ANALYSIS OF THE ROLES OF TRICHOMES ON THE COTYLEDON SURFACE OF CUCUMBER (CUCUMIS SATIVUS L.) SEEDLINGS AGAINST CONTINUOUS UV-B IRRADIATION

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Article History: **Received:** 10.09.2022 **Revised:** 18.10.2022 **Accepted:** 07.11.2022

Abstract: Continuous ultraviolet-B (UV-B; 290-320 nm) irradiation can induce rapid expansion and toluidine blue O staining in epidermal cells surrounding the trichomes on the surface of cucumber (Cucumis sativus L.) cotyledons. To directly evaluate the roles of these trichomes against UV-B irradiation, the trichomes were removed without affecting the growth of cucumber seedlings and the effect of UV-B irradiation over 7 days on the cucumber seedlings without trichomes was analyzed. After UV-B exposure for 7 days, the leaf area of the cotyledons, fresh weight of the aerial parts, and chlorophyll content of the cotyledons in the cucumber seedlings without trichomes were significantly lower than those in cucumber seedlings with trichomes. The cucumber seedlings without trichomes showed neither expansion nor toluidine blue O staining in the epidermal cells surrounding the trichomes after UV-B exposure for 7 days. Thus, trichomes on the surface of cucumber cotyledons protect the cucumber seedlings from UV-B-induced senescence. These trichomes may be necessary for UV-B-induced rapid expansion and toluidine blue O staining, namely, accumulation of polyphenolic compounds in the epidermal cells surrounding the trichomes on the surface of cucumber cotyledons. The trichomes may act as sensors to mediate the defense responses against UV-B irradiation in cucumber seedlings.

Keywords: cucumber, trichome, toluidine blue O, UV-B.

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DOI: 10.31838/ecb/2022.11.10.018

INTRODUCTION

Ultraviolet-B (UV-B; 290-320 nm) is an environmental factor that induces diverse responses at several levels in higher plants.1,2 For example, UV-B irradiation can cause DNA and membrane damage and reduce photosynthetic ability by causing reduction of chlorophyll content, inhibition of hypocotyl elongation, stunting, reduction of leaf area, 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 (Rubisco) activity, and damage to the thylakoid membrane.7 The reduction in chlorophyll content is thought to be the result of induction of senescence by UV-B irradiation.8 Thus, UV-B is harmful to higher plants, and the adverse effects of solar UV-B irradiation on farm crop productivity is a known problem in agriculture.

In response to UV-B irradiation, higher plants have developed pleiotypic protective mechanisms.9 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, the activity of CPD photolyase is correlated with UV-B sensitivity in rice (Oryza sativa L.).10-14 In addition, to reflect and scatter UV-B irradiation, higher plants have evolved cuticular waxy layers, leaf hairs (trichomes), and leaf bladders.10,15,16 Furthermore, to attenuate the penetration of UV-B irradiation, higher plants produce various secondary metabolites, including UV-B-absorbing compounds such as flavonoids, anthocyanins, tannins, and lignins.16-18 Trichomes from a variety of species have been shown to contain UV-B-absorbing compounds.19-21 Therefore, trichomes are thought to reflect and absorb UV-B irradiation in higher plants. Mutant analyses in cucumber (C.

sativus L.), a representative farm crop, recently revealed that both trichome formation and flavonoid and anthocyanin biosynthesis are interrelated.22,23 Thus, the relationship of trichomes with specific metabolic pathways has been elucidated at the molecular level.

In cucumber, the aerial 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.24-28 We had previously shown that continuous exposure of cucumber cotyledons to UV-B irradiation induces rapid cellular expansion and toluidine blue O staining, i.e., the accumulation of polyphenolic compounds, possibly stress lignins, in epidermal cells surrounding the trichomes.18 This phenomenon is thought to be one of the protective mechanisms against UV-B irradiation in cucumber cotyledons. However, the precise roles of trichomes in protecting cucumber cotyledons against UV-B irradiation are not fully understood. More specifically, it is not clear whether trichomes are necessary for the rapid expansion and accumulation of polyphenolic compounds in the epidermal cells surrounding the trichomes on the surface of cucumber cotyledons under continuous UV-B irradiation. In the present study, to directly validate the roles of trichomes against UV-B irradiation, trichomes on the surface of the cucumber cotyledons were eliminated without affecting the growth of cucumber seedlings and the effect of UV-B irradiation for 7 days on cucumber seedlings without trichomes was analyzed.

MATERIALS AND METHODS

Plant materials

Cucumber (C. 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 seedlings 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 μmol□m-2□s-1.

Elimination of trichomes on the surface of cucumber cotyledons

When the first leaf blades were over 1.0 cm long, the cotyledon blades were over 4.0 cm long in cucumber seedlings. The trichomes of cucumber seedlings with cotyledon blades over 4.0 cm in length were eliminated. To eliminate trichomes without affecting the growth of cucumber seedlings, KimWipes were cut to a size of 3 cm in height and 20 cm in width and rolled. Next, the surface of the cucumber cotyledons was gently rubbed with the rolled KimWipes while the changes in trichomes were assessed under a stereomicroscope (SPZT-50FTM, MS5573; Carton Optical Industries, Ltd., Kanagawa, Japan). The cotyledons in which trichomes were not eliminated were designated as "trichome (+) cotyledons," whereas those in which trichomes were eliminated were designated as "trichome (-) cotyledons."

UV-B irradiation

The method used for UV-B exposure was essentially the same as that described by Yamasaki et al. in 2007.18 When the first leaf blades were over 1.0 cm long, the plants were transferred to a growth cabinet 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 μmol□m-2□s-1. Just before the transfer, elimination of trichomes on the surface of cucumber cotyledons was conducted as described above. 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). Seedlings exposed to UV-B irradiation were designated as "UV-B (+)." Seedlings that were not exposed to UV-B irradiation were grown under another sunlamp covered with a polyester film equivalent to a Mylar film, which absorbed all wavelengths below

320 nm (Melinex 516; Imperial Chemical Industries PLC, London, UK). Seedlings not exposed to UV-B irradiation were designated as "UV-B (-)." 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·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 the surface of trichome (+) or trichome (-) cotyledons in cucumber seedlings grown under UV-B (-) or UV-B (+) conditions To investigate the surface of trichome (+) and trichome (-) cotyledons in cucumber seedlings grown under UV-B (-) conditions for 7 days, the trichome (+) and trichome (-) cotyledons from cucumber seedlings grown under UV-B (-) conditions were excised on days 0, 1, 3, 5, and 7. To investigate the effects of continuous UV-B irradiation for 7 days on the surface of trichome (+) or trichome (-) cotyledons in cucumber seedlings, trichome (+) or trichome (-) cotyledons from cucumber seedlings grown under UV-B

(+) conditions were excised on days 1, 3, 5, and 7. In the present study, day 0 was defined as the day when trichomes on the surface of cotyledons were eliminated and the cucumber seedlings were transferred to the growth cabinet to grow under UV-B (-) or UV-B (+) conditions. Samples of these four groups 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, after which they were observed under

a light microscope (ECLIPSE E600W; Nikon, Co., Tokyo, Japan).

Observation of the growth of cucumber seedlings with trichome (+) or trichome (-) cotyledons grown under UV-B (-) or UV-B (+) conditions To observe the growth of cucumber seedlings with trichome (+) or trichome (-) cotyledons grown under UV-B (-) conditions for 7 days, these seedlings were photographed with a digital camera (WG-40; RICOH IMAGING COMPANY, LTD., Tokyo, Japan) on days 1, 3, 5, and 7. To investigate the effect of continuous UV-B irradiation for 7 days on the growth of cucumber seedlings with trichome (+) or trichome (-) cotyledons, cucumber seedlings with trichome (+) or trichome (-) cotyledons grown under UV-B (+) conditions were photographed with the digital camera on days 1, 3, 5, and 7.

Measurement of the leaf area of trichome (+) or trichome (-) cotyledons in cucumber seedlings grown under UV-B (-) or UV-B (+) conditions To investigate the leaf area of trichome (+) or trichome (-) cotyledons in cucumber seedlings grown under UV-B (-) conditions for 7 days, trichome (+) or trichome (-) cotyledons from cucumber seedlings grown under UV-B (-) conditions were excised on days 1, 2, 3, 4, 5, 6, and 7. To investigate the effect of continuous UV-B irradiation on the leaf area of trichome (+) or trichome (-) cotyledons in cucumber seedlings for 7 days, trichome (+) or trichome (-) cotyledons from cucumber seedlings grown under UV-B (+) conditions were excised on days 1, 2, 3, 4, 5, 6, and 7. The leaf areas of these four classes of cotyledons were calculated using a photomeasure (Kenis, Ltd., Osaka, Japan). In each class, four cotyledons were used to calculate the leaf area.

Measurement of the fresh weight of the aerial parts of cucumber seedlings with trichome (+) or trichome (-) cotyledons grown under UV-B (-) or UV-B (+) conditions To investigate the fresh weight of the aerial parts of cucumber seedlings with trichome (+) or trichome (-) cotyledons grown under UV-B (-) conditions for 7 days, the aerial parts from cucumber seedlings with trichome (+) or trichome (-) cotyledons grown under UV-B (-) conditions were excised on days 1, 2, 3, 4, 5, 6, and 7. To investigate the effect of continuous UV-B irradiation on the fresh weight of the aerial parts ofcucumber seedlings with trichome (+) or trichome (-) cotyledons for 7 days, the aerial parts from cucumber seedlings with trichome (+) or trichome (-) cotyledons grown under UV-B (+) conditions were excised on days 1, 2, 3, 4, 5, 6, and 7. For each of the four classes, four seedlings were used to measure the fresh
weight.

Measurement of the chlorophyll content of trichome (+) or trichome (-) cotyledons in cucumber seedlings grown under UV-B (-) or UV-B (+) conditions The chlorophyll meter SPAD-502 Plus has been reported to be an effective tool for rapid and nondestructive estimation of leaf chlorophyll content in tomato (Solanum lycopersicum) leaves.29 The chlorophyll content of trichome (+) or trichome (-) cotyledons in cucumber seedlings grown under UV-B (-) conditions for 7 days was estimated using a chlorophyll meter (SPAD-502 Plus, KONICA MINOLTA, INC., Tokyo, Japan) on days 0, 1, 3, 5, and 7. To investigate the effect of continuous UV-B irradiation for 7 days on the chlorophyll content of trichome (+) or trichome (-) cotyledons in cucumber seedlings, the chlorophyll content of trichome (+) or trichome (-) cotyledons of cucumber seedlings grown under UV-B (+) conditions were measured by the chlorophyll meter on days 0, 1, 3, 5, and 7. In each of the four classes, four cotyledons were used to measure the chlorophyll content.

Statistical analysis

The leaf area of cotyledons, fresh weight of the aerial parts, and chlorophyll content of the cotyledons in cucumber seedlings were expressed as mean ± standard error. Statistically significant differences were assessed with an unpaired, two-tailed Student's t-test.

RESULTS

1. Elimination of trichomes on the surface of cotyledons in cucumber seedlings grown under UV-B (-) conditions

To investigate the surface of trichome (+) or trichome (-) cotyledons in cucumber seedlings grown under UV-B (-) conditions for 7 days, trichome (+) or trichome (-) cotyledons from cucumber seedlings grown under UV-B (-) conditions were excised on days 0, 1, 3, 5, and 7. Trichomes were recognized on the surfaces of cucumber cotyledons that were not rubbed with the rolled KimWipes on days 0 (Figures 1A, B), 1, 3, 5, and 7 (Figures 2A-D), whereas they were not recognized and their trails were present on the surface
of cucumber cotyledons rubbed with the rolled KimWipes on days 0 (Figures 1C, D, black arrowheads), 1, 3, 5, and 7 (Figures 2E-H). Thus, trichomes were eliminated clearly on the surface of cucumber cotyledons rubbed with the rolled KimWipes in comparison with the surface of cucumber cotyledons that were not rubbed with the rolled KimWipes. Figure 1, 2

2. Analysis of the cucumber seedlings with trichome (+) or trichome (-) cotyledons grown under
UV-B (-) conditions

1) Observation of the growth of cucumber seedlings with trichome (+) or trichome (-) cotyledons grown under UV-B (-) conditions

To investigate the growth of cucumber seedlings with trichome (+) or trichome (-) cotyledons grown under UV-B (-) conditions for 7 days, cucumber seedlings with trichome (+) or trichome (-) cotyledons grown under UV-B (-) conditions were photographed with the digital camera on days 1, 3, 5, and 7. Cucumber seedlings with trichome (+) or trichome (-) cotyledons grew well, and no remarkable visible differences were found between the cucumber seedlings with trichome (+) and trichome (-) cotyledons grown under UV-B (-) conditions for 7 days (Figures 3A-H). Thus, elimination of trichomes on the surface of cucumber cotyledons did not affect the visible growth of the seedlings grown under UV-B (-) conditions for 7 days. Figure 3

2) Analysis of the leaf area of trichome (+) or trichome (-) cotyledons in cucumber seedlings grown under UV-B (-) conditions To investigate the leaf area of trichome (+) or trichome (-) cotyledons in cucumber seedlings grown under UV-B (-) conditions for 7 days, trichome (+) or trichome (-) cotyledons from cucumber seedlings grown under UV-B (-) conditions were excised on days 1, 2, 3, 4, 5, 6, and 7. The leaf

areas of trichome (+) or trichome (-) cotyledons in cucumber seedlings expanded favorably and showed no remarkable differences between trichome (+) and trichome (-) cotyledons over 7 days (Figure 4A). Thus, elimination of trichomes on the surface of cucumber cotyledons did not affect the leaf area of cotyledons grown under UV-B (-) conditions for 7 days. Figure 4

3) Analysis of the fresh weight of the aerial parts of cucumber seedlings with trichome (+) or trichome (-) cotyledons grown under UV-B (-) conditions To investigate the fresh weight of the aerial parts of cucumber seedlings with trichome (+) or trichome (-) cotyledons grown under UV-B (-) conditions for 7 days, the aerial parts from cucumber seedlings with trichome (+) or trichome (-) cotyledons grown under UV-B (-) conditions were excised on days 1, 2, 3, 4, 5, 6, and 7. The fresh weight of the aerial parts of cucumber seedlings with trichome (+) or trichome (-) cotyledons increased favorably and showed no remarkable differences over 7 days (Figure 4B). Thus, elimination of trichomes on the surface of cucumber cotyledons did not affect the fresh weight of the aerial parts of the seedlings grown under UV-B (-) conditions for 7 days.

4) Analysis of the chlorophyll content of trichome (+) or trichome (-) cotyledons in cucumber seedlings grown under UV-B (-) conditions To investigate the chlorophyll content of trichome (+) or trichome (-) cotyledons in cucumber seedlings grown under UV-B (-) conditions for 7 days, the chlorophyll content of trichome (+) or trichome (-) cotyledons in cucumber seedlings grown under UV-B (-) conditions was measured by the chlorophyll meter on days 0, 1, 3, 5, and 7. The chlorophyll content of trichome (+) or trichome (-) cotyledons in cucumber seedlings remained constant, and no remarkable differences were found between the chlorophyll

content of trichome (+) and trichome (-) cotyledons in cucumber seedlings grown under UV-B (-) conditions for 7 days (Figure 4C). Thus, elimination of trichomes on the surface of cucumber cotyledons did not affect the chlorophyll content of the cotyledons in the seedlings grown under UV-B (-) conditions for 7 days.

3. The effect of continuous UV-B irradiation on the surface of trichome (+) or trichome (-) cotyledons in cucumber seedlings

To investigate the effect of continuous UV-B irradiation for 7 days on the surface of trichome (+) or trichome (-) cotyledons in cucumber seedlings, trichome (+) or trichome (-) cotyledons were excised on days 1, 3, 5, and 7 from cucumber seedlings grown under UV-B (+) conditions. Under UV-B (+) conditions, trichomes and expansion and toluidine blue O staining in epidermal cells surrounding the trichomes were recognized on days 1, 3, 5, and 7 on the surface of cucumber cotyledons that were not rubbed with the rolled KimWipes (Figures 2I-L), differ from the findings for the corresponding seedlings grown under UV-B (-) conditions (Figures 2A-D) for 7 days. This phenomenon is consistent with the findings of our previous study.18,30 In contrast, neither trichomes nor expansion and toluidine blue O staining in epidermal cells surrounding the trichomes was recognized and the trails of trichomes were found on days 1, 3, 5, and 7 on the surface of cucumber cotyledons rubbed with the rolled KimWipes under UV-B (+) conditions (Figures 2M-P), similar to the findings for the corresponding seedlings grown under UV-B (-) conditions (Figures 2E-H) for 7 days. Thus, neither expansion nor toluidine blue O staining in epidermal cells surrounding the trichomes was found on the surface of trichome (-) cotyledons in cucumber seedlings grown under UV-B (+) conditions for 7 days.

4. Analysis of the cucumber seedlings with trichome (+) or trichome (-) cotyledons grown under UV-B (+) conditions

1) The effect of continuous UV-B irradiation on the growth of cucumber seedlings with trichome (+) or trichome (-) cotyledons

To investigate the effect of continuous UV-B irradiation for 7 days on the growth of cucumber seedlings with trichome (+) or trichome (-) cotyledons, cucumber seedlings with trichome (+) or trichome (-) cotyledons grown under UV-B (+) conditions were photographed with the digital camera on days 1, 3, 5, and 7. In comparison with the trichome (+) cotyledons of cucumber seedlings grown under UV-B (-) conditions, trichome (+) cotyledons of cucumber seedlings grown under UV-B (+) conditions showed inhibition of the development of the first leaf and shining of the surface of cotyledons (Figures 3A-D, 3I-L). This phenomenon is consistent with the findings of our previous study.18,30 In contrast, in comparison with the trichome (-) cotyledons of cucumber seedlings grown under UV-B (-) conditions, the trichome (-) cotyledons of cucumber seedlings grown under UV-B (+) conditions showed inhibition of the development of the first leaf and yellowing and withering of the cotyledons (Figures 3E-H, 3M-P). Thus, elimination of trichomes on the surface of cucumber cotyledons affected the visible growth of the seedlings grown under UV-B (+) conditions for 7 days.

2) The effect of continuous UV-B irradiation on the leaf area of trichome (+) or trichome (-) cotyledons in cucumber seedlings

To investigate the effect of continuous UV-B irradiation for 7 days on the leaf area of trichome (+) or trichome (-) cotyledons in cucumber seedlings, trichome (+) or trichome (-) cotyledons were excised from cucumber seedlings grown under UV-B (+) conditions on days 1, 2, 3, 4, 5, 6, and 7. The leaf area of trichome (+) cotyledons in cucumber seedlings grown under UV-B (+) conditions expanded favorably, similar to that of trichome (+) cotyledons in cucumber seedlings grown under UV-B (-) conditions for 7 days (Figure 4A). In contrast, the leaf area of trichome (-) cotyledons in cucumber seedlings grown under UV-B (+) conditions decreased gradually from day 4 to day 7 and less significantly in comparison with that in trichome (+) cotyledons in cucumber seedlings grown under UV-B (+) conditions from day 5 to day 7 ($P < 0.01$) (Figure 4A). Thus, elimination of trichomes on the surface of cucumber cotyledons affected the leaf area of cotyledons in the seedlings grown under UV-B (+) conditions for 7 days.

3) The effect of continuous UV-B irradiation on the fresh weight of the aerial parts of cucumber seedlings with trichome (+) or trichome (-) cotyledons To investigate the effect of continuous UV-B irradiation for 7 days on the fresh weight of the aerial parts of cucumber seedlings with trichome (+) or trichome (-) cotyledons, the aerial parts from cucumber seedlings with trichome (+) or trichome (-) cotyledons grown

under UV-B (+) conditions were excised on days 1, 2, 3, 4, 5, 6, and 7. The fresh weight of the aerial parts of cucumber seedlings with trichome (+) cotyledons grown under UV-B (+) conditions did not change as much and was lower than that of the aerial parts of cucumber seedlings with trichome (+) cotyledons grown under UV-B (-) conditions for 7 days because the development of the first leaf was suppressed by UV-B irradiation (Figures 3A-D, 3I-L, 4B). In contrast, in comparison with the aerial parts of cucumber seedlings with trichome (+) cotyledons grown under UV-B (+) conditions, the aerial parts of cucumber seedlings with trichome (-) cotyledons grown under UV-B (+) conditions showed a reduction in fresh weight from day 3 to day 7 and a less significant reduction from day 4 to day 7 ($P < 0.01$) (Figure 4B). Thus, elimination of trichomes on the surface of cucumber cotyledons affected the fresh weight of the aerial parts of the seedlings grown under UV-B (+) conditions for 7 days.

4) The effect of continuous UV-B irradiation on the chlorophyll content of trichome (+) or trichome (-) cotyledons in cucumber seedlings To investigate the effect of continuous UV-B irradiation for 7 days on the chlorophyll content of trichome (+) or trichome (-) cotyledons in cucumber seedlings, the chlorophyll content of trichome (+) or trichome (-) cotyledons in cucumber seedlings grown under UV-B (+) conditions were measured by the chlorophyll meter on days 0, 1, 3, 5, and 7. The chlorophyll content of trichome (+) cotyledons in cucumber seedlings grown under UV-B (+) conditions was constant and did not differ much from that of trichome (+) cotyledons in cucumber seedlings grown under UV-B (-) conditions for 7 days (Figure 4C). This result is consistent with the findings of our previous study.18 In contrast, in comparison with the cucumber seedlings with trichome (+) cotyledons grown under UV-B (-) conditions, the cucumber seedlings with trichome (-) cotyledons grown under UV-B (+) conditions showed a reduction in chlorophyll content from day 3 to day 7 and a less significant reduction from day 5 to day 7 ($P < 0.01$) (Figure 4C). Thus, elimination of trichomes on the surface of cucumber cotyledons affected the chlorophyll content of cotyledons in the seedlings grown under UV-B (+) conditions for 7 days.

DISCUSSION

Trichomes are uni- or multicellular hairs that develop on the leaves, sepals, and stems of higher plants.31 Most trichomes on the surface of cucumber cotyledons consist of three cells (Figure 1).18 In the present study, we showed that visible growth, leaf area of cotyledons, fresh weight of the aerial parts, and chlorophyll content of cotyledons in cucumber seedlings with trichome (-) cotyledons did not differ from those in cucumber seedlings with trichome (+) cotyledons grown under UV-B (-) conditions for 7 days (Figures 3A-H, 4A-C). Thus, we confirmed that elimination of trichomes on the surface of cucumber cotyledons did not affect the growth of the seedlings under UV-B (-) conditions. In contrast, visible growth, leaf area of cotyledons, fresh weight of the aerial parts, and chlorophyll content of cotyledons in cucumber seedlings with trichome (-) cotyledons were lower than those in cucumber seedlings with trichome (+)cotyledons grown under UV-B (+) conditions for 7 days (Figures 3I-P, 4A-C). Thus, elimination of trichomes on the surface of cucumber cotyledons promotes senescence of cucumber seedlings under UV-B irradiation. These findings indicate that trichomes on the surface of cucumber cotyledons protect cucumber seedlings from UV-B-induced senescence. This fact is supported by previous reports showing that trichomes afford protection against UV-B irradiation in Olea europaea and Arabidopsis thaliana.15,32-34 We had previously shown that continuous exposure of cucumber cotyledons to UV-B irradiation induces rapid cellular expansion and toluidine blue O staining, namely the accumulation of polyphenolic compounds, possibly stress lignins, in epidermal cells surrounding the trichomes.18 This phenomenon was confirmed in the present study (Figures 2I-L). In the present study, to directly validate the protective role of trichomes against UV-B irradiation, trichomes on the surface of the cucumber cotyledons were eliminated without affecting the growth of cucumber seedlings and the effect of UV-B irradiation for 7 days on cucumber seedlings without trichomes was analyzed. The trichome (-) cotyledons in cucumber seedlings grown under UV-B (+) conditions for 7 days showed neither expansion nor toluidine blue O staining in the epidermal cells surrounding the trichomes (Figures 2M-P). This is the first evidence indicating the importance of trichomes for rapid expansion and toluidine blue O staining, namely accumulation of polyphenolic compounds, in the epidermal cells surrounding the trichomes on the surface of cucumber cotyledons under UV-B (+) conditions. Recently, both trichome formation and flavonoid and anthocyanin biosynthesis were shown to be interrelated at the molecular level in A. thaliana35,36, Brassica napus37, and cucumber.22,23 Moreover, polyphenolic compounds are transferred to the cell walls of the trichomes during the short period of final trichome development, which corresponds to secondary wall thickening in the leaves of O. europaea.38 By the same token, under UV-B (+) conditions, polyphenolic compounds might be transferred to the cell walls of the expanded epidermal cells surrounding the trichomes on the surface of cucumber cotyledons as trichomes are clues. If this is indeed the case, trichomes may act as sensors to mediate the above protective responses against UV-B irradiation in cucumber seedlings. This idea is consistent with the findings of a previous study proposing that trichomes could act as sensors responding to mechanical and acoustic stimuli in A. thaliana.39 In the present study, the elimination of trichomes on the surface of cucumber cotyledons could be done easily by gently rubbing the cotyledons with rolled KimWipes (Figures 1C, 1D). Farm crops can sustain such wounds during interactions with other elements in the natural world. Moreover, the average intensity of UV-B irradiation in the present study was approximately equal to the natural UV-B irradiation on a cloudy day in May in Fukuoka. Thus, UV-B-induced senescence in farm crops may be caused easily by unexpected wounds sustained in the natural world. These findings highlight the importance of protecting farm crops from wounds sustained under natural conditions.

ACKNOWLEDGEMENTS

This work was partially supported by the Japan Society for the Promotion of Science with a Grant-in-Aid for Scientific Research (C) (no. 22K02948 to S.Y.) and the Saito Gratitude Foundation.

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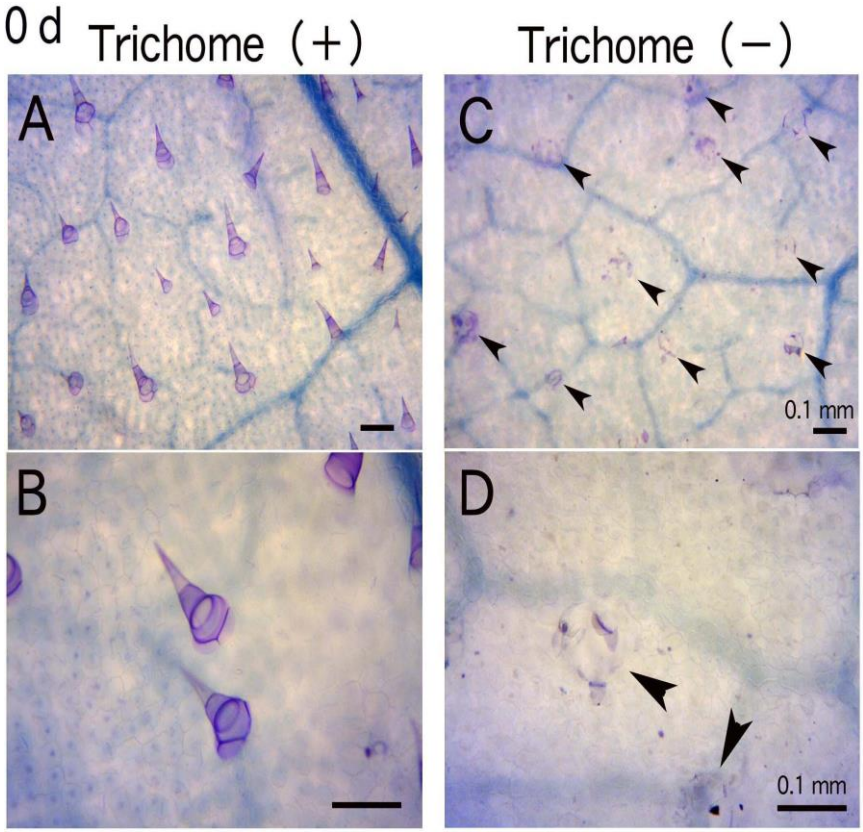

Figure 1. (A) The surface of trichome (+) cotyledons in cucumber seedlings. (B) Extended image of the surface shown in (A). (C) The surface of trichome (-) cotyledons in cucumber seedlings. (D) Extended image of the surface shown in (C). Cotyledons were stained with toluidine blue O. Black arrowheads indicate the trails of trichomes. Scale bar: 0.1 mm.

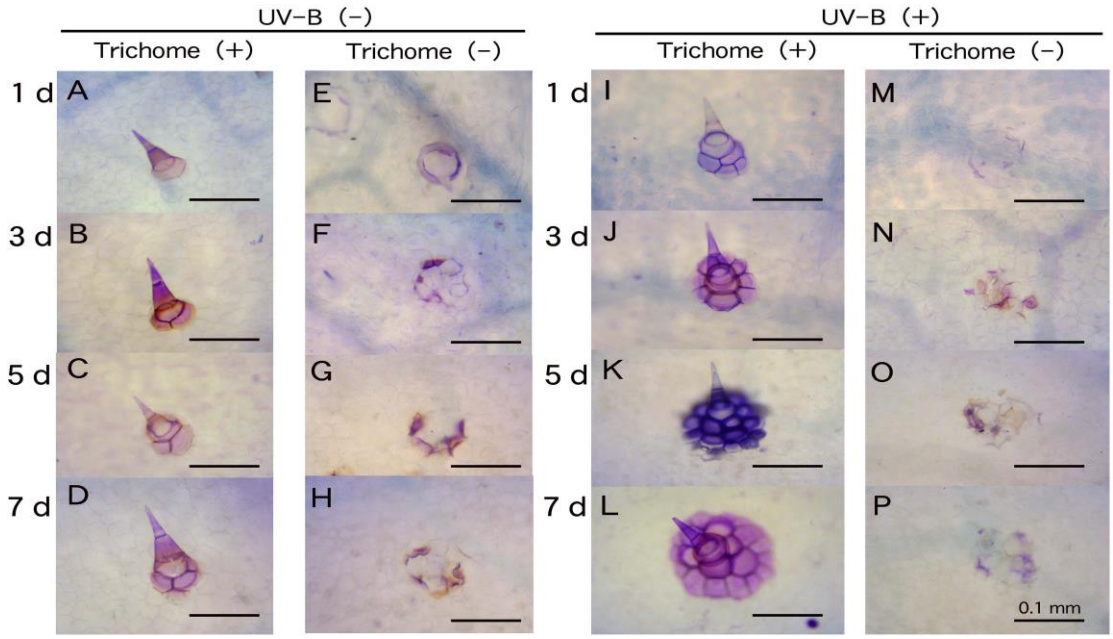

Figure 2. (A)-(D) The surface of trichome (+) cotyledons in cucumber seedlings grown under UV-B (-)conditions for 7 days. (E)-(H) The surface of trichome (-) cotyledons in cucumber seedlings grown under UV-B (-) conditions for 7 days. (I)-(L) The surface of trichome (+) cotyledons in cucumber seedlings grown under UV-B (+) conditions for 7 days. (M)-(P) The surface of trichome (-) cotyledons in cucumber seedlings grown under UV-B (+) conditions for 7 days. Cotyledons were stained with toluidine blue O and photographed at the indicated time points. Scale bar: 0.1 mm

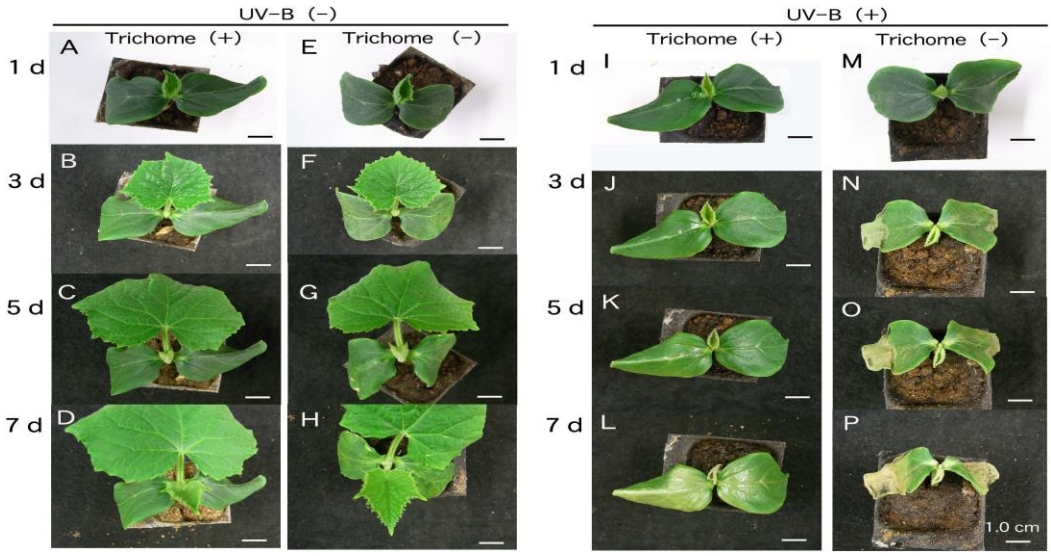

Figure 3. (A)-(D) Appearance of cucumber seedlings with trichome (+) cotyledons grown under UV-B (-)conditions for 7 days. (E)-(H) Appearance of cucumber seedlings with trichome (-) cotyledons grown under UV-B (-) conditions for 7 days. (I)-(L) Appearance of cucumber seedlings with trichome (+)cotyledons grown under UV-B (+) conditions for 7 days. (M)-(P) Appearance of cucumber seedlings with trichome (-) cotyledons grown under UV-B (+) conditions for 7 days. Cucumber seedlings were photographed at the indicated time points. Scale bar: 1.0 cm.

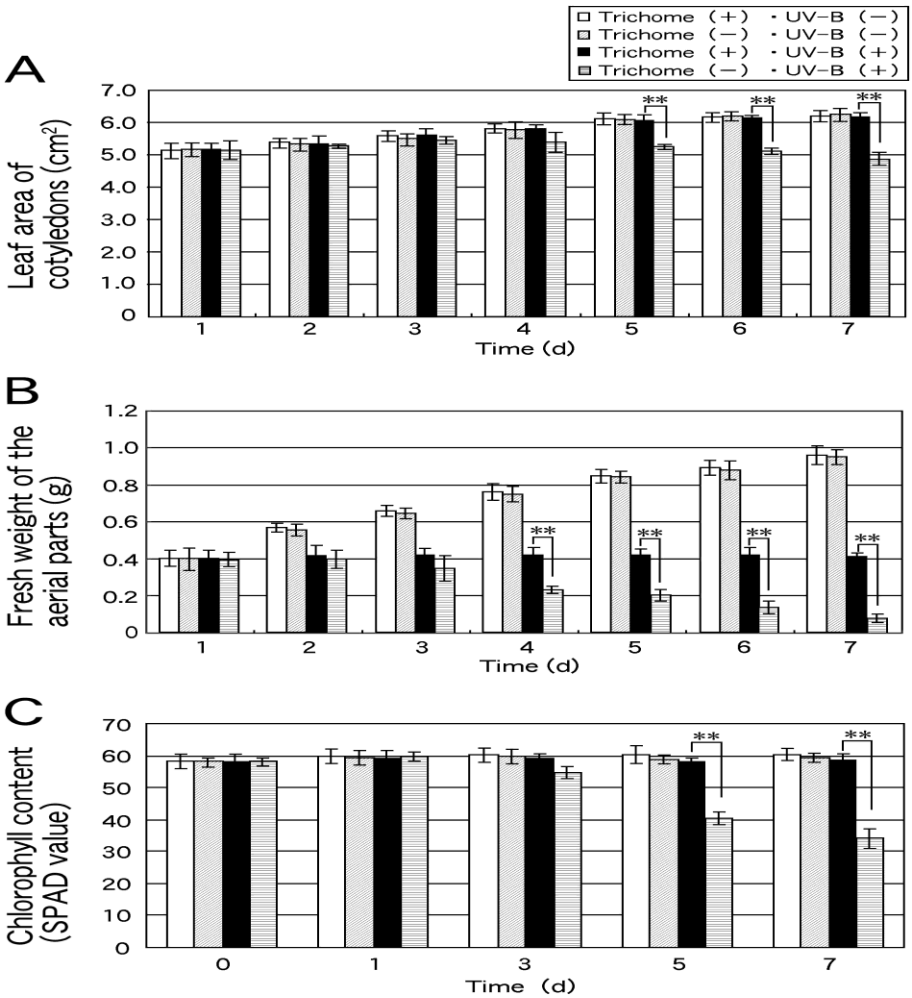

Figure 4. (A) The leaf area of trichome (+) or trichome (-) cotyledons in cucumber seedlings grown under UV-B (+) or UV-B (-) conditions for 7 days. (B) The fresh weight of the aerial parts of cucumber seedlings with trichome (+) or trichome (-) cotyledons grown under UV-B (+) or UV-B (-) conditions for 7 days. (C) The chlorophyll content of trichome (+) or trichome (-) cotyledons in cucumber seedlings grown under UV-B (+) or UV-B (-) conditions for 7 days. Statistically significant differences were determined by Student's t-test (P < 0.01).**