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Effect of short-term heat treatment of rice seedlings on sensitivity of thylakoid membranes to photoinhibition

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Abstract

The after-effects of 24 h high temperature (35 or 45 °C) treatment on the photochemical activities and photooxidative lipid peroxidation, subsequent to their irradiation were studied in 7-d-old etiolated rice (*Oryza sativa*) seedlings. Photosystem (PS) 1 and PS 2 mediated photoreactions of thylakoids isolated from the seedlings exposed to high temperature did not differ significantly from the thylakoids isolated from control seedlings (25 °C). Hence, all kinds of tested thylakoids were equally efficient in capturing and utilizing radiant energy. The high irradiance induced loss in PS 2 activity and lipid peroxidation measured in terms of malondialdehyde production was more rapid in thylakoids isolated from stressed seedlings as compared to that of control seedlings. Thus the thylakoids isolated from the stressed seedlings were more prone to photodamage than those from the control seedlings.

Additional key words: chlorophyll, lipid peroxidation, malondialdehyde, *Oryza sativa*, photosystem 1, photosystem 2.

Introduction

When leaves or isolated chloroplasts are exposed to high temperatures for short periods, their photosynthetic apparatus shows characteristic changes such as irreversible inhibition of CO_2 fixation, O_2 evolution and photophosphorylation (for reviews see Berry and Björkmann 1980, Quinn and Williams 1985). In contrast, the PS 1 mediated electron transport is stimulated by high temperature treatment (Armond *et al.* 1978, Stidham *et al.* 1982, Thomas *et al.* 1986, Sabat and Mohanty 1989). These changes occur over a wide temperature range, depending upon the plant species (Berry and Björkmann 1980, Quinn and Willims 1985).

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High temperature treatment of the chloroplasts causes disorganization of thylakoid membranes such as dissociation of PS 2 light-harvesting antenna from central core complex (Armond *et al.* 1980), destacking of grana and lipid phase separations forming aggregates of inverted lipid micelles (Gounaris *et al.* 1983, 1984). These changes lead to the decline in rate of PS 2 mediated electron transport. Heat-induced stimulation of PS 1 activity is associated with thermal uncoupling (Thomas *et al.* 1986, Sabat *et al.* 1991) as well as with certain alterations in the structure of thylakoid membrane such as increase in antenna size of PS 1 (Ivanov *et al.* 1985) and/or changes in electron donation site of $DCPIPH_2$ (Sabat and Mohanty 1989).

Efforts made so far to understand the post-heat stress induced changes in photochemistry of the photosystems and their structural alterations focus on heat treatment to leaf discs, isolated chloroplasts or PS 2 and PS 1 submembrane fractions (Katoh and San Pietro 1967, Armond *et al.* 1980, Berry and Björkmann 1980, Stidham *et al.* 1982, Quinn and Williams 1985, Sabat *et al.* 1986, Thomas *et al.* 1986, Havaux 1993). Only a few reports are available on the effect of elevated temperature on chloroplast development during seedlings establishment, *i.e.* during the transition of etioplasts to chloroplasts (Smillie *et al.* 1978, Yordanov 1978, Mohanty and Mohanty 1988).

Development of chloroplasts from etioplasts marks a critical phase in the life cycle of a plant as it characterizes the transition from heterotrophy to autotrophy. Therefore, the etiolated seedlings constitute a suitable system for studying the effect of environmental factors on photosynthesis. Earlier, investigations in our laboratory have reported that etiolated wheat seedlings subjected to 35 °C for 48 h show reduction in growth and total pigment content without any significant change in the photochemical activities (Mohanty and Mohanty 1988). However, no efforts were made to see if heat stress brings about any alteration in the sensitivity of the chloroplasts to photodamage.

In light of the above information, we attempted to investigate whether a short term exposure of etiolated rice seedlings to 45 °C could cause any permanent alteration(s) in the thylakoid membrane functions during the post-stress period. We have also compared the susceptibility of these thylakoids to photoinhibitory treatment.

Materials and methods

Plants and temperature treatment: Rice (*Oryza sativa* L. cv. Kalinga III) seeds were obtained from Central Rice Research Institute, Cuttack, India. The seeds were incubated on cotton wetted with 250 cm^3 mineral growth medium (Arora and Pardha Saradhi 1995) in glass bottles for 7 d at 25 ± 2 °C in dark. 7-d-old etiolated seedlings were then exposed to 35 ± 2 °C or 45 ± 2 °C in dark for 24 h while control seedlings were kept at 25 ± 2 °C in dark for the same period. Both the control as well as heat treated seedlings were then irradiated continuously for 3 d with white fluorescent radiation (60 μmol m^{-2} s^{-1}). Subsequently, primary and secondary leaves were excised from these seedlings and were used for various investigations.

Thylakoid membranes were isolated according to the procedure of Nakatani and Barber (1977). Leaves were homogenized in ice-cold isolation buffer containing 0.4 M sorbitol, 15 mM Tricine (pH 7.8) and 10 mM NaCl (buffer A), using a *Polytron* homogenizer *PT 3000 (Kinematica AG,* Germany). Homogenate was filtered through 4 layers of miracloth and centrifuged at 5 000 *g* for 5 min. Supernatant was discarded, and the pellet was washed in a buffer containing 10 mM Tricine (pH 7.8), 10 mM NaCl and 5 mM $MgCl_2$ (buffer B). This was then centrifuged at 5 000 *g* for 5 min, and the supernatant was discarded. The pellet was suspended in buffer containing 0.1 M sorbitol, 10 mM Tricine (pH 7.8), 10 mM NaCl, and 5 mM $MgCl_2$ (buffer C). The chlorophyll (Chl) content of the thylakoids was estimated following the procedure of Porra *et al.* (1989).

Assay of photochemical activities: The polarographic measurements of the photochemical activities were done with a Clark type O_2 electrode as described by Alia *et al.* (1992). The assay mixture for measurement of electron transfer from H_2O to *p*-benzoquinone (PBQ) contained 1.0 mM PBQ in 1 cm^3 of the buffer C. Similarly for H_2O to phenyl-*p*-benzoquinone (*p*-PBQ), H_2O to DCPIP (2,6-dichlorophenol-indophenol) or H_2O to ferricyanide (FeCN), *p*-PBQ in the above mixture was replaced by 1.0 mM *o*-PBQ, 0.1 mM DCPIP or 2 mM FeCN, respectively. The PS 1 assay mixture for $DCPIPH_2$ or $TMPDH_2$ (reduced N,N,N′,N′-tetramethyl phenylene diamine) supported methyl viologen (MV) photoreduction contained 1 cm^3 of buffer C, 0.1 mM DCPIP or 0.5 mM TMPD, 5 mM ascorbate, 2 mM sodium azide, 1 mM MV, and 0.05 mM 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). The assay mixture for whole chain electron transport from H_2O to MV contained 1 cm^3 buffer C, 2 mM sodium azide and 1 mM MV. Thylakoids equivalent to 15 μg chlorophyll (Chl) were used in all the assays. The assays were conducted at 25 °C under saturating "white light" (800 μmol m^{-2} s^{-1}). For radiant energy saturation studies of PS 2, "white light" was attenuated by using different neutral density filters (*Balzers*, USA).

Malondialdehyde estimation: Photoinhibitory damage was studied by estimation of malondialdehyde content in the thylakoids obtained from control (25 ± 2 °C) and heat-pretreated (45 ± 2 °C) seedlings. Isolated thylakoids were washed and suspended in Tris-HCl buffer (pH 7.5) containing 175 mM NaCl. Thylakoids equivalent to 1 kg m^{-3} Chl were taken in a chamber maintained at 15 °C and irradiated with strong "white light" (1200 μmol m^{-2} s^{-1}) for photoinhibition. Both MDA level and PS 2 mediated electron transport were measured at 10 min intervals up to 60 min. Simultaneously the thylakoids were kept in dark as controls and measurements were made in a similar manner. MDA estimation was done by thiobarbituric acid reaction according to Heath and Packer (1968). The absorbance was measured at 532 nm and corrected for nonspecific turbidity by subtracting the absorbance at 600 nm.

Results and discussion

Growth and chlorophyll contents: The growth of rice seedlings exposed to 35 °C did not differ significantly from that of control (25 °C) seedlings. Seedlings exposed to 45 °C were stunted and the secondary leaf formation was suppressed. However, no significant change in per cent dry mass was observed in the seedlings subjected to high temperature (Table 1). Chl (*a*+*b*) content per unit fresh mass showed a minor decline in the leaves of seedlings exposed to 45 °C as compared to the control seedlings. Also, the Chl *a*/*b* ratio remained similar in both stressed and unstressed seedlings (Table 2). In contrast, Mohanty and Mohanty (1988) observed that short term exposure of etiolated wheat seedlings to 35 °C lead to the development of photosynthetic parts with 50 - 60 % lower Chl content upon their transfer to light at 25 °C as compared to controls. Moreover, the difference in the Chl content was retained even after 6 d after their transfer to room temperature. These observations suggest that rice cv. Kalinga III has the potential to withstand even temperatures as high as 45 °C. Unlike Chl content, the concentration of carotenoids in seedlings exposed to 45 °C was significantly lower than that of the control (Table 2).

Table 1. Length and dry mass of shoots of rice seedlings exposed to short term high temperature treatment. The values are means of three independent experiments ± SE.

Temperature [°C]	Length [cm]	Dry mass [% of fresh mass]
25 ± 2	25 ± 2	15.8 ± 1
35 ± 2	25 ± 2	15.6 ± 2
45 ± 2	10 ± 2	15.7 ± 2

Table 2. Photosynthetic pigment contents [mg kg^{-1}(f.m.)] of shoots of rice seedlings exposed to short term high temperature treatment. Chl - chlorophyll the values are means of three independent experiments ± SE.

Temperature [°C]	Chl *a*	Chl *b*	Chl (*a*+*b*)	Chl *a*/*b*	Carotenoids
25 ± 2	1470 ± 17	580 ± 4	2051 ± 18	2.5	125 ± 6
35 ± 2	1466 ± 6	582 ± 4	2048 ± 19	2.5	126 ± 3
45 ± 2	1356 ± 14	541 ± 7	1864 ± 16	2.5	100 ± 6

Photochemical activities: PS 1 activity measured as $DCPIPH_2$ or $TMPDH_2$ supported MV photoreduction was significantly higher in thylakoids isolated from the 45 °C pretreated seedlings than, in those of the control (Table 3). Enhancement in PS 1 mediated electron transport on mild heat treatment of thylakoids *in vitro*, with MV as terminal acceptor has been reported by Thomas *et al.* (1986) and Sabat and Mohanty (1989). Such enhancement could be due to thermal uncoupling of electron transport from photophosphorylation and/or alteration in the absorptive cross section of PS 1

assembly (Ivanov *et al.* 1985) or even reduction of superoxide radicals by specific electron donors (Boucher and Carpentier 1993). Water oxidation capacity measured by the $H_2O \rightarrow MV$ assay did not show any difference in the thylakoids isolated from heat treated or control seedlings. Thylakoids obtained from the seedlings subjected to short term heat treatment (45 °C) registered 12 - 15 % decline in PS 2 activity expressed per unit Chl compared to the corresponding values obtained for thylakoids from control seedlings (Table 3). The changes were not significant to suggest any major alterations in membrane organization. Susceptibility of PS 2 mediated electron transport to heat stress has long been recognized (Katoh and San Pietro 1967, Yamashita and Butler 1968, Santarius 1975, Sabat *et al.* 1986). Various hypotheses explain heat-induced inactivation of PS 2 activity by the inactivation of oxygen evolving complex (Nash *et al.* 1985) and basic structural changes like blocking of PS 2 centres or physical separation of Chl *a*/*b*-light harvesting complex from the core complex (Armond *et al.* 1980). However, lack of any substantial loss in PS 2 activity as seen in the present investigation suggests that short term exposure of rice seedlings to high temperature does not alter the development of photosystems and their functions. Thus the development of photosystems in rice cv. Kalinga III seems to be resistant to high temperature (given prior to the development of the photosynthetic apparatus).

Table 3. Primary photochemical activities of thylakoids isolated from the control seedlings and seedlings exposed to short term high temperature treatment. The values are means of 3 independent experiments ± SE. For abbreviations see Materials and methods.

Photochemical reaction	25 °C	45 °C
PS 2 [mmol(O_2 evolved) kg^{-1}(Chl) s^{-1}]		
$H_2O \rightarrow$ *p*-PBQ	56.1 ± 0.8	47.8 ± 0.9
$H_2O \rightarrow$ FeCN	61.9 ± 0.6	54.4 ± 0.6
$H_2O \rightarrow$ DCPIP	51.4 ± 0.7	45.0 ± 0.7
$H_2O \rightarrow$ *o*-PBQ	47.5 ± 1.0	46.9 ± 0.7
PS 1 [mmol(O_2 consumed) kg^{-1}(Chl) s^{-1}]		
$DCPIPH_2 \rightarrow MV$	159.2 ± 1.3	178.9 ± 1.0
$TMPDH_2 \rightarrow MV$	167.2 ± 1.1	173.6 ± 1.5
whole chain [mmol(O_2 consumed) kg^{-1}(Chl) s^{-1}]		
$H_2O \rightarrow MV$	14.2 ± 0.1	14.4 ± 0.1

Saturation by radiant energy: In order to ascertain that the small decline in PS 2 activity observed in our experiments did not involve any major alteration in the organization of effective antenna size (LHC 2), we studied radiant energy saturation characteristics of PBQ supported O_2 evolution in the thylakoids obtained from untreated and treated seedlings. A similar, typical saturation curve of the Hill reaction was obtained for both types of thylakoids (Fig. 1*A*). Fig. 1*B* shows the Eadie-Hofstee type plot of the values of Fig. 1*A* (Kok 1976). This analysis also

suggests that no significant change in either R_{max} (*i.e.* V_{max}) or relative quantum yield occurred on heat-treatment of rice seedlings prior to greening period.

Therefore, all these results (Chl *a/b*, PS 2 activity and radiant energy saturation characteristics) strongly suggest the possible resistance of PS 2 photofunctions to short term exposure of high temperature during the post-stress period, even though the general growth was retarded.

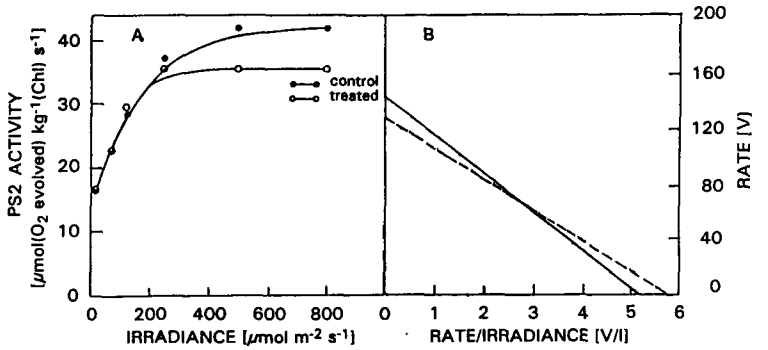

Fig. 1. (*A*) Radiant energy saturation characteristics of photosystem 2 (PS 2) activity in the thylakoids isolated from control and short term heat-treated rice seedlings. The values represent the means of three independent experiments (*B*) Eadie-Hofstee plot of rate (V) against rate/irradiance (V/I) for data in (*A*). Other details are given in materials and methods.

Photoinhibition: Exposure of thylakoids to high irradiances causes decline in PS 2 photochemical activities. The rapidity of such decline depends on the extent of photodamage caused to the pigment-protein complex (Halliwell and Gutteridge 1986, Chapman *et al.* 1990). In the present study, thylakoids isolated from seedlings exposed to high temperature showed a more rapid decline in PS 2 mediated O_2 evolution than the thylakoids from control seedlings, on exposure to high irradiance of 1200 μmol m^{-2} s^{-1} (Fig. 2). In general, the decline in PS 2 activity of thylakoids

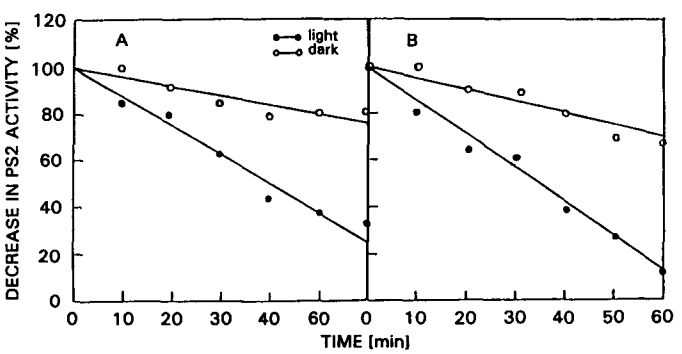

Fig. 2. Change in photosystem 2 (PS2) electron transport activity of the thylakoids, isolated from control (*A*) and short term heat treated (*B*) rice seedlings with time of incubation in dark or in strong "white light" (1200 μmol m^{-2} s^{-1}). The values represent means of three independent experiments.

exposed to high irradiance is attributed to a generation of free radicals and hence a peroxidative damage of the membranes (Halliwell and Gutteridge 1986, Alia *et al.* 1991). Exposure of thylakoids isolated from rice seedlings to high irradiance (present studies) also resulted in enhancement in MDA (a product of lipid peroxidation) contents and MDA production with time of irradiation (Fig. 3). The short term high temperature treatment increased these values (Fig. 3). Hence, the thylakoids from rice seedlings exposed to high temperature prior to greening have higher potential to generate free radicals and are more prone to photodamage when compared to the control thylakoids. This could be the main reason of the observed faster decline in PS 2 activity of thylakoids from heat treated seedlings than from control thylakoids (see Figs. 2 and 3). Therefore our experiments suggest that short term heat-exposure (45 °C) of rice seedlings might have led to alteration in the thylakoid membrane composition. High temperature induced acceleration of lipid peroxidation has been described by Simic and Karel (1980). Changes in the susceptibility of heat-treated thylakoids to lipid peroxidation might also be due to change in the lipid composition. As polyunsaturated fatty acids are the major targets of lipid peroxidation (Hattiwell 1984), we may assume that thylakoid membranes isolated from stressed plants might be richer in unsaturated fatty acids than the controls. Another probable reason behind excessive MDA production by thylakoids isolated from heat treated seedlings could be lesser carotenoid content in these seedlings (Table 2). Carotenoids play an important role in protecting the membranes against photodamage (Demming-Adams *et al.* 1989, Young and Britton 1990).

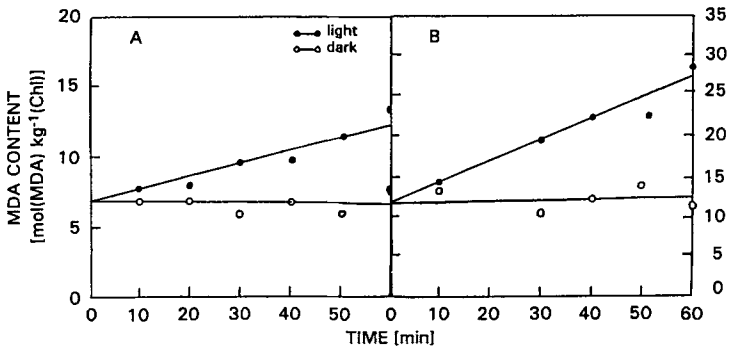

Fig. 3. Change in the malondialdehyde (MDA) content of the thylakoids, isolated from control (*A*) and short term heat treated rice seedlings (*B*) with time of incubation in dark or in strong "white light" (1200 μmol m^{-2} s^{-1}). The values represent means of three independent experiments.

In summary, our results suggest that even though no major alteration in primary photochemical activities of thylakoids isolated from rice seedlings exposed to short term high temperature stress was observed, these thylakoids are more prone to photodamage than the untreated material. However, further studies are required to analyse the actual reason(s) behind the high-susceptibility of thylakoids isolated from heat stressed rice seedlings to photodamage.

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