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The role of water-water cycle in regulating the redox state of photosystem I under fluctuating light

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ABSTRACT

The regulation of photosystem I (PSI) redox state under fluctuating light was investigated for four species using P700 measurement and electrochromic shift analysis. Species included the angiosperms *Camellia japonica*, *Bletilla striata* and *Arabidopsis thaliana* and the fern *Cyrtomium fortunei*. For the first seconds after transition from low to high light, all species showed relatively low levels of the proton gradient (ΔpH) across the thylakoid membranes. At this moment, PSI was highly oxidized in *C. japonica* and *C. fortunei* but was over-reduced in *B. striata* and *A. thaliana*. In *B. striata* and *A. thaliana*, the redox state of PSI was largely dependent on ΔpH. In contrast, the rapid oxidation of P700 in *C. japonica* was relatively independent of ΔpH, but was mainly dependent on the outflow of electrons to O_2 via the water-water cycle. In the fern *C. fortunei*, PSI redox state was rapidly regulated by the fast photo-reduction of O_2 rather than the ΔpH. These results indicate that mechanisms regulating PSI redox state under fluctuating light differ greatly between species. We propose that the water-water cycle is an important mechanism regulating the PSI redox state in angiosperms.

1. Introduction

Plants absorb light energy to drive photosynthetic electron transport that converts light energy into the chemical energy required for primary metabolism. In linear electron flow, electrons from water are transported to the cytochrome b_6/f complex and photosystem I (PSI), and ultimately to $NADP^+$, to produce NADPH, which is coupled with the formation of a proton gradient (ΔpH) across the thylakoid membranes. By comparison, cyclic electron transport around PSI (CET-PSI) forms a ΔpH without producing NADPH. The coordination of linear and cyclic electron transport balances the production of ATP and NADPH to optimize the ATP/NADPH ratio required by the Calvin cycle, photorespiration and other primary metabolism [1–3]. In addition, angiosperms also have pseudocyclic electron transport (pseudoCET) that is mediated by the photo-reduction of O_2 (Mehler reaction) [4–6]. During this pseudoCET, electrons originating from water splitting in PSII are ultimately used to reduce O_2 to water, as a result, this pseudoCET is called the water-water cycle (WWC). Because the rate of O_2 reduction is only approximately 1% of the maximum O_2 evolution in PSII during steady-state photosynthesis [7,8], the WWC is unlikely to play an important role in sustaining photosynthesis when grown under constant light. Recently, we found that the WWC was a major electron sink in *Camellia* species when CO_2 assimilation was restricted [9], which was consistent with the importance of the WWC as an electron sink during photosynthetic induction and at low temperatures [10,11]. In angiosperms, CET-PSI plays a pivotal role in photoprotection under fluctuating light [12–19]. However, the physiological function of the WWC under fluctuating light is not well known.

Under natural field conditions, plants experience a highly variable light environment [19], which is called fluctuating light. A sudden increase in light intensity can induce oxidative damage to the photosynthetic apparatus. It has been documented that fluctuating light can induce significant photoinhibition, particularly to the PSI complex, in angiosperms, such as *Arabidopsis thaliana* and rice [12,20]. PSI photoinhibition significantly suppresses photoprotection and net CO_2 assimilation [6,21–24]. Furthermore, the recovery of PSI from photoinhibition is a slow process over several days [21,25–27]. Thus, PSI photoinhibition can significantly impair plant growth [16,17,20,28]. These results indicate the importance of photoprotection of PSI in the growth of angiosperms under fluctuating light. In angiosperms, luminal acidification down-regulates the rate of electron transport through the Cyt b_6/f complex (photosynthetic control), contributing to the oxidation of PSI and thus protecting PSI from fluctuating light [16,17,20,29–31]. This process is largely dependent on CET-PSI because both the *pgr5* and *crr6* mutants showed severe PSI photoinhibition under fluctuating light [16,17,20]. Surprisingly, the activation of CET-PSI cannot prevent PSI

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photoinhibition under fluctuating light in wild-type angiosperms such as *Arabidopsis thaliana* and rice [12,20], and damage to PSI by fluctuating light can be a serious problem for angiosperms. However, the underlying mechanisms of PSI photoinhibition under fluctuating light in angiosperms have not been yet clarified.

In the water-water cycle, electrons are transported from PSI to O_2, leading to the photoreduction of O_2 by the Mehler reaction [4]. The superoxide is subsequently converted to H_2O_2 by superoxide dismutase (SOD), and ultimately, H_2O_2 is scavenged as water by ascorbate peroxidase (APX). The electron sink of the WWC in vivo was first demonstrated in algae by Radmer and Kok [32]. The electron flow of WWC alleviated PSII photoinhibition by functioning as an alternative electron sink [33]. Subsequently, Baker's group demonstrated the electron flux in the WWC in a C4 plant, maize [34]. Makino's group showed a significant contribution of the WWC to total electron flux in PSII during photosynthetic induction and at lower temperatures in rice leaves [10,11]. These results suggest that the WWC functions as an electron sink with a large electron flux in specific materials and experimental conditions. In *Camellia* species, the WWC acts as a major electron sink when CO_2 fixation is restricted [9]. Furthermore, Takagi et al. [35] reported that the angiosperm *C. japonica* showed significantly lower PSI photoinhibition during repetitive short-pulse illumination than other angiosperms, owing to its higher P700 oxidation ratio. These results suggest that the WWC may act as a safety valve in response to excitation energy in *C. japonica*. In fluctuating light, the sudden increase in light can cause over-reduction of PSI [18,36]. Because the WWC can mediate the fast photoreduction of O_2, we hypothesize that the WWC has the potential to form a large electron sink in fluctuating light, resulting in the alleviation of PSI photodamage in *C. japonica*.

In photosynthetic organisms from cyanobacteria up to gymnosperms, photoreduction of O_2 to water mediated by flavodiiron proteins (Flvs) acts as a safety valve for electrons in fluctuating light [37–41]. Flvs forms a large electron sink when the stroma is highly reduced and protects both photosystems from photodamage in fluctuating light even in the presence of PGR5-dependent CET-PSI [18]. However, the *Flv* genes are not conserved in angiosperms [42]. The introduction of *FlvA* and *FlvB* genes from the moss *Physcomitrella patens* into wild-type *Arabidopsis* relieved the over-reduction of PSI upon the transition from low to high light, making PSI resistant to fluctuating light [18,36]. If the WWC can significantly alleviate PSI photoinhibition under fluctuating light as the alternative electron flow mediated by Flvs, overexpression of the key enzymes of the WWC (SOD and APX) may help to protect PSI under fluctuating light in angiosperms.

In our pre-experiments, we have examined the redox kinetics of P700 upon dark-to-light transition in two angiosperms *Bletilla striata* and *Camellia japonica* (Fig. 1). Interestingly, *C. japonica* showed fast re-oxidation of P700. In contrast, the fast re-oxidation of P700 was clearly missing in *B. striata*. Because the fast re-oxidation of P700 indicates the outflow of electrons from PSI to O_2 [42], the fast PSI re-oxidation in *C. japonica* is attributed to the electron flow of the WWC. As a result, *C. japonica* shows high WWC activity and *B. striata* has low WWC activity. In order to examine the role of WWC in regulating PSI redox state under fluctuating light, we determined the PSI redox state and the electrochromic shift signal under fluctuating light in three angiosperms *C. japonica* (with high WWC activity), *Bletilla striata* and *Arabidopsis thaliana* (with low WWC activity) as well as a fern *Cyrtomium fortunei* (with Flvs). Our results indicate that the WWC plays a significant role in adjusting the PSI redox state and alleviating PSI photoinhibition under fluctuating light in *C. japonica*, and which is independent of the ΔpH-dependent down-regulation of plastoquiol oxidation at the Cyt b_6/f complex. This process is similar to the Flv-dependent photo-reduction of O_2 in the fern *C. fortunei*. By comparison, the regulation of PSI redox state in *B. striata* and *A. thaliana* is largely dependent on the formation of ΔpH. Therefore, the mechanisms responsible for regulating PSI redox state upon transition from low to high light differ greatly between species. The WWC has the potential to compensate for the lack of Flv-dependent electron sink in angiosperms.

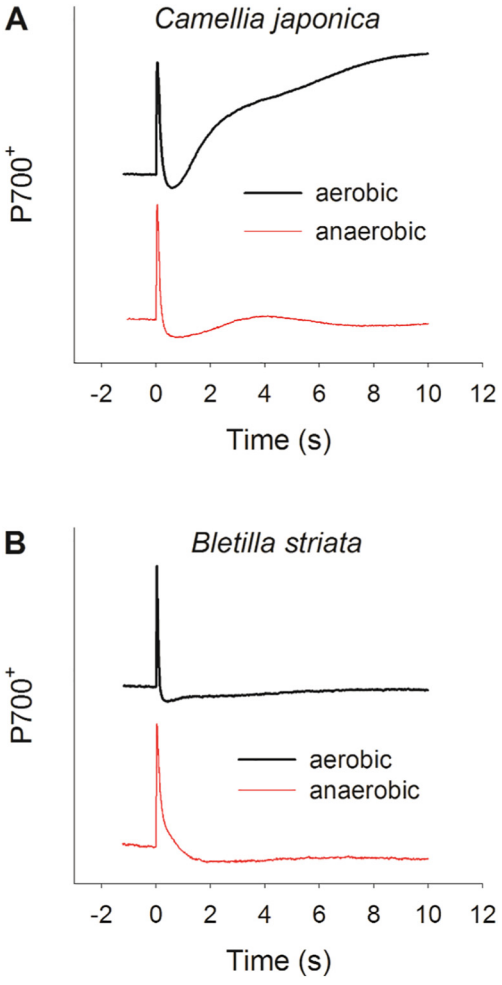

Fig. 1. Redox kinetics of P700 upon the illumination of dark-adapted leaves of *Camellia japonica* (A) and *Bletilla striata* (B). After dark adaptation under aerobic or anaerobic conditions for at least 60 min, the kinetics of redox changes were measured upon exposure to actinic light (1809 μmol photons $m^{-2} s^{-1}$).

2. Materials and methods

2.1. Plant materials and growth conditions

We used four C_3 plants including three angiosperms *Camellia japonica* (Theaceae), *Bletilla striata* (Orchidaceae) and *Arabidopsis thaliana* (Cruciferae) and one fern *Cyrtomium fortunei* (Dryopteridaceae) as experimental materials. The maximum rate of CO_2 assimilation for leaves of *B. striata* is approximately 9.5 μmol CO_2 $m^{-2} s^{-1}$ (data not shown). Plants of *C. japonica*, *B. striata* and *C. fortunei* were grown in a greenhouse with high relative air humidity (60%–70%) and 40% full sunlight in Kunming Institute of Botany, Kunming, Yunnan Province, China (elevation 1900 m, 102°41′E, 25°01′N). Plants of *A. thaliana* were grown in another greenhouse with high relative air humidity (50%–70%) and 20% full sunlight. The day/night temperatures in greenhouses were controlled at about 30/20 °C. At noontime, the maximum photosynthetic photon flux density (PPFD) of sunlight was approximately 1990 μmol photons $m^{-2} s^{-1}$. Three-year-old potted *C. japonica* and *B. striata* plants and two-year-old potted *C. fortunei* plants were used for measurements. For *A. thaliana*, fully expanded rosette leaves of plants grown for at least 5 weeks were used for experiments. All plants were cultivated without water or nutrition stresses and the mature leaves

were used for measurements.

2.2. Chlorophyll fluorescence and P700 measurements

PSI and PSII parameters were recorded simultaneously at 25 °C using a Dual-PAM 100 measuring system (Heinz Walz, Effeltrich, Germany). After dark adaptation for 30 min, a saturating pulse was applied to measure the maximum fluorescence and the maximum change in P700, and then leaves were illuminated at 1178 μmol photons $m^{-2} s^{-1}$ for 10 min to activate photosynthetic electron sinks, followed by illumination at 59 μmol photons $m^{-2} s^{-1}$ for 5 min. Afterward, the actinic light was changed to 1809 μmol photons $m^{-2} s^{-1}$, and the PSI and PSII parameters were recorded. The non-photochemical quenching in PSII was calculated as NPQ = $(F_m - F_m')/F_m'$. F_m and F_m' represent the maximum fluorescence after dark and light adaptation, respectively.

The PSI photosynthetic parameters were measured according to the method of Klughammer and Schreiber [43]. The $P700^+$ signals (P) could vary between a minimum (P700 fully reduced) and a maximum level (P700 fully oxidized). The maximum, P_m, was determined by applying a saturation pulse (300 ms and 20,000 μmol photons $m^{-2} s^{-1}$) after preillumination with far-red light for 10 s. P_m' was similarly obtained, except that actinic light was used instead of far-red light. Calculations of PSI parameters included the quantum yield of PSI non-photochemical energy dissipation due to donor side limitation, Y(ND) = P/P_m, and the quantum yield of non-photochemical energy dissipation due to acceptor side limitation, Y(NA) = $(P_m - P_m')/P_m$.

The P700 redox state during dark-to-light transition was also measured using a Dual-PAM 100. After dark-adaptation for at least 60 min, the P700 redox changes of P700 were recorded during 10 s illumination at 1809 μmol photons $m^{-2} s^{-1}$. Anaerobiosis was induced by incubation of the detached leaves in nitrogen atmosphere for at least 60 min.

2.3. Electrochromic shift (ECS) analysis

The ECS signal was monitored as the change in absorbance at 515 nm, using a Dual PAM-100 equipped with a P515/535 emitter-detector module (Heinz Walz). After dark-adaptation for 30 min, the 515-nm absorbance change induced by a single turnover flash (ECS_{ST}) was measured. Subsequently, leaves were illuminated at 1178 μmol photons $m^{-2} s^{-1}$ for 10 min, followed by illumination at 59 μmol photons $m^{-2} s^{-1}$ for 3 min. Afterward, the actinic light was changed to 1809 μmol photons $m^{-2} s^{-1}$, and then the ECS signal was measured after this light transition for 10 s. Similarly, ECS signals after the transition from 59 to 1809 μmol photons $m^{-2} s^{-1}$ for 20 s, 40 s, 60 s and 120 s were measured following a 3 min adaptation at 59 μmol photons $m^{-2} s^{-1}$. We analyzed ECS dark interval relaxation kinetics ($DIRK_{ECS}$) as described by Sacksteder et al. [44] and Takizawa et al. [45]. The difference in total *pmf* between light and dark, ECS_t, was estimated from the total amplitude of the rapid decay of the ECS signal during the dark pulse. All ECS_t levels were normalized against the magnitude of ECS_{ST}. This normalization accounted for variations in leaf thickness and chloroplast density among the leaf samples [30,45–48]. The slow relaxation of the ECS signal was measured to calculate the ΔpH and membrane potential (ΔΨ) [49,50].

2.4. Photoinhibitory treatments

In the present study, light from a 635 nm light-emitting diode (LED) equipped in a Dual-PAM-100 was used as actinic light for photoinhibitory treatments. After dark adaptation for 30 min, the initial values of F_v/F_m and P_m were measured. Subsequently, leaves were exposed to fluctuating light alternating between 59 and 1809 μmol photons $m^{-2} s^{-1}$ every 20 s for 40 min. Afterward, leaves were dark-adapted for 30 min, and the residual values of F_v/F_m and P_m were measured.

2.5. Statistical analysis

The results are displayed as the mean values of five independent experiments. One-way ANOVA was used at the $\alpha = 0.05$ significance level to determine whether significant differences existed between different treatments.

3. Results

3.1. P700 redox kinetics upon abrupt illumination of dark-adapted leaves

We first determined the P700 redox kinetics upon the illumination of dark-adapted leaves to actinic light (1809 μmol photons $m^{-2} s^{-1}$), to attempt to examine the alternative electron flow. In the angiosperm *C. japonica*, actinic light induced the initial peak of P700 oxidation, which was followed by its reduction and re-oxidation (Fig. 1A). However, this P700 re-oxidation phase was not observed in experiments performed under anaerobic conditions (Fig. 1A), indicating that the fast re-oxidation of P700 in *C. japonica* is dependent on photo-reduction of O_2. In the experiments with *B. striata*, we did not observe the P700 re-oxidation phase under both aerobic and anaerobic conditions (Fig. 1A), suggesting the lack of alternative electron flow via photo-reduction of O_2.

3.2. PSI redox state after the transition from low to high light

We next examined the PSI redox state under fluctuating light. After the transition from 59 to 1809 μmol photons $m^{-2} s^{-1}$ for 10 s, Y(ND) increased to a 0.90 and Y(NA) decreased to 0.06 in the fern *C. fortunei* (Fig. 2A and B). Concomitantly, Y(ND) rapidly increased to 0.78 in *C. japonica* but just increased to 0.33 in *B. striata* (Fig. 2A). Meanwhile, Y(NA) decreased to a low value of 0.13 in *C. japonica* but increased to 0.53 in *B. striata* (Fig. 2B). After this transition for 20 s, Y(ND) reached the maximum value (> 0.86), and Y(NA) decreased to a low value of 0.07 in *C. japonica* (Fig. 2A and B). However, the value of Y(ND) just increased to 0.49 and Y(NA) remained at a high level of 0.37 in *B. striata* (Fig. 2A and B). The over-reduction of PSI reaction centers in *B. striata* was relieved after this transition for 60 s. These results indicated that upon a sudden transition from low to high light, the PSI reaction centers were highly reduced in *B. striata*, especially during the initial 20 s. However, this over-reduction of PSI was not observed in *C. japonica* and *C. fortunei*. Furthermore, NPQ rapidly increased after this transition for 10 s in all species (Fig. 2C), suggesting the different responses of the PSI redox state and NPQ upon a sudden increase in light intensity in *B. striata*.

3.3. Change in pmf after the transition from low to high light

Because the PSI redox state can be regulated by *pmf*, we next determined the change in *pmf* after the transition from 59 to 1809 μmol photons $m^{-2} s^{-1}$ (Fig. S2). During this light transition, the total *pmf* decreased slightly (Fig. 3A), but the ΔpH component of *pmf* increased (Fig. 3B). After this transition for 10 s, the value of ΔpH in *C. japonica* was 0.51. By comparison, after this transition for 120 s, the ΔpH value increased to 1.04. These results strongly indicate that *C. japonica* cannot build up a sufficient ΔpH upon a sudden increase in light intensity, as was in experiments with *B. striata* and *C. fortunei* (Fig. 3B). The change in the ΔΨ component of *pmf* after this light transition showed a trend opposite to ΔpH (Fig. 3C). After this transition for 10 s, the ΔpH/*pmf* ratios in *C. japonica*, *B. striata* and *C. fortunei* were 0.32, 0.43 and 0.39, respectively (Fig. 3D). These ratios increased to 0.77, 0.90 and 0.82, respectively, after this transition for 120 s (Fig. 3D). These results suggested that the relatively low ΔpH upon a sudden transition from low to high light was mainly caused by the imbalanced partitioning of *pmf* into ΔpH and ΔΨ.

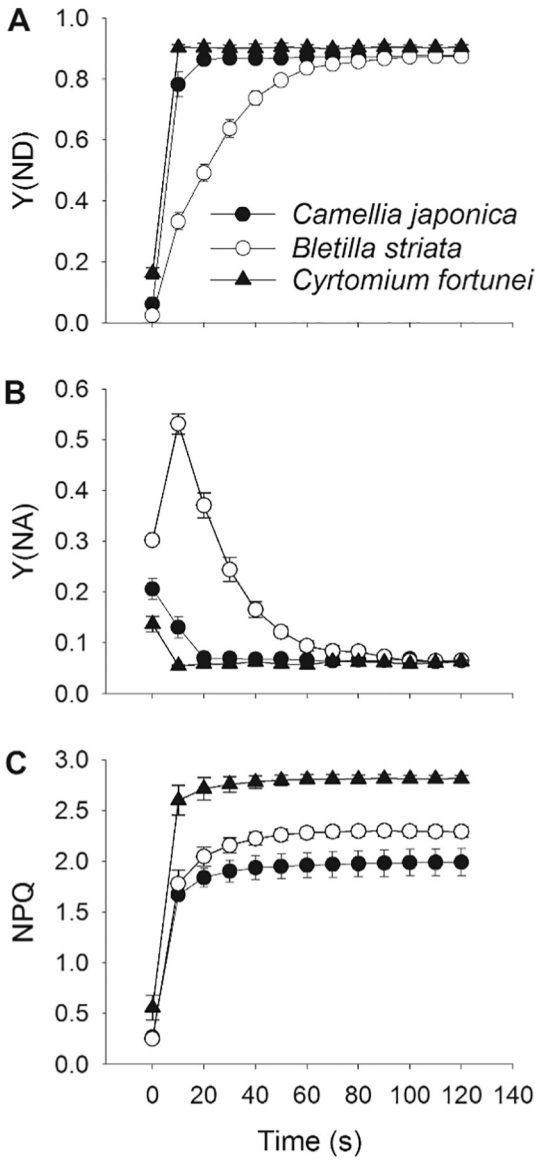

Fig. 2. Changes in PSI redox state and NPQ after the transition from low to high light. A, Quantum yield of PSI non-photochemical energy dissipation due to donor side limitation (YND). B, Quantum yield of PSI non-photochemical energy dissipation due to acceptor side limitation (YNA). C, Non-photochemical quenching in PSII (NPQ). Before this measurement, the leaves were illuminated with AL (1178 μmol photons $m^{-2}s^{-1}$) for 10 min to activate the electron sink in photosynthesis, followed by illumination at 59 μmol photons $m^{-2}s^{-1}$ for 5 min. Afterward, the actinic light was changed to 1809 μmol photons $m^{-2}s^{-1}$, and the values of Y(ND), Y(NA) and NPQ were recorded. Values are means ± SE (n = 5).

3.4. Effects of the ΔpH on the PSI redox state after the transition from low to high light

To understand the role of the ΔpH in photosynthetic regulation during transition from low to high light, we pooled the data obtained after the transition from 59 to 1809 μmol photons $m^{-2}s^{-1}$ for 10 s, 20 s, 40 s, 60 s and 120 s. We found that Y(ND) and Y(NA) values in *B. striata* were significantly controlled by the magnitude of the ΔpH (Fig. 4A and B). However, in *C. japonica* and *C. fortunei*, Y(ND) and Y (NA) were relatively independent of ΔpH (Fig. 4A and B). As a result, the important role of the ΔpH in regulating the PSI redox state was present in *B. striata* but was absent in *C. japonica* and *C. fortunei*, suggesting the diversity in strategies for regulating the redox state of PSI between species.

3.5. Photoinhibition in fluctuating light

After the transition from 59 to 1809 μmol photons $m^{-2}s^{-1}$ for 20 s, the over-reduction of PSI occurred in *B. striata* but was absent in *C. japonica* and *C. fortunei*. As a result, we speculate that the major time point for PSI photoinhibition in fluctuating light is the initial 20 s after this transition. To test this hypothesis, we examined the effect of fluctuating light alternating between 59 and 1809 μmol photons $m^{-2}s^{-1}$ every 20 s for 40 min on PSI and PSII activities in these three species. After this treatment, PSI activity decreased by 5%, 4% and 22% in *C. japonica*, *C. fortunei* and *B. striata*, respectively (Fig. 5), indicating that PSI is more sensitive to fluctuating light in *B. striata* than in *C. japonica* and *C. fortunei*. Concomitantly, PSII activity decreased slightly (Fig. 5), indicating the strong resistance of PSII to fluctuating light.

3.6. PSI redox state under fluctuating light in Arabidopsis thaliana

In the model angiosperm *Arabidopsis thaliana*, the redox state of PSI under high light is mainly regulated by the ΔpH-dependent photosynthetic control at the Cyt b_6/f complex rather than the WWC. In order to further confirm the role of WWC in regulating PSI redox state in *C. japonica*, we examined the responses of PSI redox state to a sudden increase in light intensity for leaves of *A. thaliana*. After the transition from 59 to 1809 μmol photons $m^{-2}s^{-1}$ for 10 s, Y(NA) rapidly increased from 0.22 to 0.83 (Fig. 6A), suggesting the over-reduction of PSI upon a sudden transition from low to high light. At this moment, *A. thaliana* could not build up a sufficient ΔpH (Fig. 6B). After this light transition for 60 s and 120 s, the sufficient ΔpH were accompanied with high levels of Y(ND). As a result, *A. thaliana* and *B. striata* showed the same mechanism for regulating PSI redox state under fluctuating light.

4. Discussion

4.1. The WWC alters P700 redox kinetics upon abrupt illumination of dark-adapted leaves

As indicated by Ilik et al. [42], measuring the P700 redox kinetics upon a dark-to-light transition is a simple method for monitoring the O_2 photoreduction mediated by Flvs. Because the alternative electron transport mediated by Flvs is operational in organisms from cyanobacteria up to gymnosperms, fast P700 re-oxidation was observed in the fern *C. fortunei* (Fig. S1). In the angiosperm *B. striata*, rapid P700 re-oxidation was clearly missing (Fig. 1B), suggesting the absence of Flvs. However, another angiosperm, *C. japonica*, showed fast P700 re-oxidation (Fig. 1A), which was largely different from the phenomenon in *B. striata*. Furthermore, the fast re-oxidation phase in *C. japonica* was clearly missing when measured under anaerobic conditions. Because the final re-oxidation of P700 is generally attributed to the outflow of electrons from PSI [42], these results indicate the presence of another O_2-dependent alternative electron flow in *C. japonica*. Cyclic electron flow around PSI and pseudocyclic electron transport (pseudoCET) are thought to be important to regulate PSI oxidation (see review [51]). However, the operation of CET-PSI cannot induce fast PSI re-oxidation upon a dark-to-light transition in angiosperms [42]. As a result, the fast PSI re-oxidation in *C. japonica* should be caused by the operation of pseudoCET that is responsible for the outflow of electrons from PSI to O_2. PseudoCET includes two pathways: Flv-dependent pseudoCET and the WWC. Flvs are the main mediators of pseudoCET in photosynthetic organisms, spanning from cyanobacteria to gymnosperms. However, these genes are not conserved in angiosperms. As a result, the fast PSI re-oxidation in *C. japonica* is attributed to the electron flow of the WWC. Recently, a study suggested that the WWC was a major electron sink in *Camellia* species when CO_2 assimilation is restricted [9], based on measurements of gas exchange and chlorophyll fluorescence. Therefore,

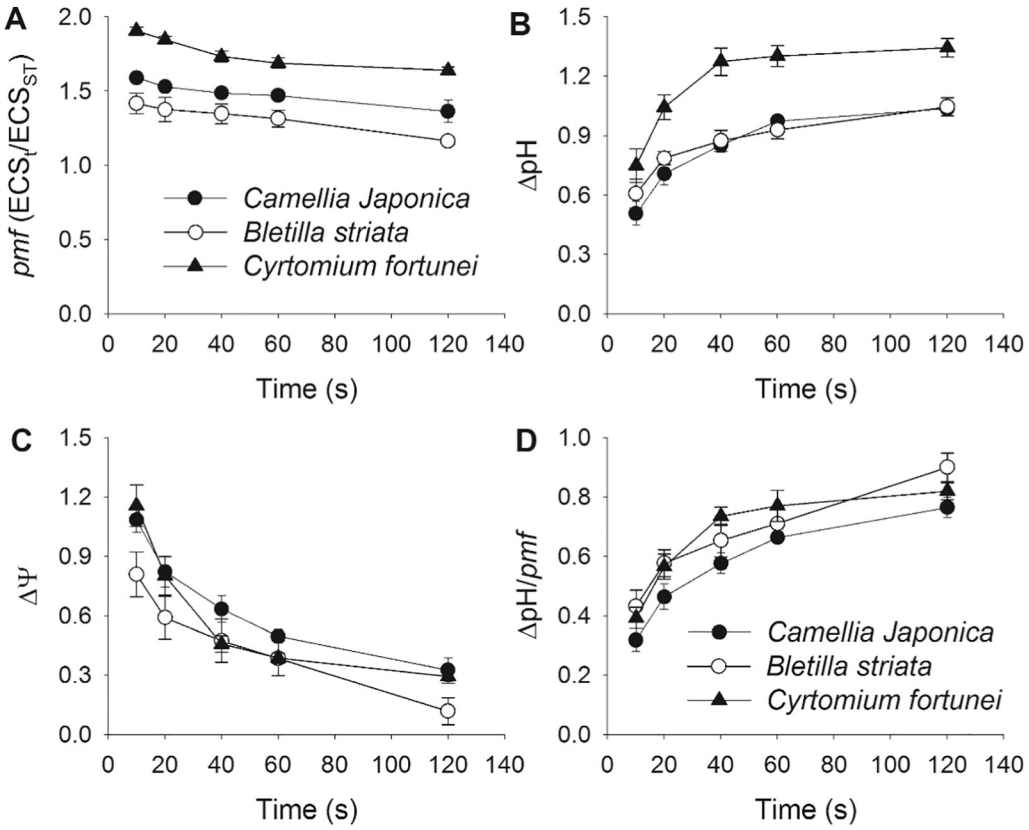

Fig. 3. Changes in proton motive force during transition from low to high light. A, Total proton motive force (*pmf*); B, proton gradient (ΔpH); C, membrane potential (ΔΨ); D, ΔpH/*pmf* ratio. After the transition from 59 to 1809 μmol photons $m^{-2} s^{-1}$ for 10 s, 20 s, 40 s, 60 s or 120 s, full ECS decay kinetics in the dark were monitored to measure *pmf*, ΔpH and ΔΨ. The 515 nm absorption change (ΔA_{ECS}) was normalized against ECS_{ST}. Values are means ± SE (n = 5).

the WWC has the potential to alter P700 redox kinetics upon abrupt illumination of dark-adapted leaves in angiosperms.

4.2. The PSI redox state in fluctuating light is regulated not only by the ΔpH but also by the WWC in angiosperms

Many studies have confirmed the important role of the ΔpH in adjusting the redox state of PSI in angiosperms [3,14–17,30], in addition to PSII photoinhibition [52]. In general, a strong ΔpH slows down the electron transfer from PSII to PSI via the Cyt b_6/f complex by inhibiting plastoquinol oxidation (photosynthetic control), leading to the oxidation of P700 and thus preventing PSI photoinhibition [16,30,53]. In mutants defective in the ability to generate enough ΔpH, excess electron flow from PSII to PSI led to the over-reduction of electron carriers in PSI, increasing the production of ROS within PSI and thus causing photodamage to PSI under high light and fluctuating light [14,16–18,30]. As a result, the acidification of the lumen is regarded as the key mechanism to avoid the over-reduction of PSI and to prevent PSI photoinhibition in response to excess excitation energy. In the present study, we found that the PSI was highly reduced in *B. striata* upon a sudden transition from low to high light (Fig. 2B), which was accompanied by the relatively low ΔpH (Fig. 3B). As a result, the PSI redox state was indeed mainly controlled by the magnitude of the ΔpH in the angiosperm *B. striata* (Fig. 4), consistent with the phenomenon in the model plant *Arabidopsis thaliana* (Fig. 6). By comparison, PSI was highly oxidized in *C. japonica* upon a sudden increase in light intensity (Fig. 2B). Because *C. japonica* also showed an insufficient ΔpH at this moment (Fig. 3B), this high level of P700 oxidation should be attributed to another regulatory mechanism rather than the ΔpH-dependent photosynthetic control at the Cyt b_6/f complex. Specifically, the WWC favored the outflow of electrons from PSI to O_2 and thus led to this fast oxidation of PSI upon the transition from low to high light, as similar to the fast re-oxidation of P700 during dark-to-light transition. As a result, the WWC could play an important role in adjusting the PSI redox state during transition from low to high light in angiosperms.

Because angiosperms are not able to build up a sufficient ΔpH upon a sudden transition from low to high light (Figs. 3B and 6B), the ΔpH-dependent regulatory mechanism cannot optimize the redox state of PSI during this transition, as indicated by the over-reduction of PSI in *B. striata* (Fig. 2B) and *Arabidopsis thaliana* (Fig. 6A). Therefore, the ΔpH-dependent regulatory mechanism displays its flaws in fluctuating light. In contrast, the WWC could induce the fast oxidation of P700 after the transition from low to high light, even at a relatively low ΔpH. Therefore, the WWC is another important strategy for regulating the redox state of PSI under fluctuating light, in addition to ΔpH-dependent photosynthetic control and PSII photoinhibition. In addition, the WWC is likely more efficient in adjusting the PSI redox state in fluctuating light than the ΔpH-dependent regulatory mechanism.

4.3. The WWC alleviates photoinhibition of PSI in fluctuating light

During steady-state photosynthesis under saturating light, the value of Y(NA) was usually maintained at a low level of approximately 0.1 in angiosperms [12,13,17,18,54–57,60], diminishing the production of ROS within PSI and thus protecting PSI from photoinhibition [58,59]. When shifted to fluctuating light, PSI photoinhibition occurred in angiosperms such as *Arabidopsis thaliana* and rice [12,20]. However, the underlying mechanisms are not well clarified. Our results indicate that upon a sudden increase in light intensity, the insufficient ΔpH induces the over-reduction of PSI electron carriers, which explains why fluctuating light treatments induce significant PSI photoinhibition in angiosperms such as *Arabidopsis thaliana*, rice and *B. striata*. As a result, although the ΔpH is thought to be the key determinant for photoprotection of PSI in fluctuating light, the ΔpH-dependent regulatory mechanism cannot prevent the photoinhibition of PSI under fluctuating light, even in wild-type plants.

Taking into consideration the changes in the PSI redox state and ΔpH during the transition from low to high light, we propose that the

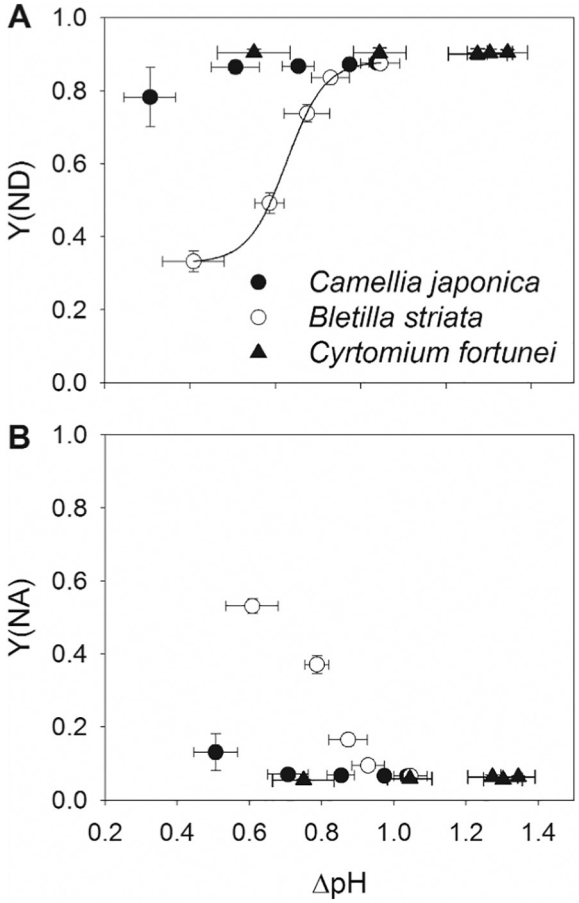

Fig. 4. Changes in Y(ND) (A) and Y(NA) (B) as a function of the ΔpH. After the transition from 59 to 1809 μmol photons $m^{-2} s^{-1}$ for 10 s, 20 s, 40 s, 60 s or 120 s, the values of Y(ND), Y(NA) and ΔpH were measured. Values are means ± SE (n = 5).

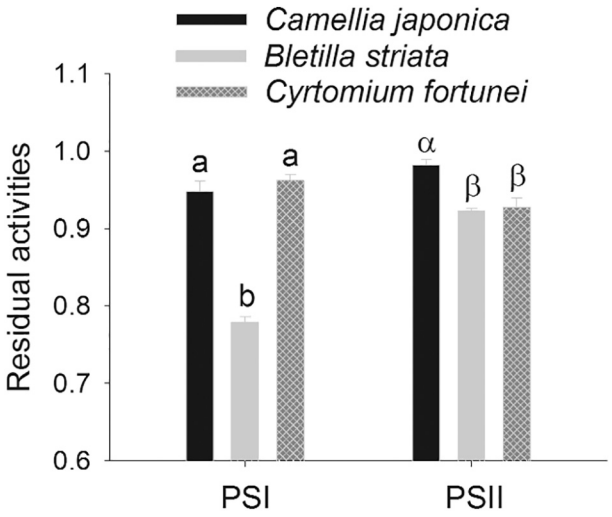

Fig. 5. Residual activities of PSI and PSII after fluctuating light treatment. After exposure to fluctuating light for 40 min (alternating between 59 and 1809 μmol photons $m^{-2} s^{-1}$ every 20 s), samples were dark-adapted for 30 min, and then F_v/F_m and P_m were measured. Data were normalized to values before treatment and are represented as the residual activities of PSI and PSII. Different letters indicate significant differences among these three species ($P < 0.05$, n = 5).

initial 20 s after this transition is the major time point of PSI photoinhibition under fluctuating light in *B. striata*. After exposure to

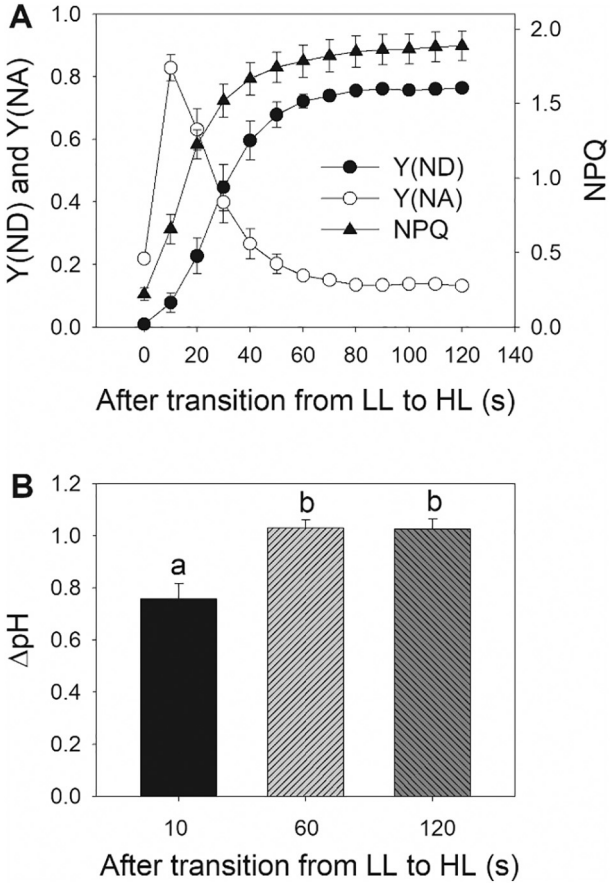

Fig. 6. Changes in PSI redox state and NPQ (A) and ΔpH (B) after the transition from 59 to 1809 μmol photons $m^{-2} s^{-1}$ measured for leaves of *Arabidopsis thaliana*. The PSI and PSII parameters were determined as mentioned in Fig. 2, and the ΔpH values were recorded as mentioned in Fig. 3. Values are means ± SE (n = 5). Different letters indicate significant differences among different treatments ($P < 0.05$).

fluctuating light alternating between 59 and 1809 μmol photons $m^{-2} s^{-1}$ every 20 s for 40 min, PSI photoinhibition was much stronger in *B. striata* when compared with *C. japonica* and *C. fortunei* (Fig. 5). This result was consistent with the changes in the PSI redox state under fluctuating light (Fig. 2). In *B. striata*, the insufficient ΔpH during the initial 20 s resulted in the over-reduction of PSI (Figs. 2 and 3), increasing the production of ROS within PSI and thus causing PSI photoinhibition (Fig. 5). By comparison, due to the operation of the WWC in *C. japonica*, PSI was highly oxidized after a sudden transition from low to high light. Under such conditions, the probability of electron donation from P700 to O_2 is suppressed. As a result, *C. japonica* can escape ROS production within PSI in fluctuating light, thus protecting PSI against photoinhibition. Therefore, we propose that the WWC is important for suppressing the production of ROS within PSI and alleviating PSI photoinhibition in fluctuating light. Because SOD and APX genes are conserved in angiosperms, over-expression of SOD and APX has the potential to enhance the capacity of the WWC and thus to alleviate the photodamage of PSI in fluctuating light. Therefore, the introduction of the WWC to enhance the resistance of PSI to fluctuating light may have broad applications in angiosperms and crops in particular. Further research is needed to explain why the WWC-dependent regulatory mechanism of the PSI redox state is not conserved in all angiosperms.

4.4. Why is Flv not retained in angiosperms?

In nonflowering photosynthetic organisms such as the fern

Cyrtomium fortunei, the alternative electron flow mediated by Flvs (Fig. S1) contributed to the rapid PSI oxidation upon the transition from low to high light (Fig. 2A), even at a relatively low ΔpH (Fig. 3B). Thus, Flvs are important for protecting PSI against photoinhibition in fluctuating light in those evolutionary groups [37–41]. However, it is unclear why *Flv* genes are not conserved in angiosperms. Here, our results provide some clues to answer this question. Upon a sudden transition from low to high light, the ΔpH-dependent regulatory mechanism (photosynthetic control) cannot make the PSI highly oxidized due to the insufficient ΔpH. Concomitantly, the operation of the WWC can rapidly deliver electrons from PSI to O_2, thus optimizing the PSI redox state and alleviating PSI photoinhibition. Therefore, the WWC can compensate for the deficiency of the ΔpH-dependent photosynthetic control.

5. Conclusions

In summary, here we have highlighted the importance of water-water cycle for PSI activity under fluctuating light in angiosperms. Upon a sudden increase in light intensity, plants cannot build up an enough ΔpH to down-regulate the electron flow from PSII. Concomitantly, the rapid oxidation of P700 is dependent on photo-reduction of O_2, which is attributed to the Flv-dependent alternative electron flow and water-water cycle in nonflowering plants and angiosperms, respectively. The reduction of water-water cycle results in the over-reduction of PSI for the first seconds after an increase in light intensity, leading to the ROS production within PSI and thus PSI photoinhibition. Taken together, the water-water cycle is a missing mechanism regulating the PSI redox state under fluctuating light in angiosperms.

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Transparency document

The Transparency document associated with this article can be found, in online version.

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Conflict of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbabio.2019.03.007.

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