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Research article

COPPER STRESS INDUCED ALTERATIONS IN THE PRIMARY PHOTOSYNTHETIC PROCESSES OF MAIZE LEAVES

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ABSTRACT: In this investigation an attempt has been made to characterize the effect of copper stress (50-200µM) on primary reactions of photosynthesis in maize seedlings. Copper stress inhibits both whole chain electron transports as well as photosystem II (PS II) catalyzed electron transport by 50% at the concentration of 100µM. In contrast to the above observations, PS I catalyzed electron transport is less sensitive (25% loss) to copper stress. Chlorophyll *a* fluorescence measurements clearly demonstrated that there is a raise in F_0 value under copper stress (100µM) in maize seedlings indicating that light harvesting complex is target for heavy metal action.

Keywords: Copper stress; Electron transport; Fluorescence; Maize plants; Photosynthesis.

INTRODUCTION

Heavy metals are known to interfere with photosynthesis in higher plants at multiple sites in electron transport [1, 2, and 3]. Copper (Cu) in the form of cupric ions at more than 1µM has been showed to inhibit the photosynthetic functions in isolated chloroplasts of higher plants [4,5,6,7 and 8] and in green algae [9]. Copper usually accumulates in the chloroplast and inhibits PS II catalyzed electron transport [4] and also it requires light for binding to chlorophyll protein and also to bring maximal inhibition in PS II catalyzed electron transport activity. Among two photosystems, PS II is more sensitive to Cu toxicity. In this present investigation, a study has been made to identify the susceptibility of photosynthetic electron transport towards Cu toxicity by taking maize seedlings as experimental material. All the electron transport activities and chlorophyll *a* (Chl *a*), fluorescence kinetic techniques have been employed to assess the Cu toxicity in maize plants.

MATERIALS AND METHODS

Healthy seeds of maize (*Zea mays*) was obtained from Acharya N.G Ranga Agriculture University, Hyderabad, Andhra Pradesh). The seedlings were randomly placed in plastic trays and watered daily with quarter strength Hoagland nutrient solution [10] and grown in a growth chamber providing with fluorescence light (Philips, India) with a light intensity of 20 Wm^{-2} at 25 ± 1^0C. Fully expanded 8th day leaf segments (4-5cm long) were cut from apical region and used for treatment. 8th day maize plants were exposed to $CuCl_2$ stress (50-200 µM) for 24h and then the primary leaves were used for biochemical investigation. Thylakoid membranes were isolated according to the procedure similar to that of [11] as described in [12]. Whole chain electron transport activity (H_2O → MV) was measured as O_2 Consumption by using MV as an electron acceptor in the thylakoid membranes. The 2ml reaction mixture contains reaction buffer [50mM HEPES-NaOH (pH 7.5), 100 mM sucrose, 2 mM $MgCl_2$ and 5 mM KCl], 0.5 mM MV, 1.0 mM Sodium azide and thylakoid membranes. PS II catalyzed electron transport assay (H_2O → pBQ) activity was measured as O_2 evolution in the thylakoid membranes.

The 2 ml reaction mixture contains reaction buffer, 0.5 mM freshly prepared pBQ and thylakoid membranes. PS I catalyzed electron transport assay was measured as O_2 consumption the 2ml reaction mixture contains reaction buffer 0.1 mM 2, 6-dichlorophenol indophenol (DCPIP), 0.5 mM MV 5mM ascorbate, 1mM sodium azide, 10 µM DCMU and thylakoid membranes. For Chl *a* fluorescence kinetics, 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) measurements were taken for kinetic studies.

RESULTS AND DISCUSSION

In this study an attempt has been made to study the effect of Cu in the form of $CuCl_2$ on the photosynthetic electron transport in relation to Chl *a* fluorescence kinetics by using O_2 electrode as well as PAM kinetic fluorimeter in maize primary leaves .Initially regarding electron transport a measurement have been made on whole chain electron transport .Control thylakoid membranes exhibited a high rate of O_2 consumption equal to 186 µM (Table 1). Cu treatment caused gradually inhibition in whole chain electron transport and almost 52% loss was noticed with the concentration of 150 µM $Cucl_2$. Further raise in the concentration caused 70% inhibition in the whole chain electron transport activity.

As Cu showed inhibition in whole chain electron transport, to find out whether the inhibition is due to PS II or PS I or both, an attempt has been made to measure the Hill activity supported by pBQ. Control thylakoid membranes exhibited PS II activity equal to 225 moles evolved (Table 2). In the presence of low concentrations (50 µM) of Cu only 28% loss was noticed. And at higher concentrations (200 µM) 70% loss was noticed. The possible reason for the loss of PS II activity may be due to alterations in the reaction centre of PS II as suggested by [6] or alterations at the level of Q_B protein as reported by [5]. To establish the susceptibility of PS I catalyzed electron transport an attempt has been made to study the PS I catalyzed electron transport using reduced DCPIP as donor (Table 3). Control thylakoid membranes exhibited PS I activity equal to 325 µ moles of O_2 consumed .Cu treatment caused less inhibition (20%) in the activity of PS I even at higher concentrations. This inhibition in PS I catalyzed electron transport could be due to inhibitory effect of Cu on the reaction centre of PS I i.e. P_{700} [4 and 13]. From this study it is clear that PS II is more sensitive to Cu stress in maize thylakoid membranes. Since Chl *a* fluorescence is an indicator of PS II photochemistry we have compared the electron transport results with Chl *a* fluorescence kinetics. Chl *a* fluorescence kinetics have been measured using PAM kinetic fluorimeter in control as well Cu treated samples. Upon excitation with weak light the fluorescence goes to F_O. After reaching the F_O to assess the photochemistry sample will be excited with strong red actinic light. Then it reaches maximum fluorescence (F_M). The difference between F_O and F_m gives the value of F_V i.e. variable fluorescence. This is an indicator of PS II photochemistry. Our results clearly demonstrated as shown in the (Table 4) there is an increase in F_O due to Cu treatment. This is an indicator of alterations in light harvesting complex. Cu treatment caused the decrease almost by 50% in F_V which is an indication for the loss of PS II catalyzed electron transport under Cu stress. Thus in this investigation we have compared the results which have obtained from polarographic study to fluorescence kinetics to have an integrated approach of the Cu stress in maize thylakoid membranes.

Table 1: Effect of copper stress on whole chain electron transport assay ($H_2O \rightarrow MV$) in the thylakoid membranes of maize primary leaves.

Copper concentrations µM	Whole chain electron transport activity ($H_2O \rightarrow MV$) µ moles of $O_2 \downarrow$ mg $Chl^{-1}h^{-1}$	Percentage inhibition
Control	186 ± 19	0
50	143 ± 10	23
100	102 ± 8	45
150	85 ± 7	55
200	62 ± 4	67

Table 2: Effect of copper on PS II catalyzed electron transport activity ($H_2O \rightarrow pBQ$) in the thylakoids of maize primary leaves.

Copper concentrations µM	PS II catalyzed electron transport activity ($H_2O \rightarrow pBQ$) µ moles of $O_2 \uparrow$ mg $Chl^{-1}h^{-1}$	Percentage inhibition
Control	225 ± 19	0
50	165 ± 14	27
100	110 ± 12	31
150	82 ± 8	64
200	72 ± 6	68

Table 3: Effect of copper on PS I catalyzed electron transport activity ($DCPIPH_2 \rightarrow MV$) in the thylakoids of maize primary leaves.

Copper concentrations µM	PS I catalyzed electron transport activity ($DCPIPH_2 \rightarrow MV$)µmoles of $O_2 \downarrow$ mg $Chl^{-1}h^{-1}$	Percentage inhibition
Control	325 ± 27	0
50	305 ± 29	6
100	292 ± 27	10
150	273 ± 24	16
200	252 ± 21	23

Table 4: Effect of copper on Chl *a* fluorescence kinetics in maize thylakoid membranes.

Copper concentrations µM	Fluorescence parameter (in terms of distance,cm)		
	F_O	F_V	F_m
Control	2.2	4.5	6.7
50	2.4	4.2	6.6
100	2.6	3.9	6.3
150	2.8	3.7	5.8
200	2.9	2.5	5.3

REFERENCES

[1] Foy, C.D., Cheney, R.L. and White, M.C. 1978. The physiology of metal toxicity in plants.*Ann.Rev. Plant Physiol.*29:511-566.

[2] Clijsters, H. and Van Assche, F. 1985. Inhibition of photosynthesis by heavy metals. Photosyn. Res. 7: 31-40.

[3] Murthy, S.D.S., and Mohanty, P. 1991. Mercury induces alterations of energy transfer in phycobilisomes by selectively affecting the pigment protein, phycocyanin, in the cyanobacterium, Spirulina Platensis. Plant Cell Physiol. 32: 231-237.

[4] Cedeno-Maldonado, A., Swader, J.A. and Heath, R.L. 1972. The cupric ions as an inhibitor of photosynthetic electron transport in isolated chloroplasts. Plant Physiol.50: 698-701.

[5] Mohanty, N., Vass, I. and Demeter, S. 1989. Copper toxicity affects photosystem II electron transfer at the secondary quinine acceptor, QB. Plant Physiol. 90: 175-179.

[6] Renganathan, M. and Bose, S. 1989. Inhibition of primary photochemistry of photosystem II by copper in isolated pea chloroplasts. Biochem. Biophys. Acta. 974: 247-253.

[7] Samuelson, G. and Oquit, G. 1980. Effect of copper chloride on photosynthetic electron transport and chlorophyll-protein complex of Spinacia oleracae. . Plant Cell Physiol. 21: 445-454.

[8] Shioi, Y., Tamai, H. and Sasa, T. 1978a. Effect of copper on photosynthetic electron transport in spinach chloroplasts. Plant Cell Physiol. 19:203-209

[9] Shioi, Y., Tamai, H. and Sasa, T. 1978b. Inhibition of photosystem II in green alga (Ankistrodesmus falcatus) by copper. Physiol. Plant.44: 434-438.

[10] Arnon, D. I. and Hoagland, D. R. 1950. A water culture method for growing with out soil. California Agric. Exp. Stn. Circ. 347: 115-123.

[11] Saha, S. and Good, N. E. 1970. Products of the photophosphorylation reaction. J. Biol. Chem. 245: 5017-5021.

[12] Swamy, P. M., Murthy, S.D.S. and Suguna, P. 1995. Retardation of dark induced invitro alterations in PS II organization of cowpea leaf discs by combination of Ca2+ and benzyladenin. Biol. Plant. 37: 457-460.

[13] Wong, D., and Govindjee 1976. Effects of lead ions on photosystem I in isolated chloroplasts: Studies on the reaction center P700. Photosynthetica. 10: 241-254.