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Characterization of alterations in photosynthetic electron transport activities in maize thylakoid membranes under zinc stress

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ABSTRACT

In this investigation an attempt has been made to study the effect of Zinc on primary reactions of photosynthesis. Photosystem II mediated electron transport activity exhibited 68% loss at 180 µM concentration. The PAM kinetic measurements revealed suppression of variable fluorescence in terms of relative distance from 5.4 to 2.7 Cm during the treatment. These measurements suggest that the targets of PSII are at water oxidation complex and Light harvesting complex as multiple sites of damage in Zinc toxicity. Another target site of zinc toxicity in photosystem II is evidenced from the loss of 33kDa protein band SDS-PAGE profiles of thylakoid membranes.

Key words: Electron transport, Maize plants, PAM fluorescence kinetics, Zinc toxicity.

INTRODUCTION

Zinc [Zn] is the second most abundant transition metal and essential nutrient for plant growth and development [1, 2].Due to industrialization and human activities there is an increase of heavy metals including Zn environment [3]. It also acts as essential component in several enzymatic functions [4,5]. Zn when present in higher concentrations in soil it becomes toxic and affects several physiological functions and photosynthesis (6). Between two photosystems, photosystem [PS] II catalyzed electron transport is more sensitive to Zn toxicity than that of PS I [4]. Tripathy group indicated that water oxidation complex could be the target site for Zn stress since exogenous donor NH_2OH is able to release the PSII inhibitors in barley thylakoid membranes [7]. But up to now very few studies are made by correlating PSII activity with PAM Chl *a* fluorescence kinetics. Therefore an attempt has been made by using maize plants as experimental material to study the effect of Zn on photosynthetic electron transport activities by comparing with Chl *a* fluorescence kinetics to identify the other target sites in PSII catalyzed electron transport, using polarographic measurements.

MATERIALS AND METHODS

Maize [*Zea mays*] seedlings were grown in a plant growth chamber with photosynthetic photon flux density of $23Wm^{-2}$ at 25 ± 2^{o} C. Maize seeds of DHM 117 variety were grown for 2 days with Hoagland nutrient solution [8] in petridishes for germination and from 3rd day onwards they are grown in the medium of different concentrations of $ZnSO_4$. A concentration of 1.2 µM $ZnSO_4$ was used as control and excess treatments were given with concentrations 60-180 µM $ZnSO_4$.Plants were allowed to grow for 7 days and fully exposed primary leaves were chosen for the

isolation of thylakoid membranes. Thylakoids have been isolated by following the procedure of Sabat *et al*., [9]. All the electron transport measurements have been assayed using Hansatech, Clark type oxygen electrode by adapting the method mentioned by Mohanty *et al*., [10]. The reaction mixture of whole chain electron transport activity contained reaction buffer, 0.5mM methylviologen [MV], 1.0 mM sodium-azide and thylakoid membranes. Similarly the reaction mixtures for PS-II catalyzed electron transport activity contained reaction buffer with 0.5mM Para-benzoquinone [PBQ] and thylakoid membranes. The PS-I catalyzed electron transport activity assay mixture contained 2mM ascorbate 0.1 mM dichlorophenol-indophenol [DCPIP] and 1mM sodium-azide,0.5mM MV and 20µM diuron [DCMU]. All the assays were measured at 25^o C by circulating water around the oxygen electrode. Whenever there is a requirement of water, a physiological donor is replaced by hydroxyl amine [0.1mM] to identify the damage at water oxidation complex [WOC]. In all electron transport assays thylakoid membranes equivalent to 30µg of chlorophyll were used in both control as well as treated samples. To identify the target sites of Zn in PSII different light intensities ranging from 24-416 Wm^{-2} were provided by passing the light through neutral density filters. Chlorophyll estimation was made by using 80% acetone and following the method of Arnon [11]. Chl *a* fluorescence kinetics was measured by using PAM kinetic fluorimeter by following the method of Murthy *et al*., [12]. To assess the alterations in the organization of polypeptides in the thylakoid membrane, SDS-PAGE was performed following the method of Lamelli [13].

RESULTS AND DISCUSSION

To analyze the effect of Zn on photosynthetic electron transport activities of maize primary leaves, the thylakoid membranes have been isolated from control and Zn treated samples after 7 days of germination. Initially the effect of Zn has been studied at whole chain electron transport activity [$H_2O \rightarrow MV$]. Control thylakoid membranes exhibited the whole chain electron transport activity equal to 124µmoles of $O_2 \downarrow$ mg Chl^{-1} h^{-1}. Zn treatment [60-180µM] caused gradual decrease in whole chain electron transport activity and at 120µM treatment 48% loss was noticed [Table-1]. Earlier similar observations have been made in barley systems by Tripathy and Mohanty [7] indicating that the reason for the loss could be due to alterations at PS-II catalyzed electron transport. To verify the above preposition an attempt has been made to characterize the effect of Zn on PS-II catalyzed electron transport assay. This activity in control samples was measured using PBQ as hill acceptor and is equal to 208 µ moles of $O_2 \uparrow$mg $Chl^{-1}h^{-1}$ [Table-2].The treatment of Zn caused concentration dependent loss in PS-II catalyzed electron transport activity and at 180µM of Zn treatment induced 68% loss in PS-II activity. The possible reason for the loss of PSII activity could be due to alterations in the WOC, which is also evidenced from the measurements of hydroxylamine mediated whole chain electron transport measurements [Table-1 & Fig-1]. Earlier reports made by Hampp *et al* [14] also indicate that Hill reaction is mainly susceptible to Zn stress. The reason for loss in PS-I photochemistry could be due to existence of inhibitory site near Q_B of PS-II as reported by both workers [14, 15, 16]. To rule out the existence of inhibitory site at reaction centre level the PS-II catalyzed electron transport has been measured at both light saturating [416Wm^{-2}] as well as at light limiting conditions [24Wm^{-2}] [Table-3]. This light intensity was obtained using neutral density filters from light source to O_2 electrode. The measurements clearly demonstrated that the inhibition of PS-II activity is more at light saturating condition that at light limiting condition almost 14% difference in inhibition of PS-II activity was observed [Table-3]. To strengthen the above results PAM chl *a* fluorescence kinetics measurements were employed to measure the Chl*a* fluorescence in thylakoid membranes [Table-4] upon excitation with weak light the fluorescence has reached to a distance called F_o in control sample. The Zn treatment caused an enhancement in F_o values from 2.1 to 3.2 Cm. This increase in F_o [Initial fluorescence] is due to alterations in the LHC of PSII. Further excitation with strong actinic red light caused further increase of fluorescence to F_m [maximum fluorescence]. The difference between F_m and F_o is known as F_v [variable fluorescence]. In control sample F_v value is 5.4 Cm. Zn toxicity caused decrease in the F_v value to 2.7 Cm. This loss is due to inhibition of PSII photochemistry by Zn in treated plants. Similar reports have been reported earlier by Murthy *et al.*, [17] in *Spirulina platensis* under mercury stress. Similar reports about suppression of Fv in Chl *a* fluorescence under Zn stress in chlorella as an indication for the loss of PS-II Photochemistry was reported by Plekhanov and Chemeris [18].

The loss in 33 kDa band protein in the Zn treated lane of SDS-PAGE image evidences the damage of water oxidation complex of PSII in maize thylakoids, where as the other bands related to reaction centre remain intact in the B [80µM] and C [120µM] lanes compared to lane A (marker), which is a protein marker lane [Fig-2]. To establish the differential sensitivity of photosynthetic activities of two photosystems an attempt has been made to characterize the effect of Zn on PS-I catalyzed electron transport using DCPIP+ Asc as donor system. Control thylakoid membranes exhibited the PS-I activity equal to 312 µmoles of O_2 consumption mg $Chl^{-1}h^{-1}$[Table-5]. High

concentrations of Zn treatment [180µM] caused partial inhibition in PS-I catalyzed electron transport [20%] which could be due to alterations in PS-I reaction centre as has been suggested by Tripathy and Mohanty [7].Thus Zn toxicity preferentially affect on PS-II rather than PS-I and the possible reason for the altered functioning of PS-II photochemistry in excess of zinc could be due to changes in LHC-II of PS-II or presence of inhibitory site at reducing side of PS-II in maize seedlings.

Table-1 Effect of zinc on whole chain electron transport activity of maize thylakoid membranes

Zinc concentration (µM)	Whole chain electron transport activity ($H_2O \rightarrow MV$) µmoles of O_2 consumed mg Chl^{-1} h^{-1}	Percent loss
Control	124±9	0
60	79±6	21
120	63±5	48
180	41±2	66

Table-2: Effect of zinc on PS-II catalyzed electron transport activity of maize thylakoid membranes

Zinc concentration (µM)	PS-II catalyzed electron transport activity ($H_2O \rightarrow PBQ$) µmoles of O_2 evolved mg Chl^{-1} h^{-1}	Percent loss
Control(1.2)	208±16	0
60	166±12	20
120	102±9	51
180	66±4	68

Table-3: Variation of inhibitory pattern of PS-II activity catalyzed electron transport activity in control and treated samples at different illumination conditions

Light intensity Wm^{-2}	PS-II catalyzed electron transport activity ($H_2O \rightarrow PBQ$) µmoles of $O_2\uparrow$ mg Chl^{-1} h^{-1}		Percent loss
	Control	Zn-Treatment (120 µM)	
416	212±4	106±3	50
212	102±6	56±4	45
102	64±8	38±	41
24	48±3	31±	36

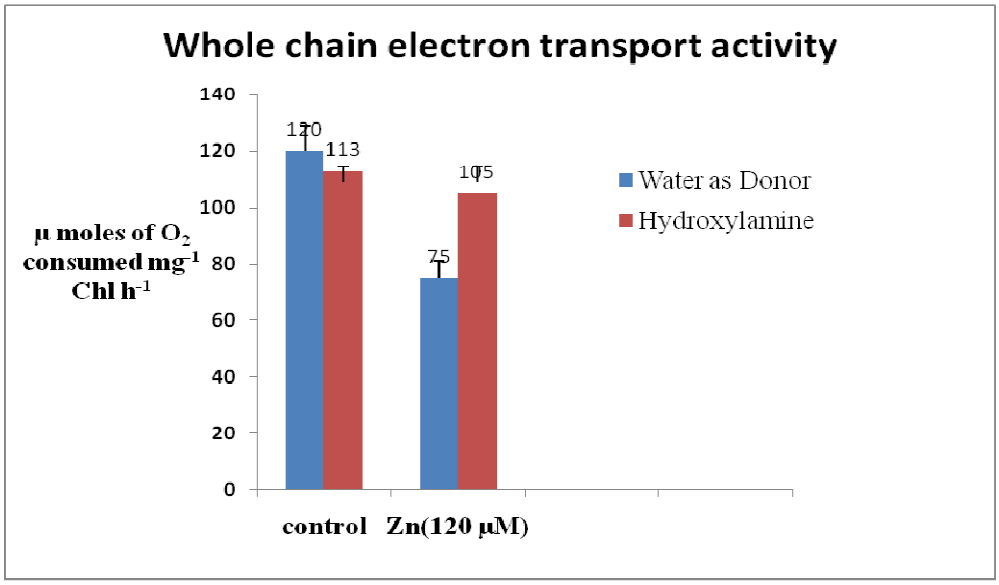

Fig: 1 Effect of zinc on whole chain electron transport activity of maize thylakoids mediated by different donors (H_2O and Hydroxylamine)

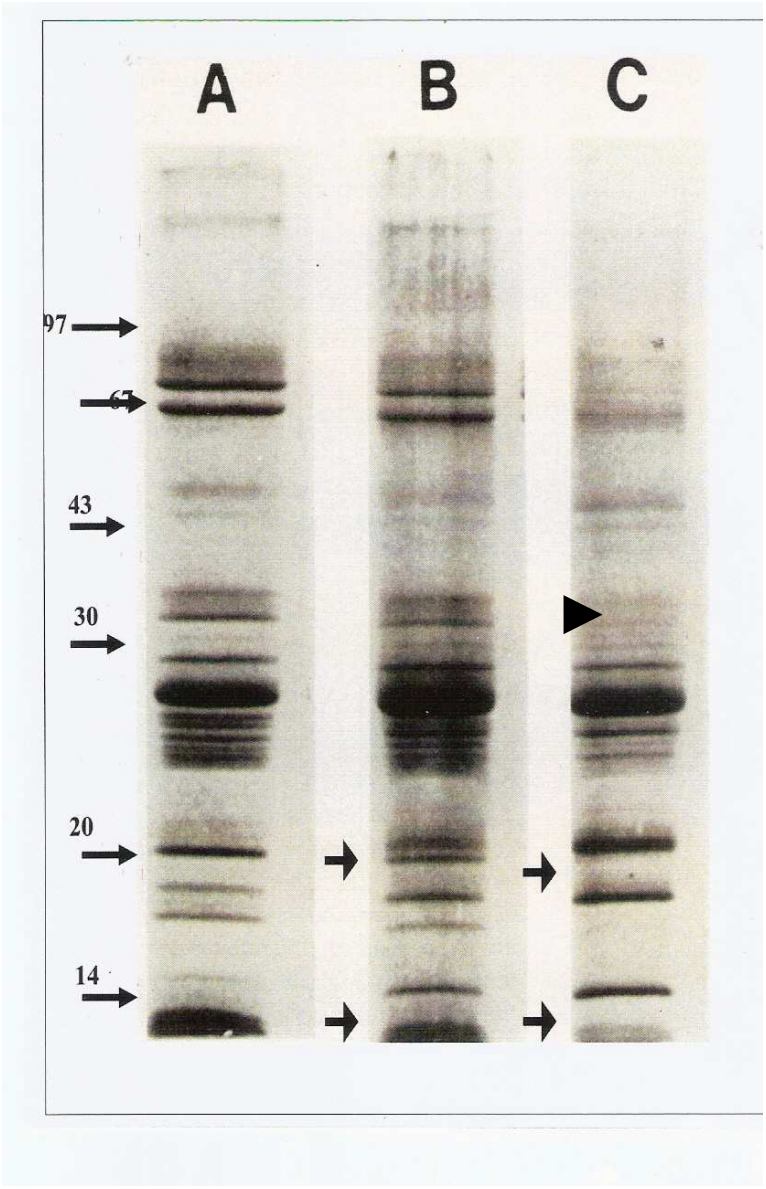

Fig: 2 Effect of zinc on Polypeptide profile in maize thylakoids
Lane A represents marker protein, Lane B represents 80 µM Zinc, Lane C represents 120 µM zinc concentration. The larger arrow in Lane C represents the loss in 33KDa protein

Table: 4 Effect of zinc on Chl *a* fluorescence kinetics of maize thylakoids
The samples were excited with very low light intensity to measure the initial fluorescence (Fo) and with strong red light to measure variable fluorescence (Fv).

Zinc concentration (µM)	Fluorescence parameters (in terms of distance, Cm)		
	Fo	Fv	Fm
Control	2.1	5.4	7.5
60	2.4	4.2	6.6
80	2.5	3.4	5.9
120	2.8	2.8	5.6
180	3.2	2.7	5.9

Table-5: Effect of Zinc on PS-I catalyzed electron transport activity of maize thylakoid membranes

Zinc concentrations (µM)	PS-I catalyzed electron transport activity (DCPIP→MV) µmoles of $O_2\downarrow$ mg Chl^{-1} h^{-1}	Percent loss
Control	312±27	0
60	284±24	9
120	265±23	15
180	252±21	19

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