A-5.4 Elucidation of Mechanism of Leaf Injury and Growth Inhibition by Enhanced UV-B

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ABSTRACT The effect of UV-B irradiation on plants was investigated using .LM8 cucumber seedlings as plant materials. First, the effect of irradiation with wide band of UV-B was investigated. The growth rate of first leaves was decreased by UV-B radiation. The degree of growth retardation of first leaves caused by UV-B light was almost identical irrespective of duration of irradiation period of UV-B a day, though the retardation was not remarkable in the case of UV-B irradiation for 4 h a day. Next, the action spectrum for growth retardation of intact plants caused by UV irradiation was examined. Cucumber first leaves were irradiated with monochromatic light, using a large spectrograph. The first leaves were severely damaged and retarded in growth rate at 280, 290 and 300 nm. From the relation between light intensity and growth rate, an action spectrum for 25% growth retardation was obtained. The inhibitory effects of 280 and 290 nm were remarkable compared with those by 300 and 310 nm.

Key Words Action spectrum, Cucumber seedling, Growth retardation, Visible injury

1. Introduction

A partial depletion of the stratospheric ozone layer would result in an enhancement of the region of radiation having wavelength from 290 to 315 nm (UV-B) in the solar ultraviolet radiation reaching the earth's surface. If so, the enhanced UV-B radiation would give some deleterious effects on plants and ecosystem because of its potential to destroy biological compounds having important roles in plant cell function[1]. UV-B radiation could cause lesions in nucleic acids and photosynthetic apparatus[2-4], which would result in several damages of physiological function. It has been reported that UV-B radiation retards the rate of growth of various plant species, for example, cucumber cotyledon[5]. On the other hand, plants would have some defense mechanisms against UV-B radiation to get out of harmful effects of UV-B radiation.

However, the mechanism of growth retardation is not yet fully clarified. Besides, any action spectrum for the growth retardation of intact plants has not yet been obtained because of technical difficulties, though a generalized action spectrum has been proposed for the effect of UV-B on plants by Caldwell[6].

2. Research Objective

The present study was mainly designed to obtain the action spectrum for plant growth retardation caused by UV-B to have some precise knowledges on the effect on plant growth of enhanced UV radiation resulted from stratospheric ozone depletion. Based on the action spectrum, we could assess the effect of enhanced UV radiation on plant growth and could presume the mechanism of UV effect on plants.

3.Research Method

3.1.Effects of UV-B Irradiation on First Leves

Cucumber (*Cucumis sativus* L. cv. Hokushin) plants were grown at 25 $\pm$ 0.5^{o}C with a relative humidity of 70 $\pm$ 5% under natural light for 7 to 10 days in environment-controlled glass house. Then, immediately before the first leaves began to develop, the plants were transferred to the artificially lit growth chamber programmed for a 20/15^{o}C day/night temperature regime and a 12h photoperiod. PPFD at the plant height was ca. 340 μmol m^{-2} s^{-1} during the light period (metal halide lamps; BOC Lamp, Mitsubishi Electric Co., Tokyo). Plants received supplemental UV-B radiation in the growth chamber. UV-B radiation was supplied by three Toshiba FL 20SE fluorescent sunlamps suspected 40 cm above the seedlings. In order to absorb all UV light having wavelengths shorter than 290 nm, 0.10 mm polyvinyl chloride sheeting (Cutting Sheet 000C, Nakagawa Chemical Inc., Tokyo) was used as a filter. UV-B treatment (ca. 10 μW cm^{-2} s^{-1}) was performed during photoperiod for a week in one experiment and for 1.5, 4 and 11.5 h a day in another one. The growth of first leaves was measured in leaf area and dry weight. Final areas of first leaves were measured with a planimeter (Model AAM-7, Hayashi Denkoh Co. Ltd., Tokyo). For the determination of dry weight, the first leaves were dried at 70^{o}C for 4 days before the measurement.

3.2.Action Spectrum for Growth Retardation of First Leaves

Cucumber plants were cultivated in environment-controlled glass house as mentioned above for 7 days, then at 20^{o}C/15^{o}C (day/night) for 2 days. When the immature first true leaves emerged and began to develop, the plants were transferred to the National Institute for Basic Biology at Okazaki and further cultivated at 19.5 - 21.5^{o}C, and illuminated with 40 W white fluorescent lamps at ca. 200 μmol m^{-2} s^{-1} during light period of 12 h every day, except the period of monochromatic light irradiation of 4 h. The first leaves were irradiated for 4 h in the middle of light period a day with monochromatic light, using a large spectrograph. The monochromatic light treatment consisted of 280, 290, 300, 310 and 320 nm, and the light intensity of each wavelength was adjusted by stepwise reduction to four different levels. Photon flux density of each monochromatic light was measured at first leaf level with a photon counter. The irradiation was repeated for 3 days.

First leaves were taken pictures together with a measure every day immediately before the commencement of monochromatic UV light irradiation. The pictures were used to determine the leaf area.

In the next experiment, a set of cucumber leaves was irradiated with visible light during monochromatic radiation at 290 and 300 nm, whereas another set was not irradiated with visible light. Visible light was obtained by xenon lamps through glass fiber, without having wavelength shorter than 360 nm, and the intensity was adjusted to 500-600 μmol s^{-1} at first leaf level. Control plants were irradiated with visible light solely or kept in the dark during the light treatments. Growth rates of first leaves of both control plants were similar each other.

Spectral flux density of UV-B and visible light was measured with a spectroradiometer (Photal MCPD-1000, Otsuka Electronics Co. Ltd., Tokyo).

4.Results

4.1.Effects of UV-B Irradiation on First Leaves

UV-B irradiation caused the growth retardation (Table 1) and visible damage, chlorosis, of the first leaves. When the plants were irradiated with UV-B for 1.5, 4 and 11.5 h a day for about 1 week, the growth retardation was observed at every UV irradiation program (Fig. 1). In some cases, 4-h irradition did not show growth inhibition . On the other hand, visible damage appeared on the first leaves of plants in 4- and 12-h irradiation plots but not in 1.5-h irradiation plot.

Table 1 Effect of UV-B irradiation on growth of cucumber first leaves at different temperature regimes

Treatment	fr. wt. (g)	dry wt. (mg)
Exp. 1 (20/15°C)		
Control	0.92 ± 0.12	123 ± 15
UV-B irradiation (7 days)	0.61 ± 0.08	82 ± 11
Exp. 2 (25°C)		
Control	1.30 ± 0.17	274 ± 41
UV-B irradiation (13 days)	1.28 ± 0.22	253 ± 43

Each figure is average of 15 samples ± SD

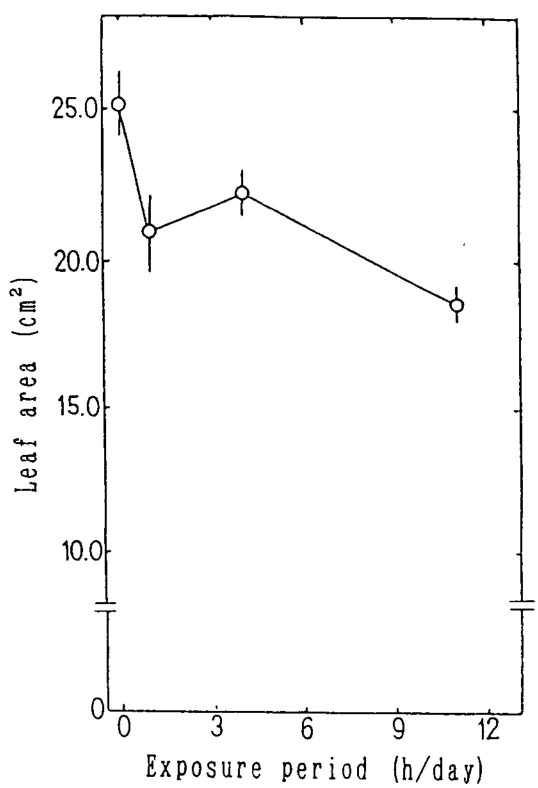

Fig. 1 Effect of exposure time of UV-B irradiation on growth

4.2. Action Spectrum for Growth Retardation of First Leaves

Leaf area of first leaves increased almost linearly during 3 days of cultivation. Monochromatic treatments with UV light at 280 to 310 nm reduced the growth rate which tended to decrease with the increase of intensity of each monochromatic irradiation. The inhibitory effect was distinct, especially, at 280 and 290 nm. UV light of 320 nm showed no effect, irrespective of light intensity examined in the present study. High light intensities of wavelengths of 280 and 290 nm gave very severe damage to leaves, i.e., water-soaked appearance and ultimately necrosis. The growth rate of leaves exposed to UV light having each wavelength was plotted against light intensities. From this relationship, the reciprocal of the quantum fluence rate necessry to 25% growth reduction was plotted against each wavelength (the relationship named action spectrum)(Fig. 2). This action spectrum clearly showed that the inhibitory effects of 280 and 290 nm on cucumber leaf growth were very remarkable compared with those of 300 and 310 nm.

Next, the effect of simultaneous irradiation with visible light and monochromatic UV light was examined on leaf growth. Visible light partially restored the inhibition caused by UV light of 300 nm, while visible light had no effect on growth retardation caused by wavelength of 290 nm.

5. Discussion

Exposure to UV-B irradiation causes reduction in the growth and the development of many plant species[7]. Thus, the enhanced UV-B radiation reaching the earth's surface has been considered to have some deleterious effects on plants and ecosystem. If we want to assess the effects of UV-B radiation on plants under natural condition, action spectra for the effects on plants are essential. Recently, Takeuchi et al.[8] reported that degree and characteristics of growth inhibition of cucumber cotyledon induced by UV-B irradiation were different due to wavelength. However, any action spectrum for UV-B-induced growth inhibition has not yet been reported. In the present study, an action spectrum was obtained for growth inhibition of cucumber first leaf. This action spectrum shows that the wavelength of 290 nm has very strong inhibitory effect compared with the wavelengths of 300 and 310 nm, which is consistent with the result obtained by Takeuchi et al.[8].

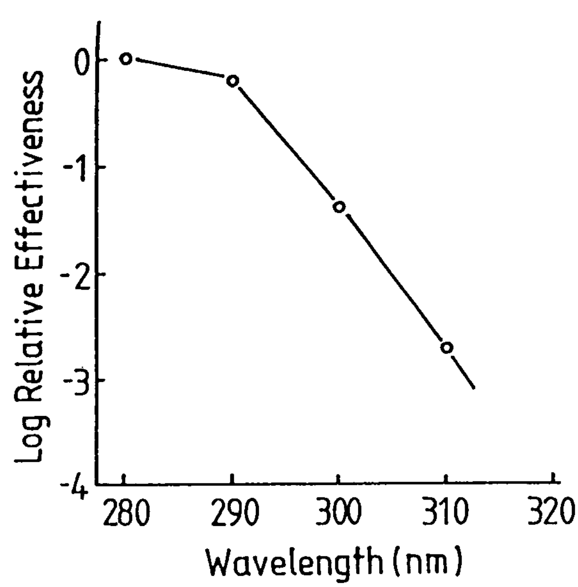

Fig.2 An action spectrum for 25% growth inhibition of the first leaf

It has been reported that the intensity of visible light supplied during plant growth and the previous UV-B irradiation would be an important factor to modulate the sensitivity of plants to UV-B radiation, and that the irradiation with high photosynthetic photon flux density (PPFD, between 400 and 700 nm) could mitigate the inhibitory effect of UV light[9,10]. Thus, it was examined in the present experiment whether simultaneous exposure to visible light and UV light influences the inhibitory effect of monochromatic UV radiation. The supplemental visible light could not mitigate the inhibitory effect of wavelength of 290 nm, though the effect of that of 300 nm was reduced by the visible light. It is predicted that a reduction of the stratospheric ozone would result in a prominent enhancement of the wavelength between 290 and 300 nm. Thus, the present result strongly suggests the possibility that stratospheric ozone reduction causes some serious damage to plants and ecosystem.

Exposure to UV-B radiation results in several physiological changes, such as growth rate and photosynthetic activity[11] and in biochemical changes in organisms, such as DNA damage[4], damage of photosystem II[3], alteration of protein composition[12], inhibition of chlorophyll synthesis[7] and alteration of gene expression[13,14]. The action spectrum obtained in the present experiment is similar to that of DNA damage reported in bacteria[15], suggesting an involvement of DNA damage in growth inhibition caused by UV-B irradiation. Oxidative stress might also participate in the deleterious effect of UV-B[14,16].

Prolonged exposure period of UV-B irradiation did not necessarily cause the increased growth inhibition as shown in Fig. 1, suggesting that some recovery and/or protective mechanisms operate in cucumber first leaves. It is known that synthesis of flavonoids, UV-absorbing substance, is enhanced by UV-B irradiation in epidermal tissue of plant leaves. Besides, UV-B irradiation induced the expression of the gene encoding some enzymes involved in defense mechanism of oxidative stress[14].

6. References

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