

Seasonal Changes in Temperature Dependence of Photosynthetic Rate in Rice Under a Free-air CO₂ Enrichment

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- **Background and Aims** Influences of rising global CO₂ concentration and temperature on plant growth and ecosystem function have become major concerns, but how photosynthesis changes with CO₂ and temperature in the field is poorly understood. Therefore, studies were made of the effect of elevated CO₂ on temperature dependence of photosynthetic rates in rice (*Oryza sativa*) grown in a paddy field, in relation to seasons in two years.
- **Methods** Photosynthetic rates were determined monthly for rice grown under free-air CO₂ enrichment (FACE) compared to the normal atmosphere (570 vs 370 µmol mol⁻¹). Temperature dependence of the maximum rate of RuBP (ribulose-1,5-bisphosphate) carboxylation (V_{cmax}) and the maximum rate of electron transport (J_{max}) were analysed with the Arrhenius equation. The photosynthesis–temperature response was reconstructed to determine the optimal temperature (T_{opt}) that maximizes the photosynthetic rate.
- **Key Results and Conclusions** There was both an increase in the absolute value of the light-saturated photosynthetic rate at growth CO₂ (P_{growth}) and an increase in T_{opt} for P_{growth} caused by elevated CO₂ in FACE conditions. Seasonal decrease in P_{growth} was associated with a decrease in nitrogen content per unit leaf area (N_{area}) and thus in the maximum rate of electron transport (J_{max}) and the maximum rate of RuBP carboxylation (V_{cmax}). At ambient CO₂, T_{opt} increased with increasing growth temperature due mainly to increasing activation energy of V_{cmax} . At elevated CO₂, T_{opt} did not show a clear seasonal trend. Temperature dependence of photosynthesis was changed by seasonal climate and plant nitrogen status, which differed between ambient and elevated CO₂.

Key words: Temperature dependence, photosynthesis, optimal temperature, activation energy, limiting step, temperature acclimation, free-air CO₂ enrichment (FACE), seasonal change, rice, *Oryza sativa*.

INTRODUCTION

Global atmospheric CO₂ concentration has risen from approx. 280 µmol mol⁻¹ in pre-industrial times to approx. 370 µmol mol⁻¹ now and may reach 570 µmol mol⁻¹ by 2050. Most global climate models predict that global surface temperature will increase by 3 °C, associated with increasing greenhouse gas emissions (IPCC, 2001). Influences of increasing CO₂ and temperature on plant growth and ecosystem function have become a major area of concern in recent decades (Mitchell *et al.*, 1995; Norby and Luo, 2004).

Photosynthesis, a key determinant of the rate of plant growth, is influenced by both CO₂ and temperature. Photosynthetic rates increase with a short-term increase in CO₂ concentration and are related parabolically to leaf temperature (von Caemmerer, 2000). These responses are mechanistically described by the biochemical model of photosynthesis (Farquhar *et al.*, 1980). The model has two major parameters, the potential rate of electron transport (J_{max}) and the maximum rate of RuBP (ribulose-1,5-bisphosphate) carboxylation (V_{cmax}).

The model of Farquhar *et al.* (1980) has contributed substantially to modelling gas exchange rates of plants and terrestrial ecosystems under changing environments. However, many modelling studies have ignored the effects of growth conditions on photosynthetic characteristics (long-term response). Photosynthesis often shows down-regulation under a long-term increase in CO₂ concentration (CO₂ acclimation; Sage, 1994; Ziska *et al.*, 1996; Seneweera *et al.*, 2002; Ainsworth *et al.*, 2003; Chen *et al.*, 2005). In many species, a long-term increase in temperature leads to an increase in the optimal temperature for maximal photosynthetic rate (temperature acclimation; Slatyer *et al.*, 1977; Berry and Björkman, 1980; Badger *et al.*, 1982; Ferrar *et al.*, 1989; Hikosaka *et al.*, 1999; Hikosaka *et al.*, 2006). Some recent studies have investigated responses in V_{cmax} and J_{max} to growth temperature (Hikosaka *et al.*, 1999; Bunce, 2000; Hikosaka, 2005; Yamori *et al.*, 2005) and to seasonal environment (Medlyn *et al.*, 2002a; Han *et al.*, 2004; Onoda *et al.*, 2005b). However, no study, as far as we know, has investigated seasonal change in temperature dependence of V_{cmax} and J_{max} under elevated CO₂ concentrations. We have investigated the effects on photosynthetic rate and seasonal acclimation of rice leaves

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grown in the field under current and increased CO₂ concentrations.

Field-grown plants were exposed to natural diurnal, seasonal and year-to-year fluctuations in leaf temperature in a free-air CO₂ enrichment (FACE) system that raises atmospheric CO₂ concentration in the field with minimal artefacts (Long *et al.*, 2004). Seasonal changes in photosynthetic characteristics were measured for two seasons. Temperature dependence of photosynthetic rates were analysed based on the model of Farquhar *et al.* (1980). Questions addressed here are: (1) does temperature dependence of photosynthesis change seasonally and, if so, how different is it between ambient and elevated CO₂? (2) What biochemical mechanisms are involved in the change in temperature dependence of photosynthesis? (3) Does growth temperature explain the seasonal change in the photosynthetic characteristics?

MATERIALS AND METHODS

The rice field was located at Shizukuishi in northern Honshu, Japan (39°38'N, 140°57'E, 200 m a.s.l.). Mean annual temperature and precipitation in 1976–2004 were 9.3 °C and 1540 mm, respectively. Elevated atmospheric CO₂ concentration (*C*_a) was created with a FACE system (Okada *et al.*, 2001), consisting of octagonal 12-m diameter CO₂ emission structures ('rings') established within the paddy. The target *C*_a at the centre of the rings was 200 μmol mol⁻¹ above ambient CO₂. The experiment was conducted over two years (2003 and 2004). The seasonal averages of *C*_a in the ambient CO₂ plots and in the elevated CO₂ plots were 384 ± 14 and 606 ± 29 μmol mol⁻¹ in 2003, and 366 ± 12 and 548 ± 28 μmol mol⁻¹ in 2004, respectively. Two ambient CO₂ (X and Z) and two elevated CO₂ plots (B and D) were used (for description of these plots see Okada *et al.*, 2001). Mean temperature and photosynthetic photon flux (PPF) during the experiment are shown in Table 1.

Rice (*Oryza sativa* L. 'Akitakomachi') plants were grown following the agronomic techniques typical of the local area (Kobayashi *et al.*, 2001; Anten *et al.*, 2003; Kim *et al.*, 2003). On 21 May 2003 and 20 May 2004, 25 d after emergence, seedlings were transplanted into paddies. Seedlings raised in a greenhouse under ambient CO₂ were planted in the ambient CO₂ plots, and those raised in another greenhouse under elevated CO₂ were planted in the elevated CO₂ plots. Distances between plants ('hills') and rows were 17.5 and 30 cm, respectively (equivalent to 19.1 hills m⁻²). The amounts of fertilizers added were: 8 g N m⁻² (25 % ammonium sulfate and 75 % LP-70) on 16 May in both 2003 and 2004, 30 g P₂O₅ m⁻² on 24 April 2003 and 19 April 2004, 15 g K₂O m⁻² on 25 April 2003 and 19 April 2004, respectively.

Photosynthetic measurements were made on the most recently fully expanded leaves in the experimental periods (leaf order and leaf age after emergence are given in Table 1). Photosynthetic rates were measured using an open gas exchange system (Model LI-6400, LiCor Inc., Lincoln, NE, USA), with an LED light source (LI-6400-02B, LiCor

TABLE 1. Climate conditions and leaf characteristics (order and age) for the photosynthetic measurements

Year	Measurement date	T _g (°C)	PPF (mol m ⁻² d ⁻¹)	Leaf order	Mean leaf age (d)
2003	18–24 June	17.7	34.9	8th	13
	15–25 July	18.5	25.3	11th	18
	20–30 August	20.9	23.7	14th	17
	11–15 September	19.1	22.0	15th	40
2004	23–28 June	18.1	36.9	9th	15
	21–26 July	21.7	22.6	12th	14
	21–23 August	21.9	31.3	13th	16
	14–18 September	19.7	24.9	14th	31

T_g and PPF are the mean daily growth temperature and mean daily photosynthetic photon flux, respectively, in the 2 weeks prior to measurements. Leaf order was numbered from the first leaf after germination.

and a dual Peltier device to regulate the PPF and temperature in the chamber (3 × 2 cm²).

Measurements were replicated using at least three leaves in each plot. CO₂ response curves of photosynthesis were determined at approx. 20, 25, 30 and 35 °C leaf temperature at PPF >1800 μmol m⁻² s⁻¹. The vapour pressure deficit (VPD) was kept at <1.5 kPa for 15–30 °C, and 1.5–2.5 kPa for 35 °C. Leaves were allowed to equilibrate for 5–10 min at each new temperature before measurement. For each CO₂ response curve, photosynthesis was first measured at the growth CO₂ concentration (ambient CO₂, 370 μmol mol⁻¹ or elevated CO₂, 570 μmol mol⁻¹; *P*_{growth}), and then the *C*_a was increased in eight steps from 50 to 1500 μmol mol⁻¹. In moving to a new CO₂ concentration, sufficient time was given (>5 min) to allow a steady-state to be attained prior to measurement of the photosynthetic rate. Immediately after gas exchange measurements were completed, the leaf was detached and 3-cm-long segments were excised (excluding the tip and base) and their width measured for calculation of area with an absolute digimatic caliper (Mitutoyo, CD-S15C, Kanagawa, Japan). The dry mass of leaf segments was determined after oven-drying at 70 °C for >72 h, and then the nitrogen content was determined using an NC analyser (Sumigraph NC-80, Shimadzu, Kyoto, Japan).

Models

The photosynthesis curve plotted against intercellular CO₂ concentration (*A*–*C*_i curve) was analysed to determine the maximum rate of RuBP carboxylation (*V*_{cmax}) and the maximum rate of electron transport (*J*_{max}) using the biochemical model of photosynthesis (Farquhar *et al.*, 1980). When ribulose-1,5-bisphosphate (RuBP) is saturated, the photosynthetic rate is determined by:

$$P_c = \frac{V_{cmax}(C_i - \Gamma^*)}{C_i + K_c(1 + O/K_o)} - R_d \quad (1)$$

where *P*_c is the photosynthetic rate limited by the Rubisco activity, *C*_i is the concentration of CO₂ at intercellular space, *Γ*^{*} is the CO₂ compensation point in the absence

of day respiration (R_d), K_c and K_o are Michaelis constants of RuBP carboxylase for CO_2 and O_2 , respectively, and O is the O_2 concentration. When RuBP regeneration limits photosynthesis, the photosynthetic rate is expressed as:

$$P_r = \frac{J_{\max}(C_i - \Gamma^*)}{4C_i + 8\Gamma^*} - R_d \quad (2)$$

where P_r is the photosynthetic rate limited by RuBP regeneration. The photosynthetic rate is the minimum of P_c and P_r .

The temperature dependence of kinetic parameters is described by the Arrhenius equation (Harley and Tenhunen, 1991; Bernacchi *et al.*, 2001):

$$f(T_k) = f(25) \exp\left[\frac{E_a(T_k - 298)}{298RT_k}\right] \quad (3)$$

where f is the value of a parameter. $f(25)$ is f at 25°C , E_a is the activation energy, R is the gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$) and T_k is leaf temperature in K.

We calculated values of K_c using eqn (3), where K_c at 25°C and E_a of K_c were assumed to be $404.9 \mu\text{mol mol}^{-1}$ and $79.43 \text{ kJ mol}^{-1}$, respectively. Similarly, K_o and Γ^* values were calculated assuming that K_o and Γ^* at 25°C were $278.4 \text{ mmol mol}^{-1}$ and $42.8 \mu\text{mol mol}^{-1}$, and E_a of K_o and E_a of Γ^* were $36.38 \text{ kJ mol}^{-1}$ and $37.83 \text{ kJ mol}^{-1}$, respectively (Bernacchi *et al.*, 2001). Using the calculated K_c , K_o and Γ^* values, eqn (1) was fitted to the C_i -response curves of photosynthesis at a lower range of CO_2 ($C_i < 300 \mu\text{mol mol}^{-1}$). R_d was assumed to be 0.02 of V_{cmax} (von Caemmerer, 2000). J_{\max} was calculated by fitting eqn (2) to a higher range of CO_2 ($C_i > 600 \mu\text{mol mol}^{-1}$). E_a of V_{cmax} and of J_{\max} were obtained from pooled data for each plot as a regression coefficient (eqn 3). Curve fitting was performed with Kaleida graph (Synergy Software, Reading, PA, USA).

Statistical analysis

Data are presented as means \pm s.e. Statistical tests were performed using SPSS 7.5.1 statistical software (SPSS Inc., Chicago, IL, USA). ANOVA (split-plot) was conducted to test the effects of year (main plot), CO_2 (subplot), months (sub-subplot) and their interactions on photosynthetic characteristics. Student's t -test was used for the effect of the CO_2 treatments.

RESULTS

The mean daily temperature (T_g) and mean daily PPF during a 2-week period prior to each measurement (Table 1) are considered as the 'growth environment' for the leaves; they varied seasonally. T_g was highest in August and lowest in June in both 2003 and 2004. When compared for the same month, T_g was slightly higher in 2004. PPF was highest in June and lowest in September in 2003 and in July in 2004.

Effects of elevated CO_2 and seasonal environment on photosynthetic characteristics

Seasonal changes in temperature dependence of the light-saturated photosynthetic rates per unit leaf area (P_{growth})

determined at the growth CO_2 concentration (Fig. 1) tended to increase to a maximum with increasing leaf temperature, and then either remained constant or decreased with further increase in leaf temperature. At any given temperature and month, P_{growth} was higher in leaves grown at elevated CO_2 . P_{growth} decreased as the growing season progressed (Table 2).

Stomatal conductance (g_s), determined at 25°C , was lower in leaves grown at elevated CO_2 (Tables 2, 3). It differed significantly between months, although no seasonal trend was observed. The average intercellular CO_2 concentration (C_i) at 25°C was 79.9% of C_a at ambient CO_2 and 80.7% of C_a at elevated CO_2 , and increased during the growing season in both ambient and elevated CO_2 (Tables 2, 3). Leaf nitrogen content per unit area (N_{area}) was not affected by CO_2 during growth, but declined during the growing season irrespective of CO_2 treatment (Tables 2, 3).

J_{\max} and V_{cmax} determined at 25°C ($J_{\max 25}$ and $V_{\text{cmax} 25}$, respectively) decreased during the season (Fig. 2A–D). Since the decrease in $V_{\text{cmax} 25}$ was greater than that in $J_{\max 25}$, the J_{\max}/V_{cmax} ratio increased (Fig. 2E, F). There was a significant effect of CO_2 on $V_{\text{cmax} 25}$ (Table 2), but was not on $J_{\max 25}$. $V_{\text{cmax} 25}$ tended to be lower at elevated CO_2 (Fig. 2C, D).

J_{\max} and V_{cmax} increased exponentially with leaf temperature: an example is shown in Fig. 3, with the curve fitted using the Arrhenius equation. The activation energy (E_a) is a measure of temperature dependence of photosynthetic rate. Since deactivation at high temperatures was not observed for either J_{\max} or V_{cmax} , we did not use a model characterized by an optimum (peak) (Medlyn *et al.*, 2002a, b). ANOVA suggested that the activation energy of J_{\max} (E_{aj}) was not different between leaves grown in different CO_2 concentrations (Table 2). However, the seasonal change in E_{aj} was not consistent across years and CO_2 conditions (Fig. 4). For example, at elevated CO_2 , E_{aj} increased seasonally in 2003 ($P = 0.004$, Fig. 4A), while it decreased in 2004 ($P = 0.003$, Fig. 4B). E_{av} was not affected by growth CO_2 but was significantly different among months (Table 2, Fig. 4C, D).

Modelling of temperature dependence of photosynthetic rate at growth CO_2 conditions

Using the above parameters C_i , J_{\max} , V_{cmax} , E_{aj} and E_{av} , we reconstructed the temperature dependence of photosynthetic rate at the CO_2 concentrations during leaf growth. There was a strong correlation between measured and estimated rates of photosynthesis ($y = 0.95x$; $r = 0.97$, $P < 0.0001$) with the regression was very close to the 1:1 line (Fig. 5). This indicates that the present photosynthesis model gave a fairly good quantitative description of the effect of years and CO_2 on growth. At ambient CO_2 , photosynthesis at optimal temperature was limited by P_c in both years, while at elevated CO_2 photosynthesis at optimal temperature was limited by P_r in earlier stages (June, July and August), and by P_c in the latest stage (September; data not shown). Temperature dependence of (relative) photosynthetic rate differed between ambient

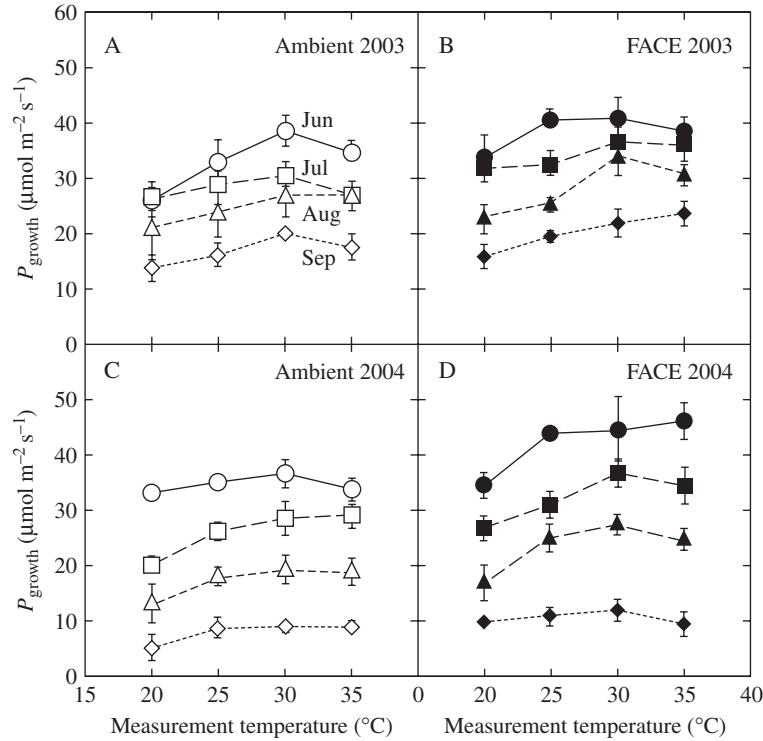


FIG. 1. Temperature dependence of the light-saturated rate of photosynthesis (P_{growth}) for rice (*Oryza sativa*) grown at (A, C) ambient CO_2 ($370 \mu\text{mol mol}^{-1}$, open symbols) and (B, D) at elevated CO_2 ($570 \mu\text{mol mol}^{-1}$, closed symbols) in 2003 (A, B) and 2004 (C, D). Measurements were made in June (circles), July (squares), August (triangles) and September (diamonds). Data from two plots are pooled and presented as mean \pm s.e. ($n = 6$).

TABLE 2. Summary of analysis of variance (ANOVA, presented as F-values) for the effects of month, CO_2 , year, and their interactions on the photosynthetic rate at 25°C under growth CO_2 concentration ($P_{\text{growth}25}$), leaf nitrogen content (N_{area}), stomatal conductance at 25°C (g_s), intercellular CO_2 concentration at 25°C (C_i), the maximum rate of electron transport at 25°C ($J_{\text{max}25}$), the maximum rate of RuBP carboxylation at 25°C ($V_{\text{cmax}25}$), the J_{max} to V_{cmax} ratio at 25°C (J/V), activation energy of J_{max} (E_{aj}), activation energy of V_{cmax} (E_{av}), and optimum temperature of photosynthesis predicted by the model (T_{opt})

Source of variation	d.f.	$P_{\text{growth}25}$	N_{area}	g_s	C_i	$J_{\text{max}25}$	$V_{\text{cmax}25}$	J/V	E_{aj}	E_{av}	T_{opt}
Year	1	10.11	0.23	19.92*	39.51*	22.58*	14.45	7.06	0.22	2.03	13.44
Main plot error	2	11.74	2.75	6.56	1.48	2.67	7.10	0.59	1.08	3.08	1.44
CO_2	1	462.73**	4.88	25.45*	1215.25***	3.19	26.71*	3.72	11.16	4.01	78.87*
$\text{CO}_2 \times \text{Year}$	1	17.19	0.41	12.83	5.44	0.19	7.61	1.88	0.46	5.11	4.16
Subplot error	2	0.10	2.28	0.18	1.72	0.83	0.49	1.25	0.97	0.73	0.80
Month	3	216.26***	175.72***	4.09*	19.87***	229.24***	307.07***	22.33***	0.83	6.41**	3.74*
Month \times CO_2	3	3.50*	6.72**	0.54	1.61	1.93	2.89	0.23	0.65	0.28	1.34
Month \times Year	3	11.01***	18.63***	4.64*	3.21	6.56**	7.91**	2.22	1.79	5.10*	5.66*
$\text{CO}_2 \times \text{Month} \times \text{Year}$	3	1.03	0.26	0.68	0.59	0.86	1.88	0.41	7.71**	10.33**	5.27*
Sub-subplot error	12										

$P_{\text{growth}25}$, N_{area} , g_s and C_i are measured values. $J_{\text{max}25}$, $V_{\text{cmax}25}$, J/V , E_{aj} , E_{av} and T_{opt} are calculated values. Significance levels: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

and elevated CO_2 (Fig. 6). The optimal temperature of photosynthesis (T_{opt} , the value where the photosynthetic rate was maximum) was significantly higher at elevated CO_2 (Table 2): it ranged from 22 to 34.5°C with an average value of 28.9°C at ambient CO_2 , and from 29.5 to 37°C with an average value of 33.5°C at elevated CO_2 . Temperature dependence of photosynthesis also showed a large seasonal change. There was a significant effect of month on T_{opt} (Table 2).

Relationship between growth temperature (T_g) and photosynthetic characteristics

There was no significant difference in E_{av} between leaves grown at the two CO_2 concentrations (Table 2). E_{av} was positively correlated with T_g across both CO_2 concentrations (Fig. 7A, $P = 0.025$). However, E_{aj} was not correlated with T_g (data not shown). There was a significant correlation between T_{opt} and T_g at ambient CO_2 ($P = 0.018$), but not at elevated CO_2 ($P = 0.122$; Fig. 7B).

TABLE 3. Stomatal conductance for water vapour (g_s) and intercellular CO_2 concentration (C_i) at $25^\circ C$ under growth CO_2 concentration, and leaf nitrogen content per unit area (N_{area}) for rice grown at ambient CO_2 ($370 \mu mol mol^{-1}$) and at elevated CO_2 ($570 \mu mol mol^{-1}$)

Year	Measurement date	g_s ($mol m^{-2} s^{-1}$)		C_i ($\mu mol mol^{-1}$)		N_{area} ($g m^{-2}$)	
		Ambient	FACE	Ambient	FACE	Ambient	FACE
2003	18–24 June	0.23 \pm 0.05	0.23 \pm 0.07	268 \pm 16	435 \pm 30***	1.91 \pm 0.26	1.95 \pm 0.17
	15–25 July	0.27 \pm 0.09	0.16 \pm 0.03*	283 \pm 13	420 \pm 15***	1.89 \pm 0.19	1.78 \pm 0.14
	20–30 August	0.28 \pm 0.07	0.18 \pm 0.03**	282 \pm 12	441 \pm 20***	2.12 \pm 0.18	1.73 \pm 0.14**
2004	11–15 September	0.27 \pm 0.09	0.20 \pm 0.07	298 \pm 17	449 \pm 30***	1.33 \pm 0.07	1.25 \pm 0.12
	23–28 June	0.34 \pm 0.05	0.32 \pm 0.04	292 \pm 3	464 \pm 9***	2.17 \pm 0.17	2.18 \pm 0.11
	21–26 July	0.32 \pm 0.06	0.30 \pm 0.04	293 \pm 12	458 \pm 18***	1.95 \pm 0.23	1.94 \pm 0.08
	21–23 August	0.41 \pm 0.07	0.44 \pm 0.10	318 \pm 4	499 \pm 10***	1.88 \pm 0.10	1.60 \pm 0.15**
	14–18 September	0.25 \pm 0.03	0.20 \pm 0.03*	329 \pm 6	509 \pm 11***	0.97 \pm 0.08	0.96 \pm 0.06

Means \pm s.e. ($n = 6$) are shown. Asterisks indicate significant differences between CO_2 treatments: ***, $P < 0.0001$; **, $P < 0.01$; *, $P < 0.05$.

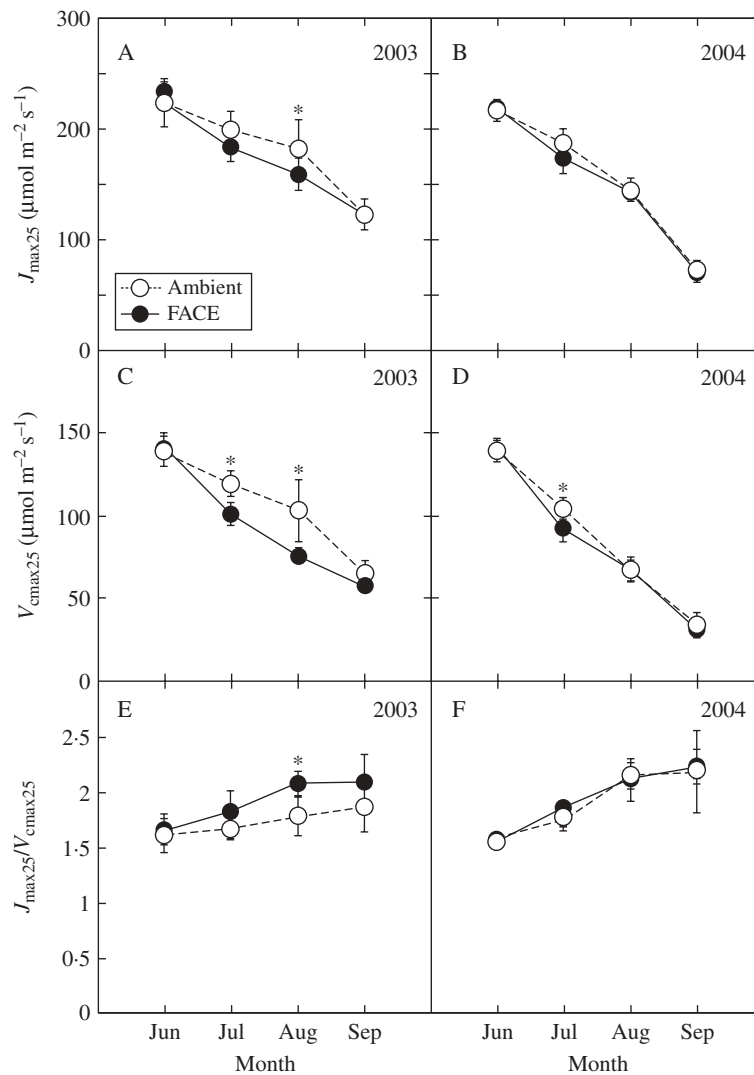


FIG. 2. Seasonal change in (A, B) the maximum rate of electron transport (J_{max25}), (C, D) the maximum rate of carboxylation (V_{cmax25}), and (E, F) the J_{max25}/V_{cmax25} ratio at $25^\circ C$ for rice (*Oryza sativa*) grown at ambient CO_2 ($370 \mu mol mol^{-1}$, open symbols) and at elevated CO_2 ($570 \mu mol mol^{-1}$, closed symbols) in 2003 (A, C, E) and 2004 (B, D, F). Data from two plots are pooled and shown as mean \pm s.e. ($n = 6$). Asterisks indicate significant differences between CO_2 treatments at $P < 0.05$.

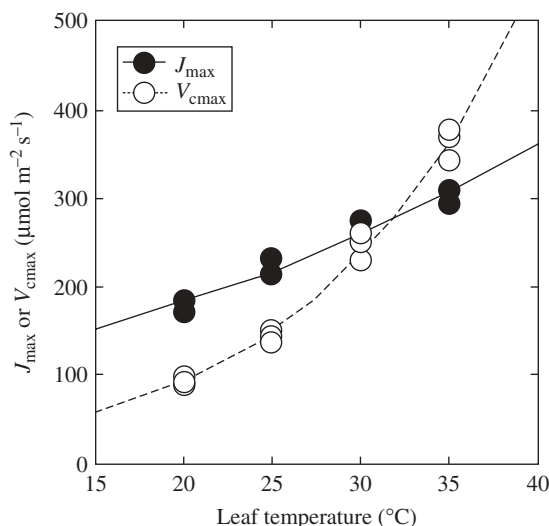


FIG. 3. An example of the temperature dependence of the maximum rate of electron transport (J_{\max}) and the maximum rate of carboxylation (V_{cmax}) of rice (*Oryza sativa*) grown at elevated CO_2 ($570 \mu\text{mol mol}^{-1}$) in June 2004. Data are fitted with the Arrhenius equation (eqn. 3). Activation energy of J_{\max} (E_{aj}) was $19.42 \text{ kJ mol}^{-1}$ and activation energy of V_{cmax} (E_{av}) was $52.93 \text{ kJ mol}^{-1}$.

DISCUSSION

The biochemical model of photosynthesis developed by Farquhar *et al.* (1980) is useful for predicting carbon exchange by plants under global environmental change because it represents a mechanism for the effects of elevated CO_2 on photosynthetic rates. The model is also useful for analysing temperature dependence of photosynthesis. As photosynthesis–temperature curves are parabolic with a broad peak, many data points are needed to obtain the optimal temperature (e.g. Cunningham and Read, 2002). However, as most of the parameters in the model of Farquhar *et al.* (1980) follow the Arrhenius equation, it is possible to describe photosynthetic response to temperature with a relatively small number of data points. The similarity between measured and estimated values (Fig. 5) suggests that the model gave a fairly good quantitative description of photosynthetic rates. Several studies have shown changes in temperature dependence of model parameters (such as E_{av} and E_{aj}) under a seasonal environment (Medlyn *et al.*, 2002a; Han *et al.*, 2004), but information is still insufficient when large acclimational change and interspecific differences are considered (Leuning, 2002; Medlyn *et al.*, 2002b). The present study is the first report showing a seasonal change in temperature dependence of photosynthetic parameters at elevated CO_2 .

Absolute photosynthetic rate (P_{growth})

Elevated CO_2 significantly increased P_{growth} (Fig. 1). This is simply ascribed to higher C_i (Table 3). However, a slight but significant decrease in $V_{\text{cmax}25}$ at elevated CO_2 (Fig. 2, Table 2) partly offset the effect of increased C_i . This down-regulation may be caused by sugar accumulation (Rey and Jarvis, 1998; Seneweera *et al.*, 2002; Rogers *et al.*, 2004) or by accelerated leaf senescence with advanced

plant development (Rogers *et al.*, 1996; Ludewig and Sonnewald, 2000; von Caemmerer *et al.*, 2001; Seneweera *et al.*, 2002).

At both CO_2 concentrations, $P_{\text{growth}25}$ (P_{growth} at 25°C) decreased as the plants grew (Fig. 1), consistent with previous studies for rice (Hasegawa *et al.*, 1996 for ambient CO_2 ; Seneweera *et al.*, 2002). This is attributed to the seasonal decrease in J_{\max} and V_{cmax} (Fig. 2), which is associated with the reduction in N_{area} (Table 3). Seasonal reduction in N_{area} may be related to plant ontogeny rather than environmental change. As plant mass increases, nutrient supply from the soil may become relatively insufficient, leading to a nitrogen deficiency in the plant body. In the later stages of the life cycle, reallocation of nitrogen to reproductive organs may also decrease nitrogen in vegetative parts. Mae and Ohira (1981) showed that about half of the nitrogen in vegetative organs was retranslocated to reproductive organs in rice.

Temperature dependence of photosynthesis

The optimal temperature of the photosynthetic rate determined by the model (T_{opt}) was higher at elevated than at ambient CO_2 (Figs 6, 7B). In earlier stages (June, July and August), this is attributed to the difference in the limiting step of photosynthesis: photosynthesis at T_{opt} was limited by P_c at ambient CO_2 and by P_r at elevated CO_2 (data not shown). In many species, P_c has a lower optimal temperature than P_r (Kirschbaum and Farquhar, 1984; Hikosaka, 1997; Hikosaka *et al.*, 1999; Onoda *et al.*, 2005b). This is because the increase in the carboxylation rate with increasing temperature is partly offset by the increase in photorespiration rate (Kirschbaum and Farquhar, 1984). In September, on the other hand, photosynthesis at T_{opt} was limited by P_c at both CO_2 concentrations. The increase in T_{opt} at elevated CO_2 is thus attributed to the effect of C_i on temperature dependence of P_c , which is directly influenced by the balance between carboxylation and photorespiration. Increasing CO_2 concentration decreases the contribution of photorespiration, which makes photosynthesis more temperature-dependent and increases the optimal temperature (Kirschbaum and Farquhar, 1984; Long, 1991).

T_{opt} showed a significant difference between months (Table 2). This may be partly explained by the increase in growth temperature at ambient CO_2 (Fig. 7B). Since P_c limited photosynthesis at ambient CO_2 , the change in T_{opt} was attributable to the change in E_{av} . According to the model, an increase in E_{av} by 10 kJ mol^{-1} leads to an increase in the optimal temperature of P_c by 5.4°C (Hikosaka *et al.*, 2006). E_{av} actually increased with growth temperature (Fig. 7A), which was consistent with other studies (Hikosaka *et al.*, 1999; Onoda *et al.*, 2005b; Yamori *et al.*, 2005). The increase in E_{av} with increasing growth temperature is a common response in C_3 species (Hikosaka *et al.*, 2006).

In contrast, at elevated CO_2 , T_{opt} showed neither a clear seasonal trend nor a dependence on T_g (Fig. 7B). This is because E_{aj} did not change with time (Table 2). In many species the temperature dependence of J_{\max} changes with

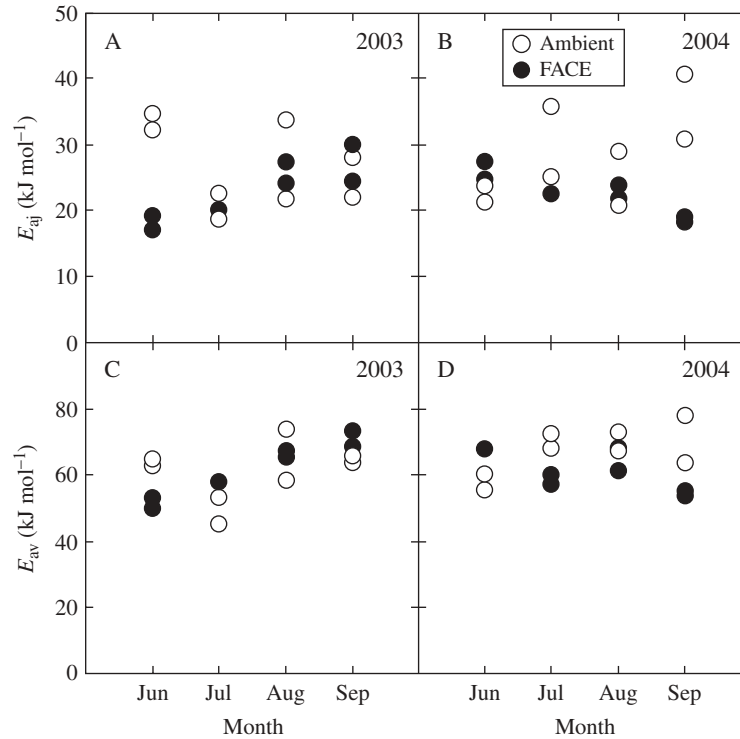


FIG. 4. (A, B) Seasonal change in the activation energy of J_{\max} (E_{aj}), and (C, D) activation energy of $V_{c\max}$ (E_{av}) for rice grown at ambient CO_2 ($370 \mu\text{mol mol}^{-1}$) and at elevated CO_2 ($570 \mu\text{mol mol}^{-1}$) in 2003 (A, C) and 2004 (B, D). One point denotes one plot.

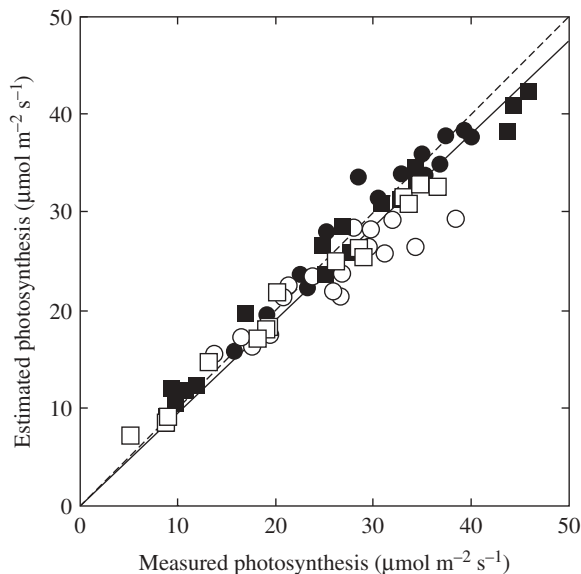


FIG. 5. Comparison between measured and estimated photosynthesis of plants grown at ambient CO_2 ($370 \mu\text{mol mol}^{-1}$, open symbols) and at elevated CO_2 ($570 \mu\text{mol mol}^{-1}$, closed symbols) in 2003 (circles) and 2004 (squares). The solid line is the regression $y = 0.95x$ ($r = 0.97$, $P < 0.0001$). The broken line indicates equivalence between measured and estimated photosynthesis.

growth temperature (Armond *et al.*, 1978; Badger *et al.*, 1982; Hikosaka *et al.*, 1999; Ziska, 2001; Yamasaki *et al.*, 2002), but in some species it does not (Sage *et al.*, 1995). The difference in T_g of less than 5°C in our study might

have been too small to detect a significant change in E_{aj} , or alternatively E_{aj} of rice was not affected by growth temperature. On the other hand, different seasonal trends in the dependence of E_{aj} between ambient and elevated CO_2 and between the two years (Fig. 4) suggest that factors other than temperature are involved in the change in E_{aj} .

At elevated CO_2 the limiting step of photosynthesis changed between the early (June, July and August) and the late stage (September), caused by a higher $J_{\max}/V_{c\max}$ ratio in September. We found a positive correlation between the $J_{\max}/V_{c\max}$ ratio and T_g (data not shown). However, this result is inconsistent with earlier studies: in some species the $J_{\max}/V_{c\max}$ ratio increased at low temperature (Hikosaka *et al.*, 1999; Hikosaka, 2005; Onoda *et al.*, 2005a; Yamori *et al.*, 2005) but in others it did not with growth temperature (Bunce, 2000; Hikosaka and Hirose, 2001; Medlyn *et al.*, 2002a; Onoda *et al.*, 2005b). For rice grown under controlled conditions, the $J_{\max}/V_{c\max}$ ratio was higher at low temperatures (Makino *et al.*, 1994). The inconsistency between earlier studies and ours may be caused by an alteration of the $J_{\max}/V_{c\max}$ ratio due to factor(s) other than T_g . Seneweera *et al.* (2002) found that flag leaves of rice had a lower $V_{c\max}$ per unit Rubisco than earlier leaves. A decrease in internal conductance of CO_2 diffusion may be involved in the seasonal change in the $J_{\max}/V_{c\max}$ ratio (von Caemmerer, 2000; Onoda *et al.*, 2005b).

CONCLUSIONS

There was an increase in the absolute value of P_{growth} and in the optimal temperature of the P_{growth} -temperature curve

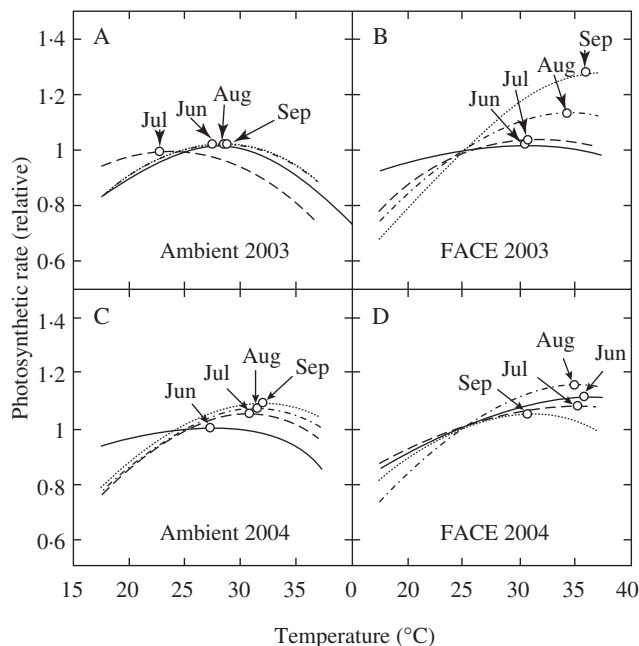


FIG. 6. Modelled temperature dependence of photosynthesis at (A, C) ambient CO_2 ($370 \mu\text{mol mol}^{-1}$), and (B, D) elevated CO_2 ($570 \mu\text{mol mol}^{-1}$) in 2003 (A, B) and 2004 (C, D) growing seasons (June, July, August and September). Values are normalized to 1 at 25°C . Circles indicate the maximum photosynthesis.

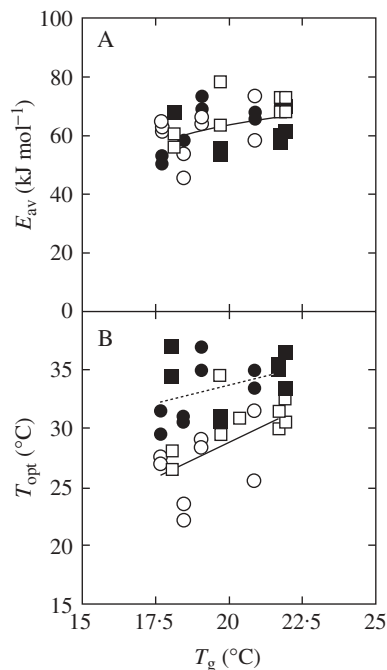


FIG. 7. Relationship between growth temperature (T_g , the mean daily temperature in the 2 weeks prior to measurements) and photosynthetic characteristics: (A) activation energy of V_{cmax} (E_{av}) and (B) the optimal temperature of photosynthesis at growth CO_2 (T_{opt}). Plants were grown at ambient CO_2 ($370 \mu\text{mol mol}^{-1}$, open symbols) and at elevated CO_2 ($570 \mu\text{mol mol}^{-1}$, closed symbols) in 2003 (circles) and 2004 (squares). Regression lines: (A) $y = 23.96 + 1.98x$ ($r = 0.40$, $P = 0.025$) and (B) $y = 4.86 + 1.21x$ ($r = 0.58$, $P = 0.018$, ambient), and $y = 20.92 + 0.64x$ ($r = 0.40$, $P = 0.122$, FACE). One point denotes one plot.

caused by elevated CO_2 concentration during growth and seasonal environment. Seasonal decrease in P_{growth} was associated with decrease in nitrogen status with plant growth, which decreased N_{area} and thus J_{max} and V_{cmax} . The seasonal change in the T_{opt} differed between the two CO_2 concentrations. At ambient CO_2 , T_{opt} increased with increasing growth temperature due mainly to increasing activation energy of V_{cmax} . At elevated CO_2 , T_{opt} did not show clear seasonal changes. This was partly caused by the seasonal increase in the $J_{\text{max}}/V_{\text{cmax}}$ ratio. Thus, the temperature dependence of photosynthesis was influenced by seasonal environment and reduction in nitrogen with plant growth, which was different between ambient and elevated CO_2 .

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