

Short Communication

Subambient growth CO₂ leads to increased Rubisco small subunit gene expression in developing rice leaves

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Received March 28, 2000 - Accepted April 12, 2000

Summary

To test the hypothesis whether low atmospheric [CO₂] can cause an increase of Rubisco small subunit gene expression, rice (*Oryza saliva* L.) growing under ambient [CO₂] at 350 μmol mol⁻¹ was switched to subambient [CO₂] at 175 μmol mol⁻¹ during late vegetative stage. After the switch, photosynthesis rate of developing leaves initially declined but partially recovered after 8 days. Transcript levels of the Rubisco small subunit gene (*rbcS*) in developing leaves increased within three days to almost twice that of ambient-CO₂ controls, followed later by an up-regulation of Rubisco total activity and protein content.

Key words: acclimation - photosynthesis - *rbcS* expression - rice - subambient CO₂

Abbreviations: DAP days after planting - PPFD p photosynthetic photon flux density - Rubisco ribulose-1,5-bisphosphate carboxylase/oxygenase - *rbcS* small subunit gene of Rubisco

Introduction

It is well established that long-term growth under elevated atmospheric [CO₂] often leads to a down-regulation of photosynthesis concomitant with Rubisco activity and protein content in terrestrial C₃ plants (Bowes 1993). However, for thousands of years prior to the industrial age plants experienced much lower [CO₂] than at present. Therefore, species exist-ing today may be adapted to growth at lower [CO₂] than they currently experience. Few studies have addressed the effects

of subambient [CO₂] on photosynthetic processes. High altitude herbaceous plants, which have evolved under atmospheric [CO₂] similar to that of pre-industrial values, often show greater photosynthetic capacity than plants from lower altitudes which are exposed to present ambient [CO₂] (Körner and Diemer 1994). Rice, which acclimates its photosynthesis to elevated [CO₂], shows rapid downward adjustments in the expression *rbcS* (Gesch et al. 1998). It was thus hypothesized that under subambient [CO₂] rice may reverse this trend and exhibit up-regulated *rbcS* expression, Rubisco activity, and protein content to optimize photosynthesis at low [CO₂]. The present study is the first to show that this does indeed occur in developing rice leaves, but not in mature leaves.

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Materials and Methods

Rice (*Oryza saliva* L. cv. Lemont) was seeded on 3 October, 1996 and grown in two sunlit controlled-environment chambers located outdoors in Gainesville, Florida. Both chambers of rice were grown at a daytime atmospheric $[\text{CO}_2]$ of 350 $\mu\text{mol mol}^{-1}$ (ambient CO_2) and day/night air and dew point temperatures were maintained at 28/21°C and 18/14°C, respectively. At 9 DAP a 0.05-m flood was applied and maintained during the experiment. Prior to planting, the soil was fertilized with 8.4 and 13.5 g m^{-2} phosphorous and potassium, respectively. Nitrogen as urea was added at 8, 18, and 25 DAP at a rate of 12.6, 6.3, and 6.3 g M^{-2} , respectively. At 34 DAP, one chamber of rice at ambient CO_2 was switched to 175 $\mu\text{mol mol}^{-1}$ (subambient CO_2). The detailed chamber characteristics and specific methods used for controlling environmental set points are described by Pickering et al. (1994).

Leaf sampling

Leaves were sampled at 1300 eastern standard time on days 1, 3, 5, and 10 of the CO_2 switching experiment when PPFD was $\sim 150 \mu\text{mol m}^{-2} \text{s}^{-1}$ (the switch was made on day 2). Developing leaves from 15 to 20 individual plants were detached, immediately immersed in liquid N_2 , ground to a fine powder and stored in liquid N_2 until analysis. The developing leaf of each plant was number 8 on the main culm and was approximately 25 % expanded when measurements began and was fully expanded by the time the last measurement was taken.

Leaf photosynthesis

The photosynthesis rate of single, attached developing leaves (the uppermost leaf during the switching experiment) was measured with a LI-6200 Portable Photosynthesis System (Li-Cor, Lincoln, NE) equipped with a 0.25-L cuvette. Measurements were made at the treatment growth $[\text{CO}_2]$ between 1130-1230 eastern standard time when PPFD was saturating at 1200-1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Rubisco assays

Rubisco total activity was assayed as previously reported (Vu et al. 1997). Approximately 150 mg of liquid N_2 -frozen leaf powder was extracted at 4°C in 3 mL of medium consisting of 50 mmol L^{-1} CO_2 -free Tricine-NaOH pH 8.0, 10 mmol L^{-1} MgCl_2 , 5 mmol L^{-1} DTT, 10 mmol L^{-1} isoascorbate, 0.1 mmol L^{-1} EDTA, and 2 % (w/v) PVP-40. Total activity was assayed at 30°C in a volume of 0.5 mL. The reaction mixture consisted of 50 mmol L^{-1} CO_2 -free Tricine-NaOH pH 8.0, 10 mmol L^{-1} MgCl_2 , 5 mmol L^{-1} DTT, 0.1 mmol L^{-1} EDTA, and 10 mmol L^{-1} $\text{NaH}^{14}\text{CO}_3$ (2 Gbq mmol^{-1}). A 0.1 mL aliquot of the leaf extract was incubated in the reaction mixture for 5 min to activate Rubisco and the reaction was initiated by the addition of 0.5 mmol L^{-1} ribulose-1,5-bisphosphate. Reactions were stopped after 45 s by adding 0.1 mL of 6N HCl. The assays were dried at 60°C and the acid-stable ^{14}C radioactivity determined by scintillation spectrometry.

Rubisco content was determined by a radioimmuno-precipitation procedure described by Vu et al. (1997). To a 0.2 mL aliquot of the leaf extract obtained from the Rubisco activity assays, NaHCO_3 was added to 10 mmol L^{-1} and the mixture allowed to incubate on ice for 20 min to activate Rubisco. A 0.025 mL aliquot of this mixture

was added to 0.05 mL of buffer (100 mmol L^{-1} Sicine pH 7.8, 20 mmol L^{-1} MgCl_2 , 1 mmol L^{-1} EDTA) containing 4 nmol $[\text{C}^{14}]$ carboxyarabinitol bisphosphate and 0.05 mL of antiserum to purified tobacco Rubisco raised from rabbits. After incubation at 37°C for 2h, the precipitate was collected on 0.45 μm pore size Millipore cellulose acetate/nitrate filters and washed with 5 mL of a solution containing 145 mmol L^{-1} NaCl and 10 mmol L^{-1} MgCl_2 . The amount of bound ^{14}C was determined by liquid scintillation spectrometry.

Rubisco transcript analysis

Total RNA was isolated from 0.5 g of liquid N_2 -frozen leaf powder using a single step method (Puissant and Houdebine 1990). Separation of total RNA (10 @ μg) on denaturing agarose-formaldehyde gels, Northern blotting and detection of *rbcS* mRNA were performed as previously described (Gesch et al. 1998). Blots were stripped and re-probed with a digoxigenin-labeled 18S rRNA probe (Nairn and Ferl 1988). The digoxigenin label was detected by chemiluminescence (CSPDO, Boehringer Mannheim Biochemicals) by exposing membranes to X-ray film at room temperature. Signal strengths were quantified by image-density scanning (IS-1000, Alpha Innotech Corp., San Leandro, CA) and normalized with respect to the amount of 18S rRNA in each lane. Northern analysis was performed twice.

Statistical methods

A repeated measures analysis of variance was used to model Rubisco and photosynthesis data according to a split-plot in time approach using the SAS Proc Mix Repeated Measures Analysis program to obtain the estimates for the model (SAS Institute Inc., Gary, NC). The factors used for the model were $[\text{CO}_2]$ treatment, day of experiment, and $[\text{CO}_2]$ treatment x day of experiment.

Results and Discussion

Developing leaves of rice plants showed photosynthetic acclimation when switched from ambient to subambient growth $[\text{CO}_2]$. One day after making the CO_2 -switch, the photosynthesis of developing leaves measured at 175 $\mu\text{mol mol}^{-1}$ was 45 % less than that of ambient 350 $\mu\text{mol mol}^{-1}$ controls measured at 350 $\mu\text{mol mol}^{-1}$ (Table 1). By day 5 it was 52 % lower. However, despite the initial decline in photosynthesis due to lower $[\text{CO}_2]$, between days 5 and 10 it increased by 35 %. Although ambient control plants also increased between days 5 and 10, the increase (7%) was much less than that of the plants switched to subambient CO_2 . However, with respect to low growth $[\text{CO}_2]$ there appears to be some variation in the photosynthetic response of C_3 plants. For instance, Tissue et al. (1995) found that *Abutilon theophrasti* Medic. showed a decreased capacity for CO_2 assimilation when grown at 150 $\mu\text{mol mol}^{-1}$ atmospheric $[\text{CO}_2]$.

The amount of *rbcS* mRNA in developing leaves of rice increased within one day after switching to subambient CO_2 (Fig. 1). Transcript levels of *rbcS* for the ambient CO_2 controls decreased with leaf age. However, amounts of *rbcS* mRNA

Table 1. Leaf photosynthetic rates for developing leaves of rice grown at ambient (350 $\mu\text{mol mol}^{-1}$) [CO₂] and switched to subambient (175 $\mu\text{mol mol}^{-1}$) [CO₂] for up to eight days. The switch was made at day 2 of the experiment. Measurements were made at the treatment [CO₂]. Values are the mean \pm SE, n = 3.

Treatment [CO ₂] ($\mu\text{mol mol}^{-1}$)	Leaf Photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$)			
	Day 1	Day 3	Day 5	Day 10
350	18.7 \pm 0.9		23.5 \pm 0.5	25.2 \pm 0.7
350 to 175		22.9 \pm 0.6	12.5 \pm 1.1*	15.3 \pm 0.7* ¹
			11.3 \pm 0.7*	

* The difference between the two treatments on the day measured was significantly different at the P<0.001 level.

¹ The difference between day 5 and 10 for the 350 to 175 treatments was significant at the P < 0.01 level, but was not significant at the P < 0.1 level for the 350 treatment.

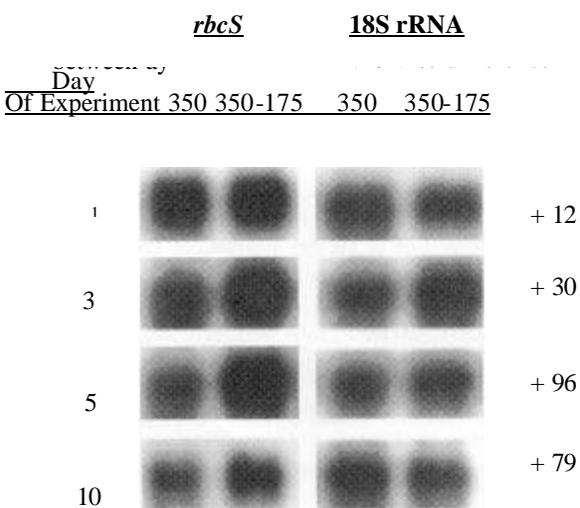


Figure 1. Northern-blot analysis of *rbcS* transcript abundance for developing leaves of rice before and after switching [CO₂]. The CO₂ - switch was made on day 2 of the experiment. Signal strengths of *rbcS* were normalized with respect to the amount of 18S rRNA. The Northern analysis was repeated a second time with similar results.

did not decline as much in plants switched to subambient CO₂, and remained considerably greater than in the ambient- CO₂ plants up to day 10. Using chamber-grown pea (*Pisum sativum* L.) exposed continuously to 160 $\mu\text{mol mol}^{-1}$ CO₂, Majeau and Coleman (1996) showed that mature leaves exhibited lower *rbcS* transcript amounts than plants grown at ambient [CO₂]. However, acclimation to [CO₂] is likely to be species-specific and dependent on leaf developmental stage. The expression of *rbcS* in leaves of wheat grown in free air carbon dioxide enrichment experiments under ambient or elevated [CO₂] differs with respect to leaf and plant development stage (Nie et al. 1995). This appears to be true for leaves of plants grown under subambient [CO₂]. In the present

study *rbcS* amounts in fully-developed leaves following the CO₂-switch did not differ greatly from those of the ambient controls (data not shown).

As with *rbcS* expression, both Rubisco total activity and the enzyme protein content increased in developing leaves of rice, but this occurred several days after the plants were switched from ambient to subambient CO₂ (Fig. 2). At day 10, Rubisco protein content was significantly (P<0.01) greater in developing leaves of the subambient- CO₂ plants than in those maintained at ambient CO₂. Although Rubisco total activity and protein content increased as the leaves developed, the increase was much greater for plants switched to subambient CO₂. Thus, between days 3 and 10, activity and protein content increased 24 and 19 % (significant at P < 0.001), respectively, in plants switched to subambient CO₂, but only 9 and 2% (not significant at P<0.1), respectively, for the ambient controls. The larger increase of Rubisco in the subambient-switched rice may explain why it had a significantly

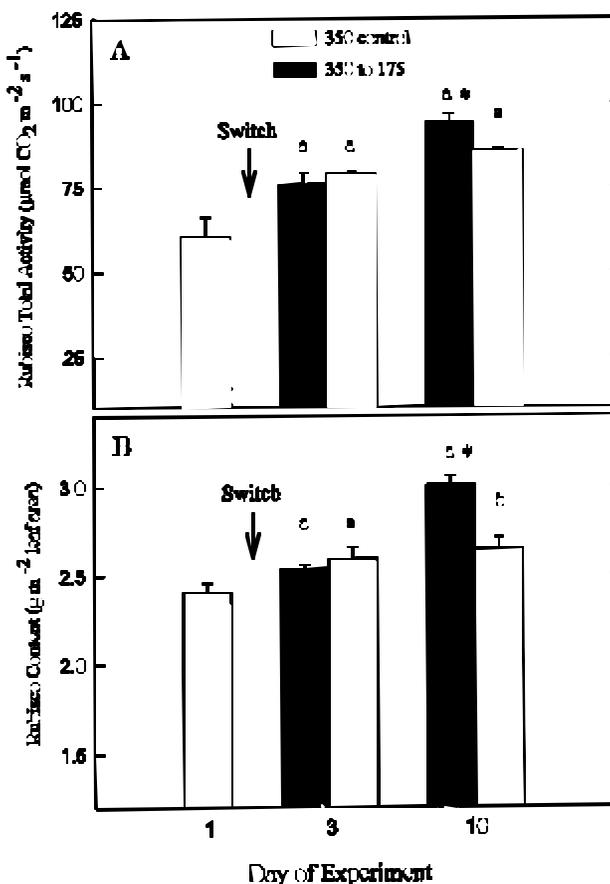


Figure 2. Rubisco total activity (A) and protein content (B) for developing leaves of rice. The CO₂-switch was made on day 2 of the experiment. Values are the means \pm SE, n = 6. Values followed by a different letter are significantly different at the P<0.01 level, * the difference between day 3 and 10 for the 350 to 175 $\mu\text{mol mol}^{-1}$ treatment was significantly different at the P<0.001 level.

greater increase in leaf photosynthesis between days 5 and 10 (Table 1) than the ambient CO₂ controls. However, this finding may not be applicable to all C₃ plants, as there are reports of either no change (Campbell et al. 1988) or even decreased amounts of Rubisco and activity (Tissue et al. 1995) when grown under atmospheric subambient [CO₂].

In the present study, the degree of difference between ambient and subambient rice leaves in the expression of *rbcS* was greater than the difference in Rubisco activity or protein content. This lack of direct correlation between *rbcS* transcript abundance and Rubisco protein content has also been observed with plants grown at elevated [CO₂] (Moore et al. 1998). It thus appears that in addition to influencing *rbcS* transcription, growth [CO₂] may affect translation and/or post-translational modification. In this regard, these other processes which also affect protein levels have yet to be fully investigated.

Our results indicate that developing leaves of rice acclimate to subambient [CO₂] by increasing their photosynthetic capacity. This response is, at least in part, due to enhanced *rbcS* expression which leads to the up-regulation in Rubisco in plants switched to subambient CO₂. The increased expression of Rubisco to low [CO₂] in rice, may have evolved as a mechanism to optimize photosynthesis and carbon balance to compete with other plants. Also, acclimation to low [CO₂] may have adaptive significance in terms of environmental stress. Cowlings and Sage (1998) showed that heat stress in *Phaseolus vulgaris* L. was aggravated by growth under subambient CO₂. Given that modern plants have been subjected to low atmospheric [CO₂] over the past several thousands of years (Barnola et al. 1987), selection pressure may have been great for increased photosynthetic capacity to enhance fitness to environmental stress.

Acknowledgements. We thank Joan Anderson for her skillful technical assistance. We would like to acknowledge the support provided by the USDA/NRICG Plant Responses to the Environment Program (Grant No. 95-37100-1597). This work is a contribution of the Agricultural Research Service, US Department of Agriculture and the Institute of Food and Agricultural Sciences, University of Florida (Florida Agricultural Experiment Station Journal Series No. R-06972).

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