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Repetitive Short-Pulse Light Mainly Inactivates Photosystem I in Sunflower Leaves

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Regular Paper

Under field conditions, the leaves of plants are exposed to fluctuating light, as observed in sunfleck. The duration and frequency of sunfleck, which is caused by the canopy being blown by the wind, are in the ranges from 0.2 to 50 s, and from 0.004 to 1 Hz, respectively. Furthermore, >60% of the sunfleck duration ranges from 0.2 to 0.8 s. In the present research, we analyzed the effects of repetitive illumination by short-pulse (SP) light of sunflower leaves on the photosynthetic electron flow. The duration of SP light was set in the range from 10 to 300 ms. We found that repetitive illumination with SP light did not induce the oxidation of P700 in PSI, and mainly inactivated PSI. Increases in the intensity, duration and frequency of SP light enhanced PSI photoinhibition. PSI photoinhibition required the presence of O_2. The inactivation of PSI suppressed the net CO_2 assimilation. On the other hand, the increase in the oxidized state of P700 suppressed PSI inactivation. That is, PSI with a reduced reaction center would produce reactive oxygen species (ROS) by SP light, leading to PSI photodamage. This mechanism probably explains the PSI photodamage induced by constant light.

Keywords: Photoinhibition • Photosystem I • Reactive oxygen species (ROS) • Water–Water Cycle (WWC).

Abbreviations: AEF, alternative electron flow; AL, actinic light; CEF, cyclic electron flow; F_o, minimum fluorescence yield; F_m, maximum fluorescence yield; F_m', maximum variable fluorescence yield; F_s, steady-state fluorescence yield; F_v/F_m, quantum yield of photochemical energy conversion in PSII; MAP pathway, Mehler–ascorbate peroxidase pathway; ML, measuring light; NPQ, non-photochemical quenching; PCO cycle, photorespiratory carbon oxidation cycle; PCR cycle, photosynthetic carbon reduction cycle; PET system, photosynthetic electron transport system; PLEF, photosynthetic linear electron flow; PQ, plastoquinone; qL, photochemical quenching of Chl fluorescence; RuBP, ribulose 1,5-bisphosphate; Rubisco, RuBP carboxylase/oxygenase; SP, short-pulse; Y(I), quantum yield of PSI; Y(II), quantum yield of PSII; Y(NA), quantum yield of non-photochemical energy dissipation due to acceptor-side limitation; Y(ND), quantum yield of non-photochemical energy dissipation due to donor-side limitation; WWC, water–water cycle.

Introduction

Photon energy absorbed by chloroplasts drives photooxidation and photoreduction cycles of reaction centers in PSI and PSII. Consequently, electron flow originating from photooxidation of water in PSII is coupled with photoreduction of $NADP^+$ in PSI through the Cyt b_6/f complex, i.e. photosynthetic linear electron flow (PLEF) begins to function. PLEF induces a proton gradient (ΔpH) across the thylakoid membrane, which is the motive force to produce ATP. In C_3 plants, NADPH and ATP produced during PLEF are used in the photosynthetic carbon reduction (PCR) cycle to drive photosynthetic CO_2 fixation and are simultaneously used in the photosynthetic carbon oxidation (PCO) cycle, which is the carbon recovery pathway for regenerating ribulose-1,5-bisphoshate (RuBP), one of the substrates for RuBP carboxylase/oxygenase (Rubisco) (von Caemmerer and Farquhar 1981).

A decrease in the production efficiency of NADPH during PLEF causes electrons to accumulate in the photosynthetic electron transport (PET) system of the thylakoid membrane. The accumulation of electrons in the PET system causes PSI and PSII photoinhibition. In general, under high light or low CO_2 conditions, PSII is photoinhibited by impairment in the PSII repair system (Nishiyama et al. 2011, Miyata et al. 2012). Under such conditions, O_2 is photoreduced to O_2^- in PSI of the thylakoid membrane in the Mehler–ascorbate peroxidase (MAP) pathway [water–water cycle (WWC)] (Asada 1999). O_2^- disproportionates to H_2O_2 and O_2, which is catalyzed by superoxide dismutase. H_2O_2 accumulates unless it is rapidly scavenged by ascorbate peroxidase (Miyake et al. 2006, Miyake 2010). The accumulated reactive oxygen species (ROS), O_2^- and H_2O_2, inactivate the elongation factor G for D1 proteins,

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and D1 synthesis is inhibited with loss of PSII activity (Ejima et al. 2012). Furthermore, PSI photoinhibition is observed in chill-stressed plants (Sonoike et al. 1994, Terashima et al. 1994, Scheller and Haldrup 2005). Net CO_2 assimilation is suppressed under low temperature, leading to low $NADP^+$ regeneration efficiency in the chloroplast stroma. Consequently, photoreduction of O_2 to O_2^- is stimulated (Asada 1999). The activities of O_2^- and H_2O_2 scavenging enzymes decrease at low temperature, which enables the accumulation of ROS. Following this, the accumulated O_2^- and H_2O_2 react with the electron transfer components in PSI, the Fe–S cluster, to produce the hydroxyl radical ·OH. ·OH reacts with the amino groups of PSI proteins, PsaA and PsaB, and degrades them (Sonoike 2011, Tikkanen et al. 2014). This inactivation in PSI has been mainly observed in cold-sensitive plants such as cucumber, with the exception of *Arabidopsis thaliana* (Zhang and Scheller 2004).

These photoinhibitions of PSII and PSI were observed in intact leaves exposed to constant light. Recently, mutants of *A. thaliana* which were deficient in the activities of cyclic electron flow around PSI (CEF-I) (*pgr5* mutant) and state transition (*stn7* mutant) were exposed to fluctuating light (Grieco et al. 2012, Suorsa et al. 2012, Kono et al. 2014). Both mutants suffered from PSI photoinhibition. These results suggest that the induction of non-photochemical quenching (NPQ) of Chl fluorescence in PSII through the formation of a ΔpH across thylakoid membranes by CEF-I, the CEF-dependent stimulated electron acceptance from PSI (Shikanai 2007, Shikanai 2014) and the preferred excitation of PSI compared with PSII by the state transition were required during fluctuating light for the protection of PSI.

Under field conditions, the leaves of plants are exposed to fluctuating light, as observed in sunfleck, where the light intensity abruptly increases (Pearcy 1983, Pfitsch and Pearcy 1989, Roden and Pearcy 1993, Pearcy et al. 1996). Before the sunfleck illumination, almost all of the reaction center Chl, P700, in PSI is thought to be in the reduced state, because the light intensity in the interval of sunfleck is low, and not saturated against photosynthesis. That is, the abrupt illumination increases the possibility that PSI donates electrons to O_2, producing ROS.

The duration and frequency of sunfleck, which is caused by the canopy being blown by the wind, are in the ranges from 0.2 to 50 s, and from 0.004 to 1 Hz, respectively. Furthermore, >60% of the sunfleck duration ranges from 0.2 to 0.8 s (Pfitsch and Pearcy 1989, Roden and Pearcy 1993). In the present research, we analyzed the effects of repetitive illumination of the leaves of sunflower with short-pulse (SP) (<300 ms) light on photosynthetic electron flow. We found that repetitive illumination mainly inactivated PSI, not PSII, only in the presence of O_2. With the oxidation of P700, the inactivation of PSI was suppressed. On this basis, we proposed the molecular mechanism of PSI inactivation. On exposure to repetitive SP light, P700 in the reduced form donated electrons to O_2, producing ROS. The ROS so produced would degrade PSI, and suppress net CO_2 assimilation.

Results

Effects of repetitive illumination with SP light on both Y(I) and Y(II) in sunflower leaves

SP light (300 ms, 20,000 µmol photons $m^{-2} s^{-1}$) illumination was produced every 10 s in the absence of actinic light (AL) under normal air conditions (**Fig. 1**). As time elapsed, the incident quantum yields of PSI, Y(I), and PSII, Y(II), decreased. Y(II) rapidly decreased from 0.8 to 0.5 at 60 min after the start of SP light illumination and then gradually decreased to 0.4 by 240 min (**Fig. 1A**). The maximum variable fluorescence yield (F_m') gradually decreased and the minimum fluorescence yield (F_o') increased during repetitive illumination with SP light (**Fig. 1B**).

In contrast, Y(I) decreased from 0.9 to approximately 0.1 at 240 min after the start of illumination with SP light (**Fig. 1D**). Y(NA), a parameter that shows the reduced state of the acceptor side of PSI, increased to 0.9 at 240 min (**Fig. 1E**). Higher Y(NA) further reduced the acceptor side of PSI. In other words, repetitive illumination with SP light caused electrons to accumulate at the acceptor side of PSI, and the efficiency of electron flow from P700 to the electron acceptor ferredoxin or $NADP^+$ decreased. Y(ND) did not increase during illumination with SP light (**Fig. 1F**).

These results indicate that repetitive illumination with SP light has different effects on PSI and PSII.

Effects of O_2 on SP light-dependent decreases in Y(II) and Y(I)

To elucidate the effects of O_2 on SP light-dependent decreases in Y(II) and Y(I), we lowered the partial pressure of O_2 (pO_2) during illumination with SP light. Lowering pO_2 from 21 to 1.5 kPa did not affect the decrease in Y(II) (**Fig. 1A**). At 1.5 kPa O_2, F_m' decreased and F_o' increased, similar to the observations under normal air conditions (**Fig. 1C**). However, the extent to which F_m' decreased at 1.5 kPa O_2 was slightly lower than that under normal air conditions.

In contrast, lowering pO_2 suppressed the decrease in Y(I) (**Fig. 1D**). Furthermore, lowering pO_2 suppressed the increase in Y(NA) (**Fig. 1E**). Y(ND) did not respond to changes in pO_2 (**Fig. 1F**). These results suggest that limiting electron flow from the P700 reaction center to the acceptor side of PSI requires O_2.

Effects of repetitive illumination with SP light on PSI and PSII activities

We analyzed the minimum yield of Chl fluorescence (F_o), the maximum yield (F_m), the quantum yield of photochemical energy conversion in PSII (F_v/F_m) and P_m, showing the maximum amount of P700 photoexcited in the dark, after repetitive illumination of the leaves with SP light for 240 min to evaluate the photoinhibitions of PSII, PSI and photosynthesis. F_m decreased and F_o increased to approximately 60% and 140% of those in control leaves, respectively (**Fig. 2A**). In other words, during repetitive illumination with SP light, the amount of Chl

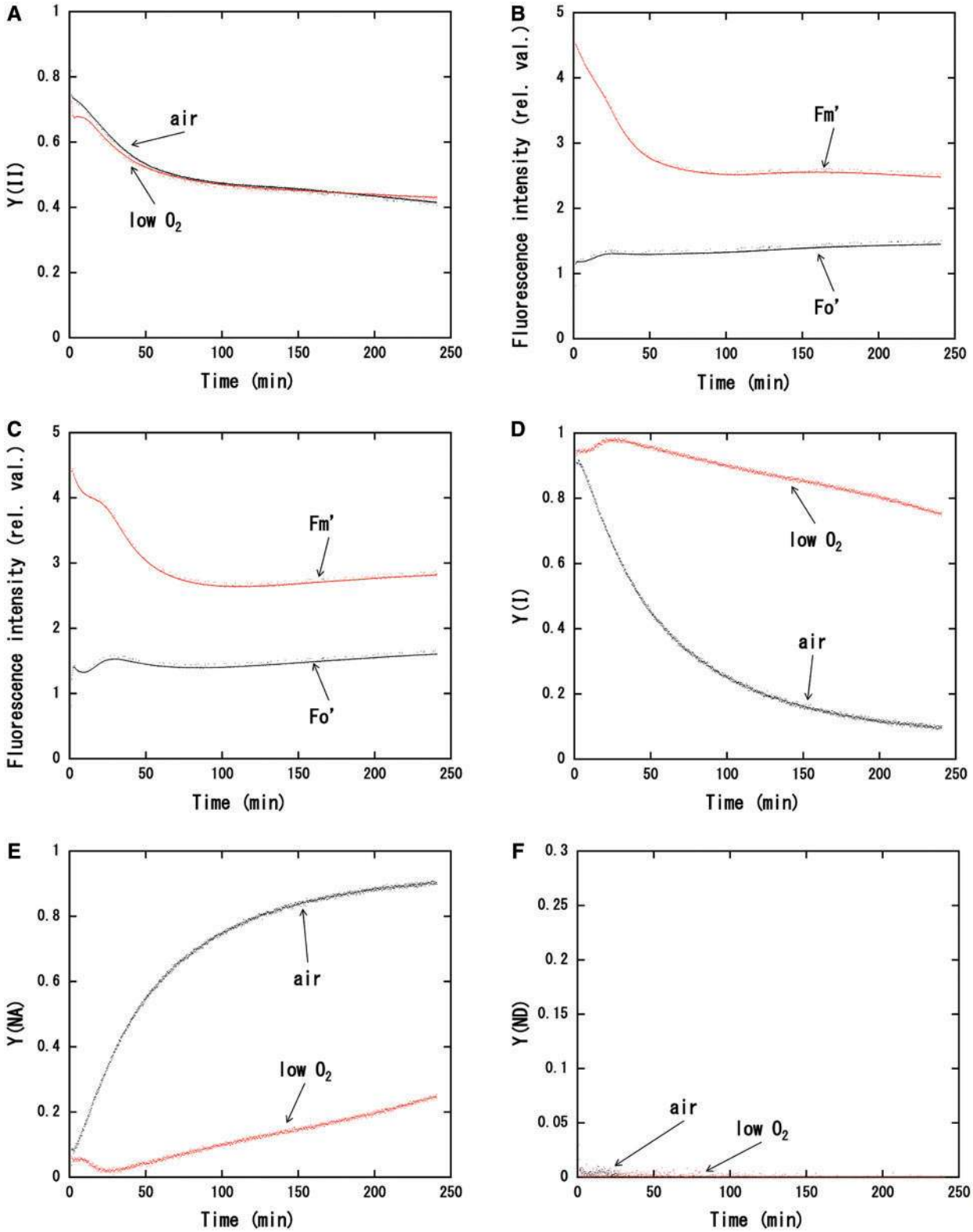

Fig. 1 Effects of repetitive illumination of sunflower leaves with short-pulse (SP) light on Chl fluorescence parameters of PSII and quantum yields of PSI. *Helianthus annuus* leaves were illuminated every 10 s with SP light (300 ms) of 20,000 μmol photons $m^{-2} s^{-1}$ under normal air (40 Pa CO_2/21 kPa O_2) and low O_2 (40 Pa CO_2/1.5 kPa O_2) conditions for 240 min. The repetitive illumination with SP light started at 0 min. (A) Y(II). Black, air; red, low O_2. (B) F_m' and F_o' under normal air conditions. Black, F_o'; red, F_m'. (C) F_m' and F_o' under low O_2 conditions. Black, F_o'; red, F_m'. (D) Y(I). Black, normal air; red, low O_2. (E) Y(NA). Black, normal air; red, low O_2. (F) Y(ND). Black, normal air; red, low O_2. Experiments were repeated three times, and typical results are shown.

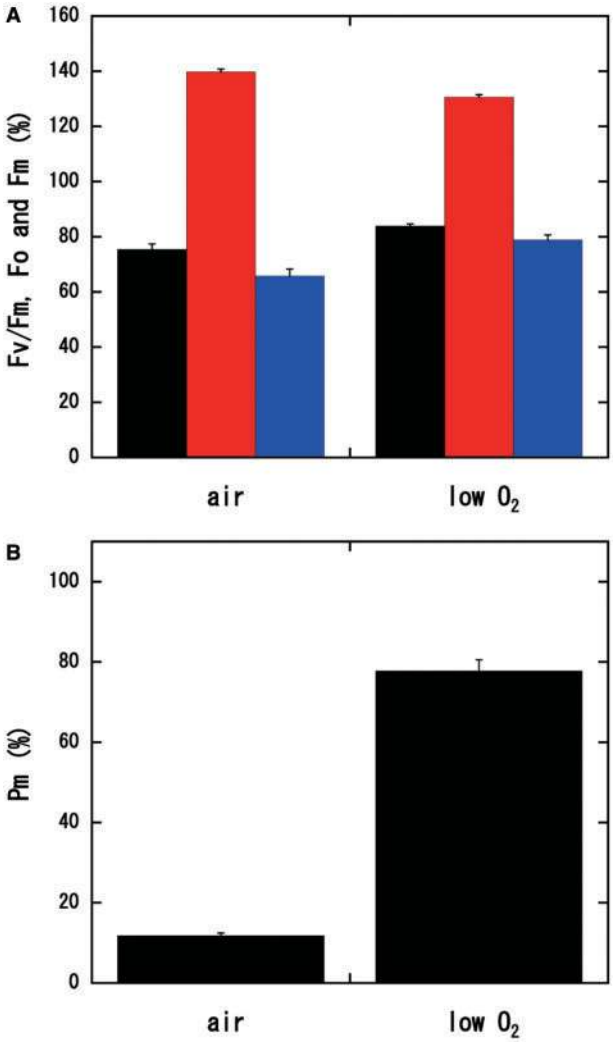

Fig. 2 Effects of repetitive illumination of sunflower leaves with short-pulse (SP) light on PSI and PSII activities, and PSII Chl fluorescence parameters. Sunflower leaves were illuminated every 10 s with SP light (300 ms) of 20,000 μmol photons $m^{-2} s^{-1}$ under normal air (40 Pa CO_2/21 kPa O_2) and low O_2 (40 Pa CO_2/1.5 kPa O_2) conditions for 240 min. (A) F_v/F_m, F_o and F_m under normal air and low O_2 conditions. These parameters were evaluated after the illuminated leaves were left for 1 h in the dark, and their values are shown against the values before the repetitive illumination treatment. F_v/F_m, black bar; F_o, red bars; F_m, blue bars. (B) P_m under normal air and low O_2 conditions. These parameters were evaluated after the illuminated leaves were left for 1 h in the dark, and their relative values are shown against the values before the repetitive illumination treatment. Experiments were repeated three times. Bars show standard errors.

fluorescent components decreased, and the photochemical quenching process in PSII was suppressed, indicating that PSII was photoinhibited (Kramer et al. 2004). Furthermore, the suppression of the photochemical quenching process in PSII might also be caused by the accumulations of electrons in plastoquinone (PQ) by the PSI photoinhibition, as described below. F_v/F_m decreased to about 75% of that in control leaves (**Fig. 2A**). F_v/F_m reflects the slope of the light curve of the photosynthetic CO_2 assimilation rate in the lower range of light intensity. The decrease in F_v/F_m means a decrease in CO_2 assimilation rate (Baker et al. 2007). Furthermore, the large decrease in F_v/F_m accompanied the decrease in the net CO_2 assimilation rate at high CO_2/high light, because the regeneration efficiency of RuBP decreased, which is due to the damage to PSII (Hikosaka et al. 2004). In the present paper, we considered that the decrease in both F_m and F_v/F_m reflects PSII photoinhibition. Lowering pO_2 suppressed the decrease in F_v/F_m by approximately 10% compared with that under normal air conditions (**Fig. 2A**). In contrast to normal air conditions, the decrease in F_m was suppressed (**Fig. 2A**), indicating that the amount of Chl fluorescent components was not lowered. Compared with normal air conditions, the increase in F_o was enhanced (**Fig. 2A**), indicating that the photochemical quenching process in PSII was suppressed. These data suggest that a part of the decrease in PSII components is the attack of ROS on the D1 repair system (Nishiyama et al. 2001) and that ROS affect the conversion process from photon energy to chemical energy driving the electron transport reaction.

However, P_m, showing the maximum amount of P700 photoexcited in the dark, decreased to approximately 10% of that in control leaves (**Fig. 2B**). Lowering pO_2 greatly suppressed the decrease in P_m by approximately 70%. These data show that compared with PSII, PSI mainly suffered from photoinhibition. Furthermore, PSI photoinhibition required O_2. These data show that the increase in Y(NA) during repetitive illumination with SP light reflected PSI photoinhibition, which caused the efficiency of electron flow at the acceptor side of P700 to decrease.

Effects of SP pulse length, intensity and frequency on PSI and PSII activities

We found that PSI and PSII were inactivated by SP light in the presence of 21 kPa O_2, as described above. Following this, we researched the effects of the duration of SP light on PSI photoinhibition. As the duration of SP light decreased from 300 to 10 ms, the inhibition of PSI and PSII was suppressed (**Fig. 3**). In particular, compared with PSII, the inactivation of PSI was greatly suppressed.

Following this, we analyzed the effects of SP light intensity on PSI and PSII photoinhibition (**Fig. 4**). Compared with a light intensity of 20,000 μmol photons $m^{-2} s^{-1}$, photoinhibition of PSI and PSII was more suppressed at a light intensity of 2,000 μmol photons $m^{-2} s^{-1}$. PSII photoinhibition was perfectly suppressed, whereas PSI was photoinhibited to approximately 10% even at 2,000 μmol photons $m^{-2} s^{-1}$.

We then analyzed the frequency of repetitive illumination with SP light on PSI and PSII photoinhibition (**Fig. 5**). As the frequency of SP light decreased from every 10 s to every 60 s, PSI and PSII photoinhibition was suppressed (**Fig. 5**). PSI photoinhibition was more suppressed than PSII photoinhibition.

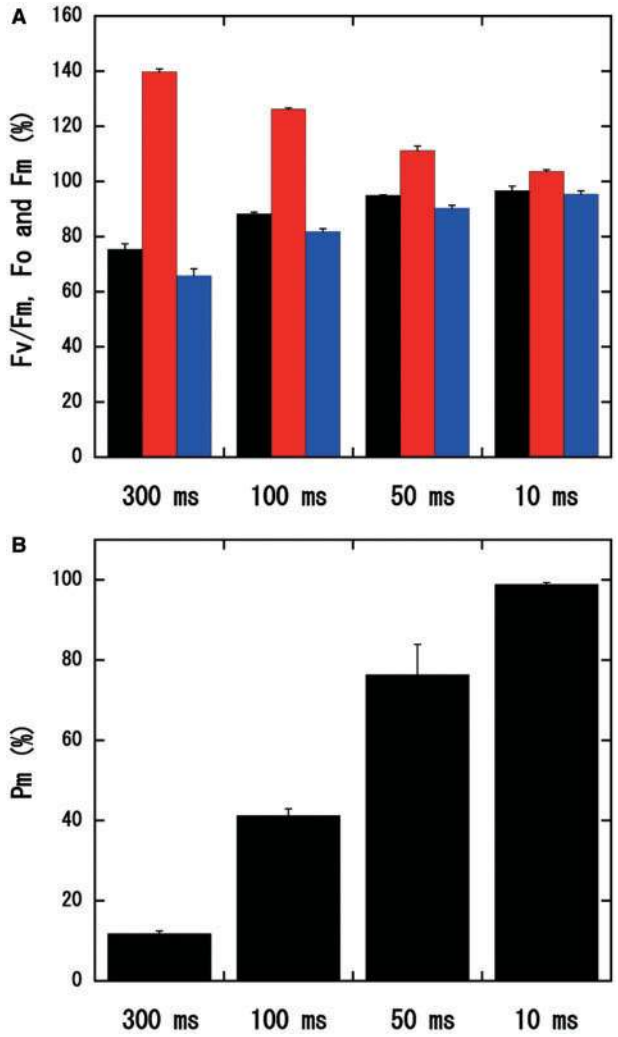

Fig. 3 Effects of duration of repetitive illumination of sunflower leaves with short-pulse (SP) light on PSI and PSII activities, and PSII Chl fluorescence parameters. Sunflower leaves were repetitively illuminated every 10 s with SP light of 20,000 µmol photons $m^{-2} s^{-1}$ under normal air (40 Pa CO_2/21 kPa O_2) conditions for 240 min. (A) F_v/F_m, black bars; F_o, red bars; F_m, blue bars. (B) P_m, black bars. The duration of SP light was changed to 300, 100, 50 and 10 ms. These parameters were evaluated after the illuminated leaves were left for 1 h in the dark, and their relative values are shown against the values before the repetitive illumination treatment. Experiments were repeated three times. Bars show standard errors.

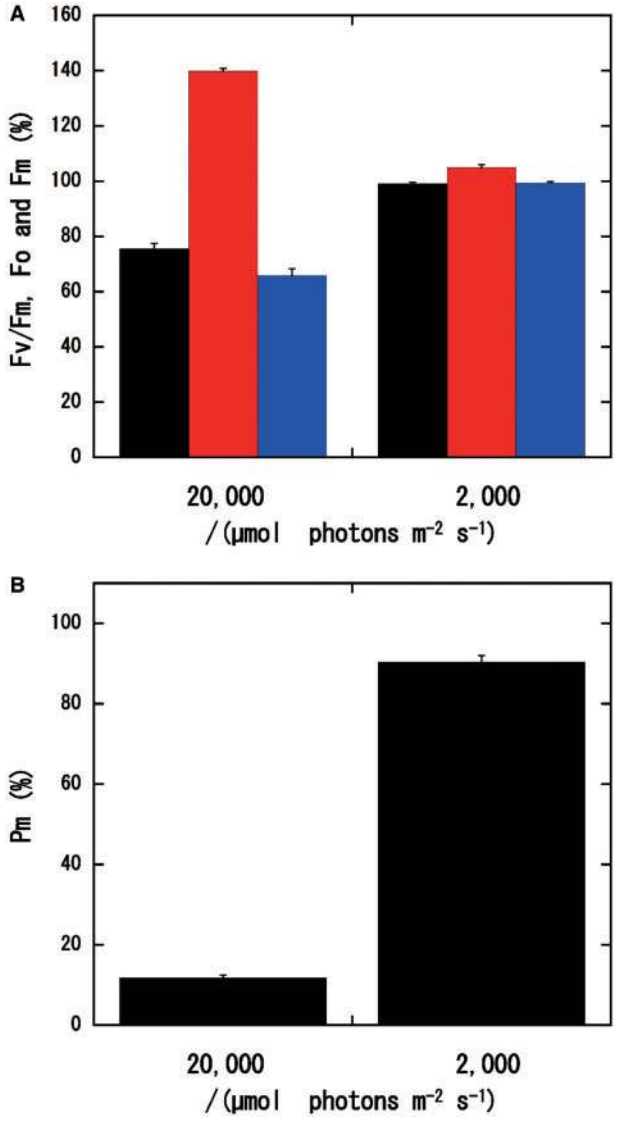

Fig. 4 Effects of intensity of repetitive illumination of sunflower leaves with short-pulse (SP) light on PSI and PSII activities, and PSII Chl fluorescence parameters. Sunflower leaves were repetitively illuminated with SP light under normal air (40 Pa CO_2/21 kPa O_2) conditions for 240 min. The intensity of SP light was changed to 20,000 and 2,000 µmol photons $m^{-2} s^{-1}$. (A) F_v/F_m, black bars; F_o, red bars; F_m, blue bars. (B) P_m, black bars. These parameters were evaluated after the illuminated leaves were left for 1 h in the dark, and their relative values are shown against the values before the repetitive illumination treatment. Experiments were repeated three times. Bars show standard errors.

These results indicate that as the interaction of PSI with O_2 became stronger, PSI was more photoinhibited than PSII.

Effects of Y(ND) on PSI and PSII activities

SP light-dependent PSI photoinhibition required O_2. The P700 Chl reaction center in PSI should be photoexcited, and the P700 charges should be separated by SP light to reduce O_2 to O_2^-. The amount of P700 that can donate electrons to the electron acceptors in PSI is reflected as Y(I). At a steady state of photosynthesis, the following equation holds: Y(I) + Y(ND) + Y(NA) = 1 (Klughammer and Schreiber 1994). We researched the effects of Y(ND) on PSI photoinhibition by changing the intensity of continuous AL. We expected that the increase in Y(ND) would suppress PSI photoinhibition because Y(I) decreases under a steady state. We illuminated sunflower leaves with AL and repetitive SP light simultaneously for 240 min (**Fig. 6**).

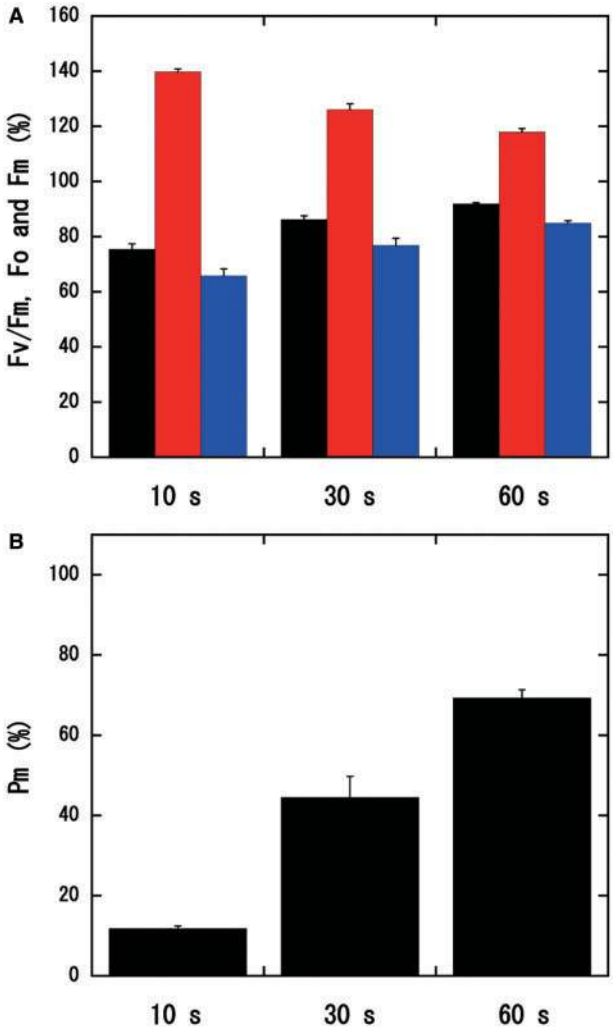

Fig. 5 Effects of frequency of repetitive illumination of sunflower leaves with short-pulse (SP) light on PSI and PSII activities, and PSII Chl fluorescence parameters. Sunflower leaves were repetitively illuminated with SP light (300 ms) of 20,000 μmol photons $m^{-2} s^{-1}$ under normal air (40 Pa CO_2/21 kPa O_2) conditions for 240 min. The frequency of repetitive illumination with SP light was changed every 10, 30 and 60 s. (A) F_v/F_m, black bars; F_o, red bars; F_m, blue bars. (B) P_m, black bars. These parameters were evaluated after the illuminated leaves were left for 1 h in the dark, and their relative values are shown against the values before the repetitive illumination treatment. Experiments were repeated three times. Bars show standard errors.

As the intensity of AL increased, Y(ND) increased (**Fig. 6A**), which reflected that the photooxidation rate of P700 became larger than the reduction rate of the oxidized P700 in PSI. Furthermore, as the AL intensity increased, NPQ of Chl fluorescence increased (**Fig. 6B**), suggesting that excess photon energy against photosynthesis was dissipated as heat in PSII (Baker 2008). After the light treatment, PSII photoinhibition did not depend on AL intensity (**Fig. 6C**), whereas PSI photoinhibition was suppressed at a higher AL intensity (**Fig. 6D**). These results show that the amount of PSI suffering from inactivation by the repetitive SP light was determined by the amount of P700 that could donate electrons to O_2 in PSI. PSI with the reduced reaction center is sensitive to repetitive SP illumination in the presence of O_2

Effects of inactivating PSI on net CO_2 assimilation

Repetitive illumination with SP light mainly inactivated PSI. Following this, we analyzed the effects of PSI photoinhibition on steady-state photosynthesis (**Fig. 7**). Repetitive illumination of sunflower leaves with SP light under normal air conditions decreased the steady-state rate of photosynthesis to approximately 20% of that in untreated leaves. In contrast, treatment of sunflower leaves with 1.5 kPa O_2 decreased the photosynthetic rate to approximately 50% of that in untreated leaves. Furthermore, decreasing the SP light intensity lowered the photosynthetic rate to approximately 90% of that in untreated leaves. These results indicate that PSI photoinhibition greatly contributed to inhibiting the net CO_2 assimilation. Even a 10% inactivation of PSI affected the net CO_2 assimilation (**Figs. 4**, **7**).

Discussion

We researched the effects of repetitive illumination with SP light on photosynthetic electron flow, where the duration of SP light was set in the range of 10–300 ms, as observed in sunfleck in field conditions. We found that the repetitive illumination by SP light induced photoinhibition of PSI (**Fig. 2**). On the other hand, the increase in Y(ND) due to AL illumination suppressed the photoinhibition of PSI during the repetitive illumination of the leaves by SP light (**Fig. 6**). Illumination by AL induces a ΔpH across thylakoid membranes by both PLEF and CEF-I, which results in the oxidation of P700, as observed in the increase in Y(ND). The acidification of the luminal side of thylakoid membranes decreases the oxidation activity of plastoquinol by the Cyt b_6/f complex (Tikkanen et al. 2012) and induces NPQ of Chl fluorescence in PSII (Niyogi 2000). Under these conditions, the electron flux in PSII is down-regulated, and the photooxidation rate of P700 in PSI exceeds the reduction rate of P700 (Miyake et al. 2005). Thus, the increase in Y(ND) shows the decrease in P700 which can drive electron flow, as reflected in a decrease in Y(I) (Klughammer and Schreiber 1994). We found that the photoinhibition of PSI required O_2 (**Figs. 1**, **2**). These facts show that the increase in the amount of P700 to donate electrons to O_2 enhances PSI photoinhibition during repetitive illumination by SP light.

Under field conditons, the leaves of plants exposed to sunfleck might suffer from PSI photoinhibition, unless the ΔpH across thylakoid membranes is induced and the oxidized P700 is accumulated. We decreased the intensity of SP light from 20,000 to 2,000 μmol photons $m^{-2} s^{-1}$, which is close to the light intensity of the sun. This lower intensity of SP light also induced PSI photoinhibition. These results indicate that plants grown under natural conditions may suffer from PSI photoinhibition by SP light from the sun. For example, plants grown in

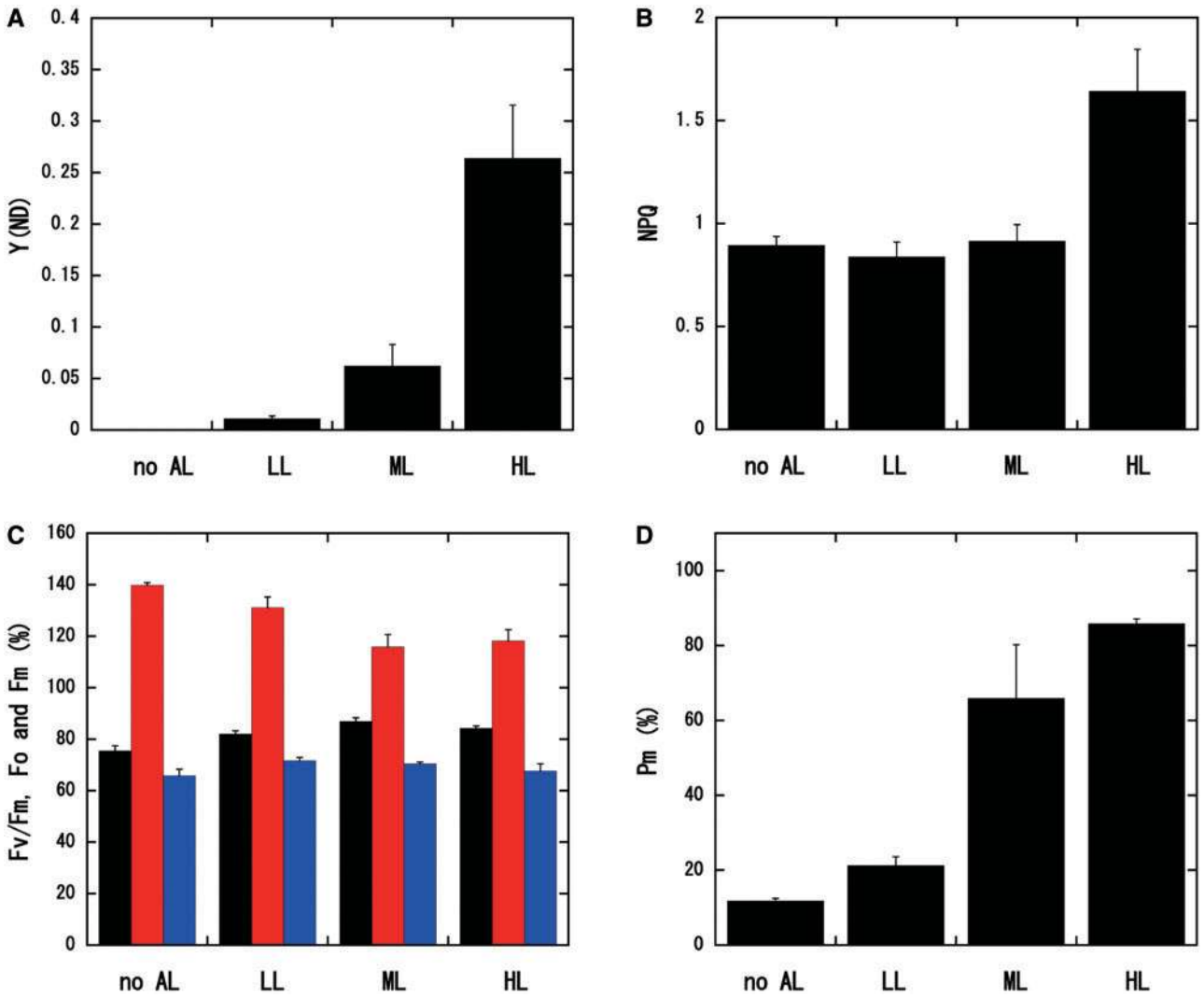

Fig. 6 Effects of actinic light (AL) on Y(ND), NPQ, PSI and PSII activities, and PSII Chl fluorescence parameters. Sunflower leaves were illuminated every 10 s with short-pulse (SP) light (300 ms) of 20,000 µmol photons $m^{-2} s^{-1}$ under normal air (40 Pa CO_2/21 kPa O_2) conditions for 240 min. (A) Y(ND). AL (no AL, 0 µmol photons $m^{-2} s^{-1}$; LL, 200; ML, 580; HL, 1,400) was illuminated during repetitive illumination with SP light. Y(ND) was determined at 240 min during the repetitive illumination treatment in **Fig. 1**. (B) Non-photochemical quenching (NPQ). AL was illuminated during repetitive illumination with SP light. NPQ was determined at 240 min during the repetitive illumination treatment in **Fig. 1**. (C) F_v/F_m, black bars; F_o, red bars; F_m, blue bars. AL was illuminated during repetitive illumination with SP light. (D) P_m, black bars. These parameters were evaluated after the illuminated leaves were left for 1 h in the dark, and their relative values are shown against the values before the repetitive illumination treatment. Experiments were repeated three times. Bars show standard errors.

the understorey of crop fields or forests repetitively receive pulse light from the sun as sunfleck (Pearcy 1983, Chazdon 1988, Knapp and Smith 1989, Vierling and Wessman 2000). Under these conditions, PSI might be damaged in the same manner as the present photoinhibition. The effects of sunfleck on PSI activity should be clarified in future work.

The decrease in the amount of P700 which is photoexcited by SP light leads to the suppression of PSI photoinhibition, as observed in the increase in Y(ND) (**Fig. 6**): PSI photoinhibition itself decreases the photoreduction of O_2 to O_2^- and suppresses the acceleration of PSI photoinhibition. How much the decrease in PSI content contributes to the protection of the photosynthetic machinery in chloroplasts from the oxidative attack by ROS is an important question to answer in order to elucidate the physiological significance of PSI photoinihibition.

In the present work, we illuminated the leaves with SP light using measuring lights (MLs), which are used to monitor both Chl fluorescence and $P700^+$ in the Dual-PAM system (Pfündel et al. 2008). MLs excite both PSI and PSII reaction centers and drive photosynthetic linear electron flow at the light-limited rate of photosynthesis. That is, the PQ pool is kept oxidized and the Chl fluorescence yield is at the minimum to yield the F_o level (Schreiber et al. 1986, Genty et al. 1989, Kramer et al. 2004). Furthermore, we showed the requirement of electrons for PSI

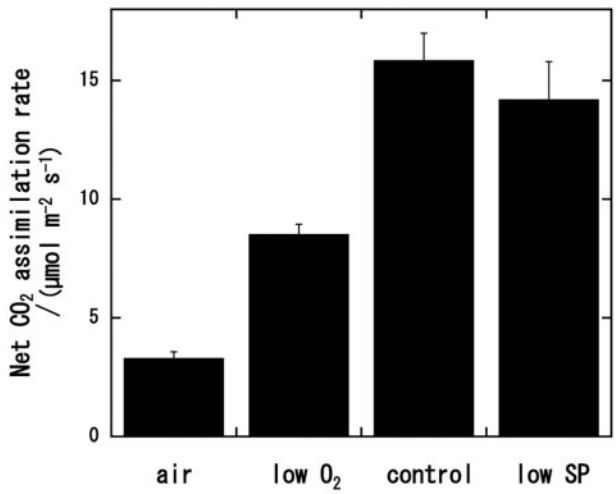

Fig. 7 Effects of repetitive illumination of sunflower leaves with short-pulse (SP) light on the net CO_2 assimilation rate. Air: Sunflower leaves were illuminated every 10 s with SP light (300 ms) of 20,000 μmol photons $m^{-2}s^{-1}$ under 40 Pa CO_2/21 kPa O_2 conditions for 240 min. Low O_2: Sunflower leaves were illuminated every 10 s with SP light of 20,000 μmol photons $m^{-2}s^{-1}$ under 40 Pa CO_2/1.5 kPa O_2 conditions for 240 min. Control: untreated sunflower leaves. Low SP: Sunflower leaves were illuminated every 10 s with SP light (300 ms) of 2,000 μmol photons $m^{-2}s^{-1}$ under 40 Pa CO_2/21 kPa O_2 conditions for 240 min. Net CO_2 assimilation rates of the leaves at steady state were measured under 400 μmol photons $m^{-2}s^{-1}$ of actinic light (AL) and 100 Pa CO_2/21 kPa O_2 conditions. Experiments were repeated three times. Bars show standard errors.

photoinhibition to photoreduce O_2 to O_2^-. The MLs might contribute to the photoreducion of O_2. Similarly to MLs, the electron flow to PQ such as chlororespiration (Rumeau et al. 2007) might also contribute to the photoreducion of O_2. On the other hand, the use of MLs in the SP treatment provides information about the parameters for Chl fluorescence {the incident quantum yield of PSII [Y(II) and NPQ)] and of PSI [Y(I), Y(NA) and Y(ND)]}. These parameters provide information on what happens in the PET system during the SP light treatments.

Regulating the excitation balance between PSI and PSII reaction centers might be important for the protection of PSI from photodamage. State transition controls the distribution of photon energy to both reaction centers by phosphorylation of light-harvesting Chl complex II (LHCII) and PSII core proteins (Bellafiore et al. 2005, Dietzel et al. 2011). In the *stn7* mutant of *A. thaliana*, which is deficient in LHCII phosphorylation activity, both the PET system and the acceptor side of PSI are more reduced in the transition from low light to high light conditions, compared with the wild type (Grieco et al. 2012). Furthermore, the *stn7* mutant showed a decrease in the amount of PSI, compared with the wild type, under fluctuating light growth conditions. These data show that the *stn7* mutant suffered from the oxidative degradation of PSI by the attack of ROS, on the basis of the present work.

Under constant light, PSI of thylakoid membranes in chilling-sensitive plants suffers from photoinhibition at low temperature (Terashima et al. 1994, Sonoike 2011). If the PLEF proceeds at the acceptor-limited rate at the chilling temperature, where P700 would be kept reduced, the possibility to produce ROS at PSI would increase. Then, PSI would be oxidatively damaged by ROS, leading to photoinhibition.

Materials and Methods

Plant materials

Sunflower (*Helianthus annuus*) plants were grown from seeds under standard air-equilibrated conditions with 25°C/20°C, light (14 h)/dark (10 h) cycles, 50–60% relative humidity, and 400 μmol photons $m^{-2}s^{-1}$ photon flux density. Seeds were sown in 0.8 dm^3 pots containing commercial peat-based compost. Plants were watered daily and fertilized (Hyponex 8-12-6; Hyponex Japan) five times a week. All measurements were made using the sixth to seventh fully expanded leaf 6–7 weeks after sowing. Measurements of photosynthetic parameters were initiated 4 h after the start of the dark period. All plants were adapted to the dark for >1 h before measurements.

Chl fluorescence, P700 and gas exchange measurements

Chl fluorescence, P700 and CO_2 exchange were simultaneously measured with Dual-PAM-100 (Heintz Walz) and IRGA measuring systems (LI-7000; Li-COR) equipped with a 3010-DUAL gas exchange chamber (Heintz Walz). Atmospheric gas (40 Pa CO_2/21 kPa O_2) and gas with the indicated mixture of pure O_2 and CO_2 prepared by mixing 20.1% (v/v) in 79.9% (v/v) N2 and 1% (v/v) CO_2 in 99% (v/v) N2 using a mass-flow controller (Kofloc model 1203; Kojima Instruments Co.) was used in gas exchange analysis. The gases was saturated with water vapor at 13.5 ± 0.1°C. The AL intensity at the upper position of the leaf in the chamber was adjusted to the indicated intensity. The leaf temperature was controlled at 25°C. Chl fluorescence parameters were calculated (Baker 2008) as follows: maximum quantum efficiency of PSII photochemistry, $F_v/F_m = (F_m - F_o)/F_m$; quantum yield of photochemical energy conversion in PSII, $Y(II) = (F_m' - F_s)/F_m'$; non-photochemical quenching, $NPQ = (F_m - F_m')/F_m'$; F_o, minimum fluorescence yield; F_m, maximum fluorescence yield; F_m', maximum variable fluorescence yield; F_s, steady-state fluorescence yield. The oxidation–reduction state of $P700^+$ was determined according to the methods of Klughammer and Schreiber (1994) and Pfündel et al. (2008) using the following acronyms: maximum oxidation level of P700 obtained by saturated pulse light under far-red illumination, P_m, which reflects the maximum amount of photooxidized P700; oxidation level of P700 under AL, P; maximum oxidation level of P700 obtained by saturated pulse light under AL illumination, P_m'; quantum yield of photochemical energy conversion,

$Y(I) = (P_m' - P)/P_m$; quantum yield of non-photochemical energy dissipation due to donor-side limitation, $Y(ND) = P/P_m$; quantum yield of non-photochemical energy dissipation due to acceptor-side limitation, $Y(NA) = (P_m - P_m')/P_m$. The three parameters sum up to 1; $Y(I) + Y(NA) + Y(ND) = 1$.

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Disclosures

The authors have no conflicts of interest to declare.

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