Rubisco activity is associated with photosynthetic thermotolerance in a wild rice (*Oryza meridionalis*)

Andrew P. Scafaro, Wataru Yamori, A. Elizabete Carmo-Silva, Michael E. Salvucci, Susanne von Caemmerer and Brian J. Atwell

*Department of Biological Sciences, Macquarie University, Sydney, New South Wales 2109, Australia*

*Plant Science Division, Research School of Biology, The Australian National University, Canberra, ACT 0200, Australia*

*USDA-ARS, Arid-Land Agricultural Research Center, Maricopa, AZ 85138, USA*

Correspondence
*Corresponding author, e-mail: brian.atwell@mq.edu.au*

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*Oryza meridionalis* is a wild species of rice, endemic to tropical Australia. It shares a significant genome homology with the common domesticated rice *Oryza sativa*. Exploiting the fact that the two species are highly related but *O. meridionalis* has superior heat tolerance, experiments were undertaken to identify the impact of temperature on key events in photosynthesis. At an ambient CO₂ partial pressure of 38 Pa and irradiance of 1500 μmol quanta m⁻² s⁻¹, the temperature optimum of photosynthesis was 33.7 ± 0.8°C for *O. meridionalis*, significantly higher than the 30.6 ± 0.7°C temperature optimum of *O. sativa*. To understand the basis for this difference, we measured gas exchange and rubisco activation state between 20 and 42°C and modeled the response to determine the rate-limiting steps of photosynthesis. The temperature response of light respiration (R_light) and the CO₂ compensation point in the absence of respiration (Γ*) were determined and found to be similar for the two species. C3 photosynthesis modeling showed that despite the difference in susceptibility to high temperature, both species had a similar temperature-dependent limitation to photosynthesis. Both rice species were limited by ribulose-1,5-bisphosphate (RuBP) regeneration at temperatures of 25 and 30°C but became RuBP carboxylation limited at 35 and 40°C. The activation state of rubisco in *O. meridionalis* was more stable at higher temperatures, explaining its greater heat tolerance compared with *O. sativa*.

Introduction

At current atmospheric CO₂ concentrations, photosynthesis is limited by two prevailing factors, the fixation of CO₂ by the enzyme rubisco and the regeneration of the rubisco sugar-phosphate substrate, ribulose-1,5-bisphosphate (RuBP) through the Calvin Cycle (Farquhar et al. 1980, Long and Bernacchi 2003, Sharkey et al. 2007). RuBP carboxylation-limited photosynthesis (A_c) has been attributed to the catalytic turnover rate of rubisco, the affinity of the enzyme for CO₂ and the competitive effects of O₂ (Jordan and Ogren 1984, Brooks and Farquhar 1985, Spreitzer and Salvucci 2002, Salvucci and Crafts-Brandner 2004a). RuBP regeneration limitation (A_r) is associated with the membrane-bound reactions of electron transport activity, the production of ATP and NADPH and the various Calvin Cycle enzymes that regenerate RuBP (von Caemmerer 2000, Sharkey 2005, Yamori et al. 2011b). As both membrane stability (Armond et al. 1980, Gounaris et al. 1984, Havaux et al. 1996) and rubisco (Law and

Abbreviations – PPFD, photosynthetic photon flux density; RuBP, ribulose-1,5-bisphosphate.
Crafts-Brandner 1999, Crafts-Brandner and Salvucci 2000) are susceptible to changes in temperature, it is not surprising that photosynthesis is significantly constrained at high temperatures. One would expect strong evolutionary pressure for thermotolerance of photosynthesis in tropical plants and indeed, it is now becoming apparent that the temperature-dependent limitation in photosynthesis is species specific and highly dynamic (Hikosaka et al. 2006, Yamori et al. 2011a). In species such as spinach (Spinacia oleracea), wheat (Triticum aestivum) and black spruce (Picea mariana), rubisco is thought to limit photosynthesis at temperatures above the photosynthetic optimum (Sage et al. 2008, Yamori et al. 2010b). In other species including tobacco (Nicotiana tabacum), sweet potato (Ipomoea batatas) and rice (Oryza sativa), RuBP regeneration is thought to be the limitation at and above the temperature optimum (Cen and Sage 2005, Makino and Sage 2007, Yamori et al. 2010b). Not only does the limiting factor change over a given temperature range within species but habitat and acclimation to growth temperatures prior to experimentation can also affect the type of limitation (Hikosaka et al. 1999, Onoda et al. 2005, Yamori et al. 2006). Considering the increase in global surface temperatures and likelihood of more extreme weather events, including heat waves, understanding what drives the limitation in photosynthetic capacity is fundamental to identifying how plants will adapt to changes in climate.

One way of gaining a better understanding of the temperature-dependent limitations of photosynthesis is by comparing similar species from contrasting climatic regimes. With this in mind, we compared the responses to temperature of two Oryza species, O. sativa L. and Oryza meridionalis Ng. There are more than 20 species in the genus Oryza (Ge et al. 1999) and nine distinct genomes; O. meridionalis shares the same genome with the two cultivated species, O. sativa from Asia and O. glaberrima from Africa (Nishikawa et al. 2005, Duan et al. 2007, Sweeney and McCouch 2007). O. meridionalis has a distribution throughout northern Australia and West Papua (Ng et al. 1981, Lu and Silitonga 1999, Henry et al. 2010), in a subtropical, monsoonal environment. It has previously been established that growth of O. meridionalis has superior tolerance to brief periods of severe heat up to 45°C relative to O. sativa ssp. japonica (Scafaro et al. 2010). In the same study it was shown that many of the proteins that change in abundance after heat exposure are related to photosynthesis. In particular, rubisco activase and cpn60, a chaperone known to interact with rubisco activase (Salvucci 2008), had highly regulated responses to heat stress in O. meridionalis. The difference in temperature susceptibility between these two highly related species provides an opportunity to evaluate photosynthesis limitations and determine any contrasting mechanisms in the response of photosynthesis to temperature.

Materials and methods

Plant material and growth

Seeds of O. meridionalis Ng. were collected from a wild accession located in the Cape York Peninsula of Australia (15°41′57″S; 145°02′48″E). O. sativa ssp. japonica cv. Amaroo seeds were obtained from the Yanco Agricultural Institute (NSW Department of Primary Industries, NSW, Australia). All plants were grown in a glasshouse at 28 ± 3°C in pots with an organic soil mix and slow release fertilizer applied following manufacturer’s instructions (Osmocote, Scotts, Baulkham Hills, Australia). All plants were well hydrated with approximately one-quarter of the bottom of pots submerged in water. Treatments were commenced after 45 days in the mid-tillering phase of development. All measurements were taken from healthy, young fully expanded leaves.

Determination of leaf mass, chlorophyll, rubisco content and rubisco activation state

For leaf mass per area (LMA), nitrogen, rubisco and chlorophyll determinations, a measured area of leaf (between 2 and 4 cm²), mid-lamina, was removed and immediately frozen in liquid nitrogen and stored at −80°C. Samples were taken from three plants from separate pots. LMA was calculated after drying the leaf material for 72 h at 70°C and weighing the dried material. Oven-dried samples were also analyzed for nitrogen content using a Leco CHN-900 gas analyzer (Leco, St Joseph, MI). Chlorophyll content and calculated chlorophyll a and chlorophyll b concentrations were determined using the method of Porra et al. (1989).

Total rubisco catalytic sites were quantified by stoichiometric binding of 14C-carboxy-arabinitol-P₂ (CABP-binding technique) according to Ruuska et al. (1998). Total sites were quantified using a CO₂-free extraction medium containing 50 mM Bicine-NaOH (pH 8.0), 10 mM MgCl₂, 15 mM NaHCO₃, 5 mM DTT, 2 mM EDTA, 1.5% (w/v) polyvinylpolypyrrolidone and 1.5% (v/v) protease inhibitor cocktail (Sigma, St Louis, MO).

For determination of rubisco activation state, leaf discs (0.5 cm²) were floated in 25 mM MES-NaOH (pH 5.5), contained within a water-jacketed beaker. The solution was flushed with a continuous gas stream (271 mol mol⁻¹ CO₂ in air) and gently stirred. The solution temperature was maintained by circulating water through the beaker jacket and monitored using a thermocouple placed just below the solution surface. After
30 min of illumination with 1000 μmol quanta m⁻² s⁻¹ at the temperatures indicated in the text, the discs were immediately frozen in liquid nitrogen. Frozen leaf discs were extracted in 100 mM Tricine-NaOH (pH 8.0, CO₂ free), 5 mM DTT, 1 mM EDTA, 5% polyvinylpyrrolidone (PVP40), 6% polyethylene glycol 3350 (PEG3350), 1 mM phenylmethylsulphonylfluoride and 10 μM leupeptin. Rubisco activities were determined by incorporation of 14CO₂ into acid-stable products at 30°C (Barta et al. 2011), immediately upon extraction (initial activity) and after incubation of the crude extract for 3 min with 10 mM MgCl₂ and 10 mM NaHCO₃ to allow carbamylation of all available catalytic sites (total activity). Duplicate assays were conducted on each sample. Four samples were measured at each temperature and the activation state was calculated as the initial activity divided by the total activity multiplied by 100.

**Gas-exchange measurements**

Net CO₂ assimilation rates (An) were measured using a Li-COR LI-6400 gas-exchange system (LI-6400, LI-COR, Lincoln, NE) similar to that of Yamori et al. (2010a). The CO₂ dependence of photosynthesis was determined by varying CO₂ partial pressure of the LI-6400, immediately upon extraction (initial activity) and after incubation of the crude extract for 3 min with 10 mM MgCl₂ and 10 mM NaHCO₃ to allow carbamylation of all available catalytic sites (total activity). Duplicate assays were conducted on each sample. Four samples were measured at each temperature and the activation state was calculated as the initial activity divided by the total activity multiplied by 100.

**Modeling of photosynthetic limitations**

The partial pressure of CO₂ at the site of chloroplast (Cc) was calculated from the relationship:

\[ C_c = C_i = \frac{A_n}{g_m} \]  (1)

where \( C_i \) (Pa) is the intercellular CO₂ partial pressure and \( g_m \) (μmol m⁻² s⁻¹ Pa⁻¹) is the mesophyll conductance. \( g_m \) was taken from values calculated by Scafaro et al. (2011) for rice over the observed temperature range. From the subsequently generated A–Cc curves, the rate-limiting step of photosynthesis was analyzed using the C₃ photosynthesis model (Farquhar et al. 1980, von Caemmerer and Farquhar 1981). RuBP carboxylation-limited photosynthesis (Aₚ) was determined from:

\[ A_p = \frac{V_{\text{cmax}}(C_c - \Gamma^* - \Gamma_{\text{R}})}{C_c + K_c(1 + O/K_o)} - R_{\text{light}} \]  (2)

where \( V_{\text{cmax}} \) (μmol m⁻² s⁻¹) is the maximum rate of RuBP carboxylation, \( K_c \) (Pa) and \( K_o \) (kPa) are the rubisco Michaelis constants for CO₂ and O₂, respectively, and \( O \) (21 kPa) is the O₂ concentration.

\( K_c \) and \( K_o \) were taken from tobacco measured over the same temperature range by Bernacchi et al. (2002). \( R_{\text{light}} \) is the light respiration and \( \Gamma^* \) is the CO₂ compensation point in the absence of respiration. Both were calculated from the Laik method (Laik 1977), where the intersection of three independent A–Cc curves with differing light irradiances corresponded to \( C^* \) (x-axis) and \( R_{\text{light}} \) (y-axis). \( C^* \), the CO₂ compensation point in the absence of respiration at an intercellular CO₂ concentration was converted to chloroplast CO₂ concentration using the mesophyll conductance \( (g_m) \) of rice determined by Scafaro et al. (2011) and the formula of von Caemmerer et al. (1994):

\[ \Gamma^* = C^* + \frac{R_{\text{light}}}{g_m} \]  (3)

The \( C_a \) range used to generate the curves was 12, 10, 7.5 and 5 Pa and the light irradiances were 100, 200 and 400 μmol quanta m⁻² s⁻¹ (Fig. S2). As \( K_c \) and \( K_o \) were taken from tobacco, we also modeled the data using tobacco \( R_{\text{light}} \) and \( \Gamma^* \) measured by Bernacchi et al. (2001, 2002) to determine if the parameter measurements we made affected the model outcome (Fig. S3). RuBP regeneration-limited photosynthesis (Aₚ) was determined from:

\[ A_r = \frac{I_g(C_c - \Gamma^*)}{4C_c + 8\Gamma^*} - R_{\text{light}} \]  (4)

where \( I_g \) (μmol m⁻² s⁻¹) is the chloroplast electron transport rate determined by gas exchange. Fitting of the model was performed using the software program GRAPHPAD PRISM (GraphPad Software Inc., San Diego, CA) and \( V_{\text{cmax}} \) and \( I_g \) were estimated from \( C_c \) observations below 20 Pa and above 30 Pa, respectively. The \( C_c \) values, at which the limitation of photosynthesis transitions from \( A_p \) to \( A_r \) (\( C_{\text{trans}} \)), were calculated using the
equation of von Caemmerer and Farquhar (1981):
\[ C_{\text{trans}} = \frac{K_c(1 + O/K_o)J_g/4V_{\text{cmax}} - 2\Gamma^*}{1 - J_g/4V_{\text{cmax}}} \]  
(5)

**Statistical analysis**

Differences between the two species in leaf properties and the temperature optimum of photosynthesis were assessed using two-sample t-tests (Table 1). A one-way ANOVA was used to assess temperature-dependent differences in photosynthesis. All graphs, curves and statistics were created using GRAPHPAD PRISM 5.0d software (GraphPad Software Inc.). All values given are means ± se of three to four plant replicates, from different pots.

**Results**

**Leaf properties, photosynthesis and respiration**

The leaves of *O. meridionalis* were characterized by significantly lower LMA as well as lower contents of nitrogen, rubisco and chlorophyll than *O. sativa* (Table 1). There was 22, 23 and 27% less nitrogen, rubisco and chlorophyll per unit leaf area, respectively, and these differences were manifested in a lower photosynthetic capacity on an area basis in *O. meridionalis* compared with *O. sativa* (see below). There was no difference in the ratio of rubisco and chlorophyll to nitrogen, or the ratio of rubisco to chlorophyll between the two species, indicating that nitrogen partitioning was similar between *O. sativa* and *O. meridionalis*. Likewise, the chlorophyll a/b ratio was not significantly different. Further evidence that the investment of nitrogen into the components of photosynthesis is similar between the species is the $J_g$ to $V_{\text{cmax}}$ ratio at $30^\circ\text{C}$, which showed no species difference, and in rice $J_g/V_{\text{cmax}}$ corresponds to the cytochrome $f$ to rubisco ratio (Yamori et al. 2011a).

The temperature response of net CO$_2$ assimilation ($A_n$) varied between domestic *O. sativa* and wild *O. meridionalis*. At a $C_\text{a}$ of 38 Pa and a PPFD of 1500 quanta m$^{-2}$ s$^{-1}$, the temperature optimum of $A_n$ ($T_{\text{opt}}$) for *O. sativa* peaked at $30.6 \pm 0.7^\circ\text{C}$, similar to previous reports (Makino and Sage 2007, Yamori et al. 2010b) and was significantly lower ($t = 2.913$, d.f. = 4, $P = 0.0436$) than the $T_{\text{opt}}$ for *O. meridionalis* at $33.7 \pm 0.8^\circ\text{C}$ (Table 1). At temperatures below or equal to $30^\circ\text{C}$, *O. meridionalis* had lower photosynthetic rates on a leaf area basis than *O. sativa* (Fig. 1A), but at temperatures of $35^\circ\text{C}$ and above there was no difference in photosynthetic rate between the species. The fact that $A_n$ decreased with an increase in temperature from 30 to $35^\circ\text{C}$ in *O. sativa* but not in *O. meridionalis* indicated that, unlike in *O. sativa*, the photosynthetic performance of *O. meridionalis* was not compromised by moderately high temperatures. Furthermore, the fall in $A_n$ with temperature in *O. sativa* was significant ($P = 0.006$), unlike *O. meridionalis* where there was no significant effect of temperature on $A_n$ ($P = 0.262$) across the temperature range. Because of the lower leaf nitrogen content of *O. meridionalis*, $A_n$ per unit of nitrogen for *O. meridionalis* matched the values for *O. sativa* at low temperatures and was greater than rates in *O. sativa* at temperatures above $35^\circ\text{C}$ (Fig. 1B), indicating greater photosynthetic nitrogen use efficiency at the higher temperatures by the wild relative.

The different temperature response of $A_n$ in the two species could not be attributed to a difference in CO$_2$ concentration in the leaves or to water relations, as $C_i$ and stomatal conductance ($g_s$) were similar in degree and response for the two species (Fig. 1C, D) and to previously reported values (Scafaro et al. 2011) over the same temperature range. Furthermore, the difference in $A_n$ between the species was not because of respiration as $R_{\text{light}}$ and its response to temperature was similar between the species, both on an area and on a nitrogen basis (Fig. 2A, B). $R_{\text{dark}}$ measured at $40^\circ\text{C}$ was also similar between the species and substantially higher than $R_{\text{light}}$ (Fig. 2C). The light to dark respiration ratio at $40^\circ\text{C}$ was 0.47 and 0.53 for *O. sativa* and *O. meridionalis*, respectively, showing that in rice $R_{\text{light}}$ is about 50% of $R_{\text{dark}}$. A lower $R_{\text{light}}$ than $R_{\text{dark}}$ is consistent with the ratios of $R_{\text{light}}/R_{\text{dark}}$ found in many other plant species (Brooks and Farquhar 1985, Peisker and Apel 2001, Pinelli and Loreto 2003, Tcherkez et al. 2005, Atkin et al. 2006).
Fig. 1. Impact of leaf temperature on the net CO₂ assimilation rate (Aₙ), per area of leaf (A) and per unit of nitrogen (B), intercellular CO₂ partial pressure (Cᵢ) (C) and stomatal conductance (gₛ) (D) for *Oryza sativa* ssp. *japonica* (closed circles/solid line) and *Oryza meridionalis* (open circles/dashed line) at an ambient CO₂ concentration (Cₐ) of 38 Pa. All measurements were made at a PPFD of 1500 μmol quanta m⁻² s⁻¹. Each point is the mean ± s.e. of measurements made on fully expanded leaves from four plants belonging to separate pots. Aₙ values were fit with quadratic equations.

Modeled rate-limiting steps of photosynthesis

Measured Γ⁺ and Rₗₐₐₜ₉ showed that the temperature response of these variables are very similar between the two rice species (Fig. 3). Γ⁺ and Rₗₐₜ₉ values were also similar to measurements made in tobacco by Bernacchi et al. (2001, 2002), which is important considering we used the Kₛ and Kₒ of tobacco for modeling. The common temperature response of Γ⁺ between tobacco and rice implies similar Kₛ and Kₒ (Brooks and Farquhar 1985) and the model parameters fit well with the observed Aₙ-Cᵢ curves (Fig. 4). At a Cₐ of 38 Pa, photosynthesis was Aₙ limited at 25 and 30°C for both *O. sativa* and *O. meridionalis* (Fig. 4A–D). For temperatures of 35 and 40°C the limitation for both species changed to an Aₖ limitation (Fig. 4E–H). When the transition point of CO₂ partial pressure from Aₙ to Aₖ limitation (C₉₆₅₈₉₉) is plotted against the recorded Cₛ at a Cₐ of 38 Pa, the similarity in photosynthetic limitations between the species is easily seen (Fig. 5). For our modeling, we used the Kₛ and Kₒ of tobacco with the Γ⁺ and Rₗₐₜ₉ measured in rice (Fig. 5–method 1). However, when we used the Γ⁺ and Rₗₐₜ₉ measured in tobacco by Bernacchi et al. (2001, 2002) the two methods gave similar results with the same limitation predicted (Fig. 5–method 2), thus affirming the reliability of the model.

Rubisco activity was measured at 271 μmol mol⁻¹ CO₂ in air because of tank availability, however, rubisco activity is insensitive to CO₂ partial pressures ranging from 36 to 6 Pa at high light irradiance (Sage et al. 2002). By excising leaf disks the temperature of the leaf material was accurately controlled and similarity between heat inactivation of leaf disks and attached leaves has been reported (Crafts-Brandner and Law 2000). The rubisco activation state of both rice species showed different responses over the temperature range of 20–42°C (Fig. 6A). For the wild *O. meridionalis*, the activation state was not affected by temperature variations between 20 and 35°C and only decreased substantially at the highest measured temperature of 42°C. In contrast, the activation state of *O. sativa* was constant between 20 and 25°C, before decreasing progressively with increasing temperatures above 25°C. At the critical temperature of 35°C close to the T₉ₖ₉₉ of *O. meridionalis* but well above the T₉ₖ₉₉ of *O. sativa*, the rubisco activation state was 66 ± 4% for *O. meridionalis*, significantly higher (P = 0.0386) than the 54 ± 3% observed in *O. sativa*. As the total activity of rubisco differed between the two species, further analysis of the initial rubisco activity (Fig. 6B) showed *O. sativa* and *O. meridionalis* to be matched at temperatures reaching 30°C before *O. sativa* values fell below *O. meridionalis* for temperatures above 30°C.
Discussion

The temperature response of An was different between O. sativa and O. meridionalis, with O. meridionalis having a greater tolerance to higher temperatures, particularly per unit of leaf nitrogen (Fig. 1). The heat-tolerant rice species O. meridionalis had a T_{opt} of 33.7°C, significantly higher than that of O. sativa and similar to the mean temperatures experienced in its natural habitat during the growing season. Data from the nearest weather station (15°50’S: 144°28’E), 60 km from the site of seed collection showed that the monthly mean daily maximum air temperatures for October, November and December were 33.7, 34.6 and 34.1°C, respectively (Australian Government Bureau of Meteorology; URL: http://www.bom.gov.au/climate/averages/tables/cw_028010.shtml). These temperatures, which exceed those typical of many rice-growing regions, may explain why O. meridionalis had a T_{opt} of 33.7°C and suboptimal photosynthetic capacity at lower temperatures. The photosynthetic temperature optima of many plants coincide with temperatures experienced in the corresponding natural habitats (Berry and Bjorkman 1980, Salvucci and Crafts-Brandner 2004b). The difference in the temperature response of photosynthesis between O. sativa and O. meridionalis was more pronounced than previously reported (Scafaro et al. 2011), however, the time exposed to each temperature was greater in this experiment and it is therefore likely that exposure time impacts upon An differentially between the rice species, with O. meridionalis having a greater ability to acclimate to high temperature.

The difference in the temperature response of An could not be attributed to differences in C_i, g_s or R_{light}, which were similar for both species (Figs 1C, D and 2). Furthermore, Scafaro et al. (2011) showed that O. sativa and O. meridionalis have a similar temperature response of g_m, which increases exponentially with temperature and results in a reduced chloroplast CO_2 drawdown at
higher temperatures. Therefore, the different temperature response of $A_n$ could not be attributed to $g_m$ either, even though this has been cited previously as a limitation to photosynthesis in rice (Makino et al. 1994, Li et al. 2009). A similar $R_{light}$ response between the two species is interesting as Atkin et al. (2006) found a close link between the temperature response of photosynthesis and respiration in the genus *Plantago*, with species from cold climates having a greater response to temperature for $R_{light}$ than for photosynthesis, relative to closely related species from warmer climates. However, temperature acclimation of respiration is highly dependent on species (Atkin et al. 2005) and, unlike *Plantago*, it seems that in *O. sativa* and *O. meridionalis* $A_n$ acclimates...
Fig. 5. Temperature limitations to photosynthesis at a $C_a$ of 38 Pa, based on transition points from $A_c$ to $A_r$ ($C_{\text{trans}}$, calculated from Eqn 5 in section Materials and methods) for *Oryza sativa* (A) and *Oryza meridionalis* (B). Estimated $C_c$ values (open circles/solid line) were plotted against $C_{\text{trans}}$ values that were modeled using the $\Gamma^*$ and $R_{\text{light}}$ parameters determined herein for rice (method 1, filled circles) and against $C_{\text{trans}}$ values obtained from the model using $\Gamma^*$ and $R_{\text{light}}$ parameters previously reported for tobacco by Bernacchi et al. (2001, 2002) (method 2, filled squares). Where $C_{\text{trans}}$ values were greater than those of $C_c$, photosynthesis is limited by RuBP carboxylation ($A_c$). This limitation is depicted by a lighter shade where only one of the modeled $C_{\text{trans}}$ curves is greater than $C_c$ and by a darker shade when both models predict an $A_c$ limitation.

The rate-limiting step of photosynthesis was the same for both species (Fig. 4). Photosynthesis was $A_c$ limited at 25 and 30°C, becoming $A_r$ limited at 35 and 40°C. Other studies indicate an $A_c$ limitation to photosynthesis in cultivated rice at similar temperatures (Makino and Sage 2007, Yamori et al. 2010b). It is thought that cold-sensitive species, including rice, are $A_c$ limited over a wide range of temperatures, while cold-tolerant species seem to be $A_r$ limited (Yamori et al. 2010b). In a similar study of Pima cotton (*Gossypium barbadense*) modeling indicated an $A_r$ limitation over a similar temperature range, attributed to inhibition of electron transport capacity (Wise et al. 2004). One possible explanation for reduced rubisco activity with heat is downregulation of $\Gamma^*$ for rice, tobacco and spinach over the temperature range measured, it seems that the temperature response of $\Gamma^*$ is relatively well conserved between species. In vitro rubisco studies by Galmés et al. (2005) showed that the rubisco specificity factor (and therefore $\Gamma^*$) and its temperature response varied among many non-cultivated species, particularly those from drier environments. However, when measured in vitro, differences in specificity factor because of drought were not found and a discrepancy between in vitro and in vivo measurements was noted (Galmés et al. 2006).

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in response to thylakoid damage inhibiting electron transport (Sharkey 2005).

In C3 species including rice it is becoming apparent that plants with lower nitrogen and rubisco concentrations have an increase in the \(I_{30}/V_{\text{max}}\) ratio, which in turn leads to a shift from \(A_e\) to \(A_c\) limitation (Yamori et al. 2011a). In rice, Makino et al. (1994) found a sharper response of \(A_n\) to increased nitrogen content when plants were grown at higher temperatures. It is, therefore, likely that nitrogen availability and the subsequent rubisco content have a significant effect on the photosynthesis limitation of rice, a possible reason for the \(A_e\) limitation at high temperatures in this study, but not in others.

Although the temperature response of respiration, CO2 conductance and water relations were not different between the rice species, a difference in the rubisco activation state at high temperatures was observed. Considering that temperature limitations to CO2 fixation by rubisco are associated with a decrease in the activation state (Law and Crafts-Brandner 1999, Crafts-Brandner and Law 2000, Salvucci and Crafts-Brandner 2004a), the ability to maintain rubisco in an active state at high temperatures would support a higher level of photosynthesis in \(O.\) meridionalis under hot conditions. This ability to maintain rubisco in an active state would explain the higher \(T_{\text{opt}}\) of photosynthesis in \(O.\) meridionalis, because rubisco was the limiting factor of photosynthesis at high temperatures for both species and any difference in rubisco activation state would affect the photosynthetic rate. A small but significant fall in rubisco activity will reduce \(A_n\) marginally. However, even a small decrease in \(A_e\) can have a relatively large impact on growth, evident in growth rate comparisons between \(O.\) sativa and \(O.\) meridionalis at relatively large impact on growth, evident in growth rate comparisons between \(O.\) sativa and \(O.\) meridionalis at high temperatures (Scafaro et al. 2010). Interestingly, the amount of rubisco activase, the protein responsible for maintaining rubisco in an active form (Crafts-Brandner and Salvucci 2000, Salvucci and Crafts-Brandner 2004b), was found to be highly regulated in response to heat stress in \(O.\) meridionalis (Scafaro et al. 2010). The highly regulated response of rubisco activase to temperature in \(O.\) meridionalis is consistent with the involvement of this protein in maintaining rubisco in an active state at high temperatures.

**Conclusions**

The wild rice \(O.\) meridionalis, adapted to a warmer climate than \(O.\) sativa, had a higher temperature optimum of photosynthesis. The rate-limiting step of photosynthesis was the same for both species, changing from RuBP regeneration to RuBP carboxylation as temperature increased. When compared with \(O.\) sativa, the activation state of rubisco in \(O.\) meridionalis was more stable at high temperatures. Considering that photosynthesis is \(A_c\) limited at high temperatures, the ability to maintain rubisco in an active state at high temperature would improve photosynthetic capability, thus accounting for the higher \(T_{\text{opt}}\) of \(O.\) meridionalis.

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Supporting Information
Additional Supporting Information may be found in the online version of this article:

Fig. S1. Determination of the optimum temperature of photosynthesis.

Fig. S2. Determination of Γ* and Rlight by the Laisk method.

Fig. S3. A–Cc models using tobacco Γ* and Rlight parameters.

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