

Effects of Drought on Primary Photosynthetic Processes of Cotton Leaves¹

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ABSTRACT

The effects of drought on Photosystem II (PSII) fluorescence and photosynthetic electron transport activities were analyzed in cotton. Water stress did not modify the amplitude of leaf variable fluorescence at room temperature in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) nor at 77 K. It is therefore concluded that photon collection, their distribution between the two photosystems, and PSII photochemistry are unaffected by the stress. In droughted leaves at room temperature under low exciting light, the transitory maximum (F_v) and steady state (F_s) fluorescence levels are increased; under high exciting light, F_v level and the rise time from the initial level (F_o) to F_v are unchanged, whereas F_v to F_s decay time is increased. These results infer that the drought slows the rate of plastoquinone reoxidation. This conclusion agrees with a larger proportion of reduced primary PSII electron acceptor Q_A measured at the steady state under low light. In thylakoids isolated from droughted leaves, PSII mediated electron flow was the same as in thylakoids from control leaves, whereas PSI mediated electron transport was inhibited. It is shown that water stress does not induce sensitization to photoinhibition in cotton.

During the past years, many workers have been interested in understanding how drought limits photosynthesis. Early experiments suggested that leaf CO_2 uptake decline observed during water stress was mainly due to stomatal closure. However, technical improvements in the analysis showed that nonstomatal component of leaf photosynthesis was also damaged during dehydration (for review, see [7]). Nonstomatal effects of water stress are well documented, particularly the effects on photosynthetic photosystems have been studied; Nir and Poljakoff-Mayber (21) and other workers (4, 10, 17, 27) have shown an inhibition of photosynthetic electron chain activity on plants subjected to drought. The activity was measured in thylakoids isolated from *in vivo* water stressed leaves. These observations were confirmed by the results of Govindjee *et al.* (11) and Newton *et al.* (20) who observed a decrease in Chl *a* fluorescence emission of leaves as the water potential decreased.

However, recent studies (2, 16) reported that in isolated intact chloroplasts, osmotic dehydration did not significantly affect electron transport. Therefore it was suggested that data obtained *in vivo* resulted from indirect effects of drought on photosynthetic electron transport.

We aimed to investigate this problem by analyzing the effects

of drought on *in vivo* fluorescence transients from cotton leaves. Indeed fluorescence induction is a difficult but powerful method of determining the functioning of photosynthesis *in vivo* (for a critical review, see [18]). We tried to distinguish between direct effects of water stress on photosynthetic apparatus and damage caused by photoinhibitory processes occurring under these conditions (3, 24). Plants were for this purpose grown at low PFD.²

MATERIALS AND METHODS

Plant Material. Cotton plants (*Gossypium hirsutum* L., cv Reba) were grown on a mixture of vermiculite and peat in 2-L pots in a controlled growth room. Conditions were: photoperiod, 14 h; day temperature, 25°C; night temperature, 20°C; RH, 65%; PFD, 300 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$ in case of cotton, 100 in case of *Nerium oleander* provided by a bank of metal halide lamps; in some experiments (Table IV) a higher PFD, 1200 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$, was used for both species. Plants were watered twice daily and fertilized once weekly. They were submitted to water stress by withholding watering 6 weeks after germination in case of cotton and 4 months after propagation by cuttings in case of *N. oleander*. Drought was achieved in 8 d. Plants were rehydrated by rewatering the pots to field capacity. Measurements were performed on fully expanded leaves (the fourth to fifth leaves from the top).

Leaf Water Potential Measurements. Leaf water potential was determined using Wescor C52 psychrometers (Wescor Inc., Logan, UT) by the dew point method or using a pressure chamber (PMS, Corvallis, OR).

Fluorescence Measurements. Room temperature fluorescence kinetics at 685 nm were measured from attached and detached leaves using a bifurcated light pipe together with a quartz light mixing rod to excite and collect the fluorescent light from the upper leaf surface. The exciting blue light was produced by a DC stabilized projector tungsten lamp passing through a combination of filters (Schott BG18+BG38+KG3) via a photographic shutter (opening time: 2 ms). The fluorescence was detected through a 685 nm interference filter (Schott PIL 0.1) and a red colored filter (Corning 2-64) using a photomultiplier tube (RTC, XP 1017). The signal was simultaneously displayed on a storage oscilloscope for initial rapid phases of the induction and on a chart recorded for slower phases. Fluorescence measurements were made on attached leaves which have been dark adapted during all the night period. Leaf discs of 1.5 cm diameter, were used for experiments with DCMU. They were infiltrated in the dark with a 20 μM DCMU solution.

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² Abbreviations: PFD, photon flux density; Q_A , primary PSII electron acceptor; MV, methyl viologen; pBQ, parabenzoquinone; Asc, ascorbate; DCPIP, dichlorophenol indophenol; PQ, plastoquinone.

During *in vivo* fluorescence measurements, a humid air stream passed around the leaf to ensure constant gaseous surroundings and high rates of photosynthesis. For fluorescence measurements in CO₂ free atmosphere, the leaf was put in a plastic chamber flushed with CO₂ free air (20 L/h). Low temperature fluorescence transients measurements at 695 nm and 735 nm (Schott PIL 0.1 interference filters) were performed with the same apparatus on leaf discs of 1.5 cm diameter frozen by immersion in liquid N₂. Discs came from one night dark adapted leaves.

Different PFD were used for fluorescence measurements, PFD at the leaf surface was estimated using a quantum sensor (Li 185B, Lambda Instrument Corp.) and adjusted with neutral density filters (Schott).

Estimation of the Contribution of Photochemical and Nonphotochemical Quenching. The redox state of Q_A was estimated according to Bradbury and Baker (5) using the fluorescence apparatus described above. Dark adapted attached leaves were exposed, at room temperature, to a low PFD (20 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$). At the steady state fluorescence level F_i (achieved after 15 min excitation) the PFD was increased to 400 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$ (light 2) by sudden removal (1 ms) of a grid used as a neutral density filter. The resulting fluorescence kinetics to F_p were recorded, and the initial F_o and variable fluorescence F_v determined. At this high PFD value, it has been verified that the transitory maximum yield F_p corresponds to a complete reduction of the PSII acceptor pool.

The redox state of Q_A at F_i was estimated by F_v/F_o . A value for F_v/F_o of zero indicates complete reduction of Q_A and increasing values of F_v/F_o indicate an increasing amounts of oxidized Q_A.

The fluorescence level FQ_A red at low PFD which corresponds to all Q_A reduced can be calculated according to the relation of Bradbury and Baker (5).

$$FQ_{A\text{red}} = F_i + F_i \times F_v/F_o.$$

Then, the photochemical quenching $q(P)$ and the nonphotochemical quenching $q(NP)$ are calculated as Bradbury and Baker (5).

Chloroplast Isolation and Electron Transport Measurements. Thylakoids were isolated from cotton leaves following the method described by Schwab and Heber (26) used for thylakoids isolation from resuspending plants. The isolation medium contained 330 mM sorbitol, 1 mM EDTA, 5 mM MgCl₂, 10 mM NaCl, 10 mM sodium pyrophosphate, 1% BSA (w/v), 1% PEG-20000 (w/v), and 50 mM Tricine, pH 8. Immediately before use, 5 mM cysteine and 5 mM sodium dithionite were added. Low concentration of sodium dithionite in the isolation medium was used to minimize an inactivation of photosynthetic electron transport during blending by binding phenolic or other interfering constituents of leaves. The resuspending medium contained 330 mM sorbitol, 1 mM MgCl₂, 1 mM MnCl₂, 2 mM EDTA, and 50 mM Hepes, pH 7.6.

Total breakage of chloroplasts was completed by osmotic shock (in resuspending medium without sorbitol) before assay in the resuspending medium.

Electron transport was followed polarographically by monitoring the O₂ evolution or uptake with a temperature controlled water jacketed Clark-type electrode assembly at 25°C. A white light illumination (PFD 2000 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$) was provided by a slide projector.

Uncoupled whole chain electron transport was measured by following the rate of O₂ uptake with 0.1 mM MV as electron acceptor in the presence of 1 mM NaN₃ and 5 mM NH₄Cl.

PSII uncoupled electron transport was determined as pBQ (1 mM) dependent O₂ evolution with 5 mM NH₄Cl.

Uncoupled PSI driven electron transport was assayed with 5 mM ascorbate using 50 μM DCPIP as electron donor in the

presence of 10 μM DCMU, 200 μM MV as electron acceptor, 1 mM NaN₃, and 5 mM NH₄Cl.

Chl concentration was determined according to Arnon (1).

RESULTS AND DISCUSSION

Figures 1 and 2 show room temperature time courses of Chl fluorescence at 685 nm in well watered (-6 bars) and water stressed (-20 bars) dark adapted cotton leaves at PFD 20 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$ and 200 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$, respectively. The strong actinic light was sufficient to reduce all Q_A then all PQ pool (at F_p level) before the onset of photoactivation of the electron flow on the reducing side of PSI. These inductions exhibited characteristic phases with fluorescence levels usually termed F_o , F_i , F_d , F_p , F_s , F_m , F_t (23). For the interpretation of these transients see (18).

We observed that water stress does not affect F_o level. As Chl *a* and *b* concentrations remain unchanged with drought (data not shown), F_o has been used to normalize the fluorescence levels and to compare samples.

At low PFD, water stress lower than -10 bars induces an increase of F_p , F_m , and F_t levels, F_i is slightly enhanced and the dip less marked.

In contrast at high PFD, only slight differences in the fast F_o to F_p transients are observed. The F_o to F_p rise time is unaffected

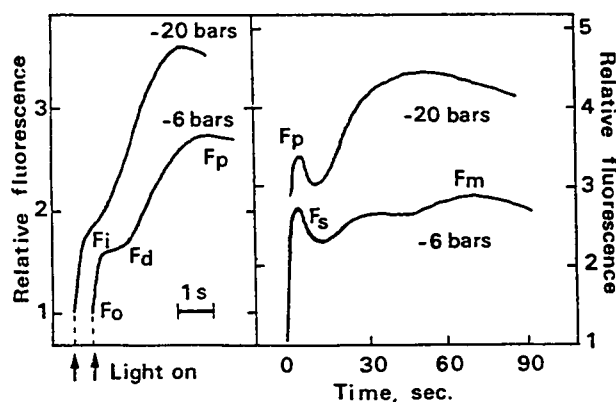


FIG. 1. Room temperature time courses of 685 nm Chl fluorescence in well watered (-6 bars) and water stressed (-20 bars) dark adapted cotton leaves at PFD 20 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$. Fast and slow time courses are normalized to F_o level of fluorescence. The characteristic fluorescence levels are also noted.

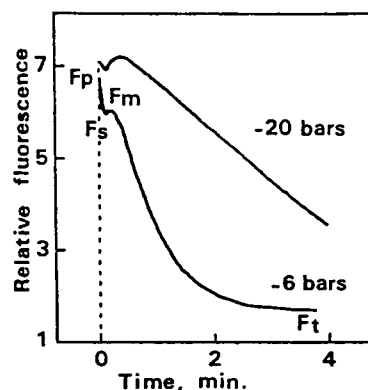


FIG. 2. Room temperature time courses of 685 nm Chl fluorescence in well watered (-6 bars) and water stressed (-20 bars) dark adapted cotton leaves at PFD 200 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$. Time courses are normalized to F_o level of fluorescence. The characteristic fluorescence levels are also noted.

by water stress. The main effect of drought is a slowdown of F_p to F_t decline which occurs at water potential lower than -10 bars.

These variations *versus* the water potential are presented in Figure 3 which shows in addition that water stress induced variations of fluorescence induction are completely reversible by rewatering the plant.

As F_o to F_p induction at high PFD is unchanged by drought, it can be concluded that the reduction by PSII of its acceptor pool is insensitive to water stress. Table I shows that characteristics of 685 nm fluorescence induction of dark adapted leaves in the presence of DCMU is unaffected by drought; these data confirmed that PSII photochemical conversion as well as charge recombination are insensitive to drought. As the DCMU infiltration is performed with an aqueous solution and as drought effect on plant is reversible, 77 K fluorescence inductions have been measured at 695 and 735 nm. The results are presented in Table

Table I. Effects of Drought on the Initial Level F_o , and Maximal Level F_{max} of Fluorescence, on the Half Rise Times for F_{max} and on the Quantum Yield of PSII Fluorescence ($1 - F_o/F_{max}$)

These parameters were determined from 685 nm room temperature fluorescence induction in dark adapted cotton leaves treated with 20 μ M DCMU at PFD 20 μ mol quanta $m^{-2}s^{-1}$. Each value is the mean for at least three plants; \pm SE is indicated.

	Water Potential		
	-6 bars	-16 bars	-29 bars
F_o	0.64 ± 0.04	0.63 ± 0.02	0.65 ± 0.03
F_{max}	3.47 ± 0.22	3.32 ± 0.27	3.09 ± 0.19
$t_{1/2}$, ms	82 ± 4	80 ± 7	79 ± 5
$1 - F_o/F_{max}$	0.81 ± 0.01	0.81 ± 0.01	0.78 ± 0.02

Table II. Effects of Drought on Initial and Maximum Levels of Fluorescence F_o , F_{max} , and on the Quantum Yield of PSII Fluorescence ($1 - F_o/F_{max}$)

These parameters were determined from 695 and 735 nm fluorescence transients in dark adapted cotton leaves frozen at 77 K at PFD 20 μ mol quanta $m^{-2}s^{-1}$. Each value is the mean for at least three plants. \pm SE is indicated.

	Water Potential		
	-5 bars	-16 bars	-27 bars
F_o 695	1.45 ± 0.04	1.49 ± 0.02	1.47 ± 0.05
F_{max} 695	9.49 ± 0.26	9.82 ± 0.29	9.86 ± 0.45
$1 - F_o$ 695/ F_{max} 695	0.84 ± 0.01	0.85 ± 0.01	0.85 ± 0.00
F_o 735	29.8 ± 1.3		31.1 ± 2.1
F_{max} 735	41.8 ± 1.6		44.0 ± 2.6

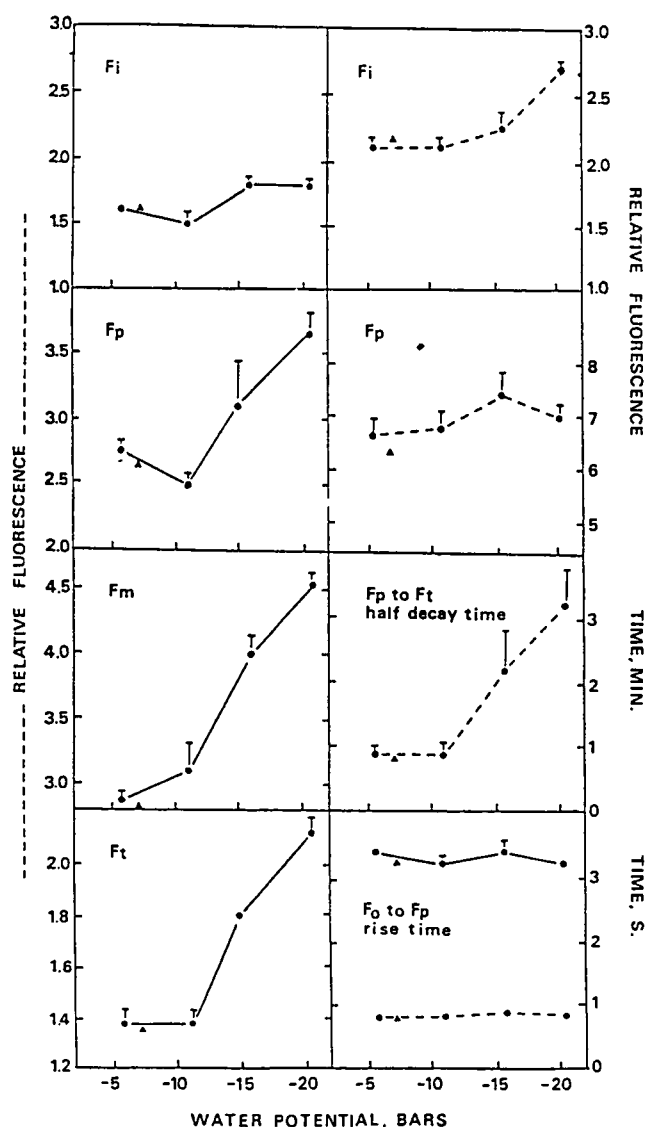


FIG. 3. Fluorescence characteristics *versus* water potential. Effect of drought on F_i , F_p , F_m , F_t , on the rate of F_p to F_t decay, and on the F_o to F_p rise time of the fluorescence transients in dark adapted leaves measured under PFD 20 μ mol quanta $m^{-2}s^{-1}$ (—) and PFD 200 μ mol quanta $m^{-2}s^{-1}$ (---). The fluorescence levels are normalized to F_o level. Each point represents the mean of four replicates. \blacktriangle , rewatered leaves; \pm SE is indicated.

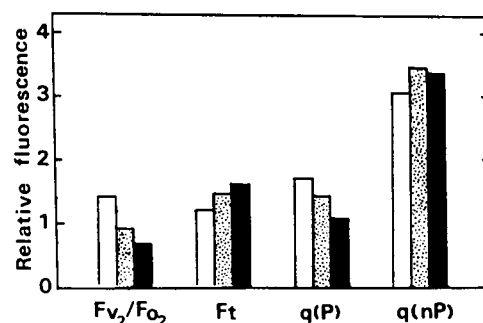


FIG. 4. Effects of drought on F_i fluorescence level, and on F_v/F_o , on the photochemical quenching $q(P)$, on the non photochemical quenching $q(nP)$, estimated at F_i as outlined in "Materials and Methods" section: estimation of $q(P)$ and $q(nP)$. Measurements were made at different water potentials: -5 bars (\square), -18 bars (\square), -26 bars (\blacksquare). F_i , $q(P)$, and $q(nP)$ are normalized to F_o . Each bar represents the mean of three replicates. The largest SE was 8% of the mean.

II which confirm that PSII photochemistry is not affected by drought. In addition this table points out that the light distribution between the two photosystems is not significantly changed by drought.

The increase of F_p , F_m , and F_i levels in weak PFD as well as the slowness of the F_p to F_t decline in high PFD suggest that reoxidation of Q_A and of the PSII acceptor pool or the light induced activation of PSI acceptor side are inhibited by water stress. To improve these conclusions, the redox state of Q_A at the steady state F_i at different water potentials have been estimated by the method of the sudden increase in intensity of the exciting beam. The relative contributions of $q(P)$ and $q(nP)$ have been deduced as described in "Materials and Methods." Figure 4 points out that water stress provokes as expected a decrease of

Table III. Electron Transport Activities of Thylakoids Isolated from Stressed (–18 Bars) and Nonstressed (–5 Bars) Cotton Leaves

Rates are the means of n isolation experiments with at least two replicate measurements per experiment; \pm SE is indicated. Rates of O_2 uptake measured in the presence of DCPIP/Asc and MV have been divided by two as two equivalents were transported per consumed O_2 mole.

Electron Donor to Acceptor	O ₂ Evolution or Uptake at Water Potential:	
	–5 bars	–18 bars
	$\mu\text{mol mg}^{-1} \text{Chl h}^{-1}$	
H ₂ O → MV	158 ± 14 ^a	116 ± 9 ^b
H ₂ O → pBQ	69 ± 7 ^a	67 ± 8 ^b
DCPIP/Asc → MV	172 ± 25 ^a	127 ± 20 ^b

^a $n = 4$. ^b $n = 3$.

F_v/F_o and a decrease of $q(P)$ which indicate an increase in reduced Q_A . It induces also a slight increase of $q(nP)$.

One of the main causes of $q(nP)$ is the acidification of the inside face of the thylakoid membrane due to the photoinduced ΔpH (18). As ΔpH increases, the reoxidation of plastoquinones by PSI is restrained in an almost logarithmic relationship (8, 12). Then even a small increase by drought of $q(nP)$, if it is due to an increase of ΔpH , could enhance the concentration in reduced Q_A .

Another possibility is an inhibition by water deficiency of the intrinsic rate of electron flow. The rate of partial photosynthetic electron flow in uncoupled thylakoids isolated from stressed or nonstressed leaves have been measured. The results reported in Table III show that there is no inhibition of PSII, whereas PSI mediated reduction of MV is diminished by 26%. These results are in agreement with those obtained by fluorescence in leaves, i.e. no inhibition of PSII electron flow and a decrease of the reoxidation of PQ by an inhibition of PSI mediated electron flow. If these data are compared to decrease of F_v/F_o presented in Figure 4, the two variations are qualitatively consistent.

The data shown here indicate that water stress induces a slowdown of photosynthetic electron transport after PQ but does not affect H₂O to PQ, PSII mediated electron flow. Particularly, we did not observe inhibition of the water donation to PSII in contrast to Govindjee *et al.* (11) on *Nerium oleander* plants. Their conclusion was based on a lowering of F_p/F_o fluorescence

ratio (same as normalized F_p) during drought. As shown in Table IV, we were not able to reproduce this result with this same species when it is grown under low PFD (100 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$). In our hands, responses of *N. oleander* and cotton plants to drought were very similar at low PFD. However, under high PFD conditions of growth (1200 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$), we found the same lowering of Chl *a* fluorescence level as did Govindjee *et al.* (11) in *N. oleander* plants. In contrast, in cotton, F_p increases with drought as in low PFD (Table IV).

Björkman and Powles (3) had also shown an interaction between light level intensity and water stress on *N. oleander*. Indeed, they showed that plants grown under full daylight and dehydrated under shaded conditions retained levels of 77 K 695 nm fluorescence during drought, while the same plants exposed to full daylight during stress exhibited a progressive decrease of this fluorescence with declining leaf water potentials. They demonstrated that the inactivation observed under full light was very similar to photoinhibition which took place when leaves of well watered *N. oleander* plants, grown under a lower light regime, were exposed to full daylight. They concluded that this was due to an impairment of the primary photochemistry associated with PSII and not to an inhibition of the donor side of this photosystem for water potentials not lower than –30 bars. As shown in Table IV, with *N. oleander* this interaction between light and water stress also exists after acclimation to the PFD level used during water stress treatment. In contrast, in cotton, 77 K fluorescence changes induced by drought are insensitive to light intensity of growth (see Tables II and IV). Therefore, in cotton there was no interaction between water stress and PFD level under our experimental conditions. A similar conclusion was reached by Sharp and Boyer (25) on sunflower. In cotton the data shown point out specific effects of drought on photosynthetic processes independent of photoinhibitory effect.

Concerning studies on isolated thylakoids (4, 10, 17, 21, 27), we believe that the contradictory data could also result from poor extractability of functional chloroplasts from leaves.

In cotton, possible causes of the drought induced slowdown of electron flow subsequent to PQ could be direct impairment of electron transport from PQ via PSI, and indirect inhibition due to a lack of reducing power utilization and of dissipation of the light induced proton gradient in relation to stress induced changes of carbon metabolism and gas diffusion pathway. Although Dietz and Heber (9) have reported that, at a high PFD, an increase of the energization of thylakoids occurred during a

Table IV. Effect of Drought on F_p Fluorescence Level and on the Quantum Yield of PSII Fluorescence ($1-F_o/F_{max}$) of Leaves from Cotton and *N. oleander* Plants Grown under Low PFD: 100 $\mu\text{mol Quanta m}^{-2}\text{s}^{-1}$ (*N. oleander*) and High PFD 1200 $\mu\text{mol Quanta m}^{-2}\text{s}^{-1}$ (Cotton, *N. oleander*)

F_p level was determined from 685 nm room temperature fluorescence induction in dark adapted leaves at PFD 20 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$ (except [*] at PFD 40 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$). The quantum yield of PSII fluorescence was determined from 695 nm fluorescence induction in dark adapted leaves frozen at 77 K at PFD 20 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$. Each value is the mean for at least three plants; \pm SE is indicated.

Species	PFD for Growth	Leaf Water Potential	F_p	$1-F_o/F_{max}$
	$\mu\text{mol quanta m}^{-2}\text{s}^{-1}$	bars		
<i>N. oleander</i>	100	–5	3.50 ± 0.29	0.85 ± 0.01
	100	–18	4.93 ± 0.31	
	100	–32	4.91 ± 0.47	0.85 ± 0.01
	1200	–5	2.44 ± 0.02	0.81 ± 0.00
	1200	–16	1.64 ± 0.03	0.73 ± 0.01
	1200	–25	1.17 ± 0.05	0.71 ± 0.01
<i>G. hirsutum</i>	1200	–6	2.32 ± 0.08*	0.83 ± 0.00
	1200	–14	3.95 ± 0.13*	0.85 ± 0.01
	1200	–22	4.09 ± 0.06*	0.85 ± 0.01

rapid dehydration, further studies are needed to characterize the effects of water stress on these phenomena for a better understanding of regulations of electron transport in this condition.

As during severe drought Q_A was not largely reduced (as indicated by F_v/F_o larger than zero [Fig. 4]) in spite of a complete inhibition of CO_2 fixation (data not shown), it is obvious that other terminal acceptor(s) could oxidize Q_A and have an important role in the regulation of electron transport in water stress as in normal physiological conditions. O_2 could play this role *in vivo* by acting as a terminal acceptor directly at the level of PSII as suggested recently (6) and/or in a Mehler reaction (13, 14). It could also be a substrate for ribulose 1,5 bis-P oxygenase (19, 22). Indeed, Bradbury *et al.* (6) have shown that O_2 plays an important role in determining the nature of the fluorescence transients by studying effects of CO_2 removal from leaves on the Chl fluorescence transients. Thus in droughted cotton leaves, these mechanisms act as a drain of reducing power. Such processes could explain, as in immature leaf tissue of monocotyledons (15), the relative insensitivity of cotton photosynthesis to photoinhibition using the hypothesis offered at present on the causes of such photoinhibition (22, 24).

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