Influence of Rising Atmospheric CO_2 and Phosphorus Nutrition on the Grain Yield and Quality of Rice (*Oryza sativa* cv. Jarrah)

S. SENEWEERA,[1] A. BLAKENEY,[2] P. MILHAM,[3] A. S. BASRA,[1] E. W. R. BARLOW,[1] and J. CONROY[1]

ABSTRACT

Raising the atmospheric CO_2 concentration from 350 µl of CO_2 per liter to a level expected by the end of the next century (700 µl/L) influenced both the grain yield and quality of the short-duration rice (*Oryza sativa*) cultivar, Jarrah. Yield was enhanced by up to 58%, primarily due to an increase in grain number, although grain size was also greater at high CO_2. Varying the supply of phosphorus influenced the magnitude of the CO_2 response with greatest responses occurring at medium rather than luxury or low phosphorus supplies. However, yield enhancement by high CO_2 was observed even when phosphorus supply was severely growth limiting. Chemical (amylose and nutrient concentration) and physical (relative paste viscosity) measurements made on the ground grain indicated that cooked rice grain from plants grown under high levels of CO_2 would be firmer. The nutritive value of grain was also changed at high CO_2 due to a reduction in grain nitrogen and, therefore, protein concentration. However, total nitrogen content per grain was unaffected by high CO_2. In contrast, phosphorus content per grain was greater at high CO_2 and there was a strong correlation between magnesium and phosphorus concentrations. These results indicate that there is a need to plan for the inevitable rise in atmospheric CO_2 concentrations by selecting genotypes that will maintain suitable quality characteristics under global change.

Cereal Chem. 73(2):239-243

Direct measurements of CO_2 concentrations in the atmosphere show clearly that the concentration has risen from 315 µl of CO_2 per liter in 1958 to 360 µl/L in 1995 and that the current rate of increase is 1.9 µl/yr (Houghton et al 1994). Given the reluctance of industrialized countries to reduce emissions from the burning of fossil fuels, it is inevitable that the CO_2 concentration will reach between 510 and 760 µl/L during the 21st century (Houghton et al 1994). While there is no uncertainty that the CO_2 level will rise, there is still debate about the climatic changes that will accompany the increase in CO_2 and other greenhouse gases. Consequently, for agriculturalists, the first step toward planning for inevitable global change is to understand how elevated CO_2 concentrations influence crop productivity and quality. Given the importance of rice (*Oryza sativa* L.) as a source of carbohydrates, protein, and mineral nutrients in human diet, this article focuses on changes in grain yield and quality of rice at high CO_2.

There is general agreement in the literature that raising the atmospheric CO_2 concentration from 350 to 700 µl/L increases grain yield of rice cultivars Niponebare and IR 30 by up to 39% (Imai et al 1985, Baker et al 1990). However, three questions remain unanswered: 1) whether all rice cultivars respond similarly to CO_2 enrichment; 2) if low soil phosphorus (P) availability moderates the response to high CO_2; and 3) whether grain quality is affected by elevated CO_2.

The CO_2 enrichment studies to date have focused on the response of rice cultivars selected for growth in Asia (Imai et al 1985, Baker et al 1990) but little is known of the response of those commonly grown in Australia. In 1994, New South Wales Agriculture released a new cultivar, Jarrah, which had been selected to suit the short growing season in South Eastern Australia (Reinke 1993). In these areas, low soil P availability has been shown to limit grain yield (Batten et al 1992). In a previous article, we investigated the influence of doubling the current CO_2 concentration on growth, development, and grain yield of cv. Jarrah grown at different levels of P supply (Seneweera et al 1994). Development was accelerated throughout the entire vegetative phase, and flowering commenced seven days earlier at high CO_2. There was a 50% increase in tiller number, and this contributed to the higher grain yield. However, neither the components of grain yield nor its quality were investigated.

The few CO_2 enrichment studies investigating changes in grain quality have concentrated on wheat rather than rice. In these studies, elevated CO_2 was shown to reduce the nitrogen (N) concentration of flour produced from wheat grain therefore influencing its quality (Conroy et al 1994). Williams et al (1994) reported that CO_2 enrichment also altered wheat grain quality by changing the lipid composition of wheat grain. Unlike wheat, rice is generally consumed as cooked whole grain; therefore, the properties of the grain itself, rather than the flour, determine quality. Consequently, the major determinants of rice quality are appearance, milling, and cooking quality (Blakeney 1992). Mineral nutritional properties are also important (Nanda and Coffman 1978).

This article reports on the effect of doubling the current CO_2 concentration on grain yield (number and weight per plant) and quality (average grain weight, amylose concentration of ground endosperm, relative paste viscosity, and mineral nutrient concentration of ground brown grain) of the short-duration, Australian rice cultivar, Jarrah. The influence of different P supplies on the CO_2 response is also reported.

MATERIALS AND METHODS

Plant Culture

Rice was grown in growth chambers under flooded conditions at either 350 or 700 µl of CO_2/L as described in Seneweera et al (1994). Briefly, P plus basal nutrients were mixed with soil collected from Mount Tomah, NSW, Australia. The soil had a low level of available P (2 mg/kg), and P (as $CaHPO_4 \cdot 2H_2O$) was added at the following rates: 0, 30, 60, 120, 240, and 480 (mg/kg of soil). There were 10 pots for each P addition rate. There was 6 kg of soil per pot (10 L) and the soil plus P and basal nutrients were mixed in separate batches for each pot. Water was then added to each pot to flood the soil. N was added as urea at the beginning of the experiment (0.26 g of N/kg of soil) and at two-week intervals (0.6 g of N/kg of soil) until the panicle initiation stage of growth was reached.

[1]School of Horticulture, University of Western Sydney, Hawkesbury, Richmond, NSW 2753, Australia.
[2]Yanco Agricultural Institute, Yanco, NSW 2703, Australia.
[3]NSW Agriculture, PMB 10, Rydalmere, NSW 2116, Australia.

Publication no. C-1996-0223-07R.

Three germinated rice seeds were sown in each pot. Five pots at each P level were then placed in one growth chamber maintained at 350 µl of CO_2/L and the other five at 700 µl/L. The temperature in the chambers was 28°C during the 12-hr light period and 21°C during the dark period. The photon flux density was 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The pots were randomly arranged in each chamber and the CO_2 treatments and the pots shifted between chambers every two weeks.

Two of the plants were removed from each pot at seven days after planting (DAP), and the remaining plant was left to grow on to maturity. The grain was harvested at 139 and 146 DAP for the 700 and 350 µl of CO_2/L, respectively. The differences between the harvest dates ensured that the grain was fully matured at both CO_2 levels. The grain was dried at room temperature, and total grain weight and number of grains per pot were then measured. Average grain weight was estimated by dividing the weight by the number of grains.

Amylose Concentration of Ground Endosperm

The grain was dehulled to produce brown rice using a Satake THU35A husker (Satake Corporation, Hiroshima, Japan). Some of the brown rice was polished to white rice using an abrasive brush mill to remove bran and lipids, which are known to interfere with amylose analysis (Juliano et al 1985). A portion of the polished white rice (2 g) was then ground to a fine powder using a ball mill.

Amylose concentration was determined using a slight modification of ISO method 6647 (Welsh and Blakeney 1992). A subsample (0.5 g) of the flour from each replicate in every treatment was placed in a Buchner funnel, defatted at room temperature using 95% (v/v) ethanol, and left in the funnel overnight to dry. The defatted flour (100 mg) was transferred to a 100-ml wide-necked volumetric flask and wetted with 1 ml of 95% (v/v) ethanol after which 9 ml of 1*M* aqueous NaOH was added to the flask. The mixture was then heated for 5 min in a sand bath held at 80°C. This removed the ethanol and gelatinized the samples with minimal decomposition by the alkali. After cooling, the volume was made up to 100 ml with distilled water. A 1-ml aliquot from each flask was transferred to tubes containing 2 ml of 0.1*M* citric acid, 1 ml of iodine/KI solution, and 16 ml of distilled water. After 20 min, the absorbance of the solution was measured at 620 nm against a reagent blank and compared with amylose standards prepared in a similar manner to the samples. Standard potato amylose was purchased from ICN Pharmaceuticals (Costa Mesa, CA).

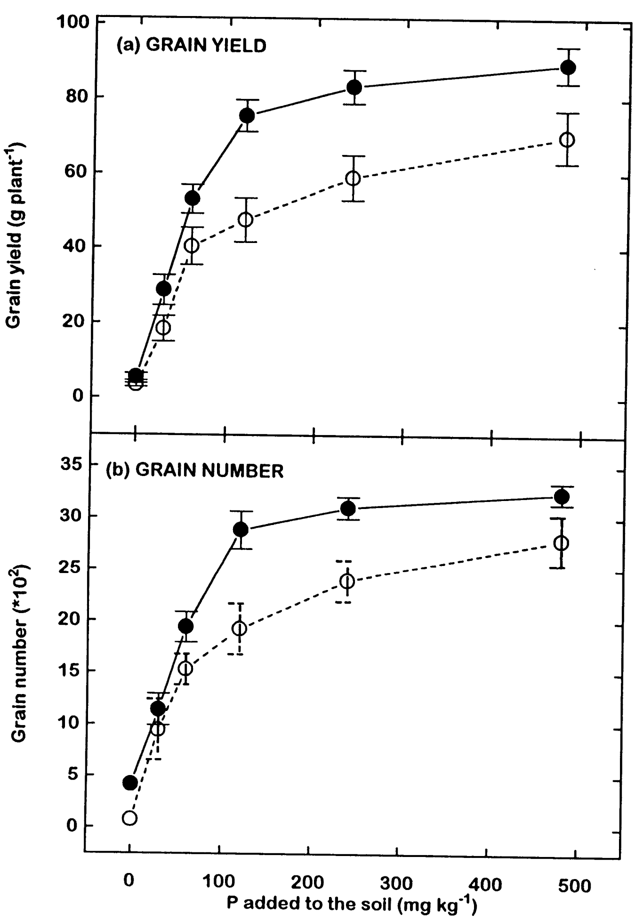

Fig. 1. Influence of elevated CO_2 and P nutrition on total grain weight (**a**) and grain number (**b**) per plant. Plants were grown in flooded soil at either 350 (○) or 700 (●) µl of CO_2/L for 146 and 139 days, respectively. Each point is the mean of five replicates. Error bars represent standard error of the treatment.

Relative Paste Viscosity and Grain Mineral Analysis of Ground Brown Rice

Amylose concentration, only, was measured on the ground endosperm because of the small sample size and the losses associated with polishing the rice. The remaining analyses were carried out on ground brown rice. The brown dehulled rice remaining after sampling for amylose concentration was ground to pass a 0.5-mm sieve in a Cyclotec 1093 mill (Tecator, Sweden). For the physical measurements, a subsample (2.5 g) of flour from each replicate in the 60 and 480 mg/kg of soil P treatments at both CO_2 concentrations was placed in a cup containing 25 ml of distilled water, and the viscosity was measured using a Newport rapid visco analyzer (RVA) (Newport Scientific, Warriewood, NSW, Australia). The data from the RVA was processed using the Thermocline and Thermoview software provided by the manufacturer.

For chemical analysis, samples from every replicate at each P and CO_2 level were digested in H_2SO_4/H_2O_2 and concentrations of mineral elements other than N was measured using an inductively coupled plasma spectrometer. After combustion, the N concentration was determined as N_2, using thermal conductivity. Total N and P content was calculated by multiplying average grain weight by the N and P concentrations of the grain.

Statistical Analysis

Treatment effects were assessed by analysis of variance. Least significant differences and standard errors were calculated (SAS User Guide 1988). Graphs were fitted by joining adjacent data points.

RESULTS AND DISCUSSION

Increasing the current atmospheric CO_2 concentration from 350 to 700 µl/L increased the total grain weight per plant of the short duration rice cultivar, Jarrah, by up to 58% (Fig. 1a). Higher P addition rates also enhanced grain yield (Fig. 1a). The greatest response to elevated CO_2 occurred at a P supply of 120 mg/kg of soil, with the responsiveness to high CO_2 being reduced at luxury P supplies. Nevertheless, CO_2 enrichment enhanced grain yield even at low P supplies, which reduced total yield by more than 100% (Fig. 1). In this respect, rice differs from other species that do not show increased productivity at high CO_2, possibly because P can be recycled more efficiently within the rice plant (Conroy et al 1992, Seneweera et al 1994).

The enhancement of grain yield by CO_2 enrichment was primarily due to an increase in grain number, although changes in grain weight also contributed (Figs. 1 and 2). Close investigation of the separate contributions of grain number and average grain weight to the CO_2 response (Figs. 1b and 2) indicated that average grain weight was more responsive to high CO_2 (24%) at medium P supplies (60 and 120 mg/kg of soil) than at the highest P supplies (480 mg/kg) (6%). There is a strong correlation between the number of cells in the endosperm of wheat and starch concentration of the grain, there being very little change in starch deposition per cell (Jenner et al 1991). Consequently, the influence of CO_2 and P supply on grain weight is likely to have resulted from changes in the number of cells in the endosperm, which, in turn, is influenced by the duration and rate of cell division in the endosperm during grain development and/or by the rate of grain filling during the ripening phase (Wardlaw 1990). We previously showed that development was accelerated by CO_2 enrichment during the vegetative phases of development so that leaf number was reduced at high CO_2 (Seneweera et al 1994). It is therefore possible that the reproductive and/or ripening phases were accelerated to such an extent by high CO_2 at the highest P supply that the duration of cell division and, therefore, cell number were reduced.

Elevated atmospheric CO_2 concentrations and P supply also affected the physical and chemical properties of the grain which influence its quality. The physical qualities affecting quality include appearance and milling quality. Appearance is influenced by grain size and shape, which is genetically determined and by uniformity of appearance. The latter, though genetically influenced, may also result from incomplete filling of the grain, which leads to some grain having a chalky appearance. Milling quality is determined by the percentage of whole grains remaining after milling. While all the factors determining milling quality have not been positively identified, Mohapatra et al (1993) suggest that incomplete filling of all the grain on each panicle leads to poor milling quality and chalky appearance. Earlier studies with the cv. Jarrah indicated that there was a greater percentage of filled grains per panicle when plants were exposed to high CO_2 at medium P supplies (Seneweera 1995). Starch, which is accumulated in the sheaths, is a major contributor to grain filling (Yoshida 1972). Both elevated CO_2 concentrations and high P supplies increased starch accumulation in the sheaths (Seneweera et al 1994), and this probably accounted for the greater capacity for grain filling at high CO_2.

The chemical properties of the rice grain influence both its cooking quality and nutritive value. When rice is harvested, it consists of approximately 20% hull (primarily lemma and palea) and 80% brown rice (embryo, endosperm, pericarp and aleurone layer) (Juliano 1992). Rice is sometimes consumed as brown rice after dehulling but is usually further polished to remove a greater amount of the outer layers of the grain (Barber and de Barber 1976). The latter results in grain consisting primarily of thinned-walled cells containing compound starch granules, which make up about 90% of the dry weight of the grain (Juliano 1992). The chemical composition of the starch in these thin walled cells has a major impact on the cooking quality of the grain. In particular, greater concentrations of amylose relative to amylopectin increases the firmness of the cooked grain (Blakeney 1992, Juliano 1992, Blakeney et al 1994).

Increases in the atmospheric CO_2 concentration are likely to increase the firmness of cooked grain because of increases in the amylose concentration of the grain (Fig. 3a). However, unlike the response of average grain weight to high CO_2 (Fig. 2), the biggest change in amylose concentration due to CO_2 enrichment occurred at the highest rate of P supply (480 mg/kg). Interestingly, there was also a greater Ca concentration at elevated CO_2, particularly at the highest P addition rate (Fig. 3b). Whether higher Ca concentration was involved in regulating the activity of enzymes involved in amylose synthesis is not known. It has been demonstrated that the amylose content in rice endosperm is related to the post-transcriptional regulation of the waxy (*Wx*) gene; rice cultivars with higher amylose content produce large amount of *Wx* mRNA and *Wx* protein (Wang et al 1995). Therefore, an understanding of the waxy gene regulation in response to CO_2 enrichment could be important in predicting how cooking quality may change under future CO_2 scenarios.

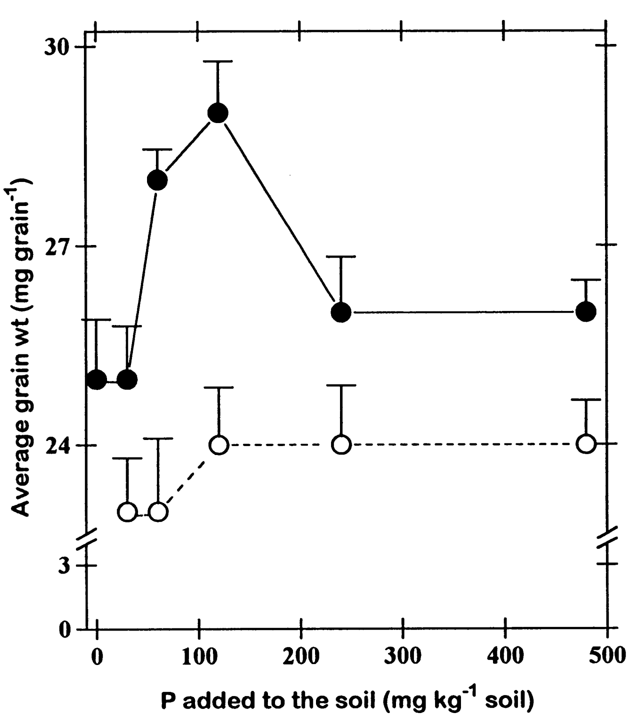

Fig. 2. Average grain weight of plants grown at either 350 (○) or 700 (●) µl of CO_2/L for 146 and 139 days, respectively. Each point is the mean of five replicates.

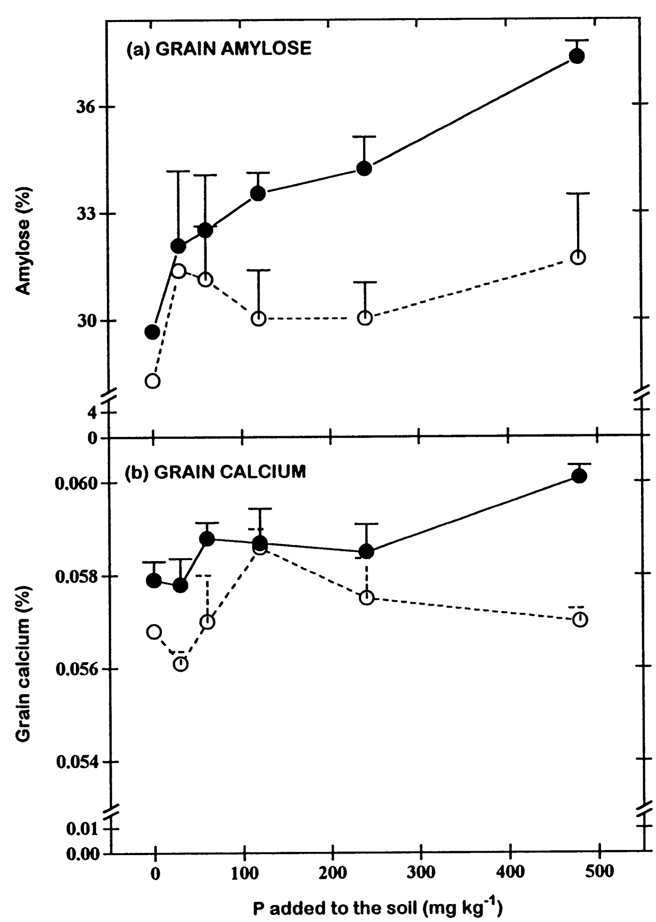

Fig. 3. Amylose concentration of ground endosperm **(a)** and Ca concentration of ground brown grain **(b).** Plants were grown at either 350 (○) or 700 (●) µl of CO_2/L for 146 and 139 days, respectively. Each point is the mean of five replicates. Error bars represent LSD at $P \leq 0.05$.

The concentration of amylose was measured in ground endosperm (polished rice) and expressed on a dry-weight basis (Fig. 3a). Amylose concentrations are generally expressed as a percentage of grain weight at 11% moisture (Juliano 1992). Consequently, the amylose concentrations reported here (Fig. 3) are higher than previously published results for this cultivar (Reinke 1993). However, correction of the results to an 11% moisture content show that the amylose concentration was 27% in ambient CO_2-grown plants, which is close to the published value of 24% (Reinke 1993).

Measurements of relative paste viscosity (Fig. 4) supported the idea that cooked grain from high-CO_2-grown plants would be firmer. The set-back value, which is calculated from the differences between the peak heights at 12 and 6 min, were 30 and 65 for the ambient and high CO_2 treatments, respectively (Fig. 4). Higher set-back values are correlated with firmer cooked grain. Due to small sample sizes, the relative paste viscosity was measured using ground brown rice, which contains not only the starchy endosperm but also the pericarp, aleurone layer, and embryo. Viscosity measurements are generally made on ground endosperm, and, hence, the peak values reported here for cv. Jarrah are lower than those reported by Blakeney (1992). Nevertheless, both the samples from the high and ambient CO_2 treatments would have been similarly affected by the presence of tissue other than endosperm. Consequently, the increase in the set-back value indicates that there will be an increase in the firmness of cooked grain from plants grown at elevated atmospheric CO_2 concentrations (Fig. 4). In contrast to the amylose measurements, the relative paste viscosity curves indicate that cooking quality will be influenced by high CO_2 at both low and high P supplies.

Higher atmospheric CO_2 concentrations are also likely to influence the nutritive value of rice grain by changing both protein and mineral concentration. Protein is located in protein bodies distributed throughout the starch granules in the endosperm and constitutes about 10% of the dry weight of polished grain. The protein content of brown rice is slightly higher because the embryo and aleurone layers contain protein (Nanda and Coffman 1978). The protein concentrations of the grain ($N \times 5.7$) from this experiment were in the range reported for other rice cultivars (Nanda and Coffman 1978). However, concentrations were reduced by CO_2 enrichment, particularly at the low P supply (Fig. 5b). The average N content per grain was unaffected by high CO_2, suggesting that the lower N concentration was caused by increases in starch accumulation.

Although the P concentration was also reduced by CO_2 enrichment due to greater starch accumulation, the total P content per grain was higher (Table I). This indicates that, in contrast to N, more P was sequestered in each grain at elevated CO_2, possibly as phytate. Much of the P in cereal grain is present in this form and binds ions such as Mg (Batten 1994). This may explain the strong correlation between grain Mg and P concentration (Fig. 6). A similar correlation was observed by Batten (1994), who analyzed the nutrient concentrations of 85 wheat grain samples collected from Australia, the United States, Canada, and the United Kingdom. Batten (1994) suggested that accumulation of phytate can cause dietary problems in areas where Mg and other mineral supplies in the diet are low. The maximum CO_2 response of grain yield and average grain weight in the rice cultivar Jarrah occurred at medium P supplies (Figs. 1 and 2). Given that total P per grain increases at high CO_2, it may be desirable to maintain P

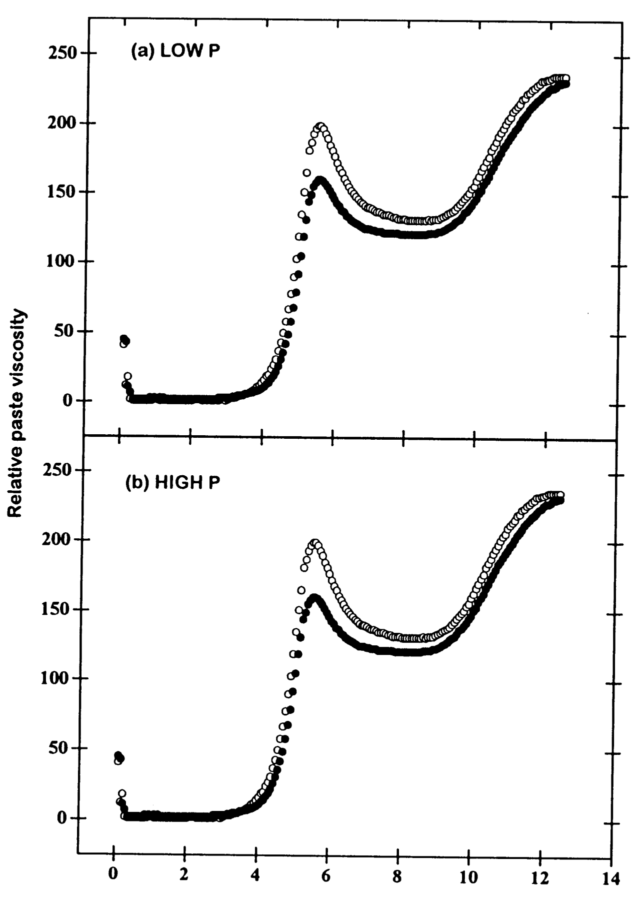

Fig. 4. Relative paste viscosity of ground brown rice grain from plants supplied with either 60 (**a**) or 480 (**b**) mg of P/kg of soil at either 350 (○) or 700 (●) µl of CO_2/L. Each point is the mean of two replicates.

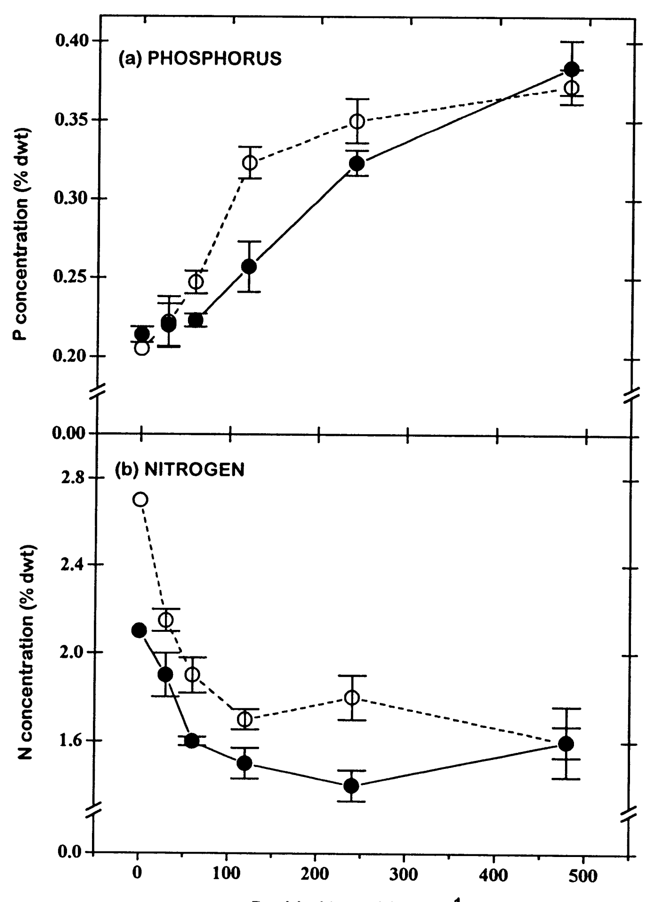

Fig. 5. P and N concentrations of ground brown rice grain. Plants were grown at either 350 (○) or 700 (●) µl of CO_2/L for 146 and 139 days, respectively. Each point is the mean of five replicates. Error bars represent the standard error of the treatment.

TABLE I
Influence of Elevated CO_2 and P Supply on Total N and P Content per Grain[a]

P (mg/kg of soil)	CO_2 (µl/L)	Total N (mg/grain)	Total P (mg/grain)
0	350	0.59	0.045
	700	0.53	0.054
30	350	0.49	0.051
	700	0.48	0.055
60	350	0.44	0.057
	700	0.45	0.062
120	350	0.41	0.078
	700	0.44	0.075
240	350	0.43	0.084
	700	0.36	0.084
480	350	0.38	0.089
	700	0.42	0.099
LSD ≥ 0.05		0.03	0.004

[a] Values are mean of five replicates.

fertilizer supplies at levels which minimize the problem of Mg binding to phytate while maintaining maximum yield and grain-filling capacity.

We conclude that a doubling of the current atmospheric CO_2 concentration is likely to alter the quality of rice grain as well as increase the total yield per plant. The challenge for chemists and plant breeders is to take advantage of the inevitable increase in atmospheric CO_2 concentrations by selecting genotypes that will be more productive and yet maintain desirable quality characteristics under future CO_2 scenarios.

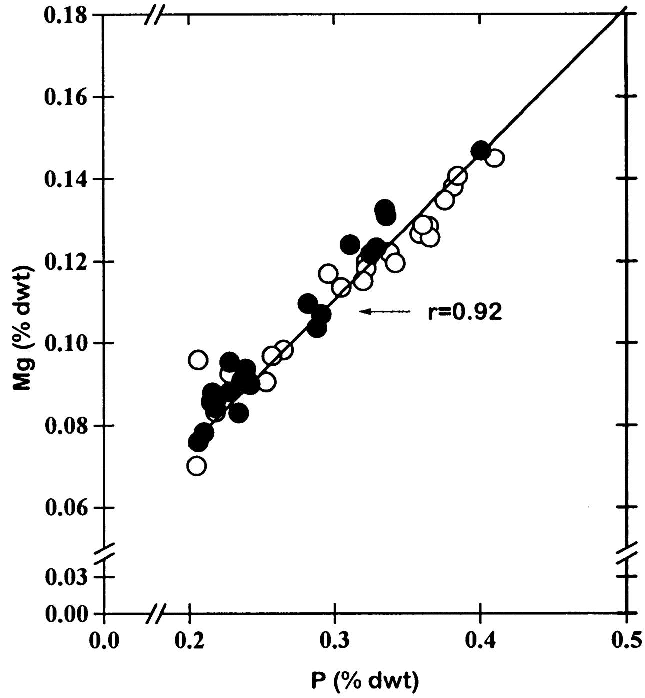

Fig. 6. Correlation between Mg and P concentrations in ground brown rice grain. Plants were grown at either 350 (○) or 700 (●) µl of CO_2/L for 146 and 139 days, respectively.

ACKNOWLEDGMENTS

Excellent technical assistance in this study by Jenelle Reece, Lynsey Welsh, and Mythri Seneweera, and funding of this work by Australian Research Council are gratefully acknowledged.

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[Received June 12, 1995. Accepted December 15, 1995.]