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Balancing light use efficiency and photoprotection in tobacco leaves grown at different light regimes

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ABSTRACT

In this study, we have compared photosynthetic regulation in tobacco leaves (*Nicotiana tabacum* L.) grown under high light (HL) and low light (LL) by measuring chlorophyll fluorescence, P700 redox state and electrochromic shift signals. Under high light, HL-plants had much higher linear electron flow and cyclic electron flow (CEF) around photosystem I (PSI) than LL-plants. Meanwhile, HL-plants showed significantly lower proton gradient (ΔpH) across the thylakoid membranes, owing to the increased activity of chloroplast ATP synthase. The relationships between ΔpH, non-photochemical quenching (NPQ) and PSI donor side limitation [Y(ND)] differed between HL- and LL-plants. At a given ΔpH, HL-plants displayed higher NPQ and Y(ND). Furthermore, at a given CEF, LL-plants showed higher ΔpH and Y(ND). These results indicate that HL-plants down-regulate ΔpH to increase light use efficiency by enhancing the activity of chloroplast ATP synthase. In contrast, LL-plants decrease the activity of chloroplast ATP synthase to up-regulate ΔpH and to favor photoprotection. Our findings suggest that the coordination of CEF and chloroplast ATP synthase balances light use efficiency and photoprotection at different growth light conditions.

1. Introduction

Plants use light to drive photosynthetic electron flow and to fix CO_2. In linear electron flow (LEF), electrons from water splitting in photosystem II (PSII) are transferred to $NADP^+$, producing NADPH. This electron transfer is coupled to the formation of proton motive force across the thylakoid membrane, which is composed of the proton gradient (ΔpH) and electrochemical gradient (ΔΨ). During cyclic electron flow (CEF), electrons from ferredoxin are cycled around photosystem I (PSI) into the plastoquinone pool, which is coupled the proton translocation from stroma to thylakoid lumen, generating ΔpH without producing NADPH (Shikanai and Yamamoto, 2017). Both ΔpH and ΔΨ drive the generation of ATP via chloroplast ATP synthase (Kramer et al., 2003). In addition, ΔpH can slow down the plastoquinol oxidation at the cytochrome (Cyt) b_6/f and thus controls electron flow from PSII to PSI (photosynthetic control) (Munekage et al., 2002; Tikkanen and Aro, 2014; Yamamoto and Shikanai, 2019). If ΔpH was too high, the electron transfer from PSII to PSI would rapidly become limited and the rates of ATP and NADPH generation would decrease, impairing CO_2 fixation and plant growth (Livingston et al., 2010; Rott et al., 2011). Alternatively, if ΔpH was too low, PSI would be over-reduced due to excess electron flow from PSII and non-photochemical quenching (NPQ) would be suppressed (Munekage et al., 2002, 2004), initiating production of reactive oxygen species (ROS) and causing photoinhibition (Takahashi et al., 2009; Suorsa et al., 2012). Although photodamaged PSII can be rapidly repaired, moderate PSII photoinhibition can significantly decrease the light use efficiency (Tikkanen et al., 2014). Furthermore, PSI photoinhibition significantly affects CO_2 assimilation and photoprotection (Sejima et al., 2014; Brestic et al., 2015, 2016; Zivcak et al., 2015). Therefore, ΔpH should be finely regulated to balance the tradeoff between CO_2 fixation and photoprotection (Armbruster et al., 2017; Alboresi et al., 2019).

In higher plants, the extent of ΔpH depends on: (1) H^+ influx activity in dependence of LEF and CEF; and (2) H^+ efflux activity through chloroplast ATP synthase (Tikkanen and Aro, 2014; Takagi et al., 2017). Generally, under low light, a low rate of photosynthetic electron flow is accompanied with a high activity of chloroplast ATP synthase, leading to a low level of ΔpH (Takagi et al., 2017; Huang et al., 2018a). Such low ΔpH in turn favors the electron transfer from PSII to PSI and increases light use efficiency. Under high light, a high rate of

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photosynthetic electron flow is accompanied with a low activity of chloroplast ATP synthase, resulting in the increase in ΔpH (Takagi et al., 2017). Consequently, electron flow from PSII to PSI is controlled at the Cyt b_6/f complex, avoiding over-reduction of PSI and preventing PSI photoinhibition (Takagi et al., 2017; Yamamoto and Shikanai, 2019). Meanwhile, the enhancement of ΔpH induces NPQ and diminishes the production of ROS in PSII, alleviating PSII photoinhibition (Takahashi et al., 2009). Many previous studies have documented that in angiosperms the ΔpH formation under high light is largely dependent on CEF (Munekage et al., 2002; Avenson et al., 2005; Wang et al., 2015). Once CEF is impaired, plants show the loss of ΔpH formation under high light, leading to severe photoinhibition of PSI and PSII (Takahashi et al., 2009; Tikkanen et al., 2014; Yamori et al., 2016). Furthermore, upon a sudden transition from low to high light, plants could not generate a sufficient ΔpH (Huang et al., 2019a; Yang et al., 2019b, 2019c), and CEF was highly stimulated to help the rapid ΔpH formation (Huang et al., 2019b; Yang et al., 2019a). Consequently, CEF is crucial for photosynthetic acclimation under fluctuating light (Suorsa et al., 2012; Yamori et al., 2016). In contrast, an *Arabidopsis* mutant with high CEF (*hcef*) showed reduction in LEF under high light due to over-acidification of thylakoid lumen (Livingston et al., 2010). Therefore, the activity of CEF should be finely regulated to optimize the ΔpH formation. In addition, chloroplast ATP synthase plays an important role in regulation of ΔpH formation (Kanazawa et al., 2017; Takagi et al., 2017). Repression of chloroplast ATP synthase induced over-acidification of thylakoid lumen and subsequently restricted CO_2 assimilation and plant growth (Rott et al., 2011). By comparison, over-enhancement of H^+ efflux activity led to the decrease in ΔpH and thus caused PSI photoinhibition under high light (Kanazawa et al., 2017; Takagi et al., 2017). Once the CO_2 assimilation was restricted, the activity of chloroplast ATP synthase was suppressed to enhance the ΔpH formation (Kanazawa and Kramer, 2002; Huang et al., 2018b, c). Therefore, coordination of CEF and chloroplast ATP synthase is essential for sustaining optimal photosynthesis.

The CEF activity depends on light intensity during plant growth. Relative to plants grown at low light intensities (LL-plants), plants grown at high light intensities (HL-plants) showed higher CEF activation when exposed to high light (Miyake et al., 2005; Huang et al., 2015). Concomitantly, HL-plants have higher levels of NPQ than LL-plants. Because the induction of NPQ in PSII is initiated by ΔpH (Takizawa et al., 2007), it was thought that HL-plants plants enhance the CEF activity to induce NPQ via increasing the ΔpH formation (Miyake et al., 2005). However, this scheme should be re-examined because ΔpH can affect the electron transfer from PSII to PSI. If HL-plants had a higher ΔpH than LL-plants, CO_2 fixation would be restricted due to limiting LEF. If fact, under high light HL-plants have a higher rate of CO_2 fixation than LL-plants (Miyake et al., 2005; Huang et al., 2015), leading to the hypothesis that HL-plants should down-regulate the ΔpH formation under high light. Owing to the fact that HL-plants have higher rates of LEF and CEF under high light, such low ΔpH can be accomplished only by increasing the activity of chloroplast ATP synthase. Therefore, we hypothesize that HL-plants enhance the activity of chloroplast ATP synthase to down-regulate ΔpH formation and to increase light use efficiency under high light.

In LEF, the ATP/NADPH production ratio is thought to be 1.29 (Sacksteder et al., 2000; Hahn et al., 2018). By comparison, the ATP/NADPH ratios required by CO_2 fixation and photorespiration are 1.5 and 1.75, respectively (Walker et al., 2016). Under ambient CO_2, oxygen, and temperature, the ATP/NADPH ratio required by primary metabolism is approximately 1.6 (Walker et al., 2014). Therefore, LEF alone cannot satisfy the energy budget for primary metabolism. Fortunately, the operation of CEF could provide additional ATP to increase the ATP/NADPH production ratio (Yamori et al., 2011, 2015; Walker et al., 2014; Murata and Nishiyama, 2018). However, the contribution of CEF to ATP synthesis at different light intensities remains controversial. For example, according to the phenotypes of *pgr5* and *ccr6*, CEF significantly helps ATP synthesis at low light but not at high light (Yamori et al., 2011, 2015; Nishikawa et al., 2012). In contrast, biochemical models of photosynthesis suggested that CEF responded to energy demand under high light but not at low light (Walker et al., 2014). Furthermore, the relative contribution of CEF on ATP synthesis was altered by growth light intensity (Huang et al., 2015), leaf developmental stages (Huang et al., 2017a) and environmental stresses (Huang et al., 2017b). In HL-plants, the higher rates of CO_2 fixation and photorespiration under high light require a large amount of additional ATP (Huang et al., 2015; Walker et al., 2016). Therefore, we speculate that in HL-plants the major role of the higher CEF is to provide additional ATP rather than to enhance ΔpH.

Under high light, plants should prevent PSI photoinhibition via maintaining high levels of P700 oxidation ratio (Munekage et al., 2002; Suorsa et al., 2016). The P700 oxidation ratio in angiosperms is mainly regulated by ΔpH (Yamamoto et al., 2016; Armbruster et al., 2017; Takagi et al., 2017). Based on the assumption that HL-plants show lower ΔpH than LL-plants under high light, a question arises whether growth light intensity changes the relationship between ΔpH and P700 oxidation ratio. Specifically, a low level of ΔpH in HL-plants leads to a higher rate of electron transfer from PSII to PSI. These final products of LEF should be immediately consumed by primary metabolism, or else PSI would be over-reduced and PSI would be damaged in HL-plants. As a result, owing to the higher rates of CO_2 fixation and photorespiration, a high P700 oxidation ratio can be maintained at a lower ΔpH in HL-plants. By comparison, because of the lower capacity of primary metabolism, LL-plants should maintain a high P700 oxidation ratio by enhancing the ΔpH-dependent photosynthetic control at the Cyt b_6/f complex. Therefore, we speculate that the relationship between ΔpH and P700 oxidation ratio can be altered by the capacity of primary metabolism.

Previous studies have documented that HL-plants increase the amount of nitrogen allocated to Calvin cycle enzymes, electron carriers and chloroplast ATP synthase (Evans, 1988; Hikosaka and Terashima, 1995). By comparison, LL-plants increase the amount of nitrogen allocated to chlorophyll-protein complexes. Furthermore, shade leaves have relatively lower connectivity among PSII and limitations in electron transport between PSII and PSI (Zivcak et al., 2014). However, the regulation of proton motive force and its relationships to photoprotection in HL- and LL-plants have not yet been clarified. In the present study, we hypothesize that coordination of chloroplast ATP synthase and CEF balances light use efficiency and photoprotection in plants. To test this hypothesis, we compared photosynthetic regulation in tobacco leaves (*Nicotiana tabacum* L.) grown under high light (HL) and low light (LL) by measuring chlorophyll fluorescence, P700 redox state and electrochromic shift signals.

2. Materials and methods

2.1. Plant materials and growth conditions

Tobacco (*Nicotiana tabacum* L. *cv*. Sumsan) plants were cultivated in a greenhouse with the relative air humidity of 50–70 %. The daily/night temperatures were about 30/20 °C. After seed germination for 1 month, seedlings were exposed to different light conditions. Growth light conditions were controlled by using non-woven shade net. Five pots were exposed to 40 % of full sunlight during their entire growth period (HL-plants, the maximum light intensity at midday is approximately 800 μmol photons m^{-2} s^{-1}), whereas four pots were exposed to 8% of full sunlight (LL-plants, the maximum light intensity at midday is approximately 160 μmol photons m^{-2} s^{-1}). Plants grown in plastic pots were irrigated with a 2/1000 dilution of a mineral nutrient solution (N:P:K = 20:20:20). During the experimental period, plants were cultivated without water or nutrient stress. After acclimation for two month, mature, but not senescent leaves from 13-week-old plants were used for photosynthetic measurements.

2.2. Chlorophyll fluorescence and P700 measurements

A Dual PAM-100 (Heinz Walz, Effeltrich, Germany) was used to simultaneously measure PSI and PSII parameters at 25 °C. After dark adaptation for 15 min, the maximum fluorescence and the maximum change in P700 was measured using a saturating pulse (20,000 μmol photons m^{-2} s^{-1}, 300 ms). Subsequently, leaves were illuminated at 923 μmol photons m^{-2} s^{-1} for 15 min to activate photosynthetic electron sinks. Afterward, light intensity dependence of PSI and PSII parameters were measured after exposure for 3 min to each light intensity (1809, 1455, 1178, 611, 330 or 132 μmol photons m^{-2} s^{-1}). PSI and PSII parameters were calculated as following: Y(I) as (Pm′ − P)/Pm, Y(ND) as P/Pm, Y(NA) as (Pm − Pm′)/Pm, Y(II) as (Fm' − Fs)/Fm', NPQ as (Fm − Fm')/Fm', Y(NO) as Fs/Fm. Y(I), quantum yield of PSI photochemistry; Y(ND), quantum yield of non-photochemical quenching due to PSI donor side imitation; Y(NA), quantum yield of non-photochemical quenching due to PSI acceptor side imitation; Y(II), effective quantum yield of PSII photochemistry; NPQ, non-photochemical quenching in PSII; Y(NO), quantum yield of non-regulated energy dissipation in PSII. Relative photosynthetic electron transport rate (ETR) was calculated as ETRI (or ETRII) = PPFD × Y(I) (or Y(II)). The rate of CEF around PSI was estimated by subtracting ETRII from ETRI.

2.3. Electrochromic shift measurements

Using a Dual PAM-100 equipped with a P515/535 emitter-detector module, the ECS signal was monitored (Klughammer et al., 2013). After dark adaptation for 30 min, the 515-nm absorbance change induced by a single turnover flash (ECS_{ST}) was measured, followed by photosynthetic induction at 923 μmol photons m^{-2} s^{-1} for 15 min. Subsequently, ECS signal was measured after exposure for 3 min to each light intensity (1809, 1455, 1178, 611, 330 or 132 μmol photons m^{-2} s^{-1}), and 30 s ECS dark interval relaxation kinetics ($DIRK_{ECS}$) were recorded to calculate the proton gradient (ΔpH) and membrane potential (ΔΨ) at different actinic light intensities (Cruz et al., 2005). All ΔpH and ΔΨ levels were normalized against the magnitude of ECS_{ST} (Livingston et al., 2010). The proton conductivity of the thylakoid membrane ($g_H{}^+$) was also calculated using DIRK analysis (Avenson et al., 2005).

2.4. Statistical analysis

Data were displayed as mean ± SD of at least four independent measurements. One-Way ANOVA test was used at $\alpha = 0.05$ significance level to determine whether significant differences existed between different treatments.

3. Results

3.1. Light intensity dependence of PSI and PSII parameters

We first examined the light-adapted PSI and PSII parameters under different light intensities. As expectedly, with the increase in light intensity, the quantum yield of PSI photochemistry [Y(I)] gradually decreased (Fig. 1A). Meanwhile, the quantum yield of non-photochemical quenching due to donor side limitation [Y(ND)] gradually increased (Fig. 1B), resulting in the low levels of acceptor side limitation in PSI [Y(NA)] (Fig. 1C). As a result, the over-reduction of PSI electron carriers was prevented, especially under high light. HL-plants showed significantly higher Y(I) under all light intensities (Fig. 1A). When exposed to light intensities below 600 μmol photons m^{-2} s^{-1}, LL-plants showed significantly higher Y(ND) than HL-plants (Fig. 1B), suggesting the stronger PSI donor side limitation in the LL-plants.

Similar to Y(I), HL-plants showed significantly higher Y(II) (effective quantum yield of PSII photochemistry) than LL-plants (Fig. 1D). The difference in NPQ induction between HL-plants and LL-plants was dependent on light intensity (Fig. 1E). At light intensities below 300 μmol photons m^{-2} s^{-1}, LL-plants displayed significantly higher NPQ than HL-plants. However, at light intensities above 600 μmol photons m^{-2} s^{-1}, HL-plants had significantly higher NPQ than LL-plants. Therefore, relative to LL-plants, HL-plants exhibited a higher NPQ capacity. Owing to the higher capacities of Y(II) and NPQ, HL-plants showed low levels of Q_A reduction state [Y(NO)], especially under high light (Fig. 1F). The higher Y(NO) in LL-plants led to the sensitivity of PSII photoinhibition under high light.

We further calculated the relative electron transport rate (ETR) under different light intensities. In general, HL-plants showed significantly higher capacities of ETRI, ETRII and CEF than LL-plants. For LL-plants, ETRI and ETRII were almost saturated at a low light of 130 μmol photons m^{-2} s^{-1} (Fig. 2A and B). By comparison, in HL-plants, ETRI and ETRII were saturated at approximately 600 and 300 μmol photons m^{-2} s^{-1}, respectively (Fig. 2A and B). At light intensities below 300 μmol photons m^{-2} s^{-1}, the CEF activity did not differ between HL-plants and LL-plants (Fig. 2C). However, when exposed to higher light intensities, HL-plants displayed much higher CEF activity than LL-plants (Fig. 2C).

3.2. Regulation of proton motive force

We next examined the formation of proton motive force (*pmf*) across the thylakoid membranes under different light intensities. At light intensities below 300 μmol photons m^{-2} s^{-1}, LL-plants showed significantly higher *pmf* than HL-plants (Fig. 3A). However, when exposed to higher illumination, HL-plants and LL-plants showed similar *pmf* (Fig. 3A). The *pmf* is composed of proton gradient (ΔpH) and membrane potential (ΔΨ). As shown in Fig. 3B, LL-plants displayed significantly higher ΔpH in the light response curve. Moreover, under a saturating light of 1809 μmol photons m^{-2} s^{-1}, the proton conductivity of chloroplast ATP synthase ($g_H{}^+$) was significantly higher in HL-plants than in LL-plants (Fig. 3C). Therefore, when exposed to high light, the relative lower ΔpH in HL-plants was caused by the enhanced H^+ efflux activity in chloroplast ATP synthase.

3.3. Contribution of CEF in regulation of photosynthesis

It has been indicated that the formation of ΔpH and P700 oxidation under high light are largely dependent on the activation of CEF. Here, we pooled the data of CEF, ΔpH, and Y(ND) obtained from light response curves (Fig. 4). The results indicated that the relationships between CEF, ΔpH, and Y(ND) differed between HL-plants and LL-plants. At a given CEF, HL-plants showed lower ΔpH and Y(ND) (Fig. 4), suggesting that in HL-plants CEF mainly contributed to ATP synthesis rather than ΔpH formation, probably owing to the enhanced $g_H{}^+$. In contrast, owing to the lower activity of chloroplast ATP synthase, CEF mainly contributed to the formation of ΔpH in LL-plants, decreasing electron flow from PSII to PSI and thus favoring photoprotection for PSI.

3.4. Relationships between lumen acidification and photoprotection

In order to understand the regulatory systems of NPQ and Y(ND), we pooled the data of ΔpH, NPQ and Y(ND) obtained from light response curves (Fig. 5). Under high light, the enhancement of NPQ and Y(ND) are both dependent on the formation of ΔpH, confirming the central role of ΔpH in protecting PSI and PSII against photoinhibition. However, the relationships between ΔpH, NPQ and Y(ND) differed significantly between HL-plants and LL-plants. At a given ΔpH, HL-plants showed higher NPQ and Y(ND) than LL-plants, suggesting that in HL-plants NPQ and Y(ND) can be affected by other regulatory mechanisms in addition to ΔpH. Therefore, growth light changes the relationships between ΔpH and photoprotection.

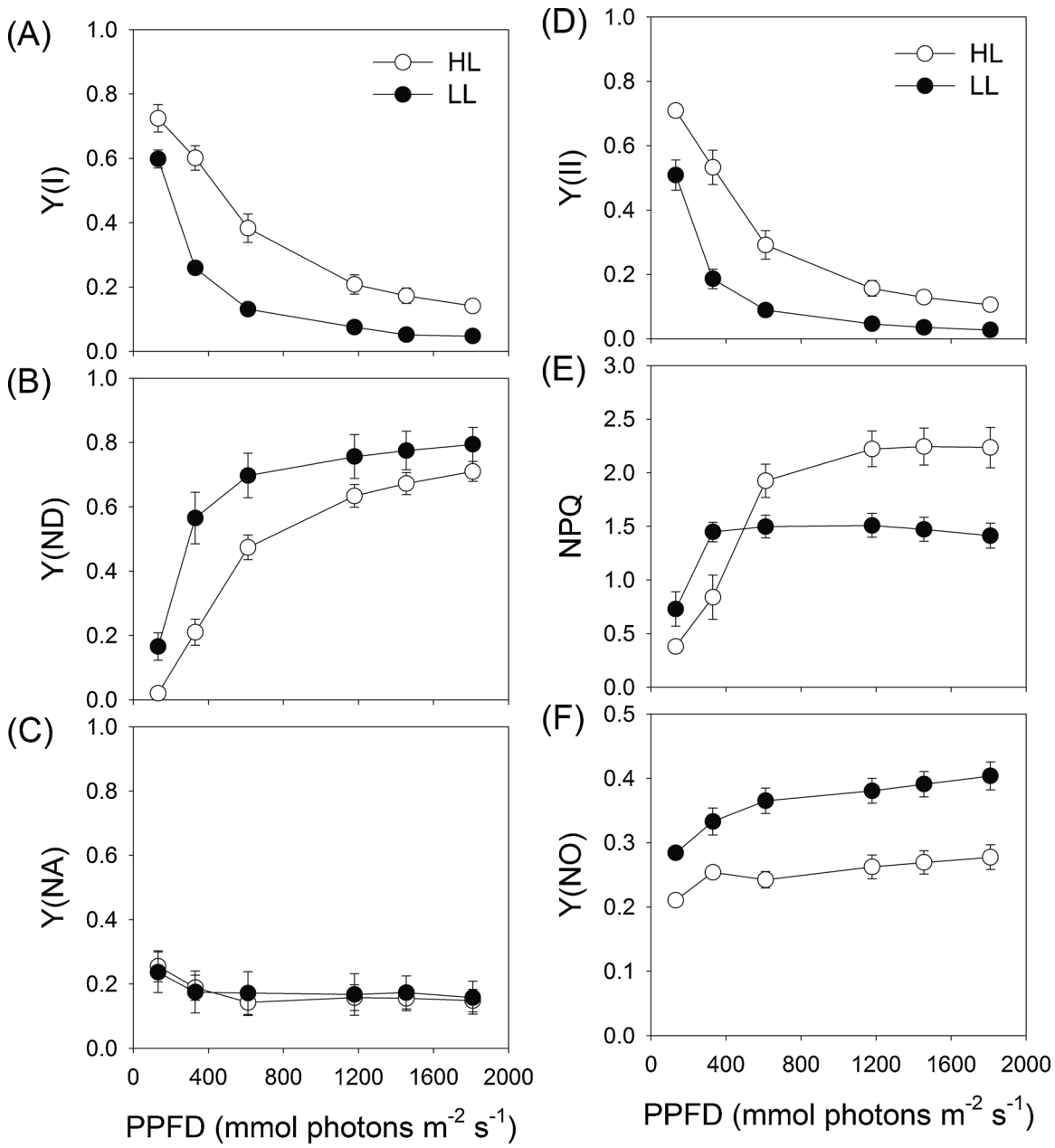

Fig. 1. Light intensity dependence of PSI and PSII parameters for HL- and LL-plants measured at 25 °C. Y(I), the quantum yield of PSI photochemistry; Y(ND), the quantum yield of non-photochemical energy dissipation due to PSI donor-side limitation; Y(NA), the quantum yield of non-photochemical energy due to PSI acceptor-side limitation. Y(II), the effective quantum yield of PSII photochemistry; NPQ, Non-photochemical quenching in PSII; Y(NO), the quantum yield of non-regulated energy dissipation in PSII. Values are means ± SD (n = 4-5).

4. Discussion

During photosynthesis, photosynthetic electron flow is coupled with the accumulation of protons in the lumen from the water-splitting activity of PSII and from the electron transfer via Cyt b_6/f, generating ΔpH across the thylakoid membranes that drives the ATP synthesis via chloroplast ATP synthase. In addition, ΔpH acts as a central signal in regulating electron transfer from PSII to PSI. Over-acidification of thylakoid lumen can down-regulate plastoquinol oxidation at the Cyt b_6/f complex, restricting LEF and impairing CO_2 fixation (Livingston et al., 2010; Rott et al., 2011). In contrast, the loss of ΔpH formation induces PSI photoinhibition under high light due to excess electron flow to PSI (Munekage et al., 2002; Suorsa et al., 2012; Takagi et al., 2017). The formation of ΔpH is determined by: (1) the rate of proton influx activity in dependence of photosynthetic electron flow; and (2) the rate of proton efflux from the lumen in chloroplast ATP synthase (Tikkanen and Aro, 2014; Takagi et al., 2017). In order to optimize the tradeoff between light use efficiency and photoprotection, we hypothesis that CEF and chloroplast ATP synthesis should be finely regulated to adjust the ΔpH formation in HL-plants and LL-plants.

When compared with LL-plants, HL-plants showed significantly higher LEF and CEF (Fig. 2), indicating the higher H^+ influx activity. Meanwhile, HL-plants showed significantly lower ΔpH and higher activity of chloroplast ATP synthase (Fig. 3). Therefore, because of the enhanced H^+ efflux activity, the higher CEF activity in HL-plants did not result in a higher ΔpH. At a low ΔpH, the photosynthetic control at the Cyt b_6/f complex is weakened, favoring the electron flow from PSII to PSI. Because HL-plants had higher capacities of CO_2 fixation and photorespiration, the increased electrons transported to PSI can be immediately consumed by primary metabolism, preventing the over-

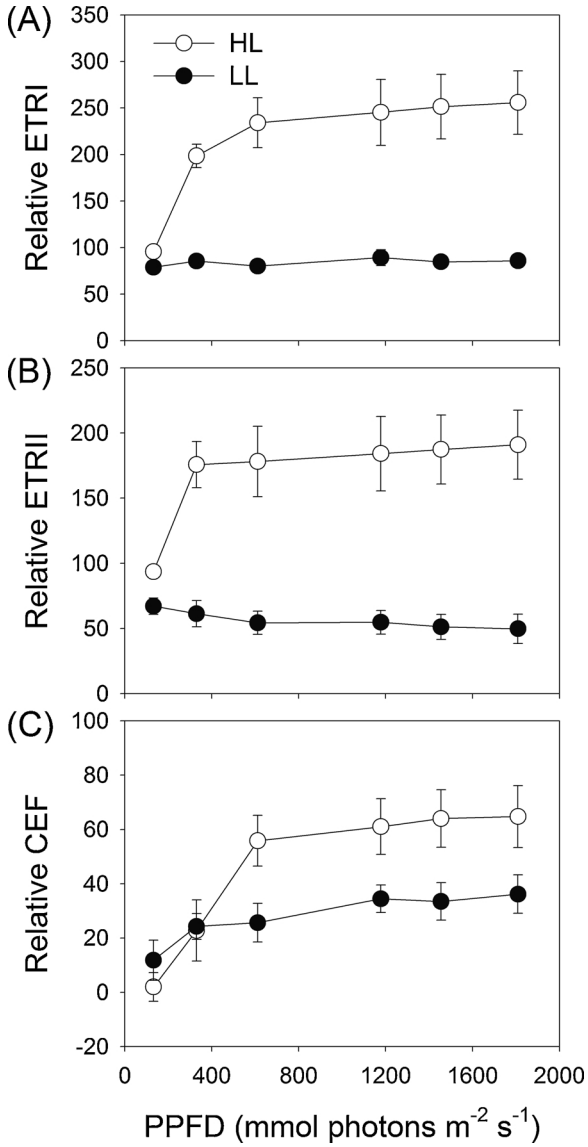

Fig. 2. Light intensity dependence of the relative electron transport rate for HL- and LL-plants measured at 25 ˚C. ETRI, electron transport rate through PSI; ETRII, electron transport rate through PSII; CEF, cyclic electron flow around PSI. Values are means ± SD (n = 4-5).

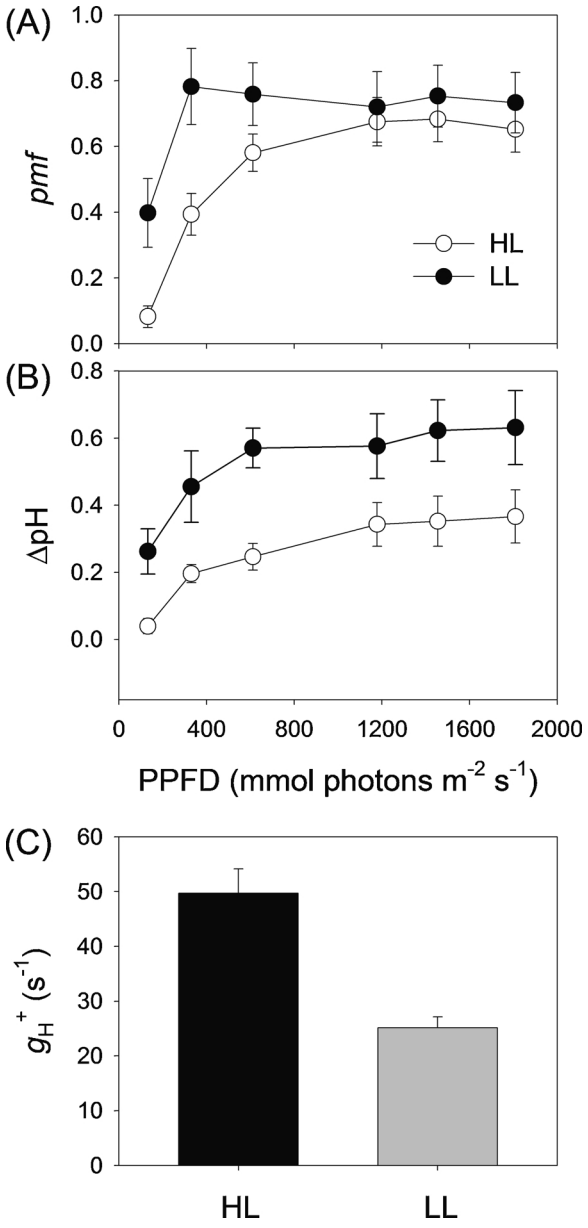

Fig. 3. (**A** and **B**) Light intensity dependence of proton motive force (*pmf*) and proton gradient (ΔpH) for HL- and LL-plants measured at 25 ˚C. (**C**) The proton conductivity of the thylakoid membrane (g_H^+) measured at 25 ˚C and 1809 μmol photons m^{-2} s^{-1}. Values are means ± SD (n = 4-5).

reduction of PSI under high light (Fig. 1C). By comparison, LL-plants had relative lower rates of CO_2 fixation and photorespiration. In order to prevent excess electron flow from PSII to PSI, LL-plants should enhance ΔpH-dependent photosynthetic control at the Cyt b_6/f complex. Indeed, we observed that LL-pants up-regulated the ΔpH formation under high light (Fig. 3B). However, such high ΔpH could not be explained by the H^+ influx activity because LL-plants had significantly lower capacities for LEF and CEF (Fig. 2). In contrast, the activity in chloroplast ATP synthase was highly down-regulated in LL-plants (Fig. 3C). Therefore, the higher ΔpH in LL-plants was caused by the lower H^+ efflux activity in chloroplast ATP synthase. Based on these findings, we propose that regulation of chloroplast ATP synthase plays an important role in adjusting ΔpH formation under different growth light conditions.

The stoichiometry of ATP/NADPH produced by LEF is thought to be 1.29 (Sacksteder et al., 2000; Hahn et al., 2018). By comparison, the ATP/NADPH ratios required by CO_2 fixation and photorespiration are 1.5 and 1.75, respectively (Walker et al., 2014, 2016). As a result, LEF alone cannot supply sufficient ATP to drive both CO_2 fixation and photorespiration (Miyake, 2010; Huang et al., 2016). Such difference between ATP/NADPH supply from LEF and demand from primary metabolism should be balanced by other alternative electron flows (Miyake, 2010; Walker et al., 2014; Yamori and Shikanai, 2016). Relative to LL-plants, HL-plants needed more additional ATP to sustain the higher rates of CO_2 fixation and photorespiration (Huang et al., 2015). Concomitantly, a higher CEF activation in HL-plants was accompanied with a lower ΔpH (Figs. 2C and 3 B). Therefore, in HL-plants the major role of CEF activity was to provide additional ATP synthesis rather than to enhance the ΔpH formation. By comparison, LL-plants had relative lower rates of CO_2 fixation and photorespiration. As a result, a relative lower CEF activity could satisfy the ATP/NADPH production ratio required by primary metabolism. Furthermore, at a given CEF, LL-plants displayed a higher ΔpH than HL-plants (Fig. 4A). There results suggest that in LL-plants CEF mainly contributes to the ΔpH formation rather than favors ATP synthesis. Therefore, the major role of CEF significantly differs between HL-plants and LL-plants.

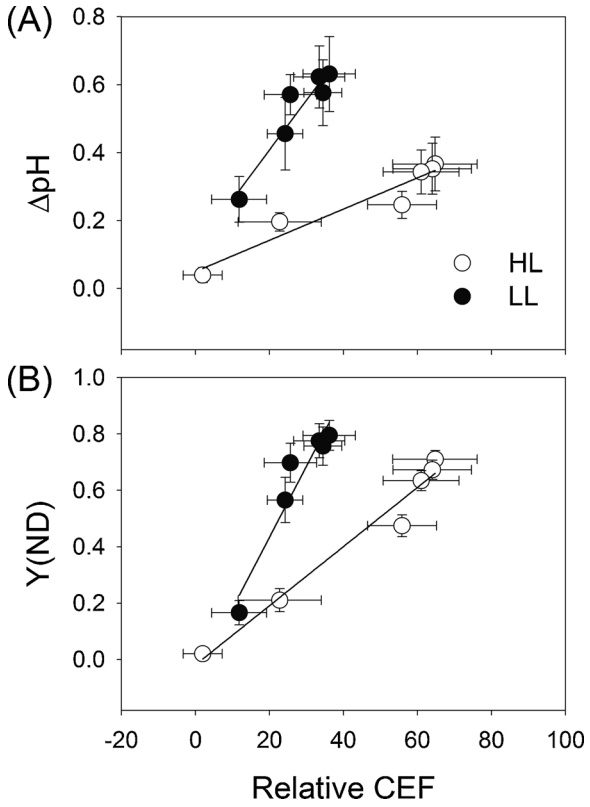

Fig. 4. Changes in ΔpH and Y(ND) as a function of relative CEF for HL- and LL-plants measured at 25 °C. Values are means ± SD (n = 4-5).

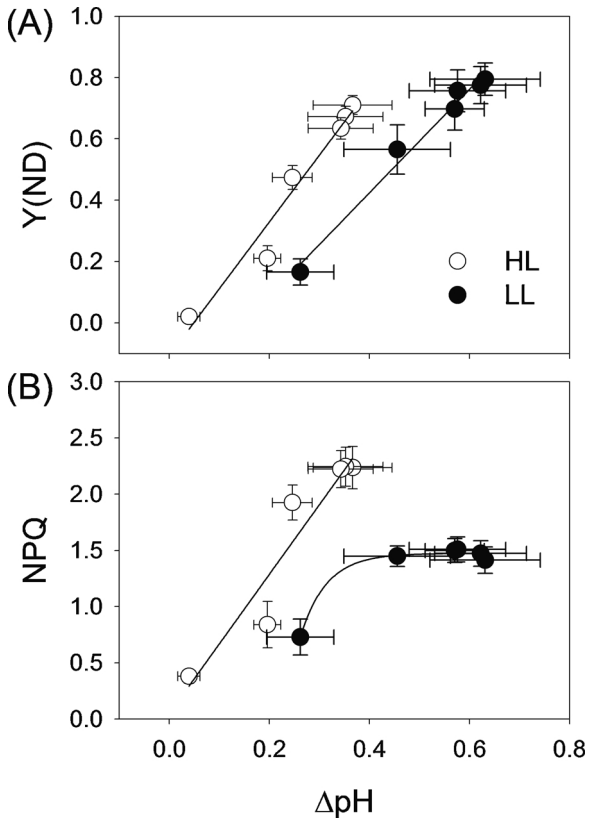

Fig. 5. Changes in Y(ND) and NPQ as a function of ΔpH for HL- and LL-plants measured at 25 °C. Values are means ± SD (n = 4-5).

Under high light, high levels of P700 oxidation are essential for safeguarding photosynthesis, especially for PSI (Shimakawa et al., 2016; Suorsa et al., 2016; Shimakawa and Miyake, 2018). As we known, ΔpH plays a seminal role in regulating PSI redox state by slowing down the electron transport through the Cyt b_6/f complex (Tikkanen and Aro, 2014; Armbruster et al., 2017; Shikanai and Yamamoto, 2017). In *pgr5* plants, the impairment of CEF and increased activity of chloroplast ATP synthase both impair the ΔpH formation under high light (Avenson et al., 2005; Wang et al., 2015). Such low ΔpH in *pgr5* plants leads to excess electron flow from PSII to PSI, resulting in the over-reduction of PSI electron carriers (Munekage et al., 2002; Suorsa et al., 2012; Kono et al., 2014). The extra reducing power in PSI triggers the production of reactive oxygen species that cause PSI photoinhibition (Munekage et al., 2002; Tikkanen et al., 2014; Yamamoto and Shikanai, 2019). This scheme is also supported by the *A. thaliana hope2* mutant with increased activity of chloroplast ATP synthase and low ΔpH (Takagi et al., 2017). Interestingly, we found that the relationship between ΔpH and Y(ND) differed between HL-plants and LL-plants. At a given ΔpH, HL-plants displayed a higher Y(ND) than LL-plants (Fig. 5A). In HL-plants, a low ΔpH results in an increase in electron flow from PSII to PSI. Concomitantly, the higher rates of CO_2 fixation and photorespiration are capable of immediately consume all the ATP and reducing power produced (Fig. 2), avoiding over-reduction of PSI (Fig. 1C). By comparison, LL-plants had relative lower capacity of primary metabolism. As a result, in LL-plants the over-reduction of PSI was prevented at the step of ΔpH-dependent photosynthetic control at the Cyt b_6/f complex (Fig. 1B). Taken together, the relationship between ΔpH and Y(ND) can be altered by the capacity of primary metabolism.

Many previous studies indicated that under high light HL-plants showed higher NPQ (Demmig-Adams and Adams, 1992; Verhoeven et al., 1997) and CEF activity (Miyake et al., 2005; Huang et al., 2015) than LL-plants. As we known, CEF helps the ΔpH formation across the thylakoid membrane, which further induces NPQ (Munekage et al., 2002, 2004; Takahashi et al., 2009). Thus, the higher CEF activity in HL-plants likely contributed to the induction of NPQ by enhancing the ΔpH formation (Miyake et al., 2005). However, this scheme is challenged by our present study. Here, we found that under high light, a higher NPQ in HL-plants was accompanied with a lower ΔpH (Figs. 1E and 3 B). Furthermore, the growth light significantly altered the relationship between ΔpH and NPQ (Fig. 5B). At a given ΔpH, HL-plants displayed a higher NPQ than the LL-plants. The induction of NPQ is dependent on not only the ΔpH formation but also the NPQ-related components such as light-harvesting complex II, PsbS, zeaxanthin and lutein (Niyogi et al., 1998; Li et al., 2002, 2009; Wilson and Ruban, 2019; Nicol et al., 2019). With the increase in light intensity, the limiting factor of NPQ induction gradually shifts from ΔpH formation to the contents of NPQ-related components (Takizawa et al., 2007). Under high light, the NPQ induction is mainly limited by the contents of NPQ-related components rather than the ΔpH formation (Takagi et al., 2017). Furthermore, leaves grown under high light usually up-regulate the zeaxanthin content to dissipate the excess energy through NPQ (Verhoeven et al., 2001; Schumann et al., 2017; Wilson and Ruban, 2019). Therefore, the higher NPQ in HL-plants is not attributed to the ΔpH formation but is probably caused by higher pools of NPQ-related components.

5. Conclusions

In the present study, we found that HL-plants down-regulated ΔpH to increase light use efficiency by enhancing the activity of chloroplast ATP synthase. In contrast, LL-plants decreased the activity of chloroplast ATP synthase to up-regulate ΔpH and to favor photoprotection. Owing to the different activities of chloroplast ATP synthase, the physiological functions of CEF differed between HL-plants and LL-plants. In HL-plants, CEF mainly contributed to maintain energy balance via

additional ATP synthesis. In LL-plants, CEF mainly favored ΔpH formation and to suppress PSI photoinhibition. These findings suggest that the coordination of CEF and chloroplast ATP synthase finely adjusts the ΔpH formation to optimize the tradeoff between light use efficiency and photoprotection.

Author contributions

WH and SBZ conceived and designed research. SLT conducted experiments. SLT, TL, SBZ and WH analyzed data. WH wrote the first draft of the manuscript, which was intensively edited by all authors.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

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