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Differential Expression of Chlorophyll Fluorescence on Heat Shock in Sorghum *Sorghum bicolor* (L.) Moench

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ABSTRACT. *Heat tolerance is a factor of a plant's tolerance to high ambient temperature. Fluorescence emitted by chlorophyll a of photosystem II (PS II) is commonly used to analyse the responses of plants to high temperature stress. The purpose of the study was to evaluate the heat tolerance in leaves of sorghum by determining the chlorophyll fluorescence. The sorghum varieties (CK 60A and IS 84) were grown in pots. Plants grown at room temperature (control), heat shocked at 42°C for an hour, heat shocked and recovered (42°C for an hour and 1 h recovery period at room temperature) were used for the study. The basal fluorescence (F_o) did not change in CK 60A (heat tolerant) but increased to 61.85% in IS 84 (heat susceptible). There was a reduction in maximum fluorescence (F_m) in both the varieties. Variable fluorescence ($F_v = F_m - F_o$) was higher in CK 60A than in IS 84 due to high Fm value of CK 60A. The fluorescence ratio (F_v / F_m) reduced slightly in CK 60A than that of in IS 84. Heat tolerance as expressed by the ratio of F_v / F_m (R_v) of heat shocked and control showed higher values for CK 60A than in IS 84. The ratios of F_o values (R_o) of heat shocked and control showed higher values for IS 84 and lower values for CK 60A. Based on the chlorophyll fluorescence parameters, CK 60A was evaluated as a more heat tolerant variety than IS 84.*

INTRODUCTION

Sorghum is fairly tolerant to drought when compared to other cereal crops like rice and wheat. Various laboratory tests like chlorophyll stability index, shoot/root ratio, germination percentage *etc.* were commonly employed for screening varieties. Another way of screening the varieties is by studying the efficiency of photosynthetic apparatus using chlorophyll fluorescence values. Photosynthesis which is a major process determining a plant's vegetative growth, is heat sensitive (Bjorkman *et al.*, 1980). Within photosynthetic apparatus PS II is the most heat sensitive function whereas photosystem I (PS I) activity, stomatal enzymes or chloroplast envelope are more thermostable (Iordandov *et al.*, 1993).

The heat tolerance limit of leaves of higher plants coincides with (and appears to be determined by) thermal sensitivity of primary phytochemical reactions occurring in the thylakoid membrane systems. The function of PS II in the thylakoid as monitored by chlorophyll fluorescence is more sensitive to heat than PS I (Weis and Berry, 1988). Changes in chlorophyll fluorescence have proven to be useful in evaluating and screening plants for heat tolerance (Wilson and Greaves, 1993; Srinivasan *et al.*, 1995). The rate of increase of the irradiation induced variable fluorescence (F_v) in heat treated leaves from different species is a useful method for determining relative heat tolerance (Burke, 1990).

Krishnaraj *et al.* (1993) utilised the induction and quenching kinetics of chlorophyll *a* fluorescence for *in vivo* salinity screening studies in wheat. The results indicated that total fluorescence quenching and maximum rates of both induction and quenching appeared to be reliable indicators for *in vivo* screening of salt tolerant wheat genotypes. Nogues *et al.* (1994) assessed the drought tolerance to evaluate 20 barley genotypes by measurement of chlorophyll fluorescence, net photosynthetic rate and leaf water potential. He suggested a combination of all the three methods necessary for evaluation. Ranney and Peet (1994) used leaf gas exchange and chlorophyll fluorescence measurements as indices for evaluating heat tolerance among five taxa of birch. The present study was undertaken to analyse the comparative heat tolerance of two contrasting sorghum genotypes (CK 60A and IS 84) based on chlorophyll fluorescence values.

MATERIALS AND METHODS

The experiment was conducted at Centre for Plant Molecular Biology, Tamil Nadu Agricultural University. Sorghum (*S. bicolor*) varieties CK 60A, IS 84, CO 25, CO 26 and 2219 A were screened for heat tolerance. One heat tolerant (CK 60A) and one heat susceptible clone (IS 84) were identified based on germination studies, electrical conductivity, chlorophyll stability index, epicuticular wax, proline content, membrane stability studies, relative water content, photosynthetic pigments and soluble protein which were used as selection indices. The selected two genotypes were used for chlorophyll fluorescence studies. The optimum temperature for heat shock for sorghum was fixed at 42°C based on germination studies. Heat shock was given by maintaining plants in controlled growth chambers. The photosynthetic efficiency of the plants was analysed using Plant Efficiency Analyser (Hansatech Instruments Kings Lynn, UK). The leaves of uniform size from intact plants (21 days old) maintained at room temperature, heat shocked (42°C for an hour) and heat shocked and recovered (42°C for an hour, and 1 h recovery period) were taken for the study. Dark adaptation was given using clips for to last 30 min during the treatment. LS 2 actinic light source was used for illumination and dark adapted leaves were illuminated for 1 min and the fluorescence was recorded. Four leaves were sampled and measured at each time. The experiment was replicated four times. The values F_o, F_m, F_v and F_v / F_m were recorded in the Fluorimeter.

RESULTS

CK 60A and IS 84 were significantly different in their fluorescence (F_o) values in control samples. There was a significant increase in F_o level of IS 84 (61.85%), but the same F_o value was retained in CK 60A after heat shock (Table 1, Fig. 1). F_o values after recovery was similar to heat shock in CK 60A. However, there was a significant reduction in F_o value after recovery in IS 84 when compared to heat shocked samples.

Maximum fluorescence (F_m) was significantly different between the genotypes CK 60A and IS 84. The reduction in F_m was also significant after heat shock in both varieties. The reduction was maintained even after the recovery period (Table 2). The reduction in F_m was greater in IS 84 (13.68%) after heat shock as compared to CK 60A (8.49%).

Table 1. Effect of heat shock and recovery on basal fluorescence (F_0) of sorghum varieties.

Varieties	Control	Heat shocked	Heat shocked and Recovered
CK 60A	0.192	0.208	0.221
IS 84	0.173	0.280	0.244
	V	T	V × T
CD (0.05)	0.009	0.011	0.015

V - Varieties T - Heat treatments

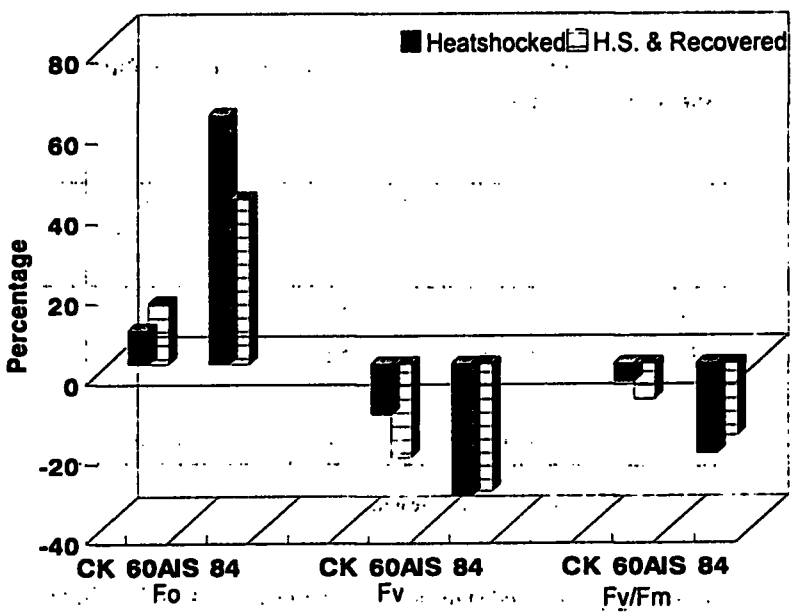

Fig. 1. Effect of heat shock on chlorophyll fluorescence (per cent over control) of sorghum varieties.

The variable fluorescence ($F_v = F_m - F_o$) was higher in CK 60A (Table 3, Fig. 1) than in IS 84 due to high F_m value of CK 60A.

Table 2. **Effect of heat shock and recovery on maximum fluorescence (F_m) of sorghum varieties.**

Varieties	Control	Heat shocked	Heat shocked and Recovered
CK 60A	0.978	0.895	0.824
IS 84	0.848	0.732	0.704
	V	T	V × T
CD (0.05)	0.022	0.027	0.038

V - Varieties T - Treatments

Table 3. **Effect of heat shock and recovery on variable fluorescence (F_v) of sorghum varieties.**

Varieties	Control	Heat shocked	Heat shocked and Recovered
CK 60A	0.786	0.687	0.603
IS 84	0.675	0.452	0.460
	V	T	V × T
CD (0.05)	0.023	0.028	0.040

V - Varieties T - Treatments

The F_v value was reduced significantly after heat shock and further reduction was noticed even after recovery in CK 60A. The variety IS 84 had lower F_v value in control and the reduction was even greater (33%) after heat shock.

There was a marginal increase in F_v value after the recovery in IS 84. The F_v / F_m ratio was almost same in both the varieties before heat shock (Table 4, Fig. 1). The reduction in the ratio was much less (4.48%) in CK 60A than that of IS 84 (22.49%) after heat shock. The reduction was significant even after recovery in CK 60A (due to high F_m value) whereas there was a slight increase in F_v / F_m value in IS 84 after recovery.

Heat tolerance, as expressed by the ratio of F_v / F_m in leaves exposed to 42°C to those maintained at control (R_v) showed wide variation. The R_v value was higher for CK 60A (0.955) than IS 84 (0.775) (Table 5).

Table 4. Effect of heat shock and recovery on fluorescence ratio (F_v / F_m) of sorghum varieties.

Varieties	Control	Heat shocked	Heat shocked and Recovered
CK 60A	0.803	0.767	0.732
IS 84	0.796	0.617	0.653
	V	T	V × T
CD (0.05)	0.013	0.016	0.023

V - Varieties T - Treatments

Table 5. Effect of heat shock and recovery on relative variable (R_v) and basal fluorescence (R_o) of sorghum varieties.

Varieties	R_v	R_o	Average	CV%
CK 60A: heat shocked and control	0.955	1.083	1.031	9.50
IS 84 : heat shocked and control	0.775	1.618	1.197	38.36
CK 60A: heat shocked + recovery and control	0.911	1.151	1.031	11.96
IS 84 : heat shocked + recovery and control	0.820	1.410	1.165	32.87

Similarly, R_v worked out between F_v / F_m of recovered plants and control and the values still were maintained at a higher level for CK 60A. The ratio of F_o values in leaves exposed to 42°C to those of controls (R_o) was on contrast to R_v, higher for IS 84 than CK 60A. The R_o value between the recovered and control also showed the same trend. In a nut shell, high R_v and low R_o values and low R_v and high R_o values were recorded for CK 60A and IS 84 respectively.

DISCUSSION

Under field conditions, plants have to tolerate combined heat and water stresses. The present experiments provided information on heat stress independent of water stress in partial process of photosynthesis, an important factor determining vegetative growth.

At 42°C and on recovery, the basal fluorescence value (F_o) did not increase much in CK 60A but increased by 1.6 times and 1.4 times in IS 84 after heat shock and recovery respectively. Similar observations were made by Bose and Ghosh (1995) in rice cultivars subjected to heat stress. They observed an initial increase in F_o in susceptible cultivar IR

Maximum fluorescence (F_m) also decreased more in IS 84 than in CK 60A during high temperature stress. Even after recovery, F_m values continued to decrease in both the varieties. Though the variable fluorescence (F_v) decreased after heat shock in both the varieties, the decline was more in IS 84 (heat susceptible) than in CK 60A (heat tolerant). The decline in F_v / F_m generally includes an increase in F_o and decrease in F_v. An increase in F_o is characteristic of destruction of PS II reaction centres (Bolhar-Nordenkampf *et al.*, 1989) whereas a decline in F_v reflects the interruption of electron donation to PS II reaction centres due to heat inactivation of the oxygen evolving system (Weis and Berry, 1988).

Maximum quantum yield of chlorophyll fluorescence (F_v / F_m) had not changed significantly after heat shock (42°C) in CK 60A and a slight reduction in F_v / F_m value was noticed after recovery. In IS 84, F_v / F_m decreased with heat shock and increased when the heat shock was relieved. In IS 84, F_v / F_m ratio decreased to 77% of that in control and on recovery, it increased to 82% of that in control. On the contrary, at 42°C maintained 95% of the F_v / F_m measured at room temperature and on recovery to 91% which might be attributed to high F_m values after recovery.

Yamada *et al.* (1996) had reported that the extent of decline in chlorophyll fluorescence ratio (F_v / F_m) caused by high temperature treatment was greater in longan than in mangoes and concluded that mango leaves were tolerant to high temperature than longan leaves. Since the present results also agreed with these results, it may be concluded that CK 60A is relatively more tolerant to heat shock than in IS 84.

The F_v / F_m ratio and ξ are good indicators for the function of PS II reaction centres suppressed by high temperature exceeding heat tolerance limit (Weis and Berry, 1988; Bolhar-Nordenkampf *et al.*, 1989). The F_v / F_m ratio has been shown to be highly correlated with the quantum yield of photosynthesis of intact leaves exposed to various levels of photo inhibition (Bolhar-Nordenkampf *et al.*, 1989). Babani (1995) also concluded that changes in fluorescence induction patterns and derived parameters could be used to estimate the damage caused by heat stress in wheat cultivars. The ratio of F_v/F_m reflected a corresponding decline in efficiency of phytochemistry in both sorghum genotypes. However, PS II reaction was more heat labile in IS 84 than in CK 60A. The greater reduction of F_v / F_m may be due to dramatic quenching of F_o with slight increase in IS 84. However, high temperature transmitted less injurious effects on PS II mediated electron transport chain in the tolerant cultivar (CK 60A) than in the sensitive one (IS 84).

Wide variation was noticed in R_v values (ratio of F_v / F_m in leaves exposed to 42°C to those of control) between genotypes. CK 60A was having higher R_v value both after heat shock and recovery than IS 84. The ratio of F_o values in the leaves exposed to 42°C to those of control (R_o) was highly and negatively correlated to R_v in a number of crops (Yamada *et al.*, 1996). They showed that pineapple, amla, cherimoya and sugar apples had low R_o values and high R_v values which were heat tolerant whereas syzygium, longan and peach which were heat sensitive had high R_o values and low R_v values. In this study, CK 60A had higher R_v and low R_o values while IS 84 recorded vice versa which confirmed the tolerant and susceptible nature of the cultivars respectively.

CONCLUSIONS

Fluorescence measurements were useful for evaluating thermotolerance of plants. Although sorghum photosystems may not be the direct location of thermal injury, fluorescence appears to be quite sensitive to cellular disruptions by high temperature. The two parameters F_v / F_m and F_o are good indicators for the function of PS II reaction centres suppressed by high temperature exceeding heat tolerance limit in sorghum. CK 60A was evaluated as a more heat tolerant type by maintenance of F_o, increased F_v / F_m values, low R_o and high R_v values than IS 84.

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REFERENCES

Babani, F. (1995). Effect of high temperature on some wheat varieties *via* chlorophyll fluorescence. pp. 797-800. *In*: Mathis, P. (Ed). Photosynthesis from light to biosphere. Vol. IV. Proc. Xth Int. Photosynthesis Congress, Montpellier, France. Aug. 20-25, 1995. Klumer Academic Publishers.

Bjorkman, O., Badger, M.R. and Armond, P.A. (1980). Response and adaptation of photosynthesis to high temperature. pp. 233-244. *In*: Turner, N.C. and Kramer, P.J. (Eds). Adaptations of Plants to Water and High Temperature Stress. Wiley Interscience, New York.

Bolhar-Nordenkamf, H.R., Long, S.P., Baker, N.R., Oquist, G., Schreiber, V. and Lechner, E.G. (1989). Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: a review of current instrumentation. Funct. Ecol. 3: 497-51.

Bose, A. and Ghosh, H. (1995). Effect of heat stress on ribulose 1, 5, bisphosphate carboxylase in rice. Phytochemistry. 38(5): 1115-1118.

Burke, J.J. (1990). Variation among species in the temperature dependence of the reappearance of variable fluorescence following illumination. Plant Physiol. 3: 652-656.

Iordandov, I.T., Moskalenko, A.A., Kuznetsova, N.Y. and Georgieva, K.M. (1993). Polypeptide composition, pigment-protein complexes and functional activity of thylakoid membranes isolated from heat-acclimated and non-acclimated pea plants. Photosynthetica. 29: 427-435.

Krishana Raj, S., Mawson, B.T., Yeung, E.C. and Thorpe, T.A. (1993). Utilisation and induction and quenching kinetics of chlorophyll fluorescence for *in vivo* salinity screening studies in wheat (*Triticum aestivum* var. kharchia-65 and Fielder). Can. J. Bot. 71: 87-92.

Nogues, S., Alegre, L., Araus, J.L., Perez-Aranda, L. and Lannoye. R. (1994). Modulated chlorophyll fluorescence and P_N gas exchange as rapid screening methods for drought tolerance in barley genotypes. Photosynthetica. 30(3): 465-474.

Ranney, T.G. and Peet, M.M. (1994). Heat tolerance of five taxa of birch. Physiological responses to supra optimal leaf temperatures. J. Am. Soc. Hort. Sc. 119(2): 243-248.

Srinivasan, A., Takeda, H. and Senbok, T. (1995). Heat tolerance in food legumes as evaluated by cell membrane thermostability and chorophyll fluorescence techniques. Euphytica. 88: 35-45.

Weis, E. and Berry, J.A. (1988). Plants and high temperature stress. pp. 329-346. In: Long, S.P. and Woodward, F.I. (Eds). Symposia of the society for experimental biology. No. XXXXII, Cambridge University Press, U.K.

Wilson, J.A. and Greaves, G.A. (1993). Development of fluorescence based screening programs for temperature and water stress on crop plants. pp. 389-398. *In*: Kuol, C.G. (Ed). Adaptation of food crops to temperature and water stress. Proc. Int. Symp., AVRDC, Taipei, Taiwan.

Yamada, M., Hidaka, T. and Fukamachi, H. (1996). Heat tolerance in leaves of tropical fruit crops as measured by chlorophyll fluorescence. Scientia Hort. 67: 37-48.