Planta (2012) 235:819–828
DOI 10.1007/s00425-011-1544-3

ORIGINAL ARTICLE

Cyclic electron flow plays an important role in photoprotection for the resurrection plant *Paraboea rufescens* under drought stress

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Received: 2 July 2011 / Accepted: 19 October 2011 / Published online: 13 November 2011

Abstract Resurrection plants could survive severe drought stress, but the underlying mechanism for protecting their photosynthetic apparatus against drought stress is unclear. Cyclic electron flow (CEF) has been documented as a crucial mechanism for photoprotection in *Arabidopsis* and tobacco. We hypothesized that CEF plays an important role in protecting photosystem I (PSI) and photosystem II (PSII) against drought stress for resurrection plants. To address this hypothesis, the effects of mild drought stress on light energy distribution in PSII and P700 redox state were examined in a resurrection plant *Paraboea rufescens*. Cyclic electron flow was not activated below the photosynthetic photon flux density (PPFD) of 400 μmol m^{-2} s^{-1} in leaves without drought stress. However, CEF was activated under low light in leaves with mild drought stress, and the effective quantum yield of PSII significantly decreased. Meanwhile, non-photochemical quenching (NPQ) was significantly stimulated not only under high light but also under low light. Compared with the control, the fraction of overall P700 that cannot be oxidized in a given state (PSI acceptor side limitation) under high light was maintained at low level of 0.1 in leaves with water deficit, indicating that the over-reduction of the PSI acceptor side was prevented by the significant stimulation of CEF. Furthermore, methyl viologen could significantly increase the PSII photo-inhibition induced by high light compared with chloramphenicol. These results suggested that CEF is an important mechanism for protecting PSI and PSII from drought stress in resurrection plants.

Keywords Cyclic electron flow · Drought stress · Photoprotection · Resurrection plant

Abbreviations

CEF	Cyclic electron flow
LEF	Linear electron flow
MV	Methyl viologen
NPQ	Non-photochemical quenching
PPFD	Photosynthetic photon flux density
PS	Photosystem
ROS	Reactive oxygen species
Y(I)	Effective quantum yield of photosystem I
Y(II)	Effective quantum yield of photosystem II
Y(NA)	Fraction of over P700 that cannot be oxidized in a given state
Y(ND)	Fraction of over P700 that is oxidized in a given state
Y(NO)	Fraction of energy that is passively dissipated in form of heat and fluorescence
Y(NPQ)	Fraction of energy dissipated in form of heat via the regulated non-photochemical quenching mechanism
Ψ_{leaf}	Leaf water potential

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Introduction

It is well known that drought stress inhibits photosynthetic carbon fixation because of stomatal limitation (Cornic 1994; Golding and Johnson 2003; Zhang et al. 2009) and non-stomatal limitation (Ortiz-Lopez et al. 1991; Jia et al. 2008), which could lead to excess light energy (Smirnoff 1993). Previous studies have indicated that the excess light energy could lead to the generation of reactive oxygen species (ROS), which not only cause oxidative damage to photosynthetic apparatus (Krieger-Liszkay et al. 2008), but also inhibit the repair cycle (Nishiyama et al. 2001, 2005; Takahashi et al. 2009). Furthermore, light-induced inactivation of the Mn cluster of water oxidation may occur in parallel with the singlet oxygen-dependent pathway (see reviews Tyystjärvi 2008; Vass 2011). It is well known that resurrection plants could survive extreme drought stress. However, the underlying mechanisms for protecting their photosynthetic apparatus against drought stress are unclear. Since drought stress could induce up-regulation of components involved in ferredoxin-dependent cyclic electron flow that is an important mechanism for protecting PSI and PSII (Lehtimaki et al. 2010), we hypothesized that resurrection plants would show strong CEF activity because they often undergo drought stress in life.

Photo-inhibition of PSI is mainly caused by the oxidation due to hydroxyl radicals which are generated by a reaction between reduced iron–sulfur centers and hydroxyl peroxide (Sonoike et al. 1997; Sonoike 2006). It is reported that the photoreduction of the iron–sulfur centers on acceptor side of PS I by light illumination is the key to photo-inhibition of PSI (Sonoike 1996; Sonoike et al. 1997; Asada 1999). Therefore, the over-reduction of PSI acceptor side is deleterious to PSI complexes. Since drought stress inhibits the photosynthetic carbon fixation, which could induce the over-reduction of PSI acceptor side, PSI probably is sensitive to drought stress. Previous studies indicated that the repair of PSI activity is a slow process needing several days (Zhang and Scheller 2004). Moreover, resurrection plants have fast recovery of photosynthesis during rehydration (Eickmeier 1979). To survive extreme drought stress, it is speculated that resurrection plants have strong ability to protect their PSI complex from photo-inhibition under drought stress. Cyclic electron flow (CEF) has been documented as a crucial mechanism for preventing PSI from excess light stress through alleviating the over-reduction of PSI acceptor side (Munekage et al. 2002, 2004, 2008; Huang et al. 2011). We speculate that CEF is strongly stimulated under drought stress in resurrection plants to protect PSI from photo-inhibition.

Excess light energy absorbed by PSII induces the generation of singlet oxygen, free radicals containing oxygen and oxidized P680 ($P680^{+}$), which damages PSII reaction centers (Chow and Aro 2005). Plants have the ability to dissipate excess light energy in PSII through non-photochemical quenching (NPQ). The activation of NPQ is dependent on the generation of a proton gradient across thylakoid membrane (ΔpH). The formation of ΔpH in chloroplast is mainly induced by linear electron flow (LEF) and CEF. It is well known that CEF is essential for protecting PSII against excess excitation pressure because CEF-dependent building-up of ΔpH across thylakoid membrane helps the activation of NPQ and stabilization of oxygen-evolving complexes (OEC) (Munekage et al. 2002, 2004; Nandha et al. 2007; Takahashi et al. 2009). Previous study indicated that CEF was significantly stimulated under high light and drought stresses to activate NPQ (Golding and Johnson 2003; Miyake et al. 2004, 2005). Sun et al. (2006) reported that small residual functional PSII population was critical for recovery of the photo-inactivated PSII complexes. Furthermore, it has been indicated that resurrection plants showed fast recovery of PSII activity during rehydration (Cooper and Farrent 2002; Deng et al. 2003). It is likely that PSII of resurrection plants is strongly protected by the stimulation of CEF under drought stress.

The resurrection plant *Paraboea rufescens* shows very strong drought-resistance. It grows on the top of limestone in marginal tropical areas (21°54′N, 101°46′E). In our present study, we determined the effect of mild drought stress on light energy distribution and P700 redox state in the resurrection plant *P. rufescens* to examine the response of CEF to drought stress in resurrection plants.

Materials and methods

Plant materials

The resurrection plant *P. rufescens* (Franch.) Burtt is native to south of China and north of Thailand, a light-demanding herbaceous species inhabiting on limestone rocks. The seedlings of it were raised in an open field in Xishuangbanna Tropical Botanical Garden (21°54′N, 101°46′E). During the study period (October 2010 and June 2011), the maximum photosynthetic photon flux density (PPFD) at mid-day was up to 1,850 μmol m^{-2} s^{-1}, and the air temperature was about 20°C at night and 32°C in the daytime.

Water rehydration and dehydration treatment

The plants of *P. rufescens* were well watered for 1 month to ensure recovery of photosynthesis. The predawn leaf water potential (Ψ_{leaf}) reached zero, and the predawn maximum quantum yield of PSII was 0.82. After measuring the light response changes in energy distribution in PSII and P700 redox state in intact leaves without drought

stress, the leaves were detached for 1 h light adaptation (200 μmol m^{-2} s^{-1}) at 25°C associated with an air humidity of 50% to dehydrate and close stomata. Ψ_{leaf} was measured by the pressure chamber (PMS 1000; PMS Instrument Company, Albany, OR, USA). One light curve measurement took 14 min, and our experience indicated that there was a slight loss of Ψ_{leaf} (about −0.1 Mpa) during light curve measurement. Leaf structure could be damaged easily by the measurement of Ψ_{leaf}. To avoid the effect of mechanical damage on photosynthetic measurement, the Ψ_{leaf} in our present study was measured after light-response-curve measurements.

Photo-inhibitory treatment

To examine the role of CEF in photoprotection in leaves of *P. rufescens* grown in an open field, detached leaves were infiltrated with either H_2O or methyl viologen (MV, 300 μM) (Chow and Hope 2004; Fan et al. 2007) for 1 h in darkness and then treated with 30°C and 1,900 μmol m^{-2} s^{-1} for 1 h on water. Detached leaves were infiltrated with chloramphenicol (CM, 1 mM) for 1 h in darkness in the presence or absence of MV and then treated with 30°C and 1,900 μmol m^{-2} s^{-1} for 1 h on water. To examine whether 1 mM CM can completely inhibit the repair of PSII activity, detached leaves with photo-inhibition of PSII (sample at noon) were infiltrated with 1 mM CM and H_2O for 1 h, respectively, and then transferred to 25°C and low light of 25 μmol m^{-2} s^{-1} for 2 h recovery.

Chlorophyll fluorescence and P700 measurements

We conducted measurements for the light responses of chlorophyll fluorescence and P700 redox state in leaves at 25°C synchronously with Dual PAM-100 (Heinz Walz, Effeltrich, Germany) connected to a computer with control software. Six mature leaves were light-adapted (340 μmol m^{-2} s^{-1}) for at least 20 min at 25°C before the measurement of light response curves, and light-adapted photosynthetic parameters were recorded after 2 min exposure to each light intensity (62, 104, 225, 348, 669, 1,037, and 1,603 μmol m^{-2} s^{-1}). In the present study, a 635 nm LED was used as actinic light.

The following chlorophyll fluorescence parameters were calculated: $F_v/F_m = (F_m - F_o)/F_m$, $F'_o = F_o/(F_v/F_m + F_o/F'_m)$ (Oxborough and Baker 1997), $F'_v/F'_m = (F'_m - F'_o)/F'_m$, $qP = (F'_m - F_s)/(F'_m - F'_o)$, $Y(II) = (F'_m - F_s)/F'_m$ (Genty et al. 1989), $Y(NO) = F_s/F_m$, $Y(NPQ) = 1 - Y(II) - Y(NO)$(Hendrickson et al. 2004; Kramer et al. 2004). F_o and F'_o are the minimum fluorescence in the dark-adapted state and the calculated value of the minimum fluorescence value in the light-adapted state, respectively. The F'_o value obtained with this method is not an independent measurement and not the same value that one can obtain by actually measuring F'_o using far-red light. F_m and F'_m are the dark-adapted and light-adapted maximum fluorescence upon illumination of a pulse (300 ms) of saturating light (10,000 μmol m^{-2} s^{-1}). F_o and F_m were determined after 30 min dark adaptation. F_s is the light-adapted steady-state fluorescence. F_v/F_m is the maximum quantum yield of PSII after dark adaptation. F'_v/F'_m is the maximum quantum yield of PSII after light adaptation. qP is the coefficient of photochemical quenching. Y(II) is the effective quantum yield of PSII. Y(NO) reflects the fraction of energy that is passively dissipated in form of heat and fluorescence, It consists of the NPQ due to photo-inactivation of PSII and constitutive thermal dissipation that is very stable despite environmental stresses (Busch et al. 2009). Y(NPQ) represents the fraction of energy dissipated in form of heat via the regulated non-photochemical quenching.

The P700 redox state was measured by Dual PAM-100 with a dual wavelength (830/875 nm) unit, following the method of Klughammer and Schreiber (1994). Saturation pulses (10,000 μmol m^{-2} s^{-1}), which were introduced primarily for PAM fluorescence measurement, were applied for assessment of P700 parameters as well. The $P700^+$ signals (P) may vary between a minimal (P700 fully reduced) and a maximal level (P700 fully oxidized). The maximum level, which in analogy to F_m is called P_m, was determined with application of a saturation pulse after pre-illumination with far-red light. At a defined optical property, the amplitude of P_m depends on the maximum amount of photo-oxidizable P700, which is a parameter for representing the quantity of efficient PSI complex. P'_m was also defined in analogy to the fluorescence parameter F'_m. P'_m was determined similarly to P_m, but with background actinic light instead of far-red illumination. The photochemical quantum yield of PSI, Y(I), is defined by the fraction of overall P700 that in a given state is reduced and not limited by the acceptor side. It is calculated as $Y(I) = (P'_m - P)/P_m$. Y(ND) represents the fraction of overall P700 that is oxidized in a given state, which is enhanced by a transthylakoid proton gradient (photosynthetic control at cytb/f complex as well as down-regulation of PSII) and photodamage to PSII. $Y(ND) = P/P_m$. Y(NA), thus represents the fraction of overall P700 that cannot be oxidized by a saturation pulse in a given state due to a lack of oxidized acceptors. $Y(NA) = (P_m - P'_m)/P_m$. Note $Y(I) + Y(ND) + Y(NA) = 1$.

Estimation of electron flow through both PSI and PSII

The electron flow through PSI and PSII were calculated as follows: $ETR(I) = Y(I) \times PPFD \times \alpha I$, $ETR(II) = Y(II) \times PPFD \times \alpha II$ (Miyake et al. 2005). αI and αII were obtained as

$\alpha I = p \times dI$ and $\alpha II = p \times dII$, where p is the absorptance (the fraction of the incident light absorbed by leaves), and dI and dII are the fractions of the absorbed light distributed to PSI and PSII, respectively. The p was determined with an USB4000 spectrasuite (Ocean Optics Inc., Dunedin, FL, USA). For each leaf, both a reference scan and a sample scan of reflection and transmittance were made from 400 to 700 nm at 0.21 nm intervals, and the results integrated over the wavelength range to obtain the average reflectance or transmittance (Chen and Cheng 2003). The value of p was calculated as: 1 – reflectance − transmittance and equaled 0.94 ± 0.002 ($n = 3$).

If CEF is activated, ETR(I) is larger than ETR(II). Therefore, the value of CEF was calculated as ETR(I) − ETR(II) (Miyake et al. 2005; Fan et al. 2007). CEF is not stimulated under very low light in *Arabidopsis* grown under a light of 50 μmol m^{-2} s^{-1} (Munekage et al. 2002, 2004). Furthermore, CEF was slightly activated under low light of 150 μmol m^{-2} s^{-1} in leaves of tobacco grown under a light of 700 μmol m^{-2} s^{-1} (Miyake et al. 2005). Plants of herb *P. rufescens* used in the present study were grown under full sunlight and well watered; and they showed good growth performance. Therefore, we assumed that CEF was not activated under very low light in leaves of *P. rufescens* without water stress and photo-inhibition because under such condition NADP+ is abundant and effectively out-competes against CEF for the electrons from reduced ferredoxin (Fd). The values of Y(I) and Y(II) under a low light of 62 μmol m^{-2} s^{-1} were measured at 25°C in intact leaves. Under such condition, we assumed that ETR(I) = ETR (II), thus $Y(I) \times PPFD \times 0.94 \times dI = Y(II) \times PPFD \times 0.94 \times dII$. From the value of Y(I) (0.53 ± 0.06) and Y(II) (0.75 ± 0.006), dI was calculated to be 0.59 ± 0.03 and dII was calculated to be 0.41 ± 0.03 ($n = 4$). Thus, we estimated ETR(I) and ETR(II) using the following equations: $ETR(I) = 0.55 \times Y(I) \times PPFD$, $ETR(II) = 0.39 \times Y(II) \times PPFD$.

Since the fractional absorbance of incident light by PSI and PSII depends on the light quality, the dI/dII was determined using the same light quality in the actual experiments. During state transition, a reduced redox state of the plastoquinone pool leads to the activation of a protein kinase, which phosphorylates light harvest complex II (LHCII) (Vener et al. 1997; Zito et al. 1999). This phosphorylation event causes the migration of the phosphorylated LHCII to PSI thus increasing the dI/dII ratio (Allen 1992; Finazzi et al. 1999, 2002; Haldrup et al. 2001; Wollman 2001; Rochaix 2007). Such a change would enhance the light absorption by PSI. If the state transition occurred under drought stress, the actual CEF would be higher than the value we estimated.

Statistical analysis

The results were displayed as mean values of four or 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 existed between different treatments.

Results

Effect of mild drought stress on light energy distribution in PSII

When the leaf water potential decreased from 0 to −0.7 Mpa, the effective quantum yield of PSII [Y(II)] decreased at all irradiances due to significant decreases in both maximum quantum yield of PSII after light adaptation [F'_v/F'_m] and coefficient of photochemical quenching (qP) (Fig. 1a–c). The decrease in qP was larger than that of F'_v/F'_m. Meanwhile, the fraction of energy dissipated as heat via the regulated non-photochemical quenching mechanism [Y(NPQ)] strongly increased at all irradiances (Fig. 1d). Under control conditions (leaf water potential = 0), NPQ was almost not activated below the photosynthetic photon flux density (PPFD) of 400 μmol m^{-2} s^{-1}. However, it was significantly activated under a low PPFD of 62 μmol m^{-2} s^{-1} in drought-stressed plants. The fraction of energy that is passively dissipated in form of heat and fluorescence in PSII [Y(NO)] was maintained stable at the baseline of 0.2 at all light intensity under the drought stress (Fig. 1e).

Effects of mild drought stress on LEF and CEF

LEF gradually increased to 136 μmol photons m^{-2} s^{-1} under a PPFD of 1,037 μmol m^{-2} s^{-1} and then decreased to 96 μmol photons m^{-2} s^{-1} under the light of 1,603 μmol m^{-2} s^{-1} in leaves without water deficit (Fig. 2a). In drought stress, LEF reached the maximum value of 47 μmol photons m^{-2} s^{-1} under a PPFD of 348 μmol m^{-2} s^{-1} and remained stable under higher light intensity (Fig. 2a). CEF was hardly activated below the PPFD of 400 μmol m^{-2} s^{-1} in leaves without water deficit and reached 100 μmol photons m^{-2} s^{-1} under the PPFD of 1,603 μmol m^{-2} s^{-1} (Fig. 2b). However, CEF was strongly stimulated not only under high light but also under low light in drought-stressed leaves. The value of CEF reached to 68 and 155 μmol photons m^{-2} s^{-1} under the PPFDs of 348 and 1,603 μmol m^{-2} s^{-1}, respectively (Fig. 2b). In leaves without water deficit, the CEF/LEF ratio

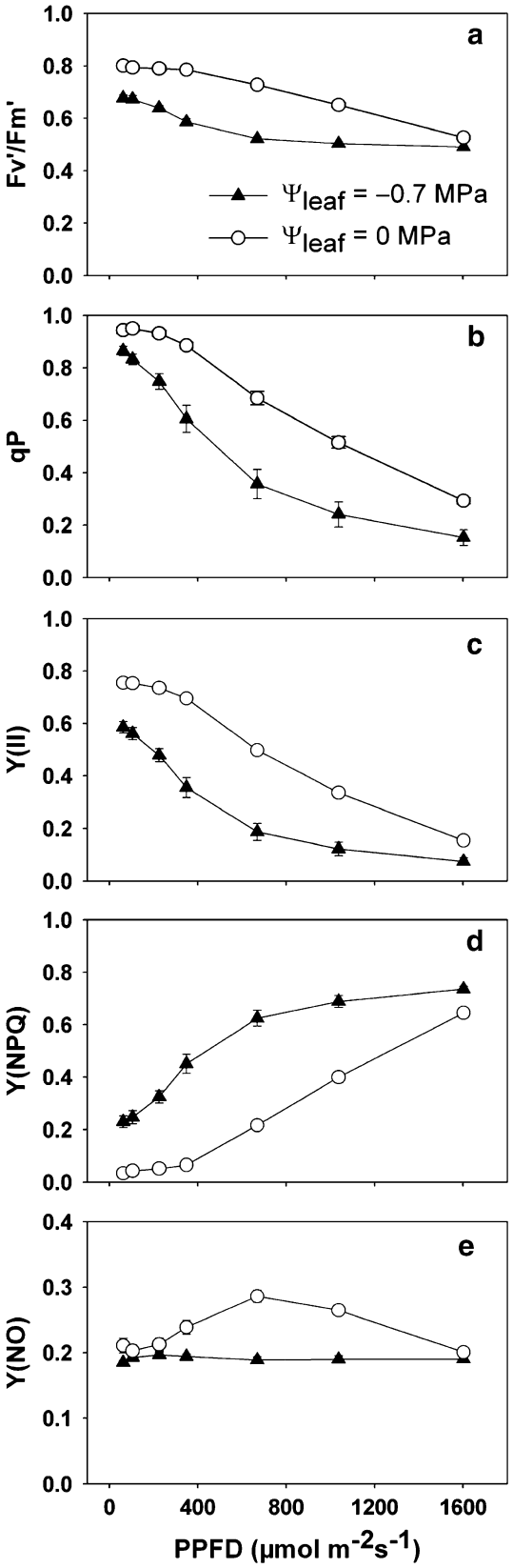

Fig. 1 Light response changes in F'_v/F'_m (**a**), qP (**b**), Y(II) (**c**), Y(NPQ) (**d**) and Y(NO) (**e**) in leaves of *Paraboea rufescens* measured at 25°C without drought stress (*open circle*) and with mild drought stress (*solid triangle*). The mean ± SE was calculated from four plants. F'_v/F'_m maximum quantum yield of PSII under light, *qP* coefficient of photochemical quenching, *Y(II)* effective quantum yield of PSII, *Y(NPQ)* fraction of energy dissipated in form of heat via the regulated non-photochemical quenching mechanism, *Y(NO)* fraction of energy that is passively dissipated in form of heat and fluorescence, Ψ_{leaf} leaf water potential

was lower than 0.1 below the PPFD of 400 μmol m^{-2} s^{-1}. However, at the low PPFD of 62 μmol m^{-2} s^{-1}, the CEF/LEF ratio was 0.9 in leaves with the drought stress (Fig. 2c). At the PPFD of 1,603 μmol m^{-2} s^{-1}, the CEF/LEF ratio of water stressed leaves reached 3.5, a value much higher than the value in leaves without drought stress (Fig. 2c).

Effects of mild drought stress on P700 redox state

Under drought stress, the effective quantum yield of PSI [Y(I)] increased slightly under low light, but was stable under moderate and high light (Fig. 3a). The fraction of overall P700 that is oxidized in a given state [Y(ND)] increased slightly in leaves under drought stress(Fig. 3b). As a result, the fraction of overall P700 that cannot be oxidized in a given state [Y(NA)] significantly decreased under low light in leaves with the water deficit and was maintained at low level of approximately 0.1 under high light (Fig. 3c), indicating that the over-reduction of PSI acceptor side was prevented under high light and mild drought stress.

Effect of methyl viologen and chloramphenicol on the decrease in PSII activity induced by high light

To examine the role of CEF in protecting PSII from photoinhibition under high light, leaves of *P. rufescens* were infiltrated with MV (to abolish any CEF) solution previously and then illuminated under high light of 1,900 μmol m^{-2} s^{-1} at 30°C for 1 h. After the high light treatment, the maximum quantum yield of PSII after dark-adaptation (F_v/F_m) decreased by 50% (Fig. 4). However, the high light stress just induced a 20% decrease in F_v/F_m in leaves infiltrated with water. After infiltration with 1 *mM* CM (to effectively inhibit protein synthesis, Fig. 5), F_v/F_m decreased by 25%, but was not significantly different from the water treatment (Fig. 4). The decrease in F_v/F_m in MV-treated leaves was not significantly different from the MV-and-CM treated leaves. These results may suggest that CEF is necessary for protecting PSII against high light stress in the resurrection plant *P. rufescens*. Moreover, under high light MV-treat leaves showed lower NPQ compared with water-treat leaves (Fig. 6a), suggesting the normal activation of NPQ in the resurrection plant *P. rufescens* was dependent on activation

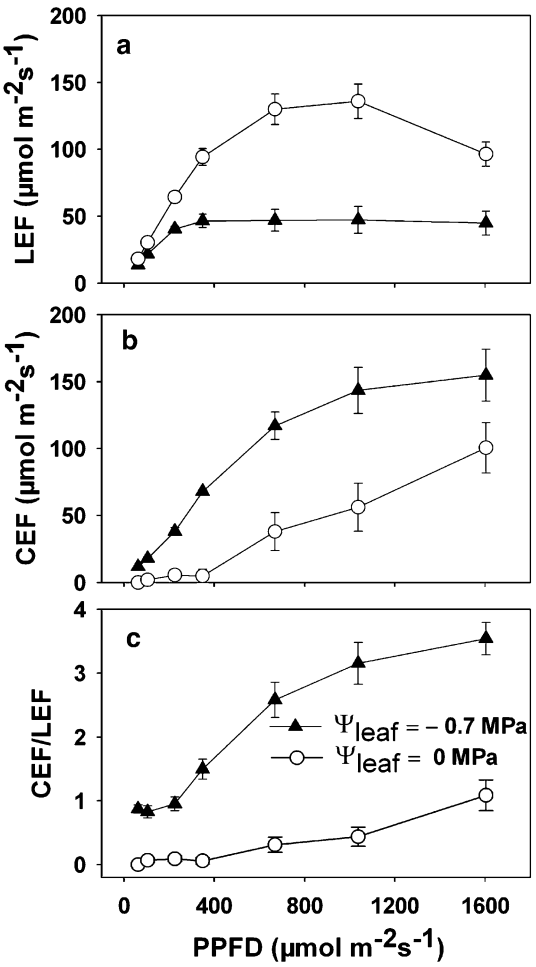

Fig. 2 Light response changes in linear electron flow (LEF) (**a**), cyclic electron flow (CEF) (**b**) and CEF/LEF (**c**) ratio in leaves of *Paraboea rufescens* measured at 25°C without drought stress (*open circle*) and with mild drought stress (*solid triangle*). The mean ± SE was calculated from four plants. The effect of drought stress on LEF, CEF and the CEF/LEF ratio was analyzed by one-way ANOVA. Statistical analysis results indicated that the drought stress significantly caused decrease in LEF and increases in CEF and the CEF/LEF ratio under all light intensities ($P < 0.05$, ANOVA, followed by Tukey's post hoc test for comparison)

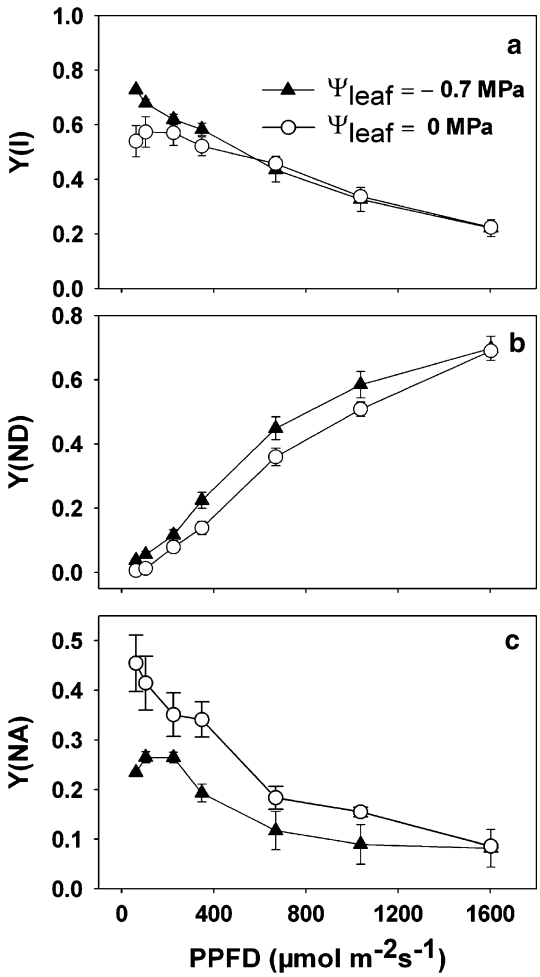

Fig. 3 Light response changes in Y(I) (**a**), Y(ND) (**b**) and Y(NA) (**c**) in leaves of *Paraboea rufescens* measured at 25°C without drought stress (*open circle*) and with mild drought stress (*solid triangle*). The mean ± SE was calculated from four plants. Y(I), effective quantum yield of PSII; 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

of CEF. MV also decreased the coefficient of photochemical quenching under high light (Fig. 6b).

Discussion

CEF-dependent formation of proton gradient across thylakoid membranes

We found that CEF was stimulated not only under high light but also under low light in leaves with mild water deficient in the resurrection plant *P. rufescens* (Fig. 2b, c), and the stimulation of CEF under high light is necessary for protecting PSII from photoinhibition (Fig. 4). The mild drought stress induced large decrease in qP (Fig. 1b), suggesting that during drought stress, more electrons returned to PSI to reduce P700 and other inter-system intermediates (consistent with greater CEF), while the greater abundance of electrons in the intermediates between the two photosystems kept the primary quinone electron acceptor of PSII (Q_A) more reduced. The light response curve indicated that LEF rates were marginally changed and similar from PPFD of 400 μmol m^{-2} s^{-1} during drought (Fig. 2a), suggesting that the mild drought limited photosynthesis under high light. Plants usually save water through closing stomata. Closure of stomata induces a decrease in the intercellular CO_2 concentration and then reduces the ability of plants to utilize the reducing power NADPH, a product of linear electron flow. The decrease in the ability of utilizing the products of linear electron flow

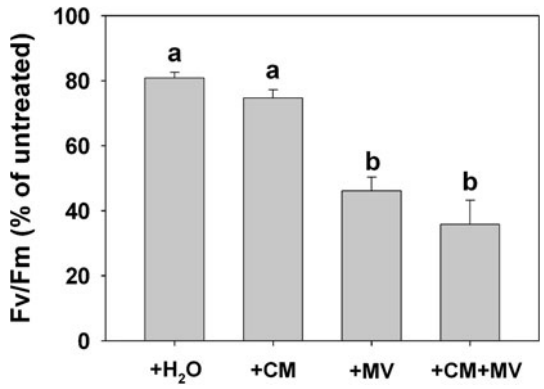

Fig. 4 Effects of methyl viologen (MV) and chloramphenicol (CM) on the decrease in F_v/F_m induced by high light treatment in leaves of *Paraboea rufescens*. After treated with chemical reagents (MV and CM) as described in "Materials and methods", leaf samples were illuminated on water at 30°C for 1 h under 1,900 μmol m^{-2} s^{-1}. All values were expressed relative to the controls with no treatment before the chilling treatment. The mean ± SE was calculated from six independent plants. Different letters represent significant differences of F_v/F_m between different treatments ($P < 0.05$, ANOVA, followed by Tukey's post hoc test for comparison)

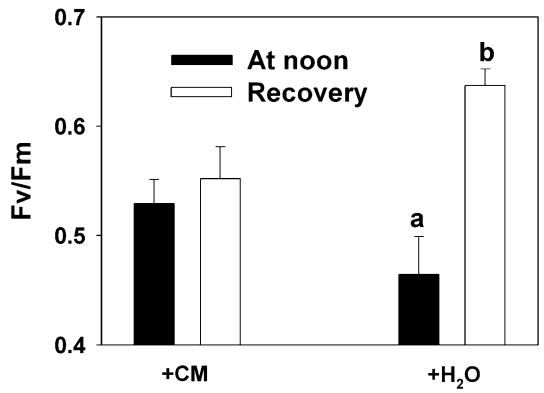

Fig. 5 Effect of chloramphenicol (CM, to inhibit the protein synthesis) on the repair of PSII activity. Detached leaves with photo-inhibition (sample at noon) were infiltrated with 1 mM CM and H_2O for 1 h, respectively, and then transferred to 25°C and low light of 25 μmol m^{-2} s^{-1} for 2 h recovery. The mean ± SE was calculated from five independent plants. Different letters represent significant differences of F_v/F_m between different treatments ($P < 0.05$, ANOVA, followed by Tukey's post hoc test for comparison)

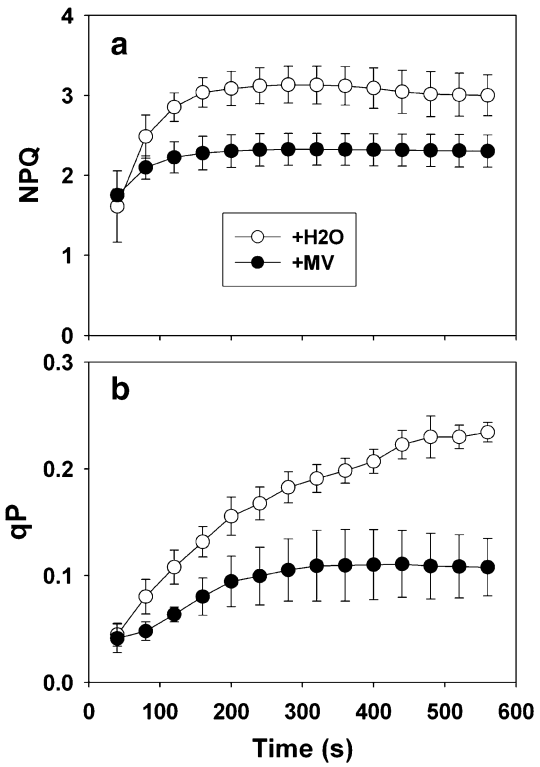

Fig. 6 Effect of methyl viologen (MV) on the induction of NPQ (**a**) and qP (**b**). After infiltraated with H_2O or 300 μM MV in the dark, detached leaves were exposed to light at 1,603 μmol m^{-2} s^{-1}. The mean ± SE was calculated from three plants. *NPQ* non-photochemical quenching, *qP* coefficient of photochemical quenching

under mild drought stress should be mainly caused by the stomatal limitation. The decrease in LEF under drought stress led to an increase in excess light energy which could induce the generation of ROS. ROS not only cause direct oxidative damage to PSII complexes, but also inhibit the de novo synthesis of the D1 protein, which is important for the repair of photodamaged PSII under strong light (Nishiyama et al. 2001; Takahashi et al. 2009). It is necessary for plants to activate NPQ to harmlessly dissipate excess light energy as heat. The activation of NPQ is dependent on the formation of proton gradient across thylakoid membranes (ΔpH). Previous studies have indicated that CEF-dependent building-up of ΔpH is necessary for the activation of NPQ (Munekage et al. 2002, 2004; Nandha et al. 2007; Takahashi et al. 2009). When LEF-dependent generation of ΔpH was limited under severe drought associated with high light, CEF was stimulated to help the formation of ΔpH and thus to help the activation of NPQ (Golding and Johnson 2003). Miyake et al. (2005) reported that in tobacco illuminated under low light, the CEF/LEF ratio was very low, and did not show any dependence on partial pressure of ambient CO_2. However, our present study indicated that NPQ was significantly activated under low light during mild drought stress (Fig. 1d), accompanied with significant stimulation of CEF (Fig. 2c). Since the activation of CEF is mainly induced by the limitation of NADP + regeneration under drought stress (Miyake et al. 2004), the decrease in Y(II) (Fig. 1c) under low light and mild drought condition suggesting large decreases in stomatal conductance and NADP + supply for LEF in resurrection plants. A possible reason is that resurrection plants close most of stomata for water conservation under drought stress and the over-closure of stomata in

resurrection plants under mild drought stress induced the decrease in LEF under low light. Meanwhile, stimulation of CEF activates NPQ which harmlessly dissipates excess light energy as heat and protects PSII from photoinhibition.

The fraction of energy that is passively dissipated in form of heat and fluorescence [Y(NO)] was maintained at the level of 0.2 under mild drought stress, which is the baseline value under low light, indicating that there was no excess excitation pressure in PSII reaction centers under the mild drought stress. Furthermore, the MV-treated leaves of *P. rufescens* showed significant larger PSII photoinhibition than water-treated and chloramphenicol-treated leaves after high light illumination (Fig. 4). MV promotes the electrons from PSI to O_2 and then induces the generation of ROS, which inhibit the protein synthesis. Thus, the effect of MV on PSII photoinhibition includes two aspects: abolishment of CEF, impairment of the repair of PSII photodamage. Since CM could inhibit the protein synthesis, the repair of PSII photodamage was blocked in CM-treat leaves illuminated under high light. The CM-treated leaves did not show significant larger PSII photoinhibition than water-treated leaves (Fig. 4), indicating the repair of PSII photodamage under high light was slow. However, the CM-MV-treated leaves showed significant larger PSII photo-inhibition compared with CM-treated leaves, indicating that the CEF-dependent formation of ΔpH is necessary for the resurrection plants to protect PSII under high light. Furthermore, the MV-treated leaves showed lower NPQ and qP under high light of 1,602 μmol m^{-2} s^{-1} than water-treated leaves (Fig. 6). There results suggested that CEF was a responsive photoprotective mechanism for PSII in the resurrection plant to resist drought stress through activating NPQ. In earlier studies, the efficiency of NPQ in protecting PSII was low (20–25%) (Tyystjärvi et al. 2005; Sarvikas et al. 2006; Takahashi et al. 2009).

Photodamage of PSII primarily occurs at the oxygen-evolving complex that locates on the luminal side of the thylakoid membrane (Hakala et al. 2005; Ohnishi et al. 2005). Previous study suggested that a high concentration of Ca^{2+} in the lumen of thylakoid membrane could stabilize the oxygen-evolving complex (OEC) against photodamage (Takahashi et al. 2009). Since acidification of the lumen could drive a Ca^{2+}/H^+ antiport to sequester Ca^{2+} in the lumen, up to about 4 mM in the lumen from an external concentration of 15 μM (Ettinger et al. 1999), the generation of ΔpH through CEF under high light stress are necessary for the stabilization of OEC (Takahashi et al. 2009). Under the mild drought stress, linear electron flow (LEF) decreased to 50 μmol photons m^{-2} s^{-1} under a PPFD of 1,603 μmol m^{-2} s^{-1}, and the CEF/LEF ratio increased to 3.5, indicating that the inhibition of LEF-dependent generation of ΔpH was compensated by the stimulation of CEF. When the CEF activity was abolished by MV, PSII was very sensitive to high light stress in the resurrection plant *P. rufescens* (Fig. 4). These results suggest that CEF-dependent building-up of ΔpH may be important for stabilizing OEC in resurrection plants with mild drought stress. Since MV induces massive production of superoxide (and therefore massive production of hydrogen peroxide) which may have a direct deleterious effect on PSII, the larger decrease in F_v/F_m in MV-treated samples compared with H_2O-treated samples suggested that the effect of MV on PSII photo-inhibition may be not only caused by abolishing CEF but also by increasing oxidative stress.

Since the CEF-dependent building-up of ΔpH not only serves for photoprotection but also help synthesis of ATP, CEF is essential for photosynthesis and growth performance (Munekage et al. 2004). Under photorespiratory conditions, CO_2 fixation requires more ATP (Osmond 1981) and the amount of ATP produced by LEF does not meet the ATP requirement for driving net CO_2 assimilation (von Caemmerer 2000). Drought stress induces a higher ATP demand, which could result in a higher NADPH/ATP ratio that might activate the NDH-mediated cyclic electron pathway. This would help dissipating excess energy and provide additional ATP to maintain active CO_2 fixation (Rumeau et al. 2007). A defect in photosynthesis induction (Burrows et al. 1998) and a growth phenotype (Horvath et al. 2000) were observed under conditions of stomatal closure in tobacco mutants defective in the NDH complex. This effect was interpreted through an involvement of the NDH-mediated cyclic electron pathway around PSI in supplying the extra ATP required in conditions of mild water stress (Horvath et al. 2000). Targeted inactivation of the plastid *ndhB* gene in tobacco results in an enhanced sensitivity of photosynthesis to moderate stomatal closure. In our present study, the value of CEF/LEF under the PPFD of 1603 μmol m^{-2} s^{-1} in mild drought stress reached 3.5 (Fig. 2b), a value much higher than that obtained under control condition (no drought stress).

CEF plays an important role in protecting PSI against mild drought stress

Our results suggested that CEF plays an important role in protecting PSI against mild drought stress in the resurrection plant *P. rufescens*. Photo-inhibition of PSI is mainly induced by the over-reduction of PSI acceptor side which leads to the generation of hydroxyl radical that damages PSI complexes (Sonoike et al. 1997; Sonoike 2006). Since drought stress induces decrease in stomatal conductance, the inhibition of CO_2 assimilation could lead to the over-reduction of PSI acceptor side, which could induce PSI photo-inhibition. Since the recovery of PSI is a slow

process requiring at least 1 week in *Arabidopsis* after chilling treatment (Zhang and Scheller 2004), the PSI of *P. rufescens* should be protected by some specific mechanisms during dehydration. Previous studies have indicated that CEF is essential for protecting PSI against high light stress through alleviating the over-reduction of PSI acceptor side (Munekage et al. 2002, 2004, 2008). The value of Y(NA) was maintained at the low level of 0.1 under high light in mild drought stress (Fig. 3c), suggesting that over-reduction of PSI acceptor side was prevented in *P. rufescens* under drought stress. Furthermore, our results displayed that the value of Y(ND) was maintained at high level under drought stress in *P. rufescens* illuminated with high light (Fig. 3b). The activation of CEF and inhibition of LEF decreased the fraction of PSII electron acceptors that were reduced and then increased the value of Y(ND). Oxidized P700 ($P700^+$) could dissipate excess light energy harmlessly as heat and thereby alleviate photo-inhibition of PSI (Nuijs et al. 1986). These results may suggest that CEF plays an important role in protecting PSI of resurrection plants through alleviating the over-reduction of PSI acceptor side.

In conclusion, we found that CEF was very responsive to mild drought stress in the resurrection plant *P. rufescens*. The mild drought stress caused large decrease in the ability of the leaves to utilize the products of linear electron flow. Meanwhile, the resurrection plant *P. rufescens* showed high CEF and NPQ to protect PSII from photo-inhibition. The over-reduction of PSI under mild drought stress was alleviated by the strong stimulation of CEF. Therefore, we conclude that CEF plays an important role in photoprotection for resurrection plants under drought stress.

Acknowledgments Xishuangbanna Station for Tropical Rain Forest Ecosystem Studies (XSTRE) provided climatic data. This work was supported by National Natural Science Foundation of China (Grant 30900174 and 30770226) and the Key Laboratory of National Forestry Bureau for Fast-growing Tree Breeding and Cultivation in Central South China (an open project grant).

References

Allen JF (1992) Protein phosphorylation in regulation of photosynthesis. Biochim Biophys Acta 1098:275–335

Asada K (1999) The water–water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Annu Rev Plant Physiol Plant Mol Biol 50:601–639

Burrows PA, Sazanov LA, Svab Z, Maliga P, Nixon PJ (1998) Identification of a functional respiratory complex in chloroplasts through analysis of tobacco mutants containing disrupted plastid *ndh* genes. EMBO J 17:868–876

Busch F, Hunter NPA, Ensminger I (2009) Biochemical constrains limit the potential of the photochemical reflectance index as a predictor of effective quantum efficiency of photosynthesis during the winter spring transition in Jack pine seedlings. Funct Plant Biol 36:1016–1026

Chen LS, Cheng L (2003) Both xanthophyll cycle-dependent thermal dissipation and the antioxidant system are up-regulated in grape (*Vitis labrusca* L. cv. Concord) leaves in response to N limitation. J Exp Bot 54:2165–2175

Chow WS, Aro EM (2005) Photo-inactivation and mechanisms of recovery. In: Wydrzynski T, Satoh K (eds) Photosystem II: The light-driven water: plastoquinone oxidoreductase advances in photosynthesis and respiration. Springer, Dordrecht, pp 627–648

Chow WS, Hope AB (2004) Electron fluxes through photosystem I in cucumber leaf discs probed by far-red light. Photosynth Res 81:77–89

Cooper K, Farrent JM (2002) Recovery of the resurrection plant *Craterostigma wilmsii* from desiccation: protection versus repair. J Exp Bot 53(375):1805–1813

Cornic G (1994) Drought stress and high light effects on leaf photosynthesis. In: Baker NR (ed) Photoinhibition of photosynthesis: from molecular mechanisms to the field. BIOS, Oxford, pp 297–313

Deng X, Hu Z-A, Wang H-X, Wen X-G, Kuang T-Y (2003) A comparison of photosynthetic apparatus of the detached leaves of the resurrection plant *Boea hygrometrica* with its non-tolerant relative *Chirita heterotrichia* in response to dehydration and rehydration. Plant Sci 165(4):851–861

Eickmeier WG (1979) Photosynthetic recovery in the resurrection plant *Selaginella lepidophylla* after wetting. Oecologia 39:93–106

Ettinger WF, Clear AM, Fanning KJ, Peck ML (1999) Identification of a Ca^{2+}/H^+ antiport in the plant chloroplast thylakoid membrane. Plant Physiol 119:1379–1385

Fan DY, Nie Q, Hope AB, Hillier W, Pogson BJ, Chow WS (2007) Quantification of cyclic electron flow around photosystem I in spinach leaves during photosynthetic induction. Photosynth Res 94:347–357

Finazzi G, Furia A, Barbagallo RP, Forti G (1999) State transitions, cyclic and linear electron flow transport and photophosphorylation in *Chlamydomonas reinhardtii*. Biochim Biophys Acta 1413:117–129

Finazzi G, Rappaport F, Furia A, Fleischmann M, Rochaix JD, Zito F, Forti G (2002) Involvement of state transitions in the switch between between linear and cyclic electron flow in *Chlamydomonas reinhardtii*. EMBO Rep 3:280–285

Genty B, Briantais JM, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta 990:87–92

Golding AJ, Johnson GN (2003) Down-regulation of linear and activation of cyclic electron transport during drought. Planta 218:107–114

Hakala M, Tuominen I, Keränen M, Tyystjärvi T, Tyystjärvi E (2005) Evidence for the role of the oxygen-evolving manganese complex in photoinhibition of photosystem II. Biochim Biophys Acta 1706:68–80

Haldrup A, Jensen PE, Lunde C, Scheller HV (2001) Balance of power: a review of the mechanism of photosynthetic state transitions. Trends Plant Sci 6:301–305

Hendrickson L, Furbank RT, Chow WS (2004) A simple alternative approach to assessing the fate of absorbed light energy using chlorophyll fluorescence. Photosynth Res 82:73–81

Horvath EM, Peter SO, Joet T, Rumeau D, Cournac L, Horvath GV, Kavanagh TA, Schafer C, Peltier G, Medgyesy P (2000) Targeted inactivation of the plastid *ndhB* gene in tobacco results in an enhanced sensitivity of photosynthesis to moderate stomatal closure. Plant Physiol 123:1337–1349

Huang W, Zhang SB, Cao KF (2011) Cyclic electron flow plays an important role in photoprotection of tropical trees illuminated at temporal chilling temperature. Plant Cell Physiol 52:297–305

Jia H, Oguchi R, Hope AB, Barber J, Chow WS (2008) Differential effects of severe water stress on linear and cyclic electron fluxes through Photosystem I in spinach leaf discs in CO2-enriched air. Planta 228:803–812

Klughammer C, Schreiber U (1994) An improved method, using saturating light pulses, for the determination of photosystem I quantum yield via $P700^{+}$-absorbance changes at 830 nm. Planta 192:261–268

Kramer DM, Johnson G, Kiirats O, Edwards GE (2004) New fluorescence parameters for the determination of Q_A redox state and excitation energy fluxes. Photosynth Res 79:209–218

Krieger-Liszkay A, Fufezan C, Trebst A (2008) Single oxygen production in photosystem II and related protection mechanism. Photosynth Res 98:551–564

Lehtimaki N, Lintala M, Allahverdiyeva Y, Aro EM, Mulo P (2010) Drought stress-induced upregulation of components involved in ferredoxin-dependent cyclic electron transfer. J Plant Physiol 167:1018–1022

Miyake C, Shinzaki Y, Miyata M, Tomizawa K (2004) Enhancement of cyclic electron flow around PSI at high light and its contribution to the induction of non-photochemical quenching of chl fluorescence in intact leaves of tobacco plants. Plant Cell Physiol 45:1426–1433

Miyake C, Miyata M, Shinzaki Y, Tomizawa K (2005) CO_2 response of cyclic electron flow around PSI (CEF-PSI) in tobacco leaves: relative electron fluxes through PSI and PSII determine the magnitude of non-photochemical quenching (NPQ) of chl fluorescence. Plant Cell Physiol 46:629–737

Munekage Y, Hojo M, Meurer J, Endo T, Tasaka M, Shikanai T (2002) *PGR5* is involved in cyclic electron flow around photosystem I and is essential for photoprotection in *Arabidopsis*. Cell 110:361–371

Munekage Y, Hashimoto M, Miyake C, Tomizawa KI, Endo T, Tasaka M, Shikanai T (2004) Cyclic electron flow around photosystem I is essential for photosynthesis. Nature 429:579–582

Munekage Y, Genty B, Peltier G (2008) Effect of PGR5 impairment on photosynthesis and growth in *Arabidopsis thaliana*. Plant Cell Physiol 49:1688–1698

Nandha B, Finazzi G, Joliot P, Hald S, Johnson GN (2007) The role of PGR5 in the redox poising of photosynthetic electron transport. Biochim Biophys Acta 1767:1252–1259

Nishiyama Y, Yamamoto H, Allakhverdiev SI, Inaba M, Yokota A, Murata N (2001) Oxidative stress inhibits the repair of photodamage to the photosynthetic machinery. EMBO J 20:5587–5594

Nishiyama Y, Allakhverdiev SI, Murata N (2005) Inhibition of the repair of photosystem II by oxidative stress in cyanobacteria. Photosynth Res 84:1–7

Nuijs AM, Shuvalov A, van Gorkom HJ, Plijter JJ, Duysens LNM (1986) Picosecond absorbance difference spectroscopy on the primary reactions and the antenna-excited states in photosystem I particles. Biochim Biophys Acta 850:310–318

Ohnishi N, Allakhverdiev SI, Takahashi S, Higashi S, Watanabe M, Nishiyama Y, Murata N (2005) Two-step mechanism of photodamage to photosystem II: step one occurs at the oxygen-evolving complex and step two occurs at the photochemical reaction center. Biochemistry 44:8494–8499

Ortiz-Lopez A, Ort DR, Boyer JS (1991) Photophosphorylation in attached leaves of *Helianthus annuus* at low water potentials. Plant Physiol 96:1018–1025

Osmond CB (1981) Photorespiration and photoinhibition. Some implications for the energetics of photosynthesis. Biochim Biophys Acta 639:77–98

Oxborough K, Baker NR (1997) Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components–calculation of qP and Fv'/Fm' without measuring Fo'. Photosynth Res 54:135–142

Rochaix JD (2007) Role of thylakoid protein kinases in photosynthetic acclimation. FEBS Lett 581:2768–2775

Rumeau D, Peltier G, Cournac L (2007) Chlororespiration and cyclic electron flow around PSI during photosynthesis and plant stress response. Plant Cell Environ 30:1041–1051

Sarvikas P, Hakala M, Pätsikkä E, Tyystjärvi T, Tyystjärvi E (2006) Action spectrum of photoinhibition in leaves of wild type and npq1–2 and npq4–1 mutants of Arabidopsis thaliana. Plant Cell Physiol 47:391–400

Smirnoff N (1993) The role of active oxygen in response of plants to water deficit and desiccation. New Phytol 125:27–58

Sonoike K (1996) Degradation of psaB gene product, the reaction center subunit of photosystem I, is caused during photoinhibition of photosystem I: possible involvement of active oxygen species. Plant Sci 115:157–164

Sonoike K (2006) Photoinhibition and protection of photosystem I. In: Golbeck JH (ed) Photosystem I: the light-driven plastocyanin: ferredoxin oxidoreductase, series advances in photosynthesis and respiration. Springer, Dordrecht, pp 657–668

Sonoike K, Kamo M, Hihara Y, Hiyama T, Enami I (1997) The mechanism of the degradation of psaB gene product, one of the photosynthetic reaction center subunits of photosystem I, upon photoinhibition. Photosynth Res 53:55–63

Sun Z-L, Lee H-Y, Matsubara S, Hope AB, Pogson BJ, Hong Y-N, Chow WS (2006) Photoprotection of residual functional photosystem II units that survive illumination in the absence of repair, and their critical role in subsequent recovery. Physiol Plant 128:415–424

Takahashi S, Milward SE, Fan DY, Chow WS, Badger MR (2009) How does cyclic electron flow alleviate photoinhibition in Arabidopsis? Plant Physiol 149:1560–1567

Tyystjärvi E (2008) Photoinhibition of photosystem II and photodamage of the oxygen evolving manganese cluster. Coord Chem Rev 252:361–376

Tyystjärvi E, Hakala M, Sarvikas P (2005) Mathematical modelling of the light response curve of photoinhibition of photosystem II. Photosynth Res 84:21–27

Vass I (2011) Role of charge recombination processes in photodamage and photoprotection of the photosystem II complex. Physiol Plant 142:6–16

Vener AV, von Kan PJM, Rich PR, Ohad I, Andersson B (1997) Plastoquinol at the quinol oxidation site of reduced cytochrome *bf* mediates signal transduction between light and protein phosphorylation: Thylakoid protein kinase deactivation by a single-turnover flash. Proc Natl Acad Sci USA 94:1585–1590

von Caemmerer S (2000) Biochemical models of leaf photosynthesis. CSIRO Publishing, Collingwood

Wollman FA (2001) State transitions reveal the dynamics and flexibility of the photosynthetic apparatus. EMBO J 20:3623–3630

Zhang SP, Scheller HV (2004) Photoinhibition of photosystem I at chilling temperature and subsequent recovery in Arabidopsis. Plant Cell Physiol 45:1595–1602

Zhang JL, Meng LZ, Cao KF (2009) Sustained diurnal photosynthetic depression in uppermost-canopy leaves of four dipterocarp species in the rainy and dry seasons: does photorespiration play a role in photoprotection? Tree Physiol 29:217–228

Zito F, Finazzi G, Delosme R, Nitschke W, Picot D, Wollman FA (1999) The Qo site of cytochrome b_6f complexes controls the activation of the LHCII kinase. EMBO J 18:2961–2969