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Synergistic effect of $AlCl_3$ and kinetin on chlorophyll and protein contents and photochemical activities in detached wheat primary leaves during dark incubation

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Abstract

Al^{3+} in combination with kinetin showed more protection against degradation of chlorophyll (Chl) and protein than Al^{3+} or kinetin alone during dark-induced senescence in wheat primary leaf segments. MV-dependent whole chain electron transport, photosystem (PS) 2 mediated oxygen evolution, and PS1 activities were also delayed to a greater extent. Absorbed excitation energy distribution was more in favour of PS1 in Al^{3+} plus kinetin-treated leaf thylakoids at 72 h.

Additional key words: aluminium; chlorophyll *a* fluorescence; electron transport; leaf senescence; photosystem.

Introduction

Leaf senescence is the final phase of leaf development during which nutrients released upon degradation of macromolecules such as Chl, protein, RNA, *etc.* are recycled to other parts of the plant (Stoddart and Thomas 1982). Among organelles, chloroplasts (from which most of the minerals are transferred to grains during senescence) are most susceptible to senescence induced degradation (Feller and Fischer 1994, Biswal and Biswal 1999). Decreased CO_2 assimilation, inhibition in photophosphorylation, and loss of photochemical activities in chloroplasts are consequential events of leaf senescence (Woolhouse 1983, Šesták 1985, Grover 1993). Therefore, the onset of leaf senescence obviously hampers the crop yield (Frith and Dalling 1980). Hence, delaying leaf senescence may help to improve crop yield (Grover 1993). Several studies reported the efficiency of metal ions in delaying senescence: Co^{2+} (Yu and Yang 1979, Geetachandra *et al.* 1981), Ni^{2+}(Mishra and Samal 1971), Ag^{+} (Aharoni and Lieberman 1979, Aharoni 1985), and Ca^{2+} (Poovaiah and Leopold 1973). Al^{3+}, one of the most abundant elements in the earth, is a plant growth-limiting factor in acid soils (Kochian 1995). Al^{3+} at low concentration stimulates PS2-mediated oxygen evolution in cyanobacteria and isolated chloroplasts (Wavare and Mohanty 1982, Wavare *et al.* 1983). Aluminium also stimulates conductance to CO_2 transfer and net photosynthetic rate (Lidon *et al.* 1998). Metal ions such as Ca^{2+} and Mg^{2+} regulate electron transport, photophosphorylation, and energy distribution between two photosystems of photosynthesis (Papageorgiou 1975, Williams 1977). Studies on the effects of Al^{3+}, particularly in combination with the senescence retardant kinetin, on pigment contents and photochemical activities during senescence are scanty. Therefore, we studied the effect of aluminium, kinetin, and the combination of both on pigment and protein contents and photochemical activities.

Materials and methods

Leaf segments (4-5-cm long) were cut from apical region of 7-d-old wheat primary leaves. Four sets of leaf segments were maintained individually on double-distilled water, 20 μM $AlCl_3$, 5 μM kinetin, and 20 μM $AlCl_3$ plus 5 μM kinetin in such a way that the bases of leaf segments were infiltrated with test solution in test tubes in dark at 25±1 °C for 96 h. Leaf segments were sampled for experiments at 24 h intervals. The content of Chl (*a*+*b*) was determined according to Arnon (1949) and total proteins were estimated using bovine serum albumin as standard following Lowry *et al.* (1951).

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Abbreviations: Al^{3+}, aluminium ion; Asc, ascorbate; Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; DCPIP, 2,6-dichlorophenol indophenol; MV, methyl viologen; p-BQ, *p*-benzoquinone; PS, photosystem.
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Electron transport activities of control and treated leaf thyla-koids were assayed polarographically following Sabat *et al.* (1989). Thylakoid membranes were isolated using a procedure similar to that of Saha and Good (1970) as described in Swamy *et al.* (1995). PS2 reaction was monitored as O_2 evolution in 2 cm^3 of reaction buffer consisting of 100 mM sucrose, 50 mM Hepes-NaOH (pH 7.5), 2 mM $MgCl_2$, 5 mM KCl, 0.5 mM p-BQ, and thylakoid membranes equivalent to 40 μg Chl. For whole chain electron transport, the reaction buffer contained 0.5 mM MV instead of p-BQ, while the PS1 reaction mixture contained 0.5 mM MV, 0.1 mM DCPIP, 5 mM ascorbate, 1 mM sodium azide, 10 μM DCMU, and thylakoid membranes equivalent to 40 μg of Chl in reaction buffer.

Fluorescence emission of thylakoid membranes of 0-h control and 72-h treated leaves were studied both at room temperature (25 °C) and low temperature (77 K). Fluorescence emission of thylakoids was measured in presence and absence of 10 μM DCMU after exciting the samples containing thylakoid membranes equivalent to 20 g m^{-3} of Chl in reaction buffer at 440 nm with a slit width of 5 nm on *Jasco FP777* spectrofluorometer. Fluorescence emission spectra at 77 K were recorded for the samples on *Hitachi MPF4* spectrofluorometer. The samples were excited at 430 nm. The slit widths of excitation and emission monochromators were 8 and 2 nm, respectively. The spectral sensitivity to the instrument was adjusted.

Results and discussion

Loss of Chl and protein contents during leaf senescence is a characteristic feature (Šesták 1985, Grover *et al.* 1986). When the primary leaves were maintained in double-distilled water in dark, a similar loss in both Chl and protein contents (74 % Chl, 64 % protein at 96 h) was observed (Table 1). However, the loss was partially retarded either by Al^{3+}or kinetin. A loss of 49 % Chl and 18 % protein was observed in leaf segments treated with Al^{3+}+kinetin. This showed the synergistic effect of Al^{3+} and kinetin. As this effect might influence photochemical functions of thylakoid membranes of primary leaf segments during dark ageing, activity of whole chain electron transport (PS2+PS1) of thylakoids at 24-h intervals was measured. Whole chain electron transport was decreased to 38 % of 0-h control in 72-h dark incubated control leaf segments, while a 24 % decrease was observed in leaf segments treated with Al^{3+}+kinetin (Table 2). Partial restoration of PS2 activity by Al^{3+} and kinetin to values of 44 and 54 % from 36 % at 96 h was observed (Table 2). A maximum of 76 % of 0-h control PS2 activity was found in Al^{3+}+kinetin-treated leaf segments at 96 h. In case of PS1, only 21 % loss at 96 h was observed in control leaf segments. The loss observed at 96 h was minimised significantly by Al^{3+}+kinetin than either of those alone (Table 2). The loss in photochemical activities is in accordance with studies of Biswal and Mohanty (1976), Sabat *et al.* (1985, 1989), Grover *et al.* (1986), Prakash *et al.* (1998), *etc.* However, the concomitant protection by Al^{3+} and kinetin synergistically could be due to stabilization of thylakoid membranes as suggested for Ca^{2+} and benzyl adenine by Swamy *et al.* (1995).

Table 1. Influence of $AlCl_3$, kinetin, and combination of $AlCl_3$ and kinetin on total chlorophyll [g(Chl) kg^{-1}(f.m.)] and total proteins [g(protein) kg^{-1}(f.m.)] in primary leaf segments during dark incubation. The concentrations of $AlCl_3$ and kinetin were 20 and 5 μM. Means±SD (n = 4). Values in parentheses indicate loss [%].

Parameter	Treatment	Dark incubation [h] 0	24	48	72	96
Chlorophyll	H_2O	2.46±0.12	1.91±0.05 (22)	1.26±0.05 (49)	0.90±0.08 (64)	0.65±0.05 (74)
	$AlCl_3$		2.35±0.08 (5)	1.77±0.08 (28)	1.13±0.09 (54)	0.86±0.02 (65)
	Kinetin		2.48±0.05 (0)	1.89±0.10 (23)	1.54±0.07 (37)	1.18±0.03 (52)
	$AlCl_3$+kinetin		2.64±0.06 (0)	2.03±0.05 (18)	1.70±0.06 (31)	1.26±0.04 (49)
Protein	H_2O	26.61±2.00	24.70±1.50 (7)	17.14±0.80 (35)	14.28±0.4 (46)	9.50±0.55 (64)
	$AlCl_3$		26.60±1.20 (0)	24.76±0.90 (7)	19.78±0.5 (26)	16.70±0.60 (38)
	Kinetin		24.80±2.00 (7)	22.86±1.00 (14)	20.90±0.70 (21)	19.00±0.40 (29)
	$AlCl_3$+kinetin		26.60±1.00 (0)	24.80±0.90 (7)	22.90±0.50 (14)	21.90±0.50 (18)

Since altered characteristics of Chl *a* fluorescence emission by DCMU may alter the primary photochemistry of PS2 under stress (Atal *et al.* 1991, Murthy *et al.* 1995), we determined fluorescence emission of thylakoid membranes at 683 nm in the presence and absence of 10 μM DCMU. DCMU, an inhibitor of electron transport from Q_A to Q_B in PS2, caused enhancement (64 %) of fluorescence emission in 0-h control thylakoids (Table 3). However, this enhancement of fluorescence emission was decreased to 14 % in 72-h control thylakoids. Combined Al^{3+}+kinetin caused retention of enhancement of the fluorescence emission by 10 μM DCMU to 45 % compared to 39 and 32 % by Al^{3+} and

Table 2. Effects of $AlCl_3$, kinetin, and combination of $AlCl_3$ and kinetin on photochemical activities: whole chain electron transport [mmol(O_2 consumed) kg^{-1}(Chl) s^{-1}], photosystem (PS) 2 [mmol(O_2 evolved) kg^{-1}(Chl) s^{-1}], and PS1 [mmol(O_2 consumed) kg^{-1}(Chl) s^{-1}] activities. The concentrations of $AlCl_3$ and kinetin were 20 and 5 μM. Means±SD (n = 4). Values in parentheses indicate loss of photochemical activities [%].

Parameter	Dark incubation [h] Treatment	0	24	48	72	96
WCE	H_2O	29±2	22±2 (24)	18±1 (38)	11±1 (62)	-
	$AlCl_3$		26±2 (10)	19±2 (35)	18±1 (38)	-
	Kinetin		24±2 (17)	22±2 (24)	19±1 (35)	-
	$AlCl_3$+knetin		28±2 (3)	26±1 (10)	22±1 (24)	-
PS2	H_2O	50±3 (0)	45±4 (10)	39±3 (22)	23±2 (54)	18±1 (64)
	$AlCl_3$		50±3 (0)	44±3 (12)	30±3 (40)	22±2 (56)
	Kinetin		49±3 (2)	47±4 (6)	31±4 (38)	27±2 (46)
	$AlCl_3$+kinetin		50±5 (0)	48±2 (4)	46±2 (8)	38±1 (24)
PS1	H_2O	136±8 (0)	127±7 (6)	120±7 (12)	113±5 (17)	107±4 (21)
	$AlCl_3$		125±8 (8)	130±6 (4.5)	118±5 (13)	107±6 (21)
	Kinetin		134±6 (1.5)	130±7 (4.5)	120±4 (12)	111±7 (18)
	$AlCl_3$+kinetin		134±6 (1.5)	133±6 (2)	127±6 (7)	116±5 (15)

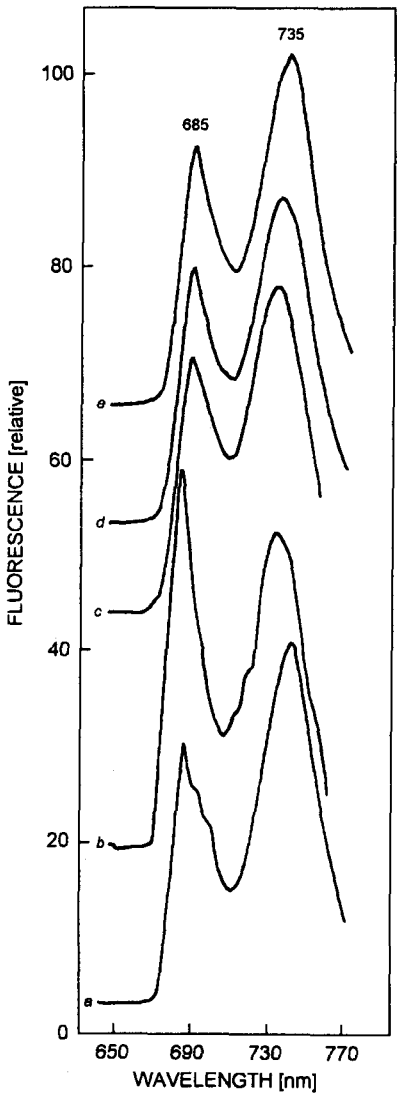

Fig. 1. 77 K emission spectra of thylakoids isolated from 0 h (*a*), 72 h control (*b*), 20 μM $AlCl_3$ (*c*), 5 μM kinetin (*d*), and 20 μM $AlCl_3$ plus 5 μM kinetin (*e*) treated leaf segments.

Table 3. Effects of $AlCl_3$, kinetin, and kinetin plus $AlCl_3$ on photosystem (PS) 2 activity [%] and ratio of F685 with added 10 μM DCMU to F685 without this addition of thylakoids of primary leaves at 0 and 72 h during dark-induced senescence. 50 mmol(O_2 evolved) kg^{-1}(Chl) s^{-1} was considered as 100 % of PS2 activity. SD was less than 10 % of mean values.

Treatment	% of PS2 activity	F685+DCMU /F685-DCMU
0 h control	100	1.645±0.020
72 h control	46	1.142±0.020
$AlCl_3$ (20 μM)	60	1.395±0.040
Kinetin (5 μM)	62	1.320±0.040
$AlCl_3$+kinetin	92	1.453±0.020

kinetin, respectively, suggesting that the combination of Al^{3+}+kinetin influenced the primary reactions of PS2 and stabilised thylakoid organisation during senescence. Assuming that the excitation energy distributed between the two photosystems might also be influenced by Al^{3+} and kinetin, the ratio of relative fluorescence emissions at 685 nm (originating in PS2) and 735 nm (originating in PS1) from 77 KChl fluorescence emission spectra was studied (Fig. 1). Dark ageing caused a decrease in the ratio F735/F685 from 1.43 at 0 h to 0.85 at 72 h, which was arrested possibly by maintaining the ratio (1.4) nearer to 0-h control ratio (1.43) compared to Al^{3+} (1.29) or kinetin (1.29) alone. Hence Al^{3+} in combination with kinetin may protect the excitation energy distribution more in favour of PS1 probably by screening negatively charged thylakoid membranes or by stabilizing thylakoid membrane organisation during dark induced

senescence (Murata 1969, Poovaiah and Leopold 1973, Barber 1982, Swamy *et al.* 1995).

In conclusion, kinetin in combination with Al^{3+} delayed the loss of pigment and protein contents and the activities of whole chain electron transport, PS2, and PS1 synergistically. These, in a synergistic manner, caused absorbed excitation energy distribution more in favour of PS1.

References

Aharoni, N.: Effect of silver ions and ethylene on auxin metabolism and auxin-induced ethylene production in tobacco leaf discs. - Physiol. Plant. **63**: 438-444, 1985.

Aharoni, N., Lieberman, M.: Ethylene as a regulator of senescence in tobacco leaf discs. - Plant Physiol. **64**: 801-804, 1979.

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

Atal, N., Pardha Saradhi, P., Mohanty, P.: Inhibition of the chloroplast photochemical reactions by treatment of wheat seedlings with low concentrations of cadmium: Analysis of electron transport activities and changes in fluorescence yield. - Plant Cell Physiol. **32**: 943-951, 1991.

Barber, J.: Influence of surface charges on thylakoid structure and function. - Annu. Rev. Plant Physiol. **33**: 261-295, 1982.

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

Biswal, U.C., Mohanty, P.: Aging induced changes in photosynthetic electron transport of detached barley leaves. - Plant Cell Physiol. **17**: 323-331, 1976.

Feller, U., Fischer, A.: Nitrogen metabolism in senescing leaves. - Crit. Rev. Plant Sci. **13**: 241-273, 1994.

Frith, G.J.T., Dalling, M.J.: The role of peptide hydrolases in leaf senescence. – In: Thimann, K.V. (ed.): Senescence in Plants. Pp. 117-130. CRC Press, Boca Raton 1980.

Geetachandra, Reddy, K.S., Mohan Ram, H.Y.: Extension of vase-life cut marigold and *Chrysanthemum* flower by the use of cobalt chloride. – Indian J. exp. Biol. **19**: 150-154, 1981.

Grover, A.: How do senescing leaves lose photosynthetic activity? - Curr. Sci. **64**: 226-231, 1993.

Grover, A., Sabat, S.C., Mohanty, P.: Effect of temperature on photosynthetic activities of senescing detached wheat leaves. - Plant Cell Physiol. **27**: 117-126, 1986.

Kochian, L.V.: Cellular mechanisms of aluminium toxicity and resistance in plants. - Annu. Rev. Plant Physiol. Plant mol. Biol. **46**: 237-260, 1995.

Lidon, F.C., Ramalho, J.C., Barreiro, M.G.: Aluminium toxicity modulates nitrate to ammonia reduction. – Photosynthetica **35**: 213-222, 1998.

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.

Mishra, D., Samal, B.: Interaction of benzimadazole and nickel in delaying senescence of detached rice leaves. - Z. Naturforsch. **26c**: 1377, 1971.

Murata, N.: Control of excitation transfer in photosynthesis. II. Magnesium ion-dependent distribution of excitation energy between two pigment systems in spinach chloroplasts. - Biochim. biophys. Acta **189**: 171-181, 1969.

Murthy, S.D.S., Mohanty, N., Mohanty, P.: Prolonged incubation with low concentrations of mercury alters energy transfer and chlorophyll (Chl) *a* protein complexes in *Synechococcus* 6301: changes in chl *a* absorption and emission characteristics and loss of the F695 emission band. - Biometals **8**: 237-242, 1995.

Papageorgiou, G.: Chlorophyll fluorescence: an intrinsic probe of photosynthesis. – In: Govindjee (ed.): Bioenergetics of Photosynthesis. Pp. 319-371. Academic Press, New York – San Francisco – London 1975.

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

Prakash, J.S.S., Baig, M.A., Mohanty, P.: Alterations in electron transport characteristics during senescence of *Cucumis* cotyledonary leaves. Analysis of the effects of inhibitors. - Photosynthetica **35**: 345-352, 1998.

Sabat, S.C., Grover, A., Mohanty, P.: Senescence induced alteration in the electron transport in wheat leaf chloroplasts. - J. Photochem. Photobiol. **B 3**: 175-183, 1989.

Sabat, S.C., Grover, A., Mohanty, P.: Alteration in characteristics of photosystem II and photosystem I catalysed electron transport in chloroplast isolated from senescing detached beet-spinach (*Beta vulgaris* L.) leaves. - Indian J. exp. Biol. **23**: 711-714, 1985.

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

Šesták, Z. (ed.): Photosynthesis During Leaf Development. – Dr W. Junk Publ., Dordrecht – Boston – Lancaster; Academia, Praha 1985.

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

Swamy, P.M., Murthy, S.D.S., Suguna, P.: Retardation of dark induced *in vitro* alterations in photosystem 2 organisation of cowpea leaf discs by combination of Ca^{2+} and benzyladenine. - Biol. Plant. **37**: 457-460, 1995.

Wavare, R.A., Mohanty, P.: Aluminium stimulation of photoelectron transport in spheroplasts of cyanobacterium *Synechococcus cedrorum*. - Photobiochem. Photobiophys. **3**: 327-335, 1982.

Wavare, R.A., Subbalakshmi, B., Mohanty, P.: Effect of Al^{3+} on electron transport catalysed by photosystem I&II of photosynthesis in cyanobacterium *Synechococcus* spheroplasts & beet-spinach chloroplasts. - Indian J. Biochem. Biophys. **20**: 301-303, 1983.

Williams, W.P.: The two photosystems and their interactions. – In: Barber, J. (ed.): Primary Processes of Photosynthesis. Pp. 99-146. Elsevier, Amsterdam – New York – Oxford 1977.

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

Yu, Y.B., Yang, S.F.: Auxin-induced ethylene production and its inhibition by aminoethoxyvinylglycine and cobalt ion. - Plant Physiol. **64**: 1074-1077, 1979.