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DIFFERENTIAL EFFECT OF HIGH TEMPERATURE ON THE PHOTOSYNTHETIC ELECTRON TRANSPORT OF MAIZE PLANTS

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ABSTRACT:

In this study an attempt has been made to establish the differential action of high temperature on photosynthetic electron transport mediated by either PS-II or PS- I or both in Maize thylakoids after giving high temperature treatment to primary leaves. The increase in the temperature from 25^0C-45^0C cause inhibition in PS-II catalysed electron transport. The possible reason for the loss of PS-II catalysed electron transport could be depletion of manganeese from water oxidation complex. In contrary high temperature induced enhancement in PS-I catalysed electron transport due to opening of new site to donate electrons before plastocyanin by reduced DCPIP. Thus high temperature exerts differential effect on PS-II as well as PS-I in Maize plants.

KEY WORDS: Chlorophyll, Electron transport, Photo system, Maize plants.

INTRODUCTION:

In PS II, the main target for high temperature stress is oxygen evolving complex. As a result, there has been loss of Mn ions and extrinsic polypeptides of water oxidation complex (Nash *et al.*, 1985; Enami *et al.*, 1994). It is suggested that the membrane permeability increases during heat treatment, which results in a decrease in the proton gradient formation across the thylakoid membrane and a suppression of the linear electron flow. The changes in the membrane viscosity could be due to the variation of grana and stroma thylakoids ratio. It was observed that the quantity of grana thylakoids in green leaves is enhanced when exposed to damaging temperatures (Gounaris *et al*, 1983). In the past, PS II was considered a key weak link (Santarius 1975; Berry and Bjorkman, 1980; Enami *et* al., 1994) but damage to PS II only occurs at high temperatures, above 35^0C (Yamane *et al.*, 1998). Heat stress is often defined as where temperatures are hot enough for sufficient time that they cause irreversible damage to plant physiological functions and development. Due to this several plant physiological processes are getting affected by temperature and one of such processes is photosynthesis (Bauer,1979; Berry and Bjorkmann, 1980). No inhibition in PS I function and intersystem electron transport was detected (Havaux, 1996). HT stress causes conformational change in the Cyt $b_6$fcomplex causing appearance of new electron donor sites (Thomas *et al.*, 1986; Mohanty *et al.*, 1987). In this investigation, an attempt has been made to analyse the effect of high temperature on photochemical activities of maize plants.

MATERIALS AND METHODS:

Healthy seeds of Maize (*Zea mays*), were obtained from Acharya N.G. Ranga Agricultural University, Hyderabad. The seedlings were raised up to 8 days in light. 4-5 long and expanded leaf segments of primary leaves were used for experimental work. Maize primary leaves were taken and given high temperature treatment in growth chamber ranging from 25-50^0C for 20 min to perform photochemical assays. After giving the high temperature treatment (35/40^0C), the seedlings were placed at growth temperature for 24h and electron transport studies were measured. Thylakoid membranes were isolated according to Swamy *et a1.*, (1995) with some modifications. Electron transport rates were measured using Clark type oxygen electrode (Hansatech, UK). These measurements were done at 4^0C. The 2 ml reaction mixture contained 50 mM Hepes-NaOH (pH 7.5), 100 mM sucrose, 2 mM $MgCl_2$ and 5 mM KCl and thylakoid membranes equivalent to 40 µg of Chl. The assay mixture was kept continuously stirred during measurements (Sabat *et al.*, 1985). Whole chain electron transport assay (H_2O to MV) was measured as molecular oxygen consumption by using MV as an electron acceptor. PS II assay (H_2O to pBQ) was studied in terms of oxygen evolution in thylakoid membranes. PS I assay ($DCPIPH_2$ to MV) was studied in terms of oxygen consumption (Mohanty *et al.*, 1987). The thylakoid membranes equivalent to 40 µg Chl were used in all electron transport assays. Intersystem electron transport assay (DQH_2 to MV) was studied in terms of oxygen consumption. Intersystem electron transport assay (DQH_2 to MV) was studied in terms of oxygen consumption (Izawa, 1977).

RESULTS:

The effect of HT on photosynthetic electron transport of Maize thylakoids has been studied by giving the treatment of HT to Maize primary leaves. After giving the treatment of HT 25-45°C, the thylakoid membranes have been isolated from the Maize primary leaves and the electron transport activities were measured by using oxygen electrode and different electron transport donors, acceptors and inhibitors by following the method of Mohanty *et a1* (1987). Methyl viologen is an artificial electron acceptor which accepts electrons from the reducing side of' PS I. Therefore, an attempt has been made to study the effect of' HT on the whole chain electron transport ($H_2O \rightarrow MV$) by using thylakoid membranes isolated from the HT treated primary leaves.

Control thylakoid membranes before giving the heat stress showed a high rate of oxygen consumption involving whole chain electron transport (160 µ moles oxygen consumed mg Chl^{-1} h^{-1}). When HT treatment was given to the primary leaves for 20 min, it caused temperature dependent inhibition in whole chain electron transport activity (Table 1). 49% inhibition was noticed at 40°C of treatment and the inhibition has gone up with the raise of temperature to 50°C by 71%. These results suggest that the extent of inhibition is dependent on the extent of HT exposure. The reason for the inhibition of whole chain electron transport activity could be either due to alterations at the level of PS II or PS I or both. Since the HT inhibited the whole chain electron transport activity under *in vivo* conditions, the effect of HT on PS II catalyzed electron transport of Maize thylakoid membranes was studied by using p-BQ as electron acceptors. The p-BQ is lipophilic in nature. Therefore, it can easily enter in to the thylakoid membranes and accepts the electrons from the PQ pool. Control thylakoid membranes exhibited appreciable rate of oxygen evolution in the Hill activity (195 µ moles oxygen evolved mg Chl^{-1} h^{-1}).

Table 1: Effect of HT on whole chain electron transport assay ($H_2O \rightarrow MV$) in Maize primary leaf segments after 20 min of incubation at respective temperatures.

Temperature (°C)	Whole chain electron transport ($H_2O \rightarrow MV$) µ moles of O_2 consumed mg Chl^{-1} h^{-1}	Percent loss
25	160 ± 16	0
30	152 ± 17	5
35	132 ± 14	17
40	76 ± 8	52
45	62 ± 7	61

The treatment of HT in leaves caused increase in the inhibition based on the extent of temperature treatment. The inhibition of 55% was noticed in the Hill reaction after giving treatment of 40°C.

Further rise to 45°C, brought enhancement in the inhibition by 78% (Table 2). At 50°C temperature treatment, there is no oxygen evolution at all which could be probably due to damage of water oxidation complex. According to literature, the inhibition in PS II catalyzed electron transport by HT could be most probably due to alterations at the level of either oxidizing side of PS II or reaction centre.

To examine whether the inhibition in Hill activity caused by HT was linked to spectral alterations of LHC II of PS II or not, the extent of inhibition caused by HT was measured at different illuminating light intensities. For this purpose, 40°C was selected as treated sample and the inhibition was measured at various light intensities (10- 400 Wm^{-2}) (Table 3).The inhibition at light saturating conditions was 13% more when compared to that of at light limiting conditions. The light intensity was varied by using different neutral density filters. In the presence of HT, the extent of inhibition under light saturating conditions (400 Wm^{-2}) was more pronounced than that under light limiting conditions (10 Wm^{-2}). The reason for the inhibition at light limiting conditions could be due to alterations in LHC II of PS II. The inhibition at high light intensities is due to alterations at the level of reaction centre. After knowing PS II is one of the targets, we have concentrated our experiments to analyze the effect of HT on PS I catalyzed electron transport.

Table 2: Effect of HT on PSII catalyzed transport assay (H_2O →p-BQ) in Maize primary leaf segments after 20 min of incubation at respective temperatures.

Temperature (°C)	PSII catalyzed electron transport assay (H_2O→p-BQ) µ moles of O_2 evolved mg Chl^{-1} h^{-1}	Percent loss
25	195 ± 21	0
30	181 ± 17	7
35	144 ± 16	26
40	87 ± 10	55
45	57 ±6	71

Table 3: Effect of illuminated light intensity on HT induced inhibition of photosystem II catalyzed electron transport activity (H_2O→ p-BQ).

Light intensity Wm^{-2}	PSII catalyzed electron transport activity µ moles of O_2 evolved mg Chl^{-1} h^{-1}		Percent Inhibition
	Control	HT treated (40°)	
10	43 ± 3	25 ± 2	41
112	85 ± 7	46 ± 3	46
205	126 ± 10	64 ± 5	49
400	203 ± 18	93 ± 7	54

Table 4: Effect of HT on PS I electron transport assay (DCPIPH_2→MV) in Maize thylakoid fragments after 20 min of incubation at respective temperatures.

Temperature (°C)	PS I catalyzed electron transport, DCPIPH_2→ MV µ moles of O_2 consumed mg Chl^{-1} h^{-1}	Percent Enhancement
25	362 ± 34	0
30	402 ± 40	11
35	452 ± 42	25
40	514 ± 49	42
45	571 ± 54	58

To perform these experiments, Asc + DCPIP has been used as a donor system and methyl viologen was used as electron acceptor (DCPIPH_2→ MV). In control thylakoid membranes the activity of PS I is equal to 362 µ moles of oxygen consumed mg Chl^{-1} h^{-1} (Table 4). The rise in the temperature to 45°C gradually caused the enhancement of PS I activity. At 45°C HT treatment, 58% enhancement in PS I catalyzed electron transport activity was noticed. The probable reason for the enhancement of PS I activity could be opening of new site for reduced DCPIP to donate the electrons to PS I. The same enhancement has been observed in spinach thylakoid membranes with reduced DCPIP a donor (Thomas *et al.*, 1986; Sabat e*t al.*, 1986). To find out the

existence of target site in intersystem for HT, analysis has been made to study the effect of HT on intersystem catalyzed PS I electron transport by using reduced duroquinone as donor and MV as acceptor. DQH_2 donates electrons near PQ in the photosynthetic electron transport. In control thylakoid membranes, the activity of intersystem electron transport was equal to 250 µmoles of oxygen consumed mg Chl^{-1} h^{-1}(Table 5). The increase in the temperature gradually caused inhibition in intersystem electron transport activity and 68% loss was noticed at 45°C HT treatment. The possible reason for the loss of intersystem electron transport activity could be the changes of PQ pool.

According to literature, spectral properties of photosynthetic pigments like absorption and fluorescence are very much related with the primary processes of photosynthesis like electron transport. Since an inhibition has been observed under HT, this could be due to alterations at the level of either LHC or reaction centre.

Table 5: Effect of HT on the intersystem catalyzed electron transport activity of the thylakoids isolated from control and HT treated Maize primary leaves after 20 min of incubation at respective temperatures.

Temperature (°C)	**Inner system electron transport** **DQH_2 to MV** **µ moles of O_2 consumed mg Chl-1 h-1**	**Percent** **Inhibition**
25	250 ± 24	0
30	232 ± 22	9
35	170 ± 11	32
40	112 ± 10	56
45	80 ± 8	68

DISCUSSION:

Temperature is one of the ecological factors which influence the plant growth and development. The temperature ranges from near freezing point in arctic zone to 50°C in the hottest deserts. In addition, due to revolution of industries mainly CO_2 is getting accumulated in the atmosphere. This accumulation of CO_2 causes greenhouse effect and raises the temperature in the atmosphere. Often plants are getting exposed or subjected to variety of seasonal temperatures due to fluctuations in the diurnal temperatures. However, some of the plants are able to grow in the high temperature by synthesizing important and new polypeptides which act as molecular chaperones. They provide thermotolerance to the organisms to overcome the stress conditions in the environment. Before discussing the temperature tolerance, it is important to know the specific alterations induced by high temperature in the selected plant system.

Up to now majority of the studies were coming out in higher plants by isolating thylakoid membranes (Thomas *et al.*, 1986). Further, most of the studies were carried out under *in vitro* conditions regarding the HT effect. Therefore, critical studies are required under *in vivo* as well as *in vitro* conditions to have an integrated approach of the problem. The above studies can provide information to identify the specific targets of action as a response to HT in the primary process of photosynthesis.

Since, Maize *(Zea mays)* is rich in proteins and related essential amino acids, the above plant system has been taken as an experimental material for the present study and HT effects were studied both under *in vitro* and *in vivo* conditions. Since, primary reactions of photosynthesis determine the plant productivity, the effect of HT was studied on the Maize system. Therefore, in the present study HT effect has been studied in the photosynthetic electron transport machinery of Maize plants of 8 days old by subjecting them for 20 min to HT ranging from 25 – 45°C (*in vivo*).

HT has great influence on the physiological processes of thylakoid membranes. HT induces alterations in the physical state of membranes and leads to the impairment of membrane linked functions. In higher plants, majority of the studies were made in thylakoid membranes in isolated conditions *(in vitro).* It has been shown that PS II catalyzed electron transport is more sensitive when compared to that of PS I (Mohanty *et al.*, 1987; Verner *et al.*, 2001; Chow *et al.*, 1991). The inhibition in PS II could be due to changes in water oxidation complex or loss of manganese (Enami *et al.*, 1994). Thomas *et a1* (1986) showed that there could be opening of new site for DCPIP donation which is responsible for enhanced PS I activity in higher plant thylakoids. To the best of our knowledge very few attempts were made under *in vivo* conditions in higher plant system. Therefore, the present work constitutes the detailed analysis of HT on Maize plant system both under *in vivo* and *in vitro* conditions to have a comprehensive view.

HT treatment initially was given to the primary leaves of Maize and after isolation of thylakoids the effect has been studied by using oxygen electrode regarding whole chain electron transport assay ($H_2O \rightarrow MV$). Table 1 clearly demonstrates that HT shows temperature dependent inhibition in whole chain electron transport and 49% inhibition was noticed at 40°C. The reason for the inhibition of whole chain electron transport could be alterations either at PS II or PS I or both. To verify the above prepositions PS II catalyzed electron transport has been measured at different temperature treatments (Table 2). 52% inhibition was observed at 40°C of HT treatment. Similar observations have been reported by earlier workers regarding the effect of HT on PS II catalyzed electron transport (Nash *et al.*, 1985; Enami *et al.*, 1994; Mohanty *et al.*, 1987). The reason for the loss of PS II

catalyzed electron transport activity by HT could be either loss of Mn ions or extrinsic polypeptides of WOC as suggested by Nash *et a1* (1985). There is another report indicating the reason for the loss of PS II activity could be the alterations of LHC II in pea thylakoids. To examine whether the inhibition in PS II catalyzed electron transport activity by HT is linked with alterations of LHC, the extent of inhibition was measured at different illuminating conditions (Table: 3). The inhibition was more at light saturating conditions than that of at light limiting conditions due to effect of HT. The possible reason for the differential inhibition could be alterations at the level of LHC II in PS II.

After analyzing the status of PS II photochemistry, further experiments were planned to study the effect of HT on PS I catalyzed electron transport (Table.4). Depending on the raise of temperature from 25-50°C there was a gradual enhancement in PS I activity and 69% enhancement was noticed at 50°C (Table; 4).The probable reason for the enhancement of PS I activity could be opening of new site for reduced DCPIP donation as suggested by Thomas *et a1* (1986) and Sabat *et al* (1986). Another possible reason for the enhancement of PS I activity could be changes in the absorption cross section of PS I which arises due to migration of LHCP from PS II to PS I. To rule out the possibility of the presence of inhibitory site in intersystem electron transport, attempts were made to measure intersystem catalyzed electron transport activity using DQH_2 as donor (Table.5). *In vivo* measurements clearly demonstrated that LHC II is main target for HT and is responsible for the altered PS II photochemistry. These results are in agreement with the observations of Sabat *et a1* (1985) who showed the similar results regarding the HT effect on beet spinach thylakoids.

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