.2 b</td>
<td>0.87 a</td>
</tr>
<tr>
<td></td>
<td>Daytime (12:00-15:00)</td>
<td>0</td>
<td>14.2 a</td>
<td>6.9 a</td>
<td>9.6 a</td>
<td>8.8 a</td>
<td>25.3 a</td>
<td>1.03 b</td>
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<tr>
<td></td>
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<td>80</td>
<td>15.5 a</td>
<td>7.0 a</td>
<td>8.7 a</td>
<td>8.2 a</td>
<td>25.0 a</td>
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<td>120</td>
<td>14.7 a</td>
<td>6.9 a</td>
<td>9.0 a</td>
<td>8.3 a</td>
<td>24.8 a</td>
<td>1.26 ab</td>
</tr>
</tbody>
</table>

* UV-B was supplied for 3 weeks from 2015/10/24 to 2015/11/5 under nighttime treatment and from 2015/12/8 to 2016/1/1 under daytime treatment in ‘Houkouakashiso’, and from 2015/10/14 to 2015/11/9 under night treatment and from 2015/11/19 to 2015/12/18 under daytime treatment in ‘Akachirimenshiso’ (n = 12).

* Means followed by same letters in the same column of the same cultivar are not significantly different among treatments at 5% by Tukey–Kramer test.
fluctuated particularly for the fresh weight of the aerial parts. This contrast to the previous study (210 and 880 mW·m$^{-2}$), suggesting that in the previous report that UV-B irradiation reduced the leaf area of perilla plants (Nishimura et al., 2008). This difference may have been due to the difference in intensity of UV-B, which is in agreement with a report that changes in plant morphology occurred in the absence of decreases in bio-
is mainly due to the difference in intensity of UV-B, though the results of the statistical analyses in both periods showed almost the same tendencies, the absolute values were different between the periods, particularly for the fresh weight of the aerial parts. This weight in the control plants in period I was 34.6% lower than that in period II, possibly owing to the difference in weather conditions. The total amount of sunlight hours and the temperature during the cultivation period were lower in period I than in period II in Kyoto (http://www.data.jma.go.jp/gmd/risk/obsdl/index.php). The fresh weight of aerial parts of perilla plants was not affected by UV-B irradiation (Table 1), in contrast to the results of a previous report that UV-B irradiation suppressed the growth of perilla plants (Nishimura et al., 2008). This difference may have been due to the difference in intensity of UV-B, given that the intensity of UV-B in this experiment (50 mW·m$^{-2}$; 0.54 kJ·m$^{-2}$·day$^{-1}$) was much lower than that in the previous study (210 and 880 mW·m$^{-2}$) with levels that are unrealistically high for pest control. The observation that both the length and width of the leaves were reduced suggested that the leaf area was reduced by UV-B irradiation (Table 1; Fig. 2). This finding agreed with that of a previous report which stated that UV-B irradiation reduced the leaf area of perilla plants (Nishimura et al., 2008). Thus, the appearance of perilla leaves, rather than their aerial growth (fresh weight), may be more sensitive to UV-B irradiation, an inference that is in agreement with a report that changes in plant morphology occurred in the absence of decreases in bio-

### Table 3. Effects of UV-B irradiation in the nighttime together with visible rays provided by fluorescent lamps on the growth and appearance of 'Houkouakashiso' and 'Akachirimenshiso'.

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Treatmentsa</th>
<th>Fresh weight of aerial parts (g)</th>
<th>Number of nodes on the main stem</th>
<th>Length of leaf blades (cm)</th>
<th>Width of leaf blades (cm)</th>
<th>Anthocyanin concentration (mg/100 mg fresh leaf)</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Houkouakashiso'</td>
<td>Control</td>
<td>10.4</td>
<td>7.0</td>
<td>9.4</td>
<td>7.5</td>
<td>29.1</td>
</tr>
<tr>
<td></td>
<td>UV-B + VIS</td>
<td>9.9</td>
<td>7.0</td>
<td>8.5</td>
<td>6.9</td>
<td>30.1</td>
</tr>
<tr>
<td></td>
<td>t-test</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>'Akachirimenshiso'</td>
<td>Control</td>
<td>13.6</td>
<td>7.2</td>
<td>8.3</td>
<td>7.5</td>
<td>27.1</td>
</tr>
<tr>
<td></td>
<td>UV-B + VIS</td>
<td>12.7</td>
<td>7.2</td>
<td>8.1</td>
<td>7.2</td>
<td>27.2</td>
</tr>
<tr>
<td></td>
<td>t-test</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

a Treatments were applied from 2016/1/8 to 2016/1/29 (n = 9).

Discussion

Before using UV-B irradiation to control spider mites in perilla production, its effect on the growth, development, and morphological appearance of perilla plants should be evaluated. Here, we investigated the effects of artificial UV-B irradiation on perilla plants grown under natural light conditions inside a plastic house. Although the results of the statistical analyses in both period I and II showed almost the same tendencies, the absolute values were different between the periods, particularly for the fresh weight of the aerial parts. This weight in the control plants in period I was 34.6% lower than that in period II, possibly owing to the difference in weather conditions. The total amount of sunlight hours and the temperature during the cultivation period were lower in period I than in period II in Kyoto (http://www.data.jma.go.jp/gmd/risk/obsdl/index.php). The fresh weight of aerial parts of perilla plants was not affected by UV-B irradiation (Table 1), in contrast to the results of a previous report that UV-B irradiation suppressed the growth of perilla plants (Nishimura et al., 2008). This difference may have been due to the difference in intensity of UV-B, given that the intensity of UV-B in this experiment (50 mW·m$^{-2}$; 0.54 kJ·m$^{-2}$·day$^{-1}$) was much lower than that in the previous study (210 and 880 mW·m$^{-2}$) with levels that are unrealistically high for pest control. The observation that both the length and width of the leaves were reduced suggested that the leaf area was reduced by UV-B irradiation (Table 1; Fig. 2). This finding agreed with that of a previous report which stated that UV-B irradiation reduced the leaf area of perilla plants (Nishimura et al., 2008). Thus, the appearance of perilla leaves, rather than their aerial growth (fresh weight), may be more sensitive to UV-B irradiation, an inference that is in agreement with a report that changes in plant morphology occurred in the absence of decreases in bio-

mass accumulation (Jansen et al., 1998). Kobayashi et al. (2013) successfully suppressed powdery mildew with 0.5–1.0 kJ·m$^{-2}$·day$^{-1}$ using nighttime UV-B irradiation in rose production. Also, irradiating at night for 2 h (0.32–1.0 kJ·m$^{-2}$·day$^{-1}$) has been recommended for powdery mildew control in strawberries (Matsuura et al., 2012) without UV-B damage. Tanaka et al. (2016) reported that 1.8 kJ·m$^{-2}$·day$^{-1}$ UV-B irradiation at night effectively suppressed its occurrence in strawberry cultures without damage. Because the cumulative irradiance of 0.54 kJ·m$^{-2}$·day$^{-1}$ used in this experiment was not as high as other studies, we can conclude that perilla is more sensitive to nighttime UV-B irradiation than roses and strawberries.

It is generally known that UV-B radiation promotes the accumulation of flavonoids, including anthocyanins, which absorb UV-B and are thought to protect plants from UV-B damage (Jansen et al., 1998). However, UV-B irradiation in this study in a plastic house reduced the a*, resulting in a change in leaf color (Table 1; Fig. 2). This change may have been due to a decrease in the concentration of anthocyanins (Table 1), pigments that range in color from pink to violet and blue. We showed that UV-B irradiation did not cause significant ultrastructural damage to the adaxial epidermal cells (Fig. 3), and it is likely that vacuole collapse was not the cause of the reduction of anthocyanins in UV-B irradiated perilla. A decrease in anthocyanin content has also been reported by Zipor et al. (2015) in flowers of Brunfelsia calycina before swelling. A vacuolar class III peroxidase was suggested to be involved in in planta anthocyanin degradation in B. calycina flowers reacting to toxic H$_2$O$_2$ (Zipor et al., 2015). Furthermore, Hosokawa et al. (2016) revealed that peroxidase activity increased in UV-B irradiated perilla leaves using semi-SDS PAGE. Thus, the lowering of the anthocyanin concentration in our study could result from its degradation, catalyzed by a vacuolar peroxidase scavenging H$_2$O$_2$. Further investigation into this effect is needed.
We investigated the effects of ambient UV-B radiation by growing perilla plants under UV cut film in a plastic house. There was almost no difference between the control and −UV treatment plants (Table 1). Accordingly, it was suggested solar UV-B under natural light conditions did not affect the growth and appearance of perilla plants. The maximum intensity of UV-B on the ground in Tokyo reaches approximately 2000 mW·m$^{-2}$ at noon in summer (Nozawa et al., 2007), and we measured approximately 300 mW·m$^{-2}$ at the plant level inside the plastic house in the afternoon at the beginning of the treatment in period 1 (data not shown). It seemed interesting that an additional 50 mW·m$^{-2}$ UV-B irradiation in the nighttime caused obvious damage to perilla plants, although intense ambient UV-B radiation in the daytime did not. We accordingly hypothesized that the extent of effects of UV-B on perilla plants differed depending on the time period of irradiation, and we conducted further experiments.

To test whether the time period of irradiation affects the extent of damage caused by UV-B, we compared the effects of UV-B irradiation between nighttime and daytime treatments under controlled light conditions. Nighttime UV-B irradiation was observed to increase the damage in both ‘Akachirimenshiso’ and ‘Houkouakashiso’ compared to daytime radiation (Table 2). In addition, the observed higher anthocyanin concentration in the daytime controls with respect to that of the nighttime controls (Table 2) may be one consequence of the lower minimum temperatures during the growth period.

One of the major differences between the nighttime and daytime treatments was the lighting conditions other than UV-B irradiation, that is, the presence of VIS provided by fluorescent lamps. In view of the finding that UV-B irradiation in the daytime caused less damage to perilla plants, we investigated the effects of VIS in mitigating UV-B damage when they were supplied together with UV-B. There was no significant difference between ‘Akachirimenshiso’ plants irradiated with both VIS and UV-B and those irradiated with VIS alone, and only the length of the leaf blades among the measured parameters in ‘Houkouakashiso’ plants was reduced by irradiation with both UV-B and VIS (Table 3). These results suggested that VIS provided by fluorescent lamps alleviated the deleterious effects of UV-B when provided in combination. One possible explanation for this phenomenon is photoreactivation. UV-B is absorbed by DNA and induces the dimerization of adjacent pyrimidine bases on the same DNA strand. This damage is repaired by photolyases in a process known as photo reactivation and requires visible light (Britt, 1999). Moreover, Takahashi et al. (2002) reported that UV-B irradiation had less effect on the growth of cucumber (Cucumis sativus) leaves when they were irradiated in the middle of the light period than when they were irradiated early in the morning or late afternoon. Takahashi et al. (2002) showed that in the middle of the light period, photolyase transcript levels and photoreactivation were relatively high, suggesting that photoreactivation has a positive effect on the growth of plants grown with UV-B radiation. However, few studies have reported the alleviating effects of artificial VIS (and its association with photoreactivation) on UV-B-induced damage in plants. The effects of irradiation with VIS provided by fluorescent lamps together with the UV-B observed in the present study offer a window to further investigate the role of photoreactivation.

In conclusion, we observed the damage caused by artificial UV-B irradiation to perilla plants, such as reduction in leaf area and change in leaf color. Nighttime irradiation may be preferable for horticultural crop production from the viewpoint of farmers’ safety. However, nighttime irradiation with UV-B caused more severe damage than daytime irradiation. Irradiation with VIS reduced the extent of the damage caused by UV-B. As a future prospect, we should aim to determine the light spectrum that reduces UV-B-induced damage in perilla leaves. The results obtained in this study will lead to the improvements in UV-B irradiation systems for controlling pests in horticultural crop production and techniques that cause less damage to plants.

**Literature Cited**


