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Research paper

Differences in the responses of photosystem I and photosystem II of three tree species *Cleistanthus sumatranus*, *Celtis philippensis* and *Pistacia weinmannifolia* exposed to a prolonged drought in a tropical limestone forest

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Drought stress can induce closure of stomata, thus leading to photoinhibition. The effects of prolonged severe drought under natural growing conditions on photosystem I (PSI), photosystem II (PSII) and cyclic electron flow (CEF) in drought-tolerant tree species are unclear. In spring 2010, southwestern China confronted severe drought that lasted several months. Using three dominant evergreen species, *Cleistanthus sumatranus* (Miq.) Muell. Arg. (Euphorbiaceae), *Celtis philippensis* Bl. (Ulmaceae) and *Pistacia weinmannifolia* J. Poisson ex Franch. (Anacardiaceae) that are native to a tropical limestone forest, we investigated the influence of this stress on PSI and PSII activities as well as light energy distribution in the PSII and P700 redox state. By the end of the drought period, predawn leaf water potential (Ψ_{pd}) largely declined in each species, especially in *C. sumatranus*. Photosystem I activity strongly decreased in the three species, especially in *C. sumatranus* which showed a decrease of 65%. The maximum quantum yield of PSII after dark adaptation remained stable in *P. weinmannifolia* and *C. philippensis* but significantly decreased in *C. sumatranus*. Light response curves indicated that both linear electron flow and non-photochemical quenching were severely inhibited in *C. sumatranus* along with disappearance of CEF, resulting in deleterious excess light energy in PSII. We conclude that PSI is more sensitive than PSII to prolonged severe drought in these three drought-tolerant species, and CEF is essential for photoprotection in them.

Keywords: cyclic electron flow, drought stress, non-photochemical quenching, photoinhibition, photosystem I, photosystem II.

Introduction

Under natural environmental conditions, the occurrence of drought can inhibit photosynthetic carbon fixation by limiting either metabolism (non-stomatal limitation; Ortiz-Lopez et al. 1991, Jia et al. 2008) or the entry of CO_2 into the leaf (stomatal limitation; Cornic 1994). These scenarios can lead to excess light excitation that cannot be consumed by photosynthetic carbon fixation and non-photochemical quenching (NPQ) (Smirnoff 1993). Although plants can harmlessly dissipate excess light energy through photorespiration and NPQ (Demmig-Adams 1990, Ortiz-Lopez et al. 1991, Niyogi et al. 1998, 2001, Li et al. 2002, Rumeau et al. 2007, Zhang et al. 2009), drought stress can cause an over-reduction of the photosynthetic electron transport chain (Golding and Johnson

2003), leading to greater oxidative stress. Reactive oxygen species (ROS) may not only directly damage the photosynthetic apparatus (Asada 1999, Chow and Aro 2005), but also inhibit protein synthesis, which is necessary for the repair of photodamage (Nishiyama et al. 2001, 2005, 2006, 2011, Ohnishi et al. 2005).

Photoinhibition of photosystem I (PSI) is mainly caused by the oxidation of hydroxyl radicals that are generated by a reaction between reduced iron–sulfur centers and hydroxyl peroxide (Sonoike et al. 1997, Sonoike 2006). Therefore, over-reduction of the PSI acceptor side is the main cause of PSI photoinhibition (Munekage et al. 2002, 2004). Drought stress leads to a decrease in stomatal conductance and then inhibits the photosynthetic carbon fixation, which also can induce over-reduction of the PSI acceptor side. In *Arabidopsis* and tropical tree species, repair of PSI photoinhibition is a slow process that requires several days (Zhang and Scheller 2004, Huang et al. 2010*a*). Therefore, the reaction centers of PSI are likely to be damaged under prolonged, severe drought stress. Photosystem I photoinhibition can be alleviated by blocking linear electron flow (LEF). This is because the generation of hydroxyl radicals at the PSI acceptor side is based on active electrons being transported from PSII (Sonoike 2006). Furthermore, cyclic electron flow (CEF) can protect PSI by alleviating over-reduction of the PSI acceptor side (Munekage et al. 2002, 2004). Therefore, it is unclear whether PSI is sensitive to prolonged, severe drought stress. Because CEF is based on PSI activity, severe PSI photoinhibition can negatively affect CEF stimulation. The effect of long-term, severe drought on CEF in drought-tolerant tree species is also unknown.

Plants have several mechanisms for protecting photosystem II (PSII) from photoinhibition, such as NPQ and CEF (Munekage et al. 2004, Takahashi et al. 2009). Activation of NPQ is based on the generation of proton gradient across the thylakoid membrane (ΔpH) (Munekage et al. 2002, 2004). Because the water–water cycle in leaves is not a major alternative electron sink for dissipation of excess excitation energy when CO_2 assimilation is restricted (Driever and Baker 2011), LEF and CEF are the two major pathways for the formation of ΔpH. Under drought stress, LEF is usually limited and, thus, the LEF-dependent generation of ΔpH is inhibited (Golding and Johnson 2003, Huang et al. 2012*a*). Meanwhile, CEF is stimulated to activate NPQ to alleviate PSII photoinhibition (Jia et al. 2008, Takahashi et al. 2009, Huang et al. 2012*a*). Previous studies have indicated that CEF is necessary for the activation of NPQ under high-light or low-CO_2 stresses (Munekage et al. 2002, 2004, Miyake et al. 2004, 2005, Nandha et al. 2007). Stimulation of CEF can enable quick repair of PSII activity under low light (Huang et al. 2010*b*), but strong photoinhibition of PSII cannot be repaired when PSI activity has been severely damaged (Huang et al. 2010*a*). This demonstrates that CEF is necessary for the fast repair of PSII (Allakhverdiev et al. 2005). Prolonged drought stress over several months likely causes PSI photoinhibition and impairs CEF stimulation. However, its effect on PSII activity has not been known in drought-tolerant tree species.

We examined how long-term natural drought influences stomatal conductance, PSI and PSII activities and light energy distribution in the PSII and P700 redox state in three dominant evergreen tree species, *Cleistanthus sumatranus* (Miq.) Muell. Arg. (Euphorbiaceae), *Celtis philippensis* Bl. (Ulmaceae) and *Pistacia weinmannifolia* J. Poisson ex Franch. (Anacardiaceae) that are native to tropical limestone forests. Pressure–volume (*P–V*) curve measurements in a normal dry season had indicated that the leaves of all three species are very drought-tolerant, with relative water content at the turgor loss point being as low as –3 MPa (Zhu et al. 2009). We addressed the following questions: (i) Are PSI and PSII activities inhibited by prolonged drought; (ii) Is CEF affected by such severe stress?

Materials and methods

Study site and plant materials

This study was conducted in a limestone forest ~3 km from the Xishuangbanna Tropical Botanical Garden (21°54′N, 101°46′E), southern Yunnan Province, China. The climate in this region is dominated by the south-west monsoon, with a distinct dry season between November and April. The mean annual temperature is 21.7 °C and the mean annual precipitation (data averaged from 1959 to 2009) is ~1500 mm, of which ~84% occurs in the rainy season (May through October). In late 2009 and early 2010, southwestern China experienced a prolonged drought (Stone 2010). Three evergreen tree species, *C. sumatranus*, *C. philippensis* and *P. weinmannifolia*, were chosen for the study, which are dominant (in number) tree species at the top of a limestone hill. The selected plants of the three species are grown on top of the same hill with a similar microenvironment and the maximum distance between them usually does not exceed 20 m. Because it is difficult to measure the accurate age of the study species in a nature reserve, we just measured the diameters of the selected plants. The diameters of selected plants are in a range of 15–40 cm. The height of the study plants is at least 5 m for the three species. Individuals of each species with similar diameters and heights were chosen. Furthermore, we measured the ecophysiology of the three species using their mature sunlit leaves, which facilitated the comparison among different species.

Measuring the predawn leaf water potential, leaf turgor loss point and gas exchange

In August (rainy season in 2009) and in March (dry season in 2010), the predawn leaf water potential (Ψ_{pd}) was measured during 6:00–8:00 before sunrise. In August 2009, the maximum quantum photosynthetic photon flux density

(PPFD) at midday was up to 1850 μmol m^{-2} s^{-1}, and the mean air temperature was ~24 °C at night and 32 °C in daytime. At the end of the dry season in 2010 (March), the maximum quantum PPFD at midday was up to 2000 μmol m^{-2} s^{-1}, with air temperatures of ~22/32 °C night/day. Two leaves or twigs from each of four to six individuals per species were cut from the trees and wrapped with wet tissue in plastic bags for transport to the laboratory. The water potentials were determined with a pressure chamber (PMS, Corvallis, OR, USA), and all samples were measured within 1 h in the laboratory.

Data for leaf *P–V* curves that had been obtained in the rainy season of 2006 and the dry season of 2007 were used to indicate leaf tolerance to desiccation for each species, as well as to represent their osmotic adjustment during periods of normal drought. These *P–V* curves were constructed according to the bench dry method (Turner 1988). Briefly, leaves were sampled early in the morning from each of four to six individuals per species. In the laboratory, their petioles were held under water for 24 h for re-hydration. The leaves were put into a black bag and the leaf water potential was measured with a pressure chamber, and the fresh weights of leaves were weighed using a balance. Then the leaves were dried on the bench, and the water potential (Ψ) and fresh weight (W_f) were measured periodically. During the measurement of Ψ, the increasing of pressure in the chamber was at the rate of 0.025 MPa s^{-1} (unpublished data) and the gas was released slowly after each measurement. When their potentials no longer continued to decline, we placed those leaves in an oven at 70 °C for 24 h before determining their dry weight (W_d). The plateau effect of water release curves was omitted according to the method of Kubiske and Abrams (1991). The saturated leaf weight (W_s) was estimated graphically by extrapolating the relationship between fresh weight and water potential. Relative water content (RWC) was calculated as follows:

$$RWC = (W_f - W_d) / (W_s - W_d)$$

The *P–V* curve parameter, i.e., turgor loss point water potential (Ψ_{tlp}), was evaluated by using a pressure–volume analysis spreadsheet developed by Sack and Pasquet-Kok (2011).

In August 2009 and March 2010, the maximum photosynthesis rate (A_{max}) and stomatal conductance (g_s) of leaves were measured in the morning between 09:00 and 12:00 by using a portable photosynthetic gas exchange system (LI-6400, Licor, Lincoln, NE, USA) with an ambient CO_2 concentration of 400 μmol m^{-2} s^{-1} and a PPFD of 1000 μmol m^{-2} s^{-1} that is photosynthetically saturated for the three species. Mature sun leaves that had flushed in the middle of the rainy season were chosen for measuring in situ gas exchange in both the rainy and the dry season. Average values for leaf to air vapor pressure deficit and temperature were 1.89 kPa and 26.8 °C, respectively.

Rapid dehydration treatment

To examine whether the maximum photo-oxidizable P700 (P_m) is affected by RWC, we measured P_m at different states during rapid dehydration for detached leaves from each species. Conditions for this experiment included darkness, 25 °C and an ambient humidity of 60%. To calculate RWC values, we used the initial weight of detached leaves after they were floated on water for 12 h (corresponding to the fully turgid state), the fresh weight at any given state of dehydration and the weight obtained after oven-drying at 77 °C for 48 h.

Chlorophyll fluorescence and P700 measurements

Light responses were monitored by simultaneously obtaining chlorophyll fluorescence and P700 redox state in the leaves at 25 °C. A Dual PAM-100 (Heinz Walz, Effeltrich, Germany) was connected to a computer with control software. Measurements were made in December 2009 (the end of the rainy season) and March 2010 (the end of the dry season). To investigate the effect of prolonged drought stress on activities of PSI and PSII, we chose mature sun leaves that had flushed in the middle of the rainy season. Chlorophyll fluorescence and P700 measurements were recorded in December 2009 (14/25 °C night/day) and March 2010 (22/32 °C). Six mature sunlit leaves per species were light-adapted (340 μmol photons m^{-2} s^{-1}) for at least 20 min at 25 °C before measurements of light response curves, and light-adapted photosynthetic parameters were recorded after 2 min exposure to each light intensity value.

The following chlorophyll fluorescence parameters were calculated: $F_o' = F_o/(F_v/F_m + F_o/F_m')$ (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), $Y(NPQ) = F_s/F_m' - F_s/F_m$, $Y(NO) = F_s/F_m$ (Hendrickson et al. 2004, Kramer et al. 2004), $NPQ = (F_m - F_m')/F_m'$. F_o and F_o' are the minimum fluorescence in the dark-adapted state and light-adapted state, respectively. 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}), respectively. F_o and F_m were determined after an overnight dark adaptation. F_s is the light-adapted steady-state fluorescence. F_v/F_m is the maximum quantum yield of PSII after an overnight 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. $Y(NO)$ is the fraction of energy that is passively dissipated in the form of heat and fluorescence, and it consists of NPQ due to photoinactivation of PSII and constitutive thermal dissipation that is very stable despite environmental stresses (Busch et al. 2009). High values of $Y(NO)$ reflect the inability of a plant to protect itself against damage by excess light energy.

The P700 redox state was measured by Dual PAM-100 with a dual wavelength (830/875 nm) unit (Klughammer and Schreiber 1994), which was used in Huang et al. (2010*b*, 2011, 2012*a*, 2012*b*). Saturation pulses (10,000 μmol m^{-2} s^{-1}), which were introduced primarily for PAM fluorescence measurement, were applied for the 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 the application of a saturation pulse after preillumination with far-red light. At a defined optical property, the amplitude of P_m depends on the maximum amount of photooxidizable P700, which is a good parameter for representing the quantity of efficient PSI complex (Huang et al. 2010*a*, 2010*b*). 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 $Y(I) = (P_m' - P)/P_m$. The light response change in the ratio of $Y(I)$ to $Y(II)$ serves to estimate the activation of CEF (Harbinson and Foyer 1991).

Statistical analysis

For all measurements, the mean ± SE was calculated from at least three plants. Data were subjected to analysis of variance [*T*-tests or one-way analysis of variance (ANOVA)] using SPSS 16.0 statistical software. Tukey's multiple comparison tests were used at $\alpha = 0.05$ level to determine whether significant differences in values existed between the rainy and dry seasons.

Results

The predawn leaf water potential (Ψ_{pd}) in the rainy season was −0.3 and −0.5 MPa in the three species, while by the end of the prolonged drought, Ψ_{pd} had decreased to −6.6 MPa in *C. sumatranus*, −4.5 MPa in *C. philippensis* and −2.8 MPa in *P. weinmannifolia*, respectively (Figure 1a). One-way ANOVA showed that the value of Ψ_{pd} in *C. sumatranus* was significantly lower than that in *C. philippensis* and *P. weinmannifolia* by the end of the prolonged drought period ($P < 0.001$). The values of Ψ_{pd} at the end of the prolonged drought were lower than that of leaf turgor loss points in the two species *C. sumatranus* and *C. philippensis* measured in the normal dry season ($P < 0.001$, one-way ANOVA) (Figure 1a, b). The differences in Ψ_{tlp} between the rainy and dry seasons for *C. sumatranus* and *C. philippensis* were 1.3 and 0.7 MPa, respectively, while the values of Ψ_{tlp} for *P. weinmannifolia* were not significantly different between the rainy and dry seasons ($P > 0.05$), indicating the greatest osmotic adjustment of *C. sumatranus* among the three species. In *P. weinmannifolia* the value of Ψ_{pd} at the end of the dry season was probably equal to the leaf turgor loss point. The low Ψ_{pd} at the end of the prolonged drought indicated that the leaves of these three tree species confronted severe loss of water, which induced the closure of stomata. The maximum stomatal conductance (g_s) in the middle of the rainy season was 0.14 mol m^{-2} s^{-1} in *C. sumatranus*, 0.16 mol m^{-2} s^{-1} in *C. philippensis* and 0.27 mol m^{-2} s^{-1} in *P. weinmannifolia* (Figure 1c). At the end of the dry season, it decreased down to 0.014 mol m^{-2} s^{-1} in *C. sumatranus*, 0.01 mol m^{-2} s^{-1} in *C. philippensis* and 0.02 mol m^{-2} s^{-1} in *P. weinmannifolia* (Figure 1c). Correspondingly, the maximum photosynthetic rates decreased dramatically at the end of the dry season compared with the rainy season in the three tree species ($P < 0.001$; Figure 1d).

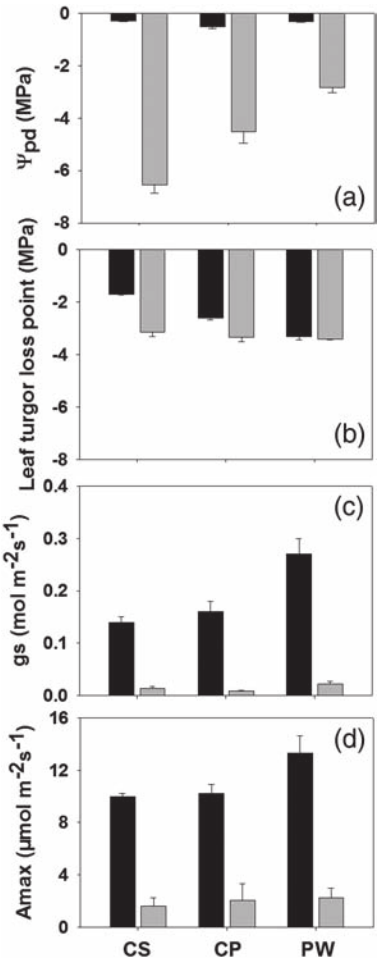

Figure 1. Seasonal changes in (a) predawn leaf water potential (Ψ_{pd}), (b) leaf turgor loss point (Ψ_{tlp}), (c) stomatal conductance (g_s) and (d) maximum photosynthetic rate (A_{max}) in *Cleistanthus sumatranus* (CS), *Celtis philippensis* (CP) and *Pistacia weinmannifolia* (PW). Black bars represent the values in the rainy season and gray bars indicate the values at the end of the prolonged dry season. The mean ± SE was calculated from at least four plants.

The maximum quantum yield of PSII (F_v/F_m) did not differ in the two species *C. philippensis* and *P. weinmannifolia* in both seasons, with F_v/F_m values being close to 0.8 (Figure 2c).

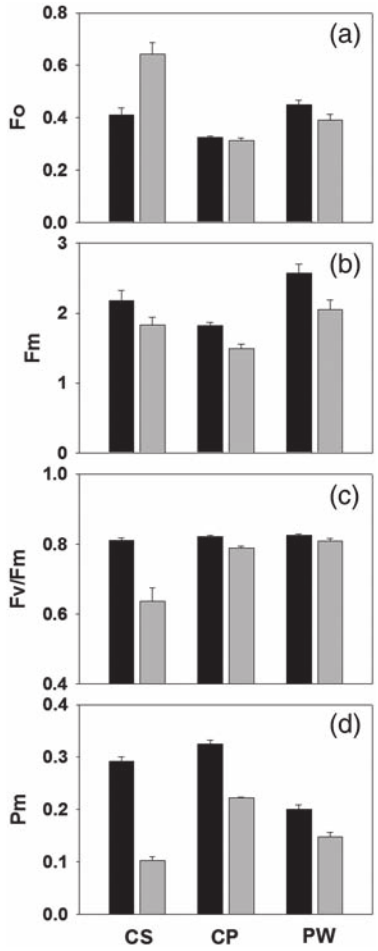

Figure 2. Seasonal changes in the minimum fluorescence in the dark-adapted state (F_o), the maximum fluorescence in the dark-adapted state (F_m), the maximum quantum yield of PSII after dark adaptation (F_v/F_m) and maximum photo-oxidizable P700 (P_m) in *Cleistanthus sumatranus* (CS), *Celtis philippensis* (CP) and *Pistacia weinmannifolia* (PW). The black bars represent the values at the end of the rainy season and gray bars represent the values at the end of the dry season. The mean ± SE was calculated from at least four plants.

However, F_v/F_m in *C. sumatranus* decreased from 0.81 in the rainy season to 0.64 at the end of the prolonged dry season (Figure 2c), which was mainly due to a large increase in the minimum chlorophyll fluorescence (F_o) (Figure 2a). The increase in F_o may be caused by the release of free chlorophyll from protein–pigment complexes. The maximum chlorophyll fluorescence (F_m) slightly decreased in the three species (Figure 2b). The maximum photo-oxidizable P700 (P_m) decreased by 65% in *C. sumatranus*, 31% in *C. philippensis* and 26% in *P. weinmannifolia* at the end of prolonged drought (Figure 2d). One-way ANOVA indicated that the decrease in P_m was significantly larger than that in *C. philippensis* and *P. weinmannifolia* ($P < 0.001$). To further examine whether P_m could reflect the quantity of PSI complexes, the effect of rapid dehydration on P_m was determined. The values of P_m in the three tree species remained very stable during the rapid dehydration treatment (Figure 6), suggesting that P_m was a reliable parameter that can reflect the change in the quantity of PSI complexes.

Light response curves indicated that at the end of the prolonged dry season, the maximum quantum yield of PSII after light adaptation (F_v'/F_m') and the coefficient of photochemical quenching (qP) largely decreased in *C. sumatranus* (Figure 3a, b), resulting in the strong decrease in effective quantum yield of PSII [*Y*(II)] (Figure 3c). In *C. philippensis*, F_v'/F_m', qP and *Y*(II) decreased slightly at the end of the dry season (Figure 3f, g, h). In *P. weinmannifolia*, F_v'/F_m' slightly changed and qP largely decreased at the end of the dry season, resulting in a decrease in *Y*(II) (Figure 3k, l, m). The maximum value of *Y*(NPQ) significantly decreased in *C. sumatranus* ($P < 0.0001$, *T*-test) but did not differ in the other two species ($P > 0.05$, *T*-test) (Figure 3d, i, n). As a result, the quantum yield of non-regulated energy dissipation in PSII [*Y*(NO)] largely increased at the end of the dry season in *C. sumatranus* but changed slightly in the other two species (Figure 3e, j, o). The increase in *Y*(NO) in *C. sumatranus* at the end of the dry season was larger than that in the other two species. At the end of the dry season, the maximum value of NPQ largely decreased in *C. sumatranus* (Figure 4a). In the other two species *C. philippensis* and *P. weinmannifolia*, the maximum value of NPQ did not change between the end of the rainy season and the end of the dry season (Figure 4b, c). The maximum value of NPQ in *C. sumatranus* at the end of the dry season was significantly lower than that in the other two species *C. philippensis* and *P. weinmannifolia* ($P < 0.0001$, one-way ANOVA).

At the end of the rainy season (December 2009), the light response curves indicated that the *Y*(I)/*Y*(II) ratio was ~1.0 under low light intensity of <100 μmol m^{-2} s^{-1} in *C. sumatranus*, but significantly increased at light intensity of >100 μmol m^{-2} s^{-1} (Figure 5a). However, at the end of the dry season (March 2010), light response curves indicated that the *Y*(I)/*Y*(II) ratio was maintained at ~1 under all light intensities in this species (Figure 5a). In the other two species *C. philippensis* and *P. weinmannifolia*, the *Y*(I)/*Y*(II) ratio was higher than 1.0 under high light in both the rainy and dry seasons (Figure 5b, c), with higher values under high PPFD in the dry season compared with that in the rainy season. The value of the *Y*(I)/*Y*(II) ratio in *C. sumatranus* at the end of the dry season was lower than that in the other two species *C. philippensis* and *P. weinmannifolia*.

Discussion

PSI is more sensitive to long-term severe drought stress than PSII

At the end of the dry season, the decrease in P_m was much larger than the decrease in F_v/F_m, suggesting that PSI is more sensitive to the long-term severe drought stress than PSII (Figure 2). The predawn leaf water potential (Ψ_{pd}) significantly

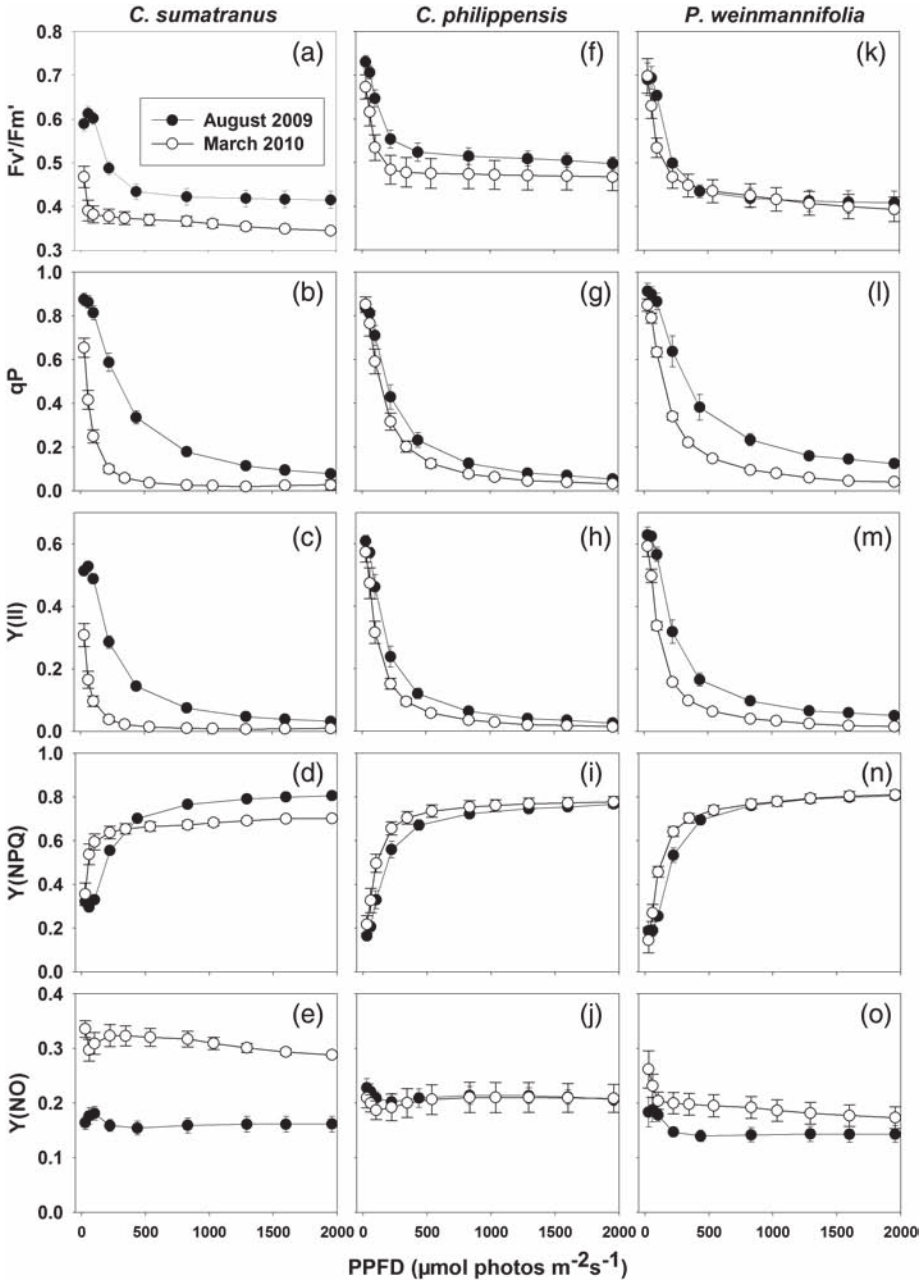

Figure 3. Light response curves of F_v'/F_m', qP, *Y*(II), *Y*(NPQ) and *Y*(NO) in *C. sumatranus*, *C. philippensis* and *P. weinmannifolia* at the end of the rainy season (closed symbols) and at the end of the dry season (open symbols). The mean ± SE was calculated from at least four plants.

decreased at the end of the dry season compared with the rainy season, especially in *C. sumatranus* (Figure 1a). The difference in Ψ_{pd} between the three species may be caused by the difference in the soil water status near the roots. The value of Ψ_{pd} at the end of the severe dry season was lower than that of the leaf turgor loss point in the two species, *C. sumatranus* and *C. philippensis*, measured in the normal dry season (Figure 1a, b). Although the value of Ψ_{pd} at the end of the severe dry season was higher than that of the leaf turgor loss point in the normal dry season in *P. weinmannifolia*, the Ψ_{pd} at the end of the dry season was still quite low (Figure 1a, b). The severe water deficit in leaves from all three species induced a strong reduction in stomatal conductance (Figure 1c), which led to a decrease in the ability of those plants to utilize NADPH (a product of LEF) and, consequently, an inhibition of their effective quantum yield of PSII [*Y*(II)] (Figure 1d, 3c), especially in *C. sumatranus*, which was characterized by the lowest values of Ψ_{pd} and F_v/F_m at the end of the dry season (Figure 1a, 2c).

There was a significant decrease in P_m at the end of the severe dry season in all three species, especially in *C. sumatranus* that showed a decrease of 65% (Figure 2d). Since P_m is a reliable parameter reflecting the quantity of maximum

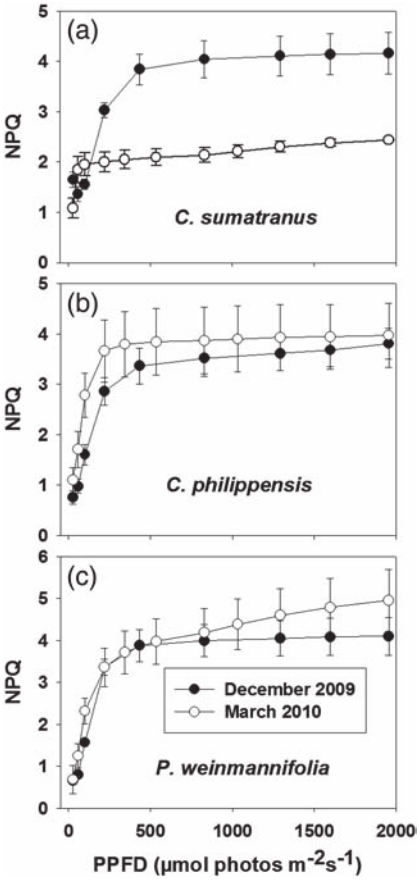

Figure 4. Light response curves of non-photochemical quenching (NPQ) in *C. sumatranus*, *C. philippensis* and *P. weinmannifolia* at the end of the rainy season (closed symbols) and at the end of the dry season (open symbols). The mean ± SE was calculated from at least four plants.

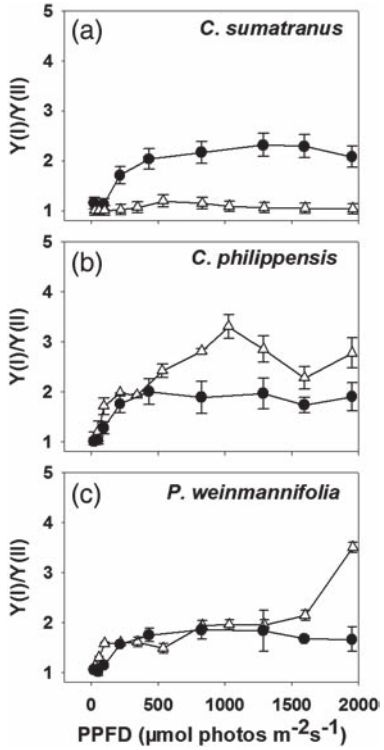

Figure 5. Light response curves of *Y*(I)/*Y*(II) in *C. sumatranus*, *C. philippensis* and *P. weinmannifolia* at the end of the rainy season (closed circle) and at the end of the dry season (open triangle). The mean ± SE was calculated from at least four plants.

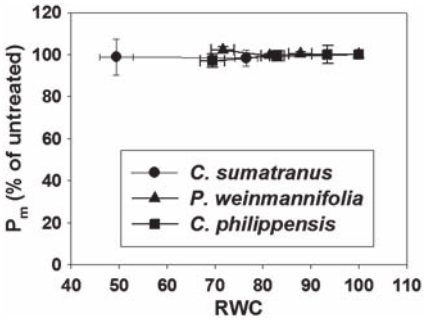

Figure 6. The value of maximum photo-oxidizable P700 (P_m) during rapid dehydration in *C. sumatranus*, *C. philippensis* and *P. weinmannifolia*. The mean ± SE was calculated from at least four plants.

photo-oxidizable P700 (Figure 6), the decrease in P_m indicates PSI photoinhibition. It is suggested that the PSI photoinhibition is mainly induced by the deleterious effect of hydroxyl radicals, which can be generated as a result of over-reduction of the PSI acceptor side and over-accumulation of reducing power NADPH on the PSI acceptor side (Sonoike et al. 1997, Sonoike 2006, 2011). The nearly complete closure of stomata in the three tree species at the end of the dry season could induce an increase in photorespiration that consumes a lot of ATP. Therefore, the NADPH/ATP ratio should be increased in the dry season and then induces PSI photoinhibition. However, plants have several mechanisms such as CEF and the antioxidant system to protect PSI from photoinhibition (Munekage et al. 2002, 2004). Our results indicated that CEF was activated in *C. philippensis* and *P. weinmannifolia* at the end of the dry season, which can alleviate PSI photoinhibition (Munekage et al. 2002, 2004). Since the recovery of PSI photoinhibition is a slow process that needs some days (Zhang and Scheller 2004), we speculate that severe PSI photoinhibition does not occur during only a few days of stress but is a result of long-term accumulated damage. Compared with PSI, the recovery of PSII is a very fast process that can be completed in several hours (Aro et al. 1994, Zhang and Scheller 2004, Huang et al. 2010*a*, 2010*b*). Therefore, the different effects of long-term severe drought on PSI and PSII activities are probably caused by differences in recovery rates between PSI and PSII.

Cleistanthus sumatranus displays more severe photoinhibition of PSI and PSII than the other two species.

Our results indicated that *C. sumatranus* experienced much greater photoinhibition of PSI and PSII than *C. philippensis* and *P. weinmannifolia* by the end of the prolonged drought, which

was consistent with its very low values for Ψ_{pd}. Although all three species are native to tropical limestone forests, they showed different amounts of sensitivity of their photosynthetic apparatus to prolonged severe drought. At the end of the prolonged drought, CEF had disappeared in *C. sumatranus* but was activated under high light in the other species (Figure 5). In *Arabidopsis*, CEF has been indicated as a crucial mechanism for protecting PSI from photoinhibition under high light (Munekage et al. 2002, 2004). It not only alleviates the over-reduction of the PSI acceptor side (Munekage et al. 2002, 2004), but also consumes excess reducing power NADPH through the NADPH dehydrogenase-dependent pathway (Wang et al. 2006, Shikanai 2007). However, we found here that CEF could not fully protect PSI against photodamage as indicated by decreases in P_m in the other two species (Figure 2d). Because activation of CEF is based on PSI activity, the severe PSI photoinhibition caused the impairment of CEF in *C. sumatranus*, which then induced the generation of hydroxyl radicals on the acceptor side of PSI and further aggravated PSI photoinhibition. The association between CEF impairment and severe PSI photoinhibition in *C. sumatranus* by the end of the prolonged drought suggested that CEF has a role in photoprotection for PSI in drought-tolerant tree species, as we previously demonstrated in a study with the resurrection plant, *Paraboea rufescens* (Franch.) Burtt. (Huang et al. 2012*a*). By the end of the dry season, light response curves indicated that the *Y*(I)/*Y*(II) ratio was maintained at ~1 under all light intensity values in *C. sumatranus* (Figure 5a), indicating that the prolonged severe drought led to the impairment of CEF in *C. sumatranus*. Since the activation of CEF is dependent on PSI activity (Peng and Shikanai 2011), the severe photoinhibition of PSI in turn inhibited the operation of CEF in *C. sumatranus*. Thus, we could conclude that the more severe photoinhibition of PSI in *C. sumatranus* was largely caused by serious damage to CEF.

By the end of the dry season, photoinhibition of PSII was much stronger in *C. sumatranus* than in the other two species. Decreases in the maximum values for *Y*(NPQ) and NPQ and an increase in *Y*(NO) at the end of the dry season in *C. sumatranus* suggested that it was unable to dissipate excess light energy and that excess ROS had been generated in the chloroplasts. Recent studies indicated that ROS have a role in inhibiting the synthesis of D1 protein, which is essential for the repair of photodamaged PSII complexes (Nishiyama et al. 2001, 2005, 2006, 2011). Therefore, the high degree of PSII photoinhibition in *C. sumatranus* compared with the other two species was partly caused by the decrease in *Y*(NPQ) and the increase in *Y*(NO).

Activation of NPQ relies on the formation of a proton gradient across the thylakoid membranes (ΔpH) (Munekage et al. 2004). In chloroplasts, LEF and CEF are the two main pathways for the generation of ΔpH. At the end of the dry season, LEF was severely inhibited in *C. sumatranus* (Figure 3c) and, thus, LEF-dependent build-up of ΔpH was inhibited. In *C. philippensis* and *P. weinmannifolia* the involvement of CEF during that period was critical to the normal activation of NPQ. However, CEF disappeared in *C. sumatranus* by the end of the dry season (Figure 5a), accompanied with low *Y*(NPQ) and NPQ (Figure 3d, 4a). Consequently, the repair cycle of PSII of *C. sumatranus* was largely inhibited by the excess light energy that cannot be harmlessly dissipated as heat. Furthermore, CEF probably protects PSII by stabilizing the oxygen-evolving complex (OEC) (Takahashi et al. 2009). Abolishment of PGR5-dependent CEF could induce larger photoinhibition of PSII compared with the impairment of NPQ (Takahashi et al. 2009), indicating that the damage to OEC is another mechanism for PSII photoinhibition induced by excess light energy (Tyystjärvi 2008). The complete impairment of CEF in *C. sumatranus* (Figure 5a) probably caused the instability of OEC and then aggravated PSII photoinhibition.

The CEF-dependent formation of ΔpH not only assists in the activation of NPQ, but also favors ATP synthesis. Under drought and high light stress, the closure of stomata would induce a decrease in the activation of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) and an increase in photorespiration (Jordan and Ogren, 1984, Salvucci and Crafts-Brandner, 2004*a*, 2004*b*). Therefore, CO_2 fixation at midday requires more ATP when photorespiration is active (Osmond, 1981). Furthermore, the selective photoinhibition of PSII caused by high light not only leads to direct LEF inhibition, but also prompts extra ATP to be consumed in the repair of PSII. Therefore, the low level of LEF-dependent ATP synthesis at midday may limit the CO_2 fixation. The high ATP demand at noon would result in a higher NADPH/ATP ratio, favoring non-photochemical reduction of the PQ pool from stromal donors, which in turn would activate the NDH-mediated CEF (Miyake et al. 2005, Rumeau et al. 2007).

In conclusion, our findings provide evidence that PSI is more sensitive than PSII to prolonged severe drought in the three tolerant tree species examined here. To a certain extent, PSI photoinhibition was caused by a slow recovery rate. Mild drought stress can induce the up-regulation of ferredoxin-dependent CEF (Lehtimäki et al. 2010). However, we found that prolonged stress induced a severe inhibition of CEF in *C. sumatranus*, the species that had experienced the greatest photoinhibition of PSI and PSII. Therefore, we have demonstrated that CEF is crucial to drought-tolerant plants for protecting their photosynthetic apparatus against severe drought stress.

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Conflict of interest

None declared.

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