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Effect of water stress on primary photosynthetic process: Interaction with light and temperature

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Effect of water stress alone and in combination with light and temperature on chlorophyll fluorescence and photosynthetic electron transport have been studied in drought-resistant wheat. It was observed that water stress alone did not modify the amplitude of variable fluorescence in leaf but showed slight decrease in photosynthetic electron tranport. However, interaction with light caused significant decrease in the efficiency of photosynthesis. This was greater when photoinhibited at 30°C than at 10°C. Results suggest that water stress alone does not lead to significant damage to the primary photochemistry but photoinhibition causes both inhibition of electron transport activity and chlorophyll fluorescence. The damage was further enhanced by the combination of water stress and high temperature.

Water stress is one of the most important environmental factors limiting photosynthesis. It, like many other environmental stresses, can predispose the primary photosynthetic reactions to damage even at moderate light[1,2]. This reduction in photosynthesis is called photoinhibition and is similar to what occurs if shade plants are suddenly exposed to bright light which results in the reduction of quantum yield of photosynthesis[3,4].

There has been sustained interest in trying to understand how drought stress affects photosynthesis. Earlier experiments[5,6] suggest that water stress affects photosynthesis in at least three ways, viz. by closure of stomata, inhibition of dark fixation process and inhibition of electron transport. While most of the reduction can be attributed to stomatal closure[7], a part of it has also been attributed to the direct effect of dehydration on the biochemistry of photosynthesis[8,9]. Severe osmotic stress of chloroplasts and cell can inhibit FBPase activity by inhibition of light activation[10] or by lowered *p*H (ref. 11). However, recent studies[12,13] have shown that osmotic dehydration does not significantly affect the electron transport activity. Earlier reports of decrease in the electron transport may have resulted from indirect effects of drought on photosynthetic electron transport activity.

It was thus considered worthwhile to investigate whether drought stress affects the primary photochemistry of photosynthesis by studying the effect of drought alone and in combination with light and temperature on photosynthetic electron transport and chlorophyll fluorescence and the results are presented here.

Materials and Methods

Plant material and growth conditions—Seeds (8 g) of wheat (*Triticum aestivum* L.; cv C306), a drought-resistant cultivar, obtained from the Indian Agricultural Research Institute, New Delhi and stored in the dark at room temperature were soaked in tap water for 1 hr and sown in a 10-cm plastic pot containing vermiculite. Plants were grown for 10 days in a controlled environment with 16 hr photoperiod (day/night temperature, 20°C/16°C ± 1°C), illuminated with a combination of fluorescent tubes and incandescent bulbs to provide a photosynthetic photon flux density (PPFD) of 60 Wm^{-2}. The water stress was applied by withholding water after germination.

Photoinhibition conditions—An apparatus for exposing the intact leaves to 60 Wm^{-2} was constructed in the University Science Instrument Centre (USIC). A tungsten halogen tube (1 kW) was mounted in a curved aluminium reflector. The light passed through a glass tank with flowing water to absorb ultra-violet and infrared radiation. The temperature during photoinhibition treatment was maintained either at 10°C in a cold room or at 30°C in an air-conditioned room. The photoinhibition treatment was given for 1-6 hr. The plotted plants were kept vertical to the light source under the water tank.

Recovery conditions—After photoinhibition treatment, plants were watered and transferred to the growth cabinet (see growth conditions).

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Leaf water potential measurements—This was determined using Wescor HR 33T psychrometer (Wescor Inc. Logan. Utah, USA) by the dew point method.

Chloroplasts isolation—Chloroplasts were mechanically isolated from 10-day-old wheat leaves following the method of Reeves and Hall[14]. Leaves (25 g) were cut into 0.5 cm pieces and ground in 200 ml of grinding medium (10 m*M* $Na_4P_2O_7$, 4 m*M* $MgCl_2$, 2 m*M* ascorbate, 0.33 *M* sorbitol (*p*H 6.5) and 0.25% (wt/vol) bovine serum albumin (BSA) for 10-15 sec using a grinder (Remi, India). The homogenate was filtered first through two la_ _ and then 8 layers of muslin. The filtrate was centrifuged at 1000 *g* for 5 min. The surface of the chloroplast pellet was washed twice with resuspending medium (50 m*M* HEPES, 0.33 *M* sorbitol, *p*H 7.6, 2 m*M* EDTA, 1 m*M* $MgCl_2$, 1 m*M* $MnCl_2$ and 0.1% BSA (wt/vol) and resuspended to give about 1 g chlorophyll ml^{-1}. All steps were performed at 0-4°C.

Assays of electron transport activities—Electron transport activities of chloroplasts were followed polarographically as O_2 evolution or uptake at 25°C using a Clark type O_2 electrode chamber (Rank Bros., Cambridge, UK). The assays were carried out according to Sharma and Singhal[15] using 2 ml of reaction buffer (0.33 *M* sorbitol, 50 m*M* tricine, *p*H 7.6, with KOH, 4 m*M* $MgCl_2$, 2 m*M* $MnCl_2$, 10 m*M* NaCl) containing chloroplasts (50 μg of chlorophyll), illuminated by a projector (Perkeo, Germany) at a PPFD of 600 Wm^{-2}. The following electron transport activities were assayed using artificial electron donors and acceptors[16].

Fluorescence measurements in intact leaves—Fluorescence induction curve from intact leaf was recorded using plant productivity fluorometer (SF-30, Richards Brancker Research Ltd, Canada) at room temperature. Leaf was kept in the dark for 30 min and fluorescence induction recorded with a time base of 10 sec and 2 min. The exciting light (670 nm) was provided using a light emitting diode at 7.5 Wm^{-2}.

Fluorescence measurements in isolated chloroplasts—These were recorded according to Delieu and Walker[17] using Hansatech (England) TR1 transient recorder. Chlorophyll (15 μg) was added to 2 ml of fluorescence buffer containing 0.4 *M* sorbitol, 100 m*M* MES (*p*H 6.8), 40 m*M* KCl and 10 m*M* $MgCl_2$ and stirred for 5 min in the dark and then 1 min with DCMU (5 μm). The experimental recording time after switching on the actinic light (40 Wm^{-2} at 660 nm, provided by a projector lamp attached to LS2; Hansatech) was 60 ms.

Results

Table 1 shows the effect of both water stress and photoinhibition individually and in combination

Table 1—Effect of water stress, photoinhibition (650 Wm^{-2} for 6 hr at 30°C and 10°C) and combination of the two on light saturated uncoupled photosynthetic electron transport in chloroplasts isolated from stressed leaves

[The reaction rates are expressed as μmole of O_2 consumed or evolved mg chlorophyll^{-1} hr^{-1}. Values are mean of three experiments. DCIP, 2, 6-dichlorophenol indophenol; DPC, 1, 5-diphenyl carbazide; FeCN, ferricyanide; MV, methyl viologen; PD, phenylenediamine; PS, photosystem; WS, water stress; PI, photoinhibition. Percent changes are given in the parentheses]

Assays	Control	WS	Photoinhibition at 30°C			Photoinhibition at 10°C		
			PI for 6 hr	WS+PI for 3 hr	WS+PI for 6 hr	PI for 6 hr	WS+PI for 3 hr	WS+PI for 6 hr
	−6	−22	−6	−23	−24	−6	−22	−22
				bars				
PS II+PS I								
H_2O to FeCN	119±8	98±6 (−18)	78±9 (−34)	41±9 (−66)	29±4 (−76)	102±10 (−14)	95±6 (−20)	60±7 (−50)
H_2O to MV	223±12	197±12 (−12)	172±9 (−23)	66±9 (−70)	43±9 (−81)	187±10 (−16)	179±12 (−20)	105±15 (−47)
PS II only								
H_2O to PD	150±15	135±9 (−10)	99±7 (−34)	41±3 (−73)	23±5 (−85)	112±8 (−25)	115±8 (−23)	88±6 (−41)
PS II+PS I−O_2 evolution								
DPC to MV	32±4	31±4 (−3)	24±6 (−25)	26±5 (−19)	17±2 (−47)	24±5 (−25)	27±5 (−16)	20±4 (−37)
PS I only								
$DCIPH_2$ to MV	159±11	191±20 (+20)	147±10 (−7)	166±19 (+4)	139±12 (+13)	197±14 (+24)	163±12 (+3)	179±10 (+13)

with each other on photosynthetic electron transport. It was observed that water stress (−22 bars) alone showed slight (10%) inhibition in PS II activity assayed as H_2O to PD compared to control (i.e., well watered plants having water potential of −6 bars). The photoinhibition of water stressed plants (−22 to −24 bars), however, caused greater degree of inhibition (73-85%) in PS II activity. PS I activity assayed as $DCIPH_2$ to MV showed an increase (20%) in water stressed plants in the absence of photoinhibition but decreased when water stressed plants were photoinhibited.

Complete recovery of electron transport activity of water stressed plans following rewatering was observed. However, recovery of water stressed plants which were photoinhibited for 6 hr at 30°C was only 72% of control even after 12 hr under normal growth conditions (Fig. 1).

Results reported in Table 1 were also substantiated by our results with chlorophyll fluorescence (Table 2). Water stress alone did not affect chlorophyll fluorescence F_o (initial or constant fluorescence), F_m (maximum fluorescence), and F_v/F_m (F_v = variable fluoresence = $F_m - F_o$) significantly. The F_o to F_p (peak fluorescence) rise time was also unaffected but F_p to F_t (terminal fluorescence) decline was slower than that observed in controls (Fig. 2). Photoinhibition of water stressed plants caused significant decrease in the F_v/F_m ratio and slight increase in F_o, which were partially recovered during 12 hr recovery.

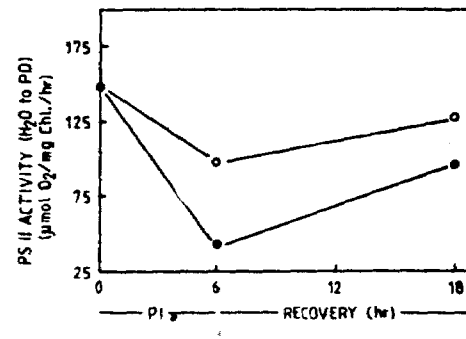

Fig. 1—Time-course of recovery of PS II activity assayed as H_2O to PD [Plants were photoinhibited at 650 Wm^{-2} at 30°C for 6 hr and then let to recover for 12 hr under growth conditions. (− ○ −), 12 hr recovery following 6 hr photoinhibition (no water stress); (− ● −), 12 hr recovery from combination of water stress (−22 bars) and photoinhibition]

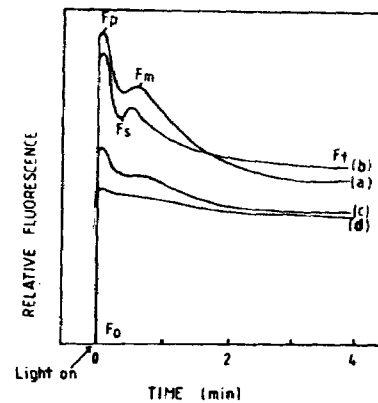

Fig. 2—Room temperature time course of 685 nm chlorophyll fluorescence (i.e., chlorophyll fluorescence from PS II associated chlorophyll only) in control (−6 bars, a); water stress (−22 bars, b); photoinhibited plants (no water stress, c); and combination of water stress, d) and photoinhibition (650 Wm^{-2} at 30°C for 6 hr [F_p, peak fluorescence; F_m, secondary maximum fluorescence; F_s, stationary fluorescence; F_o, initial fluorescence; and F_t, terminal fluorescence]

Discussion

Results presented above suggest that water stress alone (up to −22 bars) does not significantly affect the efficiency of PS II as F_v/F_m ratio remains unchanged. However, photosynthetic electron transport was affected slightly (10%, Table 1).

Table 2—Effect of water stress, photoinhibition (650 Wm^{-2} for 6 hr at 30°C and 10°C) and combination of the two on initial fluorescence (F_o), maximum fluorescence (F_m), variable fluorescence (F_v) and F_v/F_m ratio at room temperature in chloroplasts isolated from stressed leaves

Chlorophyll fluorescence (685 nm)	Control	WS	Photoinhibition at 30°C			Photoinhibition at 10°C		
			PI for 6 hr	WS + PI for 3 hr	WS + PI for 6 hr	PI for 6 hr	PI + PI for 3 hr	WS + PI for 6 hr
	−6	−22	−6	−23	−24	−6	−22	−22
				bars				
F_o	12	12	13	14	15	13	14	14
F_m	63	60	45	42	27	44	50	44
F_v	50	48	32	28	12	31	36	30
F_v/F_m	0.809	0.800	0.711	0.667	0.444	0.704	0.720	0.681

As F_o to F_p rise time remained unaffected, the efficiency of reduction by PS II of its acceptor pool is expected to be insensitive to water stress[12]. However, slowness of the F_p to F_t decline under drought suggests that reoxidation of Q_A and that of the PS II acceptor pool or the light-induced activation of PS I acceptor side are inhibited by water stress[15]. The observed decrease in F_p level due to photoinhibition and combination of photoinhibition plus water stress may suggest some damage to Q_A (ref. 18).

The fluorescence induction exhibits characteristic phases of fluorescence levels usually termed F_o, F_p, F_s, F_m and F_t (see Fig. 2). The F_o level of fluorescence is thought to represent emission by excited antenna chlorophyll, a molecule occurring before the excitation having migrated to the reaction centres[19-21], which is independent of photochemical events. The fluorescence rise after F_o is considered as variable fluorescence which reflects reduction of electron acceptor Q_A (refs 22-24). The F_p shows the state when all the PS II reaction centres are in the reduced state followed by F_s and F_m supposed to occur as a result of reoxidation of Q_A via the electron transport chain[25] and release of the *p*H gradient respectively. This is followed by F_t showing the equilibrium state of Q_A oxidation-reduction[23].

Photoinhibition of control plants causes damage to both F_v/F_m ratio and photosynthetic electron transport activity. The reduction in the electron transport activity assayed from H_2O to PD suggests that the damage due to photoinhibition is occurring to PS II while PS I was relatively insensitive to photoinhibition[27].

Photoinhibition of PS II activity is also suggested by the decrease in the room temperature fluorescence (where most of the fluorescence arise from PS II associated chlorophyll). The decrease in F_m may suggest decreased photoreduction of primary quinone acceptor Q_A or increased non-photochemical quenching[27]. The observed decrease in the F_v/F_m ratio may suggest a decrease in the efficiency of photon capture by the reaction centre[4,24].

The mechanism by which drought inhibits photosynthetic electron transport system is still unclear, although explanations have been advanced by many schools[6,12,28]. Sharkey and Seemann[7] observed that reduction in leaf (spinach) photosynthesis caused by mild water stress (-0.7 Mpa) is primarily due to stomatal closure, although probably without any damage to stomatal closure and chloroplast reactions. Ogren and Oquist[29] observed that drought-induced decline in CO_2 uptake was initially attributed equally to stomatal and non-stomatal factors, but the further decline as drought continued was solely due to non-stomatal factors.

Flowers *et al.*[30] observed that drought-resistant sorghum was able to maintain positive turgor to leaf water potential compared to drought-susceptible sorghum, but the response of stomatal conductance was similar in resistant and susceptible varieties. Similar results were observed by Stuhlfauth *et al.*[31]. Regarding the site of water stress there are various reports. Genty *et al.*[12] concluded that PS I-mediated electron transport was inhibited by water stress, whereas PS II activity was unaffected. They also reported that drought does not induce sensitiziation to photoinhibition. Contrary to this observation[29,32] there are also reports that PS II photochemistry is predisposed to photoinhibitory damage by drought stress.

Water stress along with photoinhibition treatment exacerbated the damage to PS II as well as efficiency of photon capture by PS II. This may be because water stress could predispose the photosynthetic apparatus to photoinhibition treatment thus lowering the light saturation rate of photosynthesis under water stress and thereby causing more damage[33,34]. This is also suggested by our work as the recovery from water stress and photoinhibited plants is slower than the recovery from the only photoinhibited plants (Fig. 1).

It is thus concluded that the effect of water stress alone on the primary photochemistry of photosynthesis is slight. However, in combination with other stresses (such as light and temperature) even moderate water stress exacerbates the damage many-fold by predisposing the plants to light stress damage and slowing down the recovery process.

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