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ROLE OF PHYTOHORMONES IN RETARDATION OF DARK INCUBATION INDUCED ALTERATIONS IN THE PRIMARY PROCESSES OF PHOTOSYNTHESIS IN MAIZE (*ZEA MAYS*) LEAVES.

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ABSTRACT: Phytohormones (Auxins, gibberillins and cytokinins) play a major role in regulating various physiological processes and alleviating the senescence in plants. To prove this, in this investigation an attempt has been made to compare the action of above phytohormones (25µM) individually in delaying the dark incubation (72h) induced alterations in photosynthetic electron transport and spectral properties of photosynthesis in maize plants. Among these phytohormones, cytokinin (25 µM) is able to provide more protection in terms of loss of chlorophyll (Chl) (60%), protein content (80%) and regaining of various electron transport activities (62%). Thus among phytohormones, cytokinins (25µM) is able to protect the thylakoids of maize in terms of photochemistry from dark incubation induced damage (72h).
Key words: Chl *a* fluorescence, electron transport, energy distribution, senescence, maize plants, phytohormones.

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INTRODUCTION

During leaf senescence several metabolic changes are developed which include the hydrolysis of macromolecules, such as proteins, lipids, nucleic acids and pigments that were accumulated during the growth phase [1]. The main event during the leaf senescence is the disassembly of photosynthetic apparatus within chloroplast which leads to the decrease in the photosynthetic activity [2]. The loss of function in chloroplast may be associated with the decline in the photochemical activities of photosystem I (PSI) and photosystem II (PSII) [3] but PSII is more susceptible to senescence than PSI and a greater decrease in PSII activity has been observed during senescence of leaves. Phytohormones are molecules which work as chemical messengers produced in very low concentrations but involved in the regulation of a variety of cellular processes in higher plants [4,5]. Earlier studies proved that phytohormones can extend the shelf life and some of the physiological processes alterations in banana fruit [6].They have been shown to increase growth and yield of plants and able to stabilize the cell membranes which help in the retardation of senescence [7]. Several workers showed that phytohormones are able to delay the loss of photosynthetic pigments in higher plant systems [8]. Phytohormones at high concentrations (50-100µM) are able to cause the destruction of thylakoid membranes and inhibit the photosynthetic electron transport mediated by PS II as well as PS I [9]. But the studies related to the protection of dark incubation induced changes by low concentrations (less than 50 µM) of these phytohormones are scanty. Therfore in this investigation an attempt has been made to study the protective role of phytohormones 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 continous "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 auxin or 25 µM gibberillin or 25 µM cytokinin solutions in separate test tubes. The tubes were kept at 25 ± 1^0C 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 (1949)[10] and total protein content according to Lowry *et al.* (1951)[11] 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 5mM KCl, by a procedure similar to that of Saha *et al.* (1970)[12] as described in Swamy *et al.* (1995)[13]. Electron transport activities of thylakoid membranes were assayed according to Sabat *et al* (1989)[14] in 2 ml reaction buffer (50mM Hepes-NaOH, pH 7.5, 100mM sucrose, 2mM $MgCl_2$ 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 indophenols (DCPIP), 0.5 mM MV, 5mM ascorbate (Asc), and 10mM 3-(3,5-dichlorphenyl)-1,1-dimethyl urea (DCMU), PS I activity was measured. In all the assays thylakoid membranes equivalent to 40 mg Chl were used. Chl *a* fluorescence emission of control (0h) and treated (72h) 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 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

In this investigation the protective role of phytohormones like auxin, gibberillin and cytokinin at 25 µM has been studied by incubating the 8 day old maize primary leaves in the presence and absence of above senescence redardants. Dark incubation for 72 h induced loss of Chl. In control sample the Chl observed to be 2.3 mg/g.f.w of leaf tissure (Table 1). 72 h of dark incubation brought the Chl content 0.9 mg/g.f.w of leaf segment. This loss in Chl content could be due to incubation of certain enzymes like chlorophyllases during dark incubation. When leaf segments of maize are incubated for 72h in the presence of different phytohormones. Cytokinin seems to be more protective in minimizing the loss of total Chl content [15]. 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 phytohormones during dark incubation. The studies of our results are made with metal ions in delaying the senescence induced alterations made by Murthy's group in wheat plants [16]. The protective effect of phytohormones on Chl and protein content could be due to stabilization of thylakoid membrane and inhibition of certain enzymes like chlorophyllase and proteases [17]. 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). Phytohormones are able to retain the whole chain electron transport 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 46% and PS I activity by 17% after 72 h of dark incubation. By inclusion of phytohormones during treatment maintain the loss of both electron transport activities. Earlier studies made in the maize primary leaves regarding the dark incubation control by polyamines are also in agreement with our electron transport measurements made with phytohormones [18]. Chl *a* fluorescence is an indicator of PS II photochemistry in thylakoid membranes [19]. Hence Chl *a* fluorescence was measured in the presence and absence of DCMU in different samples. The ratio in control sample seems to be 1.6 indicating the proper functioning of PS II. Dark incubation for 72 h brought the ratio from 1.6 to 1.1 indicating the impairement of PS II activity (Table 3). The treatment of phytohormones brought the ratio from 1.1 to 1.5. Prakash *et. al.,* (1988)[20] showed the similar results in seedling of *Cucumis* plants. In order to show the influence of phytohormones 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 0.69 to 0.76 in dark incubated samples during 72 h suggesting the inhibition in energy transfer from PS II to PS I [21]. However the treatment of phytohormones brought the ratio from 0.76 to 0.70 indicating the improvement of energy distribution between two photosystems. Cytokinin is able to restore the excitation energy transfer distribution from PS II to PS I in more efficient manner than auxin and gibberillic acid. Several studies have showed that cytokinin is more protective against various stress responses in wheat plants [22]. Thus phytohormones are able to stabilize thylakoid membrane and delay the loss of photochemical activity during dark incubation by shielding the negative charges of thylakoid membranes.

Table 1: Effect of phytohormones (auxin, gibberillin and cytokinin) on pigment and total protein in maize primary leaves during dark incubation. The concentration of each phytohormones was 25 µM

Incubation time (h)	mg of photosynthetic pigments or protein/g.f.w		
	Total Chl (*a*+*b*)	Carotenoids	Total proteins
Control (0h)	2.30 ± 0.10	0.060 ± 0.003	26.4 ± 1.0
Control (72h)	0.90 ± 0.07	0.031 ± 0.001	13.26 ± 0.3
Auxin (72h)	1.08 ± 0.04	0.034 ± 0.001	16.12 ± 0.6
Gibberillin (72h)	1.13 ± 0.05	0.038 ± 0.002	19.50 ± 0.5
Cytokinin (72h)	1.33±0.07	0.044 ± 0.002	20.50 ± 0.5

Table 2: Effect of 25 µM auxin, gibberillin and cytokinin 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	PS II	PS I
Control (0h)	104 ± 7	180 ± 14	489 ± 44
Conntrol (72h)	40 ± 2	83 ± 5	408 ± 38
Auxin (72h)	48 ± 2	94 ± 6	422 ± 40
Gibberillin (72h)	55 ± 3	102 ± 7	430 ± 40
Cytokinin (72h)	64 ± 4	113 ± 8	443 ± 41

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

Sample	+/- DCMU ratio of Chl *a* fluorescence
Control(0h)	1.6
Control(72h)	1.1
Auxin(72h)	1.3
Gibberillin(72h)	1.4
Cytokinin(72h)	1.5

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

Sample	F_{690}/F_{735}
Control (0h)	0.69
Control (72h)	0.76
Auxin (72h)	0.65
Gibberillins (72h)	0.66
Cytokinin (72h)	0.70

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