CO₂ Enhancement of Growth and Photosynthesis in Rice (Oryza sativa)¹

Modification by Increased Ultraviolet-B Radiation

Lewis H. Ziska² and Alan H. Teramura*
Department of Botany, University of Maryland, College Park, Maryland 20742

ABSTRACT

Two cultivars of rice (Oryza sativa L.) IR-36 and Fujiyama-5 were grown at ambient (360 microbars) and elevated CO₂ (660 microbars) from germination through reproduction in unshaded greenhouses at the Duke University Phytotron. Growth at elevated CO₂ 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 CO₂ and ultraviolet-B (UV-B) radiation, CO₂ 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 CO₂ uptake to internal CO₂ 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 CO₂ may be eliminated or reduced if UV-B radiation continues to increase.

Increases in atmospheric trace gases including CO₂, CFCs, CH₄, and N₂O may lead to substantial changes in the climate of the earth within the next century (33). Two such projected changes include increases in atmospheric CO₂ concentrations and the amount of UV-B radiation striking the earth's surface. Increases in atmospheric CO₂ 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 CO₂ will reach 600 µbars sometime within the next 30 to 75 years (29). A substantial body of evidence indicates that in C₃ plants (approximately 95% of all known plant species) increases in CO₂ will result in significant increases in growth and photosynthesis in a wide range of cultivated plant species (13).

In addition to CO₂, CFCs, CH₄, and N₂O 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 disproportionately 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 CO₂, 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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² Present address: Climate Stress Laboratory, USDA-Agricultural Research Service, Beltsville Agricultural Research Center, Beltsville, MD 20705.

1 Abbreviations: CFC, chlorofluorocarbon; CH₄, methane; N₂O, nitrous oxide; UV-B, ultraviolet-B radiation (290–320 nm); UV-Bmet, biologically effective UV-B radiation; VPD, vapor pressure defect; A, photosynthetic CO₂ assimilation rate (µmol CO₂ m⁻² s⁻¹); Aₘₙ, maximum rate of O₂ evolution at saturated light and CO₂ (µmol O₂ m⁻² s⁻¹); Pₒ, the partial pressure of CO₂ in ambient air (µbars); cₒ, partial pressure of CO₂ inside the leaf (µbars); ACE, apparent carboxylation efficiency (µmol CO₂ µL C leaf⁻¹); Fᵥ, variable component of Fₘₑₙ; Fₘₑₙ, maximum emission of PSII Chl fluorescence (arbitrary units); RuBP, ribulose bisphosphate; SLW, specific leaf weight.

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It is unclear, therefore, whether the stimulation in growth and photosynthesis provided by elevated CO₂ will persist despite increased UV-B radiation. The effects of CO₂ 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 CO₂ and UV-B radiation. Alternatively, in rice, increases in biomass and photosynthesis at elevated CO₂ 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 CO₂ (19). In both experiments, however, possible mechanism(s) by which UV-B irradiation may alter the plant response to elevated CO₂ remained unclear.

In the current study, two rice cultivars of contrasting morphologies were subjected to elevated CO₂ and CO₂/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 CO₂ 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 CO₂ enhancement. Our goal was to achieve a better mechanistic understanding of how UV-B radiation may modify the response to increased CO₂ 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 CO₂, ambient UV-B radiation (control); (b) ambient CO₂, elevated UV-B radiation; (c) elevated CO₂, ambient UV-B radiation; (d) elevated CO₂, elevated UV-B radiation. The elevated UV-B treatment (b) was included to determine the baseline effects of UV-B radiation at ambient CO₂ 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 cloudy days typically approaches 1800 to 2000 μmol m⁻² s⁻¹ at midday.

The CO₂ concentrations used in this experiment were maintained at approximately 360 or 660 μbars by using a CO₂ 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-Bₚₑₚ.

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-Bₚₑₚ, 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 sub-sample 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 CO₂ and their interaction (25). Significantly different means were
separated using the Student-Newman-Keuls multiple range test at P ≤ 0.05.

Gas exchange measurements were made after 9 weeks of CO₂ 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 CO₂ was measured in an open system using an LCA 3 system (Analytical Development Corp., Hoddesdon, UK) which allows independent control of CO₂ 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 cᵢ was determined by initially measuring A at the growth CO₂ partial pressure (cᵢ = 360 or 660 μbars) at a PPFD of 1800 μmol m⁻² s⁻¹. External CO₂ was then reduced to approximately 130 μbars and A was measured at CO₂ 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 (CO₂ = 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 CO₂ efflux.

To determine whether differences in A associated with increased CO₂ were the result of changes in stomatal conductance, rates of maximum A measured under ambient CO₂ conditions with the IRGA were compared with rates of O₂ evolution obtained at saturating PPFD in an oxygen electrode at a supersaturating CO₂ partial pressure of 50,000 μbars (Aₘₒₓ). Because little change was noted in O₂ evolution when discs were compared at 100,000 μbars, it was presumed that the CO₂ 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 cᵢ. 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 O₂ 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 Fₚ/Fₚₐ 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 Fₚ/Fₚₐ 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 A₃₀₀ 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 CO₂ partial pressures (Table I). For Fujiyama-5, no change in leaf area was noted for increased CO₂ or increased CO₂/UV-B radiation. Leaf weight did increase significantly with high CO₂ or high CO₂/UV-B radiation (Table I). SLW on average

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>CO₂ (μbar)</th>
<th>Daily UV-B Fluence</th>
<th>Leaf Area</th>
<th>Leaf Wt</th>
<th>SLW</th>
<th>Stem Wt</th>
<th>Root Wt</th>
<th>Root to Shoot Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>IR-36</td>
<td>360</td>
<td>8.8</td>
<td>0.750 a</td>
<td>56.0 a</td>
<td>74.7 b</td>
<td>30.1 b</td>
<td>25.6 c</td>
<td>0.30 d</td>
</tr>
<tr>
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<td>660</td>
<td>13.8</td>
<td>0.594 b</td>
<td>45.2 b</td>
<td>76.4 b</td>
<td>29.7 b</td>
<td>25.6 c</td>
<td>0.34 c</td>
</tr>
<tr>
<td></td>
<td>8.8</td>
<td>660</td>
<td>0.714 a</td>
<td>52.5 ab</td>
<td>73.5 b</td>
<td>37.1 a</td>
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<td>660</td>
<td>0.379 c</td>
<td>46.8 b</td>
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<td>30.9 b</td>
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<td>Fujiyama-5</td>
<td>360</td>
<td>8.8</td>
<td>0.602 b</td>
<td>31.2 c</td>
<td>51.8 b</td>
<td>20.8 c</td>
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<td>0.32 c</td>
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<td>13.8</td>
<td>360</td>
<td>0.703 a</td>
<td>30.3 d</td>
<td>43.2 b</td>
<td>17.9 d</td>
<td>16.4 c</td>
<td>0.34 b</td>
</tr>
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<td></td>
<td>660</td>
<td>8.8</td>
<td>0.580 b</td>
<td>39.2 a</td>
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<td>0.53 a</td>
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<tr>
<td></td>
<td>13.8</td>
<td>660</td>
<td>0.636 b</td>
<td>36.7 b</td>
<td>57.7 b</td>
<td>26.1 b</td>
<td>21.4 b</td>
<td>0.34 b</td>
</tr>
</tbody>
</table>
tended to be lower for Fujiyama-5 than IR-36, but no consistent pattern with respect to high CO₂ and/or UV-B was noted.

Stem weight increased significantly at elevated CO₂ in both cultivars (Table I). In IR-36, this increase in stem weight with elevated CO₂ was eliminated at the high CO₂/UV-B treatment (Table I). In Fujiyama-5, elevated UV-B radiation decreased stem weight relative to each CO₂ environment, but the stem weight in the elevated CO₂/UV-B environment was still significantly higher than the control. Similarly, the absolute increase in root weight observed in both cultivars with elevated CO₂ 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 CO₂ increased. Consequently, root to shoot (shoot = stem + leaf) ratios increased significantly with increased CO₂ (Table I). However, when the effects of increased UV-B radiation are considered at high CO₂, root weight appeared to be more sensitive than shoot weight, with a subsequent decline in the root to shoot ratio relative to the elevated CO₂ condition alone (Table I). In contrast, root to shoot ratios increased with UV-B radiation at ambient CO₂, because shoot weight was affected to a greater degree (Table I). However, in both cultivars, increased UV-B radiation at elevated CO₂ significantly reduced both root and stem weight when compared with the elevated CO₂ 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 CO₂, but this increase was eliminated if additional UV-B radiation was included (Fig. 1). For Fujiyama-5, CO₂ also resulted in a significant increase in plant biomass. This increase was still significantly greater than the control with simultaneous increases in CO₂ and UV-B radiation, although reduced when compared with the elevated CO₂ condition (Fig. 1).

Reproductive Characteristics

Yield, measured as panicle weight produced per plant, increased significantly in both IR-36 and Fujiyama-5 with enhanced CO₂ (Fig. 2). At elevated CO₂, increased UV-B radiation reduced yield in Fujiyama-5 relative to the high CO₂ condition, although, overall, yield at high CO₂/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 CO₂ was cultivar specific. For example, the increase in yield with high CO₂ 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 CO₂ in IR-36 and eliminated the CO₂-induced increase in tillers for Fujiyama-5. Significant reductions in tiller number were also noted for increased UV-B radiation at ambient CO₂ in both cultivars. Panicle weight and number were increased with high CO₂ in both cultivars. UV-B radiation eliminated any CO₂-induced increase in panicle number but had no effect on panicle weight for either cultivar (Table II). Significant increases in harvest index with elevated CO₂ were noted only for cv IR-36. Increased UV-B radiation at high CO₂ had no effect on harvest index for IR-36 or Fujiyama-5 (Table II).
Changes in CO₂ Uptake and Release

Analysis of the Response of A to cᵢ

Photosynthetic rates increased in both cultivars for single leaves grown and measured at ambient and elevated CO₂ (Fig. 3, arrows). No desensitization to high CO₂ was observed even after 9 weeks of growth in elevated CO₂. 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 cᵢ was substantially altered in both cultivars by exposure to elevated CO₂ (Fig. 3). Comparison of A at cᵢ 360 or 660 μbars with A at infinite stomatal conductance (i.e. cᵢ = 360 or 660 μbars) shows that stomatal limitations to photosynthesis declined at high CO₂ irrespective of UV-B fluence (Table III). No effect of UV-B radiation on stomatal limitation at ambient CO₂ was noted. In Fujiyama-5, increased CO₂ and/or UV-B fluence decreased the initial slope of the A/cᵢ 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 CO₂ on reducing the ACE in Fujiyama-5 (Table III). However, in Fujiyama-5, prolonged growth at elevated CO₂ increased Aₘₚ, even at elevated UV-B/CO₂ (Table III). In contrast, IR-36 showed no effect of elevated CO₂ or UV-B radiation on the initial slope of the A/cᵢ response. As with Fujiyama-5, growth at elevated CO₂ increased Aₘₚ in IR-36, but UV-B radiation

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**Table II. Changes in Reproductive Characteristics of Two Rice Cultivars with Supplemental UV-B Radiation, CO₂, or CO₂ and UV-B Radiation in Combination**

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>CO₂</th>
<th>Daily UV-B Fluence</th>
<th>No. of Tillers</th>
<th>Panicle Wt</th>
<th>No. of Panicles per Tiller</th>
<th>Harvest Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μbar</td>
<td>kJ m⁻²</td>
<td>g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IR-36</td>
<td>360</td>
<td>8.8</td>
<td>45.8 a</td>
<td>0.72 b</td>
<td>29.1 b</td>
<td>0.64 b</td>
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<td>13.8</td>
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<td>0.68 b</td>
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<td>0.95 a</td>
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<td>0.27 a</td>
</tr>
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<td>Fujiyama-5</td>
<td>360</td>
<td>8.8</td>
<td>16.7 b</td>
<td>2.18 b</td>
<td>17.5 b</td>
<td>1.05 a</td>
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<td>19.2 ab</td>
<td>1.09 a</td>
<td>0.43 a</td>
</tr>
</tbody>
</table>

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**Figure 3.** Response of A as a function of cᵢ for rice cv IR-36 and cv Fujiyama-5 (FUJI) grown at two different levels of CO₂ (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 cᵢ; at ambient or elevated CO₂ (i.e. 360 or 660 μbars) with ambient UV-B radiation.
eliminated the CO₂-induced increase (Table III) and also reduced \( A_{\text{max}} \) relative to the ambient control (Table III). Changes in \( A_{\text{max}} \) observed by oxygen electrode measurements are quantitatively similar to changes observed in the upper portion of the A/\( c_a \) curve (Table III, Fig. 3). The ratio of \( c_a \) to \( c_t \) increased with CO₂ but not with UV-B radiation alone (Table III).

### Dark Respiration

The rate of CO₂ release at night (dark respiration) declined significantly at high CO₂ 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 CO₂ and UV-B radiation in either cultivar (Fig. 4). No significant change in dark respiration was observed if UV-B fluence increased at ambient CO₂ (Fig. 4).

### 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 CO₂ 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 CO₂ were observed on the \( F_v/F_m \) ratio, although increased UV-B radiation at elevated CO₂ significantly reduced the \( F_v/F_m \) ratio when compared with the elevated CO₂ treatment (Fig. 5). No significant changes in total Chl concentration either on a weight or area basis were observed for either cultivar with increasing CO₂ 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 CO₂ 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 CO₂ 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/CO₂ 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 CO₂/UV-B treatment.
Biomass, Enhancement

5. Changes

Student-Newman-Keuls

cv sub

360

5. Changes

Student-Newman-Keuls

Different bar, different letters represent significantly different means separated by the Student-Newman-Keuls multiple range test at P ≤ 0.05, n = 10. Error bar, ± SE.

DISCUSSION

CO₂ Enhancement

Exposure to high CO₂ 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 CO₂ for these same rice cultivars (34).

No photosynthetic desensitization to elevated CO₂ was observed for rice in this experiment (i.e. plants grown at high CO₂ continued to respond to increased CO₂). Recent evidence regarding photosynthetic inhibition to high CO₂ 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 CO₂. In this study, root biomass showed the largest increase in response to elevated CO₂ 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 CO₂ was not evident, analysis of the response of A to cᵢ and changes in Aₘₐₓ suggests several physiological changes in photosynthetic capacity related to high CO₂. Increases in the upper, saturated portion of the A/cᵢ response and Aₘₐₓ values suggest that the maximum rate of RuBP regeneration is enhanced at the high CO₂ 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/cᵢ response, therefore, implies that electron transport capability is increased under a high CO₂ condition in both cultivars. Observed changes in the initial slope of the A/cᵢ 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 CO₂ in C₃ plants, a reallocation of resources (e.g. N) from Rubisco to RuBP/Pₐ regeneration could occur because lower concentrations of Rubisco would be adequate in a high CO₂ environment. This would be consistent only with changes in the A/cᵢ response observed for Fujiyama-5 in this experiment. In either cultivar, however, changes in Aₘₐₓ and the A/cᵢ response suggest an enhancement of the photosynthetic process to allow greater utilization of the increased CO₂ resource (20). This enhancement appeared to be accompanied by an overall reduction in stomatal limitation to photosynthesis and an increase in the cᵢ/cₐ ratio at high CO₂.

Increased CO₂ appeared to not only alter photosynthetic capacity but reduce CO₂ efflux as well. Reductions in dark respiration at elevated CO₂ have been observed previously in various crop species, although not in rice (3). The mechanism for possible reductions in dark respiration with high CO₂ is presently unknown. It is possible that the amount of cyanide-insensitive respiration is less at elevated CO₂ (10). Alternatively, Shaish et al. (22) cited literature demonstrating inhibition of some respiratory enzymes at high CO₂. Possible physiological mechanisms and consequences of reduced dark respiration at elevated CO₂ and the interaction with UV-B radiation merit further study.

Figure 5. Changes in Fv/Fm ratio (i.e. photochemical efficiency) for rice cv IR-36 and cv Fujiyama-5 grown at CO₂ 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 ≤ 0.05, n = 10. Error bar, ± SE.

Figure 6. Changes in UV-B-absorbing compounds for rice cv IR-36 and cv Fujiyama-5 grown at CO₂ 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 ≤ 0.05, n = 10. Error bar, ± SE.
UV-B Modification of CO₂ Enhancement

CO₂ enhancement of rice productivity is clearly altered when increased UV-B fluence is simultaneously considered. However, these changes appear to be cultivar specific. The CO₂-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 CO₂ environment, increased UV-B radiation may result in a greater relative decrease in biomass for rice than at current CO₂ levels. In this experiment biomass decreased 10.7% and 13.1% with elevated UV-B radiation at ambient CO₂ but 16.6% and 22.4% with elevated UV-B radiation at high CO₂ for IR-36 and Fujiyama-5, respectively. In contrast to biomass, yield in this experiment was still significantly higher with elevated CO₂ and UV-B radiation in combination, suggesting that yield may be a more conservative parameter with respect to CO₂/UV-B interaction.

Any modification of CO₂ enhancement effects on productivity by increased UV-B radiation will also affect the plants ability to assimilate and respire CO₂. UV-B radiation-induced decreases in the F₀/Fₚ ratio suggest that in IR-36, photochemical efficiency of PSII, and the subsequent rate of electron transport may have been reduced (regardless of CO₂ 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 response and Aₘₐₓ 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. F₀/Fₚ 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 CO₂-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 CO₂ 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 Aₘₐₓ and the upper saturated portion of the A/c response with concurrent increases in both UV-B radiation and CO₂. However, in Fujiyama-5, the production of UV-B-absorbing compounds was not adequate to prevent UV-B radiation from reducing ACE and the CO₂ enhancement of biomass.

In a number of studies, increased CO₂ 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 CO₂ environment. Thus, CO₂ 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 CO₂ 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 CO₂ 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 CO₂/UV-B radiation environment, biomass and photosynthesis were still significantly less than those obtained with high CO₂ alone. Results of this experiment suggest that current projections of increases in photosynthesis and productivity based solely on anticipated increases in atmospheric CO₂ 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 CO₂ enhancement could significantly affect future food sources. Because UV-B modification of CO₂ 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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