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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 increasein $F_o$and decreasein $F_v$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 HarvestingComplex; 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 oneofthemost phytotoxicheavymetal which causes thereduction in the photosynthetic rate, detrimental effects on chloroplast replication and cell division (Baryala *etal.*, 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 andBaszsky, 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*fluorescenceandPAMkineticsofthylakoidmembranesfrombyisolated

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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.

Materialsand 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 intensityof 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).

ResultsandDiscussion

CdeffectonPhotosystemII catalyzedelectrontransfer:

Toidentifythetarget photosystem, ameasurement ofthepartialelectrontransferreaction 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 onPS 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 catalyzedelectrontransferactivity.But,atahighconcentrationofabout750μM,Cdcaused82 %inhibition Hill activity(Table 1).

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

To studythe 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 byCd on Hill activityis linked to the Cd induced alterations in energytransfer 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).Underthelightlimitingconditioni.e.13μmolesirradianceofphotons$m^{-2}s^{-1}$caused40 %inhibitionbyCdinthePSIIcatalyzedelectrontransportactivity,whereasincreaseinthelight

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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).

EffectofCdonChl *a*fluorescencekineticsin thylakoidmembranes:

These studies indicate that there is an existence of another inhibitorysite at reducing side ofPS IInearPQ.Thereforeto identifythealterations in theLHC (Light HarvestingComplex)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 yieldremainsatlowlevel(Butler, 1977). IndarkadaptedthylakoidmembranesChl *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.ThisfluorescencerisefromF$_o$toF$_m$iscalledvariablefluorescence,F_v,becauseofitsvariable 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 fullyoxidized 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 membraneswere 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 $F_m$and$F_o$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 excitationto2.2.Thenilluminationwithstronglightcausedincreasein$F_v$to4.1,thefluorescence maximumvalue was 6.3,(F_m) under the presence of Cd at 250µM concentration. When increase inthe 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 atthedonorside, sincethedecreasein the$F_v$was observed. It clearlyindicatesthat the loss in the $F_v$and F_m are responsible for the inhibition of the PS II activity. The increase in F_o indicatesthedamage at LHC IIin PS IIphotochemistry(Campbell *et al.*, 1998). Thus,Cd isable 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 electrontransport activities in wheat primary leaves.

Concentration (µM) $Cd(NO_3)_2$	PS IIelectrontransfer activity $H_2O \rightarrow pBQ$,µmolesof 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

Table2: Effectofilluminatedlightintensityon$Cd(NO_3)_2$inducedPSIIelectrontransfer activity in the wheat primary leaves.

IrradianceµM Photons$m^{-2}s^{-1}$	PS II catalyzed electron transport activity $H_2O \rightarrow pBQ$µMofO_2evolvedmg^{-1}Chlh^{-1}		Percent inhibition
	Control	$Cd(NO_3)_2$ 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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Fig1: FluorescencekineticsofChl*a*incontrol andCdtreated wheatprimaryleaves.

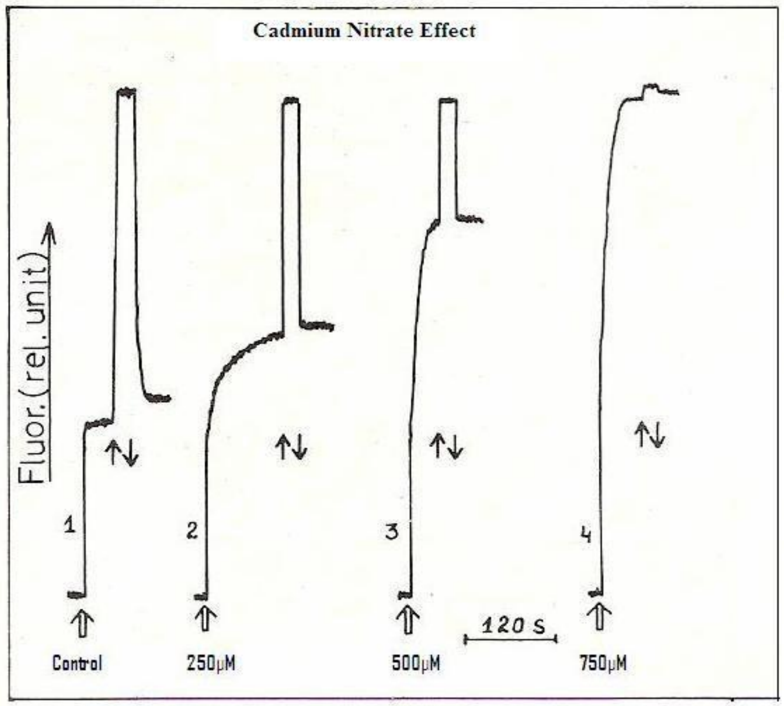

Table 3: Effect of Cd $(NO_3)_2$on Chl *a* fluorescence kinetics of wheat thylakoid membranes. The samples were excited with very low light and then increased the light intensity after the initial fluorescence (F_o) is reached. Variable fluorescence (F_v) and maximum fluorescence (F_m) measurement were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

Concentration $Cd(NO_3)_2$(μM)	Fluorescenceparameter intermsofdistance,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