한국환경과학회지 제5권(제5호), 635~642, 1996
J. of the Korean Environmental Sciences Society

Effects of UV-B radiation on carotenoids, polyamines and lipid peroxidation in rice (*Oryza sativa* L.) leaves

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(Manuscript received 13 August 1996)

=Abstract

Rice plants, cv. Koshihikari, were subjected to the biologically effective ultraviolet-B ($UV\text{-}B_{BE}$) radiation {daily dose : 0.0 (control) and 11.5 (enhanced UV-B) kJ m^{-2}} to investigate the effect of enhanced UV-B radiation on lipid peroxidation and to determine whether carotenoids and polyamines are involved in protection mechanism against enhanced UV-B radiation. Enhanced UV-B radiation significantly depressed plant dry weight. Malondialdehyde (MDA) content in rice leaves was increased by about 30% after 6 days of UV-B irradiation. Total carotenoid contents tended to slightly decrease with the UV-B irradiation, even though there was no significance. In rice leaves, 3 major polyamines, putrescine, spermidine and spermine are observed. All of the polyamine contents were increased with UV-B irradiation. The results suggest that enhanced UV-B radiation caused oxidative stress on lipids and that polyamines may serve as a biochemical protectant against increased UV-B radiation in rice plants.
Key words : carotenoid, malondialdehyde (MDA), polyamine, rice (*Oryza sativa* L.), ultraviolet-B radiation.

1. INTRODUCTION

Stratospheric ozone reduction is one of the pressing global concerns related to the climate changes. Decreases in quantity of total-column ozone, as now observed in many places in the world, tend to cause the increased penetration of solar UV-B radiation (280~320 nm) to the earth's surface (Madronich et al., 1995). In fact, there is a strong evidence of increasing in surface UV-B irradiance. It is predicted that the decrease of ozone and increase of UV-B will be continued in the future (Kerr and McElroy, 1993; Madronich et al., 1995).

Enhanced UV-B can have negative effects on plant physiological processes and biochemical metabolisms such as inhibition of photosynthesis, DNA damage and destruction of chloroplast membrane structure (Caldwell et al., 1995). It has been reported that total level of chloroplast lipids was decreased and the ratios of individual polar lipids was al tered by UV-B irradiation in several plant species (Tevini et al., 1981).

In the previous study, it was found that the enhanced UV-B radiation caused oxidative stress in both rice and cucumber (Kim et al., 1996c). Generally, lipid peroxidation is one of the potential impact on active oxygen species in plants, and the primary target can be cellular membrane lipids (Bowler et al., 1992).

In plants, there are some compounds which are responsible for preventing lipid peroxidation. For example, polyamines are potent inhibitors of many senescence-related processes in a variety of plant species (Galston and Kaur-Sawhney, 1987). Much of this antisenescent activity are related to membrane. A key process in plant senescence appears to be involved in membrane deterioration, especially via the formation of lipid hydroperoxidases (Roberts et al., 1986; Thompson et al., 1985). Polyamines are associated with membrane surface properties, presumably through interactions with anionic components of the membrane such as phospholipids. This interaction serves to stabilize the bilayer and may thus retard membrane deterioration (Roberts et al., 1986). Polyamines also have radical-scavenging properties (Bors et al., 1989). Protection of membranes from peroxidation by polyamines could be involved in their ability to interact with phospholipids and their antioxidant activity (Drolet et al., 1986; Slocum et al., 1984).

Carotenoids also have antioxidative properties and effectively protect membrane lipids from reaction with singlet oxygen (Larson and Berenbaum, 1988). Singlet oxygen is a powerful oxidizing agent which can cause the destruction of cell components mainly lipids and proteins of the organism. In general, it is accepted that carotenoids can protect plant cells from light-mediated damage through preventing the formation of singlet oxygen by quenching triplet-state chlorophyll molecule, and by scavenging any singlet oxygen produced (Young, 1991). Thus, it was suggested that carotenoids might have a specific role in protecting the membrane lipids from photodamage (Siefermann-Harms et al., 1978).

There are a number of reports concerning the effects of UV-B radiation on plants. Several experiments have been conducted to assess the effects of UV-B radiation on the growth of rice plant which is one of the most important staple food in the world (Kim et al., 1996a, b, c; Dai et al., 1992), but there are few experiments concerning the UV-B action mechanisms and protection mechanisms in rice plants to the UV-B radiation.

This study was conducted to investigate the effect of enhanced UV-B radiation on lipid peroxidation in rice plants and also to determine whether carotenoids and polyamines are involved in the protection mechanisms against enhanced UV-B radiation.

2. Materials and Methods

2.1 Plant material and culture conditions

Rice (*Oryza sativa* L. cv. Koshihikari) seeds were germinated in tap water for 3 days at room temperature, and sown in trays (30 cm deep×60 cm wide×2.6 cm high) with one seed in a cell (1.6 cm deep×1.6 cm wide×2.6 cm high). Each tray contained alluvial soil premixed with 5.7 g of mineral fertilizer ($N : P_2O_5 : K_2O = 0.21 : 0.40 : 0.39$). After incubating at 25℃ for 3 days, the seedlings were moved into a greenhouse and grown for 1 week. The seedlings were then transferred to environmentally-controlled growth chambers (2 m deep×2 m wide×1.8 m high) maintained 25 ± 0.1℃ and a relative humidity of 75 ± 5% under natural sunlight as described previously (Kim et al., 1996b). In each chamber, the seedlings in trays were immersed in water in a 30-L plastic container. The seedlings were, therefore, grown in flooded conditions.

2.2 UV-B irradiation

UV-B radiation was artificially supplied by filtered Toshiba sunlamps (FL 20 SE) as reported previously (Kim et al., 1996b). The sunlamps were wrapped with cellulose diacetate film of 0.13-mm thickness (Cadillac Plastics Co., Baltimore, Ohio, USA), to remove the UV radiation with wave length shorter than 290 nm. For the control, lamps were covered with Mylar D film (DuPont Co., Wilmington, Delaware, USA) of 0.13-mm thickness which effectively absorbs radiation below 313 nm. The filters were replaced weekly and the height of the lamps was adjusted as the plants grow to keep the lamps about 30 cm above the plant canopy. The plants were irradiated with enhanced UV-B for 6 hrs daily (from 10 : 00 to 16 : 00) for 12days. The UV-B irradiance was expressed as biologically effective UV-B ($UV\text{-}B_{BE}$) weighed with the generalized plant action spectrum normalized to 300 nm (Caldwell, 1971).

2.3 Growth measurement

To investigate the rice growth responses to the enhanced UV-B irradiation, 6 plants of each treatments were harvested at 3, 6, 9 and 12days after initiation of the UV-B irradiation. The plants were oven-dried at 70℃ and weighed.

2.4 Measurement of malondialdehyde (MDA) content

For the assay of MDA contents, samples were taken at 6 and 12days after initiation of the UV-B irradiation. MDA content in rice leaves was assayed according to the methods proposed by Heath and Packer (1968). The leaves (about 10 cm^2) were homogenized in distilled water and 3 ml of the homogenate was mixed with 5 ml of 0.5% thiobarbituric acid prepared with 20% trichloroacetic acid. The mixture was incubated in a water bath for 30 min. After centrifugation, the MDA content in the supernatants was determined by using an absorbance coefficient of 155 mM^{-1} cm^{-2} at 532 and 600 nm.

2.5 Measurement of total carotenoids

The leaves (about 10 cm^2) were extracted with 99.5% ethanol for 48hrs at 4℃ in darkness. The absorption spectra in the wavelength of 648 nm, 664 nm and 470 nm were measured with a scanning spectrophotometer (UV-1200, Shimadzu Co., Kyoto, Japan) and the total carotenoids were determined using equations described by Lichtenthaler (1987).

2.6 Measurement of polyamines contents

The leaf samples (about 200 mg) were extracted with 2 ml of 0.5 M $HClO_4$ at room temperature. Polyamines in the acid extract were derivatized with benzoyl chloride and quantitated with HPLC using diaminohexane as an internal standard according to the method described by Flores and Galston (1982). Separation and quantification of polyamine derivatives were carried out with a Shimadzu LC-6A HPLC equipped with a UV detector under the following conditions: column size (Shim-pack CLC-ODS), 6×150 mm; column temperature, 45℃; mobile phase, 63% methanol solution; liquid flow rate, 0.8 ml min^{-1}; detection, 254 nm. The aliquot of the extracted samples were hydrolyzed with 6 M HCl at 100℃ for 16hrs prior to the derivatization of polyamines to examine the presence of conjugated forms of polyamines.

2.7 Statistical analysis

Statistical differences in plant dry weight and all assay results were determined by the analysis of variances (ANOVA).

3. Results

3.1 UV-B irradiation

Mean daily integral UV-B_{BE} of each treatment is shown in Table 1. Rice plants grown under the UV-B treatment received about 11.5kJ m^{-2} day^{-1} (UV-B_{BE}). The level of UV-B_{BE} used in this study was equivalent to that which would be received at Tsukuba, Japan (36°01′N, 140°07′E) with an anticipated 36% stratospheric ozone reduction on the summer solstice, based on an empirical model (Björn and Murphy, 1985) with clear and aerosol-free sky condition and actual observation of total ozone amount at Tsukuba in 1992 (Japan Meteorological Agency, 1993). Due to the absorption characteristics of the glass walls, there was no solar UV-B_{BE} radiation in the chambers.

3.2 Effect of UV-B irradiation on growth

Rice plants did not exhibit any foliar injuries in response to the increased UV-B radiation, but plant dry weight significantly decreased by UV-B irradiation (Fig. 1). The reduction of plant dry weight was initiated at 6days of UV-B irradiation, showing about 8% of statistically significant difference in growth. The differences in plants dry weight between the enhanced UV-B and control increased as the irradiation period prolonged. In 12days of UV-B irradiation, about 15% of plant dry weight was reduced.

Table 1. Average daily integral of UV-B_{BE} (biologically effective UV-B) during the irradiation period. The UV-B irradiations was conducted for 6 hours daily (from 10 : 00 to 16 : 00).

UV-B treatments	Mean daily integral UV-B_{BE} (kJm^{-2})
Control	0.0
Enhanced UV-B	11.5

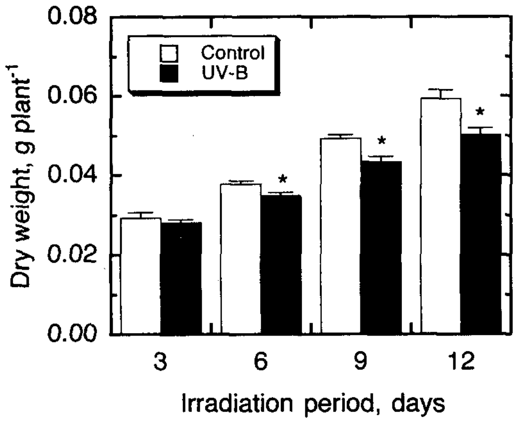

Fig. 1. Effects of enhanced UV-B radiation on dry weight of whole plant of Koshihikari. Each value is the mean of 6 plants and vertical bars represent the standard error for the mean. Statistically significant differences between the means are indicated by "*" ($p<0.05$).

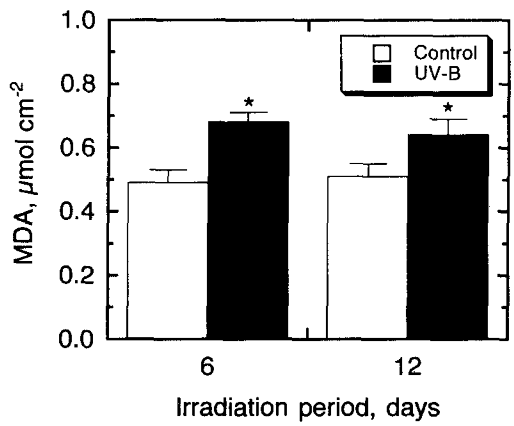

Fig. 2. Effects of enhanced UV-B radiation on MDA (malondialdehyde) content in rice leaves. Each value is the mean of 6 plants and vertical bars represent the standard error for the mean. Statistically significant differences between the means are indicated by "*" ($p<0.05$).

3.3 Effect of UV-B irradiation on lipid peroxidation

The level of lipid peroxidation in the leaf was determined by measuring changes in MDA (a product of lipid peroxidation). The changes in MDA contents of leaves as affected by enhanced UV-B irradiation are shown in Fig. 2. The enhanced UV-B irradiation resulted in statistically significant increase in MDA contents by about 30% and 28% at 6 and 12days of UV-B irradiation, respectively.

3.4 Effect of UV-B irradiation on total carotenoid contents

Changes in the total carotenoid contents by UV-B irradiation are shown in Fig. 3. The total carotenoids tended to slightly decrease with the UV-B irradiation. About 10% of total carotenoid content decreased both at 6 and 12days of UV-B irradiation, but there was not statistically significant differences.

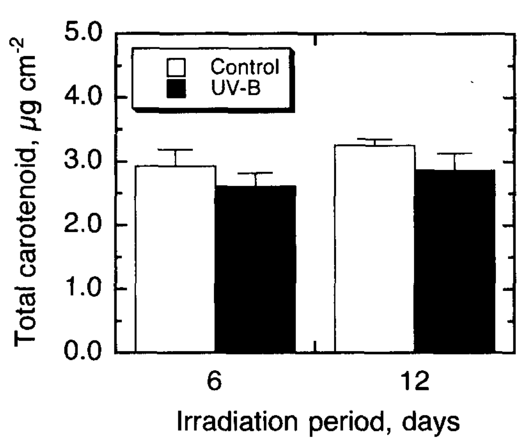

Fig. 3. Effects of enhanced UV-B radiation on total carotenoid contents in rice leaves. Each value is the mean of 4 plants and vertical bars represent the standard error for the mean.

3.5 Effect of UV-B irradiation on polyamine contents

The effect of enhanced UV-B irradiation on polyamines levels is shown in Fig. 4. Rice was composed of mainly 3 kinds of polyamines such as putrescine (Put), spermidine (Spd) and spermine (Spm). Enhanced UV-B irradiation resulted in statistically significant increases in total contents of polyamines. The contents of Spm were most significantly increased by about 43% both at 6 and 12 days of UV-B treatments. Enhanced UV-B irradiation caused a significant increase of 27% in Spd contents at 6days of UV-B treatment. After 12

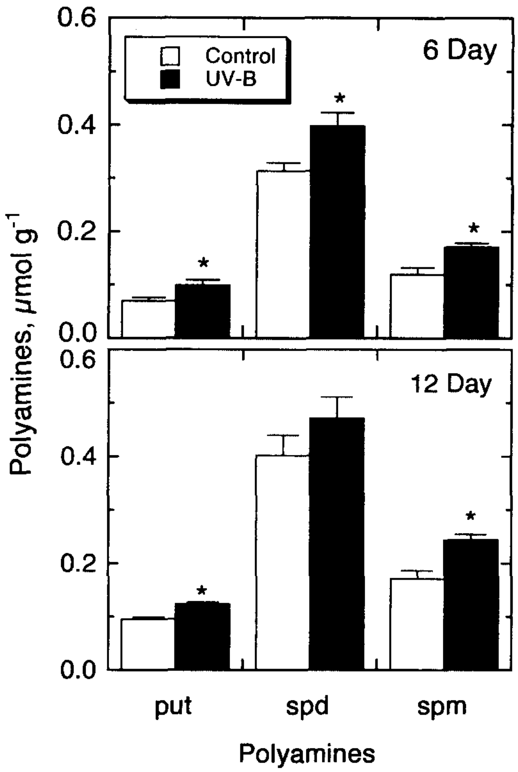

Fig. 4. Effects of enhanced UV-B radiation on polyamine levels in rice leaves. Each value is the mean of 4 plants and vertical bars represent the standard error for the mean. Statistically significant differences between the means are indicated by "*" ($p<0.05$).

days of UV-B irradiation, however, about 15% of increased Spd content was obtained, although it was not significant compared to the control.

4. Discussion

Enhanced UV-B radiation has deleterious effects on a number of plant species (Caldwell et al., 1995). Generally, rice has been classified as less sensitive to UV-B than other species such as cucumber and soybean (Krupa and Kickert, 1989). In this study, the dry weight of rice was deleteriously affected by enhanced UV-B irradiation for 12days. However, the level of reduction in dry weight of rice was not as high as that reported for other crops. Nouchi (1991) found that dry weight of cucumber decreased as much as 40% by UV-B exposure for 2 weeks. However, dry weight of rice, in this study, was reduced only 15% after 12days of UV-B irradiation.

It has been reported that the enhanced UV-B radiation caused photooxidative stress by inducing active oxygen species in plants (Larson and Berenbaum, 1988). In the previous study, It was found that the enhanced UV-B caused oxidative stress in rice plants (Kim et al., 1996c). Cellular membrane lipids can be primary targets of oxidative stress in plants (Tevini et al., 1981). In this study, there were significant increases in lipid peroxidation, as indicated by the MDA accumulation in UV-B treated rice leaves. The increase in MDA content may be responsible for alteration of membrane lipid composition by the UV-B irradiation. The same result on increased lipid peroxidation by UV-B has been observed in two cucumber cultivars (Kramer et al., 1991). UV-B tolerance of the cucumber cultivars was correlated with the degree of lipid peroxidation, which in turn could be attributed to the differences in the ability of these cultivars to protect against UV-B induced damage. These results indicate that lipids are a potential target of UV-B stress in plants.

The carotenoid content in rice leaves tended to decrease by UV-B irradiation, but the effect was not statistically significant. The carotenoid may be destroyed by UV-B, although the mechanisms were not known so far. In contrast to the carotenoid content showing only non-significant reduction by UV-B, the polyamine contents were significantly increased by the UV-B radiation. This is consistent with previous observations on polyamine increases in response to a variety of stresses on plants (Slocum et al., 1984). The potential benefit of such responses is demonstrated by the application of exogenous polyamines which result in protection from injury by ozone and chilling stresses. Such effects may be related to the ability of polyamines to stabilize membrane structures and inhibit lipid peroxidation, or to the formation of conjugates with free radical scavenging activity (Bors et al., 1989; Kramer and Wang, 1989; Slocum et al., 1984). The photoprotective pigments synthesized by plants in response to UV-B were derived from cinnamic acid (Tevini and Teramura, 1989). The inductionf polyamine conjugator by UV-B is thus a possible response to this stress, as the formation of precursors is promoted by UV-B (Kramer et al., 1991).

In conclusion, the enhanced UV-B radiation caused oxidative stress on lipids. The accumulation of polyamines may serve as a protecting response to the increased UV-B radiation in rice plants. Further studies are necessary to demonstrate conclusively that if polyamines may play a protective role in plants from UV-B stress.

Acknowledgments

Financial support for this research was provided by the Global Environment Research Program Budget of Japan Environment Agency.

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UV-B가 벼잎의 carotenoid, polyamine 및 脂質過酸化에 미치는 영향

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(1996년 8월 13일 접수)

UV-B증가가 지질과산화에 미치는 영향과 UV-B照射에 대한 carotenoid 및 polyamine의 지질과산화 방어반응을 조사하기 위해 자연광 이용의 인공기상실내에서 12일간 벼 (*Oryza sativa* L., cv. Koshihikari) 유식물에 2단계의 UV-B照射 실험을 수행했다. UV-B처리에 의한 乾物量의 감소는 照射시간의 경과와 함께 증가하였다. 지질과산화산물인 malondialdehyde (MDA)의 함량은 6일간의 UV-B처리에 의해 약 30%의 증가를 보였다. Carotenoid의 함량은 UV-B처리에 의해 약 10% 감소하는 경향을 보였으나 통계학적 유의성은 없었다. 한편, 벼에는 크게 3종류의 polyamines (putrescine, spermidine, spermine)이 존재하였으며, 이들 모두 UV-B처리에 의해 상당히 증가되었다. 이상의 결과로 볼 때, UV-B照射에 의해 생성된 활성산소가 생체막지질의 산화에 관여하는 것으로 사료되며, polyamine의 증가는 UV-B조사에 의한 세포막 파괴를 막기 위한 생화학적 방어반응으로 사료된다.