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ORIGINAL PAPER

Different responses of photosystem I and photosystem II in three tropical oilseed crops exposed to chilling stress and subsequent recovery

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Abstract

Key message **Different responses of photosystem I and II to chilling.**

Abstract Tropical crops are sensitive to chilling stress, but the underlying physiological mechanisms are unclear. We investigated the maximum quantum yield of PSII (F_v/F_m), the maximum photo-oxidizable P700 (P_m), the energy distribution in PSII, and the redox state of P700 in leaves of seedlings of three promising oilseed crops originating from tropical regions, *Plukenetia volubilis*, *Jatropha curcas* and *Ricinus communis*, during chilling treatment and subsequent recovery under a photon flux density of 450 μmol m^{-2} s^{-1}. Our results showed that F_v/F_m decreased progressively and significantly to about 44.7, 62.2 and 77.0 % of the control after chilling treatment for 3 days in *P. volubilis*, *J. curcas* and *R. communis*, respectively, mainly due to the decrease in F_m (maximum fluorescence of PSII). After recovery under 18 °C for 6 days, F_v/F_m recovered to 81.4 and 94.9 % of the control in *J. curcas* and *R. communis*, but only to 26.3 % in *P. volubilis*. Under chilling stress and subsequent recovery, P_m remained stable in *J. curcas* and *R. communis*, whereas it decreased slightly in *P. volubilis*. These results indicated that PSII was more sensitive to chilling stress than PSI under moderate light for all three species, and that *P. volubilis* was the most susceptible. Cyclic electron flow around PSI and effective quantum yield of photosystem II [$Y_{(CEF)}/Y_{(II)}$] ratio were stimulated much more in *J. curcas* and *R. communis* compared with that in *P. volubilis* under chilling conditions, resulting in more severe injury as indicated by higher accumulation of hydrogen peroxide and malondialdehyde. There was a significantly negative relationship between F_v/F_m and $Y_{(CEF)}/Y_{(II)}$, suggesting that stimulation of $Y_{(CEF)}/Y_{(II)}$ plays a pivotal role in protecting PSI and PSII from photoinhibition caused by chilling stress.

Keywords Photosystem I · Photosystem II · Chilling stress · Cyclic electron flow · Lipid peroxidation · Tropical oilseed crops

Abbreviations

A_{growth}	Actual photosynthetic rate under growth condition (μmol m^{-2} s^{-1})
A_{max}	Light-saturated photosynthetic rate (μmol m^{-2} s^{-1})
CEF	Cyclic electron flow
G_s and G_{smax}	Stomatal conductance under growth and light-saturated light, respectively (mol m^{-2} s^{-1})
H_2O_2	Hydrogen peroxide (μmol g^{-1})
F_v/F_m	The maximum quantum yield of PSII
LEF	Linear electron flow
MDA	Malondialdehyde (nmol g^{-1})
NPQ	Non-photochemical quenching
PSI	Photosystem I

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PSII	Photosystem II
ROS	Reactive oxygen species
$Y_{(I)}$	Effective quantum yield of photosystem I
$Y_{(II)}$	Effective quantum yield of photosystem II
$Y_{(NA)}$	Fraction of overall PSI reaction center P700 that cannot be oxidized in a given state
$Y_{(ND)}$	Fraction of overall PSI reaction center 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

Introduction

Oilseeds provide a major source of dietary calories for humans and are an increasingly significant source of renewable industrial material. Globally, the major oilseed crops (i.e., rapeseed, soybean, sunflower, maize, palm, linseed and olive) contribute over 90 % of all vegetable oils used for food, feed, and industrial applications. However, as the global population is growing rapidly, the requirement for edible and industrial oils is increasing (Gui et al. 2008). Thus, it is urgent to find and develop alternative energy resources for the national energy security as well as for global climate change mitigation. A potential substitute for vegetable oils is to use fallow and marginal lands for growing perennial oilseed crops because of their significant advantages of renewability, environmental friendliness, and lack of competition with cereals for land (Glover et al. 2010). Three promising perennial oilseed crops in the family Euphorbiaceae originating from tropical regions, *Plukenetia volubilis*, *Jatropha curcas*, *Ricinus communis*, have been successfully introduced and widely cultivated in Southwest China. Although seed compositions of these oilseed crops are relatively well known, to date there is a lack of detailed information about their cultivation potential (Achten et al. 2008; Cai et al. 2012).

A chilling temperature above zero is one of the most important factors that limit the survival, growth and reproduction of field crops (Allen and Ort 2011). Intensive research on the responses of tropical oilseed crops to chilling stress and subsequent recovery can contribute to the future implementation of these thermophilic crops into the agricultural systems of optimally cultivated areas. The selection of relatively chilling-resistant oilseed crops is urgently needed. However, the physiological response of tropical oilseed crops to chilling is still lacking.

Photosynthetic processes are very sensitive to low temperature (Allen and Ort 2011; Sonoike 2011). When plants are exposed to chilling stress, photosynthetic enzymes may be inactivated or degraded, and photodamage may occur, resulting in decrease of photosynthetic activity. The reduced photosynthesis may lead to accumulation of excess energy. If the excess light energy could not be harmlessly dissipated as heat through non-photochemical quenching (NPQ), a lot of reactive oxygen species (ROS) would be generated. ROS may accelerate photoinhibition not only directly through damaging the photosynthetic apparatus (Asada 2006), but also by inhibiting protein synthesis, necessary for the repair of photodamage (Danon 2012). For example, investigations of the ROS-induced inhibition of repair have demonstrated that ROS suppress the de novo synthesis of proteins and, in particular, of the D1 protein, that are required for the repair of PSII. Inhibition of the repair of PSII by ROS is accelerated by the deceleration of the Calvin cycle that occurs when the availability of CO_2 is limited (Nishiyama et al. 2006). Several mechanisms, including the anti-oxidative scavenging system, xanthophyll cycle, water–water cycle, and cyclic electron flow (CEF) around PSI may be involved in protecting photosystems (Joliot and Johnson 2011). Stimulation of CEF is an important protective mechanism against the photoinhibition of PSII under environmental stress, since CEF can generate a proton gradient across the thylakoid membrane (ΔpH) to support non-photochemical quenching and to protect the oxygen-evolving complex (Johnson 2011; Huang et al. 2012). Generally, the assimilation of CO_2 demands ATP and NADPH to be delivered in a strict stoichiometry of 3/2. It is commonly accepted that, to comply with this requirement, the photosynthetic electron transfer operates in two different modes, linear and cyclic electron flow, both being indispensable. While the former provides ATP and NADPH in a 2.6/2 ratio; the latter exclusively drives phosphorylation and thus supplies the remaining ATP (Takahashi et al. 2013). The fine tuning of these two modes is vital, but the balance will be destroyed under stressful conditions and the underlying mechanisms remain unclear.

Nowadays, variable, and even contradictory results have been found as whether PSI or PSII is more sensitive to chilling stress. There is a tendency that photoinhibition of PSI is conspicuous in some chilling-sensitive species originally grown in tropical or subtropical regions, such as cucumber (Sonoike and Terashima 1994), coffee (Ramalho et al. 1999), cotton (Kornyeyev et al. 2003) and common bean (Nakano et al. 2010). But the extent of PSI photoinhibition seems to be similar to that of PSII photoinhibition or even less in some other chilling-tolerant plant species (Govindachary et al. 2004). Moreover, the relative sensitivity of PSI vs. PSII depends on the growth

light condition, as Terashima et al. (1994) found in leaves of cucumber that the damage of PSI occurred at very low light level such as 50 μmol m^{-2} s^{-1}, whereas the damage to PSII was induced at much higher photon flux densities. Most plant materials used in previous studies on the chilling effect on photosynthetic photosystems were grown at photon flux density generally <300 μmol m^{-2} s^{-1}. Since development of protective mechanisms such as NPQ and CEF could be enhanced in plants grown under relative high-light conditions (Miyake et al. 2005), the preferential PSI photoinhibition in plants grown in low light may not reflect the natural chilling effect on plants grown in the open field, especially in tropical areas. Recently, it was reported that tropical tree seedlings grown in high light can fully acclimate to cold temperature (i.e., 4 °C), and that PSII is more sensitive to chilling and high-light stress than PSI (Huang et al. 2010, 2011). Actually, 4 °C is far lower than the minimum temperature in these natural conditions. There is another fact that should be taken into consideration: most experiments were carried out with plants that were not acclimatized to a stressful environment, in which plants are likely to respond more strongly to the sudden chilling stress than plants grown under a normal fluctuating environment (Ivanov et al. 1998). It is of great importance to clarify which photosystem is more sensitive to chilling stress and what the underlying mechanisms are to enhance crop growth and performance through providing accurate targets for traditional and genetic manipulation. Due to the existence of electron transport regulators (i.e., PGR5, PGRL1 and NAD(P)H dehydrogenase) (Murchie and Niyogi 2011), it would seem feasible to adjust the photoprotective behavior of plants via the amount of linear versus cyclic electron flow.

In this study, we investigated the maximum quantum yield of PSII (F_v/F_m), the maximum photo-oxidizable P700 (P_m), and the energy distribution in PSII and the redox state of P700 in leaves of three tropical oilseed crops exposed to chilling stress and subsequent recovery under the moderate light. The chilling temperature and light intensity were set by reference to the monthly minimum temperatures and average photosynthetic flux densities in a representative clear day during 1990–2012, respectively, trying to simulate the natural conditions in the local area. We aim to address the following questions: (1) whether PSII is susceptible to chilling stress in the oilseed crops grown under relative high-light conditions, just like some early studied tropical trees; (2) whether the three oilseed crops differ in their tolerance to chilling and whether the upregulation of CEF is more stimulated in the chilling-tolerant species; (3) whether the photoinhibition of photosystem I and II was related to oxidative stress under chilling conditions.

Materials and methods

Plant materials and treatments

Three oilseed species in the family Euphorbiaceae originating from tropical regions, *P. volubilis*, *J. curcas* and *R. communis*, were used in our study. *Plukenetia volubilis*, a woody liana species indigenous to the tropical rain forests of South America, is well known to have an edible seed with high concentrations of unsaturated fatty acids (>92 %), which is beneficial to human health (Cai 2011). *R. communis* (castor bean), a perennial shrub indigenous to north-eastern tropical Africa, is widespread throughout tropical regions; and its seed has numerous applications in transportation, cosmetics, pharmaceutical and manufacturing industries (Madankar et al. 2013). *J. curcas*, a shrub or small tree native to the American tropics, is cultivated in tropical and subtropical regions around the world, becoming naturalized in some areas. Its seed can be processed to produce a high-quality biofuel, usable in a standard diesel engine (Achten et al. 2008). In April 2012, seeds of the three species were sown into a seedbed in a greenhouse in the Xishuangbanna Tropical Botanical Garden (21°6′N, 101°5′E, 600 m a.s.l.), Chinese Academy of Sciences, Yunnan, SW China. The seedlings of each species (25–30 cm tall) were transplanted into 10 L pots with 6 kg homogenized forest topsoil in August. After 4 months of growth in open ground to acclimatize to the natural environment, the pots were moved to growth chambers for chilling treatment and subsequent recovery. During these periods, the lowest and average outdoor temperatures were 8 and 18 °C, respectively, and the average photosynthetic flux density was 450 μmol m^{-2} s^{-1} (data from the nearby Meteorological Station of Xishuangbanna Tropical Botanical Garden). To decrease the potential influence of possible environmental heterogeneity, the chamber was divided into five sections and each section contained six seedlings of each species. Totally 30 pots for each species were used. Before chilling treatment, each variable of all seedlings was determined as control, then all seedlings grew 3 days at 8 °C, and all variables were determined every day. After chilling treatment, all seedlings grew 6 days at 18 °C, and each variable was determined every 3 days.

Chlorophyll fluorescence and P700 measurements

At various time intervals during the chilling treatment and the recovery, the in vivo chlorophyll fluorescence of PSII and absorbance changes of PSI were measured by a Dual PAM-100 fluorometer (Heinz Walz, Effeltrich, Germany) connected to a computer with control software. The following parameters were calculated: $F_v/F_m = (F_m - F_o)/$

F_m, NPQ = $(F_m - F_{m'})/F_{m'}$, $Y_{(II)} = (F_{m'} - F_s)/F_{m'}$, $Y_{(NO)} = F_s/F_m$, $Y_{(NPQ)} = F_s/F_{m'} - F_s/F_m$ (Kramer et al. 2004); where F_o represents the minimum fluorescence in the dark-adapted state, and F_m and $F_{m'}$ are maximum fluorescence values upon illumination by a pulse (300 ms) of saturating light (10,000 μmol photons m^{-2} s^{-1}) in the dark-adapted state and light-adapted state, respectively. F_s is steady-state fluorescence in light. The ratio F_v/F_m, where $F_v = (F_m - F_o)$ is the variable fluorescence, is reflecting the maximum quantum yield of PSII (Kramer et al. 2004); it was measured after 30 min dark adaptation. NPQ indicates the non-photochemical quenching. $Y_{(II)}$ is the effective quantum yield of PSII and $Y_{(NPQ)}$ is the fraction of energy dissipated in the form of heat via the regulated non-photochemical quenching mechanism. $Y_{(NO)}$ is the fraction of energy that is passively dissipated in form of heat and fluorescence. It consists of the non-photochemical quenching due to photoinactivation of PSII and constitutive thermal dissipation. High values of $Y_{(NO)}$ reflect the inability of a plant to protect itself against damage by excess light energy. The chlorophyll fluorescence measured at 8 °C was corrected to that at 18 °C using the equation, F_standard $(T) = -0.003106 \times$ temperature (in K) + 1.932 according to Huang et al. (2011).

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 photons 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 (P_m), which, in analogy to F_m, was determined after a saturation pulse, was given 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 $F_{m'}$ as $P_{m'}$; it 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 trans-thylakoid proton gradient (photosynthetic control at cytb/f complex as well as down-regulation of PSII) and photodamage to PSII. $Y_{(ND)} = P/P_m$. $Y_{(NA)} = (P_m - P_{m'})/P_m$, 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. We note that $Y_{(I)} + Y_{(ND)} + Y_{(NA)} = 1$. The yield of CEF around PSI [$Y_{(CEF)}$] is calculated as $Y_{(CEF)} = Y_{(I)} - Y_{(II)}$ (Miyake et al. 2005). The ratio of the quantum yield of CEF to the LEF is calculated as $Y_{(CEF)}/Y_{(II)} = [Y_{(I)} - Y_{(II)}]/Y_{(II)}$.

Gas exchange measurement

Actual photosynthetic rates (A_{growth}) and stomatal conductance (G_s) under growth conditions were measured on the youngest fully expanded leaves using a Li-6400 portable photosynthesis system (Li-Cor, Lincoln, NE, USA) and leaf temperatures at 8 or 18 °C for the chilling and recovery treatments, respectively. For the maximum photosynthetic rate (A_{max}) and stomatal conductance (G_{smax}), leaf temperatures were maintained at 25 °C and PFD was 1,200 μmol m^{-2} s^{-1} which was found to be saturating for the three species in a preliminary experiment.

Oxidative stress measurement

Three mature leaves were collected from each sample plant on which chlorophyll fluorescence was measured and were stored under liquid nitrogen for oxidative measurement. The levels of H_2O_2 were measured by monitoring the absorbance of the titanium-peroxide complex at 415 nm as described by Brennan and Frenkel (1977). Absorbance values were calibrated to a standard curve generated using known concentrations of H_2O_2. Leaf oxidative damage to lipids was expressed as equivalents of malondialdehyde (MDA) contents. After extraction and reaction with thiobarbituric acid, the absorbance at 450, 532 and 600 nm was determined. The MDA content was calculated according to Hodges et al. (1999).

Statistical analysis

A two-way ANOVA analysis was performed for each variable using SPSS 16.0, with species and treatment as the main fixed factor. Differences in values of each species in response to chilling stress and subsequent recovery were tested by one-way ANOVA, followed by a Tukey HSD post hoc test. Prior to analysis, data were checked for normality and homogeneity of variance and, if necessary, were transformed by taking $\log_{10}$- or square root to satisfy the assumptions of ANOVA.

Results

There were significant species × treatment interactions for half of the traits including F_m, F_v/F_m, H_2O_2, MDA, $Y_{(II)}$, $Y_{(NO)}$, $Y_{(NPQ)}$, $Y_{(CEF)}$ and $Y_{(CEF)}/Y_{(II)}$, implying that their responsiveness to chilling and subsequent recovery differed

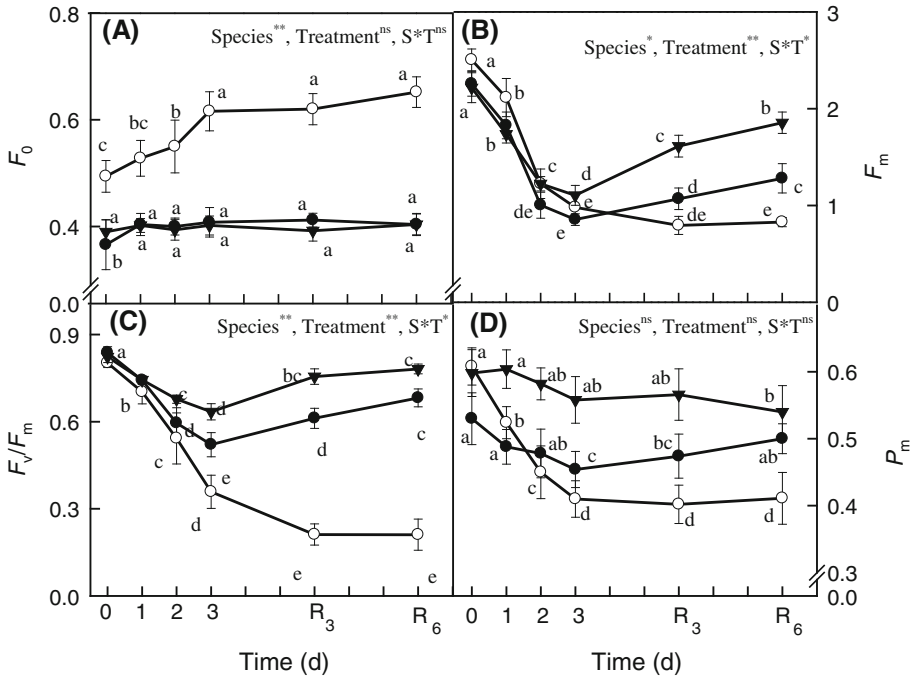

Fig. 1 Time course of F_o (**a**), F_m (**b**), F_v/F_m (**c**) and P_m (**d**) in leaves of *P. volubilis* (*open circle*) *J. curcas* (*filled circle*) and *R. communis* (*down-pointing triangle*) seedlings during the chilling treatment at 8 °C and subsequent recovery at 18 °C (R_3 for 3 days and R_6 for 6 days) under the photosynthetic flux density of 450 μmol m^{-2} s^{-1}. Values are means ($n = 5$) ± SD. Significance levels of ANOVAs testing for the effects of species, treatment and their interactions (*S*T*) are listed for each variable. *ns* not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. *Different letters* denote significant differences between treatments of each species at $P < 0.05$

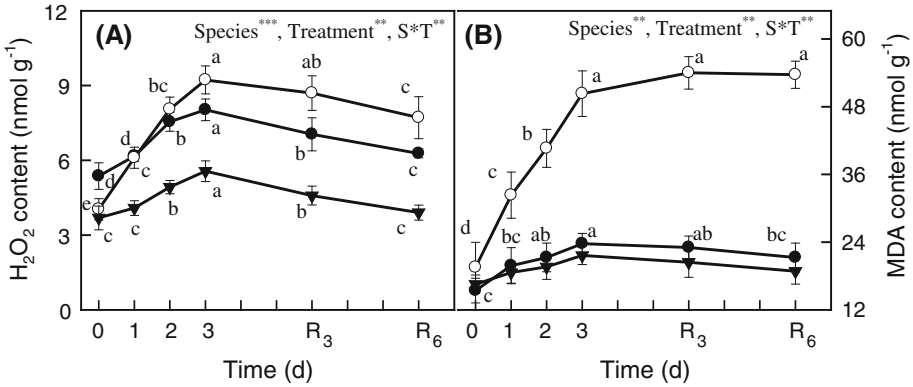

Fig. 2 Time course of in H_2O_2 (**a**) and MDA (**b**) contents in leaves of *P. volubilis* (*open circle*) *J. curcas* (*filled circle*) and *R. communis* (*down-pointing triangle*) seedlings during the chilling treatment at 8 °C and subsequent recovery at 18 °C (R_3 for 3 days and R_6 for 6 days) under the photosynthetic flux density of 450 μmol m^{-2} s^{-1}. Values are means ($n = 5$) ± SD. Significance levels of ANOVAs testing for the effects of species, treatment and their interactions (*S*T*) are listed for each variable. *ns* not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. *Different letters* denote significant differences between treatments of each species at $P < 0.05$

among species. The minimum chlorophyll fluorescence (F_o) exhibited a significant increase only in *P. volubilis*, whereas a significant decrease was observed in both *F*m and *F*v/*F*m in all three oilseed species during the chilling stress (Fig. 1a–c). After chilling treatment for 3 days, F_v/F_m was only about 44.7, 62.2 and 77.0 % of the control in *P. volubilis*, *J. curcas* and *R. communis*, respectively (Fig. 1c), indicating that *P. volubilis* was the most chilling sensitive and *R. communis* was the most tolerant. After recovery under 18 °C for 6 days, F_v/F_m recovered to 81.4 and 94.9 % of the control in *J. curcas* and *R. communis*, but further declined to 26.3 % in *P. volubilis* (Fig. 1c), again proving that *P. volubilis* was the species most sensitive to chilling. The maximum photo-oxidizable P700 (P_m) remained stable in chilling-tolerant *J. curcas* and *R. communis*, but decreased significantly in *P. volubilis* (Fig. 1d). Chilling stress resulted in increased oxidative stress in all samples, which was seen in the significant increases in H_2O_2 and MDA contents (Fig. 2a, b). During recovery, H_2O_2 decreased in all three species, whereas MDA decreased slightly only in *R. communis* and *J. curcas*. The H_2O_2 and MDA contents were higher in *P. volubilis* than those in *J. curcas* and *R. communis* regardless of treatments, and were lower in the control than during chilling stress and subsequent recovery in all three species (Fig. 2a, b). Chilling stress significantly decreased the

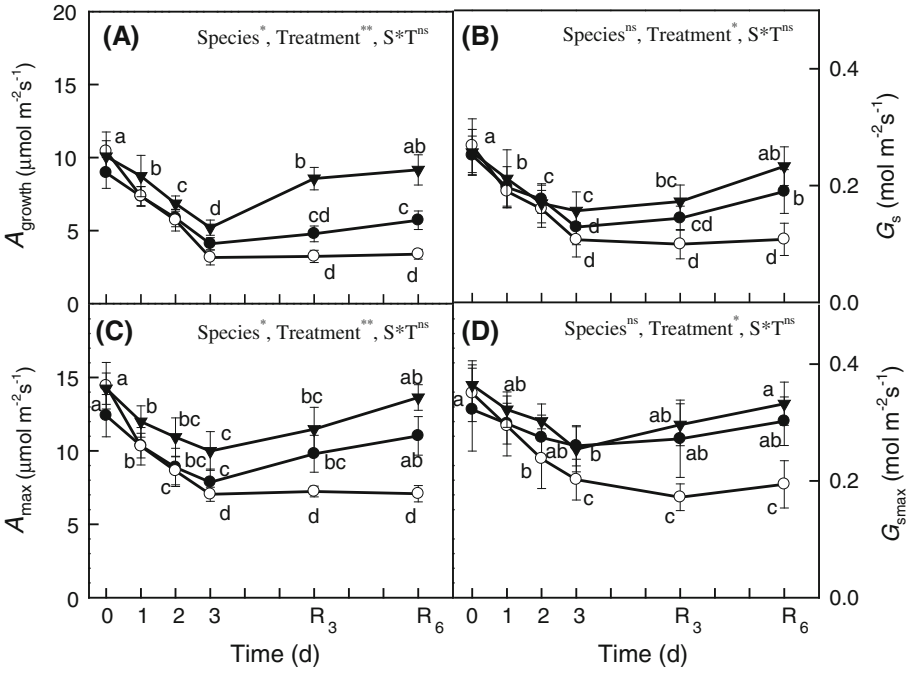

Fig. 3 Time course of A_{growth} (**a**), G_s (**b**), A_{max} (**c**) and G_{smax} (**d**) in leaves of *P. volubilis* (*open circle*) *J. curcas* (*filled circle*) and *R. communis* (*down-pointing triangle*) seedlings during the chilling treatment at 8 °C and subsequent recovery at 18 °C (R_3 for 3 days and R_6 for 6 days) under the photosynthetic flux density of 450 μmol m^{-2} s^{-1}. Values are means ($n = 5$) ± SD. Significance levels of ANOVAs testing for the effects of species, treatment and their interactions (*S*T*) are listed for each variable. *ns* not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. *Different letters* denote significant differences between treatments of each species at $P < 0.05$

actual (A_{growth}) and potential (A_{max}) photosynthetic rate (Fig. 3a, c), and also caused stomatal closure of all three species (Fig. 3b, d). There were no significant species × treatment interactions for photosynthetic rate and stomatal conductance, indicating that all three species showed a similar, modest, decrease in the gas exchange parameters in response to chilling and subsequent recovery treatments.

Effective quantum yield of photosystem II [$Y_{(II)}$] decreased significantly during the chilling period in all three species. After recovery, $Y_{(II)}$ remained stable in *J. curcas* and *R. communis*, but decreased continuously in *P. volubilis* (Fig. 4a). During the chilling treatment, significant increases were observed for $Y_{(NO)}$ in *P. volubilis* and *J. curcas*, and for $Y_{(NPQ)}$ in *R. communis*. Across all treatments, $Y_{(NO)}$ was highest in *P. volubilis*, intermediate in *J. curcas*, and lowest in *R. communis*, whereas $Y_{(NPQ)}$ exhibited a reverse pattern (Fig. 4b, c). $Y_{(I)}$ showed quite different patterns in the three species: it decreased progressively and significantly in *P. volubilis* across the chilling and recovery periods; it decreased a lot after chilling treatment but increased during the recovery process in *J. curcas*; in *R. communis*, it decreased significantly only after 3 days severe chilling stress and recovered to about 88.9 % of control in 18 °C for 6 days (Fig. 4d). *P. volubilis* displayed higher $Y_{(NA)}$ and lower $Y_{(ND)}$ than the other two species, especially under chilling stress (Fig. 4e, f). In general, $Y_{(CEF)}$ and $Y_{(CEF)}/Y_{(II)}$ increased after 3 days of chilling treatments and remained stable during the recovery in all three species (Fig. 5a, b). Across all treatments, *J. curcas* and *R. communis* had higher $Y_{(CEF)}$ and $Y_{(CEF)}/Y_{(II)}$ values than *P. volubilis* (Fig. 5a, b).

H_2O_2 and MDA contents were significantly negatively related with F_v/F_m, whereas A_{growth} and $Y_{(II)}$ were positively related with F_v/F_m across all three species (Fig. 6a–d). The correlating pattern between $Y_{(CEF)}$, $Y_{(CEF)}/Y_{(II)}$ and F_v/F_m was dependent on the species studied. With the increase of F_v/F_m, $Y_{(CEF)}$ and $Y_{(CEF)}/Y_{(II)}$ first increased then decreased in *P. volubilis*, but they continuously decreased in both *J. curcas* and *R. communis* (Fig. 6e, f).

Discussion

After exposure to low temperatures in excess of a critical period, physiological dysfunctions (i.e., alteration of metabolic processes, decrease in enzymatic activities and reduction of photosynthetic capacity) were observed in tropical plants (Allen and Ort 2011). In our study, low temperature had rapid effects on gas exchange parameters, which were seen as large decreases in net photosynthetic rate and stomatal conductance in leaves of all three studied species after only 1 day of chilling treatment (Fig. 3a, b); whereas F_v/F_m was slightly affected (Fig. 2c). This indicated that chilling inhibited the net photosynthetic rate more than the photochemical efficiencies. From days 2 to 3 of the chilling treatment, the net photosynthetic rate continued to decrease; meanwhile, F_v/F_m and $Y_{(II)}$ also markedly decreased, indicating that a photoinhibition process occurred (Figs. 1, 3). Our results were consistent with that

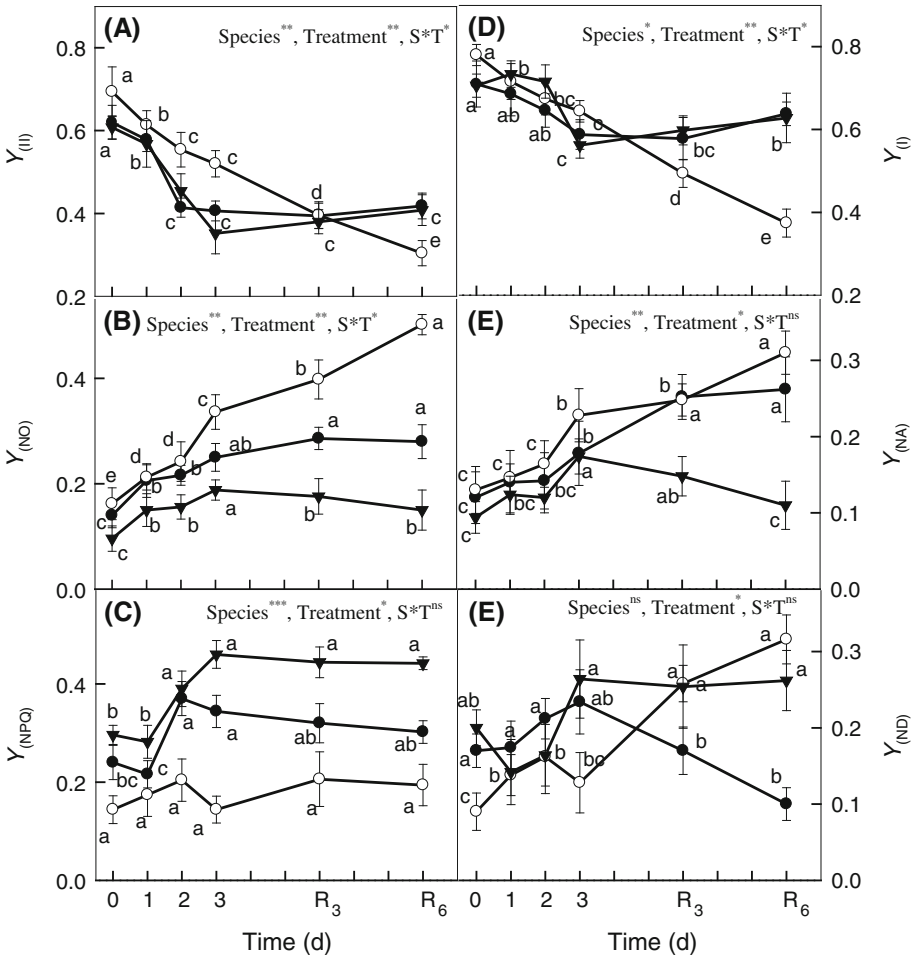

Fig. 4 Time course of $Y_{(II)}$ (**a**), $Y_{(ND)}$ (**b**), $Y_{(NPQ)}$ (**c**),$Y_{(I)}$ (**d**), $Y_{(NA)}$ (**e**) and $Y_{(ND)}$ (**f**) in leaves of *P. volubilis* (*open circle*) *J. curcas* (*filled circle*) and *R. communis* (*down-pointing triangle*) seedlings during the chilling treatment at 8 °C and subsequent recovery at 18 °C (R_3 for 3 days and R_6 for 6 days) under the photosynthetic flux density of 450 μmol m^{-2} s^{-1}. Values are means ($n = 5$) ± SD. Significance levels of ANOVAs testing for the effects of species, treatment and their interactions (*S*T*) are listed for each variable. *ns* not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. *Different letters* denote significant differences between treatments of each species at $P < 0.05$

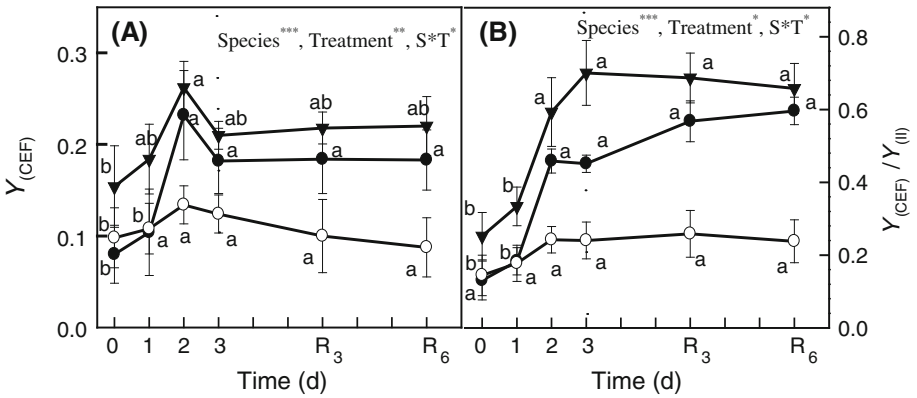

Fig. 5 Time course of $Y_{(CEF)}$ (**a**) and $Y_{(CEF)}/Y_{(II)}$ (**b**) in leaves of *P. volubilis* (*open circle*) *J. curcas* (*filled circle*) and *R. communis* (*down-pointing triangle*) seedlings during the chilling treatment at 8 °C and subsequent recovery at 18 °C (R_3 for 3 days and R_6 for 6 days) under the photosynthetic flux density of 450 μmol m^{-2} s^{-1}. Values are means ($n = 5$) ± SD. Significance levels of ANOVAs testing for the effects of species, treatment and their interactions (*S*T*) are listed for each variable. *ns* not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. *Different letters* denote significant differences between treatments of each species at $P < 0.05$

of Mai et al. (2009) who found that during 4 days of chilling treatment at 10 °C in rubber tree (*Hevea brasiliensis*), a time of 2 days was the turning point for effective stomata closure between a short- and long-term response. The impairments to photosystems are frequently related to changes in membrane permeability, as cell membrane systems are the primary sites of cold injury (Thomashow 1999). Chilling stress enhanced the production of free

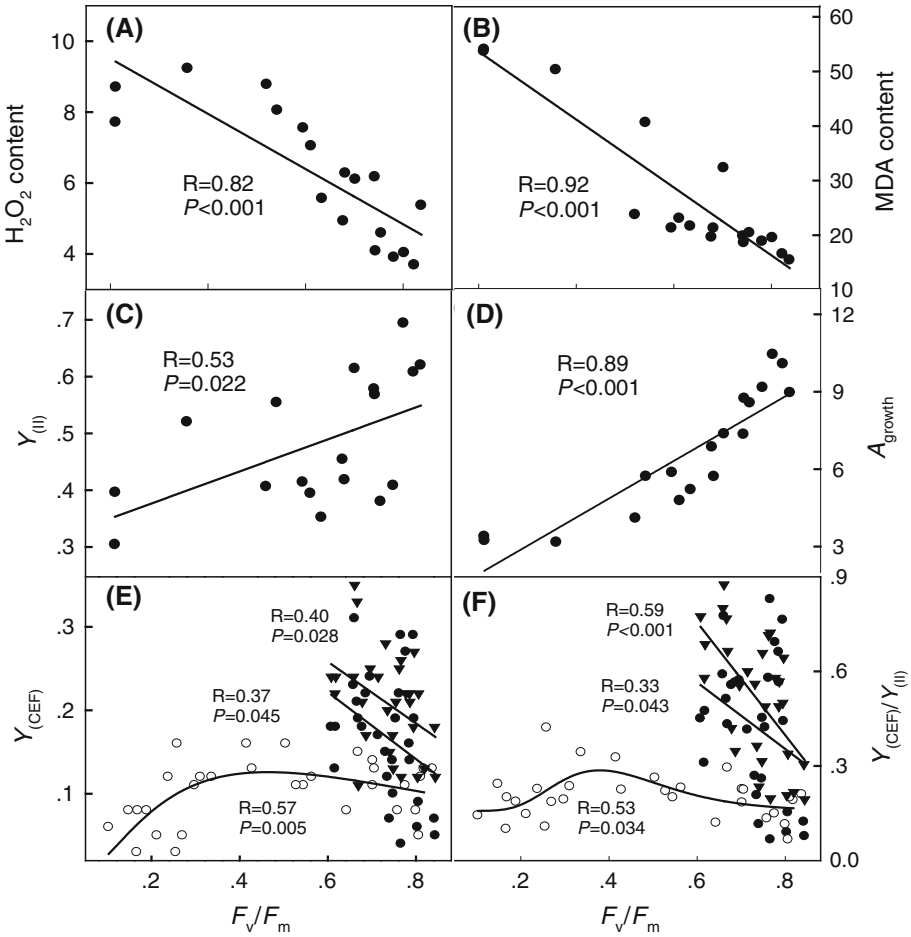

Fig. 6 H_2O_2 (**a**), MDA contents (**b**), $Y_{(II)}$ (**c**), A_{growth} (**d**),$Y_{(CEF)}$ (**e**) and $Y_{(CEF)}/Y_{(II)}$ (**f**) as a function of F_v/F_m in the seedlings of three oilseed crops during the chilling treatment at 8 °C and subsequent recovery at 18 °C under the photosynthetic flux density of 450 μmol m^{-2} s^{-1}. *P. volubilis* (*open circle*) *J. curcas* (*filled circle*), and *R. communis* (*down-pointing triangle*)

radicals (Fig. 3a), which provoked the formation of lipid radicals, thus rigidified the membranes (Almoguera et al. 2012). Malondialdehyde (MDA), one of the final products of lipid peroxidation (Mai et al. 2009), increased more pronouncedly in *P. volubilis* than the other two species during the chilling treatments (Fig. 3b), indicating that the chilling-sensitive *P. volubilis* suffered more injuries.

Photosystem II is more sensitive to chilling stress under moderate light than photosystem I in the three tropical oilseed crops

During the chilling stress, minimum chlorophyll fluorescence (F_o) exhibited a significant increase in *P. volubilis* (Fig. 1a). The rise in F_o may be due to the disconnection of the inner antennae and light harvesting complex from the core of PSII, or the release of free chlorophyll from protein-pigment complex (Briantais et al. 1996). The increase in F_o may also come from the changing microenvironment of the chlorophylls in thylakoids during the phase transition for bulk lipids, as F_o might be a rather indirect parameter for thylakoid membrane fluidity (Krumova et al. 2010; Tovuu et al. 2013). After exposure to chilling stress, rapid decreases were found in F_m and F_v/F_m in all three species. The strong decrease in F_m indicated that the PSII cooperativity was inhibited and a large proportion of PSII reaction center was destroyed (Huang et al. 2012). Both the increase in F_o and the decrease in *F*m contributed to the decrease in F_v/F_m in *P. volubilis*, whereas in the other two species, the decrease of F_v/F_m was mainly due to the decrease in F_m. Meanwhile, the maximum photo-oxidizable P700 (P_m) remained stable in *J. curcas* and *R. communis*, but showed a moderate decrease in *P. volubilis* (Fig. 1d), which implied that PSII was more sensitive than PSI under the chilling stress combined with moderate light, and *P. volubilis* was the most susceptible to chilling stress. Fast repair of PSII (i.e., recovery of F_v/F_m in 18 °C) was found in *J. curcas* and *R. communis*, not in *P. volubilis*, revealing that *P. volubilis*, very sensitive to chilling, might not be suitable for plantation in marginal lands at high altitude or latitude (Cai et al. 2012).

Compared with the other two species, the chilling-sensitive *P. volubilis* showed the lowest $Y_{(NPQ)}$ (Fig. 4c), suggesting it was unable to effectively dissipate excess light energy and excess ROS generated in the chloroplasts (Fig. 2a). So, the higher degree of PSII photoinhibition in

P. volubilis partly resulted from the decrease in $Y_{(NPQ)}$ and the increase in $Y_{(NO)}$ (Fig. 4). Since $Y_{(NA)}$ represents the overreduction of the PSI acceptor side which contributes to photoinhibition of PSI, the lower $Y_{(NA)}$ in *R. communis* indicated that its PSI was better protected than that of *P. volubilis* and *J. curcas* (Fig. 4e). These results may be related to the characteristics of PSI and PSII repair, respectively. It has been shown that the PSII complex, except for the damaged D1 subunit can be reused, but the entire PSI core complex will be degraded under the chilling combined with high-light conditions (Murchie and Niyogi 2011). Due to the irreversibility of PSI damage, it may exert a much stronger impact on the survival of plants than PSII photoinhibition (Kudoh and Sonoike 2002).

CEF plays essential roles in protecting PSI and PSII from photoinhibition

It is well known that CEF is essential for protecting PSII against excess excitation pressure because the CEF-dependent build-up of ΔpH across thylakoid membrane helps the activation of NPQ and stabilization of oxygen-evolving complexes. At chilling temperature, CEF was stimulated mainly by the overreduction of the PSI acceptor side and the imbalance of $NADP^+$/NADPH (Golding and Johnson 2003; Okegawa et al. 2008; Millaleo et al. 2013). Consequently, more P700 was oxidized to $P700^+$ and the excess accumulation of NADPH was prevented. Consistent with this, in our study, because of the strong stimulation of CEF, $Y_{(NPQ)}$ under chilling stress was much higher than that at 18 °C, especially in *J. curcas* and *R. communis* (Fig. 4c). $Y_{(CEF)}/Y_{(II)}$ was stimulated much more in *J. curcas* and *R. communis* compared with that in *P. volubilis* under the chilling conditions (Fig. 5b). Furthermore, the stimulation of CEF suppressed the production of O_2^- in the water–water cycle (Mehler-ascorbate peroxidase pathway), thus alleviated the oxidative stress (Laisk et al. 2010). That may be one of the possible reasons for the lower H_2O_2 and MDA production in leaves of *J. curcas* and *R. communis* compared to *P. volubilis* under the chilling conditions (Fig. 2a, b). These results suggest that stimulation of $Y_{(CEF)}/Y_{(II)}$ plays an important role in protecting PSI and PSII from photoinhibition caused by chilling stress at a moderate light level. In the chilling-tolerant *J. curcas* and *R. communis*, F_v/F_m was negatively related to $Y_{(CEF)}$ and $Y_{(CEF)}/Y_{(II)}$, suggesting that CEF was stimulated to repair the photodamage of PSII under chilling stress (Fig. 6e, f). Our results confirmed the findings of Huang et al. (2010), who observed a negative relationship between F_v/F_m and $Y_{(CEF)}/Y_{(II)}$ in *Dalbergia odorifera* during recovery after chilling-induced photoinhibition of PSII. Moreover, the higher correlation coefficient between F_v/F_m and $Y_{(CEF)}$ and $Y_{(CEF)}/Y_{(II)}$ in the more chilling-tolerant species *R. communis* as compared to *J. curcas* strengthened the importance of CEF in protecting PSI and PSII from photoinhibition (Fig. 6e, f).

Although CEF can serve to protect photosystem II in many circumstances, the stability of PSI is essential for the stimulation of CEF. A severe PSI photoinhibition might cause impairment of CEF, then induce the generation of hydroxyl radicals on the acceptor side of PSI and further aggravate PSI photoinhibition (Miyake 2010). Irreversible injury to photosystems I and II occurred in *P. volubilis*, which was manifested by the high accumulations of H_2O_2 and MDA, and the low values of $Y_{(II)}$, $Y_{(I)}$ and F_v/F_m even after recovering at 18 °C for 6 days (Figs. 1, 3, 5). Therefore, if the majority of PSI complexes was damaged and degraded, CEF could not be stimulated and the generation of ATP would be blocked, which would delay the complete recovery of PSII, even leading to death of leaves as in chilling-sensitive plants, e.g., *Cucumis sativus* (Kudoh and Sonoike 2002) and *Erythrophleum guineense* (Huang et al. 2010). It is hypothesized that changes in lipid composition of bio-membranes, i.e., the ratio of saturated to unsaturated fatty acids, may represent a crucial parameter to maintain optimum membrane fluidity, and thus an adaptive response to temperature variation (Kaniuga 2008). The chilling sensitivity can be manipulated by modulating levels of unsaturation of fatty acids of membrane lipids by the action of acyl-lipid desaturases and glycerol-3-phosphate acyltransferase (Nishida and Murata 1996). Further comparative studies of the function of foliar lipid composition and genetic transformation under chilling in our three oilseed species are needed.

In conclusion, our results showed that a decreased photosynthetic rate and stomatal closure were observed in our three studied tropical oilseed crops after 1 day of chilling treatment at 8 °C in moderate light, followed by progressive decrease in F_v/F_m. During the treatment, *P*m remained stable in *J. curcas* and *R. communis*, with a slight decrease in *P. volubilis*. These results implied that *P. volubilis* was the most susceptible to chilling among the three species; and PSII was more sensitive to chilling than PSI in moderate light, confirming the pattern observed by Huang et al. (2010, 2012) in tropical forest trees in high light. Cyclic electron transport was stimulated much more in *J. curcas* and *R. communis* compared with the chilling-susceptible *P. volubilis*, resulting in more injuries as indicated by the higher $Y_{(NPQ)}$ and lower $Y_{(NO)}$, along with more accumulations of H_2O_2 and MDA. *P. volubilis* might not be suitable for the plantation in marginal lands at high altitude or latitude, whereas the other two species, especially *R. communis*, would perform better in these areas.

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