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 Minár 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
isolation medium (1 g in 10 cm \(^3\)) containing 0.05 M blender homogenization of fresh leaf tissue for 5 min in Vernon (1960).

After extraction in pure acetone using the coefficients of 192 atomic absorption spectrophotometry (after dry decomposition of plant material and its peroxide. Mg, Mn, Fe, and Al contents were determined according to Hadzi-Taskovic-Sukalovic (1993) by enrich-temperature. The solutions were aerated. The nutrient solutions was adjusted to 4.6 at the time of seedling activity was determined as the change of DCIP dichlorophenolindophenol (DCIP), and 0.2 M chloroplast

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 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 termi-nated 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. 1A,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. 1C). 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. 1D). Chlorophyllase activity was significantly increased in all three inbred lines in the presence of Al (Fig. 1E), most markedly in L-2039. The lack of correlation, either positive or negative, between the changes of chlorophyllase 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 NO3- 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
1996). We found that the difference between the subdecrease of Chl content occurred than in Mn-deficient demonstrated that in Fe-deficient plants a greater Chl content [see also Garcia and Galindo (1991) who case, Fe was the most important for the maintenance of 5-ALA dehydratase activity in cucumber leaves. In our findings are in accordance with the fact that in L-2039, susceptible B-73 and the tolerant L-2039 lines. These Fe. The Mg and Mn contents were decreased both in theceptible and the tolerant genotypes was clear as concerns competition with Mg was the reason of the decrease of Chl 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. Tsujiya 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.

References

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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.

<table>
<thead>
<tr>
<th></th>
<th>B-73</th>
<th>F-2</th>
<th>L-2039</th>
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<tbody>
<tr>
<td>Chl a+b</td>
<td>2.70±0.27</td>
<td>3.40±0.19</td>
<td>3.50±0.31</td>
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<td>Chl a/b</td>
<td>3.00±0.08</td>
<td>3.20±0.17</td>
<td>3.50±0.17</td>
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<td>Mg</td>
<td>1.8±0.21</td>
<td>2.11±0.28</td>
<td>1.62±0.11</td>
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<td>Fe</td>
<td>16.06±2.25</td>
<td>34.30±4.31</td>
<td>34.20±4.07</td>
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<tr>
<td>Mn</td>
<td>4.42±0.48</td>
<td>4.90±1.57</td>
<td>3.20±1.24</td>
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<tr>
<td>N per DM</td>
<td>55.80±8.99</td>
<td>64.48±12.34</td>
<td>64.84±10.19</td>
</tr>
<tr>
<td>Al in roots</td>
<td>5.16±0.78</td>
<td>5.42±1.04</td>
<td>5.58±1.26</td>
</tr>
<tr>
<td>Al in shoots</td>
<td>5.35±0.45</td>
<td>5.27±0.90</td>
<td>9.21±0.97</td>
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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.


