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EFFECTS OF HEAT SHOCK ON LEAF CHLOROPHYLL FLUORESCENCE IN COTTON CULTIVARS

M. A. Sethar, D. L. Laidman*, J. M. Wilson* and R. B. Mirbahar
Department Physiology and Biochemistry, Sindh Agriculture University, Tandojam, Pakistan

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Four cotton cultivars *viz.* Qalandari, MNH-93, Rehmani and S-12 were analysed for their leaf chlorophyll fluorescence properties, at normal growth temperature (30°) (control), and shocked at high temperature (45°) for different time periods. There were only small variations in the values of chlorophyll fluorescence at the control temperature. However, the photosystem of the leaves was irreversibly damaged as assessed by fluorescence properties when the leaves were shocked at 45° for 30 mins or longer. All the cultivars were equally sensitive to the treatment.

Key words: Chlorophyll fluorescence, Photosynthesis, Heat shock, Cotton cultivars.

Introduction

Exposure of plants to high temperature causes a variety of changes in physiological and metabolic processes. It affects photosynthesis, respiration, translocation and membrane permeability Bjorkman, [1]. Among these processes photosynthesis appears to be especially heat sensitive, Bjorkman *et. al.*, [2]; Nash *et al.*, [3].

Under constant light conditions, the fate of light energy trapped by photosynthetic pigments is balanced and the system maintains a steady state level of activity. Under optimal conditions about 85% of this absorbed light energy is used to drive the photochemical processes of photosynthesis. A small part is dissipated as heat, or transferred out of the system to surrounding molecules in the thylakoid membrane, and the remainder (about 3%) is re-emitted as fluorescence, Papageorgiou, [4] Moffat *et al.*, [5]

Measurement of chlorophyll fluorescence has become a widely used method in plant stress physiology. The effect of certain environmental stresses, such as heat or chilling can be assessed rapidly by this method. It can be used *in vivo* or *in vitro* and it is a fast, non-destructive and cheap method of assessment.

In the present study, the method has been used to reveal the extent of damage caused by the temperature (45°) to the photosynthetic apparatus of some cotton cultivars commonly grown in Pakistan.

Materials and Methods

The study was conducted at the School of Biological Sciences, University of Wales, Bangor (UK) is the year 1991-92. The cotton seeds of different cultivars were obtained from various cotton research stations in Pakistan. Acid delinted and sterilized seeds were germinated and grown in compost in a plant growth cabinet (Fison Model Fitotran 600H) with a light intensity of 160 µmol m^{-2} sec^{-1} (400-700 nm) in a 16h/8h light/dark cycle. The day and night temperatures were 30 and 27°, respectively. The plants were allowed to grow until the first 2 true leaves were fully expanded. Leaves were then harvested and placed into a temperature-controlled sample holder in the dark for fluorescence measurement using a leaf fluorometer (Kaustky Plant Productivity Model SF-20). The analysis were carried out using a 5 sec induction period with a light intensity of 21 µmol m^{-2} sec^{-1} at 670 nm.

Chlorophyll fluorescence analysis involves the production of flouresecence induction curves Krause and Weis [6]. From these curves, F_o (initial fluorescence), F_m (maximum fluorsences), F_v (variable fluorescence = F_m F_o) and F_v/F_m can be determined. On the present study only F_v/F_m is recorded for the reason that this ratio is directly proportional to the quantum yield of oxygen evolution and there is close relationship between the F_v/F_m ratio and the photochemical efficiency of photosystem II (PS II) in the leaf chloroplast, Somersalo and Krause [7].

Leaves of the Cvs. MHH-93, Qalandari, Rehmani and S-12 were used to study the effects of heat shock applied at 45° for various periods of time. The excised leaves were pre-incubated for 30 mins at 30° before application of heat shock. They were then transferred to 45° for 5, 10, 15, 20, 25, 30 or 120 mins. After the heat shock, they were transferred back to 30° and incubated at that temperature for 6hr (recovery period). The leaves were kept in the dark in moist conditions throughout these treatments. Chlorophyll fluorescence measurements were taken at the end of the pre-incubation, at the end of the heat-shock treatment, and at 30 min, 2hr and 6hr during the recovery period.

Results and Discussion

Preliminary experiments (data not presented) showed that it was necessary to pre-incubate the excised leaves in the dark

* School of Biological Sciences, University of Wales, Bangor, U.K.

before imposition of the heat stress. This allowed the chloroplast photosynthetic system to decay to its ground energy state so that an accurate F_v/F_m ratio could be determined. This decay was complete after about 15 mins and a standard pre-incubation of 30 mins was therefore adopted for the main experiment. The F_v/F_m ratio after 15 mins remained constant at about 0.22-0.24 depending upon the cultivar.

Table 1 presents the results for the effects of heat shock on the Cv. Qalandri. The F_v/F_m ratio at the end of the pre-incubation was 0.22 and this stands as control. Shock for

Table 1. Effect of Heat-shock on Leaf Chlorophyll Fluorescence in Cv. Qalandari.

Duration of stress (min)		F_v/F_m ratio				
		After pre-incubation	After heat shock	During recovery		
				30 min	2hr	6hr
5	Mean	0.22	0.22	0.20	0.22	0.22
	sd	0.03	0.02	0.04	0.04	0.04
10	Mean	0.22	0.09	0.13	0.14	0.15
	sd	0.03	0.07	0.06	0.06	0.06
15	Mean	0.22	0.09	0.07	0.10	0.13
	sd	0.03	0.05	0.03	0.04	0.04
20	Mean	0.22	0.09	0.08	0.09	0.12
	sd	0.03	0.04	0.03	0.03	0.04
25	Mean	0.22	0.09	0.07	0.10	0.14
	sd	0.03	0.07	0.08	0.08	0.06
30	Mean	0.22	0.11	0.09	0.09	0.08
	sd	0.03	0.08	0.05	0.04	0.04
120	Mean	0.22	0.02	0.02	0.03	0.02
	sd	0.03	0.01	0.02	0.02	0.02

Each value is the mean ± sd from 8 leaves.

Table 2. Effect of Heat-shock on Leaf Chlorophyll Fluorescence in Cv. MNH-93.

Duration of stress (min)		F_v/F_m ratio				
		After pre-incubation	After heat shock	During recovery		
				30 min	2hr	6hr
5	Mean	0.23	0.22	0.21	0.22	0.22
	sd	0.03	0.02	0.03	0.04	0.03
10	Mean	0.23	0.10	0.14	0.15	0.16
	sd	0.03	0.06	0.07	0.07	0.08
15	Mean	0.23	0.11	0.11	0.12	0.14
	sd	0.03	0.06	0.04	0.05	0.06
20	Mean	0.23	0.10	0.10	0.11	0.13
	sd	0.03	0.05	0.04	0.05	0.05
25	Mean	0.23	0.09	0.08	0.11	0.11
	sd	0.03	0.06	0.05	0.06	0.06
30	Mean	0.23	0.10	0.07	0.09	0.09
	sd	0.03	0.07	0.04	0.06	0.07
120	Mean	0.23	0.03	0.02	0.02	0.02
	sd	0.03	0.02	0.03	0.02	0.02

Each value is the mean ± sd from 8 leaves.

5 mins at 45° did not change the ratio either at the end of the heat-shock treatment or during the subsequent recovery period. Longer periods of heat shock had adverse effects on chlorophyll fluorescence. However, there was a decrease of 50-60% in the F_v/F_m ratio following heat shock for periods ranging from 10-30 mins. This decrease was significant at the level of P=0.002. During subsequent recovery at 30° the ratio increase again following the 10, 15, 20 and 25 min heat-shock treatment, but it always remained at less than 50% of the starting control value. In each case, the recovery was not detectable until after 2hrs into the recovery period. A heat-shock treatment of 120 mins duration caused a large decline in the F_v/F_m

TABLE 3. EFFECT OF HEAT-SHOCK ON LEAF CHLOROPHYLL FLUORESCENCE IN CV. S-12.

Duration of stress (min)		F_v/F_m ratio After pre-incubation	After heat shock	During recovery 30 min	2hr	6hr
5	Mean	0.24	0.23	0.23	0.24	0.24
	sd	0.04	0.04	0.03	0.02	0.02
10	Mean	0.24	0.14	0.16	0.18	0.18
	sd	0.04	0.04	0.05	0.06	0.06
15	Mean	0.24	0.14	0.16	0.16	0.17
	sd	0.04	0.04	0.05	0.06	0.04
20	Mean	0.24	0.13	0.13	0.14	0.16
	sd	0.04	0.05	0.04	0.04	0.05
25	Mean	0.24	0.13	0.12	0.13	0.16
	sd	0.03	0.04	0.05	0.05	0.04
30	Mean	0.24	0.12	0.12	0.11	0.11
	sd	0.04	0.05	0.05	0.06	0.05
120	Mean	0.24	0.04	0.04	0.03	0.03
	sd	0.04	0.04	0.04	0.04	0.05

Each value is the mean ± sd from 8 leaves.

TABLE 4. EFFECT OF HEAT-SHOCK ON LEAF CHLOROPHYLL FLUORESCENCE IN CV.RAHMANI.

Duration of stress (min)		F_v/F_m ratio After pre-incubation	After heat shock	During recovery 30 min	2hr	6hr
5	Mean	0.23	0.23	0.20	0.22	0.23
	sd	0.02	0.03	0.04	0.03	0.02
10	Mean	0.23	0.13	0.12	0.14	0.15
	sd	0.02	0.02	0.02	0.03	0.03
15	Mean	0.23	0.12	0.12	0.13	0.13
	sd	0.02	0.03	0.03	0.04	0.04
20	Mean	0.23	0.12	0.13	0.14	0.15
	sd	0.02	0.03	0.04	0.03	0.03
25	Mean	0.23	0.10	0.11	0.11	0.12
	sd	0.02	0.07	0.06	0.05	0.06
30	Mean	0.23	0.09	0.09	0.09	0.08
	sd	0.02	0.05	0.05	0.04	0.04
120	Mean	0.23	0.02	0.02	0.03	0.02
	sd	0.02	0.03	0.03	0.04	0.02

Each value is the mean ± sd from 8 leaves.

ratio to about 10% of the starting value. Moreover, there was no detectable recovery from this treatment during the recovery incubation.

Measurement of F_v/F_m ratios showed that the leaves of all the cotton cultivars examined were not damaged for up to 5 mins during the imposition of heat-shock at 45°. Heat shock for longer periods of time, however, caused progressively greater damage. The leaves were able to recover, partially at least, following heat shock for up to 25 mins, but beyond this time the damage became irreversible. These results agree with those of Gounaris *et al.* [8], who showed that granal attachment sites in isolated chloroplasts from broad beans are stable for up to 5 mins at 45° but not beyond. The two results presumably reflect different but related aspects of the same damage process. It is generally agreed that the reduction in chlorophyll fluorescence in respect to temperature stress is indicative of chloroplast thylakoid damage. Either chilling or heat stress can elicit such decreases, Schreiber and Berry [9] Potvin [10]; Wolf *et al.* [11].

Photosynthetic inhibition under heat stress can normally be divided into reversible and irreversible changes. The irreversible effects are believed to be a reflection of the true susceptibility of the photosynthetic apparatus to heat, Bjorkman [1]. The ratio of reduction in fluoresecence and its recovery are also indicators of the heat tolerance of a plant and its capacity to recover from heat shock. Our results suggest that there is little difference, with respect to these parameters, between the different cotton cultivars studied. In turn, this suggests that there may be little genetic variability in the Pakistan cottons with respect to heat tolerance. Current studies in our laboratories, in which cotton plants are given acclimation treatments before the imposition of heat shock and studies using a range of heat stress temperatures, support this view.

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