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RELATION OF ALTERED PROTEIN EXPRESSION WITH CHLOROPHYLL FLUORESCENCE IN TEA UNDER WATER STRESS

P.R. JEYARAMRAJA, D. JAYAKUMAR, P.K. PIUS AND R. RAJ KUMAR*

UPASI Tea Research Foundation, Nirar Dam BPO, Valparai 642 127, Coimbatore District, Tamil Nadu

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SUMMARY

Concomitant variation was observed in chlorophyll fluorescence and protein in three nursery grown tea cultivars, ATK 1, TRF 1 and UPASI 17 subjected to different periods of water stress. The Chinery cultivar ATK 1, registered higher chlorophyll fluorescence index than TRF 1 and UPASI 17. A clear reduction in variable to maximal fluorescence ratio (Fv/Fm) with an increase in the soil moisture deficit was observed indicating a loss in the primary photochemical efficiency of the stressed leaves. Resurrection response of stressed plants to watering was observed with a drift in chlorophyll fluorescence index and differential synthesis of specific proteins. The 16 kD polypeptide synthesized by the drought tolerant clones ATK 1 and TRF 1, was absent in the drought susceptible clone UPASI 17. Therefore, it could serve as a marker for drought tolerance during the course of clonal selection.

Key words : **Chlorophyll fluorescence, drought, protein profile, tea.**

INTRODUCTION

Drought is one of the major constraints for tea cultivation. This phenomenon though initially reversible, in its severest form can lead to cell death. Its ill effects can be minimized by the use of drought tolerant planting material. Identification of tea cultivars for desired traits is extremely difficult due to their high intrinsic heterozygosity and plasticity (Bera *et al.* 1995). Biochemical markers are believed to be more reliable tools for identification when compared to conventional phenotypic assessment of genotypes.

Chlorophyll fluorescence has been used as a non-invasive probe of photochemical events taking place in intact leaves and Fv/Fm ratio has been suggested as a quantitative measure of photochemical efficiency of the photosystem II (PSII) complex and the photon yield of oxygen evolution under different environmental stresses (Bjorkman and Demmig 1987). The value of chlorophyll fluorescence index is said to be an indicator for functioning photosystem (Mohammed *et al.* 1995). Any unusual change in the overall bioenergetic status of the plant (including changes in the photosynthetic apparatus, stomatal opening etc.) can be detected by a change in chlorophyll fluorescence (OS5-FL 1997).

Drought induced-altered synthesis of large variety of proteins have been reported by Pareek *et al.* (1999). These gene products have been characterized with potential adaptive functions, such as enzymes that synthesize osmolytes and proteins that accumulate in seeds during the period of desiccation (Plant and Bray 1999). Reduction in photosynthetic efficiency by impairment of chloroplast during drought was observed by Kaiser (1987). Hence, in the present investigation, an attempt was made to study the relation of *in vivo* chlorophyll fluorescence with drought-induced protein expression.

*Corresponding author.

MATERIALS AND METHODS

The experiments were conducted with three tea cultivars of which two were drought tolerant (ATK 1 and TRF 1) and one was drought susceptible (UPASI 17). These cultivars were grown in pots (10 kg capacity). The details of different treatments are given in Table 1. On completion of each treatment, chlorophyll fluorescence measurements were taken and the samples were taken for protein extraction before watering and after 5 h of watering to study their desiccation response and resurrection ability.

Table 1. Experimental setup.

Treatment (soil moisture %)	Sampling
Control (22.73±1.2%)	watered* daily
T I (18.30±2.4%)	watered once in 3 days
T I (5 h)	5 h after watering in Treatment I
T II (16.23±0.2%)	watered once in 5 days
T II (5 h)	5 h after watering in Treatment II
T III (5.81±0.5%)	watered once in a week
T III (5 h)	5 h after watering in Treatment III
T IV (2.27±0.7%)	watered once in two week
T IV (5 h)	5 h after watering in Treatment IV

*150 ml per day

Chlorophyll fluorescence index (Fv/Fm) was measured using modulated fluorometer (OS5-FL Model). A clip was attached to the leaf and dark adapted for 30 minutes. The dark-adapted leaf was fitted with a sensor unit over the clip and shutter plate opened for fluorescence measurement. All the measurements were carried out in triplicates.

In order to analyse the low molecular weight protein profile, acetone powder was first prepared from the leaf samples. The leaf was excised and immersed immediately in ice-cold acetone. The tissue was ground with a pestle in pre-chilled mortar. The slurries were suspended in 180 ml ice-cold acetone and filtered through whatman no. 1 filter paper. The residues were washed once with ice-cold ethyl ether. Dried, pigment-free acetone powders were stored in aluminium foils at –20°C. (150 mg) Acetone powder was dissolved in 1 ml of phosphate buffer (pH 7.0) and centrifuged at 8000 rpm for 10 min at 4°C. Supernatant (200 µl) was used to estimate the protein content (Lowry *et al.* 1951). Supernatant (500 µl) was mixed with 25 mg SDS and 25 µl 2-beta mercaptoethanol and kept in a water bath for 10 min at 70°C. Equal amount of protein was loaded in all the wells of 15% polyacrylamide gel and run at 80 V for 1 hr. Gels were stained with coomassie blue and scanned with UMAX scanner (Amersham Biosciences) and the density of the bands was analysed using Image Master Total Lab software, Ver 1.0.

RESULTS AND DISCUSSION

The soil moisture content used for studying the impact of drought on plants varied from 2.27% to 22.73% and this was almost on par with the tea field soil moisture content during drought and non-drought conditions. The difference in chlorophyll fluorescence index between TRF 1 and UPASI 17 was insignificant, it was higher in the drought tolerant cultivars, ATK 1 and TRF 1 and lower in drought susceptible clone, UPASI 17 (Table 2). This supports the view that Fv/Fm is an index of PSII efficiency (Bjorkman and Demmig 1987). An apparent reduction in chlorophyll fluorescence index was observed with increased soil moisture deficit (Fig. 1). This reduction in fluorescence index was explicit after the seventh day of drought and it could be due to drought induced decrease in variable fluorescence resulting from reduced maximal fluorescence.

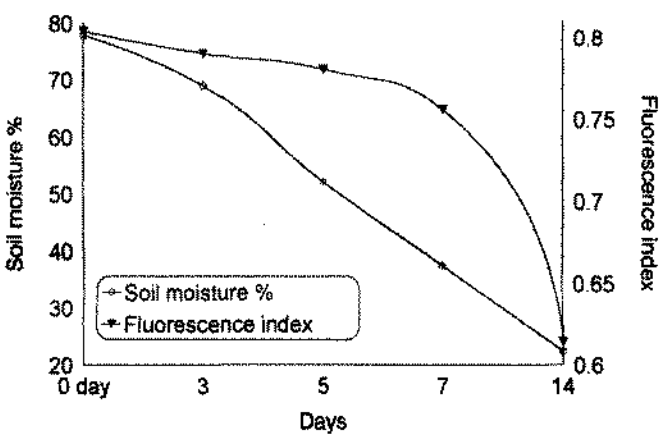

Fig. 1. Changes in Fv/Fm due to water stress

Table 2. Clonal variation in chlorophyll fluorescence.

Clone	Fo	Fv	Fm	Fv/Fm
ATK 1	222	970	1192	0.813
TRF 1	196	831	1027	0.808
UPASI 17	220	848	1068	0.793
CD (P=0.05)	5	55	56	0.009

Fo = minimal fluorescence, Fv = variable fluorescence
Fm = maximal fluorescence, Fv/Fm = ratio of the variable to maximal fluorescence

In the case of drought tolerant clones, the reduction in Fv/Fm was progressive in ATK 1 while a sudden reduction was observed with TRF 1, at T IV stage. In the susceptible cultivar UPASI 17 the fluorescence ratio came down as low as 0.59 (Table 3). It is likely that physiologically the decrease in Fv/Fm ratio indicates a reduction in the photochemical efficiency of PSII complex, which could be due to inefficient energy transfer from the light-harvesting Chl a/b complex to the reaction center (Briantais *et al.* 1986). Immediate resurrection response of stressed plants to watering with chlorophyll fluorescence index was observed (Table 3). Though the drought tolerant cultivar, TRF 1 did not suffer like UPASI 17 in terms of reduction in fluorescence index due to drought, the resurrection ability was almost lesser in TRF 1 when compared to drought susceptible cultivar, UPASI 17. Irrespective of the tolerance/susceptibility of clones to drought, the resurrection ability was more or less the same in ATK 1 and UPASI 17.

In ATK 1, the synthesis of 16, 20 and 58 kD polypeptides was peaked after watering at T III stage and inhibited at T IV stage (Fig. 2). It is likely that a moisture content of

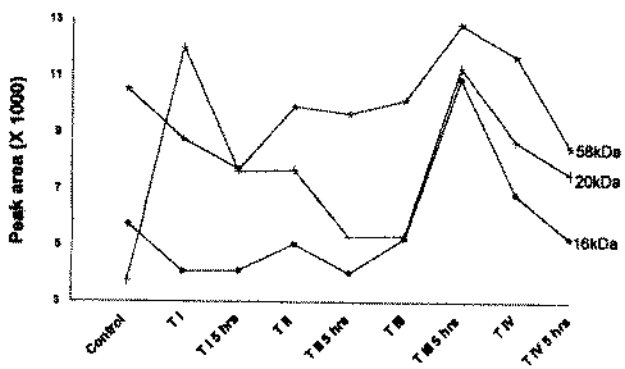

Fig. 2. Stress-induced protein expression in ATK 1

2.27% may be lethal for the synthesis of these polypeptides. An increased value of Fv/Fm was observed during resurrection after T III stage (Table 3) and so, those proteins which were synthesized higher at this stage, might have helped in the protection of photosynthetic machinery leading to an increased fluorescence index. Schneider *at al.* (1993) reported that desiccation-induced proteins are targeted to the chloroplast, and play a major role in the protection of the photosynthetic machinery.

Table 3. Drought response and resurrection ability in terms of Fv/Fm ratio of different clones.

Clone	Treatments							
	T I		T II		T III		T IV	
	B	A	B	A	B	A	B	A
ATK 1	0.796	0.804	0.777	0.780	0.745	0.760	0.627	0.641
TRF 1	0.794	0.795	0.790	0.793	0.789	0.789	0.626	0.627
UPASI 17	0.783	0.791	0.775	0.777	0.734	0.740	0.590	0.621
CD (P=0.05)	0.007	0.013	0.010	0.007	0.022	0.017	0.213	0.145

B = before watering, A = 5 h after watering, Treatment details in Table 1.

Polypeptides of 16 and 27 kD in TRF 1 were synthesized less when compared to the control in all the treatments (Fig. 3). But the 45 kD polypeptide was synthesized more after watering at T I stage and T IV stage. In could be inferred that initial and extreme drought shocks are essential for more synthesis of this polypeptide during resurrection. The resurrection ability was exhibited by an increased synthesis of all these polypeptides after 5 hr of watering in almost all the treatments. It is imperative to note here that after watering at T III stage, synthesis of these polypeptides was very less when compared to that

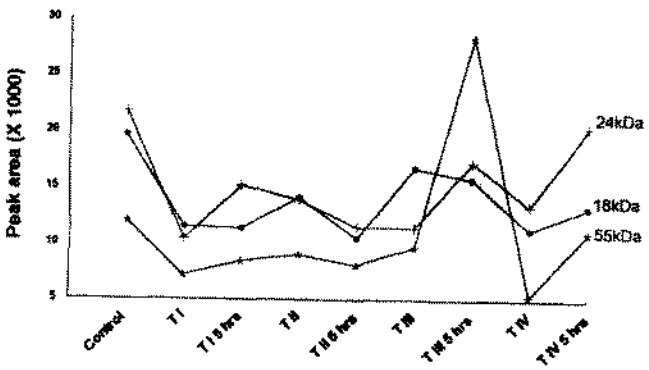

Fig. 3. Stress-induced protein expression in TRF 1

of other treatments after watering. There was more feasibility for a close relation of the synthesis of these polypeptides with chlorophyll fluorescence index and it could be proved by seeing no change in chlorophyll fluorescence index after watering at T III stage (Table 3). Augmentation of the synthesis of 24 kD and 55 kD polypeptides in UPASI 17 after watering at T III and T IV stages accompanied with drift in chlorophyll fluorescence index (Table 3; Fig. 4).

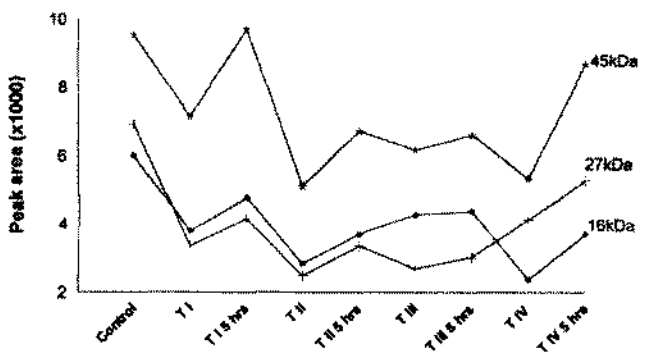

Fig. 4. Stress-induced protein expression in UPASI 17

Almost all the desiccation induced-proteins studied in this investigation showed a precipitous decrease during desiccation because of relinquishment of their innate synthesis. But it is not consistent since an increase in drought induced gene products after watering concomitantly brought about a drift in *in vivo* chlorophyll fluorescence index. These proteins could function like osmoregulators inside the chloroplasts improving photosynthetic efficiency as realized by Fv/Fm ratio. This is in agreement with the findings of Telfer and Barber (1994) who reported that degradation of D1 protein (reaction center protein of PSII) is induced during stress by oxidative damage of the protein matrix, by singlet-oxygen formed by inactivated reaction centres. The rapid repair of PSII centres during rehydration in stressed plants is due to D1 protein synthesis, which increased up to a factor 10 (Dannehl *et al.* 1996).

The 16-kD protein synthesized by the drought tolerant clones ATK 1 and TRF 1, was absent in the susceptible clone UPASI 17 (Fig. 5). Hence, the 16-kD protein traced in our experiment in the drought tolerant tea cultivars could be used as a marker during the course of clonal selection for drought tolerance. This 16-kD protein was electrophoretically similar in drought tolerant clones, it might not be precisely identical in these clones.

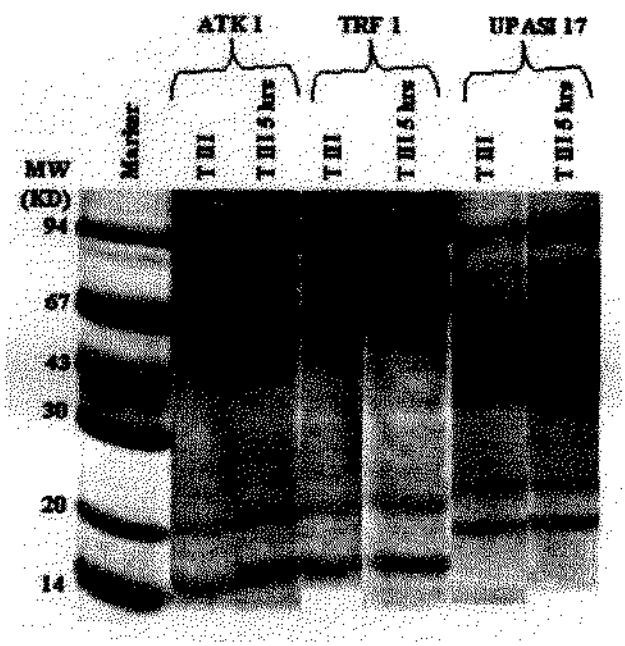

Fig. 5. Protein gel profile of three clones during desiccation and resurrection after 7 days of drought

It is evident from the study that stress responsive alterations in protein pattern showed clonal variation depending on the extent of drought. These proteins could have an important role in chlorophyll fluorescence index by protecting the photosynthetic apparatus. It is cumbersome to compare drought induced-altered protein expression in all the stages of drought treatments with changes in chlorophyll fluorescence index. Precise identification of these proteins will be a lead for understanding their function in chlorophyll fluorescence.

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