

# Short-term elevated temperature and CO<sub>2</sub> promote photosynthetic induction in the C<sub>3</sub> plant *Glycine max*, but not in the C<sub>4</sub> plant *Amaranthus tricolor*

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## ABSTRACT

The continuous increases of atmospheric temperature and CO<sub>2</sub> concentration will impact global photosynthesis. However, there are few studies considering the interaction of elevated temperature (eT) and elevated CO<sub>2</sub> (eCO<sub>2</sub>) on dynamic photosynthesis, particularly for C<sub>4</sub> species. We examine dynamic photosynthesis under four different temperature and [CO<sub>2</sub>] treatments: (1) 400 ppm × 28°C (CT); (2) 400 ppm × 33°C (CT+); (3) 800 ppm × 28°C (C+T); and (4) 800 ppm × 33°C (C+T+). In *Glycine max* L., the time required to reach 50% (*T*<sub>50%A</sub>) and 90% (*T*<sub>90%A</sub>) of full photosynthetic induction was smaller under the CT+, C+T, and C+T+ treatments than those under the CT treatment. In *Amaranthus tricolor* L., however, neither *T*<sub>50%A</sub> nor *T*<sub>90%A</sub> was not significantly affected by eT or eCO<sub>2</sub>. In comparison with the CT treatment, the achieved carbon gain was increased by 58.3% (CT+), 112% (C+T), and 136.6% (C+T+) in *G. max* and was increased by 17.1% (CT+), 2.6% (C+T) and 56.9% (C+T+) in *A. tricolor*. The increases of achieved carbon gain in *G. max* were attributable to both improved photosynthetic induction efficiency (IE) and enhanced steady-state photosynthesis, whereas those in *A. tricolor* were attributable to enhanced steady-state photosynthesis.

**Keywords:** C<sub>4</sub> photosynthesis, climate change, dynamic photosynthesis, fluctuating light, lightfleck, Rubisco activase, soybean, stomatal conductance.

## Introduction

Atmospheric CO<sub>2</sub> concentration has increased by half since the Industrial Revolution, and the annual global mean temperature has also risen by more than 0.6°C since 1950 (IPCC 2014). A large volume of studies have reported that elevated CO<sub>2</sub> concentration (eCO<sub>2</sub>) enhances photosynthetic capacity (Drake *et al.* 1997; Ainsworth and Long 2005; Ainsworth and Rogers 2007), whereas the effects of elevated temperature (eT) on photosynthetic capacity are complex, depending on both the initial leaf temperature and the degree of warming (Dusenge *et al.* 2019). However, the stress of eCO<sub>2</sub> and warming does not occur in isolation. Under climate change, the two stresses occur simultaneously (Norby and Luo 2004; Luo *et al.* 2008). Photosynthetic responses to the two stresses may be synergistic in some circumstances, but antagonistic in others (Dieleman *et al.* 2012; Smith and Dukes 2013). To project photosynthesis in a future of climate change, studies on the interactive effects of eT and eCO<sub>2</sub> on photosynthesis are urgently needed (Xu *et al.* 2013, 2014).

Most studies on photosynthesis were carried out under constant light conditions, where light was well controlled. However, in natural environments, light is constantly fluctuating, leading to fluctuations in operating leaf photosynthesis (Chazdon and Pearcy 1986; Pearcy 1990; Pearcy *et al.* 1996; Tang 1997). Investigating photosynthesis in fluctuating light; i.e. dynamic photosynthesis, will improve our understanding of photosynthesis in natural environments.

There have been some studies addressing the effects of eT or eCO<sub>2</sub> on dynamic photosynthesis in C<sub>3</sub> plants. Below the temperature optimum point, short-term eT can

promote photosynthetic induction (the time course of photosynthetic rate in response to a sudden increase in light intensity) by reducing the biochemical limitation of RuBP (ribulose-1,5-bisphosphate) regeneration and Rubisco activation (Kaiser et al. 2015). In contrast, elevating temperature above the temperature optimum point may inhibit photosynthetic induction by depressing Rubisco activation (Leakey et al. 2003; Kang et al. 2020). Short-term  $eCO_2$  can promote photosynthetic induction by reducing the biochemical limitation of Rubisco activation and stomatal limitation (Tomimatsu and Tang 2016; Kaiser et al. 2017a, 2017b; Tomimatsu et al. 2019; Kang et al. 2021).

As far as we know, there has been no study addressing the effects of the interaction of  $eT$  and  $eCO_2$  on dynamic photosynthesis. Elevating temperature decreases the solubility of  $CO_2$  in water but  $eCO_2$  can compensate this factor (Jordan and Ogren 1984; Foyer et al. 2009). Another source of uncertainty regarding the interaction of  $eT$  and  $eCO_2$  results from the inconsistent results about the effects of  $eT$  and  $eCO_2$  on stomatal limitation in previous studies (Naumburg et al. 2001; Leakey et al. 2002, 2003; Tomimatsu and Tang 2012; Kaiser et al. 2017a). Elevating  $CO_2$  reduces stomatal limitation, whereas  $eT$  could increase or reduce stomatal limitation (Kaiser et al. 2015, 2017a; Wachendorf and Küppers 2017b), probably due to the trade-off between carbon gain and evaporative cooling (Moore et al. 2021). Elevating temperature generally increases post-illumination  $CO_2$  fixation and post-illumination  $CO_2$  burst at low and medium leaf temperature (Sun et al. 1999; Foyer et al. 2009), but  $eCO_2$  decreases post-illumination  $CO_2$  burst by inhibiting photorespiration (Leakey et al. 2002). Therefore, studies are needed to address the uncertainty of the effects of the interaction of  $eT$  and  $eCO_2$  on dynamic photosynthesis.

About 3% of the Earth's angiosperm species utilise the  $C_4$  photosynthetic pathway, yet  $C_4$  plants contribute about 25% of the net terrestrial primary productivity on Earth (Sage 2004). There have been many studies on the steady-state photosynthesis in  $C_4$  plants (Furbank et al. 1990; Sage and Kubien 2007; Sage and Zhu 2011; Sage et al. 2012; Long and Spence 2013; von Caemmerer 2021), but rare on their dynamic photosynthesis (Furbank and Walker 1985; Horton and Neufeld 1998; Pignon et al. 2021; Wang et al. 2021). Kubásek et al. (2013) have shown that  $C_4$  plants lost more carbon in fluctuating light than in steady light compared with  $C_3$  plants, which they assumed was a result of an increase of bundle sheath cells (BSC) leakiness to  $CO_2$  in  $C_4$  plants in fluctuating light. However, Stitt and Zhu (2014) proposed that the two-cell Kranz system of  $C_4$  photosynthesis ensures large pools of metabolites that could drive diffusion between BSCs and mesophyll cells (MC) for buffering ATP and NADPH, which may provide powerful protection against fluctuating light intensities. Recently, Li et al. (2021) have proposed that the accumulation and diffusion of metabolites in  $C_4$  plants from MCs to BSCs take more time, which may delay the photosynthetic induction.

Therefore, the differences between  $C_3$  and  $C_4$  plants in their dynamic photosynthesis are still under debate.

In this study, we characterised photosynthetic responses to the simulated changes of light intensity in a  $C_3$  species and a  $C_4$  species under four different treatments, aiming to address: (1) the effects of the interaction of short-term  $eT$  and  $eCO_2$  on dynamic photosynthesis; and (2) the differences between  $C_3$  and  $C_4$  plants in their dynamic photosynthetic responses to  $eT$  and  $eCO_2$ . We hypothesised that: (1) the short-term  $eT$  and  $eCO_2$  would promote photosynthetic induction and enhance carbon gain; and (2) the promotion of photosynthetic induction and the enhancement of carbon gain were greater for  $C_3$  plants than for  $C_4$  plants.

## Methods and materials

### Plant materials

The seeds of the  $C_3$  species, *Glycine max* L. and the  $C_4$  species, *Amaranthus tricolor* L. (NAD-ME subtype) were sown in pots (the circular radius was 5 cm and the height was 14 cm) filled with composite soil. Each of the two species was grown within a growth chamber (E-36L1, Percival, Perry, Iowa, USA). After germination, seedlings were thinned. The plants were watered with distilled water regularly and supplied with 5 mL full concentration formula nutrient solution (nitrogen 17 g/L, phosphorus anhydride 17 g/L, potassium oxide 17 g/L, organic matter 25 g/L, and amino acid 12 g/L) every 3–5 days. These ensure that all plants can obtain sufficient nutrients and water, which helps reduce the degree of growth limitation (Poorter et al. 2012). Growth environments was kept constant and consistent throughout the experiment in both growth chambers. Photosynthetic photon flux density (PPFD) incident on the top of both canopies was about  $600 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The photoperiod was 14 h. Day/night air temperature was set to 28/22°C,  $[CO_2]$  was the same as ambient  $CO_2$  concentration and relative humidity was maintained at 70%.

### Leaf gas exchange measurements

Leaf gas exchange was measured on plants 25 days after germination using a Li-6800 portable photosynthesis system (LI-COR Biosciences, Lincoln, NE, USA) equipped with a Li-6800-01 fluorometer (90% red and 10% blue) on the most recently fully expanded leaves ( $n = 4$ ). The measured plants were moved to another growth chamber 60 min before the start of the measurement to acclimate to the target temperature and  $[CO_2]$  in advance. The measured leaves were enclosed in the Li-6800 leaf chamber, and were first acclimated in the chamber to a PPFD of  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  until steady-state net assimilation rate ( $A$ ) and stomatal conductance for  $H_2O$  ( $g_{sw}$ ) were visibly reached, after which PPFD was raised to  $600 \mu\text{mol m}^{-2} \text{s}^{-1}$  and kept

steady for 60 min. Then, PPFD was decreased to 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  until  $A$  reached steady states again. Gas exchange parameters, including  $A$ ,  $g_{\text{sw}}$ , intercellular  $\text{CO}_2$  concentration ( $C_i$ ), and transpiration rate ( $E$ ), were logged every second. To avoid any swinging from correctional changes in temperature or relative humidity, the temperature of the heat exchanger ( $T_{\text{exch}}$ ) was controlled. All measurements were repeated under four different combinations of temperature and  $\text{CO}_2$  concentration: (1) 400 ppm  $\times$  28°C, denoted as CT; (2) 400 ppm  $\times$  33°C, denoted as CT+; (3) 800 ppm  $\times$  28°C, denoted as C+T; and (4) 800 ppm  $\times$  33°C, denoted as C+T+. The vapour pressure difference between leaf and ambient air ranged from 0.9 kPa to 1.1 kPa at 28°C and from 1.1 kPa to 1.5 kPa at 33°C.

## Data analysis

Steady-state  $A$ ,  $g_{\text{sw}}$ , and  $C_i$  reached at each PPFD level were calculated by averaging the single values over the last minute of each period;  $T_{50\%A}$  and  $T_{90\%A}$  were defined as the time required to reach 50% and 90% of the differences between  $A_{100}$  and  $A_{600}$ . We calculated the induction state (IS) after Chazdon and Pearcy (1986):

$$\text{IS}(t) = \frac{A(t) - A_{100}}{A_{600} - A_{100}} \times 100\% \quad (1)$$

where  $A(t)$  is the transient  $A$  at time  $t$ .

For  $C_3$  plants, under Rubisco limitation,  $A(t)$  can be calculated after Farquhar *et al.* (1980):

$$A(t) = V_c(t) \left[ \frac{C_i(t) - \Gamma^*}{C_i(t) + K_m} \right] - R_L \quad (2)$$

where  $V_c(t)$  and  $C_i(t)$  is  $V_c$  and  $C_i$  at time  $t$ , respectively.  $K_m$  is the Michaelis–Menten constants of Rubisco and  $\Gamma^*$  is the  $\text{CO}_2$  compensation point, they were both taken from Bernacchi *et al.* (2003).  $R_L$  is mitochondrial respiration rate in the light and assumed to be 40% of dark respiration rate between 25 and 35°C (Way *et al.* 2019).

The time courses of  $V_c(t)$  were then fitted to the model proposed by Woodrow and Mott (1989), and we use  $V_c(t)$  to replace  $A(t)$  after correction of  $C_i(t)$ .

$$V_c(t) = V_{c,\text{max}} - (V_{c,\text{max}} - V_{c,\text{ini}}) \times e^{\left(\frac{-t}{\tau_R}\right)} \quad (3)$$

where  $V_{c,\text{ini}}$  and  $V_{c,\text{max}}$  are the initial and maximum  $V_c(t)$  after correction to 25°C (Bernacchi *et al.* 2003), respectively;  $\tau_R$  is the apparent time constant of Rubisco activation.

The achieved carbon gain (ACG) and ideal carbon gain (ICG) were calculated after Kang *et al.* (2021):

$$\text{ACG}(t) = \int_{t_0}^t A(t) dt - A_{100} \times (t - t_0) \quad (4)$$

$$\text{ICG}(t) = (A_{600} - A_{100}) \times (t - t_0) \quad (5)$$

where  $t_0$  is the time when PPFD was increased. The ratio of ACG to ICG is photosynthetic induction efficiency (IE), which is calculated after Yanhong *et al.* (1994):

$$\text{IE}(t) = \frac{\text{ACG}(t)}{\text{ICG}(t)} = \frac{\int_{t_0}^t A(t) dt - A_{100} \times (t - t_0)}{(A_{600} - A_{100}) \times (t - t_0)} \quad (6)$$

Eqn 4 indicates that ACG during photosynthetic induction can be decomposed into ICG, which is only influenced by steady-state  $A$ ; and IE, which is influenced mostly by the time course of photosynthetic induction. Short-term eT and e $\text{CO}_2$  can influence ACG via changes in ICG and/or those in IE. We assumed that changes in IE reflected the responses of photosynthetic induction to different treatments *per se*.

Post-illumination carbon gain (PICG) due to the simulated lightfleck was calculated as follows:

$$\text{PICG}(t) = \int_{t_1}^t [A(t) - A_{100\text{post}}] dt, \text{ when } A(t) > A_{100\text{post}} \quad (7)$$

where  $t_1$  is the time when PPFD was decreased,  $A_{100\text{post}}$  is the steady-state  $A$  at the end of post-illumination period.

Intrinsic water use efficiency (iWUE) was calculated by dividing the transient  $A$  by the transient  $g_s$  (Farquhar and Sharkey 1982):

$$\text{iWUE} = \frac{A(t)}{g_s(t)} \quad (8)$$

## Statistical analysis

To determine the effects of measurement temperature and  $\text{CO}_2$  concentration ( $[\text{CO}_2]$ ) on gas exchange parameters for two species, when the requirement of the normality and homogeneity of variances were met, we used two-way ANOVA with temperature and  $[\text{CO}_2]$  as the main factors and temperature  $\times$   $[\text{CO}_2]$  as interaction, and Duncan test was used for *post hoc* multiple comparisons. When the requirement of the normality and homogeneity of variances were not met, we used a Kruskal–Wallis test to perform the same analysis. All tests were conducted using SPSS Statistics ver. 18.0 (IBM Corp., Armonk, NY, USA) and R ver. 3.6.1.

## Results

### Steady-state photosynthesis under different temperature and $\text{CO}_2$ treatments

In *G. max*, the mean  $A_{600}$  was increased by 20.0% and 61.5% under the CT+ and C+T treatments compared to the CT treatment. However, the mean  $A_{600}$  was increased by 74.8% under the C+T+ treatment, which was less than the sum of the effects of eT and e $\text{CO}_2$  alone (Table 1). In *A. tricolor*, the mean  $A_{100}$  and  $A_{600}$  were not significantly affected by eT, e $\text{CO}_2$  or their interaction (Table 1).

**Table 1.** Steady-state photosynthetic rate ( $A$ ), stomatal conductance ( $g_s$ ), and intercellular  $\text{CO}_2$  concentration ( $C_i$ ) reached under different temperature and  $[\text{CO}_2]$  treatments in *G. max* and *A. tricolor*.

Parameter	Treatments			
	CT	CT+	C+T	C+T+
<i>G. max</i>				
$A_{100}$	$2.90 \pm 0.50\text{a}$	$3.24 \pm 0.34\text{a}$	$4.72 \pm 0.20\text{b}$	$4.78 \pm 0.22\text{b}$
$g_{s100}^A$	$0.083 \pm 0.021$	$0.118 \pm 0.024$	$0.066 \pm 0.004$	$0.066 \pm 0.006$
$C_{i100}$	$326.70 \pm 6.48\text{a}$	$338.90 \pm 5.89\text{a}$	$659.46 \pm 12.74\text{b}$	$654.25 \pm 14.20\text{b}$
$A_{600}$	$17.24 \pm 2.69\text{a}$	$20.68 \pm 1.24\text{a}$	$27.84 \pm 1.47\text{b}$	$30.14 \pm 0.32\text{b}$
$g_{s600}$	$0.243 \pm 0.053\text{ab}$	$0.396 \pm 0.058\text{b}$	$0.188 \pm 0.024\text{a}$	$0.289 \pm 0.013\text{ab}$
$C_{i600}^A$	$239.18 \pm 7.58\text{a}$	$267.21 \pm 7.30\text{a}$	$489.39 \pm 17.99\text{b}$	$560.32 \pm 6.57\text{c}$
$A_{100\text{post}}$	$4.30 \pm 0.34\text{a}$	$4.13 \pm 0.13\text{a}$	$5.48 \pm 0.31\text{b}$	$5.48 \pm 0.18\text{b}$
$g_{s100\text{post}}$	$0.209 \pm 0.051\text{a}$	$0.377 \pm 0.058\text{b}$	$0.159 \pm 0.022\text{a}$	$0.274 \pm 0.012\text{ab}$
$C_{i100\text{post}}$	$343.52 \pm 11.24\text{a}$	$366.78 \pm 3.02\text{a}$	$715.26 \pm 11.95\text{b}$	$742.49 \pm 2.69\text{c}$
<i>A. tricolor</i>				
$A_{100}$	$3.50 \pm 0.38$	$2.99 \pm 0.29$	$3.72 \pm 0.25$	$3.57 \pm 0.22$
$g_{s100}$	$0.062 \pm 0.007$	$0.072 \pm 0.017$	$0.035 \pm 0.008$	$0.049 \pm 0.007$
$C_{i100}$	$290.94 \pm 19.42\text{a}$	$308.39 \pm 15.66\text{a}$	$578.19 \pm 49.33\text{b}$	$652.24 \pm 22.39\text{b}$
$A_{600}$	$9.78 \pm 1.40$	$11.00 \pm 1.13$	$10.65 \pm 2.69$	$13.75 \pm 1.58$
$g_{s600}$	$0.126 \pm 0.014$	$0.172 \pm 0.040$	$0.075 \pm 0.018$	$0.161 \pm 0.022$
$C_{i600}$	$243.34 \pm 28.56\text{a}$	$256.11 \pm 16.85\text{a}$	$532.27 \pm 27.29\text{b}$	$614.69 \pm 24.10\text{c}$
$A_{100\text{post}}$	$3.43 \pm 0.42$	$2.89 \pm 0.23$	$3.76 \pm 0.34$	$3.45 \pm 0.25$
$g_{s100\text{post}}$	$0.094 \pm 0.019$	$0.133 \pm 0.039$	$0.055 \pm 0.012$	$0.128 \pm 0.021$
$C_{i100\text{post}}$	$318.51 \pm 20.21\text{a}$	$337.61 \pm 16.64\text{a}$	$650.54 \pm 36.29\text{b}$	$729.98 \pm 11.30\text{c}$

Values are the means of four individual plants for each species ( $\pm$ s.e.). Different letters following means indicate significant ( $P < 0.05$ ) difference across four different environment treatments within each species. Absence of letters denotes absence of significant difference.  $A_{100}$ ,  $g_{s100}$ ,  $C_{i100}$ ,  $A_{600}$ ,  $g_{s600}$ ,  $C_{i600}$ ,  $A_{100\text{post}}$ ,  $g_{s100\text{post}}$ ,  $C_{i100\text{post}}$  were steady-state photosynthetic rate (unit  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), stomatal conductance for  $\text{H}_2\text{O}$  (unit  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), and intercellular  $\text{CO}_2$  concentration (unit  $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$ ) reached before photosynthetic induction, the end of photosynthetic induction and the end of post-illumination, respectively, calculated by averaging single values over the last half-minute of each period. Abbreviations for the four treatments are: (1)  $400 \text{ ppm} \times 28^\circ\text{C}$ , denoted as CT; (2)  $400 \text{ ppm} \times 33^\circ\text{C}$ , denoted as CT+; (3)  $800 \text{ ppm} \times 28^\circ\text{C}$ , denoted as C+T; (4)  $800 \text{ ppm} \times 33^\circ\text{C}$ , denoted as C+T+.

<sup>A</sup>Statistical analysis using a Kruskal–Wallis test.

## Dynamic photosynthesis under different temperature and $\text{CO}_2$ treatments

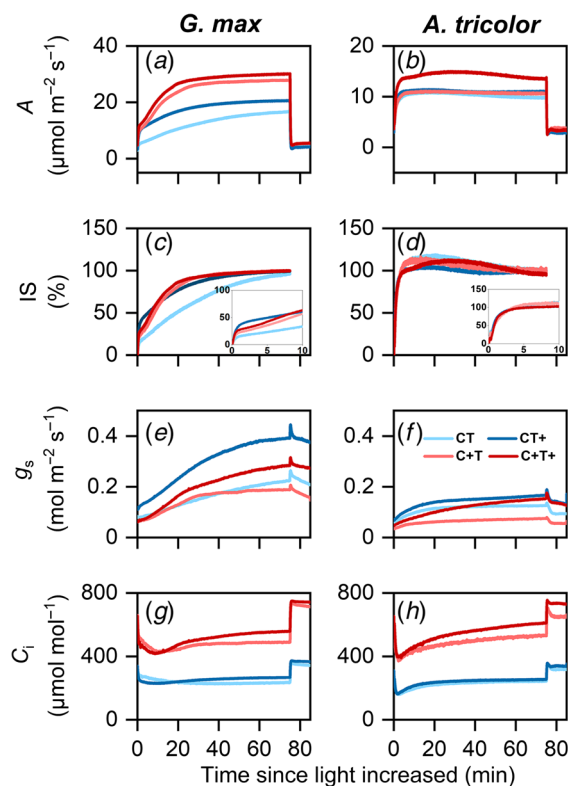
In comparison with CT treatment, the transient  $A$  in *G. max* were higher under the other three treatments, and evident difference was observed in the first minute of photosynthetic induction (Figs 1a, 2a). The transient IS was highest under the CT+ treatment during the first 5 min of induction; after that, IS was much higher under the C+T and C+T+ treatments (Fig. 1c). In *A. tricolor*, the transient  $A$  was almost the same in the first minute between all treatments; after that, the transient  $A$  was highest under the C+T+ treatment and still similar in the other three treatments (Figs 1b, 2b). The transient IS was always similar between all treatments (Fig. 1d). Bars for s.e. in Figs 1 and 2 were omitted for visual clarity. In addition, iWUE was higher under the C+T and C+T+ than other two treatments during photosynthetic induction in both species (Fig. S1).

In comparison with CT treatment, the mean  $T_{50\%A}$ ,  $T_{90\%A}$ ,  $\tau_R$ ,  $T_{50\%g}$  and  $T_{90\%g}$  in *G. max* were decreased under the other three treatments. However, effects on the mean  $T_{50\%A}$ ,  $T_{90\%A}$  and  $\tau_R$  under C+T+ treatment was less than the sum of the effects of eT and  $\text{eCO}_2$  alone (Fig. 3a, b), while effects on the mean  $T_{50\%g}$  and  $T_{90\%g}$  under C+T+ treatment was smaller than the effects of  $\text{eCO}_2$  alone (Fig. 3c, d). In *A. tricolor*, the mean  $T_{50\%A}$  and  $T_{90\%A}$  did not differ significantly between treatments (Fig. 3a, b). The mean  $T_{50\%g}$  and  $T_{90\%g}$  still had no difference between the CT, CT+ and C+T treatments, but significant higher under the C+T+ treatment (Fig. 3c, d).

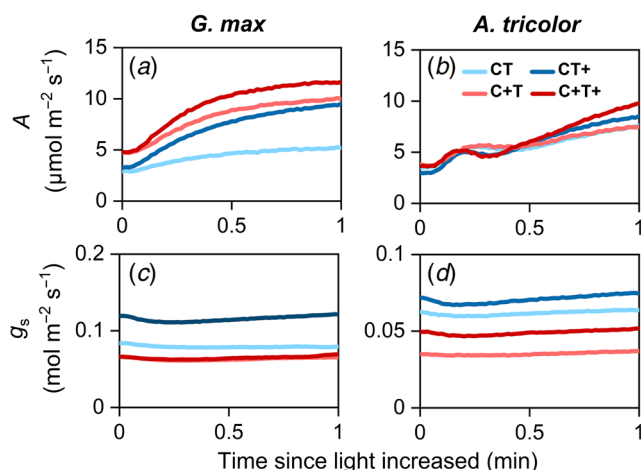
## ACG and IE

In *G. max*,  $\text{ACG}_{60 \text{ min}}$  was increased by 58.3% and 111.9% under the CT+ and C+T treatments compared to the CT treatment. However,  $\text{ACG}_{60 \text{ min}}$  was increased by 136.4% under the C+T+ treatment, which was less than the sum of the effects of eT and  $\text{eCO}_2$  alone (Fig. 4a). In *A. tricolor*,





**Fig. 1.** Time courses of  $A$  (a, b),  $IS$  (c, d),  $g_s$  (e, f), and  $C_i$  (g, h) in *G. max* (a, c, e, g) and *A. tricolor* (b, d, f, h) leaves following an increase of PPFD from 100 to 600  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and then a decrease in light intensity from 600 to 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . Values are presented as the means of four biological replicates; i.e. individual plants for each species. Bars for s.e. were omitted for visual clarity.  $A$ , photosynthetic rate;  $IS$ , induction state;  $g_s$ , stomatal conductance;  $C_i$ , intercellular  $\text{CO}_2$  concentration.



**Fig. 2.** Transient  $A$  (a, b) and  $g_s$  (c, d) in *G. max* (a, c) and *A. tricolor* (b, d) leaves during the first minute following an increase of PPFD from 100 to 600  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . Values are presented as the means of four biological replicates; i.e. individual plants for each species. Bars for s.e. were omitted for visual clarity.  $A$ , photosynthetic rate;  $g_s$ , stomatal conductance.

$ACG_{60 \text{ min}}$  under the CT treatment was also less than the other three treatments, but the promotion effect on the C+T+ treatment was larger than the sum of the effects of eT and e $\text{CO}_2$  alone (Fig. 4b). In comparison with CT treatment, PICG in *G. max* and *A. tricolor* were higher under the other three treatments, but the effects on the C+T+ treatment was less than the effects of eT alone (Fig. 4c, d).

In *G. max*, ACG and IE were higher under the other three treatments than those under the CT treatment (Fig. 5a) and ACG were always highest under the C+T+ treatment at different time points (Fig. 5c). In *A. tricolor*, IE did not differ significantly between all treatments at any time (Fig. 5b, d), despite there were changes in  $ACG_{60 \text{ min}}$  in *A. tricolor* (Fig. 4b).

To further assess the contribution of ACG and IE on increasing  $ACG_{60 \text{ min}}$ , we estimated the potential  $ICG_{60 \text{ min}}$  by assuming that eT and e $\text{CO}_2$  had no effect on photosynthetic induction (equivalent to no changes in IE). In comparison with CT treatment, the increase in  $ICG_{60 \text{ min}}$  enhanced  $ACG_{60 \text{ min}}$  by 16.9%, 55.3% and 73.1% in *G. max* and also enhanced  $ACG_{60 \text{ min}}$  by 27.7%, 11.4% and 64.5% in *A. tricolor* under the other three treatments (Fig. 6a, b). Then, we assumed no effects of eT and e $\text{CO}_2$  on steady-state  $A$  (equivalent to no changes in ICG) to assess the separate contribution of IE. Compared to the CT treatment, the increase in  $IE_{60 \text{ min}}$  increased  $ACG_{60 \text{ min}}$  by 31.5%, 31.4% and 34.3% in *G. max* under the other three treatments, but decreased  $ACG_{60 \text{ min}}$  by 6.5%, 5.4% and 3.5% in *A. tricolor* (Fig. 6a, b). In summary,  $ACG_{60 \text{ min}}$  in *G. max* was affected by both  $IE_{60 \text{ min}}$  and  $ICG_{60 \text{ min}}$ . eT had a similar effect on  $IE_{60 \text{ min}}$  as e $\text{CO}_2$  but a lower effect on  $ICG_{60 \text{ min}}$  than e $\text{CO}_2$  (Fig. 6a). However,  $ACG_{60 \text{ min}}$  in *A. tricolor* was only affected by  $ICG_{60 \text{ min}}$  and the effect of eT on it was higher than that of e $\text{CO}_2$  (Fig. 6b).

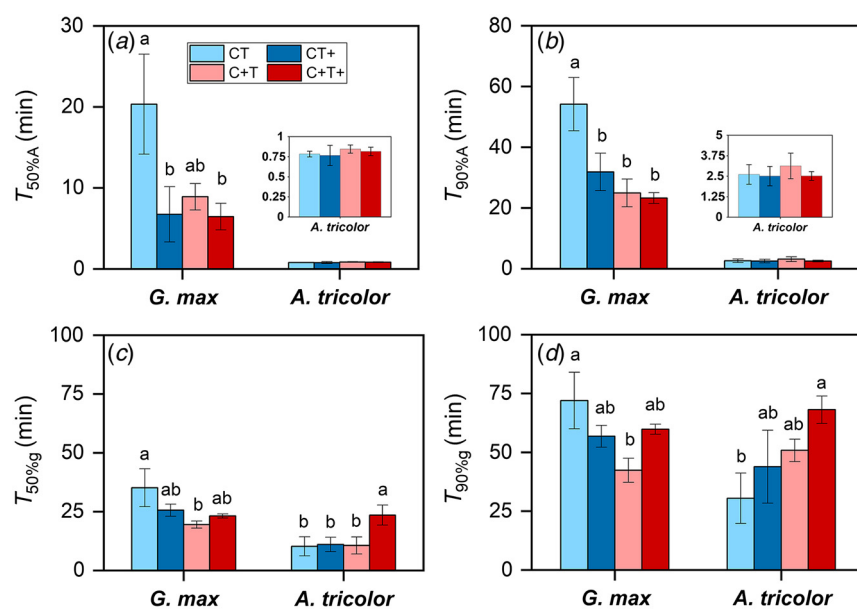
## The correlations between photosynthetic steady-state and induction parameters

We assessed the correlations between steady-state and induction parameters using Pearson's coefficients. In *G. max*,  $ACG_{60 \text{ min}}$  was not only positively correlated with  $A_{100}$  ( $P < 0.001$ ),  $A_{600}$  ( $P < 0.001$ ) and  $IE_{60 \text{ min}}$  ( $P < 0.001$ ), but also negatively related to  $T_{50\%A}$  ( $P < 0.001$ ),  $T_{90\%A}$  ( $P < 0.001$ ) and  $T_{50\%g}$  ( $P < 0.01$ ) (Fig. 7a). But in *A. tricolor*,  $ACG_{60 \text{ min}}$  was only positively correlated with  $A_{600}$  ( $P < 0.001$ ); not related to  $T_{50\%A}$ ,  $T_{90\%A}$ ,  $T_{50\%g}$  and  $IE_{60 \text{ min}}$  (Fig. 7b).

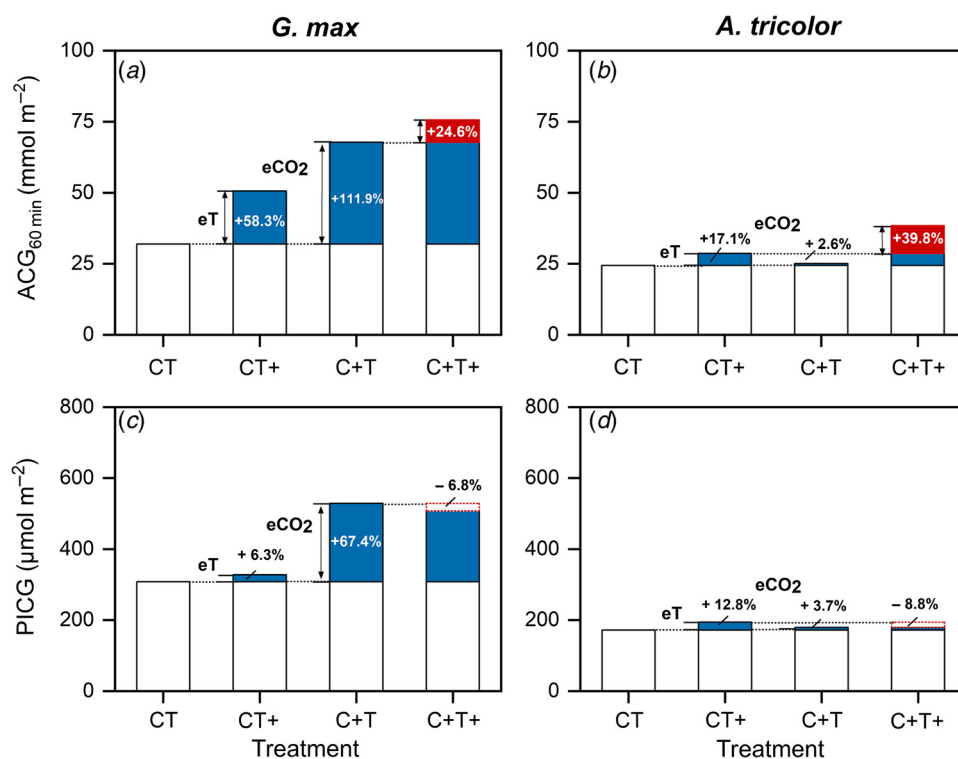
## Discussion

### Differential effects of eT and e $\text{CO}_2$ on dynamic photosynthesis in *G. max*

In *G. max*, both eT and e $\text{CO}_2$  promoted photosynthetic induction, but their effects were differential: eT alone



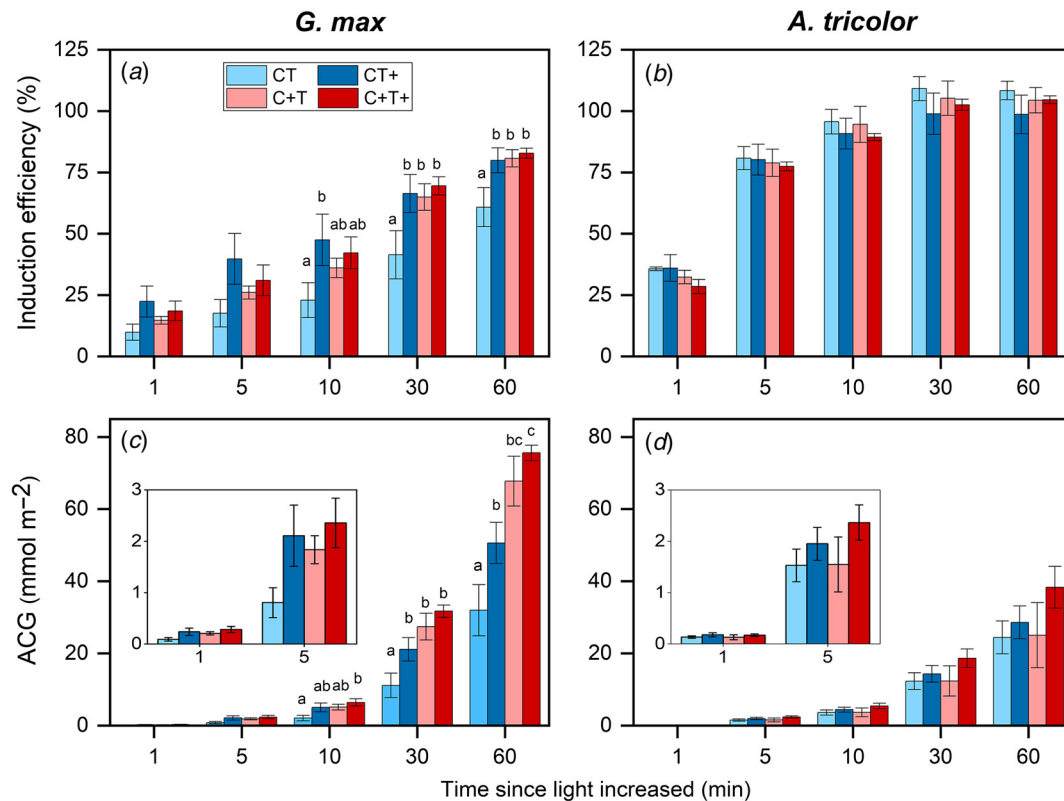
**Fig. 3.** The rates of photosynthetic induction and stomatal opening in *G. max* and *A. tricolor* leaves. (a) Time required for the photosynthetic rate to reach 50% of  $A_{600}$  ( $T_{50\%A}$ ). (b) Time required for the photosynthetic rate to reach 90% of  $A_{600}$  ( $T_{90\%A}$ ). (c) Time required for stomatal conductance to reach 50% of  $g_{s600}$  ( $T_{50\%g}$ ). (d) Time required for stomatal conductance to reach 90% of  $g_{s600}$  ( $T_{90\%g}$ ). Bars and vertical lines indicate the means and s.e. of four biological replicates; i.e. individual plants for each species, respectively. Different letters above error bars indicate significant differences between two treatments within each species. The absence of letters denotes the absence of significant difference.



**Fig. 4.** Achieved carbon gain during a time period of 60 min following an increase in light intensity from 100 to 600  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (a, b) and during the post-illumination period (c, d) in *G. max* (a, c) and *A. tricolor* (b, d) leaves. Values are means of four biological replicates; i.e. individual plants for each species. Blue-filled bars lines indicate the effect of eT and eCO<sub>2</sub>, whereas red-filled and open bars with red dotted frames indicate the interaction of eT and eCO<sub>2</sub>. Numbers indicate the extent of the effect of eT and eCO<sub>2</sub> on ACG, taking  $ACG_{60 min}$  under the CT treatment as the base value.

imposed influences on the early stage (the first 5 min) of induction, whereas eCO<sub>2</sub> alone imposed significant influences on the late stage of induction (Fig. 1a, c). It is reported

that among 193 genes related to photosynthesis in the KEGG database, expression of only nine genes changed significantly after sudden increases in irradiation received



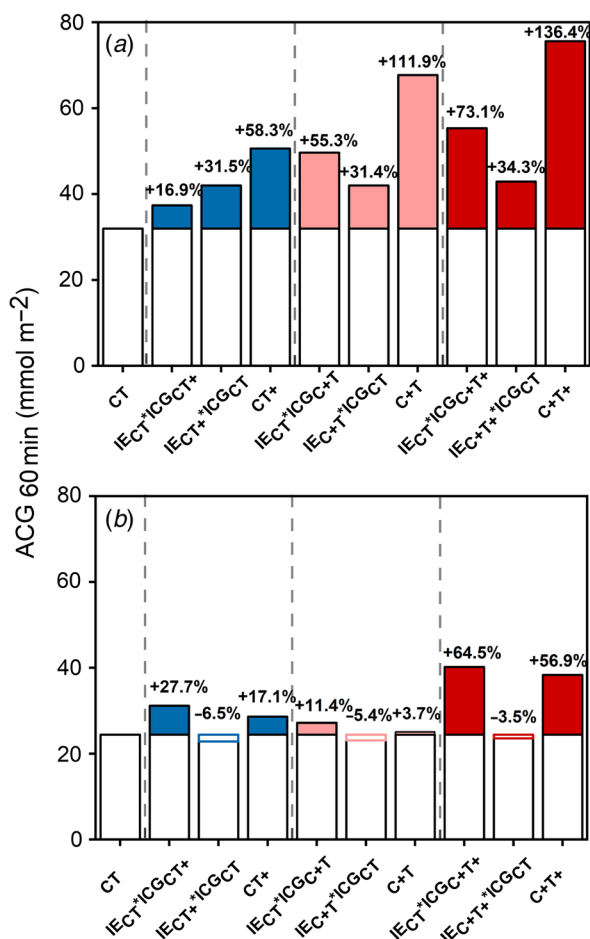
**Fig. 5.** Induction efficiency (a, b) and achieved carbon gain (c, d) in *G. max* (a, c) and *A. tricolor* (b, d) leaves. Bars and vertical lines indicate the means and s.e. of four biological replicates; i.e. individual plants for each species, respectively. Different letters above error bars indicate significant differences between treatments within each species. The absence of letters denotes the absence of significant difference.

by rice (*Oryza sativa* L.) eaves (Adachi *et al.* 2019). This result indicates that few genes are involved in photosynthetic responses to sudden changes in light. We focused on the physiological responses of photosynthetic induction to short-term eT and eCO<sub>2</sub>.

Photosynthetic induction at the early stage is widely assumed to be limited by the time lags in biochemical processes, especially RuBP regeneration and Rubisco activation (Percy *et al.* 1996; Tomimatsu and Tang 2016). We found moderately eT decrease the  $T_{50\%A}$  and  $\tau_R$  in this study (Fig. 3a; Table 2). Kaiser *et al.* (2015) reported a parabolic relationship between photosynthesis induction rate and temperature, with the fastest induction occurring at about 30°C. Such a parabolic relationship would be related to the activation rate of Rca (Rubisco activase) on Rubisco (CarmoSilva and Salvucci 2011). Elevating CO<sub>2</sub> reduces biochemical limitation by accelerating the Rubisco activation (a smaller  $\tau_R$ ), which may be ascribed to CO<sub>2</sub>-stimulated Rca upregulation (Zhao *et al.* 2019). However, in this study, eT had a greater impact on the transient IS than eCO<sub>2</sub> in the first 5 min (Fig. 1c). At a longer timescale (~60 min), photosynthetic induction is mainly limited by diffusional limitation (Way and Percy 2012; Kaiser *et al.* 2015; Lawson and Vialet-Chabrand 2019).

Diffusional limitation can be alleviated more rapidly by higher initial  $g_s$  and faster stomata opening (McAusland *et al.* 2016; Wachendorf and Küppers 2017a). However, effects of eT and eCO<sub>2</sub> on  $g_s$  and stomata opening rate are conflicting between studies; increases (von Caemmerer and Evans 2015; Urban *et al.* 2017), decreases, (Sage and Sharkey 1987) or no changes (von Caemmerer and Evans 2015) of  $g_s$  under eT have been reported before. Elevating CO<sub>2</sub> generally reduces  $g_s$  but its effect on stomatal opening can be positive (Naumburg *et al.* 2001; Leahey *et al.* 2002; Kaiser *et al.* 2017a) or negative (Tomimatsu and Tang 2012). In this study, both eT and eCO<sub>2</sub> reduced diffusional limitation by accelerating stomata opening (Fig. 3c), without significant influences on the initial  $g_s$  (Table 1).

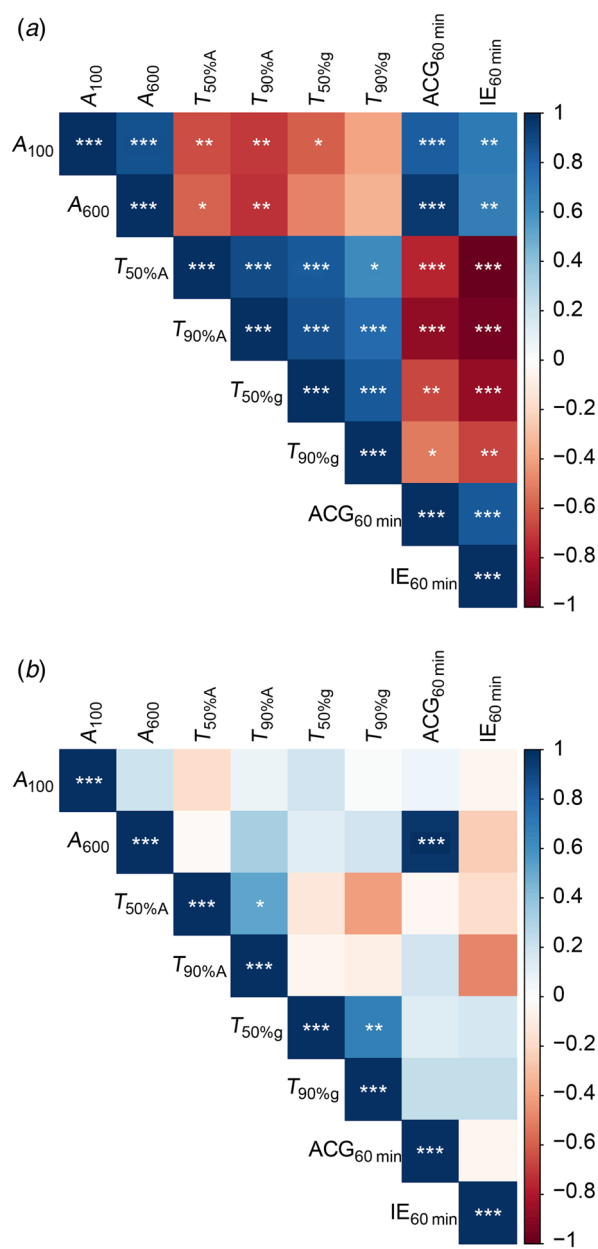
Both eT and eCO<sub>2</sub> enhanced carbon gain in fluctuating light by improving photosynthetic induction efficiency and photosynthetic capacity in *G. max*. The enhancement of ACG under eT was mainly attributable to the improved IE, while that under eCO<sub>2</sub> was mainly attributable to the improved photosynthetic capacity. The effects of eCO<sub>2</sub> were consistent with Kang *et al.* (2021), where ICG had a larger effect than IE on ACG during induction in wheat (*Triticum aestivum* L.) and rice.



**Fig. 6.** The effects of eT and eCO<sub>2</sub> on ACG<sub>60 min</sub> via changes in photosynthetic induction and steady-state photosynthesis in *G. max* (a) and *A. tricolor* (b) leaves. The ACG under the CT treatment was calculated by integrating A over a time period of 60 min following an increase in light intensity from 100 to 600  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Carbon gains under other hypothetical conditions were calculated by multiplying IE<sub>60 min</sub> and ICG<sub>60 min</sub> measured under different three treatments, as the subscripts indicate. Values are means of four biological replicates; i.e. individual plants for each species. Blue bars indicate the effect of changing ICG and red bars indicate the effect of changing IE. Numbers indicate the percentage changes in ACG<sub>60 min</sub>, taking ACG<sub>60 min</sub> under the CT treatment as the base value.

### Minor effects of eT, eCO<sub>2</sub>, or their interaction on dynamic photosynthesis in *A. tricolor*

In *A. tricolor*, photosynthetic induction was not significantly affected by eT, eCO<sub>2</sub> or their interaction (Table 3, Fig. 3b, d). In particular, during the first minute of photosynthetic induction, the time course of transient A under each of the four treatments almost overlapped in *A. tricolor* (Fig. 2b), indicating that factors insensitive to both temperature and [CO<sub>2</sub>] are dominating photosynthesis during this time. This result is consistent with the report on photosynthetic



**Fig. 7.** The correlations between photosynthetic traits measured in *G. max* (a) and *A. tricolor* (b). The numbers in the lower triangle of each matrix are the Pearson's correlation coefficients for each pair of parameters, while the sizes of the circles in the upper triangle of each matrix represent the size of the correlation coefficient. \*P < 0.05; \*\*P < 0.01; and \*\*\*P < 0.001. The numbers and the circles in red indicate negative correlations whereas those in blue indicate positive correlations.

induction at various temperatures and [CO<sub>2</sub>] in maize (Ireland et al. 1984). Rubisco activity and stomatal conductance (Fig. 2d) change little during this period (Kaiser et al. 2015; Tomimatsu and Tang 2016), thus we propose that the enhancement of photosynthetic rate is associated with the build-up of metabolite concentration



**Table 2.** The apparent time constant of Rubisco activation ( $\tau_R$ ), the ratio of the initial and maximum  $V_c$ , and the induction state (IS) under different temperature and  $[\text{CO}_2]$  treatments in *G. max* and *A. tricolor*.

Parameter	Treatments			
	CT	CT+	C+T	C+T+
<i>G. max</i>				
$\tau_R$ (min)	27.37 $\pm$ 11.78a	9.18 $\pm$ 5.34ab	12.82 $\pm$ 2.66ab	5.17 $\pm$ 1.21b
$V_{c,\text{ini}}/V_{c,\text{max}}$	31.84 $\pm$ 5.12a	46.87 $\pm$ 5.62b	32.96 $\pm$ 2.84a	43.26 $\pm$ 4.31b
IS <sub>60 s</sub>	15.02 $\pm$ 5.21	35.48 $\pm$ 9.80	22.15 $\pm$ 2.34	27.14 $\pm$ 5.60
IS <sub>30 min</sub>	64.84 $\pm$ 10.06a	86.46 $\pm$ 4.60b	92.67 $\pm$ 3.73b	92.75 $\pm$ 1.36b
<i>A. tricolor</i>				
IS <sub>60 s</sub>	64.97 $\pm$ 4.17	67.09 $\pm$ 9.52	55.70 $\pm$ 3.41	61.58 $\pm$ 3.60
IS <sub>30 min</sub>	115.08 $\pm$ 4.87	101.32 $\pm$ 10.27	107.64 $\pm$ 7.60	110.87 $\pm$ 2.37

Values are the means of four individual plants for each species ( $\pm$ s.e.). Different letters following means indicate significant ( $P < 0.05$ ) difference across four different environment treatments within each species. Absence of letters denotes absence of significant difference.  $\tau_R$  and  $V_c$  are calculated for  $C_3$  plants only. The letters and plus sign indicate four different leaf temperature and  $[\text{CO}_2]$  treatments: (1) 400 ppm  $\times$  28°C, denoted as CT; (2) 400 ppm  $\times$  33°C, denoted as CT+; (3) 800 ppm  $\times$  28°C, denoted as C+T; (4) 800 ppm  $\times$  33°C, denoted as C+T+.

gradients. In  $C_4$  photosynthesis, atmospheric  $\text{CO}_2$  is first assimilated as  $C_4$  acids in a  $C_4$  cycle before entering the Calvin-Benson cycle, or referred to as  $C_3$  cycle (Bräutigam and Weber 2011). The  $C_4$  and  $C_3$  cycle is coordinated by intracellular transport of  $C_4$  acids and  $C_3$  metabolites (Furbank *et al.* 2000), or light regulation of key enzymes (Furbank *et al.* 1997). Light regulation of the key enzymes takes minutes and should impose relatively small limitation on photosynthesis during the initial stage of the induction (Usuda *et al.* 1984). Intracellular transport of  $C_4$  acids and  $C_3$  metabolites is widely believed to occur by symplastic diffusion and require metabolite concentration gradients between the compartments (Sowiński *et al.* 2008). Isotopic labeling experiments have revealed the establishment of metabolite pools and the concentration gradients during induction (Moore and Edwards 1986a, 1986b).  $C_4$  species have low levels of RuBP and its precursors even at high light (Borghi *et al.* 2022), but maintain high levels of  $C_4$  acids at both low and high light (Moore and Edwards 1986a). Such large pools of  $C_4$  acids facilitate a fast build-up of sufficient metabolite concentration gradients (Wang *et al.* 2021) and thereby provide additional abilities to buffer the redox and energy status against fluctuating environments (Stitt and Zhu 2014).

Different from *G. max*, the increases of ACG in *A. tricolor* were almost independent of the changes of IE, but were mainly attributable to the enhancement of ideal carbon gain (ICG) (Fig. 6b). The enhancement of ICG in *A. tricolor* was less than *G. max*. The transient iWUE of  $C_4$  plants were not significantly different from those of  $C_3$  plants under C+T+ treatment (see Supplementary Fig. S1). These findings suggest  $C_3$  plants will benefit more from the simultaneous eT and e $\text{CO}_2$  under the background of future climate change than  $C_4$  plants.

## The effects of the interaction of eT and e $\text{CO}_2$ on dynamic photosynthesis

The effects of simultaneously eT and e $\text{CO}_2$  on  $T_{50\%A}$ ,  $T_{90\%A}$ , ACG<sub>60 min</sub> and IE<sub>60 min</sub> in *G. max* were lower than the sum of the sole effect of eT and e $\text{CO}_2$ , indicating that the effects of eT and e $\text{CO}_2$  on photosynthetic induction were partially offset. We hypothesised that this finding was a result of an offset in the effects of eT and e $\text{CO}_2$  on stomatal behaviour. Elevating  $\text{CO}_2$  decreased the  $g_{s600}$  but promoted the rate of the increases in  $g_s$  during induction, both of which shortened the time required for stomatal opening (Fig. 1e). The effects of e $\text{CO}_2$  on stomatal behaviour observed in this study were consistent with previous reports (Kaiser *et al.* 2017b). In contrast, eT increased the  $g_{s600}$  and promoted the rate of the increases in  $g_s$  during induction; yet the effect of the latter dominated over that of the former. We were not clear on the mechanism that underlies the increases of  $g_{s600}$  under eT. An increases of the guard cell metabolic activity or of the evaporative demand under eT could drive the increases of  $g_{s600}$ . The interaction of eT and e $\text{CO}_2$  had no influences on  $T_{50\%A}$ ,  $T_{90\%A}$  and IE<sub>60 min</sub> in *A. tricolor*.

The effects of simultaneously eT and e $\text{CO}_2$  on  $A_{600}$  in *G. max* were in close proximity to the sum of the sole effect of eT and e $\text{CO}_2$  and the effects of simultaneously eT and e $\text{CO}_2$  on  $A_{600}$  in *A. tricolor* are higher than the sum of the sole effect of eT and e $\text{CO}_2$ . These findings are different from some studies, where eT inhibited the positive effect of e $\text{CO}_2$  on steady-state photosynthetic rate and photosynthetic efficiency (Lambrevia *et al.* 2005; Cai *et al.* 2016). In contrast, Sage and Kubien (2003) found that the effects of e $\text{CO}_2$  on  $C_3$  and  $C_4$  photosynthesis were greater at warmer than at cooler temperatures. Some studies have also found the enhancement of photosynthesis by e $\text{CO}_2$  is larger at higher temperature (Long 1991; Morison and Lawlor 1999).

**Table 3.** The influences of eT and eCO<sub>2</sub> on the differences in the photosynthetic characteristics of *G. max* and *A. tricolor*.

Parameter	Factors		
	Temperature	[CO <sub>2</sub> ]	Temperature × [CO <sub>2</sub> ]
<i>G. max</i>			
A <sub>100</sub>	0.350	24.963***	0.175
A <sub>600</sub>	2.970	36.271***	0.115
A <sub>100post</sub>	0.111	25.232***	0.114
g <sub>s100</sub>	1.109	4.500	1.174
g <sub>s600</sub>	9.329*	3.804	0.402
g <sub>s100post</sub>	12.022**	3.527	0.416
T <sub>50%A</sub>	4.678	2.491	2.248
T <sub>90%A</sub>	4.117	10.293**	3.068
T <sub>50%g</sub>	0.471	4.444	2.369
T <sub>90%g</sub>	0.025	3.600	5.403*
τ <sub>R</sub>	3.796	1.958	0.632
V <sub>c,ini</sub> /V <sub>c,max</sub>	7.602*	0.074	0.266
ACG <sub>60 min</sub>	5.168*	27.197***	0.854
PICG	0.001	39.995***	0.404
IE <sub>60 min</sub>	4.218	4.930*	2.740
<i>A. tricolor</i>			
A <sub>100</sub>	1.298	1.847	0.386
A <sub>600</sub>	1.449	1.011	0.273
A <sub>100post</sub>	1.796	1.952	0.119
g <sub>s100</sub>	1.252	5.271*	0.048
g <sub>s600</sub>	6.547*	1.481	0.584
g <sub>s100post</sub>	5.079*	0.763	0.476
T <sub>50%A</sub>	0.093	0.557	0.007
T <sub>90%A</sub>	0.374	0.200	0.188
T <sub>50%g</sub>	3.313	2.923	2.576
T <sub>90%g</sub>	2.284	4.824*	0.037
ACG <sub>60 min</sub>	1.953	0.686	0.530
PICG	0.692	0.949	0.685
IE <sub>60 min</sub>	0.868	0.038	0.936

Shown are *P*-values followed by significance symbols, which are \**P* < 0.05, \*\**P* < 0.01, and \*\*\**P* < 0.001.

That is because eCO<sub>2</sub> suppresses photorespiration and mitochondrial respiration in C<sub>3</sub> plants, expanding the photosynthetic thermal optimum range (Long 1991; Way et al. 2015).

### Low steady-state photosynthetic rate in *A. tricolor*

In general, the steady state photosynthetic rate of C<sub>4</sub> plants is higher than that of C<sub>3</sub> plants. A lower A<sub>600</sub> in *A. tricolor* than that in *G. max* in this study may result from the low growth light intensity. Due to biochemical and energetic

requirement (Furbank et al. 1990; Ubierna et al. 2011), C<sub>4</sub> plants are more suitable for growing in high light. Photosynthetic rate was reduced to a greater extent in low light in the six C<sub>4</sub> grasses relative to the two C<sub>3</sub> species, and C<sub>4</sub> grasses also tended to have a lower stomatal conductance and stomatal aperture than C<sub>3</sub> species (Israel et al. 2022).

However, C<sub>4</sub> plants grown in low light or medium light still have a large metabolites pool because BSC leakiness was found to be similar for C<sub>4</sub> plants grown in different light intensity (Pengelly et al. 2010; Bellasio and Griffiths 2014; Ma et al. 2017). Above researches suggest that the CCM is still robust and the biochemical efficiency of the C<sub>4</sub> cycle does not decrease for C<sub>4</sub> plants grown in low light or medium light. Therefore, the low growth light intensity may have little influences on the effects of eT, eCO<sub>2</sub>, and their interaction on photosynthetic induction in *A. tricolor* observed in our study.

## Conclusion

By examining dynamic photosynthesis under four different temperature and [CO<sub>2</sub>] treatments, this study showed that for *G. max*, the T<sub>50%A</sub> and T<sub>90%A</sub> were significantly affected by eT and eCO<sub>2</sub>; whereas for *A. tricolor*, they were almost unaffected by eT or eCO<sub>2</sub>. This study suggests that the effects of eT and eCO<sub>2</sub> on photosynthetic induction were partially offset in C<sub>3</sub> plants and greater enhancement of photosynthesis in fluctuating light for C<sub>3</sub> plants than for C<sub>4</sub> plants in a warming and CO<sub>2</sub>-enriched future. More research is needed to address how the interaction of eT and eCO<sub>2</sub> influences dynamic photosynthesis in future experiments.

## Supplementary material

Supplementary material is available [online](#).

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**Data availability.** The original contributions presented in the study are included in the article and Supplementary material, further enquiries can be directed to the corresponding author.

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