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Role of Polyamines in Retardation of Dark Incubation Induced Changes in Thylakoid Membrane Organization and Photochemical Functions in Maize (*Zea mays)* Primary Leaves.

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ABSTRACT

In this investigation an attempt has been made to compare the action of different polyamines such as putrescine (Put), spermidine (Spd) and spermine (Spm) in retarding the changes of photochemical functions and organization of thylakoid membrane in maize plants. The treatment of PAs caused the retention of PS II catalyzed electron transport and chlorophyll *a* fluorescence properties and protected the functions of maize thylakoid membranes. Due to the treatment of PAs, mainly the polypeptides (33 and 24 kDa) related to the water oxidation complex (WOC) are retained from the damage and restored the PS II photochemistry in treated samples.

Keywords: Chlorophyll *a* fluorescence, lipid peroxidation, PS II electron transport, polyamines, maize plants.

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INTRODUCTION

Polyamines (PAs) are low molecular weight poly cations which affect the plant growth and development [1, 2]. Generally these PAs are able to stabilize the cell membranes and help in the retardation of senescence [3]. PAs have ability to increase the ion flux and restore the age induced alterations [4]. Several workers showed that PAs are able to delay the loss of photosynthetic pigments in higher plant systems [5, 6]. PAs at high concentrations (50-100µM) are able to cause the destruction of thylakoid membrane and inhibit the photosynthetic electron transport mediated by photosystem (PS) II as well as PS I [7]. But at low concentrations (less than 50 µM) these PAs are able to delay the aging process to protect the plants from senescence induced damage. Therefore in this investigation an attempt has been made to study the protective role of PAs in a comparative manner in dark incubated maize primary leaf segments. Up to now very few attempts were made regarding the study of senescence induced alterations in thylakoid lipid peroxidation and organization of thylakoid membrane.Therefore in this investigation a comparative study has been made by correlating the polarographic studies with spectral as well as electrophoretic measurements using maize primary leaves as experimental material.

MATERIALS AND METHODS

Primary leaf segments (4-5 cm long) were cut from 7 d old maize (*Zea mays*) seedlings grown under continuous "white" radiation of 30-35 µmol m^{-2} s^{-1}. Four sets consisting of 20-25 primary leaf segments were maintained in double distilled water or 25 µM putrescine or 25 µM spermidine or 25 µM spermine solutions in separate test tubes. The tubes were kept at 25 ± 1ºC in dark for 72 h. Leaf segments were sampled from each test tube and used for experimental work. Thylakoid membranes were isolated in a medium containing 50 mM Hepes-NaOH, pH 7.8, 400 mM sucrose, 2mM $MgCl_2$, and 5 mM KCl, by a procedure similar to that of [8] as described in [9]. Electon transport activities of thylakoid membranes were assayed according to [10] in 2 ml reaction buffer (50mM Hepes-NaOH, pH 7.5, 100mM sucrose, 2 mM MgCl2, and 5mM KCl) using a *Hansatech* (Kings Lynn, England) electrode. PS II activity was measured by adding freshly prepared *p*-benzoquinone (*p*-BQ) to 2 ml reaction buffer to a concentration of 0.5 mM. For chlorophyll *a* fluorescence kinetics, the samples were excited with very low light and then increased the light intensity after the initial fluorescence (F_0) is reached. Variable fluorescence (F_v) and maximum fluorescence (F_m) measurements were taken for kinetic studies. Lipid peroxidation has been measured according to the method of [11]. Polypeptide analysis of thylakoid membranes was made according to [12] using SDS-PAGE.

RESULTS AND DISCUSSION

Dark incubation for 72 h induced loss in PS II catalyzed electron transport activity mediated by pBQ by 53%. The treatment of PAs putrescine (Put) or spermidine (Spd) or spermine (Spm) at 25µM retarded the loss induced by dark incubation and protected the system from the damage **(Table 1).** Chl *a* fluorescence is an indicator of PS II photochemistry in thylakoid membranes [13, 14, 15]. PAM fluorescence kinetics were measured in control, 72 h dark incubated as well as PAs treated samples **(Table 2)**. In control sample the observed (F_0) was increased from 2.2 to 2.5 cm due to dark incubation for 72 h. This increase of (F_0) indicated the structural alterations in light harvesting complex II (LHC II). Similarly there is a decrease in (F_v) from 4.2 to 3.9 cm indicating the impairment of PS II photochemistry. But in the presence of PAs the observed loss was minimized and a protection was provided to PS II photochemistry. In the thylakoid membranes the lipids like galactolipids, phospholipids and sulpholipids are responsible for the PS II photochemistry. Any alterations in the thylakoid lipid profile can affect the photosynthetic electron transport activity and induce inhibition in PS II photochemistry.

Therefore an attempt has been made to measure the lipid peroxidation (LPO) in control, 72 h dark incubated and treated samples in the presence of PAs for 72 h through MDA formation. LPO increases the membrane permeability, which leads to leakage of ions [16]. There is a 45% increase in the LPO in dark incubated leaves for 72 h. When the leaves were dark incubated in the presence of PAs, the enhancement of LPO was minimized and the system is protected from the oxidative damage **(Table 3)**. In membranes in addition to lipids, there are several polypeptides are responsible for the functioning if photosystems (PS II and PS I). Therefore a comparative polypeptide analysis of thylakoid membranes have been made in control and 72 h dark incubated samples in the presence and absence of PAs using SDS-PAGE (**Fig**). From electrophoretogram it is clear that 33 kDa and 24 kDa are getting degraded due to 72 h of dark incubation. But the presence of PAs

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during dark incubation protected the loss of above polypeptides from the degradation in WOC of PS II. Thus PAs are able to stabilize thylakoid membrane and delay the loss of photochemical activity during dark incubation and maintained the polypeptide organization of thylakoid membranes [14].

Table 1: Effect of 25 µM putrescine, spermidine and spermine on photosystem II (O_2 evolved) catalyzed electron transport activity in maize primary leaves during dark incubation.

Incubation time (h)	PSII catalyzed electron transport activity $H_2O \rightarrow pBQ$ (µmoles of $O_2\uparrow$ mg Chl^{-1} h^{-1}).	Percentage loss
Control(0)	180±16	0
Control(72)	85±7	53
Putrescine(72)	96±8	47
Spermidine(72)	104±9	42
Spermine (72)	115±10	33

Table 2: Retarding effect of polyamines on Chl *a* fluorescence kinetics in maize thylakoid membranes.

Incubation time (h)	Fluorescence parameter (in terms of distance, cm)		
	F_O	F_V	F_m
Control(0)	2.2	4.4	6.6
Control(72)	2.5	3.7	6.2
Putrescine (72)	2.4	3.9	6.3
Spermidine (72)	2.3	4.2	6.5
Spermine (72)	2.2	4.3	6.7

Table 3: Retarding effect of polyamines on lipid peroxidation of thylakoid membranes of maize primary leaves.

Incubation time (h)	Lipid peroxidation n moles of MDA / g.f.w	Percentage enhancement
Control(0)	45 ± 3	0
Control(72)	64 ± 5	45
Putrescine(72)	50 ± 4	11
Spermidine(72)	48 ± 3	7
Spermine (72)	46 ± 3	2

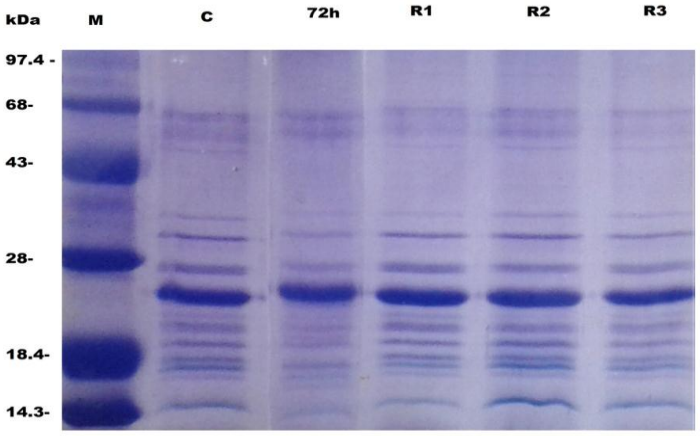

Figure 1: Protective role of polyamines on polypeptide profile of thylakoid membranes of maize primary leaves during dark incubation.

M-Standard protein markers, C- Control (H_2O), 72 h-Dark incubation (H_2O), 72 h R1-Putrescine, 72 h R2-Spermine, 72 h R3-Spermidine

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