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Research article

ALTERED ELECTRON TRANSPORT ACTIVITIES IN THE THYLAKOID MEMBRANES OF MAIZE LEAVES UNDER COPPER ION STRESS

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ABSTRACT: The Copper treatment caused loss (70 %) of chlorophyll content in maize primary leaves. The electron transport activity measurements clearly demonstrated that the existence of multiple sites inhibition in thylakoid membranes. Between two photosystems, photosystem II seems to be more susceptible to copper toxicity (50 µM), than that of photosystem II.
Key wards: Electron transport; Heavy metal (Cu); Maize.
Abbreviations: Chl-chlorophyll; PS-photosystem; WCE - wholechain electron transport

NTRODUCTION

Heavy metals inhibit photosynthetic electron transport activities at different target sites [1 and 2]. Majority of the studies was made in isolated systems. All these studies are indicating that PS II catalyzed electron transport is more sensitive to heavy metals [4 - 8]. Several people showed that Zn and Cu induced inhibition is dependent on the illuminated light intensity [9 and 6].

Compare to PS II, PS I catalyzed electron transport has been reported to be more resistant to the heavy metals [10 and 5]. Cu and Pb are able to inhibit the PS I activity at much elevated concentrations [11]. Hg is able to inhibit the PS I activity at multiple sites PCy [12 and 13], at the level of reaction center of PS I, P_{700} [14], Fd and FNR [4] and Fe-S centers [15]. Hence an attempt has been made to study the effect of heavy metal (Cu) ions in a comparative manner on the photosynthetic electron transport activities in thylakoid membranes of maize leaves.

MATERIALS AND METHODS

Plant growth and treatment:

Healthy seeds of maize were obtained from Acharya N.G. Ranga Agricultural University, Hyderabad. The seeds were surface sterilized with 0.1% $HgCl_2$ for 2 min and thoroughly washed with tap water and then with distilled water. The seeds were imbibed for 6 h and germinated in petridishes on filter paper for 3 days. The seedlings were randomly placed in plastic trays and watered daily with quarter strength Hoagland nutrient solution and grown in growth chamber providing with fluorescent light (Philips, India) with a light intensity of 30-35 µmoles $m^{-2}s^{-1}$ at 25 ± 1°C. Seedlings were treated with different concentrations of $CuCl_2$ (50,100 and 150 µM) after 4th day of germination. Plants were harvested after 3 days (7th day old) of heavy metal treatment were used for estimation of chlorophyll (Chl) and electron transport activities. Similar set of experiments were also performed for the control sample without heavy metal treatment. The experiment was repeated four times to determine each parameter.

Estimation of chlorophyll:

0.1 g of maize leaf segments were homogenized in a pre-chilled mortar and pestle in 10 ml of 80% chilled acetone. The homogenate was transferred into 15 ml centrifuge tubes and centrifuged at 3000 xg for 5 min. The Chl concentration was measured from supernatant after it dilution to a total volume of 15 ml by following the method of Arnon [16].

Photosynthetic electron transport activities

Electron transport activities of control and treated thylakoid membranes were assayed using a Clark type oxygen electrode (Hansatech, UK) following Sabat et al. [17]. Thylakoid membranes were isolated according to the procedure similar to that of Saha and Good [18] as described in Swamy et al. [19] with some modifications. PS II catalyzed electron transport activity was measured as O_2 evolution in 2 ml reaction buffer consisting of [50 mM HEPES (N-2-Hydroxyethyl Piperazine-N' Ethane Sulphonic acid) - NaOH (pH 7.5), 100 mM sucrose,2 mM $MgCl_2$ and 5 mM KCl], 0.5 mM freshly prepared p - Benzoquinone (p-BQ) and thylakoid membranes equivalent to 40 mg of Chl. For WEC activity the reaction buffer contained 0.5 mM methyl viologen (MV), 1.0 mM of Sodium (Na) - azide, while the PS I reaction mixture contained 0.5 mM MV, 1.0 mM Na-azide, 0.1 mM 2,6- dichlorophenol indophenol (DCPIP), and 10 µM dichloro methyl urea and thylakoid membranes equivalent to 40 mg of Chl in reaction buffer.

RESULTS

In this article the effect of $CuCl_2$ (50-150µM) has been studied by isolating the thylakoid membranes and estimating the Chl content. Cu treatment caused the decrease in the Chl content both *a* + *b* (upto 70%) (Table 1). The possible reason for the decrease of Chl content could be the inhibition effect of Cu on Chl biosynthetic enzymes [1]. Similarly effect of lead (Pb) in seedlings of Bajra has been reported by Prasad and Prasad [20]. After studying the effect on pigments, a study has been made regarding the effect of Cu on photosynthetic electron transport activities in maize thylakoids after Cu treatment caused 50% inhibition in whole chain electron transport activity at 100 µM. Further raise concentration brought 71%loss in the activity (Table 2). The reason for the whole chain electron transport could be alteration either at PS I and PS II [21 and 8] To verify above preposition an attempt has been made to characterize the effect of Cu on PS II catalyzed electron transport activity (Table 3). Cu treatment induced 45% of loss at 50 µM and further rise to 150 µM induced 85% inhibition. The reason for the inhibition in PS II activity could be due to alterations either at water oxidation complex (WOC) or at PS II reaction centre [6 and 8]. To role out the susceptibility of PS I, electron transport activities were measured using maize thylakoids both control and Cu treated samples (Table 4). In PS I catalyzed electron transport is less susceptible to Cu toxicity. At 150 µM concentration only 22% loss was noticed. The reason for the loss of PS I catalyzed electron transport could be due to its action on reaction centre, P_{700} in maize thylakoids. Thus Cu treatment affects photosynthetic electron transport in maize thylakoids at multiple sites.

Table 1: Effect of $CuCl_2$ on Total Chl (a+ b) (mg /g fw) content in maize leaf segments. Each value is Mean ± SE of four replications

Parameter	Concentration (µM)	Total Chl (a+ b) (mg /gm fw)	Percentage of loss
Control	-	2.43 ± 0.02	0
$CuCl_2$	50	1.88 ± 0.05	22
	100	1.17 ± 0.02	51
	150	0.72 ± 0.01	70

Table 2: Effect of $CuCl_2$ on WCE (µ moles (O_2 consumed) mg^{-1} Chl h^{-1}] activities in maize leaf segments. Each value is Mean ± SE of four replications

Parameter	Concentration (µM)	WCE activity (H_2O —> MV)	Percentage of loss
Control	-	175 ± 5	0
$CuCl_2$	50	131 ± 9	25
	100	82 ± 7	53
	150	51 ± 6	71

Table 3: Effect of $CuCl_2$ on PS II catalyzed electron transport activity [µ moles (O_2 evolved) mg^{-1} Chl h^{-1}] in maize leaf segments. Each value is Mean ± SE of four replications.

Parameter	Concentration (µM)	PS II activity (H_2O —> p-BQ)	Percentage of loss
Control	-	312 ± 4	0
$CuCl_2$	50	172 ± 8	45
	100	97 ± 9	69
	150	56 ± 3	82

Table 4: Effect of $CuCl_2$ on PS I electron transport activity [µ moles (O_2 consumed) mg^{-1} Chl h^{-1}] in maize leaf segments. Each value is Mean ± SE of four replications.

Parameter	Concentration (µM)	PS I activity ($DCPIPH_2$ —> MV)	Percentage of loss
Control	-	450 ± 13	0
$CuCl_2$	50	405 ± 10	10
	100	369 ± 16	18
	150	351 ± 10	22

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