


Article

Regulation of Chloroplast ATP Synthase Modulates Photoprotection in the CAM Plant *Vanilla planifolia*

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Abstract: Generally, regulation of cyclic electron flow (CEF) and chloroplast ATP synthase play key roles in photoprotection for photosystems I and II (PSI and PSII) in C3 and C4 plants, especially when CO₂ assimilation is restricted. However, how CAM plants protect PSI and PSII when CO₂ assimilation is restricted is largely known. In the present study, we measured PSI, PSII, and electrochromic shift signals in the CAM plant *Vanilla planifolia*. The quantum yields of PSI and PSII photochemistry largely decreased in the afternoon compared to in the morning, indicating that CO₂ assimilation was strongly restricted in the afternoon. Meanwhile, non-photochemical quenching (NPQ) in PSII and the donor side limitation of PSI (Y(ND)) significantly increased to protect PSI and PSII. Under such conditions, proton gradient (ΔpH) across the thylakoid membranes largely increased and CEF was slightly stimulated, indicating that the increased ΔpH was not caused by the regulation of CEF. In contrast, the activity of chloroplast ATP synthase (g_{H^+}) largely decreased in the afternoon. At a given proton flux, the decreasing g_{H^+} increased ΔpH and thus contributed to the enhancement of NPQ and Y(ND). Therefore, in the CAM plant *V. planifolia*, the ΔpH -dependent photoprotective mechanism is mainly regulated by the regulation of g_{H^+} rather than CEF when CO₂ assimilation is restricted.

Keywords: photosynthesis; CAM plants; photoprotection; proton gradient; cyclic electron flow



Citation: Wang, H.; Wang, X.-Q.; Xing, Y.-Z.; Zhao, Q.-Y.; Zhuang, H.-F.; Huang, W. Regulation of Chloroplast ATP Synthase Modulates Photoprotection in the CAM Plant *Vanilla planifolia*. *Cells* **2022**, *11*, 1647. <https://doi.org/10.3390/cells11101647>

Academic Editor: Marian Brestic

Received: 21 March 2022

Accepted: 10 May 2022

Published: 15 May 2022

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1. Introduction

In plants, light energy absorbed by leaves drives photosynthetic electron transport in the thylakoid membranes [1,2]. During linear electron flow (LEF), electrons split from photosystem II (PSII) are transported to NADP⁺, generating the reducing power NADPH. Meanwhile, water splitting and the translocation of protons from stroma to thylakoid lumen generate proton motive force (*pmf*) across the thylakoid membranes, producing ATP through chloroplast ATP synthase. Theoretically, LEF produces a ATP/NADPH ratio of 1.29 [3–5], while the ATP/NADPH ratio required by primary metabolism is approximately 1.6 [4,6]. Therefore, LEF alone cannot satisfy the energy budget and plants need alternative electron flow to increase the ATP/NADPH production ratio, which is mainly accomplished by cyclic electron flow (CEF) around PSI [4,7–9]. During CEF, electrons are cycled back from PSI to the plastoquinone pool, which generates *pmf* without reducing NADP⁺ [10,11]. In C3 and C4 plants, the dynamic regulation of CEF adjusts the regulation of *pmf*, which is essential for photosynthesis and photoprotection under changing environmental conditions [8,12–18]. However, the physiological functions of CEF in CAM plants are little known.

The *pmf* across the thylakoid membranes comprise a proton gradient (ΔpH) and a membrane potential ($\Delta\Psi$), both of them can drive ATP synthesis via the chloroplast ATP synthase [19–21]. In addition, ΔpH is a key signal for photosynthetic regulation [15,22,23]. Par-

ticularly, ΔpH triggers the induction of non-photochemical quenching (NPQ) in PSII, which is critical to dissipation of excess light energy and photoprotection of PSII [12,24–26]. Furthermore, ΔpH adjusts the redox state of PSI and prevents PSI photoinhibition [12,14,27–30]. Once the buildup of ΔpH was suppressed under high light, the induction of NPQ would be suppressed and PSI would be over-reduced, leading to detrimental photodamage to PSII and PSI [22,25]. If the formation of ΔpH was too high, the plastoquinone oxidation at the cytochrome (Cyt) b_6/f complex was down-regulated, restricting the operation of LEF and CO_2 assimilation [31,32]. Therefore, ΔpH should be regulated finely to optimize photosynthetic CO_2 assimilation and photoprotection.

Generally, the buildup of ΔpH is controlled by photosynthetic electron flows and chloroplast ATP synthase [11,15]. The impairment of CEF in proton gradient regulator5 (*pgr5*) mutant of *Arabidopsis thaliana* reduces ΔpH formation under high light, resulting in severe photoinhibition of PSI and PSII [12,14,25]. In *hope2* and *cfq* mutants of *A. thaliana*, the activity of the chloroplast ATP synthase (g_{H^+}) largely increased, resulting in decreased ΔpH formation and thus inducing severe loss of PSI activity under high light or fluctuating light [28,29]. In C3 plants, stimulation of CEF and down-regulation of g_{H^+} simultaneously contribute to the increase in ΔpH when CO_2 assimilation was restricted by environmental stresses, such as low CO_2 concentration [33,34], drought [35–37], low temperature [38,39], and fluctuating light [27,40–43]. By comparison, when CO_2 assimilation was restricted by low CO_2 concentration in the model C4 plant maize, the increase of ΔpH was caused by the decrease of g_{H^+} rather than stimulating CEF [34]. Therefore, C3 and C4 plants have different strategies for regulation of ΔpH formation when CO_2 assimilation was restricted. Within the first seconds after transition from dark or low light to high light, the relatively low g_{H^+} and CEF stimulation contributed to the rapid formation of ΔpH , favoring photoprotection for PSI and PSII in the CAM plant *Bryophyllum pinnatum* [44], which was similar to the phenomenon in C3 plants. In contrast to C3 and C4 plants, crassulacean acid metabolism (CAM) plants usually close stomata in daytime to survive drought habitat [45]. At daytime, CO_2 released from malic acid is assimilated by the Calvin–Benson cycle. Owing to the gradual consumption of malic acid at daytime, the chloroplast CO_2 concentration decreased in the afternoon [46,47], restricting photosynthetic light use efficiency [48]. Under such conditions, a high level of ΔpH should be formed to protect photosynthetic apparatus against photoinhibition. However, the regulatory mechanism of ΔpH formation is still poorly understood in CAM plants.

In this study, light response changes of PSI, PSII, ΔpH , and g_{H^+} were measured in the morning and in the afternoon for leaves of the CAM plant *Vanilla planifolia*. The aims are to: (1) Explore the performance of CEF when CAM photosynthesis is restricted; and (2) assess how CAM plants regulate ΔpH when light use efficiency is restricted. Our results indicated that in *V. planifolia* CEF/LEF ratio slightly increased when LEF was restricted in the afternoon. Meanwhile, the ΔpH level largely increased to trigger photoprotection, which was predominantly caused by the decrease of g_{H^+} . Therefore, regulation of g_{H^+} plays a more important role in ΔpH formation than CEF in the CAM plant *V. planifolia* upon restriction of photosynthetic CO_2 assimilation. Regulation of g_{H^+} is likely a common strategy used by C3, C4, and CAM plants to modulate photoprotection responding to environmental conditions.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

Vanilla planifolia Andrews is a famous spice plant with high commercial value. As a CAM plant native to tropical forests in Mexico and Central America, *V. planifolia* is adaptive to hot, humid, and shade environment. The studied plants of *V. planifolia* were cultivated in Spice and Beverage Research Institute, Chinese Academy of Tropical Agricultural Sciences (18°70' E, 110°18' N, altitude 10 m). The shade condition was controlled using two-layer polyester shade net, and the maximum light intensity exposed to leaves was approximately $200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. We conducted photosynthetic measurements on youngest

fully expanded but not-senescent leaves in November in 2021. During this period, the day/night air temperatures were approximately 33/25 °C and the relative air humidity approximately 80%.

2.2. PSI and PSII Measurements

Our previous study indicated that for leaves of *V. planifolia*, the light use efficiency was maximized in the morning (a.m. 10:00–12:00) but was restricted in the afternoon (p.m. 13:00–17:00) [48]. Therefore, at these two time slots, a Dual-PAM 100 measuring system (Heinz Walz, Effeltrich, Germany) was used to record PSI and PSII parameters at approximately 30 °C. After dark-adaptation for 30 min, leaves were illuminated at 923 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 15 min to reach steady state photosynthesis. Afterward, light response curves were measured. PSI and PSII parameter were calculated as follows [49,50]: The quantum yield of PSI photochemistry, $Y(\text{I}) = (P_m' - P)/P_m$; the quantum yield of PSI non-photochemical energy dissipation due to donor side limitation, $Y(\text{ND}) = P/P_m$; the quantum yield of PSI non-photochemical energy dissipation due to acceptor side limitation, $Y(\text{NA}) = (P_m - P_m')/P_m$; the quantum yield of PSII photochemistry, $Y(\text{II}) = (F_m' - F_s)/F_m'$; non-photochemical quenching, $\text{NPQ} = (F_m - F_m')/F_m'$; the quantum yield of non-regulatory energy dissipation in PSII, $Y(\text{NO}) = F_s/F_m$; the maximum efficiency of the open PSII centers in the light, $F_v'/F_m' = (F_m' - F_o')/F_m'$; the coefficient of photochemical quenching based on the “puddle model” of PSII, $qP = (F_m' - F_s)/(F_m' - F_o')$; the coefficient of photochemical quenching based on the “lake model” of PSII, $qL = qP \times F_o'/F_s$. F_o' is estimated using the following equation, $F_o' = F_o/(F_v/F_m + F_o/F_m')$ [51]. P_m , F_m , and F_o were measured after dark adaptation for 30 min. P_m' , P , F_m' , and F_s were measured after exposure to each light intensity for 3 min.

The photosynthetic electron transport rates through PSI and PSII were calculated as follows: $\text{ETRI} = \text{PAR} \times Y(\text{I}) \times 0.84 \times \text{dI}$; $\text{ETR}_{\text{II}} = \text{PPFD} \times Y(\text{II}) \times 0.84 \times \text{dII}$. PPFD is the photosynthetically active radiation, and 0.84 is the assumed light absorption of incident irradiance. Based on the assumption that the role of CEF is negligible under very low light intensity [52], the values of dI and dII were calculated using the values of $Y(\text{I})$ and $Y(\text{II})$ under a low light of 59 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in the morning according to the method of [37]. dI and dII were 0.487 and 0.513, respectively. Furthermore, these estimated values of dI and dII were also used for the calculation of ETR in the afternoon. The apparent rate of CEF was estimated by subtracting ETR_{II} from ETRI [30,37].

2.3. Electrochromic Shift Measurement

A Dual-PAM 100 measuring system equipping a P515/535 emitter-detector module was used to measure the electrochromic shift signals (ECS) [53,54]. After photosynthetic induction at 923 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 15 min, the ECS were recorded after illumination at each light intensity for 3 min. ECS dark interval relaxation kinetics were recorded to calculate the ΔpH and g_{H^+} [35,55,56].

2.4. Statistical Analysis

All data are displayed as mean values of five leaves from five independent plants. T-test was used to determine whether significant differences existed between different treatments ($\alpha = 0.05$).

3. Results

To examine the effect of restriction of CO_2 assimilation on PSI and PSII performances, we first measured the light response changes of PSI and PSII parameters in the morning and in the afternoon for leaves of *V. planifolia* (Figure 1). The quantum yield of PSI photochemistry ($Y(\text{I})$) gradually decreased with the increase in light intensity, and the values of $Y(\text{I})$ in the morning were higher than those in the afternoon (Figure 1A). Concomitantly, the PSI donor side limitation ($Y(\text{ND})$) gradually increased in both samples with higher values in the afternoon (Figure 1B), resulting in similar PSI acceptor side limitation ($Y(\text{NA})$)

between morning and afternoon (Figure 1C). The effective quantum yield of PSII photochemistry ($Y(II)$) gradually decreased with the increasing light intensity (Figure 1D). In the afternoon, $Y(II)$ significantly decreased at any light intensity when compared with in the morning. Concomitantly, non-photochemical quenching (NPQ) was significantly enhanced in the afternoon to dissipate the excess light energy (Figure 1E). As a result, the quantum yield of non-regulatory energy dissipation in PSII ($Y(NO)$) was maintained at low level (Figure 1F), diminishing the risk of PSII photoinhibition. Therefore, although $Y(I)$ and $Y(II)$ were largely restricted in the afternoon, PSI over-reduction was completely prevented and PSII photoprotection was well performed.

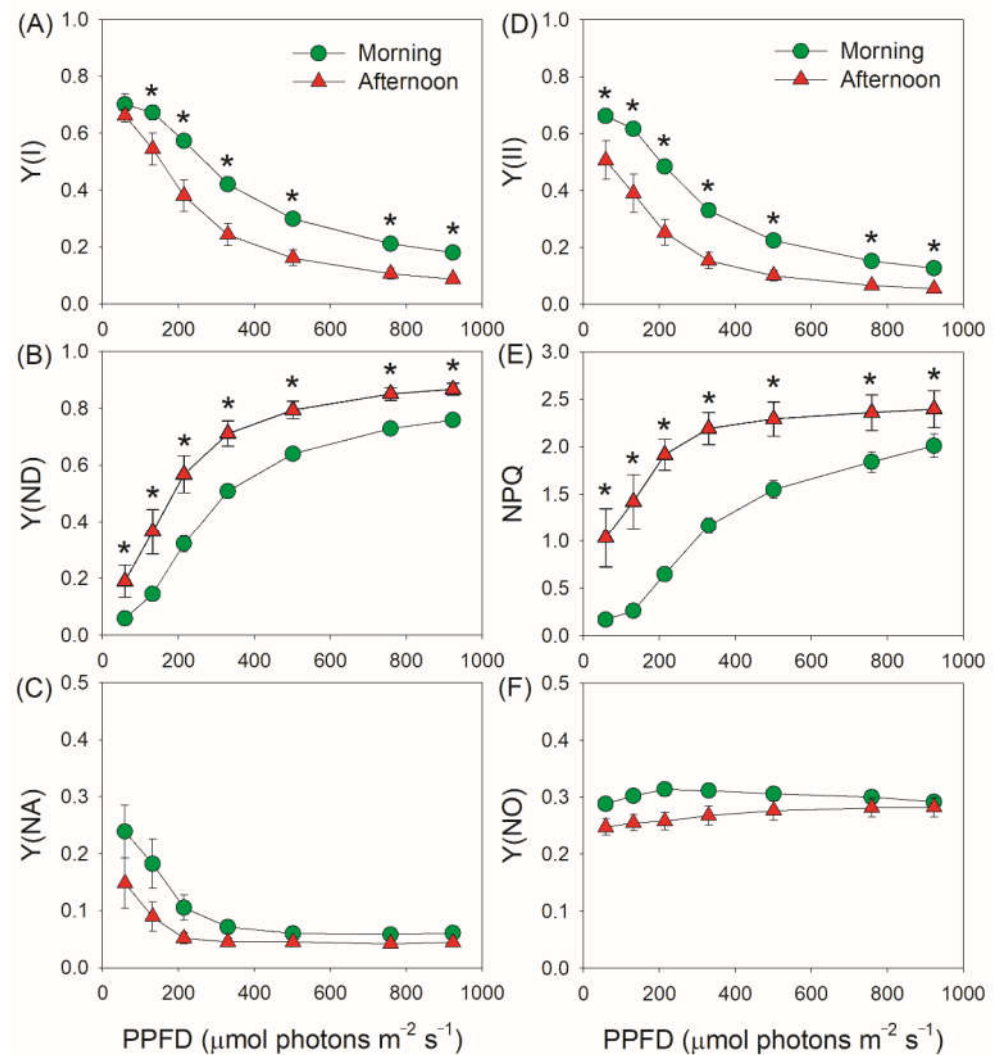


Figure 1. Light intensity dependence of PSI and PSII parameters for leaves of *Vanilla planifolia* measured in the morning and in the afternoon. $Y(I)$ (A), the quantum yield of PSI photochemistry; $Y(ND)$ (B), the quantum yield of PSI non-photochemical energy dissipation due to donor side limitation; $Y(NA)$ (C), the quantum yield of PSI non-photochemical energy dissipation due to acceptor side limitation; $Y(II)$ (D), the quantum yield of PSII photochemistry; NPQ (E), non-photochemical quenching in PSII; $Y(NO)$ (F), the quantum yield of non-regulatory energy dissipation in PSII. Data are means \pm SE ($n = 5$). Asterisk indicates a significant difference between morning and afternoon.

The value of $Y(II)$ is the product of F_v'/F_m' and qP . F_v'/F_m' represents the maximum efficiency of the open PSII centers in the light, and qP represents the coefficient of photochemical quenching based on the “puddle model” of PSII. We found that the decreased $Y(II)$ in the afternoon was mainly caused by the decrease in qP rather than the change in F_v'/F_m' (Figure 2A,B). Furthermore, we found that the coefficient of photochemical quench-

ing based on the “lake model” of PSII (q_L) also decreased in the afternoon (Figure 2C). These results indicated that the PSII reaction centers were closed to a higher extent in the afternoon. Based the data of $Y(I)$ and $Y(II)$, photosynthetic electron transport rate through PSI (ETRI) and PSII (ETRII) and the rate of CEF were calculated. Under light intensities above $200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, ETRI and ETRII largely decreased in the afternoon (Figure 3A,B). Meanwhile, the value of ETRI–ETRII was enhanced under light intensities below $330 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ but stagnated above this light intensity (Figure 3C). Furthermore, the ETRI/ETRII ratio was enhanced in the afternoon (Figure 3D). The activation of CEF is dependent on dynamic thylakoid stacking and *pgr5*-pathway [30]. The light response changes of ETRI–ETRII and ETRI/ETRII strongly indicated the activation of CEF under high light [30]. These results indicated that LEF and CEF responded differently to the restriction of CO_2 assimilation in the CAM plant *V. planifolia*. It should be noted that this method of estimating CEF has some limits. First, light intensity can affect the partitioning of light between PSI and PSII. Second, $Y(I)$ will be increased if electron supply to PSI is throttled up to a certain extent, as indicated by the lower $Y(II)$ and higher $Y(I)$ under moderate light in *pgr1* mutant compared with WT plants of *Arabidopsis thaliana* [57,58].

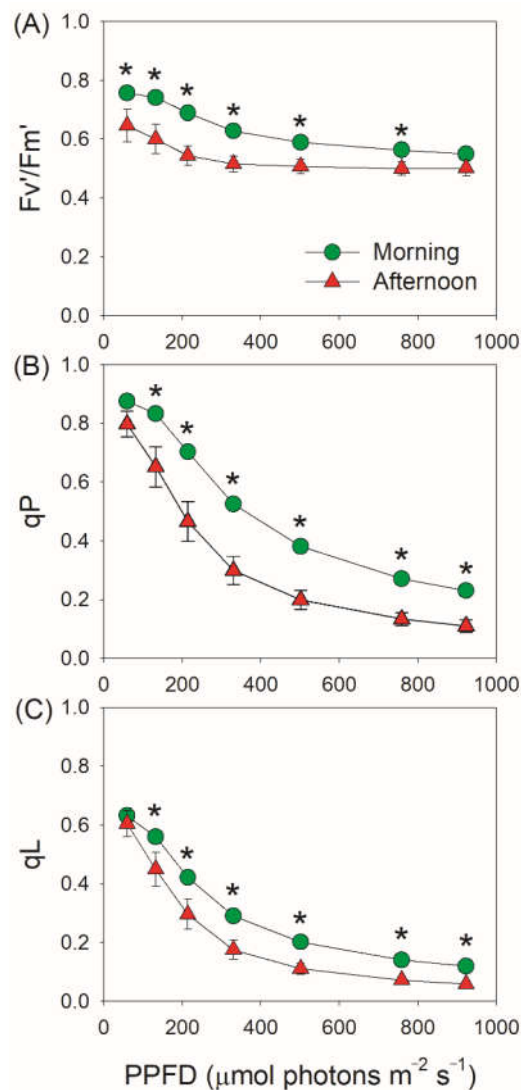


Figure 2. Light intensity dependence of F_v'/F_m' (A), q_P (B) and q_L (C) for leaves of *Vanilla planifolia* measured in the morning and in the afternoon. F_v'/F_m' , the maximum efficiency of the open PSII centers in the light; q_P , coefficient of photochemical quenching based on the “puddle model” of PSII; q_L , coefficient of photochemical quenching based on the “lake model” of PSII. Data are means \pm SE ($n = 5$). Asterisk indicates a significant difference between morning and afternoon.

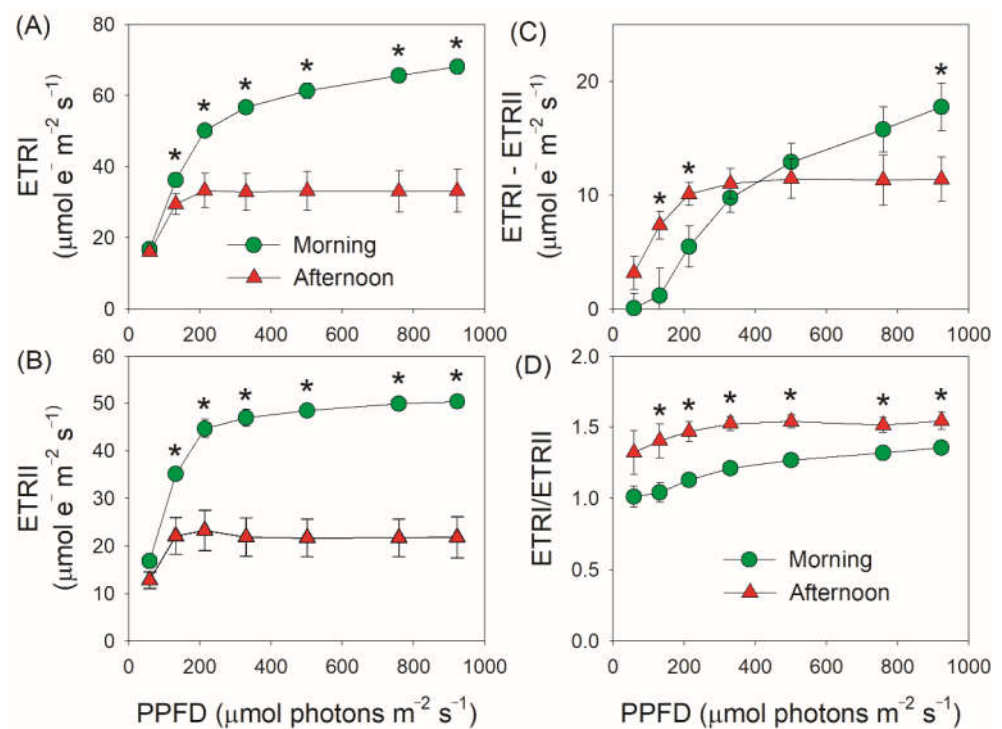


Figure 3. Light intensity dependence of electron transport rates for leaves of *Vanilla planifolia* measured in the morning and in the afternoon. ETRI (A), electron transport rate through PSI; ETRII (B), electron transport rate through PSII; ETRI–ETRII (C), estimated rate of cyclic electron flow; ETRI/ETRII (D), an indicator reflecting the contribution of cyclic electron flow to total photosynthetic electron flow. Data are means \pm SE ($n = 5$). Asterisk indicates a significant difference between morning and afternoon.

Because the activity of chloroplast ATP synthase (g_{H^+}) and ΔpH are key factors for photosynthetic regulation when CO_2 assimilation is restricted, we next measured the electrochromic shift signals for leaves of *V. planifolia*. With increasing light intensity, g_{H^+} was maintained stable in the morning but gradually decreased in the afternoon with low values (Figure 4A). Furthermore, the value of g_{H^+} in the morning was 2–3-fold than that in the afternoon. Meanwhile, the ΔpH formation was largely enhanced in the afternoon (Figure 4B). Therefore, when CO_2 assimilation was restricted in the afternoon, the activity of chloroplast ATP synthase was suppressed and the acidification of thylakoid lumen was increased. The increase of ΔpH triggered the induction of NPQ and PSI donor side regulation, as indicated by positive effects of ΔpH on NPQ and Y(ND) (Figure 5). Furthermore, when CO_2 assimilation was restricted in the afternoon, ΔpH was formed to much higher levels than that required for the NPQ induction and PSI donor side regulation.

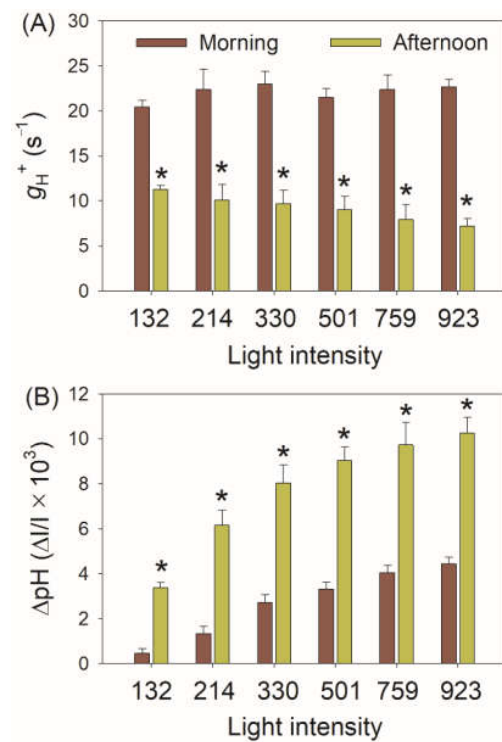


Figure 4. Light intensity dependence of g_{H^+} (A) and ΔpH (B) for leaves of *Vanilla planifolia* measured in the morning and in the afternoon. g_{H^+} , the conductivity of the chloroplast ATP synthase to protons; ΔpH , proton gradient across the thylakoid membranes. Data are means \pm SE ($n = 5$). Asterisk indicates a significant difference between morning and afternoon.

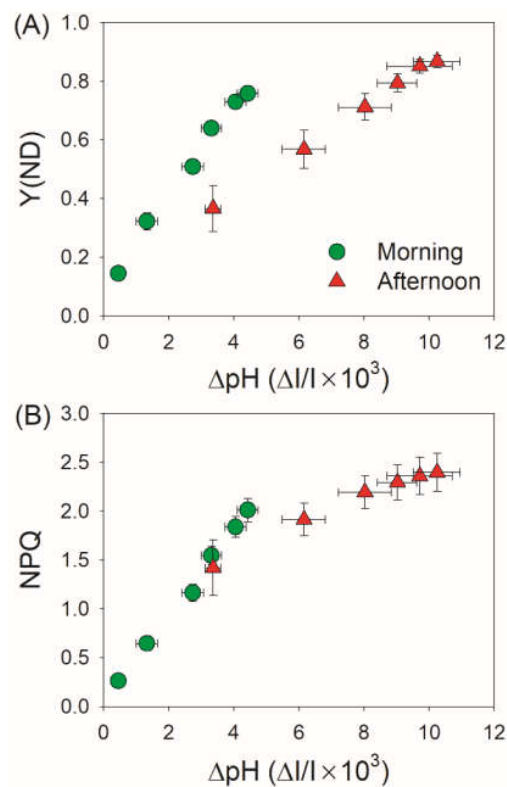


Figure 5. Changes in (A) $Y(ND)$ and (B) NPQ as a function of ΔpH for leaves of *Vanilla planifolia* measured in the morning and in the afternoon. Data are means \pm SE ($n = 5$).

To explore why the lower proton flux activity (ETRI and ETRII values) in the afternoon was accompanied by higher ΔpH , we compared the decreased amplitude of ETRI, ETRII, and g_{H^+} in the afternoon. As shown in Figure 6, the decreased amplitude of g_{H^+} in the afternoon was stronger than that of ETRI and ETRII. These results indicated that the large decrease in g_{H^+} in the afternoon compensated for the decreases in LEF and CEF under high light. In the afternoon, ETRII and CEF were saturated under a moderate light of $132 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Figure 3B,C), while ΔpH gradually increased with light intensity (Figure 4B). These results indicated that the increased ΔpH under high light was not caused by the changes in ETRII and CEF. Therefore, the lowering of g_{H^+} was the primary cause of increased ΔpH when CO_2 assimilation under high light was restricted in *V. planifolia*.

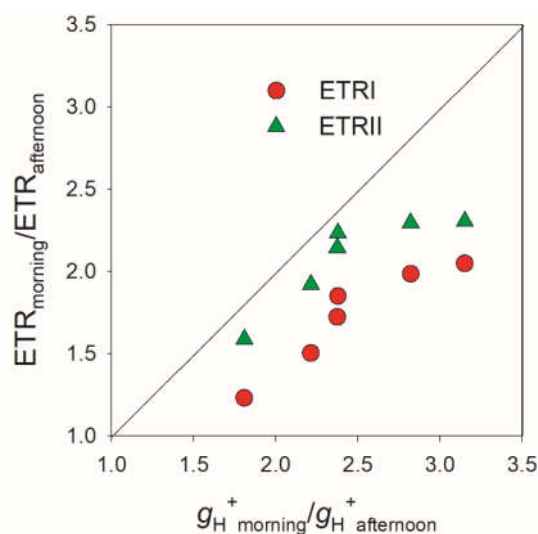


Figure 6. Changes in the decrease amplitudes of ETRI and ETRII in the afternoon as a function of the decrease amplitude of g_{H^+} for leaves of *Vanilla planifolia*. Light response data from Figures 3 and 4 were used to calculate the decrease amplitudes of ETRI, ETRII, and g_{H^+} in the afternoon when compared with those in the morning.

4. Discussion

During photosynthesis, photosynthetic electron transport in thylakoids produces ATP and NADPH for CO_2 fixation in chloroplast stroma [20,59]. PSI and PSII are two key components of photosynthetic electron transport chains, requiring finely balanced photoprotection for them [60–62]. If PSI was photodamaged, both LEF and CEF were restricted, CO_2 assimilation and photoprotection would be suppressed, impairing plant growth [14,63–65]. Alternatively, if PSII photoinhibition occurred, LEF would be reduced due to the decreased splitting of water, decreasing the regeneration of ATP and NADPH and thus declining CO_2 assimilation [25,66,67]. When CO_2 assimilation is restricted in C3 and C4 higher plants, ΔpH is the key signal for photoprotection against excess light energy [33–35,38]. However, the role of ΔpH formation in photoprotection for PSI and PSII in CAM plants is little known.

We found that ETRII largely decreased in the afternoon for leaves of the CAM plant *V. planifolia* (Figure 3B), indicating that CO_2 assimilation was restricted. In the afternoon, the stomatal closure in *V. planifolia* restricted CO_2 diffusion from air to chloroplast [48]. Therefore, the decreased ETRII in the afternoon was caused by the drying up of the malic acid pool [46,47]. At this time, $Y(\text{ND})$ largely increased and $Y(\text{NA})$ was maintained at low levels (Figure 1B,C), suggesting that PSI was highly oxidized and PSI over-reduction was prevented. The prevention of PSI over-reduction diminished the production of reactive oxygen species around PSI reaction centers and thus avoided PSI photoinhibition [14,68,69]. It has been documented that the steady-state PSI redox state under high light is largely regulated by ΔpH [11,13,28]. A high ΔpH is essential to prevent PSI over-reduction un-

der high light at donor and acceptor sides [69]. In donor side regulation, a high ΔpH restricts Qo-site activity in the Cyt b_6/f complex, such lumen pH-dependent “photosynthetic control” slows down the electron flow from PSII to PSI and thus increases Y(ND). In acceptor side regulation, a high ΔpH regulates the ATP/NADPH production ratio for CO_2 assimilation and photorespiration, which facilitates the electron sink downstream of PSI and thus prevents PSI over-reduction [70]. In rice (*Oryza sativa*) mutants defective in the PGR5/PGRL1-dependent CET or both CET pathways, CO_2 is significantly disturbed and acceptor side regulation is weakened [71,72]. As shown in Figure 5A, Y(ND) was tightly correlated to the ΔpH formation, but there were two distinct degrees of photosynthetic control in morning vs. afternoon. In the morning, a relatively low ΔpH weakened photosynthetic control at the Cyt b_6/f complex, but a high Y(ND) was achieved due to the higher light use efficiency. When CO_2 assimilation was restricted in the afternoon, a higher ΔpH -dependent photosynthetic control was necessary to maintain the high level of Y(ND). Therefore, ΔpH -dependent photosynthetic control was more important in photoprotection for PSI when photosynthesis of *V. planifolia* was restricted in the afternoon.

In addition to regulate PSI redox state, ΔpH also induces NPQ in light-harvesting complex II and the PSII core, which is essential for PSII photoprotection [13,25,26,73]. In the CAM plant *V. planifolia*, NPQ was up-regulated with the suppression of CO_2 assimilation (Figure 1E). Such enhancement of NPQ decreased the production of reactive oxygen species in PSII. Because reactive oxygen species produced within PSII inhibits the repair of PSII [74–77], the ΔpH -dependent induction of NPQ in *V. planifolia* facilitated the repair of PSII. Furthermore, a high ΔpH could sequester Ca^{2+} in the lumen through a $\text{Ca}^{2+}/\text{H}^+$ antiport, and a high Ca^{2+} concentration in the lumen could protect the oxygen-evolving complex. Once ΔpH formation under high light was suppressed, photodamage of oxygen-evolving complex further aggravated PSII photoinhibition [25,60,78]. Interestingly, we observed that *V. planifolia* generated a much higher ΔpH than that required by the induction of the maximum NPQ when CO_2 assimilation was restricted (Figure 5B). Therefore, the much higher ΔpH level in the afternoon might protect the oxygen-evolving complex in PSII. Taking together, a high ΔpH is essential for photoprotection of PSII when photosynthesis of *V. planifolia* was restricted in the afternoon.

Both LEF and CEF pump electrons from the chloroplast stroma into the thylakoid lumen and thus induce the ΔpH formation [11,59]. In addition, the splitting of water in LEF generates H^+ in the lumen of thylakoid. Therefore, the formation of ΔpH is in general primarily dependent on LEF rather CEF because LEF is usually higher than CEF [33,34,79]. However, when CO_2 assimilation and LEF are restricted under environmental stresses in C3 plants, CEF plays an essential role in the ΔpH formation under high light [43,80,81]. For example, the impairment of CEF in *A. thaliana pgr5* and *ndh* mutants decreased the ΔpH formation under high light [53,82]. In the C3 resurrection plant *Paraboea rufescens*, CEF was highly stimulated to a much higher level than LEF when leaves were dehydrated [37]. By comparison, promoting CEF played a minor role in the enhancement of ΔpH in the model C4 maize when CO_2 assimilation is restricted [34], although CEF was essential for the normal photosynthesis at ambient O_2 concentration in C4 plants [17,83,84]. This different performance of CEF between C3 and C4 plants is mainly caused by the capacity of photorespiration between them [34]. In the studied CAM plant *V. planifolia*, CEF was stimulated under low and moderate light intensities but was suppressed under high light once CO_2 assimilation was restricted (Figure 3C). Meanwhile, the CEF/LEF ratio increased at all light intensities (Figure 3D), suggesting the contribution of CEF to ΔpH formation was enhanced. Such CEF stimulation favored ΔpH -dependent photoprotective mechanisms and increased the ATP/NADPH production ratio for photorespiration [4,11]. Therefore, CEF was required to facilitate photosynthetic regulation in the CAM plants, similar to the phenotype in C3 plants but different from that in C4 plants.

The other important factor for optimizing the formation of ΔpH is the proton conductivity of chloroplast ATP synthase, as indicated by g_{H^+} measured by electrochromic shift signals in this study [28,29,31,33]. Once ATP synthase accumulation was largely

reduced in tobacco, the lowering of g_H^+ generated a much higher ΔpH and impaired plant growth [31]. On the other hand, the increasing of g_H^+ suppressed the formation of ΔpH in *A. thaliana hope2* and *cfq* mutants, resulting in severe PSI photoinhibition under high light or fluctuating light [28,29]. In C3 plants, g_H^+ decreased under drought [35,85], low CO₂ concentration [33,34], and chilling temperature [38], which is critical for the increasing of ΔpH and photoprotection. When CO₂ assimilation was suppressed, the decrease of g_H^+ was stronger than that of LEF in C3 plant *Brassica rapa* but was equal to that of LEF in maize [34]. In this study, we here documented that g_H^+ decreased greater than ETRI and ETRII in the CAM plant *V. planifolia* when CO₂ assimilation was suppressed in the afternoon (Figure 6). When illuminated at a saturating light of 923 in the afternoon, both LEF and CEF were suppressed (Figure 3B,C) but ΔpH was highly increased (Figure 4B). Therefore, the regulation of g_H^+ plays a more important role than CEF in the regulation of ΔpH formation when CO₂ assimilation is restricted in the CAM plant *V. planifolia*.

5. Conclusions

In this study, we studied how the CAM plant *V. planifolia* regulates the formation of ΔpH according to the change in light use efficiency. We found that when light use efficiency was restricted in the afternoon, g_H^+ largely decreased and CEF activity slightly increased, both of which contributed to the enhancement of ΔpH formation. Subsequently, ΔpH -dependent photoprotective mechanisms were highly activated to protect PSI and PSII. Therefore, when photosynthesis was restricted in *V. planifolia*, modulation of photoprotection was mainly regulated by g_H^+ rather than CEF. This strategy is similar to C3 plants but is different from C4 plants.

Author Contributions: Conceptualization, H.W., H.-F.Z. and W.H.; methodology, W.H.; validation, H.W., X.-Q.W., H.-F.Z. and W.H.; formal analysis, W.H.; investigation, H.W., X.-Q.W., Y.-Z.X., Q.-Y.Z. and W.H.; resources, Y.-Z.X., Q.-Y.Z. and H.-F.Z.; writing—original draft preparation, W.H.; writing—review and editing, H.-F.Z. and W.H.; funding acquisition, H.W., H.-F.Z. and W.H. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Natural Science Foundation of China (No. 31871577, 31971412), the Basic and Applied Basic Research Project of Hainan (321QN328), and the Key Research and Development Project of Hainan (ZDYF2019209).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kramer, D.M.; Avenson, T.J.; Edwards, G.E. Dynamic flexibility in the light reactions of photosynthesis governed by both electron and proton transfer reactions. *Trends Plant Sci.* **2004**, *9*, 349–357. [[CrossRef](#)] [[PubMed](#)]
2. Avenson, T.J.; Cruz, J.A.; Kanazawa, A.; Kramer, D.M. Regulating the proton budget of higher plant photosynthesis. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 9709–9713. [[CrossRef](#)] [[PubMed](#)]
3. Hahn, A.; Vonck, J.; Mills, D.J.; Meier, T.; Kühlbrandt, W. Structure, mechanism, and regulation of the chloroplast ATP synthase. *Science* **2018**, *360*, 6389. [[CrossRef](#)] [[PubMed](#)]
4. Walker, B.J.; Strand, D.D.; Kramer, D.M.; Cousins, A.B. The response of cyclic electron flow around photosystem I to changes in photorespiration and nitrate assimilation. *Plant Physiol.* **2014**, *165*, 453–462. [[CrossRef](#)] [[PubMed](#)]
5. Sacksteder, C.A.; Kanazawa, A.; Jacoby, M.E.; Kramer, D.M. The proton to electron stoichiometry of steady-state photosynthesis in living plants: A proton-pumping Q cycle is continuously engaged. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 14283–14288. [[CrossRef](#)] [[PubMed](#)]
6. Walker, B.J.; VanLoocke, A.; Bernacchi, C.J.; Ort, D.R. The costs of photorespiration to food production now and in the future. *Annu. Rev. Plant Biol.* **2016**, *67*, 107–129. [[CrossRef](#)] [[PubMed](#)]

7. Yamori, W.; Sakata, N.; Suzuki, Y.; Shikanai, T.; Makino, A. Cyclic electron flow around photosystem I via chloroplast NAD(P)H dehydrogenase (NDH) complex performs a significant physiological role during photosynthesis and plant growth at low temperature in rice. *Plant J.* **2011**, *68*, 966–976. [[CrossRef](#)]
8. Yamori, W.; Shikanai, T. Physiological functions of cyclic electron transport around photosystem I in sustaining photosynthesis and plant growth. *Annu. Rev. Plant Biol.* **2016**, *67*, 81–106. [[CrossRef](#)]
9. Tan, S.-L.; Liu, T.; Zhang, S.-B.; Huang, W. Balancing light use efficiency and photoprotection in tobacco leaves grown at different light regimes. *Environ. Exp. Bot.* **2020**, *175*, 104046. [[CrossRef](#)]
10. Joliot, P.; Johnson, G.N. Regulation of cyclic and linear electron flow in higher plants. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 13317–13322. [[CrossRef](#)]
11. Shikanai, T.; Yamamoto, H. Contribution of cyclic and pseudo-cyclic electron transport to the formation of proton motive force in chloroplasts. *Mol. Plant* **2017**, *10*, 20–29. [[CrossRef](#)] [[PubMed](#)]
12. Munekage, Y.; Hojo, M.; Meurer, J.; Endo, T.; Tasaka, M.; Shikanai, T. PGR5 is involved in cyclic electron flow around photosystem I and is essential for photoprotection in Arabidopsis. *Cell* **2002**, *110*, 361–371. [[CrossRef](#)]
13. Munekage, Y.; Hashimoto, M.; Miyake, C.; Tomizawa, K.; Endo, T.; Tasaka, M.; Shikanai, T. Cyclic electron flow around photosystem I is essential for photosynthesis. *Nature* **2004**, *429*, 579–582. [[CrossRef](#)] [[PubMed](#)]
14. Suorsa, M.; Jarvi, S.; Grieco, M.; Nurmi, M.; Pietrzykowska, M.; Rantala, M.; Kangasjarvi, S.; Paakkarinen, V.; Tikkanen, M.; Jansson, S.; et al. PROTON GRADIENT REGULATION5 is essential for proper acclimation of Arabidopsis photosystem I to naturally and artificially fluctuating light conditions. *Plant Cell* **2012**, *24*, 2934–2948. [[CrossRef](#)]
15. Tikkanen, M.; Aro, E.M. Integrative regulatory network of plant thylakoid energy transduction. *Trends Plant Sci.* **2014**, *19*, 10–17. [[CrossRef](#)]
16. Tazoe, Y.; Ishikawa, N.; Shikanai, T.; Ishiyama, K.; Takagi, D.; Makino, A.; Sato, F.; Endo, T. Overproduction of PGR5 enhances the electron sink downstream of photosystem I in a C4 plant, *Flaveria bidentis*. *Plant J.* **2020**, *103*, 814–823. [[CrossRef](#)]
17. Munekage, Y.N.; Eymery, F.; Rumeau, D.; Cuiné, S.; Oguri, M.; Nakamura, N.; Yokota, A.; Genty, B.; Peltier, G. Elevated Expression of PGR5 and NDH-H in bundle sheath chloroplasts in C4 flaveria species. *Plant Cell Physiol.* **2010**, *51*, 664–668. [[CrossRef](#)]
18. Ferroni, L.; Suorsa, M.; Aro, E.M.; Baldisserotto, C.; Pancaldi, S. Light acclimation in the lycophyte *Selaginella martensii* depends on changes in the amount of photosystems and on the flexibility of the light-harvesting complex II antenna association with both photosystems. *New Phytol.* **2016**, *211*, 554–568. [[CrossRef](#)]
19. Kramer, D.M.; Cruz, J.A.; Kanazawa, A. Balancing the central roles of the thylakoid proton gradient. *Trends Plant Sci.* **2003**, *8*, 27–32. [[CrossRef](#)]
20. Kramer, D.M.; Evans, J.R. The importance of energy balance in improving photosynthetic productivity. *Plant Physiol.* **2011**, *155*, 70–78. [[CrossRef](#)]
21. Cruz, J.A.; Avenson, T.J.; Kanazawa, A.; Takizawa, K.; Edwards, G.E.; Kramer, D.M. Plasticity in light reactions of photosynthesis for energy production and photoprotection. *J. Exp. Bot.* **2005**, *56*, 395–406. [[CrossRef](#)] [[PubMed](#)]
22. Armbruster, U.; Correa Galvis, V.; Kunz, H.H.; Strand, D.D. The regulation of the chloroplast proton motive force plays a key role for photosynthesis in fluctuating light. *Curr. Opin. Plant Biol.* **2017**, *37*, 56–62. [[CrossRef](#)] [[PubMed](#)]
23. Ruban, A.V. Nonphotochemical Chlorophyll Fluorescence Quenching: Mechanism and Effectiveness in Protecting Plants from Photodamage. *Plant Physiol.* **2016**, *170*, 1903–1916. [[CrossRef](#)]
24. Johnson, M.P.; Perez-Bueno, M.L.; Zia, A.; Horton, P.; Ruban, A.V. The Zeaxanthin-Independent and Zeaxanthin-Dependent qE Components of Nonphotochemical Quenching Involve Common Conformational Changes within the Photosystem II Antenna in Arabidopsis. *Plant Physiol.* **2008**, *149*, 1061–1075. [[CrossRef](#)] [[PubMed](#)]
25. Takahashi, S.; Milward, S.E.; Fan, D.-Y.; Chow, W.S.; Badger, M.R. How does cyclic electron flow alleviate photoinhibition in Arabidopsis? *Plant Physiol.* **2009**, *149*, 1560–1567. [[CrossRef](#)]
26. Nicol, L.; Nawrocki, W.J.; Croce, R. Disentangling the sites of non-photochemical quenching in vascular plants. *Nat. Plants* **2019**, *5*, 1177–1183. [[CrossRef](#)]
27. Kono, M.; Noguchi, K.; Terashima, I. Roles of the cyclic electron flow around PSI (CEF-PSI) and O₂-dependent alternative pathways in regulation of the photosynthetic electron flow in short-term fluctuating light in Arabidopsis thaliana. *Plant Cell Physiol.* **2014**, *55*, 990–1004. [[CrossRef](#)]
28. Takagi, D.; Amako, K.; Hashiguchi, M.; Fukaki, H.; Ishizaki, K.; Goh, T.; Fukao, Y.; Sano, R.; Kurata, T.; Demura, T.; et al. Chloroplastic ATP synthase builds up a proton motive force preventing production of reactive oxygen species in photosystem I. *Plant J.* **2017**, *91*, 306–324. [[CrossRef](#)]
29. Kanazawa, A.; Ostendorf, E.; Kohzuma, K.; Hoh, D.; Strand, D.D.; Sato-Cruz, M.; Savage, L.; Cruz, J.A.; Fisher, N.; Froehlich, J.E.; et al. Chloroplast ATP Synthase Modulation of the Thylakoid Proton Motive Force: Implications for Photosystem I and Photosystem II Photoprotection. *Front. Plant Sci.* **2017**, *8*, 719. [[CrossRef](#)]
30. Hepworth, C.; Wood, W.H.J.; Emrich-Mills, T.Z.; Proctor, M.S.; Casson, S.; Johnson, M.P. Dynamic thylakoid stacking and state transitions work synergistically to avoid acceptor-side limitation of photosystem I. *Nat. Plants* **2021**, *7*, 87–98. [[CrossRef](#)]
31. Rott, M.; Martins, N.F.; Thiele, W.; Lein, W.; Bock, R.; Kramer, D.M.; Schöttler, M.A. ATP Synthase Repression in Tobacco Restricts Photosynthetic Electron Transport, CO₂ Assimilation, and Plant Growth by Overacidification of the Thylakoid Lumen. *Plant Cell* **2011**, *23*, 304–321. [[CrossRef](#)] [[PubMed](#)]

32. Livingston, A.K.; Cruz, J.A.; Kohzuma, K.; Dhingra, A.; Kramer, D.M. An Arabidopsis mutant with high cyclic electron flow around photosystem I (hcef) involving the NADPH dehydrogenase complex. *Plant Cell* **2010**, *22*, 221–233. [[CrossRef](#)] [[PubMed](#)]
33. Kanazawa, A.; Kramer, D.M. In vivo modulation of nonphotochemical exciton quenching (NPQ) by regulation of the chloroplast ATP synthase. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 12789–12794. [[CrossRef](#)] [[PubMed](#)]
34. Shimakawa, G.; Miyake, C. Photosynthetic Linear Electron Flow Drives CO₂ Assimilation in Maize Leaves. *Int. J. Mol. Sci.* **2021**, *22*, 4894. [[CrossRef](#)] [[PubMed](#)]
35. Zivcak, M.; Kalaji, H.M.; Shao, H.B.; Olsovska, K.; Brestic, M. Photosynthetic proton and electron transport in wheat leaves under prolonged moderate drought stress. *J. Photochem. Photobiol. B Biol.* **2014**, *137*, 107–115. [[CrossRef](#)]
36. Golding, A.J.; Johnson, G.N. Down-regulation of linear and activation of cyclic electron transport during drought. *Planta* **2003**, *218*, 107–114. [[CrossRef](#)]
37. Huang, W.; Yang, S.J.; Zhang, S.B.; Zhang, J.L.; Cao, K.F. Cyclic electron flow plays an important role in photoprotection for the resurrection plant *Paraboea rufescens* under drought stress. *Planta* **2012**, *235*, 819–828. [[CrossRef](#)]
38. Huang, W.; Zhang, S.-B.; Xu, J.-C.; Liu, T. Plasticity in roles of cyclic electron flow around photosystem I at contrasting temperatures in the chilling-sensitive plant *Calotropis gigantea*. *Environ. Exp. Bot.* **2017**, *141*, 145–153. [[CrossRef](#)]
39. Huang, W.; Hu, H.; Zhang, S.B. Photosynthetic regulation under fluctuating light at chilling temperature in evergreen and deciduous tree species. *J. Photochem. Photobiol. B Biol.* **2021**, *219*, 112203. [[CrossRef](#)]
40. Huang, W.; Cai, Y.-F.; Wang, J.-H.; Zhang, S.-B. Chloroplastic ATP synthase plays an important role in the regulation of proton motive force in fluctuating light. *J. Plant Physiol.* **2018**, *226*, 40–47. [[CrossRef](#)]
41. Yang, Y.-J.; Ding, X.-X.; Huang, W. Stimulation of cyclic electron flow around photosystem I upon a sudden transition from low to high light in two angiosperms *Arabidopsis thaliana* and *Bletilla striata*. *Plant Sci.* **2019**, *287*, 110166. [[CrossRef](#)] [[PubMed](#)]
42. Tan, S.-L.; Huang, J.-L.; Zhang, F.-P.; Zhang, S.-B.; Huang, W. Photosystem I photoinhibition induced by fluctuating light depends on background low light irradiance. *Environ. Exp. Bot.* **2021**, *181*, 104298. [[CrossRef](#)]
43. Yamamoto, H.; Takahashi, S.; Badger, M.R.; Shikanai, T. Artificial remodelling of alternative electron flow by flavodiiron proteins in *Arabidopsis*. *Nat. Plants* **2016**, *2*, 16012. [[CrossRef](#)]
44. Yang, Y.-J.; Zhang, S.-B.; Huang, W. Photosynthetic regulation under fluctuating light in young and mature leaves of the CAM plant *Bryophyllum pinnatum*. *Biochim. Biophys. Acta Bioenerg.* **2019**, *1860*, 469–477. [[CrossRef](#)]
45. Yamori, W.; Hikosaka, K.; Way, D.A. Temperature response of photosynthesis in C₃, C₄, and CAM plants: Temperature acclimation and temperature adaptation. *Photosynth. Res.* **2014**, *119*, 101–117. [[CrossRef](#)]
46. Lee, S.D.; Kim, S.J.; Jung, S.I.; Son, K.-C.; Kays, S.J. Diurnal CO₂ Assimilation Patterns in Nine Species of CAM-Type Succulent Plants. *HortScience* **2006**, *41*, 1373–1376. [[CrossRef](#)]
47. Keeley, J.E.; Sandquist, D.R. Diurnal photosynthesis cycle in CAM and non-CAM seasonal-pool aquatic macrophytes. *Ecology* **1991**, *72*, 716–727. [[CrossRef](#)]
48. Wang, H.; Wang, X.-Q.; Zeng, Z.-L.; Yu, H.; Huang, W. Photosynthesis under fluctuating light in the CAM plant *Vanilla planifolia*. *Plant Sci.* **2022**, *317*, 111207. [[CrossRef](#)]
49. Schreiber, U.; Klughammer, C. Non-photochemical fluorescence quenching and quantum yields in PS I and PS II: Analysis of heat-induced limitations using Maxi-Imaging- PAM and Dual-PAM-100. *PAM Appl. Notes* **2008**, *1*, 15–18. [[CrossRef](#)]
50. Hendrickson, L.; Furbank, R.T.; Chow, W.S. A simple alternative approach to assessing the fate of absorbed light energy using chlorophyll fluorescence. *Photosynth. Res.* **2004**, *82*, 73–81. [[CrossRef](#)]
51. Oxborough, K.; Baker, N.R. Resolving chlorophyll a fluorescence of photosynthetic efficiency into photochemical components—Calculation of qP and Fv'/Fm' without measuring Fo'. *Photosynth. Res.* **1997**, *54*, 135–142. [[CrossRef](#)]
52. Miyake, C.; Miyata, M.; Shinzaki, Y.; Tomizawa, K.I. CO₂ response of cyclic electron flow around PSI (CEF-PSI) in tobacco leaves—Relative electron fluxes through PSI and PSII determine the magnitude of non-photochemical quenching (NPQ) of Chl fluorescence. *Plant Cell Physiol.* **2005**, *46*, 629–637. [[CrossRef](#)] [[PubMed](#)]
53. Wang, C.; Yamamoto, H.; Shikanai, T. Role of cyclic electron transport around photosystem I in regulating proton motive force. *Biochim. Biophys. Acta* **2015**, *1847*, 931–938. [[CrossRef](#)] [[PubMed](#)]
54. Klughammer, C.; Siebke, K.; Schreiber, U. Continuous ECS-indicated recording of the proton-motive charge flux in leaves. *Photosynth. Res.* **2013**, *117*, 471–487. [[CrossRef](#)]
55. Sacksteder, C.A.; Jacoby, M.E.; Kramer, D.M. A portable, non-focusing optics spectrophotometer (NoFOSpec) for measurements of steady-state absorbance changes in intact plants. *Photosynth. Res.* **2001**, *70*, 231–240. [[CrossRef](#)]
56. Takizawa, K.; Kanazawa, A.; Kramer, D.M. Depletion of stromal Pi induces high “energy-dependent” antenna exciton quenching (qE) by decreasing proton conductivity at CFO-CF1 ATP synthase. *Plant Cell Environ.* **2008**, *31*, 235–243. [[CrossRef](#)]
57. Okegawa, Y.; Basso, L.; Shikanai, T.; Motohashi, K. Cyclic electron transport around PSI contributes to photosynthetic induction with thioredoxin f. *Plant Physiol.* **2020**, *184*, 1291–1302. [[CrossRef](#)]
58. Okegawa, Y.; Tsuyama, M.; Kobayashi, Y.; Shikanai, T. The pgr1 mutation in the Rieske subunit of the cytochrome b6f complex does not affect PGR5-dependent cyclic electron transport around photosystem I. *J. Biol. Chem.* **2005**, *280*, 28332–28336. [[CrossRef](#)]
59. Allen, J.F. Photosynthesis of ATP-electrons, proton pumps, rotors, and poise. *Cell* **2002**, *110*, 273–276. [[CrossRef](#)]
60. Takahashi, S.; Badger, M.R. Photoprotection in plants: A new light on photosystem II damage. *Trends Plant Sci.* **2011**, *16*, 53–60. [[CrossRef](#)]
61. Sonoike, K. Photoinhibition of photosystem I. *Physiol. Plant.* **2011**, *142*, 56–64. [[CrossRef](#)]

62. Alboresi, A.; Storti, M.; Morosinotto, T. Balancing protection and efficiency in the regulation of photosynthetic electron transport across plant evolution. *New Phytol.* **2019**, *221*, 105–109. [[CrossRef](#)] [[PubMed](#)]
63. Sejima, T.; Takagi, D.; Fukayama, H.; Makino, A.; Miyake, C. Repetitive short-pulse light mainly inactivates photosystem I in sunflower leaves. *Plant Cell Physiol.* **2014**, *55*, 1184–1193. [[CrossRef](#)] [[PubMed](#)]
64. Zivcak, M.; Brestic, M.; Kunderlikova, K.; Sytar, O.; Allakhverdiev, S.I. Repetitive light pulse-induced photoinhibition of photosystem I severely affects CO₂ assimilation and photoprotection in wheat leaves. *Photosynth. Res.* **2015**, *126*, 449–463. [[CrossRef](#)] [[PubMed](#)]
65. Shimakawa, G.; Miyake, C. What quantity of photosystem I is optimum for safe photosynthesis? *Plant Physiol.* **2019**, *179*, 1479–1485. [[CrossRef](#)] [[PubMed](#)]
66. Suorsa, M.; Rossi, F.; Tadini, L.; Labs, M.; Colombo, M.; Jahns, P.; Kater, M.M.; Leister, D.; Finazzi, G.; Aro, E.-M.; et al. PGR5-PGRL1-dependent cyclic electron transport modulates linear electron transport rate in *Arabidopsis thaliana*. *Mol. Plant* **2016**, *9*, 271–288. [[CrossRef](#)] [[PubMed](#)]
67. Huang, W.; Zhang, S.-B.; Liu, T. Moderate photoinhibition of photosystem II significantly affects linear electron flow in the shade-demanding plant *Panax notoginseng*. *Front. Plant Sci.* **2018**, *9*, 637. [[CrossRef](#)]
68. Yang, Y.-J.; Tan, S.-L.; Sun, H.; Huang, J.-L.; Huang, W.; Zhang, S.-B. Photosystem I is tolerant to fluctuating light under moderate heat stress in two orchids *Dendrobium officinale* and *Bletilla striata*. *Plant Sci.* **2021**, *303*, 110795. [[CrossRef](#)]
69. Yamamoto, H.; Shikanai, T. PGR5-dependent cyclic electron flow protects photosystem I under fluctuating light at donor and acceptor sides. *Plant Physiol.* **2019**, *179*, 588–600. [[CrossRef](#)]
70. Takagi, D.; Takumi, S.; Hashiguchi, M.; Sejima, T.; Miyake, C. Superoxide and singlet oxygen produced within the thylakoid membranes both cause photosystem I photoinhibition. *Plant Physiol.* **2016**, *171*, 1626–1634. [[CrossRef](#)]
71. Nishikawa, Y.; Yamamoto, H.; Okegawa, Y.; Wada, S.; Sato, N.; Taira, Y.; Sugimoto, K.; Makino, A.; Shikanai, T. PGR5-dependent cyclic electron transport around PSI contributes to the redox homeostasis in chloroplasts rather than CO₂ fixation and biomass production in rice. *Plant Cell Physiol.* **2012**, *53*, 2117–2126. [[CrossRef](#)] [[PubMed](#)]
72. Wada, S.; Yamamoto, H.; Suzuki, Y.; Yamori, W.; Shikanai, T.; Makino, A. Flavodiiron protein substitutes for cyclic electron flow without competing CO₂ assimilation in rice. *Plant Physiol.* **2018**, *176*, 1509–1518. [[CrossRef](#)] [[PubMed](#)]
73. Wilson, S.; Ruban, A.V. Enhanced NPQ affects long-term acclimation in the spring ephemeral *Berteroa incana*. *Biochim. Biophys. Acta Bioenerg.* **2020**, *1861*, 148014. [[CrossRef](#)] [[PubMed](#)]
74. Nishiyama, Y.; Yamamoto, H.; Allakhverdiev, S.I.; Inaba, M.; Yokota, A.; Murata, N. Oxidative stress inhibits the repair of photodamage to the photosynthetic machinery. *EMBO J.* **2001**, *20*, 5587–5594. [[CrossRef](#)]
75. Murata, N.; Takahashi, S.; Nishiyama, Y.; Allakhverdiev, S.I. Photoinhibition of photosystem II under environmental stress. *Biochim. Biophys. Acta Bioenerg.* **2007**, *1767*, 414–421. [[CrossRef](#)]
76. Murata, N.; Allakhverdiev, S.I.; Nishiyama, Y. The mechanism of photoinhibition in vivo: Re-evaluation of the roles of catalase, α -tocopherol, non-photochemical quenching, and electron transport. *Biochim. Biophys. Acta Bioenerg.* **2012**, *1817*, 1127–1133. [[CrossRef](#)]
77. Allakhverdiev, S.I.; Murata, N. Environmental stress inhibits the synthesis de novo of proteins involved in the photodamage–repair cycle of Photosystem II in *Synechocystis* sp. PCC 6803. *Biochim. Biophys. Acta Bioenerg.* **2004**, *1657*, 23–32. [[CrossRef](#)]
78. Huang, W.; Yang, Y.-J.; Hu, H.; Zhang, S.-B.; Cao, K.-F. Evidence for the role of cyclic electron flow in photoprotection for oxygen-evolving complex. *J. Plant Physiol.* **2016**, *194*, 54–60. [[CrossRef](#)]
79. Miyake, C. Alternative electron flows (water-water cycle and cyclic electron flow around PSI) in photosynthesis: Molecular mechanisms and physiological functions. *Plant Cell Physiol.* **2010**, *51*, 1951–1963. [[CrossRef](#)]
80. Zivcak, M.; Brestic, M.; Balatova, Z.; Drevenakova, P.; Olsovska, K.; Kalaji, H.M.; Yang, X.; Allakhverdiev, S.I. Photosynthetic electron transport and specific photoprotective responses in wheat leaves under drought stress. *Photosynth. Res.* **2013**, *117*, 529–546. [[CrossRef](#)]
81. Livingston, A.K.; Kanazawa, A.; Cruz, J.A.; Kramer, D.M. Regulation of cyclic electron flow in C₃ plants: Differential effects of limiting photosynthesis at ribulose-1,5-bisphosphate carboxylase/oxygenase and glyceraldehyde-3-phosphate dehydrogenase. *Plant Cell Environ.* **2010**, *33*, 1779–1788. [[CrossRef](#)] [[PubMed](#)]
82. Nakano, H.; Yamamoto, H.; Shikanai, T. Contribution of NDH-dependent cyclic electron transport around photosystem I to the generation of proton motive force in the weak mutant allele of *pgr5*. *Biochim. Biophys. Acta Bioenerg.* **2019**, *1860*, 369–374. [[CrossRef](#)] [[PubMed](#)]
83. Munekage, Y.N.; Taniguchi, Y.Y. Promotion of Cyclic Electron Transport Around Photosystem I with the Development of C4 Photosynthesis. *Plant Cell Physiol.* **2016**, *57*, 897–903. [[CrossRef](#)] [[PubMed](#)]
84. Ivanov, B.; Asada, K.; Kramer, D.M.; Edwards, G. Characterization of photosynthetic electron transport in bundle sheath cells of maize. I. Ascorbate effectively stimulates cyclic electron flow around PSI. *Planta* **2005**, *220*, 572–581. [[CrossRef](#)] [[PubMed](#)]
85. Kohzuma, K.; Cruz, J.A.; Akashi, K.; Hoshiyasu, S.; Munekage, Y.N.; Yokota, A.; Kramer, D.M. The long-term responses of the photosynthetic proton circuit to drought. *Plant Cell Environ.* **2009**, *32*, 209–219. [[CrossRef](#)] [[PubMed](#)]