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Does aluminium affect root growth of maize through interaction with the cell wall – plasma membrane – cytoskeleton continuum?

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Abstract

The mechanism of aluminium-induced inhibition of root elongation is still not well understood. It is a matter of debate whether the primary lesions of Al toxicity are apoplastic or symplastic. The present paper summarises experimental evidence which offers new avenues in the understanding of Al toxicity and resistance in maize. Application of Al for 1 h to individual 1 mm sections of the root apex only inhibited root elongation if applied to the first 3 apical mm. The most Al-sensitive apical root zone appeared to be the 1–2 mm segment. Aluminium-induced prominent alterations in both the microtubular (disintegration) and the actin cytoskeleton (altered polymerisation patterns) were found especially in the apical 1–2 mm zone using monoclonal antibodies. Since accumulation of Al in the root apoplast is dependent on the properties of the pectic matrix, we investigated whether Al uptake and toxicity could be modulated by changing the pectin content of the cell walls through pre-treatment of intact maize plants with 150 m*M* NaCl for 5 days. NaCl-adapted plants with higher pectin content accumulated more Al in their root apices and they were more Al-sensitive as indicated by more severe inhibition of root elongation and enhanced callose induction by Al. This special role of the pectic matrix of the cell walls in the modulation of Al toxicity is also indicated by a close positive correlation between pectin, Al, and Al-induced callose contents of 1 mm root segments along the 5 mm root apex. On the basis of the presented data we suggest that the rapid disorganisation of the cytoskeleton leading to root growth inhibition may be mediated by interaction of Al with the apoplastic side of the cell wall – plasma membrane – cytoskeleton continuum.

Abbreviations: CEC – cation exchange capacity; CMT – cortical microtubule; DFT – distance from root tip; DTZ – distal part of the transition zone; FITC – fluorescein isothiocyanate; GaE – galacturonic acid equivalent; MF – microfilament; NS – nutrient solution; PBS – phosphate buffered saline; PE – Pachyman equivalent; TZ – transition zone

Introduction

The root apex plays a major role in aluminium (Al) perception and response in plants (Delhaize and Ryan, 1995; Horst, 1995; Kochian, 1995; Taylor, 1995 for recent reviews). The mechanism of Al-induced inhibition of root elongation is still not well understood. Evidence has been presented that Al enters the root symplast in appreciable quantities (Zhang and Taylor, 1990; Tice et al., 1992; Lazof et al., 1994). These

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results have been challenged by Rengel (1996). Horst (1995) focused the attention on the role of the apoplast in Al toxicity regarding short-term inhibition of root elongation by Al. Aluminium strongly binds to the cell wall of root epidermal and cortical cells (Delhaize et al., 1993a). This is mainly due to the negative charge properties of the pectic matrix of cell walls (Blamey et al., 1990) which determine cation binding and distribution in the apoplast and thus at the plasma-membrane surface (Kinraide et al., 1992). Consideration of the charge properties has greatly contributed to a better understanding of the toxicity of Al species (Kinraide, 1997) and cation interactions in the expression of Al and other mineral-element toxicity (Kinraide, 1994). Generally, conditions that reduce the negativity of the apoplast will alleviate Al toxicity. The root CEC as a measure of the negativity of the root apoplast has only partly been successfully used to characterise genotypic differences in Al resistance (Blamey et al., 1990; Büscher et al., 1990). The major differences in root CEC between monocots and dicots are not related to their respective Al resistance (Grauer and Horst, 1992) clearly indicating that other factors such as release of Al-binding root exudates (Delhaize et al., 1993b; Basu et al., 1994; Pellet et al., 1995) are equally or even more important for genotypic differences in Al resistance. Whether Al-induced inhibition of root elongation is due to direct interaction of Al with cell-wall constituents or plasma-membrane and cytosolic lesions cannot be decided yet.

The debate on symplastic versus apoplastic lesions of Al toxicity urgently needs to be re-evaluated taking increasing evidence on the cell wall – plasma membrane – cytoskeleton continuum into consideration (see the review of the state of knowledge meeting — report by Miller et al., 1997). The role of the cytoskeleton in the regulation of root growth and development is now well established (Barlow and Parker, 1996; Baluška et al., 1997). The effect of Al on the cytoskeleton in plant cells has rarely been investigated until now. Alfano et al. (1993) studied effects of Al on the actin cytoskeleton in plants, investigating long-term Al treatments on the liverwort *Riccia fluitans*. Later, Grabski and Schindler (1995) reported that exposure to Al increased rigor in actin MFs in suspension-cultured cells of soybean. Sasaki et al. (1997) presented preliminary evidence of Al-induced alteration in the stability of cortical microtubules in wheat roots. Just recently, Blancaflor et al. (1998) concluded from their study that stabilisation of microtubules and reorientation of actin MFs in the elongation zone coincided with root growth inhibition in maize. A decade ago, MacDonald et al. (1987) reported that Al directly influenced tubulin polymerisation *in vitro*. Aluminium is known to interact either directly and/or indirectly with factors reported to affect the organisation of the cytoskeleton such as levels of cytosolic Ca^{2+}, Mg^{2*} and calmodulin (Haug, 1984; Martin, 1988), cell surface electrical potential (Kinraide et al., 1992), callose formation (Wissemeier et al., 1987), and plasma-membrane lipids (Zhang et al., 1997). In a study inspired by Ryan et al. (1993) we recently presented evidence that in the Al-sensitive maize cultivar 'Lixis' the distal part of the transition zone (DTZ) is the most Al-sensitive apical root zone (Sivaguru and Horst, 1998). In the present study, we extended the characterisation of the sensitivity of the root apex to the structural integrity of the cytoskeleton especially in this zone and characterised the role of pectin/Al interaction for Al uptake and Al sensitivity by using Al-induced callose formation as a sensitive indicator (Horst et al., 1997).

Materials and methods

Seeds of the equally Al-sensitive maize (*Zea mays* L.) cultivars Lixis or Helix were germinated between filter paper for 4 days. Uniform seedlings were transferred to aerated nutrient solution (NS) having the following composition [in μM]: $CaSO_4$, 250; KNO_3, 400; NH_4NO_3, 200; $MgSO_4$, 100; FeEDDHA, 20; $MnSO_4$, 1; $ZnSO_4$, 0.2; $CuSO_4$, 0.2; KH_2PO_4, 10; H_3BO_3, 8; $(NH_4)_6Mo_7O_{24}$, 0.1. Plants were cultivated in a growth chamber under controlled environmental conditions with 70% relative humidity, 30 and 25 °C day/night temperature and 300 μmol m^{-2} s^{-1} photon flux density during the 16 hour day.

In order to modify the pectin content of roots, seedlings were grown in presence and absence of 150 mM NaCl. After 5 days the plants were transferred to NS without NaCl for one day and the pH was adjusted stepwise to 4.3 within 12 h. Plants were then treated for 24 h with 0, 25 or 50 μM Al, added to the NS from an Al atomic spectroscopy standard solution ($AlCl_3 \cdot 6H_2O$, 1000 mg L^{-1}, Fluka, Germany).

For the study of the cytoskeleton, the seedlings were transferred to fresh NS to which Al was added from the stock solution to achieve a final monomeric Al concentration of 90 μM (measured using the aluminon method according to Kerven et al., 1989). Seedlings were removed after various Al treatment

periods (0, 1, 6, 12, 24, 48 h) for immunofluorescence analysis.

Using a modified PVC-block method described in detail earlier (Sivaguru and Horst, 1998), which allowed the measurement of the root elongation-rate even more sensitively with a microscope, 1 mm zones of the root apex were subjected individually to Al (90 μM monomeric Al in 0.6% agarose). Aluminium contents of root segments were quantified by AAS (UNICAM 939 QZ graphite furnace atomic absorption spectrophotometer, Analytical Technologies Inc., Cambridge, UK) after digestion in concentrated ultra-pure HNO_3. Callose contents were determined according to Köhle et al. (1985).

For the determination of the pectin contents, roots were washed three times and homogenised (RW 20, Jahnke and Kunkel) in alcohol (96%) and dried at 60 °C. The remaining cell-wall material was weighed and hydrolysed according to Ahmed and Labavitch (1977) varying the incubation times to 5 min in H_2SO_4 (concentrated) and to 5 h after each step of water addition. The uronic acid content was determined according to Blumenkrantz and Asboe-Hansen (1973) using galacturonic acid as reference. For the study of the cytoskeleton, after Al treatments, 1 cm root apices were excised and processed as described by Baluška et al. (1992). Briefly, longitudinal sections (8 μm thick) embedded in low-melting point (37 °C) Steedman's wax were mounted on slides coated with Meyer's albumen. The sections were dried overnight at room temperature, dewaxed in ethanol, rehydrated in a graded ethanol/PBS series, and allowed to stand in SB buffer (SB: 50 m*M* PIPES, 5 m*M* EGTA and 5 m*M* $MgSO_4$, pH 6.9) for 30 min. After digestion of cell walls with 1% hemicellulase, the median sections were incubated with a mouse monoclonal antibody raised against chicken brain α-tubulin (Amersham International, Buckinghamshire, UK) diluted 1:200 with phosphate buffered saline (PBS) for 60 min at room temperature. Then, sections were stained with fluorescein isothiocyanate (FITC) conjugated anti-mouse IgG raised in goat (Sigma Chemical Co., St. Louis, MO, USA) diluted 1:20 with PBS. For the localisation of actin MFs, the dewaxed and rehydrated sections were treated for 10 min with absolute methanol at −20 °C and transferred to SB for 30 min. They were then incubated with mouse-monoclonal anti-actin antibody (clone C4) raised against chicken gizzard actin (ICN Biomedicals, Costa Mesa, CA, USA), diluted 1:200 in PBS for 90 min at room temperature. After a further rinse with SB, the actin antibody-conjugated sections were stained with FITC conjugated anti-mouse IgG raised in goat as described above for tubulin. After staining with toluidine blue (to quench autofluorescence) and mounting in an anti-fade mountant (Baluška et al., 1992), microtubules and actin MFs were visualised in an Axiovert 405 m inverted microscope (Zeiss, Oberkochen, Germany) equipped with epifluorescence and standard FITC exciter and barrier filters (BP 450-490, LP 520). Photographs were taken on Kodak T-Max film rated at 400 ASA.

Results

The distal part of the transition zone (DTZ) is the most Al-sensitive apical root zone

In agreement with our recent report (Sivaguru and Horst, 1998), application of Al to individual 1 mm segments of the root apex led to severe inhibition of the root elongation-rate only when the first two mm of the apex and especially the 1–2 mm zone were exposed to Al for 1 h (Figure 1). Application of Al to root zones >2.5 mm from the root tip had no effect on root growth. Treatment with Al of the entire root apex was equally inhibitory to root elongation as application only to the 1–2 mm zone.

Aluminium induces disorganisation of the cytoskeleton in apical root zones

In control (-Al) root apices, cortical microtubules (CMTs) were well organised especially in the root epidermis and the outer cortical cells (Figure 2A). Treatment of the roots with Al for 12 up to 24 h (Figure 2B, C) led to increasing randomisation and disintegration of CMTs. These Al-induced alterations of the CMT structure were particularly severe in the DTZ in agreement with the high Al sensitivity of this apical root zone. After 48 h (Figure 2D) most outer cortical cells were lacking distinct cortical and endoplasmic microtubules around nuclei, and unusual periclinal divisions had been induced which could explain the observed and frequently described swelling of Al-treated root tips. First structural modifications on CMTs were visible as early as 1 h after Al and only occurred in the epidermal and outermost cortical cells of the DTZ (not shown). Effects of Al on the actin microfilament (MF) organisation could be detected especially in the distal and middle part of the TZ as increased actin fluorescence not only in the root epidermis and outer (Figure 3A–D) but also in inner

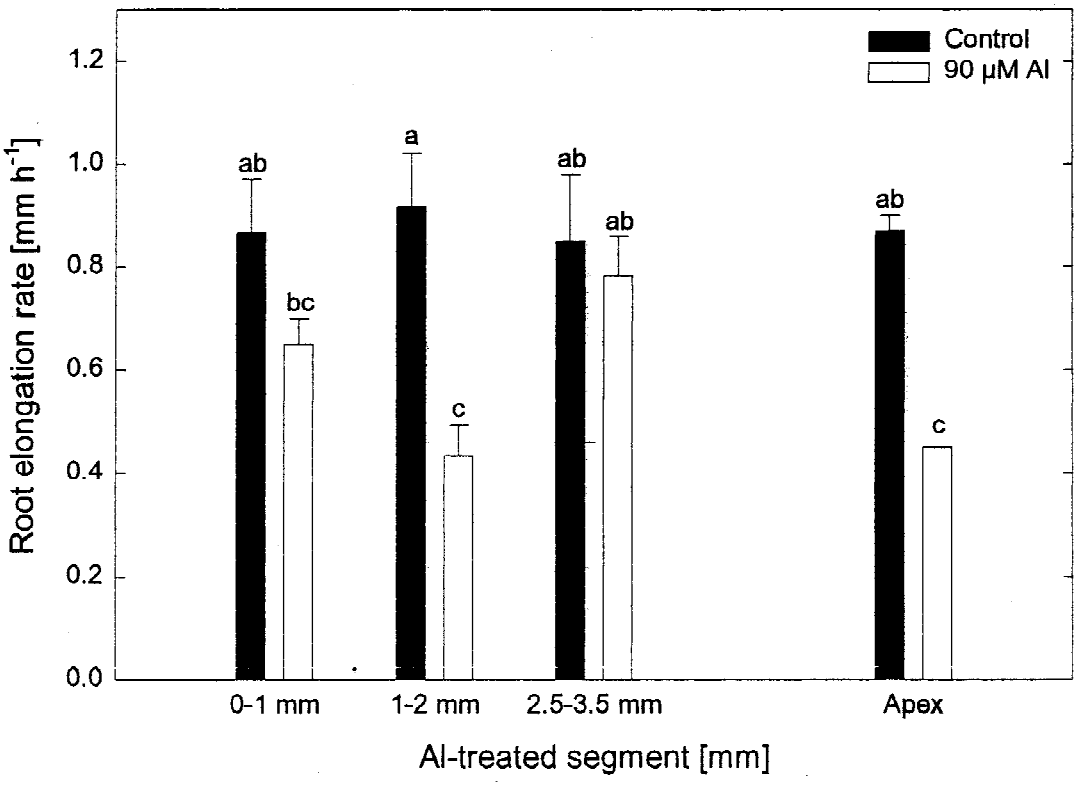

Figure 1. Effect of aluminium (90 μM) application in agarose medium to individual 1 mm zones of the root apex on the root-elongation rate of intact plants of Al-sensitive maize (cv. Lixis). Means with different letters are significantly different ($P \leq 0.05$, Tukey test) with regard to the Al effect; ±SD of five independent replicates.

cortex cell layers (Figure 3E, F) and in the metaxylem (not shown). Such modifications of actin MF were evident already after 1 h Al treatment (not shown), whereas peculiar 'actin-positive dots' (or fragmented actin filaments) (Figure 3F) appeared only after 6 h.

Aluminium uptake and toxicity is modulated by the cell-wall pectin-content in root apices of intact plants

Modification of the cytoskeleton after short-term Al treatment does not necessarily require direct Al interaction in the symplast. Increasing evidence of the existence of a cell wall – plasma membrane – cytoskeleton continuum in plant cells suggests that the deterioration of the cytoskeleton could be induced through Al interference from the apoplastic side, where Al is rapidly bound and accumulated. For this accumulation, Al binding to the pectic matrix is particularly important (see Introduction). Hence, we investigated whether Al uptake and toxicity could be modulated by manipulating the pectin content of the cell walls. Pre-treatment of the intact maize plants with 150 mM NaCl for 5 days resulted in significantly ($P \leq 0.05$) higher pectin contents of the cell walls of the 5-mm root apices, 60 mg GaE (g CWM)$^{-1}$ in the controls compared to 78 mg GaE (g CWM)$^{-1}$ after NaCl pre-treatments. Accumulation of both total (Figure 4A) and $BaCl_2$-non-exchangeable Al (Figure 4B), representing more specifically bound Al, in the root apex was enhanced in the NaCl-adapted plants with higher pectin content. Plants adapted to NaCl were more Al-sensitive as indicated by more severe inhibition of root elongation (Figure 5A) and enhanced relative callose induction by Al (Figure 5B). Aluminium-induced callose induction relative to digitonin-induced callose induction takes into consideration possible differences in the general callose-producing capability of the roots due to pre-treatment with NaCl, and therefore best characterises the Al-specific effect.

Since Al accumulation by cells and the root apex is modulated by the pectin content we tested the hypothesis that the earlier observed gradient in Al and Al-induced callose contents of 1 mm zones of the root apex (Sivaguru and Horst, 1998) is due to differences

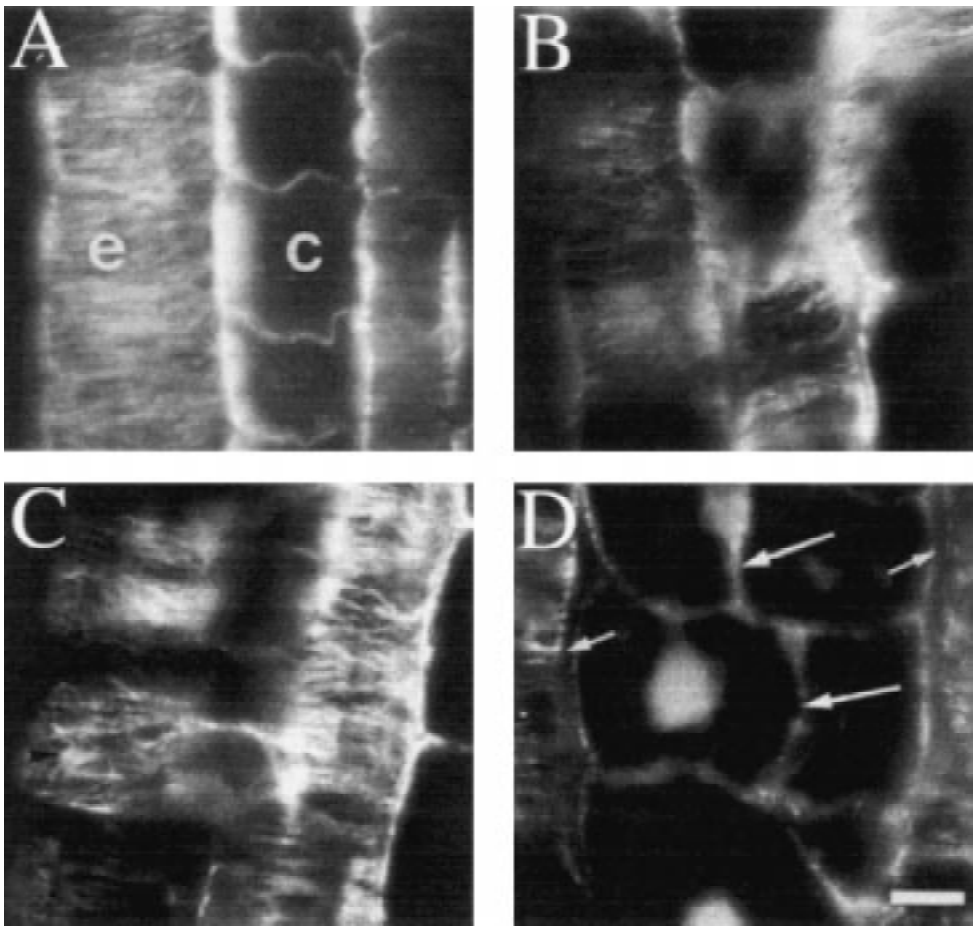

Figure 2. Aluminium-mediated alterations of the CMT cytoskeleton and altered cellular morphology of the root apex of an Al-sensitive maize (cv. Lixis). The DTZ region of roots treated with 90 μM monomeric Al for 0 (control) (A) and 12 h (B), 24 h (C), and 48 h (D) (e – epidermis, c – outer cortex cells). (A) Well organised CMTs of controls. (B, C) Al treatment induced altered polymerisation and disintegration of CMTs. (D) After 48 h Al treatments induced periclinal divisions in the outer cortex cells, cell widening increased cell-cell gaps and complete disintegration of CMTs (arrows). Bar represents 10 μm.

in the pectin content of these root zones. Indeed, pectin contents showed a decreasing gradient from the apex to more basal root zones (Figure 6) resulting in a corresponding gradient in Al contents (Figure 7A). Aluminium-induced callose formation (Figure 7B) increased with increasing pectin content, confirming the above made statement, that the 1–2 mm apical root zone (DTZ) is the most Al-sensitive zone (Figure 7B). However, the lower callose formation of the apical 0-1 mm meristematic zone in spite of the highest pectin and thus Al content is not in agreement with this conclusion.

Discussion

Externally applied Al rapidly binds to the root cell walls (Zhang and Taylor, 1989; Blamey et al., 1990). The extent to which Al^{+n} is bound depends on the cation exchange capacity of the roots (CEC) resulting primarily from the negative charges of the pectic matrix. These charges create electrical potential gradients which determine binding and distribution of ions in the root apoplast. This concept has been applied by Kinraide et al. (1992) to explain amelioration by H^+ and other cations through reduction of Al^{3+} activity at the plasma membrane. Blamey et al. (1992) and Grauer and Horst (1993) developed principally similar but conceptually different approaches assuming that binding of Al to Al-sensitive binding sites (pectins)

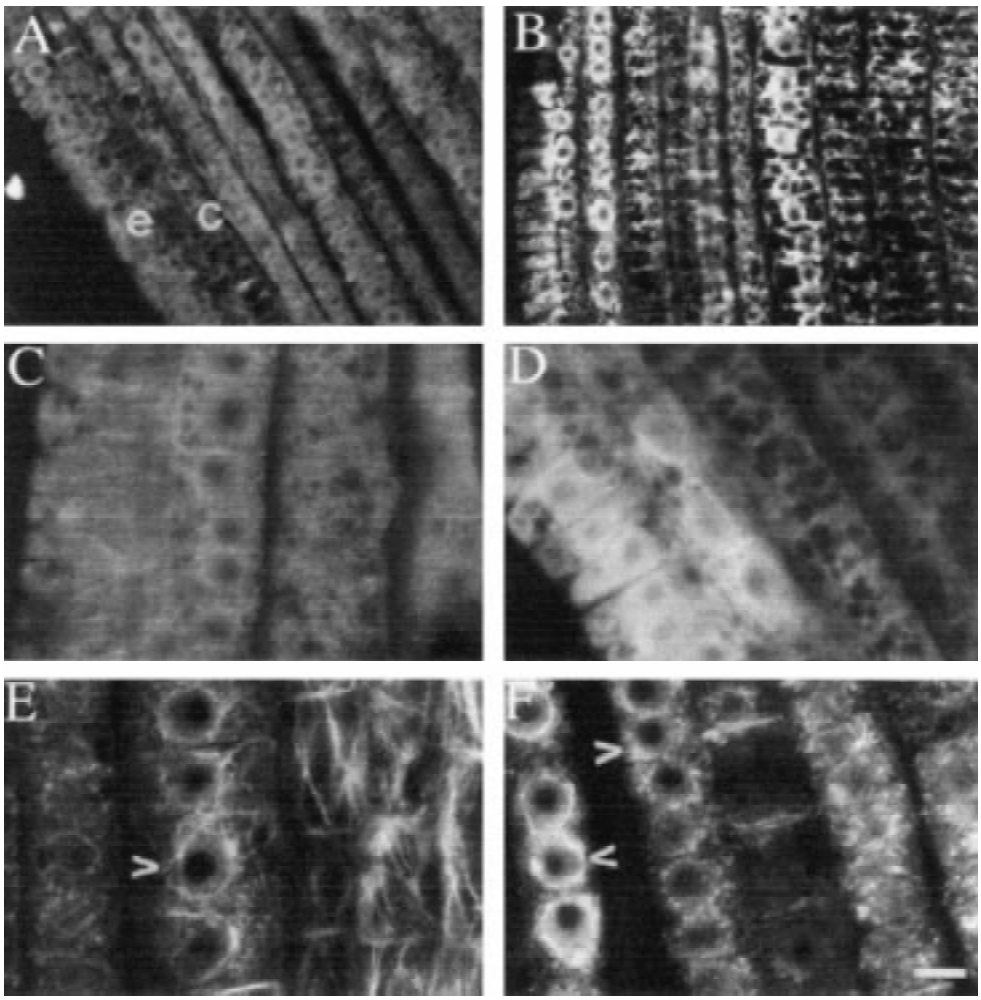

Figure 3. Effect of aluminium on the actin MFs in intact root cells of an Al-sensitive maize (cv. Lixis). All images are from the DTZ region of the root apex. Comparable regions of control (A, C, E) and treatment with 90 μM monomeric Al for 6 h (B, D, F) (e – epidermis, c – outer cortex cells). (A, B, C, D) Note the increase in the actin fluorescence intensity especially in the epidermis and outer cortex cells after Al treatment compared to control. (E, F) The filamentous nature of actin MFs (arrow head in E) in the middle and inner cortex cells after Al treatments appear as fine dots or fragmented MFs (arrow heads in F). Bar represents 23 μm for A and B, and 10 μm for C–F.

determines Al-induced inhibition of root elongation. This may lead to impairment of physical properties of the cell wall necessary for cell elongation such as extensibility and permeability (Blamey et al., 1993; Pritchard, 1994). The role of the root CEC in modulating Al toxicity is further supported by the negative relationship between root CEC/Al accumulation in roots and the adaptation of genotypes to acid soils with high Al supply and/or Al resistance (Blamey et al., 1990; Büscher et al., 1990; Maison and Bertsch, 1997). However, across a larger range of plant genotypes a clear relationship between root CEC and Al resistance could not be found (Grauer, 1992). Among other reasons (Horst, 1995), this might be due to the fact that the role of the CEC/Al is masked by other genotype-specific mechanisms of Al resistance which are equally or even more important. Recently, Yermiyahu et al. (1997) concluded that surface negativity of plasma membrane vesicles could only partly explain the differences in Al resistance of two wheat cultivars.

To our knowledge, direct evidence of the role of root cell-wall pectin-content in modulation of Al toxicity/Al resistance is clearly lacking. We therefore subjected maize plants for 5 days to an elevated NaCl concentration. In agreement with previous reports (Iraki et al., 1989a,b; Zhong and Läuchli, 1993) this treatment led to increased pectin contents in the

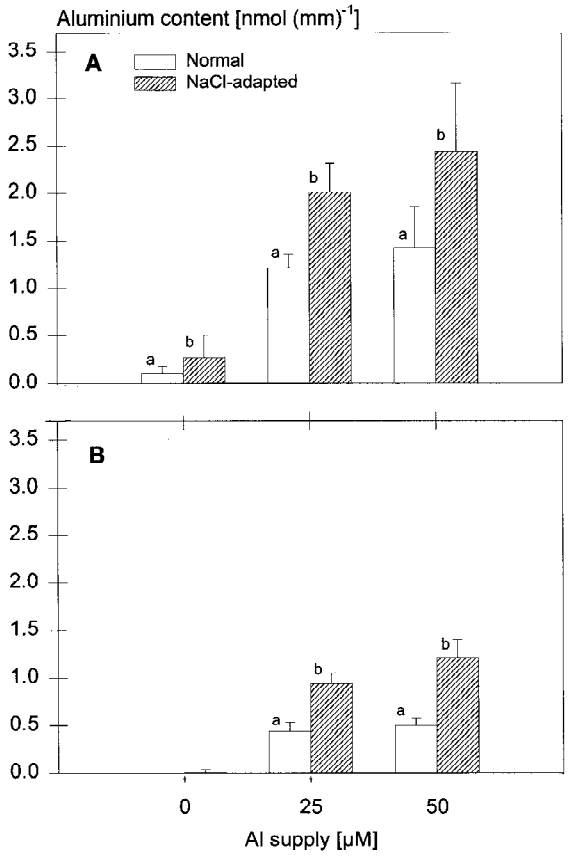

Figure 4. Total (A) and Ba^{2+}-non-exchangeable (B) aluminium contents of root tips (5 mm) of normal and NaCl-adapted roots of Al-sensitive maize (cv. Lixis). Incubation with 0, 25 or 50 μM Al, at pH 4.3 for 1 d, exchange with 50 mm $BaCl_2$ at pH 4.3 for 15 min. Means with different letters are significantly different ($P \leq 0.05$, Tukey test) with regard to the NaCl pre-treatment effect; ±SD of seven independent replicates, each comprising two 5 mm root apices.

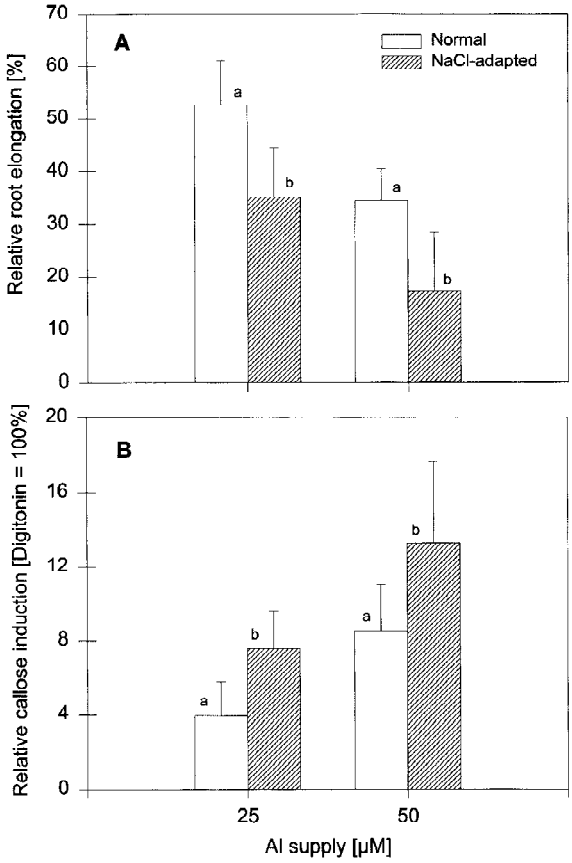

Figure 5. Relative root elongation (A) and relative callose induction of root tips (5 mm) (B) of normal and NaCl-adapted maize roots (cv. Lixis). Incubation at 0, 25, 50 μM Al, or 10 μM Digitonin at pH 4.3 for 1 d. Means with different letters are significantly different ($P \leq 0.05$, Tukey test) with regard to the NaCl pre-treatment effect; ±SD of 20 (root elongation) or 6 (callose) independent replicates.

cell walls of the root apices (see above). The modification of the cell wall composition by NaCl has been attributed to inhibition of polysaccharide degradation (Zhong and Läuchli, 1993) and reduced cellulose synthesis through disturbance of plasma membrane integrity (Zhong and Läuchli, 1988). It has to be emphasised that in the study presented here the plants were grown for 1 day without NaCl after the salt treatment prior to Al exposure. Therefore, direct interference of Al with NaCl and physiological effects related to the presence of NaCl in the apoplast such as modification of the plasma membrane properties were less likely to be important. However, reduced callose formation and slightly inhibited root growth indicated that the NaCl-pretreated plants still suffered some physiological lesions. Therefore, digitonin-induced callose induction was used as a reference for Al-induced callose formation both in control and NaCl-adapted plants. Digitonin induces callose formation strongly (Waldmann et al., 1988).

Three parameters for Al toxicity/Al resistance were evaluated: Al concentration in the root tips which has been shown to be positively related to Al toxicity (Rincon and Gonzales, 1992; Delhaize et al., 1993a; Llugany et al., 1994), induction of callose formation in root apices as a sensitive indicator of Al sensitivity (Wissemeier et al., 1987; Zhang et al., 1994; Horst et al., 1997) and root elongation which is a direct measure for the effect of Al on root growth. All three parameters consistently show that plants with enhanced pectin content due to NaCl pretreatment exhibit higher Al sensitivity (Figures 6 and 7). Based on

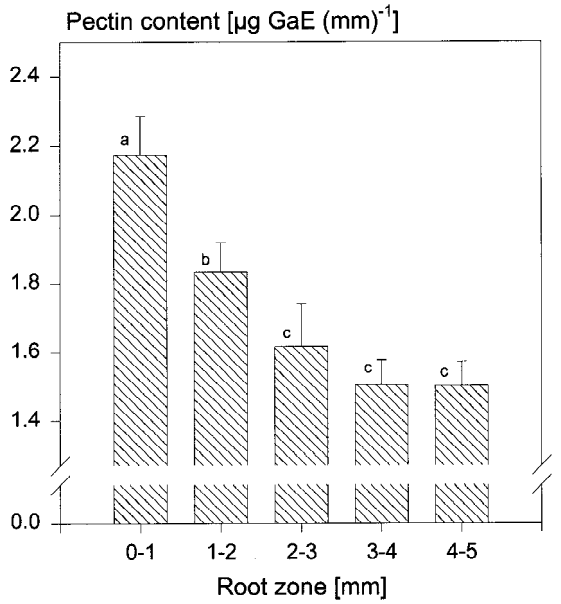

Figure 6. Pectin contents of different zones of the root apex of maize (cv. Lixis). Means with different letters are significantly different ($P \leq 0.05$, Tukey test); ±SD of 12 independent replicates, each comprising fifteen 1 mm segments of corresponding root zones.

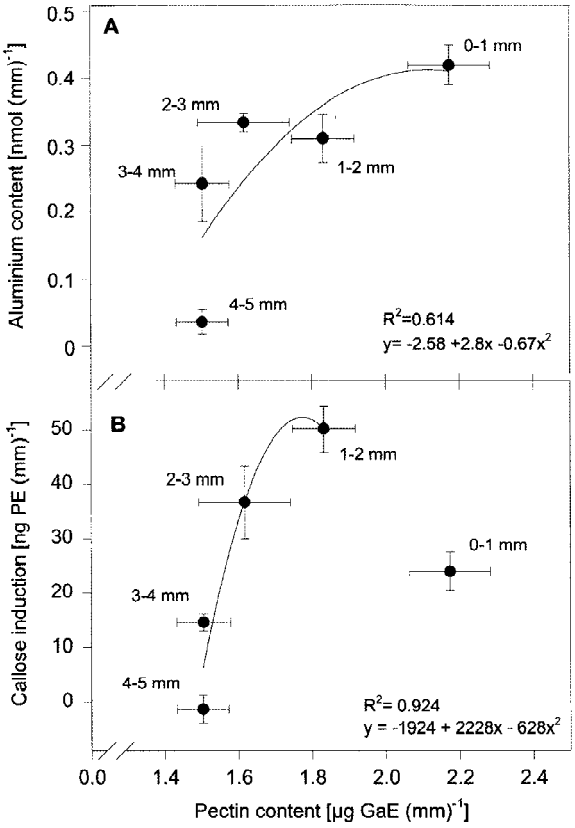

Figure 7. Correlation between Al contents (A) and Al-induced callose formation (B) and pectin contents of different zones of the root apex of maize (cv. Lixis). Incubation at 50 μM Al and pH 4.3 for 3 h. Means ± SD of 7 (Al) or 12 (pectin) independent replicates, each comprising 10 (Al) or 15 (pectin) 1 mm segments of corresponding root zones.

these results we tested the hypothesis that the spatial Al sensitivity of the root apex shown in Figure 1 and Figure 2 and by Sivaguru and Horst (1998) could be related to the pectin content of the different apical root zones. In fact, pectin content showed a steep gradient along the root tip with its maximum in the apical 1 mm (Figure 6). The positive correlation between pectin and Al content of the apical root zones after short-term Al treatment suggests that the pectin content is a factor contributing to differences in short-term Al accumulation by different root zones. High pectin content and thus Al accumulation could explain the greatest Al sensitivity of the 1–2 mm apical root zone (Figure 1). This is also supported by the highest Al-induced callose content in this zone (Figure 7B). However, the highest pectin content and thus Al content in the 1 mm apical section found in this experiment (Figure 7B) is not consistent with the lower Al-induced callose content (Figure 7B). We attribute this discrepancy to the fact that in the latter experiment in nutrient solution, the 1-mm root apex comprised more mucilage containing a high percentage of uronic acids (Peretto et al., 1990 and references therein) strongly binding Al (Archambault et al., 1996) and thus protecting the root apex from Al injury (Horst et al., 1982). Also, total pectin content may only partly reflect the Al-binding properties of cell walls because they depend on the degree of esterification (Ostatek-Boczynski et al., 1995) which may vary from 50–80% (McCann et al., 1994) depending on cell development. Cell-wall loosening occurs in maize roots in the area of 1-3 mm (Pritchard, 1994). The wall loosening seems to be related to a high degree of esterification which is modulated through pectin methylesterase (Liners and Van Cutsem, 1992). Therefore, a better correlation between pectin content and Al binding of different apical root zones could be expected if the degree of esterification of the pectin and its modification by Al is taken into consideration. There is no doubt that the indirect evidence for a special role of cell-wall pectin in modulating Al toxicity provided here and further supported by ongoing work using maize cell-suspension cultures with experimentally manipulated pectin contents, needs to be

supported by a direct chemical characterisation of the binding of Al to pectin.

The observation of Al-induced structural modifications in the CMTs and the actin MF networks shown in Figures 2 and 3 especially for the DTZ, confirms the special role of this apical root zone in Al perception and response of maize. Baluška et al. (1997) presented a detailed account of actin and its dynamics in the growth and development of maize root apex. The present observations of disturbed CMTs and increased actin fluorescence indicating alteration in polymerisation of cytoskeleton, could be detected in the early hours of Al treatment. In contrast to our results, Grabski and Schindler (1995) reported that Al induced the rigor within the actin network in suspension-cultured soybean cells. However, in accordance with our results, Sasaki et al. (1997) presented preliminary evidence for Al-induced depolymerisation or stabilisation of CMTs in wheat roots. Just recently, Blancaflor et al. (1998) concluded from their study that stabilisation of microtubules and reorientation of actin microfilaments coincided with root growth inhibition in maize. Furthermore, they could not detect Al-induced alteration in the cytoskeleton earlier than after 3 h of Al treatment. We attribute this to the fact that they did not focus their study on the DTZ (Sivaguru and Horst, 1998) but on the central elongation zone.

The exact mechanism by which Al affects the structural organisation of the cytoskeleton is not yet known. A direct interaction in the symplast cannot be excluded on the basis of the reported rapid uptake of Al into the cytoplasm (Lazof et al., 1994). However, considering the very low transfer of Al across the plasma membrane reported by Rengel (1996) an apoplastic lesion of Al toxicity appears to be more likely. The rapidly increasing understanding of a cell wall – plasma membrane – cytoskeleton continuum in higher plants offers alternative explanations (Wyatt and Carpita, 1993; Miller et al., 1997a,b). There are several pathways by which Al might affect the cytoskeleton from the apoplastic side:

(i) Al disturbs the phytohormone equilibrium. In this context the similarity between Al and auxin excess or auxin transport-inhibitors on root morphology (subapical root swelling, unusual periclinal divisions) (Ruegger et al., 1997) and on the organisation of the cytoskeleton (Blancaflor and Hasenstein, 1995; Baluška et al., 1996) appears to be especially important. Interestingly, Hasenstein et al. (1988) and Hasenstein and Evans (1988) presented evidence that the observed effect of localised Al supply on root curvature was related to local inhibition of basipetal auxin transport in the root cortex. This interference of Al with the epidermal and/or cortical auxin flow could explain the rapid effect of Al in the DTZ on cell elongation in the central elongation zone.

(ii) Al depolarises the plasma membrane. Depolarisation of the plasma membrane may trigger modifications of membrane-bound cytoskeleton organisation (Giddings and Staehelin, 1991). We found that Al supply led to rapid depolarisation of the plasma membrane only in the DTZ of an Al-sensitive maize cultivar (Sivaguru et al., 1999). A similar effect of Al on plasma-membrane properties has been reported by Takabatake and Shimmen (1997) in *Chara*. However, Papernik and Kochian (1997) found such depolarisation only in Al-resistant and not in Al-sensitive wheat cultivars and attributed this partly to enhanced organic acid excretion which is a major mechanism of Al detoxification (Delhaize et al., 1993b; de la Fuente et al., 1997).

(iii) Al interferes with the phosphoinositide signal transduction cascade involving cytosolic [Ca^{+2}]. There are contrasting reports about the effect of Al on cytosolic [Ca^{+2}]. Whereas Jones et al. (1998a) reported an Al-induced decrease in the levels of cytosolic [Ca^{+2}] in tobacco suspension cells, they showed that Al increased cytosolic [Ca^{+2}] in root hairs of *Arabidopsis* (Jones et al., 1998b) which is in agreement with studies by Lindberg and Strid (1997) who presented evidence that Al induces an increase in cytosolic [Ca^{+2}] in wheat root cells. This could explain Al-induced callose formation (Figure 7) which in addition to structural modification of the plasma membrane requires increased cytosolic [Ca^{+2}] as a prerequisite (Kauss et al., 1990). Callose deposition has been related to disorganisation of CMT arrays of pollen tubes (Pierson and Cresti, 1992). Recent work of Grabski et al. (1998) suggests that Al may affect the cytoskeleton via Ca^{2+}-regulated phosphatases and kinases which is in agreement with results of Baskin and Wilson (1997) showing that inhibition of protein kinases and phosphatases induced disorganisation of CMTs, inhibition of root elongation, and subapical swelling in *Arabidopsis*. Working with wheat roots, Jones and Kochian (1995) provided evidence for the inhibition of phospholipase-C by Al, a key enzyme of the phosphoinositide signal transduction cascade located in the plasma membrane. This supports the conclusions by Haug et al. (1994) derived from their work with neuroblastoma cells that phospholipase C is a major target of Al.

(iv) Al modifies the physical properties of the cell wall. It can be anticipated that Al binding to the pectic matrix of the cell wall will affect physical properties of the cell wall. Al-induced reduction in cell wall extensibility has been proposed repeatedly. However, the experimental evidence for this is weak (Kruger and Sucoff, 1989; Gunse et al., 1997). In vitro experiments with an artificial pectic membrane revealed that binding of Al to the membrane substantially reduced water permeability (Blamey et al., 1993). Whether this applies to intact cell walls needs to be established. Our own unpublished work clearly shows that Al reduces the permeability of the root apoplast for macromolecules. There is evidence that the structural organisation of the cell wall, particularly the ordered configuration of the cellulose microfibrils, and thus cell differentiation is under the control of the cytoskeleton (Williamson, 1991). Less is known about the control of the spatial arrangement of the cytoskeleton. Using an inhibitor of cellulose synthesis, recent information provided by Fisher and Cyr (1998) indicates that CMT alignment in elongating tobacco cells requires cellulose microfibril production, and hence cell elongation. They propose to extend the microtubule/microfibril paradigm to include a bi-directional flow of information between cell wall and cytoskeleton. Since Al rapidly induces callose formation through callose synthase, which is assumed to produce cellulose under undisturbed conditions (Amor et al., 1995; Robinson, 1996), a similar reaction pattern might be assumed. Furthermore, Al might directly interfere with the apoplastic end of the extracellular matrix connected with the cytoskeleton as proposed by Wyatt and Carpita (1993) and Miller et al. (1997).

The results presented as well as our own unpublished work with maize cell-suspension cultures clearly show the importance of the pectic matrix in cell walls for the accumulation of Al and the modulation of Al toxicity. On the basis of the data we presented we suggest that the rapid disorganisation of the cytoskeleton we observed as early as 1 h after Al application to the roots may be mediated by direct interaction of Al with the apoplastic side of the cell wall – plasma membrane – cytoskeleton continuum.

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