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

Z. XIONG, Q.W. LUO, D.L. XIONG, K.H. CUI, S.B. PENG, and J.L. HUANG+

National Key Laboratory of Crop Genetic Improvement, Ministry of Agriculture Key Laboratory of Crop Ecophysiology and Farming System in the Middle Reaches of the Yangtze River, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, 430070 Hubei, China

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 (WUE_i). 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 WUE_i. 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 CO_2 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 WUE_i

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+Corresponding author
phone: +86 2787284131
e-mail: jhuang@mail.hzau.edu.cn

Abbreviations: C_i – intercellular CO_2 concentration; C_{if} – final intercellular CO_2 concentration; g_s – stomatal conductance; $g_{s,300}$ – stomatal conductance at 300 s of induction; g_{sf} – final stomatal conductance; g_{si} – 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_{50} of g_s – the time taken for g_s to increase 50% of the difference between the first and final values; P_{90} of g_s – the time taken for g_s to increase 90% of the difference between the first and final values; P_{50} of P_N – the time taken for P_N to increase 50% 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_{300} – 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; WUE_i – intrinsic water-use efficiency; Γ^* – CO_2-compensation point in the absence of photorespiration.

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temperature, humidity, and ambient CO_2 concentration (Lawson *et al*. 2012, Kaiser *et al*. 2015, 2016, 2017; Adachi *et al*. 2019). Among those environmental factors, the light might be the most dynamic one, as its signals influence the response of both photosynthetic rate and stomatal conductance. In nature, incident irradiance on plant leaves often fluctuates due to changes in sun angle and cloud cover in addition to shading from overlapping leaves and neighboring plants (Pearcy *et al*. 1990, Kaiser *et al*. 2015). The acclimation of plants to light has been studied extensively and plants that grow under constant environmental conditions tend to have different morphology and biomass compared with the fluctuating environment (Poorter *et al*. 2016, Vialet-Chabrand *et al*. 2017a). Also, many studies have investigated the short-term acclimation of leaf gas-exchange parameters to changes of light intensity, which dominate the leaf carbon assimilation and water-use efficiency under fluctuating light (Lawson and Blatt 2014, Vialet-Chabrand *et al*. 2017b).

Stomatal aperture is controlled by guard cell turgidity, which is sensitive to light intensity. Thus, the kinetics of stomata play an important role in balancing the mesophyll demands for CO_2 against the need to maintain leaf water content under fluctuating irradiance (Lawson *et al*. 2014). However, the underlying mechanism of light-induced stomatal movement is still not fully understood (Kübarsepp *et al*. 2020, Lawson and Matthews 2020). Moreover, there is controversy about physical attributes affecting stomatal response times following environmental perturbations, since opposite relationships between g_s kinetics and stomatal morphology have been reported (Lawson and Blatt 2014, Elliott-Kingston *et al.* 2016, Vialet-Chabrand *et al.* 2016, Durand *et al*. 2019, Eyland *et al*. 2021). On the other hand, leaves with a higher initial or final steady state of the stomatal aperture also show a faster response rate to light fluctuations (Drake *et al*. 2013, Zhang *et al*. 2019), which is also consistent with the hypothesis that pre-dawn stomatal opening contributes to the faster response of stomata at early daytime (Auchincloss *et al*. 2014). In contrast, Acevedo-Siaca *et al.* (2021) showed that there is no correlation between steady- and non-steady-state gas exchange. In addition, De Souza *et al*. (2020) and Soleh *et al*. (2016) also showed a lack of significant correlation between steady- and non-steady-state photosynthesis in cassava and soybean, respectively. Therefore, further evidence is still needed to elucidate the relationship between steady- and non-steady-state gas exchange.

Previous studies have demonstrated a strong correlation between photosynthetic rate (P_N) and stomatal conductance (g_s) under a steady state (Farquhar and Sharkey 1982, Peguero-Pina *et al*. 2017, Xiong and Flexas 2020). However, plants are often confronted with a wide range of light intensity at the spatial and temporal level under field conditions. Upon a step increase in irradiation, photosynthesis and stomatal conductance exhibit a typically delayed response until reaching a new steady state (Scafaro *et al*. 2012). Stomatal conductance has a magnitude slower response than that of the photosynthetic rate to fluctuating light, which may be determined by initial and final g_s 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_s can cause a decrease in WUE_i (intrinsic water-use efficiency) towards the end of induction, when P_N has reached its steady state, whilst g_s 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_s 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_s 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 (YLY6), 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_s) 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_N 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 μmol m^{-2} s^{-1} for 700 s of light induction. During the measurement, the CO_2 concentration in the reference chamber, the leaf temperature, and the VPD were 400 μmol m^{-2} s^{-1}, 28°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 = $(P_N - P_i)/(P_f - P_i) \times 100$, where P_N [μmol m^{-2} s^{-1}] is the value at 60 s, P_f represents the final rate of induction (mean value of 50 s), and P_i 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_N/g_s, and the integrated amount of CO_2 assimilation (carbon gain) was calculated as $P_t \times d_t$, where P_t 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):

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

where P^* represents the rate of CO_2 assimilation without stomatal limitation, C_{if} is the final C_i at the end of the induction period, Γ^* 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 Γ^* value of 40 μmol mol^{-1} and R_d value of 1 μmol m^{-2} 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: $LS = (P^* - P_N)/(P_f + R_d)$, $LB = (P_f - P^*)/(P_f + R_d)$, where P_f 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. 2*C*). The photosynthetic induction and stomatal opening were independent of their initial and/or final values (Fig. 3*A*,*B*). The carbon gain during photosynthetic induction differed significantly between genotypes (Fig. 2*F*). The values of both $P_f - P_i$ and $g_{sf} - g_{si}$ positively correlated with carbon gain during the light induction (Fig. 4*A*,*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_f and P_{300} were positively correlated with g_{sf} and $g_{s,300}$, but no positive correlation was observed between

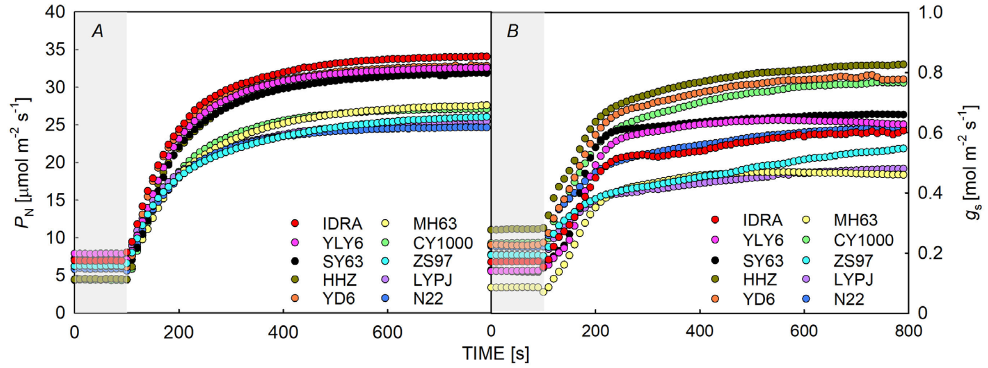

Fig. 1. Response of gas exchange to a step increase of light intensity among ten rice cultivars. (*A*) Photosynthetic rate (P_N), (*B*) stomatal conductance (g_s). Low light (*shade area*) and high light (*open area*) were 100 and 1,500 μmol m^{-2} s^{-1}, respectively. Each point represents the mean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

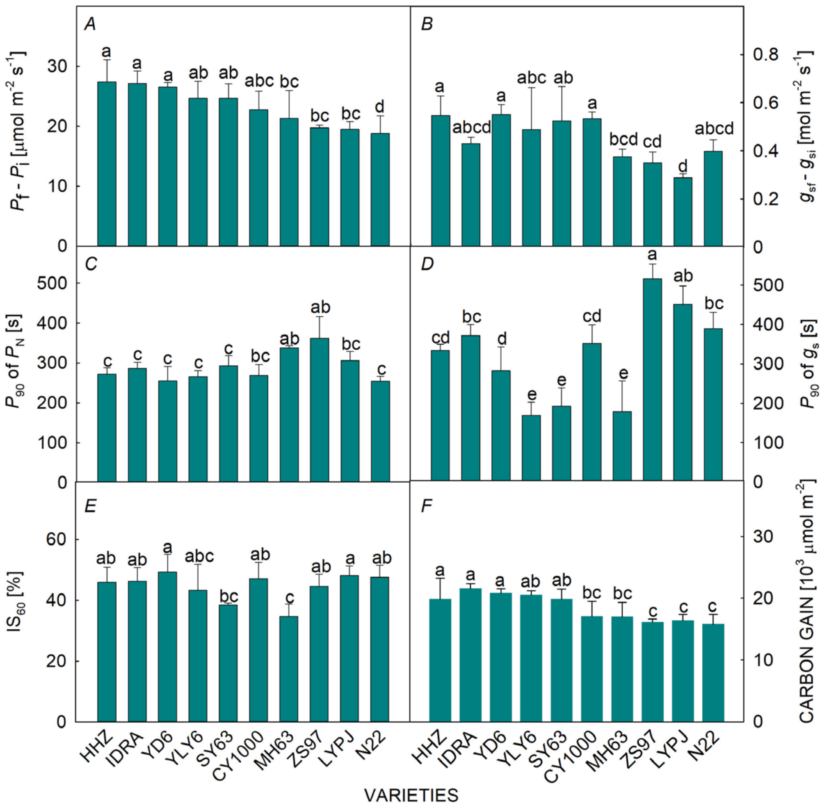

Fig. 2. Calculations of gas-exchange parameters after a step increase in light intensity across ten rice genotypes. (*A*,*B*) Variations of range from minimum values to maximum values of photosynthesis and stomatal conductance, (*C*,*D*) the time taken for P_N and g_s to increase 90% of the difference between the first and final values (P_{90} of P_N, P_{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.

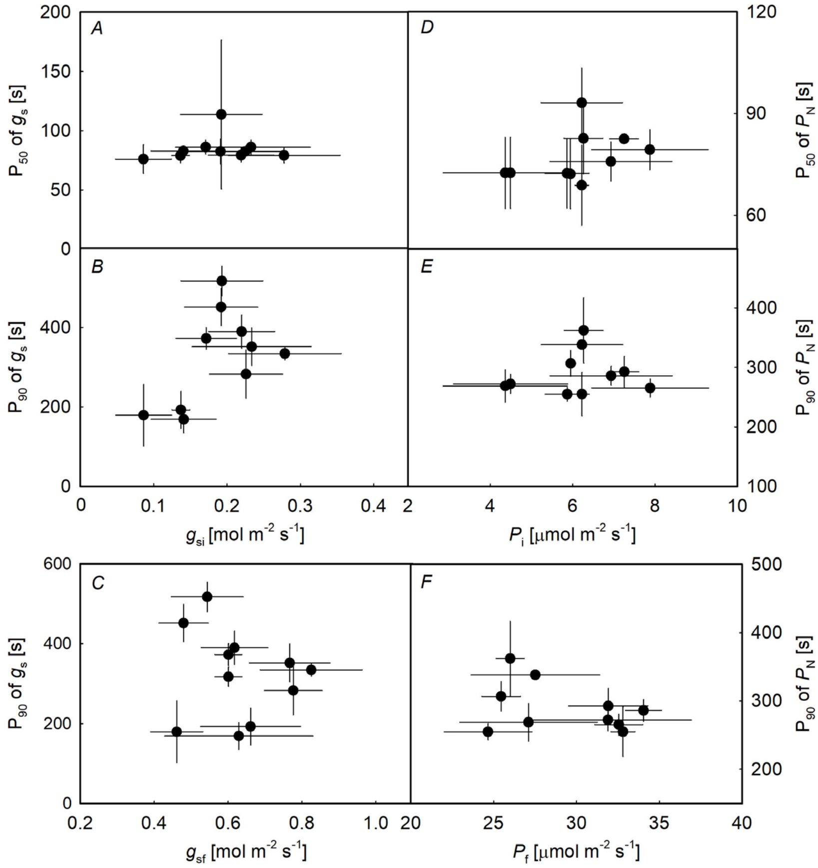

Fig. 3. Relationship between steady-state and dynamic response rate of stomatal conductance and photosynthesis. (*A–D*) Relationship between dynamic response rate of gas exchange and initial values, (*E*,*F*) relationship between dynamic response rate of gas exchange and final values. Each point represents the mean (+ SD) of three replications.

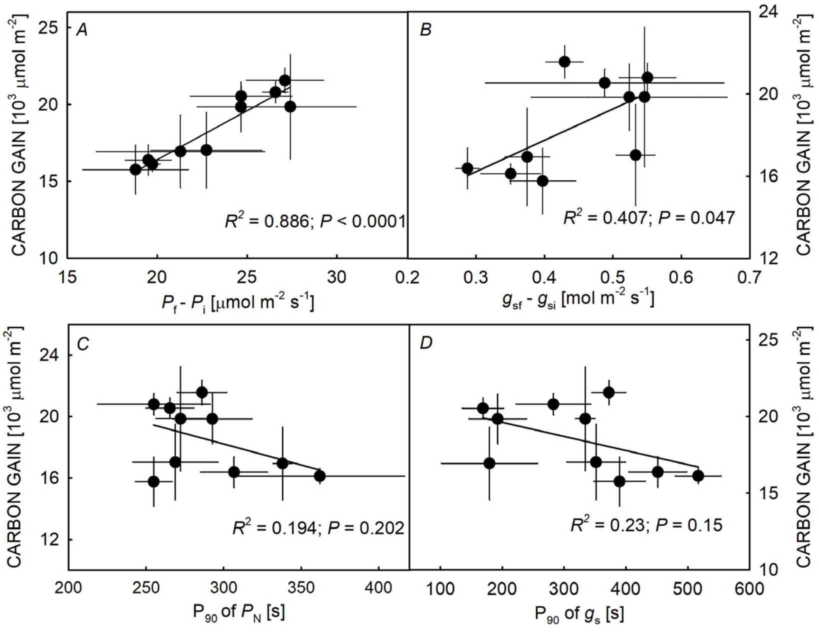

Fig. 4. Relationship between carbon gain during light induction and gas exchange. (*A*,*B*) Relationship between carbon gain and variations from the initial phase to the final phase of stomatal conductance (g_s), (*C*,*D*) relationship between carbon gain and variations from the initial phase to the final phase of photosynthetic rate (P_N). Each point represents the mean (+ SD) of three replications.

P_i and g_{si}, indicating the nonsynchronization of P_N 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_{si} ranged from 0.09 to 0.28 mol m^{-2} s^{-1} and g_{sf} ranged from 0.46 to 0.82 mol m^{-2} s^{-1}, respectively (Table 1). Consistently, across the investigated genotypes, the P_f ranged from 24.7 to 34.0 μmol m^{-2} s^{-1}, and P_i from 4.36 to 7.88 μmol m^{-2} s^{-1}, respectively. The difference between initial and final gas-exchange parameters ($P_f - P_i$, $g_{sf} - g_{si}$) was calculated. Substantial variations in the value of $P_f - P_i$ (18.8–27.4 μmol m^{-2} s^{-1}) and $g_{sf} - g_{si}$ (0.29–0.55 mol m^{-2} s^{-1}) were observed across rice genotypes (Fig. 2*A*,*B*; Table 1). The genotypes with higher $g_{sf} - g_{si}$, including Huanghuazhan, IDRA, Yangdao6, Yangliangyou6, Shanyou63, tended to have higher $P_f - P_i$ values. The significant difference was observed in WUE$_i$ among ten rice genotypes under different light conditions, particularly under low light (W_i) (Table 1). Moreover, W_i and W_f were strongly correlated with g_{si} and g_{sf}, 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 (IS$_{60}$) and response rate (P$_{50}$ of P_N, P$_{90}$ of P_N) 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$_{90}$ of g_s) (Fig. 3*D*). 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 $P_f - P_i$ under fluctuating light (Fig. 2*A*,*B*). Interestingly, the genotypes with higher $P_f - P_i$ values (HHZ, IDRA, YLY6, YD6, SY63) also exhibited faster photosynthetic responses to light fluctuations, especially for P$_{90}$ of P_N and P$_{90}$ of g_s, which would result in higher carbon assimilation (Fig. 2*F*). Furthermore, great variations were also observed in the $g_{sf} - g_{si}$ values. The rice genotypes with higher $g_{sf} - g_{si}$ values, including HHZ, YD6, YLY6, and SY63, exhibited a faster response

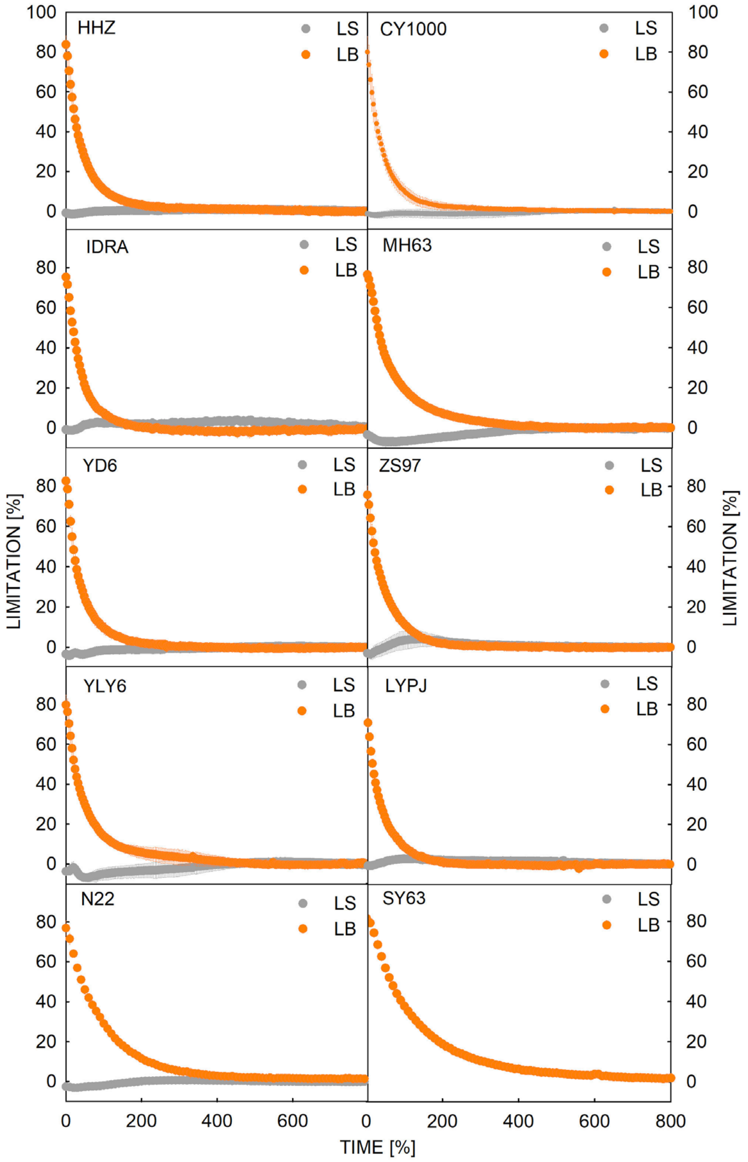

Fig. 5. Transient stomatal (LS) and biochemical limitation (LB) during photosynthetic induction of ten rice cultivars. *The gray points* represent the stomatal limitation, and *the orange points* are biochemical limitation to photosynthesis after a step increase in light intensity. Each curve represents the mean of three replications.

rate of stomatal opening to light fluctuations (Fig. 2*B*,*D*), which is significant for breeding research, as these genotypes may have stronger adaptability to fluctuating light (Fig. 2*A*,*C*), as well as higher carbon assimilation and WUE$_i$ in the field. A higher photosynthetic rate has always been a major target for improving crop performance (Yamori *et al.* 2016). A faster response rate can help maintain higher photosynthetic efficiency under increasing irradiation and therefore contribute to higher biomass in a natural environment.

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

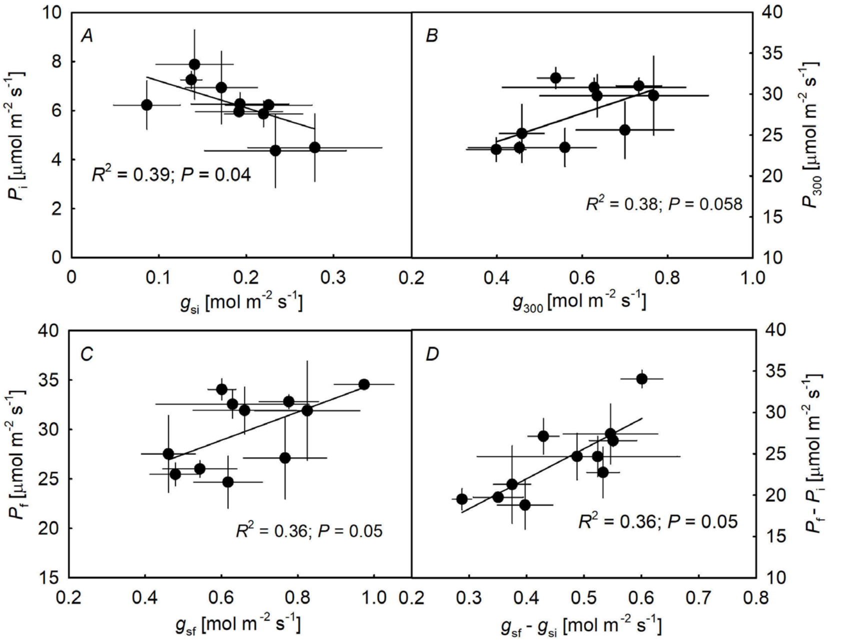

Fig. 6. Relationship between photosynthesis and stomatal conductance under different light intensity. (*A*,*C*) Relationship of stomatal conductance and photosynthesis under low light level and high light level, (*B*) relationship of stomatal conductance and photosynthesis after 300 s of induction, and (*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.

Table 1. Gas-exchange parameters of initial photosynthetic rate (P_i), final photosynthetic rate (P_f), initial stomatal conductance (g_{si}), final stomatal conductance (g_{sf}), initial water-use efficiency (W_i), and final water-use efficiency (W_f) 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.

Genotypes	P_i [µmol m^{-2} s^{-1}]	P_f [µmol m^{-2} s^{-1}]	g_{si} [mol m^{-2} s^{-1}]	g_{sf} [mol m^{-2} s^{-1}]	W_i [µmol mol^{-1}]	W_f [µmol mol^{-1}]
HHZ	4.49 ± 1.39^{c}	31.9 ± 5.0^{abc}	0.28 ± 0.08^{a}	0.82 ±0.14^{a}	16.1 ± 3.1^{d}	38.7 ± 0.4^{cd}
IDRA	6.93 ± 1.48^{ab}	34.0 ± 1.1^{a}	0.17 ± 0.04^{bc}	0.60 ± 0.04^{bc}	43.0 ± 16.4^{bcd}	56.7 ± 1.7^{ab}
YD6	6.23 ± 0.16^{abc}	32.8 ± 0.7^{a}	0.23 ± 0.05^{ab}	0.78 ± 0.08^{bc}	28.5 ± 6.3^{bcd}	42.5 ± 3.9^{bcd}
YLY6	7.88 ± 1.42^{a}	32.6 ± 1.4^{ab}	0.14 ± 0.04^{bc}	0.63 ± 0.20^{abc}	61.2 ± 25.0^{ab}	55.4 ± 17.5^{ab}
SY63	7.26 ± 0.35^{ab}	31.9 ± 2.4^{abc}	0.14 ± 0.01^{bc}	0.66 ± 0.1^{4ab}c	53.3 ± 7.2^{abc}	49.3 ± 7.2^{abcd}
CY1000	4.36 ± 1.51^{c}	27.1 ± 4.2^{cd}	0.23 ± 0.08^{ab}	0.77 ± 0.11^{ab}	22.6 ± 17.0^{cd}	36.4 ± 11.4^{d}
MH63	6.23 ± 0.99^{abc}	27.5 ± 3.9^{bcd}	0.09 ± 0.04^{c}	0.46 ± 0.07^{c}	83.2 ± 37.5^{a}	59.7 ± 0.8^{a}
ZS97	6.27 ± 0.47^{abc}	26.0 ± 0.8^{d}	0.19 ± 0.06^{ab}	0.54 ± 0.10^{c}	47.7 ± 12.8^{bcd}	49.1 ± 10.8^{abcd}
LYPJ	5.95 ± 0.12^{abc}	25.5 ± 1.2^{d}	0.19 ± 0.05^{ab}	0.48 ± 0.07^{c}	32.6 ± 9.3^{bcd}	54.1 ± 10.9^{abc}
N22	5.87 ± 0.54^{bc}	24.7 ± 2.7^{d}	0.22 ± 0.04^{ab}	0.62 ± 0.09^{abc}	27.7 ± 7.6^{cd}	40.1 ± 1.9^{bcd}

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 CO_2 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 g_{si} and the response rate of stomatal conductance (P_{50} of g_s, P_{90} of g_s), as well as P_i and the response rate of photosynthesis (P_{50} of P_N, P_{90} of P_N) (Fig. 3*A*,*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 CO_2 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 Vialet-Chabrand 2019). However, Acevedo-Siaca *et al.* (2020, 2021) recently suggested that there is still a lack of further evidence for the correlation between

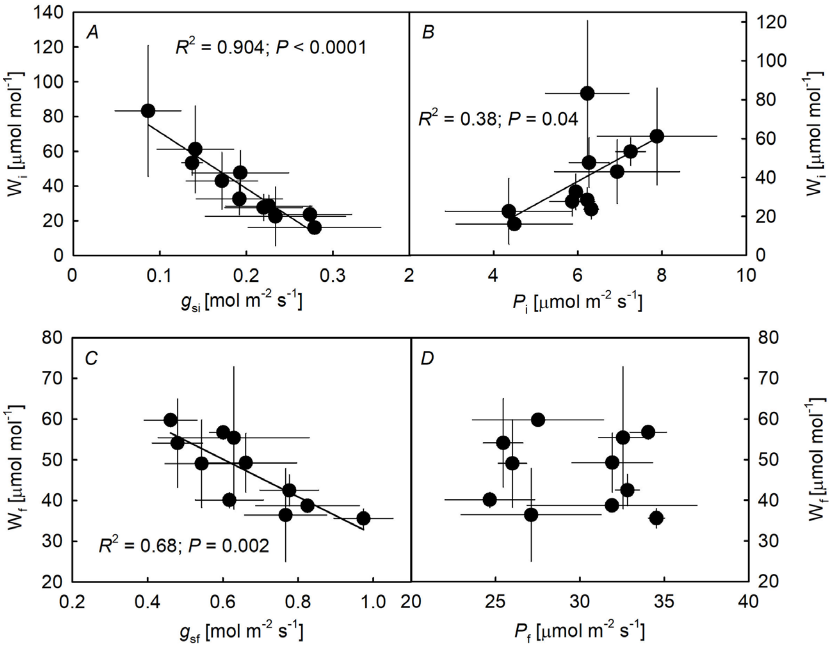

Fig. 7. Relationship between water-use efficiency and gas exchange. (*A*,*B*) Relationship between W_i and g_{si}, as well as W_i and P_i under low light level, (*C*,*D*) relationship between W_f and g_{sf}, as well as W_f and P_f under high light level. Each point represents the mean (+ SD) of three 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. 3*C*). 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 CO_2 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 CO_2 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_i (Ooba and Takahashi 2003, Vico *et al.* 2011, McAusland *et al.* 2016). Delay in the increase or decrease in g_s 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 g_s is significantly correlated with P_N between species in a natural environment, as a higher CO_2 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 (g_{sf}) was positively correlated with the final photosynthetic rate (P_f), P_{300}, and $g_{s,300}$ as well (Fig. 6*B*,*C*). Differently, no positive correlation was observed between the initial stomatal conductance (g_{si}) and initial photosynthetic rate (P_i), which might indicate that nonsynchronous stomatal conductance and photosynthesis existed at the beginning of photosynthetic induction (Fig. 6*A*) and this nonsynchronicity after a step change in light intensity is consistent with previous results (Lawson and Blatt 2014).

C_i decreased rapidly at first and then reached a steady state gradually with a step increase in irradiance. Compared with the initial phase, C_i was lower at the steady state (Fig. 1S, *supplement*), which, to some extent, suggested g_{si} 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 g_{si} 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_i was lower during the initial phase and mainly dominated by stomatal conductance (Fig. 7*A*,*B*; Fig. 2S, *supplement*), which might indicate that higher stomatal conductance during the initial phase decreased leaf W_i and had little influence on photosynthetic induction. Modeled synchrony behavior in stomatal conductance and photosynthesis has been shown to theoretically increase WUE_i 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_i under natural conditions. As it has been shown above, leaf W_i and W_f 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_i. 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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