Pak.J.Bot., 27(1): 165-168, 1995.

HARDENING POTENTIAL IN COTTON (*GOSSYPIUM HIRSUTUM L.*) AT HIGH TEMPERATURE STRESS

M.A. SETHAR, *D.L. LAIDMAN, *J.M. WILSON AND R.B.MIRBAHAR

Department of Physiology and Biochemistry, Sindh Agriculture University Tandojam, Pakistan.

Abstract

Leaves of cotton cultivar S-12 were acclimated at temperatures ranging from 35 to 44°C for different time periods followed by heat stress at 45 or 46°C. The heat acclimation potential was analyzed by Chlorophyll Fluorescence technique. The recovery of F_v/F_m ratio after application of heat stress was the parameter for determination of the acclimation potential. Very small fluorescence (F_v/F_m) values were recorded after heat stress at 46°C with fairly good response when acclimated leaves were heat stressed at 45°C.

Introduction

One of the main factors determining the ability of plants to adapt to different growth temperatures is their ability to modify their membrane constituents in order to function normally under new conditions. Heat damage to biomembranes may be prevented by the synthesis or accumulation of protective compounds in or near the membrane. There are reports that various water soluble compounds such as sugars and proteins are able to protect sensitive cell structures against heat inactivation (Santarius, 1973; Krause & Santarius, 1975; Santarius & Muller, 1979). Feldman & Ageeva (1973) have found that hardening increased the thermostability of membrane-bound (photosynthetic) ferridoxin in pea plants.

The capability of plants to harden to high temperature is an important factor in determining the plant's performance in high temperature environments. Plants that adjust to high temperature environments also show adaptation in physiological and morphological characters. Physiological adaptations to high temperature includes adaptation to photosynthesis, respiration, distribution of assimilates and reproduction. Huner *et al.*, (1984); Quinn & Williams (1985) reported that many plants are able to harden to high temperatures by increasing the optimum temperature for photosynthesis. Since photosynthetic thylakoid membranes are particularly heat sensitive, exposure of plants to supra-optimal, sub-lethal temperatures may result in acclimation of the photosynthetic apparatus and improved photosynthetic ability. Experiments were therefore carried out to study the hardening potential in cotton leaves subjected to high temperature stress.

*School of Biological Sciences, University of Wales, Bangor, UK.

Table 1. Effect of hardening treatments on chlorophyll fluorescence (F_v/F_m) in leaves of cotton cv. S-12.

Hardening temperatures (°C)		Pre-incubation 1h	Hardening 1h	Hardening 2h	Recovery 6h	Heat stress 1h	Heat stress 2h	Recovery 12h	Recovery 24h
30 (control)	Mean	0.23	0.22	0.22	0.24	0.24	0.22	0.23	0.23
	Sd±	0.04	0.04	0.04	0.03	0.03	0.06	0.03	0.02
42	Mean	0.23	0.12	0.12	0.19	0.03	0.02	0.04	0.04
	Sd±	0.04	0.04	0.04	0.07	0.03	0.03	0.04	0.05
44	Mean	0.23	0.08	0.06	0.08	0.02	0.02	0.03	0.03
	Sd±	0.04	0.03	0.04	0.06	0.03	0.04	0.06	0.07

The mean values are F_v/F_m ratios, Each mean ±Sd is from 20 leaves. Heat stress was at 46°C and pre-incubation or recovery was at 30°C.

Materials and Methods

The study was carried out at School of Biological Sciences, University of Wales, Bangor, UK during 1991-92. Acid-delinted, sterilized seeds of cotton Cv. S-12 obtained from Cotton Research Station Multan, Pakistan were germinated in a growth cabinet, Fitotran 600 with a day/night temperatures of 30/27°C and 16h light and 8h dark photoperiod cycle.

Expt. 1. Leaves from 15 day old plants placed on a fluorometer sample holder were dark acclimated for 1h at 30°C on a temperature plate then transferred to 42 or 44°C for 2h for hardening treatment. The sample holder was then transferred to 30°C for 6h recovery period followed by heat stress at 46°C for 2h then transferred to 30°C for 24h recovery.

Expt. 2. The plants were grown for 12 days as described above. On the 13th day, the plants were transferred to another growth cabinet with a temperature of 35, 40 or 42°C for 2 days for hardening treatment. The plants were then again transferred to 30/27°C light/dark growth cabinet. On the next day the leaves were excised and dark acclimated for 1h at 30°C followed by heat stress at 46°C for 2h. The leaves were then transferred to 30°C for 24h recovery.

Expt. 3. The leaves were hardened in a dark incubator instead of growth cabinet at 44°C for 4h (single treatment) or 2h daily for 7 days. The leaves were then excised and dark acclimated for 1h at 30°C followed by a 2h heat stress at 46°C. The leaves were then transferred to 30°C for 24h recovery.

Expt. 4. Since there was no evidence of hardening obtained from above experiments, the heat stress temperature was therefore slightly lowered from 46 to 45°C. The plants were given a single hardening treatment at 43°C for 2h in a dark incubator and on the following day the excised leaves were dark acclimated for 1h at 30°C followed by a 2h heat stress at 45°C. The leaves were then transferred to 30°C for 24h recovery.

All the movements of fluorometer sample holder alongwith leaves, from one temperature plate to another temperature plate were made in dark. The temperature of temperature plates were controlled by circulating water baths. The fluorescence readings were made in photographic bags. The fluorescence induction curves were obtained using the portable Kaustky apparatus (Model SF-20, Plant productive Fluorometer). At the time of reading, the leaves were illuminated for 5 seconds with a light emitting diode (LED) and the resulting fluorescence curves recorded over the same 5 second period on a potentiometric recorder. From the curve F_0 (initial fluorescence), F_m (maximum fluorescence), F_v (variable fluorescence, F_m-F_0) and the ratio of variable and maximum fluorescence (F_v-F_m) were recorded since it gives the quantum yield of oxygen evolution.

Results and Discussion

From experiments 2 and 3 negligible values of F_v/F_m were recorded after 24h recovery at 30°C. A 2h of hardening at 42°C decreased the F_v/F_m ratio by approximately 50% of the value recorded at the end of 1h pre-incubation (Table 1). The F_v/F_m values however, were approximately 83% of the pre-incubation fluorescence value at the end of first 6h recovery period. Subsequent heat stress for 2h at 46°C decreased the value to about 8% of the original value slightly increased during the final recovery period. Hardening for 2h at 44°C resulted in a greater decrease in the F_v/F_m value than observed in the hardening at 42°C treatment. The subsequent recovery at 30°C also did not improve the fluorescence status of the leaves. It seemed that the hardening at temperatures between 42-44°C followed by heat stress at 46°C may have produced a general deterioration in the leaves and poor acclimation.

The fluorescence values for hardened plants decreased from 0.23 at the end of pre-incubation period to 0.03 at the end of 2h heat stress at 45°C which then increased to 0.09 after 12h recovery and remained unchanged upto 24h recovery period at 30°C (Table 2). This represents a recovery to about 39% of the starting fluorescence value.

Table 2. Effect of whole plant hardening treatments on leaf fluorescence.

Hardening temperature (°C)		Pre-incubation	Heat stress		Recovery		
		1h	1h	2h	2h	12h	24h
Control (no hardening)	Mean	0.23	0.02	0.01	0.02	0.02	0.02
	Sd±	0.04	0.03	0.01	0.03	0.03	0.02
43	Mean	0.23	0.05	0.03	0.04	0.09	0.09
	Sd±	0.04	0.05	0.05	0.03	0.04	0.05

The mean values are F_v/F_m ratios. Each mean ±Sd is from 20 leaves. Heat stress was at 45°C and pre-incubation or recovery was at 30°C.

In the control with no hardening a more severe reduction in the fluorescence values occurred at the end of 2h stress to a value of 0.01. During the subsequent 24h recovery treatment there was a little evidence of recovery and the F_v/F_m value remained to 0.02. This is in sharp contrast to the leaves from the conditioned plants. Chen *et al.*, (1982) reported that acclimation at 35-37°C for 24h period reduced subsequent heat injury in beans, soybean, potato and tomato. Similar results have also been reported by Ageeva & Lutova (1971) for pea, and by Santarius & Muller (1979) for spinach plants where hardening for between 4 and 18h increased the heat tolerance of photosynthesis. Extended hardening for upto 3 days caused no further increase in hardiness as reported by Santarius & Muller (1979) who found that hardening took place in light as well as in dark. The poor hardening shown by single or multiple treatments to cotton leaves reported in this study was probably due to subsequent heat stress being carried out at too high temperature of 46°C which could not be tolerated even by acclimated plants. The results might have been positive if the heat stress temperature had been slightly lower. This was proved in the final experiment when the heat stress temperature was lowered by 1°C to 45°C. Although the leaves were not fully recovered from the heat stress but the relatively improved F_v/F_m values are obvious.

Acknowledgements

Financial assistance through Bilateral Linkage Programme under the ODA between Sindh Agriculture University, Tandojam, Pakistan and the University of Wales, Bangor, UK is gratefully acknowledged. The study was a part of the Ph.D. thesis of M.A. Sethar.

References

Ageeva, O.G. and M.I. Lutova. 1971. Effect of heat tempering of the pea on photosynthesis and photochemical reactions. *Bot. ZH*, 56:1365-1373. (Cited *Biol. Abst.* 55: 22309).

Chen, H.H., Z.Y. Shen and P.H. Li. 1982. Adaptability of crop plants to high temperature stress. *Crop Sci.*, 22:719-725.

Feldman, N.L. and O.A. Ageeva. 1973. Thermostability of ferridoxin from heat hardened pea leaves. *Dokl. Akad. Nauk. SSSR Ser Biol.*, 28: 479-482. (Cited *Biol. Abst.*, 56: 68985).

Huner, N.P.A., B. Elfman, M.Krol and A. Mcintosh. 1984. Growth and development at cold hardening temperatures. Chloroplast ultrastructure, pigment content and composition. *Can.J.Bot.*, 62:53-60.

Krause, G.H. and K.A. Santarius. 1975. Relative thermostability of the chloroplast envelope. *Planta*, 127:285-299.

Quinn, P.J. and W.P. Williams. 1985. Environmentally induced changes in chloroplast membranes and their effects on photosynthetic functions. In: *Photosynthetic mechanisms and environment.* (Ed.): J. Barber and N.R. Baker, pp, 1-47. Elsevir Sc. Pub. Amsterdam.

Santarius, K.S. 1973. The protective effects of sugars on chloroplast membranes during temperature and water stress and its relationship to frost, desiccation and heat resistance. *Planta*, 113:105-114.

Santarius, K.S. and M. Muller. 1979. Investigations on heat resistance on spinach leaves. *Planta*, 146:529-538.

(Received for Publication 16 December 1993)