Two distinct strategies of cotton and soybean differing in leaf movement to perform photosynthesis under drought in the field

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Abstract. This paper reports an experimental test of the hypothesis that cotton and soybean differing in leaf movement have distinct strategies to perform photosynthesis under drought. Cotton and soybean were exposed to two water regimes; drought stressed and well watered. Drought-stressed cotton and soybean had lower maximum CO_2 assimilation rates than well-watered (control) plants. Drought reduced the light saturation point and photorespiration of both species – especially in soybean. Area-based leaf nitrogen decreased in drought-stressed soybean but increased in drought-stressed cotton. Drought decreased PSII quantum yield (Φ_{PSII}) in soybean leaves, but increased Φ_{PSII} in cotton leaves. Drought induced an increase in light absorbed by the PSII antennae that is dissipated thermally via ΔpH- and xanthophylls-regulated processes soybean leaves, but a decrease in cotton leaves. Soybean leaves appeared to have greater cyclic electron flow (CEF) around PSI than cotton leaves and drought further increased CEF in soybean leaves. In contrast, CEF slightly decreased in cotton under drought. These results suggest that the difference in leaf movement between cotton and soybean leaves gives rise to different strategies to perform photosynthesis and to contrasting photoprotective mechanisms for utilisation or dissipation of excess light energy. We suggest that soybean preferentially uses light-regulated non-photochemical energy dissipation, which may have been enhanced by the higher CEF in drought-stressed leaves. In contrast, cotton appears to rely on enhanced electron transport flux for light energy utilisation under drought, for example, in enhanced nitrogen assimilation.

Additional keywords: diaheliotropic, paraheliotropic, photoprotection, water deficit.

Introduction

Cotton (*Gossypium hirsutum* L.) and soybean (*Glycine max* L.) track sunlight continually throughout the day. Cotton exhibits diaheliotropic leaf movement (turning perpendicular to the light), maintaining leaf diaheliotropic movement up to the wilting point of the plant. Soybean exhibits both diaheliotropic and paraheliotropic leaf movements, exhibiting increasing paraheliotropic movement (intercepting less light) as leaf water potential declines (Ehleringer and Forseth 1980; Ehleringer and Hammond 1987; Kao and Tsai 1998; Pastenes *et al*. 2004; Pastenes *et al*. 2005). This results in quite different incident solar radiation levels and microclimatic conditions for soybean and cotton, especially during drought (Isoda and Wang 2002). Similar phenomena were also reported on the desert winter annuals, Arizona lupine (*Lupinus arizonicus* S.Watson) and Desert Fivespot (*Malvastrum rotundifolium* A.Gray) (Forseth and Ehleringer 1982*b*).

The capacity to utilise the light energy absorbed by the photosynthetic apparatus typically decreases in plants subjected to drought, resulting in absorption of light energy in greater excess of that needed for carbon assimilation. Excessive light energy absorption is likely to induce photoinhibition of photosynthesis and to cause damage from an over-energisation of the photosynthetic apparatus (e.g. Oguchi *et al*. 2011). However, plants have evolved various photoprotection mechanisms to protect against photodamage (Long *et al*. 1994). Cotton leaves have a strong capacity, for example, via photorespiration and thermal emission, to dissipate excessive light energy that can otherwise damage the photosynthetic apparatus (Perry *et al*. 1983; Björkman and Demmig-Adams 1994; Kornyeyev *et al*. 2005). Furthermore, a higher electron transport capacity occurs in cotton leaves developed under long-term drought (Kitao and Lei 2007; Massacci *et al*. 2007). In contrast, soybean adapts to drought by dissipating the excess excitation energy thermally with the downregulation of PSII activity to protect its photosynthetic apparatus from the photodamage. This photoprotective mechanism may be assisted by paraheliotropic leaf movement, which reduces incident light on the leaf (Kao and Forseth 1992; Bielenberg *et al*. 2003; Inamullah and Isoda 2005*a*, 2005*b*; Arena *et al*. 2008). Therefore, as a consequence of this difference leaf orientational movement, the two crops should exhibit different acclimation strategies in response to drought in terms of

tranpiration and photoprotection (Inamullah and Isoda 2005*a*, 2005*b*). Sailaja and Ramadas (1996) proposed that the mechanisms of protection are different in diaheliotropic and parahelitropic plants for improving photosynthetic performance. Previous work on *L. arizonicus* and *M. rotundifolium* (the former has diaheliotropic leaf movement, whereas the latter has diaheliotropic and parahelitropic leaf movement) has shown that the two species exhibits different photosynthetic acclimation response to growth temperature and drought (Forseth and Ehleringer 1982*a*, 1983). Therefore, it seems likely that photosynthetic activity of cotton and soybean differing in leaf movement would also respond differently to drought due to differences in light interception by the leaves.

Our study was undertaken to test this hypothesis by comparing the photosynthetic characteristics of soybean and cotton. Three main facets were investigated in this study, namely, the differences between cotton and soybean under drought in terms of (1) the ability of the photosynthetic apparatus to utilise light energy, (2) the partitioning of absorbed light energy and (3) the distribution of photosynthetic electron flow between linear and cyclic transport.

Materials and methods

The experiment was conducted in an experimental field of Shihezi Agricultural College, Shihezi University, Shihezi, Xinjiang, China (45°19′N, 86°03′E) in 2008. Cotton (*Gossypium hirsutum* L. cv. Xinluzao 13) and soybean (*Glycine max* L. cv. Xindadou 1) seeds were sown on 24 April and plants were grown under field conditions with under-mulch drip irrigation. Figure 1 shows the meteorological conditions in terms of maximum and minimum temperature and precipitation during the crop growing season. The experimental design was a split-plot in randomised complete blocks (three replications) with the two
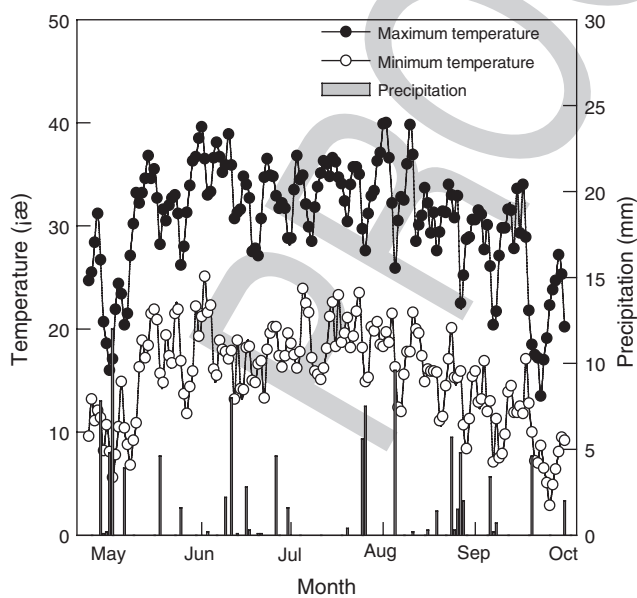

Fig. 1. Maximum and minimum temperature and percipitation at the study area.

water regimes as the main plot and two crops as the subplot and the subplot area was 60 m^2. There were two levels of irrigating water, namely, well-watered treatment and drought-stressed treatment. The well-watered plots were irrigated according to standard local practice whereas the drought-stressed plots were irrigated to the extent of 20% of the well-watered plots after sowing. Weeds and pests were controlled in the field using standard management practices.

Measurements were conducted at the seed development stage in soybean and at the boll formation stage in cotton. For the measurements, plants were selected at random and from each selected plant, the terminal leaflet of the main stem in soybean and the topmost fully expanded leaf on the main stem in cotton were chosen.

Leaf water potential

Leaf water potential was measured with a pressure chamber (SKPM 1400; Skye Instruments, Llandrindod Wells, UK) at predawn. The leaf lamina was enclosed in the chamber and subjected to increasing pressure from a compressed nitrogen cylinder until free sap was visible at the petiole outside the chamber.

Dual measurements of PSII and PSI photochemical efficiency

Simultaneous measurements of PSII and PSI photochemical efficiency were made using a saturation-pulse Dual-PAM-100 fluorometer (Walz, Effeltrich, Germany). Prior to measurement, leaves were dark-adapted sufficiently (for ~1 h). F_o, F_m and P_m were recorded. Each leaf was illuminated with an actinic light of 1033 µmol m^{-2} s^{-1} for 4–5 min. F_s, F_m' and P_m' were then measured, the latter two with a saturating light pulse. The intensity and the width of saturating pulse were 10 000 µmol m^{-2} s^{-1} and 600 ms, respectively. Then, rapid light curves were obtained with the Dual-PAM 100 using an internal program and PAR supplied by red light-emitting diodes. Nine discrete PAR steps were used (20 s each): 11, 42, 131, 344, 536, 830, 1292, 1599 and 1957 µmol m^{-2} 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'.

PSII quantum yield (Φ_{PSII}) in the light was calculated as $(F_m' - F_s)/F_m'$ (Genty *et al.* 1989). PSI quantum yield, Y(I), of photochemical energy conversion was calculated as $1 - Y(ND) - Y(NA)$. Y(ND) and Y(NA) were directly determined by the saturation pulse method. $Y(ND) = 1 - P700red.$ and $Y(NA) = (P_m - P_m')/P_m$ (Schreiber and Klughammer 2008). ETR (II) and ETR (I) was calculated according to the formula ETR (II) $= \Phi_{PSII} \times PAR \times 0.5 \times 0.84$ and ETR (I) $= Y(I) \times PAR \times 0.5 \times 0.84$, where PAR is the photosynthetically active radiation, 0.5 is a correction factor based on the assumption that the incident photons are absorbed equally by the two photosystems, and 0.84 is the proportion of incident photons absorbed by the leaf (Schreiber *et al.* 1994; Schreiber and Klughammer 2008). Photochemical quenching coefficient (qP) was calculated as $(F_m' - F_s)/(F_m' - F_o')$ (Krause and Weis 1991). Photochemical quantum yield of open PSII centers in the light-adapted state (F_v'/F_m') was calculated as $(F_m' - F_o')/F_m'$ (Schreiber *et al.* 1994). 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 fractions of light absorbed by the PSII antennae that is lost by constitutive thermal dissipation and via fluorescence ($\Phi_{f,D}$) and the fraction of light absorbed by the PSII antennae that is dissipated thermally via ΔpH- and xanthophylls-regulated processes (Φ_{NPQ}) were calculated as F_s/F_m and $(F_s/F_m') - (F_s/F_m)$, respectively (Hendrickson *et al.* 2004).

Gas exchanges and leaf nitrogen content

Leaf CO_2 assimilation rates were determined with a Li-6400 IRGA (Model LI-6400, Li-Cor Inc., Lincoln, NE, USA). Leaf CO_2 assimilation rate was measured after equilibration (obtain the steady-state of photosynthesis rate) at each PAR in a sequence of 2000, 1800, 1500, 1200, 800, 500, 300, 200, 100, 50, 30 and 0 μmol photons m^{-2} s^{-1} at leaf temperature ~33°C. Illumination was provided by a red/blue LED source (LI 6400–02B, Li-Cor Inc.).

CO_2 assimilation rate was measured at two O_2 concentrations (21% O_2 + ~360 μmol CO_2 mol^{-1}, and 2% O_2 + ~360 μmol CO_2 mol^{-1}) under 1600 μmol photons m^{-2} s^{-1} to calculate photorespiration. Photorespiration rate was estimated as CO_2 assimilation rate at low O_2 concentrations minus CO_2 assimilation rate at normal O_2 concentrations.

The Kjeldahl method was used to determine leaf nitrogen content (Schuman *et al.* 1972).

Data analyses

Significant effects due to water treatment, crop and the interaction terms were tested using two-way ANOVA using SPSS 16.0 for Windows (Chicago, IL, USA).

Results

Light response curves, photorespiration and derived parameters

Figure 2 shows typical photosynthesis–PAR response curves of both soybean and cotton. Cotton had higher CO_2 assimilation
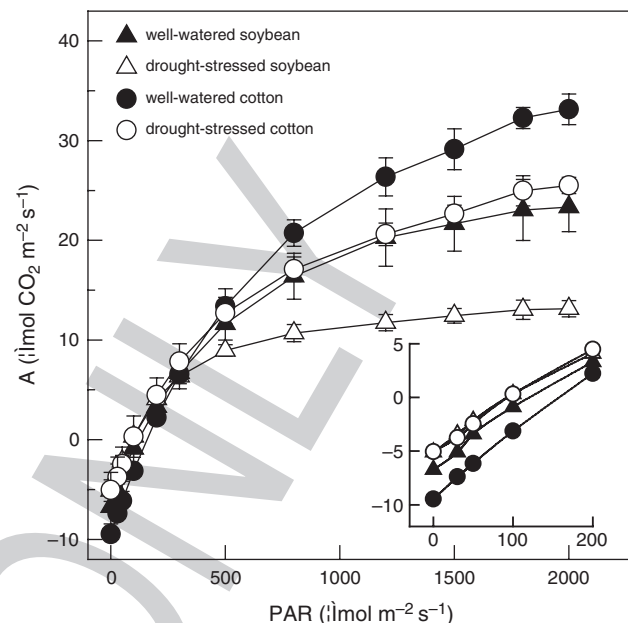

Fig. 2. Light response curves of well-watered soybean, drought-stressed soybean, well-watered cotton and drought-stressed cotton. Values are means ± s.e. ($n = 3$).

rate than soybean. CO_2 assimilation rates in both species decreased significantly in drought-stressed treatments, soybean showing a larger decrease than cotton. Light saturation point (LSP), light compensation point (LCP), maximum CO_2 assimilation rate (A_{max}), apparent quantum efficiency (AQE) and dark respiration (R_d) derived from light response curves are presented in Table 1. LSP was significantly higher ($P < 0.01$) in cotton plants than in soybean plants under control conditions, and was reduced by 43 and 13% in drought-stressed soybean and cotton plants, respectively. Both LCP and R_d were decreased by drought-stress treatment in both species, but especially in cotton. Neither LCP nor R_d were significantly

Table 1. Predawn water potential, light saturation point (LSP), light compensation point (LCP) and maximum CO_2 assimilation rate (A_{max}) derived from light response curves, area-based leaf nitrogen (N_{area}), dark respiration (R_d), photorespiration (PR), water use efficiency (WUE) and photosynthetic nitrogen utilisation efficiency (PNUE) in leaves of well-watered or drought-stressed soybean, well-watered cotton or drought-stressed cotton

WUE and PNUE were calculated as the ratio of CO_2 assimillation rate to transpiration rate and the ratio of A_{max} to N_{area}, respectively. Significance level of main effects analysed by ANOVA for species (Cv), water stress treatment (W), and their interaction term are indicated: NS, $P > 0.05$; *, $P \leq 0.05$; **, $P < 0.01$

	Soybean		Cotton		ANOVA		
	Well-watered	Drought-stressed	Well-watered	Drought-stressed	Cv	W	Cv × W
Water potential (MPa)	−1.0 ± 0.1	−1.9 ± 0.1	−1.0 ± 0.1	−2.2 ± 0.1	NS	**	NS
N_{area} (g m^{-2})	2.38 ± 0.06	1.98 ± 0.06	2.58 ± 0.13	3.35 ± 0.17	**	**	**
A_{max} (μmol m^{-2} s^{-1})	29.9 ± 3.0	17.6 ± 1.8	43.1 ± 1.3	29.8 ± 0.9	**	**	**
LSP (μmol m^{-2} s^{-1})	1736 ± 50	984 ± 38	2304 ± 88	1996 ± 118	**	*	**
LCP (μmol m^{-2} s^{-1})	127 ± 28	92 ± 13	161 ± 13	98 ± 38	NS	*	NS
AQE (μmol mol^{-1})	0.053 ± 0.007	0.055 ± 0.006	0.059 ± 0.005	0.046 ± 0.001	NS	NS	*
R_d (μmol m^{-2} s^{-1})	−6.72 ± 1.72	−5.10 ± 1.08	−8.79 ± 0.28	−4.50 ± 1.78	NS	*	NS
PR (μmol m^{-2} s^{-1})	10.3 ± 0.5	2.4 ± 0.1	12.7 ± 0.7	7.2 ± 0.6	**	**	**
WUE (μmol mmol^{-1})	1.99 ± 0.29	3.95 ± 0.8	2.49 ± 0.01	2.68 ± 0.33	NS	NS	*
PNUE (μmol g^{-1} s^{-1})	12.6 ± 1.27	8.88 ± 0.93	16.69 ± 0.49	8.89 ± 0.26	**	**	**

different, either between species or species × treatment interactions. Drought had a significant effect on A_{max} in both plants, reducing A_{max} by 41 and 31% compared with well-watered soybean and cotton plants, respectively. A_{max} was significantly different between the two plant species ($P < 0.01$) and between treatments ($P < 0.01$), showing a significant species × treatment interaction ($P < 0.01$). AQE was not significantly different either between species or treatments, but species × treatment interaction was significantly different ($P < 0.05$). A significantly ($P < 0.01$) lower photorespiration rate was found in drought-stressed plants than in well-watered plants; cotton plants had higher photorespiration than soybean in both treatments. Drought significantly increased water use efficiency (WUE) in soybean leaves. WUE was no different between the two plant species or between treatments, but showed a significant species × treatment interaction ($P < 0.05$).

Leaf water potential, area-based nitrogen and photosynthetic nitrogen utilisation efficiency

As shown in Table 1, predawn leaf water potential revealed a significant decrease ($P < 0.01$) from well-watered to drought-stressed conditions in both soybean and cotton plants. Cotton had higher area-based leaf nitrogen (N_{area}) and photosynthetic nitrogen utilisation efficiency (PNUE) than soybean. N_{area} of soybean decreased in drought-stressed plants whereas that of cotton increased in drought-stressed plants. PNUE in both plants decreased significantly in drought-stressed treatments, cotton showing a larger decrease than soybean. N_{area} and PNUE were significantly different between cotton and soybean plants ($P < 0.01$) and between water treatments ($P < 0.01$), and showed a significant species × treatment interaction ($P < 0.01$).

Photochemistry

The partitioning of absorbed light energy in cotton and soybean leaves was evaluated in response to drought (Fig. 3). In soybean and cotton leaves, Φ_{PSII} decreased with increase in PAR, Φ_{PSII} being higher in cotton than in soybean. Drought decreased Φ_{PSII} in soybean leaves (Fig. 3*a, b*), but increased Φ_{PSII} in cotton leaves (Fig. 3*c, d*). Φ_{NPQ} increased with PAR, and was higher in soybean than in cotton. Drought induced an increase in Φ_{NPQ} in soybean leaves (Fig. 3*a, b*), but a reduction in Φ_{NPQ} in cotton leaves (Fig. 3*c, d*). $\Phi_{f,D}$ increased marginally at intermediate light in both plants, and drought induced lower $\Phi_{f,D}$ in soybean leaves, whereas an increase was observed in $\Phi_{f,D}$ in cotton leaves.

The rate of electron transport through PSII, namely ETR (II), increased with PAR (Fig. 4). ETR (II) was greater in cotton than in soybean. Further, drought increased ETR (II) in cotton, but decreased it in soybean. Since ETR (II) is calculated from the product of Φ_{PSII} and PAR, where $\Phi_{PSII} = qP \times F_v'/F_m'$, we plotted each component of Φ_{PSII} in Fig. 5. Most notable features of these plots are a decrease of qP in soybean (Fig. 5*a*) and an increase of F_v'/F_m' in cotton (Fig. 5*b*) due to drought.

The ETR (I)/ETR (II) ratio exceeded 1, suggesting that CEF around PSI was functioning (Miyake *et al*. 2005). As shown in Fig. 6, soybean leaves had higher CEF than cotton leaves, and drought induced an increase in CEF in soybean leaves, but not in cotton.

Discussion

The difference between cotton and soybean leaves in leaf movement determines a different interception and utilisation of light energy under drought

In this study, soil drought reduced leaf water potential of cotton and soybean (Table 1). As previously reviewed by Chaves (1991), drought reduces leaf CO_2 assimilation rate. Expectedly, drought-stressed cotton and soybean plants both had lower maximum CO_2 assimilation rates than well-watered plants (Table 1); the extent of decrease for soybean was greater than that for cotton, indicating a greater loss of carbon assimilation capacity. According to the concept of excess energy (for details see Björkman and Demmig-Adams 1994; Oguchi *et al*. 2011), and given the similar initial slope (AQE) of the light response curve (Table 1; Fig. 2), for a given PAR, greater excess of excitation energy would be expected in the drought-stressed plants, especially in drought-stressed soybean. LCP in cotton and soybean decreased under drought to comparable extents. The lower LCP of both plants could be simply due to a lower dark respiration rate under drought (Table 1).

We noted that drought reduced LSP of both crop plants – more so in soybean than in cotton – which is in accordance with the observed differential diminution of light interception associated with leaf movement. Soybean exhibited paraheliotropic movement whereas cotton maintained diaheliotropic movement under drought, indicating that soybean leaves intercepted less sunlight than cotton leaves. Therefore, under drought, the photosynthetic apparatus of cotton leaves could utilise more light energy than those of soybean leaves. Thus, even though soybean had a lower capacity to utilise light energy, the excess light energy of soybean may be no higher than that of cotton in the field. As a result, leaf paraheliotropic movement in concert with photosynthetic characteristics can reduce the risk of photoinhibition under drought (Kao and Tsai 1998; Pastenes *et al*. 2004, 2005). Furthermore, Kao and Forseth (1991, 1992) have documented that regulating incident light on leaves to levels near the photosaturation level through paraheliotropic movement has the benefit of enhancing resources (e.g. water and nitrogen) use efficiency and photosynthetic carbon gain. Indeed, drought-stressed soybean had higher WUE than drought-stressed cotton and well-watered plants of both crops. Though drought reduced the PNUE of both crop plants (by 29% for soybean and 47% for cotton, Table 1), a smaller drop of the PNUE of soybean by drought was observed, indicating that soybean had the advantage of optimising nitrogen utilisation under drought.

Soybean preferentially uses regulated non-photochemical energy dissipation whereas cotton uses electron transport flux for light energy dissipation under drought

There are three main pathways of allocation of photons absorbed by the PSII antennae: photochemical conversion; light-regulated non-photochemical energy dissipation; and light-independent constitutive non-photochemical energy dissipation (Hendrickson *et al*. 2004). As shown in Fig. 3, the quantum yield of PSII was decreased and the regulated non-photochemical energy dissipation increased in soybean under drought. This means that under drought, in moderate or high light, regulated non-photochemical energy dissipation was the main
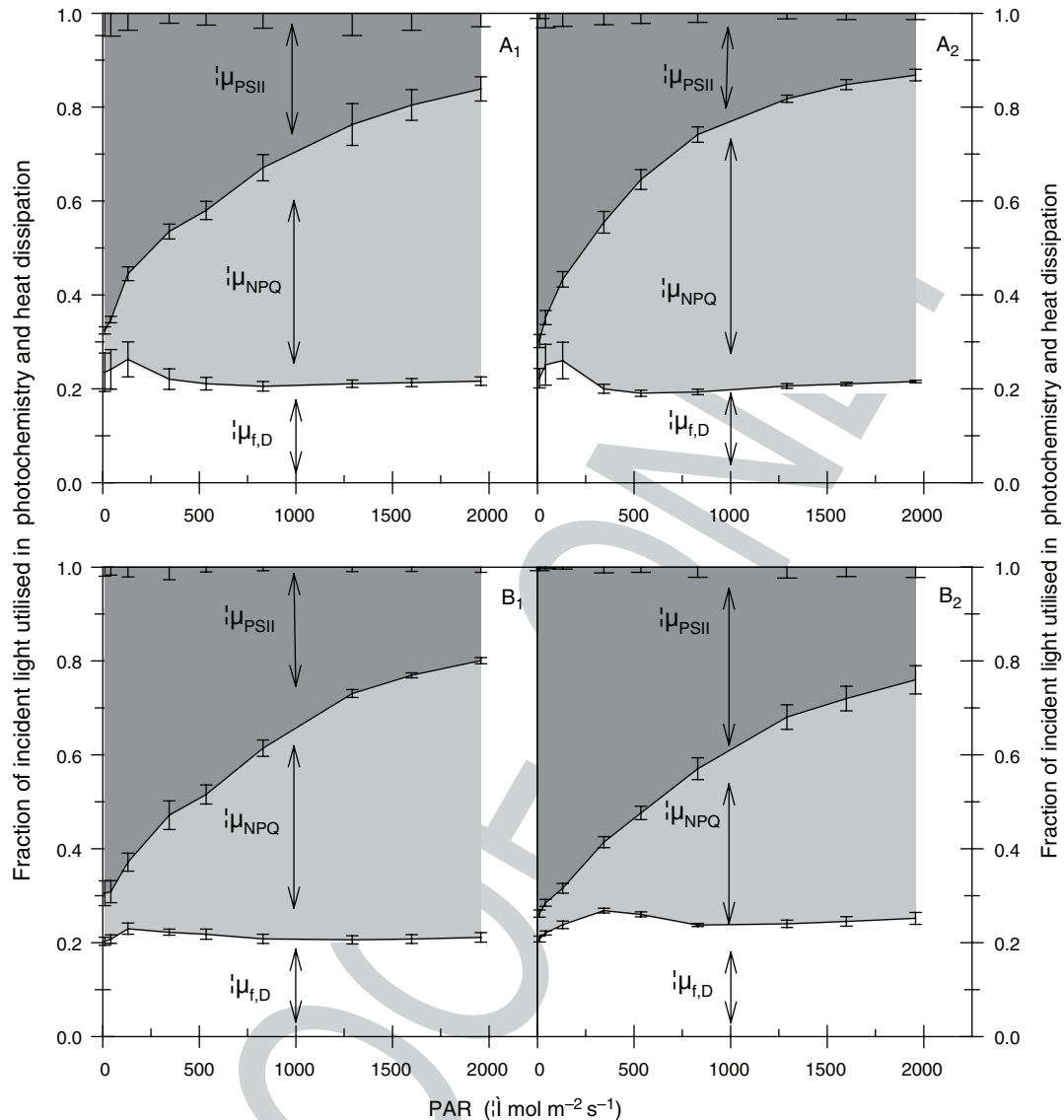
Fig. 3. Estimated fraction of absorbed PAR consumed via PSII photochemistry (Φ_{PSII}), ΔpH- and xanthophyll-regulated thermal dissipation (Φ_{NPQ}), and the sum of fluorescence and light-independent constitutive thermal dissipation ($\Phi_{f,D}$), in (*a*) leaves from well-watered soybean, (*b*) drought-stressed soybean, (*c*) well-watered cotton and (*d*) drought-stressed cotton illuminated at varying PAR. Values are means ± s.e. (*n* = 4).

pathway for light energy dissipation to provide photoprotection in soybean leaves. A similar result was reported by Inamullah and Isoda (2005*b*) in soybean. By contrast, the quantum yield of PSII was increased and the regulated non-photochemical energy dissipation was decreased in cotton under drought. Genty *et al.* (1987) have reported that PSII photochemistry of pot-grown cotton leaves is unaffected by drought, whereas Inamullah and Isoda (2005*b*) and Ennahli and Earl (2005) have shown that the PSII quantum yield of photochemistry in pot-grown cotton leaves are sometimes significantly affected and sometimes not, depending on the intensity of drought. However, in the field experiment, a higher efficiency of PSII photochemistry in cotton leaves under drought has been reported (Kitao and Lei 2007; Massacci *et al.* 2007). These results were consistent with our data.

Compared with pot-grown plants, field-grown plants generally suffer from multiple constraints because under field conditions; drought is often accompanied by other stresses such as heat stress and strong light (Havaux 1992). Furthermore, there was antagonism/synergism between heat, light and water deficit (Havaux 1992; Lu and Zhang 1999). In contrast, pot-grown plants, which generally suffer from 'short-term drought', do not include the phenomenon of acclimation which is usually well developed in field-grown plants (Panković *et al.* 1999). Therefore, the behaviour of pot-grown plants might be quite different from responses of plants in the field, and the capacity of field-grown plants to acclimate to drought can be greater than that of plants grown in pots (Plaut and Federman 1991; Havaux 1992). Thus, we propose that in the field, photochemical conversion is
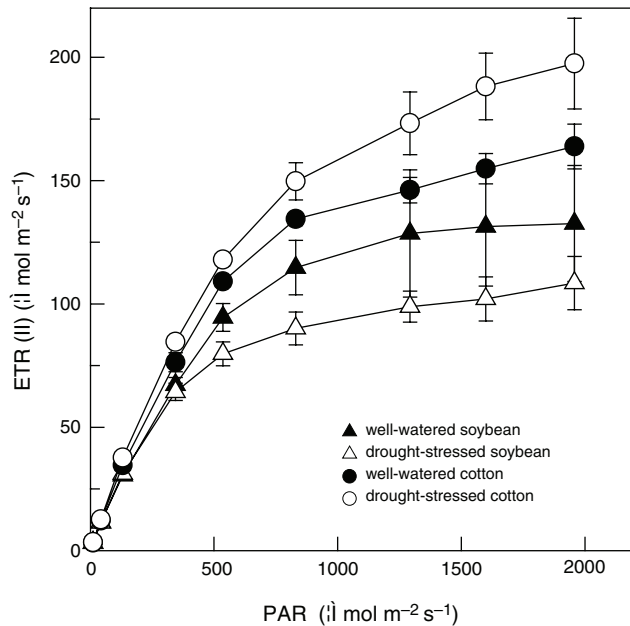
Fig. 4. Rapid light response curves of well-watered soybean, drought-stressed soybean, well-watered cotton and drought-stressed cotton. Values are means ± s.e. ($n = 4$).

the main pathway for light energy dissipation to provide photoprotection in drought-stressed cotton leaves.

Using various indicators of non-photochemical energy dissipation, either no change (Genty *et al*. 1987; Inamullah and Isoda 2005*b*; Kitao and Lei 2007) or an enhancement (Massacci *et al*. 2007) was shown in cotton leaves under drought. In our study, regulated non-photochemical energy dissipation expressed as $\Phi_{NPQ} = (F_s/F_m') - (F_s/F_m)$ was decreased in drought-stressed cotton (Fig. 3*d*). Φ_{NPQ} represents the fraction of the light energy dissipated thermally via ΔpH- and xanthophylls-regulated processes, and is not identical with NPQ, the magnitude of which has no upper limit and sometimes over-evaluates its relative importance to photoprotection (Hendrickson *et al*. 2004). Taken together, our results demonstrate that the photosynthetic apparatus of cotton and soybean has different pathways to use or dissipate the energy absorbed by PSII antennae.

The difference in the distribution of photosynthetic electron transport flow between cotton and soybean under drought

Electron flow through PSII is consumed mainly by carbon assimilation, photorespiration and nitrogen assimilation (Badger 1985; Champigny 1995; Biehler and Fock 1996; Park *et al*. 1996). As shown in Figs 2, 4 and Table 1, carbon assimilation, photorespiration and electron transport rate of soybean leaves decreased in parallel under drought. By contrast, leaf electron transport rate in cotton responded differently to drought from soybean, increasing under drought conditions, despite the partial inhibition of CO_2 assimilation capacity. Higher electron transport rate in cotton under drought has been reported previously (Kitao and Lei 2007;
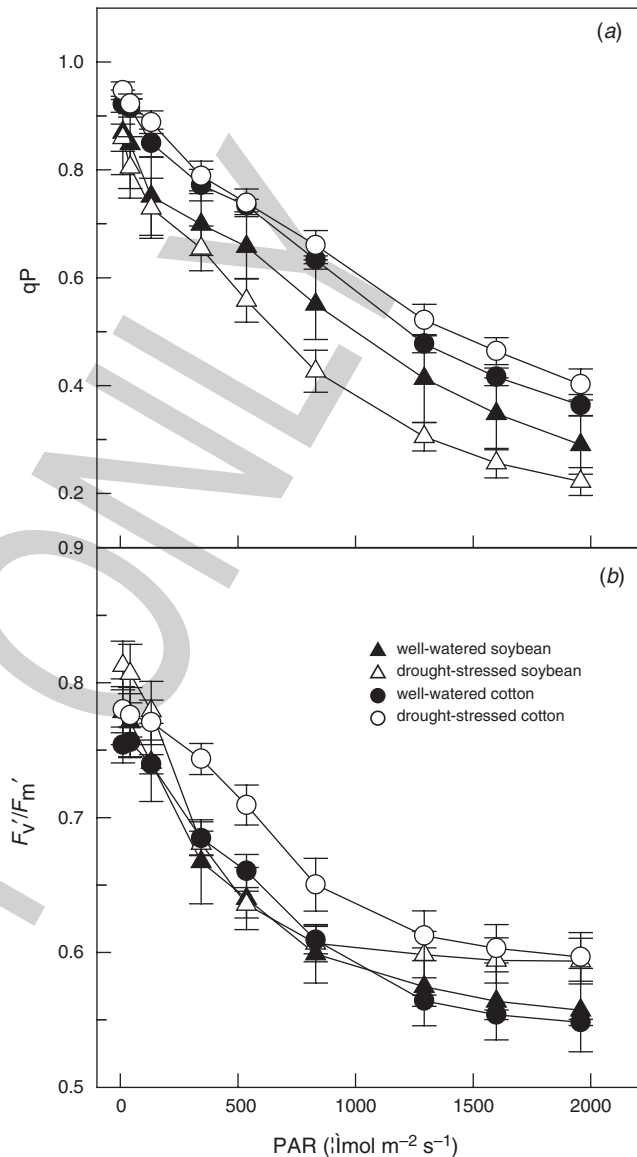

Fig. 5. Variation of qP (A) and F_v'/F_m' with PAR in well-watered or drought-stressed soybean and well-watered or drought-stressed cotton. Values are means ± s.e. ($n = 4$).

Massacci *et al*. 2007). As discussed by Massacci *et al*. (2007), it may be that photorespiration was enhanced by drought. In their experiment, however, photorespiration rate was estimated using combined measurements of gas exchange in the whole-leaf tissue and chlorophyll fluorescence. Since the measured chlorophyll fluorescence signal is predominantly emitted by chloroplasts nearer to the (adaxial) leaf surface, this method may not correctly represent the photorespiration rate in the whole-leaf tissue. In our study, we observed lower photorespiration as evaluated by the low oxygen (2%) method, which was meant to give a qualitative estimation of photorespiration (Table 1); it appears that electron flow through PSII consumed by photorespiration was not increased but, if anything, decreased under drought-stressed cotton leaves, as was A_{max}. If so, what
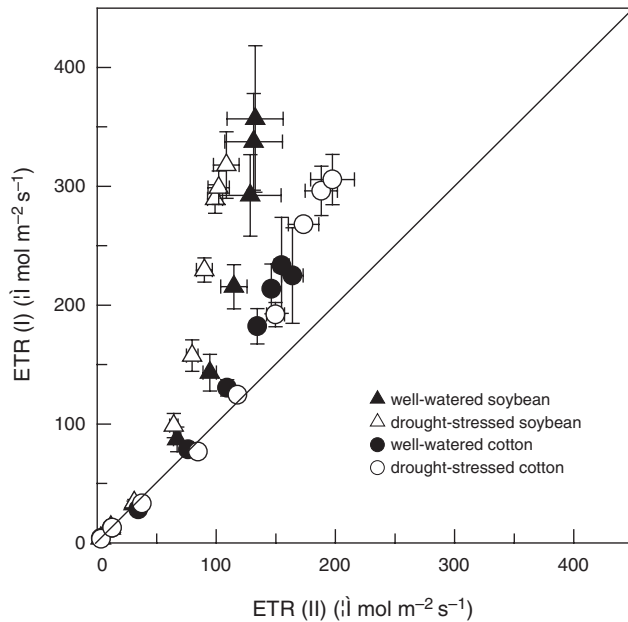
Fig. 6. ETR (II) and ETR (I) were determined simultaneously at varying PAR in leaves from well-watered soybean, drought-stressed soybean, well-watered cotton and drought-stressed cotton. Values are means $\pm$ s.e. ($n = 4$).

caused the increase in ETR (II) in drought-stressed cotton? We propose that electron flow through PSII is consumed mainly by nitrogen assimilation in drought-stressed cotton leaves. Nitrogen metabolism provides another sink for the energy harvested in photosynthesis (Larsson *et al*. 1982). For example, Kitao and Lei (2007) have shown that drought-stressed cotton leaves can maintain higher electron transport due to higher N_{area} even while carbon assimilation was reduced. In our study, higher N_{area} was indeed found in drought-stressed cotton leaves (Table 1). Nevertheless, lower PNUE was found as well. Feng *et al*. (2007, 2009) reported that PNUE was associated with not only A_{max} and N_{area} but also nitrogen allocation and partitioning among different photosynthetic component. As the nitrogen allocated to photosynthesis was decreased, PNUE would decrease. Thus, further studies conducted in nitrogen allocation under drought-stressed both plants are necessary to explore the relationship between PNUE and nitrogen allocation for clarifying the distribution of photosynthetic electron flow in photosynthetic apparatus. Although further research is needed to elucidate activities of the enzymes involved, when taken together, we propose that nitrogen assimilation may be one of the main pathways that consume electron flow through PSII in drought-stressed cotton leaves, thereby protecting the photosynthetic apparatus from photodamage.

Enhanced nitrite reduction in the chloroplasts of drought-stressed cotton leaves, for example, could divert reduced ferredoxin away from CEF with some predictable consequences. First, there should be no increase in ferredoxin-dependent CEF (as observed in drought-stressed cotton, see below). Second, the diminution of CEF would minimise the contribution of CEF to the trans-membrane pH gradient, thereby restricting Φ_{NPQ}, as observed (Fig. 3*d*). Third, due to the restricted Φ_{NPQ}, the photochemical efficiency of open PSII

traps (F_v'/F_m') should be increased, as observed (Fig. 5*b*). Fourth, the PQ pool should be slightly more oxidised when electrons are not fed into it from reduced ferredoxin, resulting in a more oxidised state of Q_A, or an increased qP, as observed (Fig. 5*a*).

Cyclic electron flow around PSI is also suggested to have an important role in photoprotection (Miyake *et al*. 2005). CEF may be involved in generating or maintaining a ΔpH that is necessary for downregulation of PSII by thermal dissipation of excess absorbed light energy (Heber and Walker 1992). The CEF-dependent photoprotection may occur via two mechanisms: one is linked to thermal energy dissipation and prevents the inhibition of the repair of photodamaged PSII at the step of protein synthesis; the other is independent of thermal energy dissipation and suppresses photodamage to PSII (Takahashi *et al*. 2009).

The ETR (I)/ETR (II) ratio in Fig. 6 was greater than 1 in both plants, more so in soybean than in cotton leaves. Since ETR (II), calculated from the product of Φ_{PSII} and PAR is based on a chlorophyll fluorescence measurement, it represents the linear electron transport rate of chloroplasts nearer to the adaxial leaf surface, where a given incident light is not yet markedly attenuated; therefore, qP (a measure of the oxidation state of the primary quinine acceptor in PSII) is lower than the average for the whole tissue. Consequently, since $\Phi_{PSII} = qP \times F_v'/F_m'$ is under-estimated, ETR (II) obtained from the product of Φ_{PSII} and PAR tends to be an under-estimate for the tissue as a whole. However, ETR (I) is based on a P700 measurement using a measuring beam (820 nm) that readily penetrates and undergoes multiple scattering within the whole leaf tissue; it is a whole-tissue measurement (Losciale *et al*. 2008) of the total (linear + cyclic) electron flux through P700. Therefore, the ETR (I)/ETR (II) ratio is likely to be an overestimate of the ratio of (cyclic + linear) to linear electron flow. Even so, the observation that the ETR (I)/ETR (II) ratio was greater than unity in both plant species suggests that cyclic electron flow was occurring, particularly in soybean under drought. In soybean, the higher CEF in drought-stressed leaves may have partly caused the enhanced regulated non-photochemical energy dissipation (Φ_{NPQ}, Fig. 3*b*) to confer photoprotection. The enhanced feedback of electrons to the PQ pool in CEF in soybean under drought, with restricted sinks such as nitrite reduction for draining electrons, would result in a more reduced PQ pool, and a lower qP, as observed (Fig. 5*a*).

In summary, soybean preferentially uses light-regulated non-photochemical energy dissipation, which may have been enhanced by the higher CEF in drought-stressed leaves. In contrast, cotton appears to rely on enhanced electron transport flux for light energy utilisation under drought, for example, in enhanced nitrogen assimilation. Although the distributional mechanism of the electron transport chain under drought-stressed leaves of both crops requires further study, it can be concluded that soybean and cotton plants rely on contrasting photoprotective mechanisms to cope with drought.

Acknowledgements

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