Plant Soil (2015) 388:323–335
DOI 10.1007/s11104-014-2334-z

REGULAR ARTICLE

Differential aluminium-impaired nutrient uptake along the root axis of two maize genotypes contrasting in resistance to aluminium

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Received: 6 March 2014 / Accepted: 12 November 2014 / Published online: 23 November 2014

Abstract

Background and aims The sensitivity of root cells and root processes to toxic aluminium ions (Al^{3+}) varies along the root axis. This study was established to assess the sensitivity of nutrient uptake to Al along the main root axis of maize genotypes that differ in resistance to Al and to test whether citrate, an Al-complexing compound that is unevenly released along the root axis, can play a role in protecting the root from Al-impaired nutrient uptake.

Methods A divided-root-chamber technique was used to measure net fluxes of calcium (Ca^{2+}), magnesium (Mg^{2+}), and potassium (K^{+}) along intact roots of two maize genotypes differing in resistance to Al. The accumulation of Al along their main root axis was also measured in short-term experiments. Results of these experiments were compared with those of a previous study, where citrate exudation had been measured along identical maize root axes.

Results Aluminium affected nutrient uptake widely along the root with strong effects in the apical region, reducing total Ca^{2+} and Mg^{2+} uptake, but not K^{+} uptake. The negative effects of Al^{3+} were more pronounced in the Al sensitive genotype than in the resistant one. The former also accumulated more Al in its roots than the latter, but this differential accumulation was observed only in the apical part of the root. The spatial pattern of nutrient uptake, irrespective of Al treatment, did not match that of Al-stimulated citrate exudation.

Conclusion Based on the differential sensitivity of the root axis of the two maize genotypes and especially on the extent of the root zones where these differences are expressed, it is suggested that the less Al-disturbed nutrient uptake of a genotype is associated with its resistance to Al.

Keywords Aluminium toxicity · Calcium · Magnesium · Plant root · Potassium · Tolerance

Responsible Editor: Yong Chao Liang.

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Introduction

The uptake of nutrients by plant roots starts with the movement of molecules from the external solution into

the cell walls and solution-filled intercellular spaces of the root cortex (i.e. the root apoplast). The uptake rate varies along the root axis depending on nutrient, plant nutritional status, plant species and developmental stage of the root tissue (Ferguson and Clarkson 1975, 1976; Harrison-Murray and Clarkson 1973; Marschner et al. 1987). The formation and subsequent suberization of the endodermis may impose a marked restriction to the absorption of nutrients that move inwards the stele in an extracellular (i.e. apoplastic) pathway (e.g. calcium [Ca^{2+}], magnesium [Mg^{2+}]). Consequently, uptake rates of such nutrients are normally higher in apical (without endodermis) than in basal (with endodermis) root zones (Ferguson and Clarkson 1976; Harrison-Murray and Clarkson 1973; Ranathunge et al. 2005). For nutrients that move in the root cortex preferentially via the symplast (e.g. potassium [K^+], phosphorus [usually as $H_2PO_4^-$]), the uptake rates tend to be more homogeneously distributed along the longitudinal axis of the root (Ferguson and Clarkson 1975; Häussling et al. 1988; Marschner 1995).

Aluminium (Al) ions in the rooting medium are able to inhibit the uptake of several nutrients in many plant species (Foy 1984). The trivalent Al^{3+} species compete with nutrient cations (mainly Ca^{2+} and Mg^{2+}) for binding sites in cell walls and on plasma membranes and displace these cations adsorbed on these sites. This may decrease the Ca^{2+} and Mg^{2+} concentration around the membrane transport sites, decrease their uptake and lead to deficiencies in the plant (Bose et al. 2011; Horst et al. 2010; Keltjens 1995; Postma et al. 2005; Rengel and Robinson 1989a).

Differences in sensitivity of nutrient uptake to the adverse effects of Al are observed among genotypes of a species (Baligar et al. 1993; Rengel and Robinson 1989b). The genotype specific ability to maintain normal nutrient fluxes and transmembrane potential of root cells in the presence of Al^{3+} is thought to be associated with or to determine Al resistance of genotypes (Ahn et al. 2001; Bose et al. 2013; Miyasaka et al. 1989; Rengel and Robinson 1989a). Huang et al. (1992) reported that Al, at concentrations of 5–20 μM $AlCl_3$, inhibited Ca^{2+} uptake significantly in the Al sensitive wheat cultivar Scout 66 but not in the Al resistant cultivar Atlas 66. This genotypic difference was larger in the first 5 mm of the root than in the region 5–20 mm behind the root tip.

Al-stimulated release of organic acid anions (OAA) (e.g. $citrate^{3-}$, $malate^{2-}$, $oxalate^{2-}$) by plant roots has been hypothesised as a mechanism of detoxification of Al^{3+} in the root apoplast and rhizosphere and thus in protection of the roots (Kochian et al. 2005). Studying genotypes of maize (*Zea mays* L.) that differ in resistance to Al, Mariano and Keltjens (2003) observed root citrate exudation rates that were 1.7–3.0 times higher in the Al resistant genotype CMS36 than in the Al sensitive BR106 across a range of external Al concentrations. The authors then hypothesised that the great amounts of citrate exuded into the root apoplast and rhizosphere of CMS36 could, apart from protecting the root in terms of growth, also protect it from the adverse effects of Al^{3+} on the uptake of nutrients. However, unlike the uptake of nutrients that may occur along the whole extent of the root, significant Al-stimulated exudation of OAA is strictly confined to root apices (Mariano and Keltjens 2003; Pellet et al. 1995). The protection to be conferred by OAA is therefore more likely at apices, possibly leaving the rest of the root unprotected against the adverse effects of Al^{3+} ions.

This study was established to investigate (i) the sensitivity of the nutrient uptake process along the root axis to the adverse effects of Al, (ii) the accumulation of Al along the root axis, and (iii) the potential role of exuded citrate to protect the root axis from Al-impaired nutrient uptake.

The sensitivity of the root nutrient uptake process and the accumulation of Al along the root axis were investigated in this study with seedlings of the two maize genotypes CMS36 and BR106 and compared with the spatial pattern of Al-stimulated citrate exudation of identical maize root axes reported by Mariano and Keltjens (2003).

Materials and methods

Plant material and growth conditions

The plant material consisted of two maize genotypes that differ significantly in resistance to Al: CMS36 (Al resistant) and BR106 (Al sensitive) (Mariano and Keltjens 2004). Subsequently they will be referred to as CM and BR, respectively. Maize seedlings of both genotypes were grown in a basal nutrient solution with pH 4.0 and the following chemical composition (mM): 1.0 NH_4NO_3, 0.005 NaH_2PO_4, 0.5 K_2SO_4, 0.5 $CaCl_2$, 0.125 $MgSO_4$, and (μM): 46 B, 0.3 Cu, 286 Fe (as Fe-EDTA), 0.1 Mo, 9.2 Mn, 0.8 Zn. They were grown in a

controlled environment chamber at 20 °C under a regime of 16 h light (light intensity 80 W m^{-2})/8 h dark.

Nutrient flux measurements

The divided-root-chamber technique, described by Ryan et al. (1993), was used to study the nutrient fluxes along the longitudinal axis of the whole primary seminal root of the seedlings. Roots of seedlings of the two genotypes were grown axenically and all materials used in the experiments were autoclaved and successive treatments were carried out in a laminar-flow hood. To sterilise the surface of the seeds, they were immersed for 1 min in 96 % ethanol, soaked for 1 h in a solution containing 1.5 % sodium hypochlorite (from commercial bleach) + 1 % Tween 20, and subsequently incubated for 15 min in a 1.5 % sodium hypochlorite solution. After each treatment, the seeds were rinsed three times with sterile demineralised water. To check for eventual microbial contamination, surface-sterilised seeds were germinated on nutrient agar plates prepared with a 1 mM $CaSO_4$ solution. The plates were placed in a dark chamber at 25 °C for 90 h. After germination, uncontaminated seedlings were individually transferred to Petri dishes (Ø 94 mm) containing 50 ml of sterile nutrient solution. The nutrient solution was adjusted to pH 4.0 with 0.1 M HCl and autoclaved before the addition of filter-sterilised Fe-EDTA stock solution.

In each Petri dish, the roots of one seedling were grown horizontally oriented whereas the shoot was grown vertically through a small notch made in the edge of the lid. The notch was sealed with lanolin and the Petri dish wrapped with foil and transferred to the growth chamber where the seedlings were grown for 3 days.

Aluminium was added to the sterile nutrient solution without Fe-EDTA to reach concentrations of 0 or 40 μM Al. Aluminium was diluted from a filter-sterilised stock solution containing 10 mM $AlCl_3$ and 0.1 mM HCl. The Al treatment solutions (50 ml) were applied to the seedlings in the same Petri dishes in which they had been growing during the preceding 3 days, replacing the old nutrient solutions. Seedlings were grown for 24 h in the Al treatment solutions (0 or 40 μM Al) before the uptake measurements started.

After 24 h of Al treatment, seedlings were transferred to large Petri dishes (Ø 145 mm) where they had their roots spatially divided using plastic rings (Ø 13 mm). These plastic rings were placed all over the main seminal root of each seedling, covering it from the root apex to the most basal section of the root (Fig. 1). A thin layer of vaseline was used to seal the space between the plastic ring and the bottom of the Petri dish and a layer of agar was poured around each plastic ring to hold it over the root. Each plastic ring isolated either an apical 10-mm or a 13-mm long root section from the rest of the root system forming an individual chamber. An aliquot of 0.5 ml of Al treatment solution (0 or 40 μM Al) was applied to each chamber and with 50 ml of the same treatment solution the rest of the root system was covered. The Petri dish holding the system was closed with a lid to prevent contamination and evaporation of the treatment solutions, wrapped with foil, and transferred to the growth chamber. The solutions enclosed by the rings were collected after 6 h, weighed, and analysed on Ca, Mg, and K. The net flux of nutrients was calculated for each root segment enclosed by one plastic ring for the period of 6 h from the volume and change in nutrient

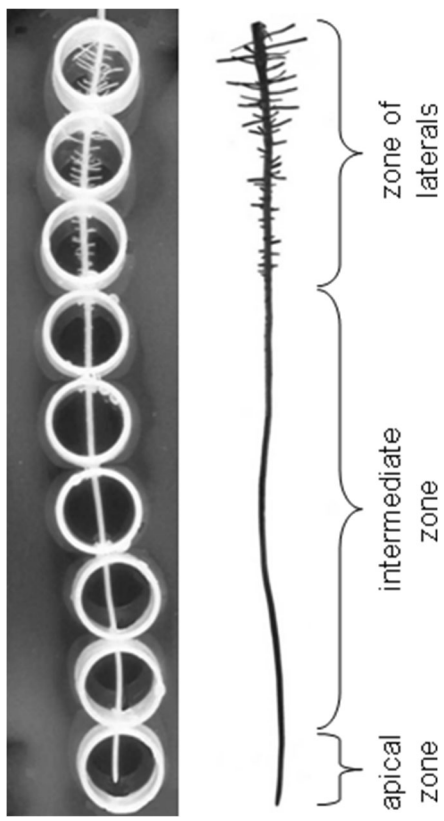

Fig. 1 Aspects of the divided-root-chamber technique showing the plastic rings over the whole root (*left*) and a typical root axis as used in the experiments, with three distinct zones: apical, intermediate, and zone of laterals (*right*). Average length of the root axes studied =12 cm

concentration of the solutions in the distinct rings. The latter was measured by inductively coupled plasma mass spectrometry (ICP MS). Changes in volume of the treatment solutions applied to the root chambers were used to detect any leakage of the root-chamber system. At the end of the experiment the number of lateral roots on the basal zone of the seminal root was counted under a binocular (3.2 × magnification) and the root segment in each plastic ring was excised, gently blotted, and weighed.

Aluminium accumulation and nutrient uptake by entire roots measurements

Short-term experiments were performed to study the accumulation of Al along the root axis and to measure the uptake of Ca^{2+}, Mg^{2+}, and K^{+} by intact entire roots of both maize genotypes. Maize seeds were germinated as previously described and after germination, uniform seedlings were transferred to polystyrene discs (covers) placed on 1.8-L pots (12 seedlings pot^{-1}) with aerated nutrient solution, and grown in the controlled environment chamber. After an acclimation period of 24 h, the number of seedlings was thinned to nine per pot and the basal nutrient solution was replaced by a simpler, sulphate- and phosphate-free nutrient solution with pH 4.0 and composition (mM): 1.0 NH_4NO_3, 1.0 KCl, 0.5 $CaCl_2$, and 0.125 $MgCl_2$. Aliquots of a freshly prepared stock solution of 10 mM $AlCl_3$ and 0.1 mM HCl were added to this solution to reach concentrations of 0 or 40 μM Al. Sulphate and phosphate were left out to avoid their complexation with Al and the subsequent precipitation of the complexes at the root surface, what could mask the results of Al accumulation in the root.

In the Al accumulation experiment, four different root segments along the main seminal root axis were excised after 24 h of exposure to Al. Seedlings were removed from the Al treatment solution and their roots were rinsed briefly in a HNO_3 solution of pH 4.0 to remove Al and other elements present in the treatment solution adhered to the root. The roots were then gently blotted between layers of tissue paper and the segments were excised. From the tip to the base of the root, the four segments were excised with the following lengths and at the respective positions along the axis: 0–10 mm, 11–23 mm, the last 13-mm long segment before the appearance of the laterals, and a 13-mm long segment in the middle of the zone of laterals. The segments were weighed and immediately transferred to Eppendorf vials where Al was desorbed from the root with a 5.0 mM Na_3-citrate solution of pH 7.0 for 60 min at room temperature. The Na_3-citrate solutions collected were analysed on Al by using an inductively coupled plasma optical emission spectrometer (ICP OES) (Varian Vista AX, Mulgrave, Australia), with an axially viewed configuration.

In the nutrient uptake experiment, each pot was weighed and had its nutrient solution sampled after 24 h of exposure of the plants to Al. The root system of the maize plants was harvested, gently blotted between layers of tissue paper, and weighed. The solutions were analysed on Ca, Mg, and K by ICP OES and the nutrient uptake in each pot was calculated from the volume and change in nutrient concentration.

Statistical analyses

There were four experimental treatments. They resulted from a factorial combination of two maize genotypes (CM and BR) and two concentrations of Al (0 and 40 μmol $AlCl_3$ L^{-1}) in the nutrient solution. In the nutrient flux experiments there were three replicate seedlings per treatment per experiment and the entire experiment was carried out twice. Because the seedlings used did not have roots of the same length, the number of plastic rings used to cover the entire root varied among replicate seedlings. To normalise the length of the roots, the length of the root segment that each plastic ring covered was expressed relatively to the total length of that root. For the regression analyses and graphs, the nutrient flux values were plotted in the middle of the root segments in which they were measured.

Linear statistical models were used to describe the functional relation between the fluxes of the plant nutrients studied (dependent variable) and the relative position of a root zone along the root length (independent variable). Univariate regression analyses were applied to the individual nutrients. Because of the heterogeneous variances found between the genotypes, a weighted regression analysis was used, with the weights calculated as inversely proportional to the square root of the mean square error (MSE) of each treatment.

In the Al accumulation and nutrient uptake experiments there were three replicates per treatment and each experiment was carried out once. Results of each of the four different root segments analysed were compared individually between the two genotypes with the nonparametric Wilcoxon Signed Rank test. This nonparametric test was also applied to the nutrient uptake data.

Residual analysis was performed to confirm the adequacy of the statistical model and to detect violations of the assumptions underlying the random errors. We found that errors had a constant variance, were independent (i.e. not correlated) and normally distributed. All statistical analyses were computed with the SAS software (SAS Institute 1990).

Results

Characterisation of the root material used The maize seedlings used had one long seminal root derived from the radicle and typically three to five shorter seminal roots at the time they were used for the nutrient flux measurements (or they had only the main seminal root in the Al accumulation experiments). Only the main (i.e. longest) seminal root of each seedling was studied. It was a root about 120 mm in length, with short (<5–6 mm) lateral roots in its most mature (basal) part (Fig. 1). This part of the root represented about 35 % of the total length. The remaining part of the root consisted of the root apice and an intermediate zone, between the root apice and the zone with laterals. The apical zone of the root, enclosed by one plastic ring, represented about 10 % and the intermediate zone about 55 % of the total root length.

Spatial pattern of nutrient fluxes along the main seminal root We measured net fluxes (i.e. the net difference between influx and efflux) of Ca^{2+}, Mg^{2+}, and K^+ along the whole main root of the maize seedlings. A net positive flux (i.e. uptake) was more often observed than a net negative flux (i.e. efflux), especially with Ca^{2+} and Mg^{2+} (Figs. 2, 3, and 4). Linear functions fitted well to the data, although there was not a unique pattern of nutrient flux along the root with all treatments studied. The model most often fitted was a piecewise linear regression with one break (Neter et al. 1996):

$$Net\ \left(Ca^{2+}, Mg^{2+}, K^{+}\right)\ flux = \beta_0 + \beta_1 root + \beta_2 laterals \quad (1)$$

where

root is the relative position of a root zone (from 0 to 10) along the root axis

laterals is equal to $\begin{cases} 0(zero)\ when\ root \le 6.13 \\ (root - 6.13)\ when\ root\ > 6.13 \end{cases}$

6.13 is the mean relative position of appearance of lateral roots on the root axes studied

β_0 is a parameter employed for goodness of fit

β_1 represents the unit of variation on the net flux due to a unit variation of relative root length

β_2 represents an extra net flux (positive or negative) due to the existence (root > 6.13) or not (root ≤ 6.13) of lateral roots

The relative position of the break was initially assumed to coincide with the appearance of the lateral roots on the main root axis, i.e. at 61.3 % of the root length. Subsequently its position was varied around 61.3 % of the root length and the equations fitted were evaluated. The best fits were those obtained with the break coinciding with the appearance of laterals. Influence analyses were performed and four outliers were detected. Two of them were aberrant values for Ca^{2+} and Mg^{2+} simultaneously (CM at 0 and 40 μM Al) while the other two were aberrant only for K^+ (BR at 0 μM Al). The values of Ca^{2+}, Mg^{2+}, and K^+ fluxes of each measurement were left out separately and were only removed where they were found to influence the results strongly and negatively.

The effects of Al on the net fluxes of nutrients varied among the treatments and nutrients studied. Al effects that resulted in lower uptake rates or even in net losses of nutrients by the root were more often observed with Ca^{2+} and Mg^{2+} than with K^+. Roots grown in solution with Al had more positive K^+ flux values than roots grown without Al. Furthermore, higher uptake rates of K^+ were observed at 40 μM Al than at 0 μM Al.

The spatial distribution of the effects of Al on root fluxes was assessed by comparing mean net fluxes of nutrients estimated with the mathematical models of Figs. 2, 3, and 4 between the two Al treatments at various positions along the root axis (Table 1). The adverse effects of Al were observed along the entire length of the root with strong negative effects on Ca^{2+} and Mg^{2+} uptake particularly in the apical region of the root (Table 1). Reductions in estimated Ca^{2+} and Mg^{2+} fluxes were in almost all cases notably higher for BR than for CM. Genotypic differences were also observed with K^+ fluxes, where the genotype CM had a more pronounced change due to Al than the genotype BR.

Effects of external Al on root nutrient uptake Seedlings grown without added Al absorbed similar amounts of Ca^{2+} and Mg^{2+} during the 24-h period studied (Fig. 5),

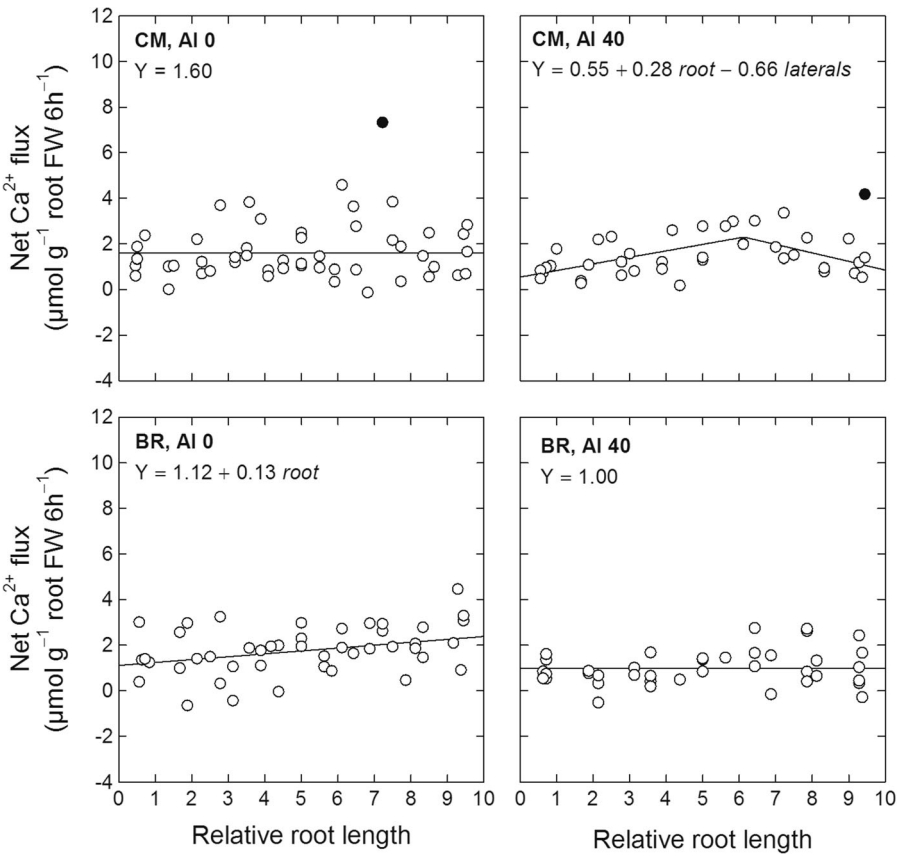

Fig. 2 Net fluxes of Ca^{2+} along the main seminal root of two maize genotypes exposed to 0 or 40 μM Al. Positive values denote uptake whereas negative ones denote efflux. The position of the fluxes along the root is expressed relatively to the length of the root axis. The root tip is positioned at 0 and the root base at 10 on the X-axis. *Black circles* denote outliers

with no significant differences between the two maize genotypes for these nutrients (Table 2).

Exposure of seedlings of both genotypes to Al reduced the uptake of Ca^{2+}, Mg^{2+}, and K^+, except for K^+ in CM (Table 2 and Fig. 5). The adverse effects of Al ions on Ca^{2+}, Mg^{2+}, and K^+ fluxes differed between the two maize genotypes. Aluminium inhibited Ca^{2+} and Mg^{2+} uptake more severely in the Al sensitive genotype BR than in the Al resistant CM. While Al caused a significant reduction of about 50 % in the Ca^{2+} and Mg^{2+} uptake and 20 % in the K^+ uptake of the genotype BR, CM showed no significant reduction (Table 2).

Spatial pattern of Al accumulation along the main seminal root The four root segments that showed the highest values of Al-impaired Ca^{2+} and Mg^{2+} uptake or in which the largest differences in these variables were observed between the two genotypes were selected to study the accumulation of Al. The positions at which the segments were collected refer to the following relative positions along the root axis (from the tip to the base of the root): 0.5, 1.5, 6.0, and 8.0 (Fig. 6).

The amount of Al per unit of root fresh weight decreased from the tip to the more mature region of the root including the segment with the lateral roots and their own tips. This pattern was similar to both genotypes. However, the genotype BR had a mean concentration of Al in the root tip that was significantly higher than that of the genotype CM. Besides the concentration in the root tip, the two genotypes did not differ in concentrations of Al in any other root segment analysed (statistics not shown).

Spatial patterns of nutrient fluxes and citrate exudation The spatial distribution of net Ca^{2+} and Mg^{2+} fluxes (the current study) and that of Al-stimulated citrate exudation (Mariano and Keltjens 2003) of intact roots of genotype CM were summarised

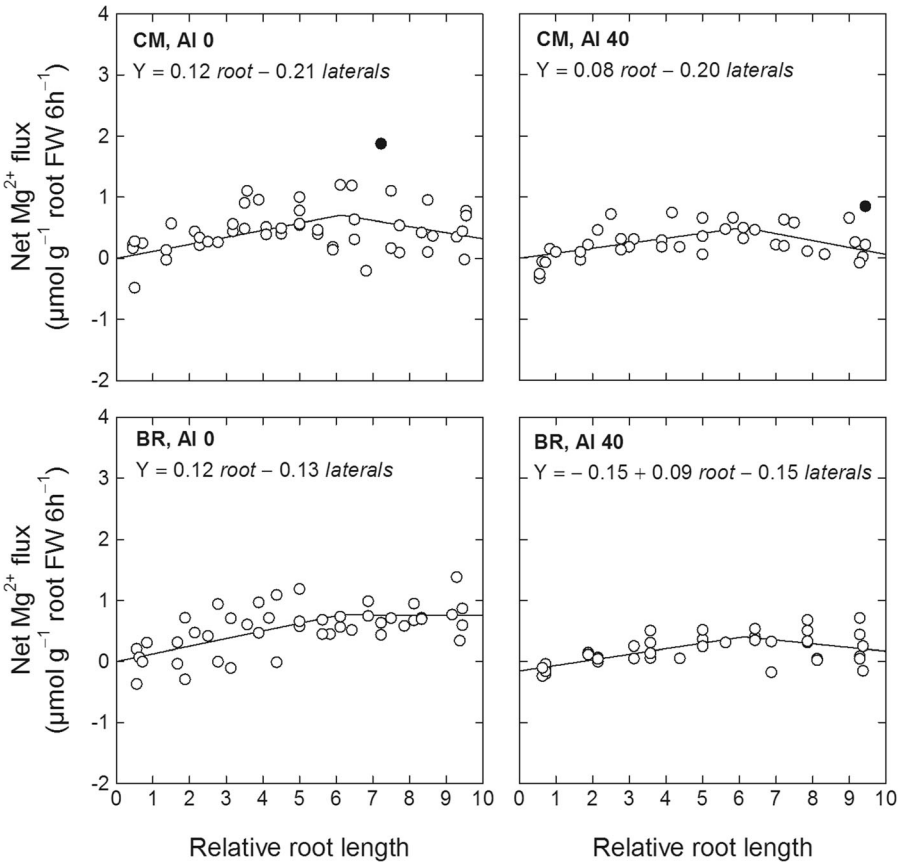

Fig. 3 Net fluxes of Mg^{2+} along the main seminal root of two maize genotypes exposed to 0 or 40 μM Al. Positive values denote uptake whereas negative ones denote efflux. The position of the fluxes along the root is expressed relatively to the length of the root axis. The root tip is positioned at 0 and the root base at 10 on the X-axis. *Black circles* denote outliers

in Fig. 7. It is seen that the two patterns did not match. They were actually very distinct from each other since the highest rates of citrate exudation were observed in root regions where uptake of Ca^{2+} and Mg^{2+} was relatively low. This disagreement was most evident in the apical zone of the root.

Discussion

The Al resistant maize genotype CM maintained higher nutrient uptake rates than the Al sensitive BR when at exposure to Al (Table 1; Figs. 2, 3, 4, and 5). The differences in rates may appear small, but differences considered in terms of total uptake can become larger and more significant when the rates are integrated in time and when they are considered together with the differential ability of the genotype CM to sustain root growth under Al stress. Grown at 40 μM Al, the genotype CM had a reduction of 26 % in the root elongation rate whereas the genotype BR had a reduction of 60 % relatively to its control (Mariano and Keltjens 2004). Yet at this same concentration of 40 μM Al, these differences in Al-inhibited root growth coincided with differences in the release of citrate as stimulated by Al. The root apices of genotype CM released citrate at rates three times as high as those of genotype BR (in pmol/root apice.hour: CM = 247, BR = 82) (Mariano and Keltjens 2003). These greater amounts of citrate exuded into the root apoplast and rhizosphere of genotype CM may explain the smaller amounts of Al adsorbed to its root tips in the current study. Present in a less charged or electroneutral complex, Al no longer adsorbs in great amounts at root exchange sites and less Al accumulates in the root (Keltjens 1995; Postma et al. 2005; Klug and Horst 2010).

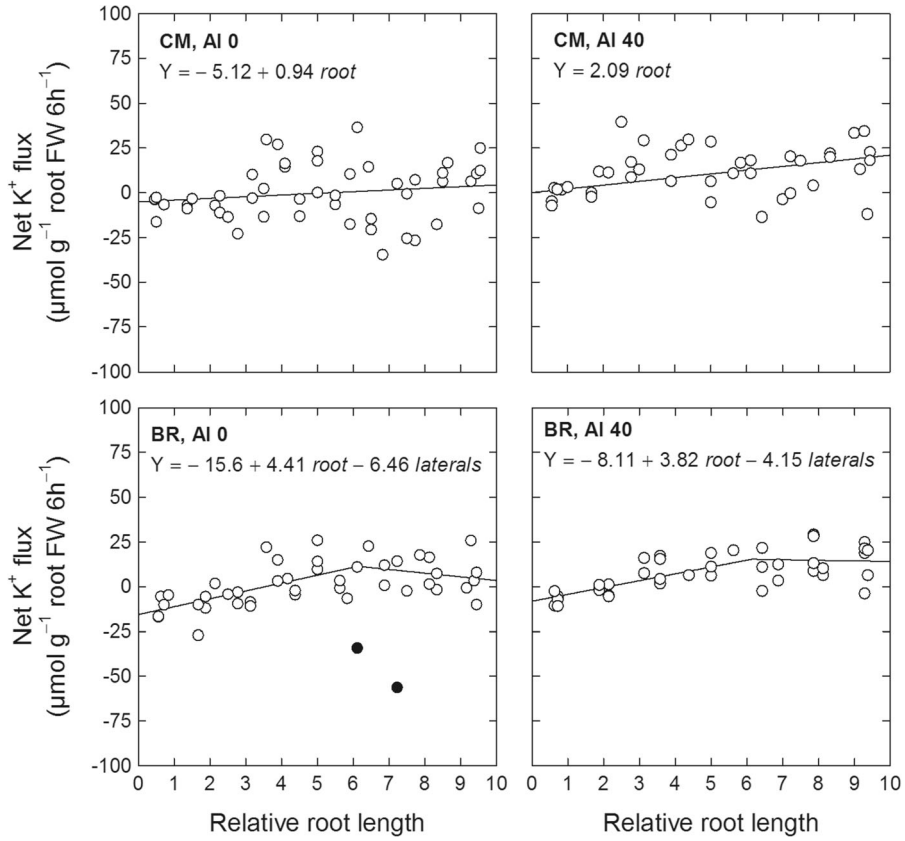

Fig. 4 Net fluxes of K^+ along the main seminal root of two maize genotypes exposed to 0 or 40 μM Al. Positive values denote uptake whereas negative ones denote efflux. The position of the fluxes along the root is expressed relatively to the length of the root axis. The root tip is positioned at 0 and the root base at 10 on the X-axis. *Black circles* denote outliers

Despite a significant difference in Al accumulation in the apical region, there were no other differences in Al accumulation in the roots of the two maize genotypes that could be related to the relatively higher nutrient uptake rates of CM than of BR when exposed to Al (Table 1; Figs. 2, 3, 4, and 5). The sensitivity of nutrient uptake processes to Al in more mature parts of the root seems genotype-specific and does not seem to be related to the capacity of the root to exclude Al. Significant correlations between resistance to Al, generally assessed by measuring the relative root growth under Al stress, and degree of Al-impaired nutrient uptake have invariably been found with plant germplasms showing interspecific or intraspecific variation for Al resistance (Baligar et al. 1993; Rengel and Robinson 1989a). The results of the current study suggest therefore that the ability of a genotype to maintain a less disturbed nutrient uptake when exposed to toxic concentrations of Al^{3+} is associated to its resistance to Al. While the Al-impaired uptake of the apical region may affect primarily the local metabolism and processes like cell division and growth, that of more mature root regions, which are the dominant regions of uptake and translocation of nutrients (Ferguson and Clarkson 1976; Huang et al. 1993), may have implications for translocation of nutrients to the plant upper parts and, therefore, negative impacts on the mineral nutrition of the plant. In the event of mineral nutrient deficiency, malfunctioning of the plant will not only inhibit growth but also disturb the process of nutrient uptake (Marschner 1995).

The results of our study also support the conclusion of Piñeros et al. (2005), who proposed that although OAA release may play an important role in Al resistance, it is not the unique or the main mechanism of resistance to Al in maize. Piñeros et al. (2005) drawn this conclusion after they had studied several potential mechanisms of resistance to Al, although none of them related to mineral nutrient uptake, and had failed to

Table 1 Estimated mean net fluxes of Ca^{2+}, Mg^{2+}, and K^+ based on models of Figs. 2, 3, and 4 at six selected positions along roots of each maize genotype at 0 and 40 μM Al and relative changes in these estimated fluxes due to Al addition (40 μM Al) relative to control (0 μM Al). Position 0.5 refers to the apical zone whereas position 9.5 refers to the basal zone of the root axis

Treatment	Net nutrient flux (μmol g^{-1} root FW 6 h^{-1})					
	Relative root length					
	0.5	1.5	3.0	6.0	8.0	9.5
	Ca^{2+}					
CM						
Al 0	1.60	1.60	1.60	1.60	1.60	1.60
Al 40	0.69	0.98	1.40	2.26	1.60	1.00
Change (%)	−57	−39	−13	+41	0	−38
BR						
Al 0	1.18	1.31	1.50	1.88	2.13	2.32
Al 40	1.00	1.00	1.00	1.00	1.00	1.00
Change (%)	−15	−24	−33	−47	−53	−57
	Mg^{2+}					
CM						
Al 0	0.058	0.173	0.346	0.691	0.521	0.372
Al 40	0.041	0.123	0.246	0.492	0.290	0.120
Change (%)	−29	−29	−29	−29	−44	−68
BR						
Al 0	0.062	0.187	0.374	0.747	0.756	0.751
Al 40	−0.109	−0.019	0.117	0.390	0.288	0.198
Change (%)	−276	−110	−69	−48	−62	−74
	K^+					
CM						
Al 0	−4.65	−3.71	−2.31	0.51	2.38	3.79
Al 40	1.05	3.14	6.28	12.56	16.74	19.88
Change (%)	+123	+185	+372	+2363	+603	+425
BR						
Al 0	−13.40	−8.99	−2.38	10.84	7.56	4.48
Al 40	−6.20	−2.37	3.37	14.84	14.74	14.25
Change (%)	+54	+74	+242	+37	+95	+218

establish a significant correlation between the operation of the mechanisms studied and Al resistance in a collection of six distinct maize genotypes.

It has been proposed that the resistance to Al ions of plasma membrane (PM) properties involved in transport and adsorption of ions in the cell (e.g. transmembrane potential, H^+-ATPase activity, surface negativity) can contribute to genotypic differences in Al resistance. That is based on evidences that depolarization of the PM and inhibition of H^+-ATPase activity by Al occurs more strongly in Al sensitive than in Al resistant genotypes of species like squash and wheat (Ahn et al. 2001; Miyasaka et al. 1989). Independently of the genotype, these adverse effects of Al are more expressed in apical than in basal root zones (Ahn et al. 2001). The Al-inhibited uptake of Ca^{2+} and Mg^{2+} observed with roots of maize in the current study, including the higher sensitivity of the root apices than of the other root segments (Table 1; Figs. 2 and 3), might be explained by a differential resistance of PM properties to Al of the genotypes studied. Al can also effectively block Ca^{2+} channel in the root cell plasma membrane (Huang et al.

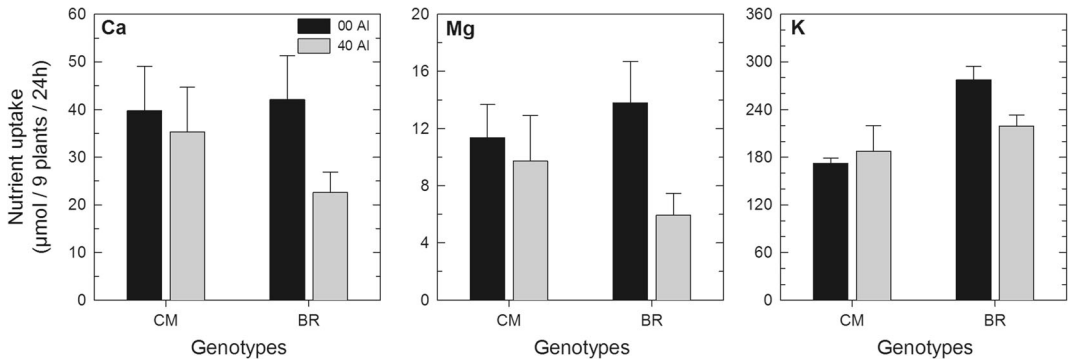

Fig. 5 Net uptake of Ca^{2+}, Mg^{2+}, and K^+ by the main seminal root of two maize genotypes exposed to 0 or 40 μM Al. *Bars* show means of three values whereas *vertical lines* show one standard error

1996; Piñeros and Tester 1993), thus reducing Ca^{2+} influx into the root.

In the case of K^+ uptake, where almost no inhibition was observed, depolarization of PM may be involved too. Sasaki et al. (1994) reported smaller values of membrane depolarization caused by H^+ with Al than without Al in roots of wheat. The smaller depolarization observed in the presence of Al resulted in lower values of K^+ efflux by roots than those observed at the same low pH solution, but without Al. It is also possible that interactions between Al^{3+} and K^+ ions occurred at the root exchange sites and that these interactions resulted in displacement of the monovalent ions and in increase in their absorption through the PM. Roots grown without added Al had small net negative fluxes of K^+ ions and, on the contrary, those grown with 40 μM Al had most often net positive K^+ fluxes (Fig. 4). The mechanistic relation between adsorption of ions in the root apoplast and their absorption into the cell is not yet understood. Rengel and Robinson (1989b) observed that more K^+ and Na^+ as well as less Ca^{2+} and Mg^{2+} were desorbed from roots of ryegrass cultivars exposed to 74 μM Al than from control roots (0 μM Al) whereas Godbold and Jentschke (1998) found high amounts of Al adsorbed to cell walls of Norway spruce roots without finding differences in Mg^{2+} uptake between Al-treated and non-treated roots. The results of these authors seem to suggest that the effects of Al^{3+} on adsorption and absorption of cations might be therefore distinct and independent from each other. If true, this can explain displacement of nutrient cations from the exchange sites without reduction, or even with increase, as shown here for K^+, in their uptake by the root cells.

We found that the spatial patterns of two root processes analysed, net nutrient fluxes and citrate exudation, were remarkably unconnected in root axes of maize (Fig. 7). The disagreement observed was particularly striking in the apical part of the root. Although accounting for 62 % of the total citrate exuded by the whole root (Mariano et al. 2005), the apical part absorbed no more than 8 % of the total Ca^{2+} and 3 % of the total Mg^{2+} taken up by the root axis. The root is therefore exuding

Table 2 Relative changes in total uptake of Ca^{2+}, Mg^{2+}, and K^+ of roots at 40 μM Al (compared to the control, 0 μM Al), and results of the F test applied to the hypotheses for Al treatment within each genotype (CM and BR) (H_0: total $uptake_{Al40}$/total $uptake_{Al0}$=1; H_a: total $uptake_{Al40}$/total $uptake_{Al0} \neq 1$), and for genotype within each Al treatment (0 and 40 μM Al) (H_0: total $uptake_{CM}$/total $uptake_{BR}$ =1; H_a: total $uptake_{CM}$/total $uptake_{BR} \neq 1$). Statistically significant tests are those with a *P*-value ≤ 0.1

Treatment		Change (%)	*P*-value	Decision	Treatment		*P*-value	Decision
Ca	CM	−11	1.0000	Not reject H_0	Ca	Al 0	1.0000	Not reject H_0
	BR	−46	0.0809	Reject H_0		Al 40	0.3827	Not reject H_0
Mg	CM	−14	0.6625	Not reject H_0	Mg	Al 0	0.6625	Not reject H_0
	BR	−57	0.0809	Reject H_0		Al 40	0.3827	Not reject H_0
K	CM	+09	0.6625	Not reject H_0	K	Al 0	0.0809	Reject H_0
	BR	−21	0.0809	Reject H_0		Al 40	0.6625	Not reject H_0

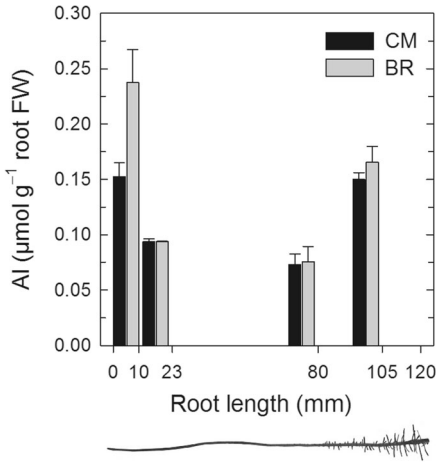

Fig. 6 Al concentration in different segments of the main seminal root of two maize genotypes exposed to 40 μM Al for 24 h. Al was extracted from the root with a 5.0 mM Na_3-citrate solution of pH 7.0. Four segments were collected along the root axis. The Al values were plotted considering a representative root of 12 cm of length. A representative root axis as used in the experiments is shown for reference. *Bars* show means of three replicates whereas *vertical lines* show one standard error

most of its citrate from a region where Ca^{2+} and Mg^{2+} uptake is fairly the lowest.

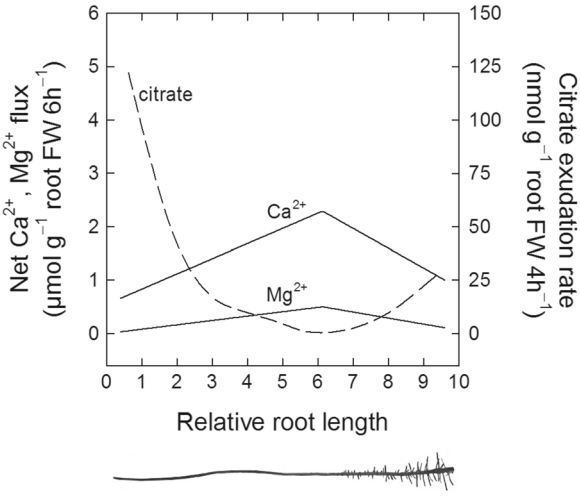

Fig. 7 Net fluxes of Ca^{2+} and Mg^{2+} and exudation rates of citrate along the main root of the Al resistant maize genotype CM. The measurements were done in two independent and separate experiments. Data on net fluxes of Ca^{2+} and Mg^{2+} are from Figs. 2 and 3 of the present work (CM at 40 μM Al) whereas data on citrate exudation were calculated from Fig. 4 of Mariano and Keltjens (2003), where the experimental conditions were exactly the same as the present work. The position of the fluxes along the root is expressed relatively to the length of the root axis. The root tip is positioned at 0 and the root base at 10 on the X-axis. A representative root axis as used in the experiments is shown for reference

The mechanism of root citrate exudation stimulated by Al does not seem to be directly involved in protecting the root from Al-impaired nutrient uptake, because the highly localized citrate exuded is not connected to root regions of high nutrient uptake rates. This lack of concordance suggests that citrate locally exuded at Al exposure is primarily involved in detoxifying Al^{3+} around the root meristem, and thus, in protecting this Al sensitive part for root growth (Ryan et al. 1993). However, with plants grown in soil systems, the intermediate root segment (i.e. the zone with almost no citrate exudation) may benefit from the citrate exuded before by the root apex. The intermediate root zone may get protection from this citrate against Al when following the apex through a citrate-enriched soil environment. This could confer to citrate, exuded by the apex, a positive role in nutrient uptake under acid soil conditions. Maintaining the uptake of nutrients on a higher rate might be a secondary and additional effect of root citrate exudation to make plants more resistant to Al.

The maize roots showed the capacity to absorb Ca^{2+}, Mg^{2+}, and K^+ along their whole extent, but at higher rates in the more mature parts than in the apical zone (Figs. 2, 3, and 4). The roots used in the current study were very young and, with an average length of 12 cm, had much probably their endodermis not yet completely developed and/or suberised at the time they were used in the experiments. The formation of suberised lamellae in the walls of endodermal cells begins in maize plants at 5 to 10 cm from the root tip and is concluded at around 20 cm when all cells in the endodermis become suberised (Ferguson and Clarkson 1975). The developmental stage of the root tissue can, therefore, explain the high uptake rates observed along the root axis in our study. Other factors that additionally can explain the high rates of uptake in a region some distance behind the root tip include the continued permeability of the endodermis to divalent cations (e.g. Ca^{2+}, Cu^{2+}) despite its complete suberization (Ferguson and Clarkson 1975; Ranathunge et al. 2005) and the transient leakages in its structure in the region where lateral roots are initiated (Häussling et al. 1988; Marschner 1995).

In conclusion, based on the differential sensitivity of nutrient uptake to Al of the root axis of the two maize genotypes and especially on the extent of the root zones where these differences are expressed, it is suggested that the less disturbed nutrient uptake pattern of a genotype in the presence of Al is associated with its resistance to Al. Whether the less Al-impaired nutrient

uptake causes resistance to Al or it is a consequence of the Al resistance phenomenon remains to be determined.

Acknowledgments We thank Dr. Sidney N. Parentoni (CNPMS-Embrapa, Brazil) for kindly supplying the maize seeds and Dr. Ellis Hoffland (Wageningen University, The Netherlands) for valuable suggestions and comments on the manuscript. We also greatly appreciated constructive comments from two anonymous reviewers. EDM gratefully acknowledges financial support from the Brazilian Institutions CNPq (grant #477231/08-0) and FAPESP (grants #04/04821-0 and #11/00189-0).

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