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Combined effect of $AlCl_3$ and GA on Biochemical Characterization of Wheat Primary Leaves during Dark Incubated Senescence

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Abstract

Senescence is the age-dependent end of the life span. In plants, it can be visualized by yellowing of leaves that accompanies the mobilization of leaf nutrients to the reproductive structures. The yellowing of senescing leaves is correlated with a series of biochemical changes such as loss of chlorophyll contents, degradation of proteins, RNA and a decline in photosynthetic activity. Senescence limits crop yields in annual crops like maize, rice and wheat. Delayed leaf senescence is a desirable agronomic trait to improve crop yield. In this study the combination of $AlCl_3$ and Gibberellic acid reduced the loss of pigments, proteins, spectral properties, electron transport activities in wheat primary leaves under incubated dark conditions. The restoration of whole chain electron transport activity by the combination of $AlCl_3$ and GA was closely associated with the restoration of PS II activity when compared with PS I. The combination of Gibberellic acid and $AlCl_3$ treated leaf thylakoid membranes showed an increase in absorption at 680 nm moderate increases at 480 nm and 440 nm at 72 h during dark incubation. The $AlCl_3$ and Gibberellic acid protected the degradation of water oxidation complex polypeptides (33, 23, 17 KDa) of PS II and slightly protected the 68 kDa of PS I polypeptides.

Keywords: Senescence retardation; PSII and PS I activity; water oxidation complex polypeptides

Introduction

Senescence is defined as a time-dependent, gradual decay of multiple biological functions (López-Otín et al., 2013). In plants, senescence is a highly controlled and active process involved in disintegration and remobilization of valuable resources (Maillard et al., 2015). Senescence is followed by a massive degradation of chlorophyll and by inhibition of photosynthetic processes in leaf including photosystem II (PS II) (Janečková et al., 2018) and photosystem I (PS I) activity (Krieger Liszkay et al., 2015). The clearly visible form of senescence in leaf is yellowing caused by chloroplast pigment-protein complexes (Smart, 1995). Therefore, with the onset of senescence in leaf curtails the economic yield of crop plants to a significant extent. Senescence induced by dark is being used experimentally to study the progress of leaf senescence in an easy manner. Some metal ions like Co^{2+} (Geetha Chan-

dra, 1981), Ni (Mishra and Samal, 1971) Al^{3+} (Sudhan and Murthy, 2001) delay senescence in various crop plants. Plant hormones play a major influencing role in each stage of leaf senescence, including the initiation, progression and terminal phase of senescence. Gbberellic acid (GA) delay leaf senescence (Zhang et al., 2013; Li et al., 2014).

Over the last few years, the study on delay in the senescence of leaf is being carried out widely with high-interest but the studies related to photosynthetic activities are scanty. Hence biochemical characterization of GA +$AlCl_3$ helped to elucidate few processes that are occurring.

Materials and Methods

The healthy seeds of wheat were obtained from RARS, Tirupati, Andhra Pradesh, India. 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 incubated for 6 h and germinated in petri dishes on filter paper for 3 days. The seedlings were randomly placed in plastic trays and watered daily with quarter strength Hoagland nutrient solution and grown in a growth chamber providing a fluorescent light intensity of 30-35 µ moles m^{-2} S^{-1} at 25±1 °C. Fully expanded 8th day leaf segments (4-5 cm long) were cut from the apical region and used for treatment. To study the combined effect of phytohormone 5 µM Gibberellic acid (GA) and metalion 20 µM $AlCl_3$ (Al^{3+}) was used. Leaf segments in test solutions were kept in dark at 25 °C for 24-96 h. During the period of treatment, the test solutions were regularly replaced every 24 h with fresh ones.

Estimation of Chlorophyll and Protein Content

The total Chl and protein content was measured using the method of Arnon (1949). The protein content in the leaf segment was determined using Lowry et al. (1951) method.

Electron Transport and Spectral activities

Thylakoid membranes were isolated according to the procedure similar to that of Saha and Good (1970) as described in Swamy et al. (1995).

The Whole chain electron transport activity(WCE) was measured as O_2consumption by using Methyl Viologen (MV) as an electron acceptor in the thylakoid membranes. The 2 ml reaction mixture contained reaction buffer 50 mM HEPES-NaOH, (pH 7.5), 100 mM Sucrose, 2 mM $MgCl_2$ and 5 mM KCl, 0.5 mM MV 1.0 mM sodium -azide and thylakoid membranes equivalent to 40 µg of Chl. PS II catalyzed electron transport assay was measured as (H_2O →p-BQ) as O_2evolution in the thylakoid membranes. The 2 ml reaction mixture contains reaction buffer 50 mM HEPES-NaOH, (pH 7.5), 100 mM Sucrose, 2 mM $MgCl_2$, 5 mM KCl, 0.5 mM freshly prepared p-BQ and thylakoid membranes equivalent to 40 µg of Chl. PS I catalyzed electron transport assay was measured as O_2 consumption. The 2 ml reaction mixture contains reaction buffer 50 mM HEPES-NaOH, (pH 7.5), 100 mM Sucrose, 2 mM $MgCl_2$, 5 mM KCl, 0.1 mM 2,6-dichlorophenol indophenols (DCPIP), 0.5 mM MV, 5 mM ascorbate, 1 mM sodium azide, 10 µM DCMU and thylakoid membranes equivalent to 40 µg of Chl.

Polypeptide Analysis

Polypeptide analysis of thylakoid membranes was made according to Laemmli (1970) using SDS-PAGE mini gel apparatus.

Statistical Analysis

All the treatments data are represented as mean± SE of five replications. Students T-test was performed to identify the time points at which the mean for GA and corresponding control values are considered significant at $p< 0.01$.

Results and Discussion

Chlorophyll and Protein Content

Total Chl steadily declined to 31% in control leaves segments at 96 h dark incubation. The combined application of GA+ $AlCl_3$ significantly reduced this loss to 48% at 96 h ($p < 0.05$) (Fig. 1). Total protein content steadily decreased to 34% in control leaves segments at 96 h during dark incubation (Fig. 2). The combined application of GA+ $AlCl_3$ significantly reduced this loss to 79 % at 96 h ($p < 0.05$). Degradation of chlorophyll is a signal in the process of senescence (Krieger –Liszkay et al., 2019). Our results were supported by Doorn et al. (2013) in restoration of protein and chlorophyll content in iris flower senescence by using cytokinins and jasmonates. Reduction of protein and chlorophyll loss by Al^{3+} indicates valency dependent protection by metal ions.

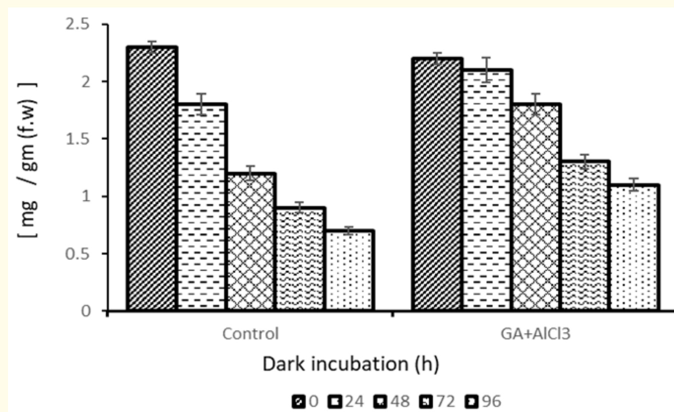

Figure 1: Effect of GA+ $AlCl_3$ on total chlorophyll content in wheat primary leaf segments under dark-induced senescence. Each value is mean± SE of five replications.

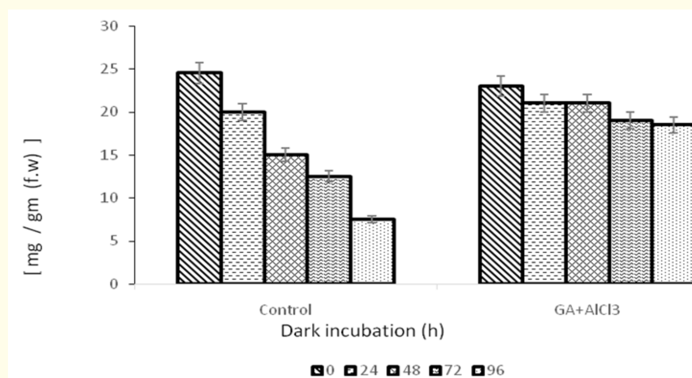

Figure 2: Effect of GA+ $AlCl_3$ on protein content in wheat primary leaf segments under dark-induced senescence. Each value is mean± SE of five replications.

Electron Transport Activities

To relate the persistants of pigments and proteins by GA + $AlCl_3$ to photochemical activities of thylakoid membranes, WCE, PS II and PS I were assayed. The WCE activity was measured by using MV as an electron acceptor. In the control thylakoid membranes WCE decreased to 42% at 72 h, while the activity was not found at 96 h dark incubation (Table 1). The combined application of GA+ $AlCl_3$ significantly reduced the WCE loss to 75% at 72h ($p < 0.01$). p-BQ supported control PS II activity decreased to 38% during dark incubation at 96 h and this loss was reduced significantly to 62% by GA+ $AlCl_3$ at 96 h (Table 1) ($p < 0.01$). PS I activity slightly decreased to 78% in control thylakoid membranes at 96h. The combined application of GA+ $AlCl_3$ reduced this loss to 89% at 96 h (Table 1) ($p < 0.01$). Our results were supported by Sudan and Murthy (2001) in restoration of PS II, PS I and WCE by combined application of kinetin and aluminium in wheat. In cucumber cotyledons, GA and kinetin influence the functional site of PS I and PS II reaction centers, thereby encouraging the development of the photosynthetic electron transport system (Pedhadiya et al., 1987). Similarly, in broad bean protoplasts, short-term GA-3 treatment increased the net photosynthetic rate and O_2 evolution (Yuan and Xu, 2001).

Photosynthetic activity	***Treatment***	***Incubation time (h)***				
		0	***24***	***36***	***72***	***96***
WCE	Control	115±2	91±2	73±3	48±3	-
		(100)	(79)	(63)	(42)	
	GA+ $AlCl_3$	115±2	110±5	99±4	85±7	-
		(100)	(96)	(86)	(75)	
PS II	Control	190±4	170±11	151±4	91±4	73±8
		(100)	(89)	(79)	(48)	(38)
	GA+ $AlCl_3$	190±4	188±15	175±12	151±12	118±11
		(100)	(99)	(92)	(79)	(62)
PS I	Control	480±10	447±16	430±11	399±12	399±11
		(100)	(93)	(90)	(83)	(78)
	GA+ $AlCl_3$	480±10	470±8	459±15	448±16	425±69
		(100)	(98)	(96)	(93)	(89)

Table 1: Effect of GA+ $AlCl_3$ on WCE [μM (O_2 consumed) mg^{-1} Chl h^{-1}], PS II [μM (O_2 evolved) mg^{-1} Chl h^{-1}] and PS I [μM (O_2 consumed) mg^{-1} Chl h^{-1}] activities in wheat primary leaf segments under dark incubated senescence.
Each value is mean± SE of five replications.
Values in parenthesis indicate % residual activities.

Spectral Activities

The chlorophyll a absorption and fluorescence derived parameters can be suggested as tool to monitor the leaf senescence. In this connection absorption and fluorescence activities were assayed. Absorption spectra of 0h control thylakoid membranes showed two prominent peaks at 680 nm and 440 nm for the absorption of Chl a and humps at 650 and 480 nm for Chl b and carotenoids respectively. At 72h drastic suppression of peaks took place in control thylakoid membranes (Fig. 3). The suppression of peak heights at 680 nm without being shifted to either side of the spectra and suppression of humps at 480 and 440 nm at 72 h were marginalized in GA+ $AlCl_3$ treated thylakoid membranes. The above finding suggests an alteration in the primary photochemistry of PS II at 72 h is responsible for the decrease of fluorescence emission ratio in both situations with and without DCMU.

Compared to 0h control, 72h thylakoid membranes showed the loss in fluorescence emission (Fig. 4). GA+ $AlCl_3$ treated thylakoid membranes reduced this loss at 72 h. This trend was observed in the both situations i.e, with and without DCMU. The ratio of Chl a fluorescence emission in both the situations i.e, with and without DCMU at 0h control thylakoid membrane is 1.67 whereas this value

is decreased to 1.15 in 72 h control thylakoid membranes during dark incubation. GA+ $AlCl_3$ reduced this loss in fluorescent emission to 1.60 at 72 h (data not shown).

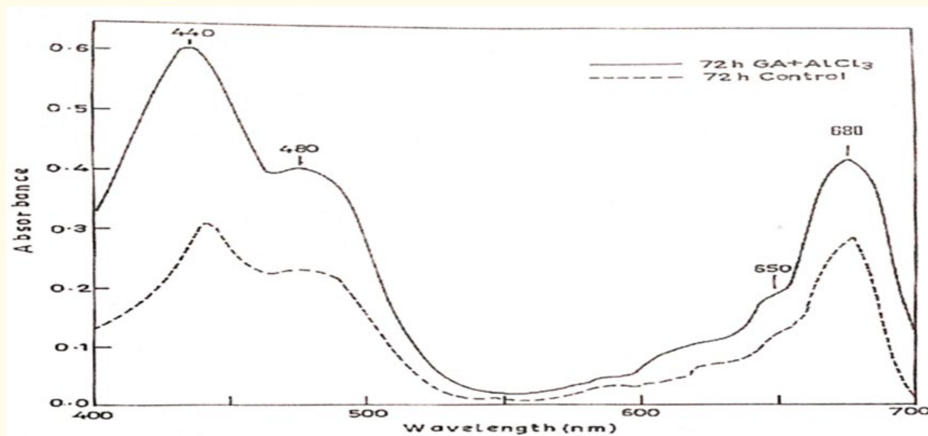

Figure 3: Effect of GA+ $AlCl_3$ on room temperature absorption spectra of thylakoid membranes in wheat primary leaf segments under dark-induced senescence.

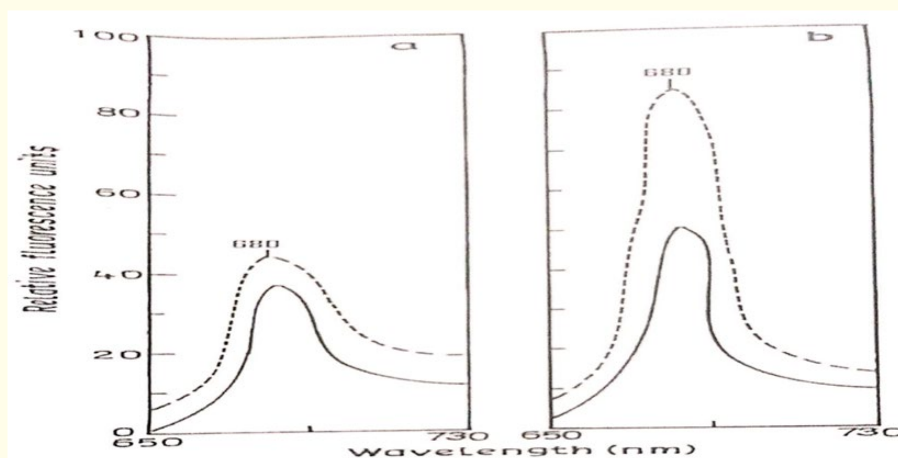

Figure 4: Effect of GA+ $AlCl_3$ at room temperature Chl a fluorescence emission spectrum of (a) 72 h control and (b) 72 h GA+ $AlCl_3$ treated thylakoid membranes in wheat primary leaf segments under dark-induced senescence in the presence and absence of 10 µM DCMU.

Polypeptide Analysis

In SDS-PAGE polypeptide analysis of control thylakoid membrane, polypeptides degrade at 72 h during dark incubation. However, they were protected from degradation by GA+ $AlCl_3$ at 72 h (Fig. 5). Polypeptides in the region of 68 KDa protected by GA+ $AlCl_3$ at 72 h, restored the loss in Ps I activity. Polypeptides with molecular weight 43, 33, 23 and 17 KD appeared to be degraded in 72 h dark incubated control leaf thylakoid membrane as the intensity of bands in this region decreased. They were protected by GA+ $AlCl_3$ at 72

h of dark incubation (Lane 4). Since GA+ $AlCl_3$ caused retention of protein in the thylakoid membrane, they can be seen as increased intensity of bands in Lane-4.

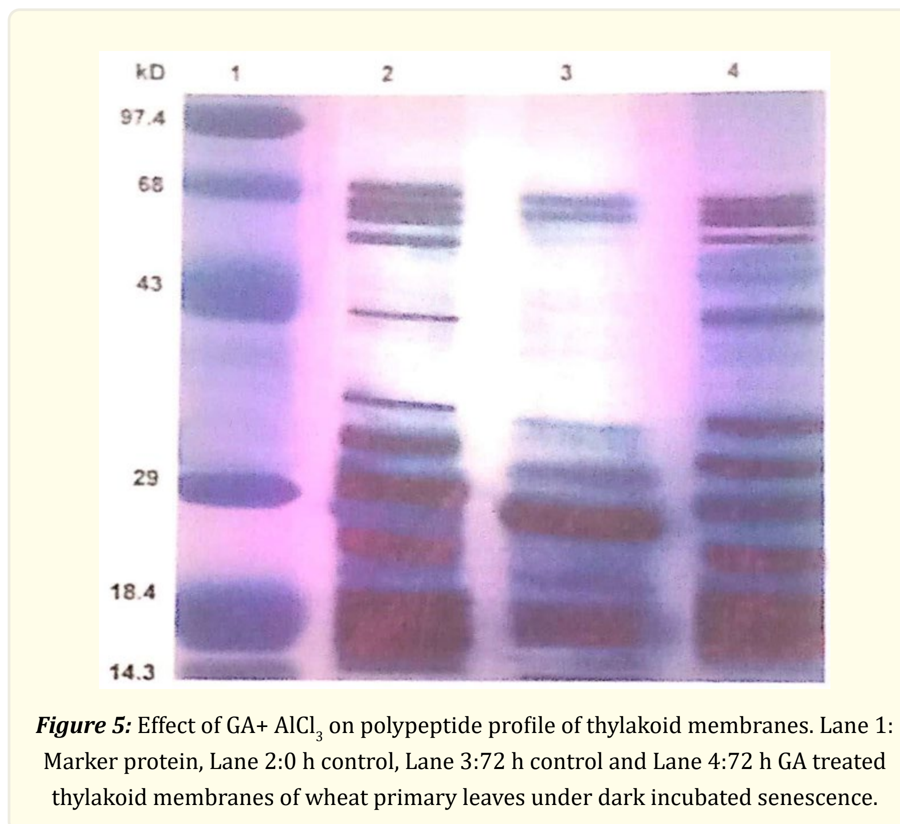

Figure 5: Effect of GA+ $AlCl_3$ on polypeptide profile of thylakoid membranes. Lane 1: Marker protein, Lane 2:0 h control, Lane 3:72 h control and Lane 4:72 h GA treated thylakoid membranes of wheat primary leaves under dark incubated senescence.

Conclusion

In conclusion it was found that the GA+ $AlCl_3$ reduced the loss of pigments, proteins, electron transport activities, spectral properties. The restoration of whole chain electron transport activity by GA+ $AlCl_3$ was closely associated with the restoration of PS II activity compared to that of PS I. GA+ $AlCl_3$ treated leaf thylakoid membranes showed an increase in absorption at 680 nm moderate increase at 480 nm and 440 nm at 72 h during dark incubation. GA+ $AlCl_3$ protected the degradation of water oxidation complex polypeptides (33, 23, 17 KDa) of PS II and slightly protected the 68 kDa of PS I polypeptides. In combination GA+ $AlCl_3$ restored the photochemical activities and stabilized the thylakoid membranes during dark incubated senescence.

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