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Biochemical Characterization on Photosynthetic Activities during Dark Incubated Senescence of Wheat Primary Leaves by Gibberellic Acid

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Authors' contributions

This work was carried out in collaboration between both authors. Author GF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SDSM managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

In annual crop plants like maize, rice and wheat etc. Senescence limits crop yields of annual crops like maize rice and wheat. Delayed leaf senescence is a desirable agronomic trait to improve crop yield. In this study 10 µM GA reduced the loss of wheat primary leaves under incubated dark conditions. GA reduced the loss of pigments, proteins, electron transport activities, spectral properties. The restoration of WCE activity by GA was closely associated with the restoration of PS II activity compared to that of PS I. GA 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 protected the degradation of water oxidation complex polypeptides (33, 23, 17 KDa) of PS II and slightly protected the PS I polypeptides.

Keywords: GA; PS II activity; electron transport chain; polypeptide analysis; senescence.

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1. INTRODUCTION

Senescence in plants is a complex, highly regulated process that involves a decline in photosynthesis, dismantling of chloroplasts, degradation of macromolecules such as proteins, nucleic acids and lipids, loss of chlorophyll [1]. Leaf senescence is a key developmental state in plants that leads to massive mobilization and export of nitrogen and minerals to developing seeds to prepare for the next generation which can be influenced by several phytohormones. Auxin act as a senescence retarding compound due to increased indole acetic acid levels in S_2 phase [2]. Over the last few years, the study on delaying of leaf senescence has been carried out Widely with high-interest interest but the studies related to photosynthetic activities are scanty. Hence biochemical characterization of these has helped to elucidate some of the processes that are occurring.

2. 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 8^{th}-day leaf segments (4-5 cm long) were cut from the apical region and used for treatment. To study the effect of phytohormone 10 µM Gibberellic acid **(**GA) was used. Leaf segments in test solutions were kept in dark at 25ºC for 24-96 h. During the period of treatment, the test solutions were regularly replaced every 24 h with fresh ones.

2.1 Estimation of Chlorophyll and Protein Content

The total Chl and protein content was measured using the method of Arnon [3]. The protein content in the leaf segment was determined using Lowry et al. [4] method.

2.2 Electron Transport and Spectral Activities

Thylakoid membranes were isolated according to the procedure similar to that of Saha and Good [5] as described in Swamy et al. [6].

The Whole chain electron transport activity (WCE) was measured as O_2 consumption 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_2 evolution 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.

2.3 Polypeptide Analysis

Polypeptide analysis of thylakoid membranes was made according to Laemmli [7] using SDS-PAGE mini gel apparatus.

2.4 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 $p< 0.01$.

3. RESULTS AND DISCUSSION

3.1 Chlorophyll and Protein Content

Total Chl steadily declined to 31% in control leafs segments at 96 h dark incubation. GA significantly reduced this loss to 33% at 96 h ($p< 0.01$). Total protein content steadily decreased to 34% in control leafs segments at 96 h during dark incubation (Fig. 1). GA significantly reduced this loss to 54 % at 96 h ($p< 0.01$). Chl is essential to the vital process of photosynthesis in green plants. A biochemical relation between leaf senescence and GA was first reported by Fletcher and Osborne [8] showing that GA retarded senescence of *Taraxacum officinale* by

maintaining Chl levels. The progressive loss in protein content throughout dark incubation is probably due to higher activity of proteases which might be degrading cellular proteins during senescence.

3.2 Electron Transport Activities

The WCE activity was measured 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). GA significantly reduced the WCE loss to 49% at 72 h ($p< 0.01$). *p*-BQ supported control PS II activity decreased to 38% during dark incubation at 96 h and this loss was significantly reduced to 45% by GA at 96 h (Table 1) ($p< 0.01$). PS I activity slightly decreased to 78% in control thylakoid membranes at 96 h, GA significantly reduced this loss to 82% at 96 h (Table 1) ($p< 0.01$). The susceptibility of PS II may be due to alteration in the oxidizing side / or reducing side of PS II [9].

3.3 Spectral Activities

Absorption spectra of 0 h 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 72 h drastic suppression of peaks took place in control thylakoid membranes (Fig. 2). GA treated leaf segments showed an increase in absorption at 680, a moderate increase at 480 and 440 nm at 72 h control during dark incubation. 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 the presence and absence of DCMU [10].

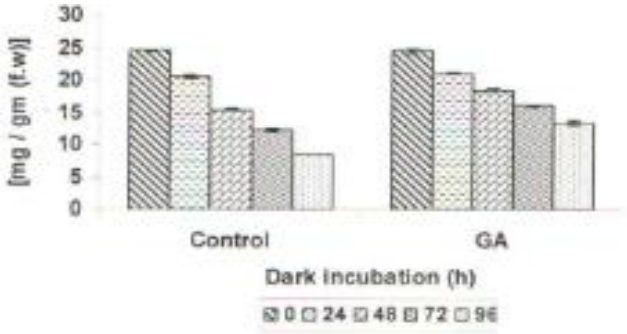

Fig. 1. Effect of 10 µM GA on protein content in wheat primary leaf segments under dark-induced senescence. Each value is mean± SE of five replications

Table 1. Effect of 10 µM GA 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

Photosynthetic activity	Treatment	Incubation time (h)				
		0	24	36	72	96
WCE	Control	115±2 (100)	91±2 (79)	73±3 (63)	48±3 (42)	-
	GA	115±2 (100)	94±5 (81)	76±4 (66)	56±2 (49)	-
PS II	Control	190±4 (100)	170±11 (89)	151±4 (79)	91±4 (48)	73±8 (38)
	GA	190±4 (100)	174±10 (92)	157±11 (83)	109±9 (57)	86±9 (45)
PS I	Control	480±10 (100)	447±16 (93)	430±11 (90)	399±12 (83)	399±11 (78)
	GA	480±10 (100)	454±8 (95)	442±15 (91)	411±9 (86)	394±10 (82)

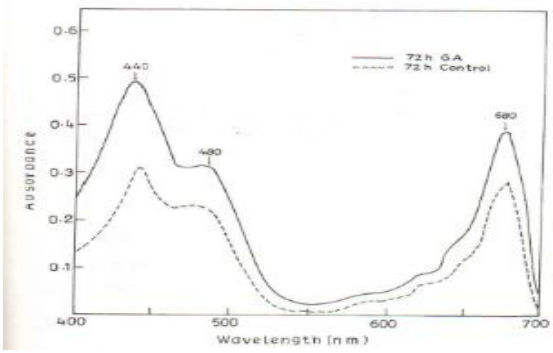

Fig. 2. Effect of 10 µM GA on room temperature absorption spectra of thylakoid membranes in wheat primary leaf segments under dark-induced senescence

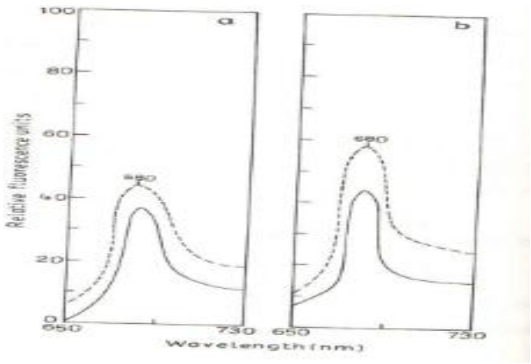

Fig. 3. Effect of 10 µM GA at room temperature Chl *a* fluorescence emission spectra of (a) 72 h control and (b) 72 h GA treated thylakoid membranes in wheat primary leaf segments under dark-induced senescence in the presence and absence (—) of 10 µM DCMU

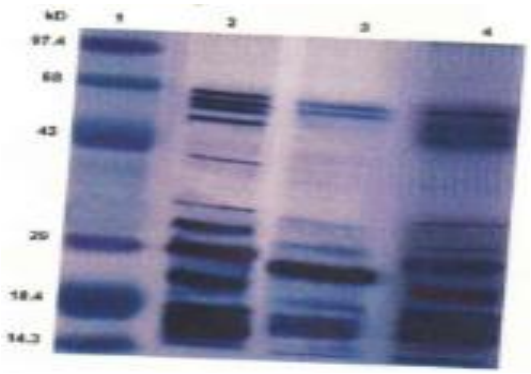

Fig. 4. Effect of 10 µM GA on polypeptide profile of thylakoid membranes. Lane 1: Marker protein, Lane 2:0 h control, Lane 3:72 h control and Lane 4:72 h GA treated thylakoid membranes of wheat primary leaves under dark incubated senescence

Compared to 0 h control, 72 h thylakoid membranes showed the loss in fluorescence emission (Fig. 3). GA treated thylakoid membranes reduced this loss at 72 h. This trend was observed in the presence and absence of DCMU. The ratio of Chl *a* fluorescence emission in the presence and absence of DCMU at 0 h control thylakoid membrane is 1.67 whereas this value is decreased to 1.15 in 72 h control thylakoid membranes during dark incubation. GA reduced this loss in fluorescent emission to 1.31 at 72 h (data not shown).

3.4 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 at 72 h (Fig. 4) above. Polypeptides in the region of 68 KDa indicated the loss in PS I activity. Polypeptides with molecular weight 43, 33, 23 and 17 KDa appeared to be degraded in 72 h dark incubated control leaf thylakoid membrane as the intensity of bands in this region decreased. They were slightly protected by GA at 72 h of dark incubation (Lane 4). Since GA caused retention of protein in the thylakoid membrane, they can be seen as increased intensity of bands in Lane-4 compared to 72 h during dark incubation (Lane 3).

4. CONCLUSION

In conclusion it was found that the gibberellic acid reduced the loss of pigment concentration, proteins, electron transport activities, spectral properties. The restoration of whole chain electron transport activity by gibberellic acid was closely associated with the restoration of PS II activity compared to that of PS I. Gibberellic acid 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 protected the degradation of water oxidation complex polypeptides (33, 23, 17 KDa) of PS II and slightly protected the PS I polypeptides.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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