Plant Physiol. (1994) 105: 173–179

Responses of Ribulose-1,5-Bisphosphate Carboxylase, Cytochrome *f*, and Sucrose Synthesis Enzymes in Rice Leaves to Leaf Nitrogen and Their Relationships to Photosynthesis[1]

Amane Makino*, Hiromi Nakano, and Tadahiko Mae

Department of Applied Biological Chemistry, Faculty of Agriculture, Tohoku University, Tsutsumidori-Amamiyamachi, Sendai 981, Japan

The photosynthetic gas-exchange rates and various biochemical components of photosynthesis, including ribulose-1,5-bisphosphate carboxylase (Rubisco) content, cytochrome (Cyt) *f* content, and the activities of two sucrose synthesis enzymes, were examined in young, fully expanded leaves of rice (*Oryza sativa* L.) grown hydroponically in different nitrogen concentrations. The light-saturated rate of photosynthesis at an intercellular CO_2 pressure of 20 Pa (CO_2-limited photosynthesis) was linearly dependent on leaf nitrogen content, but curvilinearly correlated with Rubisco content. This difference was due to a greater than proportional increase in Rubisco content relative to leaf nitrogen content and the presence of a CO_2 transfer resistance between the intercellular air spaces and the carboxylation sites. CO_2-limited photosynthesis was proportional to Cyt *f* content, one of the key components of electron transport, but was not proportional to the activities of cytosolic fructose-1,6-bisphosphatase and sucrose phosphate synthase, the two regulatory enzymes of sucrose synthesis. Light-saturated photosynthesis above an intercellular CO_2 pressure of 60 Pa (CO_2-saturated photosynthesis) was curvilinearly dependent on leaf nitrogen content. This CO_2-saturated photosynthesis was proportional to Cyt *f* content in the low- and normal-nitrogen leaves, and correlated better with the activities of cytosolic fructose-1,6-bisphosphatase and sucrose phosphate synthase in the high-nitrogen leaves. The increase in the activities of these two enzymes with increasing leaf nitrogen was not as great as the increase in Cyt *f* content. Thus, as leaf nitrogen increased, the limitation caused by the activities of sucrose synthesis enzymes came into play, which resulted in the curvilinear relationship. However, this limitation by sucrose synthesis enzymes did not affect photosynthesis under normal ambient air.

The photosynthetic capacity of leaves is closely related to their nitrogen content. As leaf nitrogen content increases, the photosynthetic rate at any partial pressure of CO_2 is enhanced (von Caemmerer and Farquhar, 1981; Evans, 1983; Evans and Terashima, 1988; Sage et al., 1990b; Makino et al., 1992). According to the photosynthetic model of Farquhar and von Caemmerer (1982), the photosynthetic rate at low CO_2 partial pressures is limited by Rubisco capacity, whereas the rate at high CO_2 is limited by electron transport capacity. In addition, photosynthesis under saturating CO_2 conditions can also be limited by the capacity of starch and Suc synthesis to regenerate Pi for photophosphorylation (Sharkey, 1985; Stitt, 1986). Therefore, the increase in photosynthesis at all CO_2 partial pressures suggests that the capacity of each limiting process increases with increasing leaf nitrogen content. However, the increase in the capacity of each limiting process does not necessarily occur to the same relative degree. For example, the ratio of Rubisco to electron transport activities increases with increasing leaf nitrogen in many C_3 species (see Makino et al., 1992). This relative increase in Rubisco is considered to be required to maintain the balance between the in vivo capacities of Rubisco and electron transport because of the presence of a CO_2-transfer resistance between intercellular air spaces and the carboxylation sites (Evans and Terashima, 1988).

Sage et al. (1990a), working with *Chenopodium album*, found that the CO_2-saturation point of photosynthesis decreases with increasing leaf nitrogen. Based on the assumption that photosynthesis at the CO_2-saturation point can be limited by the capacity of starch and Suc synthesis, Sage et al. (1990b) have argued that the increase in the capacity of starch and Suc synthesis with increasing nitrogen is not as great as the increase in the capacity of Rubisco and electron transport. However, none of the underlying biochemical reasons for the decline in the CO_2-saturation point have been reported. In addition, it is largely unknown how leaf nitrogen affects the capacity of starch and Suc synthesis.

In this study, we used young, fully expanded leaves of rice (*Oryza sativa* L.) grown hydroponically with different nitrogen concentrations and focused on the in vivo balance among the capacities of Rubisco, electron transport, and Suc synthesis in response to leaf nitrogen content. Using a gas-exchange system, we first measured rates of CO_2-limited and CO_2-saturated photosynthesis and then compared them with the amounts of Rubisco and Cyt *f* proteins and the activities of cytosolic FBPase and SPS. The limitation of Cyt *f* content for electron transport capacity under high light has been reported repeatedly (Holloway et al., 1983; Evans, 1987; Terashima

[1] Research was supported by Grants-in-Aid for Scientific Research (Nos. 03304004 and 04660064) from the Ministry of Education, Science and Culture, Japan.

* Corresponding author; fax 81–22–272–1870.

Abbreviations: FBP, fructose-1,6-bisphosphate; FBPase, fructose-1,6-bisphosphatase; G3P, glyceraldehyde-3-phosphate; G3P-DH, G3P-dehydrogenase; pCc, chloroplastic CO_2 partial pressure; pCi, intercellular CO_2 partial pressure; RuBP, ribulose-1,5-bisphosphate; SPS, sucrose phosphate synthase.

and Evans, 1988; Heber et al., 1989), and cytosolic FBPase and SPS are considered to be limiting factors for in vivo Suc synthesis (Huber, 1983; Stitt et al., 1987; Stitt, 1988). Finally, we quantitatively deduced the change in the in vivo balance between the capacities of the above three processes limiting photosynthesis with increasing leaf nitrogen.

MATERIALS AND METHODS

Plant Culture

Rice (*Oryza sativa* L. cv Kinuhikari and Asahi) plants were grown hydroponically in an environmentally controlled growth chamber. Irradiance was provided by a combination of metal halide lamps (Toshiba, Yoko DF, Tokyo, Japan) and high-output fluorescent lamps (National FPR 96EX-N/A, Tokyo, Japan) and maintained at a PPFD of 1000 μmol quanta m^{-2} s^{-1} at plant level. Photoperiod was 14 h, with 1 h of metal halide lamps only at the beginning of each day. Temperature was controlled at 23°C during the day and 18°C at night. RH was maintained at 60%. The basal hydroponic solution used was described by Makino and Osmond (1991) and was continuously aerated. The solution was renewed once a week and its pH was adjusted to 5.0. All measurements were made on young, fully expanded leaves of 80- to 95-d-old plants. From 16 to 20 d before the measurements, plants were grown with three nitrogen concentrations (mM): 0.5 (0.25 mM NH_4NO_3), 2.0 (1.0 mM NH_4NO_3), and 8.0 (2.5 mM NH_4NO_3 plus 3.0 mM $NaNO_3$).

Bean (*Phaseolus vulgaris* L. cv Tendergreen) plants were also grown hydroponically with a nutrient solution containing 4 mM $NaNO_3$ in a greenhouse under natural sunlight conditions (Makino et al., 1992).

Gas Exchange

Gas exchange was determined with an open gas-exchange system using a temperature-controlled chamber equipped with two fans. The system was detailed by Makino et al. (1988). Differences in the partial pressures of CO_2 and H_2O entering and exiting the chamber were measured with an IRGA (Horiba ASSA-1110, Horiba, Kyoto, Japan) and a dew point hygrometer (EG&G model 911, EG&G, Waltham, MA), respectively. The PPFD at the position of the leaf in the chamber was measured with a Li-Cor quantum sensor (LI-190SA and LI-1000, Li-Cor, Lincoln, NE) and was adjusted to 1800 μmol quanta m^{-2} s^{-1}. Leaf temperature was controlled at 25°C. Gas-exchange parameters were calculated according to the equations of von Caemmerer and Farquhar (1981).

Determination of Chl, Total Leaf Nitrogen, Rubisco, and Cyt *f*

A leaf blade (19.4 ± 1.3 cm^2) was homogenized in 50 mM Na-phosphate buffer (pH 7.0) containing 120 mM 2-mercaptoethanol, 2 mM iodoacetic acid, and 5% (v/v) glycerol at a leaf:buffer ratio of 1:9 (g mL^{-1}) in a chilled mortar and pestle. The total Chl and leaf nitrogen contents were measured from part of this homogenate (Makino et al., 1992).

For determination of Rubisco, a Triton X-100 solution to a final concentration of 0.1% (v/v) was added to a portion of the leaf homogenate. After centrifugation of 10,000*g* for 3 min, the supernatant fluid was treated with a lithium dodecylsulfate solution (1.0% [w/v], final concentration) at 100°C for 1 min. This preparation was stored at −35°C until analyzed by SDS-PAGE. The amount of Rubisco was determined spectrophotometrically after formamide extraction of Coomassie brilliant blue R-250-stained subunit bands separated by SDS-PAGE (Makino et al., 1986). A calibration curve was made with Rubisco purified from rice leaves (Makino et al., 1983a).

The remaining leaf homogenate was used for the determination of Cyt *f*. The crude extract was passed through four layers of cheesecloth and centrifuged at 2500*g* for 3 min. The pellet was suspended in 100 mM Tris-HCl buffer (pH 8.6) and treated with a lithium dodecylsulfate solution, added to a final concentration of 2.0% (w/v). This preparation was heated immediately at 100°C for 1 min and centrifuged at 10,000*g* for 1 min at room temperature. Triton X-100 was added to the supernatant fraction to a final concentration of 12.5% (v/v), followed by storage at −35°C. The amount of Cyt *f* in this preparation was determined by rocket immunoelectrophoresis according to the method of Plumley and Schmidt (1983) with slight modifications. Monospecific antibodies against Cyt *f* were prepared previously (Hidema et al., 1991). Agarose gels (1%, w/v) without antibodies were cast onto a glass plate (8.4 × 10 cm) to a thickness of 1 mm, and an appropriately sized area was excised with a razor blade. Next, agarose gels containing antibodies to allow detection of 0.5 to 2 pmol of Cyt *f* were cast onto the excised area. The gel solution consisted of 80 mM Tris, 40 mM Na-acetate, 1 mM EDTA, 4% (w/v) PEG-4000, and 0.1% (v/v) Triton X-100 (pH 8.6 at 20°C with acetic acid). Wells of 3 mm diameter were punched, and 2 μL of a sample was applied to each well. Electrophoresis was conducted at 20 mA for about 5 h using the above buffer without PEG-4000 or Triton X-100. A standard curve was made with samples containing known concentrations of Cyt *f*, prepared as described by Evans and Terashima (1987). These Cyt *f* concentrations were estimated from the difference between the hydroquinone-reduced and the ferricyanide-oxidized spectra of rice thylakoid fractions solubilized with 1% (v/v) Triton X-100 in 50 mM Mes-NaOH (pH 6.5), 5 mM $MgCl_2$, and 1 mM EDTA according to the method of Bendall et al. (1971). A millimolar extinction coefficient of 20 mM^{-1} cm^{-1} was used (Evans and Terashima, 1987).

Enzyme Assays

NADP-G3P-DH activity was measured at 25°C according to the method described by Makino et al. (1983b). A leaf blade was homogenized in 50 mM Hepes-NaOH buffer (pH 7.5) containing 5 mM DTT, 0.5 mM EDTA, and 5% (v/v) glycerol at a leaf:buffer ratio of 1:9 (g/mL) in a chilled mortar and pestle. Chl content was measured in a subsample of this crude homogenate. After centrifugation at 10,000*g* for 2 min, the supernatant fluid was used for enzyme assay. The enzyme reaction was carried out in a buffer of 100 mM Bicine-NaOH (pH 8.5), 5 mM DTT, 20 mM Na_2HAsO_4, 20 mM NaF, 1 mM EDTA, 0.5 mM NADP, and 1 mM D-G3P. The assay was started by adding D-G3P.

Cytosolic FBPase was assayed at 30°C according to Sharkey et al. (1991). The grinding buffer consisted of 50 mM Hepes-NaOH (pH 7.5), 0.5 mM EDTA, and 5% (v/v) glycerol. The assay mixture contained 50 mM Hepes-NaOH (pH 7.2), 5 mM $MgCl_2$, 100 mM KCl, 0.5 mM NADP, 2 units mL^{-1} phosphoglucoisomerase, 2 units mL^{-1} Glc-6-P dehydrogenase, and 0.1 mM FBP. The reaction was initiated by adding FBP.

SPS was assayed at 25°C by the method of Huber et al. (1989). A leaf blade was homogenized in 50 mM Hepes-NaOH buffer (pH 7.5) containing 2.0 mM DTT, 10 mM $MgCl_2$, 1 mM EDTA, and 0.1% (v/v) Triton X-100. After centrifugation at 10,000*g* for 2 min, a 1.0-mL portion of the supernatant fraction was immediately passed through a 5-mL column of Sephadex G-25 previously equilibrated with the homogenization buffer without Triton X-100. The eluate was used for enzyme assay. SPS activity was measured under V_{max} substrate conditions as Fru-6-P-dependent formation of Suc from UDP-Glc. Substrate concentrations were 10 mM Fru-6-P, 10 mM UDP-Glc, and 40 mM Glc-6-P. Suc was determined by the anthrone method.

RESULTS

The light response of photosynthesis at several pCi in the leaf of rice was first examined. The purpose of this analysis was to elucidate the dependence of the photosynthetic light-response curve on pCi. If the light response depends on pCi, it is possible that the maximum photosynthetic rate at high CO_2 partial pressures for the high-nitrogen leaf may be underestimated relative to photosynthesis at low CO_2 when measured at the same irradiance. In fact, for some species such as *C. album* (Sage et al., 1990b) and *Eucalyptus maculata* (Ögren and Evans, 1993), the light-saturation point is enhanced with increasing pCi. This means that higher irradiances are progressively required to reach light saturation at high pCi.

Our results with rice, as compared with those in bean, are shown in Figure 1. The photosynthetic rate at any given pCi increased with increasing irradiance in both species (upper panels), but there was a difference in the light-response curves of the relative rates between these two C_3 species (lower panels). In rice the dependence of photosynthesis on light was the same irrespective of pCi, whereas in bean the light-response curve of photosynthesis was appreciably affected by pCi. The light-saturation point in bean increased with increasing pCi, as with C. *album* (Sage et al., 1990b) and *E. maculata* (Ögren and Evans, 1993). The response in rice was similar to that found with wheat (data not shown). In the present study with rice, therefore, we considered that there was no difference in the rate of photosynthesis caused by a difference in the dependence of the light response on pCi although we measured the subsequent CO_2-assimilation rates at the same irradiance.

The rate of CO_2 assimilation was examined as a function of pCi in leaves of rice grown at different nitrogen concentrations (Fig. 2). The rate of CO_2 assimilation at any given pCi increased with increasing nitrogen supply (upper panel). When the CO_2 response curve for each nitrogen treatment was normalized to the rate of CO_2 assimilation at 20 Pa, the

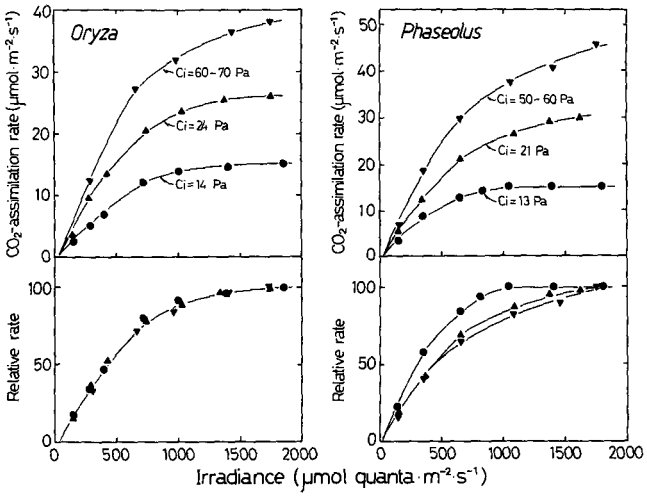

Figure 1. Response of the CO_2-assimilation rate to irradiance at the indicated pCi in the leaves of rice (left panel) and bean (right panel). Upper panels, Response curves expressed in μmol m^{-2} s^{-1}. Lower panels, Response curves of relative rates to those at the respective highest irradiances. Measurements were made at a leaf temperature of 25°C and the indicated pCi. Rice and bean plants were grown with 2.0 and 4.0 mM nitrogen concentrations, respectively.

respective curves collapsed to almost identical curves over a wide range of pCi (lower panel). Above approximately 40 Pa of pCi, however, the relative rates of photosynthesis in the high nitrogen-grown plants were lower than in the low nitrogen-grown plants, and the CO_2-saturation point in the high nitrogen-grown plants appeared at lower CO_2 partial pressures. These results were similar to those with *C. album* reported by Sage et al. (1990a), despite the difference in the dependence of the light response curve on pCi between these two species.

According to the photosynthesis model of Farquhar and von Caemmerer (1982), the photosynthetic rate at high CO_2 partial pressures is limited by electron transport capacity. However, in some cases, the photosynthetic rate at high CO_2 is also suggested to be limited by the capacity of Pi regeneration during starch and Suc synthesis (Sharkey, 1985; Stitt, 1986). Both limitations at high CO_2 pressures eventually determine RuBP regeneration for in vivo Rubisco activity, but Sharkey et al. (1988) reported that they can be distinguished. According to their theory, Pi-regeneration-limited photosynthesis is insensitive to increasing CO_2 or decreasing O_2, whereas photosynthesis limited by electron transport capacity is still stimulated by increasing CO_2. In addition, the Pi-regeneration limitation also occurs at normal pCi when the partial pressure of O_2 is reduced to about 2 kPa (Sage and Sharkey, 1987; Sharkey et al., 1988). Therefore, Sage et al. (1989) reported that the O_2 sensitivity of photosynthesis at normal pCi to a reduction in $[O_2]$ from atmospheric partial pressures to 2 kPa can be an indicator of the relative capacity of Pi regeneration during starch and Suc synthesis.

We examined the O_2 sensitivity of rice photosynthesis at pCi = 20 Pa when the partial pressure of O_2 was reduced from 21 to 2 kPa, according to Sage and Sharkey (1987). The

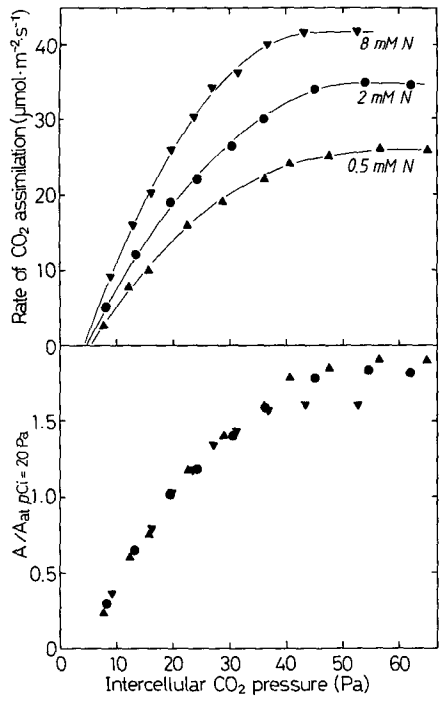

Figure 2. Rate of CO_2 assimilation (A) as a function of pCi in leaves of rice grown with 0.5 (▲), 2.0 (●), and 8.0 (▼) mM nitrogen concentrations. Upper panel, Response curves expressed in μmol m^{-2} s^{-1}. Lower panel, Response curves that have been normalized relative to the CO_2-assimilation rate at pCi = 20 Pa. Measurements were made at a leaf temperature of 25°C and a PPFD of 1800 μmol quanta m^{-2} s^{-1}.

0.5 mM nitrogen-grown plants exhibited a 34 ± 1% O_2 sensitivity, whereas the 8.0 mM nitrogen-grown plants showed 27 ± 2% sensitivity. This suggested that a relative decrease in Pi-regeneration capacity during starch and Suc synthesis occurred in the high nitrogen-grown plants.

Figure 3 shows the relationships between the photosynthetic rates at pCi of 20 Pa and above 60 Pa, and the ratio of their rates, versus total leaf-nitrogen content. The photosynthetic rate at pCi = 20 Pa was linearly correlated with leaf nitrogen content, whereas the rate above 60 Pa CO_2 was curvilinearly correlated. Consequently, the ratio of the photosynthetic rates at pCi > 60 Pa to pCi = 20 Pa decreased with increasing leaf nitrogen content. This was not caused by a difference in the light-saturation point between low and high CO_2 pressures (see Fig. 1). The decline in this ratio means that as the photosynthetic capacity increased with leaf nitrogen content, CO_2-saturated photosynthesis did not increase to the same relative extent as CO_2-limited photosynthesis.

We next examined several key components and enzymes of C_3 photosynthesis, which are located in the chloroplast stroma and thylakoids and the cytosol. All data are expressed on a leaf nitrogen basis (Fig. 4). Rubisco content per unit leaf nitrogen increased with increasing leaf nitrogen content. Although NADP-G3P-DH is also located within the chloroplast stroma, its activity remained constant. This response was almost identical to those of Chl and Cyt f contents. Among the other stromal enzymes examined, including ribulose-5-phosphate kinase, chloroplastic FBPase, and carbonic anhydrase, only carbonic anhydrase showed a similar response to that of Rubisco (data not shown). In contrast, the activities of two key enzymes of Suc synthesis, FBPase and SPS, decreased on a unit leaf-nitrogen basis with increasing leaf nitrogen content.

The increase in the ratio of Rubisco to total leaf nitrogen with nitrogen supply is frequently found in many C_3 species (see Makino et al., 1992). This relative increase in Rubisco content is considered to be required because the partial pressure of CO_2 at the carboxylation sites is reduced with increasing Rubisco content by a CO_2-transfer resistance (Evans and Terashima, 1988). The presence of a significant CO_2-transfer resistance between the intercellular air spaces and the carboxylation sites was originally pointed out by Evans (1983) because of a curvilinear relationship between CO_2-limited photosynthesis and the in vitro Rubisco activity. In addition, the presence of this resistance has been confirmed by concurrent measurements of gas exchange and ^{13}C discrimination during CO_2 uptake (Evans et al., 1986; von Caemmerer and Evans, 1991) and by the analysis of the CO_2 sensitivity of photosynthesis using gas-exchange and Chl fluorescence measurements (Harley et al., 1992; Loreto et al., 1992).

We also found a curvilinear relationship between the pho-

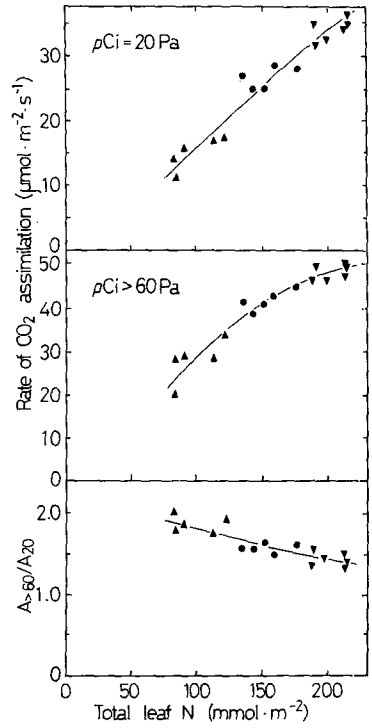

Figure 3. Rates of CO_2 assimilation at pCi = 20 Pa and pCi > 60 Pa, and the ratio of the rates at pCi > 60 Pa to pCi = 20 Pa, versus total leaf nitrogen content. Symbols are the same as in Figure 2. Measurements were made at a leaf temperature of 25°C and a PPFD of 1800 μmol quanta m^{-2} s^{-1}.

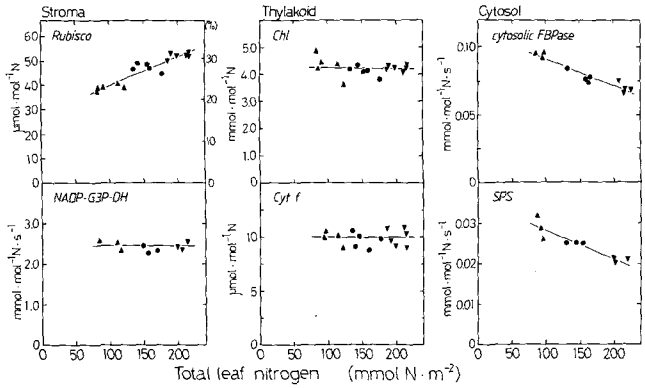

Figure 4. Ratios of Rubisco content, NADP-G3P-DH activity, total Chl content, Cyt *f* content, cytosolic FBPase activity, and SPS activity to total leaf nitrogen content versus total leaf nitrogen content. Symbols are the same as in Figure 2. The percent (%) values in the Rubisco panel show the proportion of total leaf nitrogen in Rubisco.

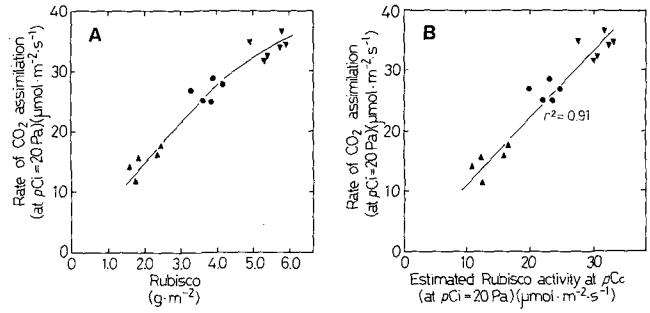

Figure 5. Rates of CO_2 assimilation at pCi = 20 Pa versus Rubisco content (A). Rate of CO_2 assimilation at pCi = 20 Pa versus estimated Rubisco activity at pCc obtained at pCi = 20 Pa (B). $y = 1.12x$, $r^2 = 0.91$. The pCc was calculated using the CO_2 transfer conductance reported with rice (von Caemmerer and Evans, 1991). The equation between the CO_2 transfer conductance (g_w) and the photosynthetic rate (A) was calculated to be $g_w = (8.1 \times 10^3) A + 0.24$ (mol m^{-2} s^{-1}). Rubisco activity was estimated from the Rubisco content and its kinetic constants from rice (Makino et al., 1988), using the equations in Makino et al. (1985). Symbols are the same as in Figure 2.

tosynthetic rate at pCi = 20 Pa and Rubisco content (Fig. 5A). The slope of the relationship decreased significantly above 4 g m^{-2} of Rubisco content. To extrapolate to the in vivo Rubisco activity, we calculated the Rubisco activity at the carboxylation sites at pCi = 20 Pa using the CO_2 transfer conductance estimated by von Caemmerer and Evans (1991) with rice. Since they reported that the CO_2 transfer conductance can vary with leaf nitrogen content, we used the equation of the linear relationship between the CO_2 transfer conductance and the photosynthetic rate calculated from their data with rice. Although the estimated Rubisco activity at pCc was about 10% too low to account for the photosynthetic rate at pCi = 20 Pa, the relationship between them was almost linear and its regression line passed through the origin (Fig. 5B). These results indicate that the photosynthetic rate at pCi = 20 Pa reflects the in vivo Rubisco activity.

Figure 6 shows the changes in the ratios of the photosynthetic rates at pCi = 20 Pa and pCi > 60 Pa to Cyt *f* content, cytosolic FBPase, and SPS activities versus leaf nitrogen content. The ratio of the photosynthetic rate at pCi = 20 Pa to Cyt *f* content remained almost constant, but that to cytosolic FBPase or SPS activity increased steadily with increasing leaf nitrogen content. Since the photosynthetic rate at pCi = 20 Pa reflects the in vivo Rubisco activity (see above and Fig. 5B), this means that the in vivo balance between Rubisco activity and Cyt *f* is constant regardless of nitrogen treatment, but the balance between Rubisco activity and Suc synthesis is affected by nitrogen treatment.

The ratio of the photosynthetic rate at pCi > 60 Pa to Cyt *f* content was relatively constant in the 0.5-mM- and 2.0-mM-nitrogen leaves, but declined in the 8.0-mM-nitrogen leaves (Fig. 6). In contrast, the ratio at pCi > 60 Pa to cytosolic FBPase or SPS activity was lower in the 0.5-mM-nitrogen leaves but remained almost constant in the 2.0-mM- and 8.0-mM-nitrogen leaves. These results suggest that photosynthesis at high CO_2 in the low-nitrogen leaves was limited by Cyt *f* content, but as leaf nitrogen increased, the limitation by the activity of Suc synthesis came into play. Thus, the relative decrease in CO_2-saturated photosynthesis at high

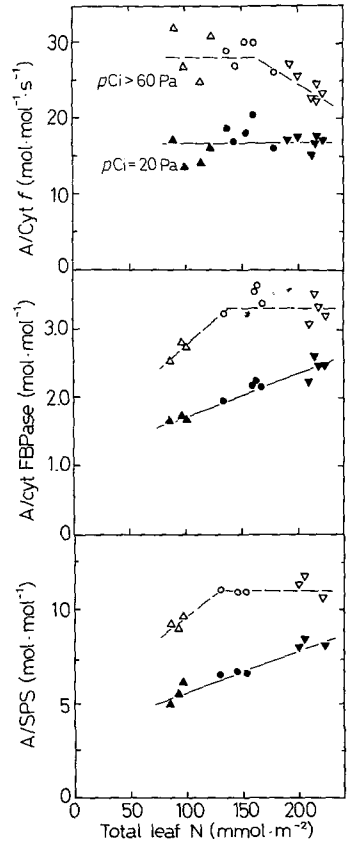

Figure 6. Ratios of the CO_2-assimilation rates (A) at pCi = 20 Pa (closed symbols) and pCi > 60 Pa (open symbols) to Cyt f content, cytosolic FBPase activity, and SPS activity versus total leaf nitrogen content. Symbols are the same as in Figure 2.

leaf nitrogen content may have been caused by the decline in the ratio of these two key enzymes of Suc synthesis to Rubisco and Cyt *f*.

DISCUSSION

CO_2-Limited Photosynthesis Versus Leaf Nitrogen Content

The relationship between CO_2-limited photosynthesis and leaf nitrogen content is presumably reflected by the relationship between Rubisco activity and leaf nitrogen content (von Caemmerer and Farquhar, 1981; Seemann and Berry, 1982; Makino et al., 1988, Sage et al., 1990a). However, as shown in Figures 3 and 5A, the relationship between photosynthetic rate at pCi = 20 Pa and leaf nitrogen content was almost linear, whereas Rubisco content was curvilinearly related to CO_2-limited photosynthesis, as has been seen for wheat (Evans, 1983) and spinach (Evans and Terashima, 1988). Although such a curvilinear relationship was not found in our previous work with rice (Makino et al., 1985), this was probably because the present rice variety can have greater nitrogen contents. The curvilinear relationship between CO_2-limited photosynthesis and Rubisco content (Fig. 5A) may have been due mainly to the presence of a CO_2 transfer resistance. Evans and Terashima (1988) have argued that with increasing leaf nitrogen content, Rubisco activity in vivo is progressively limited to a greater extent by the CO_2 transfer resistance.

The CO_2 transfer conductance was originally estimated from the curvilinear relationship on the assumption that all the leaves had a similar CO_2 transfer conductance (Evans, 1983). When we estimated the CO_2 transfer conductance from the curvature in Figure 5A by a method similar to that of Evans (1983), according to the statistical analysis of Wilkinson (1961), its calculation gave a value of 0.50 ± 0.08 mol m^{-2} s^{-1}. This value is quite similar to that of the CO_2 transfer conductance that von Caemmerer and Evans (1991) estimated for rice grown with high nitrogen using the combined techniques of conventional gas analysis and concurrent measurements of carbon isotope discrimination during CO_2 uptake. However, von Caemmerer and Evans (1991) used this technique and found that the CO_2 transfer conductance does vary with leaf nitrogen content. This means that our estimate of CO_2 transfer conductance (0.50 mol m^{-2} s^{-1}) is not necessarily valid because it was based on the assumption that all the leaves had a similar CO_2 transfer conductance. According to their data, however, the change in the CO_2 transfer conductance with leaf nitrogen content was smaller than that in photosynthetic rate (e.g. 1.5-fold in rice). Therefore, the relationship shown in Figure 5A may still have been curvilinear.

Recently, Quick et al. (1992), using transgenic tobacco plants with antisense genes for the small subunit of Rubisco, considered that in plants grown in high nitrogen, Rubisco is a nitrogen store that can also be a functional protein for a slightly higher water-use efficiency and/or for photosynthesis to respond to temporarily high irradiance. In our studies with rice, however, the ratio between the photosynthetic rate at pCi = 20 Pa (corresponding to the in vivo Rubisco activity) and Cyt *f* content remained constant regardless of leaf nitrogen content (Fig. 6). These results at the in vitro level strongly support the view of Evans and Terashima (1988) that the activities of Rubisco and electron transport remain balanced in vivo even in leaves with high nitrogen contents. At the same time, these findings suggest that Rubisco in the present rice plants is never in excess, even in plants grown at high nitrogen.

CO_2-Saturated Photosynthesis Versus Leaf Nitrogen Content

It is largely unknown what determines the relationship between CO_2-saturated photosynthesis and leaf nitrogen content. The results in Figure 3 clearly show the existence of a curvilinear relationship between these two parameters. Similar trends are found in the data reported by Sage et al. (1990a). Terashima and Evans (1988) also reported a curvilinear relationship between CO_2-saturated O_2 evolution and leaf nitrogen content. We suggest that this curvilinear relationship is caused by a smaller increase in the capacity of Suc synthesis relative to that of electron transport with increasing leaf nitrogen. As shown in Figure 6, CO_2-saturated photosynthesis was proportional to Cyt *f* content in the low- and normal-nitrogen leaves, and correlated better with the activities of cytosolic FBPase and SPS in the high-nitrogen leaves. Thus, as leaf nitrogen increased, the limitation by the activities of Suc synthesis enzymes came into play, which resulted in the curvilinear relationship.

Although the relationships between CO_2-saturated photosynthesis and electron transport components or activities have been frequently reported (von Caemmerer and Farquhar, 1981; Evans, 1987, Evans and Terashima, 1988; Terashima and Evans, 1988; Hidema et al., 1991), there are few reports examining the relationship between photosynthesis and cytosolic FBPase and/or SPS. Sharkey et al. (1992) reported that a mutant line of *Flaveria linearis* lacking most of the cytosolic FBPase showed a much reduced O_2 sensitivity of photosynthesis at normal CO_2. Socias et al. (1993) found a positive correlation between CO_2-saturated photosynthesis and SPS activity when bean plants were grown in high CO_2 (65 Pa). The above results, including our own, indicate that the limitation by Suc synthesis rarely occurs in plants grown under normal conditions.

In conclusion, our results show that as leaf nitrogen content increased, the capacity of Suc synthesis, as indicated by the activities of cytosolic FBPase and SPS, increased to a lesser extent than that of Rubisco and Cyt *f* at both the in vivo and in vitro levels. This means that the balance between the capacities of Suc synthesis and other processes limiting photosynthesis depends on leaf nitrogen content. However, the change in this balance did not affect photosynthesis under normal ambient air.

ACKNOWLEDGMENTS

We thank Jun Hidema and Yoshito Nishimori for the use of the Cyt *f* antiserum. We are also grateful to Dr. John Evans for his valuable comments and critical reading of the manuscript.

Received November 15, 1993; accepted February 9, 1994.
Copyright Clearance Center: 0032-0889/94/105/0173/07.

LITERATURE CITED

Bendall DS, Davenport HF, Hill R (1971) Cytochrome components in chloroplasts of the higher plants. Methods Enzymol **23:** 327–344

Evans JR (1983) Nitrogen and photosynthesis in the flag leaf of wheat (*Triticum aestivum* L.). Plant Physiol **72:** 297–302

Evans JR (1987) The relationship between electron transport components and photosynthetic capacity in pea leaves grown at different irradiances. Aust J Plant Physiol **14:** 157–170

Evans JR, Sharkey TD, Berry JA, Farquhar GD (1986) Carbon isotope discrimination measured concurrently with CO_2 exchange to investigate CO_2 diffusion in leaves of higher plants. Aust J Plant Physiol **13:** 281–292

Evans JR, Terashima I (1987) Effects of nitrogen nutrition on electron transport components and photosynthesis in spinach. Aust J Plant Physiol **14:** 59–68

Evans JR, Terashima I (1988) Photosynthetic characteristics of spinach leaves grown with different nitrogen treatments. Plant Cell Physiol **29:** 157–165

Farquhar GD, von Caemmerer S (1982) Modelling of photosynthetic response to environmental conditions. *In* OL Lange, PS Nobel, CB Osmond, H Ziegler, eds, Physiological Plant Ecology II. Water Relations and Carbon Assimilation, Vol 12B. Springer-Verlag, Berlin, pp 550–587

Harley PC, Loreto F, DiMarco G, Sharkey TD (1992) Theoretical considerations when estimating the mesophyll conductance to CO_2 flux by analysis of the response of photosynthesis to CO_2. Plant Physiol **98:** 1429–1436

Heber U, Neimanis S, Dietz K-J (1989) Fractional control of photosynthesis by the Q_B protein, the cytochrome f/b_6 complex and other components of the photosynthetic apparatus. Planta **173:** 267–274

Hidema J, Makino A, Mae T, Ojima K (1991) Photosynthetic characteristics of rice leaves aged under different irradiances from full expansion through senescence. Plant Physiol **97:** 1287–1293

Holloway PJ, Maclean DJ, Scott KJ (1983) Rate-limiting steps of electron transport in chloroplasts during ontogeny and senescence of barley. Plant Physiol **72:** 795–801

Huber SC (1983) Role of sucrose-phosphate synthase in partitioning of carbon in leaves. Plant Physiol **71:** 818–821

Huber SC, Nielsen TM, Huber JLA, Pharr DM (1989) Variations among species in light activation of sucrose-phosphate synthase. Plant Cell Physiol **30:** 277–285

Loreto F, Harley PC, DiMarco G, Sharkey TD (1992) Estimation of mesophyll conductance to CO_2 flux by three different methods. Plant Physiol **98:** 1437–1443

Makino A, Mae T, Ohira K (1983a) Purification and storage of ribulose 1,5-bisphosphate carboxylase from rice leaves. Plant Cell Physiol **24:** 1169–1173

Makino A, Mae T, Ohira K (1983b) Photosynthesis and ribulose 1,5-bisphosphate carboxylase in rice leaves. Changes in photosynthesis and enzymes involved in carbon assimilation from leaf development through senescence. Plant Physiol **73:** 1002–1007

Makino A, Mae T, Ohira K (1985) Photosynthesis and ribulose-1,5-bisphosphate carboxylase/oxygenase in rice leaves from emergence through senescence. Quantitative analysis by carboxylation/oxygenation and regeneration of ribulose-1.5-bisphosphate. Planta **166:** 414–420

Makino A, Mae T, Ohira K (1986) Colorimetric measurement of protein stained with Coomassie brilliant blue R on sodium dodecyl sulfate-polyacrylamide gel electrophoresis by eluting with formamide. Agric Biol Chem **50:** 1911–1912

Makino A, Mae T, Ohira K (1988) Differences between wheat and rice in the enzymic properties of ribulose-1,5-bisphosphate carboxylase/oxygenase and the relationship of photosynthetic gas exchange. Planta **174:** 30–38

Makino A, Osmond B (1991) Effects of nitrogen nutrition on nitrogen partitioning between chloroplasts and mitochondria in pea and wheat. Plant Physiol **96:** 355–362

Makino A, Sakashita H, Hidema J, Mae T, Ojima K, Osmond B (1992) Distinctive responses of ribulose-1,5-bisphosphate carboxylase and carbonic anhydrase in wheat leaves to nitrogen nutrition and their possible relationships to CO_2 transfer resistance. Plant Physiol **100:** 1737–1743

Ögren E, Evans JR (1993) Photosynthetic light-response curves. I. The influence of CO_2 partial pressure and leaf inversion. Planta **189:** 182–190

Plumley FG, Schmidt GW (1983) Rocket immunoelectrophoresis of protein solubilized with sodium dodecyl sulfate. Anal Biochem **134:** 86–95

Quick WP, Fichtner K, Schulze E-D, Wendler R, Leegood RC, Mooney H, Rodermel SR, Bogorad L, Stitt M (1992) Decreased ribulose-1,5-bisphosphate carboxylase-oxygenase in transgenic tobacco transformed with "antisense" *rbc*S. IV. Impact on photosynthesis in conditions of altered nitrogen supply. Planta **188:** 522–531

Sage RF, Sharkey TD (1987) The effect of temperature on the occurrence of O_2 and CO_2 insensitive photosynthesis in field grown plants. Plant Physiol **84:** 658–664

Sage RF, Sharkey TD, Pearcy RW (1990a) The effect of leaf nitrogen and temperature on the CO_2 response of photosynthesis in the C_3 dicot *Chenopodium album* L. Aust J Plant Physiol **17:** 135–148

Sage RF, Sharkey TD, Seemann JR (1989) Acclimation of photosynthesis to elevated CO_2 in five C_3 species. Plant Physiol **89:** 590–596

Sage RF, Sharkey TD, Seemann JR (1990b) Regulation of ribulose 1,5-bisphosphate carboxylase activity in response to light intensity and CO_2 in the C_3 annuals *Chenopodium album* L. and *Phaseolus vulgaris* L. Plant Physiol **94:** 1735–1742

Seemann JR, Berry JA (1982) Interspecific differences in the kinetic properties of RuBPCase protein. Carnegie Inst Washington Year Book **81:** 78–83

Sharkey TD (1985) Photosynthesis in intact leaves of C_3 plants: physics, physiology and rate limitations. Bot Rev **51:** 53–105

Sharkey TD, Berry JA, Sage RF (1988) Regulation of photosynthetic electron-transport in *Phaseolus vulgaris* L., as determined by room-temperature chlorophyll a fluorescence. Planta **176:** 415–424

Sharkey TD, Savitch LV, Vanderveer PJ, Micallef BJ (1992) Carbon partitioning in a *Flaveria linearis* mutant with reduced cytosolic fructose bisphosphatase. Plant Physiol **100:** 210–215

Sharkey TD, Vassey TL, Vanderveer PJ, Vierstra RD (1991) Carbon metabolism enzymes and photosynthesis in transgenic tobacco (*Nicotiana tabacum* L.) having excess phytochrome. Planta **185:** 287–296

Socias FX, Medrano H, Sharkey TD (1993) Feedback limitation of photosynthesis of *Phaseolus vulgaris* L. grown in elevated CO_2. Plant Cell Environ **16:** 81–86

Stitt M (1986) Limitation of photosynthesis by carbon metabolisms. I. Evidence for excess electron transport capacity in leaves carrying out photosynthesis in saturating light and CO_2. Plant Physiol **81:** 1115–1122

Stitt M (1988) Control of sucrose synthesis. *In* J Barber, R Malkin, eds, Techniques and New Developments in Photosynthetic Research. Plenum, London, pp 365–386

Stitt M, Huber S, Kerr P (1987) Control of photosynthetic sucrose formation. *In* MD Hatch, NK Boardman, eds, The Biochemistry of Plants, Vol 10, Photosynthesis. Academic Press, New York, pp 327–409

Terashima I, Evans JR (1988) Effect of light and nitrogen nutrition on the organization of the photosynthetic apparatus in spinach. Plant Cell Physiol **29:** 143–155

von Caemmerer S, Evans JR (1991) Determination of the average partial pressure of CO_2 in chloroplasts from leaves of several C_3 plants. Aust J Plant Physiol **18:** 287–305

von Caemmerer S, Farquhar GD (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta **153:** 376–387

Wilkinson GN (1961) Statistical estimations in enzyme kinetics. Biochem J **80:** 324–332