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RESEARCH ARTICLE

Cadmium Stress-Induced Alterations In The Photosystem II Photochemistry Of Maize Primary Leaves.

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ABSTRACT

Exposure of maize plants to cadmium chloride (0.1-0.4 mM) caused inhibition in whole chain electron transfer as well as PS II catalyzed electron transfer. The chlorophyll fluorescence measurements in the presence and absence of DCMU indicated that cadmium is affecting the photo-synthetic electron transfer of PS II at the reducing side of PS II. Thus cadmium is not only affecting the oxidizing side but also it exerts inhibitory effects on the reducing side of PS II.
Keywords: Cadmium, Chlorophyll, Electron transfer, Photosystem II, Maize plants.

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INTRODUCTION

Heavy metals are phytotoxic, which leads to environmental pollution and impairs the physiological process [1,2,3]. Cadmium is one of the phytotoxic heavy metal which causes the reduction in the photosynthetic rate, detrimental effects on chloroplast replication and eel I division [4,5,6,7] chloroplast structure [8], as well as the water splitting apparatus of photosystem (PS) II and photosynthetic electron transfer [9,10]. The over production and rapid accumulation of reactive oxygen species (ROS), is considered as one of the important mechanisms of heavy metal toxicity in plants [11,12,13]. Studies related to the effect of cadmium on PS II catalyzed electron transfer in relation to chlorophyll (Chl) fluorescence are scanty, hence in this study an attempt has been made to correlate the cadmium induced PS II catalyzed electron transfer activities with chlorophyll fluorescence in the presence and absence of DCMU in maize plants.

MATERIALS AND METHODS

Maize (Zea mays) seedlings were raised on petri plates under continuous white light (160 μmoles m^{-2} s^{-1}) at 25°C. Hoagland's solution was supplied at 2 days intervals to the seed lings. 8-day-old seedlings were exposed to different concentrations of $CdCl_2$ (0.05 mM-0.2 mM) for 24 hrs. The corresponding $CdCl_2$, solutions were prepared and applied to the seedlings along with Hoagland's solution. After 24 hrs of exposure the plates were washed to remove the traces of $CdCl_2$, then the primary leaves of both control and cadmium treated seedlings were sampled and thylakoid membranes were isolated. For isolation of thylakoid membranes primary leaves were cut into pieces and homogenized in the presence of isolation buffer which contains 25 mM Hepes buffer (pH 6.8), 400 nm of sucrose and 1 mM of $MgCl_2$. After homogenization the content was filtered through the three layered cheese cloth and the filtrate was centrifuged at 6000 x g for 20 min at 4°C in a cool centrifuge.

After isolation the thylakoid membranes were used for measurement of photochemical activities by following the procedure of Sabat *et al.,* (1986) [14] with slight modification i.e., the suspension of isolated thylakoids in the reaction buffer 25 mM Hepes and 3 mM $MgCl_2$. In reaction buffer sucrose is totally eliminated.

The assay mixture for whole chain electron transfer activity contained 0.5 mM methyl viologen (MV) and 1 mM sodium azide in three ml of the 25 mM Hepes reaction buffer (pH 6.8). For PS II mediated oxygen evolution measurement, the reaction mixture consisted of 0.5 mM para benzoquinone (pBQ) in three ml reaction buffer. Chl fluorescence measurements were made by using the Jasco fluorimeter both in the presence and absence of DCMU at 25°C. Thylakoid membranes equivalent to 20 μg Chl/3 mL were suspended in the reaction buffer and the samples were excited at 440 nm to excite the Chl to record the emission spectra.

RESULTS AND DISCUSSION

Cadmium induced alterations in whole chain electron transfer

MV is known accept the electrons from A in photosynthetic electron transfer chain. Therefore, whole chain electron transfer activity has been measured in thylakoid membranes using MV as terminal electron acceptor ($H_2O \rightarrow MV$). Control thylakoids without heavy metal treatment exhibited a high rate of oxygen consumption (186 μ moles $O_2 \downarrow mg^{-1}$ Chl h^{-1}). Increase in the Cd concentration from 0.05 mM to 0.2 mM brought enhancement in the inhibition of whole chain electron transfer. Almost 50 % loss was noticed above 0.1 mM of Cd treatment (Table 1). The reason for the loss of whole chain electron transfer could be either alterations at the level of PS II or PS I catalyzed electron transfer [15,16,9]. Thus, Cd stress induced inhibition of whole chain electron transfer could be due to either alterations at PS II or PS I. To identify the target photosystem, we have measured the partial electron transfer reactions mediated by individual photosystems.

Inhibitory effect of Cd on photosystem II catalyzed electron transfer

Since Cd inhibited the whole chain electron transfer, to find out whether the alterations are due to changes in PS II or PS I, an attempt has been made to study the Cd effect on PS II catalyzed pBQ supported Hill reaction (Table 2). pBQ is known to accept the electrons from PQ pool. Being lipophilic in na-

ture pBQ can easily enter into thylakoid membrane and reach PQ pool. Control thylakoids exhibited a rate of oxygen evolution activity (265 μ moles of O_2 ↓ mg^{-1} Chl h^{-1}). Cadmium treatment caused gradual increase in the inhibitory pattern and maximum loss was observed after giving the treatment with 0.2 mM of $CdCl_2$. 52% loss was noticed at 0.1 mM of $CdCl_2$. The reason for the loss of PS II catalyzed electron transfer could be either due to alterations at water oxidation complex or due to changes in and polypeptides or due to alteration at the level of reducing side of PS II [16,17,18].

Chlorophyll fluorescence can be used as an indicator to evaluate the status of PS II functioning. Therefore the Chl a fluorescence measurements were made in control and cadmium treated thylakoid membranes in the presence as well as in the absence of DCMU. To know the functions of PS II, DCMU has been added to the control sample and after 2 minutes of dark incubation thylakoids were excited with 440 nm light specifically to excite Chl a in the control samples. In the absence of DCMU 62 rel units of Chl a fluorescence was observed. After the addition of DCMU it has been raised to 99 rel units (Table 3). As a result the ratio between presence and absence became 1.6. The treatment of Cd gradually caused the decrease in the ratio fluorescence DCMU to 1.08 in a concentration dependent manner. This clearly shows the existence of inhibitory site at the reducing end of PS II. Thus cadmium exerts multiple effects on PS II catalyzed electron transfer our results are in agreement with the observation of Murthy (1991) [19] who showed the effects of mercury on both oxidizing and reducing side of PS II in the cyanobacterium, Spirulina platensis.

Table 1: Effect of Cd on the whole chain electron transfer activity of the thylakoids isolated from control and Cd treated maize primary leaves

Concentration of $CdCl_2$ mM	Whole chain electron transfer activity ($H_2O \rightarrow MV$) μ moles of 0_2 consumed mg^{-1} Chl h^{-1}	Percentage loss
Control	186 ± 17	0
0.05	132 ± 12	29
0.1	100 ± 9	46
0.15	67 ± 6	64
0.2	50 ± 4	73

Table 2: Effect of Cd on the photosystem II catalyzed electron transfer activity of the thylakoids isolated from control and Cd treated maize primary leaves

Concentration of $CdCl_2$ mM	PS II catalyzed electron transport activity ($H_2O \rightarrow pBQ$) μ moles of 0_2 evolved mg^{-1} Chl h^{-1}	Percentage loss
Control	265 ± 25	0
0.05	201 ± 19	24
0.1	127 ± 11	52
0.15	95 ± 8	64
0.2	82 ± 7	69

Table 3: Effect of $CdCl_2$ on room temperature chlorophyll fluorescence in the presence and absence of DCMU in maze thylakoid membranes.

Concentration of $CdCl_2$ mM	Fluorescence intensity (rel. units) F68S (-DCMU)	F68S (+ DCMU)	Ratio +/-
Control	62 ± 3.2	99 ± 7.2	1.6
0.05	58 ± 4.1	80 ± 6.4	1.4
0.1	50 ± 3.3	60 ± 5.3	1.2
0.15	40 ± 2.2	45 ± 4.2	1.1
0.2	35 ± 2.4	38 ± 3.4	1.08

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