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Impact of Optimal and Superoptimal Temperatures on the Photosynthetic Apparatus of Cotton Leaves

M. A. Sethar, V. Mala Pahoja, Q. I. Chachar, [1]D. L. Laidman
Department of Plant Physiology and Biochemistry, Sindh Agriculture University, Tandojam, Pakistan
[1]School of Biological Sciences, University of Wales Bangor, UK

Abstract: The leaves of cotton cultivars Qalandri, MNH-93, Rehmani and S-12 were stressed for 2 h at 30 (control), 35, 38, 40, 42, 43, 44, 45 and 46°C to quantify the damage caused to chloroplast photosynthetic apparatus by chlorophyll fluorescence technique. There was 10-30% decrease in Fv/Fm ratio up to the temperature stress of 40°C. But at 44°C the cultivars showed considerably higher damage to photosynthesis process by recovering only 10, 13, 37 and 50% of the Fv/Fm ratio by the cultivars MNH-93, Qalandri, Rehmani and S-12 respectively.

Key words: Chlorophyll fluorescence, temperature, cotton, stress leaves

Introduction

The response of plants to temperature are governed by the direct effects of temperature upon the chemical reactions occurring in the plant and indirect effect brought about via genetic and environmental interactions (Hochachka and Somero, 1973; Sutcliffe, 1977). There is moreover a limit of temperature that any crop can withstand. Higher or lower temperature beyond the acceptable range may lead to damaging effects. Global temperature is becoming higher day by day due to green house effects. Lawlor (1997) reported that global temperature would increase 4° C by 2100 AD and it would probably decrease the production of most crops about 25% for a 4°C rise in air temperature. High temperature is a stress that has a very strong impact on the natural geographical distribution and seasonal growth of plants. It is an important limiting factor for plant production in tropical and subtropical regions. The symbiotic nitrogen fixation was severely inhibited in soybean at 45°C (Keerio, 2001). Crop plants are especially vulnerable during the seedling stage because of their small size and soft and delicate nature of their tissues. In cotton, heat stress during the early growth phase is an important yield limiting-factor in countries such as Pakistan, where temperatures are often high at the time of germination and seedling emergence. The reactions of the plant thylakoid membranes are among the first processes to be damaged by heat (Mukohata *et al.*, 1973; Santarius, 1975; Simillie, 1979). In particular, high temperatures severely damage the water splitting photosystem II (PSII) activity and ATP formation (Santarius and Muller, 1979). Early detection of heat stress is important since prolonged heating usually causes a more extensive cellular damage. Moreover, in some cases heat injury may only become visibly apparent when the stress concerned is removed. By that time the valuable information on the early responses of the photosynthetic process to unfavourable factors can be provided by analysis of chlorophyll fluorescence of attached or detached leaves. Furthermore, chlorophyll fluorescence analysis enables the detection and quantification of stress injury before any visible symptoms occur.

Chlorophyll fluorescence has become one of the most widely used methods in plant stress physiology. The effects of certain environmental stresses, such as heat or chilling can be assessed rapidly. It can be used *in vivo* or *in vitro* (Larcher, 1980) and it is a fast, non-destructive and cheap method of assessment. Other alternative methods such as delayed light emission (DLE), restricted ferricyanide reduction caused by inhibition of water splitting photosystem II, changes in chlorophyll absorption, CO_2 exchange, respiration and membrane integrity are usually more costly and more time consuming.

The objectives of the study were to assess the utility of the chlorophyll florescence technique and to screen cotton cultivars for high temperature tolerance. The other purpose was to reveal the extent of damages caused by optimal and superoptimal temperatures to different cotton cultivars at the seedling stage.

Materials and Methods

The study was conducted at School of Biological Sciences, University of Wales, Bangor, (UK) in 1992-93. Seeds of cotton cultivar Qalandri, MNH-93, Rehmani and S-12 were obtained from various cotton research institute of Pakistan. Acid delinted and sterilized seeds were planted in germination trays for 15 days in a plant growth cabinet at 30/27°C with photo period of 16/8h day/night, respectively.

Under optimal conditions 85% of the light intercepted by a plant leaf is absorbed by photosynthetic pigments (in thylakoid membrane of chloroplast) and is used in photosynthesis. The remainder is lost as heat or is radiated as fluorescence. Most of the fluorescence is emitted from the chlorophyll photosystem II (PSII) and provides a non-destructive probe for examining the photochemical events of photosynthesis (Papageorgiou, 1975) and can be effectively used to monitor changes in the activities and organizations of the photosynthesis apparatus *in vivo* (Baker and Bradbury, 1981; Bilger *et al.*, 1987).

Measurement of chlorophyll fluorescence: The method of measurement of chlorophyll fluorescence has been described in Sethar *et al.*, 1994. The heat-stress treatments were carried out at 30 (control), 35, 38, 40, 42, 43, 44 or 46°C for cultivars Qalandri and MNH-93, while for other two cultivars i.e., Rehmani and S-12 the stress treatment of 45°C was also included. The fluorescence observations were recorded after 1 h of pre-incubation at 30°C, after 1 and 2 h of temperature stress, and after 2, 6, 12 and 24 h of the recovery at 30° C. Only Fv/Fm ratio are given in the tabular and graphical forms, since this ratio indicates the oxygen evolution during the process of photosynthesis.

Results and Discussion

The chlorophyll fluorescence (Fv/Fm ratio) decreased with increasing heat stress temperature (Table 1). This pattern was similar in all cultivars. Thus, during the first hour of stress the chlorophyll fluorescence ratio dropped sharply and it continued

Table 1: Effect of stress temperatures on leaf fluorescence in cotton (each Mean ± SE is from 20-40 leaves)

Stress temperature (°C)		Pre incubation	Heat stress		Recovery			
		1h	1h	2h	2h	6h	12h	24h
Fv/Fm ratio				Cv. Qalandri				
30	Mean	0.23	0.22	0.230	0.22	0.22	0.24	0.23
	(control) ±SE	0.01	0.01	0.010	0.01	0.01	0.01	0.01
35	Mean	0.23	0.22	0.210	0.23	0.24	0.22	0.20
	±SE	0.01	0.004	0.010	0.01	0.004	0.01	0.02
38	Mean	0.23	0.19	0.170	0.21	0.21	0.21	0.20
	±SE	0.01	0.01	0.010	0.01	0.01	0.01	0.02
40	Mean	0.23	0.14	0.130	0.15	0.19	0.21	0.17
	±SE	0.01	0.01	0.010	0.01	0.02	0.01	0.02
42	Mean	0.23	0.09	0.070	0.11	0.15	0.19	0.15
	±SE	0.01	0.01	0.010	0.01	0.01	0.01	0.02
43	Mean	0.23	0.09	0.080	0.10	0.14	0.16	0.13
	±SE	0.01	0.01	0.010	0.01	0.01	0.02	0.02
44	Mean	0.23	0.04	0.020	0.03	0.03	0.04	0.03
	±SE	0.01	0.004	0.004	0.01	0.01	0.01	0.02
46	Mean	0.23	0.01	0.004	0.01	0.01	0.01	0.01
	±SE	0.01	0.002	0.002	0.002	0.01	0.01	0.002
Percentage of control								
35		100	100	91	104	109	92	87
38		100	86	74	95	95	88	87
40		100	64	57	68	86	88	74
42		100	41	30	50	68	79	65
43		100	41	35	45	64	67	57
44		100	18	09	14	14	17	13
46		100	05	02	05	01	04	04
Fv/Fm ratio				Cv. MNH-93				
30	Mean	0.22	0.22	0.21	0.22	0.22	0.22	0.21
(control)	±SE	0.01	0.01	0.01	0.01	0.01	0.01	0.01
35	Mean	0.22	0.20	0.19	0.20	0.22	0.20	0.19
	±SE	0.01	0.01	0.01	0.01	0.01	0.01	0.01
38	Mean	0.22	0.18	0.17	0.19	0.21	0.20	0.20
	±SE	0.01	0.01	0.01	0.01	0.01	0.004	0.01
40	Mean	0.22	0.15	0.13	0.15	0.18	0.19	0.18
	±SE	0.01	0.01	0.01	0.01	0.01	0.01	0.01
42	Mean	0.22	0.12	0.10	0.12	0.17	0.18	0.16
	±SE	0.01	0.01	0.01	0.01	0.01	0.01	0.01
43	Mean	0.22	0.09	0.08	0.10	0.14	0.17	0.15
	±SE	0.01	0.01	0.01	0.01	0.01	0.02	0.02
44	Mean	0.22	0.04	0.02	0.01	0.02	0.02	0.02
	±SE	0.01	0.01	0.01	0.002	0.01	0.004	0.01
46	Mean	0.22	0.02	0.004	0.01	0.01	0.01	0.01
	±SE	0.01	0.002	0.002	0.002	0.002	0.01	0.004
Percentage of control								
35		100	91	90	91	100	91	90
38		100	82	81	86	95	91	95
40		100	68	62	68	82	86	86
42		100	55	48	55	77	82	76
43		100	41	38	45	64	77	71
44		100	18	10	05	09	09	10
46		100	09	02	05	05	05	05
Fv/Fm ratio				Cv. Rehmani				
30	Mean	0.24	0.24	0.26	0.270	0.260	0.250	0.270
(control)	±SE	0.01	0.01	0.01	0.004	0.001	0.004	0.002
35	Mean	0.24	0.24	0.23	0.250	0.250	0.250	0.220
	±SE	0.01	0.01	0.01	0.004	0.004	0.004	0.010
38	Mean	0.24	0.19	0.18	0.220	0.230	0.230	0.210
	±SE	0.01	0.01	0.01	0.010	0.010	0.010	0.004
40	Mean	0.24	0.18	0.16	0.190	0.220	0.200	0.190
	±SE	0.01	0.01	0.01	0.010	0.010	0.010	0.010
42	Mean	0.24	0.12	0.09	0.110	0.150	0.160	0.140
	±SE	0.01	0.01	0.01	0.020	0.020	0.020	0.020
44	Mean	0.24	0.06	0.04	0.070	0.090	0.080	0.100
	±SE	0.01	0.01	0.01	0.010	0.020	0.02	0.01
45	Mean	0.24	0.03	0.02	0.020	0.020	0.02	0.02

	±SE	0.01	0.01	0.01	0.010	0.010	0.01	0.01
46	Mean	0.24	0.02	0.01	0.010	0.000	0.02	0.02
	±SE	0.01	0.01	0.01	0.010	0.004	0.01	0.01
Percentage of control								
35		100	100	88	93	96	100	81
38		100	79	69	81	88	91	78
40		100	75	62	70	85	80	70
42		100	50	35	41	58	64	52
44		100	25	15	26	35	32	37
45		100	13	08	07	08	08	07
46		100	08	04	04	04	08	07
Fv/Fm ratio			Cv. S-12					
30	Mean	0.24	0.25	0.25	0.25	0.25	0.23	0.20
(control)	±SE	0.01	0.01	0.004	0.01	0.004	0.004	0.01
35	Mean	0.24	0.21	0.20	0.21	0.23	0.22	0.19
	±SE	0.01	0.01	0.01	0.01	0.004	0.004	0.01
38	Mean	0.24	0.17	0.17	0.18	0.20	0.20	0.19
	±SE	0.01	0.01	0.01	0.01	0.01	0.01	0.01
40	Mean	0.24	0.13	0.12	0.15	0.19	0.20	0.18
	±SE	0.01	0.01	0.01	0.01	0.01	0.01	0.01
42	Mean	0.24	0.10	0.09	0.12	0.16	0.17	0.16
	±SE	0.01	0.01	0.01	0.01	0.01	0.01	0.01
44	Mean	0.24	0.07	0.06	0.07	0.12	0.12	0.10
	±SE	0.01	0.01	0.01	0.01	0.01	0.01	0.01
45	Mean	0.24	0.02	0.01	0.02	0.02	0.02	0.02
	±SE	0.01	0.01	0.002	0.01	0.004	0.01	0.002
46	Mean	0.24	0.01	0.003	0.01	0.01	0.01	0.01
	±SE	0.01	0.002	0.01	0.01	0.004	0.004	0.01
Percentage of control								
35		100	84	80	84	92	96	95
38		100	68	68	72	80	87	95
40		100	52	48	60	76	87	90
42		100	40	36	48	64	74	80
44		100	28	24	28	48	52	50
45		100	08	04	08	08	09	10
46		100	04	01	04	04	04	05

up to the end of 2h of stress. When the leaves were put back to 30°C for recovery the chlorophyll fluorescence tended to increase. This increase was confined to certain temperature treatments, however. Those temperatures were from 35 to 43°C for cultivars Qalandri and MNH-93, and from 35 to 44°C for cultivars Rahmani and S-12. Beyond this upper limit of 43-44°C the ratio did not recover in any of the cultivars. At stress temperatures above 44°C there was not only a negligible recovery in the Fv/Fm ratio but also 95% leaves turned brown in colour either completely or partially. It is also worth mentioning that both the large and small leaves turned brown. There was however, a positive correlation between the failure of chlorophyll fluorescence to recover and the browning of the leaves. Table 1 also show the Fv/Fm ratio in terms of percentage of the control (30°C) values. Temperature stress up to 38°C has little or no permanent effect on the Fv/Fm values in all the cultivars. In cultivars Qalandri and MNH-93 (after 2h stress) the Fv/Fm ratio decreased to 30 and 48% respectively of the control when they were stressed at 42°C. The recovery after 24h was considerably higher, rising to 65 and 76% respectively. Even though stress at 43°C strongly lowered the fluorescence values in both cultivars, recovery after 24h was nearly equal to the recovery observed following stress at 42°C. Above 43°C, both cultivars responded poorly and they were unable to recover more than 13% of their control fluorescent values after 24h. Cultivars Rehmani and S-12 gave a relatively better performance up to a stress temperature of 44°C where their fluorescence decreased to 37 and 50% respectively of their controls following 24h recovery at 30°C.

Fig. 1 presents the results more clearly that the decrease in the Fv/Fm value was rapid during the 1st h of heat stress and that it decreased more slowly during the 2nd h of stress. The fluorescence loss during this period also increased with increasing stress temperature. When the leaves were put back to 30°C for recovery, there was a general increase in the Fv/Fm values for about 12h. There then followed a decline in the Fv/Fm between 12 and 24 hour. The improvement during the first 12h of the recovery period is most obvious following stress up to 43°C. At higher stress temperatures there was clearly little recovery however. The statistical analysis of temperature stress treatments were significant ($p < 0.001$) and the cultivar differences were significant at $p < 0.05$.

The cultivar differences can be seen more clearly when their Fv/Fm values for stress at 44°C are plotted together (Fig. 2). It can be seen that cultivars S-12 and Rehmani have a better performance throughout the experimental period. Although the Fv/Fm values for cv. Rehmani are generally lower than those of cv. S-12, they approach the cv. S-12 values at the end of 24h recovery period. Cultivars Qalandri and MNH-93 gave significantly lower values than those of Rehmani and S-12 throughout the experimental period at this temperature treatment.

Cotton leaves were not damaged up to 5 min. during the incubation at 45°C but a 30 minutes stress at this temperature by 60-75% in cotton cultivars (Sethar *et al.*, 1994). Heat stress at longer times however, caused a progressive decline in fluorescence (Fv/Fm ratio). These results agreed well with

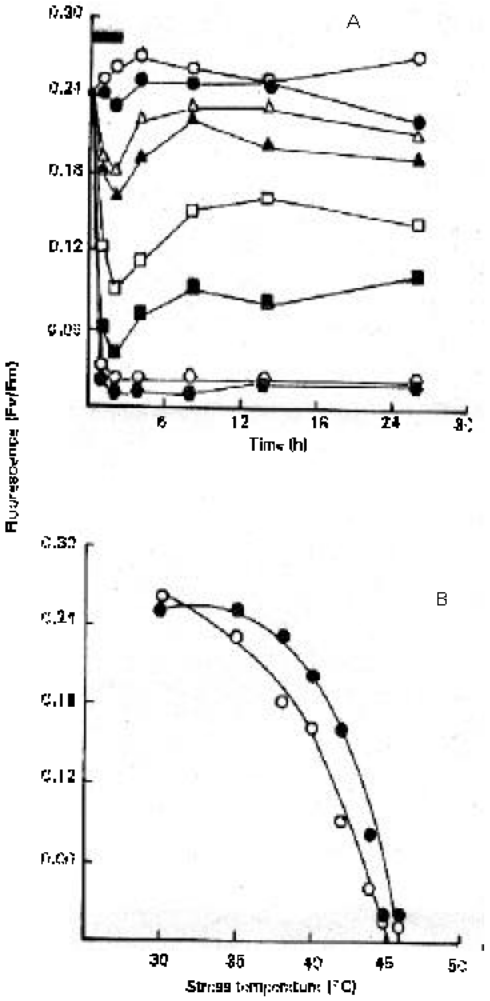

Fig. 1: Effect of temperature on leaf fluorescence in cv Rehmani. A, time course of fluorescence during and after heat stress at given temperatures. ○, 30°C; ●, 35°C; △, 39°C; ▲, 42°C; □, 43°C; ■, 44°C; ○, 45°C; ●, 47°C. B, Plots of fluorescence against stress temperature °C, at the end of 2 h heat stress (●) and after 12 h recovery. The bar at the top left of Figure A represents the period of heat stress.

the findings of Gounaris *et al.* (1984) that granal attachment sites in isolated chloroplast from broad bean were stable for up to 5 min when incubated at 45°C, but not beyond. It is generally agreed that reduction in chlorophyll fluorescence in respect to temperature stress are indicative of chloroplast thylakoid damage. Either chilling or heat stress applied to leaves elicits such decreases in induced chlorophyll fluorescence (Schreiber and Berry, 1977; Smillie, 1979; Simillie and Gibbons, 1981; Hetherington *et al.*, 1983; Potvin, 1985; Wolf *et al.*, 1986).

Photosynthetic inhibition under heat stress can normally be divided into reversible and irreversible changes. Reversible changes are observed at sub-lethal temperature, while irreversible effects at higher temperatures are believed to be a reflection of the true susceptibility of the photosynthetic apparatus to heat. The rate of reduction in fluorescence during the course of heat stress and its recovery at subsequent lower temperatures is a good indicator of the degree of heat

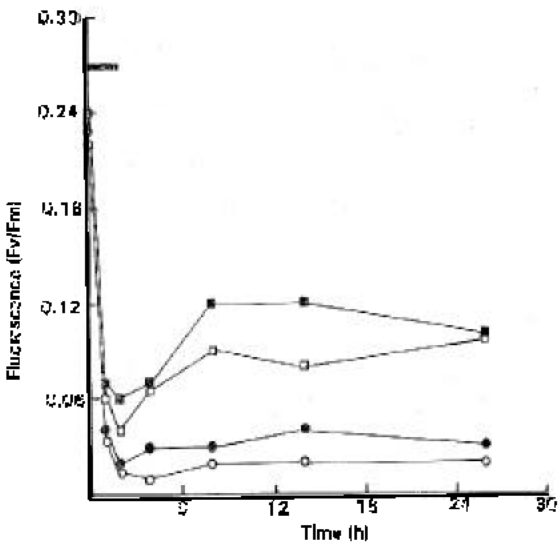

Fig. 2: Effect of heat stress at 44°C on leaf fluorescence. ○, MNH-93; ●, Qalandari; □, Rehmani; ■, S-12. The bar at the left of the figure represents the period of heat stress.

tolerance of a plant and its capacity to recover. The results obtained fit this general pattern. The decline in the fluorescence values following 24h recovery (compared with 12h recovery) might be due to aging of the stressed leaves or due to some other kind of deterioration.

Evaluation of fluorescence parameters recorded in the cotton cultivars suggested that the "optimum" temperature lies between 30 and 40°C and, plants can tolerate these temperatures and make compensating adjustments to their photosynthetic membranes. Substantial changes in fluorescence characteristics were revealed in the range between 40 and 44°C, indicating that more permanent thermally-induced alterations of the thylakoid membrane had taken place. At heat stress temperatures between 40 and 44°C, cultivars Rehmani and S-12 proved more stable than cultivars Qalandri and MNH-93. At 45°C, the fluorescence values in all the cultivars dramatically decreased with no recovery at 30°C. Bukhov *et al.* (1987) reported similar results for pea, barley and wheat leaves where the variable fluorescence sharply decreased at temperatures above 37°C with little ability to recover. This indicates the irreversible suppression of the reaction centers of PS II (Schreiber and Armond, 1978; Havaux *et al.*, 1988). The results reported by Berry and Raison (1981) working on macrophytes and Bilger *et al.* (1987) with *Arbutus unedo* also supported the findings reported here and concluded that at superoptimal temperatures above 40°C, thermal disruption of the thylakoid membranes and the related impairment of photosynthesis occurs. The photosynthetic rate in leaves of soybean was decreased by 12.5% with the increase in the leaf temperature of 2.83°C (from 35.1 to 39.93°C) as stated by Begum Samsun Nahar and Ikeda, 2001. Larcher *et al.* (1990) similarly reported that Fv/Fm ratio was unchanged up to 40°C in *Vigna unguiculata*, but perturbations occurred above this temperature. The leaves of the desert shrub *Lorrea divaricata* and of *Spinacia oleracea* exposed to high temperatures between 35 and 45°C exhibited characteristic temperature-

dependent damage to their photosynthetic apparatus (Armond *et al.*, 1978; Downton and Berry, 1982). It has also been noted by Gounaris *et al.* (1984) that granal membranes tend to destack in broad bean chloroplasts at temperatures above 45°C; incubation at temperatures from 45 to 55°C resulted in complete breakdown of the thylakoid membrane to form isolated vesicles. It is concluded that there was little damage occurred up to 38°C, beyond this temperature the damage seems to be considerably high. At stress of 45°C and above the photosynthetic damage was about 90%. Cultivars Rehmani and S-12 showed good tolerance at 44°C than cultivars Qalandri and MNH-93.

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