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ALTERED THYLAKOID MEMBRANE PHOTOFUNCTIONS UNDER HIGH TEMPERATURE STRESS IN WHEAT PRIMARY LEAVES

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Electron transport measurements of thylakoids isolated from elevated temperature treated 8-day-old wheat primary leaves indicated that photosystem II lost its ability to donate electrons. In addition, a significant increase in the reduced DCPIP mediated photosystem I mediated methyl viologen photoreduction activity was observed. However the electron transport supported by intersystem (Duroquinone to methyl viologen) was extremely sensitive to high temperature. Lipid peroxidation measurements indicate that there is an inverse relation to lipid peroxidation and inhibition of photosystem II and intersystem supported electron transport. Thus alterations in the thylakoid lipids are mainly responsible for altered functions of photosynthetic electron transport in wheat thylakoids.

Keywords : Electron transport; High temperature; Lipid peroxidation; Wheat thylakoids.

Introduction

Plants are generally getting exposed to various fluctuations in temperatures when they are grown in varied environmental conditions. Due to this there will be disruption of functional integrity of photosynthetic apparatus at the level of chloroplast[1-3]. The thylakoid membranes have been shown to be more heat sensitive than other biomemebranes[4]. High temperature (HT) causes not only alterations in electron transport but also it affects the photophosphorylation[5,6]. They also reported the stimulation in PS I activity when the isolated chloroplasts are subjected to high temperatures. There is a preferential sensitivity of thylakoid membranes depending on the nature of sample whether it is *in vivo* or *in vitro* towards high temperatures. Majority of the work is related to the *in vitro* studies. Only few studies of high temperature has been concentrated on the whole leaves[3] and excised leaves[7]. Up to now studies related to the effects of HT on photochemical activities in relation to lipid peroxidation are scanty. Therefore in the present investigation, we have made an attempt to investigate the effect of HT on partial electron transport activities and lipid peroxidation in chloroplasts isolated from the intact wheat seedling after giving temperature shocks for 10 Min from 35°C to 47°C.

Materials and Methods

Wheat (*Triticum aestivum* L.C.V. Kalyana sona) seedlings were grown on petriplates at 25° C under continuous white light (~16 Wm^{-2}). Nutrient solution was supplied in the form of Hoagland at 4-day intervals to seedlings. After 8th day, seedlings were exposed to various elevated temperature for 10 min in low light. The primary leaves of both control and high temperatures (HT) treated seedlings were sampled for chloroplast isolation and assay of photochemical activities.

The chloroplast isolation and electron transport measurements of the partial electron transport activities were done as described earlier[8]. The primary leaves were 1.0mM MV homogenized in 25mM Hepes isolation buffer (pH 7.5) 1.0mMV containing 400mM sucrose and 10mM $MgCl_2$ and 5mM KCl. The assay mixture for whole chain electron transport activity contained 1.0mM MV (Methyl viologen) and sodium azide in three ml of the 25mM HEPES reaction buffer (pH 7.8). For PS II mediated oxygen evolution, the reaction mixture consisted of 0.3 mM FeCN (Ferricyanide) and 0.2 mM PD (Phenyl diimine) in three ml reaction buffer. PS I catalyzed assay mixture contained 0.1 mM DCPIP (2, 6-Dichlorophenol-indophenol), 2 mM azide, lmM MV and 5 µM DCMU. Lipid peroxidations of thylakoid membranes have been measured by following the method of Heath and Packer[9]. Chlorophyll (Chl) was estimated according to method of Arnon[10]. In all the electron transport assays chloroplasts equivalent to 30µg of Chl was used. The assays were conducted at 25°C under saturating light intensity (400Wm^{-2}).

Results and Discussion

Photosystem II catalyzed electron transport measured ($H_2O \rightarrow PD \rightarrow FeCN$) polarographically in heat stressed thylakoids of 8 day old wheat primary leaves showed decline in electron transport activity with the increase of temperature from 35°C to 50°C(Table 1). No measurable oxygen evolution activity was observed in chloroplasts isolated from 47°C treated plants. This indicates that there is a complete loss of water oxidation capacity. Several workers also showed that water oxidation complex is sensitive to high temperature[3,11-13]. Ascorbate electron donation depends on the integrity of chloroplasts[14].

Table 1. Effect of high temperature on PS II catalyzed electron transport activity of wheat thylakoids.

Temperature °C	PS II catalyzed electron transport $H_2O \rightarrow PD \rightarrow FeCN$ µmoles of O_2 evolved mg Chl^{-1} hr^{-1}	Percent Loss
25	192±19	7
30	180±18	9
35	150±17	22
40	92±9	52
45	42±9	78
50	-	-

Table 2. Effect of high temperature on whole chain electron transport activity supported by ascorbate (Asc→MV).

Temperature °C	Whole chain electron transport (Asc→MV) µmoles of O_2 consumed mg Chl^{-1} hr^{-}	Percent Loss
25	160±16	0
30	162±17	0
35	162±16	0
40	82±8	50
45	65±7	60
48	47±4	70

Table 3. Effect of high temperature on PS I catalyzed electron transport activity in wheat thylakoid membranes.

Temperature °C	PS I catalyzed electron transport ($DCPIPH_2 \rightarrow$ MV) enhancement µmoles of O_2 consumed mg Chl^{-1} hr^{-1}	Percent enhance-ment
25	375±36	0
30	390±30	5
35	415±40	11
40	450±45	20
45	500±49	33
50	635±63	69

Table 4. Effect of high temperature on inter system electron transport activity ($DQH_2 \rightarrow$MV) in intact cells of wheat.

Temperature °C	Inter system electron transport Activity ($DQH_2 \rightarrow$MV) µmoles of O_2 consumed mg Chl^{-1} hr^{-1}	Percent inhibition
25	250±24	0
30	232±22	9
35	170±11	32
40	112±10	56
45	80±8	68

Table 5. Effect of high temperate on lipid peroxidation of thylakoid membranes of wheat plants.

Temperature °C	Lipid peroxidation n mol MDA mg $protein^{-1}$	Percent increase
25	35±3.5	0
30	48±4.7	37
35	58±5.7	66
40	65±6.4	86
45	63±6.2	80

Therefore we have studied the ascorbate supported whole chain electron transport in chloroplasts isolated from control and HT treated leaves. Table 2 shows that the ability of ascorbate to support PS II activity and PS II supported hill activity in chloroplasts of high temperature treated leaves declined with the increase of temperature. Failure of ascorbate to donate electrons above 45° C is suggestive of impairment of activity of PS II most probably at the level of OEC. Other workers also made similar conclusions using oxygen electrode measurements and Chl fluorescence emission spectra of HT stresses chloroplasts[1-2].

In Table 3, there is a clear indication regarding the effect of HT on reduced DCPIP supported PS I mediated MV photoreduction activity. Chloplasts isolated from HT treated leaves exhibited a siginificant increase in the PS I activity. HT induced stimulations of PS I activity has been reported under *in vitro* conditions both in the presence and absence of uncouplers[2,8,13,15,16]. The mechanism of heat-induced stimulations of PS I activity *in vivo* is not yet clear.

To find out the target site of HT in intersystem electron transport before DCPIP donation, the electron transport assay has been measured using DQH_2 as donor

to plastoquinone (Table 4). HT treatment caused gradual loss in intersystem electron transport activity suggesting the existence of site between plastoquinone and plastocyanin. Since plastoquinone is diffusible electron carrier which is lipophilic in nature and the altered lipid environment could be one of the reasons for the loss of PS II activity. To confirm this, lipid peroxidation of thylakoid membranes of both control and treated samples has been measured. Table 5 shows the lipid peroxidation patterns and HT effect on thylakoid lipid properties. The increase in the temperatures from 25°C to 45°C caused gradual increase in the MDA (Melonaldehyde) fractions by 86%. At 45°C, it has come down to 80%. These results clearly indicate that the enhancement of HT caused more lipid peroxidation fractions which can alter the lipid protein interaction of thylakoid membranes. In this way alterations in the thylakoid membrane lipids are partially responsible for the changes in the electron transport activities mediated by either PS II or PS I in the wheat thylakoids.

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