ASPECTS OF ALUMINIUM TOXICITY IN SUNFLOWER. I. ALUMINIUM STRESS INDUCED IN NUTRIENT SOLUTIONS

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ABSTRACT

The aim of this paper was to study the effects of Al on the main root growth, the plant response to Al stress, and to determine whether the response is specific for Al stress. Three sunflower hybrids, stressed with different Al concentrations, in nutrient solutions containing $CaSO_4$ (0.5 mM), in aerated and non aerated experimental systems were used. The results showed a good relative root elongation rate at low levels of Al (even stimulation of root growth at Turbo hybrid), and the inhibition of growth at high levels of Al, after 72 h of culture. Under non aerated growth conditions, the relative root elongation rates were lower than in aerated systems (Alcazar hybrid was the most affected one, at lower concentration of Al; at higher concentrations, no difference was between the studied hybrids). By addition of citric acid in the stress solutions, the relative elongation rate of the roots was improved, with the increasing of the concentration of this acid up to 150 mM.

Key words: aluminium, toxicity, sunflower

INTRODUCTION

Soil acidification represents a world-wide agricultural problem, as up to 40% of the world's arable lands are already acid (Kochian, 1995). On acid soils, Al represents the major limiting crop productivity factor (Foy et al., 1978; Huang, 1984).

Aluminium is the most abundant metal and the third most common element in the earth's crust. At neutral pH, aluminium forms insoluble aluminosilicates or oxides. If the pH of the soil decreases, phytotoxic forms of aluminium are released into the soil solution, until it riches levels that affect root and the whole plant growth (Kochian, 1995). The initial and the most dramatic symptom of aluminium toxicity is the inhibition of root growth (Delhaize et al., 1993), which can occur within 1-2 hours after exposure to aluminium. It is often difficult to separate primary responses related to inhibition of growth from secondary responses that arise as the result of a damaged root system (inhibition of mineral and water uptake) (Kochian, 1995). So root apex is the primary site of aluminium toxicity.

Many mechanisms of aluminium resistance have been proposed in the literature, but most of these are speculative, with little evidence supporting them (Kochian, 1995). Resistance mechanisms were divided in 2 groups tough: aluminium exclusion mechanisms and mechanisms conferring plants the ability to tolerate aluminium in symplasm (known as aluminium tolerance mechanisms). Resistance to aluminium is, as Kochian's definition, the ability of plants to exibit superior root growth and enhanced plant vigor on aluminium toxic soils and solutions.

The most documented exclusion mechanisms are: release of Al-chelating ligands and increases in rhizosphere pH. Numerous studies support the Al-chelating ligands releasing (Delhaize et al., 1993; Miyasaka et al., 1991; Pellet et al., 1995 etc.).

In the literature is a lack of information regarding Al-toxicity in sunflower. So the aim of this paper is to elucidate some aspects on this topics using nutrient solutions supplemented with Al to determine the root growth at different Al concentrations, organic acid content at different levels in plant and in radicular exudates. As there are papers suggesting that the response at P deficiency at some plants consists in organic acid exudation, another goal of this paper is to separate this stress responses and also to find out whether the exudation of organic acids at sunflower is caused mainly by Al stress or P deficiency.

MATERIALS AND METHODS

Plant material and growth conditions

Three Romanian sunflower hybrids were used: Alcazar, Select, Turbo.

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Seeds were germinated at darkness, at 25°C, on filter paper rolls, moistned with 60 mL $CaSO_4$ (2 mM).

Plants were grown in controlled environment-chambers (16 h daylight, with 22°C, and 8 h darkness with 18°C), in nutrient solutions, and stress solutions.

Nutrient solutions, Al treatments, and material preparation

A. Organic acids analysis.

The nutrient solution used for plant growth had the following content: 0.7 mM K_2SO_4, 0.1 mM KCl, 0.5 mM $MgSO_4$, 2 mM $Ca(NO_3)_2$, 0.1 mM KH_2PO_4, 2 µM Fe EDTA, 10 µM H_3BO_3, 0.5 µM $ZnSO_4$, 0.2 µM $CuSO_4$, 0.01 µM $(NH_4)6Mo_7O_{24}$. The pH was adjusted to 6.5. After 10 days of cultivation in this solutions (4 leaf stage) plants were stressed with a 0.5 mM $CaSO_4$ solution supplemented with 50 µM $AlCl_3$, at a Ph = 4.5. Control plants were cultivated in a 0.5 mM $CaSO_4$ solution at the same pH.

The exudates were collected in distilled water, for 2 hours, and analysed at HPLC.

Also, leaves and roots content was extracted in 5% H_3PO_4, and analysed at HPLC

The same nutrient solution, except P, was used for testing the plant response at P deficiency. Plants were cultivated also for 10 days, exudates were collected in distilled water after 5 and 10 days, and HPLC analysed. Plants were stressed furthermore with Al (50 µM $AlCl_3$) in a 0.5 mM $CaSO_4$ solution for 24 hours in order to determine whether there are differences in the responses of plants grown on normal solutions and plants grown on P deficient solutions. The exudates were collected in the same way and HPLC analysed.

HPLC conditions:

- column: 250 × 4 mm Merck Lichrospher 100 5 µ, RP18;
- eluent: 18 mM KH_2PO_4, pH adjusted at 2.1-2.5 with o-phosphoric acid, flow rate 0.5 mL/min.;
- temperature: 20°C;
- detection: UV 220 nm.

As standard, a mixture of 12 organic acids was used (tartaric acid –2.5 ppm, formic acid – 2.5 ppm, malic acid – 2.5 ppm, isocitric acid – 2.5 ppm, lactic acid – 2.5 ppm, acetic acid – 2.5 ppm, maleic acid – 0.025 ppm, citric acid – 2.5 ppm, fumaric acid – 0.025 ppm, succinic acid – 2.5 ppm, cis-aconitic acid – 0.025 ppm, transaconitic acid – 0.025 ppm)

B. Root growth measurements

After germination plantlets with root length between 4-8 cm were cultivated on 0.5 mM $CaSO_4$ solution at a pH = 4.5. Stress was induced by adding different levels of $AlCl_3$ in solutions (25 µM, 50 µM, 75 µM, 100 µM). Root length was measured after 24 h, 48 h, and 72 h. For this experiment aerated and non aerated cultivation systems were used.

Root elongation rate was calculated using the formula proposed by Parker, 1995:

RER = Lal + final - Lal + initial/L control, final - L control, initial × 100;

Lal + final = root length after a period of stress;

Lal + initial = root length before stress application ;

L control final = root length after a period of time at control plants;

L control initial = initial root length at control plants.

Under the same experimental conditions and using the same calculation formula, the efficiency of citric acid in Al detoxification was tested. For this purpose 50 µM, 100 µM, 150 µM. 200 µM citric acid was added in the stress solutions containing 100 µm Al.

RESULTS AND DISCUSSIONS

Al addition in the solutions induced some modifications in organic acid content. The weakest response, as expected, was found at the leaf level where citric, malic and fumaric acid concentration decreased, but not significant differences were recorded between control and stressed plants. There are some differences in genotypic reaction tough. Select hybrid recorded the lowest citric and malic acid content (Figure 1, A).

In the basal part of the roots, only fumaric and citric acids were found. The concentration of those acids decreased significantly at all 3

hybrids, but there are no differences in genotypic response (Figure 1, B). At the root tip level the response reaction to the stress was more diverse. So fumaric and malic acid concentration decreased (significant differences at all studied hybrids between control and stressed plants), and citric acid concentration increased significantly. Very closed values were found at all studied hybrids (Figure 1, C).

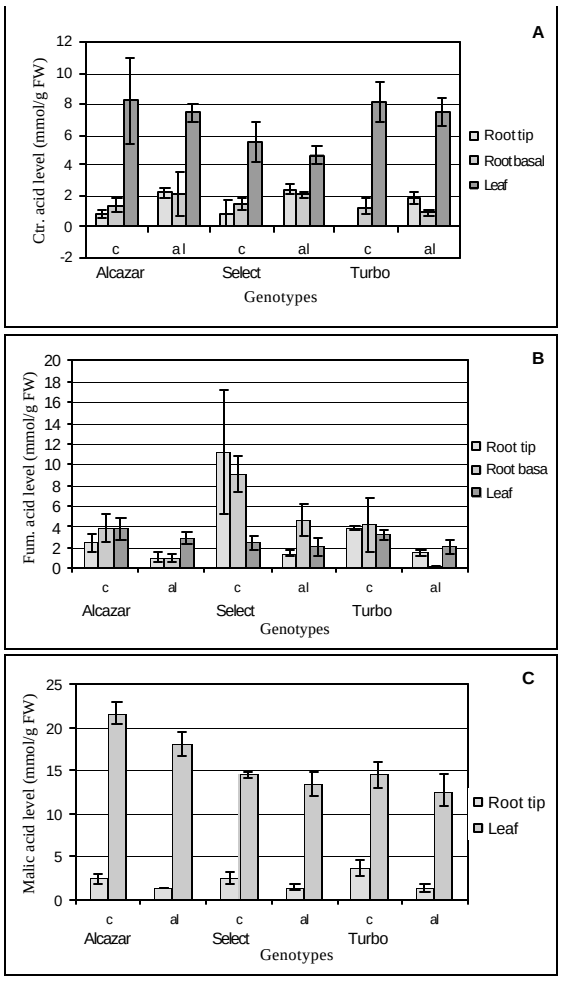


Figure 1. Internal levels of citric acid (A), fumaric acid (B), and malic acid (C)

Analyses of the root exudates revealed a strong response to the Al stress. Citric and fumaric acids were determined at this level. Only traces of citric acid were found in the exudates of control plants at all the studied hybrids, while in the exudates of stressed plants were found relatively big concentrations. A dif-ference between genotypical response was also noticed (Figure 2).

The presence of malic acid in the root tips, and the absence of this acid in the root exudates indicate that citric and fumaric acid exudation is a typical stress response reaction (metabolic response), not a passive process following the cell membrane breaking. Also a certain response to Al stress is the accumulation of citric acid in the root tips.

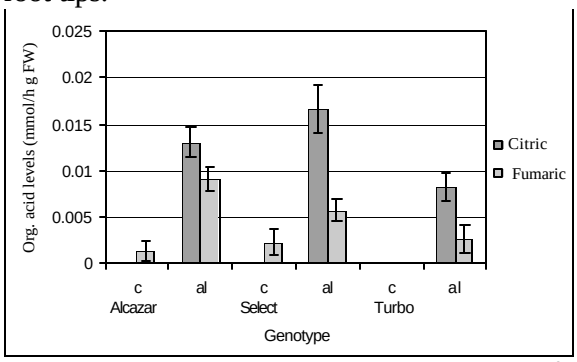


Figure 2. Organic acid levels in radicular exudates of Al stressed plants

There are no published studies to confirm our results at sunflower, but exudation of organic acids triggered by Al was found at many plants. At maize, a 24-48 h Al exposure induced a dramatic stimulation of citrate release (3.5-7 fold increase) by roots of Al-tolerant South American 3, while in Al sensitive Tuxpeno, there was no significant stimulation of citrate after Al exposure (Pellet et al., 1995)

Also citric acid exudation triggered by Al was mentioned at some other plant species. At tobacco, different lines exuded between 2-5 more citric acid under stress conditions (De la Fuente et al., 1997). Ma et al. (1997) performed some studies on *Cassia tora* L. (a plant resistant to Al), by exposing the roots to 50 µM Al. Again citric acid was the main organic acid in the exudates of stressed plants (10.1 µM/12h). Also, Pellet et al. (1995) found that citric acid was the primary organic acid released as a response to Al stress at maize.

At other plant species, malic (wheat - Ryan et al., 1995; Delhaize et al., 1993) and oxalic acid (buckwheat, Zheng et al., 1998; taro, Ma et al., 1998) exudation was triggered by Al.

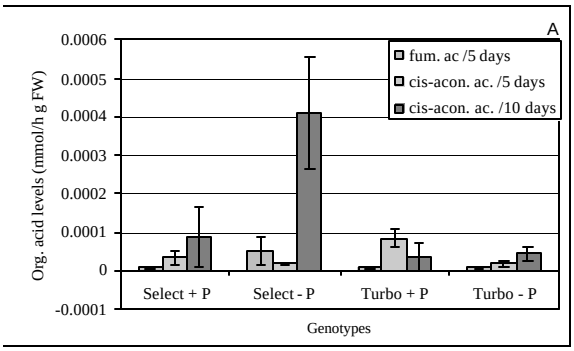
The increasing of internal malic acid content was noticed by Delhaize et al. (1993), at wheat root tips of Al stressed ET3 resistant line, while at the ES3, a sensitive line a decreasing was found. So, we consider our results confirmed by literature.

A comparision between the amount of malic acid exudated by the roots of wheat tolerant line ET3 (3.57 nmol/h seedling) (Delhaize et al., 1993), and the amount of citrate exudated by sunflower roots (0.008-0.0166 µM/h g FW), shows that all sunflower genotypes we have studied released big amounts of citric acid, when compared with Al resistant wheat genotypes. On the other hand, Ma et al. (1997) working on *Cassia tora* L (a plant resistant to Al) found a release of citric acid of 0.83 µmol/h g DW, and 0.38 µmol/h g DW at Atlas 66 (wheat genotype resistant to Al), both bigger than the exudation rates we found in sunflower.

The specificity of Al-stimulated release of citric acid was tested. Stress solutions without P were used for stress inducing. It could have been possible that P deficiency stimulate citric acid exudation. After 5 days of P starvation a stimulation of fumaric and cis-aconitic acids exudation was found, but no citric acid was identified in the exudates. The same situation was present after 10 days of P starvation (Figure 3, A)

Delhaize et al. (1993) and Ma et al. (1997) obtained the same results working with wheat and taro cultivars.

By stressing the P starved plants with 50 µM Al, a different genotypical response was found. Select hybrid exudated more citric acid, and Turbo hybrid, on contrary, less citric acid, compared with plants grown on normal nutrient solutions and than stressed in the same conditions (Figure 3, B).



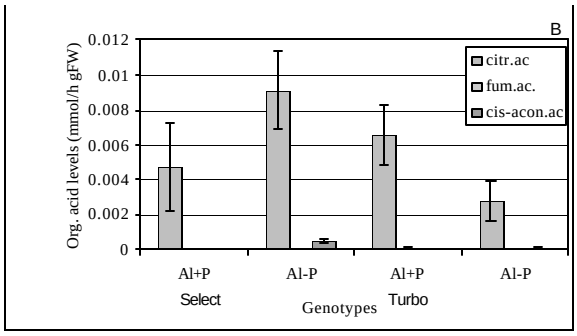


Figure 3. Organic acid levels in the radicular exudates of P straved plants (A), and P starved plants stressed with Al (B)

Delhaize et al. (1993) using ET3 line (resistant to Al), obtained a decreasing of citric acid exudation by adding P in stress solutions. Ma et al. (1997) by adding a small amount of P (0.3 µM) obtained a small increase of oxalate exudation, but 100uM P decreased the oxalate exudation.

The relative root elongation rate decreased with the increasing of Al levels and the time of stress (Figure 4, A,B,C).

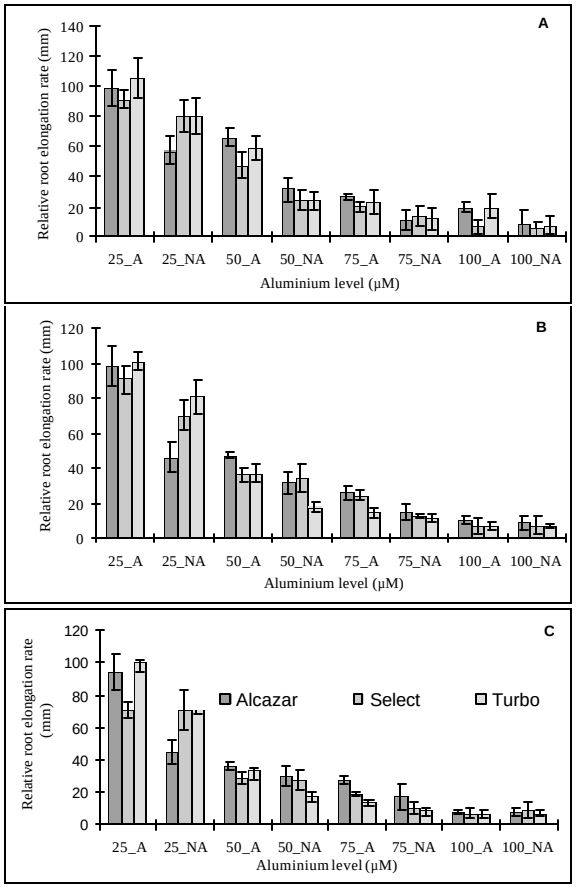


Figure 4. Relative elongation root of main root after 24 h (A), 48h (B) and 72 h(C) of stress,

in aerated (_A) and nonaerated (_NA) solutions

At a 25 mM Al level in the stress solutions, in the first 24 h, RER was around 100% (98.955% Alcazar hybrid, 91.587% Select hybrid), evan more at Turbo hybrid (105%), slightly decreasing after 48, and 72 hours of stress. After 72 h of stress the most affected was Select hybrid, RER decreasing at less than 80%. Low concentrations of Al have been observed to enhance root growth of some other plant species (Clune and Copeland, 1999, Kinraide, 1993, Grauer and Horst, 1990) but the mechanism of this effect is not yet understood. Kinraide (1993) proposed that hexahydrated Al ions stimulate growth of wheat roots by relieving a proton stress at the root surface. Clune and Copeland (1999) consider unlikely that the stimulation of canola root growth be attributed to the alleviation of a proton stress at the root surface as the increasing on pH in the solutions from 4.5-4.8 did not increase the root growth. Also, we didn't observe an increase of the pH in the stress solutions (data not shown).

In Clune opinion the enhancement of root growth did not seem to be due to the relief of stress caused by other ions in the solutions, such as ammonium, as Grauer and Horst (1990) proposed.

Parker (1995) using resistant Atlas 66 and sensitive Scout 66, wheat genotypes, found after 48 h of stress a 94% RER at 25 µM Al, and 65% RER at 50 µM Al level in stress solution (at the resistant genotype, while at the sensitive genotype root growth was inhibited at 10 µM Al. These results confirm our data obtained in the same experimental conditions (almost 100% RER at 25 µM Al., and 37-47% RER at 50 µM Al)

With the increasing of Al levels at 75 and 100 µM, RER decreased dramatically. The same results obtained Lazof and Holland (1999), at soybean, after 72 h of stress (20% RER at 50 µM Al). In non aerated systems, RER was smaller than in aerated systems at the same Al levels. The most affected hybrid in non aerated conditions, at low Al levels was Alcazar. So, probably, on Romanian acid soils, this would be the less adapted genotype.

If citric acid has a role in Al tolerance it should be able to chelate Al in stress solutions. To prove this, different citric acid levels were added in a stress solution containing 100 µM Al. The results shown that 150 µM citric acid was enough to improve RER to 60 % at Alcazar hybrid and to almost 80% at the other two studied hybrids (Figure 5 A, B). Delhaize et al. (1993) found that root growth at Al sensitive wheat seedlings was almost restored to control levels by adding 400 µM malic acid to a stress solution containing 50 µM Al.

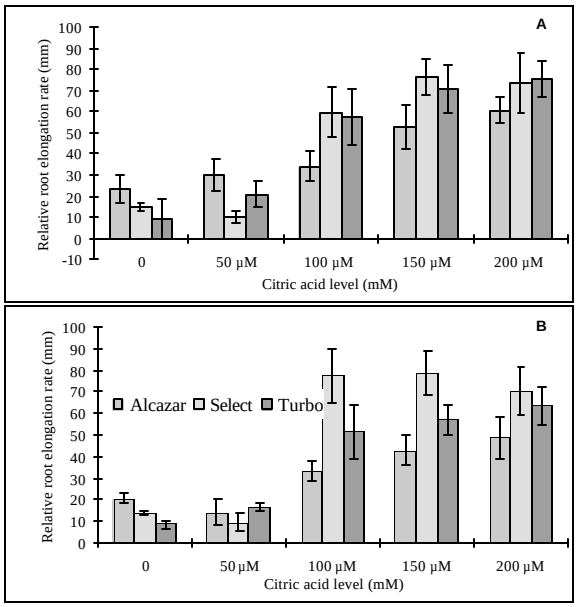


Figure 5. The effect of citric acid addition in the stress solution (A-24h, B-48h)

It is a big difference between the amount of citric acid, used for restoring the sunflower root growth, and the malic acid amount used to restore the wheat root growth. But different authors mentioned that the detoxifying capacity of citric acid is better. This might be attributed to their different stability constants with Al (bigger at citric acid) (Zheng et al., 1998).

CONCLUSIONS

We found at sunflower the same response at aluminium stress (organic acid exudation) as is mentioned in literature at some other plants: wheat, maize, soybean, taro etc. The amount of citric acid exudated by roots is approximately in the same range found at some other plants studied, such as wheat.

Citric acid exudation is a specific response to Al stress, and is not triggered by other stress factors as P starvation, and also is not a passive migration due to membrane damage.

Sunflower hybrids studied exhibited a good RER at low levels of Al, as compared with wheat resistant cultivars.

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Table 1. Influence of aluminium ions, in reaction mixture, on the level of saccharasic activity in a reddish-brown soil fertilized with compost with different quantities (glucose+fructose-mg/100 g soil dw/24 hours)

A- Factor	B – Factor – COMPOST (t/ha)								Average (A)	
	b1-0	%	b2-0	%	b3-0	%	b4-0	%		%
a1–without Al^{3+}	b 3287	100	b 4028	100	b 2579	100	b 3472	100	b 3341	100
a2- with Al^{3+}	a 4228	129	a 5019	125	a 3472	135	a 4528	130	a 4312	129
Average (B)	3757 c		4523 a		3025 d		4000 b			

LD P	5%	1%	0,1%
A	291	673*	2143
B	101	142	201*
AB	302	628*	1799
BA	144*	201	284

Table 2. Influence of aluminium ions, in reaction mixture, on the level of saccharasic activity in a chernozem mineral fertilized or manured with farmyard compost (glucose+fructose-mg/100 g soil dw/24 hours)

A- Factor	B – Factor – COMPOST (t/ha)										Average (A)	
	b1-0	%	b2-$N_{32}P_{32}$	%	b3-$N_{94}P_{96}$	%	b4-$N_{128}P_{128}$	%	b5 compost	%		%
a1–without Al^{3+}	b 1564	100	b 1496	100	b 1459	100	b 1401	100	b 1732	100	b 1530	100
a2- with Al^{3+}	a 1686	108	a 1581	106	a 1684	115	a 1589	113	a 1864	108	a 1681	110
Average (B)	1625 b		1538 d		1571 c		1495 e		1798 a			

LD P	5%	1%	0,1%
A	7	17	54*
B	14	20	27*
AB	19	28	45*
BA	20*	28	39