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HEAT ACCLIMATION POTENTIAL OF CHLOROPHYLL FLUORESCENCE OF COTTON CULTIVARS

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Abstract

Cotton cultivars Qalandri and MNH-93 were analysed for their heat acclimation potential. The 8-12 days old leaves were given repeated hardening treatments at 42°C for 2h for upto 7 days and after every treatment the leaves were heat stressed at 44°C for 2h. The heat acclimation potential was analysed after 24h recovery period at 30°C. The results showed that cv. MNH-93 has fairly good acclimation potential than cv. Qalandri by recovering 63% Fv/Fm ratio as compared 50% in cv. Qalandri indicating that adaptive changes in PS-II were more effective in cv. MNH-93 than cv. Qalandri.

Introduction

In most living organisms including plants, when environmental temperatures reach a certain level, an acclimation mechanism is triggered resulting in increased heat tolerance. The capability of plants to harden to high temperature is an important factor in determining the plants performance in high temperature environments. Chen *et al.*, (1982) observed significant differences in heat tolerance in beans, soybean, potato and tomato species after plants had been hardened. They suggested that the most efficient genotypes were those which possessed high potential for heat acclimation. It may be mentioned that not all the species responded to the acclimation process. Some species acclimate extensively in response to environmental stress while others acclimate only to a small extent and some species do not acclimate at all (Burke *et al.*, 1976).

Plants that adjust to high temperature environments also show adaptation in physiological and morphological characters. Physiological adaptations to high temperature include adaptation to photosynthesis, respiration, distributions of assimilates and reproduction. Huner *et al.*, (1984); Quinn & Williams (1985) found that many plants are able to harden to high temperatures by increasing the optimum temperature for photosynthesis. Since photosynthesis thylakoid membranes are particularly heat sensitive, exposure of a plant to supra-optimal, sub-lethal temperatures may result in acclimation of the photosynthetic apparatus and improved photosynthetic activity. Feldman & Ageeva (1973) found that hardening increased the thermostability of membrane bound (photosynthetic) ferredoxin in pea plants. Quinn & Williams (1985); Tarzaghi *et al.*, (1989) further suggested that high temperature adaptation occurs due to alterations in the lipid and/or protein composition of the photosynthetic membranes.

Experiments were carried out to evaluate the effectiveness of short temperature treatments on the hardening capabilities of two promising Pakistani cotton cultivars at sub-lethal high temperatures. The acclimation potential was analysed by the measurement of chlorophyll fluorescence which has proved to be a useful and nondestructive method.

Materials and Methods

The study was undertaken at School of Biological Sciences, University of Wales, Bangor (UK) in the year 1992-93. The delinted, sterilized cotton seeds of cultivars Qalandri and MNH-93 obtained from Cotton Research Institutes Multan and Sakrand, Pakistan were germinated in growth cabinet (Fitotran 600) with a day/night temperatures of 30/27°C and 16/8h light and dark photoperiod cycle. At 8 to 12 days after sowing the plants were transferred at midday (being 6h into the light period) to another incubator for hardening treatment at 42°C in dark for a period of 2h. The plants were then returned to the growth cabinet at 30°C. These hardening treatments were repeated for upto 7 days. Leaves from heat hardened plants were then subjected to heat stress for 2h at 44°C before being allowed to recover for upto 24h at 30°C. The heat stress temperature of 44°C was so selected that it had been proved almost lethal for the above said cultivars which did not recover after stressed for 2h (Sethar *et al.*, 2001). Chlorophyll fluorescence was determined at the end of pre-incubation period (for 30 min at 30°C) after each hour during heat stress and after 2, 6, 12 and 24h during the recovery period. The chlorophyll fluorescence measurement procedure has been described by Sethar *et al.*, (1995). The ratio of variable and maximum fluorescence (Fv/Fm) is reported since it gives the quantum yield of oxygen evolution during the process of photosynthesis in plant leaves. The experiment was conducted in 3 replications.

Results and Discussion

In tables the fluorescence data are presented in 2 ways, one as the absolute Fv/Fm values and the other as the percentage of the values recorded at the end of the dark pre-incubation period (Figs. 1A and 2A, Tables 1 and 2) . In the cv. Qalandri there was some evidence of induced hardening between 4-7 days of hardening treatments (Table 1). Only 25-29% of the original Fv/Fm values was recovered after 3 days hardening and 12h of recovery. After 4 more days of hardening however, this cultivar showed greater heat hardiness and the Fv/Fm value after a recovery period of 12h had recovered to 42-50% of the value recorded at the end of the preincubation period.

The cv. MNH-93 had been severely damaged by a 2h heat stress following a single hardening treatment (Table 2). In this treatment however, a gradual improvement was observed during the recovery period when the plants regained 38% of their starting Fv/Fm value. Plants subjected to 2 hardening treatments showed a significantly better performance in comparison to control leaves which had received no hardening. Although there was a sharp decline in the Fv/Fm ratio to 38% at the end of the 2h heat stress period, it increased to 58% after 12h recovery. After 3 days of hardening treatment the plants performed poorly compared with 2 days of treatment but they improved again after being hardened for 4 days. In the 4-day treatment the fluorescence dropped to 42% at the end of 2h heat stress and recovered to 63 and 67%, respectively, after 6 to 12h recovery periods. The value dropped slightly again to 63% after 24h recovery. Hardening treatments for more than 4 days produced lower fluorescence ratios compared with the 1-4 days treatments.

The derivative plots were prepared using the Fv/Fm values from 2h heat stress and from 12h in the recovery period (Figs. 1A and 2A, Figs. 1B and 2B) where the Fv/Fm values for each hardening treatment are plotted against the number of hardening days. The figures

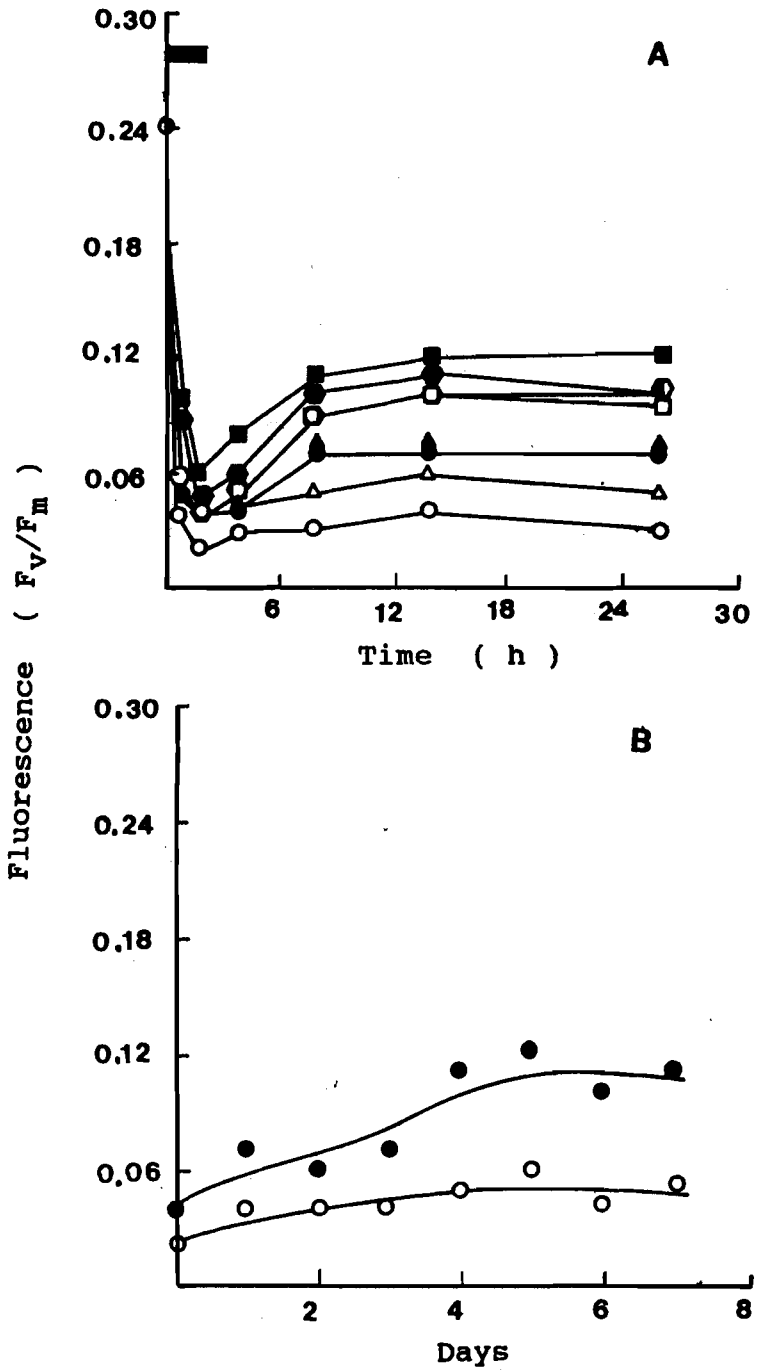

Fig. 1. Effect of hardening of leaf fluorescence in cv Qalandari A, time course of fluorescence during and after heat hardening for 0 to 7 days. ○, no hardening (control); ●, 1 day; Δ, 2 days; ▲, 3 days; , 4 days; ■, 5 days; ⬡, 6 days; ⬢, 7 days; B, plot of fluorescence against hardening period. O, after 2 h heat stress; ●, after 12 h recovery. The bar at the top left of Figure A represents the period of heat stress.

Table 1. Effect of whole plant hardening on leaf fluorescence in cotton cv. Qalandri.

Hardening days		Preincu-bation	Heat stress 44°C		Recovery 30°C			
		1h	1h	2h	2h	6h	12h	24h
FV/Fm ratio								
Control (no hard.)	Mean	0.24	0.04	0.02	0.03	0.03	0.04	0.03
	±se	0.01	0.004	0.004	0.01	0.01	0.01	0.01
1	Mean	0.24	0.05	0.04	0.04	0.07	0.07	0.07
	±se	0.01	0.01	0.01	0.01	0.01	0.01	0.01
2	Mean	0.24	0.05	0.04	0.04	0.05	0.06	0.05
	±se	0.01	0.01	0.01	0.01	0.01	0.02	0.02
3	Mean	0.24	0.05	0.04	0.04	0.07	0.07	0.07
	±se	0.01	0.01	0.01	0.01	0.02	0.02	0.02
4	Mean	0.24	0.09	0.05	0.05	0.10	0.11	0.10
	±se	0.01	0.01	0.01	0.01	0.02	0.02	0.02
5	Mean	0.24	0.10	0.06	0.08	0.11	0.12	0.12
	±se	0.01	0.01	0.01	0.01	0.02	0.02	0.02
6	Mean	0.24	0.06	0.04	0.05	0.09	0.10	0.10
	±se	0.01	0.01	0.004	0.01	0.01	0.01	0.01
7	Mean	0.24	0.08	0.05	0.06	0.10	0.11	0.10
	±se	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Percentage of pre-incubation (Fv/Fm) values								
Control (no hard.)		100	17	08	13	13	17	13
1		100	21	17	17	29	29	29
2		100	21	17	17	21	25	21
3		100	21	17	17	29	29	29
4		100	38	21	21	42	48	42
5		100	42	25	33	46	50	50
6		100	25	17	21	38	42	42
7		100	33	21	25	42	46	42

Each mean ±se is from 20-40 leaves.

display clearly the differences in the results obtained for both cultivars. The curve relating to cv. Qalandri shows a substantial increase between 3 and 5 days. A small increase is evident for the Fv/Fm values recorded at the end of the 2h stress period. In Fig. 2B for cv. MNH-93 the scatter in the data points makes it difficult to plot accurate curves. Nevertheless, the general trend is clear and it is obvious that the best hardening response was obtained in leaves which had been hardened for 2 to 4 days. Statistically the results are significant ($P<0.05$).

During the study the general appearance of the leaves also gave valuable information regarding the effects of hardening. In the leaves given no hardening treatment, about 90-95% of them turned brown either fully or partially following heat stress, but little or no browning was noted in the heat hardened leaves.

Hardening treatments repeated over a number of days gave markedly better heat tolerance than the control treatment (unhardened leaves) and differences were detected between cultivars. In cv. Qalandri the recovery in leaf fluorescence after heat stress was

Table 2. Effect of whole plant hardening on leaf fluorescence in cotton cv. MNH-93.

Hardening days		Preincu-bation	Heat stress 44°C		Recovery 30°C			
		1h	1h	2h	2h	6h	12h	24h
FV/Fm ratio								
Control (no hard.)	Mean	0.02	0.02	0.01	0.02	0.02	0.02	0.02
	±se	0.01	0.01	0.004	0.01	0.01	0.004	0.004
1	Mean	0.24	0.09	0.04	0.05	0.08	0.09	0.09
	±se	0.01	0.01	0.01	0.01	0.02	0.02	0.02
2	Mean	0.24	0.11	0.09	0.09	0.12	0.14	0.13
	±se	0.01	0.01	0.01	0.02	0.02	0.02	0.02
3	Mean	0.24	0.11	0.07	0.05	0.08	0.10	0.10
	±se	0.01	0.01	0.01	0.01	0.02	0.02	0.02
4	Mean	0.24	0.12	0.10	0.09	0.15	0.16	0.15
	±se	0.01	0.01	0.02	0.02	0.02	0.02	0.01
5	Mean	0.24	0.11	0.07	0.07	0.09	0.11	0.09
	±se	0.01	0.01	0.02	0.01	0.02	0.02	0.02
6	Mean	0.24	0.08	0.07	0.07	0.10	0.12	0.12
	±se	0.01	0.01	0.01	0.01	0.02	0.02	0.01
7	Mean	0.24	0.09	0.06	0.07	0.11	0.11	0.10
	±se	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Percentage of pre-incubation (Fv/Fm) values								
Control (no hard.)		100	08	04	08	08	08	08
1		100	38	17	21	33	38	38
2		100	46	38	38	50	58	54
3		100	46	29	21	33	42	42
4		100	50	42	38	63	67	63
5		100	46	29	29	38	46	38
6		100	33	29	29	42	50	50
7		100	38	25	29	46	46	42

Each mean ±se is from 20-40 leaves.

found to be significantly lower than that in cv. MNH-93; where cv. Qalandri recovered only about 50% of the Fv/Fm ratio, while cv, MNH-93 regained 63% of Fv/Fm values of the control. This indicates that the hardening process was not working at its full capacity in cv. Qalandri.

Bauer & Senser (1979) in a similar experiment on ivy leaves, reported that the Hill reaction in heat hardened plants was inactivated immediately after heat stress at 46°C to about the same extent as that of unhardened plants. The heat hardened plants however, recovered four times faster as compared to unhardened plants. Similar results were reported by Sethar *et al.*, (1995) in cotton leaves that very poor hardening potential was observed at heat stress of 46°C (leaves were hardened at 42 or 44°C for 2h). However, fairly good response was obtained where leaves were stressed at only 1°C lower temperature i.e 45°C (leaves were hardened at 43°C for 2h). Such similar observations have been made with *Tradescantia fluminesis* (Barbalchuck & Chernyavskaya, 1974) that heat hardening in the range of 34-44°C resulted in increased thermo-resistance and the most effective acclimation occurred after hardening in the 38-42°C range.

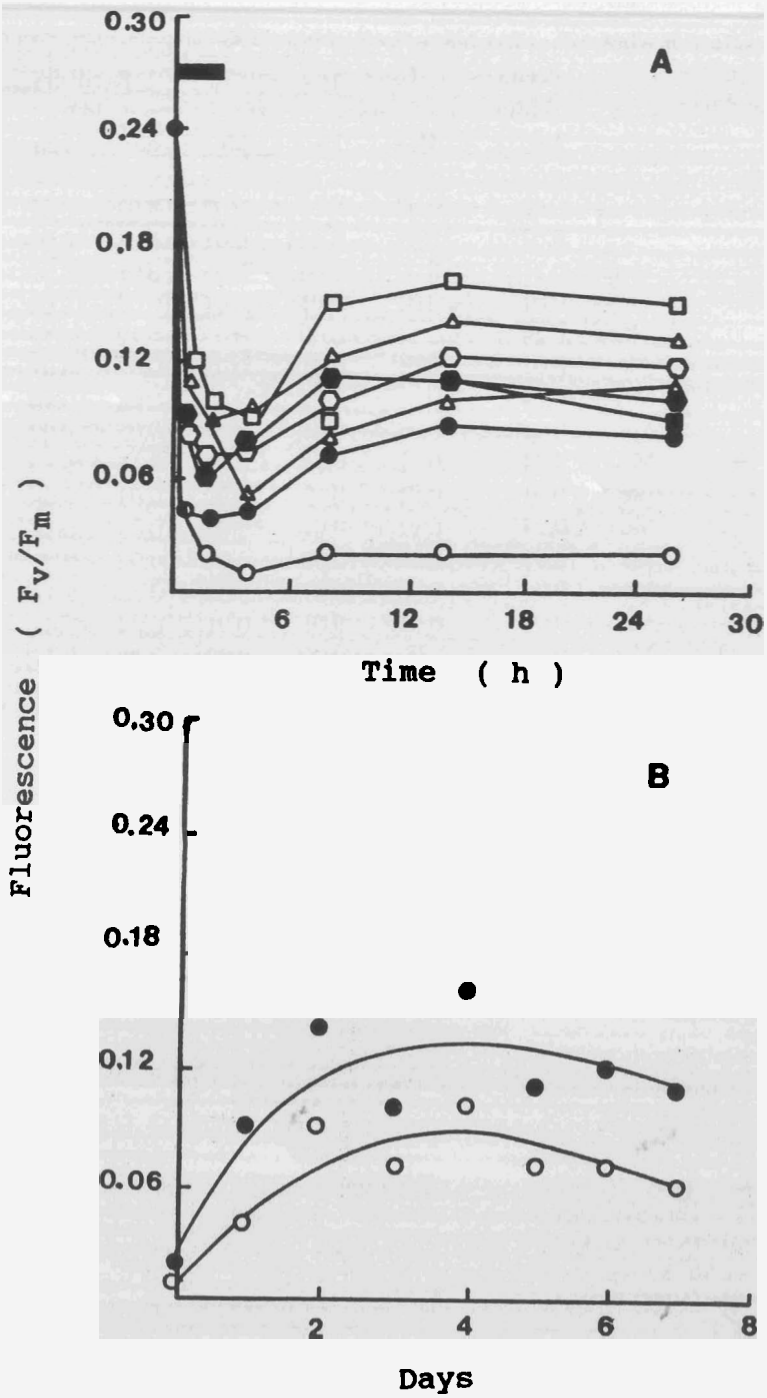

Fig. 2. Effect of hardening of leaf fluorescence in cv MNH-93 A, time course of fluorescence during and after heat hardening for 0 to 7 days. ○, no hardening (control); ●, 1 day; Δ, 2 days; ▲, 3 days; , 4 days; ■, 5 days; ⬡, 6 days; ⬡, 7 days; B, plot of fluorescence against hardening period. O, after 2 h heat stress; ●, after 12 h recovery. The bar at the top left of Figure A represents the period of heat stress.

Due to the natural selection of genotypes, each species has an optimum temperature range for growth, but this can be shifted substantially in some species by applying short term acclimation treatments. Alexandrov (1964) stated that heat hardening is a specific reaction of the plant cell towards the injurious action of the heat. The acclimation mechanism however, works only within a narrow range of temperatures and became less efficient at temperatures above or below this (Blum, 1986; Guye *et al.*, 1987; Yelenosky & Guye, 1989).

There is evidence regarding the acclimation of photosynthesis at low temperatures. Woledge & Dennis (1982) reported that although temperature has a well known direct effect on the rate of leaf photosynthesis where, the light saturated rate in rye grass and white clover grown at 15°C reduced by half when measurements were made at 5°C. There was some acclimation in that the amount by which photosynthetic rate decreased by low temperature was less in plants which have been grown at low temperature (Woledge & Dennis, 1982; Nosberger *et al.*, 1983). The temperature acclimation may have increased the photosynthesis of canopies of rye grass and white clover (Woledge *et al.*, 1989). Howarth (1991) found that acclimation of a plant at sub-lethal temperature may also induce the synthesis of heat shock proteins (hsp) which protect the plant from the potentially damaging effects of the later and more severe high temperature stress. Burke *et al.*, (1985) further stated that quite a few hsp accumulated in cotton leaves at canopy temperature of 40°C for several weeks.

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