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Influence of supplemental UV-B radiation on photosynthetic characteristics of rice plants

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Abstract

In a field experiment with rice (*Oryza sativa* L. cv. Saket 4) grown under ambient and supplemental ultraviolet-B (UV-B) radiation at 20 % ozone depletion, differences in gas exchange, concentrations of photosynthetic pigments, anthocyanins and flavonoids, biomass accumulation, catalase and peroxidase activities, and contents of ascorbic acid and phenol were determined. Decline in photosynthesis was associated with reductions in stomatal conductance and concentrations of photosynthetic pigments. Enhanced UV-B radiation (eUV-B) increased the contents of flavonoid and phenolic compounds in leaves. Peroxidase activity increased and catalase activity was always lower at eUV-B. The total plant biomass decreased at eUV-B.

Additional key words: biomass; carotenoids; catalase; chlorophyll; flavonoids; *Oryza sativa*; peroxidase; phenols; stomatal conductance; transpiration rate.

Introduction

Most of the reports on effect of eUV-B on rice plants are from those grown in greenhouse and growth chambers where the irradiance is totally different from the normal sunlight, and therefore the sensitivity of plants may vary (Teramura *et al.* 1991, He *et al.* 1993, Dai *et al.* 1994, 1995, Murthy and Rajagopal 1995). Rice is variably sensitive to eUV-B under growth chambers (Krupa and Kickert 1989, Teramura *et al.* 1991, Haung *et al.* 1993, He *et al.* 1993). Dai *et al.* (1992) and Barnes *et al.* (1993) have shown drastic reduction in the net photosynthetic rate (P_N) in rice plants grown in a glasshouse. Teramura *et al.* (1991) found a reduction in total plant biomass in six out of 16 cultivars exposed to UV-B simulating a 20 % ozone depletion at the equator in a glasshouse.

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Tropics receive higher UV fluxes as compared to temperate regions. Rice is one of the major food grain crops of the world particularly in Asia, so the present investigation was conducted to quantify the physiological and biochemical responses of rice (*Oryza sativa* L. cv. Saket 4) plants to eUV-B grown in the field at natural photosynthetically active radiation (PAR).

Materials and methods

Plants: The experiments were performed from July through September in 1993 and 1994 at the Botanical Garden of Banaras Hindu University, Varanasi (25°18'N latitude and 83°1'E longitude, at an elevation of about 76 m a.s.l.), situated in the eastern part of Gangetic plains in India. The climate of the region is tropical monsoonic. The soil of the study site was sandy loam (sand 45 %, silt 28 %, and clay 27 %) and neutral in reaction (pH 7.0 to 7.2). During the experiment, average temperature ranged from 25 to 38 °C, relative humidity from 70 to 98 %, and rainfall was 642 mm. The PAR averaged 827 μmol m^{-2} s^{-1}. In six field plots of 1.5×1.5 m, each prepared using standard agronomic practices, rice bunches of three seedlings (25 d-old) were transplanted in rows spaced 0.3 m apart. Recommended doses of nitrogen, phosphorus, and potassium as urea, superphosphate, and muriate of potash (8 : 4 : 2 g m^{-2}) were added to all plots as basal dressing at 40 d. At 60 and 80 d ages, urea was twice amended (0.6 g m^{-2}) as top dressing. Watering was done at regular intervals to maintain the moisture at field capacity. For the control and eUVB-treatments, always three replicate plots were maintained.

eUV-B was artificially provided by *Q Panel UV-B 313* fluorescent lamps (*Q Panel*, Cleveland, U.S.A.). Four lamps per bank fitted 30 cm apart on a wooden frame were suspended above and perpendicular to the planted rows. The lamp banks were equipped with dimming ballasts and dimmer switches to control the UV-B irradiance. Each lamp was covered with 0.13 mm thick cellulose diacetate film (*Cardillac Plastics*, Baltimore, U.S.A.) which absorbed radiation emitted by lamps below 290 nm. For control, lamps were covered with 0.13 mm thick polyester film (*Cardillac Plastics*, Baltimore, U.S.A.) which absorbed radiation emitted by lamps below 320 nm. Plastic filters were aged 5 h under unfiltered *Q Panel UV-B* lamps prior to use and changed twice a week to avoid ageing effects on the spectral transmission of UV-B. The 0.45 cm distance between the top of plant canopy and UV-B lamps was kept constant. Plants were artificially irradiated after 5 d of transplantation for 5 h per day in the middle of the photoperiod till the maturity.

The UV-B irradiance at the top of the canopy under the lamps was measured by an Ultraviolet Intensity Meter (*UVP*, San Gabriel, U.S.A.). The readings were converted to UV-B$_{BE}$ values by comparing with the Spectro Power Meter (*Scientech*, Boulder, U.S.A.). Plants under polyester filtered lamps received only ambient UV-B (9.6 kJ m^{-2} UV-B$_{BE}$) on the summer solstice weighted against the generalized plant response action spectrum of Caldwell (1971). The plants beneath cellulose diacetate film received eUV-B (+7.1 kJ m^{-2}) that mimicked 20 % reduction in stratospheric ozone at Varanasi (25 °N) during clear sky condition on the summer solstice (Green *et al.*

1980) normalised at 300 nm. These authors assumed that the O_3 column thickness was 3.0 mm, the albedo was 0, and the scatter was 1.0.

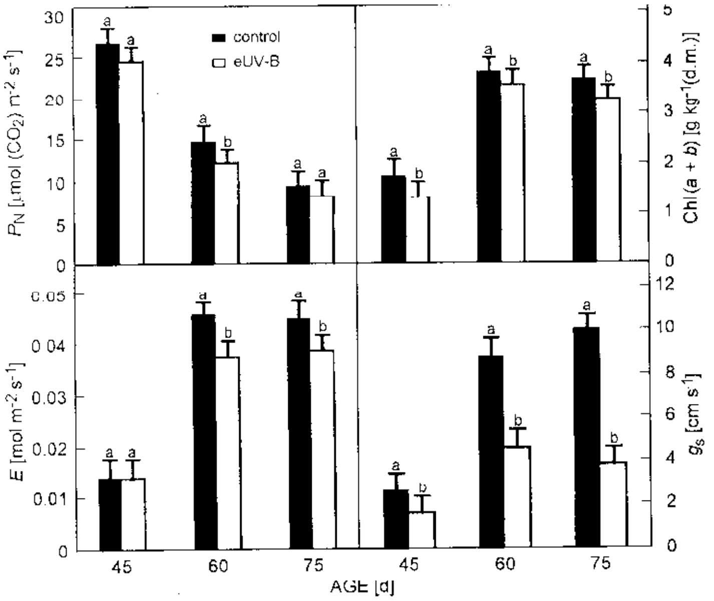

Fig. 1. Effects of enhanced UV-B radiation (eUV-B) on net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and chlorophyll (Chl) ($a+b$) content of *Oryza sativa* L. plants at different growth stages (means ± S.E.). Values marked with the same letter were not significantly different from each other ($p < 0.05$).

Various analyses: Three plants were always sampled randomly from each replicate plot after 45, 60 and 75 d. The *LI-COR 6200* portable photosynthesis system (*LI-COR*, Lincoln, U.S.A.) under ambient conditions was used for measurements at a PAR between 1000 1200 µmol m^{-2} s^{-1} on the third leaf below the apex of the stem, and mean ambient CO_2 concentration of 342 g m^{-3}. For biomass determination, plants were oven dried at 80 °C to constant mass. Total chlorophyll (Chl) and carotenoid contents were extracted from leaf discs with 80 % acetone and quantified as described by Maclachlan and Zalik (1963) and Duxbury and Yentsch (1956), respectively. Ascorbic acid and phenolic substances in fresh leaves were determined using the methods of Keller and Schwager (1977) and Bray and Thorpe (1954), respectively. Catalase and peroxidase activities were determined using the methods of Kar and Mishra (1976) and Britton and Mehley (1955), respectively. Estimations of anthocyanin and flavonoid contents were done following the methods of Beggs and Wellmann (1985) and Flint *et al.* (1985), respectively.

Quantitative changes of different parameters at different periods of plant growth were analysed through analysis of variance (ANOVA). The Duncan's Multiple Range Test was applied for mean separation for significant differences among treatments.

Results

The P_N did not change significantly due to eUV-B treatment at 45 and 75 d ages (Fig. 1). However, at 60-d age, P_N declined significantly as compared to the control. The transpiration rate (E) at 45 d did not differ significantly between the control and the eUV-B plants, but with the increasing age and cumulative UV-B dose, it declined significantly (by 13 to 17 %) in the treated plants. Stomatal conductance (g_s) was significantly lower due to eUV-B at all plant ages (Fig. 1). Chl ($a + b$) concentration was significantly lower (by 10.5, 14.2, and 19.5 % at 45, 60, and 75 d, respectively) under eUV-B as compared to the control plants (Fig. 1). Carotenoid and anthocyanin contents decreased significantly in eUV-B leaves as compared to control at all ages of growth, with the exception of anthocyanins at 75 d when a surprising increase in content was found (Table 1). Absorption profiles of flavonoids showed higher values in eUV-B plants as compared to control at all wavelengths (Fig. 2), but the increase was higher at increasing plant age.

Table 1. Age-wise variation in concentrations of carotenoid and anthocyanin [g kg^{-1}(f.m.)] in control and eUV B *Oryza sativa* plants at different growth stages (means ± S.E.). Values followed by the same letter were not significantly different from each other at $p < 0.05$. Values in bracketts give % change to control.

Plant age [d]	Treatment	Carotenoids	Anthocyanins
45	control	0.350 ± 0.006^a	0.021 ± 0.005^a
	eUV-B	0.230 ± 0.005^b [-34.3]	0.003 ± 0.003^b [-85.7]
60	control	0.700 ± 0.012^a	0.019 ± 0.006^a
	eUV-B	0.610 ± 0.011^b [-12.8]	0.012 ± 0.001^b [-36.8]
75	control	1.280 ± 0.011^a	0.016 ± 0.001^a
	eUV-B	1.090 ± 0.170^b [-14.8]	0.063 ± 0.008^b [+293.8]

The catalase activity declined significantly, whereas peroxidase activity increased significantly in eUV-B plants as compared to the control ones (Fig. 3). Maximum catalase activity was recorded at the age of 45 d, whereas maximum peroxidase activity was observed at 75 d. The content of phenolic compounds was higher in eUV-B plants as compared to the control (Fig. 3), the maximum content was observed at 60 d age. The ascorbic acid content decreased significantly in eUV-B plants (Fig. 3), the maximum reduction was 75.7 % at 75 d age. Total plant biomass decreased significantly in eUV-B plants at 60 and 75 d age, the reduction being 25.2 and 58.3 %, respectively (Fig. 4).

Discussion

Exposure of rice plants to eUV-B caused reduction in P_N, E, and g_s. Teramura *et al.* (1991) and He *et al.* (1993) have also shown reduction in P_N in rice plants. The reduction in P_N was correlated with reduction in g_s and total Chl concentration. A similar response to UV-B for two rice cultivars grown in glasshouse has been described by He *et al.* (1993) at 25 % O_3 depletion. Kim *et al.* (1996) also showed reduction in Chl content at eUV-B in three pot-grown rice cultivars in the field at 38 % O_3 depletion. The reduction in total Chl content was due to reduction in both Chl *a* and Chl *b*. Carotenoid concentration also showed significant reduction due to UV-B exposure. Iwanzik and Tevini (1982) found that Chl *b* was less affected by UV-B than the carotenoids. Anthocyanin concentration declined significantly in UV-B exposed plants up to 60 d but thereafter a significant increase was observed. Takahashi *et al.* (1991) showed an inverse correlation between anthocyanin concentration and UV-B sensitivity. An increase in UV-B absorbing compounds such as flavonoids is a consistent response to eUV-B, as already reported in two cultivars of rice grown in glasshouse by Teramura *et al.* (1991), by He *et al.* (1993) at 20 and 25 % O_3 depletion, and in pot-grown field plants by Kim *et al.* (1996) at 38 % O_3 depletion. Liu *et al.* (1995) report that UV-B markedly increases flavonoid accumulation in barley plants at 25 % O_3 depletion.

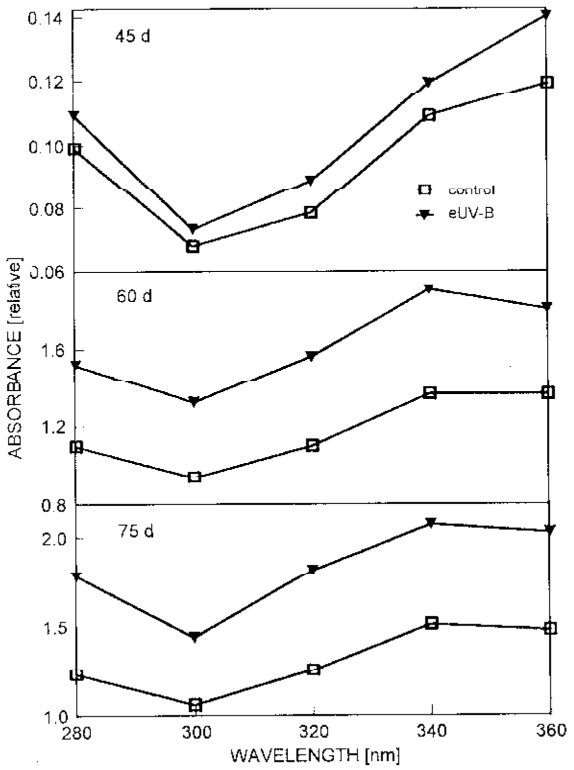

Fig. 2. Effect of UV-B radiation on absorbance profile of flavonoids in *Oryza sativa* L. plants at 45, 60, and 75 d of age. Absorbances were related to leaf fresh mass.

Catalase and peroxidase are important in antioxidant defense system for scavenging H_2O_2. We found that eUV-B resulted in significant reduction of catalase activity in exposed plants as compared to control. A similar reduction was observed in 3rd leaf of cucumber seedlings (Krizek *et al.* 1993). During stress the tetrameric catalase molecules may degrade into monomeric catalase subunits having peroxidase-like activity (Nandi *et al.* 1984). The decreased activity of catalase would result in

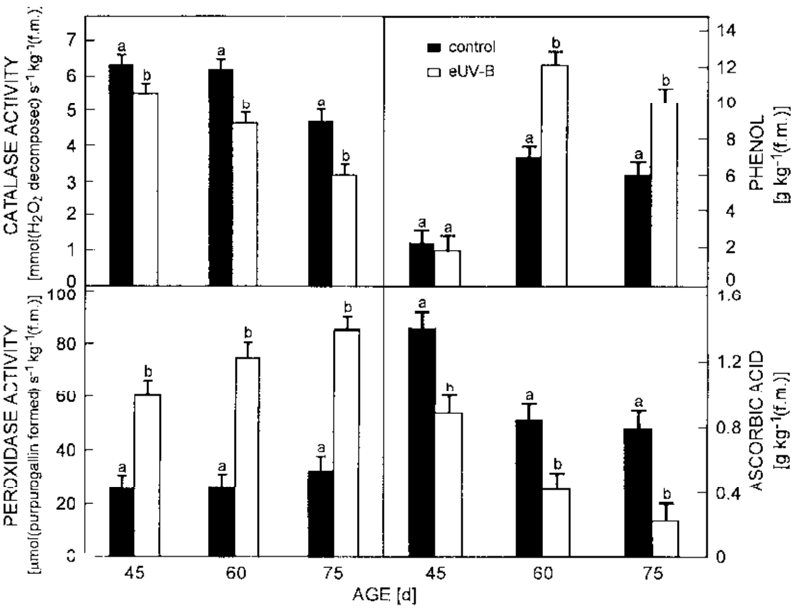

Fig. 3. Effects of UV-B radiation on catalase and peroxidase activities and contents of phenolic compounds and ascorbic acid in field grown *Oryza sativa* L. plants at different growth stages (means ± S.E.). Values followed by the same letter were not significantly different from each other ($p < 0.05$).

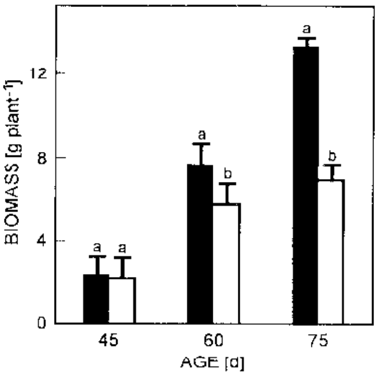

Fig. 4. Effect of UV-B radiation on biomass content of *Oryza sativa* L. plants at different growth stages (means ± S.E.). Values followed by the same letter were not significantly different from each other ($p < 0.05$).

elevated steady state contents of free radicals which would in turn result in an increased lipid peroxidation. Peroxidase is involved in secondary phenolic conversions which include degrading flavonoid glycones (Stafford 1990). Peroxidase activity is often increased under stresses (Siegel and Siegel 1986). In our experiments, peroxidase activity increased significantly in eUV-B plants. A similar response was reported in barley plants by Liu and McClure (1995) who also suggested that it reduced the sensitivity of plants to UV-B radiation.

Ascorbic acid content was low in eUV-B plants. This reduction further suggested generation of oxy-radicals due to UV-B exposure. Ascorbic acid acts as an *in vivo* biological antioxidant and functions as a co-substrate of plant peroxidases (Halliwell 1982).

An increase was observed in the contents of phenolic substances in eUV-B plants after an initial reduction at 45 d age, since at this age rice plants only received UV-B exposure for 15 d, which may not be enough to alter the phenolic metabolism. However, at later stages the content of phenolics increased in eUV-B plants. A similar response was observed in primary leaves of barley exposed to eUV-B (Liu and McClure 1995). The increase in phenolics may be connected with a reduction of CO_2 fixation, disintegration of chloroplasts and ATP synthesis, *etc.* (Howell 1974). In our experiments both peroxidase and phenol contents increased in plants due to eUV-B, and thus their metabolism was interrelated.

The eUV-B exposure resulted in significant reduction of biomass accumulation at 60 and 75 d age. Teramura *et al.* (1991) also showed significant reduction in total biomass of one third of 16 rice cultivars grown in glasshouse at eUV-B simulating a 20 % O_3 depletion. He *et al.* (1993), Haung *et al.* (1993), and Dai *et al.* (1995) also showed a similar reduction in UV-B exposed rice plants grown in glasshouses.

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