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HIGH LIGHT INDUCED ALTERATIONS IN THE PHOTOSYNTHETIC ELECTRON TRANSPORT ACTIVITIES OF THE WHEAT PRIMARY LEAVES

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ABSTRACT
The main source of energy which helps to perform the primary process of photosynthesis is light. In excess, this light in high intensities causes damage to the photosynthetic process. In this investigation an attempt has been made to study the effect of high light (100-430 Wm^{-2}) on photochemical activities of thylakoid membranes isolated from wheat primary leaves. The increase in the intensity of light caused gradual decrease in both whole chain and photosystem (PS) II catalyzed electron transport activities. The PS I catalyzed electron transport gets inhibited by 13% even at high intensities of light. Light intensity measurements clearly demonstrated that the inhibition is more at light saturating conditions than at light limiting conditions. The possible reason for the inhibition of PS II activity at light limiting conditions could be the alterations at light harvesting pigment protein complex in PS II of wheat primary leaves

INTRODUCTION

Photoinhibition occurs under all light intensities of visible region ranging from 400 - 700 nm. In photoinhibition there will be a series of reactions which cause inhibition in photosynthetic electron transport activities (Ohnishi *et al*., 2005). The reason for the inhibition of PS II catalyzed electron transport would be alterations of PS II reaction centre *i.e.* P680 (Styring *et al.,* 1990; Kruse *et al*., 1997). Mattoo *et al.,* (1984) suggested that the formed oxyradicals from molecular oxygen is responsible for the damage of D_1 protein of PS II under high light stress. The main reason for the inhibition of PS I activity could be inactivation of Fe-S centers present at the reducing side of PS I (Rajagopal *et al*., 2003). The inhibition of PS I activity is also dependent on the temperature at which the plants are exposed to high light (Terashima *et al*., 1994). Upto now studies related to the effect of high light intensity on economically important cereal crops are scanty. Hence in this investigation, an attempt has been made to analyze the effect of photoinhibition on photosynthetic electron transport reactions on wheat primary leaves by exposing them to 100-430 Wm^{-2} of high light.

MATERIALS AND METHODS

Wheat variety' *Triticum aestivum* 'was grown on soil in trays at 25°C by adding Hoagland media according to the method of Hoagland and Arnon (1950). The plants were grown for seven days under regulatory conditions at 15Wm^{-2} light intensity. Wheat plants were exposed to different intensities of light (100 – 430 Wm^{-2}) for 10-40 min. Electron transport activities of control and treated thylakoid membranes were assayed using a Clark type Oxygen electrode (Hansatech, UK) following Sabat *et al*., (1989). Thylakoid membranes were isolated according to the procedure to that of Saha and Good (1970) as described in Swamy *et al*., (1995) with some modifications. Photosystem II catalyzed electron transport activity was measured as O_2 evolution in 2 mL reaction buffer consisting of [50 mM HEPES - NaOH (pH 7.5), 100 mM sucrose, 2 mM $MgCl_2$ and 5 mM KCl], 0.5 mM freshly prepared p-benzoquinone (p-BQ) and thylakoid membranes equivalent to 40 μg of Chl. For WEC activity the reaction buffer contained 0.5 mM methyl viologen (MV), 1.0 mM of Na-azide, while the PS I reaction mixture contained 0.5 mM MV, 1.0 mM Na-azide, 0.1 mM 2,6- dichlorophenol indophenol (DCPIP), and 10 μM dichloro dimethyl urea (DCMU) and thylakoid membranes equivalent 40 μg of Chl in reaction buffer. Neutral density filters have been used to provide both low and high intensities of light while measuring the electron transport activities of PS II.

RESULTS AND DISCUSSION

In this present study the wheat plants were exposed to different intensities of saturating white light (100-430 Wm^{-2}) for 30 min and thylakoid membranes have been isolated and photosynthetic electron transport measurements were made. Therefore initially whole chain electron transport activity has been

Table 1: Effect of different high light intensities on whole chain electron transport assay ($H_2O \rightarrow MV$) in the thylakoid membranes of wheat primary leaves. Three mL of reaction mixture contains reaction buffer (25 mM HEPES-NaOH) (pH 7.5) containing 20 mM NaCl, 0.5 mM MV, 1mM Sodium azide and thylakoid cells equivalent to 15 μg of Chl. The values are average of three separate experiments and SD is not more than 10%

High light Wm^{-2}	Whole chain electron transport activity ($H_2O \rightarrow MV$) μ moles O_2 consumed / mg Chl /h	Percent loss
Control	125 ± 10	0
100	91 ± 7	27
220	65 ± 5	48
350	56 ± 4	55
430	36 ± 3	71

Table 2: Effect of different high light intensities on PS II catalyzed electron transport assay ($H_2O \rightarrow pBQ$) in the thylakoids of wheat primary leaves. Three mL of reaction mixture contains reaction buffer (25 mM HEPES-NaOH) (pH 7.5) containing 20 mM NaCl, 0.5 mM MV, 1mM Sodium azide and thylakoid cells equivalent to 15 μg of Chl. The values are average of three separate experiments and SD is not more than 10%

Intensity of light Wm^{-2}	PS II electron transport activity ($H_2O \rightarrow pBQ$) μ moles O_2 evolved / mg Chl /h	Percent loss
Control	226 ± 19	0
100	163 ± 14	28
220	131 ± 11	42
350	102 ± 9	55
430	70 ± 5	69

Table 3: Effect of different high light intensities on PS I catalyzed electron transport assay ($DCPIPH_2 \rightarrow MV$). Thylakoid fragments were incubated in the presence of high light. The reaction mixture of PS I catalyzed electron transport assay contained reaction buffer and other ingredients as mentioned in the Materials and methods. The values are average of three separate experiments and SD is not more than 10%

Intensity of high light (Wm^{-2})	Photosystem I catalyzed electron transport activity ($H_2O \rightarrow DCPIPH_2$) μ moles O_2 consumed / mg Chl /h	Percent loss
Control	392 ± 32	0
100	356 ± 29	9
220	333 ± 21	15
350	310 ± 19	21
430	278 ± 221	29

Table 4: Effect of different illuminated light intensities on high light induced inhibition of PS II catalyzed electron transport activity. Other details were mentioned in the material and methods

Intensity of high light $Wm^{-2)}$	Control	PS II catalyzed electron transport ($H_2O \rightarrow pBQ$) N moles of $O_2 \uparrow$ mg Chl^{-1} h^{-1} (350 Wm^{-2})30 min	Percentage of inhibition
420	228 ± 21	102 ± 10	55
350	141 ± 13	82 ± 7	42
200	106 ± 11	58 ± 4	45
50	58 ± 4	35 ± 3	40

measured using MV as electron acceptor (Table 1). The treatment with white light for 30 min caused gradual decrease in whole chain electron transport activity by 71% with 430 Wm^{-2}. The inhibition in whole chain electron transport activity could be due to alterations at the level of PS II (Theg *et al*., 1986; Choquet and Vallon, 2000) or changes at the level of PS I (Satoh and Fork, 1982; Rajagopal *et al.,* 2003). To verify the above possibility PS II catalyzed electron transport has been measured using pBQ as Hill acceptor. In PS II catalyzed electron transport 55% loss was noticed after exposure of level to 350 Wm^{-2} for 30 min (Table 2). The observed inhibition in PS II catalyzed electron transport could be either due to fine radical mediate damage to D_1 protein of PS II or due to inactivation of water oxidation complex as suggested by earlier workers (Kyle *et al.,* 1984; Ohad *et al.,* 1994). Compared to PS II activity, PS I activity seems to be less sensitive to high light when the stress is given at room temperature. High light treatment caused 29% inhibition in PS I catalyzed electron transport activity with 430 $Wm^{-2}$of white light (Table 3). PS I mediated electron transport activity to photoinhibition when the stress given at room temperature is on study also. The possible reason for the observed inhibition in PS I catalyzed electron transport could be due to alterations at the level of PS I reaction centre, P700 or at the level of iron-sulfur centre as earlier reported by Sonoike group (Sonoike *et al.,* 1995; Sonoike *et al*., 1997). Recently Rajagopal *et al.,* (2003) re-

ported that high light shows more inhibition in PS I activity at chilling temperature, due to effect on LHC I in PS I membrane particles. To identify whether high light induced inhibition of Hill activity is related to the spectral changes of LHC II or not the effect was studied at different illuminating conditions (Table 4). The measurement of Hill activity in thylakoids at different illuminating conditions indicates that high light induced inhibition was more at light saturating conditions than that at light limiting conditions. This indicates that the reason for the inhibition at light limiting conditions could be alterations in light harvesting complex (LHC) of PS II. Thus high light stress exerts multiple effects on photosynthetic electron transport in wheat thylakoid membranes.

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