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Changes in activities of both photosystems and the regulatory effect of cyclic electron flow in field-grown cotton (*Gossypium hirsutum* L) under water deficit

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Abstract

To clarify the influence of water deficit on the functionality of the photosynthetic apparatus of cotton plants, leaf gas exchange, chlorophyll *a* fluorescence, and P700 redox state were examined in field-grown cotton *Gossypium hirsutum* L. cv. Xinluzao 45. In addition, we measured changes in the P515 signal and analyzed the activity of ATP synthase and the trans-thylakoid proton gradient (ΔpH). With increasing water deficit, the net CO₂ assimilation rate (A_N) and stomatal conductance (g_s) significantly decreased, but the maximum quantum efficiency of PSII photochemistry (F_v/F_m) did

not change. The photochemical activity of photosystem II (PSII) was reflected by the photochemical quenching coefficient (qP), quantum efficiency of photosystem II [$Y(II)$], and electron transport rate through PSII [ETR(II)], while the activity of photosystem I (PSI) was reflected by the quantum efficiency of photosystem I [$Y(I)$] and the electron transport rate through PSI [ETR(I)]. Both activities were maintained under mild water deficit, but were slightly decreased under moderate water deficit. Under moderate water deficit, cyclic electron flow (CEF), the fraction of absorbed light dissipated thermally via the ΔpH - and xanthophyll-regulated process [$Y(NPQ)$], and the fraction of P700 oxidized under a given set of conditions [$Y(ND)$] increased. Our results suggest that the activities of both photosystems are stable under mild water deficit and decrease only slightly under moderate water deficit. Moderate water deficit stimulates CEF, and the stimulation of CEF is essential for protecting PSI and PSII against photoinhibition.

Abbreviations: ΔpH , trans-thylakoid proton gradient; $\Delta\psi$, trans-thylakoid membrane potential difference; A_N , net CO₂ assimilation rate; C_i , intercellular carbon dioxide concentration; CEF, cyclic electron flow; ETR(II), electron transport flow through PSII; ETR(I), electron transport flow through PSI; F_m , maximal fluorescence in the dark-adapted state; F_m' , maximal fluorescence in the light-adapted state; F_o , minimal fluorescence in the dark-adapted state; F_o' , minimal fluorescence in the light-adapted state; F_s , steady-state fluorescence in the light-adapted state; F_v/F_m , maximum quantum efficiency of PSII; F_v'/F_m' , photochemical quantum yield of open PSII centers in the light-adapted state; g_s , stomatal conductance; PSII, photosystem II; PSI, photosystem I; qP , photochemical quenching; Ψ_w , leaf water potential; $Y(II)$, quantum efficiency of PSII; $Y(I)$, quantum efficiency of PSI; $Y(NA)$, the fraction of P700 that cannot be oxidized by a saturating pulse under a given set of conditions; $Y(ND)$, the fraction of P700 oxidized under a given set of conditions; $Y(NO)$, the fraction of absorbed light lost by either constitutive thermal dissipation or via fluorescence; $Y(NPQ)$, the fraction of absorbed light dissipated thermally via ΔpH - and xanthophyll-regulated process; Z , zeaxanthin.

Keywords: cotton; water deficit; photosystems; cyclic electron flow; thermal dissipation

Introduction

Water deficit is the most prominent environmental factor limiting plant growth and yield formation (Boyer, 1982; Flexas et al., 2006). It is well known that water deficit inhibits CO₂ assimilation through stomatal limitation (Flexas et al., 2006; Chaves et al., 2009) and/or non-stomatal limitation (Lawlor and Cornic, 2002). Since CO₂ assimilation is a major sink for absorbed light energy, a decrease in CO₂ assimilation could lead to excess absorbed light energy. Accumulation of excitation energy in closed PSII traps could generate excited triplet states of chlorophyll (³Chl*) and then excited singlet oxygen (¹O₂), causing photo-oxidative damage to PSII (Chow and Aro 2005). In addition, the excess light energy could result in the electron transport chain becoming highly reduced, and in electron transport to oxygen molecules at the acceptor side of PSI producing superoxide radicals (O₂⁻), which could give rise to the subsequent formation of hydrogen peroxide (H₂O₂). H₂O₂, in turn, can react with reduced iron-sulfur centers of PSI and generate hydroxyl radicals (HO•), which can cause damage to PSI (Sonoike, 2011). PSII is often regarded as the primary and major site of photoinhibition (Anderson et al., 1998; Takahashi and Badger, 2011), including chronic and dynamic photoinhibition (Osmond, 1994). Chronic photoinhibition is assessed as a sustainable decrease in predawn maximum quantum efficiency of PSII photochemistry (F_v/F_m), whereas dynamic photoinhibition is determined from the fully reversible diurnal decline in F_v/F_m (Werner et al., 2002). PSI is insensitive to high light stress and has often been considered more stable than PSII (Scheller and Haldrup, 2005). In order to prevent and alleviate photodamage, plants have developed several photoprotective pathways, such as thermal dissipation (Demmig-Adams and Adams, 1996) and cyclic electron flow (CEF) (Heber and Walker, 1992; Wei et al., 2009; Huang et al., 2012; Kou et al., 2013). Huang et al. (2012) demonstrated that CEF is essential for protecting PSII against photoinhibition

due to CEF-dependent generation of a proton gradient across thylakoid membranes (ΔpH), with ΔpH helping the activation of thermal dissipation. Moreover, CEF can protect PSI from photoinhibition by alleviating the over-reduction of the PSI acceptor side (Munekage et al., 2002; Tikkanen et al., 2015).

Cotton (*Gossypium hirsutum* L.) is a drought-tolerant crop (Turner et al., 1986; Kitao and Lei, 2007; Yi et al., 2016b). When it is exposed to water deficit conditions, the CO_2 assimilation rate and stomatal conductance decrease significantly, but the functionality of PSII and the photosynthetic electron transport systems show a relatively high stability (Genty et al., 1987; Inamullah and Isoda, 2005; Massacci et al., 2008; Snider et al., 2014; Yi et al., 2016b). Zhang et al. (2010) reported that under severe water deficit (leaf water potential of about -4.0MPa) the activity of PSII is temporarily reduced during the daytime but can fully recover in the night. Snider et al. (2014) proposed that electron transport through PSII is not limited by a wide range of water-deficit conditions in field-grown cotton. This is mainly because cotton plants can dissipate excess absorbed light energy through other photoprotection pathways. For example, Björkman and Schäfer (1989) reported that about 56% of the absorbed light is dissipated as thermal energy in well-watered cotton plants, increasing up to 70-82% under moderate and severe drought. Furthermore, photorespiration can also protect water-deficit cotton plants against photoinhibition (Massacci et al., 2008; Chastain et al., 2014; Yi et al., 2014, 2016a). Compared with the photoinhibition of PSII of cotton plants, much less is known about the activity of PSI under water deficit. Our previous study reported that water deficit increases the electron flux for the Mehler reaction (Yi et al., 2014); the reduction of O_2 in this reaction may result in the formation of harmful reactive oxygen species (ROS) at the acceptor side of PSI. Will these ROS lead to the photoinhibition of PSI? Can CEF, as an important alternative electron sink, effectively dissipate excess electrons on the side of PSI? Singh et al. (2014) investigated the effect of short-term water deficit on CEF in cotton plants grown in a growth chamber; the results showed that the CEF/ETR(II) ratio gradually increased with increasing water deficit. It is difficult to extrapolate the results to field

conditions because water deficit is usually accompanied by other limiting factors, such as high temperature and irradiance under field conditions. Moreover, short-term water deficit cannot induce the phenomenon of acclimation, which is usually well developed in mature field-grown plants.

In order to better elucidate the influence of water deficit on photosynthesis in field-grown cotton plants, gas-exchange parameters, chlorophyll *a* fluorescence, and the P700 redox state were measured to assess carbon assimilation and the functionality of both photosystems. In addition, P515 signal changes were simultaneously analyzed to evaluate the activity of ATP synthase and the generation of ΔpH in water-deficit cotton plants.

Materials and methods

Our study was conducted at an experimental field of Shihezi Agricultural College, Shihezi University, Xinjiang, China (45°19'N, 86°03'E). Cotton (*Gossypium hirsutum* L. cv. Xinluzao 45) seeds were sown on 25 April, 2016, in rows 12 cm apart at a plant density of $1.8 \times 10^5 \text{ ha}^{-1}$. The experiment was constructed following a completely random block design (three replicates), and the plot area was 40.8 m². The same three levels of irrigating water were used as in our previous research (Yi et al., 2016a): well-watered (CK, in which water was maintained at between 75% and 85% of soil water capacity), mild water deficit (Mild, in which the plots were irrigated to the extent of 60% of the well-watered plots), and moderate water deficit (Mod, in which the plots were irrigated to the extent of 20% of the well-watered plots). At approximately 55 d after sowing, plants were subjected to water-deficit treatments. The plot was drip-irrigated once a week, and all measurements were performed randomly on the topmost west-facing leaf on the main stem using four replicates during flowering and boll-setting stages.

Water potential measurement

Leaf water potential (Ψ_w) was measured with a pressure chamber (SKPM 1400; Skye

Instruments, Llandrindod Wells, UK) at pre-dawn as described by Yi et al. (2016a). Each fully expanded third (upper) leaf was excised from the cotton plant, and the leaf petiole was immediately sealed in a compression gasket with the cut surface of the petiole exposed. The leaf blade was sealed in the pressure chamber, which was subjected to increasing pressure from a compressed nitrogen cylinder until free sap was visible at the cut surface of the petiole.

Measurement of gas exchange

Instantaneous gas exchange was carried out between 11:00 and 13:00 h, using an open infrared gas-exchange analyzer system (Li-6400, Li-Cor Inc., Nebraska, USA). Leaf temperature was kept at $\sim 30^{\circ}\text{C}$ via the temperature control device of the Li-6400. Illumination was provided by a LED source and was adjusted to $1,800 \mu\text{mol m}^{-2} \text{s}^{-1}$, while the CO_2 concentration in the cuvette was $400 \mu\text{mol CO}_2 \text{ mol}^{-1}$ air. Net CO_2 assimilation rate (A_N), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were recorded.

Measurement of chlorophyll a fluorescence and P700 redox state

Chlorophyll *a* fluorescence and P700 redox state measurements were conducted simultaneously using a saturation-pulse Dual-PAM-100 (Heinz Walz, Effeltrich, Germany). Each leaf was dark-adapted for at least 30 min before the measurements of F_o , F_m , and P_m . Each leaf was light-adapted ($1,033 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 4-5 min before the measurement of rapid light response curves. F_s , F_m' , and P_m' were recorded after 20 s exposure to each light intensity (30, 119, 240, 555, 849, 1,052, 1,311, 1,618, and $1,976 \mu\text{mol m}^{-2} \text{s}^{-1}$). Each light increment was followed by the measurement of F_s and by a saturating pulse for the measurement of F_m' and P_m' . In the present study, the intensity and the width of the saturating pulse were $10,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ and 300 ms, respectively. A 627nm LED was used as an actinic light source.

The maximum quantum efficiency of photosystem II (F_v/F_m) was measured at predawn where F_v is variable fluorescence, calculated as $F_v = F_m - F_o$ (Krause and

Weis, 1991). Quantum efficiency of PSII Y(II) in the light was calculated as $(F_m' - F_s)/F_m'$ (Genty et al., 1989). Photochemical quenching (qP) was calculated as $(F_m' - F_s)/(F_m' - F_o')$ (Schreiber et al., 1995). The photochemical quantum yield of open PSII centers in the light-adapted state (F_v'/F_m') was calculated as $(F_m' - F_o')/F_m'$ (Genty et al., 1989). Minimal fluorescence under light exposure (F_o') was calculated according to Oxborough and Baker (1997) using the equation $F_o' = F_o/(F_v/F_m + F_o/F_m')$. The fraction of absorbed light lost by either constitutive thermal dissipation or via fluorescence was calculated as $Y(NO) = F_s/F_m$, and the fraction of absorbed light dissipated thermally via ΔpH - and xanthophyll-regulated process was calculated as $Y(NPQ) = (F_s/F_m') - (F_s/F_m)$ (Kramer et al., 2004).

The quantum efficiency of PSI was calculated as $Y(I) = 1 - Y(ND) - Y(NA)$, where $Y(ND)$ is the fraction of P700 oxidized under a given set of conditions, and $Y(NA)$ is the fraction of P700 that cannot be oxidized by a saturating pulse under a given set of conditions. $Y(ND)$ and $Y(NA)$ were directly determined by the saturation pulse method: $Y(ND) = P/P_m$, where P is the $P700^+$ signal under a given set of conditions, and $Y(NA) = (P_m - P_m')/P_m$ (Schreiber and Klughammer, 2008a). The electron transport rate through PSII ETR(II) and that through PSI ETR(I) were calculated according to the formula $ETR(II) = Y(II) \times \text{incident PPFD} \times 0.84 \times 0.5$ and $ETR(I) = Y(I) \times \text{incident PPFD} \times 0.84 \times 0.5$, respectively (where 0.84 is a usual leaf absorptance for C_3 plants and 0.5 assumes equal distribution of excitation between the two photosystems) (Schreiber and Klughammer, 2008a).

Measurement of P515 signal changes

The dual beam 550-515 nm difference signal change was monitored simultaneously using the P515/535 module of the Dual-PAM-100 and the automated routines provided by the Dual-PAM software (Schreiber and Klughammer, 2008b). After 1 h of dark adaptation, P515 signal changes induced by a saturating single turnover flash were recorded to evaluate the integrity of the thylakoid membrane. After 10 min of pre-illumination at $1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ and 4 min of dark adaptation,

P515 signal changes induced by the flash were recorded to analyze the activity of ATP synthase. Slow dark-light-dark induction transients of the 550-515 nm signals reflect changes in both the membrane potential and the zeaxanthin (Z) content. These transients were measured after several hours of dark adaptation. Then the actinic light ($1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$) was turned on for 5 s and off after 10 min. Determination of Z content, transmembrane potential ($\Delta\psi$), and ΔpH using the dark-light-dark induction transients was done as described previously by Schreiber and Klughammer (2008b).

Statistical analysis

Significant effects due to water-deficit treatments, species, and the interaction terms were tested with *SPSS 16.0* for Windows (SPSS, Chicago, IL, USA) by one-way analysis of variance (ANOVA) of means of four replicates. The data are presented as the means \pm standard error. The significance of differences between means was determined using the Duncan test at the $p \leq 0.05$ level.

Results

Effect of water deficit on leaf water potential (Ψ_w) and photosynthetic parameters

Table 1 shows that, with increasing water deficit, Ψ_w in cotton leaves declined significantly, from -0.87 MPa in well-watered plants to -1.24 and -1.82 MPa in mild and moderate water-deficit plants, respectively. In addition, with increasing water deficit, net CO₂ assimilation rate (A_N), stomatal conductance (g_s), and intercellular CO₂ concentration (C_i) gradually decreased, and there was an obvious difference between well-watered and water-deficit plants. Compared with well-watered plants, A_N decreased by 34% and 59%, g_s decreased by 59% and 76%, and C_i decreased by 21% and 26% for mild water deficit and moderate water deficit, respectively.

Effect of water deficit on the functionality of PSII and PSI

PSII function can be assessed by using F_v/F_m . As shown in Table 1, there was no difference in F_v/F_m between well-watered and water-deficit plants, indicating that water deficit had no effect on the activity of PSII. With increasing light intensity, the photochemical quantum yield of open PSII centers in the light-adapted state (F_v'/F_m') and photochemical quenching (qP) gradually decreased. There was no difference in F_v'/F_m' and qP between well-watered plants and plants under mild water deficit, but qP significantly decreased under moderate water deficit (Fig. 1). The quantum efficiency of PSII Y(II) significantly decreased under moderate water deficit; according to $Y(II) = qP \times F_v'/F_m'$, the decrease in Y(II) was mostly due to the decrease in qP , not in F_v'/F_m' . In addition, we found that the decrease in Y(II) under moderate water deficit was compensated for by an increase in the fraction of absorbed light dissipated thermally via ΔpH - and xanthophyll-regulated process Y(NPQ) and in the fraction of absorbed light lost by either constitutive thermal dissipation and via fluorescence Y(NO) (Fig. 2A, C, E).

With increasing light intensity, the quantum efficiency of PSI Y(I) gradually decreased, while the fraction of overall P700 oxidized under a given set of conditions

Y(ND) gradually increased. Under moderate water deficit, the decrease in Y(I) was compensated for by an increase in Y(ND), and the fraction of P700 that cannot be oxidized under a given set of conditions Y(NA) was lower than that of well-watered plants (Fig. 2B, D, F).

Effect of water deficit on the electron transport rate of PSII and PSI

Fig. 3 presents the light response curves of the electron transport rate through PSII, ETR(II), and that through PSI, ETR(I). With increasing light intensity, ETR(II) and ETR(I) gradually increased. There was no difference in ETR(II) and ETR(I) between well-watered and mild-water-deficit plants, but moderate water deficit induced an obvious decrease in ETR(II) and ETR(I). However, ETR(II) and ETR(I) began to decrease under the PPFDs of 240 and 850 $\mu\text{mol m}^{-2} \text{s}^{-1}$, with ETR(II) decreasing more than ETR(I). Would this lead to an increase in CEF? As shown in Fig. 4, the ETR(I)/ETR(II) ratio and CEF were significantly elevated under moderate water deficit compared with well-watered conditions and mild water deficit. ETR(I)/ETR(II) increased from 1.8 in well-watered plants to 3.3 in moderate-water-deficit plants at 1,300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD. CEF was activated at 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD. This result indicated that moderate water deficit activated the activity of CEF in cotton plants.

Single turnover saturating flash induced P515 signal change under water deficit

Fig. 6 presents P515 signal changes induced by a saturating single turnover flash in cotton leaves. The changes of P515 signal reflect the membrane potential difference across the thylakoid membrane (Schreiber and Klughammer, 2008b). After 1 h of dark adaptation, the flash induced a rapid rise phase, followed by a slow rise phase, and then by a slow decline phase (Bailleul et al., 2010). The rapid rise phase reflects transmembrane charge separation in PSII and PSI reaction centers, the slow rise phase indicates the transmembrane electron transport in Cyt *b₆f*, and the slow decline phase reflects the integrity of the thylakoid membrane (Bailleul et al., 2010), but also depends on the activation state of the ATP synthase. As shown in Fig. 6, the changes

in the P515 signal were similar in all treatments after 1 h dark adaptation. After 10 min illumination at $1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ actinic light and 4 min dark adaptation, the flash also induced a rapid rise phase, followed by a faster decline phase compared with that after 1 h dark adaptation, while the slow rise phase seemingly disappeared. The initial rate in the fast decline phase reflects the H^+ conductivity of the membrane. The stronger H^+ conductivity is associated with the stronger ATP synthase activity (Bailleul et al., 2010). As shown in Fig. 6, with increasing water deficit the initial rate in the fast decline phase gradually decreased, indicating that the activity of ATP synthase decreased under water deficit, especially under moderate water deficit.

Slow dark-light-dark induction transients of the 550-515 nm signal under water deficit

Fig. 7 shows the recording of dark-light and light-dark induced changes in the 550-515 nm signal in cotton leaves. The light-induced P515 signal changes not only reflect the proton motive force, but also the Z formation (Schreiber and Klughammer, 2008b). The relative extent of Z formation can be judged from the increase of the “dark baseline” apparent after light-off. Due to the high stability of the 550-515 nm difference signals, the observed slow changes in the “dark baseline” can be reliably evaluated in terms of reversible changes of Z content. Fig. 7 shows that the “dark baseline” gradually decreased with increasing water deficit, suggesting that Z formation gradually decreased under water deficit. The rapid light-off response reflects H^+ efflux from the lumen to the stroma of chloroplasts via the thylakoid ATP synthase. The rapid signal decline is followed by a biphasic signal increase to an apparent “dark baseline”. According to Schreiber and Klughammer (2008b), the relative amplitudes of $\Delta\psi$ and ΔpH can be estimated from the characteristic levels observed during the light-off response. The difference between the steady-state signal and the “dark baseline” reflects a substantial $\Delta\psi$ during steady-state illumination. The “undershoot” below the “dark baseline” is considered a measure for the steady-state ΔpH , which sets the transient maximum H^+ diffusion potential difference across the thylakoid membrane on cessation of illumination. As indicated in Fig. 7, with

increasing water deficit ΔpH gradually increased, especially under the moderate water deficit condition.

Discussion

PSII activity of cotton leaves under water deficit

Our results show that water deficit significantly decreased A_N , and the decrease in A_N was mainly due to a decrease in g_s and C_i (Table 1). Compared with CO_2 assimilation, electron transport through PSII was relatively stable under mild water deficit, because there was no change in ETR(II) (Fig. 3A). This result is supported by Snider et al. (2014), who suggested that electron flow through PSII in cotton is insensitive to a wide range of water-deficit conditions. However, under moderate water deficit ETR(II) decreased significantly (Fig. 3A). The decreased capacity for CO_2 assimilation and the diminished electron transport may result in increased excitation pressure in PSII under moderate water deficit. Our results show that $Y(NO)$ under moderate water deficit was slightly higher than that of well-watered and mild-water-deficit cotton plants, indicating that the PSII super-complex may have been damaged and/or the turnover of D1 may have been disturbed under moderate water deficit. Fortunately, our results indicated that water deficit did not induce chronic photoinhibition of PSII in cotton plants (Table 1). This result is in accordance with previous reports that PSII is insensitive to water deficit (Genty et al., 1987; Kitao and Lei, 2007; Massacci et al., 2008; Zhang et al., 2010; Chastain et al., 2014; Snider et al., 2015; Yi et al., 2014, 2016a, 2016b). Therefore, we infer that cotton plants have developed multiple photoprotective mechanisms to maintain the stability of PSII. Our previous study found that the Mehler-peroxidation reaction, photorespiration, and nitrate reduction are efficient electron sinks under water deficit (Yi et al., 2014, 2016).

Our present results show that $Y(NPQ)$ significantly increased under moderate water deficit (Fig. 2E), suggesting that thermal energy dissipation was activated in order to dissipate excess light energy, thereby avoiding ROS generation and

maintaining the balance between the absorption and consumption of light energy under moderate water deficit. Kornyejev et al. (2005) and Massacci et al. (2008) reported that non-photochemical energy dissipation serves as the major photoprotective mechanism when light energy absorption becomes excessive in cotton plants. However, Takahashi et al. (2009) considered that thermal dissipation is not the main mechanism to protect PSII from photodamage, and that CEF is a more important photoprotection pathway than thermal dissipation. The main reason is that the stimulation of thermal dissipation is dependent on ΔpH , and CEF can stimulate the generation of ΔpH (Heber and Walker, 1992). A few studies have indicated that CEF-dependent generation of ΔpH is necessary for the activation of thermal dissipation (Munekage et al., 2002, 2004; Nandha et al., 2007; Takahashi et al., 2009). Golding and Johnson (2003) reported that when LEF-dependent generation of ΔpH was limited under drought stress, CEF was stimulated to help the formation of ΔpH and thus to help the activation of thermal dissipation. Our results show that with increasing water deficit, ΔpH gradually increased, especially under the moderate water deficit condition (Fig. 7). Therefore, we can infer that the higher ΔpH is associated with the higher CEF and lower ATP synthase activity under moderate water deficit (Figs. 4B, 6C, 7). Miyake et al. (2004) reported that the activation of CEF is accompanied by higher thermal dissipation in tobacco leaf under high light. Our results show that there was an approximate linear correlation between $Y(\text{NPQ})$ and CEF (Fig. 5A), demonstrating that the activation of thermal dissipation was positively correlated with that of CEF.

The xanthophyll-cycle-dependent thermal dissipation is considered to be the major mechanism for dissipating excess energy (Demmig-Adams and Adams, 1996; Niyogi, 1999). Demmig et al. (1987) found a correlation between NPQ and Z: under low light, violaxanthin (V) was present and no NPQ was observed, whereas under high light, V was converted to Z, in parallel with the development of NPQ. However, in the present study, we found that the Z content significantly decreased under water deficit (Fig. 7), suggesting that xanthophyll-cycle-dependent thermal dissipation in

water-deficit cotton plants was impaired. This conclusion is supported by Inamullah and Isoda (2005), who reported that there is no significant change in the photochemical reflectance index (PRI) (which is correlated with the epoxidation state of the xanthophyll cycle) in cotton under water deficit. Therefore, our results suggest that the activation of thermal dissipation is related to ΔpH which is stimulated by CEF.

PSI activity of cotton leaves under water deficit

As described above, water deficit induced stomatal closure and then decreased A_N (Table 1). The reduced capacity for CO_2 assimilation may result in the over accumulation of NADPH, which can enhance the formation of $HO\cdot$, resulting in damage to PSI (Mi et al., 2000). However, we found that the activity of PSI shows a similar or only slightly lower value under moderate water deficit and high light conditions (Fig. 2F), indicating that the PSI complex in cotton leaf has strong resistance to water deficit. In addition, the decrease in $Y(NA)$ showed that PSI was not affected substantially under moderate water deficit. This result is in accordance with our previous reports that PSI of cotton leaf is insensitive to water deficit (Yi et al., 2016b).

A large number of studies have demonstrated that CEF plays an important role in protecting PSI against drought (Huang et al., 2012, 2013), high light (Munekage et al., 2002, 2004), and low temperatures (Zhang et al., 2014). CEF could alleviate the reduction of the PSI acceptor side by oxidizing the acceptor-side components of PSI by recycling the electrons from PSI to the PQ pool and Cyt b_6/f (Munekage et al., 2002; Sonoike, 2011; Tikkanen et al., 2015). Our results showed that the value of $Y(ND)$ was maintained at a high level while the value of $Y(NA)$ was maintained at a low level under moderate water deficit (Fig. 2F). The relationship between $Y(ND)$ and CEF showed approximate linearity in all treatments, and water deficit showed a higher CEF with a higher $Y(ND)$ (Fig. 5B); this means that the activation of CEF increased the value of $Y(ND)$. In addition, the higher $Y(ND)$ was also due to the inhibition of ETR(II) (Fig. 3A), which decreased the electron transport from PSII to

PSI: a large number of P700⁺ entities do not receive electrons, resulting in the P700 population being in a more oxidized state. Moreover, the Mehler reaction could also alleviate the reduction of the PSI acceptor side by dissipating excessive electrons through the photoreduction of O₂ in PSI (Radmer and Kok, 1976). The greater abundance of P700⁺, a quencher of excitation energy (Karapetyan, 2008), helps to dissipate excess light energy harmlessly as heat, while the lower level of Y(NA) avoids the oxidation-reduction reaction between reduced P700 and hydroxyl radicals (Nuijs et al., 1986). Therefore, the activation of CEF effectively alleviated photoinhibition of PSI under moderate water deficit.

In higher plants, there are two pathways of cyclic electron flow, one dependent on the proton gradient regulation 5 protein (PGR5) and the PGR5-like protein 1 (PGRL1) and the other pathway dependent on the nicotinamide adenine dinucleotide dehydrogenase-like complex (NDH). The *PRG5* and *PGRL1* genes could have been up-regulated in cotton under water-deficit-conditions, as has been reported for *Arabidopsis thaliana* by Lehtimäki et al. (2010). Wei et al. (2009) reported that the effect of cell water amount on photosynthetic yield in the cyanobacterium is related to NDH-1-mediated cyclic electron transport. While, in higher plants, the former pathway is more important, the NDH-mediated pathway of CEF seems less important, and NDH may actually aid the operation of the antimycin A-sensitive, PGR5-dependent pathway (Kou et al., 2015).

P515 signals of cotton leaves under water deficit

By analyzing the light-off kinetics of the P515 signal, we can obtain the two components of the proton motive force (pmf), $\Delta\psi$ and ΔpH , which play an important role in regulating photosynthesis and plant growth in higher plants (Zhang et al., 2014; Yamori et al., 2011; Yamori and Shikanai, 2016). First, both $\Delta\psi$ and ΔpH drive ATP generation and balance the ATP/NADPH energy budget (Walker et al., 2014). Especially under environmental stress conditions, the demand of ATP for photosynthetic CO₂ assimilation would be increased because environmental stresses cause stomatal closure, which decreases CO₂ availability in the mesophyll, resulting

in enhanced photorespiration (Flexas et al., 2002; Yi et al., 2014, 2016a). Second, ΔpH helps to down-regulate the light harvesting efficiency of photosynthetic antennae via the energy-dependent quenching (qE) mechanism (Ioannidis et al., 2012). Under normal conditions the lumen is maintained at a moderate pH and low qE, while under environmental stress conditions stomatal closure causes the ratio of CO_2/O_2 to decrease, so that the proton motive force is predominantly in the form of ΔpH , maximizing lumen acidification (Ioannidis et al., 2014). Ioannidis et al. (2014) suggested that acidification of the lumen controls photosynthetic electron transport by slowing the rate of plastoquinone (PQ) oxidation at the Cyt *b₆f*. Our results show that with increasing water deficit, ΔpH gradually increased, especially under the moderate water deficit condition (Fig. 7), indicating that the lumen maintained a lower pH and preventing the accumulation of highly reducing species on the acceptor side of PSI. ΔpH can drive ATP generation catalyzed by the ATP synthase (Yamori and Shikanai, 2016). Did the increased ΔpH under moderate water deficit enhance the formation of ATP? Our results showed that the activity of ATP synthase appeared to decrease under moderate water deficit (Fig. 6C). However, it is difficult to assess whether the ATP content increased or decreased, because we did not measure the ATP content. Therefore, the relationship between ΔpH and ATP generation requires further study.

In conclusion, water deficit decreased CO_2 assimilation capacity but did not induce photoinhibition of both photosystems in field-grown cotton plants. CEF served as an efficient sink for excess electrons and maintained the stability of both photosystems by alleviating the over-reduction of the acceptor side of PSI and promoting thermal dissipation of excitation energy in PSII.

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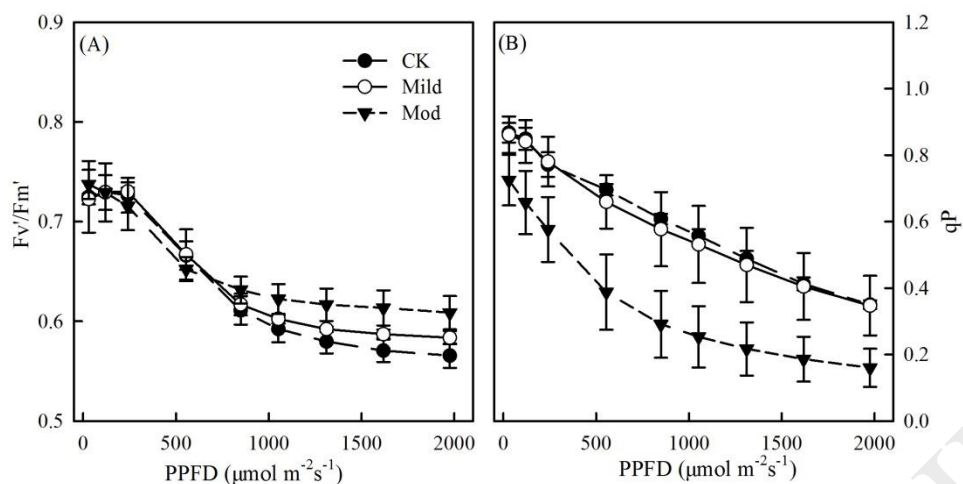


Fig.1. Light response curves of photochemical quantum yield of open PSII centers in the light-adapted state (F_v'/F_m') (A), and photochemical quenching coefficient (qP) (B). Leaves of Xinluzao 45 plants, grown under well-watered (CK, closed circles), mild water deficit (Mild, open circles), and moderate water deficit (Mod, closed triangles) conditions, were illuminated at varying PPFD. Vertical bars indicate standard errors of the means.

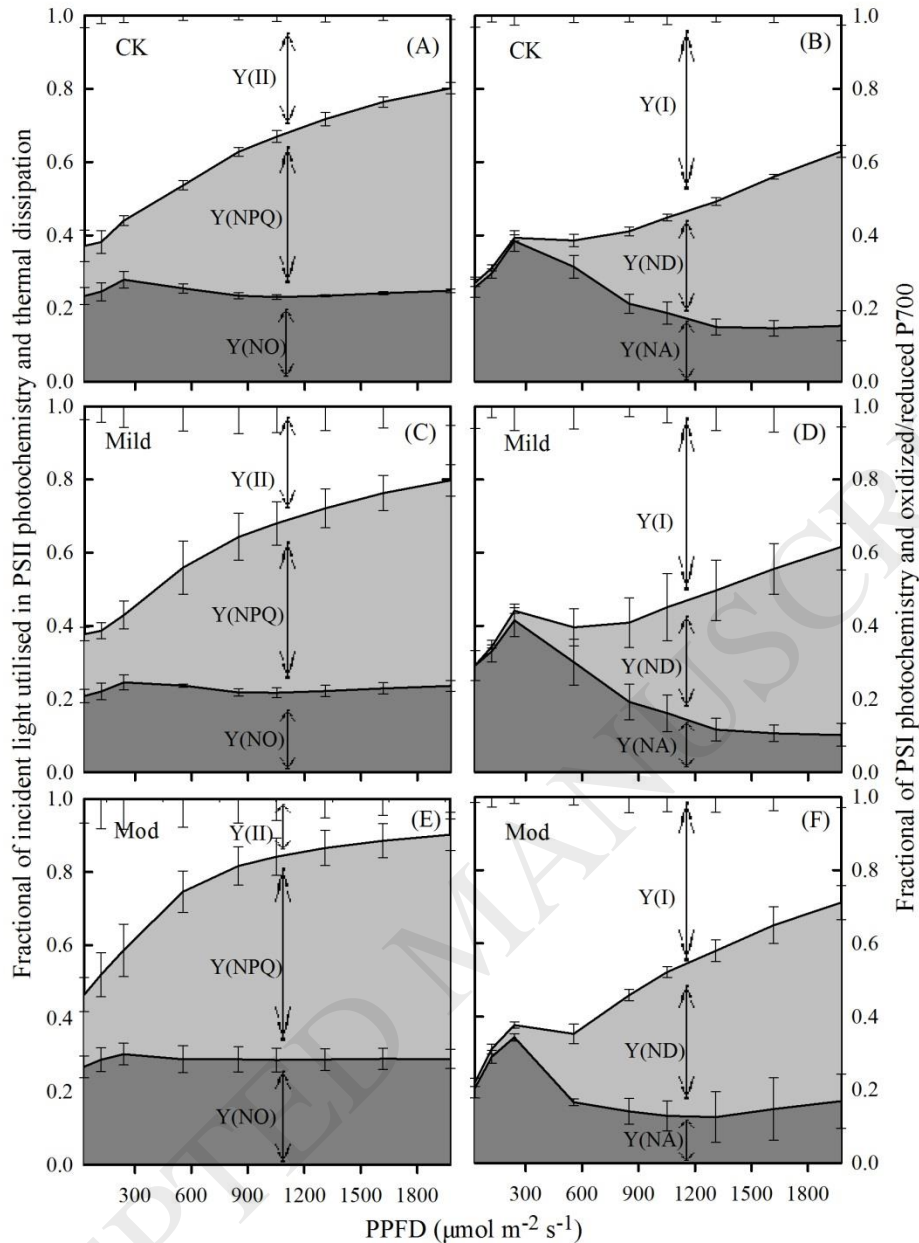


Fig.2. Light response changes in quantum efficiency of PSII, the fraction of absorbed light dissipated thermally via ΔpH - and xanthophyll-regulated process Y(NPQ), the fraction of absorbed light lost by either constitutive thermal dissipation and via fluorescence Y(NO), and quantum efficiency of PSI Y(I), the fraction of P700 oxidized in a given state Y(ND), and the fraction of P700 that cannot be oxidized by a saturating pulse in a given state Y(NA). Leaves of Xinluzao 45 plants, grown under well-watered (CK, A and B), mild water deficit (Mild, C and D), and moderate water deficit (Mod, E and F) conditions, were illuminated at varying PPFD. Vertical bars indicate standard errors of the means.

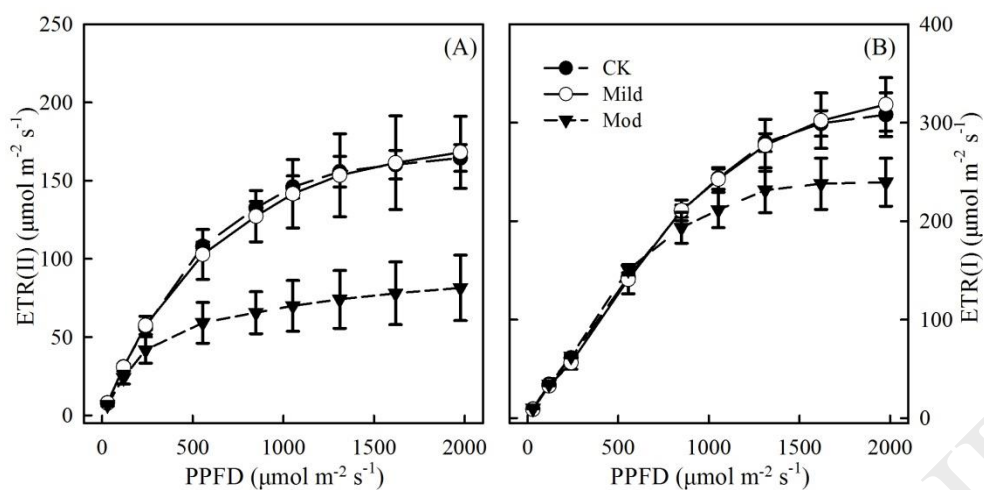


Fig.3. Light response curves of electron transport rate through PSII, ETR(II) (A), and PSI ETR(I) (B). Leaves of Xinluzao 45 plants, grown under well-watered (CK, closed circles), mild water deficit (Mild, open circles), and moderate water deficit (Mod, closed triangles) conditions, were illuminated at varying PPFD. Vertical bars indicate standard errors of the means.

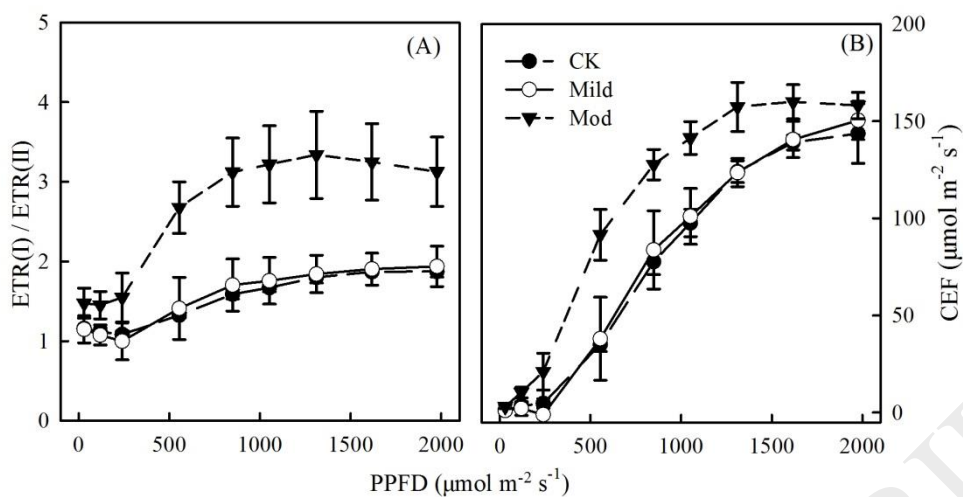


Fig.4. Light response changes in the ratio of electron transport flow through PSI, ETR (I), to electron transport flow through PSII, ETR (II) (A), and cyclic electron flow (CEF) (B). Leaves of Xinluzao 45 plants, grown under well-watered (CK, closed circles), mild water deficit (Mild, open circles), and moderate water deficit (Mod, closed triangles) conditions, were illuminated at varying PPFD. Vertical bars indicate standard errors of the means.

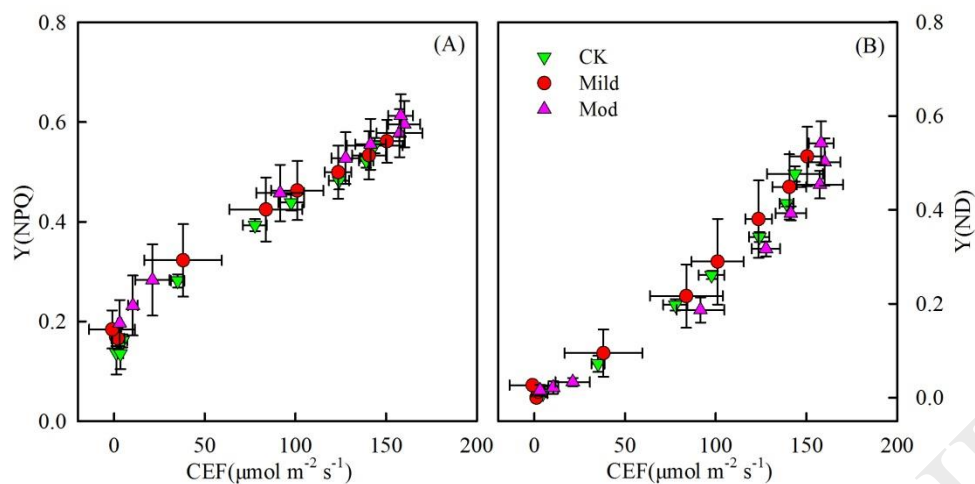


Fig.5. Relationship between cyclic electron flow (CEF) and the fraction of absorbed light dissipated thermally via ΔpH - and xanthophyll-regulated process Y(NPQ) (A) and the fraction of P700 oxidized in a given state Y(ND) (B). Leaves of Xinluzao 45 plants which grown under well-watered (CK, green triangles down), mild water deficit (Mild, red circles), and moderate water deficit (Mod, pink triangles up) conditions. Vertical and horizontal bars indicate standard errors of the means.

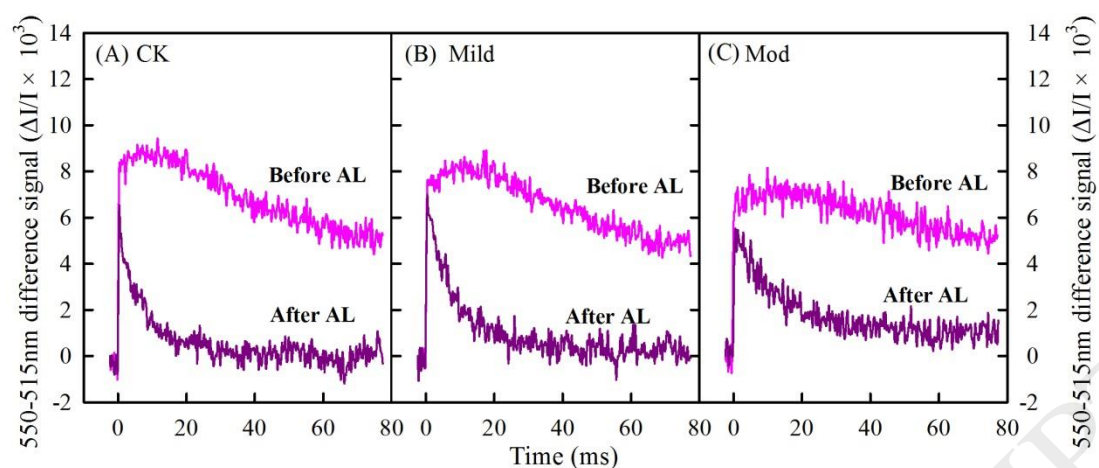


Fig.6. The saturating single turnover flash-induced change in the 550–515 nm difference signal before and after 10 min of actinic light ($1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$) in leaves of Xinluzao 45 plants grown under well-watered (CK) (A), mild water deficit (Mild) (B), and moderate water deficit (Mod) (C) conditions. A single turnover saturation flash ($10,000 \mu\text{mol m}^{-2} \text{s}^{-1}$) was applied at time 0 ms.

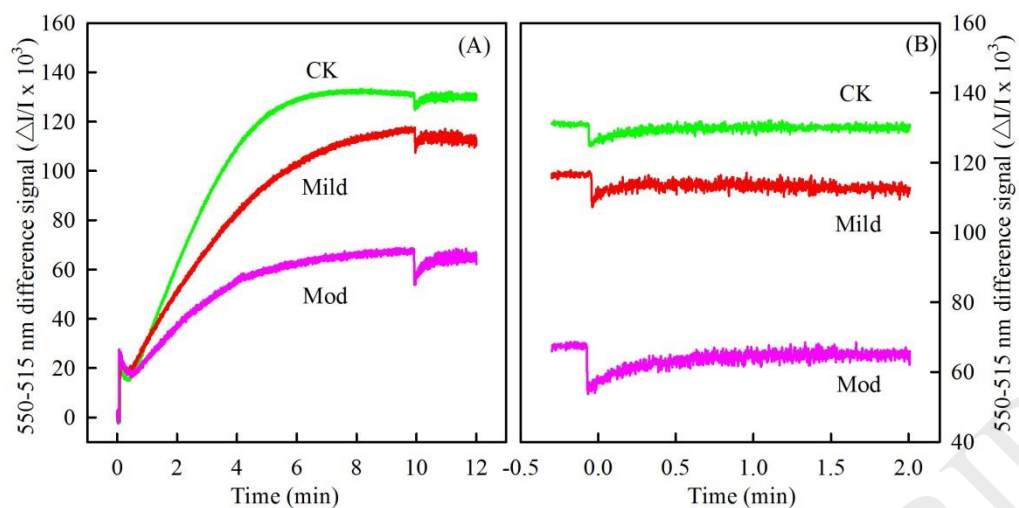


Fig.7. Slow dark-light-dark induction transients of the 550–515 nm difference signal in leaves of Xinluzao 45 plants grown under well-watered (CK), mild water deficit (Mild), and moderate water deficit (Mod) conditions. Actinic light (AL, $1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$) was turned on at 5 s and off after 10 min.

Table 1. Effects of water deficit on the leaf water potential (Ψ_w , MPa), maximal photochemistry of PSII (F_v/F_m), net CO₂ assimilation rate (A_N , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance (g_s , $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), and intercellular carbon dioxide concentration (C_i , $\mu\text{mol CO}_2 \text{ mol}^{-1}$). Photosynthetic gas exchange was measured under a PPFD of $1,800 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and at a temperature of about $30 \text{ }^\circ\text{C}$. Values are the means \pm SE. Values followed by different letters are significantly different at $p \leq 0.05$.

Treatments	Ψ_w (MPa)	A_N ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	F_v/F_m	g_s ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	C_i ($\mu\text{mol CO}_2 \text{ mol}^{-1}$)
CK	$-0.87 \pm 0.02\text{a}$	$34.9 \pm 1.83\text{a}$	$0.84 \pm 0.004\text{a}$	$0.58 \pm 0.09\text{a}$	$235.8 \pm 13.8\text{a}$
Mild water deficit	$-1.24 \pm 0.07\text{b}$	$23.1 \pm 2.25\text{b}$	$0.85 \pm 0.01\text{a}$	$0.24 \pm 0.06\text{b}$	$186.6 \pm 22.2\text{b}$
Moderate water deficit	$-1.82 \pm 0.15\text{c}$	$14.2 \pm 0.83\text{c}$	$0.83 \pm 0.02\text{a}$	$0.13 \pm 0.02\text{c}$	$174.2 \pm 19.1\text{b}$