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Senescence Retarding Effect of CA and Al Metal Ions: Induced Alterations in Pigment, Protein Content and Photochemical Activities in Rice (Oryza Sativa) Leaves

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ABSTRACT:

Al^{3+} significantly delayed the loss of chlorophyll (Chl), protein and carotenoids when compared to Ca^{2+} during dark-induced senescence of detached primary leaves of Rice (*Oryza sativa*). Thylakoid membranes isolated from Al^{3+} treated leaves showed a better retention of Photosystem (PS II), Photosystem (PS I) and whole chain electron (WCE) transport activities than thyalokoid of Ca^{2+} treated leaves.

Key words: Chlorophyll, Carotenoids, electron transport, Senescence, calcium , proteins.

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INTRODUCTION:

Investigation discovered that at least 32-45 inorganic elements are required for plant growth and development. Numerous studies reported the competence of metal ions in delaying senescence: Ni^{2+} (Mishra and Samal, 1971), Mg^{2+} (Hemanthrajan and Vaishampayan, 1984) and Ca^{2+} (Poovaiah and Leopold, 1973; Cheour *et al.,* 1992). Ca^{2+} acts as an intracellular signal to a number of basic processes like cytoplasmic streaming, cell division, differentiation and defense (Reddy, 2001; Sanders *et al.,* 2002). Raise in intracellular Ca^{2+} have also been noted for a series of abiotic stresses, including chilling, heat shock, anaerobic stress, salinity and drought (Bowler and Fluhr, 2000; Chinnusamy *et al.,* 2004). Ca^{2+} is identified to protect the reliability of cell membranes, membrane permeability and prevents ion leakage caused by environmental stress (Lin *et al.,* 2008). The effects of Ca^{2+} on the senescence process and photosynthetic activity are dependent on its cytosolic concentration, which is governed by the activity of ion-channels in the plasma membrane (Knight, 2000).

Al^{3+} is one of the mainly profuse element on the earth is a growth limiting factor in acid soils (Kochian, 1995; Mosser-Pietraszewska, 2001). A great deal of the previous research on Al^{3+} toxicity has focused on monocotyledonous crop plants such as Rice and maize (Sung *et al.,* 2001). At high metal ion concentration, Al^{3+} inactivates the photochemical reaction (Tripathy and Mohanty, 1980; Wavare and Mohanty, 1982). At low concentrations, Al^{3+} stimulates the electron transport catalyzed by PS II of cyanobacterial systems and isolated beet-spinach chloroplsts (Wavare and Mohanty, 1982; Wavare *et al.,* 1983). Paliwal *et al.,* (1994) reported that Al^{3+} induces decrease in biomass accumulation in the cowpea genotypes differing in their sensitivity to Al^{3+}. Hence a study on delay of senescence by Ca^{2+} and Al^{3+} on pigments, proteins, photochemical activities in Rice primary leaf segments was made during dark incubation.

MATERIALS AND METHODS:

Plants and treatment: Healthy seeds of Rice (*Oryza sativa*) were obtained from Acharya N. G. Ranga Agricultural College, Hyderabad. The seeds were surface sterilized with 0.1 % $HgCl_2$ for 2 min and thoroughly washed with tap water and then with distilled water. The seeds were imbibed for 6 h and germinated in petridishes on filter paper for 4 days. The seedlings were randomly placed in plastic trays and watered daily with quarter strength Hoagland nutrient solution (Arnon and Hoagland, 1950) and grown in a growth chamber providing with fluorescence light (Philips, India) with a light intensity of 30-35 µ moles m^{-2} s^{-1} at 25 ±1 ^{0}C. Fully expanded 10 th day leaf segments (4-5 cm long) were cut from apical

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region and used for treatment. Treatment of leaf segments for 96 h in dark at 25 ^{0}C was given with 50µM $CaCl_2$ and $AlCl_3$. Studies were made on senescence induced alterations in pigments, proteins and photochemical activities by keeping the leaf segments in double distilled water in dark at 25 ^{0}C for 24 - 96 h as control.

ESTIMATION OF PIGMENTS AND PROTEINS:

0.1 gm leaf segments were homogenized in a pre-chilled mortar and pestle in 10 ml of 80 % chilled acetone. The homogenate was transferred into 15 ml centrifuge tubes and centrifuged at 3000 xg for 5 min. The Chl and carotenoid concentration was measured in supernatant after dilution to a total volume of 15 ml by following the method of Arnon (1949). Carotenoid concentration was calculated according to the method of MacKinney (1941) .The protein content in the leaf segments was determined according to the method of Lowry *et al.* (1951). 0.1 g of leaf segments add 10 % TCA and homogenized incubated at 4 ^{0}C for 8 h. The TCA precipitates were ether-dried, dissolved in 1N NaOH. Protein content was determined using bovine serum albumin (BSA) as standard.

Isolation of thylakoid membranes: Thylakoid membranes were isolated according to the procedure similar to that of Saha and Good (1970) as described in Swamy *et al.* (1995). One g of leaf segments were homogenized with a prechilled mortar and pestle in 10 ml of ice-cold isolation buffer containing 50 mM HEPES-NaOH (pH 7.8), 400 mM sucrose, 2 mM $MgCl_2$ and 5 mM KCl. The homogenate was filtered through two layers of miracloth and centrifuged at 6000 xg for 5 min. The pellet was washed twice with washing buffer containing 5 mM HEPES-NaOH (pH 7.8), 100 mM sucrose, 2 mM $MgCl_2$ and 5 mM KCl and centrifuged briefly at 2000 xg for 30 s. The supernatant was pelleted again at 6000 xg for 5 min and the pellet was finally suspended in a minimal volume of suspension buffer containing 20 mM HEPES-NaOH (pH 7.8), 100 mM sucrose, 2 mM $MgCl_2$ and 5 mM KCl. They were immediately used for measuring the photosynthetic electron transport activities. All the operations were carried out at 4 ^{0}C in dim light. Chl was estimated according to Arnon (1949).

Photosynthetic electron transport activities: Electron transport rates were measured using a Clark type oxygen electrode (Hansatech, UK) in the thylakoid membranes. The measurements were done at 25 ^{0}C under saturating intensity of white light. The 2 ml assay reaction buffer contained 50 mM HEPES-NaOH (pH 7.5), 100 mM sucrose, 2 mM $MgCl_2$ and 5 mM KCl according to Subhan and Murthy (2000). WCE and PS I mediated activities were measured as molecular consumption. The reaction mixture for WCE contained 0.5mM MV methyl viologen (MV) while that for the PS I determination contained 5mM

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ascorbate, 1mM sodium azide,0.5mM MV, 0.1 mM DCPIP and 10µM DCMU. PS II mediated electron transport was studied as molecular oxygen evolution using 0.5mM p-BQ. Thylakoid membranes equivalent to 40µg Chl were used in all assays.

RESULT:

Effect of Ca^{2+} and Al^{3+} on pigments and proteins

The rate of dark induced senescence in control and treated Rice primary leaf segments was estimated through determining the quantitative changes in pigments. Total Chl was steadily declined to 30 % in control leaf segments during dark incubation at 96 h (Fig. 1). Ca^{2+} and Al^{3+} significantly reduced this loss to 35 % and 42 % respectively at 96 h ($p< 0.05$). Almost the same trend can be seen in degradation profile of Chl *a* during dark incubation. Chl *a* was decreased to 28 % at 96 h (Fig. 2). Ca^{2+} and Al^{3+} significantly reduced this loss to 30 % and 40 % respectively at 96 h ($p< 0.05$). Al^{3+} caused a maximum retention of Chl *a* than Ca^{2+} at 96 h. The degradation pattern of Chl *b* in dark control was different from Chl *a* degradation (Fig. 3). The Chl *b* degraded to 42 % at 96 h during dark incubation. Ca^{2+} and Al^{3+} significantly reduced this loss to 52 % and 58 % at 96 h respectively ($p< 0.05$).

Carotenoid content was steadily declined to 48 % whereas Ca^{2+} and Al^{3+} significantly reduced this loss to 53 % and 57 % during dark incubation at 96 h (Fig. 4) ($p< 0.05$). Since protein degradation is the main criteria in senescence an estimation of total protein content has been made. The total protein content was decreased to 34 % in 0 h control leaf segments (Fig. 5). Ca^{2+} and Al^{3+} significantly reduced this loss to 58 % and 73 % during dark incubation at 96 h ($p< 0.05$).

EFFECT OF CA^{2+} AND AL^{3+} ON PHOTOSYNTHETIC ACTIVITIES

Since alteration in pigments may influence the photochemical activities, an attempt has been made to analyze the electron transport activities (WCE, PS II and PS I) of thylakoid membranes. In control leaf segments WCE ($H_2O \rightarrow MV$) decreased to 41 % at 72 h, while the activity was not found at 96 h dark incubation (Table 1). Ca^{2+} and Al^{3+} significantly reduced the WCE activity loss to 50 % and 58 % respectively at 72 h ($p< 0.05$). It seems there is a valency dependent protection of WCE activity by Ca^{2+} and Al^{3+} against loss during dark incubation. To find out the susceptibility of photosystems both PS II and PS I 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 50 % by Ca^{2+} and Al^{3+} respectively at 96 h during dark incubation (Table 1) ($p< 0.05$). The retention of PS II activity by Al^{3+} was more than

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that of Ca^{2+}. PS I activity was slightly decreased to 78 % in control at 96 h, Ca^{2+} and Al^{3+} significantly reduced this loss to 82 % and 86 % during dark incubation (Table 1) ($p< 0.05$). In both the photosystems, PS II activity decreased more than that of PS I.

DISCUSSION:

This study deals with the effect of Ca^{2+} and Al^{3+} in delaying dark incubated senescence. In control leaf segments total Chl loss was observed at 96 h during dark incubation. Ca^{2+} and Al^{3+} reduced the total Chl loss at 96 h (Fig. 13). A major protection of Chls by Al^{3+} at 96 h was observed when evaluate to Ca^{2+}. This indicates valency dependent protection of Chl loss. The screening of the fixed negative membrane surface charges is measured to be dependent on the cations valency and not on their chemical nature of their co-ions (Barber and Mills, 1976). Thus the order of effectiveness is assumed to be M^{3+}, M^{2+}, M^{+}. Chl contents showed a significantly lessening trend during dark incubation with increasing of M^{2+}, M^{3+}valency (Malathi *et al.,* 2002).

Chl *a* degraded more than Chl *b* during dark incubation (Fig. 14 & 15). Chl *a* level in PS I complex is greater than PS II complex (Fujita and Murakami, 1987). Hence any fall in Chl *a* levels indicates the loss of PS I pigment protein complexes during dark induced senescence. Chl *b* has fallen equally with 10 % dissimilarity to Chl *a* indicating degradation of Chl *a/b* polypeptides namely LHC II (Biswal and Biswal, 1999). This has also been observed during drought-induced senescence in *Salvia officinalis* (Munne-Bosch *et al.,* 2001). Carotenoids assumed to protect photochemical reactions due to high concentrations in dark incubation (Fig. 4). Decrease in carotenoid content can be attributed to enzymatic degradation (Srichandan *et al.,* 1989). Ca^{2+} and Al^{3+} treatment added further constancy to carotenoids during dark incubation. This may result in a relatively higher stability of Chl during senescence. The loss in carotenoids was observed in control leaf segments throughout dark incubation. However the relative rate of degradation of carotenoids was low as compared to Chl (Fig. 4). According to Thimann (1980) the leaves turn to yellow seems to show that carotenoids are more stable than Chls. However carotenoid, cloud not fully protects the pool of Chl molecule of membranes during senescence. The spacing for energy to transfer from Chl triplets to carotenoids on membrane is damaged during senescence, this may cause the transfer of some energy from triplet Chl to O_2 rather than to carotenoid resulting in the formation of O_2', which decomposes Chl molecules (Krieger-Liszkay, 2005).

The total protein content was decreased in control leaf segments during dark incubation (Fig. 5). Ca^{2+} and Al^{3+} reduced this loss at 96 h. Mukherjee (2003) reported the decline in the protein content in

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Cajanus cajan leaves during their senescence. Penarrubia and Moreno (1995) recommended that increased protein breakdown may result from different mechanisms such as denovo synthesis of proteolytic enzymes activation of pre existing proteases, decompartmentalization of proteases and their substances or stabilization of protein substrates to degradation. Proteases are the enzymes responsible for hydrolysis of proteins (Huffaker, 1990). Metal ions screen surface negative charges on membranes and may reduce the loss of protein during leaf senescence (Mc Carty 1980).

To find out whether the loss of pigments is related to photochemistry or not, electron transport measurements have been made. There was a progressive loss in WCE during dark incubation (Table 1). The loss in WCE is due to alterations in PS II and /or PS I. However the Ca^{2+} and Al^{3+} mediated protection of WCE could be due to restoration of PS II activity when compare to PS I activity. Since the extent of restoration of PS II activity by these Ca^{2+} and Al^{3+} in Rice leaves at 72 h was more than that of PS I (Table 1). One of the first symptoms of leaf senescence is a deterioration of the chloroplast; it may be pertinent to note that the chloroplast is the location for more than half of the Ca^{2+} content in leaves (Stocking and Ongun, 1962). A possible role of Ca^{2+} on the donor side of OEC of PS II mimicked the action of 23 kDa of PS II system (Preston and Critchley, 1985). According to Keren *et al.* (2000) depleted PS II preparations were not active in water splitting. Al^{3+} restored the photochemical activities to a significant extent probably by delaying the loss of PS II and PS I activities during dark incubation compared to Ca^{2+}. In Ca^{2+}, and also in Mn- depleted PS II not only the water splitting activity is inhibited, but also the midpoint potential of the Q_A/Q_A- redox couple is up shifted by about 150 mV (Krieger-Liszkay, 2005). Protection of the activity by Ca^{2+} and Al^{3+} is in valency dependant manner indicates the possible role in stabilization of electrostatic repulsive/ attractive forces.

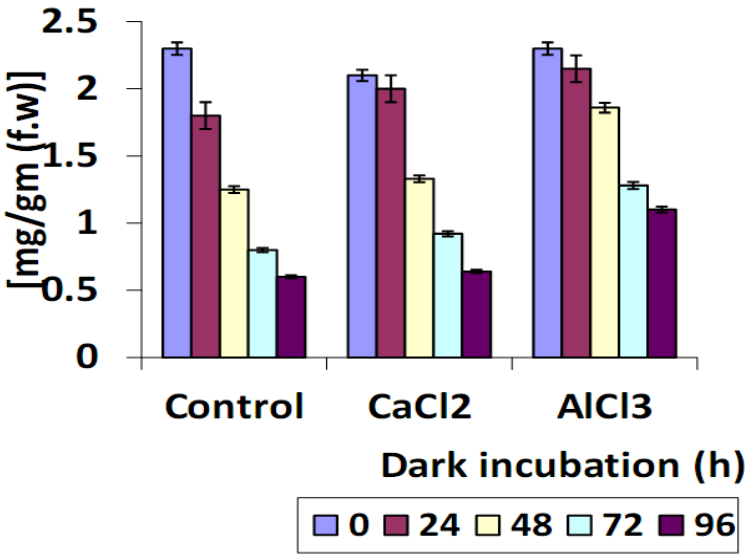

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Fig. 1: Effect of 50 µM $CaCl_2$ and 50 µM $AlCl_3$ on total Chl content in Rice primary leaf segments under dark induced senescence. Each value is mean ± SE of five replications.

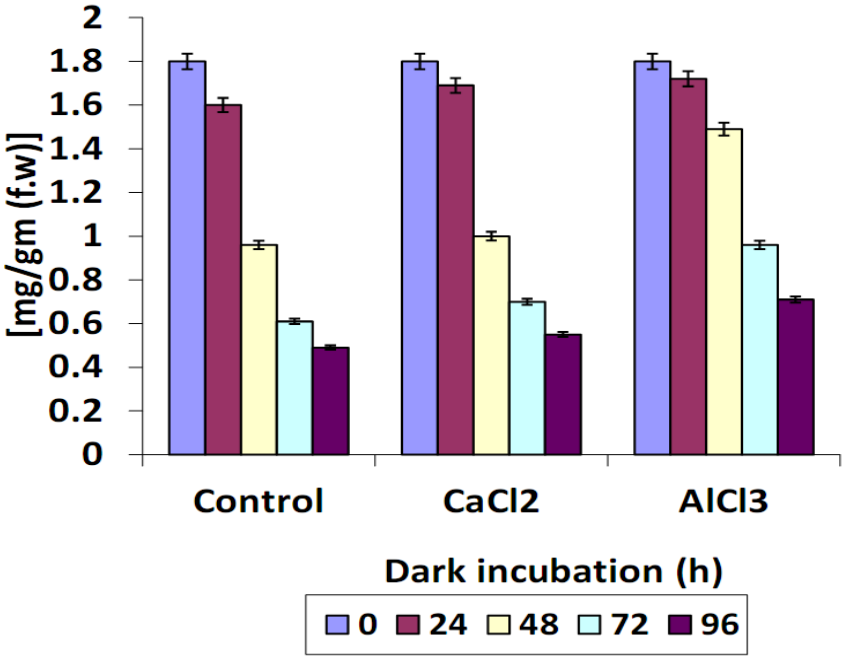

Fig.2: Effect of 50 µM $CaCl_2$ and 50 µM $AlCl_3$ on Chl *a* content in Rice primary leaf segments under dark induced senescence. Each value is mean ± SE of five replications.

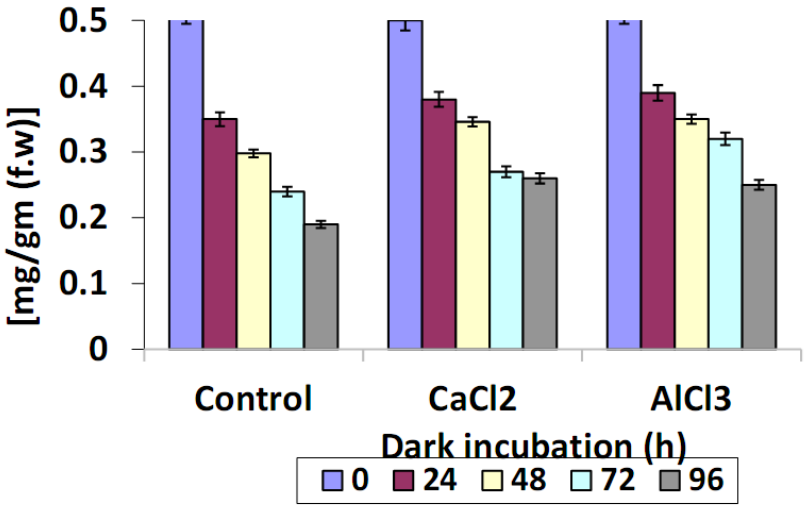

Fig. 3: Effect of 50 µM $CaCl_2$ and 50 µM $AlCl_3$ on Chl *b* content in Rice primary leaf segments under dark induced senescence. Each value is mean ± SE of five replications.

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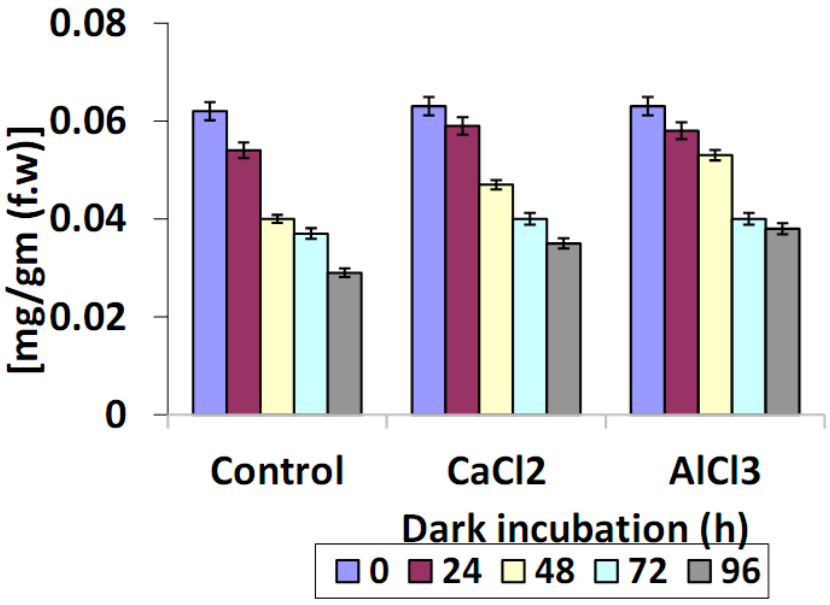

Fig. 4: Effect of 50 µM $CaCl_2$ and 50 µM $AlCl_3$ on carotenoid content in Rice primary leaf segments under dark induced senescence. Each value is mean ± SE of five replications.

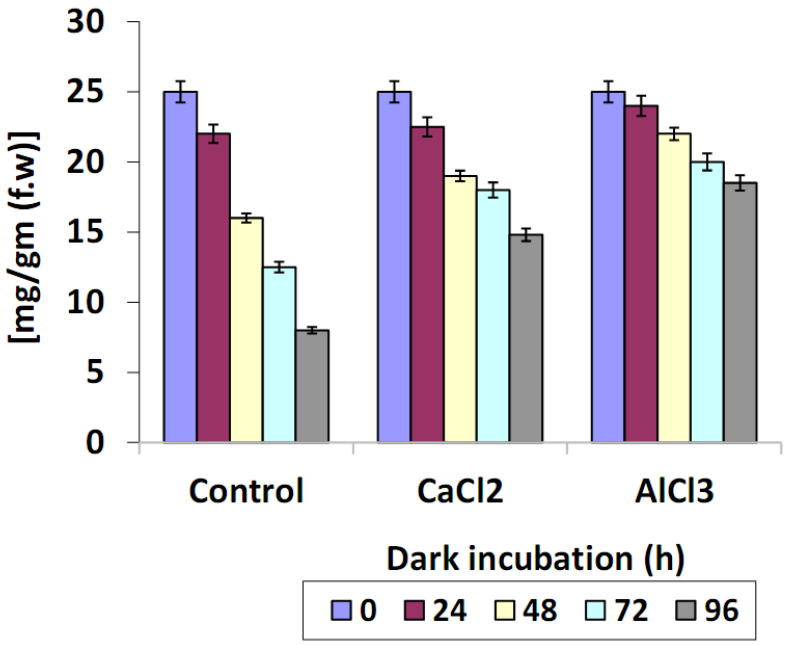

Fig. 5: Effect of 50 µM $CaCl_2$ and 50 µM $AlCl_3$ on protein content in Rice primary leaf segments under dark induced senescence. Each value is mean ± SE of five replications.

Table 1: Effect of 50 µM $CaCl_2$ and 50 µM $AlCl_3$ on WCE [µ moles (O_2 consumed) mg^{-1} Chl h^{-1}] , PS II [µ moles (O_2 evolved) mg^{-1} Chl h^{-1}] and PS I [µ moles (O_2 consumed) mg^{-1} Chl h^{-1}] activities in

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Rice primary leaf segments under dark incubated senescence. Each value is mean ± SE of five replications. Values in parentheses indicate % residual activities.

Parameters / Treatment		Dark incubation [h]				
		0	24	48	72	96
WCE	Control	115 ±2(100)	91± 2(79)	73 ± 3(63)	47 ± 3(41)	-
	$CaCl_2$		97 ± 4(84)	79 ± 2(69)	58 ± 4(50)	
	$AlCl_3$		103 ± 5(90)	91 ± 4(79)	67 ± 8(58)	-
PSII	Control	190 ±4(100)	170 ± 11(89)	151± 4(79)	91 ± 4(48)	73 ± 8(38)
	$CaCl_2$		175 ± 1 (92)	159 ± 8(84)	115 ± 9(60)	86 ± 11(45)
	$AlCl_3$		178 ± 7(94)	165 ± 12(87)	127 ± 9(67)	96 ± 10(50)
PS I	Control	480± 1(100)	447 ± 16(93)	430 ±11(90)	399 ± 12(83)	375 ±11(78)
	$CaCl_2$		458± 13(95)	442 ± 9(92)	418 ± 15(87)	398 ± 16(82)
	$AlCl_3$		462 ± 11(96)	450 ± 17(94)	430 ± 16(90)	411 ± 12(86)

CONCLUSION:

Ca^{2+} and Al^{3+} delayed the degradation of pigments, proteins and maintained the photosynthetic electron transport activities of Rice primary leaves during dark incubation. Al^{3+} was more effective than Ca^{2+} indicating valency dependent protection of pigments, proteins and electron transport activities during dark incubated senescence. The activities of WCE, PS II were drastically declined when compared to that of PS I.

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