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Chlorophyll Fluorescence in the Leaves of Soybean Affected by High Temperature Stress

[1]Mohammad Ibrahim Keerio,[1]Saghir Ahmed Sheikh
[1]Shanila Yasmeen Chang and [2]Samiullah K.Agha
[1]Dept. of Plant Physiology & Biochemistry, [2]Dept. of Agronomy
Sindh Agriculture University, Tandojam, Pakistan

Abstract: The chlorophyll fluorescence has been widely used to study the activity of the photosynthetic machinery. The first trifoliate leaf was used to study the effects of high temperature on the leaves using the chlorophyll fluorescence method (Fv/Fm ratio). No effects were found on the ratio when leaves were stressed for 2 or 4h at 35 °C or following subsequent recovery at 24 °C. After 4h stress at 40 °C, however, there was an approximately 50% decrease in the Fv/Fm ratio. The Fv/Fm ratio was more severely affected by heat stress at 42.5 °C, but effects were greater in cultivars Mago-80 and Bragg. At 45 °C there were approximately 80% decreases in the Fv/Fm ratios in all cultivars.

Keywords: Soybean, Heat stress, Chlorophyll fluorescence

Introduction

Chlorophyll fluorescence is now routinely used as a probe of thylakoid membrane activity in many aspects of photosynthesis research. Reactions of the plant thylakoid membrane are considered to be the first processes to be damaged by heat, (Smillie, 1979). Several fields of plant stress physiology have used chlorophyll fluorescence technique as a tool to assess the degree of environmental injury incurred by plants and genotypic differences in response to stress exposure. These include high temperature stress (Bilger *et al.*, 1987; Moffat *et al.*, 1990), chilling (Neuner and Larcher, 1990), freezing (Greaves and Wilson, 1987), and salinity (Larcher *et al.*, 1990). The thermal environment in which a plant is grown also markedly affects the temperature dependency of photosynthesis (Bjorkman *et al.*, 1975). These heat stress effects can be assessed rapidly by the chlorophyll fluorescence technique. It is a fast and cheap method that can be used *in vivo* or *in vitro* (Larcher, 1980). Alternative methods are often more costly and time consuming. Thus, chlorophyll fluorescence has been widely used to study the activity of the photosynthetic machinery. In particular, the linear relationship between quantum yield and the ratio of variable fluorescence to maximum fluorescence (Fv/Fm) (Adam *et al.*, 1990) has proved especially useful. The Fv/Fm ratio must be measured in dark-adapted photosynthetic systems and with an actinic light bright enough to saturate all the electron acceptors of photosystem II (PS II) at the P-peak. The dark adaptation usually takes 5-10 min to ensure that all energy- dependent quenching is relaxed (Krasue and Weis, 1984). The present experiment was conducted to assess the utility of the chlorophyll fluorescence technique for our purposes and to evaluate soybean cultivars for high temperature tolerance.

Materials and Methods

Effect of heat stress on chlorophyll fluorescence in leaves: The study was conducted at School of Biological Sciences, University of Wales, Bangor, U.K. The seed of soybean cultivars Bragg, was supplied by ARI, Oil Seed Section, Tandojam, Williams-82 and Davis by PARC, Islamabad, and Mago-80, Sable and Hardee by ARI, Tarnab, Peshawar. Seeds were grown in a growth room in sterilized pots (8, 10 cm diameter) filled with pre-washed vermiculite (Vermiperl Horticultural Grade). The room was maintained at 24°C throughout with a 16h photoperiod (light intensity 82-85 μmols m^{-2} s^{-1}). The pots were irrigated with half-strength Long Ashton nutrient solution every other day for 4 weeks. After 4 weeks, the first trifoliate leaf was determined using the chlorophyll fluorescence method (Fv/Fm ratio). For this analysis, detached leaves from 14 plants were placed into a sample holder. A constant temperature plate was placed under the sample holder and connected to a circulating water bath to maintain a constant required temperature. The sample holder consisted of an aluminum plate, covered with two layers of filter paper (Whatman No.1), moistened with 30 cm^3 of distilled water. Leaves in the sample holder were completely moistened, because any water stress may have altered the severity of the effects of temperature on the chloroplast thylakoids (Wilson, 1976). Thus, the leaves were placed onto the filter paper and covered by a layer of plastic film, which was permeable to air but not to water. Finally, a plastic grid plate with 3.3 mm diameter holes was placed on top of the film, (The probe head of the fluorometer could be placed into these holes for the fluorescence measurements). The sample holder and the constant temperature plate were put into a black photographic bag for 30 min dark acclimation and the control chlorophyll fluorescence (Fv/Fm ratio) was then measured. The temperature of the sample holder was monitored by inserting the probe of an electronic thermometer under one of the leaves. Heat stress treatments of 2 and 4h at 35, 40, 42.5, or 45 °C were then given to the leaves by moving the sample plates and their contents (under a green safe light) to another constant temperature plate at the higher temperature. The chlorophyll fluorescence (Fv/Fm ratio) was recorded before the heat stress and after 2 and 4h of heat-stress treatment. After the heat stress, the plates were returned to the initial temperature (24°C) for recovery and chlorophyll fluorescence was measured at 12, 24, and 48h into the recovery period.

Statistical analysis: All means, standard deviation

Table 1: High temperature effects on chlorophyll fluorescence (Fv/Fm) ratio in the leaves of soybean cultivars

		Stress		Recovery		
Temp. °C	Control	2h	4h	12h	24h	48h
Cv. Williams-82						
35	0.872 ±0.005	0.859 ±0.002	0.858 ±0.003	0.884 ±0.002	0.881 ±0.002	0.890 ±0.001
40	0.87 ±0.002	0.673 ±0.07	0.485 ±0.09	0.768 ±0.05	0.855 ±0.006	0.868 ±0.003
42.5	0.89 ±0.004	0.552 ±0.03	0.436 ±0.04	0.583 ±0.05	0.752 ±0.02	0.800 ±0.02
45	0.864 ±0.005	0.124 ±0.02	0.104 ±0.02	0.00 ±0.0	0.00 6±0.0	0.00 ±0.0
Cv.Sable						
35	0.873 ±0.004	0.864 ±0.001	0.864 ±0.002	0.889 ±0.002	0.873 ±0.003	0.858 ±0.01
40	0.881 ±0.003	0.719 ±0.05	0.444 ±0.08	0.806 ±0.02	0.837 ±0.01	0.839 ±0.01
42.5	0.868 ±0.002	0.692 ±0.01	0.433 ±0.08	0.509 ±0.08	0.753 ±0.02	0.820 ±0.01
45	0.866 ±0.002	0.153 ±0.01	0.161 ±0.02	0.00 ±0.0	0.00 ±0.0	0.00 ±0.0
Cv.Mago-80						
35	0.865 ±0.003	0.845 ±0.001	0.839 ±0.003	0.865 ±0.002	0.849 ±0.002	0.853 ±0.002
40	0.857 ±0.03	0.748 ±0.01	0.724 ±0.02	0.724 ±0.03	0.755 ±0.02	0.756 ±0.02
42.5	0.851 ±0.002	0.321 ±0.02	0.291 ±0.01	0.016 ±0.01	0.040 ±0.04	0.040 ±0.04
45	0.861 ±0.004	0.237 ±0.02	0.070 ±0.02	0.020 ±0.01	0.00 ±0.0	0.00 ±0.0

Values are the mean of 14 replicates.
± = S.E

and standard error values were calculated using a pocket scientific calculator and confirmed using a computer. Statistical analysis of variance (ANOVA) was done using Minitab for Windows (version 10.2).

Results and Discussion

The results reveal that the same value of Fv/Fm ratio (approximately 0.87) was recorded in the controls of all the cultivars. Furthermore, no changes were found in the ratio when leaves were stressed for 2 or 4h at 35 ° C or following subsequent recovery at 24 ° C. After 4h stress at 40 ° C, however, there was an approximately 50% decrease in the Fv/Fm ratio of Williams-82 and Sable cultivars (Table 1). Smaller decreases occurred in Mago-80 (Table 1), Bragg, Davis and Hardee cultivars (Table 2). The Fv/Fm ratio recovered fully in all the cultivars following stress at 40 ° C, although slower recovery appeared to take place in leaves. The Fv/Fm ratio was more severely affected by heat stress at 42.5 ° C. At this temperature, the effects were greater in cultivars Mago-80 (Table 1) and Bragg (Table 2) compared with the other cultivars. Leaves appeared to be affected more than those from Mago-80 and Sable, but in the cultivar Davis the leaves seemed to be more sensitive to temperature stress. The Fv/Fm ratio recovered fully from the 42.5°C treatments in Williams-82 and Sable. Slower recoveries were found in Davis and Hardee cultivars. The leaves of Bragg showed only a poor ability to recover from the treatments. Even slower recovery was found in cultivar Mago-80. In all the cultivars except Davis and Hardee, the leaves apparently recovered slow. It is clear that chlorophyll fluorescence was severely affected in all the cultivars when the temperature was raised to 45 ° C (Table 1-2). There were approximately 80% decreases in the Fv/Fm ratios after 2h stress at this temperature. Further reductions were found after 4h stress in Mago-80 (Table 1) and Bragg (Table 2), but no further changes were found in Williams-82, Sable (Table 1) or Hardee (Table 2). After this heat stress, many leaves were visibly very badly damaged. The data were analyzed using the ANOVA test, which showed that the Fv/Fm ratio was strongly dependent ($P<0.05$) upon the cultivar, temperature, stress period and the recovery period. Also, there was significant interaction ($P<0.05$) between the cultivar and temperature. In the present investigation, the ratio of Fv to Fm has been used to quantify thylakoid membrane damage for a variety off reasons. In particular, Somersalo and Krause (1989, 1990) reported that there is a close relationship between the Fv/Fm ratio and the photochemical efficiency of PS II. It is further stated that this ratio is directly proportional to the quantum yield of oxygen evolution (Oqiust and Wass, 1988). The present results are in general agreement with those of Sethar (1993) working on cotton, who found that the chlorophyll fluorescence values in several cultivars dramatically decreased at 45 ° C with no recovery at all during subsequent 24h recovery at 30 ° C. It is generally agreed that reductions in chlorophyll fluorescence with respect to temperature stress are indicative of chloroplast thylakoid damage. Either chilling or heat stress, when applied to leaves, elicits decreases in induced chlorophyll fluorescence in several species (Smillie and Gibbons, 1981; Potvin, 1985). Yucel *et al.* (1992) have reported that PS II chlorophyll fluorescence transients in wheat seedlings were inhibited by 40-50% following 30 min heat stress at 37 ° C. Continued heat treatment of the seedlings for up to 5h inhibited PS II fluorescence by around 80%. Complete recovery of PS II variable fluorescence

Table 2: High temperature effects on chlorophyll fluorescence (Fv/Fm) ratio in the leaves of soybean cultivars

Temp. °C	Control	Stress 2h	Stress 4h	Recovery 12h	Recovery 24h	Recovery 48h
Cv.Bragg						
35	0.858 ±0.003	0.833 ±0.002	0.836 ±0.004	0.856 ±0.003	0.837 ±0.007	0.85 ±0.003
40	0.860 ±0.001	0.802 ±0.005	0.767 ±0.007	0.801 ±0.01	0.807 ±0.01	0.818 ±0.01
42.5	0.861 ±0.002	0.390 ±0.02	0.299 ±0.01	0.00 ±0.0	0.00 ±0.0	0.142 ±0.06
45	0.863 ±0.003	0.235 ±0.01	0.050 ±0.02	0.00 ±0.0	0.00 ±0.0	0.00 ±0.0
Cv.Davis						
35	0.847 ±0.004	0.853 ±0.003	0.853 ±0.002	0.721 ±0.01	0.861 ±0.002	0.853 ±0.005
40	0.860 ±0.004	0.799 ±0.003	0.779 ±0.005	0.838 ±0.002	0.801 ±0.006	0.819 ±0.005
42.5	0.861 ±0.001	0.692 ±0.01	0.635 ±0.01	0.673 ±0.02	0.685 ±0.01	0.613 ±0.02
45	0.866 ±0.002	0.126 ±0.005	0.157 ±0.006	0.00 ±0.0	0.00 ±0.0	0.00 ±0.0
Cv.Hardee						
35	0.846 ±0.003	0.854 ±0.002	0.861 ±0.002	0.727 ±0.02	0.860 ±0.003	0.863 ±0.005
40	0.873 ±0.003	0.779 ±0.01	0.751 ±0.01	0.841 ±0.005	0.836 ±0.003	0.835 ±0.006
42.5	0.863 ±0.002	0.622 ±0.01	0.548 ±0.02	0.646 ±0.02	0.650 ±0.02	0.566 ±0.02
45	0.875 ±0.002	0.146 ±0.01	0.166 ±0.02	0.010 ±0.01	0.00 ±0.0	0.00 ±0.0

Values are the mean of 14 replicates.
± = S.E

was found after the seedlings were returned to 22 °C for 24h. Kislyuk (1979) has found that, when photosynthesis is not totally inhibited by heat stress on cucumber and tradescantia leaves, recovery of the photosynthetic apparatus after removal of the heat stress is faster in light than in the dark. Protein dissociation and denaturation is the most common explanation for heat injury in biological systems. Christiansen (1978) has suggested that, in plants, the proteins of two major kinds, enzymes and membranes proteins, are potentially the most vulnerable to high temperatures. Heat-induced blocking of PS II reaction centers, combined with dissociation of the light-harvesting chlorophyll a/b protein complex, has been postulated to occur at temperatures corresponding to those affecting fluorescence decrease in *Larrea divaricta* leaves (Armond *et al.*, 1978). Aoki (1990) has reported that chlorophyll fluorescence values in detached cucumber leaves decreased with increasing temperature and duration of the heat treatment.Membrane disruption in plants may alter water, ion and organic solute movement, as well as photosynthesis and respiration (Christiansen, 1978). Havaux *et al.* (1991) have reported that, in pea leaves, heat stress in darkness increases the capacity for cyclic electron flow around PS I, while heat stress in the light reduces PS I-driven cyclic electron transport. Heat stress in darkness results in the progressive closure of PS I reaction centers, whereas PS II centers under steady illumination remain open, reflecting adjustment of the photochemical efficiency of un-damaged PS I to the reduced activity in PS II. There is very little published work on the effects of high temperature on soybean. Most research on the species has been done on low temperature effects.

References

Adams, W.W., B. Demmig-Adams, K. Winter and U. Schrieber, 1990. The ratio of variable to maximum chlorophyl lfluorescence from photosystem II, measured in leaves at ambient temperature and at 77K, as an indicator to the photon yield of photosynthesis. Planta, 180: 166-174.

Aoki, S., 1990. Measurement of heat sensitivity in cucumber leaves by chlorophyll fluorescence method. Tropical Agric. Res. Series., 23: 239-247.

Armond, P.A., U. Schrieber and O. Bjorkman, 1978. Photosynthetic acclimation to temperature in the desert shrub, *Larrea divaricta*. Plant Physiol., 61:411-415.

Bilger, W., U. Schrieber and O.L. Lange, 1987. Chlorophyll fluorescence as a indicator of heat induced limitation of photosynthesis in Arbutus unedo. In "Plant response to environmental stress". (eds. J.D. Tenhunen, F.M. Catarino, O.L. Lange and W.C. Oechel). Nato ASI Series, Vol. G.15: pp 391-399.

Bjorkman, O., H.A. Mooney and J. Ehleringer, 1975. Comparison of photosynthetic characteristics of intact plants. Year book of the Carnegie Inst. Washington, 74: 748-751.

Christiansen, M.N., 1978. The physiology of plant tolerance to temperature extremes. In "Crop tolerance to suboptimal land conditions". (ed. G.A. Jung). Am. Soc. Agron. Madison, Wis., pp. 173-191.

Greaves, J.A. and J.M. Wilson, 1987. Assessment of the frost sensitivity of wild and cultivated potato species by chlorophyll fluorescence analysis. Potato Res., 30: 381-395.

Havaux, M., H. Greppin and R.J. Strasser, 1991. Functioning of photosystem I and II in pea leaves exposed to heat stress in the presence and absence of light. Analysis using *in vivo* fluorescence, absorbance, oxygen and photoacoustic measurements. Planta., 186: 88-98.

Kislyuk, I.M., 1979. Protecting and injurious effects of light on photosynthetic apparatus during and after heat treatment on leaves. Photosynthetica, 13: 386-391.

Krause, G.H. and E. Weis, 1984. Chlorophyll fluorescence as tool in plant physiology. II. Interpretation of fluorescence signals. Photosyn. Res., 5: 139-157.

Larcher, W., J. Wagner and A. Thammathaworn, 1990. Effects of superimposed temperature stress on *in vivo* chlorophyll fluorescence of Vigna unguiculata under saline stress. J. Plant Physiol., 136: 92-102.

Larcher, W., 1980. In "Physiological plant ecology". (ed. W. Larcher). Springer-Verlag, Berlin., pp: 10-11.

Moffat, J.M., R.G. Sears and G.M. Paulsen, 1990. Wheat high temperature tolerance during reproductive growth. 1. Evaluation by chlorophyll fluorescence. Crop Sci., 30: 881-885.

Neuner, G. and W. Larcher, 1990. Determination of differences in chilling susceptibility of two soybean varieties by means of *in vivo* chlorophyll fluorescence measurements. J. Agron. and Crop Sci., 164: 73-80.

Oquist, G. and R. Wass, 1988. A portable microprocessor operated instrument for measuring chlorophyll fluorescence kinetics in stress physiology. Physiologia plantarum, 73: 211-217.

Potvin, C., 1985. Effects of leaf detachment on chlorophyll fluorescence during chilling experiments. Plant Physiol., 78: 883-886.

Sethar, M.A., 1993. A physiological study of heat stress in cotton seedlings. Ph.D Thesis, Univ. of Wales, U.K.

Smillie, R.M. and G.C. Gibbons, 1981. Heat tolerance and heat hardening in crop plants measured by chlorophyll fluorescence. Carlsberg Res. Commun., 46: 395-403.

Smillie, R.M., 1979. The useful chloroplast: a new approach for investigating chilling stress in plants. In "Low temperature stress in crop plants: The role of membrane". (eds. J.M. Lyons, D. Graham and J.K. Raison). Academic Press, London., pp: 187-202.

Somersalo, S. and G.H. Krause, 1989. Photoinhibition at chilling temperatures. Fluorescence characteristics of unhardened and cold-acclimated spinach leaves. Planta, 177: 409-416.

Somersalo, S. and G.H. Krause, 1990. Photoinhibition at chilling temperatures and effects of freezing stress on cold-acclimated spinach leaves in the field. A Fluorescence , study. Physiologia Plantarum, 79: 617-622.

Wilson. J.M., 1976. The mechanism of chill and drought hardening of *Phaseoulus vulgaris* leaves. New Phytol., 76: 257-270.

Yucel, M., J.J. Burke and H.T. Nguyen, 1992. Inhibition and recovery of photosystem II following exposure of wheat to heat shock. Envir. and Exp. Bot., 32: 125-135.