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Effects of UV-B Radiation on Chl Fluorescence of Greening Barley (*Hordeum vulgare* L.) Seedling

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Effects of UV-B radiation on the developing chloroplast of barley (*Hordeum vulgare* L.) seedling during greening were determined by Chl contents, Fo, Fv and fluorescence quenching coefficients. In greening of etiolated barley seedling, the value of Fo was greatly increased after the initiation of greening. However Fv and Fv/Fo were gradually increased. In greening with the additional irradiation of UV-B radiation, the value of Fo was strikingly decreased than that of the control after the initiation of greening, but Fv was gradually decreased from than that of the control during the greening period. These results suggest that the function of light-harvesting Chl are more sensitive than photosynthetic electron transport system by UV-B. Chl contents, Fv/Fo, qP and qNP, were decreased from than that of the control during the 72 h greening, especially, qR was strikingly decreased, but qE was slightly decreased by UV-B. These suggest that the sites of inhibition by UV-B are PSII and all sites of photosynthetic electron transport system. But PQ pool seems to be slightly inhibited by UV-B.

Keywords: UV-B, barley seedling, greening, Chl fluorescence, PSII.

The radiation of UV-B, in spite of about 1.5% of total solar radiant energy (Frederick *et al.*, 1989), can be changed by many factors such as the solar angle, the latitude, the amount of clouds, the turbidity of the atmosphere and the ozone density of the stratosphere. UV-B radiation changes metabolic pathway, the structure and the development of life, genetic information and the interaction between species in the individual ecosystem because it affects the capability for absorbing light of various biomolecules including DNA, protein, photosynthetic pigments and electron transport mediator such as quinone (Melis *et al.*, 1992; Takeuchi *et al.*, 1993). The most sensitive region to UV-B radiation in the process of photosynthesis is generally reported in the PSII (Sission, 1981; Tevini and Pfister, 1985) but there's no agreement on the exact target region yet. Until now as the target region of the PSII by UV-B radiation, Renger *et al.* (1989) reported the water oxidation site, Melis *et al.* (1992) did quinone, the receptor region, Hideg *et al.* (1993) did the water oxidation site and the quinone receptor. Besides Tevini and Pfister (1985) reported the damage of the reaction center of PSII by UV-B radiation. The study of the influence of UV-B radiation on plant community, individuals and their leaves has concentrated in that of plant productivity, the change of photosynthetic pigments contents, and the rate of photosynthesis and the growth for a long time. In other words it was the study about the accumulated influence on plants by UV-B radiation for a long time. And the method of treating thylakoid membrane of the extracted chloroplast with UV-B radiation has been used mostly in order to find out the target region of photosystem by UV-B radiation.

Chloroplast starts to develop by light and the development of photosynthetic capability in greening process are receiving much attention (Binder and Bachofen, 1979). There's little knowledge about the study with the measurement of Chl fluorescence to find out the influence of UV-B radiation on the development of chloroplast, so a new method and access are still required. Though the photochemical activity and the change around PSII can be observed very sensitively and fast by the analysis of Chl fluorescence (Hipkins and Baker, 1986), the influence of UV-B radiation on the development of chloroplast in greening has never been studied by the change of Chl fluorescence.

In this study, when an etiolated barley seedling was greened by the light of 100 $\mu mol \cdot m^{-2}s^{-1}$ and

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simultaneously was irradiated by UV-B radiation of 0.2 W m^{-2}, the influence of UV-B radiation on the development of chloroplast was examined by analyzing the Chl contents and Chl fluorescence of chloroplast.

MATERIALS AND METHODS

Plant Materials

Barley (*Hordeum vulgare* L, cv, Olbori) seeds distributed from the agricultural agent in Seung-ju Kun were sowed after washing them in distilled water and then culturing in the dark at the temperature of 22± 2°C and relative humidity of 70+5%. Then the etiolated barley seedlings were used as the materials of this experiment and irradiated by the light of 100 $\mu mol \cdot m^{-2}s^{-1}$ in greening. For the measurement of Chl contents and fluorescence, the first leaf with the 2 cm long cut without the end of the 1 cm of the leaf.

UV-B Treatment

To find out the influence of UV-B radiation on the development of chloroplast, the etiolated barley seedling was treated with UV-B radiation of 0.2 W m^{-2} and also greened by being irradiated by the light of 100 $\mu mol \cdot m^{-2}s^{-1}$ with fluorescence lamps and incandescent lamps. As the light resource of UV-B radiation, 15W-BLE-1T158 (Spectronics Corp., Westburg, NY) UV-B lamp was used. Ultraviolet-B treatment and greening were carried out in the Plant Growth Cabinet (KGW-1400-S1) at the temperature of 22±2°C and the relative humidity of 70+5%.

Measurement of Chl Contents

Chlorophyll was extracted by Hiscox and Israelstam (1979); 0.1 g leaf in 10 mL dimethyl sulfoxide (DMSO) was put in the thermostat at the temperature of 65°C for 3 h. The contents of Chl a and b were quantified by measuring the degree of absorbing light at 663 nm and 645 nm each according to Arnon (1949) and that of carotenoid at 480 nm according to Liaaen-Jensen and Jensen (1971).

Measurement of Chl Fluorescence

The fluorescence of Chl a was measured by PAM Chl Fluorometer of Walz Corp. After the dark-adaptation of an intact leaf for 20 min, its sample hold of 0.3×0.9 cm in size was irradiated by the measured light (the weak red light modulated into 1.6 kHz) and then Fo (the fluorescence when all the reaction center of PSII were opened) was measured. In the same way the sample hold was irradiated by the saturated light (3,000 $\mu mol \cdot m^{-2}s^{-1}$) and Fm (the fluorescence when all the reaction center of PSII were closed and the electron receptor, quinone are completely reduced). Fo and Fm were used as the indicators of PSII activity. The ratio of Fv to Fo was calculated with Fv which equals Fm minus Fo and was compared and analyzed (Driesenaar *et al.*, 1994). To get the fluorescence quenching coefficient, the sample was irradiated continuously by actinic light (1,330 $\mu mol \cdot m^{-2}s^{-1}$) and simultaneously pulsed by saturated light for a second every 20 s. The fluorescence quenching coefficient was calculated as photochemical quenching (qP), nonphotochemical quenching (qNP), fluorescence quenching that is not reversed by 3(3,4-dichlrophenyl)-1, 1-dimethylurea (qR) and energy-dependent fluorescence quenching (qE) by Oxborough and Horton (1988).

RESULTS

Chl Contents

In greening of an etiolated barley seedling by the light of 100 $\mu mol \cdot m^{-2}s^{-1}$, UV-B radiation of 0.2 W m^{-2} was irradiated at the same time. The change of Chl contents were measured every 6 h for total 72 h greening (Fig. 1). In greening of etiolated barley seedling, the contents of Chl a, b and carotenoid and the ratio of Chl a to b increased quickly for about 24 h after the initiation of greening and after then did slowly. In greening with the additional irradiation of UV-B radiation, the contents of Chl a, b and carotenoid were much less than that of the control. In 72 h greening, Chl a specially decreased as most as 43%, Chl b 36%, carotenoid 27% (Fig. 1A, B and C) and the ratio of Chl a to b 11% than that of the control (Fig. 1D).

Chl Fluorescence

Fig. 2 shows the relative Chl fluorescence yield to find out the influence on the development of chloroplast by the additional irradiation of the UV-B radiation in greening of etiolated barley seedling. In greening of etiolated barley seedling, Fo was detected but at relatively low level even in the

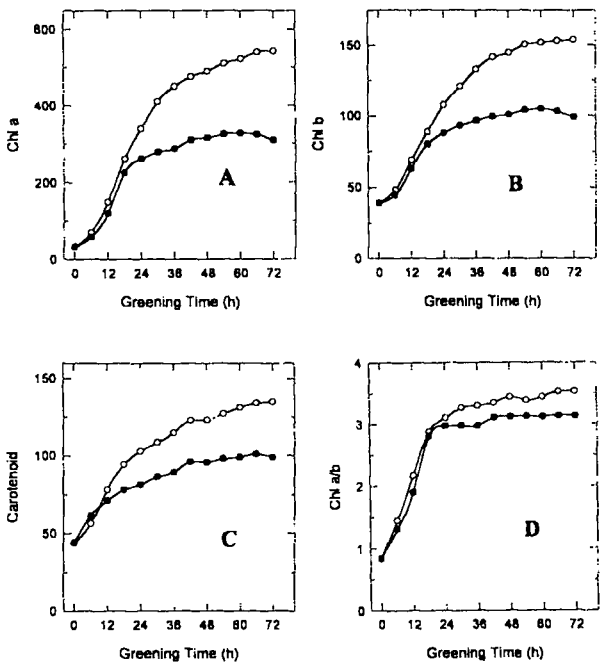

Fig. 1. Changes of the Chl contents and Chl a/b ratio of barley seedling treated with 0.2 W m^{-2} UV-B during greening period. a, Chl a content (μg/g fr wt); B, Chl b content (μg/g fr wt); C, carotenoid content (μg/g fr wt); D, Chl a/b ratio. ○, control; ●, 0.2 W m^{-2} UV-B. The values are the means of three independent experiments with three measurements.

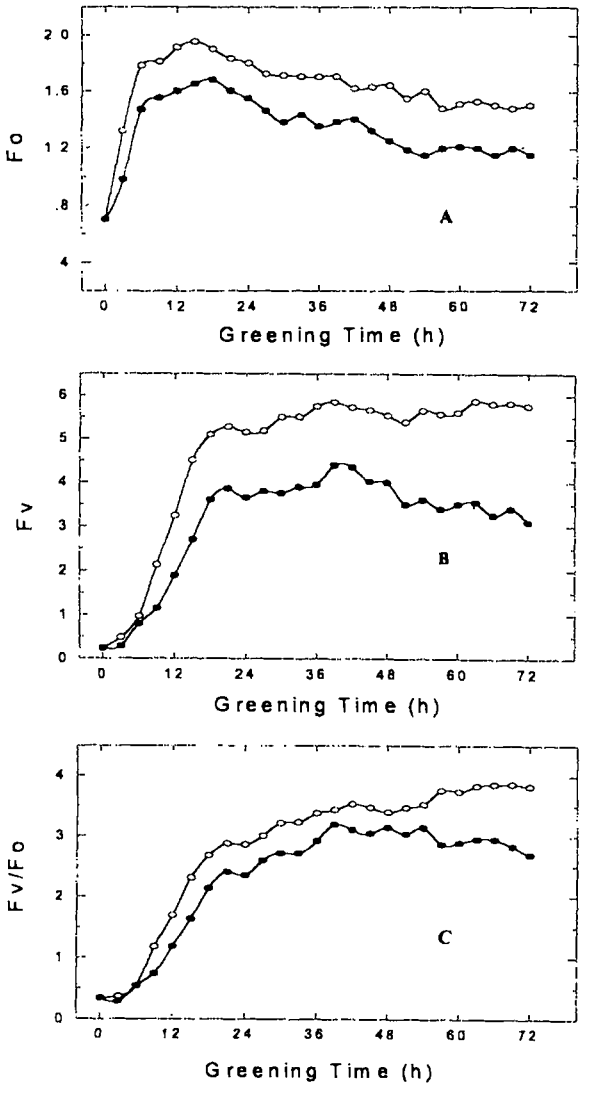

Fig. 2. Changes of relative Chl fluorescence of greening barley seedling treated with 0.2 W m^{-2} UV-B. A, Fo; B, Fv; C, Fv/Fo, ○, control; ●, 0.2 W m^{-2} UV-B. The values are the means of three independent experiments with three measurements.

etiolated state. The level of Fo was increased markedly for 6 h after the initiation of greening and then the rate of increase was slow down until 15 h, after that it was decreased slowly. In greening with the additional irradiation of UV-B radiation, the pattern of Fo was a great difference from that of control, especially by the early stage of greening. The difference of the level of Fo between the treatment and control did not change significantly from 12 h to 72 h. Fo of treatment seedling was 77% of that of control at 72 h after the initiation of greening (Fig. 2A). In greening of etiolated barley seedling, the value of Fv increased quickly for 21 h after the initiation of greening and then changed very little with greening time. In greening with the additional irradiation of UV-B radiation, the value of Fv made more and more difference and decreased as much as 46% in 72 h greening than that of the control (Fig. 2B). In greening of etiolated barley seedling, the ratio of Fv to Fo increased with time of greening but kept constant after about 57 h greening. In greening with the additional irradiation of UV-B radiation, there's little difference from the control for 54 h but the ratio of Fv to Fo has decreased gradually as much as 30% after 72 h greening than that of the control (Fig. 2C).

Fig. 3 is the analysis of Chl fluorescence quenching in order to find out the influence of UV-B radiation on the development of chloroplast in greening of an etiolated barley seedling by the light of 100 $\mu mol \cdot m^{-2}s^{-1}$. In greening of etiolated barley seedling, qP increased quickly for 21 h and kept almost constant in spite of the slight decrease later. In greening with the additional irradiation of UV-B radiation, qP decreased with little difference from that of control after 18 h greening. With time, the qP made more

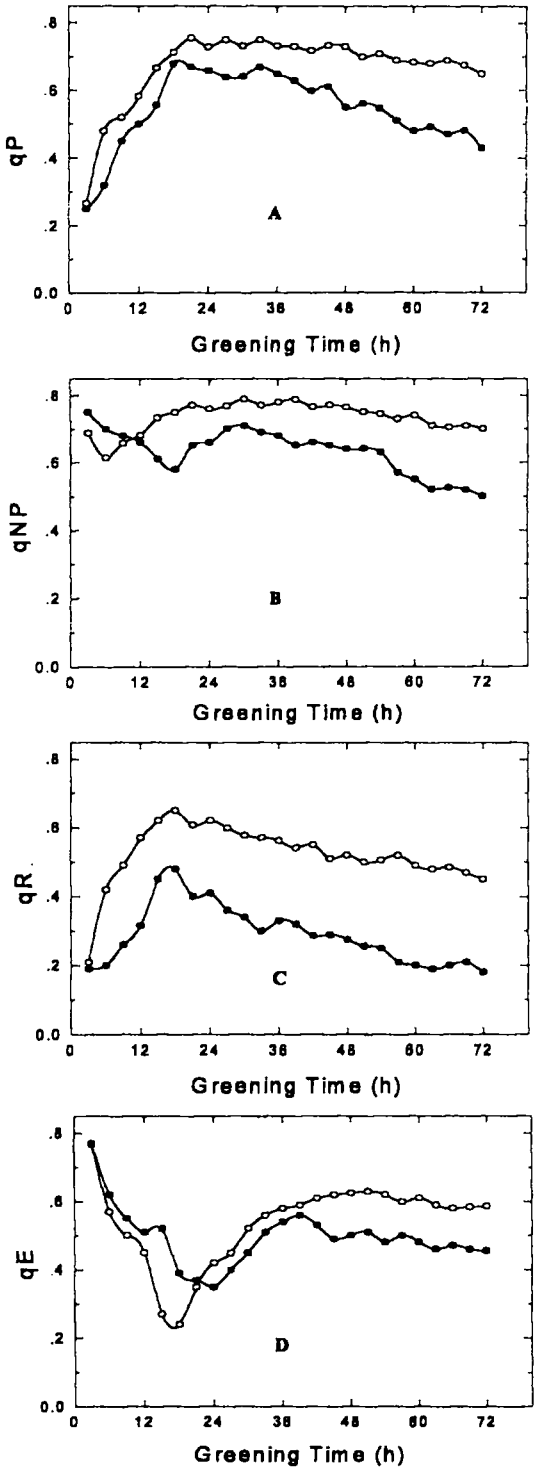

Fig. 3. Changes of fluorescence quenching coefficients of barley seedling treated with 0.2 W m^{-2} UV-B during greening period. These values were calculated by the way described in meterials and methods. A, qP; B, qNP; C, qR; D, qE. ○, control; ●, 0.2 W m^{-2} UV-B. The values are the means of three independent experiments with three measurements.

and more difference from that of control and the value of qP decreased 35% after 72 h greening than that of the control (Fig. 3A). In greening of etiolated barley seedling, qNP decreased for 6 h after the initiation of greening but increased for 24 h and then decreased gradually again. In greening with the additional irradiation of UV-B radiation, qNP decreased for 18 h and increased for another 12 h and finally after 72 h greening decreased 34% than that of the control (Fig. 3B). In greening of etiolated barley seedling, qR increased rapidly until 18 h, and then decreased gradually. The experiment with the additional irradiation of UV-B radiation had similar graph to the control but the value of qR decreased very much from that of control and decreased 67% after 72 h greening than that of the control (Fig. 3C). In greening of etiolated barley seedling, the value of qE decreased rapidly for 18 h, and then increased quickly until 36 h, after that it kept constant. In greening with the additional irradiation of UV-B radiation, qE decreased for 24 h after the initiation of greening. The level of qE was increased for another 15 h and decreased gradually again until 16% decrease than that of the control after total 72 h greening (Fig. 3D).

DISCUSSION

In this experiment, the changes of Chl contents related to Chl fluorescence were examined in order to find out the influence of UV-B radiation on the development of barley chloroplast by the measurement of Chl fluorescence. With the result in Fig. 1, the influence of UV-B radiation on Chl contents was slight in short treatment of UV-B radiation but increased very much as the UV-B treatment continued. The influence on Chl a was greater than that on Chl b. Takeuchi *et al.* (1993) has reported that Chl synthesis was decreased strongly by UV-B radiation, too. And the result in Fig. 1 was the same as Strid *et al.* (1990) that the 7 d irradiation of UV-B radiation in greening of pea leaf decreased greatly the ratio of Chl a to Chl b.

In Fig. 2A, Fo increased quickly for 6 h after the initiation of greening, in spite of relatively slow increase of Chl a (Fig. 1A) after the greening. The ratio of Fv to Fo increased quickly after the low situation for 6 h after the initiation of greening. It means that the energy absorbed in light harvesting pigments of the reaction center of PSII couldn't transport easily into the reaction center of PSII at the early stage of the greening. After 6 h greening, Fo

decreased gradually after slow increase in spite of the rapid increase of Chl a and the ratio of Fv to Fo increased rapidly. So it explains that electron transport capability of PSII develops quickly after 6 h greening. The UV-B radiation treatment resulted in similar Chl contents to that of the control and in very different Fo value from that of the control at the early stage of greening. It shows that the UV-B treatment had relatively little influence on the contents of Chl but great influence on the function of light harvesting pigments at the early stage of greening. As the UV-B treatment continued, the level of Fo kept constant from that of the control, but the level of Fv had great difference from that of the control in spite of slight difference at the early stage of the treatment. It means that there's no difference in the efficiency of energy excited from pigment molecules of PSII antenna but the efficiency of energy transported into the reaction center of PSII and used for photosynthesis decreased relatively very much. It was the same result as Larkum and Wood (1993)'s that UV-B irradiation to algae decreased the value of Fv and as Melis *et al.* (1992) that the UV-B irradiation to the thylakoid membrane extracted from spinach made the reaction center of PSII inactive. By the additional irradiation of UV-B radiation in greening an etiolated barley seedling, Chl a decreased 43% for 72 h than that of the control but Fo was relatively less decrease, only 23%. Fo was expected to decrease very much because of the 43% decrease of Chl a content. It seems that the little decrease of Fo is due to the damage of PSII. qP, indicating the redox situation of quinone, shows the opening of the reaction center of PSII. Thus the quick increase of qP for 21 h after greening in Fig. 3A means the rapid development of the electron transport system after quinone. At the early stage of UV-B treatment, the difference between qP and that of control shows the delay of development of the electron transport system after quinone because of UV-B. The decrease of qP of treated plants with UV-B compared to control plants resulted from the inhibition of Q_A oxidation since qP reflected the oxidation of Q_A (Chun *et al.*, 1993). Melis *et al.* (1992) reported that the UV-B irradiation to thylakoid membrane extracted from spinach damaged easily the reduction region such as Q_A and plastoquinone. Hideg *et al.* (1993) reported that the UV-B irradiation to thylakoid membrane extracted from spinach damaged the redox region of PSII. In Fig. 3A and 3B about the continuous irradiation of UV-B, both of the photochemical and nonphotochemical quenching decreased than that of control. It means that UV-B had a serious influence on various non-photochemical factors as well as photosynthetic electron transport pathway. In Fig. 3C, qR decreased as much as 67% than that of the control after the additional irradiation of UV-B for 72 h greening. In Fig. 3D, the decrease of qE by UV-B treatment was relatively little and it is possible that the treatment had relatively little influence on water splitting of PSII or PQ pool and as a result the influence on forming of pH gradient bordered on thylakoid membranes was little.

In the above experiment, as an etiolated barley seedling was greened by the light of 100 $\mu mol \cdot m^{-2}s^{-1}$ with the additional UV-B irradiation, the ratio of Fv to Fo decreased 30% than that of the control for 72 h, and qP and qNP did 35% and 34% each, specially, qR about 67% but qE only about 16%. These results show that the UV-B treatment restrained not one special region of photosynthetic electron transport system but all the region of photosystem. Since the decrease of qE was relatively small, PQ pool as the reduction region of PSII was not affected significantly.

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