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ORIGINAL ARTICLE

PSI photoinhibition is more related to electron transfer from PSII to PSI rather than PSI redox state in *Psychotria rubra*

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Abstract Although it has been believed that wild-type plants are capable of protecting photosystem I (PSI) under high light, our previous study indicates that PSI is sensitive to high light in the shade-established tree species *Psychotria rubra*. However, the underlying physiological mechanisms are unclear. In this study, we examined the roles of electron transfer from PSII to PSI and PSI redox state in PSI photoinhibition in *P. rubra* by treatments with lincomycin (Lin), diuron (DCMU), and methyl viologen (MV). After exposure to 2000 μmol photons m^{-2} s^{-1} for 2 h, PSI activity decreased by 35, 29, 3, and 49 % in samples treated with H_2O, Lin, DCMU, and MV, respectively. Meanwhile, the MV-treated samples showed higher P700 oxidation ratio than the H_2O-treated samples, suggesting the PSI photoinhibition under high light was accompanied by high levels of P700 oxidation ratio. PSI photoinhibition was alleviated in the DCMU-treated samples but was accelerated in the MV-treated samples, suggesting that PSI photoinhibition in *P. rubra* was mainly controlled by electron transfer from PSII to PSI. Taking together, PSI photoinhibition is more related to electron transfer from PSII to PSI rather than PSI redox state in *P. rubra*, which is different from the mechanisms of PSI photoinhibition in *Arabidopsis thaliana* and cucumber.

Keywords Electron transfer · High light · PSI photoinhibition · P700 redox state · Shade-established species

Introduction

Under conditions in which absorbed light is in excess of the requirements for photosynthesis, photoinhibition occurs in leaves (Powles 1984). In wild-type plants, high light usually causes selective photodamage of photosystem II (PSII) (Barber and Andersson 1992; Aro et al. 1993). It is generally recognized that that wild-type plants are capable of protecting photosystem I (PSI) under high light (Barth and Krause 1999; Barth et al. 2001; Munekage et al. 2002; Suorsa et al. 2012; Tikkanen et al. 2014), excluding at chilling temperature (Havaux and Davaud 1994; Terashima et al. 1994; Sonoike 1995; Barth and Krause 1999; Zhang and Scheller 2004). However, our recent study indicated that the shade-established species *Psychotria rubra* displayed significantly PSI photoinhibition under high light at 25 °C (Huang et al. 2015a). Cyclic electron flow (CEF) around PSI prevents PSI from photoinhibition under high light at normal temperatures in the model plant *Arabidopsis thaliana* (Munekage et al. 2002, 2004; Suorsa et al. 2012; Kono et al. 2014). However, *P. rubra* showed highly activation of CEF under high light (Huang et al. 2015a). These results suggest that the mechanism of PSI photoinhibition may be different between *P. rubra* and *A. thaliana*.

A reaction between reduced iron–sulfur centers and hydroxyl peroxide generates hydroxyl radicals that cause

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oxidative damage to PSI complexes (Sonoike 1995, 2006, 2011). Therefore, over-reduction of PSI acceptor side and production of hydroxyl peroxide at PSI acceptor side are regarded as two main causes of PSI photoinhibition (Sonoike et al. 1997; Munekage et al. 2002; Tikkanen et al. 2014). At chilling temperature, the water–water cycle induces production of hydroxyl peroxide at PSI acceptor side, which further leads to PSI photoinhibition in chilling-sensitive species such as cucumber, spinach, and *Arabidopsis thaliana* (Havaux and Davaud 1994; Terashima et al. 1994; Sonoike 1995, 1996; Kudoh and Sonoike 2002; Hwang et al. 2004; Zhang and Scheller 2004). Once the electron transfer from PSII to PSI was blocked with DCMU and DBMIB, photoinhibition of PSI was not observed in chilled potato, cucumber, and spinach (Sonoike 1996). At normal temperature (for example at 25 °C), high light induces significantly PSI photoinhibition in *pgr5* (a CEF-deficient mutant) plants of *Arabidopsis thaliana* (Munekage et al. 2002; Suorsa et al. 2012). However, the sensitivity of PSI to high light in *pgr5*-mutant could be avoided when electron flow from PSII to PSI was limited in the addition of DCMU (Suorsa et al. 2012) or upon moderate PSII photoinhibition (Tikkanen et al. 2014). These results mean that excess electron flow from PSII is essential for photoinhibition of PSI. Our previous study indicated that in *P. rubra*, after exposure to 2000 μmol photons m^{-2} s^{-1} for 2 h, electron flow from PSII to PSI was depressed due to severe PSII photoinhibition, and PSI activity decreased very slightly during further high light treatment. This result also suggests that PSI photoinhibition in *P. rubra* is mainly dependent on the active electron flow from PSII to PSI.

Additionally, P700 redox state is another important factor determining the response of PSI to high light (Munekage et al. 2002). The wild-type and *pgr5* plants of *Arabidopsis thaliana* showed similar linear electron flow under high light (Takahashi et al. 2009; Kono et al. 2014), but the P700 redox state under high light differed largely between them (Munekage et al. 2002, 2004). In *pgr5* plants, P700 oxidation ratio was nearly zero under all light intensities (Munekage et al. 2002, 2004). By comparison, the WT plants had high levels of P700 oxidation ratio when exposed to high light (Munekage et al. 2002, 2004; Kono et al. 2014; Kou et al. 2015). These results indicated that PSI acceptor was over-reduced in *pgr5* plants being exposed to high light. Once the over-reduction of PSI acceptor side was alleviated, PSI activity in *pgr5* plants was insusceptible to high light (Suorsa et al. 2012; Tikkanen et al. 2014). These results indicate that over-reduction of PSI acceptor side is the main reason for PSI photoinhibition in *pgr5* plants exposed to high light. In the shade-established species *P. rubra*, P700 oxidation ratio was maintained at high levels during exposure to a strong light of 2000 μmol photons m^{-2} s^{-1}, but PSI activity was still photoinactivated at this high light (Huang et al. 2015a). As a result, we hypothesize that high-light-induced PSI photoinhibition in *P. rubra* is less related to P700 redox state.

To further understand the mechanisms of PSI photoinhibition under high light in the shade-established tree species *P. rubra*, we examined the responses of PSI activity and P700 redox state to a strong light of 2000 μmol photons m^{-2} s^{-1} in the presence of lincomycin (Lin), diuron (DCMU), and methyl viologen (MV). We found that DCMU protected PSI activity by blocking electron transfer from PSII to PSI. Furthermore, MV enhanced PSI photoinhibition rather than protected PSI from photoinhibition. Therefore, our results strongly suggest that PSI photoinhibition is more related to electron transfer from PSII to PSI rather than PSI redox state in *P. rubra*. The differences in mechanisms of PSI photoinhibition between *P. rubra* and *Arabidopsis thaliana* were discussed.

Materials and methods

Plant materials

The tropical tree species *Psychotria rubra* (Lour.) Poir. (Rubiaceae) was studied in our present study. *P. rubra* is a shade-established shrub species of rain forests native to south of China, Indonesia, Vietnam, Laos, and Malaysia. In the present study, we used plants of *P. rubra* grown naturally in tropical rain forest in Xishuangbanna tropical botanical garden (21°54′N, 101°46′E) that is located in the northern boundary of tropical zone. Photosynthetic parameters were measured in mature leaves grown at an understory environment with deep shade (light intensity <5 % of sunlight).

PSI and PSII measurements

In our present study, the PSI and PSII parameters were measured simultaneously by Dual-PAM-100 (Heinz Walz GmbH, Effeltrich, Germany). To measure the light responses of the steady-state PSI and PSII parameters at various light intensities, intact mature leaves were light adapted at a high light (1033 μmol photons m^{-2} s^{-1}) for 25 min. Afterward, photosynthetic parameters were evaluated at 2-min intervals at photosynthetic photon flux densities (PPFDs) of 1957, 1599, 1292, 830, 536, 344, 221, 100, 42, and 11 μmol photons m^{-2} s^{-1}.

The maximum quantum yield of PSII, $F_v/F_m = (F_m - F_o)/F_m$, was measured to monitor PSII activity. Other fluorescence parameters were calculated as follows

(Genty et al. 1989; Hendrickson et al. 2004; Kramer et al. 2004): $Y(II) = (F_m' - F_s)/F_m'$; $Y(NPQ) = F_s/F_m' - F_s/F_m$; $Y(NO) = F_s/F_m$; $NPQ = (F_m - F_m')/F_m'$. Y(II) represents the effective quantum yield of PSII; Y(NPQ) and Y(NO) represent the quantum yield of regulated and non-regulated energy dissipation in PSII, respectively. NPQ indicates the non-photochemical quenching. F_o and F_m are the minimum and maximum fluorescence measured after 30 min dark adaptation. F_m' is the maximum fluorescence after light adaptation and measured upon illumination of a saturating pulse (300 ms and 10,000 μmol photons m^{-2} s^{-1}), F_s is the steady-state fluorescence after light adaptation. Note that $Y(II) + Y(NPQ) + Y(NO) = 1$.

The PSI photosynthetic parameters were evaluated by Dual PAM-100, based on P700 oxidation signal (i.e., the difference in intensities of 830 and 875 nm pulse-modulated measuring light reaching the photodetector) (Klüghammer and Schreiber 2008). The $P700^+$ signals (P) may vary between a minimal (P700 fully reduced) and a maximal level (P700 fully oxidized). The maximum level (P_m) was determined with application of a saturation pulse (300 ms and 10,000 μmol photons m^{-2} s^{-1}) after pre-illumination with far-red light (Klüghammer and Schreiber 1994, 2008). P_m' was determined similar to P_m but with actinic light instead of far-red light. P_m was recorded to estimate the maximum photo-oxidizable P700 (Huang et al. 2010a, 2010b, 2013; Suorsa et al. 2012; Tikkanen et al. 2014). In our present study, P_m was measured after 20 min dark adaptation. The quantum yield of PSI was calculated as $Y(I) = (P_m' - P)/P_m$, and the P700 oxidation ratio in a given actinic light was calculated as $Y(ND) = P/P_m$. The fraction of P700 that cannot be oxidized by an SP to the overall P700 was calculated as $Y(NA) = (P_m - P_m')/P_m$. Note that $Y(I) + Y(ND) + Y(NA) = 1$.

Photoinhibitory treatments

To determine the roles of electron transfer from PSII and PSI redox state in PSI photoinhibition in *P. rubra*, detached leaves were infiltrated with H_2O, Lin (1 mM), DCMU (70 μM), and MV (300 μM) for 3 h in darkness and then exposed to 2000 μmol photons m^{-2} s^{-1} at 25 °C for 2 h. Subsequently, Y(ND) and Y(NA) were measured after 3 min adaptation at 1804 μmol photons m^{-2} s^{-1} and 25 °C. To examine the effect of DCMU on electron transport through PSII, the light induction of Y(II) at 606 μmol photons m^{-2} s^{-1} was measured in detached leaves infiltrated with H_2O or DCMU for 3 h in darkness. The photoinhibitory treatments were conducted in our laboratory with air temperature being controlled at 25 °C.

Statistical analysis

The results were displayed as mean values of six independent experiments. The data were subjected to analysis of variance (ANOVA) using the SPSS 16.0 statistical software. Tukey's multiple comparison test was used at $\alpha = 0.05$ significance level to determine whether significant differences exist among different treatments.

Results

Cyclic electron flow in *P. rubra*

In higher plants, the NDH and PGR5 pathways explained most of the CEF judging from the phenotype of double mutants (Shikanai et al. 1998; Munekage et al. 2002; Johnson 2011). The transient post-illumination increase in chlorophyll fluorescence has been documented as a reliable method to monitor NDH activity (Shikanai et al. 1998; Yamori et al. 2011), as a result of NDH-dependent reduction of the plastoquinone pool in darkness (Shikanai et al. 1998; Yamori et al. 2011). In wild type of *Arabidopsis thaliana*, tobacco, and rice, the transient increase in chlorophyll fluorescence was observed. However, in mutants lacking in NDH activity, the transient increase in chlorophyll fluorescence was not observed. In *P. rubra*, the transient increase in chlorophyll fluorescence was observed (Fig. 1), suggesting that NDH cyclic pathway maybe present in leaves of *P. rubra*. However, we cannot conclude that the studied species *Psychotria rubra* has NDH-dependent cyclic pathway for lack of *ndh* mutant data.

Light response curves indicated that Y(I) and Y(II) gradually declined with increasing light intensity (Fig. 2a, b). Meanwhile, with the increase in PPFD, Y(ND) and Y(NPQ) markedly increased (Fig. 2a, b). From 11 to 830 μmol photons m^{-2} s^{-1}, Y(NA) gradually decreased with increasing light intensity (Fig. 2a). At a high light of 1957 μmol photons m^{-2} s^{-1}, values for Y(ND) and Y(NA) were 0.83 and 0.08, respectively. With the increase in light intensity, Y(NO) changed slightly and was maintained at approximately 0.23 (Fig. 2b). The value of NPQ largely increased when light intensity increased from 11 to 536 μmol photons m^{-2} s^{-1} (Fig. 2c). At PPFDs >536 μmol photons m^{-2} s^{-1}, NPQ changed slightly. At a low light <100 μmol photons m^{-2} s^{-1}, the value of Y(I)/Y(II) was maintained at low levels being approximately 0.7. However, the value of Y(I)/Y(II) reached to 1.9 at 830 μmol photons m^{-2} s^{-1} and increased slightly under higher PFFDs (Fig. 2c). Because a high level of Y(ND) and a low level of Y(NA) is dependent on PGR5 cyclic pathway (Munekage

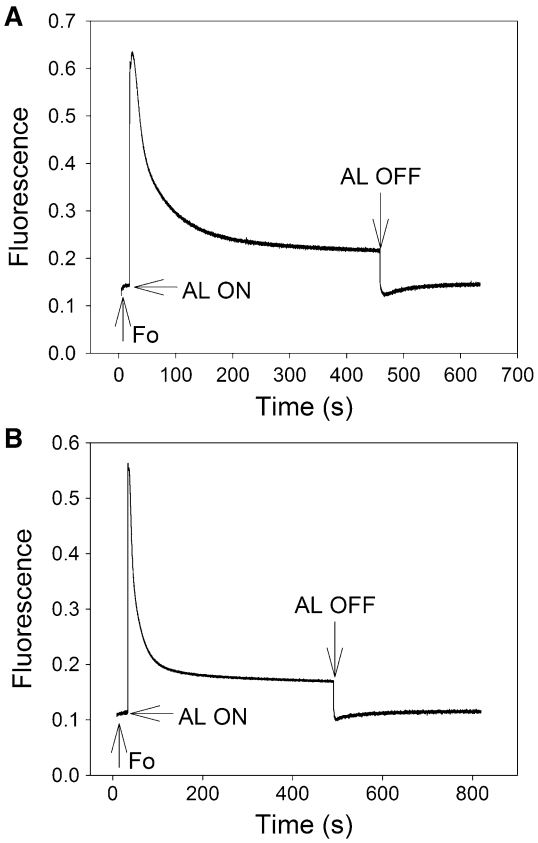

Fig. 1 Monitoring of NDH cyclic pathway by chlorophyll fluorescence analysis. The *curve* shows a typical trace of chlorophyll fluorescence in the mature leaves of *Psychotria rubra*. A mature leaf of *Psychotria rubra* was exposed to actinic light (AL) (**a** 209 μmol photons m^{-2} s^{-1}; **b** 1804 μmol photons m^{-2} s^{-1}) after the measuring light was turned on (F_o, the minimum level of chlorophyll fluorescence). The AL was turned off and the subsequent change in chlorophyll fluorescence was monitored as an indicator of NDH cyclic pathway. The mature leaf was dark-adapted at 25 °C for 30 min, and the chlorophyll fluorescence was measured at 25 °C

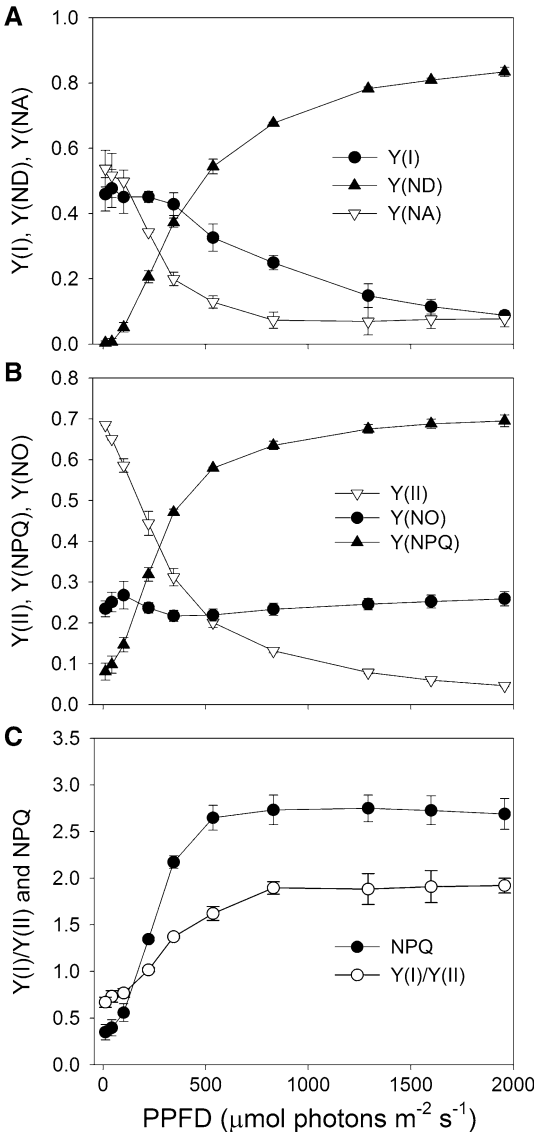

Fig. 2 Light response changes in PSI and PSII parameters in *Psychotria rubra* measured at 25 °C. **a** Y(I), Y(ND), and Y(NA); **b** Y(II), Y(NPQ), and Y(NO); **c** Y(I)/Y(II) ratio and NPQ. The mean ±SE were calculated from six independent plants. Y(II), effective quantum yield of PSII; Y(NPQ), quantum yield of regulated energy dissipation in PSII; Y(NO), quantum yield of non-regulated energy dissipation in PSII; Y(I), effective quantum yield of PSII; Y(ND), fraction of overall P700 that is oxidized in a given state; Y(NA), fraction of overall P700 that cannot be oxidized in a given state

et al. 2002, 2004; Suorsa et al. 2012; Kono et al. 2014), the light response changes in Y(ND) and Y(NA) suggested the presence of PGR5 cyclic pathway in *P. rubra*. Furthermore, the light response change in Y(I)/Y(II) ratio also implied the activation of PGR5 cyclic pathway under high light (Yamori et al. 2011; Kono et al. 2014).

Effects of Lin, DCMU, and MV on P700 redox state under high light

After exposure to 2000 μmol photons m^{-2} s^{-1} at 25 °C for 2 h, values for Y(I) in detached leaves treated with H_2O, Lin, DCMU, and MV were 0.064, 0.046, 0.035, and 0.024, respectively (Fig. 3a). Meanwhile, all these samples showed high levels of Y(ND) (>0.85) at 1804 μmol photons m^{-2} s^{-1} (Fig. 3a). The samples treated with DCMU and MV had significantly higher Y(ND) than the H_2O-treated samples. The value of Y(NA) at 1804 μmol photons m^{-2} s^{-1} was maintained at low levels in samples treated with H_2O, Lin, and MV. Interestingly, Y(NA) at 1804 μmol photons m^{-2} s^{-1} was zero in the DCMU-treated samples.

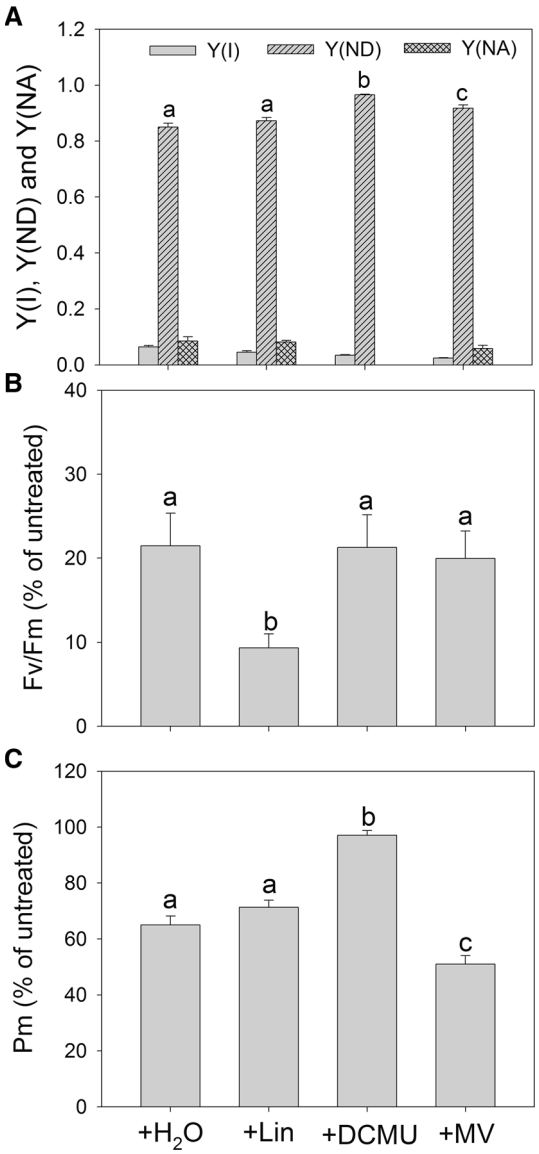

Fig. 3 Effects of lincomycin (Lin), DCMU, methyl viologen (MV) on P700 redox state (**a**), F_v/F_m (**b**), and P_m (**c**) in leaves of *Psychotria rubra*. After infiltration with chemical reagents (H_2O, Lin, DCMU, MV) in darkness for 3 h, leaf samples were illuminated at 2000 μmol photons m^{-2} s^{-1} and 25 °C for 2 h in the presence of these chemical solutions. Subsequently, values for Y(I), Y(ND), Y(NA), F_v/F_m, and P_m were measured as described in "Materials and methods." The mean ±SE were calculated from six independent plants. Different letters indicate significant differences among the different treatments ($P < 0.05$, One-way ANOVA)

Effects of Lin, DCMU, and MV on photoinhibition of PSI and PSII under high light

After 2 h exposure to 2000 μmol photons m^{-2} s^{-1} at 25 °C, F_v/F_m decreased by approximately 80 % in samples treated with H_2O, DCMU, and MV (Fig. 3b). Meanwhile, F_v/F_m decreased by 91 % in the Lin-treated samples (Fig. 3b). In samples treated with H_2O, Lin, DCMU, and MV, the value of P_m decreased by 35, 29, 3, and 49 %, respectively (Fig. 3c), suggesting the sensitivity of PSI to high light in *P. rubra*. Infiltration with DCMU significantly protected PSI activity against photoinhibition in *P. rubra* when exposed to high light. However, infiltration with MV significantly enhanced PSI photoinhibition rather than protected PSI against photoinhibition. After infiltration with DCMU in darkness for 3 h, the electron flow through PSII was blocked compared with the H_2O-treated samples (Fig. S1). Because DCMU inhibits the production of reactive oxygen species (ROS) at the acceptor side of PSI, and MV accepts electrons from PS I and reduces oxygen to the superoxide anion radical, these results strongly suggest that the extent of PSI photoinhibition in *P. rubra* can be significantly affected by the production of ROS at the acceptor side of PSI.

Discussion

In this study, we examined the roles of electron transfer from PSII to PSI and P700 redox state in determining PSI photoinhibition in *P. rubra*. We found that photoinhibition of PSI under high light in *P. rubra* was accompanied with high levels of P700 oxidation ratio. Furthermore, active electron transport to PSI was an important determinant of PSI photoinhibition in *P. rubra*. Comparing *Arabidopsis thaliana*, *P. rubra* showed different mechanisms of PSI photoinhibition. Our present study reveals some insights concerning the interspecific difference of mechanisms underlying PSI photoinhibition.

PSI photoinhibition in *P. rubra* is independent of P700 oxidation ratio

Our results showed that the activation of CEF under high light induced high P700 oxidation ratio in the shade-established species *P. rubra* (Fig. 2). However, PSI activity still decreased by 35 % in the H_2O-treated samples after exposure to 2000 μmol photons m^{-2} s^{-1} for 2 h at 25 °C (Fig. 3c). These results indicate that the occurrence of PSI photoinhibition in *P. rubra* is accompanied with high levels of P700 oxidation ratio, suggesting that over-reduction of the acceptor side of PSI is not necessary for PSI photoinhibition in *P. rubra*. On the other hand, CEF cannot prevent PSI activity from photoinhibition at high light in *P. rubra*, which is inconsistent with the role of CEF in preventing PSI photoinhibition at normal temperatures reported in previous studies (Munekage et al. 2002; Suorsa et al. 2012; Tikkanen et al. 2014).

Several studies have shown that CEF is essential for photosynthesis and photoprotection in plants (Munekage et al. 2002, 2004), especially in conditions of excess light

energy (Takahashi et al. 2009; Huang et al. 2012; Suorsa et al. 2012; Kono et al. 2014; Tikkanen et al. 2014). Under saturating light conditions, CEF-dependent generation of ΔpH is primarily involved in photoprotection for PSI and PSII (Nishikawa et al. 2012; Huang et al. 2015b). Because PSI tends to be damaged when electron flow from PSII to PSI exceeds the capacity of PSI electron acceptors (Tikkanen et al. 2014), the rise in ΔpH due to CEF activation under saturating light conditions can control the electron flow from PSII to PSI via the Cyt b_6/f complex, thereby protecting PSI against photodamage (Tikkanen and Aro 2014). Furthermore, because over-reduction on the PSI acceptor side can lead to PSI photodamage, CEF activation under high light can protect PSI through preventing the over-reduction of the PSI acceptor side (Munekage et al. 2002; Sonoike 2006; Suorsa et al. 2012). In *pgr5* plants of *Arabidopsis thaliana*, low levels of P700 oxidation ratio under high light lead to severe photodamage to PSI (Munekage et al., 2002; Suorsa et al., 2012; Tikkanen et al. 2014). Our present study indicated that the higher PSI photoinhibition in the MV-treated samples than the H_2O-treated samples was accompanied with higher P700 oxidation ratio in the MV-treated samples (Fig. 3a, c), suggesting that the extent of PSI photoinhibition in *P. rubra* is not controlled by P700 oxidation ratio. As a result, although to some extent CEF-dependent photosynthetic control maybe alleviates PSI photoinhibition in *P. rubra*, photoinhibition of PSI under high light in *P. rubra* cannot be avoided by the activation of CEF.

It has been indicated that hydroxyl radicals immediately destroy the iron–sulphur centers and cause PSI photoinhibition (Sonoike et al. 1995). Hydroxyl radicals are produced by the Fenton reaction between hydrogen peroxide and reduced metal ions (i.e., reduced iron–sulphur centers in PSI) (Sonoike et al. 1997). Under high light, although the proportion of reduced P700 reaction centers was low in the H_2O-treated samples, PSI photoinhibition was inevitable. Similarly, in the MV-treated samples, the low proportion of reduced P700 reaction centers was accompanied with significant PSI photoinhibition. However, in the DCMU-treated samples, P700 reaction centers were completely oxidized due to block of electron flow from PSII to PSI, and PSI photoinhibition could be prevented. These results suggested that, when leaves of *P. rubra* was exposed to high light, electron flow PSII to PSI induced the generation of hydroxyl radicals even though the proportion of reduced reaction centers in PSI was low.

PSI photoinhibition in *P. rubra* is controlled by electron transfer from PSII to PSI

We found that electron transfer from PSII to PSI played an important role in determining PSI photoinhibition in *P. rubra*. When the electron transport from PSII was blocked by DCMU (Fig. S1), PSI activity was maintained stable in *P. rubra* (Fig. 3c). Furthermore, when the electron transfer from PSI to O_2 was accelerated in the addition of MV, *P. rubra* showed stronger PSI photoinhibition (Fig. 3c). These results are consistent with the theory that the excess electron transfer from PSII is essential for the photoinhibition of PSI under environmental stresses (Sonoike 1995; Suorsa et al. 2012; Tikkanen et al. 2014).

At low light, electrons from PSII can be transported efficiently to $NADP^+$, resulting in the production of NADPH that is used by primary metabolism such as the Calvin cycle and photorespiration. At saturating light, CO_2 assimilation is limited by RuBP regeneration and/or RuBP carboxylation, causing depletion of $NADP^+$. Under such conditions, excess electrons transported from PSII to the acceptor side of PSI result in the formation of superoxide anion radicals (Asada 1999; Murata et al. 2007). The dismutation of superoxide anion radicals produces hydroxyl peroxide, which reacts with reduced iron–sulphur centers to form hydroxyl radicals that immediately destroy the iron–sulfur centers of PSI complexes (Sonoike 2006). Our results showed that DCMU and MV showed different effects on PSI activity under high light. In cucumber, the addition of MV protects PSI from photoinhibition rather than enhancing it in isolated thylakoid membranes (Sonoike 1996). However, the addition of MV accelerated PSI photoinhibition under high light in *P. rubra*. This controversy result may be caused by different mechanisms of PSI photoinhibition between cucumber and *P. rubra*. In *P. rubra*, PSI photoinhibition under high light was mainly controlled by electron flow from PSII to O_2 via PSI.

Conclusion

Our present study indicates that PSI photoinhibition is more related to electron transfer from PSII to PSI rather than PSI redox state in the shade-established plant *Psychotria rubra*. In cucumber and *Arabidopsis thaliana*, over-reduction of PSI acceptor side plays a dominant role in PSI photoinhibition (Munekage et al. 2002; Sonoike 2006, 2011). However, excess electron transfer from PSII to PSI induces the production and the accumulation of hydroxyl radicals at the acceptor side of PSI and then causes PSI photoinhibition in *P. rubra*, irrespective of P700 oxidation ratio. Therefore, the major mechanisms of PSI photoinhibition are probably different in *P. rubra* compared to *Arabidopsis thaliana* and cucumber.

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