Acclimation of biochemical and diffusive components of photosynthesis in rice, wheat and maize to heat and water deficit: implications for modeling photosynthesis.

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Abstract

The impact of the combined effects of heat stress, increased vapor pressure deficit (VPD) and water deficit on the physiology of major crops needs to be better understood to help identify the expected negative consequences of climate change and heat waves on global agricultural productivity. To address this issue, rice, wheat and maize plants were grown under control temperature (CT, 25°C, VPD 1.8 kPa), and a high temperature (HT, 38°C, VPD 3.5 kPa), both under well-watered (WW) and water deficit (WD) conditions. Gas-exchange measurements showed that, in general, WD conditions affected the leaf conductance to CO_2, while growth at HT had a more marked effect on the biochemistry of photosynthesis. When combined, HT and WD had an additive effect in limiting photosynthesis. The negative impacts of the imposed treatments on the processes governing leaf gas-exchange were species-dependent. Wheat presented a higher sensitivity while rice and maize showed a higher acclimation potential to increased temperature. Rubisco and PEPC kinetic constants determined i*n vitro* at 25°C and 38°C were used to estimated V_{cmax}, J_{max} and V_{pmax} in the modeling of C_3 and C_4 photosynthesis. The results here obtained reiterate the need to use species-specific and temperature-specific values for Rubisco and PEPC kinetic constants for a precise parameterization of the photosynthetic response to changing environmental conditions in different crop species.

Abbreviations

A_G, gross CO_2 assimilation rate; A_N, net CO_2 assimilation rate; C_a, atmospheric CO_2 concentration; C_c, chloroplastic CO_2 concentration; C_i, intercellular CO_2 concentration; CT, control temperature; F_o, basal fluorescence of a dark adapted leaf; F_s, steady-state fluorescence signal; g_{bs}, bundle sheath conductance to CO_2; g_m, mesophyll conductance to CO_2; g_s, stomatal conductance to CO_2; HT, high temperature; J_{max}, maximum photosynthetic electron transport rate; K_c, Michaelis-Menten constant of Rubisco for CO_2; k_{cat}^c, reaction turnover rate for carboxylation activity of Rubisco; K_o, Michaelis-Menten constant of Rubisco for O_2; K_P, Michaelis-Menten constant of phosphoenolpyruvate carboxylase for HCO_3; R_{dark}, mitochondrial respiration at pre-dawn; R_L, non-photorespiratory CO_2 evolution in the light; R_m, mesophyll mitochondrial respiration; PAR, photosynthetic active radiation; PSII, photosystem II; $S_{c/o}$, *in vitro* Rubisco specificity factor for CO_2/O_2; TAI, temperature acclimation index; TSI, temperature sensitivity index; V_{cmax}, maximum velocity of Rubisco carboxylation activity; V_{pmax}, maximum velocity of PEPC carboxylation activity; VPD, vapour pressure deficit; WD, water deficit; WW, well-watered; Γ*, photorespiratory CO_2 compensation point.

Keywords

Photosynthesis, high temperature, water deficit, crops, C_3, C_4.

Introduction

Mean global air temperatures are predicted to rise on average 0.3-0.6°C per decade over the next century, with heat waves becoming more frequent, intense and persistent (IPCC, 2013). In certain geographical regions, increased annual temperatures and heat wave frequency might be accompanied by decreased precipitation, causing decreased water availability for plants. While predicted increases in the concentration of atmospheric CO_2 may be positive for plant productivity (Long et al., 2006), in some agricultural regions these beneficial effects are likely to be offset by negative impacts of increased temperature and water deficit (Gornall et al., 2010). Hence, future predicted environments will compromise agricultural productivity and food security for the increasing world population. A more detailed understanding of the capacity of major crops, which sustain most of the human caloric intake, to respond and acclimate to water deficit and high temperature is key to mitigate the negative impacts of climate change on plant productivity.

Decreased crop productivity under water deficit and high temperature tend to be primarily caused by limited photosynthetic carbon assimilation and persisting mitochondrial respiration (Ainsworth and Ort, 2010; Atkin et al., 2006; Flexas et al., 2006a). Under water deficit, stomatal conductance (g_s) decreases, minimizing water loss, with parallel decreases in mesophyll conductance (g_m) (Flexas et al., 2013; Théroux-Rancourt et al., 2014). As a consequence, the capacity of the leaf to transfer CO_2 from the atmosphere to the sites of carboxylation in the chloroplast stroma decreases under drought conditions (Galmés et al., 2011). On the other hand, for most species, the photosynthetic machinery is robust enough to be largely unaffected by conditions of mild to moderate water deficit (Flexas et al., 2006a; Galmés et al., 2007b, 2013). Therefore, limitations in water availability decrease CO_2 assimilation mainly through diffusive rather than metabolic limitations (Flexas et al., 2006b; Galmés et al., 2007b). Increased temperature often results in increased vapor pressure deficit, which may exacerbate even more the diffusional limitations (Perez-Martin et al., 2009).

Photosynthetic processes are strongly temperature dependent, and moderate increases above the thermal optimum cause decreases in photosynthetic CO_2 uptake. Contrarily to water deficit, the negative impact of high temperature on the rate of CO_2 assimilation (A) is mostly due to biochemical limitations (Carmo-Silva et al., 2012; Scafaro et al., 2011). Rubisco activase is extremely heat-sensitive and this results in deactivation of Rubisco catalytic sites at moderately high temperatures (Salvucci and Crafts-Brandner, 2004; Yamori et al., 2011). Moreover, increases in the maximum catalytic rate of carboxylation (k_{cat}^c) with temperature are offset by decreases in the affinity of Rubisco for CO_2 (i.e., increases in the Michaelis-Menten constant for CO_2, K_c, and decreases in the specificity factor, $S_{c/o}$) and the lower CO_2/O_2 ratio in solution, which increase photorespiration (Sage and Kubien, 2007). On the other hand, mitochondrial respiration appears to be relatively unaffected by water availability but increases

with temperature compared to photosynthesis (Atkin and Macherel, 2009; Atkin and Tjoelker, 2003; Galmés et al., 2007c; Rodríguez-Calcerrada et al., 2010; Silim et al., 2010). Thus, the response of mitochondrial respiration to combined heat and drought would tend to further decrease the leaf carbon balance.

The above responses correspond to general trends observed when stresses are applied over relatively short periods of 15-20 days. In nature plants face long-term exposure to water deficit and high temperature, and photosynthesis and mitochondrial respiration have been shown to acclimate to both water (Flexas et al., 2009; Galmés et al., 2006; Walters, 2005) and heat stress (Berry and Björkman, 1980; Campbell et al., 2007; Sage and Kubien, 2007; Yamori et al., 2005), although the capacity and mechanisms of plant acclimation may differ between species (Cheesman and Winter, 2013; Dillaway and Kruger, 2011; Hikosaka et al., 2006; Kattge and Knorr, 2007; Scafaro et al., 2011). In semi-arid climates like the Mediterranean, drought and heat stress occur simultaneously and exert a combined effect on plant functioning (Mittler, 2006).

Most mechanistic models of carbon uptake and release in C_3 and C_4 leaves currently do not account for long-term responses to changes in the environmental conditions (e.g., von Caemmerer, 2000; Hu et al., 2010; Pittermann and Sage, 2001). Further, these models are usually parameterized with invariable values and temperature responses for the Rubisco kinetic parameters and g_m among different species. In fact, the use of Rubisco kinetics and g_m values experimentally measured in *Nicotiana tabacum* have been employed in most of the studies modelling leaf gas exchange responses to variations in the environment (Bernacchi et al., 2001, 2002, 2003; von Caemmerer, 2013; Diaz-Espejo, 2013). However, Rubisco kinetic constants and g_m, as well as their dependence on temperature vary among species. These species-specific differences in Rubisco parameters explain differences in photosynthetic responses to temperature, and significantly bias modeling of C_3 photosynthesis (von Caemmerer and Evans, 2015; Diaz-Espejo, 2013; Flexas and Diaz-Espejo, 2015; Galmés et al., 2014; Walker et al., 2013). Further, C_4 photosynthesis modelling at variable temperatures has received little attention (von Caemmerer, 2013; Massad et al., 2007; Sage and Kubien, 2007). It is important that future approaches incorporate the temperature dependence of phosphoenolpyruvate carboxylase (PEPC) activity and the thermal response of the underlying kinetic parameters, such as the affinity of PEPC for CO_2 (K_P).

Rice (*Oryza sativa*), wheat (*Triticum aestivum*) and maize (*Zea mays*) are major commercially important crops. Together, these species account for ~85% of global cereal production and contribute the majority of the energy in food of humans eaten directly as staple foods or indirectly through consumption of livestock fed with grain (Grassini et al., 2013). The three cereals were domesticated in different climates and differ largely in their growth environments: rice and maize are cultivated in tropical hot and wet climates, whereas wheat

tends to be grown in cooler temperate climates (Makino, 2011). Further, these species differ in their photosynthetic mechanism, maize is a C_4 crop, and rice and wheat are C_3 crops. The objectives of the present study were: i) to analyze the patterns of response of leaf photosynthesis and respiration to long-term drought, vapor pressure deficit (VPD) and temperature stress in these three crops; ii) to compare the sensitivity and acclimation capacity of leaf photosynthesis and respiration to these stresses in the three species; and iii) to compare the effect on C_3 and C_4 photosynthetic models of using species-specific kinetics of Rubisco and PEPC, and their species-specific response to temperature.

Material and methods

Plant material, growth conditions and treatments

Plants of rice (*Oryza sativa* L. cv. Bomba), wheat (*Triticum aestivum* L. cv. Cajeme) and maize (*Zea mays* cv. Carella) were grown from seeds in a greenhouse in 3.5 L pots containing a 70:30 mixture (v:v) of horticultural substrate (Projar S.A, Spain) and perlite (granulometry A13, Projar S.A, Spain). After 2 weeks, seedlings were selected to uniform size and were moved to a controlled environment room. Light was provided by metal halide lamps (OSRAM, Germany) placed at specific distances from the plants to obtain a photosynthetically active photon flux density (PPFD) of 500 µmol m^{-2} s^{-1}, with a photoperiod of 12 h day/12 h night. Ambient temperature and relative humidity were monitored with portable sensors Testo 175-H1 data logger (Gerilab, Spain). Relative humidity (RH) was maintained at 40-60% using humidifiers. For logistical reasons, assays were performed in two consecutive experiments with two sets of plants of identical age. For the first experiment a first set of plants of the three species was grown under control conditions (CT, 25/20°C day/night), which combined with the set RH resulted in a vapor pressure deficit (VPD) of 1.8/1.0 kPa day/night. A second set of plants, for the second experiment, was grown at higher temperature (HT, 38/33°C, resulting in VPD 3.5/2.3 kPa day/night). Only temperature and VPD differed between the two sets of plants or experiments, while all other environmental conditions (e.g. light intensity and quality, air removal, photoperiod duration) were identical and computer-controlled.

For each set of plants, i.e. for each growing temperature and VPD treatment, ten pots per species were grown at soil field capacity until plants presented fully expanded leaves (typically two weeks). Thereafter, twenty days after germination, pots of all species were randomly assigned to two irrigation treatments: five pots per species were maintained at field capacity throughout the experiment (well-watered treatment, WW) and five were maintained at 45% of field capacity (moderate water deficit treatment, WD). The level of water availability was determined gravimetrically by weighing the pots daily and maintained by compensating water losses with 50% Hoagland's solution. Plants were considered to be under water deficit when g_s

was decreased by 40% compared to the well-watered plants; g_s was considered as a good indicator of the water deficit status, as previously demonstrated (Medrano et al., 2002).

New leaves were allowed to develop and expand under the two irrigation treatments for a minimum of 30 days. All measurements and samples were taken 40-50 days after the water treatment was initiated (i.e., 60-70 days after germination), on new leaves developed completely under the temperature and/or water treatments (Perdomo, 2015).

Gas exchange and chlorophyll* a *fluorescence measurements

Leaf gas exchange and chlorophyll *a* fluorescence measurements were performed with a portable photosynthesis system (Li-6400; Li-Cor Inc., USA) equipped with a leaf chamber fluorometer (Li-6400-40, Li-Cor Inc.), the latter using the multi-flash protocol (Loriaux et al., 2013). The response of net CO_2 assimilation rate (A_N) to varying intercellular airspace CO_2 concentration (C_i) was measured on the youngest fully expanded leaf at a saturating photosynthetic active radiation (PAR) of 1500 µmol m^{-2} s^{-1} (10% blue light), a relative humidity of the incoming air between 40 and 50% and at two leaf temperatures: 25°C and 38°C. A_N-C_i curves were initiated by allowing the leaf to reach steady-state A_N and stomatal conductance (g_s) at a CO_2 concentration in the leaf chamber (C_a) of 400 µmol CO_2 mol^{-1} air, before varying the C_a between 50 and 2000 µmol CO_2 mol^{-1} air. Corrections for the leakage of CO_2 into and out of the leaf chamber were applied to all gas-exchange data (Flexas et al., 2007).

The photochemical efficiency of photosystem II (Φ_{PSII}) was determined according to Genty et al. (1989):

$$\Phi_{PSII} = (F_m' - F_s) / F_m' \quad [1]$$

where F_s is the steady-state fluorescence yield and F_m' the maximum fluorescence yield obtained with a light-saturating pulse of 8000 µmol m^{-2} s^{-1}.

Φ_{PSII} was used for the calculation of the linear rate of electron transport (ETR) according to Krall and Edwards (1992):

$$ETR = \Phi_{PSII} \cdot PPFD \cdot \alpha \cdot \beta \quad [2]$$

where α is the leaf absorptance and β is the partitioning of absorbed quanta between photosystems I and II. β was assumed to be 0.5 (Laisk and Loreto, 1996; Tosens et al., 2012). α was measured for all species grown under each treatment inside a dark chamber using the light source from the Li-6400 and a spectroradiometer (HR2000CG-UV-NIR; Ocean Optics Inc., USA), as described by Schultz (1996). All values obtained for α were 0.86-0.87, with non-significant differences between species and species × treatment combinations.

Modelling C_3 photosynthesis in wheat and rice

From combined gas-exchange and chlorophyll *a* fluorescence measurements, mesophyll conductance to CO_2 (g_m) was estimated for wheat and rice according to the variable J method (Harley et al., 1992):

$$g_m = A_N / (C_i - (\Gamma^* (ETR + 8 (A_N + R_L)) / (ETR - 4 (A_N + R_L)))) \quad [3]$$

where A_N and C_i were obtained from gas exchange measurements at saturating light. The rate of non-photorespiratory CO_2 evolution in the light (R_L) was determined as half of the mitochondrial respiration at pre-dawn (R_{dark}), which was measured at a C_a of 400 µmol CO_2 mol^{-1} air and leaf temperatures of 25°C or 38°C. The chloroplast CO_2 compensation point in the absence of mitochondrial respiration (Γ^*) was calculated from the *in vitro* measurements of Rubisco specificity factor ($S_{c/o}$) as:

$$\Gamma^* = \frac{0.5\,O}{S_{c/o}} \quad [4]$$

A_N-C_i curves were converted into A_N-C_c curves using the values of g_m:

$$C_c = C_i - (A_N / g_m) \quad [5]$$

Maximum velocity of Rubisco carboxylation (V_{cmax}) and maximum electron transport rate (J_{max}) were calculated from A_N-C_c curves according to Bernacchi et al. (2002), but using the Rubisco kinetic constants (the Michaelis-Menten constants for CO_2 and O_2 and the $S_{c/o}$) measured for each species at 25°C and 38°C. For comparative purposes, V_{cmax} and J_{max} were also calculated for rice and wheat using the values for the Rubisco kinetics parameters and respective temperature dependencies reported for tobacco by Bernacchi et al. (2001, 2002).

Modelling C_4 photosynthesis in maize

The C_4 photosynthesis model described by von Caemmerer (2000) was applied to the A_N-C_i curves measured for maize as detailed by Massad et al. (2007), with the modifications of Carmo-Silva et al. (2008). The maximum velocity of Rubisco carboxylation (V_{cmax}) and the maximum velocity of PEPC carboxylation (V_{pmax}), as well as the CO_2 concentrations in the bundle sheath (C_s) and in the mesophyll cells (C_m) were estimated from the hyperbolic function describing the A_N-C_i curves using a C_i step-size of 5 µmol mol^{-1}, by applying the equations:

$$A_N = g_m(C_i - C_m) \quad [6]$$

$$A_N = \frac{C_s V_{cmax}}{C_s + K_c\left(1 + O/K_o\right)}\left(1 - \frac{\Gamma^* O}{C_s}\right) - R_L \quad [7]$$

$$A_N = \frac{C_m V_{pmax}}{C_m + K_p} - g_{bs}(C_s - C_m) - R_m \quad [8]$$

In these equations, the oxygen partial pressure in the bundle sheath and mesophyll cells (O), the bundle sheath conductance to CO_2 (g_{bs}) and the mesophyll conductance to CO_2 (g_m)

were assumed to be invariable between water and temperature treatments, as in Carmo-Silva et al. (2008) and Massad et al. (2007), respectively. Constant values for these parameters were O = 210 mbar, g_{bs} = 3 mmol m^{-2} s^{-1} and g_m = 2 mol m^{-2} s^{-1} (von Caemmerer 2000).

The model also requires values for kinetic constants of Rubisco and PEPC: the Rubisco specificity for CO_2/O_2 ($S_{c/o}$, from which Γ* is calculated as 0.5 $O/S_{c/o}$), the Michaelis-Menten constants of Rubisco for CO_2 (K_c) and O_2 (K_o), and the Michaelis-Menten constant of PEPC for CO_2 (K_p). V_{cmax} and V_{pmax} values calculated using the *in vitro* kinetic constants of maize Rubisco and PEPC at 25°C and 38°C were compared to V_{cmax} and V_{pmax} calculated using the values at 25°C for Γ* (0.000193), K_c (65 Pa) K_o (45 kPa) and K_p (8 Pa) reported in von Caemmerer (2000). The temperature equations provided by Bernacchi et al. (2001, 2002) were used to calculate values for the Rubisco kinetic constants at 38°C, while K_p was assumed to be invariable with temperature changes.

Determination of Michaelis–Menten constants of Rubisco and PEPC for their gaseous substrates

The Michaelis-Menten constants of Rubisco for CO_2 (K_c) and O_2 (K_o) were determined at 25°C and 38°C using leaf samples of rice, wheat and maize, as previously described Galmés et al. (2015). In the present study, assays were done under either 0% O_2 (100% N_2) or 21% O_2 (in 79% N_2), and thus K_o was estimated using the equation:

$$K_c\,(21\%\ O_2) = K_c\,(0\%\ O_2)\cdot(1+[O_2]/K_o) \quad [9]$$

The Michaelis-Menten constant of PEPC for CO_2 (K_p) was determined for maize at 25°C and 38°C, essentially as described by Uedan and Sugiyama (1976). PEPC was extracted from leaf samples (1.2 cm^2) by grinding in a mortar with 46 mg insoluble PVPP and 2 mL of ice-cold extraction buffer containing 50 mM Bicine-NaOH (pH 8.2), 1 mM EDTA, 0.18% (w/v) PEG4000, 11 mM ε-aminocaproic acid, 2.2 mM benzamidine, 1.8 mg bovine serum albumin (BSA), 2.8% (v/v) Tween and 1.8 mM $Na_2H_2PO_4$. The homogenate was centrifuged for 4 min at 13,000 *g* and 4°C. Eight 7 mL septum-sealed vials containing 990 µL assay buffer (50 mM Bicine-NaOH (pH 8.2), 5 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 100 mM phosphoenolpyruvate (PEP), 20 mM NADH, 100 mM malic dehydrogenase (MDH), 100 mM glucose-6-phospate) and varying concentrations of $NaH^{14}CO_3$ (0 to 10 mM, 1.3×10^{10} Bq mol^{-1}) were equilibrated with nitrogen (N_2) for 30 min. Reactions were started by the addition of 10 µl leaf extract and quenched after 1 min with 10 M formic acid. Acid stable ^{14}C was measured by liquid scintillation counting. To convert K_c, K_o and K_p values from concentration to partial pressures, solubilities for CO_2 of 0.0334 mol (L bar)$^{-1}$ at 25°C and 0.0243 mol (L bar)$^{-1}$ at 38°C and for O_2 of 0.00126 mol (L bar)$^{-1}$ at 25°C and 0.00102 mol (L bar)$^{-1}$ at 38°C were used.

Rubisco specificity factor determination

Rubisco specificity for CO_2/O_2 ($S_{c/o}$) was measured at 25°C and 38°C for rice, wheat and maize (n = 6-12) using purified leaf extracts obtained as in Galmés et al. (2006) and the oxygen electrode (Model DW1; Hansatech, Kings Lynn., UK) method described by Parry et al. (1989). Reaction mixtures contained (final concentrations) 100 mM Bicine-NaOH (pH 8.2), 10 mM $MgCl_2$, 0.15 mg mL^{-1} carbonic anhydrase, 2 mM $NaH^{14}CO_3$ (18.5 kBq mol^{-1}), activated Rubisco from purified extracts (20 µL) and 2.5 µM RuBP. The basic buffer was pre-equilibrated with CO_2-free air at the temperature of measurement. RuBP oxygenation was calculated from the oxygen consumption and carboxylation from the amount of ^{14}C incorporated into PGA when all the RuBP had been consumed. To convert the $S_{c/o}$ values from concentration to partial pressures, the CO_2 and O_2 solubilities were used as described above for the Rubisco and PEPC kinetics.

Temperature/VPD sensitivity and acclimation

The effect of temperature/VPD on the main leaf gas exchange parameters was examined using two indexes. The temperature sensitivity index (TSI), to assess the impact of an increase in the measuring temperature on a given parameter (Y) in plants grown at 25°C (CT), was calculated as:

$$TSI = \frac{Y_{\mathrm{CT-25}}}{Y_{\mathrm{CT-38}}} \qquad [10]$$

The temperature acclimation index ratio (TAI) of the same leaf gas exchange parameters measured and grown at a specific temperature (Silim et al., 2010) was calculated as:

$$TAI = \frac{Y_{HT-38}}{Y_{CT-25}} \qquad [11]$$

Statistical analysis

The statistical significance of trait variation was tested by factorial ANOVA, with species, irrigation and temperature/VPD regimes, and the interaction between treatments, as fixed factors. Post hoc comparison between treatments was performed with Duncan test ($P < 0.05$) using Statistica 6.0 software package (StatStof Inc., USA). Regressions coefficients were calculated with Sigma Plot 11.0 software package.

Results

Leaf CO_2 conductances and assimilation in rice, wheat and maize grown under water deficit and elevated temperature and VPD

Plants of rice, wheat and maize grown at 25°C and 1.8 kPa VPD with optimal water supply (CT-WW) had similar values of net CO_2 assimilation rate (A_N) at 25°C (Fig. 1a). By comparison, when A_N was measured at 38°C in the same plants it had similar values to that in maize at 25°C in maize, but was decreased slightly in rice and substantially in wheat. In plants

grown at 38°C and 3.5 kPa VPD with optimal water supply (HT-WW), A_N measured at 38°C was higher in maize than in rice or wheat, and A_N measured at 25°C was largely decreased in maize, and slightly decreased in rice and wheat as compared to measurements at the higher temperature (Fig. 1b).

Growth under conditions of water deficit (CT-WD and HT-WD) had a negative impact on A_N for all plants except for maize grown at HT, mostly as a consequence of decreased stomatal conductance (g_s, Fig. 1c, d). Effects of water deficit and growth temperature on mesophyll conductance (g_m) estimated for the C_3 species showed less obvious trends (Fig. 1e, f). Comparison of the results for g_m, obtained with the three different methods, (Fig. S1), showed some scattering in the data, but significant positive correlations ($P < 0.01$) were obtained between the method of Harley (Harley et al., 1992; adopted in this work for subsequent comparisons and modeling) and two alternative methods (Ethier and Livingston, 2004; Yin et al., 2009). No clear pattern was observed for the 4 treatments out of 16 showing discrepancies, e.g. in some cases measurements were at 25°C and in others at 38°C. The unexpected increase in g_m in wheat plants grown at 25°C under WD compared to WW conditions was confirmed by the three estimation methods (Fig. S1).

Decreases in g_s and g_m largely explained the limitation of A_N in rice and wheat plants under WD conditions (Fig. 1), so that a tight correlation was observed between the total leaf conductance to CO_2 (g_t, calculated from integration of g_s and g_m) and A_N (Fig. S2). A similar trend was observed for g_s in maize (Fig. S2), supporting the conclusion that diffusive limitations to photosynthesis predominate in plants exposed to moderate water deficit conditions. Conversely, in wheat plants grown at 25°C and measured at 38°C, A_N was much decreased even though g_t was mostly unaffected (Fig. S2).

Leaf mitochondrial dark respiration (R_{dark}) in rice, wheat and maize grown under water deficit and elevated temperature and VPD

Plants of all three species grown at CT showed similar responses of mitochondrial dark respiration rate (R_{dark}) to the imposed treatments (Fig. 2). These responses consisted of a boost of R_{dark} after a sudden increase in the temperature of measurement. The effects of WD on R_{dark} in CT plants were non-significant at the measurement temperature of 25°C in all three species, but became significant in wheat and maize measured at 38°C. In HT grown plants, the patterns of response of R_{dark} to the imposed treatments were radically different to those displayed by CT plants. In HT plants, R_{dark} became sensitive to the irrigation treatment in the C_3 crops (except for wheat measured at 25°C), but not in maize. In addition, in HT plants the effects of the measuring temperature on R_{dark} were less evident than in CT plants, with significant changes only observed in maize and in HT-WW wheat.

Long-term effects of water deficit and high temperature and VPD stress on the photosynthetic biochemistry of the three crops

The response of photosynthesis to increasing CO_2 concentration was analyzed in the three species on the basis of the CO_2 concentration in the chloroplastic stroma (i.e. A_N–C_c curves in rice and wheat, and A_N–C_s in maize). All crops displayed the well-described response of A_N to increasing C_c or C_s (Figs. S3, S4 and S5).

In general, for rice and wheat, the effect of temperature was more evident than that of water availability on the shape of the A_N-C_c curves (Figs. S3 and S4). This observation suggests a higher resilience of photosynthetic biochemistry to water deficit than to high temperatures. The biochemical parameters derived from A_N-C_c curves confirmed this same pattern. In CT plants, the maximum velocity of Rubisco carboxylation (V_{cmax}) was more responsive to the increase in measuring temperature from 25°C to 38°C in rice than in wheat (Fig. 3a). By contrast, both species showed decreased V_{cmax} in HT plants when lowering the measuring temperature from 38°C to 25°C (Fig. 3b). Significant effects of WD on V_{cmax} were observed in rice under CT-25°C and HT-38°C, and were absent in wheat.

Compared to V_{cmax}, the maximum rate of electron transport (J_{max}) was less affected by changes in the temperature of measurement, but similarly by changes in the irrigation treatment (Fig. 3c, d). In consequence, in both rice and wheat, the ratio J_{max}/V_{cmax} was lower when measured at 38°C compared to 25°C, irrespective of the growth temperature (data not shown). The effect of the growth temperature on J_{max}/V_{cmax} ratio was significant when plants were measured at 38°C in the two species. By contrast, significant effects of WD were restricted to CT-25°C rice and CT-38°C wheat.

Long-term growth under WD had more evident effects on the shape of A_N-C_s curves in maize compared to the effects on the A_N-C_c curves in the C_3 crops (Fig. S5). However, these effects were restricted to the linear part of the A_N-C_s curve, informative of PEPC activity. Accordingly, the maximum rate of PEPC carboxylation (V_{pmax}) was affected by WD under all treatments except HT-25°C (Fig. 3c, d). The effect of the measuring temperature on V_{pmax} was dependent on the growth temperature: no effects were observed in CT-grown plants, but V_{pmax} dramatic decreased dramatically in HT-grown plants when the measurement temperature decreased from 38°C to 25°C. V_{pmax} was also highly responsive to the growth temperature, showing a high capacity for thermal acclimation (i.e., highest values when V_{pmax} was measured at the respective growth temperature). In maize, V_{cmax} was also significantly affected by the irrigation treatment in plants grown at CT, while at HT-25°C V_{cmax} increased under WD (Fig. 3a, b). Likewise, V_{cmax} in maize increased with the measuring temperature at both growth temperatures irrespective of water availability (Perdomo, 2015).

Kinetic properties of Rubisco and PEPC and their relevance for modelling photosynthesis of C_3 and C_4 plants

The gross CO_2 assimilation rate (A_G) was calculated from the sum of A_N and half of the mitochondrial respiration in the dark (R_{dark}). In rice and wheat, A_G increased linearly with the ratio of CO_2 and O_2 concentrations in the chloroplast (C_c/O) (Fig. 4). For a given temperature treatment, WD plants showed a lower A_G due to decreased C_c/O, in both rice and wheat. It is remarkable that rice plants measured at the same temperature but grown at different temperatures (e.g., compare CT-25°C and HT-25°C) presented different A_G values for a given C_c/O, suggesting that the carboxylase/oxygenase activity of Rubisco was sensitive to the growth temperature.

Rubisco and PEPC kinetic constants, required for photosynthesis modeling, were measured *in vitro* at the two temperatures of measurement to enable a more accurate modeling. As expected, all kinetic constants increased at 38°C with respect to 25°C in the three species (Table 1). Differences between the two C_3 crops and maize were significant for the Michaelis-Menten constant of Rubisco for CO_2 (K_c) at both temperatures and for the Michaelis-Menten constant of Rubisco for O_2 (K_o) at 38°C.

The V_{cmax} estimated for the C_3 species by applying the model of Farquhar et al. (1980), and using the values of the *in vitro* Rubisco kinetics specific for each species at each measurement temperature/VPD (Table 1) tended to be lower than the V_{cmax} estimated using the kinetic parameters from Bernacchi et al. (2001, 2002) (Table 2). However, the estimates obtained by each method for the different species under each treatment were highly related. None of the differences between V_{cmax} values estimated using specific kinetics and kinetics from Bernacchi et al. (2001, 2002) were significant in rice, and only in 3 cases the differences were significant in wheat (CT-WD-25°C, HT-WW-25°C and HT-WD-25°C). Indeed, the correlation between the two V_{cmax} estimates was high ($r^2 > 0.99$) in both species, however the slope in wheat (0.86) was lower than in rice (0.98) and thus more distant to a 1:1 relationship (data not shown). Significant differences between values of J_{max} estimated using specific and Bernacchi kinetics (Bernacchi et al. 2001, 2002) were observed for three treatments in rice (CT-WW-25°C, CT-WD-25°C and HT-WD-25°C) and two in wheat (CT-WW-25°C and CT-WD-25°C) (Table 2). To strengthen these observations, the estimates for V_{cmax} and J_{max} obtained using the species-specific values of Rubisco kinetics measured in the present study and those reported for tobacco in Bernacchi et al. (2001, 2002) were compared by applying the method described by Ethier and Livingston (2004); significant differences in V_{cmax} were observed in two cases for each species, while significant differences in J_{max} were only observed in CT-WD-25°C wheat (Table S1).

Regarding C_4 modeling, the comparison was established between V_{cmax} and V_{pmax} estimates using the Rubisco and PEPC kinetics reported in the present study for maize and those

reported in von Caemmerer (2000) (Table 3). von Caemmerer (2000) used the temperature dependence of Rubisco kinetic constants reported by Bernacchi et al. (2002), while K_p was assumed to be invariable with temperature. Differences in V_{cmax} estimates between the two approaches were non-significant under all treatments, while significant differences in V_{pmax} were found in CT-grown plants, irrespective of the irrigation treatment and the measuring temperature (Table 3).

Sensitivity and acclimation capacity to high temperature and water deficit in rice, wheat and maize

A temperature sensitivity index (TSI, Table 4) was calculated for the main photosynthetic parameters as the ratio between the value at CT-25°C and that at CT-38°C. The photosynthetic machinery of maize was particularly insensitive to the sudden increase in the measuring temperature in CT-grown plants, both under WW and WD. By contrast, A_N, g_s and g_m were affected by short-term heat stress in rice and wheat (as denoted by the asterisks), although relative sensitivity was dependent on the irrigation treatment. Irrespective of the irrigation treatment, wheat was the unique species with TSI > 1 for J_{max}, and rice presented the lowest TSI for V_{cmax} (i.e., the largest increment due to the increase in the temperature of measurement). R_{dark} was the most sensitive parameter to the increase in the temperature of measurement, particularly under WW conditions.

The temperature acclimation index (TAI, Table 5) provides a tool for comparison of plants grown and measured at the same temperature (CT-25°C and HT-38°C). Under WW, wheat was the species with the lowest TAI for A_N, and both rice and wheat presented TAI > 1 for V_{cmax}, while maize TAI for V_{pmax} was also > 1 (Table 5). Under WD, maize was the unique species with TAI for A_N significantly higher than 1, all three species presented TAI > 1 for g_s and V_{cmax}, and maize for V_{pmax}. TAI for R_{dark} was not significantly different from 1 under WD but increased under WW conditions in all species. In wheat WW, TAI < 1 for A_N and TAI > 1 for R_{dark}, suggesting a lower capacity of acclimation to increased temperature (Perdomo, 2015).

Discussion

Long-term responses to increased temperature, VPD and drought stress were compared in rice, wheat and maize, to improve our understanding of how these three major global crops will respond to future the climate. In addition, we used the data obtained as well as the Rubisco *in vitro* kinetics for each species and treatments to check the validity of commonly employed 'universal Rubisco constants' to parameterize photosynthesis models in different species. These two objectives are discussed independently in the next sections.

Long-term acclimation to high temperature and drought stress in three important global crops

Plants grown and measured at 25°C under well-watered conditions (CT-WW-25°C) showed similar values for A_N in the three species (Fig. 1). However, WD resulted in a significant decrease of A_N in all three species, the strongest effect being observed in rice and the mildest in maize. In the two C_3 species, these limitations were mostly due to stomatal conductance (g_s), which largely decreased in both species, while the mesophyll conductance to CO_2 (g_m) decreased under water deficit in rice but apparently increased in wheat (Choluj et al., 1997; Kalaji and Nalborczyk, 1991). Parameters reflecting photosynthetic activity (V_{cmax} and J_{max}) were largely unaffected by WD (Fig. 3), as is often found in C_3 species (Flexas et al., 2002, 2006b; Galmés et al., 2007b). In C_4 maize, the drought stress-induced decrease in A_N was due to combined decreases on V_{cmax} and V_{pmax} plus g_s only when measured at 38°C (Figs. 1 and 3); this is in agreement with previous reports in C_4 plants (Carmo-Silva et al., 2010; Lal and Edwards, 1996).

When WW plants grown at CT were measured at 38°C, significant decreases of photosynthesis were observed in all species but maize, although these effects were of smaller magnitude than those induced by WD except in wheat, where the two stresses resulted in responses of similar magnitude (Fig. 1). These results suggest that, of the three crops, rice was the most sensitive species to drought stress and wheat was the most sensitive to increased measurement temperature, while maize was the least sensitive to both drought stress and increased measurement temperature (see also Table 4). In many studies, short-term responses are taken as evidence to predict the future photosynthetic performance of a given species under a changing climate. However, there are at least two factors that can bias these responses: (i) interactions between stresses, and (ii) acclimation in the long-term (Centritto et al., 2002; Cheesman and Winter, 2013; Flexas et al., 2006a; Vile et al., 2012). Regarding interactions, these are evidenced by measuring at 38°C plants grown at CT and subjected to water deficit (CT-WD-38°C). In rice, A_N values were somewhat larger under WD when plants were measured at 38°C than at 25°C, however, the effect of WD at 38°C was not significant (Fig. 1). In other words, in rice the high measurement temperature and drought stress interacted somehow to increase photosynthesis as compared to measuring drought plants at the lower temperature. In wheat, in contrast, the interaction of high measurement temperature and WD resulted in a cumulative effect of both stresses, so that photosynthesis under WD-38°C was about half of the value observed when stress treatments were applied independently (Fig. 1).

Considering the comparison of WW-CT plants measured at 25°C and WD-CT plants measured at 38°C, it can no longer be considered that rice is more drought stress sensitive and wheat more temperature sensitive. Instead, both species are similarly sensitive to the combination of high measurement temperature and drought stress. This result illustrates how

short-term studies observing the response to isolated stresses may fail to reproduce plant responses to the most complex, combined stress conditions that are often experienced in the field (Prasad et al., 2008; Shah and Paulsen, 2003; Tozzi et al., 2013; Vile et al., 2012). In maize, the combination of high temperature/VPD and WD resulted in photosynthesis rates only marginally lower than those displayed by WW plants measured at low temperature, as expected for a C_4 species (Crafts-Brandner and Salvucci, 2002; Edwards et al., 2001; Osborne and Beerling, 2006).

Long-term acclimation responses may further confound the predictive value of short-term observations. Acclimation was evident for the three species. Values of A_N were very similar between plants grown and measured at 25°C and those grown and measured at 38°C (i.e. TAI close to 1, Table 5), both under WW and WD conditions (Fig. 1a and 1b). Only in WW wheat A_N was lower in plants grown and measured at high temperature (HT-WW-38°C) than in plants grown at control temperature and measured at 25°C (CT-WW-25°C), and in WD maize A_N was higher in plants grown and measured at 38°C than at 25°C, confirming the adaptation of these two species to cool and hot temperature conditions, respectively (Hikosaka et al., 2006; Nagai and Makino, 2009; Yamori et al., 2009). A similar acclimation to growth temperature – i.e., A_N is kept constant – has also been observed in poplar (Silim et al., 2010). Interestingly, acclimation of photosynthesis to high temperature in the three species was achieved through different homeostatic mechanisms. For instance, in both WW and WD rice and WD wheat, the same A_N at the two temperatures was achieved by increasing g_s and V_{cmax} but decreasing g_m (Figs. 1 and 3). Contrarily, in WW wheat, a lower A_N was observed at high temperature despite increased V_{cmax}, which was in part attributable to large increases in respiration (Fig. 2). These results indicate that changing climate results in species-dependent changes in the ratios between biochemical and diffusive parameters even in cases where net photosynthesis does not change.

In summary, the present results illustrate that the photosynthetic responses to climate conditions – e.g. drought stress and increased temperature and VPD – differ when analyzed in the short- or long-term, in a species-specific manner. Therefore, it is necessary to be cautious when deriving generalizations or predictions from short-term studies with few species subjected to isolated stress conditions. Rather, detailed long-term experiments with different species and stress interactions are urged for a better understanding of crop responses to withstanding climate change conditions.

Species-specific Rubisco kinetics and their effects on accurate parameterization of C_3 and C_4 photosynthesis models

Photosynthesis models such as that of Farquhar et al. (1980) for C_3 plants, or that of von Caemmerer (2000) for C_4 plants, allow the estimation of biochemical traits such as the maximum velocity of Rubisco carboxylation (V_{cmax}) and the maximum rate of electron transport

(J_{max}) in C_3 plants, and the maximum velocities of PEPC (V_{pmax}) and Rubisco (V_{cmax}) carboxylation in C_4 species (von Caemmerer, 2013). While this parameterization was originally applied on a C_i basis (Berry and Björkman, 1980; Farquhar et al., 1980), it is now widely recognized that correct parameterization should take into account the CO_2 concentration at the Rubisco site inside chloroplasts (C_c), for which knowledge of the mesophyll conductance to CO_2 (g_m) is required. For instance, a recent survey on 130 species reveals that assuming infinite g_m underestimates, on average, V_{cmax} by as much as 75% and J_{max} by 60% (Gu and Sun, 2014; Sun et al., 2014). Under severe drought stress conditions the underestimations may be even larger (Flexas et al., 2006b).

On the other hand, g_m typically decreases under drought stress (Flexas et al., 2002; Gallé et al., 2009; Galmés et al., 2007a; Hu et al., 2010) and increases with temperature, at least up to a certain threshold (Evans and von Caemmerer, 2013; Silim et al., 2010; Walker et al., 2013). The response of g_m to temperature has recently been shown to be strongly species-dependent (von Caemmerer and Evans, 2015), although the mechanisms for this are still unclear (von Caemmerer and Evans, 2015; Flexas and Diaz-Espejo, 2015). Therefore, to correctly parameterize photosynthesis, g_m should be precisely determined for plants at each experimental condition and measurement temperature. In this study, g_m decreased in rice after the increase in the measurement temperature, but this decrease was not significant in wheat. In contrast, an increase in g_m with measurement temperature was reported by von Caemmerer and Evans (2015) and supported by data from Xiong et al. (2015) in rice. A recent reported showed a decline in g_m as leaves aged from fully expanded to senescing (Barbour et al., 2016), supporting that the above discrepancies reflect the importance of the experimental conditions and leaf age.

Several problems with existing methods for the estimation of g_m have been raised recently (Gu and Sun, 2014; Tholen et al., 2012). On one hand, the estimated g_m may not reflect purely a diffusion conductance, because A_N reflects a CO_2 net flux that combines photosynthesis, photorespiration and mitochondrial respiration, and these three processes move CO_2 along different distances and diffusion pathways (Tholen et al., 2012). On the other hand, apparent responses of g_m to varying light and CO_2 may be artefactual, resulting from analysis of g_m dependence on variables that are explicitly included in the equations used to calculate g_m (Gu and Sun, 2014). These type of errors should affect only methods that estimate different g_m values at any given C_i (i.e., Harley et al., 1992 and Yin et al., 2009), but not methods that solve for a single g_m estimate along a C_i gradient (i.e. Ethier and Livingston, 2004). Since, in most cases, the estimates based on these three different methods of estimation show a significant agreement (Fig. S1) we may dismiss the importance of these errors in the present study, but since different values were obtained with the different methods for some treatments potential errors cannot be completely ruled out.

Hence, while recognizing that some of the values presented may represent an approximation to the *true* g_m, we can still use these predictions to check for the effects of species-specific differences in Rubisco kinetic constants and their temperature response and acclimation on the parameterization of photosynthesis models. This is because in addition to precise knowledge on g_m and its temperature dependency, *a priori* knowledge of Rubisco kinetic constants ($S_{c/o}$, K_c and K_o), as well as their temperature dependencies, is required to parameterize photosynthesis models. Since these constants are unknown for Rubiscos from many species, it is becoming a common practice to use 'standard' constants for any given species. The most commonly used 'standard' Rubisco kinetics and temperature functions are those for tobacco as obtained by Bernacchi et al. (2002). However, it is well documented that significant differences occur among species in Rubisco kinetics (e.g., Galmés et al., 2005, 2015; Orr et al., 2016; Prins et al., 2016; Savir et al., 2010), and these differences result in significant bias in model parameterization (Walker et al., 2013). These authors also indicate that *in vitro* Rubisco kinetics may not accurately describe the operation of Rubisco under physiological conditions, due to degradation and/or inactivation of the enzyme during extraction or differences in the *in vitro* assay conditions compared to the chloroplast stroma. While the latter may be true, degradation or activation of Rubisco during the extraction may affect quantitatively absolute parameters, such as the maximum Rubisco activity or Rubisco concentration, but should not affect $S_{c/o}$, K_c and K_o.

Determination of *in vivo* kinetics for a large number of species with different functional types, as urged by Walker et al. (2013), may not be accomplished in the short term, as such experiments require the use of mutants with low Rubisco contents for each species, growth under low CO_2 concentrations and the use of gas exchange measurements at different oxygen partial pressures, i.e. plant material that is yet to be created and techniques that are not readily available except in very few laboratories. In contrast, measuring *in vitro* kinetic constants of Rubisco is easier and less time consuming, so that a number of different species can be characterised in a reasonable time (Hermida-Carrera et al., 2016; Orr et al., 2016; Prins et al., 2016). Therefore, we propose using Γ* derived from *in vitro* $S_{c/o}$ measured in each species at different temperatures to first estimate g_m and, then, parameterize photosynthesis from A_N-C_c curves using the species and temperature specific *in vitro* kinetics of Rubisco rather than 'standard' values determined for model species.

The *in vitro* values for Γ* and K_c are within the range of values obtained *in vivo* for tobacco and Arabidopsis by Walker et al. (2013), supporting the use of *in vitro* values as a valid approach to estimate Rubisco constants comparable to those operating *in vivo*. Nevertheless, K_o was almost the double in rice and wheat regarding the tobacco and Arabidopsis also reported by Walker et al. (2013), which demonstrates the differences in the Rubisco kinetic parameters

among species and point out the importance of considering the species-specific values instead of general "consensus values".

Rubisco from C_4 maize had a lower affinity for CO_2 (i.e., higher K_c) than the Rubisco from C_3 rice and wheat (Table 1), in agreement with previous reports (Carmo-Silva et al., 2010; Christin et al., 2008; Cousins et al., 2010; Savir et al., 2010; Whitney et al., 2011). At 25°C, differences in Γ* and K_o between species and photosynthetic mechanisms were non-significant, indicative that maize Rubisco presents a higher maximum catalytic turnover for the carboxylation reaction (k_{cat}^c) than the two C_3 species, which is in agreement with recent studies (Sharwood et al., 2016a, 2016b). The response of Rubisco kinetics to increased temperature followed trends already described in the literature, both *in vivo* (Bernacchi et al., 2001; Brooks and Farquhar, 1985; Walker et al., 2013) and *in vitro* in C_3 and C_4 species (Badger and Collatz, 1977; Boyd et al., 2015; Galmés et al., 2005; Jordan and Ogren, 1984), with increases in K_c, K_o, K_c/K_o and Γ* (Table 1). The relative increase in Γ* with temperature was lower in wheat than in maize and rice. There are few reported measurements of the Michaelis-Menten constant for PEPC (K_p) in C_4 species (Bauwe, 1986; Boyd et al., 2015; Pfeffer and Peisker, 1998) and limited studies on the temperature dependence of K_p (Boyd et al., 2015). K_p increased with temperature in *Setaria viridis* (Boyd et al., 2015) and in maize (this study) to a lesser extent than the increase of K_c. This fact, together with the higher temperature-driven increase of V_{pmax} as compared to that of V_{cmax} (Fig. 3), suggests increased Rubisco limitations for C_4 photosynthesis at high temperatures. That the K_p values for *Setaria viridis* are higher than those reported here for maize, corroborates the need to use species-specific kinetic constants for both Rubisco and PEPC for greater accuracy in C_4 photosynthetic modelling.

Using each species Rubisco constants resulted in model parameterization estimates that in some cases differed significantly from those obtained using the 'standard' constants by Bernacchi et al. (2002) in C_3 plants. These differences represented on average 10-20% overestimation of V_{cmax} and a largely variable underestimation of J_{max} (Table 2), with strongly biased V_{cmax}/J_{max} ratios. The magnitude of these discrepancies, similar to that found by Walker et al. (2013), is remarkable, especially considering that the species compared (tobacco and Arabidopsis in Walker's study, rice and wheat here) are all herbaceous angiosperms. It is likely that even broader deviations would occur when using 'standard' tobacco kinetics to parameterize more distant species, like woody angiosperms, gymnosperms, ferns or mosses. Part of this bias in the parameterization of V_{cmax} and J_{max} is due to bias in the estimation of g_m, as indicated by the significant differences obtained between the g_m values estimated using the Rubisco kinetic values from the present study and those reported in Bernacchi et al. (2001, 2002). These differences were observed regardless of the g_m estimation method (i.e., Harley, Ethier and Livingstone or Yin; Table S2). These results also demonstrate that species- and

temperature-specific kinetic parameters for PEPC and Rubisco are required for accurate photosynthesis parameterization in C_4 plants, in particular for estimating V_{pmax} (Table 3).

In summary, the present results confirm and extend the conclusion by Walker et al. (2013) that species-specific differences in *in vivo* Rubisco parameters are large enough to significantly bias modelling of C_3 photosynthesis. It is further shown, for the first time, that differences in species-specific kinetics are large enough to bias modelling of C_4 photosynthesis. It is thus strongly recommended that the use of "standard" Rubisco kinetics from tobacco is avoided when modelling photosynthesis in other species. As obtaining *in vivo* Rubisco kinetics for different species is not achievable in the short-term, we propose to use *in vitro* kinetics as determined by the methods explained here and elsewhere (Galmés et al., 2016; Kane et al., 1994; Orr et al., 2016; Parry et al., 2007; Perdomo, 2015; Prins et al., 2016; Ruuska et al., 1998; Shay and Kubien, 2013) as a first proxy for *in vivo* kinetics.

Authors contribution

JP performed the experiment, analysed the data and wrote the paper.

ECS contributed to the design, analysis and interpretation of the C_4 gas-exchange model data.

CH was involved in the acquisition of the Rubisco kinetic data.

JF is an expert in plant physiology, contributed substantially to write the paper and critically revised the work.

JG obtained funding for the project and was a substantial contributor to the conception and design of the work.

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References

Ainsworth, E.A., and Ort, D.R. (2010). How do we improve crop production in a warming world? *Plant Physiol.* 154, 526–30. doi:10.1104/pp.110.161349.

Atkin, O.K., and Macherel, D. (2009). The crucial role of plant mitochondria in orchestrating drought tolerance. *Ann. Bot.* 103, 581–97. doi:10.1093/aob/mcn094.

Atkin, O.K., and Tjoelker, M.G. (2003). Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends Plant Sci.* 8, 343–51. doi:10.1016/S1360-1385(03)00136-5.

Atkin, O.K., Scheurwater, I., and Pons, T. (2006). High thermal acclimation potential of both photosynthesis and respiration in two lowland Plantago species in contrast to an alpine congeneric. *Glob. Chang. Biol.* 12, 500–515. doi:10.1111/j.1365-2486.2006.01114.x.

Badger, M., and Collatz, G. (1977). mechanism of ribulose-1,5-bisphosphate carboxylase and oxygenase reactions, with particular reference to the effect of temperature on kinetic parameters. *Carnegie Inst. Washington, Year B.* 76, 355–361.

Barbour, M.M., Evans, J.R., Simonin, K.A., and von Caemmerer, S. (2016). Online CO_2 and H_2O oxygen isotope fractionation allows estimation of mesophyll conductance in C_4 plants, and reveals that mesophyll conductance decreases as leaves age in both C_4 and C_3 plants. *New Phytol.* 210, 875–889. doi:10.1111/nph.13830.

Bauwe, H. (1986) An efficient method for the determination of K_m values for HCO_3 of phosphoenolpyruvate carboxylase. *Planta* 169, 356–360. doi:10.1007/BF00392131.

Bernacchi, C.J., Pimentel, C., and Long, S.P. (2003). *In vivo* temperature response functions of parameters required to model RuBP-limited photosynthesis. *Plant. Cell Environ.* 26, 1419–1430. doi:10.1046/j.0016-8025.2003.01050.x.

Bernacchi, C.J., Portis, A.R., Nakano, H., von Caemmerer, S., and Long, S.P. (2002). Temperature response of mesophyll conductance. Implications for the determination of Rubisco enzyme kinetics and for limitations to photosynthesis *in Vivo*. *Plant Physiol.* 130, 1992–1998. doi:10.1104/pp.008250.water.

Bernacchi, C.J., Singsaas, E.L., Pimentel, C., Portis, Jr A.R., and Long, S.P. (2001). Improved temperature response functions for models of Rubisco-limited photosynthesis. *Plant. Cell Environ.* 24, 253–259. doi:10.1046/j.1365-3040.2001.00668.x.

Berry, J., and Björkman, O. (1980). Photosynthetic response and adaptation to temperature in higher-plants. *Physiol. Plant Mol. Biol.* 31, 491–543.

Boyd, R.A., Gandin, A., and Cousins, A.B. (2015). Temperature response of C_4 photosynthesis: Biochemical analysis of Rubisco, phosphoenolpyruvate carboxylase and carbonic anhydrase in *Setaria viridis*. *Plant Physiol.* 169, 1850–1861. doi:10.1104/pp.15.00586.

Brooks, A., and Farquhar, G.D. (1985). Effect of temperature on the CO_2/O_2 specificity of ribulose-1,5-bisphosphate carboxylase/oxygenase and the rate of respiration in the light. *Planta* 165, 397–406.

von Caemmerer, S. (2000). *Biochemical models of leaf photosynthesis*. Collingwood: CSIRO.

von Caemmerer, S. (2013). Steady-state models of photosynthesis. *Plant. Cell Environ.* 36, 1617–1630. doi:10.1111/pce.12098.

von Caemmerer, S., and Evans, J.R. (2015). Temperature responses of mesophyll conductance differ

greatly between species. *Plant. Cell Environ.* 38, 629–637. doi:10.1111/pce.12449.

Campbell, C., Atkinson, L., Zaragoza-Castells, J., Lundmark, M., Atkin, O.K., and Hurry, V. (2007). Acclimation of photosynthesis and respiration is asynchronous in response to changes in temperature regardless of plant functional group. *New Phytol.* 176, 375–89. doi:10.1111/j.1469-8137.2007.02183.x.

Carmo-Silva, A.E., Gore, M.A., Andrade-Sanchez, P., French, A.N., Hunsaker, D.J., and Salvucci, M.E. (2012). Decreased CO_2 availability and inactivation of Rubisco limit photosynthesis in cotton plants under heat and drought stress in the field. *Environ. Exp. Bot.* 83, 1–11. doi:10.1016/j.envexpbot.2012.04.001.

Carmo-Silva, A.E., Keys, A.J., Andralojc, P.J., Powers, S.J., Arrabaça, M.C., and Parry, M.A.J. (2010). Rubisco activities, properties, and regulation in three different C_4 grasses under drought. *J. Exp. Bot.* 61, 2355–66. doi:10.1093/jxb/erq071.

Carmo-Silva, A.E., Powers, S.J., Keys, A.J., Arrabaça, M.C., and Parry, M.A.J. (2008). Photorespiration in C_4 grasses remains slow under drought conditions. *Plant. Cell Environ.* 31, 925–940. doi:10.1111/j.1365-3040.2008.01805.x.

Centritto, M., Lucas, M.E., and Jarvis, P.G. (2002). Gas exchange, biomass, whole-plant water-use efficiency and water uptake of peach (*Prunus persica*) seedlings in response to elevated carbon dioxide concentration and water availability. *Tree Physiol.* 22, 699–706.

Cheesman, A.W., and Winter, K. (2013). Growth response and acclimation of CO_2 exchange characteristics to elevated temperatures in tropical tree seedlings. *J. Exp. Bot.* 64, 3817–3828. doi:10.1093/jxb/ert211.

Christin, P.A., Salamin, N., Muasya, A.M., Roalson, E.H., Russier, F., and Besnard, G. (2008). Evolutionary switch and genetic convergence on *rbc*L following the evolution of C_4 photosynthesis. *Mol. Biol. Evol.* 25, 2361–2368. doi:10.1093/molbev/msn178.

Choluj, D., Kalaji, M.H., and Niemyska B. (1997) Analysis of the gas exchange components in chilled tomato plants. *Photosynthetica.* 34, 583–589.

Cousins, A.B., Ghannoum, O., von Caemmerer, S., and Badger, M.R. (2010). Simultaneous determination of Rubisco carboxylase and oxygenase kinetic parameters in *Triticum aestivum* and *Zea mays* using membrane inlet mass spectrometry. *Plant. Cell Environ.* 33, 444–452. doi:10.1111/j.1365-3040.2009.02095.x.

Crafts-Brandner, S.J., and Salvucci, M.E. (2002). Sensitivity of photosynthesis in a C_4 plant, maize, to heat stress. *Plant Physiol.* 129, 1773–1780. doi:10.1104/pp.002170.or.

Diaz-Espejo, A. (2013). New challenges in modelling photosynthesis: temperature dependencies of Rubisco kinetics. *Plant. Cell Environ.* 36, 2104–2107.

Dillaway, D.N., and Kruger, E.L. (2011). Leaf respiratory acclimation to climate: comparisons among boreal and temperate tree species along a latitudinal transect. *Tree Physiol.* 31, 1114–1127. doi:10.1093/treephys/tpr097.

Edwards, G., Furbank, R., Hatch, M., and Osmond, C. (2001). What does it take to be C_4? Lessons from the evolution of C_4 photosynthesis. *Plant Physiol.* 125, 46–49.

Ethier, G.J., and Livingston, N.J. (2004). On the need to incorporate sensitivity to CO_2 transfer

conductance into the Farquhar-von Caemmerer-Berry leaf photosynthesis model. *Plant. Cell Environ.* 27, 137–153. doi:10.1111/j.1365-3040.2004.01140.x.

Evans, J.R., and von Caemmerer, S. (2013). Temperature response of carbon isotope discrimination and mesophyll conductance in tobacco. *Plant. Cell Environ.* 36, 745–756. doi:10.1111/j.1365-3040.2012.02591.x.

Farquhar, G.D., von Caemmerer, S., and Berry, J.A. (1980). A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta* 149, 78–90.

Flexas, J., Barón, M., Bota, J., Ducruet, J-M., Gallé, A., Galmés, J., et al. (2009). Photosynthesis limitations during water stress acclimation and recovery in the drought-adapted *Vitis* hybrid Richter-110 (*V. berlandierixV. rupestris*). *J. Exp. Bot.* 60, 2361–2377. doi:10.1093/jxb/erp069.

Flexas, J., Bota, J., Escalona, J., Sampol, B., and Medrano, H. (2002). Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. *Funct. Plant Biol.* 29, 461–471.

Flexas, J., Bota, J., Galmés, J., Medrano, H., and Ribas-Carbó, M. (2006a). Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. *Physiol. Plant.* 127, 343–352. doi:10.1111/j.1399-3054.2005.00621.x.

Flexas, J., Díaz-Espejo, A., Berry, J.A., Cifre, J., Galmés, J., Kaldenhoff, R., Medrano, H., and Ribas-Carbó, M. (2007). Analysis of leakage in IRGA's leaf chambers of open gas exchange systems: quantification and its effects in photosynthesis parameterization. *J. Exp. Bot.* 58, 1533–43. doi:10.1093/jxb/erm027.

Flexas, J., and Díaz-Espejo, A. (2015). Interspecific differences in temperature response of mesophyll conductance: food for thought on its origin and regulation. *Plant. Cell Environ.* 38, 625–628 doi:10.1111/pce.12476.

Flexas, J., Niinemets, Ü., Gallé, A., Barbour, M. M., Centritto, M., Diaz-Espejo, A., et al. (2013). Diffusional conductances to CO_2 as a target for increasing photosynthesis and photosynthetic water-use efficiency. *Photosynth. Res.* 117, 45–59. doi:10.1007/s11120-013-9844-z.

Flexas, J., Ribas-Carbó, M., Bota, J., Galmés, J., Henkle, M., Martínez-Cañellas, S., and Medrano, H. (2006b). Decreased Rubisco activity during water stress is not induced by decreased relative water content but related to conditions of low stomatal conductance and chloroplast CO_2 concentration. *New Phytol.* 172, 73–82. doi:10.1111/j.1469-8137.2006.01794.x.

Gallé, A., Florez-Sarasa, I., Tomas, M., Pou, A., Medrano, H., Ribas-Carbo, M., and Flexas, J. (2009). The role of mesophyll conductance during water stress and recovery in tobacco (*Nicotiana sylvestris*): acclimation or limitation? *J. Exp. Bot.* 60, 2379–2390. doi:10.1093/jxb/erp071.

Galmés, J., Aranjuelo, I., Medrano, H., and Flexas, J. (2013). Variation in Rubisco content and activity under variable climatic factors. Photosynth Res 117: 73–90. doi: 10.1007/s11120-013-9861-y.

Galmés, J., Flexas, J., Keys, A.J., Cifre, J., Mitchell, R., Madgwick, P.J., Haslam, R., Medrano, H., and Parry, M.A.J. (2005). Rubisco specificity factor tends to be larger in plant species from drier habitats and in species with persistent leaves. *Plant. Cell Environ.* 28, 571–579. doi:10.1111/j.1365-3040.2005.01300.x.

Galmés, J., Flexas, J., Savé, R., and Medrano, H. (2007a). Water relations and stomatal characteristics of

Mediterranean plants with different growth forms and leaf habits: responses to water stress and recovery. *Plant Soil* 290, 139–155. doi:10.1007/s11104-006-9148-6.

Galmés, J., Hermida-Carrera, C., Laanisto, L., and Niinements, Ü. (2016). A compendium of temperature responses of Rubisco kinetic traits: variability among and within photosynthetic groups and impacts on photosynthesis modeling. *J. Exp. Bot.* doi:10.1093/jxb/erw267.

Galmés, J., Kapralov, M.V., Copolovici, L., Hermida-Carrera, C., and Niinemets, Ü. (2015). Temperature responses of the Rubisco maximum carboxylase activity across domains of life: phylogenetic signals, trade-offs and importance for carbon gain. *Photosynth. Res.*123, 183–201. doi: 10.1007/s11120-014-0067-8.

Galmés, J., Kapralov, M.V, Andralojc, P.J., Conesa, M.À., Keys, A.J., Parry, M.A.J., and Flexas, J. (2014). Expanding knowledge of the Rubisco kinetics variability in plant species: environmental and evolutionary trends. *Plant. Cell Environ.* 37, 1989–2001. doi:10.1111/pce.12335.

Galmés, J., Medrano, H., and Flexas, J. (2006). Acclimation of Rubisco specificity factor to drought in tobacco: discrepancies between *in vitro* and *in vivo* estimations. *J. Exp. Bot.* 57, 3659–67. doi:10.1093/jxb/erl113.

Galmés, J., Medrano, H., and Flexas, J. (2007b). Photosynthetic limitations in response to water stress and recovery in Mediterranean plants with different growth forms. *New Phytol.* 175, 81–93. doi:10.1111/j.1469-8137.2007.02087.x.

Galmés, J., Ribas-Carbó, M., Medrano, H., and Flexas, J. (2007c). Response of leaf respiration to water stress in Mediterranean species with different growth forms. *J. Arid Environ.* 68, 206–222. doi:10.1016/j.jaridenv.2006.05.005.

Galmés, J., Ribas-Carbó, M., Medrano, H., and Flexas, J. (2011). Rubisco activity in Mediterranean species is regulated by the chloroplastic CO_2 concentration under water stress. *J. Exp. Bot.* 62, 653–665. doi:10.1093/jxb/erq303.

Genty, B., Briantais, J., and Baker, N. (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta* 990, 87–92.

Gornall, J., Betts, R., Burke, E., Clark, R., Camp, J., Willett, K., and Wiltshire, A. (2010). Implications of climate change for agricultural productivity in the early twenty-first century. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 365, 2973–89. doi:10.1098/rstb.2010.0158.

Grassini, P., Eskridge, K.M., and Cassman, K.G. (2013). Distinguishing between yield advances and yield plateaus in historical crop production trends. *Nat. Commun.* 4, 2918. doi:10.1038/ncomms3918.

Gu, L., and Sun, Y. (2014). Artefactual responses of mesophyll conductance to CO_2 and irradiance estimated with the variable J and online isotope discrimination methods. *Plant. Cell Environ.* 37, 1231–1249. doi:10.1111/pce.12232.

Harley, P.C., Loreto, F., Di Marco, G., and Sharkey, T.D. (1992). Theoretical considerations when estimating the mesophyll conductance to CO_2 flux by analysis of the response of photosynthesis to CO_2. *Plant Physiol.* 98, 1429–36.

Hermida-Carrera, C., Kapralov, M.V., and Galmés, J. (2016) Rubisco catalytic properties and temperature

response in crops. *Plant Physiol.*

Hikosaka, K., Ishikawa, K., Borjigidai, A., Muller, O., and Onoda, Y. (2006). Temperature acclimation of photosynthesis: mechanisms involved in the changes in temperature dependence of photosynthetic rate. *J. Exp. Bot.* 57, 291–302. doi:10.1093/jxb/erj049.

Hu, L., Wang, Z., and Huang, B. (2010). Diffusion limitations and metabolic factors associated with inhibition and recovery of photosynthesis from drought stress in a C_3 perennial grass species. *Physiol. Plant.* 139, 93–106. doi:10.1111/j.1399-3054.2010.01350.x.

IPCC (2013). Working Group I Contribution to the IPCC Fifth Assessment Report Climate Change 2013: The Physical Science Basis Summary for Policymakers.

Jordan, D.B., and Ogren, W.L. (1984) The CO_2/O_2 specificity of ribulose 1,5-bisphosphate carboxylase/oxygenase: : Dependence on ribulosebisphosphate concentration, pH and temperature. *Planta*. 161, 308–313. doi:10.1007/BF00398720.

Kalaji, H., and Nalborczyk, E. (1991) Gas-exchange of barley seedlings growing under salinity stress. *Photosynthetica.* 25, 197–202.

Kane, H., Viil, J., Entsch, B., Paul, K., Matthew, K., and Andrews, T. (1994). An improved method for measuring the CO_2/O_2 specificity of ribulosebisphosphate carboxylase-oxygenase. *Aust. J. Plant Physiol.* 21, 449–461.

Kattge, J., and Knorr, W. (2007). Temperature acclimation in a biochemical model of photosynthesis: a reanalysis of data from 36 species. *Plant. Cell Environ.* 30, 1176–90. doi:10.1111/j.1365-3040.2007.01690.x.

Krall, J., and Edwards, G. (1992). Relationship between photosystem II activity and CO_2 fixation in leaves. *Physiol. Plant.* 86, 180–187.

Laisk, A., and Loreto, F. (1996). Determining Photosynthetic Parameters from Leaf CO_2 Exchange and Chlorophyll Fluorescence. *Plant Physiol.* 110, 903–912.

Lal, A., and Edwards, G. (1996). Analysis of inhibition of photosynthesis under water stress in the C_4 species *Amaranthus cruentus* and *Zea mays*: electron transport, CO_2 fixation and carboxylation. *Funct. Plant Biol.* 23, 403–412.

Long, S., Ainsworth, E.A., Leakey, A., Nösberger, J., and Ort, D. (2006). Food for thought: lower-than-expected crop yield stimulation with rising CO_2 concentrations. *Science.* 312, 1918–1921.

Loriaux, S.D., Avenson, T.J., Welles, J.M., McDermitt, D.K., Eckles, R.D., Riensche, B., and Genty, B. (2013). Closing in on maximum yield of chlorophyll fluorescence using a single multiphase flash of sub-saturating intensity. *Plant. Cell Environ.* 36, 1755–1770. doi:10.1111/pce.12115.

Makino, A. (2011). Photosynthesis, grain yield, and nitrogen utilization in rice and wheat. *Plant Physiol.* 155, 125–129. doi:10.1104/pp.110.165076.

Massad, R.S., Tuzet, A., and Bethenod, O. (2007). The effect of temperature on C_4-type leaf photosynthesis parameters. *Plant. Cell Environ.* 30, 1191–1204. doi:10.1111/j.1365-3040.2007.01691.x.

Medrano, H., Escalona, J.M., Bota, J., Gulías, J., and Flexas, J. (2002). Regulation of photosynthesis of C_3 plants in response to progressive drought: stomatal conductance as a reference parameter. *Ann Bot.* 89, 895–905. doi: 10.1093/aob/mcf079.

Mittler, R. (2006). Abiotic stress, the field environment and stress combination. *Trends Plant Sci.* 11, 15–19. doi:10.1016/j.tplants.2005.11.002.

Nagai, T., and Makino, A. (2009). Differences between rice and wheat in temperature responses of photosynthesis and plant growth. *Plant Cell Physiol.* 50, 744–55. doi:10.1093/pcp/pcp029.

Orr, D.J., Melao Alcantara, A., Kapralov, M.V., Andralojc, P.J., Carmo-Silva, A.E., and Parry, M.A.J. (2016). Surveying Rubisco diversity and temperature response to improve crop photosynthetic efficiency. *Plant Physiol*. 172, 707–717. doi: http://dx.doi.org/10.1104/pp.16.00750

Osborne, C.P., and Beerling, D.J. (2006). Nature's green revolution: the remarkable evolutionary rise of C_4 plants. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 361, 173–194. doi:10.1098/rstb.2005.1737.

Parry, M.A.J., Madgwick, P.J., Carvalho, J.F.C., and Andralojc, P.J. (2007). Prospects for increasing photosynthesis by overcoming the limitations of Rubisco. *J. Agric. Sci.* 145, 31–43. doi:10.1017/S0021859606006666.

Parry, M.A.J., Keys, A.J., and Gutteridge, S. (1989). Variation in the specificity factor of C_3 higher plant rubisco determined by the total consumption of ribulose-P_2. *J. Exp. Bot.* 40, 317–320.

Perdomo, J.A. (2015) Acclimation of photosynthesis to water deficit and high temperature: physiological and biochemical aspects. [PhD thesis]. [Palma de Mallorca (IB)]: University of the Balearic Islands.

Perez-Martin, A., Flexas, J., Ribas-Carbó, M., Bota, J., Tomás, M., Infante, J. M., and Díaz-Espejo, A. (2009). Interactive effects of soil water deficit and air vapour pressure deficit on mesophyll conductance to CO_2 in *Vitis vinifera* and *Olea europaea*. *J. Exp. Bot.* 60, 2391–2405. doi:10.1093/jxb/erp145.

Pfeffer, M., and Peisker, M. (1998). CO_2 gas exchange and phosphoenolpyruvate carboxylase activity in leaves of *Zea mays* L. *Photosynth Res.* 58, 281–291. doi:10.1023/A:1006188705423.

Pittermann, J., and Sage, R.F. (2001). The response of the high altitude C_4 grass *Muhlenbergia montana* (Nutt.) A.S. Hitchc. to long- and short-term chilling. *J. Exp. Bot.* 52, 829–838.

Prins, A., Orr, D.J., Andralojc, P.J., Reynolds, M.P., Carmo-Silva, A.E., and Parry, M.A.J. (2016). Rubisco catalytic properties of wild and domesticated relatives provide scope for improving wheat photosynthesis. *J. Exp. Bot.* 67, 1827–1838. doi: 10.1093/jxb/erv574.

Prasad, P., Staggenborg, S., and Ristic, Z. (2008). "Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants," in Response of Crops to Limited Water: Understanding and Modeling Water Stress Effects on Plant Growth Processes, Advances in Agricultural Systems Modeling, eds. L. Ahuja, V. Reddy, S. Saseendran, and Q. Yu (Madison, Wisconsin: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America), 301–355.

Rodríguez-Calcerrada, J., Atkin, O.K., Robson, T.M., Zaragoza-Castells, J., Gil, L., and Aranda, I. (2010). Thermal acclimation of leaf dark respiration of beech seedlings experiencing summer drought in high and low light environments. *Tree Physiol.* 30, 214–224. doi:10.1093/treephys/tpp104.

Ruuska, S., Andrews, T., Badger, M., Hudson, G., Laisk, A., Price, G., and von Caemmerer, S. (1998). The interplay between limiting processes in C_3 photosynthesis studied by rapid-response gas exchange using transgenic tobacco impaired in photosynthesis. *Aust. J. Plant Physiol.* 25, 859–870.

Sage, R.F., and Kubien, D.S. (2007). The temperature response of C_3 and C_4 photosynthesis. *Plant. Cell Environ.* 30, 1086–1106. doi:10.1111/j.1365-3040.2007.01682.x.

Salvucci, M.E., and Crafts-Brandner, S.J. (2004). Relationship between the heat tolerance of photosynthesis and the thermal stability of Rubisco activase in plants from contrasting thermal environments. *Plant Physiol.* 134, 1460–1470. doi:10.1104/pp.103.038323.1460.

Savir, Y., Noor, E., Milo, R., and Tlusty, T. (2010). Cross-species analysis traces adaptation of Rubisco toward optimality in a low-dimensional landscape. *Proc. Natl. Acad. Sci. U. S. A.* 107, 3475–3480. doi:10.1073/pnas.0911663107.

Scafaro, A.P., von Caemmerer, S., Evans, J.R., and Atwell, B. J. (2011). Temperature response of mesophyll conductance in cultivated and wild *Oryza* species with contrasting mesophyll cell wall thickness. *Plant. Cell Environ.* 34, 1999–2008. doi:10.1111/j.1365-3040.2011.02398.x.

Schultz, H. (1996). Leaf absorptance of visible radiation in *Vitis vinifera* L.: estimates of age and shade effects with a simple field method. *Sci. Hortic. (Amsterdam).* 66, 93–102.

Shah, N.H., and Paulsen, G.M. (2003). Interaction of drought and high temperature on photosynthesis and grain-filling of wheat. *Plant Soil* 257, 219–226. doi:10.1023/A:1026237816578.

Sharwood, R.E., Ghannoum, O., and Whitney, S.M. (2016a). Prospects for improving CO_2 fixation in C_3-crops through understanding C_4-Rubisco biogenesis and catalytic diversity. *Curr. Opin. Plant Biol.* 31, 135–142. doi:10.1016/j.pbi.2016.04.002.

Sharwood, R.E., Sonawane, B.V., Ghannoum, O., and Whitney, S.M. (2016b). Improved analysis of C_4 and C_3 photosynthesis via refined *in vitro* assays of their carbon fixation biochemistry. *J. Exp. Bot.* 67, 3137–3148. doi:10.1093/jxb/erw154.

Shay, P.E., and Kubien, D. S. (2013). Field analysis of photoprotection in co-occurring cool climate C_3 and C_4 grasses. *Physiol. Plant.* 147, 316–328. doi:10.1111/j.1399-3054.2012.01662.x.

Silim, S.N., Ryan, N., and Kubien, D.S. (2010). Temperature responses of photosynthesis and respiration in *Populus balsamifera* L.: acclimation versus adaptation. *Photosynth. Res.* 104, 19–30. doi:10.1007/s11120-010-9527-y.

Sun, Y., Gu, L., Dickinson, R.E., Pallardy, S.G., Baker, J., Cao, Y., et al. (2014). Asymmetrical effects of mesophyll conductance on fundamental photosynthetic parameters and their relationships estimated from leaf gas exchange measurements. *Plant. Cell Environ.* 37, 978–994. doi:10.1111/pce.12213.

Théroux-Rancourt, G., Éthier, G., and Pepin, S. (2014). Threshold response of mesophyll CO_2 conductance to leaf hydraulics in highly transpiring hybrid poplar clones exposed to soil drying. *J. Exp. Bot.* 65, 741–753. doi:10.1093/jxb/ert436.

Tholen, D., Ethier, G., Genty, B., Pepin, S., and Zhu, X-G. (2012). Variable mesophyll conductance revisited: theoretical background and experimental implications. *Plant. Cell Environ.* 35, 2087–2103. doi:10.1111/j.1365-3040.2012.02538.x.

Tosens, T., Niinemets, Ü., Vislap, V., Eichelmann, H., and Castro-Díez, P. (2012). Developmental changes in mesophyll diffusion conductance and photosynthetic capacity under different light and water availabilities in *Populus tremula*: how structure constrains function. *Plant. Cell Environ.* 35, 839–856. doi:10.1111/j.1365-3040.2011.02457.x.

Tozzi, E.S., Easlon, H.M., and Richards, J.H. (2013). Interactive effects of water, light and heat stress on

photosynthesis in Fremont cottonwood. *Plant. Cell Environ.* 36, 1423–1434. doi:10.1111/pce.12070.

Uedan, K., and Sugiyama, T. (1976). Purification and characterization of phosphoenolpyruvate carboxylase from maize leaves. *Plant Physiol.* 57, 906–10.

Vile, D., Pervent, M., Belluau, M., Vasseur, F., Bresson, J., Muller, B., Granier, C., and Simonneau, T. (2012). Arabidopsis growth under prolonged high temperature and water deficit: independent or interactive effects? *Plant. Cell Environ.* 35, 702–718. doi:10.1111/j.1365-3040.2011.02445.x.

Walker, B., Ariza, L.S., Kaines, S., Badger, M.R., and Cousins, A.B. (2013). Temperature response of *in vivo* Rubisco kinetics and mesophyll conductance in *Arabidopsis thaliana*: comparisons to *Nicotiana tabacum*. *Plant. Cell Environ.* 36, 2108–2119. doi:10.1111/pce.12166.

Walters, R.G. (2005). Towards an understanding of photosynthetic acclimation. *J. Exp. Bot.* 56, 435–447. doi:10.1093/jxb/eri060.

Whitney, S.M., Sharwood, R.E., Orr, D., White, S.J., Alonso, H., and Galmés, J. (2011). Isoleucine 309 acts as a C_4 catalytic switch that increases ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) carboxylation rate in *Flaveria*. *Proc. Natl. Acad. Sci. U. S. A.* 108, 14688–14693. doi:10.1073/pnas.1109503108.

Xiong, D., Liu, X., Liu, L., Douthe, C., Li, Y., Peng, S., et al. (2015). Rapid responses of mesophyll conductance to changes of CO_2 concentration, temperature and irradiance are affected by N supplements in rice. *Plant. Cell Environ.* 38, 2541–2550. doi:10.1111/pce.12558.

Yamori, W., Noguchi, K., Hikosaka, K., and Terashima, I. (2009). Cold-tolerant crop species have greater temperature homeostasis of leaf respiration and photosynthesis than cold-sensitive species. *Plant Cell Physiol.* 50, 203–15. doi:10.1093/pcp/pcn189.

Yamori, W., Noguchi, K., and Terashima, I. (2005). Temperature acclimation of photosynthesis in spinach leaves: analyses of photosynthetic components and temperature dependencies of photosynthetic partial reactions. *Plant. Cell Environ.* 28, 536–547. doi:10.1111/j.1365-3040.2004.01299.x.

Yamori, W., Takahashi, S., Makino, A., Price, G.D., Badger, M.R., and von Caemmerer, S. (2011). The roles of ATP synthase and the cytochrome b6/f complexes in limiting chloroplast electron transport and determining photosynthetic capacity. *Plant Physiol.* 155, 956–62. doi:10.1104/pp.110.168435.

Yin, X., Struik, P.C., Romero, P., Harbinson, J., Evers, J.B., VAN DER Putten, P.E.L., and Vos, J. (2009). Using combined measurements of gas exchange and chlorophyll fluorescence to estimate parameters of a biochemical C_3 photosynthesis model: a critical appraisal and a new integrated approach applied to leaves in a wheat (*Triticum aestivum*) canopy. *Plant. Cell Environ*. 32, 448–464. doi:10.1111/j.1365-3040.2009.01934.x.

Supplementary Material

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

Figure S1. Comparison of the mesophyll conductance (g_m) estimated from three different methods: Harley et al. (1992), Ethier and Livingston (2004), Yin et al. (2009).

Figure S2. The relationship between: net CO_2 assimilation rate (A_N) and total leaf conductance (g_t).

Figure S3. Relationship between the net CO_2 assimilation rate (A_N) and the chloroplastic CO_2 concentration (C_c) in rice.

Figure S4. Relationship between the net CO_2 assimilation rate (A_N) and the chloropastic CO_2 concentration (C_c) in wheat.

Figure S5. Relationship between the net CO_2 assimilation rate (A_N) and the bundle sheath CO_2 concentration (C_s) in maize.

Table S1. Comparison of the maximum velocity of Rubisco carboxylation (V_{cmax}) and maximum rate of electron transport (J_{max}), estimated with the method described by Ethier and Livingston (2004), using the Rubisco kinetic parameters (K_c, K_o and $S_{c/o}$) measured in the present study for rice and wheat (own kinetics), and using the parameters described in Bernacchi et al. (2001, 2002).

Table S2. Comparison of the mesophyll conductance (g_m, mol m^{-2} s^{-1}) estimated from three different methods: Harley et al. (1992), Ethier and Livingston (2004), Yin et al. (2009) using the Rubisco kinetic parameters measured *in vitro* in the present study for rice and wheat, and using the Rubisco kinetic parameters reported for tobacco by Bernacchi et al. (2001, 2002).

Table 1. Kinetic parameters of Rubisco and PEPC from rice, wheat and maize measured at 25°C and 38°C. Four replicates were used for the Michaelis–Menten constants of Rubisco for CO_2 and O_2 (K_c and K_o) and the Michaelis–Menten constant of PEPC for CO_2 (K_P), and five replicates for the Rubisco specificity factor ($S_{c/o}$). The chloroplast CO_2 compensation point in the absence of mitochondrial respiration (Γ*) was calculated from $S_{c/o}$ as explained in the Material and Methods. Different letters and asterisks denote statistically significant differences (Duncan analysis, $P < 0.05$) among species within the same temperature and between measurement temperatures within the same species, respectively.

Species	**K_c (Pa)**		**K_o (kPa)**		**Γ* (Pa)**		**K_P (Pa)**	
	25°C	**38°C**	**25°C**	**38°C**	**25°C**	**38°C**	**25°C**	**38°C**
Rice	29.1±1.6^{a}	87.7±5.2^{a*}	45.7±4.6^{a}	58.3±5.7^{a*}	4.19±0.19^{a}	6.34±0.36^{b*}	-	-
Wheat	31.6±1.1^{a}	89.3±3.6^{a*}	39.2±3.8^{a}	50.3±4.5^{a*}	3.87±0.21^{a}	5.32±0.32^{a*}	-	-
Maize	85.8±7.0^{b}	188.3±5.7^{b*}	39.8±3.5^{a}	74.5±7.5^{b*}	4.30±0.30^{a}	6.47±0.26^{b*}	8.0±3.0	13.2±5.0*

Table 2. Comparison of the maximum velocity of Rubisco carboxylation (V_{cmax}) and maximum rate of electron transport (J_{max}) in plants grown at CT and HT, under WW and WD, and measured at 25°C and 38°C, using the Rubisco kinetics parameters (K_c, K_o and $S_{c/o}$) measured in the present study for rice and wheat (own kinetics), and using the parameters reported for tobacco by Bernacchi et al. (2001, 2002). V_{cmax} and J_{max} were calculated on a C_c basis as estimated by the method of Harley et al. (1992). Values are means ± standard errors (n = 5). Asterisks denote statistically significant differences between the two V_{cmax} or J_{max} values (Duncan analysis, $P < 0.05$) within the same treatment (growth T and irrigation treatment).

Species	**Growth T (°C)**	**Irrigation Treatment**	**Measurement T (°C)**	**Own kinetics**		**Kinetics by Bernacchi *et al.* (2001, 2002)**	
				V_{cmax}	**J_{max}**	**V_{cmax}**	**J_{max}**
Rice	CT	WW	25	102.7±6.1	184.5±9.2	116.0±6.0	112.7±22.3*
Rice	CT	WD	25	70.1±6.2	146.7±13.2	77.9±7.2	82.8±4.5*
Rice	CT	WW	38	185.1±10.8	208.3±5.9	206.2±10.8	197.6±6.9
Rice	CT	WD	38	182.2±5.0	207.3±4.2	195.8±3.1	198.5±0.3
Rice	HT	WW	25	70.2±5.4	118.8±6.6	78.0±5.8	108.8±5.1
Rice	HT	WD	25	50.2±3.3	90.0±1.6	59.2±3.8	79.3±2.7*
Rice	HT	WW	38	222.1±11.0	215.4±12.6	228.3±14.4	198.5±11.0
Rice	HT	WD	38	173.2±6.8	173.4±11.8	176.5±15.2	161.4±5.8
Wheat	CT	WW	25	105.4±7.2	181.5±7.9	125.8±9.7	101.5±15.4*
Wheat	CT	WD	25	122.9±2.1	199.8±7.1	144.1±3.3*	81.9±2.5*
Wheat	CT	WW	38	127.7±13.7	154.7±11.6	151.6±16.2	156.9±11.3
Wheat	CT	WD	38	119.2±3.3	125.3±5.4	134.5±8.9	119.7±5.8
Wheat	HT	WW	25	88.9±4.2	143.8±6.8	110.0±6.1*	133.6±5.9
Wheat	HT	WD	25	80.9±5.9	127.2±5.7	104.5±6.1*	122.8±6.6
Wheat	HT	WW	38	205.2±26.0	198.7±13.3	247.7±29.2	186.8±10.9
Wheat	HT	WD	38	197.8±13.5	181.1±7.6	230.3±15.9	174.2±8.6

Table 3. Comparison of the maximum velocity of Rubisco carboxylation (V_{cmax}) and the maximum velocity of PEPC carboxylation (V_{pmax}) in plants grown at CT and HT, under WW and WD, and measured at 25°C and 38°C, using Rubisco and PEPC kinetic parameters (K_c, K_o, $S_{c/o}$ and K_p) measured in the present study for maize, and using the parameter values reported by von Caemmerer (2000). Values are means ± standard errors (n = 5). Asterisks denote statistically significant differences between the two V_{cmax} or V_{pmax} values (Duncan analysis, $P < 0.05$) within the same treatment (growth T and irrigation treatment).

Species	**Growth T (°C)**	**Irrigation Treatment**	**Measurement T (°C)**	**Own kinetics**		**Kinetics by von Caemmerer (2000)**	
				V_{cmax}	**V_{pmax}**	**V_{cmax}**	**V_{pmax}**
Maize	CT	WW	25	29.9±2.3	133.8±4.3	29.7±2.2	112.8±3.8*
Maize	CT	WD	25	22.5±0.3	81.1±3.5	22.3±0.3	69.5±2.6*
Maize	CT	WW	38	38.7±1.0	141.1±5.9	41.5±0.9	117.5±6.4*
Maize	CT	WD	38	33.6±1.7	82.6±6.9	37.5±0.8	62.0±4.4*
Maize	HT	WW	25	14.4±1.8	44.9±4.7	14.1±1.8	38.4±4.1
Maize	HT	WD	25	22.7±1.2	52.8±8.2	22.2±1.2	46.8±7.0
Maize	HT	WW	38	31.7±0.9	219.5±23.7	32.6±1.1	182.0±15.0
Maize	HT	WD	38	31.1±2.2	158.7±18.6	32.2±2.2	141.4±10.0

Table 4. Temperature sensitivity index (TSI) for the net CO_2 assimilation rate (A_N), stomatal conductance (g_s), mesophyll conductance (g_m), maximum velocity of Rubisco carboxylation (V_{cmax}), maximum rate of electron transport (J_{max}), maximum velocity of PEPC carboxylation (V_{pmax}) and mitochondrial respiration at pre-dawn (R_{dark}). TSI was calculated, under both well-watered (WW) and water deficit (WD) conditions, as the ratio between the values from plants grown and measured at 25°C and those from plants grown at CT and measured at 38°C (TSI = (CT-25°C) / (CT-38°C)). The asterisks indicate significant differences between CT-25°C and CT-38°C. Values are means ± standard errors (n = 5).

Parameter	**WW**			**WD**		
	Rice	**Wheat**	**Maize**	**Rice**	**Wheat**	**Maize**
A_N	1.6±0.2*	1.7±0.2*	0.9±0.1	0.8±0.2	2.0±0.8*	0.8±0.2
g_s	1.5±0.3	1.0±0.2	0.7±0.1	0.8±0.3	1.5±0.3*	0.7±0.2
g_m	2.5±0.9*	1.7±0.2*	-	1.0±0.4	4.4±1.3*	-
V_{cmax}	0.6±0.1*	0.9±0.1	0.8±0.1	0.4±0.1*	1.0±0.1	0.7±0.1*
J_{max}	0.9±0.1	1.2±0.1	-	0.7±0.1*	1.6±0.1*	-
V_{pmax}	-	-	1.0±0.1	-	-	1.0±0.1
R_{dark}	0.2±0.1*	0.2±0.1*	0.1±0.1*	0.3±0.1*	0.3±0.1*	0.2±0.1*

Table 5. Temperature acclimation index (TAI) for the net CO_2 assimilation rate (A_N), stomatal conductance (g_s), mesophyll conductance (g_m), maximum velocity of Rubisco carboxylation (V_{cmax}), maximum rate of electron transport (J_{max}), maximum velocity of PEPC carboxylation (V_{pmax}) and mitochondrial respiration at pre-dawn (R_{dark}). TAI was calculated, under both well-watered (WW) and water deficit (WD) conditions, as the ratio between the values from plants grown and measured at 38°C and those from plants grown and measured at 25°C (TAI = (HT-38°C) / (CT-25°C)). The asterisks indicate significant differences between CT-25°C and HT-38°C. Values are means ± standard errors (n = 5).

Parameter	**WW**			**WD**		
	Rice	**Wheat**	**Maize**	**Rice**	**Wheat**	**Maize**
A_N	0.9±0.1	0.7±0.1*	1.0±0.1	1.0±0.4	0.9±0.1	1.3±0.1*
g_s	1.2±0.1	0.8±0.1	1.1±0.1	1.6±0.6	2.4±0.5*	1.3±0.1*
g_m	0.6±0.1	0.8±0.1	-	0.8±0.5	0.2±0.1*	-
V_{cmax}	2.2±0.1*	1.9±0.2*	1.1±0.1	2.6±0.3*	1.6±0.1*	1.4±0.1*
J_{max}	1.2±0.1	1.1±0.1	-	1.2±0.2	0.9±0.1	-
V_{pmax}	-	-	1.6±0.2*	-	-	2.0±0.4*
R_{dark}	2.1±1.0*	5.5±1.3*	2.3±0.3*	0.8±0.1	0.8±0.2	1.1±0.2

Figure legends

Figure 1. (a, b) The net CO_2 assimilation rate (A_N), (c, d) the stomatal conductance (g_s) and (e, f) the mesophyll conductance (g_m) in plants grown at CT (a, c, e) and HT (b, d, f) for rice, wheat and maize measured at ▬ WW-25°C, ▭ WD-25°C, ▬ WW-38°C and ▭ WD-38°C. Values are means ± standard error (n = 3-5). Different letters denote statistically significant differences between treatments within each species and growth temperature and asterisks between the two growth temperatures within the same species, irrigation treatment and temperature of measurement (Duncan analysis, $P < 0.05$).

Figure 2. The mitochondrial respiration at (R_{dark}) in plants grown at CT (a) and HT (b) for rice, wheat and maize measured at ▬ WW-25°C, ▭ WD-25°C, ▬ WW-38°C and ▭ WD-38°C. Values are means ± standard error (n = 3-5). Different letters denote statistically significant differences among treatments within each species and same growth temperature and asterisks between the two growth temperatures within the same species, irrigation treatment and temperature of measurement (Duncan analysis $P < 0.05$).

Figure 3. (a, b) The maximum velocity of Rubisco carboxylation (V_{cmax}), (c, d) the maximum electron transport rate (J_{max}) and the maximum velocity of PEPC carboxylation (V_{pmax}), in plants grown at CT (a, c) and HT (b, d). V_{cmax} were measured for wheat, rice and maize, J_{max} in wheat and rice, and V_{pmax} only in maize. All parameters were measured at ▬ WW-25°C, ▭ WD-25°C, ▬ WW-38°C and ▭ WD-38°C. Values are means ± standard error (n = 3-5). Different letters denote statistically significant differences among treatments within each species and same growth temperature and asterisks between the two growth temperatures within the same species, irrigation treatment and temperature of measurement (Duncan analysis ($P < 0.05$).

Figure 4. The relationship between: the gross photosynthesis rate (A_G) and the relative concentrations of CO_2 and O_2 (C_c/O) for (a) rice and (b) wheat. Symbols, treatments and lines as follows: ▲ CT-WW-25°C, △ CT-WD-25°C, solid regression (—); ▼ CT-WW-38°C, ▽ CT-WD-38°C, dashed regression (- - -); ■ HT-WW-25°C, □ HT-WD-25°C, dotted regression (·····); ● HT-WW-38°C, ○ HT-WD-38°C, dashed-dotted regression (— · —). In rice, solid regression $R^2 = 0.90$ $P < 0.001$, dashed regression $R^2 = 0.69$ $P < 0.01$, dotted regression $R^2 = 0.54$ $P < 0.05$ and dashed-dotted regression $R^2 = 0.77$ $P < 0.001$. In wheat, solid regression $R^2 = 0.79$ $P < 0.001$, dashed regression $R^2 = 0.60$ $P < 0.01$ and dotted regression $R^2 = 0.65$ $P < 0.01$.

Figure 1.

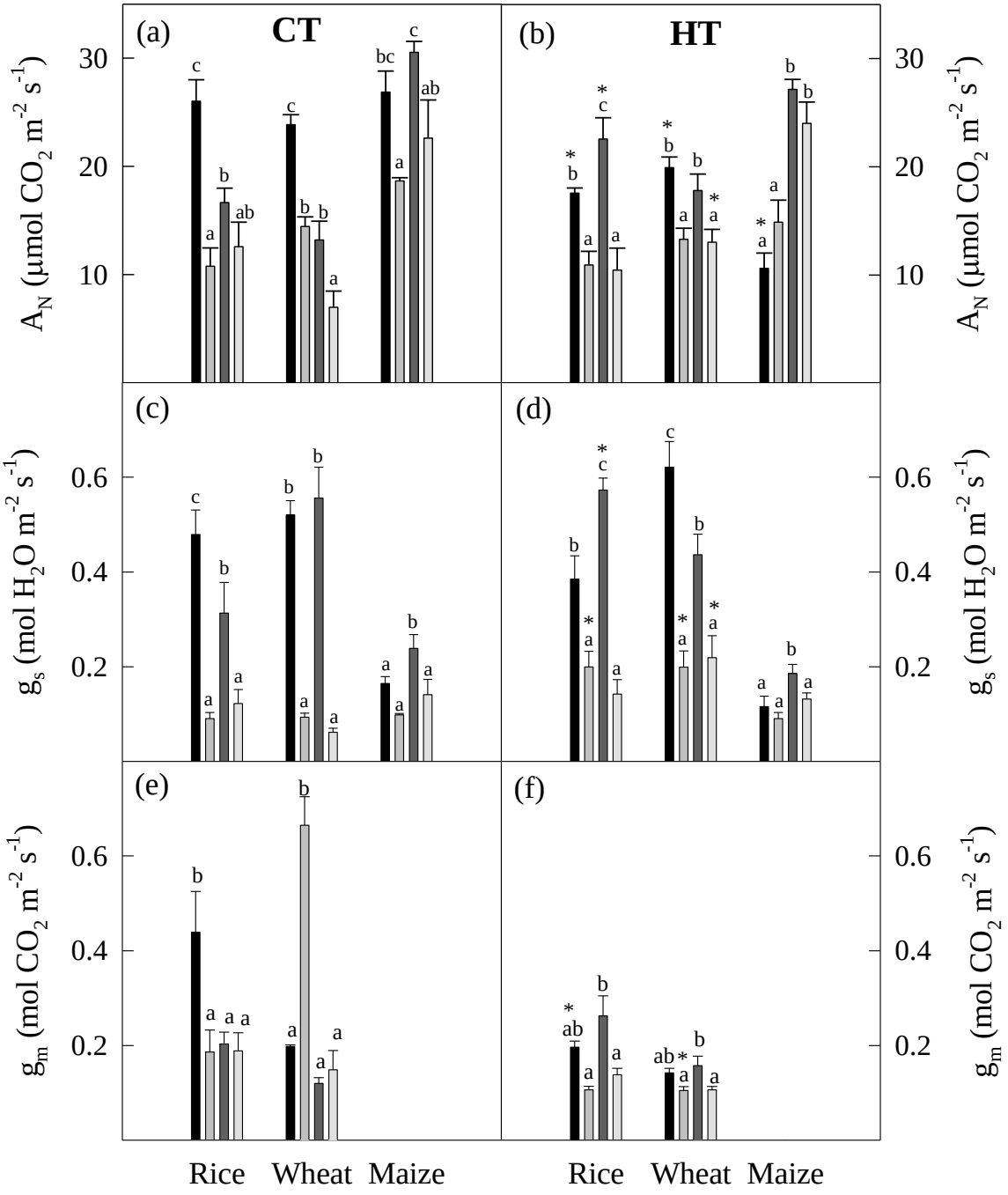

(a)
CT
(b)
HT
(c)
(d)
(e)
(f)
A_N (μmol CO_2 m^{-2} s^{-1})
g_s (mol H_2O m^{-2} s^{-1})
g_m (mol CO_2 m^{-2} s^{-1})
Rice
Wheat
Maize

Figure 2.

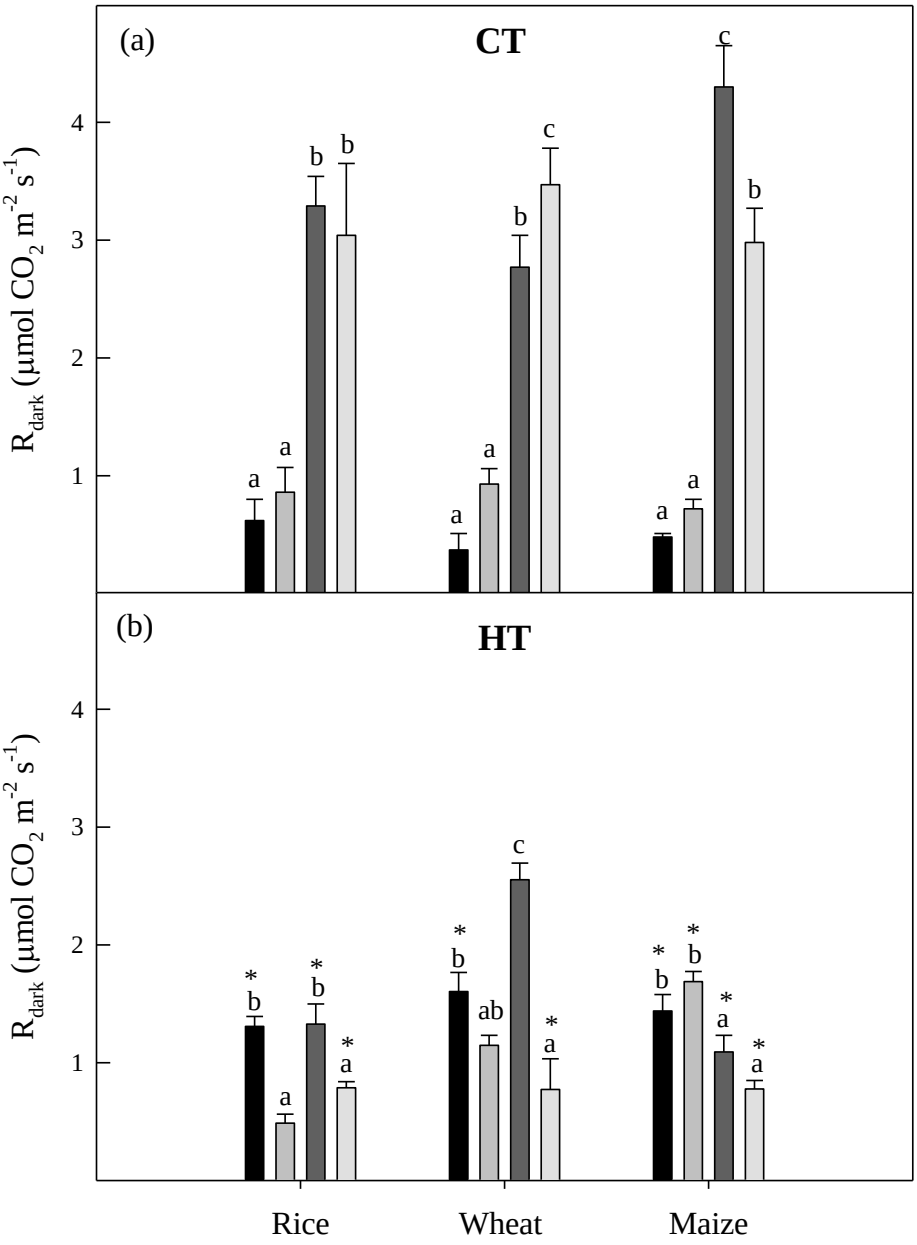

(a)
CT
(b)
HT
R_{dark} (μmol CO_2 m^{-2} s^{-1})
1
2
3
4
a
a
b
b
a
a
b
c
a
a
c
b
b
a
b
a
ab
c
a
b
b
a
a
Rice
Wheat
Maize

Figure 3.

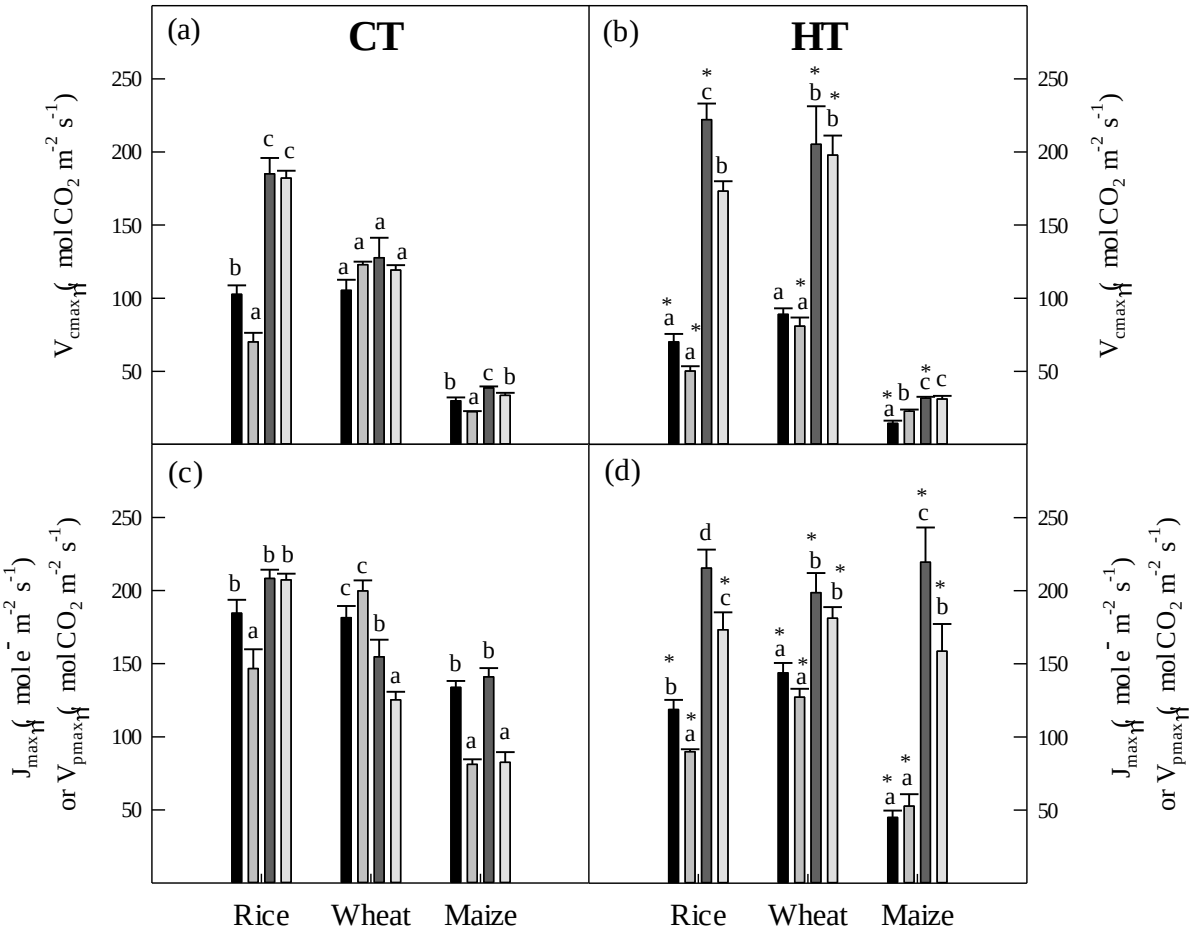
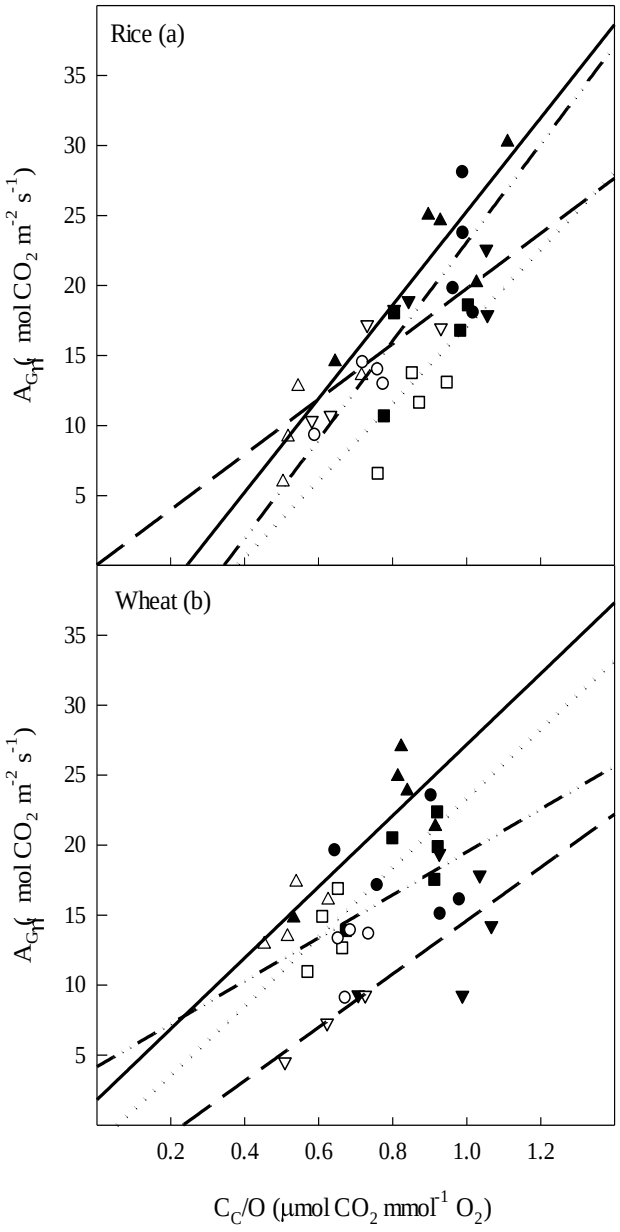

(a)
CT
(b)
HT
(c)
(d)
V_{cmax} ($mol CO_2 m^{-2} s^{-1}$)
J_{max} ($mole^- m^{-2} s^{-1}$) or V_{pmax} ($mol CO_2 m^{-2} s^{-1}$)
250
200
150
100
50
Rice
Wheat
Maize

Figure 4.


Rice (a)
Wheat (b)
A_{Gn} (mol CO_2 m^{-2} s^{-1})
C_C/O (µmol CO_2 $mmol^{-1}$ O_2)
5
10
15
20
25
30
35
0.2
0.4
0.6
0.8
1.0
1.2

Supplementary Material

Acclimation of biochemical and diffusive components of photosynthesis in rice, wheat and maize to heat and water deficit: implications for modeling photosynthesis.

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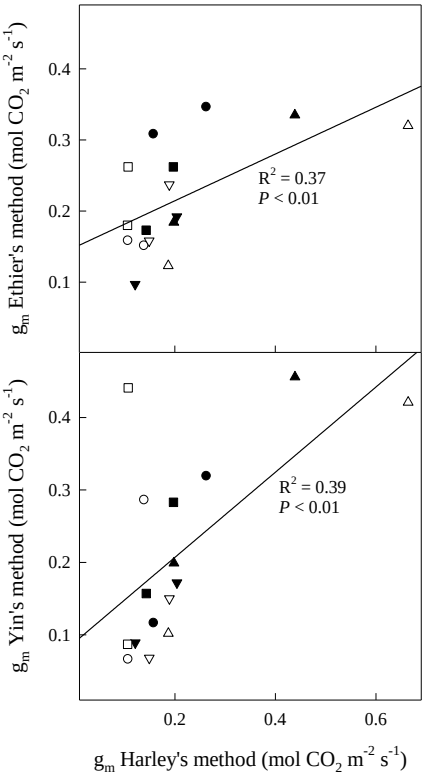
Figure S1. Comparison of the mesophyll conductance (g_m) estimated from three different methods: Harley et al. (1992), Ethier and Livingston (2004), Yin et al. (2009), in rice and wheat plants grown at CT and HT, under WW and WD conditions, and measured at 25ºC and 38ºC. The values for the Rubisco kinetic parameters required in the three methods were those measured *in vitro* in the present study for rice and wheat at the two temperatures, 25ºC and 38ºC (Table 1). Ethier method is based on gas exchange measurements, while Harley and Yin methods are based on combination of gas exchange and chlorophyll fluorescence measurements. Yin and Harley methods differ in that Yin includes the possible contributions of cyclic electron transport, pseudocyclic electron transport, and variable Q-cycle to balance H^+ and e^- supply. Differences among methods are described in more detail by Pons *et al.* (2009). Symbols and treatments as follows: ▲ CT-WW-25ºC, △ CT-WD-25ºC, ▼ CT-WW-38ºC, ▽ CT-WD-38ºC, ■ HT-WW-25ºC, □ HT-WD-25ºC, ● HT-WW-38ºC, ○ HT-WD-38ºC.



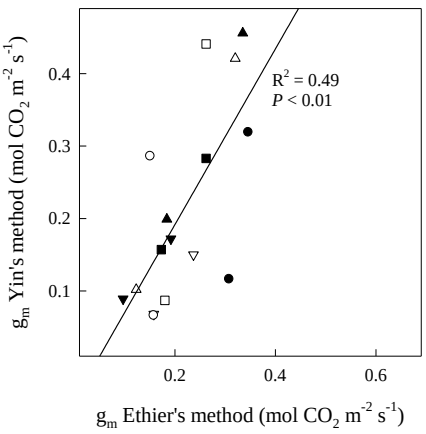


Figure S2. The relationship between the net CO_2 assimilation rate (A_N) and the total leaf conductance (g_t) (a, b) in rice and wheat, and the stomatal conductance (c) in maize. Symbols and treatments follows: ▲ CT-WW-25ºC, △ CT-WD-25ºC, ▼ CT-WW-38ºC ▽ CT-WD-38ºC, ■ HT-WW-25ºC, □ HT-WD-25ºC, ● HT-WW-38ºC, ○ HT-WD-38ºC. Lines and treatments as follows: solid regression (—) CT-25ºC, dashed regression (- - -) CT-38ºC, dotted regression (·····) HT-25ºC, dashed-dotted regression (-·-·-) HT-38ºC.

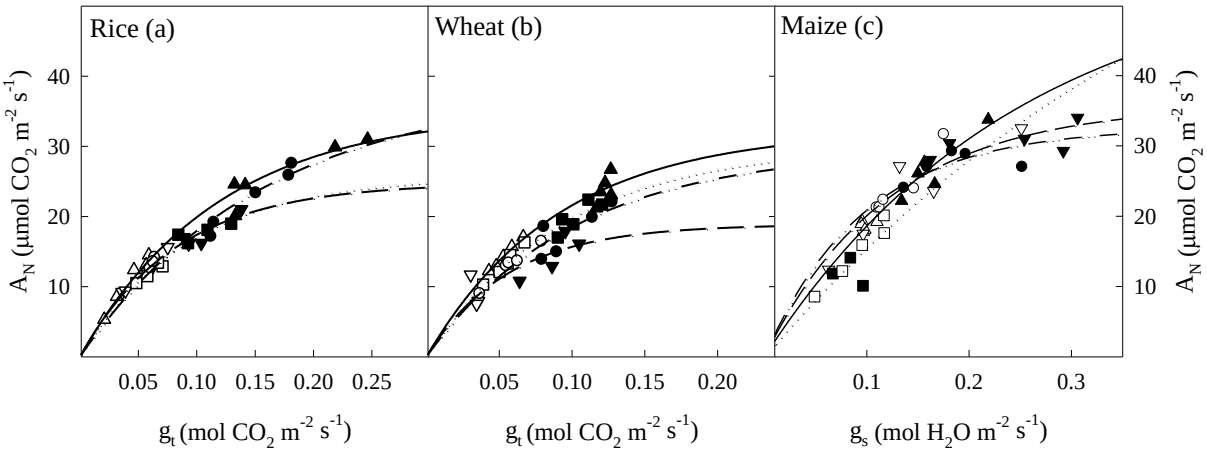


Figure S3. Relationship between the net CO_2 assimilation rate (A_N) and the chloroplastic CO_2 concentration (C_c) in rice. Values are means ± standard error (n = 5). Axis scales have been adjusted to allow comparison among panels within this figure and with Figs. S4 & S5.

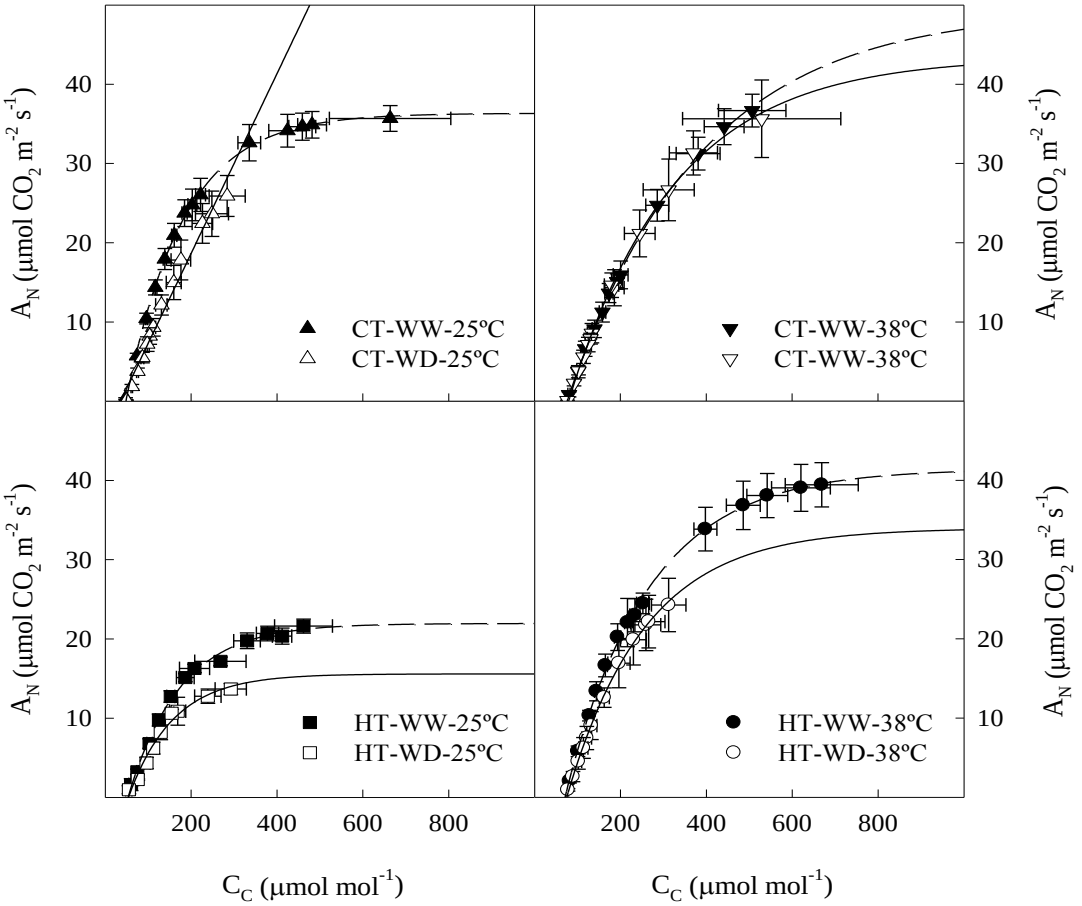


Figure S4. Relationship between the net CO_2 assimilation rate (A_N) and the chloropastic CO_2 concentration (C_c) in wheat. Values are means ± standard error (n = 5). Axis scales have been adjusted to allow comparison among panels within this figure and with Figs. S3 & S5.

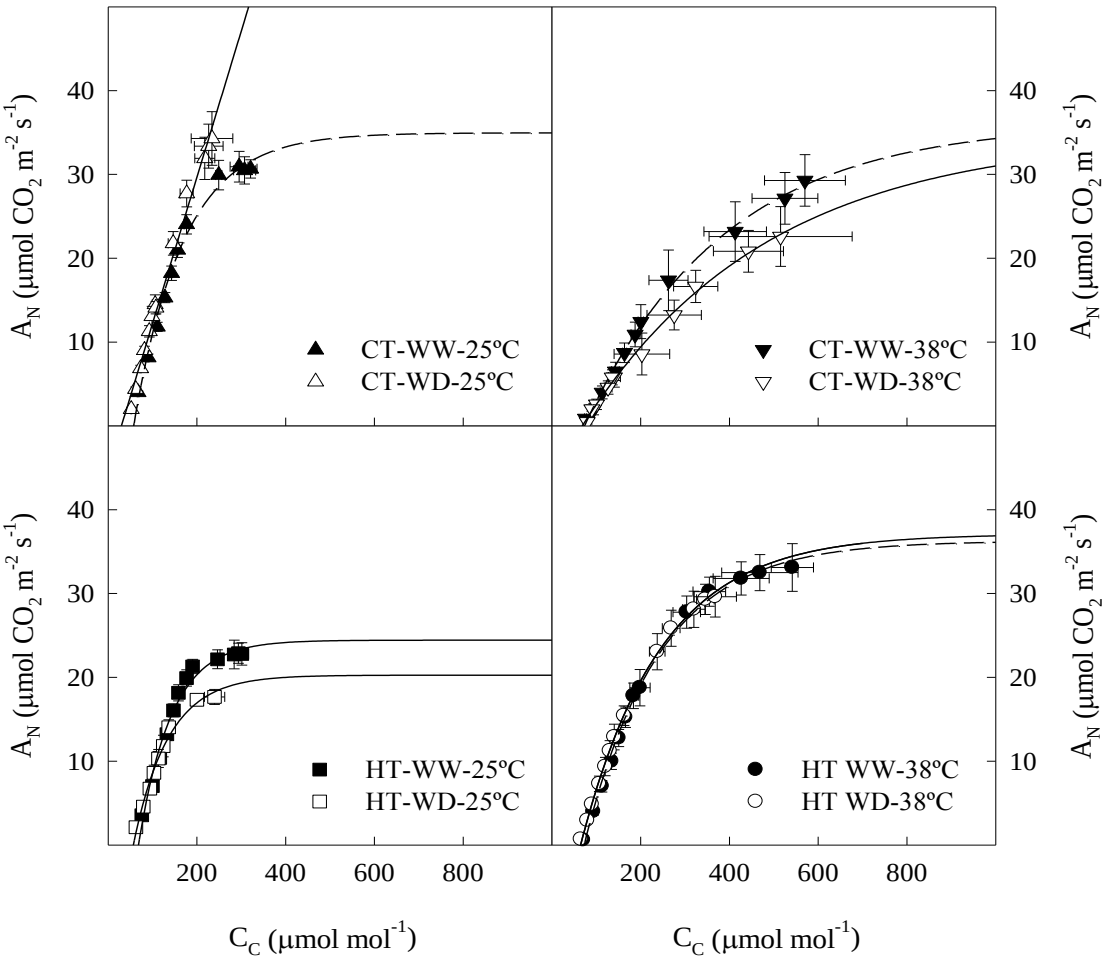


Figure S5. Relationship between the net CO_2 assimilation rate (A_N) and the bundle sheath CO_2 concentration (C_s) in maize. Values are means ± standard error (n = 5). Y-axis scale has been adjusted to allow comparison among panels within this figure and with Figs. S3 & S4.

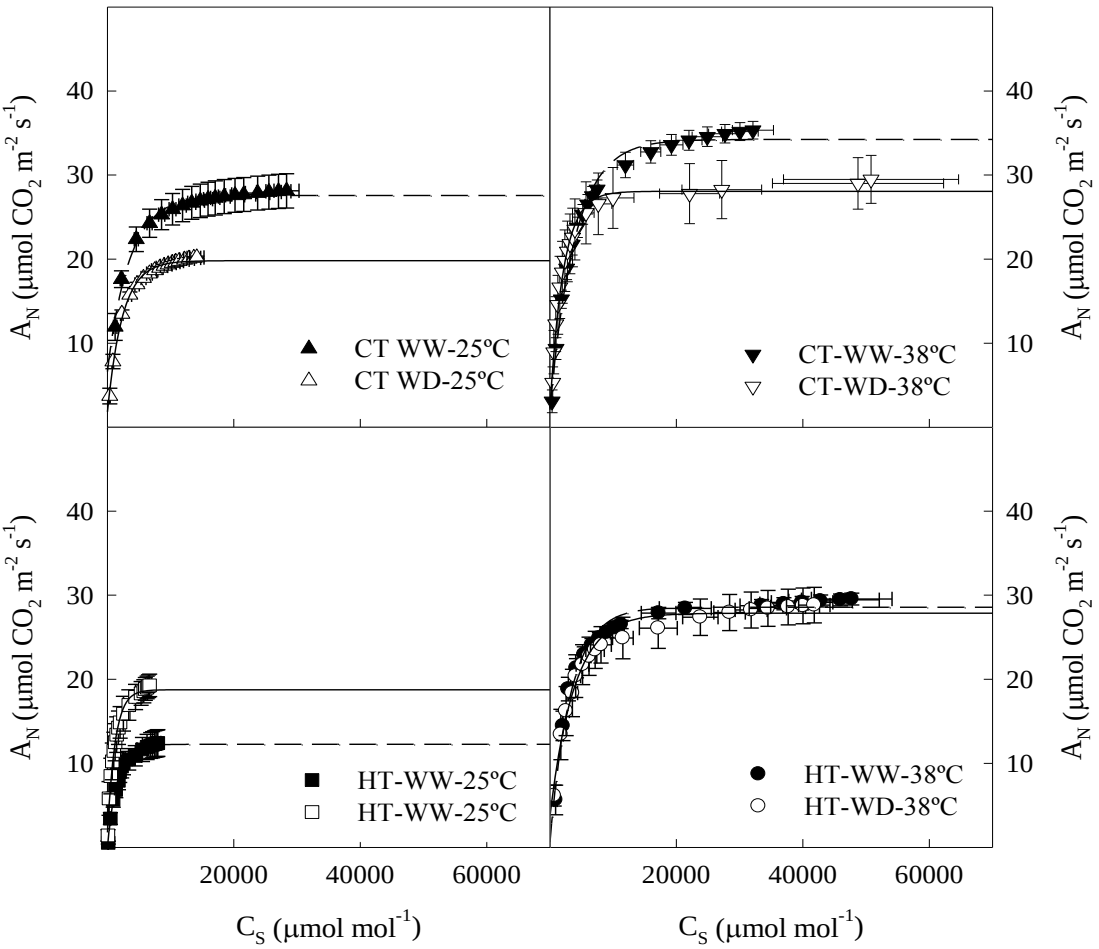


Table S1. Comparison of the maximum velocity of Rubisco carboxylation (V_{cmax}) and maximum rate of electron transport (J_{max}), estimated with the method described by Ethier and Livingston (2004), in plants grown at CT and HT, under WW and WD, and measured at 25ºC and 38ºC, using the Rubisco kinetics parameters (K_c, K_o and $S_{c/o}$) measured in the present study for rice and wheat (own kinetics), with regard to the parameters described in Bernacchi et al. (2001, 2002). Values are means ± standard errors (n = 5). Asterisks denote statistically significant differences by Duncan analysis ($P < 0.05$) between the two methods within the same treatment.

Species	**Growth T (ºC)**	**Irrigation Treatment**	**Measurement T (ºC)**	**Own kinetics**		**Kinetics by Bernacchi et al. (2001, 2002)**	
				V_{cmax}	**J_{max}**	**V_{cmax}**	**J_{max}**
Rice	CT	WW	25	165.0±9.6	186.1±13.2	183.0±17.2	179.3±12.4
Rice	CT	WD	25	108.4±4.6	120.3±17.5	146.8±7.9*	115.1±18.4
Rice	CT	WW	38	251.2±6.2	202.7±14.1	275.7±15.2	188.9±11.1
Rice	CT	WD	38	152.8±4.8	214.1±6.6	224.2±3.2*	180.3±16.6
Rice	HT	WW	25	100.7±10.0	121.8±7.7	89.0±7.5	108.5±4.5
Rice	HT	WD	25	60.3±2.9	70.0±3.5	61.2±8.6	74.1±6.6
Rice	HT	WW	38	260.0±11.1	204.6±18.2	303.1±15.3	201.7±18.8
Rice	HT	WD	38	141.6±29.5	117.9±17.3	159.2±26.0	112.2±16.8
Wheat	CT	WW	25	173.6±10.2	182.7±10.2	169.6±3.7	170.6±4.5
Wheat	CT	WD	25	169.3±7.4	201.6±7.6	178.1±0.9	169.0±1.4*
Wheat	CT	WW	38	156.8±25.4	147.7±13.9	207.4±45.1	146.7±15.4
Wheat	CT	WD	38	99.3±21.1	123.0±19.5	150.2±22.5	124.9±9.4
Wheat	HT	WW	25	159.8±10.9	145.5±13.7	149.2±12.9	136.5±12.8
Wheat	HT	WD	25	98.2±12.5	94.3±2.8	93.0±12.3	96.1±4.5
Wheat	HT	WW	38	153.8±17.6	161.6±12.0	277.5±30.6*	169.6±14.0
Wheat	HT	WD	38	142.9±9.0	156.3±14.9	220.5±23.4*	146.7±9.6

Table S2. Comparison of the mesophyll conductance (g_m, mol m^{-2} s^{-1}) estimated from three different methods: Harley et al. (1992), Ethier and Livingston (2004), Yin et al. (2009) using the Rubisco kinetic parameters measured *in vitro* in the present study for rice and wheat at the two temperatures, 25ºC and 38ºC (own kinetics), with regard to the Rubisco kinetic parameters reported for tobacco by Bernacchi et al. (2001, 2002) at 25ºC and 38ºC, in rice and wheat plants grown at CT and HT, under WW and WD conditions, and measured at 25ºC and 38ºC. Values are means ± standard errors (n = 5). Asterisks denote statistically significant differences by Duncan analysis ($P < 0.05$) between the Rubisco kinetics used (i.e., own kinetics *vs*. Bernacchi kinetics) within the same method and treatment.

Species	**Growth T (ºC)**	**Irrigation Treatment**	**Measurement T (ºC)**	**Own kinetics**			**Kinetics by Bernacchi *et al.* (2001, 2002)**		
				g_m Harley's method	**g_m Ethier's method**	**g_m Yin's method**	**g_m Harley's method**	**g_m Ethier's method**	**g_m Yin's method**
Rice	CT	WW	25	0.439±0.086	0.335±0.027	0.456±0.091	0.354±0.037	0.306±0.025	0.317±0.050
Rice	CT	WD	25	0.187±0.046	0.123±0.020	0.102±0.026	0.217±0.090	0.121±0.016	0.087±0.018
Rice	CT	WW	38	0.204±0.025	0.192±0.032	0.172±0.032	0.464±0.098*	0.188±0.025	0.132±0.020
Rice	CT	WD	38	0.189±0.038	0.237±0.037	0.150±0.049	0.152±0.011	0.242±0.055	0.114±0.031
Rice	HT	WW	25	0.197±0.013	0.262±0.023	0.283±0.063	0.273±0.025*	0.330±0.045	0.253±0.055
Rice	HT	WD	25	0.107±0.007	0.262±0.057	0.441±0.029	0.115±0.019	0.245±0.077	0.365±0.020*
Rice	HT	WW	38	0.263±0.042	0.346±0.044	0.319±0.009	0.347±0.116	0.258±0.034*	0.299±0.009*
Rice	HT	WD	38	0.139±0.013	0.151±0.039	0.286±0.016	0.170±0.050	0.234±0.080	0.215±0.027

Wheat	CT	WW	25	0.198±0.004	0.184±0.011	0.199±0.005	0.203±0.010	0.196±0.017	0.170±0.005
Wheat	CT	WD	25	0.664±0.060	0.320±0.012	0.421±0.068	0.661±0.069	0.382±0.030	0.423±0.038
Wheat	CT	WW	38	0.121±0.012	0.097±0.013	0.089±0.017	0.218±0.044*	0.219±0.076*	0.109±0.023
Wheat	CT	WD	38	0.149±0.041	0.158±0.012	0.068±0.018	0.097±0.041	0.269±0.068*	0.085±0.026
Wheat	HT	WW	25	0.143±0.009	0.173±0.022	0.157±0.010	0.137±0.007	0.181±0.019	0.136±0.009
Wheat	HT	WD	25	0.106±0.008	0.180±0.031	0.087±0.013	0.107±0.008	0.161±0.007	0.078±0.012
Wheat	HT	WW	38	0.158±0.020	0.308±0.023	0.116±0.024	0.258±0.061*	0.231±0.032*	0.141±0.033
Wheat	HT	WD	38	0.107±0.007	0.158±0.020	0.066±0.008	0.139±0.010*	0.189±0.038	0.073±0.010