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Downregulation of PSII activity and increased cyclic electron transport in cotton prevents PSI from photoinhibition due to night chilling

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Abstract

The objective of this experiment was to study the effects of night chilling on the photosynthetic characteristics of cotton (*Gossypium hirsutum* L.) at a boll-forming stage. The results suggest that overreduction of PSII after night chilling (≤ 10°C) led to excess excitation energy in cotton leaves. The night chilling (compared to 22°C) reduced PSI acceptor side limitation under moderate and high light intensity and increased maximum photooxidizable P700. This suggests that in contrast to PSII, PSI was protected from photoinhibition due to night chilling. However, PSII activity and linear electron transport were not significantly affected by the 30/16°C treatment. In addition, the night chilling (≤ 10°C) increased the quantum yield of cyclic electron transport. This suggests that cyclic electron transport around PSI might be important to prevent photoinhibition of PSI and PSII in cotton under night chilling stress.

Additional key words: chlorophyll fluorescence; photoprotection; photosynthesis; stomatal limitation.

Introduction

Chilling (*i.e.*, low temperatures during either day or night) is a major factor limiting the productivity and geographical distribution of agricultural crops (Allen and Ort 2001). Photosynthetic processes are very sensitive to low temperature and the response of photosynthesis to chilling in light has been widely studied (Sonoike and Terashima 1994, Kornyeyev *et al.* 2003). Less is known, however, about the effects of night chilling.

When plants are exposed to chilling stress, photosynthetic enzymes may be inactivated or degraded, and photoinhibition may occur (Sonoike 1998, Liu *et al.* 2012, Lei *et al.* 2014). Photoinhibition is a light-induced reduction in photosynthetic capacity under the conditions, when the light energy absorbed by pigments exceeds the requirement for photosynthesis (Sonoike 2011). Chilling can induce photoinhibition not only under high light intensity but also under low light intensity (Powles 1984, Liu *et al.* 2012, Zhang *et al.* 2014).

The photosystems are the primary targets for chilling-induced photoinhibition (Bertamini *et al.* 2005). PSII has long been considered the original site and primary target of photoinhibition due to chilling (Powles 1984, Havaux *et al.* 1991). Chilling under light results in the absorption of excessive energy by PSII pigments, thus increasing the potential for oxidative damage, especially to the D1 protein at the core of the PSII reaction center (Allen and Ort 2001, Takahashi and Badger 2011). The PSII repair mechanism is inhibited if plants are exposed to low temperatures during night (Allen and Ort 2001, Liu *et al.* 2012). Chilling in light generally reduces photochemical reaction rates, therefore limiting the sinks for absorbed excitation energy, particularly CO_2 fixation and photorespiration (Huner *et al.* 1998, van Heerden *et al.* 2003). Chilling in light generally inhibits PSII activity, whereas PSI is severely photodamaged in some species, such as potato (Havaux and Davaud 1994), cucumber (Terashima *et al.* 1994), and *Arabidopsis* (Zhang and Scheller 2004). However, there is still insufficient evidence from intact leaves to classify PSI as the primary target of a chilling stress (Allen and Ort 2001).

Previous research has shown that photoinhibition to PSI in some chilling-sensitive plants (*e.g.* cucumber) is

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Abbreviations: C_a – external CO_2 partial pressure; CEF – cyclic electron flow; C_i – intercellular CO_2 concentration; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; LEF – linear electron flow; P_N – net photosynthetic rate; P_{Nmax} – light-saturated net photosynthetic rate; PQ – plastoquinone; q_P – photochemical quenching coefficient; Y_I – effective quantum yield of PSI; Y_{II} – effective quantum yield of PSII; Y_{CEF} – effective quantum yield of CEF; Y_{NA} – nonphotochemical quantum yield of PSI caused by acceptor side limitation; Y_{ND} – nonphotochemical quantum yield of PSI caused by donor side limitation; Y_{NO} – quantum yield of nonregulated energy dissipation; Y_{NPQ} – quantum yield of regulated energy dissipation.
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attributable to oxidation by reactive oxygen species (ROS) (Sonoike 1996a), especially, by hydroxyl radicals, the most reactive species of active oxygen. Low temperature reduces CO_2 fixation, leading to large declines in $NADP^+$. The $NADP^+$ is a major acceptor of electrons in PSI. Declines in $NADP^+$ accelerate both overreduction on the PSI acceptor side and the production of hydroxyl radicals (Mi *et al.* 2000). Therefore, electron flow from PSII is essential to PSI photoinhibition. Researchers have also demonstrated that recovery from photoinhibition is much slower in PSI than that in PSII, because the turnover rate of PSI subunits is not as high as that of D1 protein (Sonoike 2011).

Effective defense mechanisms have evolved in higher plants to prevent damage to the photosynthetic apparatus under excess excitation energy. Cyclic electron flow (CEF) is widely considered to be an important protective mechanism of PSI in some plants such as *Arabidopsis* (Munekage *et al.* 2002), cucumber (Kim *et al.* 2001), and tropical trees (Huang *et al*. 2011). CEF is essential for balancing the ATP/NADPH ratio so as to prevent overreduction of the stroma (Munekage *et al.* 2004, Yamori and Shikanai 2016). Moreover, some studies suggest that interruption of linear electron flow (LEF) from PSII to PSI can greatly suppress PSI photoinhibition in intact leaves and in isolated thylakoid membranes (Sonoike 1995, Kudoh and Sonoike 2002a, Zhang *et al.* 2011). Admittedly, inhibition of LEF is an important protective mechanism against PSI photoinhibition. However, the inhibition of LEF and the limitation of CO_2 fixation induced by chilling stress results in excessive generation of ROS which suppress the PSII repair cycle. This leads to severe PSII damage (Takahashi and Murata 2008). Therefore, it is important to clarify the mechanisms by which the photosynthetic apparatus adapts to night chilling so as to enhance growth and adaptability under adverse environmental stresses.

Cotton (*Gossypium hirsutum* L.) grows best in climates with warm days and relatively warm nights (Gipson 1986). However, in many cotton growing areas, especially arid ones, the night temperature may fall to a suboptimal level. This can have important consequences on cotton growth and yield, especially at the boll-forming stage (Guo *et al.* 1991, Bange and Milroy 2004). Previous research in tomato has shown that low night temperature treatment induced the reversible photoinhibition of PSII and aggravated the photoinhibition of PSI by increasing the acceptor side limitation of PSI (Liu *et al*. 2012). However, little is known about how photosystems of cotton at the boll-forming stage respond and adapt to night chilling. The P700 and fluorescence measuring system *Dual-PAM-100* is capable of detecting imbalanced rates of photochemistry of PSI and PSII with a high accuracy by simultaneously recording quantum yields of both photosystems. In this study, by using this instrument, we focused on the influence of night chilling on energy distribution and activity in PSII and PSI.

Materials and methods

Plant materials and night chilling treatments: The experiment was conducted at Shihezi University, Shihezi, China (45°19'N, 86°03'E). The chilling-sensitive cotton in this study, *Gossypium hirsutum* L. cv. 'Xinluzao 45', is commonly grown in northwest China. The cotton was germinated and grown in pots (24.8 cm in diameter, 26 cm in height) filled with commercially rich soil or Hoagland nutrient solution. Rich soil could supply sufficient nutrients to cotton. Enough water was supplied to the plants grown in soil. Nutrient solution was renewed every day in order to avoid any potential nutrient deficiency. There was one plant per pot. The plants were planted in a heated greenhouse (day/night temperatures of 28–35/20–28°C) with natural light and relative humidity of 60–70%.

The cotton plants were divided randomly into four groups at the boll-forming stage, where 20 pots were in each group. The first group of plants was used as control in a phytotron (*BRS-10*, Nan Jing HengYu, China). The second, third, and fourth group was subjected to night chilling treatments at 16, 10, and 4°C by transferring to another phytotron. During the day time, temperature was the same in the both control and night-chilling treatments, in order to carefully investigate the effect of night chilling on photosynthetic parameters in cotton leaves. The treatment was done for 10 h a day (from 22:00 to 08:00 h) for 2 d. The environmental conditions were as follows: the photoperiod was 14-h light/10-h dark, PPFD was 1,000 μmol m^{-2} s^{-1}, and the relative humidity was 60–70%, day/night temperatures of 30/22°C (control), night-chilling temperatures were set as 30/16, 30/10, and 30/4°C (day/night) by referring to the temperature records of research area at the site (https://shzqx.gov.cn/). During the experiment, the measurements involved in gas exchange and chlorophyll (Chl) fluorescence and PSI parameters were performed using the fourth uppermost main-stem leaf on each plant. At least four plants of each test index were made in each treatment. The experiment was repeated twice and the data were pooled.

Gas-exchange: In the morning, when the cotton plants achieved the highest photosynthetic rate, gas-exchange parameters were measured using a portable photosynthesis system (*LI-6400*, *Li-Cor Inc.*, Nebraska, USA) equipped with CO_2 control modules. The external CO_2 partial pressure (C_a) was 400 μmol mol^{-1}. Blue-red light-emitting diodes (*6400-02B*, *Li-Cor Inc.*, Nebraska, USA) were used as the light source. Leaf net photosynthetic rate (P_N) and stomatal conductance (g_s) were measured after equilibration in order to obtain a steady-state photosynthetic rate. Light-response curves were created by reducing PAR in the following order: 1,500; 1,200; 1,000; 800, 600, 400, 200, 150, 100, 50, 20, and 0 μmol(photon) m^{-2} s^{-1}. The stomatal limitation (L_s) was calculated using the equation: $L_s = 1 - C_i/C_a$ (Berry and Downton 1982). P_{Nmax} was determined from the P_N-PAR curves using *Photosynthesis Assistant* (*Dundee Scientific*, Scotland, UK). The measurements of photosynthetic response of the plants to PPFD were performed on the third day at 10:00 h and the temperature during the measurements was 30°C.

Chl fluorescence and P700: The *in vivo* Chl fluorescence of PSII and the P700 redox state of PSI were simultaneously

measured using a saturation-pulse *Dual-PAM-100* fluorometer (*Walz*, Effeltrich, Germany) connected to a computer with *Wincontrol* software using pulse amplitude modulation. At 09:00 h on the third day (light-adapted for 1 h during day time at 30°C), the leaves were kept in the dark for 30 min and the Chl fluorescence and P700 redox state were measured simultaneously. First, dark-adapted minimum fluorescence (F_0) and dark-adapted maximum fluorescence (F_m) were recorded upon illumination by a 400-ms pulse of saturating light [15,000 μmol(photon) m^{-2} s^{-1}]. Next, each leaf was illuminated with an actinic light [1,031 μmol(photon) m^{-2} s^{-1}] for 5–6 min. The light-adapted steady-state fluorescence (F_s) and light-adapted maximum fluorescence (F_m') were then measured with the same pulse of saturating light as that in the dark-adapted state. The leaves were light adapted at least 10 min and then used to generate light-response curves. The data for the curves were obtained using an internal program in the *Dual-PAM-100*. The PAR was supplied by red light-emitting diodes. Eleven discrete PAR steps were used (20 s each): 30, 61, 94, 150, 240, 363, 555, 849; 1,311; 1,618; and 1,976 μmol(photon) m^{-2} s^{-1}. Each light increment was followed by the measurement of F_s, and then by a saturating pulse for measurement of F_m' and P_m' in the light-adapted state.

The following Chl parameters were calculated: the maximum quantum yield of PSII after dark adaptation for 30 min, $F_v/F_m = (F_m - F_0)/F_m$, where F_v is variable fluorescence; the light-adapted maximum quantum yield of PSII, $F_v'/F_m' = (F_m' - F_0')/F_m'$, where F_v' is the light-adapted variable fluorescence, F_0' is the light-adapted minimum fluorescence and F_0 is the dark-adapted minimum fluorescence; and the coefficient of photochemical quenching, $q_P = (F_m' - F_s)/(F_m' - F_0')$. The effective quantum yield of PSII (Y_{II}) was calculated using the equation $Y_{II} = (F_m' - F_s)/F_m'$ (Genty *et al.* 1989). The fraction of energy passively dissipated in form of heat and fluorescence (Y_{NO}) was calculated using the equation $Y_{NO} = F_s/F_m$ (Hendrickson *et al.* 2004, Kramer *et al.* 2004). The Y_{NO} consists of nonphotochemical quenching due to photoinactivation and constitutive thermal dissipation that are very stable despite environmental stresses (Busch *et al.* 2009). The fraction of energy dissipated in the form of heat *via* the regulated nonphotochemical quenching mechanism (Y_{NPQ}) was calculated using the equation $Y_{NPQ} = F_s/F_m' - F_s/F_m$ (Kramer *et al.* 2004).

To study the effects of night-time chilling on CEF, the redox state of PSI was determined using a *Dual-PAM-100* with a dual wavelength (830/875 nm) unit (difference of intensities of 875 and 830 nm pulse modulated measuring light reaching the photodetector) (Klughammer and Schreiber 2008). A saturation pulse [15,000 μmol(photon) m^{-2} s^{-1}] was primarily introduced to measure PAM fluorescence. The P700 parameters were also determined. The P700 signals may vary between a minimal level (P700 fully reduced) and a maximal level (P700 fully oxidized). The maximum P700 oxidation (P_m, analogous to F_m), a parameter representing the quantity of efficient PSI complex, was determined by applying a saturation pulse after pre-illumination with far-red light. Light-adapted maximum P700 oxidation (P_m', analogous to F_m') was measured in a way similar to P_m, except that actinic light was used instead of far-red illumination. The fraction of total P700 that is reduced in a given state ($P700_{red}$) which was determined with a saturation pulse. The photochemical quantum yield of PSI (Y_I) is defined as the fraction of total P700 that is reduced in a given state and not limited by the acceptor side. The Y_I is calculated from the complementary PSI quantum yields of nonphotochemical energy dissipation (*i.e.*, Y_{ND} and Y_{NA}). The Y_{ND} (*i.e.*, donor side limitation calculated by the equation $1 - P700_{red}$) represents the fraction of overall P700 that is oxidized in a given state. The Y_{ND} is enhanced by a trans-thylakoid proton gradient (photosynthetic control at the cytochrome *b*/*f* complex as well as downregulation of PSII) and photodamage to PSII. The Y_{NA} [*i.e.*, acceptor side limitation calculated by the equation $(P_m - P_m')/P_m$] represents the fraction of overall P700 that cannot be oxidized by a saturation pulse in a given state due to a lack of acceptors. The Y_{NA} is enhanced by dark adaptation (*i.e.*, deactivation of key enzymes of the Calvin-Benson cycle) and by damage at the site of CO_2 fixation. The equation for calculating Y_I is $1 - Y_{ND} - Y_{NA}$.

It has been recently pointed that Y_{II} and Y_I may have been determined from different parts of the leaf tissues (Klughammer and Schreiber 1994). The Chl fluorescence signal is mainly measured from leaf mesophyll near the leaf surface, whereas the P700 signal comes from the whole tissue; therefore, it is possible that LEF is underestimated and, consequently, CEF is overestimated. Even though CEF may be overestimated, we believe that the CEF/LEF ratio is a reliable indicator of changes in cyclic electron transport. Therefore, two parameters, Y_{CEF} and Y_{CEF}/Y_{II} were used (*1*) to show changes in the quantum yield distribution between the two photosystems and (*2*) to estimate changes in the ratio of quantum yield of CEF to that of LEF (Huang *et al.* 2010, Li and Zhang 2015). The equations for calculating the parameters were as follows: $Y_{CEF} = Y_I - Y_{II}$ (Miyake *et al.* 2005, Huang *et al.* 2010); $Y_{CEF}/Y_{II} = (Y_I - Y_{II})/Y_{II}$. Photosynthetic electron flow through PSI and PSII (ETR_I and ETR_{II}, respectively) was also calculated by the *Dual-PAM* software as follows: $ETR_I = Y_I \times PPFD \times 0.84 \times 0.5$ and $ETR_{II} = Y_{II} \times PPFD \times 0.84 \times 0.5$, where 0.84 represents the leaf absorptance and 0.5 is the proportion of absorbed light energy allocated to PSI or PSII (Genty *et al.* 1989).

Statistical analysis: Results were analyzed by one-way analysis of variance (*ANOVA*) using *SPSS v. 16.0* software (*SPSS*, Chicago, USA). Means were compared using least significant difference tests at the 0.05 probability level. The figures were plotted using *Origin version 9.0* software. The data are presented as the mean ± SE.

Results

P_N was significantly affected by night-time chilling temperatures. P_N values decreased considerably during the day after night chilling at 4°C (Fig. 1). The greatest P_{Nmax} (16.08 ± 1.2 μmol m^{-2} s^{-1}) was observed in the control treatment (Table 1). After night chilling below 16°C, P_{Nmax} and g_s

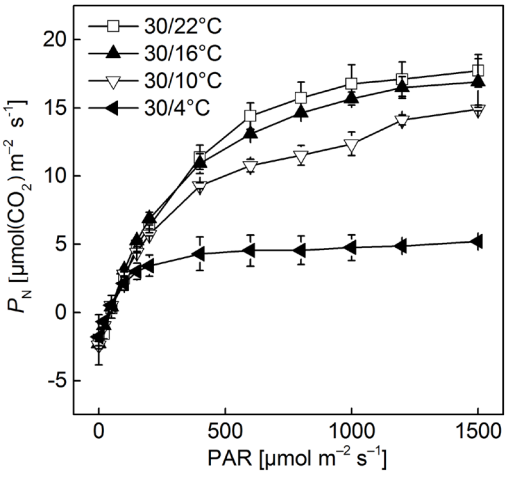

Fig. 1. Light-response curves of 'Xinluzao 45' cotton as affected by night-time chilling treatments. During a 2-d period, the plants were exposed to four temperature treatments: 30/22, 30/16, 30/10, and 30/4°C. The 30/22°C treatment was used as the control. Data are means ± SE, $n = 4$.

values during the day decreased significantly, whereas L_s increased significantly. The latter result indicated that stomatal limitation was one of the reasons for the decline in P_{Nmax} due to night chilling.

PSII: The Chl fluorescence parameters were significantly affected by night-time chilling temperatures. The F_v/F_m values remained normal in all treatments, although the value decreased with lowering night-chilling temperature (Fig. 2*A*). Night chilling at 4 and 10°C significantly decreased F_v'/F_m' at low PAR levels and q_P at all PAR levels (Fig. 3). The effective quantum yield of PSII (Y_{II}) is the product of F_v'/F_m' multipled by q_P; therefore, the results indicated that the marked decrease in Y_{II} observed during the day time after night chilling was mainly due to decreases in q_P rather than F_v'/F_m'.

The Y_{NO} and Y_{NPQ} values increased with increasing PAR (Fig. 4*B*,*C*). Interestingly, the lowest Y_{NPQ} was observed after night chilling at 16°C, although night chilling did not significantly affect q_P, F_v'/F_m', Y_{II}, or Y_{NPQ} at any treatment and PAR level (Figs. 3; 4*A*,*C*).

Quantum yields of PSI and PSII in the light-adapted state were recorded after illumination for 6 min. The Y_{II} values after night chilling at 4 and 10°C were significantly lower than that of control (Table 2). However, the greatest Y_{II} value was observed after the night chilling at 16°C, where Y_{NO} and Y_{NPQ} values were the lowest among the night-chilling treatments, thus corresponding to the changes in the light-response curves (Fig. 4*A*–*C*).

PSI: The P_m, which estimates the maximum amount of photooxidizable P700, can be used to estimate PSI activity (Takagi *et al*. 2017). The results showed that the P_m value increased with decreasing the night-chilling temperature, to attain the maximal value (0.57) after night chilling at 10°C. However, Y_I was significantly lower in the 30/10 and 30/4°C treatments than in the 30/22°C treatments at all PAR levels (Fig. 4*D*). The Y_{ND} gradually increased with light intensity (Fig. 4*E*). Furthermore, Y_{ND} was also much lesser at high night temperature (30/22°C) than at low night temperature (30/10 and 30/4°C). This meant that the rapid declines in Y_I with light intensity at temperatures ≤ 10°C were mainly due to increases in donor side limitation (Y_{ND}). More importantly, Y_{NA} at 0–400 μmol(photon) m^{-2} s^{-1} was significantly greater in the 30/4°C treatment than in the other treatments (Fig. 4*F*). Interestingly, the 30/16°C treatment had the greatest Y_{NA} among all treatments at PAR between 400 and 2,000 μmol(photon) m^{-2} s^{-1}. The value of Y_{NA} was stable among all treatments at PAR between 400 and 2,000 μmol(photon) m^{-2} s^{-1}. The quantum yields of energy conversion in PSI in the light-adapted state showed that night chilling-induced declines in Y_I were due to increased donor side limitation (Y_{ND}). The results in Table 2 also suggest that the 30/16°C treatment had the highest Y_{NA} among all treatments.

Electron transport rate of PSII and PSI: The electron transport rates of both PSII and PSI (ETR_{II}, ETR_I, respectively) increased as PAR increased in the rapid light curves (Fig. 5). The ETR_{II} and ETR_I values were significantly lesser in the 30/10 and 30/4°C treatments than that in control, especially under moderate and high PAR. It is noteworthy that a reduction in night-time temperature from 22 to 16°C had no significant effect on either ETR_{II} or ETR_I. In addition, ETR_I compared to ETR_{II} required greater light intensity to reach a maximum. This suggests the existence and response of CEF in PSI. Interestingly, in the light-adapted state, ETR_{II} was greatest in the 30/16°C treatment (144.14 ± 15.6 μmol m^{-2} s^{-1}).

Table 1. The major photosynthetic characteristics of 'Xinluzao 45' cotton leaves obtained by *LI-6400* at 1,000 μmol(photon) m^{-2} s^{-1} at 30°C after two days of three different night-chilling treatments: 30/22, 30/16, 30/10, and 30/4°C day/night temperature. The 30/22°C treatment was used as a control. Values are means ± SE. Values within a row followed by *a different letter* are significantly different at the 0.05 level of significance. P_{Nmax} – net photosynthetic rate under light-saturated conditions; g_s – stomatal conductance; L_s – stomatal limitation.

Characteristic	Temperature treatment			
	30/22°C	30/16°C	30/10°C	30/4°C
P_{Nmax} [μmol m^{-2} s^{-1}]	16.08 ± 1.20^a	15.7 ± 1.0^a	11.0 ± 1.4^b	5.3 ± 1.4^c
g_s [mol(H_2O) m^{-2} s^{-1}]	0.21 ± 0.02^a	0.17 ± 0.02^b	0.11 ± 0.02^c	0.05 ± 0.01^d
L_s	0.33 ± 0.01^d	0.39 ± 0.01^c	0.44 ± 0.02^b	0.53 ± 0.03^a

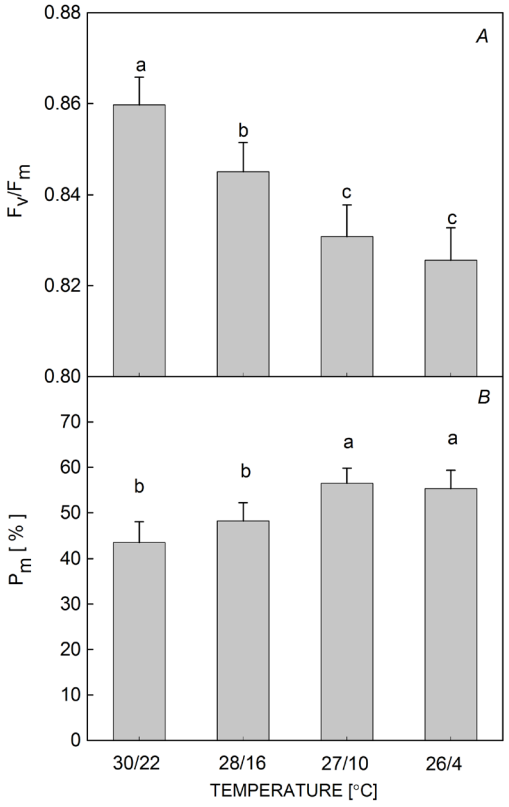

Fig. 2. The dark-adapted maximum quantum yield of PSII (F_v/F_m) (*A*) and the maximum photooxidizable P700 (P_m) (*B*) of 'Xinluzao 45' cotton leaves as affected by short-term night chilling temperatures. The leaves were dark-adapted for 30 min and the chlorophyll fluorescence and P700 redox state were measured simultaneously. Values are means ± SE (n = 4). *Different letters* indicate significant differences between the treatments (P<0.05, one-way *ANOVA*).

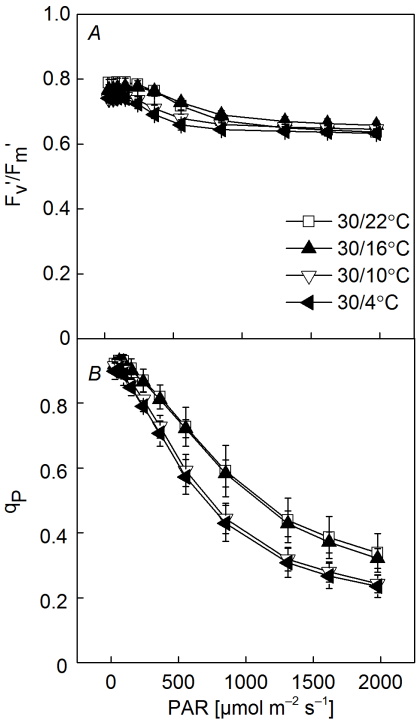

Fig. 3. Light-response changes in the light-adapted maximum quantum yield of PSII (F_v'/F_m') (*A*) and photochemical quenching coefficient (qP) (*B*) of 'Xinluzao 45' cotton leaves as affected by night-time chilling temperatures. The leaves were dark-adapted for 30 min and the chlorophyll fluorescence was measured. Values are means ± SE (n = 4).

The role of CEF in photoprotection under night chilling stress: Night chilling significantly affected CEF during the day. Specifically, CEF under moderate and high PAR was significantly greater in the 30/10 and 30/4°C treatments than that of control (Fig. 6). However, the 30/16°C treatment had the lowest Y_{CEF} among all treatments (Fig. 6). The Y_{CEF}/Y_{II} ratios under moderate and high PAR were significantly enhanced by the gradual reduction in night temperature (Fig. 6). The Y_{CEF}/Y_{II} ratios at moderate and high PAR were much greater in the 30/10 and 30/4°C treatments than that of control treatments (Fig. 6*A*). However, the 30/16°C treatment had the lowest Y_{CEF}/Y_{II}, mainly due to low Y_{CEF} and high Y_{II} values.

Discussion

PSII performance in response to night chilling stress: The present results suggest that night chilling decreased efficiency of light energy absorbed by photosynthetic pigments and used for reduction of Q_A, resulting in excessive excitation and photoinhibition of PSII during the day (Figs. 2*A*, 3). Previous research proposed that low environmental temperature can inhibit carbon fixation capacity and LEF, potentially leading to excess light excitation pressure and ROS (Allen and Ort 2001, Liu *et al.* 2012), which then aggravates the photoinhibition of PSI (Sonoike 2011) and PSII (Murata *et al.* 2007, Takahashi and Badger 2011). The results of the present study confirmed that Y_{II} was much lesser at night temperatures ≤ 10°C than at night temperatures of 22°C (Fig. 4*A*). This can be attributed to the inhibition of LEF from PSII to PSI under night chilling stress. The results in our experiment indicated that the decline in Y_{II} was accompanied by similar decreases in both q_P and F_v'/F_m' (Fig. 3), suggesting that the inhibition of LEF from PSII to PSI during the day resulted in potential excess light excitation pressure in PSII reaction centers after night chilling stress. When carbon assimilation capacity was inhibited by low night temperature, a strong downregulation of PSII photochemical activity played an essential photoprotection role in cotton (Table 1).

Although the status of D1 protein in this study was not determined, one parameter for Y_{NO} is considered a good indicator of PSII photodamage (Takahashi *et al.* 2009, Liu *et al.* 2012, Huang *et al.* 2016). Here, we found that the night chilling (compared to 22°C) increased Y_{NO} values (Fig. 4*B*), suggesting that night chilling stress may have accelerated photodamage of the PSII supercomplex and disrupted D1 protein turnover in the PSII repair cycle. This may be attributed to the low activity of antioxidant

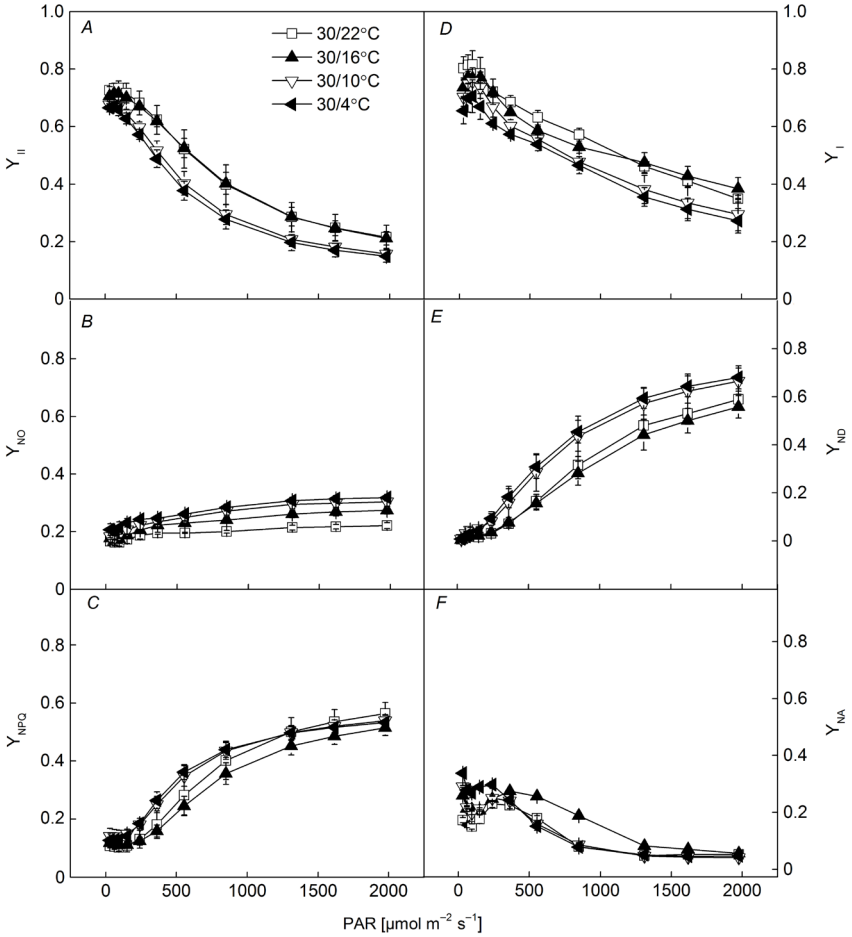

Fig. 4. Rapid light-response changes in the effective quantum yield of PSII (Y_{II}) (*A*), quantum yield of nonregulated energy dissipation (Y_{NO}) (*B*), yield of regulated energy dissipation of PSII (Y_{NPQ}) (*C*), quantum yield of PSI (Y_I) (*D*), donor side limitation of PSI (Y_{ND}) (*E*), and acceptor side limitation of PSI (Y_{NA}) (*F*) in 'Xinluzao 45' cotton leaves as affected by night chilling treatments. The leaves were dark-adapted for 30 min and the chlorophyll fluorescence and P700 redox state were measured simultaneously. Values are means ± SE (n = 4).

enzymes, especially ascorbate peroxidase (APX) (Kornyeyev *et al.* 2003). In addition, plants have evolved complicated mechanisms that can quickly and effectively repair photodamaged PSII (Takahashi and Badger 2011). Our results also suggest that the night chilling (≤ 10ºC) significantly increased Y_{NPQ} values compared with 22°C under moderate PAR (Fig. 4*C*). This suggests that photochemical energy conversion and protective regulatory mechanisms (*e.g.,* heat dissipation) played important roles in protecting PSII from photodamage by dissipating excess light energy under night chilling stress (Takahashi *et al.* 2009). It is interesting to note that in the night-chilling experiment, Y_{NPQ} was significantly lesser but Y_{II} significantly greater in the 30/16°C treatment than in the 30/22°C treatment. It seems plausible that night cooling to 16°C did not cause PSII photodamage. These results further indicated that PSII in cotton was more sensitive to night chilling temperature.

PSI performance in response to night chilling stress: No studies have been done about the effects of low night temperature on PSI activity in cotton. We investigated the direct performance of PSI *in vivo* of cotton plants using a *Dual-PAM-100* fluorometer. One reliable parameter, Y_{NA}, can be used to reflect the acceptor side limitation of PSI and as an indicator of PSI photoinhibition. Here, during the treatment period, we found that the night chilling (≤ 10°C) significantly decreased Y_{NA} (Fig. 4*F*). This suggests that the acceptor side limitation of PSI was low, thus protecting PSI from photoinhibition due to night chilling. In addition, the night chilling (≤ 10°C) significantly increased P_m compared with 30/22°C treatment (Fig. 2*B*). The P_m is an indicator of the maximum amount of photooxidizable P700 (Klughammer and Schreiber 1994, 2008). These results suggest that night chilling at 10 or 4°C had less limitation on the acceptor side of PSI (Figs. 4*F*; 5*B*,*D*) and that the availability of functional $P700^+$ increased with decreasing night chilling temperature, which is expected to enhance cyclic electron transport. The increase of P_m might be induced by the optical structure of leaf, which can be changed by the stress. The response activity of PSI to low night temperatures has been described previously in tomato (Liu *et al.* 2012). In some representative species, such as cucumber (Sonoike 1999) and *Arabidopsis thaliana* (Zhang and Scheller 2004), photoinhibition of PSI is induced prior to PSII under chilling stress. However, the extent of photoinhibition is not always greater in PSI than that in PSII. For example, in tropical tree species and *Cymbidium tracyanum*, PSII is much more sensitive to chilling/light stress than PSI (Huang *et al*. 2011, Li and Zhang 2015). In this study, we found that PSI activity remained stable as indicated by slightly increased P_m and lesser limitation on the acceptor side of PSI after night chilling treatment. However, Kornyeyev *et al.* (2001) pointed out that the PSI

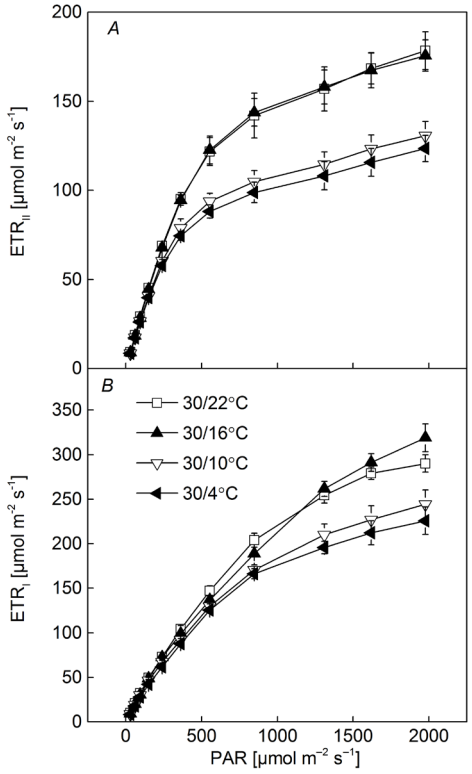

Fig. 5. Rapid light-response changes in the electron transport rate of PSII (ETR_{II}) (*A*) and PSI (ETR_I) (*B*) in 'Xinluzao 45' cotton leaves as affected by night chilling temperatures. The leaves were dark-adapted for 30 min and the chlorophyll fluorescence and P700 redox state were measured simultaneously. Values are means ± SE (n = 4).

is more sensitive to chilling-induced photoinactivation than PSII in cotton leaves. This high sensitivity of PSI to chilling may be related to lesser PSII photodamage due to the extremely short treatment time (*i.e.*, at 10°C for 20 min in the chamber) compared with our treatment time for 48 h. It is indicated that downregulation of PSII activity is essential for protecting PSI from photoinhibition (Kudoh and Sonoike 2002b, Sonoike 2011). It is possible that downregulation of Y_{II} due to night chilling stress (Figs. 4*A*,*F*; 5*A–D*, Table 2) alleviated the photoinhibition of PSI under moderate or high PAR. In the present study, we proposed that the increase in Y_{ND} after night chilling at 10 and 4°C may help to alleviate the photoinhibition of PSI of cotton by reducing the ETR_I and Y_I (Figs. 4, 5). The results indicated that 30/4°C treatment had the highest Y_{NA} among all treatments when PAR was < 250 μmol(photon) m^{-2} s^{-1} (Fig. 4*F*). This is consistent with the report of Kim *et al.* (2005) who observed that the Y_{NA} of cucumber under weak light was much greater at chilling temperature than at moderate temperature. Our results further confirmed that weak illumination is essential for selective photoinhibition of PSI in chilling-sensitive plants (Sonoike 1996b). As the prominent electron sink pathway, most electrons transported from PSII to PSI are used for CO_2 assimilation. However, low night temperature may cause a large accumulation of carbohydrate in cotton leaves resulting in the impaired regeneration of the carboxylation substrate ribulose-1,5-bisphosphate by decreasing the concentration of Pi in the chloroplast, thereby inhibiting the rate of CO_2 assimilation (Azcón-Bieto 1983, Liu *et al.* 2012). In the present study, indeed, low night temperature increased stomatal limitation and reduced CO_2 assimilation (Table 1). Previous researchers observed that excessive accumulation of electrons on the PSI acceptor side can cause the generation of hydroxyl radicals and ultimately photoinhibition of PSI (Sonoike 1996a, 2011; Asada 1996). It is worth noting that high Y_{NA} was observed in the 30/16°C treatment but not in the 30/10°C and 30/4°C treatments. However, PSII was not affected by the 30/16°C treatment as evidenced by the high Y_{II} and ETR_{II} values (Table 2). This suggests that high PSII activity and LEF may exacerbate damage to PSI as night temperatures decrease. Previous studies have indicated that PSI photoinibition can be alleviated by blocking LEF from PSII to PSI (Kudoh and Sonoike 2002a, Zhang *et al.* 2011, Huang *et al.* 2016). For cucumber leaves, dark-chilling pretreatment reduced PSI photoinhibition under light-chilling conditions (Kudoh and Sonoike 2002a), probably because dark chilling severely limits the oxygen-evolving activity of PSII (Higuchi *et al.* 2003). Li and Zhang (2014) have also proposed that the relative stability of PSI can largely be attributed to the depression of LEF and/or photodamage of PSII. Therefore, based on our results and published studies, we deduce that rapid downregulation of PSII photochemical activity plays a prominent photoprotective role in alleviating PSI photoinhibition

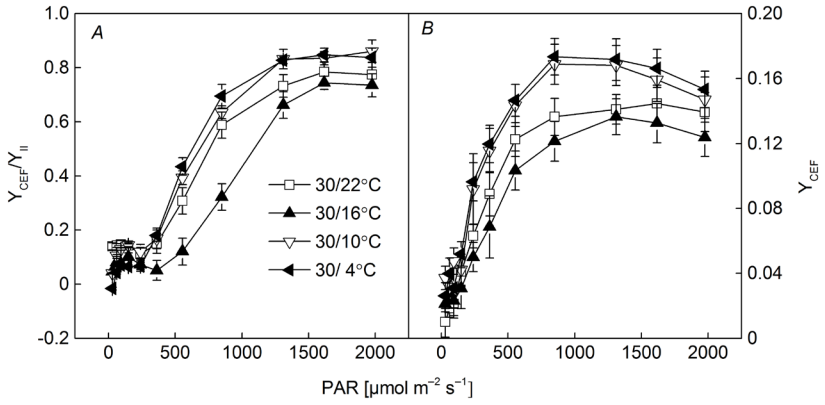

Fig. 6. The rapid light-response changes of the ratio of the effective quantum yield of cyclic electron transport (Y_{CEF}) to the effective quantum yield of PSII (Y_{II}) (Y_{CEF}/Y_{II}) (*A*) and the effective quantum yield of cyclic electron transport (Y_{CEF}) (*B*) in 'Xinluzao 45' cotton leaves as affected by night chilling temperatures. The leaves were dark-adapted for 30 min and the chlorophyll fluorescence and P700 redox state were measured simultaneously. Values are means ± SE (n = 4).

caused by night chilling in cotton. Further studies are needed to explore the photoprotective mechanism between PSI and PSII under night chilling stress.

The role of CEF in photoprotection under night chilling stress: Cyclic electron transport plays a vital role in protecting PSII and PSI from photoinhibition (Munekage *et al.* 2002, Shikanai 2007, Li and Zhang 2014). The present study suggested in cotton plants that (*1*) CEF is involved in protecting PSII from photoinhibition due to night chilling stress and (*2*) CEF can also alleviate PSI photoinhibition by regulating PSII activity. The night chilling (≤ 10°C) significantly increased Y_{CEF} under moderate and high PAR (Fig. 6). Interestingly, the 30/16°C treatment compared with the other treatments had the lowest CEF activity at all light intensities. The photochemical reactions may consume energy absorbed by the photosynthetic pigments under moderate night temperature, no severe photodamage occurred. Consequently, CEF was low at moderate night temperature. However, photosynthesis was inhibited by night chilling stress (Table 1), leading to the accumulation of excess energy and increasing the risk of PSII photoinhibition (Fig. 3). Excess light energy can be dissipated as heat by PSII antenna proteins through regulated energy dissipation. This is induced by acidification of the thylakoid lumen (Müller *et al.* 2001). It has been shown that CEF can sustain a large proton gradient across the thylakoid membrane under chilling stress (Munekage *et al.* 2004). In the present study, we observed that CEF significantly increased when night temperatures were low (*i.e.*, at 10 or 4°C). Low night temperatures also increased Y_{NPQ} under moderate PAR compared with control. Interestingly, the 30/16°C treatment had the lowest Y_{CEF} and the lowest Y_{NPQ} at all PAR levels in this study. The results suggest that CEF during night chilling stress was crucial to the normal activation of NPQ. This may explain how PSII was protected from photoinhibition during night-time chilling stress.

Our findings also suggested that CEF also played an important role in protecting PSI from photoinhibition. It has been widely assumed that PSI photoinhibition is mainly caused by NADPH accumulation leading to overreduction of the PSI acceptor side and the generation of hydroxyl radicals which destroy the PSI complex (Sonoike 1996a, Yamori and Shikanai 2016). In the present study, night chilling stress reduced stomatal conductance and inhibited CO_2 assimilation (Table 1). This could lead to NADPH accumulation and PSI photoinhibition as described above. Previous studies have indicated that CEF can protect PSI against chilling stress by alleviating overreduction of the PSI acceptor side and by balancing the $NADP^+$/NADPH ratio (Munekage *et al.* 2002, 2004; Yamori and Shikanai 2016). In our study, Y_{NA} under moderate and high PAR was maintained at a low level after exposure to night chilling at 10 or 4°C. This suggests that overreduction of the PSI acceptor side was prevented during the day time. The stimulation of CEF could result in more P700 being oxidized to $P700^+$ (Figs. 2*B*,4*E*). This reaction would prevent NADPH accumulation (Shikanai 2007) and result in the harmless dissipation of excess light energy as heat (Nuijs *et al.* 1986). Our findings agree with the previous report that stimulation of CEF and inhibition of LEF decreased the fraction of PSII electron acceptors that were reduced resulting in greater Y_{ND} (Huang *et al.* 2012). The light-response curves showed that night chilling at 10 or 4°C significantly increased Y_{ND}. The increase in Y_{ND} can be attributed to higher Y_{CEF} (Figs. 4*E*, 6). Overall, these results suggest that CEF around PSI might be one of the most important factors allowing cotton to tolerate night chilling stress and prepare for recovery of photosynthetic function during the transition from the cool night to the warm day.

Conclusion: In the present study, we examined the effects of night chilling on the photosynthetic gas exchange and on energy distribution and activity in PSII and PSI of cotton (*Gossypium hirsutum* L.) at the boll-forming stage. We found that night chilling caused closure of PSII reaction centers and the inhibition of LEF in cotton. This led to PSII photoinhibition due to the accumulation of excessive excitation energy. In contrast to PSII, the quantum yield of PSI was lowered mainly due to increases in donor side limitation (Y_{ND}) after night chilling. The night chilling increased the quantum yield of cyclic electron transport. Therefore, CEF around PSI may be one of the most important factors preventing PSI and PSII photoinhibition, thus allowing cotton to tolerate night chilling stress.

References

Allen D.J., Ort D.R.: Impacts of chilling temperatures on photosynthesis in warm-climate plants. – Trends Plant Sci. **6**: 36-42, 2001.

Asada K.: Radical production and scavenging in the chloroplasts. – In: Baker N.R. (ed.): Photosynthesis and the Environment. Advances in Photosynthesis and Respiration. Pp. 123-150. Springer, Dordrecht 1996.

Azcón-Bieto J.: Inhibition of photosynthesis by carbohydrates in wheat leaves. – Plant Physiol. **73**: 681-686, 1983.

Bange M.P., Milroy S.P.: Impact of short-term exposure to cold night temperatures on early development of cotton (*Gossypium hirsutum* L.). – Aust. J. Agr. Res. **55**: 655-664, 2004.

Berry J.A., Downton W.J.S.: Environmental regulation of photosynthesis. – In: Govindjee (ed.): Photosynthesis. Volume II. Development, Carbon Metabolism, and Plant Productivity. Pp. 263343. Academic Press, New York 1982.

Bertamini M., Muthuchelian K., Rubinigg M. *et al*.: Photoinhibition of photosynthesis in leaves of grapevine (*Vitis vinifera* L. cv. Riesling). Effect of chilling nights. – Photosynthetica **43**: 551-557, 2005.

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

Genty B., Briantais J.M., Baker N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – BBA-Gen. Subjects **990**: 87-92, 1989.

Gipson J.R.: Temperature effects on growth, development, and fiber properties. – In: J.R. Mauney (ed.): Cotton Physiology. Pp. 47-56. Cotton Foundation, Memphis, TN, USA 1986.

Havaux M., Davaud A.: Photoinhibition of photosynthesis in chilled potato leaves is not correlated with a loss of photosystem-II activity. Preferential inactivation of photosystem I. – Photosynth. Res. 40: 75-92, 1994.

Havaux M., Greppin H., Strasser R.J.: Functioning of photosystems I and II in pea leaves exposed to heat stress in the presence or absence of light. – Planta **186**: 88-98, 1991.

Hendrickson L., Furbank R.T., Chow W.S.: A simple alternative approach to assessing the fate of absorbed light energy using chlorophyll fluorescence. – Photosynth. Res. **82**: 73-81, 2004.

Higuchi M., Noguchi T., Sonoike K.: Over-reduced states of the Mn-cluster in cucumber leaves induced by dark-chilling treatment. – BBA-Bioenergetics **1604**: 151-158, 2003.

Huang W., Yang S.J., Zhang S.B. *et al*.: Cyclic electron flow plays an important role in photoprotection for the resurrection plant *Paraboea rufescens* under drought stress. – Planta **235**: 819-828, 2012.

Huang W., Yang Y.J., Zhang J.L. *et al*.: PSI photoinhibition is more related to electron transfer from PSII to PSI rather than PSI redox state in *Psychotria rubra*. – Photosynth. Res. **129**: 85-92, 2016.

Huang W., Zhang S.B., Cao K.F.: Stimulation of cyclic electron flow during recovery after chilling-induced photoinhibition of PSII. – Plant Cell Physiol. **51**: 1922-1928, 2010.

Huang W., Zhang S.B., Cao K.F.: Cyclic electron flow plays an important role in photoprotection of tropical trees illuminated at temporal chilling temperature. – Plant Cell Physiol. **52**: 297-305, 2011.

Huner N.P., Öquist G., Sarhan F.: Energy balance and acclimation to light and cold. – Trends Plant Sci. **3**: 224-230, 1998.

Kim J.H., Kim S.J., Cho S.H. *et al*.: Photosystem I acceptor side limitation is a prerequisite for the reversible decrease in the maximum extent of P700 oxidation after shortterm chilling in the light in four plant species with different chilling sensitivities. – Physiol. Plantarum **123**: 100-107, 2005.

Kim S.J., Lee C.H., Hope A.B., Chow W.S.: Inhibition of photosystems I and II and enhanced back flow of photosystem I electrons in cucumber leaf discs chilled in the light. – Plant Cell Physiol. **42**: 842-848, 2001.

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

Klughammer C., Schreiber U.: Saturation pulse method for assessment of energy conversion in PSI. – PAM Appl. Notes **1**: 11-14, 2008.

Kornyeyev D., Logan B.A., Allen R.D., Holaday A.S.: Effect of chloroplastic overproduction of ascorbate peroxidase on photosynthesis and photoprotection in cotton leaves subjected to low temperature photoinhibition. – Plant Sci. **165**: 1033-1041, 2003.

Kornyeyev D., Logan B.A., Payton P. *et al*.: Enhanced photochemical light utilization and decreased chilling-induced photoinhibition of photosystem II in cotton overexpressing genes encoding chloroplast-targeted antioxidant enzymes. – Physiol. Plantarum **113**: 323-331, 2001.

Kramer D.M., Johnson G., Kiirats O., Edwards G.E.: New fluorescence parameters for the determination of QA redox state and excitation energy fluxes. – Photosynth. Res. **79**: 209-218, 2004.

Kudoh H., Sonoike K.: Dark-chilling pretreatment protects PSI from light-chilling damage. – J. Photosci. **9**: 59-62, 2002a.

Kudoh H., Sonoike K.: Irreversible damage to photosystem I by chilling in the light: cause of the degradation of chlorophyll after returning to normal growth temperature. – Planta **215**: 541-548, 2002b.

Lei Y.B., Zheng Y.I., Dai K.J. *et al*.: Different responses of photosystem I and photosystem II in three tropical oilseed crops exposed to chilling stress and subsequent recovery. – Trees **28**: 923-933, 2014.

Li J.W., Zhang S.B.: Differences in the responses of photosystems I and II in *Cymbidium sinense* and *C. tracyanum* to long-term chilling stress. – Front. Plant Sci. **6**: 1097, 2014.

Liu Y.F., Qi M.F., Li T.L.: Photosynthesis, photoinhibition, and antioxidant system in tomato leaves stressed by low night temperature and their subsequent recovery. – Plant Sci. **196**: 8-17, 2012.

Mi H., Klughammer C., Schreiber U.: Light-induced dynamic changes of NADPH fluorescence in *Synechocystis* PCC 6803 and its *ndhB*-defective mutant M55. – Plant Cell Physiol. **41**: 1129-1135, 2000.

Miyake C., Horiguchi S., Makino A. *et al*.: Effects of light intensity on cyclic electron flow around PSI and its relationship to nonphotochemical quenching of Chl fluorescence in tobacco leaves. – Plant Cell Physiol. **46**: 1819-1830, 2005.

Munekage Y., Hashimoto M., Miyake C. *et al*.: Cyclic electron flow around photosystem I is essential for photosynthesis. – Nature **429**: 579-582, 2004.

Munekage Y., Hojo M., Meurer J. *et al*.: PGR5 is involved in cyclic electron flow around photosystem I and is essential for photoprotection in *Arabidopsis*. – Cell **110**: 361-371, 2002.

Murata N., Takahashi S., Nishiyama Y., Allakhverdiev S.I.: Photoinhibition of photosystem II under environmental stress. – BBA-Bioenergetics **1767**: 414-421, 2007.

Müller P., Li X.P., Niyogi K.K.: Non-photochemical quenching. A response to excess light energy. – Plant. Physiol. **125**: 1558-1566, 2001.

Nuijs A.M., Shuvalov V.A., van Gorkom H.J. *et al*.: Picosecond absorbance difference spectroscopy on the primary reactions and the antenna-excited states in Photosystem I particles. – BBA-Bioenergetics **850**: 310-318, 1986.

Powles S.B.: Photoinhibition of photosynthesis induced by visible light. – Annu. Rev. Plant Physiol. **35**: 1544, 1984.

Shikanai T.: Cyclic electron transport around photosystem I: genetic approaches. – Annu. Rev. Plant Biol. **58**: 199-217, 2007.

Sonoike K.: Selective photoinhibition of photosystem I in isolated thylakoid membranes from cucumber and spinach. – Plant Cell Physiol. **36**: 825-830, 1995.

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

Sonoike K.: Photoinhibition of photosystem I: its physiological significance in the chilling sensitivity of plants. – Plant Cell Physiol. **37**: 239-247, 1996b.

Sonoike K.: Various aspects of inhibition of photosynthesis under light/chilling stress: "Photoinhibition at chilling temperatures" versus "chilling damage in the light". – J. Plant Res. **111**: 121-129, 1998.

Sonoike K.: The different roles of chilling temperatures in the photoinhibition of photosystem I and photosystem II. – J. Photoch. Photobio. B **48**: 136-141, 1999.

Sonoike K.: Photoinhibition of photosystem I. – Physiol. Plantarum **142**: 56-64, 2011.

Sonoike K., Terashima I.: Mechanism of photosystem-I photoinhibition in leaves of *Cucumis sativus* L. – Planta **194**: 287-293, 1994.

Takagi D., Amako K., Hashiguchi M. *et al*.: Chloroplastic ATP synthase builds up a proton motive force preventing production of reactive oxygen species in photosystem I. – Plant J. **91**: 306-324, 2017.

Takahashi S., Badger M.R.: Photoprotection in plants: a new light on photosystem II damage. – Trends Plant Sci. **16**: 53-60, 2011.

Takahashi S., Milward S.E., Fan D.Y. *et al*.: How does cyclic electron flow alleviate photoinhibition in *Arabidopsis*? – Plant Physiol. **149**: 1560-1567, 2009.

Takahashi S., Murata N.: How do environmental stresses accelerate photoinhibition? – Trends Plant Sci. **13**: 178-182, 2008.

Terashima I., Funayama S., Sonoike K.: The site of photoinhibition in leaves of *Cucumis sativus* L. at low temperatures is photosystem I, not photosystem II. – Planta **193**: 300-306, 1994.

van Heerden P.D.R., Krüger G.H.J., Loveland J.E. *et al*.: Dark chilling imposes metabolic restrictions on photosynthesis in soybean. – Plant Cell Environ. **26**: 323-337, 2003.

Xingxian G , Wei Z .: Study on relationship between temperature and cotton fiber development in Xinjiang [China]. – Cotton Sci. **3**: 43-52, 1991.[In Chinese]

Yamori W., Shikanai T.: Physiological functions of cyclic electron transport around photosystem I in sustaining photosynthesis and plant growth. – Annu. Rev. Plant Biol. **67**: 81-106, 2016.

Zhang G., Liu Y., Ni Y. *et al*.: Exogenous calcium alleviates low night temperature stress on the photosynthetic apparatus of tomato leaves. – PLoS ONE **9**: e97322, 2014.

Zhang S., Scheller H.V.: Photoinhibition of photosystem I at chilling temperature and subsequent recovery in *Arabidopsis thaliana*. – Plant Cell Physiol. **45**: 1595-1602, 2004.

Zhang Z., Jia Y., Gao H. *et al*.: Characterization of PSI recovery after chilling-induced photoinhibition in cucumber (*Cucumis sativus* L.) leaves. – Planta **234**: 883-889, 2011.