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CO_2 Enhancement of Growth and Photosynthesis in Rice (*Oryza sativa*)[1]

Modification by Increased Ultraviolet-B Radiation

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ABSTRACT

Two cultivars of rice (*Oryza sativa* L.) IR-36 and Fujiyama-5 were grown at ambient (360 microbars) and elevated CO_2 (660 microbars) from germination through reproduction in unshaded greenhouses at the Duke University Phytotron. Growth at elevated CO_2 resulted in significant decreases in nighttime respiration and increases in photosynthesis, total biomass, and yield for both cultivars. However, in plants exposed to simultaneous increases in CO_2 and ultraviolet-B (UV-B) radiation, CO_2 enhancement effects on respiration, photosynthesis, and biomass were eliminated in IR-36 and significantly reduced in Fujiyama-5. UV-B radiation simulated a 25% depletion in stratospheric ozone at Durham, North Carolina. Analysis of the response of CO_2 uptake to internal CO_2 concentration at light saturation suggested that, for IR-36, the predominant limitation to photosynthesis with increased UV-B radiation was the capacity for regeneration of ribulose bisphosphate (RuBP), whereas for Fujiyama-5 the primary photosynthetic decrease appeared to be related to a decline in apparent carboxylation efficiency. Changes in the RuBP regeneration limitation in IR-36 were consistent with damage to the photochemical efficiency of photosystem II as estimated from the ratio of variable to maximum chlorophyll fluorescence. Little change in RuBP regeneration and photochemistry was evident in cultivar Fujiyama-5, however. The degree of sensitivity of photochemical reactions with increased UV-B radiation appeared to be related to leaf production of UV-B-absorbing compounds. Fujiyama-5 had a higher concentration of these compounds than IR-36 in all environments, and the production of these compounds in Fujiyama-5 was stimulated by UV-B fluence. Results from this study suggest that in rice alterations in growth or photosynthesis as a result of enhanced CO_2 may be eliminated or reduced if UV-B radiation continues to increase.

Increases in atmospheric trace gases including CO_2, CFCs[3], CH_4, and N_2O may lead to substantial changes in the climate of the earth within the next century (33). Two such projected changes include increases in atmospheric CO_2 concentrations and the amount of UV-B radiation striking the earth's surface.

Increases in atmospheric CO_2 have been monitored directly for the last three decades and appear to be primarily associated with the burning of fossil fuels and to a lesser extent deforestation. It is anticipated that the atmospheric partial pressure of CO_2 will reach 600 μbars sometime within the next 30 to 75 years (29). A substantial body of evidence indicates that in C_3 plants (approximately 95% of all known plant species) increases in CO_2 will result in significant increases in growth and photosynthesis in a wide range of cultivated plant species (13).

In addition to CO_2, CFCs, CH_4, and N_2O are also increasing with industrialization. The increase of these trace gases is expected to deplete the stratospheric ozone column with a subsequent increase in the amount of solar UV-B radiation reaching the earth (4, 24). Although UV-B radiation represents only a small proportion of the total electromagnetic spectrum, UV-B radiation has a disproportionally large photobiological effect, primarily due to its absorption by proteins and nucleic acids (9). Given the long atmospheric lifetime of the chlorine species (approximately 100 years) and the continued use of CFCs in manufacturing by many countries, the extent of stratospheric ozone depletion is difficult to predict. Recent measurements from the National Aeronautics and Space Administration, in fact, indicate that stratospheric ozone depletion over temperate latitudes is increasing at twice the predicted rate (1). In contrast to CO_2, increased UV-B radiation has been shown to reduce growth and photosynthesis in a number of cultivated and native plant species (24, 27).

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[3] Abbreviations: CFC, chlorofluorocarbon; CH_4, methane; N_2O, nitrous oxide; UV-B, ultraviolet-B radiation (290–320 nm); UV-B_{BE}, biologically effective UV-B radiation; VPD, vapor pressure defect; A, photosynthetic CO_2 assimilation rate (μmol CO_2 m^{-2} s^{-1}); A_{max}, maximum rate of O_2 evolution at saturated light and CO_2 (μmol O_2 m^{-2} s^{-1}); c_a, the partial pressure of CO_2 in ambient air (μbars); c_i, partial pressure of CO_2 inside the leaf (μbars); ACE, apparent carboxylation efficiency (μmol CO_2 μL c_i^{-1}); F_v, variable component of F_m; F_m, maximum emission of PSII Chl fluorescence (arbitrary units); RuBP, ribulose bisphosphate; SLW, specific leaf weight.

It is unclear, therefore, whether the stimulation in growth and photosynthesis provided by elevated CO_2 will persist despite increased UV-B radiation. The effects of CO_2 and UV-B on changes in plant productivity and photosynthesis have been determined separately for a large number of species, but little work has focused on the interaction of these factors. Teramura *et al.* (25) reported that total biomass and photosynthesis were significantly higher in wheat and soybean with concurrent increases in CO_2 and UV-B radiation. Alternatively, in rice, increases in biomass and photosynthesis at elevated CO_2 were eliminated when supplemental UV-B radiation was applied simultaneously (25). Similarly, UV-B radiation eliminated any potential increase in the growth of pea, tomato, or aster caused by elevated CO_2 (19). In both experiments, however, possible mechanism(s) by which UV-B irradiation may alter the plant response to elevated CO_2 remained unclear.

In the current study, two rice cultivars of contrasting morphologies were subjected to elevated CO_2 and CO_2/UV-B radiation in combination. The objectives of the experiments described here were (a) to further examine changes in vegetative and reproductive parameters with and without supplemental CO_2 and UV-B radiation and (b) to analyze possible changes in the photosynthetic apparatus with increased UV-B radiation, which could explain the lack of response to CO_2 enhancement. Our goal was to achieve a better mechanistic understanding of how UV-B radiation may modify the response to increased CO_2 in rice.

MATERIALS AND METHODS

Seeds of two rice (*Oryza sativa* L.) cultivars, IR-36 and Fujiyama-5, were obtained from the International Rice Research Institute (Los Banos, Philippines). Fujiyama-5 is a japonica-type rice that produces few tillers and obtains heights in excess of 1 m. IR-36, in contrast, is a short japonica-indica hybrid (<60 cm), producing many tillers.

Seeds of each cultivar were planted in 20-L pots in unshaded greenhouses at the Duke University phytotron, Durham, NC, on July 17, 1990. A pot size of 20 L was used to prevent potential feedback limitations to photosynthesis resulting from restricted root growth (28). Plants were grown in a potting medium of pea gravel (≤4 mm diameter), sand, and peat (2:2:1, v:v:v) and were thinned after emergence to two plants per pot. All pots were placed on carts and rotated daily, and the carts were rotated weekly to reduce the effects of greenhouse heterogeneity and shading on plant growth. Rice was watered four times daily and fertilized once daily with half-strength Hoagland solution (6). No symptoms of iron deficiency were observed.

Twenty plants of each cultivar were subjected to the following treatments from germination until ripening (approximately 16 weeks): (a) ambient CO_2, ambient UV-B radiation (control); (b) ambient CO_2, elevated UV-B radiation; (c) elevated CO_2, ambient UV-B radiation; (d) elevated CO_2, elevated UV-B radiation. The elevated UV-B treatment (b) was included to determine the baseline effects of UV-B radiation at ambient CO_2 levels. Five replicates of four pots each were randomly distributed within a treatment (*i.e.* 20 plants per treatment per cultivar). Growing conditions in each of the two unshaded phytotron greenhouses were 31°C day/23°C night temperature, 65% RH and a total daily PPFD (between 400 and 700 nm) of approximately 80 to 85% of ambient (25). Ambient visible irradiance at Durham, NC, on cloudless days typically approaches 1800 to 2000 μmol m^{-2} s^{-1} at midday.

The CO_2 concentrations used in this experiment were maintained at approximately 360 or 660 μbars by using a CO_2 injection system. UV-B radiation was provided by Q-panel UVB-313 sunlamps (Q-panel, Cleveland, OH) suspended above and perpendicular to the plants. Sunlamps were filtered either with presolarized 0.08-mm thick cellulose diacetate (transmission down to 290 nm) or polyester films (no transmission below 320 nm) according to the procedure described by Lydon *et al.* (15). The spectral irradiance at plant height under the lamps was measured with an Optronic Laboratories Inc. (Orlando, FL) model 742 spectroradiometer interfaced with a Hewlett-Packard (Cupertino, CA) model 85 printing calculator. The spectroradiometer was equipped with a dual holographic grating and modified to maintain constant temperature by the addition of Peltier heat exchange units. The spectroradiometer was calibrated against a National Institute of Standards and Technology traceable 1000-W tungsten filament quartz halogen lamp, and wavelength alignment was checked at 253.7, 296.7, 302.2, and 334.1 nm using mercury vapor emission lines from a mercury arc lamp. The absolute spectral irradiance was weighted with the generalized plant response action spectrum (4) and normalized to 300 nm to obtain the daily UV-B_{BE}.

Greenhouse glass does not transmit UV-B radiation; consequently, all UV-B radiation was artificially supplied by lamps. UV-B lamps provided a UV-B fluence that approximated the UV-B radiation received at Durham, NC (35°N), under present stratospheric ozone conditions (control) and that anticipated at Durham with a 25% stratospheric ozone depletion under clear sky conditions during the summer solstice (8.8 and 13.8 effective kJ m^{-2} UV-B_{BE}, respectively, according to the empirical model of Green *et al.* [11] and weighted with the generalized plant action spectrum of ref. 4). The different irradiances were obtained by adjusting the heights of the lamps above the tops of the plants to maintain a fixed distance of 0.50 and 0.35 m for the ambient and elevated UV-B treatments, respectively. Daily UV-B radiation was supplied throughout an 8-h period (0900–1700 h, eastern standard time) during the experiment, and cellulose acetate filters were changed weekly to avoid aging effects on the UV-B spectral transmission through the filters.

All plants were harvested 113 d after planting. Plants were separated into reproductive organs (panicles), vegetative organs (leaves and tillers), and roots, dried at 65°C for 7 to 10 d, and then weighed. A subsample of 20 leaves per cultivar per treatment were placed in moistened plastic bags to prevent leaf rolling, and leaf area was determined using a Li-Cor area meter (model 3100, Lincoln, NE). These leaves were then dried for 48 h at 65°C, and total leaf area per plant and SLW were estimated by the regression of leaf area to leaf dry weight ($r^2 = 0.89$). All data were analyzed by a two-way analysis of variance that tested main effects of UV-B radiation and CO_2 and their interaction (25). Significantly different means were separated using the Student-Newman-Keuls multiple range test at $P \leq 0.05$.

Gas exchange measurements were made after 9 weeks of CO_2 exposure (from September 19 through October 5) on the flag leaf of four replicate plants from each treatment. Rice plants entered the reproductive phase approximately 8 to 10 weeks after germination. The flux of CO_2 was measured in an open system using an LCA 3 system (Analytical Development Corp., Hoddeson, UK) which allows independent control of CO_2 and humidity reaching the leaf. All gas exchange parameters were determined according to the calculations of von Caemmerer and Farquhar (30).

The response of A to c_i was determined by initially measuring A at the growth CO_2 partial pressure (c_a = 360 or 660 μbars) at a PPFD of 1800 μmol m^{-2} s^{-1}. External CO_2 was then reduced to approximately 130 μbars and A was measured at CO_2 partial pressures of 130, 260, 390, 650, 1040, and 1430 μbars. Average leaf temperature during measurement was 33.7°C ± 1.4°C. Natural sunlight was supplemented with a Westinghouse 300-W cool-beam floodlight on cloudy days. The airstream (CO_2 = 360 or 660 μbars) was humidified to a given dew point to maintain a vapor pressure deficit of <2.0 kPa within the leaf chamber. The same IRGA was used to measure dark respiration rates at night (2000–2400 h) in six to eight flag leaves from each cultivar and treatment. Measurements were made at night to obtain a steady-state CO_2 efflux.

To determine whether differences in A associated with increased CO_2 were the result of changes in stomatal conductance, rates of maximum A measured under ambient CO_2 conditions with the IRGA were compared with rates of O_2 evolution obtained at saturating PPFD in an oxygen electrode at a supersaturating CO_2 partial pressure of 50,000 μbars (A_{max}). Because little change was noted in O_2 evolution when discs were compared at 100,000 μbars, it was presumed that the CO_2 inside the leaf was sufficiently high to saturate photosynthesis. Leaf discs from six to eight replicate plants of each cultivar and treatment were sampled for those same leaves used in determining the response of A to c_i. A PPFD of approximately 1800 μmol m^{-2} s^{-1} during the measurement was supplied by a Bjorkman lamp connected to a Hansatech LS-2 light source (Hansatech Ltd., Kingslynn, UK). PPFD values >1800 μmol m^{-2} s^{-1} did not increase rates of O_2 evolution; consequently, 1800 μmol m^{-2} s^{-1} was considered saturating. Temperature of the electrode was maintained at 34°C by circulating water through a water bath. Chl was determined on the leaf discs used in oxygen evolution measurements according to the procedure of Knudsen *et al.* (14).

The maximum photochemical efficiency of PSII is directly proportional to the ratio of F_v/F_m of dark-adapted tissue (8). Induction of fluorescence emission was measured *in situ* using a portable fluorescence meter (Plant Stress Meter; Biomonitor, Charleston, SC) on 10 leaves per cultivar per treatment over a 3-d period during the afternoon (1300–1600 h) 1 week after photosynthetic measurements. The proper amount of dark adaptation and actinic excitation was selected by experimentation. No further increases in the ratio of F_v/F_m were noted when the length of dark adaptation was increased beyond 10 min or if the actinic excitation beam increased from 200 to 400 μmol m^{-2} s^{-1}.

The concentration of methanol-extractable UV-B-absorbing compounds (primarily flavonoids) was determined at the time of harvest for each cultivar and treatment. Additional details of this procedure are given by Mirecki and Teramura (16). The A_{300} was arbitrarily chosen for comparative analysis of the extract.

RESULTS

Changes in Productivity

Vegetative Characteristics

Cultivar IR-36 showed significant declines in both leaf area and leaf weight with elevated UV-B radiation at both CO_2 partial pressures (Table I). For Fujiyama-5, no change in leaf area was noted for increased CO_2 or increased CO_2/UV-B radiation. Leaf weight did increase significantly with high CO_2 or high CO_2/UV-B radiation (Table I). SLW on average

Table I. *Changes in Vegetative Characteristics of Two Rice Cultivars with Supplemental UV-B Radiation, CO_2, or CO_2 and UV-B Radiation in Combination*

Characteristics are measured on a dry weight per plant basis. Different letters for each column and cultivar represent significantly different means separated by the Student-Newman-Keuls multiple range test at $P \leq 0.05$, n = 10.

Cultivar	CO_2	Daily UV-B Fluence	Leaf Area	Leaf Wt	SLW	Stem Wt	Root Wt	Root to Shoot Ratio
	μbar	*kJ* m^{-2}	m^{-2}	*g*	*g* m^{-2}	*g*	*g*	
IR-36	360	8.8	0.750 a	56.0 a	74.7 b	30.1 b	25.6 c	0.30 d
		13.8	0.594 b	45.2 b	76.4 b	29.7 b	25.6 c	0.34 c
	660	8.8	0.714 a	52.5 ab	73.5 b	37.1 a	44.9 a	0.50 a
		13.8	0.379 c	46.8 b	123.4 a	30.9 b	33.3 b	0.43 b
Fujiyama-5	360	8.8	0.602 b	31.2 c	51.8 b	20.8 c	16.3 c	0.32 c
		13.8	0.703 a	30.3 d	43.2 b	17.9 d	16.4 c	0.34 b
	660	8.8	0.580 b	39.2 a	67.5 a	32.8 a	38.5 a	0.53 a
		13.8	0.636 b	36.7 b	57.7 b	26.1 b	21.4 b	0.34 b

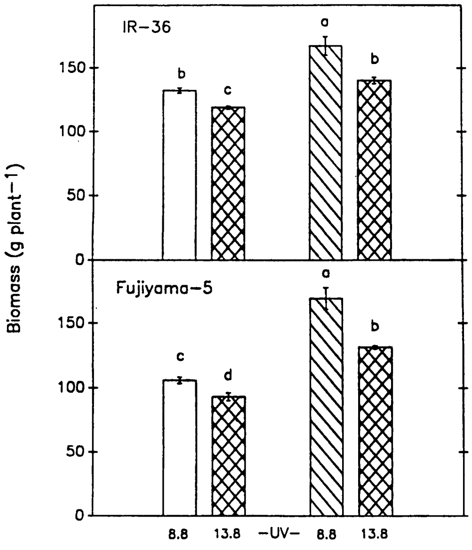

Figure 1. Change in average dry biomass per plant at harvest for rice cv IR-36 and cv Fujiyama-5 grown at CO_2 partial pressures of 360 and 660 μbars and daily UV-B fluences of 8.8 and 13.8 kJ m^{-2}. Different letters represent significantly different means separated by the Student-Newman-Keuls multiple range test at $P \leq 0.05$, $n = 10$. Error bar, ± SE .

tended to be lower for Fujiyama-5 than IR-36, but no consistent pattern with respect to high CO_2 and/or UV-B was noted.

Stem weight increased significantly at elevated CO_2 in both cultivars (Table I). In IR-36, this increase in stem weight with elevated CO_2 was eliminated at the high CO_2/UV-B treatment (Table I). In Fujiyama-5, elevated UV-B radiation decreased stem weight relative to each CO_2 environment, but the stem weight in the elevated CO_2/UV-B environment was still significantly higher than the control. Similarly, the absolute increase in root weight observed in both cultivars with elevated CO_2 was reduced when the effects of UV-B radiation were included but was still significantly higher than the control condition. In both cultivars, root weight showed the largest relative increase in any vegetative characteristic as CO_2 increased. Consequently, root to shoot (shoot = stem + leaf) ratios increased significantly with increased CO_2 (Table I). However, when the effects of increased UV-B radiation are considered at high CO_2, root weight appeared to be more sensitive than shoot weight, with a subsequent decline in the root to shoot ratio relative to the elevated CO_2 condition alone (Table I). In contrast, root to shoot ratios increased with UV-B radiation at ambient CO_2, because shoot weight was affected to a greater degree (Table I). However, in both cultivars, increased UV-B radiation at elevated CO_2 significantly reduced both root and stem weight when compared with the elevated CO_2 at ambient UV-B treatment (Table I).

Changes in plant biomass integrate biotic and/or abiotic factors that influence plant productivity. In this experiment, IR-36 showed a significant increase in plant biomass with a doubling of atmospheric CO_2, but this increase was eliminated if additional UV-B radiation was included (Fig. 1). For Fujiyama-5, CO_2 also resulted in a significant increase in plant biomass. This increase was still significantly greater than the control with simultaneous increases in CO_2 and UV-B radiation, although reduced when compared with the elevated CO_2 condition (Fig. 1).

Reproductive Characteristics

Yield, measured as panicle weight produced per plant, increased significantly in both IR-36 and Fujiyama-5 with enhanced CO_2 (Fig. 2). At elevated CO_2, increased UV-B radiation reduced yield in Fujiyama-5 relative to the high CO_2 condition, although, overall, yield at high CO_2/UV-B radiation was still significantly greater than the ambient controls in both cultivars (Fig. 2).

The response of reproductive characteristics to increased UV-B radiation and/or CO_2 was cultivar specific. For example, the increase in yield with high CO_2 in Fujiyama-5 is due, in part, to an increase in tiller number, whereas tiller number in IR-36 remained unchanged (Table II). UV-B radiation reduced tiller number at elevated CO_2 in IR-36 and eliminated the CO_2-induced increase in tillers for Fujiyama-5. Significant reductions in tiller number were also noted for increased UV-B radiation at ambient CO_2 in both cultivars. Panicle weight and number were increased with high CO_2 in both cultivars. UV-B radiation eliminated any CO_2-induced increase in panicle number but had no effect on panicle weight for either cultivar (Table II). Significant increases in harvest index with elevated CO_2 were noted only for cv IR-36. Increased UV-B radiation at high CO_2 had no effect on harvest index for IR-36 or Fujiyama-5 (Table II).

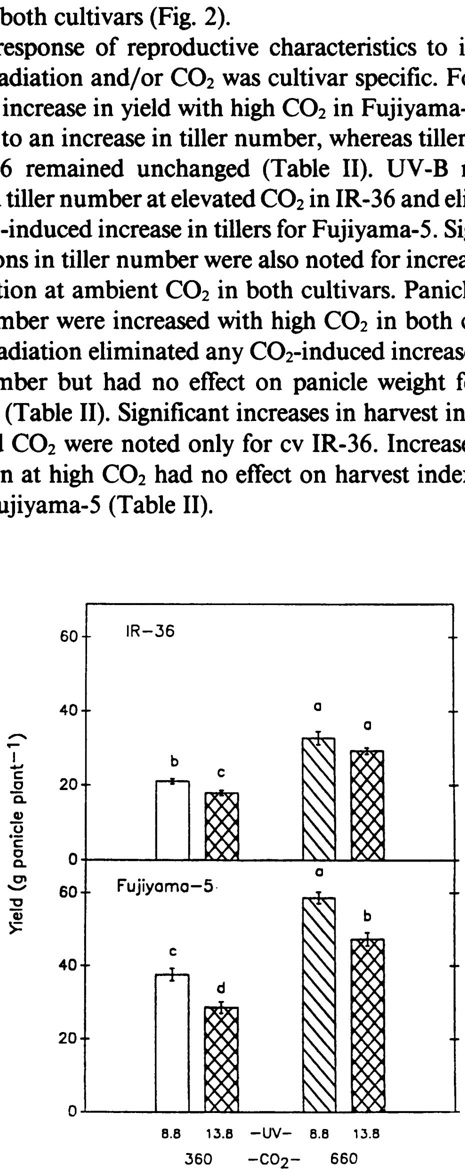

Figure 2. Same as Figure 1 but for yield measured as panicle dry weight per plant at harvest.

Table II. *Changes in Reproductive Characteristics of Two Rice Cultivars with Supplemental UV-B Radiation, CO_2, or CO_2 and UV-B Radiation in Combination*

Harvest index is the ratio of panicle weight to above-ground biomass. Panicle weight is the average panicle weight (total panicle weight/panicle number). Characteristics are measured on a per plant dry weight basis. Different letters for each column and cultivar represent significantly different means separated by the Student-Newman-Keuls multiple range test at $P \leq 0.05$, $n = 10$.

Cultivar	CO_2	Daily UV-B Fluence	No. of Tillers	Panicle Wt	No. of Panicles	No. of Panicles per Tiller	Harvest Index
	μbar	*kJ m^{-2}*		*g*			
IR-36	360	8.8	45.8 a	0.72 b	29.1 b	0.64 b	0.19 b
		13.8	41.5 b	0.68 b	27.1 c	0.66 b	0.20 b
	660	8.8	44.5 a	0.97 a	34.6 a	0.78 a	0.27 a
		13.8	38.8 b	0.95 a	30.8 b	0.79 a	0.27 a
Fujiyama-5	360	8.8	16.7 b	2.18 b	17.5 b	1.05 a	0.42 a
		13.8	14.0 c	1.95 b	14.7 c	1.05 a	0.37 a
	660	8.8	23.0 a	2.70 a	21.7 a	0.95 a	0.45 a
		13.8	17.8 b	2.45 a	19.2 ab	1.09 a	0.43 a

Changes in CO_2 Uptake and Release

Analysis of the Response of A to c_i

Photosynthetic rates increased in both cultivars for single leaves grown and measured at ambient and elevated CO_2 (Fig. 3, arrows). No desensitization to high CO_2 was observed even after 9 weeks of growth in elevated CO_2. This is similar to previous results obtained with these same cultivars (34), but it is in contrast to the rapid desensitization (2-6 weeks) observed by other experimenters for different crop species (5, 21).

The response of A to changing c_i was substantially altered in both cultivars by exposure to elevated CO_2 (Fig. 3). Comparison of A at c_a 360 or 660 μbars with A at infinite stomatal conductance (*i.e.* c_i = 360 or 660 μbars) shows that stomatal limitations to photosynthesis declined at high CO_2 irrespective of UV-B fluence (Table III). No effect of UV-B radiation on stomatal limitation at ambient CO_2 was noted. In Fujiyama-5, increased CO_2 and/or UV-B fluence decreased the initial slope of the A/c_i curve (the ACE), which is dependent on the concentration and/or activity of RuBP carboxylase (7). There was also a significant interaction of UV-B radiation and CO_2 on reducing the ACE in Fujiyama-5 (Table III). However, in Fujiyama-5, prolonged growth at elevated CO_2 increased A_{max}, even at elevated UV-B/CO_2 (Table III). In contrast, IR-36 showed no effect of elevated CO_2 or UV-B radiation on the initial slope of the A/c_i response. As with Fujiyama-5, growth at elevated CO_2 increased A_{max} in IR-36, but UV-B radiation

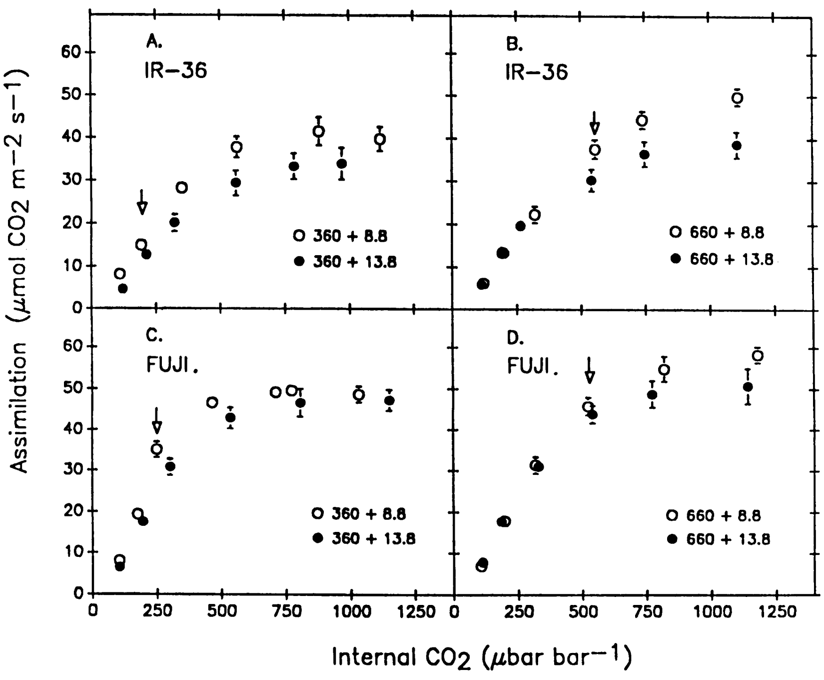

Figure 3. Response of A as a function of c_i for rice cv IR-36 and cv Fujiyama-5 (FUJI.) grown at two different levels of CO_2 (360 and 660 μbars) and daily UV-B fluences (8.8 and 13.8 kJ m^{-2}). Each data point is an average of four values; error bars, ± SE. Arrows are actual c_i at ambient or elevated CO_2 (*i.e.* 360 or 660 μbars) with ambient UV-B radiation.

Table III. *Changes in Photosynthetic Parameters of Two Rice Cultivars with Supplemental UV-B Radiation, CO_2, and CO_2 and UV-B Radiation in Combination*

Stomatal limitation was determined as described by Farquhar *et al.* (7). Different letters for each column and cultivar represent significantly different means separated by the Student-Newman-Keuls multiple range test at $P \leq 0.05$, $n = 10$.

Cultivar	CO_2	Daily UV-B Fluence	Stomatal Limitation	ACE	A_{max}	c_i/c_a
	μbar	*kJ m^{-2}*	%	*μmol CO_2 μL c_i^{-1}*	*μmol O_2 m^{-2} s^{-1}*	
IR-36	360	8.8	29.4 a	0.084 a	44.2 b	0.61 b
		13.8	31.1 a	0.077 a	39.2 c	0.64 b
	660	8.8	19.2 b	0.081 a	49.5 a	0.84 a
		13.8	16.6 b	0.087 a	42.4 b	0.82 a
Fujiyama-5	360	8.8	25.2 a	0.156 a	55.1 b	0.67 b
		13.8	21.6 a	0.121 b	53.1 b	0.78 ab
	660	8.8	12.8 b	0.115 bc	64.5 a	0.80 a
		13.8	13.8 b	0.106 c	61.4 a	0.83 a

eliminated the CO_2-induced increase (Table III) and also reduced A_{max} relative to the ambient control (Table III). Changes in A_{max} observed by oxygen electrode measurements are quantitatively similar to changes observed in the upper portion of the A/c_i curve (Table III, Fig. 3). The ratio of c_i to e_2 increased with CO_2 but not with UV-B radiation alone (Table III).

Dark Respiration

The rate of CO_2 release at night (dark respiration) declined significantly at high CO_2 in both cultivars when compared with the control (Fig. 4). However, in this experiment, no change in dark respiration compared with the control was observed at high CO_2 and UV-B radiation in either cultivar (Fig. 4). No significant change in dark respiration was observed if UV-B fluence increased at ambient CO_2 (Fig. 4).

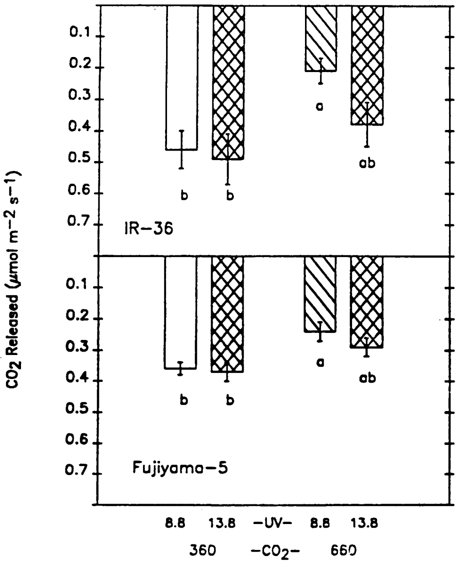

Figure 4. CO_2 released at night (*i.e.* dark respiration) for rice cv IR-36 and cv Fujiyama-5 grown at CO_2 partial pressures of 360 and 660 μbars and daily UV-B fluences of 8.8 and 13.8 kJ m^{-2}. Different letters represent significantly different means separated by the Student-Newman-Keuls multiple range test at $P \leq 0.05$, $n = 10$. Error bar, ± SE.

Chl Fluorescence—F_v/F_m

Environmental stress significantly increases susceptibility to photoinhibition. Photoinhibition is characterized by damage to PSII and a subsequent decline in the fluorescence ratio of F_v/F_m. In this experiment, growth at increased CO_2 and ambient UV-B radiation significantly increased the F_v/F_m ratio in Fujiyama-5, suggesting a decrease in the degree of photoinhibition (Fig. 5). However, this decrease in photoinhibition was eliminated when the effects of elevated UV-B radiation were considered (Fig. 5). For IR-36, no effects of increased CO_2 were observed on the F_v/F_m ratio, although increased UV-B radiation at elevated CO_2 significantly reduced the F_v/F_m ratio when compared with the elevated CO_2 treatment (Fig. 5). No significant changes in total Chl concentration either on a weight or area basis were observed for either cultivar with increasing CO_2 regardless of UV-B level (data not shown).

UV-B-Absorbing Compounds

Production of flavonoid compounds that absorb UV-B radiation has been shown to provide some degree of photoprotection in a variety of plant species (26). In this experiment, growth at high CO_2 increased the concentration of UV-absorbing compounds in IR-36, but exposure to increased UV-B radiation had no effect (Fig. 6). In Fujiyama-5, elevated CO_2 also resulted in significant increases in UV-absorbing compounds, but a significantly greater concentration of these compounds was observed with UV-B radiation, with a significant interaction noted for the elevated UV-B/CO_2 treatment (Fig. 6). Relative to the control, IR-36 and Fujiyama-5 had a 3 and 25% increase in UV-absorbing compounds, respectively, with the high CO_2/UV-B treatment.

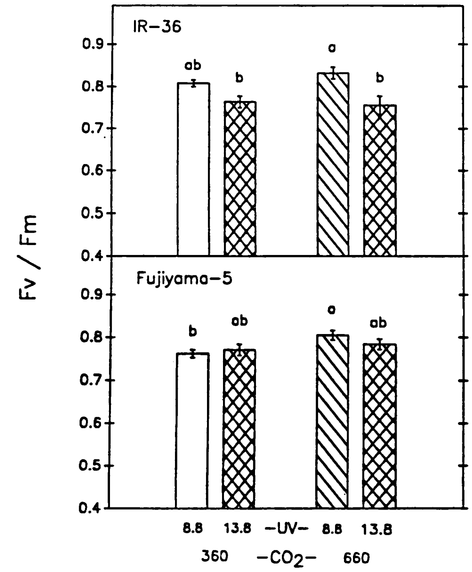

Figure 5. Changes in F_v/F_m ratio (*i.e.* photochemical efficiency) for rice cv IR-36 and cv Fujiyama-5 grown at CO_2 partial pressures of 360 and 660 μbars and daily UV-B fluences of 8.8 and 13.8 kJ m^{-2}. Different letters represent significantly different means separated by the Student-Newman-Keuls multiple range test at $P \leq 0.05$, $n = 10$. Error bar, ± SE .

DISCUSSION

CO_2 Enhancement

Exposure to high CO_2 resulted in significant increases in biomass, yield, and photosynthesis in rice. The observed increases in these parameters are consistent with previous results obtained at high CO_2 for these same rice cultivars (34).

No photosynthetic desensitization to elevated CO_2 was observed for rice in this experiment (*i.e.* plants grown at high CO_2 continued to respond to increased CO_2). Recent evidence regarding photosynthetic inhibition to high CO_2 argues that inhibition could be related to restrictions in pot volume with subsequent restrictions on root growth and sink size (28). Changes in root restrictions suggest that sink strength may influence photosynthetic response to high CO_2. In this study, root biomass showed the largest increase in response to elevated CO_2 for both rice cultivars. Large pots (approximately 20 L in volume) were used to grow plants from germination through harvest to prevent any possible root restrictions. Water, light, and temperature were also optimal, which would presumably promote active growth and sink activity throughout the experiment.

Although desensitization to high CO_2 was not evident, analysis of the response of A to c_i and changes in A_{max} suggests several physiological changes in photosynthetic capacity related to high CO_2. Increases in the upper, saturated portion of the A/c_i response and A_{max} values suggest that the maximum rate of RuBP regeneration is enhanced at the high CO_2 condition in both cultivars (see Caemmerer and Farquhar 30). The maximum rate of RuBP regeneration has been related to the maximum rate of coupled electron transport *in vivo* (7) but may also be restricted by the balance of chloroplast sugar phosphate export and Pi import (23). The A/c_i response, therefore, implies that electron transport capability is increased under a high CO_2 condition in both cultivars. Observed changes in the initial slope of the A/c_i response (*e.g.* ACE) in Fujiyama-5, using the same von Caemmerer-Farquhar analysis, would suggest a decline in either the content and/or specific activity of Rubisco *in vivo*.

It has been suggested (20, 34) that, at high CO_2 in C_3 plants, a reallocation of resources (*e.g.* N) from Rubisco to RuBP/P_i regeneration could occur because lower concentrations of Rubisco would be adequate in a high CO_2 environment. This would be consistent only with changes in the A/c_i response observed for Fujiyama-5 in this experiment. In either cultivar, however, changes in A_{max} and the A/c_i response suggest an enhancement of the photosynthetic process to allow greater utilization of the increased CO_2 resource (20). This enhancement appeared to be accompanied by an overall reduction in stomatal limitation to photosynthesis and an increase in the c_i/c_a ratio at high CO_2.

Increased CO_2 appeared to not only alter photosynthetic capacity but reduce CO_2 efflux as well. Reductions in dark respiration at elevated CO_2 have been observed previously in various crop species, although not in rice (3). The mechanism for possible reductions in dark respiration with high CO_2 is presently unknown. It is possible that the amount of cyanide-insensitive respiration is less at elevated CO_2 (10). Alternatively, Shaish *et al.* (22) cited literature demonstrating inhibition of some respiratory enzymes at high CO_2. Possible physiological mechanisms and consequences of reduced dark respiration at elevated CO_2 and the interaction with UV-B radiation merit further study.

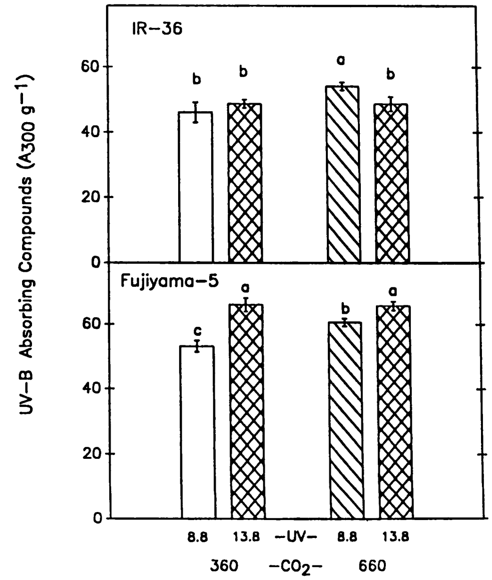

Figure 6. Changes in UV-B-absorbing compounds for rice cv IR-36 and cv Fujiyama-5 grown at CO_2 partial pressures of 360 and 660 μbars and daily UV-B fluences of 8.8 and 13.8 kJ m^{-2}. Different letters represent significantly different means separated by the Student-Newman-Keuls multiple range test at $P \leq 0.05$, $n = 10$. Error bar, ± SE.

UV-B Modification of CO_2 Enhancement

CO_2 enhancement of rice productivity is clearly altered when increased UV-B fluence is simultaneously considered. However, these changes appear to be cultivar specific. The CO_2-enhanced increase in biomass is entirely eliminated in IR-36 but only partially reduced in Fujiyama-5. Results obtained in this experiment are similar to biomass data obtained previously by Teramura *et al.* (25) using IR-36, and they are consistent with the observations of Rozema *et al.* (19) on pea, tomato, and aster. The biomass data suggest that, under a future high CO_2 environment, increased UV-B radiation may result in a greater relative decrease in biomass for rice than at current CO_2 levels. In this experiment biomass decreased 10.7 and 13.1% with elevated UV-B radiation at ambient CO_2 but 16.6 and 22.4% with elevated UV-B radiation at high CO_2 for IR-36 and Fujiyama-5, respectively. In contrast to biomass, yield in this experiment was still significantly higher with elevated CO_2 and UV-B radiation in combination, suggesting that yield may be a more conservative parameter with respect to CO_2/UV-B interaction.

Any modification of CO_2 enhancement effects on productivity by increased UV-B radiation will also affect the plants ability to assimilate and respire CO_2. UV-B radiation-induced decreases in the F_v/F_m ratio suggest that in IR-36, photochemical efficiency of PSII, and the subsequent rate of electron transport may have been reduced (regardless of CO_2 environment). Because electron transport is necessary for RuBP regeneration, increased UV-B radiation may reduce the capacity for RuBP regeneration. This proposed decline in RuBP regeneration would be consistent with the decrease in the upper portion of the A/c_i response and A_{max} values observed in IR-36. Previous experiments have shown that damage to PSII has been associated with increased UV-B exposure in isolated chloroplasts (12, 18) and thylakoids (2) for different plant species.

In contrast to IR-36, Fujiyama-5 showed no significant effect of UV-B radiation on the RuBP regeneration limitation (*e.g.* F_v/F_m was not affected by UV-B radiation) but did show a significant decline in ACE as UV-B radiation increased. Because ACE reflects changes in Rubisco capacity (and ultimately photosynthesis and growth), a decrease in Rubisco kinetics with increasing UV-B radiation is indicated. Previous results with soybean, pea, and tomato have also demonstrated that UV-B radiation can reduce RuBP carboxylase activity (31).

Differences between cultivars with respect to UV-B-induced reductions of CO_2-enhanced effects on photosynthesis may be related to the production of UV-B-absorbing compounds. In the present study, increasing UV-B fluence had no effect on the concentration of UV-B-absorbing compounds (presumably flavonoids) in IR-36. In contrast, Fujiyama-5 had a higher concentration of these compounds overall, and significant increases in these compounds were observed as UV-B radiation increased in both CO_2 environments. In Fujiyama-5, increased production of UV-B-absorbing compounds could partially explain maintenance of photochemical efficiency in PSII as UV-B radiation fluence increased. The ability to maintain photochemical efficiency may, in turn, have allowed sufficient electron transport to produce the necessary amounts of ATP and NADH required to regenerate RuBP. This may explain why this cultivar was able to maintain significant increases in A_{max} and the upper saturated portion of the A/c_i response with concurrent increases in both UV-B radiation and CO_2. However, in Fujiyama-5, the production of UV-B-absorbing compounds was not adequate to prevent UV-B radiation from reducing ACE and the CO_2 enhancement of biomass.

In a number of studies, increased CO_2 has been shown to maintain relative increases in photosynthesis and biomass even if abiotic factors such as water (17) or N (32) were limiting. However, this experiment suggests that depending on the rice cultivar, UV-B radiation may directly damage PSII reaction centers, limit RuBP regeneration, or reduce ACE even in an elevated CO_2 environment. Thus, CO_2 enhancement may be significantly reduced by increased UV-B radiation with a subsequent change in photosynthetic capacity and/or productivity.

CONCLUSIONS

Results from this study indicate that increased UV-B radiation may reduce or eliminate any CO_2 enhancement effects with respect to photosynthesis and productivity in rice. For IR-36, UV-B radiation may directly damage the photosynthetic apparatus by affecting the PSII reaction centers or the ACE to the extent that supplemental additions of CO_2 do not increase A or plant biomass. Although increased UV-B-absorbing compounds in Fujiyama-5 were associated with significant increases in biomass and photosynthesis at a high CO_2/UV-B radiation environment, biomass and photosynthesis were still significantly less than those obtained with high CO_2 alone. Results of this experiment suggest that current projections of increases in photosynthesis and productivity based solely on anticipated increases in atmospheric CO_2 may be overestimated and that interactions with other environmental parameters should be considered. Because rice is a substantial source of calories, UV-B-induced modifications of CO_2 enhancement could significantly affect future food sources. Because UV-B modification of CO_2 enhancement appears to be cultivar specific, additional data over a wide range of cultivars would be necessary to determine how combinations of abiotic factors anticipated with future changes in the global environment may affect rice production.

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