

Leaf Wilting Movement Can Protect Water-Stressed Cotton (*Gossypium hirsutum* L.) Plants Against Photoinhibition of Photosynthesis and Maintain Carbon Assimilation in the Field

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Abstract Under severe water stress, leaf wilting is quite general in higher plants. This passive movement can reduce the energy load on a leaf. This paper reports an experimental test of the hypothesis that leaf wilting movement has a protective function that mitigates against photoinhibition of photosynthesis in the field. The experiments exposed cotton (*Gossypium hirsutum* L.) to two water regimes: water-stressed and well-watered. Leaf wilting movement occurred in water-stressed plants as the water potential decreased to -4.1 MPa, reducing light interception but maintaining comparable quantum yields of photosystem II (PS II; Yield for short) and the proportion of total PS II centers that were open (qP). Predrawn F_v/F_m (potential quantum yield of PS II) as an indicator of overnight recovery of PS II from photoinhibition was higher than or similar to that in well-watered plants. Compared with water-stressed cotton leaves for which wilting movement was permitted, water-stressed cotton leaves restrained from such movement had significantly increased leaf temperature and instantaneous CO_2 assimilation rates in the short term, but reduced Yield, qP, and F_v/F_m . In the long term, predrawn F_v/F_m and CO_2 assimilation capacity were reduced in water-stressed leaves

restrained from wilting movement. These results suggest that, under water stress, leaf wilting movement could reduce the incident light on leaves and their heat load, alleviate damage to the photosynthetic apparatus due to photoinhibition, and maintain considerable carbon assimilation capacity in the long term despite a partial loss of instantaneous carbon assimilation in the short term.

Keywords Carbon assimilation · Chlorophyll fluorescence · Leaf wilting movement · Photoprotection · Water stress

Abbreviations

A	CO_2 assimilation rate (in micromoles of CO_2 per square meter per second)
F_m	maximal chlorophyll fluorescence yield
F_o	ground chlorophyll fluorescence yield
F_v, F_v'	maximum variable fluorescence in the dark- and light-adapted state, respectively
F_v/F_m	potential quantum yield of photosystem II
F_m'	maximum light-adapted fluorescence
F_s	steady-state fluorescence yield during illumination
NPQ	nonphotochemical quenching
PAR	photosynthetically active radiation (in micromoles per square meter per second)
PS II	photosystem II
qP	photochemical quenching coefficient
Yield	PS II quantum yield in the light

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Introduction

Photoinhibition has an adverse impact on plant growth and productivity (Long et al. 1994). However, plants can

minimize photoinhibition and avoid photodamage through various morphological adjustments and physiological alterations on shorter or longer time scales (Long et al. 1994). One of the most efficient ways of avoiding excessive light absorption in leguminous species is the active paraheliotropic movement of leaves (Ludlow and Björkman 1984; Kao and Tsai 1998; Pastenes et al. 2005; Arena et al. 2008). However, a photoprotective mechanism of this type is not universal in higher plants.

Water stress often accompanies high light stress in the field. On one hand, water stress limits leaf transpiration, resulting in an increase in leaf temperature, thereby intensifying the photoinhibition of photosynthesis caused by strong irradiation (Powles 1984). On the other hand, water stress lowers the water potential of leaves and the osmotic potential of petiole cells—in extreme cases, the leaves wilt. This passive, drought-induced drooping of leaves may have multiple physiological significance for plants in that it may reduce both water loss from the leaf and the amount of light incident on the leaf surface, the latter lowering the energy load on a leaf (Begg 1980; Chiariello et al. 1987; Björkman and Demming-Adams 1994). Supposedly, the reduced energy load helps to alleviate photoinhibition and to accelerate net recovery. Although such drought-induced drooping of leaves is quite general in higher plants, very few studies, to our knowledge, have provided direct experimental evidence focused on its role in photoprotective mechanisms.

Cotton (*Gossypium hirsutum* L.) is an important fiber crop worldwide. Under favorable conditions, cotton turns its leaves towards sunlight to maximize light interception—the so-called leaf diaheliotropic movement—(Lang 1973; Ehleringer and Hammond 1987), indicating that cotton is a species acclimatized to high-intensity sunlight. However, under moderate drought, cotton leaves wilt like those of any higher plant, and the diaheliotropic leaf movement disappears. Thus, the present study sought to assess the role of leaf wilting movement in photoprotection in field-grown cotton subjected to water stress.

Materials and Methods

The experiment was conducted at an experimental field of Shihezi Agricultural College, Shihezi University, Xinjiang, China (45°19' N, 86°03' E) in 2008. The experimental design was a randomized complete block with three replications with two plots per block. Each plot in a block corresponded to a water regime: well-watered and water-stressed and was 30×3.5 m. Cotton (*G. hirsutum* L. cv. Xinluzao 13) was grown under field conditions with undermulch drip irrigation. Seeds were sown on 24 April 2008 in rows 12 cm apart and the plant density was 1.8×

10⁵ ha⁻¹. Two water regimes were followed, namely, well-watered and water-stressed. The well-watered plots were irrigated according to standard local practice, whereas the water-stressed plots were not irrigated after sowing in order to induce the leaves to wilt. As expected, leaves began to wilt in mid-June. Measurements were conducted three times in July and only 1-day data are present here due to similarity of the results. For the measurements, plants in each plot were selected at random and, from each selected plant, the topmost west-facing leaf on the main stem was chosen.

On the day of measurement, when leaves wilted, to eliminate the effect of passive leaf movement caused by wilting, some of them were brought to a horizontal position and retained in that position by sandwiching them between nylon strips in a metal frame, as described by Pastenes et al. (2005). (From now on, these are referred to as stress-restrained leaves. Leaves that were allowed passive wilting movement are referred to as stress-free leaves.) To protect stress-restrained leaves from wind, windbreaks were used around the experimental plot where stress-restrained leaves and stress-free leaves were located.

Environmental Conditions

Ambient photosynthetically active radiation (PAR) was measured with an external quantum sensor of a Li-6400 infrared gas exchange analyzer (IRGA; Li-Cor Inc., Lincoln, USA) by positioning the sensor perpendicular to the sunlight or horizontal to the ground. Air temperature and relative humidity were measured with a HOBO hygrometer (Onset, USA) 1 m above the ground.

Leaf Movement

Leaf movement was measured as a change in the midrib angle using an inclinometer and a protractor. The leaf position and orientation can be determined quantitatively by measuring three separate angles: azimuth angle, lamina angle, and midrib angle, which were described by Ceulemans and Isebrands (1996). Because azimuth angle and lamina angle remained more or less constant, only data on midrib angle are presented here.

Leaf Water Potential

Leaf water potential was measured with an SKPM 1400 pressure chamber (Skye, UK) four times in a day: at predawn (0600 hours), at midmorning when leaves in the water-stressed plants had wilted (about 1100 hours), at midday (1400 hours), and in late evening (2100 hours). 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. In each treatment, at least four leaves in each plot were chosen at random for measurement.

Chlorophyll Fluorescence

Chlorophyll fluorescence was measured in diurnal time courses using a portable saturation pulse fluorometer PAM-2100 equipped with a 2030-B leaf clip holder (Walz, Effeltrich, Germany). PAR incident on the leaf surface was measured with a microquantum sensor on the leaf clip. Leaf temperatures were recorded simultaneously with a Ni/NiCr thermocouple of the leaf clip holder appressed to the underside (abaxial surface) of the leaves. For each treatment, nine to ten individual leaves in each plot were sampled and labeled. Care was taken not to disturb the position of the leaf being measured and its exposure to light. Three measurements were made at different points on each leaf.

Maximal (F_m) and ground (F_o) fluorescence yields of dark-adapted leaves were measured at predawn. F_o was obtained with a measuring light of $0.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ at a frequency of 0.6 kHz, while the F_m was measured with a 0.8-s saturating pulse at $>8,000 \mu\text{mol m}^{-2} \text{s}^{-1}$. Potential quantum yield of photosystem II (PS II; F_v/F_m) was calculated, where F_v is the maximum variable fluorescence ($F_v = F_m - F_o$). During the diurnal time courses, F_s (fluorescence of the light-adapted leaf) and F_m' (the maximum light-adapted fluorescence) were measured. F_s was obtained by setting the measuring light to a frequency of 20 kHz; F_m' was measured with a 0.8-s saturating pulse at $>8,000 \mu\text{mol m}^{-2} \text{s}^{-1}$. PS II quantum yield in the light (Yield) was calculated as $(F_m' - F_s)/F_m'$ (Genty et al. 1989). Photochemical quenching coefficient (qP) was calculated as $(F_m' - F_s)/(F_m' - F_o')$ (Krause and Weis 1991). Minimal fluorescence under light exposure (F_o') was calculated according to Oxborough and Baker (1997) using the equation $F_o' = F_o/(F_v/F_m + F_o/F_m')$. Nonphotochemical quenching (NPQ) was calculated as $(F_m - F_m')/F_m'$, where F_m is the value of the predawn measurements (Bilger and Björkman 1990).

The maximum quantum yield after 30 min dark adaptation using leaf clips, measured as F_v/F_m , was determined by means of a nonmodulated fluorimeter PEA (Hansatech, UK); the leaves were the same as those used for the measurements during the day. For each treatment, at least five leaves in each plot were chosen for measurement. Different leaves were used for the fluorescence quenching parameters and gas exchange parameters in that dark adaptation affect the leaf light exposure status.

After keeping the wilted leaves horizontal for 4 days, predawn F_v/F_m of leaves was determined using PAM 2100 in the case of both stress-free leaves and stress-restrained

leaves (those that had been subjected to stress but were maintained in the horizontal position by providing adequate support).

CO₂ Assimilation Rate

Leaf CO₂ assimilation rate was measured in diurnal time courses using a Li-6400 IRGA. Measurements were done on the same previously labeled leaves used for the fluorescence quenching in the same day, taking care not to disturb the leaf position. Red–blue light (Li-Cor Inc., Lincoln, USA) was used for tracking the PAR incident on leaves measured by 2010-B leaf holder of PAM 2100. Although the effect of light spectrum on photosynthesis needs to be considered, particularly the lack of far-red light present in artificial light, red–blue artificial light sources have been widely used to conduct photosynthesis research.

After keeping the wilted leaves horizontal for 4 days, steady-state leaf CO₂ assimilation rates and C_i/C_a of both stress-restrained leaves and stress-free leaves were determined under natural conditions with PAR of $1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ and a leaf temperature of 31–33°C.

Date Analyses

Statistical analysis of the data was performed by one-way analysis of variance according to Duncan's multiple range test, based on a significance level of $P < 0.05$.

Results

Climate

As shown in Fig. 1a, at 0800 hours, PAR measured with the sensor perpendicular to the sun's rays reached $900 \mu\text{mol m}^{-2} \text{s}^{-1}$, but was only $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ when the sensor was horizontal. Both increased rapidly, reaching 2,020 and $1,880 \mu\text{mol m}^{-2} \text{s}^{-1}$ at midday, respectively, and decreased in the afternoon. Air temperature rose from 19°C early in the morning to 27.5°C at midday. At 1800 hours, the air temperature reached the highest value 34°C and then fell gradually in the afternoon (Fig. 1b). As expected, relative humidity fell throughout the day as air temperature increased (Fig. 1c).

Leaf Water Potential, Leaf Movement, Light Interception, and Leaf Temperature

Water-stressed plants had a significantly lower predawn water potential than well-watered plants (−3.4 and −0.9 MPa respectively, Fig. 2). During the day, water-stressed plants had consistently lower water potential than

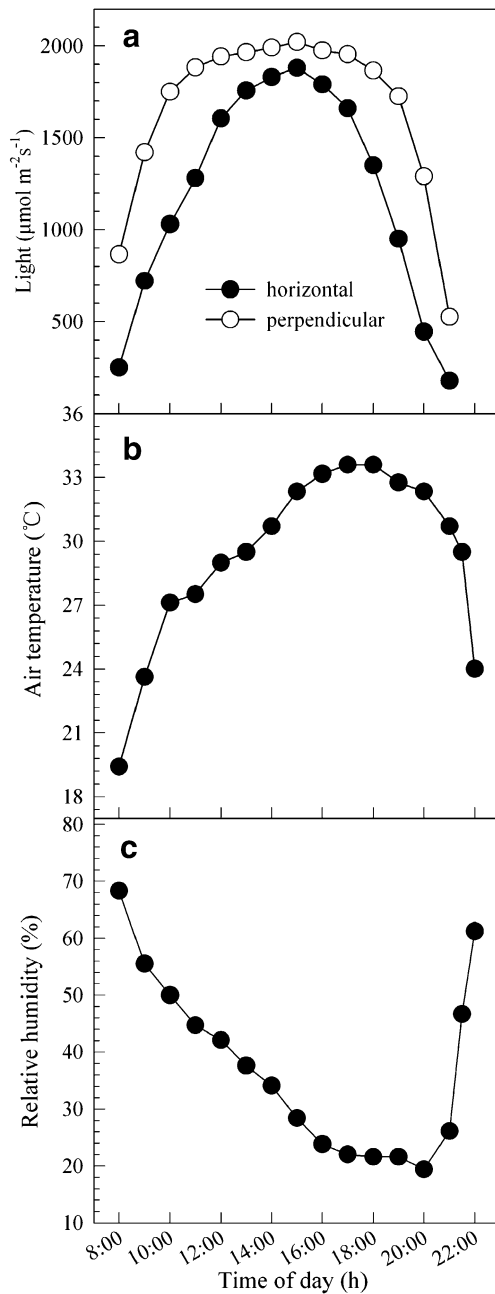


Fig. 1 Diurnal time course of **a** PAR on a surface horizontal to the ground or perpendicular to sunlight, **b** air temperature, and **c** relative humidity

well-watered plants. At about 1100 hours, when leaves had wilted in water-stressed plants (Fig. 2), water potential was -4.1 MPa; no difference was observed on water potential between stress-free and stress-restrained leaves.

As shown in Fig. 3, diheliotropic movement of leaves was observed in the field-grown cotton, which maximized the incident light. Leaves from the water-stressed plants, however, only exhibited diheliotropic movement in the morning, after which they drooped considerably. As expected, with the increase in sunlight and air temperature,

leaf water potential declined further and leaves from the water-stressed plants wilted at about 1100 hours.

Associated with leaf wilting, interception of light by a water-stressed leaf also decreased, as shown by the PAR incident on a leaf (Fig. 4a). Leaf temperature was higher in water-stressed leaves than in well-watered leaves, but wilting without restrained leaf movement ameliorated the temperature rise (Fig. 4b).

Chlorophyll Fluorescence

As shown in Fig. 5, predawn F_v/F_m values were above 0.8 in water-stressed and well-watered plants. During the day, water-stressed (but unrestrained) leaves had higher or similar F_v/F_m values after 30 min dark adaptation compared to those from well-watered plants. However, stress-restrained leaves had significantly lower F_v/F_m compared to stress-free leaves. By late evening, the F_v/F_m values had recovered to above 0.8 in leaves from all the treatments. Even so, compared to stress-free leaves, the stress-restrained leaves had significantly lower values of F_v/F_m .

The diurnal time courses of Yield of PS II photochemistry differed markedly between treatments, which showed the usual inverse relation to PAR (Figs. 4a and 6a). From 0800 to 1000 hours, no significant differences were observed between well-watered and water-stressed plants. When leaves from water-stressed plants wilted (about 1100 hours), the stress-free leaves recorded significantly higher Yield than well-watered leaves did. However, stress-restrained leaves had significantly lower Yield. From midday to afternoon, the stress-free leaves had a consistently higher Yield than that recorded in well-watered plants

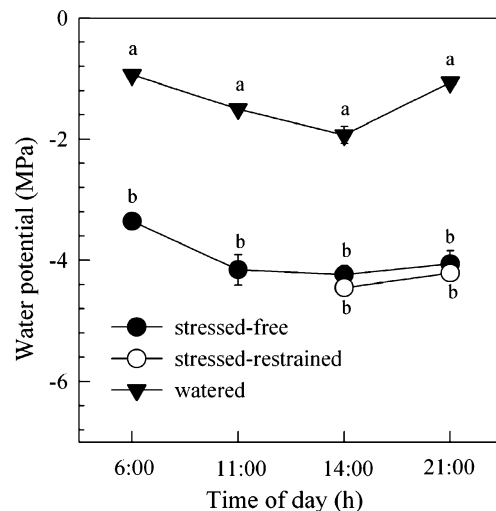


Fig. 2 Diurnal time course of leaf water potential in well-watered leaves, stress-free leaves, and stress-restrained leaves. Different letters indicate differences between treatments at the same time of day at $P < 0.05$ according to Duncan's multiple range test. Bars represent the standard error (SE)

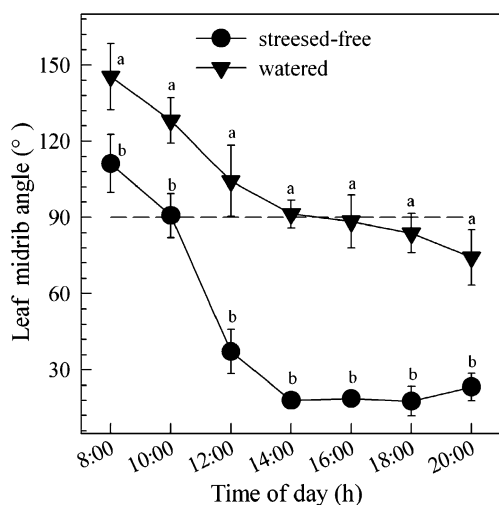


Fig. 3 Diurnal time course of leaf midrib angle in well-watered leaves, and stress-free leaves. Dashed line represents horizontal leaves. Different letters indicate differences between treatments at the same time of day at $P < 0.05$ according to Duncan's multiple range test. Bars represent the SE

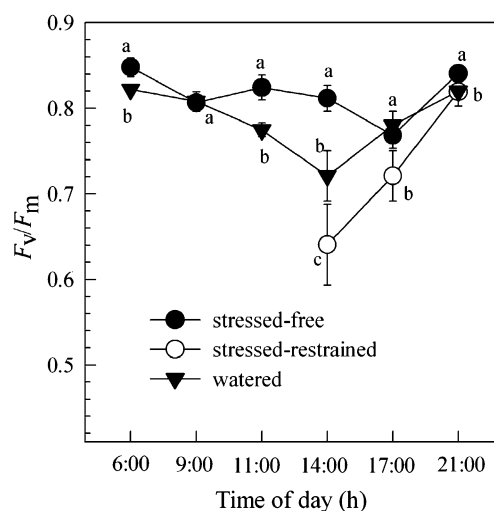


Fig. 5 Diurnal time course of F_v/F_m in well-watered leaves, stress-free leaves, and stress-restrained leaves. Different letters indicate differences between treatments at the same time of day at $P < 0.05$ according to Duncan's multiple range test. Bars represent the SE

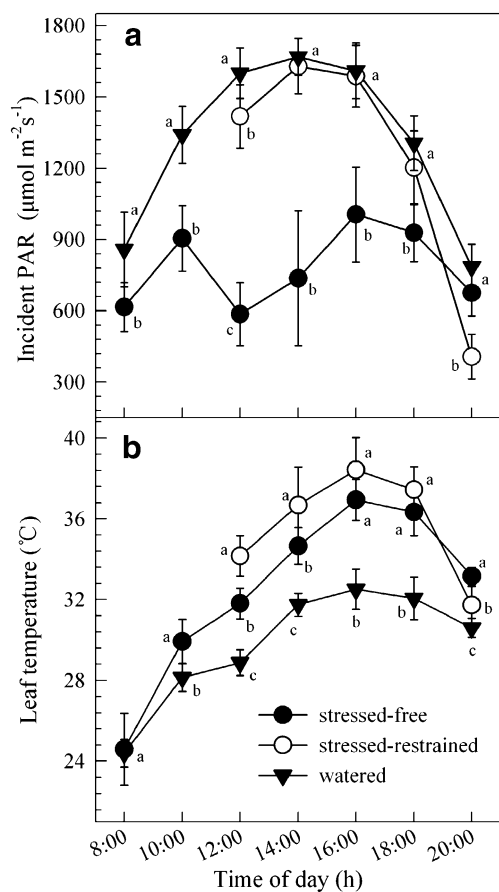


Fig. 4 Diurnal time course of **a** leaf incident PAR light and **b** leaf temperature in well-watered leaves, stress-free leaves, and stress-restrained leaves. Different letters indicate differences between treatments at the same time of day at $P < 0.05$ according to Duncan's multiple range test. Bars represent the SE

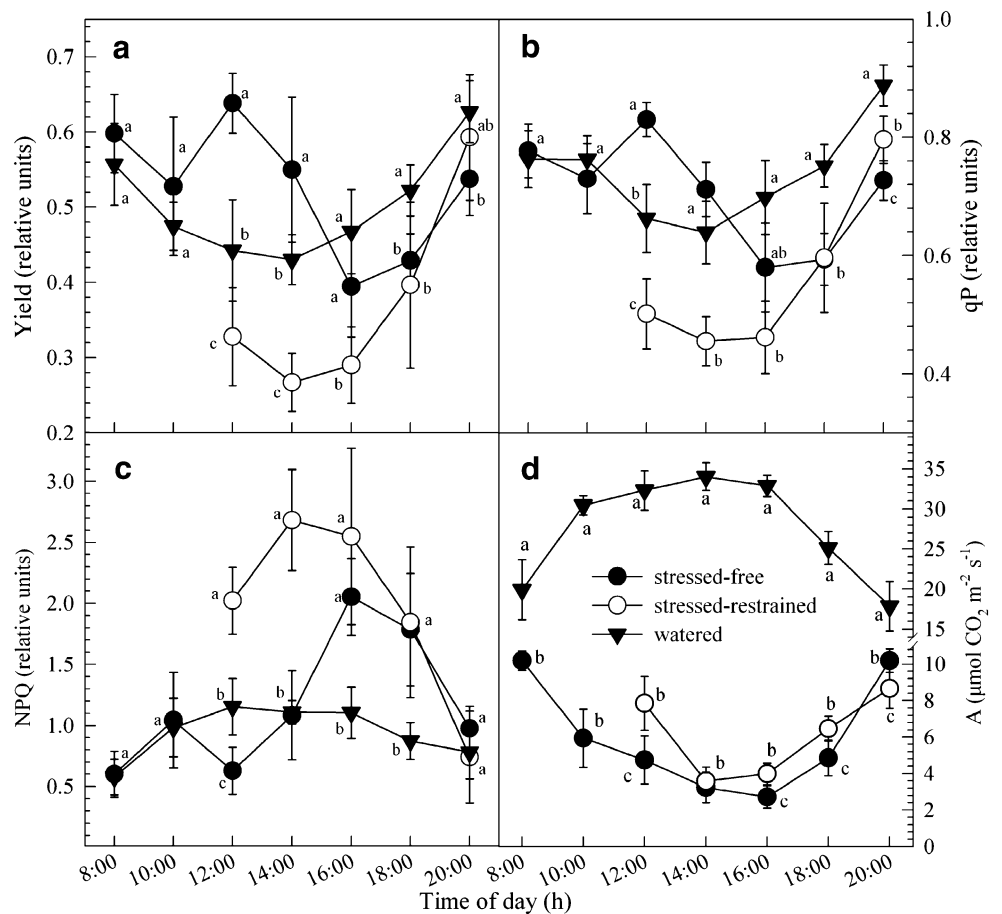
because more light was incident on the well-watered leaves; both were higher than that recorded in stress-restrained leaves, except at 2000 hours.

As expected, the diurnal time courses of qP also showed marked differences between treatments (Fig. 6b). The patterns were similar to those shown for Yield and F_v/F_m . As shown in Fig. 6c, the diurnal time course of NPQ showed a pattern that followed the PAR incident on leaves with a very clear gradient between treatments. Although the stress-free leaves had received consistently lower incident light compared to leaves from well-watered plants, the free leaves had a higher or similar NPQ during the day, except at 1200 hours. In particular, NPQ was the highest in stress-restrained leaves.

CO₂ Assimilation Rate

Measurements of gas exchange confirmed the expected effects of water deficit on carbon assimilation (Fig. 6d). Leaves from well-watered plants maintained higher CO₂ assimilation rates during the day than those from water-stressed plants. In well-watered plants, the diurnal pattern of CO₂ assimilation rates followed that of PAR in the morning, but after midday, CO₂ assimilation rates began to decline. Leaves from water-stressed plants, however, recorded their highest CO₂ assimilation rates early in the morning, but the rates fell sharply at midday to below $3 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. Furthermore, although the PAR incident on leaves declined, CO₂ assimilation rates recovered in the stress-free leaves during the afternoon. In the case of stress-restrained leaves, CO₂ assimilation rates were significantly higher than those in stress-free leaves except at 1400 and 2000 hours.

Fig. 6 Diurnal time course of **a** Yield, **b** qP, **c** NPQ, and **d** CO₂ assimilation rate *A* in well-watered leaves, stress-free leaves, and stress-restrained leaves. Different letters indicate differences between treatments at the same time of day at *P*<0.05 according to Duncan's multiple range test. Bars represent the SE



Predawn F_v/F_m , CO₂ Assimilation Rates, and C_i/C_a 4 Days After the Wilted Leaves had been Restrained

As shown in Fig. 7, predawn F_v/F_m in the stress-free leaves remained at about 0.84. However, a significantly lower predawn F_v/F_m value (0.59) was observed in the stress-restrained leaves.

As expected, CO₂ assimilation rates and C_i/C_a in the stress-free leaves were significantly higher and lower than that in the stress-restrained leaves, respectively (Fig. 7).

Discussion

Leaf Wilting Movement Reduced the Light Incident on Leaves and their Heat Load, Thereby Alleviates Damage from Photoinhibition

Leaf movement, or diaheliotropism, to maximize the capture of sunlight during the day is well known in cotton (Lang 1973; Ehleringer and Hammond 1987). As shown in Figs. 2 and 3, well-watered plants showed diaheliotropic leaf movement during the day. Water-stressed plants, however, showed the movement only in the early morning and the extent of diaheliotropic leaf movement was smaller.

When leaf water potential declined further in water-stressed plants, the leaves wilted at about 1100 hours. As expected, wilting movement significantly affected the PAR incident on leaves and leaf temperature (Fig. 4a, b). After wilting,

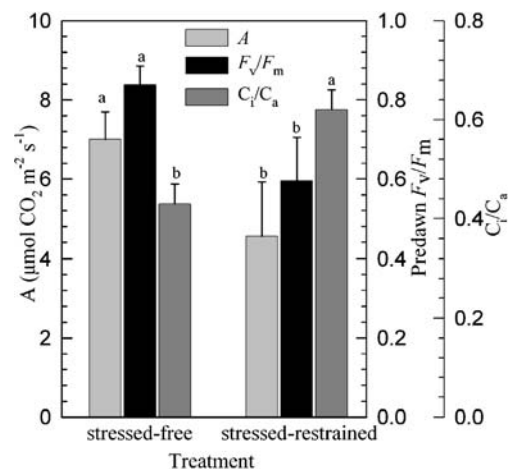


Fig. 7 CO₂ assimilation rate (left axis), predawn F_v/F_m (right axis) and C_i/C_a (right offset axis) of stress-free leaves (left column) and stress-restrained leaves that had been held horizontal for 4 days (right column). Different letters indicate differences between treatments at *P*<0.05 according to Duncan's multiple range test. Bars represent the SE

the PAR incident on leaves from water-stressed plants decreased significantly and, although it increased after 1200 hours with change in the angle of incident sunlight, the incident PAR during the day was lower. A similar phenomenon was reported in sunflower (*Helianthus argophyllus* L.) in an earlier study (Rawson 1979). Furthermore, when the wilted leaves were supported, they recorded greater incident light (Fig. 4a). In the case of leaf temperature, water-stressed plants, as expected, recorded higher leaf temperature (Fig. 4b). Although wilting movement reduced the light energy load, leaf temperature in stress-free leaves was consistently higher than that in well-watered leaves. However, stress-restrained leaves recorded higher leaf temperature than stress-free leaves, directly demonstrating that wilting movement reduces heat load. High temperature affects photosynthesis and has been shown to intensify photoinhibition (Mamedov et al. 1993; Long et al. 1994). Therefore, wilting movement of leaves is an effective mechanism to relieve light stress and heat stress when plants are under water stress, as mentioned in earlier reviews (Begg 1980; Björkman and Demming-Adams 1994).

Values of F_v/F_m below 0.8 generally indicate photoinhibition (Björkman and Demmig 1987). Chronic photoinhibition, the loss of PS II photochemical efficiency that results from the generation of nonfunctional centers and is slowly reversible via D1 protein synthesis (Osmond and Grace 1995), can be indicated by the parameter of predawn F_v/F_m . As shown in Fig. 5, predawn F_v/F_m values in both well-watered and water-stressed plants were above 0.8. However, when wilted leaves were supported horizontally for 4 days, predawn F_v/F_m values in such leaves were significantly lower (the average value was 0.6; Fig. 7). It means that, under water stress, stress-restrained leaves experienced chronic photoinhibition overnight, but stress-free leaves did not.

During the afternoon, F_v/F_m in stress-free leaves was comparable to or only slightly lower than that in well-watered leaves (Fig. 5), indicating that water stress did not substantially enhance the degree of photoinhibition when leaf movement was permitted. However, when wilted leaves in water-stressed plants were fixed in a horizontal position, F_v/F_m was significantly lower compared to that in stress-free leaves. This is because the leaf wilting movement that normally lowers the PAR incident on leaves as well as leaf temperature, as discussed above, was not permitted. Wilting movement in water-stressed plants, therefore, helped to alleviate the degree of photoinhibition.

The contention that wilting movement confers protection against photoinhibition is further supported by measurements of Yield and qP (Fig. 6a, b). From 0800 to 1600 hours, Yield in stress-free leaves was higher than or comparable to that in leaves from well-watered plants;

from 1600 to 2000 hours, it was lower in stress-free leaves than that in well-watered leaves because of the large decrease in PAR incident on the well-watered leaves. By comparison, when the wilted leaves were restrained, Yield was significantly decreased. A similar trend was also observed in qP (Fig. 6b). The stress-free leaves had higher qP than stress-restrained leaves due to an increase in the PAR incident on the stress-restrained leaves. It is, therefore, concluded that wilting movement in water-stressed plants helps to avoid exposure to excess light that would otherwise lead to PS II photoinhibition.

Interestingly, the patterns of variation of Yield (Fig. 6a) and qP (Fig. 6b) bore a striking resemblance. Given that $\text{Yield} = \text{qP} \times F_v'/F_m'$, where F_v'/F_m' is the photochemical quantum yield of open PS II centers in the light-adapted state, the resemblance of Yield and qP implies that F_v'/F_m' was relatively constant through the day. Sailaja and Ramadas (1996) suggested that many diheliotropic species have a characteristic ability to maintain constancy of photochemical efficiency through the day.

It is well documented that water stress enhances the dissipation of thermal energy to ensure photoprotection (Scheuermann et al. 1991; Lu and Zhang 1999; Flexas et al. 2002). Higher values of NPQ were observed in stress-free leaves, and especially in stress-restrained leaves (Fig. 6c), indicating that dissipation of thermal energy of PS II was also enhanced in water-stressed leaves as protection against photodamage.

Leaf Wilting Movement Reduces Carbon Assimilation in the Short Term but Helps to Maintain it in the Long Term

It is well known that water stress reduces CO₂ assimilation rates (Chaves 1991). In this study, water-stressed plants had consistently lower CO₂ assimilation rates than well-watered plants during the day (Fig. 6). Although water-stressed plants had CO₂ assimilation rates in the early morning and in the late afternoon that approached those of well-watered plants, well-watered plants attained even higher CO₂ assimilation rates around midday.

A striking situation occurred in stress-free and stress-restrained leaves. The protective effect of wilting movement in water-stressed leaves, as manifested in higher values of qP and Yield in stress-free leaves compared to that in stress-restrained leaves, did not result in higher instantaneous CO₂ assimilation rates in the short term. Although the stress-restrained leaves recorded higher leaf temperature and lower Yield and qP, CO₂ assimilation rates in those leaves were higher than those in stress-free leaves (Fig. 6d). This is due to the stress-restrained leaves receiving more incident light energy than stress-free leaves (Fig. 4a), resulting in increased electron flow even if PS II had low photochemical efficiency (Yield). Nevertheless, in

the long term, after 4 days of having been restrained in a horizontal position, the stress-restrained leaves recorded significantly lower steady-state CO₂ assimilation rates than the stress-free leaves did (Fig. 7), indicating more severe damage to the photosynthesis apparatus. Therefore, in the long term, leaf wilting movement may help to maintain considerable carbon assimilation.

In the long-term treatment, both the predawn F_v/F_m and the steady-state CO₂ assimilation rates (Fig. 7) decreased to a similar extent (by about 40%) in stress-restrained leaves compared with stress-free leaves. This coincidence, however, does not necessarily mean that the CO₂ assimilation rate was being limited by PS II functionality. Indeed, work with isolated intact chloroplasts (Heber et al. 1988) and leaves (Lee et al. 1999) showed that the light- and CO₂-saturated photosynthetic capacity is not limited by PS II until >40% of the PS II centers are inactivated. It is more likely that the ~40% loss of predawn F_v/F_m in stress-restrained leaves is an overestimation for the leaf tissue as a whole. Since the measured chlorophyll fluorescence signal is predominantly emitted by chloroplasts near the (adaxial) leaf surface receiving direct sunlight or measurement light, the decrease in F_v/F_m is certainly greater than for the whole leaf tissue and does not correctly represent the loss of active PS II in the whole leaf tissue (Losciale et al. 2008). Therefore, the loss of CO₂ assimilation rates, which was measured for the whole tissue, cannot be completely accounted for by the limited loss of active PS II centers in the whole tissue. Considering that C_i/C_a in stress-restrained leaves was higher than that in stress-free leaves (Fig. 7), we propose that the CO₂ assimilation rate was decreased due to impairment of downstream processes, such as in the Calvin–Benson cycle (Feller et al. 1998). We note that leaf temperature reached the highest levels in stress-restrained leaves (Fig. 4b). Perhaps, over the period of 4 days, critical temperature thresholds were reached, resulting in the loss of carbon assimilation activity. If so, the most important function of leaf wilting movement during water stress may be to limit the leaf temperature to a tolerable level.

The Ecophysiological Significance of Passive Wilting Leaf Movement in Protection of Leaves from Photoinhibition

Active paraheliotropic leaf movement, generally in leguminous species, is not universal in higher plants. This active leaf movement, responding rapidly to an unfavorable environment, is induced by high light, and water stress often reduces the threshold for that response (Koller and Shak 1990; Björkman and Demming-Adams 1994). Furthermore, this active leaf movement not only occurs in water-stressed plants but also in well-watered plants when both leaf water potential and potential photosynthetic capacity are normal (Pastenes et al. 2005; Arena et al. 2008). Thus, it seems that

paraheliotropic leaf movement in response to water stress are far from a terminal action preceding senescence (Rawson 1979). Passive leaf wilting movement, on the other hand, occurs quite generally in higher plants (including leguminous species) and is induced by water stress. Furthermore, leaf wilting movement becomes apparent only after several anatomical and physiological adaptations as water stress develops (Hsiao 1973; Begg 1980). Therefore, passive wilting leaf movement seem to be a final step of morphological adaptation for water-stressed leaves to mitigate not only against photoinhibition, but also senescence (Rawson 1979); without it, the photosynthesis apparatus will be damaged, and in the extreme case, leaf will die. Indeed, we observed that wilted free leaves maintained comparable carbon assimilation capacity, whereas wilted restrained leaves died at the end of the experiment.

Conclusions

In this study, we found that the wilting movement of leaves could protect water-stressed cotton plants against photoinhibition and could maintain considerable carbon assimilation in the long term in the field. Therefore, we have provided direct evidence that passive wilting leaf movement can be a strategy of photoprotection that enables plants to survive combined high light and water stress that frequently occur together in the field.

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