Speed of light-induced stomatal movement is not correlated to initial or final stomatal conductance in rice


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Abstract

In nature, plants are often confronted with wide variations in light intensity, which may cause a massive carbon loss and water waste. Here, we investigated the response of photosynthetic rate and stomatal conductance to fluctuating light among ten rice genotypes and their influence on plant acclimation and intrinsic water-use efficiency (WUEi). Significant differences were observed in photosynthetic induction and stomatal kinetics across rice genotypes. However, no significant correlation was observed between steady-state and non-steady-state gas exchange. Genotypes with a greater range of steady-state and faster response rate of the gas exchange showed stronger adaptability to fluctuating light. Higher stomatal conductance during the initial phase of induction had little effect on the photosynthetic rate but markedly decreased the plant WUEi. Clarification of the mechanism influencing the dynamic gas exchange and synchronization between photosynthesis and stomatal conductance under fluctuating light may contribute to the improvement of photosynthesis and water-use efficiency in the future.

Keywords: fluctuating light; gas exchange; intrinsic water-use efficiency; non-steady state; steady state.

Introduction

Canopy photosynthesis is considered a major target for improving crops because of its importance for supporting plant growth and grain yield formation (Long et al. 2006, Lawson et al. 2012, Wu et al. 2019). Over the last decades, the steady-state leaf photosynthesis (amount of CO₂ assimilated per leaf area per time under a given environmental condition) has been widely studied and significant knowledge gaps have been filled. However, canopy photosynthesis in natural conditions is not always stable, due to environmental fluctuations, such as light,

Highlights

- Faster photosynthetic induction contributes to a stronger adaptation to fluctuating light
- No significant correlation was observed between steady-state and non-steady-state gas exchange
- Higher stomatal conductance during the initial phase of light induction decreased plant WUEi

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Abbreviations: $C_i$ – intercellular CO₂ concentration; $C_if$ – final intercellular CO₂ concentration; $g_s$ – stomatal conductance; $g_{s,300}$ – stomatal conductance at 300 s of induction; $g_{st}$ – final stomatal conductance; $g_{st,300}$ – initial stomatal conductance; LB – transient biochemical limitation; LS – transient stomatal limitation; $P_f$ – final photosynthetic rate; $P_i$ – initial photosynthetic rate; $P_{N}$ – photosynthetic rate; $P_{90}$ of $g_s$ – the time taken for $g_s$ to increase 90% of the difference between the first and final values; $P_{90}$ of $P_{N}$ – the time taken for $P_{N}$ to increase 90% of the difference between the first and final values; $P_{fr}$ – photosynthetic rate at 300 s of induction; $R_d$ – dark respiration rate; $W_{f}$ – final intrinsic water-use efficiency; $W_{i}$ – initial intrinsic water-use efficiency; $\Gamma^{*}$ – CO₂-compensation point in the absence of photorespiration.

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rate to fluctuating light, which may be determined by initial and final g, and the response rate of stomatal movement, causing a stomatal limitation to photosynthetic rate under fluctuating light (Lawson and Vialet-Chabrand 2019). Adachi et al. (2019) suggested that the higher stomatal conductance during photosynthetic induction is the primary factor for the rapid response of photosynthesis in rice under fluctuating light. Also, this nonsynchronization between $P_{n}$ and $g$ can cause a decrease in WUE (intrinsic water-use efficiency) towards the end of induction, when $P_{n}$ has reached its steady state, whilst $g$ continues to increase at the end of light induction (McAusland et al. 2016).

In the present study, ten rice genotypes were pot-grown in a natural environment with sufficient nutrition. The objectives of this study were to investigate: (1) the potential variations of dynamic $P_{n}$ and $g$, among rice genotypes, and their influence on leaf acclimation under fluctuating light, (2) the relationship between the steady and non-steady state of photosynthesis and stomatal conductance, and (3) the influence of nonsynchronization of $P_{n}$ and $g$, on plant water-use efficiency during light induction.

**Materials and methods**

**Plant growth conditions:** Ten genotypes of conventional and hybrid rice, including Huanghuazhan (HHZ), IDRA, ShanYou63 (SY63), YangLiangYou6 (YL6Y), MingHui63 (MH63), YangDao6 (YD6), LiangYouPeiJiu (LYPJ), ChaoYou1000 (CY1000), ZhenShan97 (ZS97), and N22, were used in this study (Table 1S, supplement). Rice seeds were sown in plates with holes and filled with soil in a growth chamber with a 12-h light (28°C) and 12-h dark (23°C) cycle, and PAR of 400 μmol(photon) m$^{-2}$ s$^{-1}$ at the soil surface. Three fifteen-day-old seedlings were transplanted to 10-L pots filled with 10 kg crushed dry field paddy soil in March 2017. The nitrogen fertilizer application was 3 g(N) per pot and split-applied at a ratio of 4:3:3 at three phases including basal, tillering stage, and panicle initiation, which was applied in the form of urea. Respectively, 1.5 g of phosphorus (P) and potassium (K) were mixed into each pot as basal fertilizer and in the form of superphosphate and potassium chloride. For each genotype, three pots were prepared, and the pots were randomly rearranged weekly. Plants were grown outdoor (at the campus of Huazhong Agricultural University, Wuhan, China), and watered daily to avoid water deficit.

**Leaf gas-exchange measurements:** Photosynthetic rate ($P_{n}$) and stomatal conductance to water vapor ($g$) were measured on the youngest fully expanded leaves using a Li-6400XT portable photosynthesis system equipped with a 6400-40 leaf chamber (Li-Cor Inc., Lincoln, NE, USA). One day before the measurement, the pots were moved into a Conviron growth chamber (Controlled Environments Limited, Manitoba, Canada), and the air temperature, PPFD on the top canopy, and the relative humidity were set to 28°C, 400 μmol m$^{-2}$ s$^{-1}$, and 75%, respectively. To investigate the dynamics of photosynthesis, the leaves were first equilibrated at a PPFD of 100 μmol m$^{-2}$ s$^{-1}$ until
$P_90$ and $g_s$ reached the ‘steady state’, which was defined as $g_s$ at a $< 1\%$ change in rate during a 5-min period. Once the steady state was reached, PPFD was increased to $1,500 \mu\text{mol} \text{ m}^{-2} \text{s}^{-1}$ for 700 s of light induction. During the measurement, the $\text{CO}_2$ concentration in the reference chamber, the leaf temperature, and the VPD were $400 \mu\text{mol} \text{ m}^{-2} \text{s}^{-1}$, $28^\circ\text{C}$ (± 1), and 1.3 ± 0.1 kPa, respectively. Gas-exchange parameters were recorded every 10 s. All measurements were conducted on the youngest fully expanded leaves at the tillering stage.

**Photosynthetic induction:** The response of photosynthetic induction was calculated with a previously reported method (Chazdon and Pearcy 1986, Kaiser et al. 2017) as follows: photosynthetic induction $= (P_N - P_s)(P_t - P_l) \times 100$, where $P_N$ [\mu\text{mol} \text{ m}^{-2} \text{s}^{-1}]$ is the value at 60 s, $P_l$ represents the final rate of induction (mean value of 50 s), and $P_t$ is the initial value (mean value of 50 s).

$P_{90}$ of $P_N$ and $P_{50}$ of $P_N$ was the time taken for $P_N$ to increase 90 and 50% of the difference between the initial and final values during induction within 700 s after shifting to high light. The relative rate of increase in $g_s$ ($P_{90}$ of $g_s$, $P_{50}$ of $g_s$) during photosynthetic induction was also calculated. Intrinsic water-use efficiency (WUE$_i$) was calculated as $P_{50}/g_{s_{50}}$, and the integrated amount of CO$_2$ assimilation (carbon gain) was calculated as $P_s \times d_t$, where $P_s$ represents the photosynthetic rate across the measured period from the initial to the final phase of 700 s, and $d_t$ represents the integrated amount of time during 700 s of light induction.

**Induction limitation analysis:** Transient stomatal (LS) and biochemical (LB) limitation during photosynthetic induction were calculated according to Woodrow and Mott (1989) and Urban et al. (2007):

$$P^* = \frac{(P_N + R_d)(C_{it} - \Gamma^*)}{C_i - \Gamma} - R_d$$

where $P^*$ represents the rate of CO$_2$ assimilation without stomatal limitation, $C_{it}$ is the final $C_i$ at the end of the induction period, $\Gamma^*$ is the chloroplast CO$_2$-compensation point in the absence of photorespiration, and $R_d$ is the dark respiration rate. In the present study, a $\Gamma^*$ value of 40 $\mu\text{mol} \text{ mol}^{-1}$ and $R_d$ value of 1 $\mu\text{mol} \text{ m}^{-2} \text{s}^{-1}$ were used for rice leaves (Yamori et al. 2011, Xiong et al. 2015). Subsequently, LS and LB during the photosynthetic induction phase were calculated as: $LS = (P^* - P_N)/(P_t - P_s)$, $LB = (P_t - P^*)/(P_t - P_d)$, where $P_t$ is the final photosynthetic rate of light induction.

**Statistical analysis:** One-way analysis of variance (ANOVA) and the least-significant difference (LSD) test were used to assess the measured parameters among different genotypes using SPSS 21.0 (SPSS for Windows, Chicago, Illinois, USA). Linear regression was analyzed to test the correlation among measured parameters using SigmaPlot 12.5 (Systat Software Inc., San Jose, CA, USA).

**Results**

**Photosynthetic induction under fluctuating light:** After a step increase in light intensity, $P_N$ increased and rapidly reached the maximum value. However, the stomatal opening was rather slow and the $g_s$ did not reach the maximum after 700 s of high light exposure (Fig. 1). The $P_{90}$ of $P_N$ varied from 224 to 307 s and that of $g_s$ varied from 134 to 434 s (Fig. 2C). The photosynthetic induction and stomatal opening were independent of their initial and/or final values (Fig. 3A,B). The carbon gain during photosynthetic induction differed significantly between genotypes (Fig. 2F). The values of both $P_t - P_s$ and $g_{s90} - g_{s50}$ positively correlated with carbon gain during the light induction (Fig. 4A,B), but there was a lack of a link between gas-exchange induction (represented by $P_{50}$ or $P_{90}$) and carbon gain. Limitation analysis showed that during the initial phase, biochemical limitation accounted for approximately 80%, but declined rapidly at high light level (Fig. 5). Conversely, the stomatal limitation was low at the initial phase and increased gradually after exposure to high light. $P_t$ and $P_{90}$ were positively correlated with $g_{s90}$ and $g_{s50}$, but no positive correlation was observed between

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**Fig. 1.** Response of gas exchange to a step increase of light intensity among ten rice cultivars. (A) Photosynthetic rate ($P_N$), (B) stomatal conductance ($g_s$). Low light (shade area) and high light (open area) were 100 and 1,500 $\mu\text{mol} \text{ m}^{-2} \text{s}^{-1}$, respectively. Each point represents the mean of three replications.

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Fig. 2. Calculations of gas-exchange parameters after a step increase in light intensity across ten rice genotypes. (A,B) Variations of range from minimum values to maximum values of photosynthesis and stomatal conductance. (C,D) the time taken for $P_n$ and $g_s$ to increase 90% of the difference between the first and final values ($P_{90}$ of $P_n$, $g_{90}$ of $g_s$). (E) the rate of photosynthetic induction at 60 s ($IS_{60}$), and (F) carbon assimilation during 700 s of photosynthetic induction. Each bar represents the mean (+ SD) of three replications across two pairs of diploid and tetraploid rice. Different letters indicate statistically significant differences ($P<0.05$) between rice genotypes.

Fig. 3. Relationship between steady-state and dynamic response rate of stomatal conductance and photosynthesis. (A–D) Relationship between dynamic response rate of gas exchange and initial values, (E,F) relationship between dynamic response rate of gas exchange and final values. Each point represents the mean (+ SD) of three replications.
$P_i$ and $g_{st}$, indicating the nonsynchronization of $P_i$ and $g_s$ in the initial phase of induction (Fig. 6).

Variation of initial and final gas exchange across rice genotypes: The steady-state gas-exchange parameters varied significantly among rice genotypes. The $g_s$ ranged from 0.09 to 0.28 mol m$^{-2}$ s$^{-1}$ and $g_{st}$ ranged from 0.46 to 0.82 mol m$^{-2}$ s$^{-1}$, respectively (Table 1). Consistently, across the investigated genotypes, the $P_i$ ranged from 24.7 to 34.0 μmol m$^{-2}$ s$^{-1}$, and $P_f$ from 4.36 to 7.88 μmol m$^{-2}$ s$^{-1}$, respectively. The difference between initial and final gas-exchange parameters ($P_i - P_f, g_{st} - g_s$) was calculated. Substantial variations in the value of $P_i - P_f$ (18.8–27.4 μmol m$^{-2}$ s$^{-1}$) and $g_{st} - g_s$ (0.29–0.55 mol m$^{-2}$ s$^{-1}$) were observed across rice genotypes (Fig. 2A, B; Table 1). The genotypes with higher $g_{st} - g_s$, including Huanghuazhan, IDRA, Yangdao6, Yangliangyou6, Shanyou63, tended to have higher $P_i - P_f$ values. The significant difference was observed in WUE, among ten rice genotypes under different light conditions, particularly under low light (W1) (Table 1). Moreover, W1 and W2 were strongly correlated with $g_s$ and $g_{st}$, respectively, but not with $P_f$ (Fig. 7).

Discussion

The steady-state gas exchange varies greatly among rice genotypes: In nature, plants usually experience a wide range of spatial and temporal variations in light intensity, which leads to simultaneous fluctuations in leaf carbon assimilation and water loss (Pearcy et al. 1990, Lawson and Blatt 2014). When a shaded leaf is suddenly exposed to irradiation, the photosynthesis will slowly increase to reach a new stable steady state. This process is called photosynthetic induction, which takes seconds to hours and depends on stomatal and biochemical limitations (Kaiser et al. 2017, Zhang et al. 2018). Significant differences were observed between rice genotypes in their response rate of photosynthesis to light fluctuations, especially in the early phase of induction (Acevedo-Siaca et al. 2020). Moreover, no correlation was found between different growth stages in steady and dynamic gas-exchange parameters in rice (Acevedo-Siaca et al. 2021). Similarly, we observed significant differences in photosynthetic induction (ISo) and response rate ($P_{50}$ of $P_i$, $P_{90}$ of $P_s$) across ten rice genotypes under a stepwise increase in irradiance (Fig. 2). However, the significant differences were more likely to be found during the whole process, rather than only in the initial phase (Fig. 1). Consistently, significant differences were also observed in the response rate of stomatal conductance to fluctuating light ($P_{50}$ of $g_s$) (Fig. 3D). Generally, stomatal response to changing conditions is an order of magnitude slower than the photosynthetic response in some plant species, which possibly causes a 10–15% stomatal limitation on photosynthesis (McAusland et al. 2016, Lawson and Vialet-Chabrand 2019).

In this study, the rate of steady-state leaf photosynthesis varied widely among rice cultivars (Table 1), which is consistent with previous results (Kanemura et al. 2007). However, little research has noticed the scope of photosynthetic rate and stomatal conductance ranges from low light to high light conditions. Significantly, we observed great variations in $P_i - P_f$ under fluctuating light (Fig. 2A, B). Interestingly, the genotypes with higher $P_i - P_f$ values (HHZ, IDRA, YLY6, YD6, SY63) also exhibited faster photosynthetic responses to light fluctuations, especially for $P_{50}$ of $P_i$ and $P_{90}$ of $g_s$, which would result in higher carbon assimilation (Fig. 2F). Furthermore, great variations were also observed in the $g_{st} - g_s$ values. The rice genotypes with higher $g_{st} - g_s$ values, including HHZ, YD6, YLY6, and SY63, exhibited a faster response...
The influences of initial stomatal opening state on light-induced stomatal kinetics: Previous studies have suggested that light-induced stomatal kinetics is related to stomatal morphology including stomatal size, density, and shape (Franks and Beerling 2009, Drake et al. 2013, Raven 2014, Lawson and Blatt 2014, McAusland et al. 2016). It has also been demonstrated that plant species with a higher density of small stomata tend to have a faster stomatal response rate to environmental fluctuations (Franks and Beerling 2009, Drake et al. 2013, Vialet-Chabrand et al. 2016). However, Elliott-Kingston et al. (2016) suggested that darkness-induced stomatal closing rate was not correlated with stomatal size but related to atmospheric CO$_2$ concentration at the time of taxa diversification (Elliott-Kingston et al. 2016). In addition, plant species with dumbbell-shaped guard cells have much faster stomatal kinetics under fluctuating light than those species with elliptical-shaped guard cells (McAusland et al. 2016), since dumbbell-shaped guard cells require lower energy to change the stomatal aperture than elliptical-shaped guard cells (Hetherington and Woodward 2003, Franks and Farquhar 2007, Raven 2014). Recently, several studies have noticed that stomatal kinetics may be related to minimum and maximum stomatal conductance during light induction (Zhang et al. 2019). One hypothesis
concerning nocturnal transpiration is that ‘pre-opening’ at dawn may help the stomata reach the maximum aperture more rapidly, and reduce the diffusional limitation of CO₂ uptake in the early daytime (Dawson et al. 2007, Drake et al. 2013). In a previous study, one-hour low-humidity treatments to reduce predawn nocturnal stomatal aperture do affect the response rate of stomatal conductance and photosynthesis at the first several minutes after dawn (Auchincloss et al. 2014). However, in the present study, no correlation was found between gₛᵢ and the response rate of stomatal conductance (P₅₀ of gₛ) or photosynthesis (P₅₀ of Pₐ) (Fig. 3A,B,D,E). The disconnection between initial and response rate suggested that more research attention should be paid to the specific mechanisms of these dynamic processes, which largely determine the carbon assimilation of plants in the natural environment.

Stomatal size and density are potential determinants of leaf diffusive conductance to CO₂ and water vapor (Franks et al. 2009). There is usually a negative relationship between stomatal size and density (Xiong et al. 2018). Smaller stomata are generally coupled with a higher maximum stomatal conductance and higher photosynthetic capacity (Franks and Beerling 2009), enhance plant fitness in a broader range of environments, and are capable of achieving a faster response rate (Hetherington and Woodward 2003, Raven 2014, Lawson and Violett-Chabrand 2019). However, Acevedo-Siaca et al. (2020, 2021) recently suggested that there is still a lack of further evidence for the correlation between

Table 1. Gas-exchange parameters of initial photosynthetic rate (Pᵢ), final photosynthetic rate (Pₐ), initial stomatal conductance (gₛᵢ), final stomatal conductance (gₛₐᵢ), initial water-use efficiency (Wᵢ), and final water-use efficiency (Wᵢ) during the initial and final phases of light induction. All data are shown as mean ± SD of three replications. The data with different lowercase letters in each column were significantly different at P<0.05 level.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Pᵢ [μmol m⁻² s⁻¹]</th>
<th>Pₐ [μmol m⁻² s⁻¹]</th>
<th>gₛᵢ [mol m⁻² s⁻¹]</th>
<th>gₛₐᵢ [mol m⁻² s⁻¹]</th>
<th>Wᵢ [μmol mol⁻¹]</th>
<th>Wₐ [μmol mol⁻¹]</th>
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</thead>
<tbody>
<tr>
<td>HHZ</td>
<td>4.49 ± 1.39c</td>
<td>31.9 ± 5.0abc</td>
<td>0.28 ± 0.08a</td>
<td>0.82 ± 0.14a</td>
<td>16.1 ± 3.1d</td>
<td>38.7 ± 0.4cd</td>
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<tr>
<td>IDRA</td>
<td>6.93 ± 1.48ab</td>
<td>34.0 ± 1.1a</td>
<td>0.17 ± 0.04ac</td>
<td>0.60 ± 0.04bc</td>
<td>43.0 ± 16.4bed</td>
<td>56.7 ± 1.7b</td>
</tr>
<tr>
<td>YD6</td>
<td>6.23 ± 0.16abc</td>
<td>32.8 ± 0.7a</td>
<td>0.23 ± 0.05ab</td>
<td>0.78 ± 0.08bc</td>
<td>28.5 ± 6.3bed</td>
<td>42.5 ± 3.9b</td>
</tr>
<tr>
<td>YLY6</td>
<td>7.88 ± 1.42a</td>
<td>32.6 ± 1.4ab</td>
<td>0.14 ± 0.04bc</td>
<td>0.63 ± 0.20bc</td>
<td>61.2 ± 25.0ab</td>
<td>55.4 ± 17.5b</td>
</tr>
<tr>
<td>SY63</td>
<td>7.26 ± 0.35ab</td>
<td>31.9 ± 2.4abc</td>
<td>0.14 ± 0.01bc</td>
<td>0.66 ± 0.14cd</td>
<td>53.3 ± 7.2abc</td>
<td>49.3 ± 7.2bed</td>
</tr>
<tr>
<td>CV1000</td>
<td>4.36 ± 1.51c</td>
<td>27.1 ± 4.2abc</td>
<td>0.23 ± 0.08ab</td>
<td>0.77 ± 0.11bc</td>
<td>22.6 ± 17.0ed</td>
<td>36.4 ± 11.4c</td>
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<tr>
<td>MH63</td>
<td>6.23 ± 0.99abc</td>
<td>27.5 ± 3.9bcd</td>
<td>0.09 ± 0.04bc</td>
<td>0.46 ± 0.07bc</td>
<td>83.2 ± 37.5a</td>
<td>59.7 ± 0.8b</td>
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<tr>
<td>ZS97</td>
<td>6.27 ± 0.47abc</td>
<td>26.0 ± 0.8a</td>
<td>0.19 ± 0.06ab</td>
<td>0.54 ± 0.10b</td>
<td>47.7 ± 12.8bcd</td>
<td>49.1 ± 10.8bcd</td>
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<tr>
<td>LYPJ</td>
<td>5.95 ± 0.12abc</td>
<td>25.5 ± 1.2a</td>
<td>0.19 ± 0.05bc</td>
<td>0.48 ± 0.07bc</td>
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<td>54.1 ± 10.9gbc</td>
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<td>N22</td>
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<td>0.22 ± 0.04ab</td>
<td>0.62 ± 0.09bc</td>
<td>27.7 ± 7.6d</td>
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Fig. 6. Relationship between photosynthesis and stomatal conductance under different light intensity. (A,C) Relationship of stomatal conductance and photosynthesis under low light level and high light level, (B) relationship of stomatal conductance and photosynthesis after 300 s of induction, and (D) relationship between variations of stomatal conductance and photosynthetic rate from the initial phase to the final phase. Each point represents the mean (+ SD) of three replications.
a steady-state and dynamic gas exchange since little correlation was found between the maximum value and the response rate. This is consistent with the present study (Fig. 3C). One possible explanation may be the distribution of resources for photosynthetic proteins, including the content of Rubisco and Rubisco activase, which may dominate the steady-state and dynamic process of photosynthesis (Acevedo-Siaca et al. 2021). Similarly, no correlation was found between the maximum value and response rate of stomatal conductance under fluctuating light in this study. This might be partly attributed to the mechanism underlying light-induced stomatal movement, in which red light induction is believed to connect stomatal kinetics and mesophyll CO₂ assimilation (Matthews et al. 2020), though the exact ‘mesophyll signals’, which are transferred from mesophyll or chloroplast to guard cells and trigger the guard cell function, have not been fully elucidated (Lawson et al. 2014). Besides, the supply of osmoticum and energy by guard cell photosynthesis may also contribute to the stomatal movement under fluctuating light (Santelia and Lawson 2016). Overall, the light-induced stomatal behavior was not correlated with steady-state values and might be associated with the inside ‘signals’ stimulated by a fluctuation of environments outside.

Stomatal kinetics and the implications for carbon and water economics under light fluctuation: Stomata are micropores composed of pairs of guard cells, which control nearly all CO₂ absorption and water loss of plant leaves (Caird et al. 2007). The stomatal movement under fluctuating light plays a key role in leaf carbon assimilation and WUE (Ooba and Takahashi 2003, Vico et al. 2011, McAusland et al. 2016). Delay in the increase or decrease in gs response after a step change in irradiance has been reported in many experiments, which may result in a nonsynchronous stomatal conductance and photosynthetic rate (Lawson et al. 2010, Vico et al. 2011, Lawson and Blatt 2014). The gs is significantly correlated with Ps between species in a natural environment, as a higher CO₂ assimilation rate may require a larger pore aperture (Peguero-Pina et al. 2017). This is consistent with our result under high light level, as final stomatal conductance (gsf) was positively correlated with the final photosynthetic rate (Pf), P300, and gs300 as well (Fig. 6B,C). Differently, no positive correlation was observed between the initial stomatal conductance (gsi) and initial photosynthetic rate (Pi), which might indicate that nonsynchronous stomatal conductance and photosynthesis existed at the beginning of photosynthetic induction (Fig. 6A) and this nonsynchronicity after a step change in light intensity is consistent with previous results (Lawson and Blatt 2014).

C decreased rapidly at first and then reached a steady state gradually with a step increase in irradiance. Compared with the initial phase, C was lower at the steady state (Fig. 1S, supplement), which, to some extent, suggested gs was higher than needed for carboxylation. The stomatal limitation was lower approximately less than 10% during photosynthetic induction across ten rice genotypes, especially at the beginning of induction (Fig. 5), again indicating that gs was exorbitant. This is consistent with Acevedo-Siaca et al. (2020) and photosynthetic induction was strongly limited by nonstomatal limitations, and stomatal limitation only increased gradually from 2% to 10–15% over the first 300 s. Furthermore, W was lower during the initial phase and mainly dominated by stomatal conductance (Fig. 7A,B; Fig. 2S, supplement), which might indicate that higher stomatal conductance during the initial phase decreased leaf W and had little influence on photosynthetic induction. Modeled synchrony behavior in stomatal conductance and photosynthesis has been shown to theoretically increase WUE, by 20% in a bean
leaf exposed to dynamic light (Lawson and Blatt 2014). Improving synchronous photosynthesis and stomatal conductance at the beginning of induction will, to some extent, benefit the improvement of plant WUE under natural conditions. As it has been shown above, leaf Wt and Wf were mainly determined by stomatal conductance at low light and high light levels (Fig. 7). The results suggested that decreasing stomatal conductance during the initial phase of induction might benefit the balance between carbon assimilation and water loss under fluctuating light.

**Conclusion:** This study demonstrates significant differences between ten rice genotypes in steady-state and dynamic photosynthesis and stomatal conductance. No significant correlation was observed between steady-state and non-steady-state gas exchange. The genotypes with greater variations in steady-state gas exchange and faster response rate of dynamic gas exchange could have higher carbon assimilation and may have stronger adaptability to the natural environment than other genotypes. Higher stomatal conductance during the initial phase of induction has little influence on photosynthetic rate but reduces plant WUE. The findings of the present study might contribute to the exploration of the deeper mechanism of dynamic photosynthetic rate and stomatal movement under fluctuating light.

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RESPONSE OF PHOTOSYNTHETIC RATE AND STOMATAL CONDUCTANCE TO FLUCTUATING LIGHT

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