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CHARACTERIZATION OF CADMIUM STRESS INDUCED ALTERATIONS IN PRIMARY PHOTOCHEMISTRY OF PHOTOSYSTEM II USING CHLOROPHYLL *a* FLUORESCENCE KINETICS AS A TOOL

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Abstract

Chlorophyll *a* fluorescence has been used as a major technique to monitor the photochemical reactions of photosynthesis. Generally at room temperature chlorophyll *a* fluorescence will be generated from photosystem (PS) II. Therefore chlorophyll *a* fluorescence can be used as a probe to study the alterations in PS II photochemistry. In this investigation a study has been made by using PAM kinetic spectrofluoremeter to characterize the alterations induced by Cd $(NO_3)_2$ in thylakoid membranes isolated from wheat (*Triticum aestivum*) primary leaves. The treatment of Cd (250-750 μM) caused an increase in F_o and decrease in F_v depending on the concentration. The increase in F_o clearly indicates the alterations in light harvesting complex whereas decrease in F_v shows the inhibition in the PS II catalyzed electron transport. Thus increase in F_o of thylakoid membranes can be used a tool identify the above heavy metal toxicity.

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Keywords: Cadmium, Chlorophyll *a* fluorescence, Light harvesting complex, PAM fluorescence kinetics, photochemistry, Photosystem II.

Abbreviations: Chl – Chlorophyll; F_o – Initial Fluorescence; LHC – Light Harvesting Complex; F_m – Maximal fluorescence; pBQ – Para-benzoquinone; PHC – Photochemistry; PS I – Photosystem I; PS II – Photosystem II; PQ – Plastoquinone; PAM – Pulse Amplitude Modulation; Q – Quinone; F_v – Variable fluorescence.

Introduction

The heavy metals are spread in the environment due to human interaction with soil and water through industrial activities, agriculture and the disposal of sewage sludge, threaten all living organisms, especially plants. Heavy metals are phytotoxic, lead to environment pollution and impair the physiological process (De Fillips and Pallghy, 1994; Heng *et al.*, 2004; Lamia *et al.*, 2005). Cadmium is one of the most phytotoxic heavy metal which causes the reduction in the photosynthetic rate, detrimental effects on chloroplast replication and cell division (Baryala *et al.*, 2001;Cheng *et al.*, 2002; Kupper *et al.*, 2007; Liu *et al.*, 2008) and water splitting apparatus of photosystem (PS) II and photosynthetic electron transports (Mallick and Mohn 2003; Faller *et al* 2005). Cd damages the photosynthetic apparatus, is particular the PS II (Siedleck and Baszsky, 1993; Siedleck and Krupa 1996). Earliar studies showed that PS II catalyzed electron transport is more sensitive when compared to that of PS I (Chow *et al.*, 1987). The inhibition in PS II could be due to changes in water oxidation complex or loss of manganese (Enami *et al.*, 1994). The loss of in the PS II catalyzed electron transport activity by Cd may be due to the alteration in the reaction centre as suggested by Renganathan and Bose (1990) or at the level of Q_B protein as reported by Mohanty *et al*., (1989). Therefore a study was carried out regarding to chlorophyll (Chl) *a* fluorescence and PAM kinetics of thylakoid membranes from by isolated

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thylakoids from wheat primary leaves. Studies related to the compare of Chl fluorescence kinetics with PS II catalyzed and electron transports are scanty.

Materials and Methods

Wheat (*Triticum aestevum*) seedlings were rised on Petri plates which were arranged in completely randomized block design, factorial design with there replicates for each plants growing in sterilized plastic tray. The experiment was conducted in a growth chamber under continous white light 12 h light /12 h dark under light intensity 15 Wm^{-2} (produced by neon lamps, Philips T-40 W/55) at plant level with a day/night temperature 24 ± 2 / 25 ± 3^{o}c and relative humidity of 65 ± 2 to 75 ± 2 % and watered daily with quarter strength Hoagland's nutrient solution. 7^{th} day old wheat (*Triticum aestevum*) primary leaves were used to present study. The plant samples from each container were separately harvested after 7 days old plants which were treated with Cd $(NO_3)_2$ in different concentrations (250 µM - 750 µM).

Thylakoid membranes were isolated according to Saha and Good (1970) as described in Swamy *et al.*, (1995) with some modifications. PS II catalyzed electron transport activity was measured as O_2 evolution in thylakoid membranes according to Mohanty *et al.*, (1989). PS II catalyzed electron transport activity was measured at different light intensity ranges from 13 - 410 µ moles irradiance. Fluorescence emission spectra of thylakoids were recorded by following the procedure of Mohanty *et al*, (1989). Chlorophyll *a* fluorescence induction kinetics was measured in PAM Chl fluoremeter which was developed by Schreiber (1986). The intensity of weak modulated light was 1 m wm^{-2} with a modulation frequency of 100 kHz and the intensity of red actinic light (>689 nm) was 60 m wm^{-2}. Cell suspension equivalent to 20 µg of Chl was used for kinetic measurements. Chlorophyll was estimated before measuring photochemical activities by following Arnon (1949).

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Results and Discussion

Cd effect on Photosystem II catalyzed electron transfer:

To identify the target photosystem, a measurement of the partial electron transfer reaction mediated by individual photosystems was made. Hence an attempt has been made to study the effect of Cd on PS II catalyzed electron transport activity. The study was made to the effect on PS II catalyzed pBQ Hill reaction in the thylakoid membranes. pBQ is an artificial electron acceptor ($H_2O \rightarrow pBQ$) and it accepts electron from PQ pool (Trebst, 1974). Control thylakoid membranes exhibited a high rate of PS II dependent 312 µ moles of O_2 evolved mg^{-1} Chl h^{-1}, in the absence of Cd heavy metal. But in the presence of Cd at a concentration 250 µM there was about 45 % inhibition in the PS II catalyzed electron transport activity in treated samples. The increase in the concentration of Cd from 250 to 500 µM caused 69 % inhibition of PS II catalyzed electron transfer activity. But, at a high concentration of about 750 µM, Cd caused 82 % inhibition Hill activity (Table 1).

Characterization of the site of inhibition in PS II catalyzed electron transport activity by Cd:

To study the maximal alterations in photosynthetic electron transport in terms of spectral features, wheat plants were treated with Cd in a particular concentration. To study whether the inhibition by Cd on Hill activity is linked to the Cd induced alterations in energy transfer with in chlorophylls. Therefore, a measurement was made regarding the extent of inhibition caused by Cd toxicity at different intensities of light. For this study, concentration of Cd of about 250 µM was selected. 250 µM of Cd was able to cause nearer to 50 % inhibition in Hill activity ($H_2O \rightarrow$ pBQ). Under the light limiting condition i.e. 13 µ moles irradiance of photons m^{-2} s^{-1} caused 40 % inhibition by Cd in the PS II catalyzed electron transport activity, whereas increase in the light

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intensities of about 120 µ moles and 230 µ moles irradiance caused 46 % and 47 % inhibition respectively under the presence of Cd at 250 µM concentration.. The increase in the saturating intensity of light above 410 µ moles irradiance of photons $m^{-2}s^{-1}$ did not change the extent of inhibition significantly with 250 µM of Cd. But, with the higher intensity of about 410 µ moles irradiance photons m^{-2} s^{-2} the inhibition with 250 µM of Cd was around 48 % (Table 2).

Effect of Cd on Chl *a* fluorescence kinetics in thylakoid membranes:

These studies indicate that there is an existence of another inhibitory site at reducing side of PS II near PQ. Therefore to identify the alterations in the LHC (Light Harvesting Complex) II, Chl fluorescence kinetic (PAM) measurements were made. The inhibition at the acceptor side of PS II caused by diuron abruptly raises the yield of variable fluorescence to the maximal level (Butler, 1977). However, during impairment of electron flow from donor side of PS II, the fluorescence yield remains at low level (Butler, 1977). In dark adapted thylakoid membranes Chl *a* fluorescence transient was observed upon illumination (Papageorgious, 1975).

The fluorescence emission increases from an initial level called, F_o, to a maximal level, F_m. This fluorescence rise from F_o to F_m is called variable fluorescence, F_v, because of its variable nature associated with redox reaction of PS II stable acceptor Q_A. A portion of absorbed light is lost and appears as fluorescence or initial fluorescence level, F_o (Mathis and Paillotin, 1981) (Fig 1). The true F_o can be observed at the onset of illumination when the Q_A is in fully oxidized state (dark adapted samples) or with a very weak modulated light (1m wm^{-2}) which is incapable of causing PS II photochemistry (Schreiber, 1986). After dark adaptation thylakoid membranes were excited with low modulated light to measure F_o followed by red actinic and strong additional white light to measure the F_m. The difference between F_m andF_o is F_v (Fig 1).

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In control spectrum, weak modulated light caused a rise upon excitation which is nothing but F_o (2.0). Further illumination with strong light caused enhancement in the signal to F_v (4.5), the maximum fluorescence variable was, $F_{m,}$ 6.5 (Table 3). But in the Cd treated Chl *a* fluorescence kinetics of thylakoid membranes weak modulated light caused enhancement of F_o excitation to 2.2.Then illumination with strong light caused increase in F_v to 4.1, the fluorescence maximum value was 6.3, (F_m) under the presence of Cd at 250 µM concentration. When increase in the concentration of Cd to 500 µM caused increase in F_o (2.5), F_v value was 3.2 which leads to F_m value was about 5.7. Cd caused alterations in fluorescence kinetics of 750 µM treated sample brought F_o value to 3.2, F_v value to 1.8, the maximal value, $F_{m,}$ was 5.0. There were a significant changes of values of fluorescence kinetics of Chl *a* as F_o value (2.0 to 3.2), F_v value (4.5 to 1.8) and F_m value (6.5 to 5.0). The above results indicate that the alterations by Cd may be due to the inhibition at the donor side, since the decrease in the F_v was observed. It clearly indicates that the loss in the F_v and F_m are responsible for the inhibition of the PS II activity. The increase in F_o indicates the damage at LHC II in PS II photochemistry (Campbell *et al.*, 1998). Thus, Cd is able to cause alterations in the PS II photochemistry i.e. electron transport activity under this toxic conditions. The alterations in PS II photochemistry is related to changes in water oxidation complex and PHC II of PS II. Thus Cd exerts multiple effects on photosynthetic electron transport activities depending on its concentrations.

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Table 1: Effect of Cd $(NO_3)_2$ on PS II [µ moles of O_2 evolved mg^{-1} Chl h^{-1}] catalyzed electron transport activities in wheat primary leaves.

Concentration (µM) $Cd(NO_3)_2$	PS II electron transfer activity $H_2O \rightarrow$ pBQ, µ moles of O_2 evolved mg^{-1} Chl h^{-1}	Percent Inhibition
Control	312 ± 4	0
250	172 ± 8	45
500	97 ± 9	69
750	56 ± 3	82

Table 2: Effect of illuminated light intensity on Cd $(NO_3)_2$ induced PS II electron transfer activity in the wheat primary leaves.

Irradiance µM Photons m^{-2} s^{-1}	PS II catalyzed electron transport activity $H_2O \rightarrow$ pBQ µM of O_2evolved mg^{-1} Chl h^{-1} $Cd(NO_3)_2$		Percent inhibition
	Control	treated(250µM)	
13	55 ± 5	33 ± 3	40
120	130 ± 13	70 ± 6	46
230	180 ± 17	95 ± 10	47
410	300 ± 26	154 ± 14	48

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Fig 1: Fluorescence kinetics of Chl *a* in control and Cd treated wheat primary leaves.

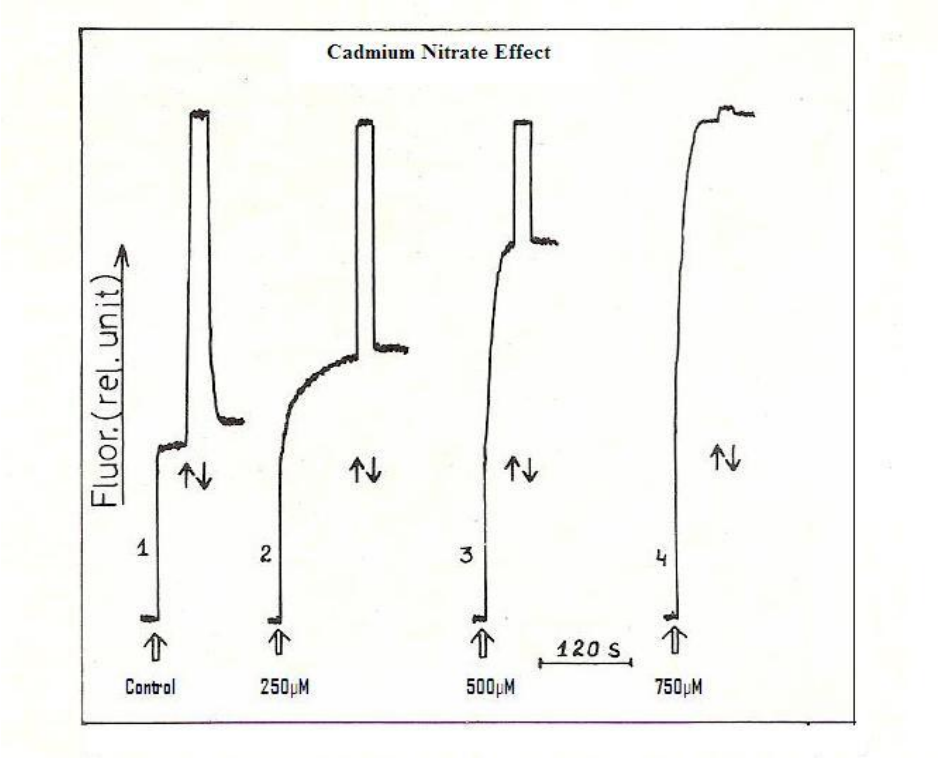

Table 3: Effect of Cd $(NO_3)_2$ on Chl *a* fluorescence kinetics of wheat thylakoid membranes. The samples were excited with very low light and then increased the light intensity after the initial fluorescence (F_o) is reached. Variable fluorescence (F_v) and maximum fluorescence (F_m) measurement were taken.

Concentration $Cd(NO_3)_2$ (µM)	Fluorescence parameter in terms of distance, cm		
	F_o	F_v	F_m
Control	2.0	4.5	6.5
250	2.2	4.1	6.3
500	2.5	3.2	5.7
750	3.2	1.8	5.0