PCP
PLANT & CELL PHYSIOLOGY

Cyclic Electron Flow Plays an Important Role in Photoprotection of Tropical Trees Illuminated at Temporal Chilling Temperature

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(Received August 18, 2010; Accepted October 28, 2010)

Our previous study indicated that PSII is more sensitive to chilling and light stress than PSI in tropical trees, and *Erythrophleum guineense* is more sensitive to chilling stress than *Dalbergia odorifera* and *Khaya ivorensis*, but the underlying physiological mechanisms are unclear. Although recent studies have reported that cyclic electron flow (CEF) plays an important role in photoprotection, the role of CEF in protecting PSI and PSII of tropical tree species remains unclear. We investigated the effect of temporal chilling temperature on energy distribution in PSII, the redox state of P700 and CEF in the above-mentioned tropical evergreen tree species grown in an open field. Our results indicated that the overclosure of PSII reaction centers at chilling temperature led to excess excitation pressure in PSII. At the temporal chilling temperature under low light, PSI acceptor side limitation [Y(NA)] was lower than those at 25°C for all species. Although the effective quantum yield of CEF [Y(CEF)] was not significantly stimulated in *E. guineense* and *K. ivorensis* under temporal chilling at low light levels, the ratio of Y(CEF) to the effective quantum yield of PSII [Y(II)] significantly increased. Under chilling conditions Y(CEF)/Y(II) was stimulated much more in *K. ivorensis* and *D. odorifera* compared with that in the chilling-sensitive *E. guineense*. These results suggested that stimulation of Y(CEF)/Y(II) plays an important role in protecting PSI and PSII from photoinhibition caused by chilling stress.

Keywords: Chilling temperature • Cyclic electron flow • Photoprotection • PSI • Tropical trees.

Abbreviations: CEF, cyclic electron flow; LEF, linear electron flow; NPQ, non-photochemical quenching; PFD, photon flux density; PQ, plastoquinone; Y(CEF), effective quantum yield of CEF; Y(II), efficient quantum yield of PSII; Y(I), efficient quantum yield of PSI; Y(NA), PSI acceptor side limitation; Y(ND), PSI donor side limitation; Y(NO), yield of non-regulated energy dissipation; Y(NPQ), yield of regulated energy dissipation.

Introduction

Photoinhibition that is regarded as a decrease in photosynthetic efficiency under excessive light (Powles 1984, Barber and Andersson 1992) takes place not only under high light but also under low illumination at chilling temperature (Aro et al. 1990, Aro et al. 1993, Havaux and Davaud 1994, Sonoike 1995, Sonoike 1996, Tjus et al. 1998, Sonoike 1999, Zhang and Scheller 2004, Huang et al. 2010). Previous studies have indicated that under chilling temperature at low light the damage to PSII is negligible whereas PSI is severely photodamaged in chilling-sensitive species such as cucumber, potato, pumpkin and Arabidopsis (Havaux and Davaud 1994, Terashima et al. 1994, Barth and Krause 1999, Zhang and Scheller 2004). However, almost all the plant materials used in previous studies on the chilling effect on PSI were grown at low photon flux density (PFD) between 100 and 300 $\mu mol\, m^{-2}\, s^{-1}$ or even lower. Since development of protective mechanisms such as non-photochemical quenching (NPQ) and cyclic electron flow (CEF) could be enhanced in tobacco leaves grown under high growth light (Miyake et al. 2005), the preferential PSI photoinhibition in plants grown under low or moderate light may not reflect the natural chilling effect on plants grown in an open field. Furthermore, in previous studies on the effect of chilling on PSI, the plant materials were grown at a warm temperature such as 25°C before chilling treatment; as a result, the effect of natural cold acclimation was ignored. Our previous study has indicated that PSII is more sensitive to chilling and light stress than PSI in tropical tree species that were grown in high light and allowed to acclimate fully to natural cold temperatures (Huang et al. 2010). This is in contrast to the previous findings that PSI is more sensitive to short-term chilling and light stress than PSII. However, it is unclear why PSI in tropical trees grown under high light is not susceptible to chilling stress.

In the chilling-sensitive herbaceous plants cucumber and potato, the preferential damage to PSI was suggested to be

Plant Cell Physiol. 52(2): 297–305 (2011) doi:10.1093/pcp/pcq166, available online at www.pcp.oxfordjournals.org

caused by the oxidation by hydroxyl radicals (Sonoike 1996, Sonoike 2006). The hydroxyl radicals, the most reactive species of active oxygen, which are generated by the reaction between hydrogen peroxide and photoreduced iron–sulfur centers, destroy PSI at the site of production of hydroxyl radicals (Sonoike 1996, Asada 1999, Sonoike 2006). Low temperature causes a large decrease in the rate of carbon dioxide fixation which could lead to an accumulation of reducing power NADPH on the acceptor side of PSI (Mi et al. 2000). NADPH is an essential biochemical energy carrier for carbon dioxide fixation, but its accumulation enhances the generation of hydroxyl radicals. Furthermore, when the PSI acceptor side is over-reduced, the recombination between the radical pairs $P700^+/A0^-$ or $P700^+/A1^-$ can generate the triplet state of P700 (Shuvalov et al. 1986, Golbeck 1987, Golbeck and Bryant 1991). Chl triplets can react with molecular oxygen to produce very toxic singlet oxygen that could cause photoinhibitory damage to PSI. Therefore, there are two main mechanisms for the PSI photodamage: the accumulation of reducing power NADPH at the PSI acceptor side and the over-reduction of the PSI acceptor side. Previous studies have indicated that photoinhibitory damage to PSI could be prevented by the inhibition of linear electron flow (LEF) from PSII to PSI (Satoh 1970, Sonoike 1995, Kudoh and Sonoike 2002). Furthermore, CEF has been documented as an important mechanism for protecting PSI in cucumber and Arabidopsis under chilling stress (Kim et al. 2001, Munekage et al. 2002, Takahashi et al. 2009). CEF could not only oxidize P700 into $P700^+$ but could also consume excess reducing power NADPH (Shikanai 2007). $P700^+$ is able to dissipate excess excitation energy to harmless heat and thereby to quench deleterious effects of excess light effectively (Nuijs et al. 1986). Based on these previous studies and our previous finding on the greater sensitivity of PSII than PSI under light and chilling conditions in tropical trees, we speculate that the inhibition of LEF and stimulation of CEF are two crucial mechanisms for the protection of PSI from chilling injury in tropical trees. Our previous study also indicated that PSI is much more sensitive to chilling and light stress in *Erythrophleum guineense* than in two other tropical tree species studied (*Khaya ivorensis* and *Dalbergia odorifera*) (Huang et al. 2010); we speculate that the stimulation of CEF at chilling temperature is much weaker in *E. guineense* than in the other two species.

Although inhibition of LEF can protect PSI from photoinhibition, it is harmful to PSII because of excessive excitation pressure accumulated on PSII reaction centers. If the excessive excitation energy could not be dissipated harmlessly through the NPQ system and other electron sink pathways, photoinhibition of PSII would result from excess generation of reactive oxygen species (Asada 1996, Asada 1999, Niyogi 2000). In addition to NPQ, it has been reported that CEF is another important mechanism for the protection of PSII under excessive light conditions (Munekaga et al. 2002, Takahashi et al. 2002, Miyake et al. 2004, Takahashi et al. 2009). CEF-dependent generation of a proton gradient across the thylakoid membrane helps to alleviate photoinhibition of PSII by at least two main mechanisms (Takahashi et al. 2009): one is linked to NPQ that prevents direct photodamage to PSII and the repair of photodamaged PSII reaction centers at the step of protein synthesis, and the other is independent of NPQ. Although CEF has been documented as an important mechanism for protecting PSII from photoinhibition in herbaceous plants such as Arabidopsis, no information has been documented on the role of CEF in photoprotection in woody plants such as tropical tree species (Cruz et al. 2005).

The trees that are distributed in the low tropics are very sensitive to chilling temperature, but little is known about the photosynthetic mechanisms of this sensitivity to chilling stress. In the present study, we investigated the effect of chilling temperature on energy distribution in PSII, the P700 redox state and CEF in three tropical tree species grown in an open field in a marginal tropical area, one of which is very chilling sensitive. The following questions were addressed. (i) Is CEF, relative to LEF, stimulated in tropical trees illuminated under chilling temperature? (ii) Is the stimulation of CEF at chilling temperature much weaker in the very chilling-sensitive species?

Results

Effects of temporal chilling conditions on energy distribution of light absorbed by PSII

Compared with that at 25°C, the light-adapted maximum quantum yield of PSII (F_v'/F_m') at 4°C decreased only slightly under low light on the light response curves for all three species (**Fig. 1A**). However, the photochemical quenching coefficient (qP) showed large decreases at all light intensities on the light response curves (**Fig. 1B**) (Huang et al. 2010). Since the effective quantum yield of PSII [Y(II)] is the product of (F_v'/F_m') multipled by qP, our results indicated that the significant decrease in Y(II) at 4°C was mainly due to the decrease in the PSII qP but not the decrease in F_v'/F_m'.

The energy distribution in PSII varied significantly between 4 and 25°C for all three species. Y(II) at 4°C was much lower than at 25°C (**Fig. 2A**). In contrast, the quantum yield of regulated energy dissipation in PSII [Y(NPQ)] at 4°C was much higher than at 25°C especially under low light (**Fig. 2B**). Nevertheless, the quantum yield of non-regulated energy dissipation in PSII [Y(NO)] at 4°C was significantly higher than at 25°C (**Fig. 2C**).

Effect of temporal chilling temperature on the redox state of P700 in the light

The redox state of P700 was significantly affected by the temporal chilling conditions. Under the chilling temperature of 4°C, on the light response curves the photochemical quantum yield of PSI [Y(I)] rapidly decreased with light intensity at low light range, always with much lower values at all light dosages on the light response curves than at 25°C (**Fig. 2D**). The fast decrease in Y(I) with light intensity at 4°C was mainly due to

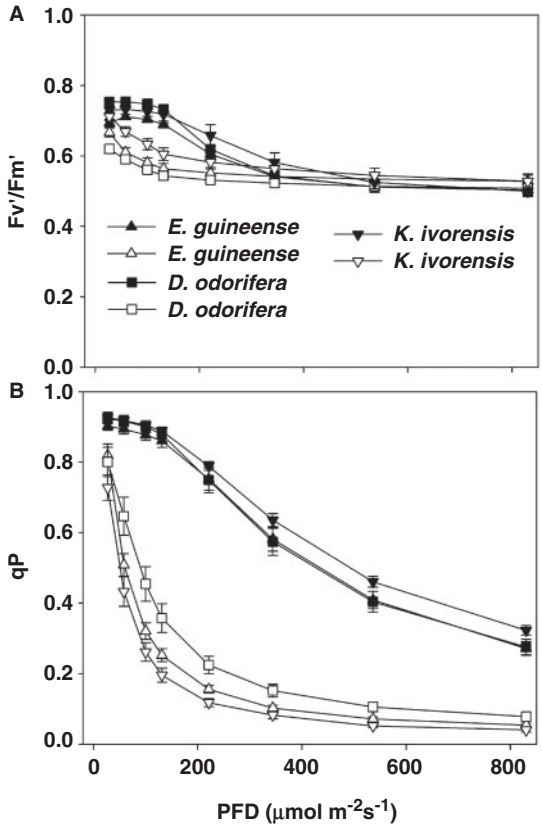

Fig. 1 Difference in the light response changes of F_v'/F_m' and qP at 4°C (open symbols) and 25°C (filled symbols) among the three tree species *Erythrophleum guineense*, *Dalbergia odorifera* and *Khaya ivorensis*. The means ± SE were calculated from 6–8 plants. The data about qP were referred from Huang et al. (2010).

the increase in the donor side limitation [Y(ND)]. Under the chilling temperature, Y(ND) increased rapidly with light intensity in the low light range and almost reached the maximum value of 0.8 at the PFD of 344 μmol m^{-2} s^{-1} (**Fig. 2E**). Y(ND) gradually increased with light intensity at 25°C, always with much lower values at all light dosages on the light response curves than at 4°C. More importantly, the value of PSI acceptor side limitation [Y(NA)] under low light at 4°C was lower than at 25°C in the three species, especially in *D. odorifera* (**Fig. 2F**).

Response of CEF to temporal chilling temperature

CEF was not stimulated in *D. odorifera* illuminated under low light at 25°C (**Fig. 3C**). However, in *E. guineense* and *K. ivorensis*, CEF already attained quite high values under low light at 25°C, especially in *E. guineense* (**Fig. 3A, B**). Under the temporal chilling temperature of 4°C, CEF was significantly stimulated in *D. odorifera* illuminated under low light (**Fig. 3C**). However, the value of the effective quantum yield of CEF [Y(CEF)] under low light at 4°C in *E. guineense* was lower than that at 25°C (**Fig. 3A**). Under low light, there was no significant difference in Y(CEF) between 25 and 4°C in *K. ivorensis* (**Fig. 3B**). At high light on the light response curves, Y(CEF) was more strongly reduced at 4°C than at 25°C in all species.

The value of Y(CEF)/Y(II) under low light was significantly stimulated by the temporal chilling temperature in all three species (**Fig. 3D–F**). Compared with that in the chilling-sensitive *E. guineense*, under temporal chilling conditions this ratio was much higher in *K. ivorensis* at all light dosages on the light response curve and significantly higher in *D. odorifera* at low light dosages. In *K. ivorensis*, the value of Y(CEF)/Y(II) at 4°C was much higher than that at 25°C (**Fig. 3E**). An analysis of the chilling effect on Y(CEF)/Y(II) among the three species by one-way analysis of variance (ANOVA) indicated that the stimulation of Y(CEF)/Y(II) at 4°C associated with PFDs of 131 and 221 μmol m^{-2} s^{-1} in *D. odorifera* and *K. ivorensis* was significantly greater than in *E. guineense* ($P < 0.001$) (data not shown).

Discussion

PSII is susceptible to chilling and light stress

Our results indicated that the excessive excitation in PSII induced the severe reversible photoinhibition of PSII under temporal chilling and light stress. Low temperature decreased the capacity for carbon fixation and LEF, which led to potential excess light excitation pressure in PSII reaction centers (Xu et al. 1999, Clarke and Johnson 2001, Hendrickson et al. 2003). It is well known that excess light could cause the generation of singlet oxygen and other reactive oxygen species which are deleterious to PSII (Asada 1996, Asada 1999, Niyogi 2000). Our results indicated that the Y(II) at 4°C was much lower than that at 25°C (**Fig. 2A**). This could come about if electron delivery from PSII to $P700^+$ was slowed at the lower temperature. Perhaps diffusion of plastoquinone (PQ) or plastocyanin (PC) to convey electrons to PSI, or the oxidation of plastoquinol (PQH_2) at the cytochrome *b/f* complex, was inhibited by the chilling temperature of 4°C. Meanwhile, the yield of regulated energy dissipation of PSII [Y(NPQ)] was strongly stimulated (**Fig. 2B**). Nevertheless, the yield of non-regulated energy dissipation [Y(NO)] at 4°C was significantly higher than at 25°C (**Fig. 2C**). Furthermore, Casano et al. (1997) reported that the activity of the antioxidant system was negatively affected by chilling temperature, which aggravated the reversible photoinhibition of PSII. This indicated that the PSII reaction center activities were strongly down-regulated or reversibly damaged by excess light energy which was induced by the chilling stress. It is concluded that the reversible photoinhibition of PSII in tropical tree species was caused by a high level of non-regulated energy dissipation which was induced by short-term chilling and light stress.

PSI is not sensitive to chilling and light stress

We found that the lower limitation of the PSI acceptor side [Y(NA)] at 4°C than that at 25°C prevented PSI from photoinhibition under chilling and light stress (**Fig. 2F**). It has been reported that the photoinhibition of PSI is due to the oxidation of hydroxyl radicals that are induced by active excess electron transport from PSII to PSI (Havaux and Davaud 1994,

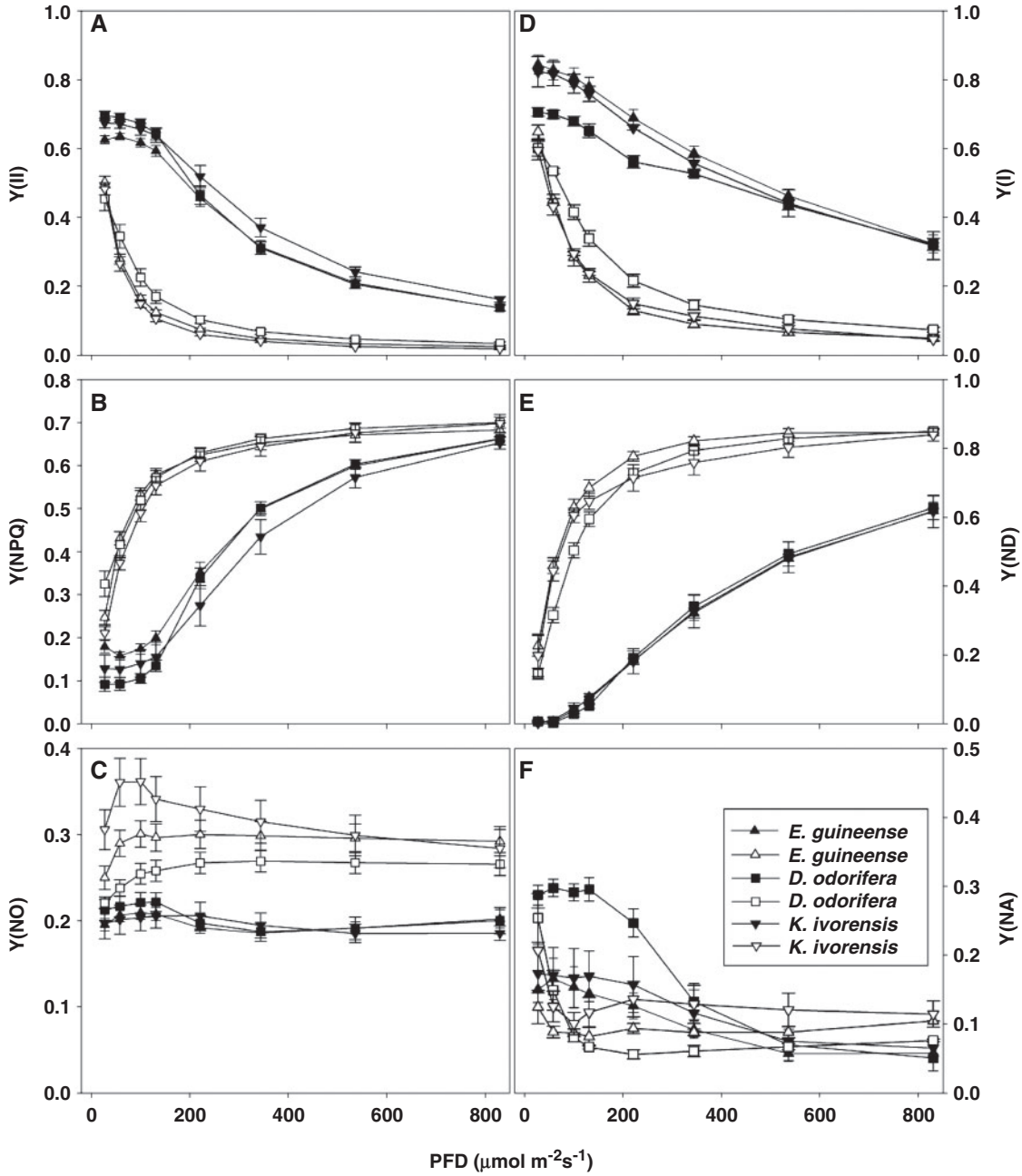

Fig. 2 Difference in the light response changes of Y(II), Y(NPQ), Y(NO), Y(I), Y(ND) and Y(NA) at 4°C (open symbols) and 25°C (closed symbols) among the three tree species *Erythrophleum guineense*, *Dalbergia odorifera* and *Khaya ivorensis*. The means ± SE were calculated from 6–8 plants. Y(II) is the efficient quantum yield of PSII; Y(NPQ) is the yield of regulated energy dissipation of PSII; Y(NO) is the yield of non-regulated energy dissipation of PSII; Y(I) is the quantum yield of PSI; Y(ND) is the donor side limitation of PSI; and Y(NA) is the acceptor side limitation of PSI.

Kudoh and Sonoike 2002). The electrons transported from PSII to PSI could be consumed by downstream electron sink pathways such as carbon fixation. Since low temperature decreased the rate of carbon fixation, the excess light energy resulted in over-reduction of the PSI acceptor side and accumulation of reducing power NADPH at the PSI acceptor side, and then caused the generation of hydroxyl radicals (Sonoike 1996). Accordingly, the photoinhibition of PSI could be prevented by blocking the LEF from PSII to PSI (Havaux and Davaud 1994, Sonoike 1995, Kudoh and Sonoike 2002). In our present study, at 4°C LEF from PSII to PSI was inhibited mainly through closure of PSII reaction centers (**Fig. 1**) and possibly photodamage to PSII, as indicated by an increase of Y(NO) (**Fig. 2C**). As a result, PSI was protected.

The over-reduction of the PSI acceptor side is a prerequisite for the generation of hydroxyl radicals (Sonoike 1996). Thus the

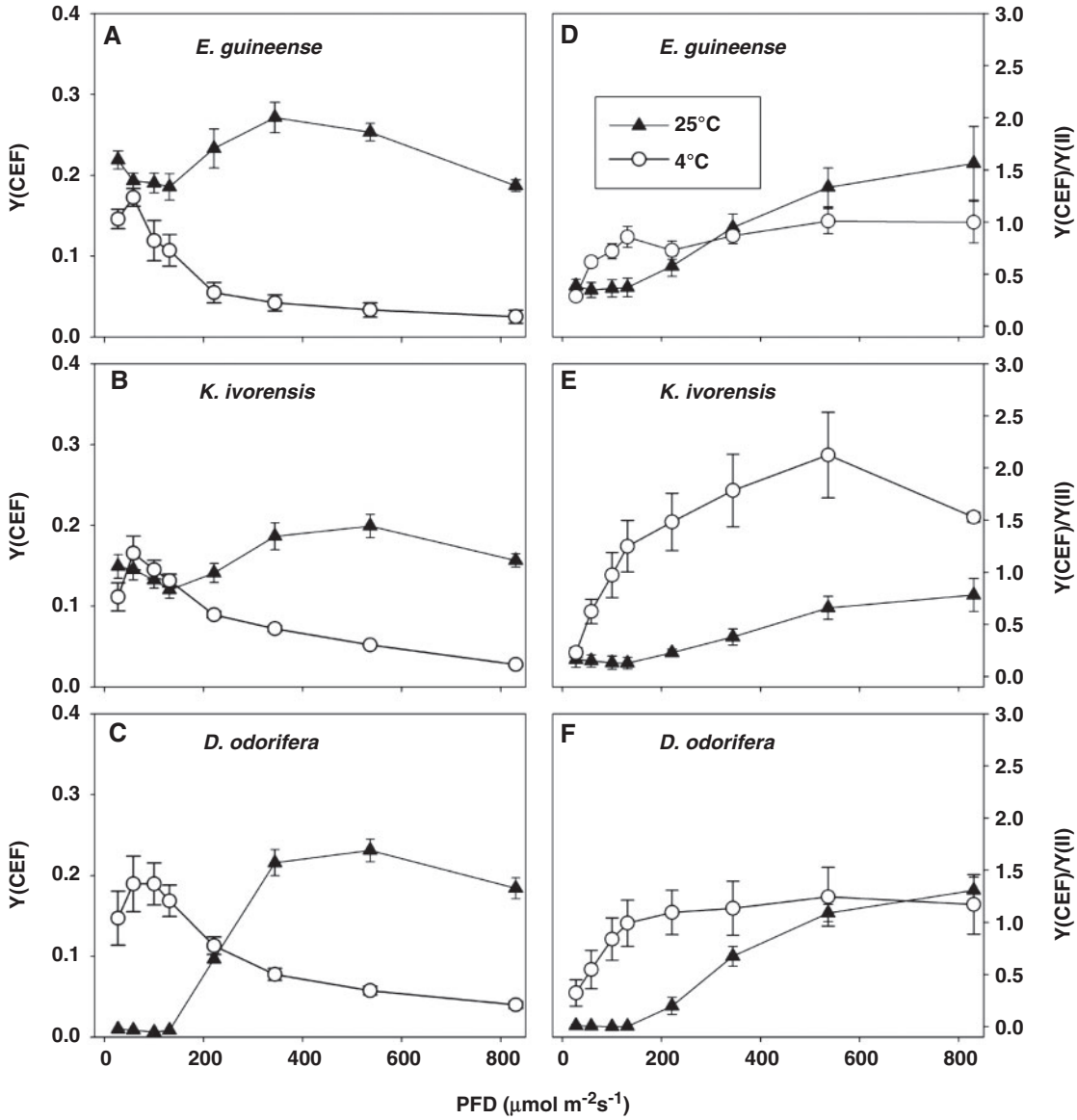

Fig. 3 The light response changes of Y(CEF) and Y(CEF)/Y(II) at 4 and 25°C in the three tree species *Erythrophleum guineense*, *Dalbergia odorifera* and *Khaya ivorensis*. The means ± SE were calculated from 6–8 plants. An analysis of the chilling effect on Y(CEF)/Y(II) among the three species by one-way ANOVA indicated that the stimulation of Y(CEF)/Y(II) at 4°C associated with PFDs of 131 and 221 μmol m^{-2} s^{-1} in *D. odorifera* and *K. ivorensis* was significantly greater than in *E. guineense* ($P < 0.001$).

proportion of reduced electron carriers on the acceptor side of PSI which could not be oxidized by a saturating pulse [Y(NA)] can be used as an indicator of the photoinhibition of PSI. Our results showed that the Y(NA) at 4°C was significantly lower than at 25°C at a PFD of 0–221 μmol m^{-2} s^{-1}. Therefore, the photoinhibition of PSI could be prevented at the chilling temperature of 4°C associated with a PFD of <250 μmol m^{-2} s^{-1} in the two species *K. ivorensis* and *D. odorifera* (Huang et al. 2010). Our finding of decreased Y(NA) under low light at the temporal chilling temperature of 4°C in the tropical tree species is in contrast to the results of Kim et al. (2005), who reported that under low light at chilling temperature Y(NA) in cucumber was much higher than at moderate temperature. Our results indicated that the down-regulation of Y(II) was a crucial cause for the non-susceptibility of PSI to chilling stress associated with moderate light intensity in the tropical trees. Furthermore, CEF is another mechanism for protecting PSI against chilling and light stress in the tropical tree species, as discussed below.

The role of CEF in protecting PSI and PSII from photoinhibition

The present study revealed that CEF played an important role in protecting PSII in the tropical tree species exposed to chilling

temperature, which supports the results of previous studies on the herbaceous plant Arabidopsis. In *D. odorifera* in the present study, Y(CEF) was strongly stimulated by the temporal chilling of 4°C under low light (**Fig. 3C**). Since at 25°C under low light the absorbed light energy could be consumed through photosynthesis, the excessive light energy was negligible and no photoinhibition occurred. Consequently, CEF was maintained low under low light at 25°C. However, at 4°C, the photosynthetic efficiency greatly decreased, resulting in excessive excitation pressure in PSII and consequently increasing risk of photoinhibition of PSII. Plants could dissipate excess light energy harmlessly to heat in the antenna proteins of PSII via the regulated energy dissipation that is dependent on the proton gradient across thylakoid membranes. Since CEF could generate a proton gradient across the thylakoid membrane (Munekage et al. 2002) through transferring electrons from PSI to PQ, it is important for protecting PSII through dissipating excess light energy (Takahashi et al. 2009). Consistent with this, in our study, because of the strong stimulation of CEF, Y(NPQ) under low light at 4°C was much higher than at 25°C. As a result, PSII in the three tropical tree species was well protected from photoinhibition at the chilling temperature associated with a low PFD of 0–150 μmol m^{-2} s^{-1} (Huang et al. 2010).

Our study indicated that CEF also played an important role in protecting PSI from photoinhibition under temporal chilling and light stress in the three tropical tree species. The photoinhibition of PSI is caused by the over-reduction of the PSI acceptor side and excess accumulation of reducing power NADPH. At chilling temperatures, a decrease in carbon fixation capacity could lead to these situations. CEF could not only alleviate the over-reduction of the PSI acceptor side through transporting electrons from the PSI acceptor side to PQ, but could also consume excess reducing power NADPH through the NADPH dehydrogenase-dependent pathway (Shikanai 2007). At the chilling temperature, CEF was stimulated mainly by the over-reduction of the PSI acceptor side and the imbalance of $NADP^+$/NADPH (Okegawa et al. 2008). Consequently, more P700 was oxidized to $P700^+$ and the excess accumulation of NADPH was prevented. $P700^+$ could harmlessly dissipate excess light energy to heat (Nuijs et al. 1986). Therefore, the rapid increase in Y(ND) with light intensity on the light response curve at 4°C under low light in the three tropical tree species should be mainly caused by the stimulation of CEF and contribute to protecting PSI from photoinhibition. Our previous study indicated that in the three tropical tree species PSI was insensitive to the chilling temperature of 4°C with a low PFD of 0–150 μmol m^{-2} s^{-1} (Huang et al. 2010); the present study revealed that CEF played a crucial role in protecting PSI from this chilling and light stress.

Our results indicate that the extent of stimulation of Y(CEF)/Y(II) affects the sensitivity of PSI to chilling and light stress. Our previous study indicated that the PSI and PSII in *E. guineense* were more sensitive to the chilling temperature of 4°C with a PFD of 250 μmol m^{-2} s^{-1} than in *D. odorifera* and *K. ivorensis*, but the underlying mechanism is unclear (Huang et al. 2010). Our present study indicated that in *E. guineense* the value of Y(CEF)/Y(II) was not stimulated very much under a PFD of 221 μmol m^{-2} s^{-1} at 4°C compared with at 25°C (**Fig. 3A**). In contrast, in *D. odorifera* and *K. ivorensis* the value of Y(CEF)/Y(II) remained very low under very low light at 25°C but was significantly stimulated at 4°C, especially under low and moderate light on the light response curves. We consider that the deficiency of CEF under the chilling temperature in *E. guineense* is responsible for its exacerbated photoinhibition of PSI and PSII induced by chilling and light stress. In addition, we regard CEF as being a constitutive protective mechanism in *E. guineense* as indicated by the relatively high Y(CEF) at low light at 25°C (**Fig. 3A, D**) but a derivational protective mechanism in *D. odorifera* and *K. ivorensis*. CEF could be stimulated to provide significant additional protection in *D. odorifera* and *K. ivorensis* when they are confronted with chilling and light stress. We speculate on whether CEF is a constitutive or a derivational protective mechanism that could affect the sensitivity of PSI and PSII to chilling and light stress.

In conclusion, we found that the selective photoinhibition of PSII at chilling temperature under moderate light intensity is mainly caused by the strong excitation pressure due to the overclosure of PSII reaction centers. The non-susceptibility of PSI in two of the three tropical tree species to the chilling and light stress is largely due to the strong inhibition of LEF and the significant stimulation of CEF which prevented the over-reduction of the PSI acceptor side and excess accumulation of reducing power NADPH. Under chilling temperature associated with light, the low stimulation of CEF could cause severe photoinhibition of PSI and PSII in tropical trees.

Materials and Methods

Plant materials and growth conditions

The following three evergreen tropical tree species were chosen for the study: *K. ivorensis* A. Chev (Meliaceae) is a large canopy species found in various habitat types in west and central tropical Africa but is most abundant in wet undisturbed evergreen forests; *D. odorifera* T. Chen (Fabaceae) is native to the Hainan Island of China and is a light-demanding tree species that inhabits secondary forests; and *E. guineense* G. Don (Fabaceae) is a large canopy species native to tropical Africa. All of these species produce high-quality timber and their seedlings exhibit good growth performance in Xishuangbanna Tropical Botanical Garden (21°54′N, 101°46′E) that is located in the northern boundary of the tropical zone. The 3-year-old seedlings of the three species were cultivated in an open field. The highest PFD at midday is up to 1,850 μmol m^{-2} s^{-1} in summer and 1,350 μmol m^{-2} s^{-1} in winter.

Chl fluorescence and P700 measurements

In order to understand why PSII is severely photoinhibited by the chilling temperature of 4°C under illumination of a moderate light intensity in these tropical trees, in January 2010 when

the plant were fully cold acclimated, we determined the light responses of Chl fluorescence and P700 from the leaves at 4 and 25°C synchronously with a Dual PAM-100 (Heinz Walz) connected to a computer with control software. During this period, the outdoor air temperatures at night and noon are ~12°C and ~25°C, respectively. Six to eight detached mature leaves placed on wet paper were light adapted (450 μmol m^{-2} s^{-1}) for 20 min at 25°C before measurement of light response curves at 25 and 4°C, and light-adapted fluorescence parameters were recorded after 2 min exposure to each of the PFDs (27, 58, 100, 131, 221, 344, 536 and 830 μmol m^{-2} s^{-1}). Before measurement at 4°C, leaves were dipped in chilled water (4°C) for 2 min to decrease the leaf temperature to 4°C. The temporal chilling conditions represent the effect of chilling temperature on the light energy distribution if PSI and PSII before irreversible photoinhibition take place.

Since the Dual PAM-100 instrument was placed in thermostatic 4°C chilling storage, the leaf temperature can be kept at 4°C during measurement. Our preliminary experiments indicated that after 15 min light adaptation under a PFD of 450 μmol m^{-2} s^{-1}, 2 min for each PFD during the light response measurements is enough to make the photosynthetic measurement stable. Moreover, 2 min moderate light did not cause significant photoinhibition of either PSI or PSII. After one series of light response curve measurements, both PSI and PSII were not significantly photoinhibited. Since the standard foil delivered with the DUAL-PAM-100 is temperature sensitive, according to experience, the Chl fluorescence measured at 4°C was corrected to that at 25°C using the equation, F_standard(T) = −0.003106 × temperature (in K) + 1.932; therefore, the data were normalized by measuring temperature so that the fluorescence value at 300 K is 1.

The following Chl fluorescence parameters were calculated: $F_v'/F_m' = (F_m' - F_o')/F_m'$, $qP = (F_m' - F_s)/(F_m' - F_o')$, $Y(II) = (F_m' - F_s)/F_m'$ (Genty et al. 1989), $Y(NO) = F_s/F_m$, $Y(NPQ) = 1 - Y(II) - Y(NO)$ (Kramer et al. 2004). F_o' is the minimum fluorescence in the light-adapted state. F_m and F_m' are the dark-adapted and light-adapted maximum fluorescence upon illumination with a pulse (300 ms) of saturating light (10 000 μmol m^{-2} s^{-1}). F_m was determined after an overnight dark adaptation. F_s is the light-adapted steady-state fluorescence; Y(II) is the effective quantum yield of PSII. Y(NO) is the quantum yield of non-regulated energy dissipation of PSII. Y(NO) consists of the non-photochemical quenching due to photoinactivation and constitutive thermal dissipation that is very stable despite environmental stresses (Busch et al. 2009). A high Y(NO) value indicates that both photochemical energy conversion and protective regulatory mechanisms are inefficient, and thus indicative of the plant having serious problems coping with the incident radiation. Y(NPQ) is the quantum yield of regulated energy dissipation of PSII.

In order to understand the effects of chilling temperature on CEF and the redox state of P700, we determined the light response Y(I), Y(NA) and Y(ND) at 4 and 25°C by Dual PAM-100 with a dual wavelength (830/875 nm) unit. A saturation pulse (10 000 μmol m^{-2} s^{-1}), which was introduced primarily for PAM fluorescence measurement, was applied for assessment of P700 parameters as well. The P700 signals may vary between a minimal level (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. P_m' was also defined in analogy to the fluorescence parameter F_m'. P700red, which was determined in a given state with the help of a saturation pulse, represents the fraction of overall P700 that is reduced in a given state. 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 from the complementary PSI quantum yields of non-photochemical energy dissipation Y(ND) and Y(NA): $Y(I) = 1 - Y(ND) - Y(NA)$. $Y(ND) = 1 - P700red$, $Y(NA) = (P_m - P_m')/P_m$. 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 the cytochrome *b*/*f* complex as well as down-regulation of PSII) and photodamage to PSII. Y(NA) represents the fraction of overall P700 that cannot be oxidized by a saturation pulse in a given state due to a lack of acceptors, and is enhanced by dark adaptation (deactivation of key enzymes of the Calvin–Benson cycle) and damage at the site of CO_2 fixation. Y(CEF) around PSI was estimated from the difference between Y(I) and Y(II), $Y(CEF) = Y(I) - Y(II)$ (Miyake et al. 2005, Fan et al. 2008).

Some experts recently pointed that Y(II) and Y(I) may have been determined from different parts of the leaf tissues. The Chl fluorescence signal is mainly measured from leaf mesophyll near the leaf surface, while the P700 signal comes from the whole tissues, and therefore LEF is possibly underestimated and consequently CEF would be overestimated. The method for measuring the P700 redox state was originally reported in Klughammer and Schreiber (1994) and has been widely referred to in previous studies on CEF estimation (Golding and Johnson 2003, Chow and Hope 2004, Miyake et al. 2005, Fan et al. 2008, Jia et al. 2008). Even though there might be some overestimation of CEF, we believe that the trend of change in the ratio of CEF to LEF is reliable.

Funding

This work was supported by the National Natural Science Foundation of China [grants 30900174 and 30770226]; the Key Laboratory of National Forestry Bureau for Fast-growing Tree Breeding and Cultivation in Central South China [an open project grant].

Acknowledgments

The CAS-XTBG plant germplasm bank provided the cold storage room for the chilling experiment, and the Xishuangbanna Station for Tropical Rain Forest Ecosystem Studies (XSTRE) provided climatic data.

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