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Original Article

Continuous UV-B irradiation inhibits abscission of initial long trichomes on the surface of the young first leaves of cucumber (*Cucumis sativus* L.) seedlings

Seiji Yamasaki* and Rie Tateishi

Laboratory of Plant Physiology, Department of Science Education, Faculty of Education, University of Teacher Education Fukuoka, 1-1 Akamabunkyomachi, Munakata, Fukuoka, 811-4192, Japan.

ABSTRACT

The young first leaves (blade length, approximately 1.1 cm) of cucumber (*Cucumis sativus* L.) seedlings are covered by long and dense trichomes. Abscission of these long trichomes and growth of other new trichomes, a process referred to as molting of trichomes, occurs over the first 7 days of leaf development. We examined the effect of continuous ultraviolet-B (UV-B; 290-320 nm) irradiation on the development of the young first leaves of cucumber seedlings for 7 days. The first leaves of UV-B-irradiated cucumber seedlings at day 7 were shorter than those of the control seedlings. The density, length and cell number of trichomes on the surface of the first leaves of UV-B-irradiated cucumber seedlings at day 7 were greater than those in the control seedlings. We found that the initial long and dense trichomes do not abscise and other new trichomes grow on the surface of the first leaves of UV-B-irradiated cucumber seedlings for 7 days. Thus, continuous UV-B irradiation inhibits abscission of initial long trichomes, thereby maintaining the hairy surface of the young first leaves in cucumber seedlings.

KEYWORDS: abscission, cucumber, toluidine blue O, trichome, UV-B.

*Corresponding author: yamasaki@fukuoka-edu.ac.jp

INTRODUCTION

Ultraviolet-B (UV-B; 290-320 nm) is a key environmental factor that initiates diverse responses at numerous levels in higher plants [1, 2]. For example, UV-B irradiation causes DNA and membrane damage, reduction of photosynthetic ability, inhibition of hypocotyl elongation, stunting, leaf area reduction, bronzing and necrosis [3-5]. UV-B-induced DNA damage is mediated by photolesions such as cyclobutane pyrimidine dimers (CPDs) and pyrimidine-(6-4)-pyrimidone photoproducts [(6-4) photoproducts] [6]. The reduction of photosynthetic ability is caused by the degradation of photosystem II D1 and D2 proteins, reduction of ribulose 1,5-bisphosphate carboxylase/oxygenase activity and damage to the thylakoid membrane [7]. Thus, UV-B is harmful to higher plants, and an increase in solar UV-B can conceivably decrease farm productivity in agriculture. Thus, a thorough understanding of the various effects of UV-B irradiation on farm crops is essential.

In response to UV-B irradiation, plants have developed various effective protective mechanisms [8]. For example, a complex set of repair mechanisms, including photolyase induction, excision and recombinational repair, are initiated to eliminate CPDs or (6-4) photoproducts. Indeed, CPD photolyase activity determines UV-B sensitivity in rice [9-13]. In addition, to reflect

and scatter UV-B irradiation, plants develop cuticular waxy layers, leaf hairs (trichomes) and leaf bladders [9, 14, 15]. Moreover, to attenuate the penetration of UV-B irradiation, plants produce various secondary metabolites, including UV-B-absorbing compounds such as flavonoids, anthocyanins, tannins and lignins [15-17]. Trichomes from a variety of species have been shown to contain UV-B-absorbing compounds [18, 19]. Therefore, trichomes reflect and absorb UV-B irradiation in higher plants. Thus, trichomes have been shown to play an important role in the protection of leaf tissues from UV-B irradiation in higher plants.

In cucumber (*Cucumis sativus* L.), a representative farm crop, aboveground parts of the seedlings consist of the cotyledons, hypocotyl and shoot apical meristem (SAM). These parts are often used in experimental studies of UV-B irradiation due to their high sensitivity [20-24]. We had previously shown that continuous exposure of cucumber cotyledons to UV-B irradiation induces rapid cellular expansion and the accumulation of polyphenolic compounds, possibly stress lignins, in epidermal cells surrounding the trichomes [17]. This phenomenon is thought to be one of the protective mechanisms against UV-B irradiation in cucumber cotyledons. However, the effects of UV-B irradiation on the SAM, namely the young expanding first leaves, in cucumber seedlings are not fully understood. Because cell division does not occur in open cotyledons while remaining active in the SAM, the responses against UV-B irradiation are expected to be different between the cotyledons and SAM in cucumber seedlings.

Young expanding leaves are known to show a hairy surface, with the number of trichomes reducing gradually as the leaves develop [25]. This pattern of trichome development seems to be rather common [26]. On the other hand, some studies have indicated that UV-B exposure increases trichome density or length [27-31]. These findings raise questions about the actual influence of UV-B irradiation on trichome development in young expanding leaves. To address these questions, we examined the effect of UV-B irradiation on trichome development of the young first leaves in cucumber seedlings.

MATERIALS AND METHODS

Plant materials

Cucumber (*Cucumis sativus* L. cv. 'Santo-suyo No. 2') seeds were purchased from Nakahara Seed Co. Ltd. (Fukuoka, Japan). The seeds were germinated on wet filter paper in a Petri dish at 26 °C in the dark for 2 to 3 days, and the seedlings were then transferred to plastic celltrays containing the soil composite Kumiai-Engei-Baido (0.4 g N, 1.2 g P, and 0.2 g K per kg; Seishin Sangyo Co. Ltd., Kitakyushu, Japan). The plants were grown under continuous fluorescent light (FLR40SW/M/36-B; Hitachi, Ltd., Tokyo, Japan) in an incubator (LH-200RDS; Nippon Medical & Chemical Instruments Co. Ltd., Osaka, Japan) at 26 °C. The photosynthetic photon flux density (PPFD) at the plant surface was approximately 213 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

UV-B irradiation

The method for UV-B exposure was essentially the same as that described by Yamasaki *et al*. [17]. When the first leaf blades were over 1.0 cm long, the plants were transferred to a growth cabinet furnished with continuous fluorescent light (FLR40SW/M/36-B; Hitachi, Ltd., Tokyo, Japan) at 25 °C, and the PPFD at the plant surface was approximately 160 $\mu mol \cdot m^{-2} \cdot s^{-1}$. For continuous UV-B irradiation, a sunlamp (FL-20E; Tozai Densan, Ltd., Osaka, Japan) was suspended 7 cm above the cotyledons. Wavelengths below 290 nm were absorbed by covering the sunlamp with a polyvinyl chloride film (cutting sheet 000C; Nakagawa Chemical Inc., Tokyo, Japan). Control plants that were not exposed to UV-B irradiation were grown under another sunlamp covered with a polyester film equivalent to a Mylar film, which absorbs all wavelengths below 320 nm (Melinex 516; Imperial Chemical Industries PLC, London, UK). The films were replaced weekly to account for reductions in transmittance. UV-B irradiation was conducted for 7 days. The UV intensity was measured using digital UV intensity meters (UV-5.7, UV-6.2, and UV-8.0; MK Scientific, Inc., Yokohama, Japan). The average intensity of UV-B irradiation was 0.57 ± 0.16 $W \cdot m^{-2}$, which was approximately equal to the natural UV-B irradiation on a cloudy day in May in Fukuoka (data not shown). Since the UV-A intensity was

0.33 ± 0.05 W·m^{-2} in the control plants and 0.40 ± 0.04 W·m^{-2} in the UV-B-irradiated plants, the UV-A intensities were similar between the treatment groups. Thus, the effects of UV-A irradiation were not considered in the present study.

Observation of trichomes on the surface of the first leaves of cucumber seedlings

To investigate the process of trichome development, first leaves of various lengths over 1.0 cm were excised from both UV-B-irradiated and control seedlings during the 7-day UV-B irradiation experiment. In addition, to investigate the effect of continuous UV-B irradiation for 7 days on trichome development, the first leaves on day 7 were excised from both UV-B-irradiated and control seedlings. These samples were immersed in 50% (v/v) commercial bleach (Kitchen kirei-kirei; Lion, Co., Tokyo, Japan) at 65 °C until they were decolorized. After washing with distilled water, the samples were stained with 0.005% toluidine blue O while deaerating them in an aspirator (EYELA A-1000S; Tokyorikakikai, Co., Tokyo, Japan) for 20 min and were observed under a light microscope (ECLIPSE E600W; Nikon, Co., Tokyo, Japan).

Measurement of the length of the first leaves and the density, length and cell number of trichomes

To investigate the effect of continuous UV-B irradiation on the development of the young first leaves of cucumber seedlings, the length of the first leaves irradiated with UV-B for up to 7 days was measured once a day using a scale. Measurements obtained for four plants were used to calculate the average length of the first leaves.

To investigate the effect of continuous UV-B irradiation on trichome development of the young first leaves of cucumber seedlings, the density, length and cell number of trichomes in the UV-B-irradiated first leaves at day 7 were measured. Trichome density was estimated by determining the number of trichomes randomly measured 8 times under a light microscope with 10 × 10 magnification divided by the area of the field-of-vision (1.1 mm × 1.1 mm × 3.14 = 3.7994 mm^2) (n = 3). Thus, measurements of trichome density were performed 24 times. The cell number of the trichomes was measured randomly for 40 trichomes under a light microscope with 10 × 10 magnification (n = 3). Thus, trichome cell number measurements were performed 120 times. Trichome length measurements were obtained randomly for 10 trichomes under a light microscope with 10 × 10 magnification (n = 3) using ocular and stage micrometers. Thus, trichome length measurements were performed 30 times.

Statistical analysis

The length of the first leaves and trichome density and length were expressed as mean ± standard error. Statistically significant differences were assessed with an unpaired, two-tailed Student's t-test.

RESULTS

The effect of continuous UV-B irradiation on the development of the young first leaves of cucumber seedlings

To investigate the effect of continuous UV-B irradiation on the development of the young first leaves of cucumber seedlings, the lengths of the first leaves irradiated with UV-B for up to 7 days were measured. In the control seedlings, the young first leaves developed favorably, and the blade length reached 5.6 ± 0.1 cm on day 7 (Figure 1A, B). In contrast, in the UV-B-irradiated seedlings, the blade length reached 3.4 ± 0.3 cm on day 7 (Figure 1A, B). Thus, continuous UV-B irradiation inhibited the development of the young first leaves of cucumber seedlings. This result is consistent with the findings of our previous study [17, 32, 33].

Trichome development on the surface of the young first leaves in the control seedlings

To investigate the normal process of trichome development, first leaves of various lengths over 1.0 cm were excised from the control seedlings during the 7-day experimental period and subjected to microscopic observation. Initial long trichomes developed on the surface of the 1.1–3.2-cm-long first leaves (Figure 2A-D). Most of these initial long trichomes consisted of 3–5 cells. The surface of the 3.6–4.6-cm-long first leaves showed many circular trails formed by the abscission of the

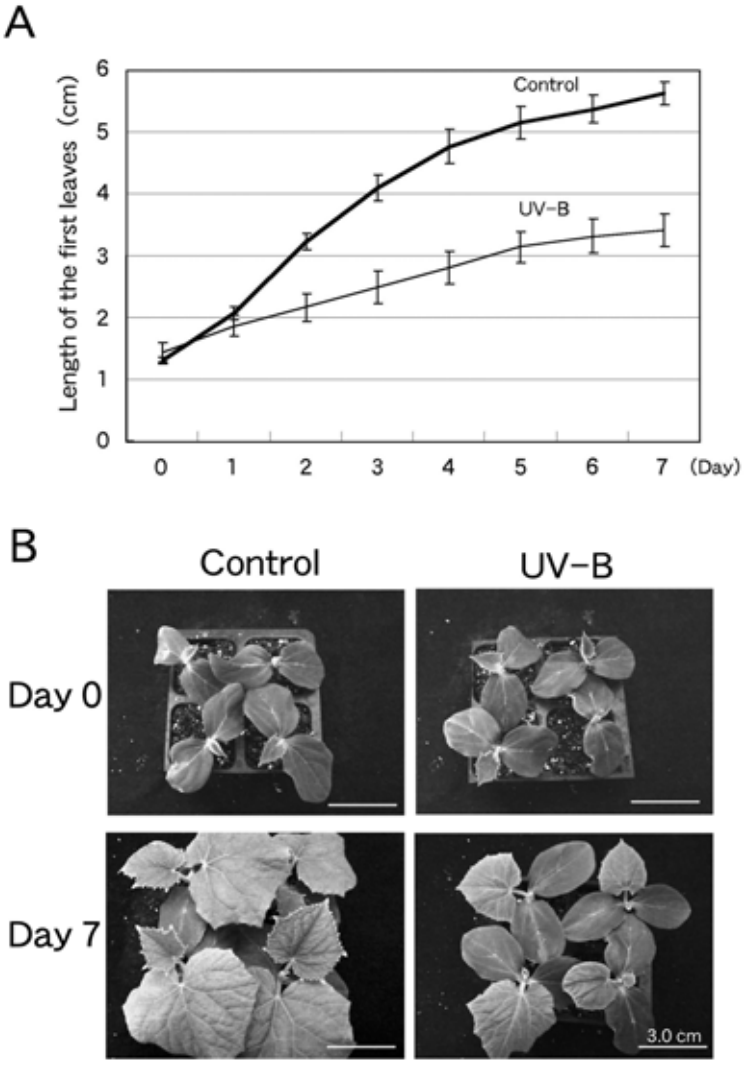

Figure 1. (A) Effect of continuous UV-B irradiation on the length of the first leaves of cucumber seedlings for 7 days. **(B)** Effect of continuous UV-B irradiation for 7 days on the growth of cucumber seedlings. Scale bar: 3.0 cm.

initial long trichomes (Figure 2E-G, black arrowheads). In fact, the 4.1-cm-long first leaf had a circular trail accompanied by an initial long trichome which was just starting to abscise (Figure 2F, white arrowhead bordered in black). These leaves also showed the growth of another new set of short trichomes (Figure 2C-H, vertical black arrows). Most of these newer short trichomes consisted of 1-2 cells. On the surface of the 5.5-cm-long first leaves, the circular trails indicating the abscission of the initial long trichomes had disappeared, and the developing new trichomes were present sparsely (Figure 2H). Thus, molting of trichomes with a reduction in trichome density occurs with the growth of the first leaves in cucumber seedlings.

The effect of continuous UV-B irradiation on trichome development of the young first leaves of cucumber seedlings

To investigate the effect of continuous UV-B irradiation on trichome development of the young first leaves of cucumber seedlings, the density, length and cell number of trichomes on the surface of the UV-B-irradiated first leaves at day 7 were measured. Representative images of the

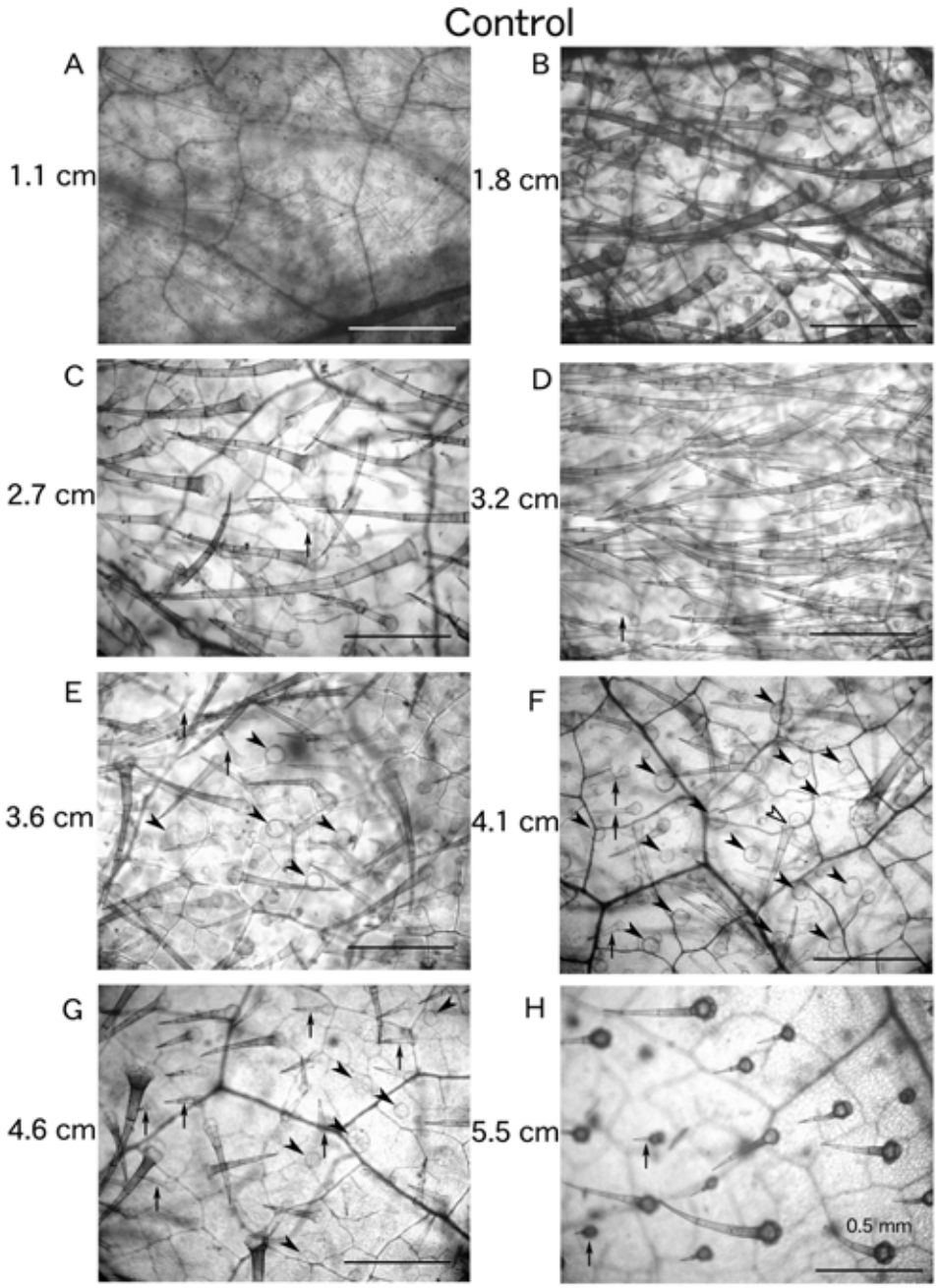

Figure 2. Developmental state of the trichomes on the 1.1-cm- **(A)**, 1.8-cm- **(B)**, 2.7-cm- **(C)**, 3.2-cm- **(D)**, 3.6-cm- **(E)**, 4.1-cm- **(F)**, 4.6-cm- **(G)** and 5.5-cm-long **(H)** first leaves of cucumber seedlings during the 7-day experimental period. Black arrowheads indicate circular trails formed by the abscission of the initial long trichomes. White arrowhead bordered in black **(F)** indicates the circular trail and just abscised initial long trichome. Vertical black arrows indicate growing new short trichomes. Scale bar: 0.5 mm.

surface of the first leaves at day 7 in both control and UV-B-irradiated seedlings are shown in Figure 3A. Trichome density was 4.5 ± 0.4/mm^2 on the surface of the first leaves in control seedlings and 14.7 ± 0.7/mm^2 on the surface of the first leaves in the UV-B-irradiated seedlings (Figure 3B). Thus, the trichome density on the surface of the first leaves in UV-B-irradiated

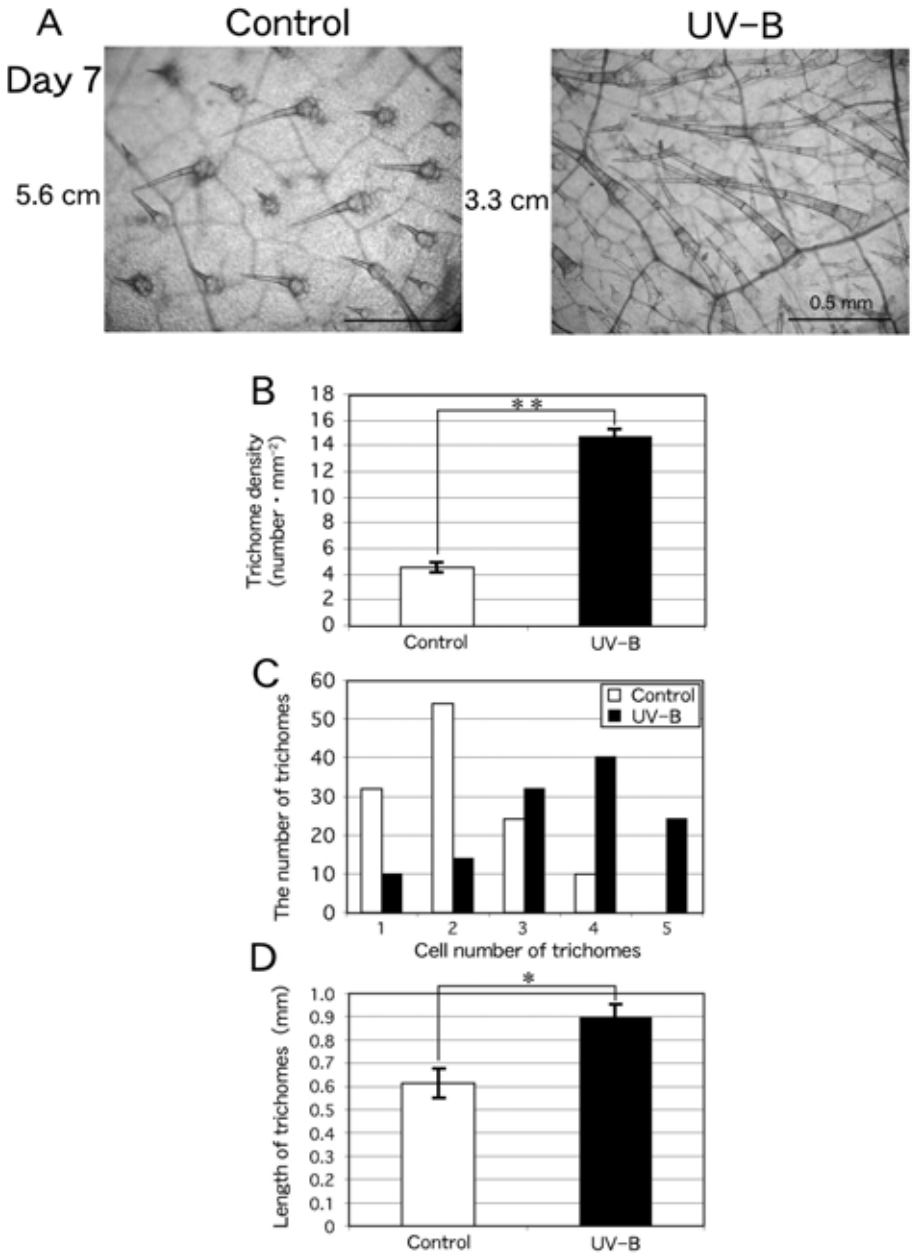

Figure 3. (A) Effect of continuous UV-B irradiation on the first leaves of cucumber seedlings for 7 days. "5.6 cm" and "3.3 cm" indicate the lengths of the first leaves of cucumber seedlings on day 7. Scale bar: 0.5 mm. **(B)** Effect of continuous UV-B irradiation on the trichome density on the surface of the first leaves of cucumber seedlings for 7 days. Statistically significant differences were determined by Student's *t*-test (***P* < 0.01). **(C)** Effect of continuous UV-B irradiation on the cell number of trichomes on the surface of the first leaves of cucumber seedlings for 7 days. **(D)** Effect of continuous UV-B irradiation on the length of trichomes on the surface of the first leaves of cucumber seedlings for 7 days. Statistically significant difference was determined by Student's *t*-test (**P* < 0.05).

seedlings was greater than that in the control seedlings ($P < 0.01$). The control seedlings showed trichomes consisting of 1–4 cells ($n = 120$), with trichomes consisting of one ($n = 32$) and two ($n = 54$) cells appearing most frequently (Figure 3C). In contrast, the UV-B-irradiated seedlings showed trichomes consisting of 2–5 cells ($n = 120$), with trichomes consisting of three

(n = 32), four (n = 40), and five (n = 24) cells appearing most frequently (Figure 3C). Thus, the cell number of trichomes in UV-B-irradiated seedlings was also greater than that in the control seedlings. The trichome length was 0.61 ± 0.06 mm on the surface of the first leaves in the control seedlings whereas it was 0.89 ± 0.06 mm on the surface of the first leaves in the UV-B-irradiated seedlings (Figure 3D). Thus, the trichome length on the surface of the first leaves in UV-B-irradiated seedlings was greater than that in the control seedlings ($P < 0.05$).

Trichome development on the surface of the young first leaves in UV-B-irradiated seedlings

To investigate the effect of UV-B irradiation on trichome development, first leaves of various lengths over 1.0 cm were excised from the UV-B-irradiated seedlings over the 7-day experimental period and assessed microscopically. The initial long trichomes did not abscise but continued to exist on the surface of the 1.4–3.4-cm-long first leaves (Figure 4A-H). Most of the initial long trichomes consisted of 3–5 cells. Moreover, the leaves also showed the growth of newer short trichomes (Figure 4C-H, vertical black arrows). Most of these newer trichomes consisted of 1-2 cells. Thus, abscission of the initial long trichomes was not found on the surface of the first leaves of UV-B-irradiated seedlings during the 7-day experimental period.

DISCUSSION

Trichomes are uni- or multicellular hairs that develop on the leaves, sepals and stems of higher plants [34]. Although trichomes on the surface of the young first leaves in cucumber seedlings consist of 1-5 cells, their developmental process is still unclear. In this study, we confirmed that the trichome density on the surface of the first leaves of control seedlings decreases as the first leaves develop over 7 days (Figure 2). Specifically, we found that abscission of the initial long trichomes and growth of new trichomes, i.e., the process of molting of trichomes, occurs during the reduction in trichome density on the surface of the first leaves of control seedlings (Figure 2). These findings extend our existing knowledge regarding molting of trichomes, which stated that young expanding leaves have a hairy surface and show a gradual decrease in the number of trichomes as they develop [25].

In this study, the effect of continuous UV-B irradiation on the development of trichomes on the surface of the young first leaves of cucumber seedlings was analyzed. We found that the initial long trichomes do not abscise and continue to exist on the surface of the first leaves of UV-B-irradiated seedlings (Figure 4), in contrast to the control seedlings (Figure 2), throughout the 7-day experimental period. Moreover, the developmental state of trichomes on the surface of the 1.8-, 2.7- and 3.2-cm-long first leaves of UV-B-irradiated seedlings (Figure 4B, D, F) was similar to that of the 1.8-, 2.7- and 3.2-cm-long first leaves of the control seedlings (Figure 2B, C, D). Thus, there is a high correlation between the developmental state of trichomes on the surface of the first leaves and the blade length of the first leaves in both control and UV-B-irradiated cucumber seedlings (Figures 2, 4). The control seedlings did not show abscission of the initial long trichomes on the surface of first leaves less than 3.2 cm in length (Figure 2D), but they showed abscission on the surface of 3.6-cm-long first leaves (Figure 2E). Therefore, UV-B irradiation is thought to suppress the length of the first leaves to less than 3.4 cm, restraining the developmental state of trichomes before the initial long trichomes start abscission. As a result of the UV-B-induced inhibition of both the development of the first leaves and the abscission of initial long trichomes, the surface of the young first leaves in cucumber seedlings continues to show a state of relatively high trichome density. This is the cause for the differences in the density, cell number and length of trichomes between control and UV-B-irradiated cucumber seedlings on day 7 (Figure 3).

Trichomes have been reported to protect higher plants from UV-B irradiation, drought, pathogens and herbivores [19, 35-40]. In this study, two possible roles of the long and dense trichomes against UV-B irradiation could be estimated. First, long and dense trichomes are thought to physically reflect UV-B irradiation in cucumber seedlings, similar to the findings of a previous report in which dense trichomes increased the reflectance of UV-B irradiation in two

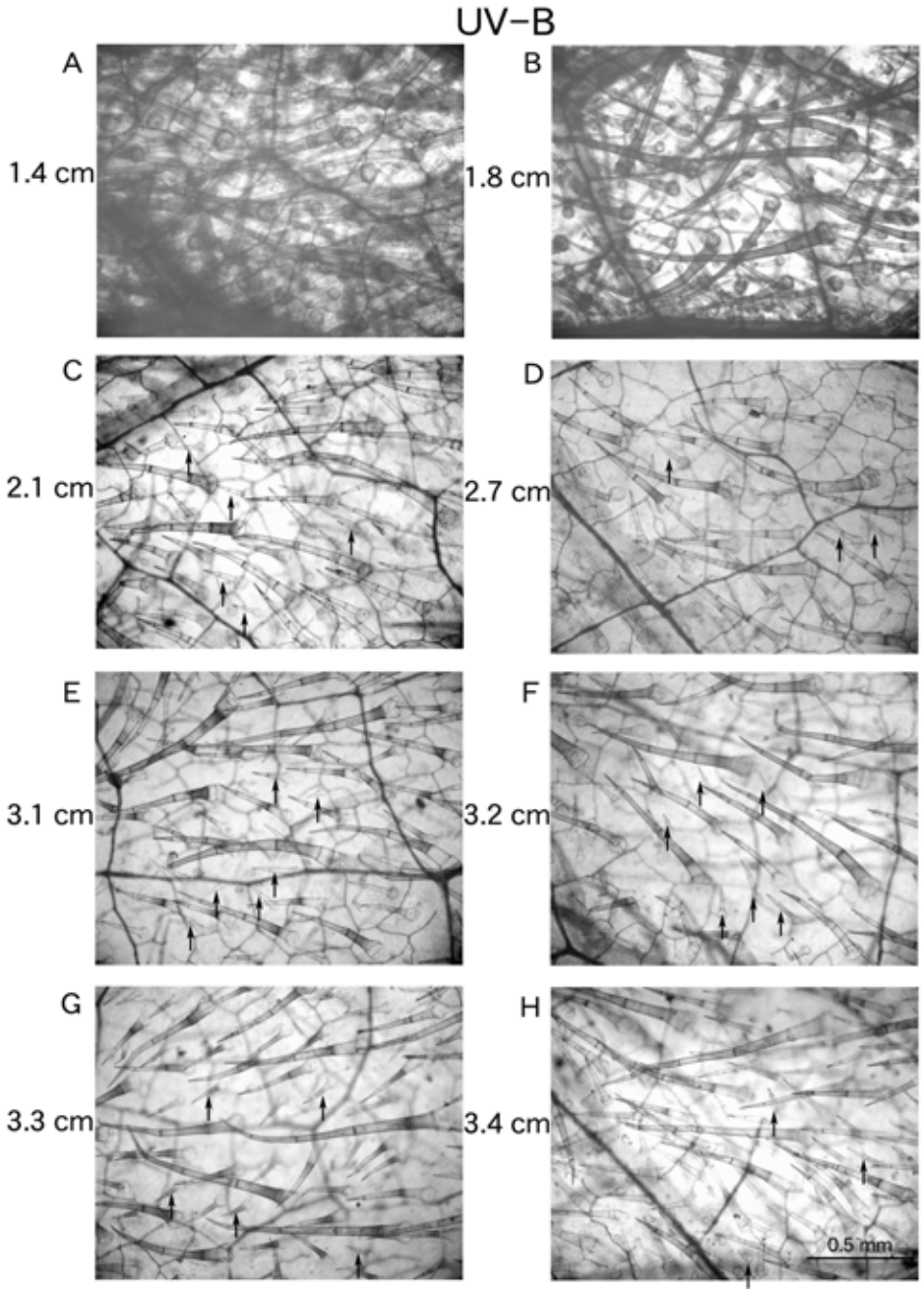

Figure 4. Effect of continuous UV-B irradiation on the developmental state of trichomes on 1.4-cm- **(A)**, 1.8-cm- **(B)**, 2.1-cm- **(C)**, 2.7-cm- **(D)**, 3.1-cm- **(E)**, 3.2-cm- **(F)**, 3.3-cm- **(G)** and 3.4-cm-long **(H)** first leaves of cucumber seedlings over the 7-day experimental period. The vertical black arrows indicate growing new short trichomes. Scale bar: 0.5 mm.

xeromorphic plants (olive and oak) and Sonoran Desert plants [15, 37, 41]. Second, long and dense trichomes are thought to increase the absorbance of UV-B irradiation. In higher plants, polyphenolic compounds such as flavonoids, cinnamate esters, lignins and tannins play multiple roles in signal transduction, structural integrity and pathogen defense [8]. Among other functions of polyphenolic compounds, absorption of UV-B irradiation is evolutionarily important for plants to

adapt to terrestrial environments [8]. Our histochemical data showed that the trichomes on the surface of the first leaves of cucumber seedlings were stained by toluidine blue O (Figures 2, 4). Since toluidine blue O stains polyphenolic compounds [42], it is conceivable that the trichomes on the surface of the first leaves of cucumber seedlings contain polyphenolic compounds. This idea is consistent with the findings of previous reports showing the presence of UV-B-absorbing compounds in trichomes from a variety of species [18, 19]. Thus, the long and dense trichomes may effectively absorb UV-B irradiation in cucumber seedlings. In summary, the long and dense trichomes physically reflect and effectively absorb UV-B irradiation, thereby protecting the underlying tissues in cucumber seedlings. This might be one of the natural protective mechanisms for young expanding leaves in cucumber seedlings.

In farm crops, strong UV-B irradiation is expected to restrain growth and increase the number of long trichomes. The presence of trichomes on the edible parts of farm crops will affect the texture, and the increased levels of polyphenolic compounds can be expected to affect the taste of the crops. These findings may indicate the need to control the exposure of farm crops to UV-B irradiation.

CONCLUSION

The young first leaves of cucumber seedlings are covered by long and dense trichomes. Abscission of these long trichomes and growth of other new trichomes, a process referred to as molting of trichomes, occurs over the first 7 days of leaf development. We examined the effect of continuous UV-B irradiation on the development of the young first leaves of cucumber seedlings for 7 days. We found that the initial long and dense trichomes do not abscise and other new trichomes grow on the surface of the first leaves of UV-B-irradiated cucumber seedlings for 7 days. Thus, continuous UV-B irradiation inhibits abscission of initial long trichomes, thereby maintaining the hairy surface of the young first leaves in cucumber seedlings. It is possible that the long and dense trichomes physically reflect and effectively absorb UV-B irradiation, thereby protecting the underlying tissues in cucumber seedlings. This might be one of the natural protective mechanisms for young expanding leaves in cucumber seedlings.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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