

# What Quantity of Photosystem I Is Optimum for Safe Photosynthesis?<sup>1</sup>

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PSI has the potential to generate reactive oxygen species and be oxidatively inactivated by the reactive oxygen species. The photo-oxidative damage of PSI (also called PSI photoinhibition) causes the inhibition of the plant growth and is a lethal event for plants. It has been reported that PSI photoinhibition does not occur as long as the reaction-center chlorophyll (P700) remains oxidized, even in excess light conditions. This process is termed P700 oxidation and is supported by various regulatory mechanisms and likely also by the stoichiometric quantities of photosynthetic apparatus. In this study, we assessed how decreased photochemically active PSI in *Arabidopsis* (*Arabidopsis thaliana*) affected a variety of photosynthetic parameters, including P700 oxidation. Inactivation of PSI was rapidly and selectively induced by repetitive short-pulse illumination. PSI photoinhibition correlated linearly with decreases in effective quantum yield of PSII and nonphotochemical quenching; however, the photosynthetic CO<sub>2</sub> assimilation rate was less affected, as exemplified by ~50% of the normal CO<sub>2</sub> assimilation rate maintained with an 80% loss in PSI photochemical activity. In contrast, effective quantum yield of PSI was enhanced following PSI photoinhibition, mainly owing to a decrease in the electron donor-side limitation of PSI. Based on these results, we propose that the stoichiometric quantity of PSI is optimized to induce P700 oxidation for dissipating excess light energy in PSI, thus avoiding inhibition of photosynthetic CO<sub>2</sub> assimilation caused by PSI photoinhibition.

Plants carry out photosynthesis, at the risk of photo-oxidative damage, by converting the energy from sunlight to reducing power in the photosynthetic electron transport system, which begins with photo-excitation of the reaction-center chlorophylls in PSII and PSI, i.e. P680 and P700, respectively, in the thylakoid membrane in chloroplasts. Electrons originating from the oxidation of water on the luminal side of PSII are transported into PSI *via* the plastoquinone (PQ) pool, the cytochrome *b<sub>6</sub>/f* complex (Cyt *b<sub>6</sub>/f*), and

plastocyanin, accompanied by the formation of a proton gradient across the thylakoid membrane, to produce ATP. Photo-excited P700 gives electrons to the acceptor side of PSI and finally to NADP<sup>+</sup>. Both ATP and NADPH are consumed in the Calvin-Benson cycle for photosynthetic CO<sub>2</sub> assimilation. However, the production and consumption of reducing power is easily unbalanced because of various environmental fluctuations. If photo-excitation energy originating from P700 is more than the demand for NADPH production, it can cause reactive oxygen species-derived photo-oxidative damage to PSI (Terashima et al., 1994; Sejima et al., 2014). Once reactive oxygen species are generated, PSI is rapidly inactivated and thereafter takes days or even weeks to recover completely, thus inhibiting photosynthesis and growth of oxygenic photoautotrophs (Zivcak et al., 2015a; Shimakawa et al., 2016). This photo-oxidative damage to PSI is termed PSI photoinhibition and is recognized as a lethal event for oxygenic photoautotrophs.

Generally, PSI photoinhibition rarely occurs because plants suppress excessive photo-excitation of P700 by keeping it in its oxidized form that functions as the quencher of light energy (Foyer et al., 1990; Klughammer and Schreiber, 1994; Trissl, 1997; Bukhov and Carpentier, 2003; Golding and Johnson, 2003; Miyake et al., 2005). This is the mechanism by which oxygenic photoautotrophs safely carry out photosynthesis under the sun. Oxidation of P700 is controlled by various molecular mechanisms, known collectively as

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the P700 oxidation system (Shimakawa et al., 2017; Takagi et al., 2017; Shimakawa and Miyake, 2018). Conversely, it is supposed that P700 can be easily kept oxidized, even without any regulatory mechanisms, due to the stoichiometric quantities of photosynthetic apparatus, including PSII, Cyt  $b_6/f$ , and PSI. The stoichiometric quantity (mol per leaf area) of Cyt  $b_6/f$  is modulated and kept lower than those of PSII and PSI in plant leaves (Yamori et al., 2011; Schöttler and Tóth, 2014), which is one of the reasons why the electron transport in Cyt  $b_6/f$  is usually recognized as the limiting step in the photosynthetic electron transport system (Anderson, 1992; Schöttler et al., 2015). Oxidized P700 is reduced with electrons predominantly from PSII, which clearly suggests that PSII quantity larger than that of PSI potentially impairs the maintenance of P700 in an oxidized state. Indeed, Bukhov et al. (2004) observed a faster reduction rate of oxidized P700 in those plant leaves in which PSI photoinhibition occurred under chilling stress (Bukhov et al., 2004). Additionally, a barley mutant having a decreased amount of PSII showed higher P700 oxidation than that of the wild type (Pfundel et al., 2008), whereas a chlorophyll  $b$ -deficient mutant showing a low PSI/PSII ratio was impaired in P700 oxidation (Brestic et al., 2015). Furthermore, PSI photoinhibition was reported in a mutant that contained a lower quantity of PSI than that of PSII (Ivanov et al., 2015). These facts imply that the stoichiometric quantity of PSI is optimized to induce P700 oxidation to protect PSI from photoinhibition and ensure robust photosynthesis. In this study, we sought to characterize the effects of quantitative decrease of the photochemically active PSI on a variety of photosynthetic parameters using the intact leaves of the Arabidopsis wild type.

## RESULTS

### Relationship between the Quantity of the Photochemically Active PSI and Photosynthetic Parameters of PSI in Arabidopsis

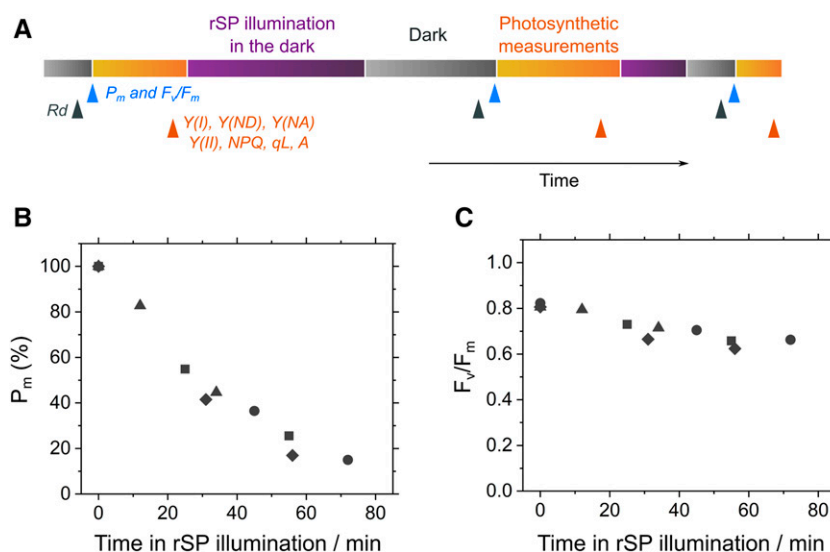
For experimentally modifying the quantity of photochemically active PSI in the plant leaves, we used the method "repetitive short-pulse (rSP) illumination," which facilitates rapid and selective induction of PSI photoinhibition in the plant leaves *in vivo*. For this method, rSP (300 ms, 20,000  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ , every 10 s) is applied to the plant leaves of angiosperms in darkness, which instantaneously fills the photosynthetic electron transport system with electrons in the absence of stimulus for any regulatory mechanism of P700 oxidation (Sejima et al., 2014; Zivcak et al., 2015a, 2015b; Kono et al., 2017; Takagi et al., 2017; Shimakawa et al., 2019; Tikkanen and Grebe, 2018). Using rSP illumination, we created plant leaves partially impaired in PSI and evaluated the relationship between the quantity of photochemically active PSI with various photosynthetic parameters in Arabidopsis, as follows.

In Arabidopsis, rSP illumination caused PSI photoinhibition (Fig. 1). The quantity of photochemically active PSI was spectroscopically evaluated *in vivo* as the total quantity of photo-oxidizable P700 ( $P_m$ ) before and after rSP illumination (Fig. 1A; Klughammer and Schreiber, 1994). In the leaves of Arabidopsis wild-type plants,  $P_m$  decreased to  $\sim 20\%$  of the intact value after rSP illumination in the dark (300 ms, 20,000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , every 10 s) for 1 h in ambient air (Fig. 1B). In contrast, maximum quantum efficiency of PSII photochemistry ( $F_v/F_m$ ) was sustained, i.e.  $\sim 80\%$  of the intact value, even after rSP illumination (Fig. 1C), indicating that the method selectively induces inactivation of PSI in Arabidopsis like in other angiosperms (Sejima et al., 2014). After rSP illumination, we incubated the plants in darkness for 30 min so as to relax qE quenching and state transition (Baker, 2008) before the determination of  $P_m$  and  $F_v/F_m$  (Fig. 1A).

Photosynthetic parameters of PSI were measured in Arabidopsis at a steady state of photosynthesis under red actinic light (820  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) after the determination of  $P_m$  and  $F_v/F_m$  (Fig. 1A). Commonly in  $C_3$  plants, photorespiration functions as a large alternative electron sink (Badger et al., 2000; Driever and Baker, 2011; Hanawa et al., 2017), likely affecting a variety of photosynthetic parameters. In this study, the measurements of photosynthetic parameters were performed under high  $\text{CO}_2$  conditions (21 kPa  $\text{O}_2$ , 100 Pa  $\text{CO}_2$ ) to exclude the effects of photorespiration on these parameters. With a 80% loss of PSI photochemical activity, effective quantum yield of PSI [ $Y(\text{I})$ ] increased from 0.4 to 0.9 (Fig. 2A). The photochemical reaction in PSI is limited at both electron donor and acceptor-sides. Quantum yield of nonphotochemical energy dissipation due to donor-side limitation [ $Y(\text{ND})$ ] indicates the ratio of oxidized P700 to total photo-oxidizable P700; that is,  $Y(\text{ND})$  is the indicator of P700 oxidation. At the steady state of photosynthesis, in intact leaves of Arabidopsis under actinic light,  $Y(\text{ND})$  was calculated as 0.5, which reflects that 50% of P700 remained oxidized (Fig. 2B). However,  $Y(\text{ND})$  dramatically decreased from 0.5 to 0.1 with a decrease in  $P_m$  from 100% to 60% (Fig. 2B). These data suggest that PSI photoinhibition in this range prevented oxidation of P700. Additionally, we plotted quantum yield of nonphotochemical energy dissipation due to acceptor-side limitation [ $Y(\text{NA})$ ] against  $P_m$ . When  $P_m$  decreased from 100% to 60%,  $Y(\text{NA})$  increased with the inactivation of PSI (Fig. 2C). However, when the amount of photochemically active PSI was less than 20% of the intact value, we could not detect  $Y(\text{NA})$  at all (Fig. 2C).

### Relationship between the Quantity of the Photochemically Active PSI and Photosynthetic Parameters of PSII in Arabidopsis

Next, we assessed the impact of the quantity of photochemically active PSI on the photosynthetic parameters of PSII in Arabidopsis. Effective quantum



**Figure 1.** Effects of PSI photoinhibition on the photochemical activities of PSI and PSII. A, Schematic representation of the experiment to induce PSI photoinhibition. Repetitive short-pulse (rSP) illumination (300 ms, 20,000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , every 10 s) was applied to Arabidopsis leaves in the dark as indicated by purple bars. Total oxidizable P700 ( $P_m$ ), maximum quantum efficiency of PSII photochemistry ( $F_v/F_m$ ), and dark respiration rate ( $R_d$ ) were periodically determined after a 30-min dark incubation (gray bars), with the timing shown by blue ( $P_m$  and  $F_v/F_m$ ) and black ( $R_d$ ) arrows, and thereafter a variety of photosynthetic parameters were measured in the light (orange bars), with the timing indicated by orange arrows. The abbreviations of photosynthetic parameters were defined as follows: Y(I), effective quantum yield of PSI; Y(ND) and Y(NA), quantum yield of nonphotochemical energy dissipation of PSI due to the donor- and acceptor-side limitation; Y(II), effective quantum yield of PSII; NPQ, nonphotochemical quenching; qL, inferred oxidation level of PQ pool; and A, net photosynthetic  $\text{CO}_2$  assimilation rate. B, Time course of  $P_m$  during rSP illumination. C, Time course of  $F_v/F_m$  during rSP illumination. Data are independent measurements, with quadruple replicates indicated by circles, triangles, squares, and diamonds.

yield of PSII [Y(II)] showed an approximately linear relationship with  $P_m$  (Fig. 3A), which suggests that the operating efficiency of the photochemical reaction in PSII is correlated to the quantity of the photochemically active PSI in plant leaves. Similarly, nonphotochemical quenching of chlorophyll fluorescence in PSII (nonphotochemical quenching), the indicator of a thermal dissipation of excess light energy in PSII, was proportional to  $P_m$  (Fig. 3B). Furthermore, photochemical quenching of chlorophyll fluorescence based on the lake model of the antenna system in PSII (represented as qL), reflecting the oxidation level of  $Q_A$  and possibly the PQ pool, showed a trend similar to that of Y(II) (Fig. 3C).

#### Relationship between the Quantity of the Photochemically Active PSI and Photosynthetic $\text{CO}_2$ Assimilation in Arabidopsis

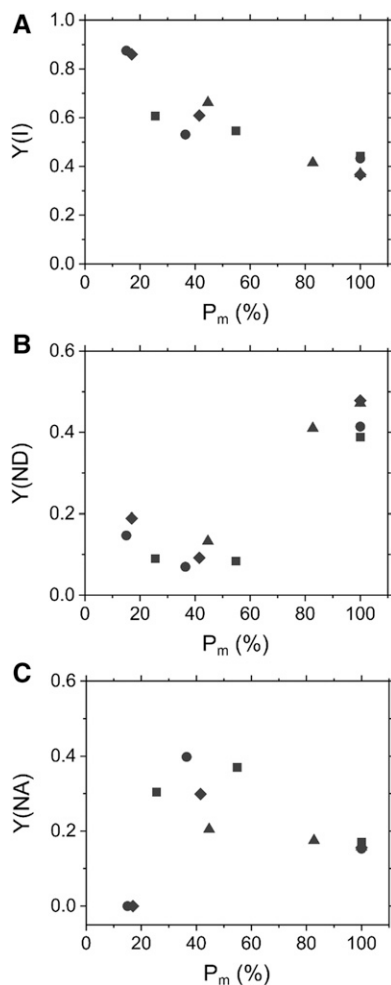
We measured photosynthetic  $\text{CO}_2$  assimilation rate, calculated as the sum of net  $\text{CO}_2$  assimilation rate (A) and dark respiration rate, in intact and damaged leaves of Arabidopsis. When compared with how Y(II), NPQ, and qL responded to PSI photoinhibition, a less linear correlation was observed in the inactivation of photosynthetic  $\text{CO}_2$  assimilation when  $P_m$  decreased (Fig. 4A). The inhibition of  $\text{CO}_2$  assimilation rate might

be suppressed until the residual  $P_m$  was less than 60%, and thereafter, it started to decrease with the inactivation of PSI (Fig. 4A). However,  $\sim 50\%$  of the normal  $\text{CO}_2$  assimilation rate was maintained even with an 80% reduction in PSI photochemical activity in the leaves of Arabidopsis wild-type plants (Fig. 4A). Additionally, when Y(II) was plotted against the  $\text{CO}_2$  assimilation rate (Fig. 4B), the resultant relationship indicated that the effective quantum yield of PSII can relatively reflect the photosynthetic  $\text{CO}_2$  assimilation rate when PSI photoinhibition occurs.

## DISCUSSION

### Sufficient Quantity of PSI Supports P700 Oxidation in Plant Leaves

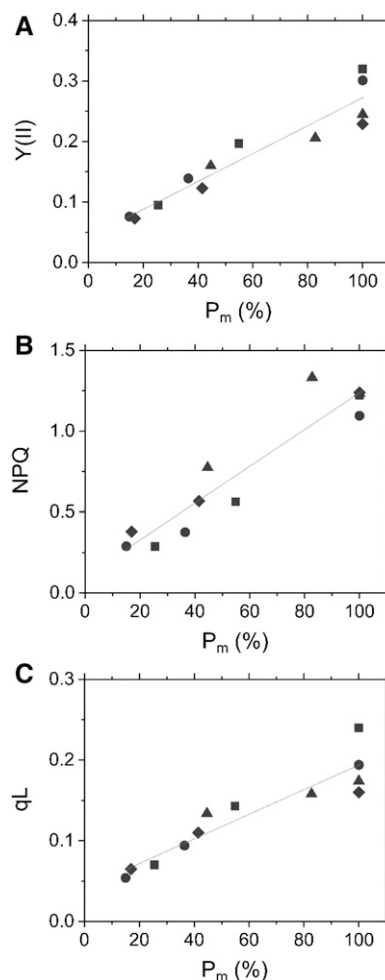
In the current study, we investigated the relationship between the stoichiometric quantity of the photochemically active PSI and P700 oxidation in vivo during steady-state photosynthesis in Arabidopsis. Although diverse regulatory mechanisms for the oxidation of P700 have been reported in oxygenic photoautotrophs (Shimakawa and Miyake, 2018), the effect of the quantity of PSI on P700 oxidation was poorly understood. rSP illumination is a useful tool to rapidly and selectively induce PSI photoinhibition in plant leaves



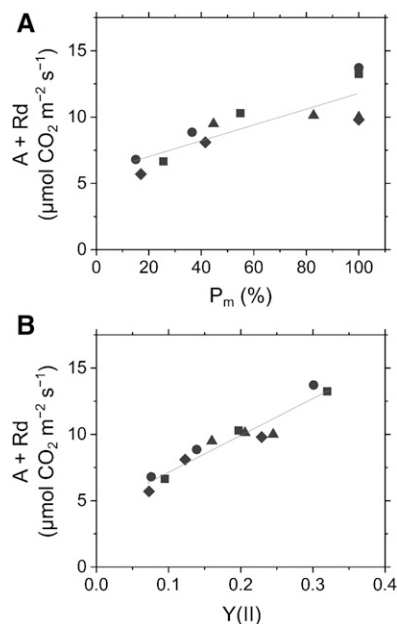
**Figure 2.** Effects of PSI photoinhibition on photosynthetic parameters of PSI in Arabidopsis. A, Relationship between effective quantum yield of PSI [ $Y(I)$ ] and residual total oxidizable P700 ( $P_m$ ). B and C, Relationship between quantum yield of nonphotochemical energy dissipation of PSI due to the donor- and acceptor-side limitation [ $Y(ND)$  and  $Y(NA)$ , respectively] and  $P_m$ . Evaluations were made before and after rSP illumination (300 ms, 20,000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , every 10 s). Photosynthetic parameters of PSI were measured at the steady state of photosynthesis (820  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) under 21 kPa  $\text{O}_2$  and 100 Pa  $\text{CO}_2$ . Data are independent measurements, with quadruplicate replicates indicated by circles, triangles, squares, and diamonds.

(Sejima et al., 2014; Zivcak et al., 2015a, 2015b; Kono et al., 2017; Takagi et al., 2017; Shimakawa et al., 2019; Tikkanen and Grebe, 2018). This method enabled us to decrease the quantity of active PSI in the leaves of Arabidopsis wild-type plants. With PSI inactivated,  $Y(I)$  in principle increased (Fig. 2A); that is, the residual photochemically active PSI efficiently drives the photooxidation/reduction cycle, which is reasonable considering that the electron donor (i.e. PSII) and acceptor (i.e. the Calvin-Benson cycle) of PSI are relatively larger in these situations. In contrast, when  $P_m$  was decreased from 100% to 60%,  $Y(ND)$  decreased from 0.5 to 0.1 (Fig. 2B), indicating that plant leaves cannot

keep P700 oxidized when 40% of PSI is inactivated even in the presence of a variety of regulatory mechanisms (i.e. P700 oxidation system). In other words, the leaves of Arabidopsis contain a quantity of PSI that is likely sufficient to enable P700 oxidation in excess light conditions, which may be regulated at the genetic level and modulated to suit any variation in environmental conditions. In the illustrated model of the photosynthetic electron transport system in the processes of PSI photoinhibition in Arabidopsis leaves (Fig. 5), we summarized the effects of a decrease in the stoichiometric quantity of PSI on P700 oxidation as well as on the other physiological states as follows.



**Figure 3.** Effects of PSI photoinhibition on photosynthetic parameters of PSII in Arabidopsis. A, Relationship between effective quantum yield of PSII [ $Y(II)$ ] and residual total oxidizable P700 ( $P_m$ ). B, Relationship between nonphotochemical quenching (NPQ) and  $P_m$ . C, Relationship between inferred oxidation level of PQ pool (qL) and  $P_m$ . Evaluations were made before and after rSP illumination (300 ms, 20,000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , every 10 s). Photosynthetic parameters of PSII were measured at the steady state of photosynthesis (820  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) under 21 kPa  $\text{O}_2$  and 100 Pa  $\text{CO}_2$ . Data are independent measurements, with quadruplicate replicates indicated by circles, triangles, squares, and diamonds. Red lines show linear regressions (A,  $R^2 = 0.896$ ; B,  $R^2 = 0.897$ ; C,  $R^2 = 0.860$ , respectively).



**Figure 4.** Effects of PSI photoinhibition on photosynthetic CO<sub>2</sub> assimilation of PSI in Arabidopsis. A, Relationship between photosynthetic CO<sub>2</sub> assimilation rate (A + dark respiration rate [Rd]) with residual total oxidizable P700 (P<sub>m</sub>). B, Relationship between A + Rd and effective quantum yield of PSII [Y(II)]. Evaluations were made before and after rSP illumination (300 ms, 20,000 μmol photons m<sup>-2</sup> s<sup>-1</sup>, every 10 s). Photosynthetic parameters of PSI were measured at the steady state of photosynthesis (820 μmol photons m<sup>-2</sup> s<sup>-1</sup>) under 21 kPa O<sub>2</sub> and 100 Pa CO<sub>2</sub>. Data are independent measurements, with quadruple replicates indicated by circles, triangles, squares, and diamonds. Red lines show linear regressions (A, R<sup>2</sup> = 0.718; B, R<sup>2</sup> = 0.920, respectively).

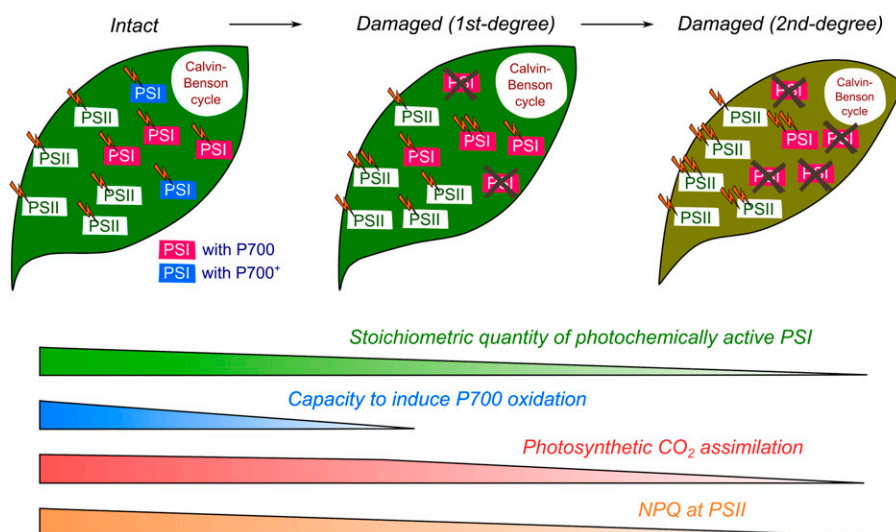
**Capacity of Remaining PSI Can Suppress the Inhibition of Photosynthesis**

When P<sub>m</sub> decreased to 60% of that present in untreated leaves, the photosynthetic CO<sub>2</sub> assimilation rate was likely to show a smaller decrease than that in the

phase whereby P<sub>m</sub> further decreased from 60% to 20% of normal levels, as supported by the low linear correlation for the relationship between P<sub>m</sub> and CO<sub>2</sub> assimilation rate (Fig. 4A). The former phase of PSI photoinhibition, termed as “first-degree” in Figure 5, corresponds to the phase in which oxidized P700 can be still observed as reflected in Y(ND). In the latter phase (“second-degree” in Fig. 5), P700 oxidation is no longer maintained in the steady-state photosynthesis (Fig. 2B). In principle, oxidized P700 should not cause charge separation in PSI, and rather it should engage in thermal dissipation of light energy (Trissl, 1997; Bukhov and Carpentier, 2003). This may be the reason why the decrease in photosynthetic CO<sub>2</sub> assimilation rate was alleviated when P<sub>m</sub> ranged from 100% to 60% (Fig. 4A); that is, the fractions of PSI with oxidized P700 reflect the capacity of remaining PSI complexes following PSI photoinhibition and can be recognized as surplus PSI for photosynthesis in intact leaves (Fig. 5).

**Oxidized P700 Functions as a Quencher of Light Energy to Balance the Photo-Excitation between PSII and PSI**

Theoretically, oxidized P700 thermally dissipates the light energy in PSI, which can mitigate the pressure of photo-excitation in PSII. As PSI photoinhibition advanced and P<sub>m</sub> ranged from 100% to 40%, Y(NA) slightly increased; and thereafter, it disappeared (Fig. 2C). As mentioned above, with a decrease in photochemically active PSI, the limitation on the electron acceptor side should in principle be relieved. Therefore, the slight increase in Y(NA; Fig. 2C) suggests that the electron influx into PSI on the donor side was enhanced. Light energy absorbed by chloroplasts in the plant leaves is distributed into PSII and PSI (Fig. 5), which suggests that a decrease in the quantity of the photochemically active PSI can cause an increase in the light energy flow into PSII. Indeed, Bukhov et al. (2004) reported accelerated rereduction of oxidized



**Figure 5.** Hypothetical overview of the progression of PSI photoinhibition in plant leaves. Stoichiometric quantity of photochemically active PSI decreases with the degree of PSI photoinhibition. As PSI photoinhibition progresses, P700 oxidation is not maintained, which is the turning point between first and second degree PSI photoinhibition.

P700 in PSI-damaged cucumber leaves. In this study, the observed increase in  $Y(\text{NA})$  when  $P_m$  ranged from 100% to 40% (Fig. 2C) supports the theory that oxidized P700 functions as the quencher of light energy in PSI. In PSII,  $Y(\text{II})$  decreased with PSI photoinhibition (Fig. 3A), but did not show a linear relationship with photosynthetic  $\text{CO}_2$  assimilation rate passing through the graph origin, different from that in intact  $C_3$  plant leaves (Fig. 4A; Genty et al., 1989). This can be explained by an increase in the absorbance of PSII fraction (Genty et al., 1989; Baker et al., 2007). These data also demonstrate the effects of the change in the distribution of light energy between PSII and PSI on the chlorophyll fluorescence parameters. Simultaneously, the decrease in  $qL$  is assumed to reflect two effects: a decrease in the electron acceptor (i.e. PSI) and an increase in the photo-excitation pressure (Fig. 3C). Evidence for the hypothesis that oxidized P700 suppresses the charge separation of PSII by quenching the light energy in PSII through light harvesting complex and/or spillover-dependent mechanisms (Ueno et al., 2018; Yokono and Akimoto, 2018) might be also present within the results of the current study.

#### Decrease in PSI Is Correlated with a Decrease in NPQ

NPQ showed a linear inverse correlation with the extent of PSI photoinhibition in *Arabidopsis* (Fig. 3B). In the chlorophyll fluorescence measurement, NPQ reflects a thermal dissipation of excess light energy in PSII to protect PSII against photoinhibition (Li et al., 2002). Thus, a reduction of the electron acceptor side of PSII at steady-state photosynthesis, following PSI photoinhibition, as reflected in a decrease in  $qL$  (Fig. 3C), might be contributed also by the decrease in NPQ. These results suggest that PSI photoinhibition possibly causes PSII photoinhibition as a secondary effect under illuminated conditions. To our knowledge, the linear relationship between PSI/PSII ratio and NPQ was first characterized by Brestic and coworkers in early growth stages of chlorophyll *b*-deficient wheat mutant lines (Brestic et al., 2015). In  $C_3$  plants, NPQ is mainly composed of  $qE$  quenching, which is believed to correlate with the lumen acidification produced by photosynthetic linear and possibly cyclic electron flow, both of which depend on PSI (Kanazawa and Kramer, 2002; Li et al., 2002). Therefore, a loss of PSI can decrease the thylakoid membrane potential to lower  $qE$  quenching. In fact, PSI photoinhibition causes a decrease in the proton gradient across the thylakoid membrane in wheat leaves (Brestic et al., 2015; Zivcak et al., 2015a). On the other hand, the thylakoid membrane potential is modulated also by the narrowing proton conductance of chloroplast ATP synthase and thylakoid ion transporters (Rott et al., 2011; Armbruster et al., 2014). However, proton conductance is not affected by PSI photoinhibition (Zivcak et al., 2015a). In this study, we present a correlation between the amount of photochemically active PSI and NPQ in the

wild-type plant leaves (Fig. 3B). The contribution of PSI to NPQ should be further quantitatively characterized in future works.

## MATERIALS AND METHODS

### Arabidopsis Growth Conditions

*Arabidopsis* (*Arabidopsis thaliana*) wild type plant (Columbia ecotype) was grown under a long-day condition (16-h light, 23°C, 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , white fluorescent lamp; 8-h dark, 21°C) with a relative humidity of 60%  $\pm$  10%. Seeds were planted in pots that contained a 1:1 mixture of vermiculite and Metro-Mix 350 (Sun Gro Horticulture); 1000-fold diluted Hyponex solution (Hyponex) was used as a watering solution.

### Measurements of Gas Exchange, Chlorophyll Fluorescence, and P700

Gas exchange ( $\text{CO}_2$  and water) was measured using a LI-7000 gas analyzer (Li-COR) equipped with a 3010 DUAL gas exchange leaf chamber (Walz, Effeltrich, Germany), in which the flowing gas was saturated with water vapor at 18.0  $\pm$  0.1°C and the leaf temperature was maintained at 25°C. Chlorophyll fluorescence and oxidized P700 were simultaneously measured using a Dual-PAM-100 fluorometer (Walz).

The photosynthetic parameters of PSII were calculated using chlorophyll fluorescence parameters as follows (Baker, 2008): PSII operating efficiency (quantum yield of photochemical energy conversion in PSII),  $Y(\text{II}) = (F_m' - F') / F_m'$ ; nonphotochemical quenching,  $\text{NPQ} = (F_m - F_m') / F_m'$ ; fraction of "open" PSII centers (with  $Q_A$  oxidized), on the basis of a lake model, for the PSII photosynthetic apparatus,  $qL = [(F_m' - F') / (F_m' - F_o')] \times (F_o' / F')$ , where,  $F_o$  represents minimum fluorescence from a dark-adapted leaf;  $F_o'$ , minimum fluorescence from a light-adapted leaf;  $F_m$ , maximum fluorescence from a dark-adapted leaf;  $F_m'$ , maximum fluorescence from a light-adapted leaf; and  $F'$ , fluorescence emission from a light-adapted leaf. Pulse-amplitude modulated red measuring light (620 nm, 0.1  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) was used to determine  $F_o$ . Red actinic light was supplied using a chip-on-board light emitting diode (LED) array (635 nm). Short-saturation pulse light (8,000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , 300 ms) was also provided by the LED array for the determinations of  $F_m$  and  $F_m'$ .

The photosynthetic parameters of PSI were calculated from the redox state of P700 as follows (Klughammer and Schreiber, 1994; Schreiber and Klughammer, 2008): quantum yield of photochemical energy conversion in PSI,  $Y(\text{I}) = (P_m' - P) / P_m'$ ; quantum yield of nonphotochemical energy dissipation due to donor-side limitation,  $Y(\text{ND}) = P / P_m'$ ; quantum yield of nonphotochemical energy dissipation due to acceptor-side limitation,  $Y(\text{NA}) = (P_m - P_m') / P_m'$ , where,  $P_m$  represents total quantity of photo-oxidizable P700;  $P_m'$ , maximum quantity of photo-oxidized P700 by a saturation pulse; and  $P$ , quantity of photo-oxidized P700 at steady state. Pulse-amplitude modulated near-infrared measuring lights (830 and 870 nm) were applied to measure the transmittance of oxidized P700.

### Statistical Analysis

All statistical analyses were performed using Origin 2017 (LightStone).

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