BRIEF COMMUNICATION

Effects of aluminium on photosynthetic performance in Al-sensitive and Al-tolerant maize inbred lines

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Abstract

Maize plant inbred lines, one Al-sensitive (B-73) and two Al-tolerant (F-2 and L-2039), were grown hydroponically in the presence of 200 μM Al. After 13 d of growth, root and shoot lengths, photosystem 2 (PS2) activity, chlorophyll (Chl) content, 5-aminolevulinic acid (5-ALA) synthesis rate, chlorophyllase (Chlase) activity, and N, Mg, Fe, and Mn contents in leaves were determined. PS2 activity and Chl content were most severely affected by Al in B-73, but F-2 was almost unaffected. This was in accordance with Al-accumulation in the plants. The observed changes in B-73 coincided with 5-ALA synthesis inhibition, Chlase activation, and leaf deprivation of Fe and Mg. In Al-treated L-2039 plants, the leaf Mg and Mn contents were decreased. Also, an excessive Chlase activation was found in Al-treated L-2039, without a substantial Chl loss. This may indicate the activation of different enzyme pools in tolerant and sensitive genotypes under low-stress conditions.

Additional keywords: 5-aminolevulinic acid; chlorophyll; chlorophyllase; Fe; genotype susceptibility; Mg; Mn; N; photosystem 2; root; shoot.

Metabolic changes in Al-affected plants are induced by both direct and indirect Al actions (Haug 1984, Lazof *et al*. 1997, Barcelo and Poschenrieder 2002). Although it is considered that Al^{3+}, the toxic form of Al, is very scarce at plant cytosolic pH values, this ion may still be dangerous for the symplast due to its very high affinity towards metabolically important molecules. Al also induces substantial disturbances in the trans-membrane transport of ions (NO_3^-, PO_4^{3-}, K^+, Ca^{2+}, Mg^{2+}) in plant roots (Vicherková and Minář 1987, Taylor 1991, Nichol *et al.* 1993, Kochian 1995), thereby being indirectly responsible for the impairments of root-shoot transport and metabolic processes in shoots.

The impact of Al on photosynthesis is probably indirect. Hoddinott and Richter (1987) found a decrease in photosynthesis and in the translocation of photosynthates in beans after direct injection of Al into the xylem. Moustakas *et al.* (1996) found that Al (1 mM) indirectly caused significant disturbances in the chloroplast architecture in *Thinopyrum bessarabicum*, as well as a decrease in photosynthesis due to a reduction of electron transport in photosystem 2 (PS2) (most likely due to membrane impairment). Ohki (1986) found in a C_3 (*Triticum aestivum)* and a C_4 plant (*Sorghum bicolor*) that increasing the concentration of Al in the growth medium from 50 to 300 μM caused a proportional decrease in photosynthesis and the chlorophyll (Chl) content. Pereira *et al.* (2006) found that Al decreased 5-ALA dehydratase activity in cucumber. Heavy metals affect the content of 5-ALA (Padmaja *et al.* 1990), Chlase (Abdel-Basset *et al.* 1995) or ALA dehydratase (Morsch *et al.* (2002) activities.

Within each investigated species, Al-sensitive and Al-tolerant genotypes have been identified. Al-induced slower root growth is in conjunction with higher Al and reactive oxygen species accumulation (Darko *et al.* 2004, Meriga *et al.* 2004) as well as greater DNA damage in the roots of Al-sensitive genotypes (Meriga *et al.* 2004). Until lately, there was very little information concerning the influence of Al on metabolic processes in leaves and on genotype differences in Al-tolerance. In two recent studies (Zhang *et al.* 2007, Ali *et al.* 2008), Al induced the decrease in Chl content and photosynthetic rate.

We investigated the influence of Al on photosynthetic

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performance with respect to tolerant and sensitive genotypes. Assuming that the influence of Al in leaves is most probably exerted indirectly, through the inhibition of ion uptake or root-shoot transport, contents of nutrients essential for Chl synthesis and chloroplast assembly and maintenance (N, Mg, Fe, and Mn) were also determined.

Three maize inbred lines produced by the Maize Research Institute "Zemun-Polje" (Zemun, Serbia) were chosen on the basis of an earlier screening of 62 maize inbred lines for their Al-susceptibility (Lazic-Jancic *et al.* 1991). Only five tested lines fulfilled the set criterion (root shortening in the presence of 250 μM Al by less than 60 %). We used an Al-sensitive inbred line B-73 and two (F-2 and L-2039) Al-tolerant lines. The seeds were surface sterilized and germinated for 3 d between two moist filter-papers in the dark at 25 °C. After germination the seedlings were transferred to nutrient solution and grown in a growing chamber for 13−14 d under 70 % relative humidity, 12/12-h day/night cycle, 150 μmol(photon) m^{-2} s^{-1}, and 25/18 °C day/night temperature. The solutions were aerated. The nutrient solution for the plant growth was Knop solution modified according to Hadzi-Taskovic-Sukalovic (1993) by enrichment with N compounds, *i.e.* $[NO_3^-]/[NH_4^+]$ = 16.4/7.8 mM; the solutions contained 100 μM $Al_2(SO_4)_3$. pH of all solutions was adjusted to 4.6 at the time of seedling placement.

The total N content was determined by the micro-Kjeldahl procedure after plant material decomposition at 230 °C in the presence of sulphuric acid and hydrogen peroxide. Mg, Mn, Fe, and Al contents were determined after dry decomposition of plant material and its subsequent re-suspension in diluted HCl using flame atomic absorption spectrophotometry (*Pye Unicam SP 192*). Chl content was determined spectrophotometrically after extraction in pure acetone using the coefficients of Vernon (1960).

PS2 activity was determined according to Lewandowska *et al.* (1976). Plastids were isolated by blender homogenization of fresh leaf tissue for 5 min in isolation medium (1 g in 10 cm^3) containing 0.05 M HEPES-NaOH, pH 7.6, 0.4 M sucrose, 0.01 M NaCl, 0.005 M $MgCl_2$, and 20 % polyethyleneglycol 4000 (m/v). The homogenate was filtered through gauze and centrifuged at 1 000×*g* for 10 min. The upper layer of the sediment was re-suspended in the same medium and centrifuged once more. The sediment was re-suspended in 0.05 MES-NaOH, pH 6.4, with 0.4 M sucrose, 0.01 M NaCl, 0.005 $MgCl_2$, and 20 % PEG 4000. The incubation mixture for the determination of PS2 activity contained 0.2 M MES-NaOH pH 6.4, 4 mM NaCl, 2 mM $MgCl_2$, 0.76 M sucrose, 1.2 mM $(NH_4)_2SO_4$, 0.08 mM 2,6-dichlorophenolindophenol (DCIP), and 0.2 M chloroplast suspension. The mixture was divided between two tubes, one of which was the control kept in the dark. The PS2 activity was determined as the change of DCIP absorption at 600 nm after exposing one of the tubes to "white light" of 2 300 μmol m^{-2} s^{-1}.

To test 5-ALA synthesis, plastids were isolated according to Kannangara *et al.* (1977). Fresh leaf tissue was homogenized in homogenization medium (1 g per 3 cm^3) containing 0.5 M glycerol, 0.1 M tricine (pH 8.0), 1 mM dithiothreitol, 1 mM $MgCl_2$, and 1 mM EDTA. The homogenate was filtered through gauze and centrifuged at 100×*g* for 2 min. The supernatant was then centrifuged at 1 500×*g* for 8 min. The sediment was applied to 40 (v/v) *Percoll* in homogenization medium and centrifuged at 10 000×*g* for 10 min. Intact plastids settled at the bottom and the sediment was re-suspended in a small quantity of homogenization buffer and then used for the determination of enzymatic activity according to Dei and Tsui (1987). The reaction mixture contained 0.3 M glycerol, 0.1 M *Tricine* (pH 8.0), 1 mM EDTA, 25 mM $MgCl_2$, 1 mM L-glutamate, 5 mM ATP, 1.5 mM NADPH, 10 mM levulinic acid, and isolated plastids (equivalent to 2−4 mg of proteins) and was incubated for 60 min at 30 °C in the growing chamber. The reaction was terminated by adding of 0.1 cm^3 of 40 % trichloroacetic acid. Control reaction was terminated immediately. After the addition of Ehrlich reagent, the difference in absorbance at 553 nm of the sample and the control was determined.

Chlase (EC 3.1.1.14; Ellsworth 1971, Ellsworth *et al.* 1976) was extracted from fresh leaves that were blended in 67 mM phosphate buffer (pH 6.2) which contained 5 mM cysteine, 2.5 mM EDTA, and *Triton X-100* (1 g per 10 cm^3). Chl was extracted using *n*-butanol and discarded. Proteins were precipitated with acetone, dried, weighed, and re-suspended in 5 cm^3 of the phosphate buffer. Chl, the substrate for Chlase, was extracted from spinach leaves in methanol/dioxan (7 : 1) after which it was precipitated with potassium phosphate buffer (pH 8.0) and re-suspended in acetone. The incubation mixture of 10 cm^3 (40 % acetone in the buffer) contained ≈250 μg Chl and 2 cm^3 of enzyme extract. Blanks contained only substrate and buffer. Reactions lasted 30 min under weak irradiance at 30 °C. Aliquots of reaction mixtures and the blanks were taken before and after the reaction and transferred to the measuring mixture (3 cm^3 *n*-hexane, 0.75 cm^3 of 1 M NaOH, and 2.25 cm^3 of acetone). The remaining Chl partitioned in the upper layer (*n*-hexane); its concentration was determined spectrophotometrically.

The Al sensitivity of the B-73 inbred line and Al-tolerance of the two other lines were confirmed by basic growth parameters (Fig. 1*A,B*). The Chl content of the sensitive inbred line and of the tolerant L-2039 decreased in the presence of Al (to a different degree). However, Chl *a/b* ratio was not affected. The activity of PS2 in the B-73 inbred line was severely affected by the presence of Al (Fig. 1*C*). In addition to slightly changed Chl content in the presence of Al (Table 1), chloroplast function in the L-2039 line was also somewhat impaired by an indirect Al mechanism.

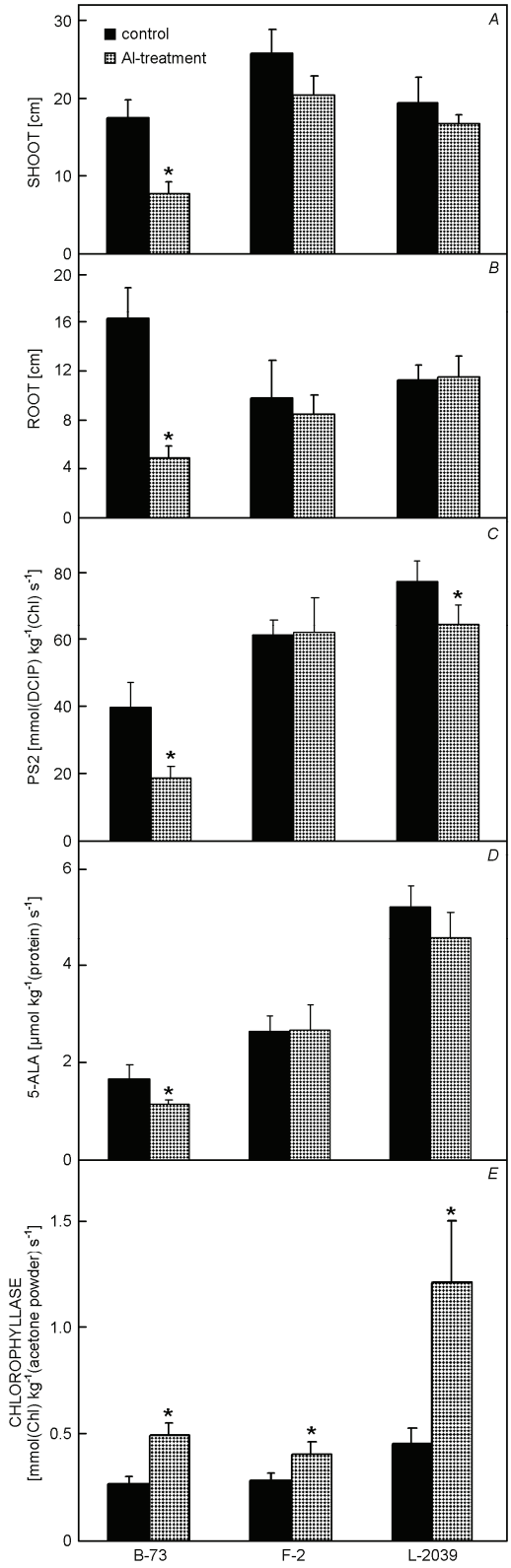

Fig. 1. Shoot (*A*) and root (*B*) lengths, photosystem 2 activity in isolated chloroplasts (*C*), rate of 5-ALA synthesis (*D*), and chlorophyllase activity (*E*) of control and Al-treated maize plants. Means of 6 (*C*–*E*) or 20 (*A, B*) replicates ± S.D., $^{*}p<0.05$.

The rate of 5-ALA synthesis was significantly affected by Al only in the Al-sensitive inbred line. However, the decrease in this activity in B-73 was not as great as the decrease in Chl content (Fig. 1*D*). Chlase activity was significantly increased in all three inbred lines in the presence of Al (Fig. 1*E*), most markedly in L-2039. The lack of correlation, either positive or negative, between the changes of Chlase activity and the changes of Chl content confirmed the complex nature of this activity.

Al induced a decrease in the Mg and Mn contents both in B-73 (by 50 %) and L-2039 (by ≈30 %) (Table 1). The Fe content was decreased only in B-73 line, whereas the L-2039 showed a stimulated accumulation of Fe under the influence of Al. The presence of Al decreased the N content in leaves of the B-73 (Table 1). In L-2039, the presence of Al induced a stimulation of total N uptake. Such stimulation may represent a part of adaptation mechanism to Al and is a result of Al effect on NO_3^- transporters in root plasma-membranes.

F-2 had a good Al-exclusion mechanism (Table 1). Al accumulation in roots of L-2039 was lower than in B-73. A slightly changed Al-content was found in shoots of B-73 and L-2039 in the presence of Al.

Although Al content in shoots increased less than in roots (Table 1), in the susceptible line a serious impairment occurred and the Chl content was 50 % lower in the presence of Al (Table 1). This was not accompanied with visible stains and necrosis. The finding is in accordance with the results of Moustakas *et al.* (1996) who found great thylakoid impairments in *Thinopyrum bessarabicum*, which were ascribed to indirect Al action in the absence of actual Al accumulation in leaves.

One of the key mechanisms to decrease the Chl content is the inhibition of its synthesis. We showed that Al acted differently on the key step of Chl synthesis (5-ALA synthesis) in the susceptible and in the tolerant inbred lines. Inhibition of 5-ALA synthesis had been demonstrated in the presence of Cd (Padmaja *et al.* 1990). Whereas Cd induced this effect by direct binding to the enzymes responsible for 5-ALA synthesis, the effect of Al was mostly indirect. The synthesis of 5-ALA depends on the influx of glutamate (Kumar *et al.* 1996) and exogenous glutamate activates Chl synthesis (Averina *et al.* 1989). The Chl content directly reflects the N content in the plant within a wide range. Although we found that the total amount of absorbed N decreased only in the sensitive line in the presence of Al, N influx was not the limiting factor for Chl synthesis, because Al induced stronger reduction of elongation than of N accumulation in this line (Table 1). The increase in N content found in L-2039, however, did not lead to Chl content increase, probably due to other limiting factors.

5-ALA synthesis may be inhibited by a decreased availability of reducing equivalents and accumulation of subsequent intermediates in Chl synthesis (Kumar *et al.* 1996). Both changes may arise from the impairment of thylakoid membrane arrangement, which was found by

Table 1. Effect of Al treatment on contents of chlorophyll (Chl) [g kg^{-1}(FM)], Mg [g kg^{-1}(DM)], Fe [mg kg^{-1}(DM)], Mn [mg kg^{-1}(DM)], N [g kg^{-1}(DM)] or [mg per plant] in leaves, and Al [mg kg^{-1}(DM)] in roots or shoots. Means of 4 or 6 replicates. $^{*}p<0.05$. C = control, DM = dry mass, FM = fresh mass.

	B-73		F-2		L-2039	
	C	Al	C	Al	C	Al
Chl *a+b*	2.70±0.27	1.30±0.22*	3.40±0.19	3.50±0.31	3.50±0.11	3.20±0.14*
Chl *a/b*	3.00±0.08	2.90±0.05	3.20±0.17	3.50±0.17	3.50±0.17a	3.30±0.11
Mg	1.81±0.21	0.30±0.04*	2.11±0.28	1.62±0.11	3.52±0.44	1.63±0.21*
Fe	16.06±2.25	2.61±0.57*	34.30±4.31	34.20±4.07	51.41±5.30	66.60±7.16*
Mn	4.42±0.48	1.15±0.29*	4.90±1.57	3.20±1.24	8.08±1.84	2.89±1.51*
N per DM	55.80±8.99	35.81±4.02*	64.48±12.34	64.84±10.19	115.67±17.54	116.15±8.32
N per plant	5.16±0.78	2.59±0.29*	5.42±1.04	5.58±1.26	7.71±1.16	9.58±0.68*
Al in roots	5.35±0.45	23.63±4.88*	5.27±0.90	9.21±0.97*	10.71±1.58	15.02±2.08*
Al in shoots	1.69±0.32	2.24±0.52	1.96±0.39	1.67±0.15	2.39±0.37	3.27±0.13*

Moustakas *et al.* (1996) in Al-treated plants. Our measurements of PS2 activity inhibition (greatest in B-73) confirmed this assumption. Photosynthesis inhibition by Al has been recently confirmed in soybean (Zhang *et al.* 2007) and mung bean (Ali *et al.* 2008). This has been ascribed to enzyme inactivation and membrane injuries most probably caused by oxidative stress. Such stress in leaves may be caused indirectly, by Al-generated water stress in the plants.

The presence of cations (primarily Mg, Fe, and Mn) is necessary for the maintenance of the thylakoid membrane ultrastructure as well as for thylakoid membrane arrangement (Milivojevic and Rastovic 1983, Horton *et al.* 1996). We found that the difference between the susceptible and the tolerant genotypes was clear as concerns Fe. The Mg and Mn contents were decreased both in the susceptible B-73 and the tolerant L-2039 lines. These findings are in accordance with the fact that in L-2039, regardless of its comparative Al-tolerance, Al accumulation occurred in roots that disturbed ion transport from roots to shoots. This was also in accordance with a slight drop of PS2 activity in this line in the presence of Al because PS2 activity also depends on thylakoid membrane structure that is maintained by divalent cations. Similarly, Pereira *et al.* (2006) assumed that Al competition with Mg was the reason of the decrease of 5-ALA dehydratase activity in cucumber leaves. In our case, Fe was the most important for the maintenance of Chl content [see also Garcia and Galindo (1991) who demonstrated that in Fe-deficient plants a greater decrease of Chl content occurred than in Mn-deficient plants]. The actual increase of Fe content in L-2039 leaves, accompanied by a significant increase in the roots (unpublished data), may be explained by possible stimulation of citrate exudation, which serves to precipitate Al in root zone of tolerant plants, but also increases solubility and uptake of Fe (Neumann and Römheld 2000).

In our experiments, Chlase activity increased under the influence of Al, but did not show a direct relation with Chl content changes. The increase of this enzyme activity was most obvious in the Al-tolerant L-2039. This was probably due to a more complex function of the enzyme that is usually described as a catalyst of the first steps of Chl degradation during senescence or under unfavourable conditions. Ellsworth *et al.* (1976) found that Chlase is a mixture of enzymes, one of which has a synthetic and the other a catalytic role. Tsuchiya *et al.* (1997) purified three enzymes with Chlase activity from *Chenopodium album*; some of them had a synthetic role. In our previous experiments with wheat, ammonium-treated plants under drought showed a several times higher Chlase activation than nitrate-treated plants. This was accompanied by Chl content increase in ammonium-treated plants under moderate drought (Mihailovic *et al.* 1997). We proposed a possible protective and synthetic role for some Chlase fractions under drought. Nevertheless, in the present experiments we determined only the total Chlase activity in maize plants. It is therefore possible that individual components of Chlase were stimulated to various degrees in different inbred lines, which would explain the lack of correlation between Chl content and Chlase activity.

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