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Research article

ROLE OF POLYAMINES IN RETARDATION OF DARK INCUBATION INDUCED ALTERATIONS IN THE PRIMARY PROCESSES OF PHOTOSYNTHESIS IN MAIZE (*ZEA MAYS)* 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 delaying the dark incubation induced alterations in primary reactions of photosynthesis in maize plants. Among these polyamines spermine (25µM) is able to provide more protection in terms of loss of chlorophyll (Chl), protein content and regaining of various electron transport activities. In addition to this, these PAs stabilize the thylakoid membranes of 72 h dark incubated samples in valency dependent manner and restore the excitation energy distribution between two photosystems (PS II and PS I).
Key words: Chl *a* fluorescence, energy distribution, electron transport, polyamines, maize plants

INTRODUCTION

Polyamines (PAs) are unique chemical agents 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 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. The parameters studied are contents of photosynthetic pigments, protein, electron transport activities and spectral properties.

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.The Chl content was estimated according to Arnon [8] and total protein content according to Lowry *et al* [9] using bovine serum albumin as standard. 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 Saha *et al* [10] as described in Swamy *et al* [11]. Electon transport activities of thylakoid membranes were assayed according to Sabat *et al* [12] 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.

Whole chain electron transport was assayed with 0.5 mM methyl viologen (MV). By using 0.1 mM 2,6-dichlorophenol indophenol (DCPIP), 0.5 mM MV, 5mM ascorbate (Asc), and 10 mM 3-(3,5-dichlorphenyl)-1,1-dimethyl urea (DCMU), PS 1 activity was measured. In all the assays, thylakoid membranes equivalent to 40 µg Chl were used. Chl *a* fluorescence emission of control (0 h) and treated (72 h) leaf thylakoid membranes equivalent to 20g (Chl) m^{-3} were investigated in presence or absence of 10 µM DCMU in a medium containing 50mM Hepes-NaOH, PH 7.5, 100 mM sucrose, 2 mM $MgCl_2$ and 5 mM KCl on a *Jasco-FP 777* (Tokyo, Japan) spectrofluorometer at 25 ± 1 ºC with an excitation wavelength of 440nm. The excitation and emission slit width was 5nm. Besides, 77 K fluorescence emission spectra were recorded on a *Hitachi MPF4* (Tokyo, Japan) spectrofluorometer in samples prepared by mixing equal volume of 60 % glycerol and thylakoid membrane suspension. The samples were excited at 430 nm. The slit width of excitation and emission was 8 and 2 nm, respectively.

RESULTS AND DISCUSSION

Dark incubation for 72 h induced loss of Chl. In control sample the Chl observed to be 2.4 mg/g.f.w of leaf tissue (Table 1). 72 h of dark incubation brought the Chl content 0.9 mg/g.f.w of leaf segment. This loss in Chl could be due to induction of certain enzymes like chlorophyllases during dark incubation. When leaf segments of maize are incubated for 72 h in the presence of different polyamines. Spermine seems to be more protective in minimizing the loss of total Chl content [13]. Similarly there is a loss of carotenoids and protein content in 72 h dark incubated samples and was regain to 80% of the original content due to the application of PAs during dark incubation. The protective effect of PAs on Chl and protein content could be due to stabilization of thylakoid membrane and inhibition of certain enzymes like chlorophyllases and proteases [14, 15]. After studying pigment, protein contents an attempt has been made to analyze the photochemical activities of the thylakoid membranes during dark incubation in terms of oxygen exchange measurements (Table 2). PAs are able to retain the whole chain electron activity even 72 h of incubation. In order to ascertain the target photosystems, both PS II and PS I catalyzed transport activities were measured after isolating thylakoid membranes from different samples. There is a decrease of PS II activity by 53% and PS I activity by 17% after 72 h of dark incubation. By inclusion of PAs during treatment maintain the loss of both electron transport activities depending on the valency of PAs. Chl *a* fluorescence is an indicator of PS II photochemistry in thylakoid membranes [16,17,18]. Hence Chl fluorescence was measured in the presence and absence of DCMU in different samples. The ratio in control sample seems to be 1.7 indicating the proper functioning of PS II. Dark incubation for 72 h brought the ratio from 1.7 to 1.2 indicating the impairment of PS II activity (Table 3). The treatment of PAs brought the ratio from 1.2 to1.6 depending upon the valency of PAs. Prakash *et al* [19] showed the similar results in seedling of *Cucumis* plants. In order to show the influence of PAs on an energy distribution between the two photosystems, an attempt has been made to study the Chl *a* low temperature emission spectra by estimating the ratio of F_{690}/F_{735} (Table 4). The ratio was increased from 0.70 to 0.78 in dark incubated samples during 72 h suggesting the inhibition in energy transfer from PS II to PS I [20]. However the treatment of PAs brought the ratio from 0.78 to 0.71 indicating the improvement of energy distribution between two photosystems. Spm is able to restore the excitation energy transfer distribution from PS II to PS I in more efficient manner than spd and put. Thus PAs are able to stabilize thylakoid membrane and delay the loss of photochemical activity during dark incubation by shielding the negative charges of thylakoid membranes [21].

Table 1: Effect of polyamines (putrescine, spermidine and spermine) on pigment and total protein in maize primary leaves during dark incubation. The concentration of each polyamine was 25 µM.

Incubation time (h)	mg of photosynthetic pigments or protein/g.f.w.		
	Total Chl (*a*+*b*)	Carotenoids	Total roteins
H_2O (0 h)	2.40±0.12	0.061±0.004	26.66±2.0
H_2O (72 h)	0.91±0.08	0.032±0.001	14.28±0.4
Putrescine (72 h)	1.10±0.06	0.036±0.001	18.12±0.8
Spermidine (72h)	1.15±0.07	0.040±0.002	20.50±0.5
Spermine (72 h)	1.35±0.09	0.046±0.002	21.50±0.5

Table 2: Effect of 25 µM putrescine, spermidine and spermine on electron transport activities [Whole Chain (O_2 consumed), Photosystem II (O_2 evolved), Photosystem I (O_2 consumed)] in maize primary leaves during dark incubation.

Incubation time (h)	Electron transport activities (µM of O_2↑ (evolved) or↓ (consumed)/mg Chl/h).		
	WCE	PSII	PSI
H_2O (0h)	105±8	180±16	491±46
H_2O (72h)	40±3	85±7	409±38
Putrescine (72 h)	49±3	96±8	423±40
Spermidine (72 h)	56±4	104±9	432±41
Spermine (72 h)	66±5	115±10	445±42

Table 3: Retarding effect of polyamines in the presence and absence of DCMU on Chl *a* fluorescence in maize thylakoid membranes.

Sample	+/- DCMU ratio of Chl *a* fluorescence
Control(0)	1.7
Control(72)	1.2
Putrescine(72)	1.4
Spermidine(72)	1.5
Spermine (72)	1.6

Table 4: Retarding effect of polyamines on low temperature Chl a fluorescence relative intensity of PS II and PS I in maize thylakoid membranes.

Sample	F_{690}/F_{735}
Control(0)	0.70
Control(72)	0.78
Putrescine (72)	0.67
Spermidine (72)	0.68
Spermine (72)	0.71

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