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BRIEF COMMUNICATION

Retardation of dark induced *in vitro* alterations in photosystem 2 organisation of cowpea leaf discs by combination of Ca^{2+} and benzyladenine

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Abstract

Cowpea (*Vigna unguiculata* L.) leaf discs incubated in the dark in solutions with $CaCl_2$ and benzyladenine (BA) maintained higher concentration of chlorophylls and proteins and higher activity of photosystem 2 (PS2) than controls. The effect of $CaCl_2$ and BA in combination was additive.

Key words: calcium chloride, chlorophyll, polyacrylamide gel electrophoresis, protein, *Vigna unguiculata* L.

Leaf photosynthetic activity declines steadily during senescence because of the extensive degradation of both structural and functional components of chloroplasts (Stoddart and Thomas 1982, Biswal and Biswal 1988, Grover and Mohanty 1992, Choudhury *et al.* 1993). Loss of pigments, photochemical activities, inhibition of photophosphorylation and loss in ribulose-1,5-bisphosphate carboxylase/oxygenase activity are the consequential events in senescence (Woolhouse 1983). PS2 is more sensitive to degrading effects than PS1 (Grover and Mohanty 1992). The loss in PS2 activity during senescence could be either due to the loss of cofactors responsible for O_2 evolution or due to the structural alterations in PS2 organization in thylakoid membrane (Biswal and Biswal 1988). In leaf discs of maize and *Rumex* addition of Ca^{2+}, gibberellic acid or BA retarded the senescence during *in vitro* dark incubation (Poovaiah and Leopold 1973). However, the knowledge on combined action of Ca^{2+} and BA on the components of PS2 electron transport *in vitro* is scanty. Therefore we studied separately and in combination the influence of Ca^{2+} and BA on

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Abbreviations: BA - benzyladenine; Chl - chlorophyll; DCPIP - 2,6-dichlorophenolindophenol; PS - photosystem; SDS-PAGE - polyacrylamide gel electrophoresis after sodium dodecyl sulphate treatment.

photochemical functions and organizational changes in PS2 during the *in vitro* dark incubation of cowpea leaf discs.

Primary leaves from 12 d-old seedlings of cowpea (*Vigna unguiculata* L.) were harvested, washed and blotted dry. 50 leaf discs (10 mm diameter were floated on 50 cm^3 of distilled water or on solutions of 10 mM $CaCl_2$ or 15 μM BA or Ca^{2+} and BA in combination at 25 °C in dark. The samples were collected after 24 to 96 h. Thylakoids were isolated from control and treated leaf discs according to Sabat *et al.* (1986). The leaves were homogenized in 10 mM Hepes buffer (pH 7.5) containing 400 mM saccharose, 2 mM $MgCl_2$ and 5 mM KCl. Oxygen evolving PS2 preparations were made according to Dunhay *et al.* (1984) by using Triton X-100. Chl content was estimated according to Arnon (1949). The assay mixture for measurement of PS2 mediated oxygen evolution activity contained 20 mM Hepes buffer (pH 7.8), 100 mM saccharose, 2 mM $MgCl_2$, 0.1 mM DCPIP, 5 mM KCl and PS2 particles equivalent to 6.7 mg(Chl) cm^{-3}. The polypeptide analysis of O_2 evolving PS2 particles was made using SDS-PAGE (Laemmli 1970). After destaining the gels were scanned at 550 nm in a scanning spectrophotometer (*Shimadzu UV-240*, Japan). The protein estimation was done according to Lowry *et al.* (1951).

Table 1. Influence of $CaCl_2$, benzyladenine (BA) and $CaCl_2$ plus BA on the total chlorophyll content [g kg^{-1}(f.m.)] and total protein content [g kg^{-1}(f.m.)] and photosystem 2, (PS2) ($H_2O \rightarrow$ DCPIP) activity [nmol(O_2) kg^{-1}(Chl) s^{-1}] of cowpea leaf discs during dark incubation. Means ± SD (n = 5). The concentrations of $CaCl_2$ and BA were 10 mM and 15 μM, respectively.

Parameters	Treatment	Incubation time [h] 0	24	48	72	96
Chlorophyll	Control	2.70 ± 0.10	1.84 ± 0.14	1.59 ± 0.08	1.01 ± 0.16	0.81 ± 0.09
	$CaCl_2$		2.05 ± 0.08	2.00 ± 0.10	1.95 ± 0.08	1.85 ± 0.10
	BA		1.92 ± 0.05	1.85 ± 0.05	1.62 ± 0.05	1.42 ± 0.01
	$CaCl_2$ + BA		2.67 ± 0.15	2.52 ± 0.13	2.31 ± 0.07	2.02 ± 0.04
Protein	Control	20.82 ± 0.20	14.60 ± 0.50	12.20 ± 0.40	9.50 ± 0.10	7.40 ± 0.50
	$CaCl_2$		17.50 ± 0.20	16.90 ± 0.50	16.10 ± 0.50	15.80 ± 0.50
	BA		17.30 ± 0.40	16.50 ± 0.30	16.70 ± 0.30	16.60 ± 0.20
	$CaCl_2$ + BA		20.80 ± 0.30	19.50 ± 0.50	18.50 ± 0.20	18.20 ± 0.50
PS2 activity	Control	60.0 ± 3.00	44.00 ± 3.00	21.00 ± 2.00	0	0
	$CaCl_2$		51.00 ± 4.00	37.00 ± 3.00	0	0
	BA		48.00 ± 4.00	37.00 ± 3.00	0	0
	$CaCl_2$ + BA		55.00 ± 5.00	45.00 ± 4.00	0	0

Chl content declined rapidly from 0 to 96 h in leaf discs floated in the dark in distilled water (Table 1). The loss of Chl was retarded partially either in the presence of Ca^{2+} ions or BA. When the two senescence retardants were used in combination, the loss of Chl (25 %) was observed only after 96 h of incubation (Table 1). These results are in agreement with the observation of additive effect of Ca^{2+} and BA in delaying dark Chl degradation in *Rumex* and maize (Poovaiah and Leopold 1973). Similarly a loss of total leaf soluble proteins was observed. The decrease in protein content in the dark was less rapid in all the treatments when compared to control leaf

discs. Only a 20 % loss was observed with the BA + Ca^{2+} treatment at the end of 96 h incubation (Table 1). The loss in protein content during senescence could be due to the increase in proteolytic enzyme activities in chloroplasts (Wittenbach 1978). The decline in PS2 activity was much slower in the PS2 particles isolated from Ca^{2+} + BA treated leaf discs than in the other variants (Table 1). Thus the Ca^{2+} + BA was more effective in protecting Chl and proteins as well as the functional ability of PS2 during dark incubation than the individual application of either Ca^{2+} or BA. Generally, any decrease in PS2 activity could be due to three possible reasons: (*1*) alterations at oxidizing side of PS2, (*2*) structural changes in reaction centre, (*3*) alterations at the reducing side of PS2. According to Roberts *et al.* (1987) differential degradation of thylakoid proteins is responsible for the loss of photochemical functions during senescence. The SDS-PAGE analysis of polypeptides related to PS2 indicates that the 32, 30, 24 and 17 kDa polypeptides are responsible for water oxidation (Ghanotakis and Yocum 1990). Our analysis (Fig. 1)

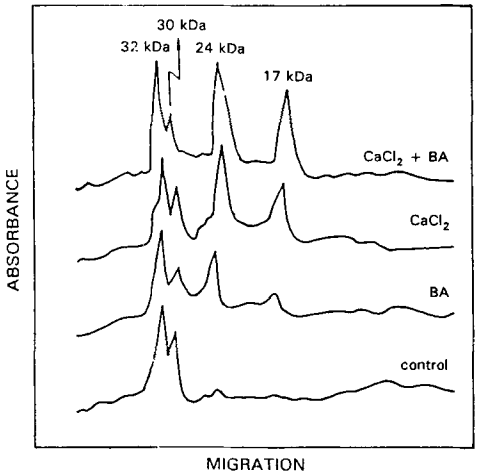

Fig. 1. Scanning polypeptide pattern of PS2 particles isolated from cowpea leaf discs.

showed the presence of 32 and 30 kDa polypeptides in PS2 particles of control leaf discs. BA, $CaCl_2$ and $CaCl_2$ + BA treatments showed the presence of further two polypeptides (24 and 17 kDa): their relative concentration increased in the mentioned sequence. These polypeptides are responsible for water oxidation and their loss during dark incubation of control leaf discs is probably responsible for the loss in PS2 activity.

References

Arnon, D.I.: Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris* L. - Plant Physiol. **24**: 1-15, 1949.

Biswal, U.C., Biswal, B.: Ultrastructural modifications and biochemical changes during senescence of chloroplasts. - Int. Rev. Cytol. **113**: 271-321, 1988.

Choudhury, N.K., Swain, N.K., Raval, M.K., Biswal, U.C.: Stability of chlorophyll, carotenoids and protein of thylakoid membranes during *in vitro* aging of chloroplasts. - Photosynthetica **29**: 631-634, 1993.

Dunahay, T.G., Staehelin, L.A., Seibert, M., Ogilvie, P.D., Berg, S.P.: Structural, biochemical and biophysical characterization of four oxygen-evolving Photosystem II preparations from spinach. - Biochim. biophys. Acta **764**: 179-193, 1984.

Grover, A., Mohanty, P.: Leaf senescence induced alterations in structure and function of higher plant chloroplasts. - In: Abrol, Y.P., Wattal, A., Gnaram, A., Govindjee, Ort, D.R., Teramura, A.H. (ed.): Proceedings of Global Climatic Changes in Plant Productivity. Pp. 227-235. Academic Press, New Delhi 1991.

Laemmli, U.K.: Clevage of structural proteins during the assembly of the head of bacteriophage T_4. - Nature **227**: 680-685, 1970.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with the Folin phenol reagent. - J. biol. Chem. **193**: 265-275, 1951.

Ghanotakis, D.F., Yocum, C.F.: Photosystem II and the oxygen evolving complex. - Annu. Rev. Plant Physiol. **41**: 255-276, 1990.

Poovaiah, B.W., Leopold, A.C.: Defferal of leaf senescence with calcium. - Plant Physiol. **52**: 236-239, 1973.

Roberts, D.R., Thompson, J.E., Dumbroff, E.B., Gepstein, S., Mattoo, A.K.: Differential changes in the synthesis and steady-state levels of thylakoid proteins during bean leaf senescence. - Plant mol. Biol. **9**: 343-353, 1987.

Sabat, S.C., Mohanty, N., Mohanty, P.: Heat induced alteration in electron donation sites of ascorbate and ascorbate reduced catechol in the electron transport chain of *Amaranthus* chloroplasts. - J. Biochem. Biophys. **23**: 266-269, 1986.

Stoddart, J.L., Thomas, H.: Leaf senescence. - In: Boulter, D., Parthier, B. (ed.): Nucleic Acids in Plants I. Structure, Biochemistry, and Physiology of Proteins. Pp. 592-636. Springer-Verlag, Berlin - Heidelberg - New York 1982.

Wittenbach, V.A: Breakdown of ribulose bisphosphate carboxylase and changes in proleolytic activity during dark-induced senescence of wheat seedlings. - Plant Physiol. **62**: 604-608, 1978.

Woolhouse, H.W.: Foliar senescence. - In: Zieberman, M. (ed.): Post-Harvest Physiology and Crop Preservation. Pp. 1-43. Plenum Press, New York 1983.