Journal of Advances in Science and Technology

Vol. III, No. VI, August-2012, ISSN 2230-9659

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*Journal of Advances in Science and Technology*
*Vol. III, No. VI, August-2012, ISSN 2230-9659*

# Effect of Gibberellic Acid in Delay Senescence and Protecting the Dark Incubation Mediated Changes in Primary Processes of Photosynthesis in Rice (Oryza Sativa) Leaves

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***Abstract:*** ***Gibberellic Acid (GA) at 15 µM concentration retarded the loss of pigments, proteins, electron transport activities under dark incubation at 25ºC for 24-96 hr. The restoration of whole chain electron transport (WCE) activity by GA was closely associated with the restoration of photosystem II (PS II) activity compared to that of photosystem (PS I).***

***Key words: Gibberellic Acid (GA), pigments, proteins, Photosystem.***

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## INTRODUCTION:

Leaf senescence is a key developmental state in the life of plants that leads to a massive mobilization and export of nitrogen and minerals to younger tissues to prepare for the next generation and/or to allow plant survive under adverse environmental conditions (Buchanan *et al.,* 1997). Leaf senescence serves to remobilize nutrients (especially nitrogen) from the oldest leaves to the youngest ones (Diamantoglou and Kull, 1988). The most remarkable event in leaf senescence is the disassembly of the photosynthetic apparatus within chloroplasts and thus leads to the drop off in photosynthetic activity (Grover and Mohanty, 1992). The process of senescence brings about both qualitative and quantitative changes in the composition of pigments, macromolecules, structure and organization of thylakoids, damage of electron transport system (Biswal, 1997). Senescence has been shown to modify the structural components of thylakoid complexes like PS II, PS I, Cyt b/f and ATP synthase and cause their quantitative loss (Matile, 1992; Grover, 1993).

The senescence programmed is the final developmental phase of a leaf which can be influenced by several phytohormones. GA inhibits *in vitro* senescence of oat leaf segments (Tetley and Thimann, 1974) and *Rumex* leaf segments respectively (Goldthwaite and Laetsch, 1968; Whyte and Luckwill, 1966). Mutations in the F-box protein (SLY1), result in a block of GA-responsive genes (Dill *et al.,* 2004), delay senescence when crossed to *abi1* (Richards *et al.,* 2001). Studies related to the photosynthesis in the delay of senescence by GA are scanty. Hence a study on the effect of GA on pigments, proteins and 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 region and used for treatment. Treatment of leaf segments for 96 h in dark at 25 $^0$C was given with 15µM GA. Studies were made on senescence induced alterations in pigments, proteins and photochemical activities by keeping the leaf segments in double distilled water in dark as control for 24-96hrs.

**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 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 GA on pigments and proteins

Total Chl was steadily declined to 30 % in control leaf segments at 96 h during dark incubation (Fig. 26). GA significantly reduced this loss to 32 % at 96 h ($p< 0.01$). Chl *a* was decreased to 27 % at 96 h during dark incubation (Fig. 27). GA significantly reduced this loss to 29 % at 96 h ($p< 0.001$). The Chl *b* degraded to 42 % at 96 h during dark incubation (Fig. 28). GA significantly reduced this loss to 51 % at 96 h ($p< 0.01$). Carotenoid content was steadily declined to 48 % whereas GA significantly reduced this loss to 53 % at 96 h (Fig. 29) ($p< 0.05$). The total protein content was decreased to 35 % in control leaf segments at 96 h during dark incubation (Fig. 30). GA significantly reduced this loss to 55 % at 96 h ($p< 0.01$).

### Effect of GA on photosynthetic activities

The WCE activity has been measured using MV as an electron acceptor. In control thylakoid membranes WCE decreased to 42 % at 72 h, while the activity was not found at 96 h during dark incubation (Table 1). GA significantly reduced the WCE activity loss to 50 % 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 was slightly decreased to 78 % in control thylakoid membranes at 96 h, GA significantly reduced this loss to 82 % (Table 1) ($p< 0.01$).

## DISCUSSION:

This experiment deals with the effect of GA in delaying dark incubated senescence. Chl is essential to the vital process of photosynthesis in green plants. Total Chl was steadily declined in control leaf segments at 96 h during dark incubation (Fig. 1). GA slightly reduced this loss at 96 h. Chl *a* degraded more than Chl *b* during dark incubation (Fig. 2 & 3). GA slightly reduced this loss in Chl *a* and Chl *b* at 96 h. Chls are needed for the use of light energy in photosynthesis. A biochemical relation between leaf senescence and GA was first reported by Fletcher and Osborne (1965) showing that GA retarded senescence of excised leaf tissue from *Taraxacum officinale* by maintaining Chl levels. According to Richmond and Lang (1957) application of cytokinin could retard leaf senescence by preventing the Chl breakdown. In barley primary leaves the number of chloroplasts per mesophyll cells was found to be particularly unchanged during the period of degradation of Chl and chloroplast proteins (Martinoia *et al.,* 1983). Corresponding findings have been reported for senescent *Festuca* and Rice leaves (Camp *et al.,* 1982; Thomas, 1983; Wardley *et al.,* 1984). According to Matile *et al.,* (1988)

chloroplast constituents including the thylakoid pigments are within the senescent plastids and these intermediary products of Chl breakdown might be expected to accumulate in the chloroplasts. Treatment with cytokinin in permanent darkness resulted in low contents of pink pigments representing intermediary products of natural Chl breakdown (Matile *et al.,* 1987).

Carotenoid content was steadily declined during dark incubation whereas GA slightly reduced the loss in carotenoid content at 96 h (Fig. 4). During senescence, neoxanthin and β-carotene decreased concomitantly with Chl, whereas lutein and xanthophylls cycle pigments were less affected, leading to increase in lutein/ Chl and xanthophylls cycle pigments/ Chl ratios (Lu *et al.,* 2001). The total protein content was decreased in control leaf segments during dark incubation whereas GA reduced this loss at 96 h (Fig. 5). The progressive loss in protein content throughout dark incubation is probably due to higher activity of proteases which might degrade cellular proteins during senescence. However, the protein degradation was retarded by GA which was seen as maintenance of protein content in GA treated leaf segments. The degradation of Chl and proteins in senescent leaves are biochemical expressions of chloroplasts into gerontoplasts (Matile *et al.,* 1988).

WCE decreased at 72 h, while the activity was not found at 96 h during dark incubation (Table 1). GA reduced the WCE activity loss at 72 h. *p*-BQ supported control PS II activity decreased at 96 h during dark incubation and this loss was reduced by GA at 96 h (Table 1). PS I activity was slightly decreased in control leaf segments at 96 h during dark incubation and GA reduced the loss in PS I activity at 96 h. (Table 1). The susceptibility of PS II may be due to alterations in oxidizing side (Choudhry and Imaseki, 1990; Swamy *et al.,* 1995) or reducing side of PS II (Prakash *et al.,* 1998, 1999).

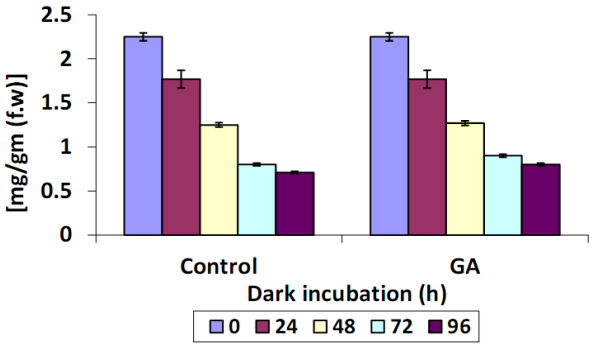


**Fig. 1: Effect of 15 µM GA on total Chl content in Rice primary leaf segments under dark induced senescence. Each value is mean ± SE of five replications.**

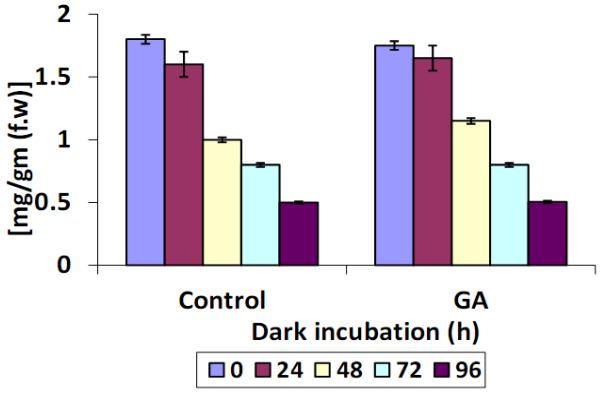


**Fig. 2: Effect of 15 µM GA on Chl *a* content in Rice primary leaf segments under dark induced senescence. Each value is mean ± SE of five replications.**

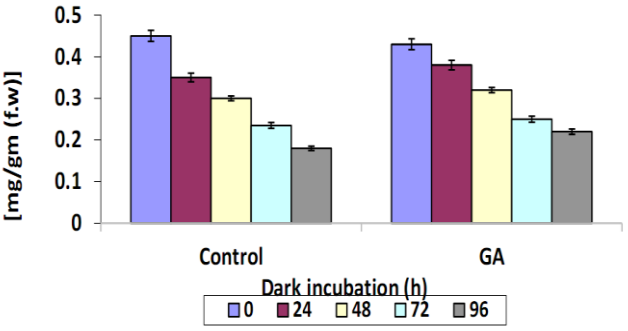


**Fig. 3: Effect of 15 µM GA on Chl *b* content in Rice primary leaf segments under dark induced senescence. Each value is mean ± SE of five replications.**

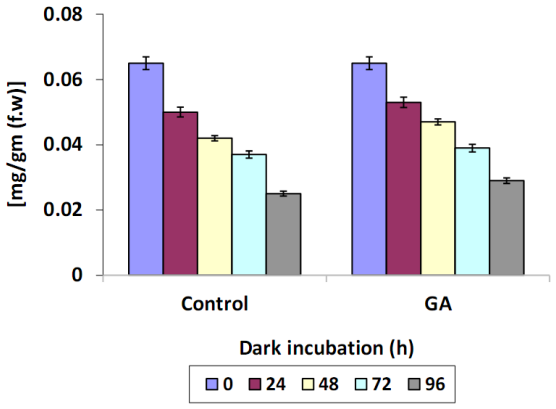


**Fig. 4: Effect of 15 µM GA on carotenoid content in Rice primary leaf segments under dark induced senescence. Each value is mean ± SE of five replications.**

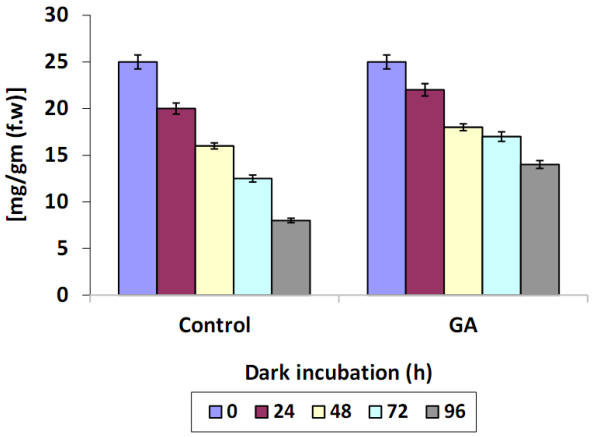


**Fig. 5: Effect of 15 µM GA on protein content in Rice primary leaf segments under dark induced senescence. Each value is mean ± SE of five replications.**

**Table 1: Effect of 15 µM GA 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 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) | 48 ± 3(42) | - |
| | GA | | 94± 5(81) | 76 ± 4(66) | 51± 3(50) | - |
| PSII | Control | 190 ± 4(100) | 170 ± 11(89) | 151± 4(79) | 91 ± 4(48) | 73 ± 8(38) |
| | GA | | 174 ±10(92) | 157 ±11(83) | 109 ± 9(57) | 86 ± 9(45) |
| PS I | Control | 480± 1(100) | 447 ± 16(93) | 430 ±11(90) | 399 ± 12(83) | 375 ±11(78) |
| | GA | | 454 ± 8(95) | 442 ±15(91) | 411 ± 9(86) | 394 ±10(82) |

## CONCLUSION:

GA retarded the loss of pigments, proteins, electron transport activities. The restoration of WCE activity by GA was closely associated with the restoration of PS II activity compared to that of PS I.

## REFERENCES:

Buchanan-Wollaston, V. and Ainsworth, C. (1997). Leaf senescence in *Brassica napus*: Cloning of senescence related genes by subtractive hybridization. *Plant Mol. Biol*. **33**: 821-834.

Diamantoglou, S. and Kull, U. (1988). Seasonal variations of nitrogen components in Mediterranean evergreen *Sclerophyllous* leaves. *Flora.* **180:** 377-390.

Grover, A. and Mohanty, P. (1992). Leaf senescence induced alterations in structure and function of higher plant chloroplasts. In: Y. P. Abrol, P. Mohanty and Govindjee, (eds.), Photosyhthesis: Photoreactions to plant productivity, pp. 225-255. Kluwer Academic Publishers, The Netherlands.

Biswal, B. (1997). Chloroplast metabolism during leaf greening and degreening. In: M. Pessarakli, (eds.), Handbook of photosynthesis, pp. 71-81. Marcel Dekker, New York.

Matile, P. (1992). Chloroplast senescence. In: N. R. Baker and H. Thomas, (eds.), Crop photosynthesis: spatial and temporal determinants, pp. 413-440. Elsevier, Amsterdam.

Grover, A. (1993). How do senescing leaves lose photosynthetic activity? *Curr. Sci.* **64:** 226-234.

Tatley, R. M. and Thimann, K. V. (1974). The metabolism of oat leaves during senescence. I. Respiration, carbohydrate metabolism and the action of cytokinins. *Plant Physiol.* **54:** 294-303.

Goldthwaite, J. J. and Laetsch, W. M. (1968). Control of senescence in *Rumex* leaf discs by gibberellic acid. *Plant Physiol.* **43:** 1855-1858.

Whyte, P. and Luckwill, L. C. (1966). A sensitive bioassay for gibberellins based on retardation of leaf senescence in *Rumex obtusifolius. Nature.* **210:** 1360.

Dill, A., Thomas, S. G., Hu, J., Steber, C. M. and Sun, T. (2004). The *Arabidopsis* F-box protein SLEEPY1 targets gibberellin signaling repressors for gibberellin-induced degradation. *Plant Cell.* **16:** 1392-1405.

Richards, D. E., King, K. E., Ait-ali, T. and Harberd, N. P. (2001). How gibberellin regulates plant growth and development: A molecular genetic analysis of gibberellin signalling. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **52:** 67-88.

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.* **24:** 1-15.

MacKinney, G. (1941). Absorption of light by chlorophyll solutions. *J. Biol. Chem.* **140:** 315-322.

Lowry, O. H., Rosebrought, J. H. Farr, A. L. and Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193:** 265-275.

Saha, S. and Good, N. E. (1970). Products of the photophosphorylation reaction. *J. Biol. Chem.* **245:** 5017-5021.

Swamy, P. M., Murthy, S. D. S. and Suguna, P. (1995). Retardation of dark induced invitro alterations in PS II organization of cowpea leaf discs by combination of $Ca^{2+}$ and benzyladenin. *Biol. Plant.* **37:** 457-460.

Subhan, D. and Murthy, S. D. S. (2000). Synergistic effect of $AlCl_3$ and kinetin on chlorophyll and protein contents and photochemical activities in detached wheat primary leaves during dark incubation. *Photosynthetica.* **38:** 211-214.

Barbar, J. and Mills, J. (1976). Control of chlorophyll senescence by the diffuse double layer. *FEBS Lett.* **68:** 288-292.

Malathi, N., Indira, P., Sarethy and Kailash, P. (2002). Effect of aluminium on the growth and physiology of *Acacia nilotica* seedlings. *J. Plant. Biol.* **29:** 29-32.

Fujita, Y. and Murakami, A. (1987). Regulation of electron transport composition in cyanobacterial photosynthetic system: Staichiometry among photosystem I and II complexes and their light harvesting antennae and Cyt $b_6f$ complex. *Plant Cell Physiol.* **28:** 1547-1553.

Biswal, B. and Biswal, U. C. (1999). Leaf senescence: physiology and molecular biology. *Curr. Sci.* **77:** 775-782.

Munne-Bosch, S. Alegre, L. (2000). The xanthophylls cycle is induced by light irrespective of water status in field-grown lavender. *Physiol. Plant.* **108:**147-151.

Srichandan, S. C. Choudhury, N. K. and Biswal, U. (1989). Carotenoid degradation during *invitro* aging of wheat chloroplast. *Photosynthetica.* **23:** 687-690.

Thimann, K.V. (1980). The senescence of leaves In: K. V. Thimann, (eds.), Senescence in Plants. CRC Press, Boca Raton, Florida.

Krieger-Liszkay (2005). Singlet oxygen production in photosynthesis. *J. Exp. Bot.* **56:** 337-346.

Mukherjee, D. (2003). Physiological changes in leaves, flowers and pods during development and senescence in *Cajanus cajan* L. *J. Indian Bot. Soc.* **82:** 78-84.

Penarrubia, L. and Moreno, J. (1995). Senescence in plants and crops. In: M. Pessarkli (eds.), Hand book of plant and crop physiology. Marvel Dekker, Inc, USA.

Huffaker, R. C. (1990). Proteolytic activity during senescence of plants. *New Phytol* **116:** 199-231.

Mc Carty, R. E. (1980). Photosynthetic phosphorylation by chloroplasts of higher plants. In: K. C. Smith, (eds.), Photochemical and Photobiological Reviews, Vol. 5, pp. 1-47, Newyork, London.

Stocking, C. R. and Ongun, K. (1962). The cellular distribution of some metallic elements in leaves. *Amer. J. Bot.* **49**: 284-289.

Preston, C. and Critchley, C. (1985). $Ca^{2+}$ requirement for photosynthetic oxygen evolution of spinach and mangrove photosystem II membrane preparation. *FEBS Lett.* **184**: 318-322.

Keren, N., Ohad, I., Rutherford, A. W., Drepper, F., Krieger-Liszkay, A. (2000). Inhibition of PS II activity by saturating single turnover flasher in calcium depleted and active photosystem II. *Photosynthesis. Res.* **63:** 209-216.