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UV Effect on Plant Growth

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UV-B radiation gives harmful effects on plants, such as production of several types of DNA lesions, and growth inhibition. On the other hand, plants have some protective mechanisms, including filtering effect due to accumulation of phenolic compounds in epidermal cells and reactivation of DNA lesions, which are enhanced by UV-B irradiation. We have investigated the mechanism of UV-B effects on plants using cucumber seedlings as plant materials. Cucumber plants were cultivated in an artificially lit growth chamber. Supplemental UV-B irradiation, of which intensity was almost equal to the level of natural sunlight, retarded the growth of first leaves. The growth retardation must result from the inhibition of cell division and/or cell growth. Microscopical observation of leaf epidermis suggested that the growth retardation might be mainly caused by cell growth inhibition. The retardation was, however, restored within 2 or 3 days after the termination of UV-B irradiation. It is known that UV-B irradiation lowers the activity of photosystem II (PS II). In the present experimental conditions, however, UV-B irradiation has little effect on PS II activity as estimated by chlorophyll fluorescence. The stomatal conductance, a major factor determining photosynthetic rate, of first leaves increased during the growth. The increase of stomatal conductance was suppressed by UV-B irradiation and restored by termination of the irradiation. It has not been clear, however, what mechanisms are involved in the suppression of increase of stomatal conductance.

Key words: UV-B, cucumber, growth retardation, photosystem II, stomatal conductance, cytokinin, cortical microtubules

INTRODUCTION

It is known that UV-B irradiation causes the growth retardation of a lot of plants [1-3]. We have examined the UV-B effects on plant growth using cucumber seedlings as plant material in order to clarify the mechanism of growth retardation of plant caused by UV-irradiation [4]. Cucumber seedlings are sensitive to UV-B and grow rapidly. In addition, cucumber leaves are oriented in horizontal direction. Thus, the leaves can be irradiated uniformly with light. From these characteristics, cucumber seedlings seem to be one on the most suitable plant materials for the experiment of UV-B effects.

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MATERIALS AND METHODS

Cucumber was cultivated in a naturally lit growth chamber first. After 6 days, the seedlings were transferred to an artificially lit growth chamber, and cultivated and irradiated with UV-B. Visible light was obtained from metal-halide lamps (BOC lamp, Mitsubishi). When plants were irradiated with UV-B, UV-B light was supplementary added to the visible light. UV-B light was obtained from UV-fluorescent lamp, of which the wavelength below 290 nm was cut off with a filter, set in white plastic boxes [2]. Light period was 12 hrs. UV-B irradiation was given during light period. Temperature was regulated to be 20 and 15°C, light and dark periods each. UV-B intensity was 0.2 W m^{-2}.

Leaf area of first leaves was obtained following the procedure by Murase et al.[4]. Using this method, we can obtain the leaf area easily without destruction.

In order to investigate the effect of UV-B on the size of epidermal cells, SUMP method was used. The surface of SUMP plate, which is celluloid plates with 1.5 cm diameter, deliquesced with SUMP 1 solution. Then the surfaces of SUMP were cohered to abaxial surfaces of the first leaves. After caked, they were peeled off and observed with optical

microscope.

For analysis of cytokinin, the leaves were extracted with 80% methanol. After the extract was washed out with chloroform, the sample was acidified by acetic acid and then partially purified with Cellulose Phosphate P11 (Whatman) column. The sample was partitioned to butanol layer and then purified with TLC (Merck), followed by analysis with HPLC using inertsil ODS-3 column.

For observation of cortical microtubules in epidermal cells, adaxial epidermal strips were carefully prepared with razor blade and fixed with formalin. The cell wall of the strips was partially digested with Pectlyase Y-23 (Seishin Pharmaceutical Co.). After washing, microtubules in the samples were stained by successive incubation with an equal mixture of monoclonal antibodies against chick brain α-tubulin and β-tubulin (Amersham), and then FITC-conjugated antibodies developed in sheep against mouse IgG (Amersham) [5]. Stained preparations were examined under fluorescence microscope.

RESULTS AND DISCUSSION

The growth of first leaves continued linearly during the experimental period, but it was distinctively retarded by UV-B irradiation (Fig.1). This growth retardation was restored 2 or 3 days after the termination of the irradiation (data not shown).

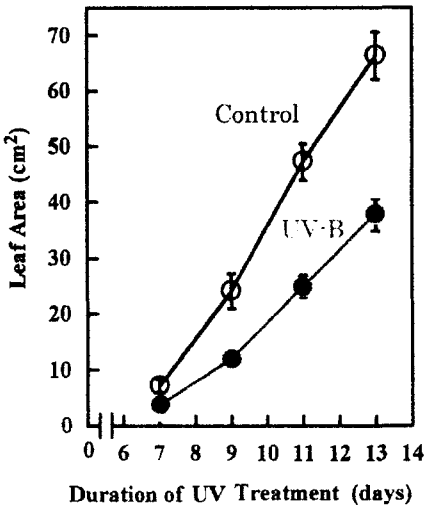

Figure 1. Growth retardation of cucumber first leaves. UV-B irradiation was commenced at day 0 (7 days after the sowing).

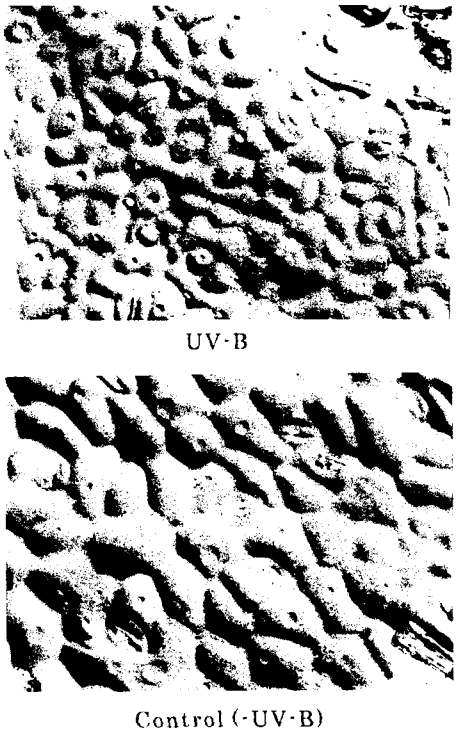

Figure 2. Micrographs of replica of adaxial (upper) surface of first leaves 18-day-old cucumber seedlings.

Figure 2 shows appearance of epidermis. Lower is control plant and upper is UV-B irradiated one. Size of epidermal cells was markedly decreased by UV-B irradiation. Leaf growth is determined by cell division and cell growth. The present result suggests that the growth retardation by UV-B irradiation might be mainly resulted from the inhibited cell growth. It is probable that the cell division of first leaves has already terminated before the start of irradiation with supplemental UV-B.

Then, we investigated some factors regulating plant growth. One of the factors is content or activity of phytohormone. In the present study, the level of cytokinin was compared. It is known that cytokinin enhances the expansion growth of leaf cells [6-8]. The second is the orientation of cortical microtubules in epidermal cells. It is known that orientation of cortical microtubules is regulated by phytohormone such as cytokinin and gibberellin and that cortical microtubules regulate the orientation of cellulose microfibrills newly synthesized in cell wall resulting in the regulation of cell growth [9, 10].

Figure 3 shows chromatograms of HPLC for measurement of cytokinin. The peak indicated by Z is that of zeatin. The peak indicated by ZR is that of zeatin

riboside. These are the major components of cytokinin in plant cells. The amounts of these cytokinins were rather increased by UV-B irradiation. Thus, we could not explain the growth retardation by the level of cytokinin. On the other hand, auxin is prerequisite to cytokinin-dependent growth promotion. It has been reported that auxin is oxidized to inacitive form by UV-B irradiation [11, 12], although auxin was not analyzed in the present work. Therefore, the possibility cannot be omitted yet that the growth retardation by UV-B might be due to the inactivation of phytohormones.

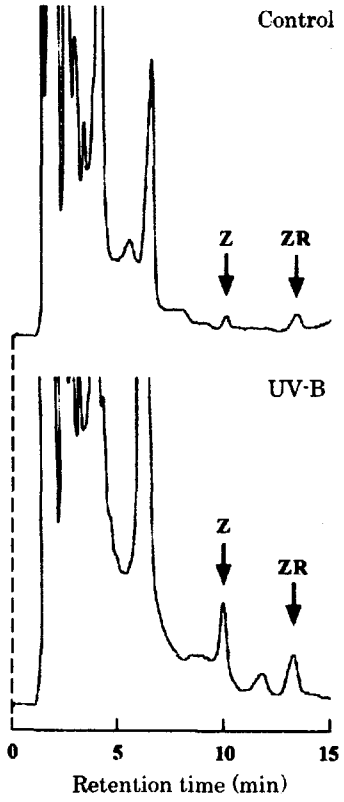

Figure 3. HPLC chromatograms of endogenous cytokinins in cucumber first leaves. Cytokinin was extracted from the leaves 3days after the start of UV-B irradiation. Z: zeatin, ZR: zeatin riboside.

Figure 4 shows the picture of cortical microtubules in epidermal cells. We can see relatively clear microtubule orientation. It seems likely that the cortical microtubules were not affected by UV-B irradiation.

It has been reported that UV-B inhibits the photosystem II of photosynthesis. We measured the PSII activity as measured by chlorophyll fluorescence. PSII activity increased during growth period, but the activity was not noticeably affected by UV-B irradiation in the present experiment (data not shown). We have already reported that UV-B irradiation gave only a little effect on photosynthetic activity measured with chlorophyll fluorescence [13].

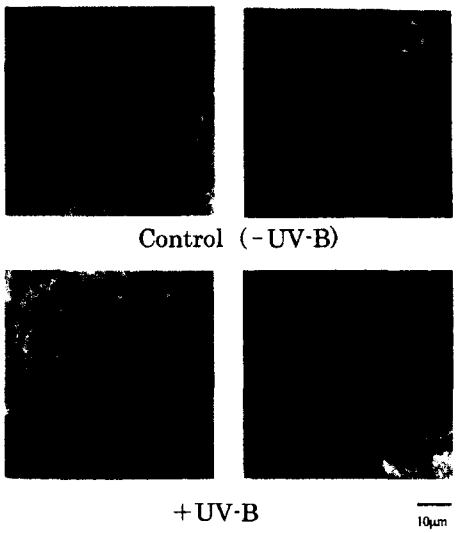

Figure 4. Cortical microtubules in epidermal cells. First leaves were irradiated by UV-B for 6days. Microtubules were microscopically observed by immunofluorescence method.

In the next experiment, we measured the stomatal conductance. Photosynthetic rate is determined by stomatal conductance. The conductance increased during the growth period. But the increase was markedly suppressed by UV-B irradiation in both leaf surfaces. However, after the termination of UV-B irradiation, the suppression was clearly restored (data not shown). This result shows that the change of growth rate of first leaves due to UV-B irradiation is parallel with the change of stomatal conductance, suggesting that the retarded increase of stomatal conductance may be at least a factor determining the growth retardation caused by UV-B irradiation. It has not been investigated yet what mechanisms are involved in the effects of UV- B irradiation on stomatal conductance. The clarification of this point should be most important in order to make clear the mechanism of growth retardation caused by UV-B irradiation.

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