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Effects of Growth Temperature on the Responses of Ribulose-1,5-Bisphosphate Carboxylase, Electron Transport Components, and Sucrose Synthesis Enzymes to Leaf Nitrogen in Rice, and Their Relationships to Photosynthesis[1]

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Effects of growth temperature on the photosynthetic gas-exchange rates and their underlying biochemical properties were examined in young, fully expanded leaves of rice (*Oryza sativa* L.). The plants were grown hydroponically under day/night temperature regimes of 18/15°C, 23/18°C, and 30/23°C and all photosynthetic measurements were made at a leaf temperature of 25°C and an irradiance of 1800 μmol quanta m^{-2} s^{-1}. Growth temperature affected the photosynthetic CO_2 response curve. The relative ratio of the initial slope to the CO_2-saturated photosynthesis increased with rising growth temperature. This was caused mainly by an increase in CO_2-limited photosynthesis for a given leaf nitrogen content with rising growth temperature. However, there was no difference in ribulose-1,5-bisphosphate carboxylase (Rubisco) content at any given leaf nitrogen content among temperature treatments. In addition, the activation state and catalytic turnover rate of Rubisco were not affected by growth temperature. The increase in CO_2-limited photosynthesis with rising growth temperature was the result of an increase in the CO_2 transfer conductance between the intercellular airspaces and the carboxylation sites. The amounts of total chlorophyll and light-harvesting chlorophyll *a/b* protein II increased for the same leaf nitrogen content with rising growth temperature, but the amounts of cytochrome *f* and coupling factor 1 and the activities of cytosolic fructose-1,6-bisphosphatase and sucrose-phosphate synthase were the same between plants grown at 23/18°C and those grown at 30/23°C. Similarly, CO_2-saturated photosynthesis was not different for the same leaf nitrogen content between these treatments. For the 18/15°C-grown plants, a slight decrease in the amounts of cytochrome *f* and coupling factor 1 and an increase in the activities of cytosolic fructose-1,6-bisphosphatase and sucrose-phosphate synthase were found, but these were not reflected in CO_2-saturated photosynthesis.

The photosynthetic capacity of leaves is strongly affected by temperature. However, the dependence of each photosynthetic limiting process on temperature is not necessarily the same. With short-term (minutes to hours) temperature change, some key components of the photosynthetic apparatus seem to be affected more than others. For example, temperature strongly influences CO_2-saturated photosynthesis, whereas it had only a slight effect on CO_2-limited photosynthesis (Kirschbaum and Farquhar, 1984; Sage and Sharkey, 1987; Labate and Leegood, 1988; Sage et al., 1990). According to the photosynthetic model of Farquhar and von Caemmerer (1982) and Sharkey (1985), these results suggest that temperature affects the rate of RuBP regeneration limited by electron transport and/or starch and Suc synthesis to a greater relative extent than the rate limited by Rubisco capacity. In fact, the difference between them has been accounted for mainly by the differences in the temperature response between Rubisco kinetic constants and electron transport capacity (Berry and Björkman, 1980). Recent simultaneous analysis by Chl *a* fluorescence and gas exchange also showed that the in vivo ratio of electron transport to CO_2 fixation clearly increases with rising temperature (Oberhuber and Edwards, 1993). In addition, Labate and Leegood (1988) have noted the importance of Pi recycling during starch and Suc synthesis on the temperature response of photosynthesis. Thus, photosynthesis at normal CO_2 is relatively limited by Rubisco capacity under high-temperature conditions, whereas under low-temperature conditions the limitation shifts to RuBP regeneration by electron transport capacity and/or Pi-regeneration capacity during starch and Suc synthesis.

However, in spite of these understandings of the short-term response of photosynthesis to temperature, little is known about how growth temperature affects the in vivo balance between the capacities of the respective photosynthetic limiting processes. Changes in activities of Rubisco, other Calvin cycle enzymes, or electron transport with growth temperature have been frequently reported (Badger et al., 1982; Maruyama et al., 1990; Holaday et al., 1992). However, these changes cannot be necessarily attributed to the observed change in photosynthesis that results from acclimation to growth temperature, although some gas-exchange studies

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Abbreviations: CF1, coupling factor 1; FBPase, fructose-1,6-bisphosphatase; LHC II, light-harvesting chlorophyll *a/b* protein II; *pCc*, chloroplastic CO_2 partial pressure; *pCi*, intercellular CO_2 partial pressure; RuBP, ribulose-1,5-bisphosphate; SPS, sucrose-phosphate synthase.

suggest that change in RuBP regeneration with growth temperature is relatively great (Mooney et al., 1978; Badger et al., 1982; Ferrar et al., 1989). In addition, it is largely unknown whether the changes in Rubisco and/or other key components are caused directly by the nitrogen partitioning for them or are caused secondarily by relative change in leaf area, dry weight, or other growth indexes. Since Rubisco is the largest source for nitrogen among the photosynthetic components, changes in its content will have the greatest effect on nitrogen partitioning for other photosynthetic components.

The purpose of this study was to elucidate the effects of growth temperature on the in vivo balance among the capacities of Rubisco, electron transport, and Suc synthesis, and their underlying biochemical and physiological mechanisms. We used young, fully expanded leaves of rice (*Oryza sativa* L.) grown at three day/night temperature regimes of 18/15°C, 23/18°C, and 30/23°C, and compared the patterns of nitrogen allocation into several key components of each photosynthetic limiting process. We measured Rubisco as a determinant for CO_2-limited photosynthesis (von Caemmerer and Farquhar, 1981; Evans, 1983; Makino et al., 1985b), Chl and LHC II as light-harvesting components, Cyt *f* and CF1 as rate-limiting factors for electron transport (Leong and Anderson, 1984; Evans, 1987; Heber et al., 1988), and cytosolic FBPase and SPS as key enzymes during Suc synthesis (Huber, 1983; Stitt, 1988; Daie, 1993). To deduce the quantitative contributions of these key enzymes and components to photosynthesis, we next analyzed their relationships to the gas-exchange data measured at 25°C, and then characterized the photosynthetic system acclimated to growth temperature.

MATERIALS AND METHODS

Plant Culture

Rice (*Oryza sativa* L. cv Kinuhikari and Asahi) plants were grown hydroponically in an environmentally controlled growth chamber (Makino et al., 1994). The chamber was operated with a 14-h photoperiod, 23/18°C day/night temperature, 60% RH, and a PPFD of 1000 μmol quanta m^{-2} s^{-1} at plant level during the day periods. Irradiance was provided by a combination of metal halide lamps (Toshiba, Yoko DF, Tokyo, Japan) and high-output fluorescent lamps (National FPR 96 EX-N/A, Tokyo, Japan). The basal hydroponic solution used was described by Makino and Osmond (1991) and was continuously aerated. All measurements were made on young, fully expanded leaves of 80- to 105-d-old plants. From 16 to 28 d before the measurements, plants were grown at three day/night temperature regimes of 18/15°C, 23/18°C, and 30/23°C. Nitrogen concentrations in the hydroponic solutions were (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 $NaNO_3$) for each temperature treatment.

Gas-Exchange Measurements

Gas exchange was determined with an open gas-exchange system using a temperature-controlled chamber equipped with two fans (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, Natick, MA), respectively. Measurements were made at a leaf temperature of 25°C, a PPFD of 1800 μmol quanta m^{-2} s^{-1}, and leaf-to-air vapor pressure difference of 1.0 to 1.2 kPa. Gas-exchange parameters were calculated according to the equations of von Caemmerer and Farquhar (1981).

Biochemical Assays

Determinations of Chl, total leaf nitrogen, Rubisco, and Cyt *f* contents were according to Makino et al. (1994). The α and β subunits of CF1 and LHC II contents were also determined by rocket immunoelectrophoresis after solubilization with dodecyl sulfate according to Plumley and Schmidt (1983) with slight modification (Makino et al., 1994). Polyclonal-specific antibodies against the α and β subunits of CF1 and LHC II were used according to Hidema et al. (1991, 1992, respectively).

Activation state of Rubisco was determined according to Sage et al. (1993). The leaf was rapidly frozen in liquid nitrogen when the steady-state rate of photosynthesis at *pCi* = 20 Pa was attained with the gas-exchange system. Rubisco was quickly extracted at 0 to 4°C in 50 mM Hepes-NaOH (pH 8.0) containing 20 mM $MgCl_2$, 5 mM DTT, and ambient CO_2 concentrations in a chilled mortar and pestle with acid-washed quartz sand. The protease inhibitors were not added to the extraction buffer because they had no effect. After centrifugation at 0 to 2°C for 10 s, the initial activity of Rubisco was assayed at 25°C for 1 min in 100 mM Bicine-NaOH (pH 8.15) containing 25 mM $MgCl_2$, 5 mM $NaH^{14}CO_3$, and 0.6 mM RuBP. This procedure required about 120 s from the start of extraction to the start of an assay. Another portion of the extract was used for the assay of total Rubisco activity. The enzyme was injected into the above assay buffer without RuBP and then incubated at 25°C for 5 min. The total activity was started by adding RuBP. The activation state was taken as the ratio of the initial to total Rubisco activities. The amount of Rubisco protein in the extract was determined as described by Makino et al. (1985a).

The activities of cytosolic FBPase and SPS were determined by the methods of Sharkey et al. (1991) and Huber et al. (1989), respectively, as described by Makino et al. (1994). The SPS activity was measured under V_{max} substrate conditions.

Calculations of the CO_2 Transfer Conductance

The RuBP-saturated rate of CO_2 assimilation, A, (μmol m^{-2} s^{-1}) can be expressed by the equation (Farquhar and von Caemmerer, 1982):

$$A = (1 - \Gamma_*/pCc)\, v_c - Rd \qquad (1)$$

where v_c is the rate of RuBP carboxylation, Rd is the day respiration, and Γ_* is the partial pressure of CO_2 in the chloroplast at which photorespiratory CO_2 evolution equals the rate of carboxylation:

$$\Gamma_* = 0.5\, V_o\, K_c\, O/V_c\, K_o \qquad (2)$$

where V_c and V_o denote the maximum Rubisco activity of carboxylation and oxygenation (mol mol^{-1} Rubisco s^{-1}) and O is the partial pressure of O_2 in the chloroplast but assumed to be the same as in the atmosphere.

The RuBP-saturated rate of carboxylation, v_c, is given by:

$$v_c = E\ V_c\ pCc/[pCc + K_c\ (1 + O/K_o)] \quad (3)$$

where K_c and K_o are the Michaelis-Menten constants for CO_2 and O_2 and E is the amount of the activated Rubisco protein (μmol m^{-2}). Combining Equations 1 and 3, A is given by:

$$A = E\ V_c\ (pCc - \Gamma_*)/[pCc + K_c\ (1 + O/K_o)] - Rd. \quad (4)$$

The CO_2 transfer conductance from the intercellular air spaces to the carboxylation sites, g_w, is given by:

$$g_w = A/(pCi - pCc). \quad (5)$$

When $A + Rd$ is replaced with A and pCc is cancelled by combining Equations 4 and 5, g_w can be calculated by:

$$g_w = A\ (E\ V_c - A)/[pCi\ (E\ V_c - A) - A\ K_c\ (1 + O/K_o) - E\ V_c\ \Gamma_*]. \quad (6)$$

RESULTS

Gas-Exchange Characteristics among Temperature Treatments

The rate of CO_2 assimilation at 25°C was examined as a function of pCi in leaves of rice grown at different temperatures. Typical examples are shown in Figure 1. Growth temperature strongly influenced the CO_2 response curves of photosynthesis. The difference in the photosynthetic rate at high pCi between temperature treatments was considerably greater than that of the initial slope (upper panel). When the CO_2 response curve for each temperature treatment was normalized to the rate at pCi = 20 Pa, the respective curves at high pCi were greatly different from one another (lower panel). The ratio of the rate at high pCi to the initial slope was appreciably lower in the high-temperature-grown plants than in the low-temperature-grown plants. According to the photosynthetic model of Farquhar and von Caemmerer (1982) and Sharkey (1985), these results suggest that change in the in vivo balance between RuBP carboxylation and RuBP regeneration did occur with growth temperature and the RuBP regeneration capacity decreased relatively in the high-temperature-grown plants. Similar trends were also apparent with some *Eucalyptus* species (Ferrar et al., 1989).

Table I shows the O_2 sensitivity of photosynthesis at pCi = 20 Pa to a reduction of [O_2] from 21 to 2 kPa and the ratio of the photosynthetic rate at pCi = 20 Pa and 2 kPa O_2 to the CO_2-saturated maximum photosynthesis at 21 kPa. The high-temperature-grown plants exhibited lower O_2 sensitivity than the low-temperature-grown plants. The ratio of the rate at pCi = 20 kPa in 2 kPa O_2 to $pCi > 60$ Pa was higher in the high-temperature-grown plants, and the rate at pCi = 20 Pa in 2 kPa O_2 was already close to the CO_2-saturated rate in the 30/23°C-grown plants.

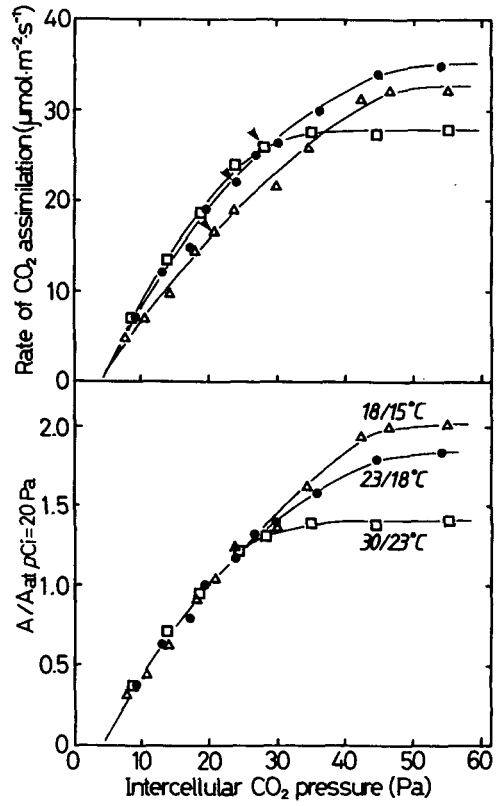

Figure 1. Rate of CO_2 assimilation (A) as a function of pCi in the leaves of rice grown at day/night temperature regimes of 18/15°C (△), 23/18°C (●), and 30/23°C (□). Upper, Response curves expressed in μmol m^{-2} s^{-1}. The arrowheads indicate the points obtained at an external CO_2 partial pressure of 35.5 Pa. Lower, 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}.

Photosynthesis and Leaf Nitrogen

Figure 2 shows the relationships between the photosynthetic rates at pCi = 20 Pa and $pCi > 60$ Pa, and stomatal conductance at pCi = 20 Pa versus total leaf nitrogen content. There was a considerable difference in the photosynthetic rate at pCi = 20 Pa for the same nitrogen content between temperature treatments. The photosynthetic rate at 20 Pa of the 30/23°C-grown plants was the highest and the rate of the 18/15°C-grown plants was the lowest. In addition, the relationships in the 30/23°C- and 23/18°C-grown plants were almost linear, whereas the relationship in the 18/15°C-grown plants was curvilinear. In contrast, there was no difference between the 30/23°C- and 23/18°C-grown plants in the photosynthetic rate at $pCi > 60$ Pa at any given leaf nitrogen content, but the rate from the 18/15°C-grown plants was a little low. Thus, the relative decrease in the photosynthesis at high pCi in the high-temperature-grown plants (Fig. 1) was not caused by the decrease in the rate at high pCi but was caused mainly by the increase in CO_2-limited photosynthesis for a given leaf nitrogen content. In stomatal conductance, there was a great difference among treatments. The 30/

Table I. *O_2 sensitivity of the CO_2 assimilation rate at pCi = 20 Pa and the ratio of the CO_2 assimilation rate at pCi = 20 Pa and 2 kPa O_2 ($A_{20/2}$) to the CO_2 assimilation rate at pCi > 60 Pa and 21 kPa O_2 ($A_{>60}$)*

Growth Temperature	O_2 Sensitivity[a]	$A_{20/2}/A_{>60}$
	%	%
18/15°C	35 ± 3[b]	80 ± 4
23/18°C	32 ± 1	84 ± 3
30/23°C	29 ± 1	98 ± 2

[a] Percent O_2 sensitivity equals (1 − *A* at 21 kPa O_2 divided by *A* at 2 kPa O_2) × 100% (Sage and Sharkey, 1987). [b] Means ± SE (n = 3).

23°C-grown plants showed the highest stomatal conductance and the 18/15°C-grown plants exhibited the lowest.

We next examined the relationships between several key photosynthetic enzymes and components and total leaf nitrogen content (Figs. 3 and 4). Rubisco content was the same at any given leaf nitrogen content irrespective of temperature treatment (Fig. 3). This relationship was clearly different from that between the photosynthetic rate at *pCi* = 20 Pa and leaf nitrogen content (Fig. 2). In contrast, there was a big difference in total Chl content among treatments. The Chl content was higher in the high-temperature-grown plants and lower in the low-temperature-grown plants. This relationship was also similar to that between LHC II and leaf nitrogen contents. Considering the change in Chl *a*/*b* ratio with growth temperature, however, the response of Chl to growth temperature was not caused only by that of LHC II. The Cyt *f* and CF1 contents and cytosolic FBPase and SPS activities at any given leaf nitrogen content did not differ between the 30/23°C-

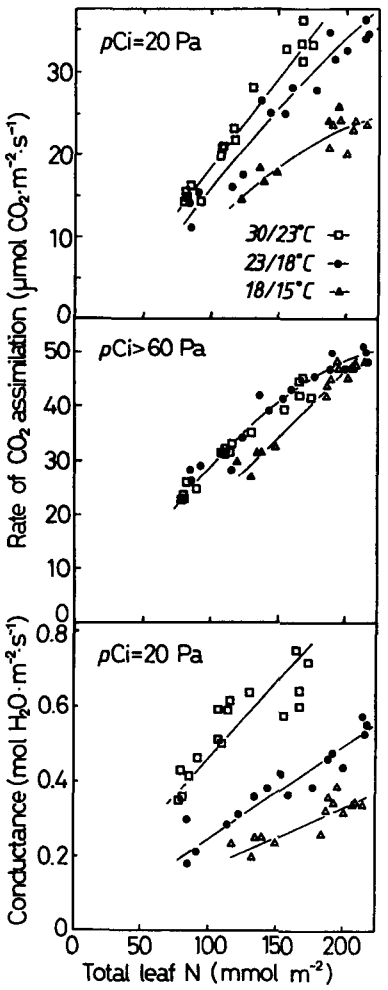

Figure 2. Rates of CO_2 assimilation at *pCi* = 20 Pa and *pCi* > 60 Pa and stomatal conductance at *pCi* = 20 Pa versus total leaf nitrogen content. Symbols are the same as in Figure 1. Measurements were made at a leaf temperature of 25°C and a PPFD of 1800 μmol quanta m^{-2} s^{-1}.

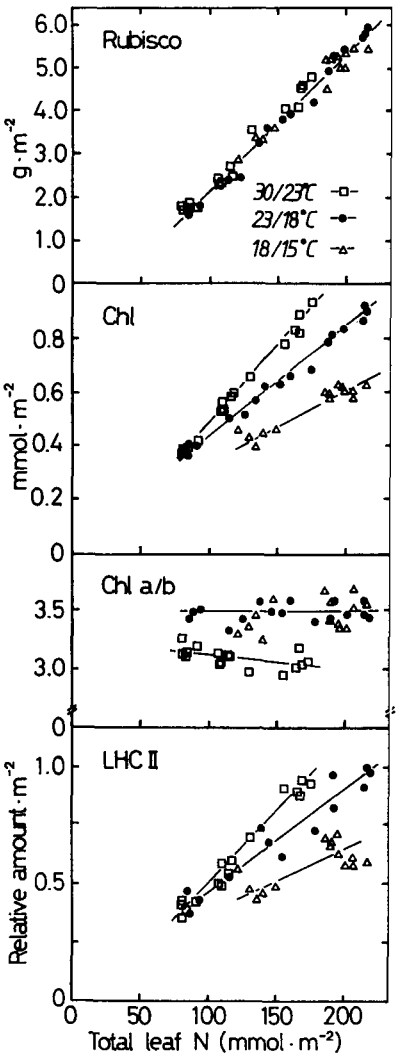

Figure 3. Rubisco content, total Chl content, Chl *a*/*b* ratio, and LHC II content versus total leaf nitrogen content. Symbols are the same as in Figure 1.

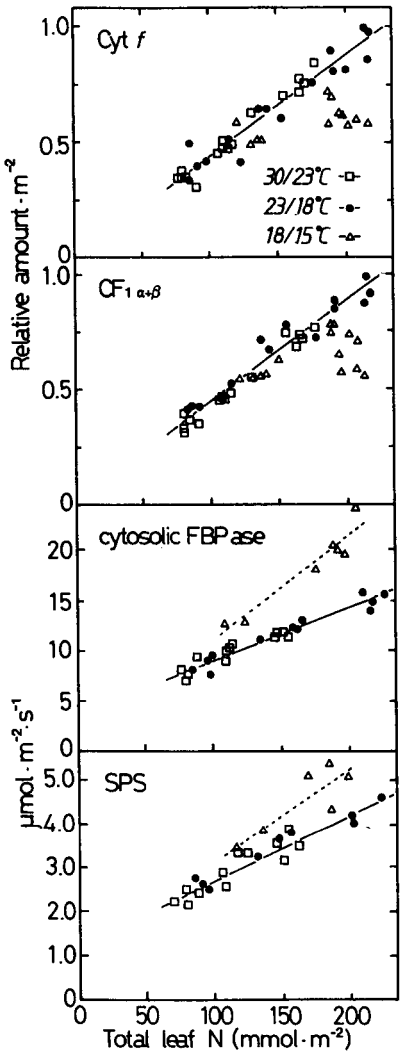

Figure 4. Cyt *f* and CF1 (α and β subunits) contents, and cytosolic FBPase and SPS activities versus total leaf nitrogen content. Symbols are the same as in Figure 1.

and 23/18°C-grown plants (Fig. 4). However, in high-nitrogen leaves of the 18/15°C-grown plants, the Cyt *f* and CF1 contents decreased, and in the same treatment cytosolic FBPase and SPS activities increased.

CO_2-Limited Photosynthesis versus Rubisco

We analyzed the relationship between the photosynthetic rate at pCi = 20 Pa and Rubisco content (Fig. 5). Although Rubisco is a limiting factor for CO_2-limited photosynthesis, there was a significant difference in the photosynthetic rate at pCi = 20 Pa for the same Rubisco content among temperature treatments. The photosynthetic rate per Rubisco content was the highest in the 30/23°C-grown plants and its rate was the lowest in the 18/15°C-grown plants. This difference was not essentially caused by a change in the activation state and/or catalytic turnover rate of Rubisco (Table II). Although the activation state of Rubisco was slightly lower in leaves

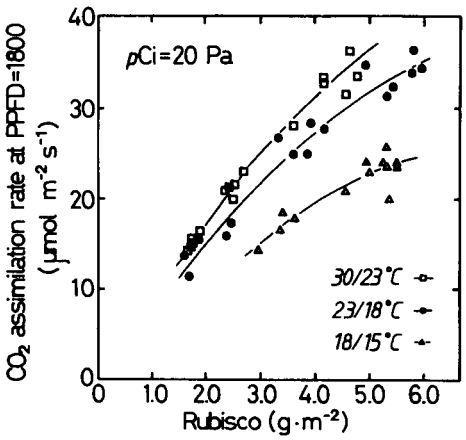

Figure 5. Rate of CO_2 assimilation at pCi = 20 Pa versus Rubisco content. Symbols are the same as in Figure 1.

from the 18/15°C-grown plants, its activation state was appreciably higher than that from the dark control. Thus, Rubisco was substantially activated under the conditions of 1800 μmol quanta m^{-2} s^{-1}, 20 Pa CO_2, and 25°C, irrespective of temperature treatment. In addition, growth temperature also had no effect on the catalytic turnover rate of Rubisco, although about 50% inhibition was found for the dark control (Table II). Therefore, we considered that the difference in the photosynthetic rate for the same Rubisco content among temperature treatments lies in a difference in the CO_2 transfer conductance between the intercellular airspaces and the carboxylation sites. We next tried to estimate the CO_2 transfer conductance in each treatment from the activated amount and kinetic constants of Rubisco and the gas-exchange data. However, since the in vitro catalytic turnover rate of Rubisco is generally lower than that predicted from gas-exchange measurements (von Caemmerer and Evans, 1991; Makino et al., 1994), the calculated absolute values are overestimated. Therefore, the values are given in relative terms, referenced to the 2 mM nitrogen treatment grown at 30/23°C (Table III). The estimated CO_2 transfer conductances were greatly different among temperature treatments, being the highest in the 30/23°C-grown plants and the lowest in the 18/15°C-grown plants. Thus, difference in CO_2-limited photosynthesis

Table II. *The activation state and catalytic turnover rate (k_{cat}) of Rubisco in leaves under the conditions of a PPFD of 1800 μmol quanta m^{-2} s^{-1}, a pCi of 20 Pa, and a leaf temperature of 25°C*

All enzyme assays were carried out at 25°C and pH 8.15.

Growth Temperature	Activation State	k_{cat}
	%	*mol CO_2 mol^{-1} s^{-1}*
18/15°C	91.3 ± 6.3[a]	13.6 ± 0.3
23/18°C	100.1 ± 3.3	14.1 ± 0.1
30/23°C	99.0 ± 2.5	14.0 ± 0.2
Dark control[b]	71.5 ± 3.7	7.5 ± 0.2

[a] Means ± SE (n = 3). [b] Samples were collected from the 30/23°C-grown plants kept at 25°C for 12 h in darkness.

Table III. *Estimated CO_2 transfer conductance at pCi = 20 Pa*

The CO_2 transfer conductance was estimated at the average values from each nitrogen treatment, using Equation 6 and the Rubisco kinetic constants from rice in Makino et al. (1988). The amount of activated Rubisco was estimated using the data in Table II.

Nitrogen Treatment	Growth Temperature		
	18/15°C	23/18°C	30/23°C
	Relative value		
0.5 mM	17	31	66
2.0 mM	23	45	100[a]
8.0 mM	23	46	75

[a] 100 corresponded to 1.44 mol CO_2 m^{-2} s^{-1}.

for the same nitrogen content among temperature treatments was caused by the difference in the CO_2 transfer conductance.

CO_2-Saturated Photosynthesis versus Electron Transport Components and Suc Synthesis Enzymes

Figure 6 shows the relationships between CO_2-saturated photosynthesis, Cyt *f* and CF1 contents, and cytosolic FBPase and SPS activities. No differences between the 30/23°C- and 23/18°C-grown plants were found for these relationships. Whereas Cyt *f* and CF1 contents were curvilinearly correlated with photosynthesis, cytosolic FBPase and SPS activities were linearly correlated. As well, the regression lines from Cyt *f* and CF1 almost passed through the origin, but those from FBPase and SPS crossed the *x* axis at positive values of enzyme activities. This means that at low rates of photosynthesis, Cyt *f* and CF1 contents are more closely related to CO_2-saturated photosynthesis, and at high rates of photosynthesis, the limitation by cytosolic FBPase and SPS activities come into play (for detailed discussion, see Makino et al., 1994). In the 18/15°C-grown plants, Cyt *f* and CF1 contents were poorly correlated with photosynthesis (r^2 = 0.45 and r^2 = 0.24, respectively; data omitted). Cytosolic FBPase and SPS activities were considerably higher than those in the other treatments for the same photosynthetic rate. Thus, these factors in the 18/15°C-grown plants did not limit CO_2-saturated photosynthesis.

DISCUSSION

It has been proposed by some researchers that at high irradiances, atmospheric CO_2 partial pressures, and normal temperatures close to the growth conditions, photosynthesis is co-limited by both the capacities of Rubisco and electron transport (von Caemmerer and Farquhar, 1981; Evans, 1986; Brooks, 1986; Evans and Terashima, 1988). At this time, nitrogen is considered to be optimally distributed between RuBP carboxylation and RuBP regeneration including electron transport (for a review, see Evans, 1989). However, short-term environmental change disrupts this balance between Rubisco and electron transport, and in some cases photosynthesis can be also limited by another factor, Pi regeneration during starch and Suc synthesis (Sharkey, 1985; Stitt, 1986; Sage et al., 1990; Makino et al., 1994). As described in the introduction, when plants are exposed to high temperatures photosynthesis is limited by Rubisco capacity. When plants are exposed to low temperatures photosynthetic limitation shifts to electron transport and/or starch and Suc synthesis. Therefore, if plants have a positive acclimation to temperature change, growth at high temperature should cause a relative increase in Rubisco capacity and the growth at low temperature should promote RuBP regeneration capacity.

In this study, the gas-exchange data suggested that rice was apparently acclimated to growth temperature (Fig. 1). The high-temperature-grown plants showed a relative increase in the initial slope, and the low-temperature-grown plants exhibited a relative increase in the photosynthesis at high *pCi*. However, the analysis of the relationship between gas-exchange rate and leaf nitrogen content showed that CO_2-limited photosynthesis increased with rising growth temperature, but CO_2-saturated photosynthesis was negatively affected by growth temperature (Fig. 2). In addition, it was a surprise that despite such a response of CO_2-limited photosynthesis, there was no difference in Rubisco content at any given leaf nitrogen content among temperature treatments (Fig. 3). This difference was the result of different CO_2 transfer conductance among temperature treatments (Table III). Furthermore, the existence of this difference in the CO_2 transfer conductance among temperature treatments was also supported by the difference in the O_2 sensitivity of photosynthesis at *pCi* = 20 Pa (Table I). Von Caemmerer and Evans (1991) have demonstrated that the O_2 sensitivity at low *pCi*

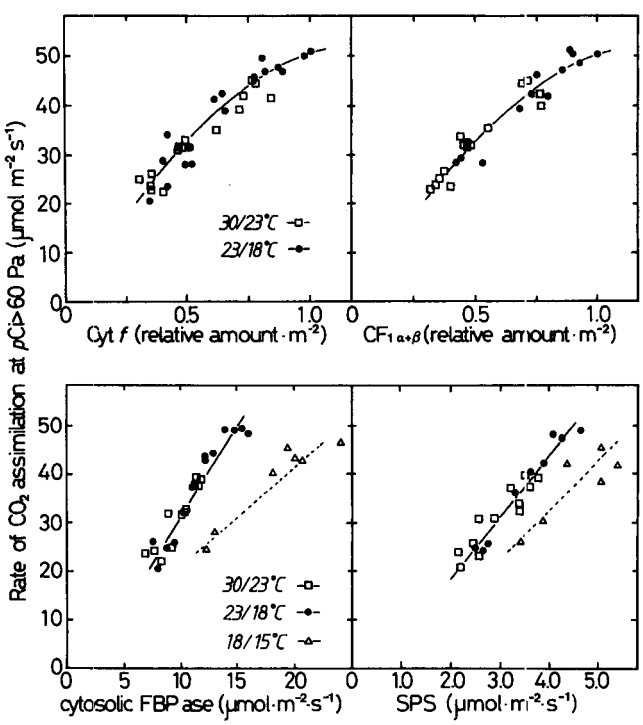

Figure 6. Rate of CO_2 assimilation at *pCi* > 60 Pa versus Cyt *f* and CF1 (α and β subunits) contents, and cytosolic FBPase and SPS activities. Symbols are the same as in Figure 1.

values at which Rubisco is rate limiting can be an indicator of the CO_2 transfer conductance. According to their theory, the plants having a greater CO_2 transfer conductance should show a more reduced O_2 sensitivity. This relationship between the CO_2 transfer conductance and the O_2 sensitivity can be found for our results in Tables I and III, although the O_2 sensitivity in the 30/23°C-grown plants may have been beyond *pCi* values at which Rubisco is rate limiting. Thus, growth temperature positively influenced the CO_2 diffusive conductance from stomata to the carboxylation sites.

On the other hand, the difference in growth temperature between 23/18°C and 30/23°C had no effect on the photosynthetic biochemical factors except Chl and LHC II. The response of Chl and LHC II can be regarded as a positive acclimation to growth temperature. Since the light-saturation point is enhanced with increasing leaf temperature (e.g. Ludlow and Wilson, 1971), the light requirement of plants may increase relatively with rising temperature. Although we found slight increases or decreases in some key components for the 18/15°C-grown plants (Fig. 4), these were not reflected in photosynthesis (Fig. 6).

The 25 to 30% difference in the photosynthetic rate at atmospheric CO_2 partial pressures between the 23/18°C- and 30/23°C-grown plants without changes in the biochemical limiting factors gives some doubt about the co-limitation by Rubisco and RuBP regeneration of photosynthesis at saturating irradiance and atmospheric CO_2. The studies of transgenic tobacco with reduced Rubisco (Hudson et al., 1992) clearly indicate that stomatal function is independent of Rubisco capacity and its balance to RuBP regeneration. This means that stomatal conductance is not linked to the flux of CO_2 and *pCi* at which co-limitation occurs. Our results also indicate that photosynthesis in the 23/18°C-grown plants was strongly limited by Rubisco capacity and that the limitation shifted toward RuBP regeneration in the plants grown at 30/23°C. Thus, the capacities of Rubisco and RuBP regeneration may not necessarily result in a similar potential rate at atmospheric air. In fact, there are some reports suggesting that maximum photosynthesis at atmospheric air is relatively limited by Rubisco capacity (Makino et al., 1985; 1988; Seemann et al., 1987; Hidema et al., 1991; Hudson et al., 1992; Masle et al., 1993).

In this study, growth temperatures appeared to strongly affect the CO_2 diffusion conductance from stomata to the carboxylation sites. There are many reports on the response of stomata to growth temperature, but much disagreement exists among the findings (see Berry and Björkman, 1980, for a review). This is because stomatal response to temperature is closely related to other factors such as the internal plant water status and the difference in the water vapor pressure between the leaf and the ambient air. Generally, in well-watered plants, stomata tend to open with rising temperature (Schluze et al., 1973; Lösch, 1977). Since our rice was grown by water culture, stomatal conductance may have increased depending on rising growth temperature. However, there is little information about the CO_2 diffusion inside the leaf. Boese and Huner (1990) reported that growth at low temperature resulted in an increase in leaf thickness. The leaf thickness might affect a CO_2 diffusion. Park and Tsunoda (1979) observed with EM images that when the rice plants were exposed to low temperature, their chloroplasts swelled and then accumulated excess starch. Starch accumulation with growth at low temperature was also found for other plants such as spinach (Guy et al., 1992) and *Solanum* species (Chen and Li, 1980). Although there are no available data on the causal relationship between starch accumulation and the CO_2 transfer conductance, it is possible that large starch grains hinder CO_2 diffusion in the chloroplast and reduce chloroplast surface area adjacent to plasma membrane. In addition, changes in lipid properties of the membranes with growth temperature might affect the permeability of CO_2. Thus, several morphological and/or physical factors would be related to the change in the CO_2 transfer conductance.

Rice is said to originate in southeast Asia as a summer-season crop, and essentially belongs to chilling-sensitive crops. Kabaki et al. (1982) reported that the growth rate and metabolism in rice are drastically changed in the range of 12 to 18°C. Similarly, Kishitani and Tsunoda (1974) found that a positive correlation between photosynthesis and leaf nitrogen was lost when rice was grown at a day/night temperature regime of 17/12°C. In our study with the 18/15°C-grown rice plants, gas-exchange data could not be theoretically analyzed from photosynthetic biochemistry and physiology. Unfortunately, in rice little is known about the biochemical mechanism(s) and factor(s) underlying the depression of potential photosynthesis under chilling temperatures (Terashima et al., 1989).

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