Photorespiration plays an important role in the regulation of photosynthetic electron flow under fluctuating light in tobacco plants grown under full sunlight

Wei Huang1,2, Hong Hu1,2 and Shi-Bao Zhang1,2*

1 Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China, 2 Yunnan Key Laboratory for Wild Plant Resources, Kunming, China

Plants usually experience dynamic fluctuations of light intensities under natural conditions. However, the responses of mesophyll conductance, CO2 assimilation, and photorespiration to light fluctuation are not well understood. To address this question, we measured photosynthetic parameters of gas exchange and chlorophyll fluorescence in tobacco leaves at 2-min intervals while irradiance levels alternated between 100 and 1200 µmol photons m−2 s−1. Compared with leaves exposed to a constant light of 1200 µmol photons m−2 s−1, both stomatal and mesophyll conductances were significantly restricted in leaves treated with fluctuating light condition. Meanwhile, CO2 assimilation rate and electron flow devoted to RuBP carboxylation at 1200 µmol photons m−2 s−1 under fluctuating light were limited by the low chloroplast CO2 concentration. Analysis based on the C3 photosynthesis model indicated that, at 1200 µmol photons m−2 s−1 under fluctuating light, the CO2 assimilation rate was limited by RuBP carboxylation. Electron flow devoted to RuBP oxygenation at 1200 µmol photons m−2 s−1 under fluctuating light remained at nearly the maximum level throughout the experimental period. We conclude that fluctuating light restricts CO2 assimilation by decreasing both stomatal and mesophyll conductances. Under such conditions, photorespiration plays an important role in the regulation of photosynthetic electron flow.

Keywords: CO2 assimilation, fluctuating light, photorespiration, photosynthetic electron flow, mesophyll conductance

Introduction

In nature, plants grown in open habitats usually experience changes in light intensities because of clouds. Even on clear days, the leaves of understory plants are frequently exposed to short-term fluctuating light levels due to movements by the leaves and stems of other plants above them. To cope with fluctuating light conditions, plants must regulate their photosynthetic processes.

Abbreviations: A, CO2 assimilation rate; Cc, chloroplastic CO2 concentration; Fv/Fm′, the maximum quantum yield of PSII after light adaptation; Ctrans, the chloroplastic CO2 concentration at which the transition from RuBP carboxylation to RuBP regeneration occurred; ΦPSII, effective quantum yield of PSII; gmax, mesophyll conductance; gc, stomatal conductance; Jc, electron flow devoted to RuBP carboxylation; Jo, electron flow devoted to RuBP oxygenation; Js, total electron flow through PSII; Jmax, the maximum rate of RuBP regeneration; NPQ, non-photochemical quenching; PSI, photosystem I; PSII, photosystem II; qP, coefficient of PSI photochemical quenching; Vcmax, the maximum rate of RuBP carboxylation.
Under constant low light, most of the absorbed light energy can be used to drive photosynthesis, even when stomatal conductance ($g_s$) is reduced. Under constant high light, the rate of CO$_2$ assimilation ($A_n$) is maintained at an elevated level due to high $g_s$ and mesophyll conductance ($g_m$) (Yamori et al., 2010, 2011). Fluctuating light restricts both $g_s$ and CO$_2$ assimilation rate (Fay and Knapp, 1993; Kirschbaum et al., 1998). However, it is unclear how $g_m$ and photorespiration respond to those changes in irradiance.

Under natural conditions, $g_m$ is an important determinant of the CO$_2$ assimilation rate, especially at high light levels (Carriqui et al., 2015). Several environmental factors, such as water status and temperature, can affect $g_m$ (Flexas et al., 2002; Scafaro et al., 2011; Walker et al., 2013). For tobacco (Nicotiana tabacum) plants grown with adequate water and optimum temperature, $g_m$ is mainly dependent upon the growth light intensity (Yamori et al., 2010). Plants exposed to strong light have higher values of $g_m$ when compared with those grown under low light. When light levels are constant, $g_m$ does not appear to be dependent upon light intensity (Yamori et al., 2010). However, the effect of fluctuating light condition on $g_m$ is unclear. According to the model of Farquhar et al. (1980), CO$_2$ assimilation in C$_3$ plants is limited by either the carboxylation or the regeneration of ribulose-1,5-bisphosphate (RuBP). In tobacco, a model C$_3$ plant, the rate of CO$_2$ assimilation under high light is influenced by leaf nitrogen (N) content. CO$_2$ assimilation rate under high light tends to be limited by RuBP regeneration for plants grown at high N concentration (Yamori et al., 2010, 2011). However, that presumption is based on high values of $g_s$ and $g_m$. Once $g_s$ and $g_m$ decrease because of environmental stresses such as drought, CO$_2$ assimilation rate is then partially constrained by RuBP carboxylation (Flexas et al., 2002; Flexas and Medrano, 2002). Therefore, if fluctuating light levels restrict $g_m$, then $A_n$ likely tends to be limited by RuBP carboxylation.

Photorespiration, an inevitable process in photosynthesis, plays a supporting role in photosynthetic CO$_2$ assimilation (Timm et al., 2012; Busch et al., 2013; Weber and Bauwe, 2013). This process is initiated by the oxygenation of RuBP, in which one molecule of glycolate-2-phosphate and one molecule of glycerate-3-phosphate are produced (Ogren, 1984). Although glycolate-2-phosphate cannot be used by plants for biosynthetic reactions, and is also a potential inhibitor of chloroplast functioning (Anderson, 1971), it can be converted into glycerate-3-phosphate through the photorespiratory pathway (Leegood et al., 1995). When $g_s$ and $g_m$ are diminished, the decreased chloroplast CO$_2$ concentration increases the specificity of Rubisco to O$_2$ and then induces a rise in the rate of RuBP oxygenation (Wingler et al., 1999, 2000).

Plants avoid those detrimental effects of glycolate-2-phosphate and other photorespiratory intermediates by activating the photorespiratory pathway when chloroplast CO$_2$ concentration is low. In Arabidopsis thaliana plants grown under low irradiance, photorespiration plays a minor role in regulating photosynthetic electron flow after exposure to short-term fluctuating light (Kono et al., 2014). The growth light intensity significantly affects the development of the photorespiratory pathway (Huang et al., 2014). For example, plants such as tobacco grown under bright light have a greater capacity than those under low light (Huang et al., 2014). However, little is known about how the photorespiratory pathway functions in the acclimation to fluctuating light by plants grown under high light. Because this pathway is critical to the control of $A_n$ and photosynthetic electron flow (Takahashi et al., 2007; Timm et al., 2012; Huang et al., 2014), it is important that research focused on photosynthetic regulation under fluctuating light should include growth light intensity as an experimental variable.

In this study, we measured the photosynthetic parameters of gas exchange and chlorophyll fluorescence to investigate the responses of $g_m$, $A_n$, and photosynthetic electron flow to fluctuations in light levels. We also examined the limiting step of CO$_2$ assimilation and the role the photorespiratory pathway has in modulating photosynthetic electron flow under alternating light conditions. Our objective was to improve our understanding of how photosynthesis is regulated when sun-grown plants are exposed to changes in irradiance. The following questions were addressed: (1) Is $g_m$ restricted by fluctuating light? (2) What is the limiting step of $A_n$ under fluctuating light? and (3) Does the photorespiratory pathway play an important role in regulating photosynthetic electron flow under fluctuating light?

### Materials and Methods

#### Plant Materials and Growing Conditions

Following seed germination, seedlings of tobacco cv. y87 were cultivated in phytotron for 7 weeks. Afterwards, they were grown in plastic pots in an open field at Kunming Institute of Botany, Yunnan, China (elevation 1900 m, 102°41'E, 25°01'N). During our experiment period (10 May to 24 June 2013), none of the plants experienced any water or nutrient stresses. The average temperature at Kunming was 20.9°C in May and 20.6°C in June. Fully expanded mature leaves on 13-week-old plants were used for photosynthetic measurements.

#### Analyses of Gas Exchange, Chlorophyll Fluorescence, and Mesophyll Conductance

Photosynthetic parameters for gas exchange and chlorophyll fluorescence were monitored with an open gas exchange system that incorporated infrared CO$_2$ and water vapor analyzers (Li-6400XT; Li-Cor Biosciences, Lincoln, NE, USA) and a 2-cm$^2$ measuring head (6400-40 Leaf Chamber Flurometer; Li-Cor Biosciences). Measurements were made in a phytotron where relative air humidity (60%) and air temperature (25°C) were controlled. The atmospheric CO$_2$ concentration was maintained at 400 µmol mol$^{-1}$ by the Li-6400XT. To generate a light response curve, we initially exposed the mature leaves to strong irradiance (2000 µmol photons m$^{-2}$ s$^{-1}$) for 20 min to obtain steady, high levels of $g_s$ and CO$_2$ assimilation. Afterward, photosynthetic parameters were evaluated at 2-min intervals at photosynthetic photon flux densities (PPFDs) of 2000, 1600, 1200, 800, 500, 300, 200, 100, 50, 20, or 0 µmol photons m$^{-2}$ s$^{-1}$. To investigate the responses of $g_s$, $g_m$, CO$_2$ assimilation...
rate, and photosynthetic electron flow to fluctuating light, we also evaluated those photosynthetic parameters under light levels that alternated every 2 min between 100 and 1200 μmol photons m⁻² s⁻¹ after dark-adaptation for 30 min. Photosynthetic induction curves were also developed at 1200 μmol photons m⁻² s⁻¹ after 30 min of darkness. Values for those parameters were recorded automatically by the Li-6400XT at 2-min intervals.

The CO₂ assimilation rate vs. chloroplast CO₂ concentration (Cₜ) was examined at 1200 μmol photons m⁻² s⁻¹ (von Caemmerer and Farquhar, 1981). For each Aₙ/Cₜ curve, the photosynthetic rate reached a steady state at 400 μmol mol⁻¹ CO₂, then decreased to a lower limit of 50 μmol mol⁻¹ before increasing stepwise to an upper limit of 1600 μmol mol⁻¹. Each stepwise measurement was completed within 2–3 min. Using those Aₙ/Cₜ curves, we calculated the maximum rates of RuBP regeneration (Jₘₐₓ) and RuBP carboxylation (Vₖₐₚₐₜ) according to the method of Long and Bernacchi (2003).

The fluorescence parameters F’ₜ, F’ₘ, and Fₜ were evaluated as previously described in Baker and Rosenqvist (2004). Here, F’ₜ and F’ₘ represented the minimum and maximum fluorescence after light-adaptation, respectively. Fₜ indicated the light-adapted steady-state fluorescence. The maximum quantum yield of PSII after light adaptation (F’ₜ/F’ₘ) was calculated as (F’ₘ−F’ₜ)/F’ₘ. Coefficient of PSII photochemical quenching (qP) was calculated as (Fₘ−Fₜ)’/(Fₘ−F’ₜ). Effective quantum yield of PSII (Φₚₛₛᵦ) was calculated as (Fₘ−Fₜ)/Fₘ (Genty et al., 1989).

Total photosynthetic electron flow through PSII was calculated as Jₜ = Φₚₛₛᵦ × PPFD × Lₐₜ茝 × 0.5 (Krall and Edwards, 1992), where Lₐₜ茝 represented leaf absorbance and was assumed to be 0.85 for sun-grown tobacco leaves that receive high-nitrogen nutrition (Miyake et al., 2005). The constant of 0.5 was applied based on the assumption that photons were equally distributed between photosystem I (PSI) and PSII (Miyake et al., 2005). Following the assumption that the water–water cycle is not a major alternative electron sink when CO₂ assimilation is limited (Driever and Baker, 2011), we allocated the electron flow through PSII to RuBP carboxylation (Jₖₚₐₜ) and oxygenation (Jₒ). Values for Jₖₚₐₜ and Jₒ were estimated according to the method of Valentini et al. (1995):

\[
\begin{align*}
J₀ &= 2/3 \times (Jₜ - 4 \times (Aₙ + Rₚ)) \\
Jₖₚₐₜ &= 1/3 \times (Jₜ + 8 \times (Aₙ + Rₚ))
\end{align*}
\]

where Aₙ was the net rate of CO₂ assimilation and Rₚ represented the rate of mitochondrial respiration as measured after 30 min of dark-adaptation.

We recorded values for mesophyll conductance (gₘₚ) at 1200 μmol photons m⁻² s⁻¹ after plants were exposed to either fluctuating or constant light for 60 min. For our comparisons, gₘₚ was also estimated at 1200 μmol photons m⁻² s⁻¹ in light response curves. Values for gₘₚ were estimated through a combination analysis of gas exchange and chlorophyll fluorescence, and according to the following equation (Harley et al., 1992; Loreto et al., 1992; Warren and Dreyer, 2006; Yamori et al., 2010, 2011):

\[
gₘₚ = \frac{Aₙ}{Cₜ - Γₚ*(Jₜ + 8 \times (Aₙ + Rₚ))/(Jₜ - 4 \times (Aₙ + Rₚ))}
\]

where Aₙ was the net rate of CO₂ assimilation, Cₜ was the intercellular CO₂ concentration, Jₜ was total photosynthetic electron flow through PSII, Rₚ was the rate of mitochondrial respiration, and Γₚ was the CO₂ compensation point in the absence of daytime respiration (Farquhar et al., 1980; Brooks and Farquhar, 1985), with the latter assumed to be 32.2 at 25°C (Long and Bernacchi, 2003). Using the estimated gₘₚ, we calculated the chloroplast CO₂ concentration with the following equation (Long and Bernacchi, 2003; Warren and Dreyer, 2006; Yamori et al., 2010, 2011):

\[
Cₜ = Cₙ - Aₙ/gₘₚ
\]

where Cₜ was the intercellular CO₂ concentration, Aₙ was the net rate of CO₂ assimilation, and gₘₚ was mesophyll conductance. To identify the limiting step of CO₂ assimilation under fluctuating light, we applied the method of Yamori et al. (2010, 2011) to determine Cₖₚₐₜ, the chloroplast CO₂ concentration at which the transition from RuBP carboxylation to RuBP regeneration occurred:

\[
Cₖₚₐₜ = \frac{Kₖ(1 + OK₀)Jₘₚₐₜ/4Vₖₚₐₜmax - 2Γₚ}{1 - Jₘₚₐₜ/Vₖₚₐₜmax}
\]

where Kₖ (μmol mol⁻¹) and K₀ (mmol mol⁻¹) were the Michaelis constants for CO₂ and O₂, respectively (Farquhar et al., 1980), and were assumed to be 406.7 μmol mol⁻¹ and 277 mmol mol⁻¹ at 25°C, respectively (Long and Bernacchi, 2003); Jₘₚₐₜ was the maximum rate of RuBP regeneration; Vₖₚₐₜmax was the maximum rate of RuBP carboxylation; and Γₚ was the CO₂ compensation point in the absence of daytime respiration. The limiting step of CO₂ assimilation was then determined by comparing the values of Cₜ and Cₖₚₐₜ.

**Statistical Analysis**

The results were displayed as mean values of four independent measurements. We used One-Way ANOVA and SPSS 16.0 software (SPSS Inc., Chicago, IL, USA) to examine differences among treatments involving fluctuating vs. constant light. Those differences were considered significant at P < 0.05.

**Results**

Light response curves indicated that gₘₚ was maintained at high levels (>0.3 mol m⁻² s⁻¹) when plants were exposed to light intensities above 100 μmol photons m⁻² s⁻¹ (Figure 1A). When light levels were reduced from 100 to 0 μmol photons m⁻² s⁻¹, values for gₘₚ decreased sharply from 0.29 to 0.14 mol m⁻² s⁻¹ within 6 min (Figure 1A). This indicated that stomatal conductance in sun-grown tobacco leaves is very sensitive to light intensity in sun-grown tobacco leaves. Under strong irradiance, i.e., 1500 μmol photons m⁻² s⁻¹, Aₙ was 28.5 μmol CO₂ m⁻² s⁻¹ (Figure 1B). At levels below 1500 μmol photons m⁻² s⁻¹, values for Jₜ, Jₖₚₐₜ, Jₒ/Jₜ gradually rose with increasing PPFD, peaking at 233 μmol electrons m⁻² s⁻¹.
Fluctuating light conditions significantly restricted the opening of stomata. After plants were alternately exposed to 100 and 1200 µmol photons m$^{-2}$ s$^{-1}$ every 2 min for 60 min, $g_s$ was 0.18 mol m$^{-2}$ s$^{-1}$ (Figure 2A). However, when plants were illuminated at a constant 1200 µmol photons m$^{-2}$ s$^{-1}$ for 60 min, $g_s$ was 0.30 mol m$^{-2}$ s$^{-1}$. After 60 min of fluctuating light, the CO$_2$ assimilation rate at 1200 µmol CO$_2$ m$^{-2}$ s$^{-1}$ was 18.1 µmol CO$_2$ m$^{-2}$ s$^{-1}$ vs. 25.4 µmol CO$_2$ m$^{-2}$ s$^{-1}$ after exposure to 1200 µmol photons m$^{-2}$ s$^{-1}$ for 60 min (Figure 2B). Those values for $g_s$ and $A_n$ differed significantly between constant and fluctuating-light treatments, demonstrating that the latter condition inhibited $g_s$ as well as CO$_2$ assimilation. This finding was consistent with those reported previously (Fay and Knapp, 1993; Kirschbaum et al., 1998).

By contrast, values for $qP$ at 1200 µmol photons m$^{-2}$ s$^{-1}$ differed only slightly between the constant and fluctuating light treatments (Figure 3A), while $F_v'/F_m'$ and $\Phi_{PSII}$ at 1200 µmol photons m$^{-2}$ s$^{-1}$ were significantly lower under fluctuating light ($P < 0.001$; Figures 3B,C). The parameter $F_v'/F_m'$ represents the maximum efficiency of PSII when all reaction centers are “open,” and $qP$ is the factor that relates maximum PSII efficiency to the operating PSII efficiency (Farage et al., 2006). Because $\Phi_{PSII}$ is the product of $qP$ and $F_v'/F_m'$, the difference in $\Phi_{PSII}$ that we found between fluctuating light and constant light resulted from the change in $F_v'/F_m'$. These results suggested that although fluctuating light had little effect on the coefficient of PSII photochemical quenching, it induced a significant decline in the maximum efficiency of PSII.

After 60 min of treatment, total electron flow through PSII ($J_T$) at 1200 µmol photons m$^{-2}$ s$^{-1}$ was significantly higher under constant illumination than under fluctuating light, i.e.,
FIGURE 3 | Responses of coefficient of PSII photochemical quenching (qP) (A), maximum quantum yield of PSII after light-adaptation (Fv’/Fm’) (B), and effective quantum yield of PSII (ΦPSII) (C) to fluctuating light levels (closed symbols) or constant bright light (open symbols).

Treatment protocol followed that described for Figure 2. Measurements were conducted at 25°C and 400 µmol mol⁻¹ CO₂. Values are means ± SE (n = 4).

225 vs. 185 µmol electrons m⁻² s⁻¹, respectively (Figure 4A). During that time period, the value for electron flow devoted to RuBP oxygenation (J₀) at 1200 µmol photons m⁻² s⁻¹ changed only slightly between constant- and fluctuating-light treatments (Figure 4B). By contrast, electron flow devoted to RuBP carboxylation (Jₐ) at 1200 µmol photons m⁻² s⁻¹ was higher under constant light (151 µmol electrons m⁻² s⁻¹) than under fluctuating light (115 µmol electrons m⁻² s⁻¹) (Figure 4C). Consequently, the ratio J₀/Jₐ at 1200 µmol photons m⁻² s⁻¹ was higher for plants treated with fluctuating light because of the lower value for Jₐ (Figure 4D). These results indicated that fluctuations in irradiance levels suppressed photosynthetic electron flow, primarily by restricting electron flow devoted to RuBP carboxylation. By comparison, electron flow devoted to RuBP oxygenation was hardly affected by fluctuating light conditions.

After pooling the photosynthesis data collected at 1200 µmol photons m⁻² s⁻¹ under fluctuating light, we determined that gₛ was linearly and positively correlated with Aₙ, Jₜ, and Jₐ (Figures 5A,B). We found it interesting that J₀ was independent of gₛ (Figure 5B), which implied that RuBP carboxylation and RuBP oxygenation responded differently to gₛ. Under fluctuating light, J₀ remained at nearly the maximum level throughout the experimental period. In the initial stage of fluctuating light treatment, electron flow attributed to RuBP oxygenation contributed largely to the total electron transport through PSII (Figure 5C).

To analyze the limiting step of CO₂ assimilation under fluctuating light, we examined the relationship between photosynthesis and chloroplast CO₂ concentration. Here, the...
ratio of the maximum rate of RuBP regeneration ($I_{\text{max}}$) to that of RuBP carboxylation ($V_{\text{cmax}}$) was 0.92, and the chloroplast CO$_2$ concentration at which the transition from RuBP carboxylation to RuBP regeneration occurred ($C_{\text{trans}}$) was 135 µmol mol$^{-1}$ (Figure 6). After exposure to fluctuating light conditions for 60 min, $g_m$ at 1200 µmol photons m$^{-2}$ s$^{-1}$ was 0.21 mol m$^{-2}$ s$^{-1}$, which was significantly lower than that found with light curves (0.29 mol m$^{-2}$ s$^{-1}$) or under constant light (0.28 mol m$^{-2}$ s$^{-1}$) (Figure 7). For the light curves, $C_c$ at 1200 µmol photons m$^{-2}$ s$^{-1}$ was 154 µmol mol$^{-1}$. After exposure to fluctuating light or constant light for 60 min, the value for $C_c$ was 105 or 131 µmol mol$^{-1}$, respectively. This indicated that fluctuating light not only decreased $g_s$ but also restricted $g_m$, leading to a decline in $C_c$. Because $C_c$ was significantly lower than $C_{\text{trans}}$ ($P < 0.0001$), the rate of CO$_2$ assimilation at 1200 µmol photons m$^{-2}$ s$^{-1}$ under fluctuating light was limited by RuBP carboxylation.

### Discussion

The limiting step of $A_n$ is mainly determined by the relative values of $C_{\text{trans}}$ and $C_c$. For tobacco plants supplied with high concentrations of nitrogen, photosynthesis tends to be limited by RuBP regeneration because $C_c$ is higher than $C_{\text{trans}}$ (Yamori et al., 2010, 2011). Such previous conclusions have been based on experiments that involved high levels of $g_s$ and $g_m$ under constant strong light. Although $g_s$ and $A_n$ can be significantly inhibited under fluctuating light, the limiting step of $A_n$ under such conditions has been unknown. Here, our results indicate that both $g_s$ and $g_m$ are significantly restricted under fluctuating light, leading to the decrease in $C_c$. After exposure to fluctuating light for 60 min, $C_c$ was 105 vs. 135 µmol mol$^{-1}$.
for \( C_{\text{trans}} \). Those data provided evidence that, at 1200 \( \mu \text{mol} \) photons m\(^{-2}\) s\(^{-1}\), the photosynthetic process is limited by RuBP carboxylation under fluctuating light. Meanwhile, the high activation of photorespiration contributed largely to the regulation of photosynthetic electron flow.

Carriquí et al. (2015) have demonstrated that \( g_m \) plays an important role in determining the \( CO_2 \) assimilation rate, especially at high light intensities. Nevertheless, the response of \( g_m \) to light intensity remains controversial. For example, in sclerophylls such as Banksia integrifolia, B. serrata, and B. paludosa, the average \( g_m \) under ambient \( CO_2 \) concentration is 22% lower at 500 than at 1500 \( \mu \text{mol} \) photons m\(^{-2}\) s\(^{-1}\) (Hassiotou et al., 2009). However, the average \( g_m \) calculated for wheat leaves is not affected by light intensity (Tazoe et al., 2009). In tobacco leaves, \( g_m \) is significantly lower at 250 than at 1000 \( \mu \text{mol} \) photons m\(^{-2}\) s\(^{-1}\) (Flexas et al., 2007). By contrast, Yamori et al. (2010) have shown that \( g_m \) differs little between constant high light and constant low light in tobacco leaves. Our results indicated that \( g_m \) at 1200 \( \mu \text{mol} \) photons m\(^{-2}\) s\(^{-1}\) under the fluctuating light was 25% lower than the level calculated at constant light of 1200 \( \mu \text{mol} \) photons m\(^{-2}\) s\(^{-1}\) (Figure 7). We believe that this difference was caused by the use of a low light regime (100 \( \mu \text{mol} \) photons m\(^{-2}\) s\(^{-1}\)) in fluctuating light. We found that, under fluctuating light, \( g_m \) was regulated by both high and low light levels; i.e., although the former induced an increase in \( g_m \), this effect could be partially reversed when plants were then exposed to reduced irradiance.

According to the photosynthesis model of Farquhar et al. (1980), \( CO_2 \) assimilation in \( C_3 \) plants is constrained by RuBP carboxylation and/or RuBP regeneration. Therefore, based on that model, the limiting step can be altered in two ways: (1) adjustments in the balance between the maximum rates of RuBP regeneration and RuBP carboxylation, or (2) changes in the chloroplast \( CO_2 \) concentration (Hikosaka et al., 2006; Yamori et al., 2011). For example, in research with tobacco plants, Yamori et al. (2011) have reported that the \( CO_2 \) assimilation rate at 380 \( \mu \text{mol} \) mol\(^{-1}\) \( CO_2 \) and 1500 \( \mu \text{mol} \) photons m\(^{-2}\) s\(^{-1}\) (A\(_{380}\)) depends upon the leaf-N content and is mainly determined by \( J_{\text{max}}/V_{\text{cmax}} \). Furthermore, at high leaf-N content, A\(_{380}\) is limited by RuBP regeneration due to the low ratio of \( J_{\text{max}}/V_{\text{cmax}} \) (Yamori et al., 2010, 2011). However, those conclusions have been drawn from experiments with plants that had high values for both \( g_s \) and \( g_m \), and which did not consider the effects of fluctuating light levels. By comparison, our photosynthetic data for \( g_s \), \( A_n \), \( F_v \), and \( J_{\text{max}}/V_{\text{cmax}} \) ratio are very similar to those that describe the performance of plants grown with a high nitrogen supply (Yamori et al., 2011), indicating that plants grown with high N concentration were used in the present study. Furthermore, \( CO_2 \) assimilation rate at 1200 \( \mu \text{mol} \) photons m\(^{-2}\) s\(^{-1}\) under constant light was limited by RuBP regeneration. When plants were exposed to fluctuating light, the declines in \( g_s \) and \( g_m \) resulted in a decrease in \( C_2 \). The low light regimes under fluctuating light decreased the Rubisco activation state (Yamori et al., 2012), which further restricted the Calvin cycle. The rate of \( CO_2 \) assimilation under high light during the fluctuating-light treatment tended to be limited by RuBP carboxylation. Fluctuating light has altered the limiting step of \( CO_2 \) assimilation in tobacco plants with high leaf-N content.

Previous studies with \( A. \text{thaliana} \) have investigated the roles of cyclic electron flow (CEF) and \( O_2 \)-dependent alternative electron sinks in regulating photosynthetic electron flow under fluctuating light (Suorsa et al., 2012; Kono et al., 2014). It is believed that CEF is essential for proper acclimation of PSI to such light condition (Suorsa et al., 2012). However, the contribution of photorespiration to photodamage under fluctuating light is small in \( Arabidopsis \) leaves sampled from plants exposed to low light (Kono et al., 2014). In tobacco, the capacity of the photorespiratory pathway is strongly influenced by the growth light intensity, with sun leaves up-regulating this pathway to control \( CO_2 \) assimilation and photosynthetic electron flow (Huang et al., 2014). However, it is unknown what role the photorespiratory pathway has in enabling plants normally grown under high light to adapt to fluctuating light conditions. Our data demonstrated that, when plants were exposed to fluctuating light, the reduction in \( C_2 \) meant that less electron flow could be devoted to RuBP carboxylation. However, we found that the flow devoted to RuBP carboxylation was completely and highly activated under such conditions.

Suppression of \( CO_2 \) fixation can cause over-acidification of lumen in the thylakoid membrane, which then activates non-photochemical quenching (NPQ) to dissipate excess light energy harmlessly as heat (Flexas and Medrano, 2002; Takahashi et al., 2007; Huang et al., 2012). At 1200 \( \mu \text{mol} \) photons m\(^{-2}\) s\(^{-1}\), \( F_v/F_m' \) were lower under fluctuating light than under constant light. Because \( F_v/F_m' \) is inversely related to NPQ, this result was evidence of the higher activation of NPQ under fluctuating light. Furthermore, an increase in the proton gradient across the thylakoid membrane can limit linear electron flow (LEF) via cytochrome b6/f (Tikkanen and Aro, 2014). Consumption of photochemical energy, such as ATP and NADPH, through the photorespiratory pathway is thought to alleviate such over-acidification. Especially in the initial stage of our fluctuating-light period, electron flow that was consumed by the photorespiratory pathway largely contributed to the operation of LEF. Therefore, for tobacco plants grown under full sunlight, photorespiratory pathway would be essential for regulating photosynthetic electron flow under fluctuating light, even though our findings contradict a previous report concerning low-light-grown \( A. \text{thaliana} \) (Kono et al., 2014).

Although photorespiratory intermediates such as glycine and glycerate inhibit the Calvin cycle (Chastain and Ogren, 1989; Eisenhut et al., 2007; Timm et al., 2012), they can be converted to glycerate-3-phosphate through the photorespiratory pathway (Peterhansel and Maurino, 2011). This process is critical for photosynthesis and photoprotection (Takahashi et al., 2007). Under fluctuating light, a reduction in \( C_2 \) will accelerate RuBP oxygenation and, ultimately, the production of those intermediates. If the photorespiratory pathway is maintained at a low level under such conditions, the accumulation of those intermediates inhibits \( CO_2 \) assimilation as well as photosynthetic electron flow, causing acceleration of photodamage (Chastain and Ogren, 1989; Eisenhut et al., 2007; Takahashi et al., 2007). In plants with a high rate of \( CO_2 \) assimilation, rapid acceleration of photorespiratory pathway results in low glycine and glycerate contents (Timm et al., 2012). Therefore, to overcome those...
detrimental effects of photorespiratory intermediates, this pathway is highly activated under fluctuating light, which then benefits photosynthetic CO₂ assimilation and photosynthetic electron flow. In addition, the operation of this pathway is necessary for the regeneration of RuBP (Takahashi et al., 2007). To optimize photosynthetic CO₂ fixation, the rates of RuBP oxygenation and RuBP regeneration through photorespiratory pathway must be balanced. Therefore, under fluctuating light conditions, strong activation of the photorespiratory pathway accelerates RuBP regeneration, preventing a decrease in the RuBP pool and favoring the Calvin cycle.

In summary, our results provide evidence that, for sun-grown tobacco leaves, fluctuating light conditions significantly decrease both stomatal and mesophyll conductances, as well as chloroplast CO₂ concentration. Consequently, the rate of CO₂ assimilation is limited by RuBP carboxylation under such conditions. Meanwhile, the photorespiratory pathway is highly activated to regulate photosynthetic electron flow and benefit photosynthetic CO₂ fixation. Thus, strong activation of this pathway is an important strategy by which sun-grown plants adapt to fluctuating light.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (Grant 31300332), funding from the China Postdoctoral Science Foundation to WH (2014T70892), and the following scientific foundations from the Yunnan Tobacco Academy of Agriculture: 110201101003 (TS-03), 2011YN02, 2011YN03.

References

Anderson, L. E. (1971). Chloroplast and cytoplasmic enzymes. II. Pea leaf triose phosphate isomerases. *Biochim. Biophys. Acta* 235, 237–244. doi: 10.1016/0005-2744(71)90051-9

Baker, N. R., and Rosenqvist, E. (2004). Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. *J. Exp. Bot.* 55, 1607–1621. doi: 10.1093/jxb/erh196

Brooks, A., and Farquhar, G. D. (1985). Effect of temperature on the CO₂/O₂ specificity of ribulose-1,5-bisphosphate carboxylase/oxygenase and the rate of respiration in the light. *Plant Physiol.* 165, 397–406. doi: 10.1007/BF00392238

Busch, F. A., Sage, T. L., Cousins, A. B., and Sage, R. F. (2013). C₃ plants enhance rates of photosynthesis by re-assimilating photorespired and respired CO₂. *Plant Cell Environ.* 36, 200–212. doi: 10.1111/j.1365-3040.2012.02567.x

Carriqui, M., Cabrera, M., Conesa, M. A., Coopman, R. E., Douthe, C., Gago, J., et al. (2015). Diffusional limitations explain the lower photosynthetic capacity of ferns as compared with angiosperms in a common garden study. *Plant Cell Environ.* 38, 448–460. doi: 10.1111/pce.12402

Chastain, C. J., and Ogren, W. L. (1989). Glyoxylate inhibition of ribulosebisphosphate carboxylase/oxygenase activation state in vivo. *Plant Physiol.* 93, 977–944.

Driever, S. M., and Baker, N. R. (2011). The water-water cycle in leaves is not necessary for the regeneration of RuBP. *Plant Physiol.* 156, 1456–1466. doi: 10.1104/pp.111.170503

Eisenhut, M., Bauwe, H., and Hagemann, M. (2007). Glycine accumulation is detrimental effects of photorespiration intermediates. *Frontiers in Plant Science* | www.frontiersin.org 8 August 2015 | Volume 6 | Article 621

Genty, B., Briantais, J. M., and Baker, N. R. (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta* 99, 87–92. doi: 10.1016/S0005-2760(00)74021-9

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

Huang, W., Yang, S. J., Zhang, S. B., Zhang, J. L., and Cao, K. F. (2012). Cyclic electron flow plays an important role in photoprotection for the resurrection plant *Paraboea rufescens* under drought stress. *Plant Cell* 235, 819–828. doi: 10.1007/s00425-011-1544-3

Huang, W., Zhang, S. B., and Hu, H. (2014). Sun leaves up-regulate the photorespiratory pathway to maintain a high rate of CO₂ assimilation in tobacco. *Front. Plant Sci.* 5:688. doi: 10.3389/fpls.2014.00688

Kirschbaum, M. U. F., K üppers, M., Schneider, H., Giersch, C., and Noe, S. (1998). Modelling photosynthesis in fluctuating light with inclusion of stomatal conductance, biochemical activation and pools of key photosynthetic intermediates. *Plant Cell Environ.* 20, 16–26. doi: 10.1043/000250505223

Kono, M., Naguchi, K., and Terashima, I. (2014). Roles of the cyclic electron flow around PSI (CEF-PSI) and O₂-dependent alternative pathways in regulation of the photosynthetic electron flow in short-term fluctuating light in *Arabidopsis thaliana*. *Plant Cell Physiol.* 55, 990–1004. doi: 10.1093/pcp/pcu033

Koza, P. J., and Edwards, G. E. (1992). Relationship between photosystem II activity and CO₂ fixation in leaves. *Physiol. Plant.* 86, 180–187. doi: 10.1111/1399-3054.1992.tb01328.x

Leepood, R. C., Lea, P. J., Adcock, M. D., and Hausler, R. E. (1995). The regulation and control of photorespiration. *J. Exp. Bot.* 46, 1397–1414. doi: 10.1093/jxb/46.46.special_issue.1397

Long, S. P., and Bernacchi, C. J. (2003). Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *J. Exp. Bot.* 54, 2393–2401. doi: 10.1093/jxb/erg262

Loreto, F., Harley, P. C., Marco, G. D., and Sharkey, T. D. (1992). Theoretical considerations when estimating the mesophyll conductance to CO₂ flux by analysis of the response of photosynthesis to CO₂. *Plant Physiol.* 98, 1429–1436. doi: 10.1104/pp.98.4.1429

Flexas, J., and Medrano, H. (2002). Energy dissipation in C₃ plants under drought. *Plant Physiol.* 128, 1284–1298. doi: 10.1104/pp.1284-1298

Hassiotou, F., Ludwig, M., Renton, M., Veneklaas, E. J., and Evans, J. R. (2009). Influence of leaf dry mass per area, CO₂, and irradiance on mesophyll conductance in sclerophylls. *J. Exp. Bot.* 60, 2303–2314. doi: 10.1093/jxb/erp021
Miyake, C., Horiguchi, S., Makino, A., Shinzaki, Y., Yamamoto, H., and Tomizawa, K. (2005). Effects of light intensity on cyclic electron flow around PSI and its relationship to non-photochemical quenching of chl fluorescence in tobacco leaves. Plant Cell Physiol. 46, 1819–1830. doi: 10.1093/tcp/pci197

Ogren, W. L. (1984). Photorespiration: pathways, regulation, and modification. Annu. Rev. Plant Physiol. 35, 415–442. doi: 10.1146/annurev.pp.35.060184.02215

Peterhansel, C., and Maurino, V. G. (2011). Photorespiration redesigned. Plant Physiol. 155, 49–55. doi: 10.1104/pp.110.165019

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

Suorsa, M., Jarvi, S., Grieco, M., Niurmi, M., Pietrzykowska, M., Rantala, M., et al. (2012). PROTON GRADIENT REGULATIONS is essential for proper acclimation of Arabidopsis photosystem I to naturally and artificially fluctuating light conditions. Plant Cell 24, 2934–2948. doi: 10.1105/tpc.112.097162

Takahashi, S., Bawie, H., and Badger, M. R. (2007). Impairment of the photorespiratory pathway accelerates photoinhibition of photosystem II by suppression of repair but not acceleration of damage processes in Arabidopsis. Plant Physiol. 144, 487–494. doi: 10.1104/pp.107.097253

Tazoe, Y., von Caemmerer, S., Badger, M. R., and Evans, J. R. (2009). Light and CO₂ do not affect the mesophyll conductance to CO₂ diffusion in wheat leaves. J. Exp. Bot. 60, 2291–2301. doi: 10.1093/jxb/erp035

Tikkkanen, M., and Aro, E. M. (2014). Integrative regulatory network of plant thylakoid energy transduction. Trends. Plant Sci. 19, 10–17. doi: 10.1016/j.tplants.2013.09.003

Timm, S., Florian, A., Arrivault, S., Stitt, M., Fernie, A. R., and Bawie, H. (2012). Glycine decarboxylase controls photosynthesis and plant growth. FEBS Lett. 586, 3692–3697. doi: 10.1016/j.febslet.2012.08.027

Valentini, R., Epron, D., De Angelis, P., Matteucci, G., and Dreyer, E. (1995). In situ estimation of net CO₂ assimilation, photosynthetic electron flow and photorespiration in Turkey oak (Q. cerris L) leaves: diurnal cycles under different levels of water supply. Plant Cell Environ. 18, 631–640. doi: 10.1111/j.1365-3040.1995.tb00564.x

von Caemmerer, S., and Farquhar, G. D. (1981). Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153, 376–387. doi: 10.1007/BF00384257

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

Warren, C. R., and Dreyer, E. (2006). Temperature response of photosynthesis and internal conductance to CO₂: results from two independent approaches. J. Exp. Bot. 57, 3057–3067. doi: 10.1093/jxb/erl067

Weber, A. P., and Bawie, H. (2013). Photorespiration – a driver for evolutionary innovations and key to better crops. Plant Biol. 15, 621–623. doi: 10.1111/plb.12036

Winger, A., Lea, P. J., Quick, W. P., and Leegood, R. C. (2000). Photorespiration: metabolic pathways and their role in stress protection. Philos. Trans. R. Soc. B Biol. Sci. 355, 1517–1529. doi: 10.1098/rstb.2000.0712

Yamori, W., Quick, W. P., Bungard, R. A., Bailey, K. J., Lea, P. J., and Leegood, R. C. (1999). The role of photorespiration during drought stress: an analysis utilising barley mutants with reduced activities of photorespiratory enzymes. Plant Cell Environ. 22, 361–373. doi: 10.1046/j.1365-3040.1999.00410.x

Yamori, W., Evans, J. R., and von Caemmerer, S. (2010). Effects of growth and measurement light intensities on temperature dependence of CO₂ assimilation rate in tobacco leaves. Plant Cell Environ. 33, 332–343. doi: 10.1111/j.1365-3040.2009.02067.x

Yamori, W., Masumoto, C., Fukayama, H., and Makino, A. (2012). Rubisco activase is a key regulator of non-steady-state photosynthesis at any leaf temperature and, to a lesser extent, of steady-state photosynthesis at high temperature. Plant J. 71, 871–880. doi: 10.1111/j.1365-311X.2012.05041.x

Yamori, W., Nagai, T., and Makino, A. (2011). The rate-limiting step for CO₂ assimilation at different temperatures is influenced by the leaf nitrogen content in several C₃ crop species. Plant Cell Environ. 34, 764–777. doi: 10.1111/j.1365-3040.2011.02280.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2015 Huang, Hu and Zhang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.