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Metal ion mediated protection in spectral properties of detached maize leaves during dark incubation

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Abstract:

In this study effect of selected metal ions (Ca^{2+}/Al^{3+}) on dark incubation induced changes in various pigment proteins through absorption spectral measurements in control 72 hr darks adopted leaves of maize in the presence and absence of Ca^{2+}/Al^{3+} were made. Calcium /Aluminum ions are able to restore the changes in Chlorophyll a, b and carotenoid absorption properties due to their bivalency dependent nature. Similar results are also noticed in energy transfer process through Chlorophyll a fluorescence measurements. In the presence and absence diuron, the observations clearly supported that Ca^{2+}/Al^{3+} are able to minimize the dark incubation induced changes in PSII photochemistry of maize leaves.

Keywords: Absorption, Chlorophyll, Fluorescence, Maize plants, Senescence.

Introduction:

In order to prepare for the next generation and/or to enable the plant to survive in unfavourable environmental conditions, leaf senescence is a crucial developmental stage in the life of plants. It causes a massive mobilisation and export of nitrogen and minerals to younger tissues (such as growing leaves, flowers, fruits, and developing seeds) (1). Senescent leaves allow nutrients, particularly nitrogen, to be transferred from the oldest to the youngest leaves (2). The plant's nitrogen nutrition status, as well as source/sink relationships is related to the rate of senescence and the remobilization of leaf nitrogen (3). Chloroplasts house up to 75% of the nitrogen found in mesophyll cells (4). In particular, glutamine synthetase activity, whose expression has been demonstrated to be connected with senescing leaves, converts nitrogen into amino acids (5, 6). The reduction in photochemical activity during leaf senescence restricts photosynthesis. In various senescing systems, it is observed that PS II, PS I, and WCE activities have drastically decreased (7, 8, 9). The modifications in two mobile electron carriers, PQ and PC, caused

higher loss in WCE transport than in PS II or PS I (10). In *Phaseolus*, it has been observed that the electron transport activities of PS II and PS I decreased by 25% and 33%, respectively, during leaf senescence. It was also found that senescing leaves have an issue with the acceptor side of PS I (9). In the cotyledonary chloroplasts of soyabean, Bricker and Newman (1982) discovered a greater reduction in PS I electron transport activity during senescence than in PS II. The PS II electron transport rates in barley decrease along with a decline in the amounts of D1 protein (11). Thus, the onset of leaf senescence clearly significantly reduces the crop's yield by changing the electron transport (12). In this study, an effort has been made to characterise how $CaCl_2$ and $AlCl_3$ delay the spectral changes caused by dark incubation in order to analyse the changes in energy transfer of primary maize leaves.

Materials and Methods

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^{o}C.Treatment of leaf segments for 96h in dark at 25^{o}C was given with $CaCl_2$or $AlCl_3$ alone in distilled water. Thylakoid membranes were isolated according to the procedure (13). Absorption spectra of thylakoids were recorded at room temperature 25^{o}C on Hitachi 557 spectrophotometer. Thylakoid membranes were suspended in a medium containing 50mM HEPES-NaOH (pH7.5), 100mM sucrose, 2mM $MgCl_2$ and 5 mM KCl. The spectra were normalized between 400 to 750 nm. The base line was corrected carefully and each spectrum was scanned at least three times. Jasco FP777 spectrofluorimeter was used to record fluorescence emission and excitation spectras in absence and in presence of 10 µM DCMU at 25^{o}C.Thylakoid membranes equivalent to 20 µg of Chl/ml were suspended in a reactionbuffer containing 50 mM HEPES-NaOH (pH 7.5), 100 mM sucrose, 2 mM $MgCl_2$ and 5 mM KCl. The samples were exited at 440 nm via a slit width of 5 nm. The emission was collected from 650 to 730 nm in the visible region via a silt width of 5 nm.

Results:

Alteration in thylakoid membrane functions could be due to alterations inthe spectral properties of thylakoid membranes. To verify the above preposition absorption, fluorescence emission properties at room temperature were recorded. Absorption spectra of 0 h control thylakoid membranes showed two prominent peaks at 680 and 440nm which are assigned to the absorption of Chl a and humps at 650 and 480 nm for Chl b and carotenoids respectively (Fig. 1). The drastic suppression of peaks took place at 72 h control during dark incubation compare to 0h control thylakoid membranes. It was found from the spectral analysis that the suppression of peak at 680 nm without being shifted to either side of the spectra and drastic suppression of peaks at 480 and 440 nm at 72h. They were marginalized by Al^{3+} treatment. Ca^{2+} treated leaf segments showed increase in absorption at 680, moderate increase at 480 and 440 nm when compare to that of 72 h control spectra. The effect was further characterized by calculating the absorption ratios of different pigment proteins such as A480/A440, A680/A440 and A680/A480. The ratio of peak heights of carotenoid

absorption at 480 and Chl a soret band absorption of 440 nm region was 0.67 in 0 h control thylakoid membranes. This ratio was increased to 0.74 at 72 h during dark incubation. Ca^{2+} and Al^{3+} reduced the loss to 0.58 and 0.70 respectively at 72 h. Al^{3+} showed more retention in the A480/A440 ratio compared to Ca^{2+} (Fig. 2).

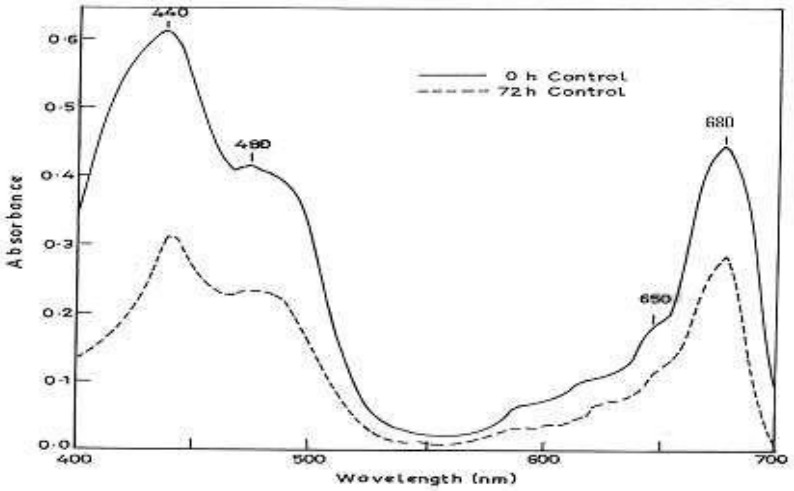


Fig. 1: Effect of dark incubation on room temperature absorption spectra of control thylakoid membranes of maize primary leaf segments under dark incubated senescence.

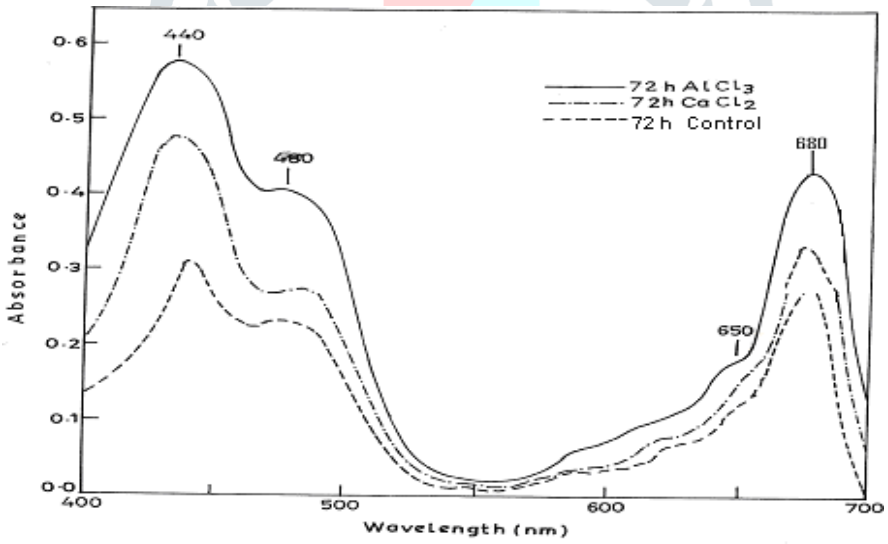
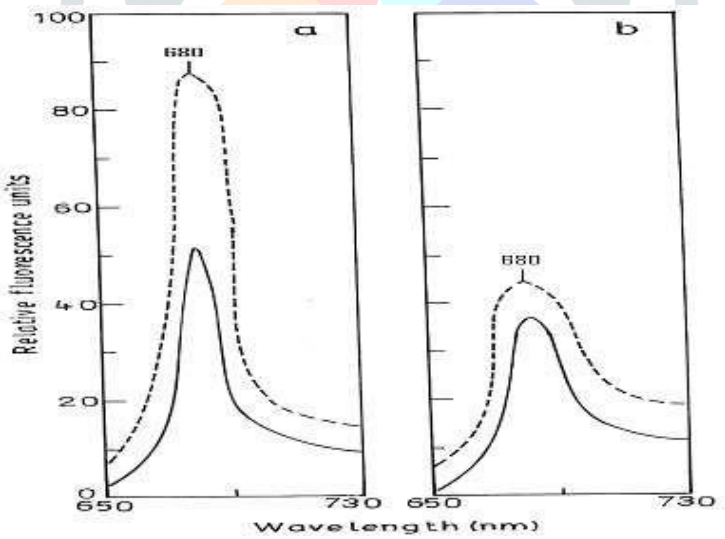


Fig. 2: Effect of 40 µM $CaCl_2$ and 40µM $AlCl_3$ on room temperature absorption spectra of thylakoid membranes of maize primary leaf segments under dark incubated senescence at 72 h.

The ratio of absorption of Chl a peak heights at red region and blue region was 0.72 in 0 h control thylakoid membranes. This ratio was increased to 0.93 at 72 h duringdarkincubation.Ca^{2+} and Al^{3+} reduced this loss to 0.79 and 0.74 respectively at 72h during dark incubation. Al^{3+} showed more retention in the A480/A440 ratio compared to Ca^{2+}. The ratio of absorption at Chl a region to the absorption at carotenoid region was 1.07 in 0 h control thylakoid membranes. This ratio was increased to 1.26 at 72h during dark incubation (Fig. 2). Ca^{2+} and Al^{3+} reduced the loss to 1.18 and 1.04 respectively at 72h. Al^{3+} showed more retention in the above photosynthetic pigments compared to Ca^{2+} treated ones. The Chl a fluorescence emission intensity was measured at 683 nm with an excitation wavelength at 440 nm in presence and absence of DCMU. Compare to 0 h control, 72h thylakoid membranes showed loss in fluorescence emission during dark incubation (Fig. 3). Ca^{2+} and Al^{3+} treated thylakoid membranes reduced the loss influorescence emission at 72h (Fig. 3). This trend was observed in presence and absence of 10µM DCMU which causes an enhancement of fluorescence emission at 683 nm and acts as inhibitor of PS II photochemistry. The ratio of Chl *a* fluorescence emission in the presence and absence of DCMU at 0 h control thylakoid membranes is 1.67 whereas this value is decreased to 1.15 in 72 h control thylakoid membranes (Table 1). Ca^{2+}/Al^{3+} reduced the loss influorescence emission to 1.31 and 1.42 respectively at 72 h (Fig. 4).

Fig. 3: Effect of dark incubation on room temperature Chl a fluorescence emission spectra of (a) 0 h control and (b) 72 h control thylakoid membranes of maize primary leaves under dark incubated senescence in presence and absence of 10µM DCMU.



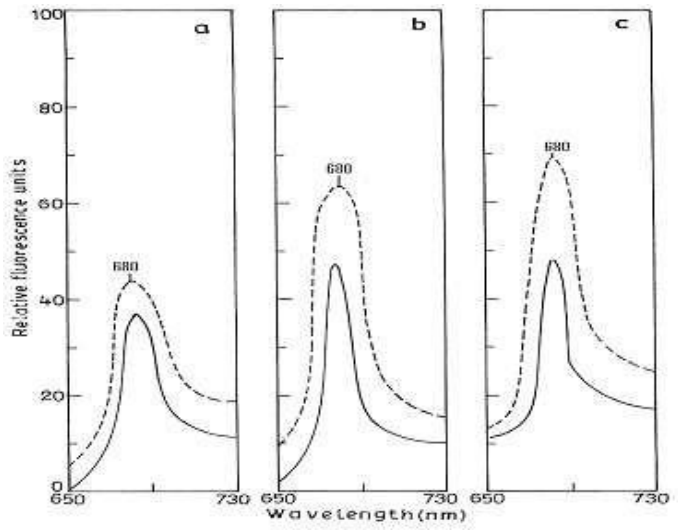


Fig. 4: Effect of 40 µM $CaCl_2$ and 40 µM $AlCl_3$ on room temperature Chl *a* fluorescence emission spectra of (a) 72 h control, (b) 72 h Ca^{2+} and (c) 72 h Al^{3+} treated thylakoid membranes of maize primary leaves under dark incubated senescence in presence and absence of 10µM DCMU.

Parameter	Treatment			
	Control 0h	Control 72h	$CaCl_2$ 72h	$AlCl_3$ 72h
F_{685}-DCMU	52	38	43	49
F_{685}+DCMU	87	44	64	70
F_{685}+DCMU/F_{685} DCMU	1.67	1.15	1.31	1.42

Table 1: Effect of 40 µM $CaCl_2$ and 40 µM $AlCl_3$ on room temperature Chl a fluorescence emission ratios in the presence and absence of DCMU in thylakoid membranes of maize primary leaf segments under dark incubated senescence.

Discussion:

Since, photosynthetic pigments absorption properties are related to the functioning of PSII, an attempt has been made to study the alterations in the absiprtion spectra of maize thylakoid membranes. In absorption spectra 680 nm is responsible for the absorption of chlorophyll a in the red region of the spectrum and 440 nm is responsible for the absorption of same pigments in the blue region of the spectrum. A hump at 650nm and 480 nm is responsible for the absorption of chlorophyll b and carotenoids (Fig. 1). Dark incubation of maize thylakoids exhibited decrease in chlorophyll a and carotenoid absorption. The inclusion of Ca^{2+}/ Al^{3+} reversed the above noticed changes in absorption spectra due to valency dependent protective nature (Fig. 2). Compare to 0h control, 72h thylakoid membranes showed loss in fluorescence emission. Ca^{2+}/ Al^{3+}

treated thylakoid membranes reduced the loss influorescence emission at 72 h during dark incubation (Fig.1). DUMU causes an enhancement of fluorescence emission in thylakoid membranes. The ratio of fluorescence emission in presence and absence of DCMU to at 0h control thylakoid membranes indicates alterations in primary photochemistry of PSII during senescence of dark incubation. At 72 h, the decrease in room temperature fluorescence emission even in presence and absence of DUMU was observed. The above finding suggests alterations in primary photochemistry of PS II at 72h. The loss in fluorescence emission enhancement in presence of DCMU arises primarily due to alterations in PS II (14, 15). Thylakoids membranes isolated from Ca^{2+}/ Al^{3+} treated leaves restored to near 0h control value of fluorescence emission in presence and absence of 10 µM DCMU (Table 1) suggesting that Ca^{2+}/Al^{3+} facilitates highest enhancement in fluorescence emission and restoration of PSII photochemistry. Thus, chlorophyll a fluorescence measurements act as an indicator to establish the changes in functioning of PSII in dark incubated maize leaves.

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