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Contribution of NDH-dependent cyclic electron transport around photosystem I to the generation of proton motive force in the weak mutant allele of *pgr5*

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ABSTRACT

In angiosperms, cyclic electron transport (CET) around photosystem I (PSI) consists of two pathways, depending on PGR5/PGRL1 proteins and the chloroplast NDH complex. In single mutants defective in chloroplast NDH, photosynthetic electron transport is only slightly affected at low light intensity, but in double mutants impaired in both CET pathways photosynthesis and plant growth are severely affected. The question is whether this strong mutant phenotype observed in double mutants can be simply explained by the additive effect of defects in both CET pathways. In this study, we used the weak mutant allele of *pgr5-2* for the background of double mutants to avoid possible problems caused by the secondary effects due to the strong mutant phenotype. In two double mutants, *crr2-2 pgr5-2* and *ndhs-1 pgr5-2*, the plant growth was unaffected and linear electron transport was only slightly affected. However, NPQ induction was more severely impaired in the double mutants than in the *pgr5-2* single mutant. A similar trend was observed in the size of the proton motive force. Despite the slight reduction in photosystem II parameters, PSI parameters were severely affected in the *pgr5-2* single mutant, the phenotype that was further enhanced by adding the NDH defects. Despite the lack of ΔpH-dependent regulation at the cytochrome b_6f complex (donor-side regulation of PSI), the plastoquinone pool was more reduced in the double mutants than in the *pgr5-2* single mutants. This phenotype suggests that both PGR5/PGRL1- and NDH-dependent CET contribute to supply sufficient acceptors from PSI by balancing the ATP/NADPH production ratio.

1. Introduction

Light reactions of photosynthesis produce ATP and NADPH, which are used for CO_2 fixation. Linear electron transport (LET) from water to $NADP^+$ generates both ATP and NADPH but their production ratio does not satisfy the requirement by the Calvin-Benson cycle and photorespiration based on the structure of ATP synthase in chloroplasts [1]. Additional ATP is considered to be provided by cyclic electron transport (CET) around photosystem I (PSI) [2]. In angiosperms, two pathways of CET operate. The main pathway depends on PROTON GRADIENT REGULATION 5 (PGR5) and PGR5-Like Photosynthetic Phenotype (PGRL1) proteins [3,4]. Based on its sensitivity to antimycin A [5,6] and the ability to produce ATP in ruptured chloroplasts [7], PGR5/PGRL1-dependent CET likely corresponds to the cyclic phosphorylation discovered by Arnon and coworkers [8]. Another CET pathway is mediated by chloroplast NADH dehydrogenase-like (NDH) complex [9]. Despite the structural similarity of chloroplast NDH to respiratory complex I, chloroplast NDH accepts electrons from ferredoxin (Fd) rather than NADH or NADPH [10].

The Arabidopsis *pgr5* mutant is defective in the antimycin A-sensitive CET and cannot induce ΔpH-dependent non-photochemical quenching (NPQ) of chlorophyll fluorescence under high light [3]. PGR5-dependent luminal acidification is also necessary to down-regulate electron transport through the cytochrome (Cyt) b_6f complex [11]. This donor-side regulation protects PSI from photodamage under fluctuating light intensity [12]. PGR5-dependent CET contributes to the generation of proton motive force (*pmf*) across the thylakoid membrane [13] and consequently ATP synthesis [7]. By balancing the ATP and NADPH production ratio, PGR5/PGRL1-dependent CET provides sufficient electron acceptors from PSI, $NADP^+$ and oxidized Fd [3]. This is

Abbreviations: AL, actinic light; CET, cyclic electron transport; ΔpH, proton concentration gradient; Δψ, membrane potential; Cyt, cytochrome; ECS, electrochromic shift; Fd, ferredoxin; LET, linear electron transport; NDH, NADH dehydrogenase-like (complex); NPQ, non-photochemical quenching; PGR5, PROTON GRADIENT REGULATION 5; PGRL1, PGR5-like Photosynthetic Phenotype 1; *pmf*, proton motive force; PQ, plastoquinone; SP, saturating pulse; WT, wild-type

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acceptor-side regulation. The function of PGR5 is required for both donor-side and acceptor-side regulations [14]. The introduction of exogenous flavodiiron protein complemented the sensitivity of the *pgr5* mutant to the WT level by forming a large electron sink from PSI [15].

The weak allele of *pgr5-2* was discovered as a reduced NPQ mutant in CO_2-free air containing 5% O_2 at 50 μmol photons $m^{-2} s^{-1}$, as well as the *dpgr* (*disturbed proton gradient regulation*) mutants, in which the regulation of H^+/K^+ antiporter KEA3 localized to the thylakoid membrane was disturbed [16]. In this artificial air, both CO_2 fixation and photorespiration were inhibited and mutants defective in the regulatory processes were picked after the screening. To distinguish two mutant alleles of *pgr5*, we call the strong allele *pgr5-1*, which has been designated *pgr5* so far. The *pgr5-1* mutant has a glycine-to-alanine alteration in the C-terminal region of protein, by which the mutant protein was largely destabilized [3]. The *pgr5-2* mutant has a serine-to-phenylalanine alteration in the middle of mature protein and accumulates higher levels of PGR5 and PGRL1 proteins than those of the *pgr5-1* mutant [14].

In contrast to the severe disturbance of photosynthetic regulation in the *pgr5-1* mutants, mutants defective in the chloroplast NDH complex do not show a strong mutant phenotype in tobacco [17,18] and Arabidopsis [19]. Some studies have focused on the involvement of the chloroplast NDH complex in stress resistance [20–24], but no evidence has been obtained to clarify how the complex alleviates the stresses. Recently, mild but clear mutant phenotypes have been observed in NDH mutants at low light intensity and under fluctuating light intensity in rice [25,26]. A similar phenotype at low light intensity was also reported in the NDH-less mutant in *Marchantia polymorpha* [27]. Because Fd-dependent PQ reduction is likely coupled with proton pumping across the thylakoid membrane [28,29], the chloroplast NDH may not be able to function efficiently in the presence of large *pmf*. The idea is consistent with a significant contribution of chloroplast NDH in the *pgr5* mutant background, in which the *pmf* is small.

The chloroplast NDH complex is essential for normal growth in the *pgr5-1* mutant background [30]. The simplest idea is that the chloroplast NDH functions according to the same mechanism with PGR5/PGRL1-dependent CET, i.e., CET around PSI providing *pmf*. Because of the strong mutant phenotype of the double mutants defective in both pathways of CET, however, an alternative idea may explain the function of the chloroplast NDH complex. Both PGR5 and the NDH complex regulate the redox state of the plastoquinone (PQ) pool in early chloroplast development and their defects alleviated the variegated leaf phenotype of the Arabidopsis *immutans* (*im*) mutant defective in plastid terminal oxidase (PTOX), which oxidizes the PQ pool in early chloroplast development [31]. The problems that occurred in early chloroplast development may secondarily affect photosynthetic electron transport in mature leaves. Because the NPQ induction was severely impaired in the *pgr5-1* single mutant, it was difficult to monitor the further contribution of the chloroplast NDH complex in double mutants [30]. In this study, we used the *pgr5-2* mutant background to analyze the function of the chloroplast NDH complex in double mutants. Plant growth was not affected and LET was only slightly affected in double mutants but we observed the clear contribution of chloroplast NDH to *pmf* formation especially at low light intensity.

2. Materials and methods

2.1. Plant materials and growth conditions

Arabidopsis thaliana WT (ecotype Columbia-0) and mutant plants were grown in soil for 6 to 8 weeks under growth chamber conditions (50 μmol photons $m^{-2} s^{-1}$, 8-h light/16-h dark cycles at 23 °C). For Fig. 1, plants were cultured under 16-h light/8-h dark cycles.

2.2. Measurements of chlorophyll fluorescence and 700 absorbance changes

Chlorophyll fluorescence and P700 absorption changes were simultaneously measured using a DUAL-pulse-amplitude modulation portable chlorophyll fluorometer (DUAL-PAM-100 MODULAR Version), as described previously [14]. Plants were dark-adapted for 20 min and then their detached leaves were used for measurement. The minimum fluorescence in the dark-adapted state (F_o) was recorded in the presence of measuring light (620 nm) at 0.05 to 0.1 μmol photons $m^{-2} s^{-1}$. A saturating pulse (SP) of light (300 ms, 20,000 μmol photons $m^{-2} s^{-1}$) was applied to determine the maximum fluorescence in the dark-adapted state (F_m) and during actinic light (AL) illumination (F_m'). To monitor the light-intensity dependence of fluorescence parameters, we used AL (635 nm) of 11, 25, 50, 100, 190, 320, 520, 650, 830 and 1050 μmol photons $m^{-2} s^{-1}$. The steady-state fluorescence level (F_s) was recorded at each AL intensity. The maximum quantum yields of PSII and NPQ were calculated as F_v/F_m and $(F_m - F_m')/F_m'$, respectively. The quantum yield of PSII, Y(II) was calculated as $(F_m - F_s)/F_m'$. Relative ETR(II) was calculated as Y(II) × 0.5 × 0.84 × light intensity (μmol photons $m^{-2} s^{-1}$). The proportion of an open PSII center that reflects the oxidized level of the PQ pool (qL) was calculated as $(F_m' - F)/(F_m' - F_o') \times F_o'/F_s$ [32]. F_o' was calculated as $F_o/(F_v/F_m + F_o/F_m')$ [33].

The redox changes of P700 were analyzed by monitoring the absorbance changes of transmission light at 830 and 875 nm. The maximum level of $P700^+$ (oxidized P700) in the dark (Pm) was determined by application of a SP in the background of far-red light (720 nm). The maximum revel of $P700^+$ during AL illumination (Pm′) was also determined by applying a SP. The steady-state $P700^+$ level (P) was recorded just before applying a SP. The quantum yield of PSI, Y(I) was calculated as (Pm′ − P)/Pm. The acceptor-side limitation of PSI, Y(NA) was calculated as (Pm − Pm′)/Pm. The donor-side regulation of PSI, Y(ND) was calculated as P/Pm. Three complementary quantum yields are defined: Y(I) + Y(NA) + Y(ND) = 1 [34]. Statistical analysis was preformed using Tukey-Kramer test.

2.3. ECS analysis

The electrochromic shift (ECS) signal was monitored as the absorbance changes at 515 nm by using a DUAL-PAM-100 (Walz, Effeltrich, Germany) equipped with a P515/535 emitter-detector module, as described previously [13]. Plants were dark adapted for 20 min and then the detached leaves were analyzed. ECS_t, which represents the difference in total *pmf* between light and dark, was estimated from the total amplitude of the rapid decay of the ECS signal during a dark pulse. All ECS_t levels were normalized against the 515-nm absorbance change induced by a single turnover flash (ECS_{ST}), as measured on dark-adapted leaves before recording. To monitor the light-intensity dependence of *pmf*, we used AL of 54, 160, 360, 760, and 1220 μmol photons $m^{-2} s^{-1}$. Statistical analysis was preformed using Tukey-Kramer test.

3. Results

3.1. NDH complex is not required for normal plant growth in the pgr5-2 mutant background

To cross with the *pgr5-2* mutant, we selected two Arabidopsis mutants specifically defective in the activity of chloroplast NDH. The *crr2-2* (*chlororespiratory reduction 2-2*) mutant is defective in a pentatricopeptide repeat (PPR) protein necessary for the expression of *ndhB* gene encoding an NDH subunit in chloroplasts [19]. The *crr2-2 pgr5-1* double mutant showed severe defects in plant growth and photosynthesis [30]. We selected the *crr2-2* mutant as a mutant relatively severely defective in NDH activity. The *ndhs/crr31* mutant is defective in the nuclear gene encoding an NDH subunit, NdhS required for high-

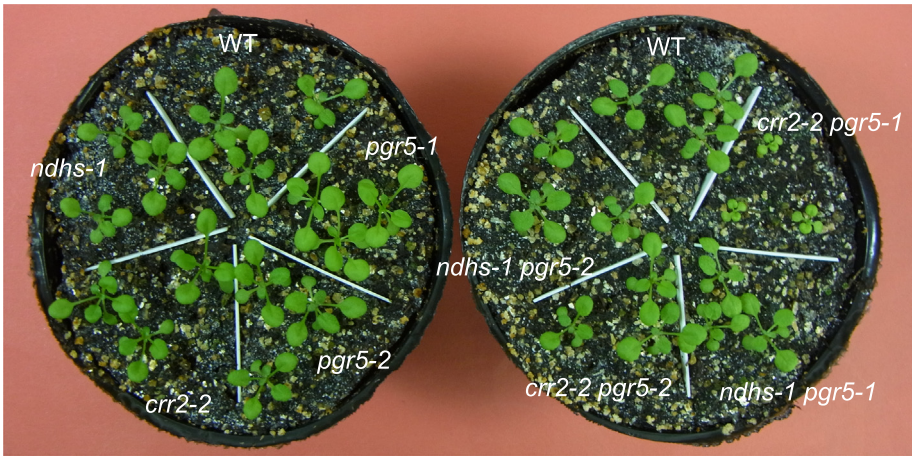

Fig. 1. Plant growth of WT and mutants defective in CET pathways. Seedlings were cultured under the long-day conditions for 16 days.

affinity binding of the NDH complex to Fd [10,35,36]. NdhS is not necessary for stabilizing the core of the NDH complex and is not absolutely necessary for NDH activity [10]. The *ndhs-1 pgr5-1* double mutant plants could grow like wild-type (WT) plants under constant low light, although the photosynthesis was severely disturbed. Mainly on the basis of the different growth rate under the *pgr5-1* mutant background, we selected the *ndhs-1* mutant as a mutant relatively weakly defective in NDH activity compared to the *crr2-2* mutant.

Fig. 1 shows the plant growth of the WT and *pgr5-1*, *pgr5-2*, *crr2-2*, *ndhs-1*, *crr2-2 pgr5-1*, *crr2-2 pgr5-2*, *ndhs-1 pgr5-1* and *ndhs-1 pgr5-2* mutants. A severe growth defect was observed in *crr2-2 pgr5-1*. Consistent with a previous report [10], no growth defect was observed in the *ndhs-1 pgr5-1* mutant. In the *pgr5-2* mutant background, the *crr2-2* defect also did not result in growth reduction.

3.2. Chloroplast NDH is required for inducing NPQ in the pgr5-2 background

The maximum quantum yield of PSII (F_v/F_m) representing the intactness of photosystem II (PSII) was not dramatically affected in any genotype (Table 1). On the contrary, F_v/F_m was reduced to < 0.5 in the *crr2-2 pgr5-1* double mutant [30]. The rate of electron transport at PSII, ETR(II) was not affected in any genotype (Fig. 2A). NPQ induction was slightly affected at moderate light intensities (190–520 μmol photons $m^{-2} s^{-1}$) in *pgr5-2* but not in *crr2-2* or *ndhs-1* mutants (Fig. 2B). NPQ induction was more severely impaired in the double mutants, *crr2-2 pgr5-2* and *ndhs-1 pgr5-2*. Because LET monitored by ETR(II) was not affected in the double mutants (The difference was not statistically significant), the reduced NPQ suggests the contribution of NDH-dependent PSI CET to ΔpH generation in the double mutants.

The qL parameter represents the oxidation state of the PQ pool. The PQ pool was more reduced in both double mutants at light intensities of 190–520 μmol photons $m^{-2} s^{-1}$ (Fig. 2C, $P < 0.05$). However, the different response of qL was observed at low light intensities < 50 μmol photons $m^{-2} s^{-1}$ (Fig. 2C, inset). In the *crr2-2* mutant, the PQ

Table 1
The maximum quantum yield of PSII.

Genotype	F_v/F_m	SD
WT	0.81	0.01
pgr5-2	0.78	0.02
crr2-2	0.79	0.01
ndhs-1	0.79	0.03
crr2-2 pgr5-2	0.77	0.03
ndhs-1 pgr5-2	0.79	0.02

$n = 5$, SD, standard deviation.

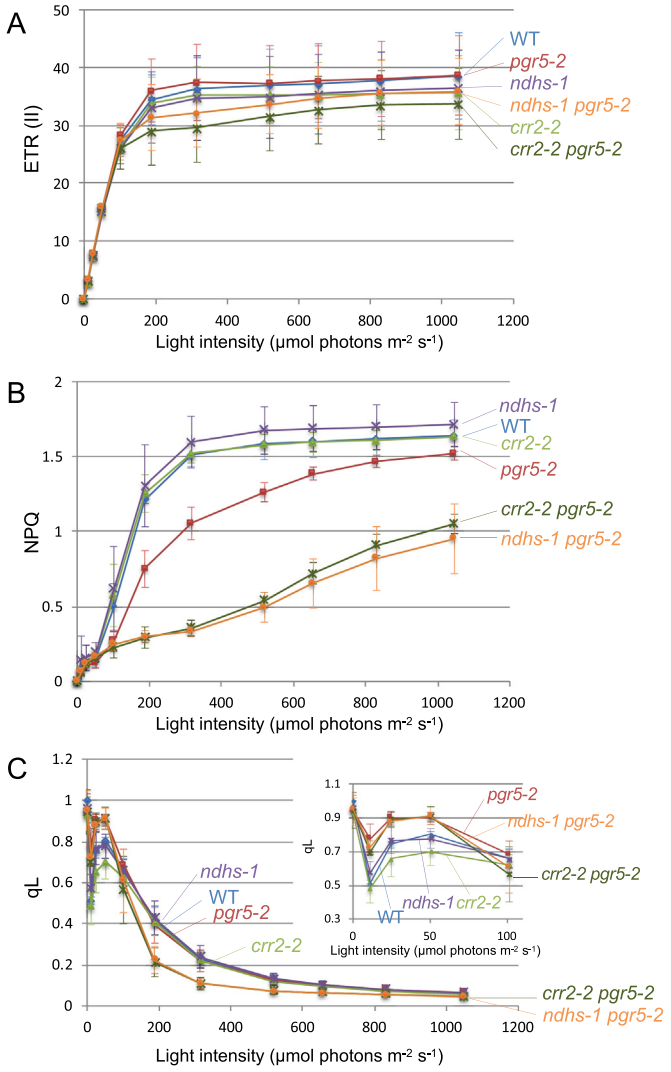

Fig. 2. Light-intensity dependence of chlorophyll fluorescence parameters. (A) ETR(II) represents the relative rate of electron transport at PSII. (B) NPQ is mainly caused by ΔpH-dependent qE. (C) qL represents oxidation of the PQ pool. The inset in C is a close-up of low light intensities (< 100 μmol photons $m^{-2} s^{-1}$). Data represent means ± SD ($n = 5$).

pool was slightly more reduced at 50 μmol photons $m^{-2} s^{-1}$, although the phenotype was not observed in the *ndhs-1* mutant. This is consistent with the observation in rice [25] and *Marchantia polymorpha* [27], although the exact mechanism for this phenotype is unclear. The NDH complex is likely important in low light also in Arabidopsis. In the *pgr5-2* mutant background, *pgr5-2*, *crr2-2 pgr5-2* and *ndhs-1 pgr5-2*, the PQ pool was more oxidized at low light intensities < 50 μmol photons $m^{-2} s^{-1}$ (Fig. 2C, inset).

3.3. The induction of donor-side regulation was severely impaired in the double mutants

Analysis of absorbance change at the reaction center chlorophyll pair (P700) of PSI provides three parameters. Y(I) represents reduced P700, which can transfer electrons to Fd. In contrast, Y(NA) represents the fraction of reduced P700, which cannot transfer electrons to Fd. Y (NA) is induced by both acceptor-limitation and the accumulation of excessive electrons at the donor side of PSI. The induction of Y(NA) leads to PSI photodamage via generating reactive oxygen species in PSI. In contrast, Y(ND) represents fractions of P700 oxidized in the light and is mainly induced by the operation of photosynthetic control at the Cyt b_6f complex. In contrast to Y(NA), oxidized P700 is stable under high light.

Although ETR(II), which is calculated as Y(II) x light intensity (μmol photons $m^{-2} s^{-1}$) were the same between the WT and the *pgr5-2* mutant, Y(I) was significantly lower in *pgr5-2* than WT at light intensities > 190 μmol photons $m^{-2} s^{-1}$ (Fig. 3A, $P < 0.05$), probably reflecting the absence of PGR5/PGRL1-dependent CET. The ratio of Y (I)/Y(II) was 1.84 and 0.83 at 1050 μmol photons $m^{-2} s^{-1}$ in the WT and *pgr5-2*, respectively. In the *crr2-2 pgr5-2* and *ndhs-1 pgr5-2* double mutants, the levels of Y(I) were slightly lower than those in the *pgr5-2* mutant, although the difference was not statistically significant (Fig. 3A).

Consistent with our recent report [14], the induction of the donor-side regulation monitored by the Y(ND) parameter was severely reduced in the *pgr5-2* mutant (Fig. 3B). At light intensities of 50–100 μmol photons $m^{-2} s^{-1}$, the Y(ND) levels were even lower in the *crr2-2 pgr5-2* and *ndhs-1 pgr5-2* double mutants than in the *pgr5-2* single mutant, although the difference was not statistically significant. In contrast, the high levels of Y(ND) were induced in the WT and the *crr2-2* and *ndhs-1* mutants at high light intensities. Although we observed slightly lower levels of Y(ND) in the *ndhs-1* mutant, which was reflected by the slight increase in the Y(NA) level in the *ndhs-1* mutant, the differences were not statistically significant (Fig. 3B and C). Reflecting the lower levels of Y(ND) in the *pgr5* mutant background, the Y(NA) levels were very high at high light intensities (Fig. 3C). The *crr2-2 pgr5-2* and *ndhs-1 pgr5-2* double mutant exhibited higher levels of Y(NA) than the *pgr5-2* single mutant, although the difference was not statistically significant. Consistent with the reduced NPQ induction, both *crr2-2* and *ndhs-1* mutations enhanced the defect in ΔpH-dependent donor-side regulation in the *pgr5-2* mutant background.

3.4. Chloroplast NDH significantly contributes to the pmf formation in low light in the pgr5-2 background

We directly monitored the size of *pmf* formed in the light (Fig. 4). ECS depends on the membrane potential formed across the thylakoid membrane [37]. ECS_t represents the light-dark difference of the size of *pmf* and is standardized by absorbance change depending on charge separation by a single turnover flash (ESC_{ST}). In the *pgr5-2* mutant, the size of *pmf* was slightly lower than the WT at light intensities > 360 μmol photons $m^{-2} s^{-1}$, although the difference was statistical significant only at 360 μmol photons $m^{-2} s^{-1}$ (Fig. 4). In the *pgr5-1* mutant, the size of *pmf* was reduced to half the WT level even at low light intensity of 130 μmol photons $m^{-2} s^{-1}$ [13]. In contrast, the size of *pmf* was not affected at any light intensities in *crr2-2* or *ndhs-1* mutants. In

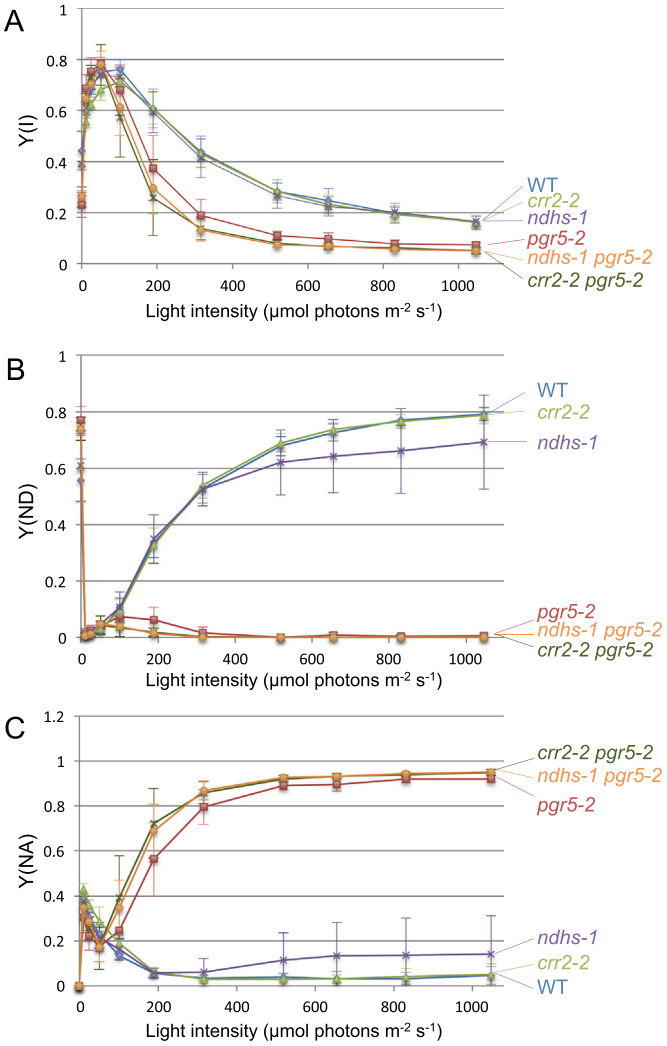

Fig. 3. Light-intensity dependence of changes in P700 absorbance parameters. (A) Y(I) is the quantum yield of PSI. (B) Y(ND) represents donor-side regulation of PSI. (C) Y(NA) represents acceptor-side limitation of PSI. Data represent means ± SD ($n = 5$).

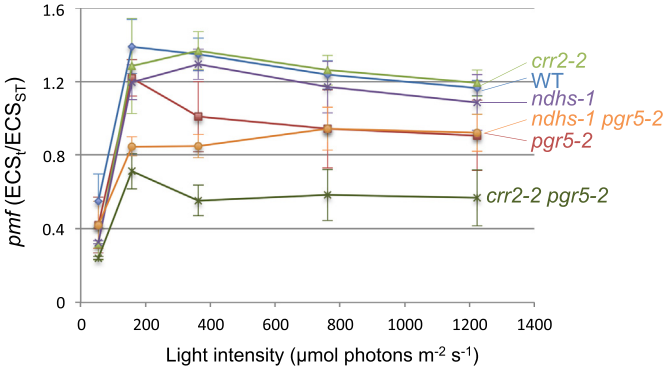

Fig. 4. Light-intensity dependence of the total size of *pmf* (ECS_t/ECS_{ST}). Data represent means ± SD ($n = 3$).

the *crr2-2 pgr5-2* and *ndhs-1 pgr5-2* double mutants, the contribution of the chloroplast NDH complex was evident at moderate light intensities of 160 and 370 μmol photons $m^{-2} s^{-1}$. Although the *pmf* levels were lower in the *crr2-2 pgr5-2* mutant than in the *pgr5-2* single mutant, the same levels of *pmf* were monitored between the *ndhs-1 pgr5-2* and *pgr5-2* mutants. Consistent with the phenotype of double mutants observed

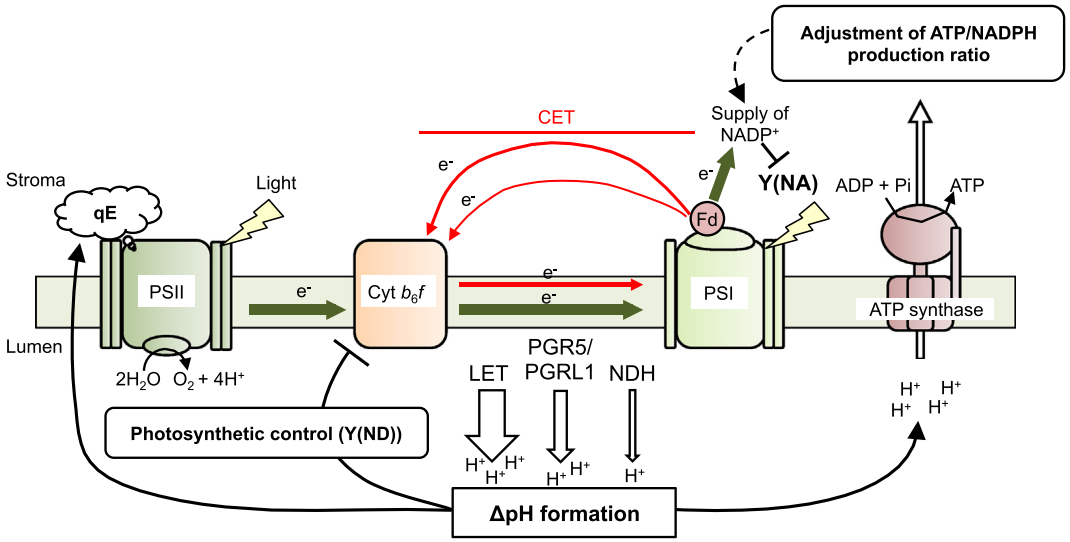

Fig. 5. Two CET pathways depending on PGR5/PGRL1 and NDH contribute to the formation of ΔpH, as well as LET. Luminal acidification induces down-regulation of electron transport by inducing qE monitored by NPQ and photosynthetic control at the Cyt b_6f complex monitored by Y(ND). Formation of ΔpH also contributes to ATP synthesis as *pmf*, as well as membrane potential. Balancing ATP/NADPH production ratio alleviated the limitation in acceptors ($NADP^+$ and oxidized Fd) from PSI, which is monitored by Y(NA). In this study, contribution of the NDH complex to the three processes is confirmed in the mutant background with normal LET-dependent ΔpH formation and mildly reduced PGR5/PGRL1-dependent ΔpH formation.

in NPQ (Fig. 3B), the significant contribution of the chloroplast NDH complex to *pmf* was confirmed in the ECS analysis (Fig. 4).

4. Discussion

Our conclusion in this study is summarized in Fig. 5. Double mutants defective in both pathways of PSI CET were previously characterized using a strong mutant allele of *pgr5-1* [30]. Here, we used a weak mutant allele of *pgr5-2*. The advantage of using *pgr5-2* is the absence of any growth defects even in double mutants (Fig. 1). Furthermore, we observed no reduction in ETR(II) in any light intensities in the *pgr5-2* single mutant (Fig. 2A). In light-intensity dependence NPQ induction, we observed a slight decline in the size of NPQ at moderate light intensities from 190 to 650 μmol photons $m^{-2} s^{-1}$ (Fig. 2B). However, the difference was even more slight in our recent report [14]. The evident reduction in NPQ was observed in the double mutants, *crr2-2 pgr5-2* and *ndhs-1 pgr5-2* (Fig. 2B). The reduction level was slightly lower than that in the *pgr5-1* single mutant (approximately 0.6 at 1000 μmol photons $m^{-2} s^{-1}$) [14]. Because ETR(II) was not significantly affected in these double mutants, this observation suggests the contribution of the chloroplast NDH complex to ΔpH formation via PSI CET. Consistent with this idea, the size of *pmf* was lower in the double mutants at light intensities of > 100 μmol photons $m^{-2} s^{-1}$ (Fig. 4). The reduction was also observed in the *pgr5-2* single mutant.

The qL parameter represents the oxidized pool of PQ and was higher at low light intensities of from 11 to 50 μmol photons $m^{-2} s^{-1}$ in the *pgr5-2* mutant background than in other genotypes (Fig. 2C, inset). This phenotype may be due to the weak operation of donor-side regulation even at low light intensity in the presence of WT PGR5. In contrast, qL was slightly lower in the *crr2-2* mutant at 50 μmol photons $m^{-2} s^{-1}$ (Fig. 2C, inset). This may reflect the function of chloroplast NDH in low light, as suggested in rice [25] and *Marchantia polymorpha* [27]. But this phenotype was unclear in the *ndhs-1* mutant.

Notably, the level of qL was lower in the double mutants at light intensities > 190 μmol photons $m^{-2} s^{-1}$, than in other genotypes (Fig. 2C). Because donor-side regulation was not induced in the double mutants, the Cyt b_6f complex did not restrict electron transport. Most likely, acceptor capacity from PSI is limited in the double mutants, resulting in accumulation of electrons in electron transport chains including the PQ pool. This phenotype suggests the importance of acceptor-side regulation depending on two CET pathways by balancing the ATP/NADPH production ratio (Fig. 5). We do not eliminate the possibility that the defect in NPQ induction affected the reduced level of the PQ pool, as observed in the Arabidopsis *npq1* mutant [38]. Although the deviation from the WT was observed approximately at 500 μmol photons $m^{-2} s^{-1}$ in the qP parameter in *npq1*, it was evident at 190 μmol photons $m^{-2} s^{-1}$ in the double mutants in this study. qL is calculated as qP × F_o'/F_s and was prosed to reflect the fraction of open PSII center [32].

In this study, we used two mutants defective in NDH activity. Based on the growth phenotype observed in the *pgr5-1* mutant background, the *crr2-2* mutant showed a stronger phenotype than the *ndhs-1* mutant [10,30]. However, we observed no clear differences in photosynthetic parameters between the two NDH mutants. One exception is the size of *pmf* at high light intensities of 760 and 1220 μmol photons $m^{-2} s^{-1}$ (Fig. 4). Although in the *ndhs-1 pgr5-2* double mutant the level of *pmf* was similar to that in the *pgr5-2* single mutant, it was more severely affected in the *crr2-2 pgr5-2* double mutant. However, NPQ induction was similarly affected in both double mutants (Fig. 2B). Because of the large standard deviation, we drew no conclusion regarding the differences in the size of *pmf* at high light intensities between *pgr5-2* and *ndhs-1 pgr5-2* (Fig. 4). We found no clear difference in the photosynthetic parameter to explain the dramatic difference in plant growth between *crr2-2 pgr5-1* and *ndhs-1 pgr5-1* [10,30]. The plant growth may not necessarily be linearly related to the photosynthetic activity and the developmental program may be dramatically affected below a certain threshold.

In the *pgr5-2* mutant, ETR(II) was not affected at any light intensity (Fig. 2A). However, the induction of donor-side regulation monitored by Y(ND) was severely impaired (Fig. 3B) and NPQ induction was also slightly affected (Fig. 2B). This phenotype is explained by the idea that the size of ΔpH was reduced to a level sufficient for inducing ΔpH-dependent NPQ (qE) partially but not for inducing donor-side regulation efficiently. The idea is consistent with the slight reduction of *pmf* in the *pgr5-2* mutant (Fig. 4). Its mechanism is also explained by the original idea that PGR5 is involved in CET [3,5,7]. This idea is supported by the reduction in Y(I) without affecting Y(II). However, we may not be able to simply rely on these parameters for the evaluation of CET activity because Y(I) was unusually lower than Y(II). This may be due to the photodamage of PSI in the *pgr5-2* mutants and we observed similar

problems in the evaluation of the *pgr5-1* mutants. However, the levels of Y(I) and Y(II) were similar in the *pgr5-1* mutant accumulating flavodiiron proteins in the artificial air (5% CO_2 and 5% O_2) used for the membrane inlet mass-spectrometry assay [15]. The oxidation of P700 complemented the WT level. Based on this concept, we discuss why the defects in the NDH-dependent CET decreased ΔpH further to a level at which NPQ induction was clearly impaired (Fig. 2B). This idea is supported by the further decline in Y(I) (Fig. 3A) and the *pmf* levels (Fig. 4) in the double mutants.

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Disclosures

The authors have no conflicts of interest to declare.

Transparency document

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