IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

METAL ION MEDIATED PROTECTION OF PHOTOSYSTEM II CATALYSED ELECTRON TRANSPORT AND THYLAKOID POLYPEPTIDES OF DETACHED *MAIZE* LEAVES DURING DARK INCUBATION

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Abstract: In this study an attempt has been made to analyze the protective role of selected two metal ions Ca^{2+}/Al^{3+} in electron transport activities of maize thylakoid membranes. To achieve these leaf segments of 7 days were incubated for 2h-96h in distilled water Ca^{2+} solution or Al^{3+} solution. The photosynthetic electron transport activities of PS II were clearly indicated that there is a valency dependent restoration in primary reactions of photosynthesis. The SDS PAGE analysis of thylakoid membranes clearly demonstrated that 33 kDa protein of water oxidation complex of PS II is susceptible for aging and metal ion incubation protested the degradation of the above polypeptide to restore the PS II activity.

Index Terms **- Chlorophyll, Maize plants, Photosystems, SDS PAGE, Senescence.**

I. INTRODUCTION

Leaf senescence is a key developmental state in the life of plants that leads to a massive mobilization and export of nitrogen and minerals to younger tissues (eg. growing leaves, flowers, fruits and developing seeds) to prepare for the next generation and/or to allow plant survive under adverse environmental conditions [1]. Leaf senescence serves to remobilize nutrients (especially nitrogen) from the oldest leaves to the youngest ones. The rate of senescence and the remobilization of leaf nitrogen are related to the nitrogen nutrition status of the plant and on source/sink relations [2]. Up to 75 % of the nitrogen present in mesophyll cells is located in the chloroplasts [3]. In particular, nitrogen is converted into amino acids via glutamine synthetase activity whose expression has been found to be associated with senescing leaves [4], [5]. During leaf senescence, the loss of photochemical activities limits the photosynthesis. The drastic decline in activities of PS II, is reported in several senescing systems [6], [7], [8]. [9] observed that the more loss in WCE transport than that of either PS II or PS I, due to the changes in two mobile electron carriers, PQ and PC. Electron transport activities of PS II and PS I have been reported to be declined during leaf senescence by 25% and 33%respectively in *Phaseolus* [10]. [8] observed that the acceptor side of PS I is affected in senescing leaves. [11] found higher loss in PS I electron transport activity as compared to that of PS II during senescence of cotyledonary chloroplasts in soyabean. Decline in the levels of D1 protein is associated with a parallel decrease in the PS II electron transport rates in barley [12]. Therefore, the onset of leaf senescence obviously curtails the yield of the crop to a significant extent by altering the electron transport [13]. In this investigation an attempt has been made to characterize the effect of $CaCl_2$ and $AlCl_3$ in delaying the dark incubation induced changes in primary processes of photosynthesis in maize leaves.

II. MATERIALS AND METHODS

Leaf senescence is a Healthy seeds of Maize were obtained from Acharya N.G. Ranga Agricultural College, Tirupati. The seedlings were randomly placed in plastic trays and watered daily with quarter strength Hoagland nutrient solution and grown in a growth chamber providing with fluorescence light (Philips, India) with a light intensity of 30-35µmoles.m^{-2} s^{-1} at 25±3°C.Treatment of leaf segments for 96h in dark at 25°C was given with $CaCl_2$or $AlCl_3$ alone in distilled water. Thylakoid membranes were isolated according to the procedure similar to that of [14] as described in [15]. PS II catalyzed electron transport assay ($H_2O \rightarrow$p-BQ) activity was measured as O_2 evolution in the thylakoid membranes. Thylakoid polypeptide profile of control and treated samples were analysed by following the procedure of SDS-PAGE [16].

III. RESULTS

Leaf senescence is a Healthy seeds of Maize were obtained from Acharya N.G. Ranga Agricultural College, Tirupati. The seedlings were randomly In this investigation an attempt has been made to analyze the protective role of metal ions in detached leaf thylakoids isolated from 24-96h dark incubated leaf segments for the measurements of electron transport activities (PS II) of thylakoid membranes. To find out the susceptibility of photosystems PSII activities has been measured individually. p-BQ supported control PS II activity decreased to 38 % at 96 h and this loss was significantly restricted to 45 % and 51 % by Ca^{2+} and Al^{3+} respectively at 96 h during dark incubation (Table 1). The retention of PSII activity by Al^{3+} was more than that of Ca^{2+}. The possible reason for the alteration of PS II activity could be either loss of cofactors (Mn/Ca/Cl) or polypeptides related to the water oscillation complex 33, 24 and 17 kDa. To verify this thylakoid polypeptide profile of control and treated samples were made using SDS-PAGE. The polypeptides are resolved into 97 and 14 KDa region (Fig 18). In dark incubation sample there was a particular loss of 33 KDa after 72 h in thylakoid membrane. This polypeptide could be related to the PS II photochemistry as per the literature. This polypeptide was maintained like normal as seen in control due to the incubation of leaf segments in the presence of Ca^{2+} or Al^{3+}. It is clearly demonstrated that metal ions can protect the structure and function of thylakoid membrane during dark incubation of leaves when they are present or available to the system.

IV. DISCUSSION

To relate the persistence loss of pigments and proteins to photochemical activity electron transport activities were studied. There was a progressive loss in WCE during dark incubation as observed by [10] and [7] during leaf senescence. However Ca^{2+}/Al^{3+} inclusion during dark incubation in delaying the WCE activity loss). The loss in WCE is due to alterations in PS II and /or PS I. A significant restoration of PS II by Ca^{2+}/Al^{3+}, both the activities were reported significantly in both the systems during dark induced senescence (Table 1).

Table 1: Effect of 40µM $CaCl_2$ or 40 µM $AlCl_3$ on PS II [µmoles (O_2evolved) mg^{-1} Chl h^{-1}] activities in maize primary leaf segments under dark incubated senescence. Each value is mean ± SE of five replications. Values in parenthesis indicate % residual activities.

Treatment	Incubation Time (h)				
	0	24	48	72	96
Control	190±4 (100)	170±11 (89)	151±4 (79)	91±4 (48)	73±8 (38)
$CaCl_2$	190±4 (100)	175±10 (92)	159±8 (84)	115±9 (60)	86±11 (45)
$AlCl_3$	190±4 (100)	178±7 (94)	165±2 (87)	127±9 (67)	97±11 (51)

Dark incubation studies revealed that PS II is susceptible and maximum protection was seen due to Ca^{2+}/Al^{3+}treatment. The loss of photosynthetic activity during senescence in maize is related to the loss of whole chloroplast activity [17]. Chloroplast in a green leaf is the earliest and major target of senescence induced catabolism [18]. There was a drastic reduction in 72h control thylakoid membranes during dark incubation. Ca^{2+}/Al^{3+} marginalized the drastic loss in electron transport activities and protect the thylakoid membrane on aging induced damage. To support our electron transport measurements of PS II, an attempt has been made to establish the structural alterations of polypeptides related to water oxidation complex. The polypeptides are resolved into 97 and 14 KDa region (Fig 1).

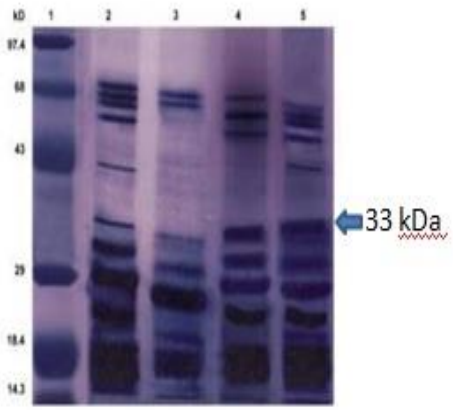

Fig. 1: Effect of 40µM $CaCl_2$ and 40µM $AlCl_3$ on polypeptides profile of thylakoid membranes by SDS-PAGE analysis. Lane 1: Marker proteins, Lane 2: 0h control, Lane 3: 72 h control, Lane 4: 72h Ca^{2+} treated and Lane 5: 72 h Al^{3+} treated maize primary leaves under dark induced senescence.

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