

Effects of aluminium on pigments and pigment-protein complexes of soybean

D.B. MILIVOJEVIĆ*, D.Đ. STOJANOVIĆ* and S.D. DRINIĆ**

Institute for the Application of Nuclear Energy, University of Belgrade-Zemun, and Maize Research Institute "Zemun Polje"**, YU-11080 Belgrade-Zemun, Yugoslavia*

Abstract

The effect of aluminium (0.5 - 1.0 mM) on contents of phosphorus, pigments, and pigment-protein complexes was studied in soybean (*Glycine max* Merril.) grown in different nutrient medium with and without P. Increased Al concentrations led to the decrease in the contents of chlorophylls (Chl) *a* and *b*, and carotenoids (Car) in soybean leaves, but Chl *a/b* ratio did not vary significantly. In long-term experiments, P ameliorates the negative effects of Al.

Additional key words: carotenoids, chlorophylls, *Glycine max*, phosphorus nutrition, polypeptides, SDS-PAGE.

Al-toxicity limits growth of many plant species grown on acid soils. As Al is a soil mineral component, its toxicity is theoretically possible in most or nearly all types of soils when soil pH is decreased to such a value that Al solubility begins to rise. Root growth inhibition followed by disturbance in development of other plant parts is the most often observed adverse effect of Al ions. These effects are studied more on roots (for review see Kochian 1995) than on shoots (Lorenc-Plucińska *et al.* 1996, Lidon *et al.* 1998). Al reduces P transport from root into shoot and thereby causes incidence of P-deficiency symptoms in shoots. Therefore, the aim of this paper was to determine the effect of Al (0.5 and 1.0 mM) in different nutrient media with or without phosphorus on contents of P in soybean organs, and on pigments, and pigment-protein complexes in chloroplasts.

After 5-d germination in darkness, plants of soybean (*Glycine max* Merril. cv. ZP S 015) were transferred into pots with nutrient medium of pH = 5 (Hogland and Arnon 1950). Plants were grown in growth chambers at a 12-h photoperiod, irradiance of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (*Sylvania* cool white lamp P9GT12-CEW-VHO, USA) and temperature of 24/20 °C. Plants grown on the complete

medium were the control ones. The P content in the second variant was half of that in the control, while the third variant had no P. Plants with and without P were exposed to AlCl_3 (0.5, 0.7, and 1.0 mM) for 12 d. The medium was occasionally shaken to prevent Al precipitation. The solution was added or replaced every second day, while pH of solution was controlled each day. Pigment contents were determined and calculated according to Lichtenthaler (1987). The method of Delepeilair and Chua (1979) was used for SDS-PAGE electrophoresis of thylakoid polypeptides. The gels of proteins were scanned densitometrically by the Ultra Scan Laser Densitometer LKB 2202 (Uppsala, Sweden). Light-harvesting complex (LHC) proteins were reported as a sum of 25 - 30 kDa proteins, photosystem 1 (PS1) as 110 and 66 kDa proteins, and photosystem 2 (PS2) as 50 - 45 kDa proteins, and as a percent of total proteins. P amount was determined upon tissue drying and destroying in the mixture of concentrated nitric and perchloric acids by A.O.A.C. method (Horwitz 1960). The analysis of variance (ANOVA) for all variables was carried out by the MStatic programme.

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Abbreviations: Al - aluminium; Car - carotenoids; Chl - chlorophyll; P - phosphorus; SDS-PAGE - sodium dodecyl sulphate polyacrylamide gel electrophoresis; PS1, PS2 - photosystems 1 and 2.

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The applied concentrations of Al did not decrease the P content in roots with complete P nutrition (0.10 M KH_2PO_4), contrary to plants grown on 0.05 M KH_2PO_4 . The amount of P in stem and leaves decreased under increased Al concentrations (0.7 to 1.0 mM) (Table 1). This confirms interaction between Al and P (by forming AlPO_4) occurring during uptake and transport of P from the root to the shoot. The content of P in organs of P-deficient plants was significantly lower (by 60 %) than in control plants. In P-deficient plants, the amount of total P did not significantly vary under Al treatments in all

organs, except in cotyledons because under P deficiency plants could use P stored in cotyledons.

The contents of Chl and Car in leaves were lowered at high Al concentrations (0.7 - 1.0 mM) in all variants. This decrease was even higher at P-deficiency. The Chl *a/b* ratio did not significantly vary, while Chl *a+b/Car* ratios was higher at higher Al concentrations regardless the presence of P. Decreased amounts of P in the medium as well as complete P-deficiency led to increase in Al-toxicity (Table 2).

Table 1. Amount of phosphorus [$\text{g}(\text{P}_2\text{O}_5) \text{ kg}^{-1}(\text{d.m.})$] in organs of 17-d old soybean plants grown under different Al concentrations (0 to 1 mM) in phosphate-sufficient (+P), half of control phosphate (1/2P), and phosphate-deficient (-P) nutrient solution. Values are means of 3 determinations from 2 - 3 plants.

Al [mM]	Leaf			Stem			Cotyledon			Root		
	+P	1/2P	-P	+P	1/2P	-P	+P	1/2P	-P	+P	1/2P	-P
0.0	2052.0	1393.0	818.5	1859.0	716.2	716.1	1129.0	720.8	829.2	1949.0	1286.0	870.3
0.5	1972.0	1236.0	787.1	1851.0	668.9	658.9	1093.0	716.2	716.1	2001.0	812.0	811.7
0.7	1383.0	1113.0	742.0	1572.0	628.2	628.2	1093.0	779.6	532.9	1820.0	820.9	791.7
1.0	1416.0	897	720.2	1450.0	402.5	402.5	1042.0	716.2	342.8	1866.0	578.7	749.9
LSD _{0.05}	332.6		268.7		264.2				286.1			
LSD _{0.01}	469.4		379.2		372.9				403.1			

Table 2. Changes in pigment concentrations [$\text{g kg}^{-1}(\text{f.m.})$], their ratios, and relative amounts [%] of polypeptides of PS1 (66 kDa), PS2 (51 and 46 kDa) and LHC2 (27 - 31 kDa) of soybean chloroplasts grown in presence or deficiency of P and under different concentrations of Al. Values are means ($n = 6$), LSD_{0.05}/LSD_{0.01} in parentheses.

P Al [mM]	+P				1/2P				-P			
	0.0	0.5	0.7	1.0	0.0	0.5	0.7	1.0	0.0	0.5	0.7	1.0
Chl <i>a+b</i> (0.29/0.41)	2.01	2.20	1.93	1.48	1.89	1.79	1.52	1.44	1.48	1.46	1.14	0.82
Chl <i>a/b</i> (0.31/0.44)	2.10	2.00	2.10	1.90	2.20	2.00	1.90	2.30	1.90	1.80	1.90	2.00
Car (0.03/0.05)	0.20	0.16	0.10	0.06	0.11	0.09	0.07	0.06	0.06	0.04	0.03	0.02
Chl/Car (10.5/12.8)	10.10	14.10	18.40	22.80	16.70	20.80	23.00	23.20	25.50	32.40	34.50	41.00
PS1 (2.59/3.65)	11.60	10.80	11.50	11.60	11.10	10.60	11.40	10.80	13.10	12.80	14.50	15.60
PS2 (2.51/3.82)	8.60	8.50	6.10	8.30	8.50	6.80	7.60	8.10	6.70	6.40	6.60	7.80
LHC2 (2.74/3.86)	17.00	18.10	18.40	18.20	17.70	18.50	17.70	18.00	18.50	12.60	11.40	10.00
(LHC2+PS2)/PS1	2.20	2.50	2.10	2.30	2.40	2.40	2.20	2.40	1.90	1.50	1.20	1.10
(0.47/0.66)												

Chl *a/b* ratio was not correlated with the content of LHC2 proteins. Plants grown on medium with P and Al contained more LHC2 proteins than plants grown on the medium with Al but without P, while the Chl *a/b* ratio did not change. The lowered content of LHC2 proteins was probably related to lowered Car content. (LHC2+PS2)/PS1 ratio indicated modulation of size of Chl-protein complexes within PS2 units. These units became smaller in P-deficient plants under Al treatments

(Table 2). These changes were similar as those known in sun adapted plants where effective photoprotection can be provided by regulation of PS2 antennae size and by the number of reaction centres (Anderson *et al.* 1988). In addition, sun-adapted plants have a large pool of the xanthophyll cycle pigments (Demmig-Adams and Adams 1996). Modification in chloroplast ultrastructure and inhibition of electron transport after short-term effects of Al ions was observed by Moustakas *et al.* (1996, 1997).

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