BIOLOGIA PLANTARUM 49 (3): 321-331, 2005

REVIEW

Target sites of aluminum phytotoxicity

S.J. ZHENG* and J.L. YANG

College of Environmental and Resource Science, Zhejiang University, Hangzhou 310029, P.R. China

Abstract

The primary phytotoxic effect of aluminum (Al) is confined to the root apex. It is a matter of debate whether the primary injury of Al toxicity is apoplastic or symplastic. This review paper summarizes our current understanding of the spatial and metabolic sites of Al phytotoxicity. At tissue level, the meristematic, distal transition, and apical elongation zones of the root apex are most sensitive to Al. At cellular and molecular level, many cell components are implicated in Al toxicity including DNA in nucleus, numerous cytoplastic compounds, the plasma membrane, and the cell wall. Although it is difficult to distinguish the primary targets from the secondary effects so far, understanding of the target sites of Al toxicity is helpful for elucidating the mechanisms by which Al exerts its deleterious effects on root growth.

Additional key words: acid soil, apoplast, callose, calmodulin, cell wall, cytoskeleton, nucleus, plasma membrane, root, symplast.

Introduction

Aluminum is the most abundant metal and the third most abundant element in the earth's crust. A large proportion of Al, however, is incorporated into soil minerals like aluminosilicate with very small quantities appearing in the soluble forms that are capable of influencing biological systems (May and Nordstrom 1991). In acid soils, however, the release of Al from Al-containing minerals is accelerated and this increases the concentration of phytotoxic forms of Al in the soil solution. The adverse effect of acid soil on plant growth in many cases is directly related to the toxicity of the dissolved Al ions.

Aluminum toxicity was implicated in yield reduction of barley (*Hordeum vulgare*) and rye (*Secale cereale*) in acid soil as early as 1918 (Hartwell and Pember). Al is now viewed as the most important growth-limiting factor in many acid soils, as micromolar concentrations of Al can inhibit root growth at organ, tissue, and cellular levels (Čiamporová 2002). While soil acidification can be a natural process the rate of acidification is increased by some farming practices and from industrial pollution *via* acid rain. These processes pose an increasing threat to agricultural production and to natural ecosystems in temperate and tropical regions (Van Breemen 1985). Therefore finding ways to alleviate the adverse effect of Al on plant growth has become an important goal.

Many wild and crop plants exhibit genetic-based difference in Al sensitivity that has allowed plant breeders to develop Al-resistant crops through genetic manipulation (Kochian 1995). Considerable research effort has been directed toward elucidating the mechanisms of Al toxicity and tolerance in plants. Although recent advances have clarified the mechanisms of Al tolerance in some species (Matsumoto 2000, Ryan *et al.* 2001), the underlying processes involved in Al toxicity are still not well understood. It is now established in several cereal species that the root apex must be directly exposed to Al for root elongation to be affected. However, the important targets for Al at the cellular and molecular levels remain controversial. This review summarizes current understanding of target sites of Al phytotoxicity from tissue, cellular and molecular levels.

Received 23 February 2004, *accepted* 20 January 2005.

Abbreviations: CaM - calmodulin; CEC - cation exchange capacity; cMTs - cortical microtubules; DTZ - distal transition zone; EZ - elongation zone; PIP_2 - phosphatidylinositol-4,5-bisphosphate; PM - plasma membrane; ROS - reactive oxygen species.

Acknowledgements: This work was partly supported by fund from the Huoyingdong Foundation, Education Ministry of China and Natural Science Foundation of China (Contact No. 30170548). The authors thank Dr. Peter Ryan, Plant Industry, CSIRO for his critical reviewing and good comments and improving the manuscript.

* Corresponding author; fax: (+86) 571 86433724, e-mail: sjzheng@zju.edu.cn

Root apex

A major consequence of Al toxicity is the inhibition of root growth which subsequently affects nutrients and water uptake (Foy 1983). In particular, it is the roots that also incur the greatest cellular damage. Roots become stunted and brittle, root hair development is poor, and the root apices become swollen and damaged (Clarkson 1965).

Root elongation is the result of division and elongation of the root cells. At the early stage of research on Al toxicity, the blockage of cell division was regarded as the primary mode of Al injury since the cessation of root elongation and the disappearance of mitotic figure was close correlated (Clarkson 1965). However, cell division is a slow process, while the inhibition of root elongation of Al-sensitive maize can occur within 30 min of Al treatment (Llugany *et al.* 1995). Therefore, it is now generally believed that the primary mechanism leading to the inhibition of root elongation is caused by the inhibition of cell elongation. Bennet and Breen (1991) attributed a major role to the root cap in wheat for the perception of Al and for the initiation of signals that lead to the inhibition of root growth. Fiskesjö (1990) found "an Al structure" in root cap cells of *Allium cepa* L. and implied that the Al-structures eventually divided into two, one on each side of the nucleus and both oriented lengthwise in the cell. However, Ryan *et al.* (1993) reported that both the onset and extent of inhibition of growth in maize roots by Al was the same in intact roots as it was in roots that had their root caps removed. Ryan *et al.* (1993) used divided-chambers and agar blocks infused with Al to apply Al to different zones of the root and concluded that the zone 0 - 3 mm behind the quiescent zone was the most Al-sensitive site in maize. This section of the root in maize seedlings includes the meristem and the beginning of the elongation zone but excluded the majority of the elongation zone. Root apices of most plant species exude a mucilaginous substance constituted mainly of polysaccharides containing uronic acid (Moody *et al.* 1988). Since this mucilage has a high binding capacity for Al some authors have suggested that it could provide protection from toxic Al ions (Horst *et al.* 1982, Archambault *et al.* 1996). To test this hypothesis, Li *et al.* (2000) demonstrated that mucilage of maize does bind Al strongly and that Al bound to mucilage is not phytotoxic. However, the total binding capacity of the mucilage was too small to confer effective protection from Al-induced root inhibition in hydroponic culture. On the other hand, Miyasaka and Hawes (2001) found that Al-induced mucilage released from root cap border cells of snap bean can protect root tips from Al-induced cellular damage. Therefore, although the root cap may not be involved directly with the Al-induced inhibition of root growth in maize, more information concerning the role of root cap in Al perception and Al signal transduction is required for other species.

Using a polyvinyl chloride block technique, Sivaguru and Horst (1998) demonstrated that the distal transition zone (DTZ, 1 - 2 mm from the root tip) is the primary target of Al in an Al-sensitive maize cultivar Lixis. While cells in this zone are undergoing a preparatory phase for rapid elongation it does not contribute significantly to root elongation. Subsequently, Sivaguru *et al.* (1999a) and Horst *et al.* (1999) showed that Al leads to an alteration in the organization of microtubules and actin microfilaments, which were most severe in the DTZ (see later). Short-term Al treatment applied solely to the DTZ inhibited root elongation in the main elongation zone (EZ, 2.5 - 5 mm from the root tip) to the same extent as treatment to the entire maize root apex. Whereas application of Al to the EZ had no effect on root elongation of either Al-resistant (cv. ATP-Y) or Al-sensitive (cv. Lixis) maize which is consistent with the previous observations of Ryan *et al.* (1993). The genotypic differences in Al resistance between these genotypes appear to be located within DTZ (Kollmeier *et al.* 2000). Kollmeier *et al.* (2000) suggested that a signalling pathway in the root apex involving the basipetal transport of auxin mediated the flow of information between the DTZ, where Al was perceived, and the EZ, where the inhibition of root growth occurred. These results implicate that the DTZ of root apex is more important target for Al toxicity.

Taken together, the meristematic, transition, and apical elongation zones along the root apex are most sensitive to Al toxicity. Although it is difficult to accurately define the critical zone due to the experimental techniques per se, we can draw such conclusion that it is there where cells are more active that Al exerts more toxic effect. Nonetheless, the specific anatomical and molecular sites within this tissue remain unclear. The results reported maintained the possibility that either extracellular or intracellular sites could account for the initial symptoms of Al toxicity. In the next part of this review, therefore, we are going to discuss the Al targets from spatial and molecular points of view.

Cell wall

Although it now appears that Al can enter the cytosol of plant cells rapidly (Lazof *et al.* 1994, Taylor *et al.* 2000), there is evidence that the apoplast play a major role in the perception of Al and the expression of toxicity (Horst

1995, Horst *et al.* 1999, Schofield *et al.* 1998, Schmohl *et al.* 2000). Al binds rapidly in the apoplast with as much as 30 - 90 % of the total absorbed Al in root tissue being localized to the extracellular compartments (Tice *et al.* 1992, Rengel 1996, Schofield *et al.* 1998).

Furthermore chemical and mechanical properties of the cell wall seem to be modified by Al toxicity (Horst *et al.* 1999, Le Van *et al.* 1994, Vázquez *et al.* 1999, Tabuchi and Matsumoto 2001) supporting the hypothesis that Al-induced inhibition of root elongation is caused by processes initiated in the apoplast of the apical root cells (Horst 1995, Rengel 1996). However, it remains unclear for many of these studies whether Al interacts directly with the cell wall or whether these changes are indirect consequences of other Al-induced changes. Most recent evidence from Schildknecht and Vidal (2002) indicates that the wall plasticity and mechanical strength from two maize cultivars can be modified by Al, and that this could affect cell elongation and cell survival.

The extent to which Al is bound to the wall depends on the density of negative charges and this determines the cation exchange capacity (CEC). Several studies have investigated the correlation between CEC of plant tissues with their sensitivity to Al. For instance, a large component of the CEC derives from the negative charges carried on pectins in the cell wall (Horst 1995). Horst *et al.* (1999) reported that plants with higher pectin content accumulated more Al in their root apices and were also more Al-sensitive. Furthermore, the density of negative charges in walls was implicated in causing the differential Al resistance in two maize cultivars (Schildknecht and Vidal 2002). However, the reports are not consistent and no robust model has emerged (Wagatsuma 1983, Ishikawa and Wagastsuma 1998). This is not surprising since different techniques have been used, different tissues extracted (whole roots *versus* apical tissues) and comparisons have often been made between plant species. It is also worth emphasizing that, *in vivo*, CEC has contributions from the plasma membrane (PM) as well as from the cell wall (Horst 1995). Thus, future research needs to determine the CEC of highly purified cell wall and cell extracts to see if any can be correlated with Al tolerance within or between species.

Another mechanism for Al toxicity targeted to the apoplast invokes a rapid and irreversible displacement of Ca from cell wall components such as calcium pectin (Blamey 2001). The displaced Ca ions are replaced by Al ions which are proposed to rigidify the cell wall and prevent its loosening for cell elongation (Gunse *et al.* 1997). The binding of Al to the pectin of the cell wall could also inhibit the movement of water and nutrients through the apoplast (Blamey and Dowling 1995). Nevertheless, Ryan *et al.* (1997) provided evidence that displacement of Ca by Al from the apoplast is unlikely to be the cause of Al toxicity in wheat because in simple nutrient solutions low concentrations of Al were able to inhibit root growth without displacing Ca from the apoplast. Schofield *et al.* (1998) came to similar conclusion with *Allium cepa* using particle-induced X-ray emission microanalysis technique to measure Al and Ca in the cell walls and they hypothesized that an Al-related signal transduction was involved in the inhibition of root growth. The displacement by Al of cell wall Ca and the involvement of this process in Al toxicity remains an ongoing area of interest. In any case, since Ca is a principal element involved in many cytosolic signalling processes, it is feasible that changes in the free and bound concentrations of Ca in the apoplast could alter cellular metabolism via a Ca-dependent signal transduction.

A significant correlation between the amounts of Al and phosphate was also found in the cell wall (Marienfeld and Stelzer 1993). The formation of Al-P complexes, especially the insoluble $Al_4(PO_4)_3$, in the cell wall may even retard the transport of Al into the cytosol. On the other hand, Vázquez *et al.* (1999) reported that tolerance in maize was relied on the active transport of Al from the cell wall to vacuoles. Interactions between Al and other cell wall components, such as xyloglucans and proteins like extensin, may also affect the function of root cell walls. For instance, Al impaired the sucrose utilization for cell wall formation in cotton seedlings (Huck 1972), induced the production of cell wall polysaccharides in squash seedlings (Le Van *et al.* 1994), increased the amount of covalently bound cell wall extensins in wheat root tips (Kenzhebaeva *et al.* 1999) and decreased the mobility of apoplast protein in soybean root tips (Kataoka *et al.* 2003).

These results suggest that: *1*) cell wall has a high affinity of Al binding due to its high density of negative charges; *2*) interactions of Al with these charged sites may reduce cell wall extensibility and have down-stream effects on Ca signalling and cellular function; *3*) more studies relating cell wall CEC with Al tolerance are required. A beneficial direction for future research would be to elucidate the effects of Al on cell wall biosynthesis and function.

Plasma membrane

Ionic Al has a very strong affinity for the PM surface (Akeson *et al.* 1989) and the binding capacity of the PM for Al was ascribed to the negativity of carboxyl groups and phosphate groups in the PM (Obi *et al.* 1989a,b, Akeson and Munns 1989). There is accumulating evidence suggesting that the PM of cells at the root apex could be a primary target for Al toxicity (Basu *et al.* 1994, Wagatsuma *et al.* 1995, Yermiyahu *et al.* 1997).

Indeed, Ishikawa *et al.* (2001) proposed that maintenance of an intact PM in root tip cell is a primary factor associated with Al tolerance. Al can also alter the structure and function of the PM by interacting with the lipids and inducing lipid peroxidation as discussed below.

Perception of Al and signal transduction pathways: The rapid inhibition of root growth by Al treatment indicates that some, even more rapid, signal transduction processes may be involved in causing this response. Special attention has been paid to the phosphoinositide-associated transduction pathway since early research with animal cells indicated that cellular mechanisms of Al toxicity could involve interactions between Al and components of the pathway (Berridge 1987). This pathway has been investigated in plant cells and it is now clear that similar components are present in plants (Coté *et al.* 1993, Kochian 1995). Recently, Osawa and Matsumoto (2001) found that K-252a, an inhibitor of certain protein kinases, reduced Al-dependent efflux of malate from Al-tolerant wheat apices, which implies that protein phosphorylation is involved in this process. Since this process occurs within several minutes, an Al-associated signal transduction process may be speculated. Whereas this remains possible the process was attributed to the direct interaction between Al and proteins controlling organic acids secretion rather than the transduction.

Membrane potential and Al stress: The PM usually possesses two types of "membrane potential". One is the trans-membrane potential that reflects the imbalance in anion and cation concentrations inside the cell. The trans-membrane potential is nearly always negative in healthy cells (negative in the symplasm with reference to the apoplasm) and it can be measured by several methods including the insertion of microelectrodes into the cytoplasm. This potential is affected by the net movement of nutrients across the PM and by the action of transport proteins such as the ATP-dependent proton pump which moves H^+ out of the cell. The second type of potential is caused by the net concentration of fixed anions and cations on the membrane surface, and specifically on the lipid side-chains and membrane-bound proteins (Kinraide 1994). This surface or "zeta" potential can be estimated by measuring the movement of vesicles in an electric field or by measuring the accumulation of charged, non-permeable dyes at the membrane surface. Al appears to be able to affect both these types of potentials. For instance 50 μM Al can bind to, or otherwise screen, the fixed anions on the PM and shift the zeta potential from -20 mV to +1 mV (Kinraide 1994, Ahn *et al.* 2001). Depolarization of the transmembrane potential was reported in cells of fibrous roots of sugar beet (Lindberg *et al.* 1991) and in Al-tolerant wheat cultivar Dade (Olivetti *et al.* 1995). The maximum depolarization was 150 mV at 150 μM $AlC1_3$. In contrast, the membrane potential of the Al-sensitive cultivar Romano was depolarized only slightly by Al (Olivetti *et al.* 1995). Furthermore, root cells of squash treated with 10 μM Al showed a reduced activity of the PM H^+-ATPase as well as a depolarized membrane surface potential (Ahn *et al.* 2001). Ahn *et al.* (2002) concluded that the impaired flux of H^+ across the PM gave rise to a positive shift in surface potential. One of the consequences on these changes is that the concentration of ions bound to, or near, the pores of channels or transport proteins could be affected and this could alter the movement of ions across the membrane. These types of interactions may explain, in part, the widely reported affects of Al on nutrient uptake (see above). For instance, Nichol *et al.* (1993) reported that Al inhibited the influx of Ca^{2+} (69 %), NH_4^+ (40 %), and K^+ (13 %) and enhanced the influx of NO_3^- (44 %) and phosphate (17 %). Moreover, there is convincing evidence that shifts in surface potential in response to pH or increased concentration of other cations and anions can change the relative toxicity of Al and other charged compounds by altering their accumulation at the membrane surface (Kinraide 1994).

Lipid peroxidation: An Al-enhanced peroxidation of lipids has been reported in various systems including phospholipids liposomes (Oteiza 1994), soybean root tips (Horst *et al.* 1992), detached rice leaves (Kuo and Kao 2003) and cultured tobacco cells (Ono *et al.* 1995), and barley roots (Šimonovičová *et al.* 2004). Also, the Al-induced genes encoding proteins that function to overcome oxidative stress (*e.g.* glutathione S-transferase, peroxidase, blue copper-binding protein, phenylalanine ammonia lyase, 1,3-(β-glucanase, or cysteine proteinase) has been previously reported (Ezaki *et al.* 1995, 1996, Cruz-Ortega *et al.* 1997). In addition, expression of these Al-induced genes in transgenic *Arabidopsis* plants conferred Al tolerance and enhanced oxidative stress (Ezaki *et al.* 2000). Basu *et al.* (2001) reported that transgenic *Brassica napus* overexpressing MnSOD gene acquires an Al resistance phenotype. However, lipid peroxidation is often observed only after prolonged treatment in Al (24 h or more) (Cakmak and Horst 1991). Furthermore, the lipid peroxidation and the uptake of Evans blue were only intensified by the inclusion of Fe^{2+} to the solution (Ono *et al.* 1995). Thus, it is likely that this process is a consequence of some other primary effects of Al on membrane structure and function. However, it has been reported recently that Al alone (without Fe supply) also enhances the production of ROS in both tobacco cells and pea roots (Yamamoto *et al.* 2002). Furthermore, Al triggered the production of ROS, inhibited respiration and depleted ATP was strongly correlated with inhibition of cell growth or root elongation. Yamamoto *et al.* (2002) concluded that the critical event in Al inhibition of cell growth in this system was the Al-dependent production of ROS in the mitochondria.

Most recently, Devi *et al.* (2003) found that Al-induced lipid peroxidation could account for the differential Al resistance of cultured tobacco cell lines SL (an Al sensitive cell line) and ALT 301 (an Al tolerant cell line). Furthermore, the intracellular antioxidant conditions play the major role in defence the cell against the Al-induced damage. Boscolo *et al.* (2003) provided the evidence that superoxide dismutase and peroxidase activities esere enhanced in S1587-17 (an Al-sensitive inbred line of maize) after 48 h treatment with 36 μM Al^{3+}, but not in Cat100-6 (an Al-tolerant inbred line of maize). But in contrast to observations from other species, Al treatment failed to induce lipid peroxidation in either of these lines but protein oxidation was detected. While this is the first report of Al inducing protein oxidation, Boscolo *et al.* (2003) concluded that although the Al-induced oxidation could lead to other stresses, it was not the primary cause for the inhibition of root growth.

Aluminum itself cannot catalyze redox reactions as it is not a transition metal. Although the process implicated in Al toxicity and the production of ROS is unknown, the involvement of oxidative stress in Al toxicity is emerging and seems to be a determining factor of root elongation inhibition by Al (Yamamoto *et al.* 2003).

Calcium and calmodulin

One of the earliest identified consequences of Al exposure to plant roots is a reduction in Ca uptake (Huang *et al.* 1992a,b, Rengel 1992a,b). Calcium uptake rapidly recovers when Al is removed from the solution. The inhibition of net $^{45}Ca^{2+}$ uptake by Al in *Amaranthus tricolor* protoplasts was studied using a range of Ca^{2+}-channel blockers as well as inhibitors of Ca^{2+}-ATPase, calmodulin and GTP-proteins (Rengel and Elliott 1992). It was concluded that Al^{3+} acts as a Ca^{2+}-channel blocker by binding to the verapamil-specific channel-receptor site and by interfering with the action of GTP-binding proteins. It is also clear the Al-induced changes to membrane surface potential may also influence the uptake of Ca and other cations by the root cells (Kinraide 1994). Evidence that Al can directly block Ca^{2+}-channels was finally provided by Piñeros and Tester (1993) using planar lipid bilayers prepared from wheat roots. Collectively, these results indicated that Al toxicity might result from the inhibition of Ca^{2+} uptake *via* channels in the PM. Ryan *et al.* (1994) also agreed that high concentrations of Al can inhibit Ca^{2+} uptake by wheat but, in contrast to the earlier studies, they found no evidence to support the hypothesis that the primary cause of Al toxicity in wheat was the inhibition of Ca uptake. Ryan *et al.* (1994) provided two reasons for this conclusion: *1*) when Ca uptake was inhibited to a similar extent with Al or with other cations (Mg^{2+} and Na^{+}), root growth was inhibited in the Al treatment but not in the other treatments; *2*) low concentrations of Al were found to inhibit root growth without any measurable affects on Ca uptake.

Cytoplasmic Ca^{2+} is known to regulate many processes in cell growth and metabolism. The disruption of cytoplasmic Ca^{2+} homeostasis is another mechanism hypothesized to cause Al injury (Taylor 1990, Delhaize and Ryan 1995, Kochian 1995). Nichol and Oliveira (1995) reported that the disturbance of cytoplasmic Ca^{2+} level in excised Al-sensitive barley roots after 2 d exposure to 50 μM Al. Evidence from Lindberg and Strid (1997) indicated that exposure of wheat root protoplasts to 80 μM Al caused an immediate, transient (2 min duration), or oscillating increase in cytoplasmic Ca^{2+} concentration. However, no differences were observed between protoplasts isolated from Al-resistant and Al-sensitive cultivars. Jones *et al.* (1998) also provided evidence that the rise in cytoplasmic Ca^{2+} concentration in root hair tips of *Arabidopsis thaliana* L. occurred as late as 20 - 30 min after exposure to Al. Likewise, the disruption is not tightly correlated with the inhibition of growth. Taken together, these results indicated that Al does influence the cytoplasmic Ca^{2+} homeostasis, but the question whether the influence is the primary cause of Al-induced inhibition of root growth or only the secondary effects remains unclear Recently, Zhang and Rengel (1999) found that Al induced a sustained increase in cytoplasmic Ca^{2+} in intact root tip cells of two near isogenic wheat lines different in Al tolerance, and that, the increases were correlated with the inhibition of root growth in both lines. Furthermore, they provided convincing evidence that the initial toxic effect of Al results from the disruption of cytoplasmic Ca^{2+} homeostasis by interference with the plasmalemma and endo-membrane functions (Rengel and Zhang 2003). Thus, the Al-dependent disruption of cytoplasmic Ca^{2+} homeostasis may be directly or indirectly involved in the inhibition of the cell division or root elongation.

Calmodulin (CaM) plays a pivotal role in cellular metabolism and there is some evidence that interactions between Al and CaM could be an important cause of toxicity (Kochian 1995). However, Haug and Vitorello (1996) argued against the displacement of Ca by Al from thermodynamic and kinetic aspects. They also suggested that Al impairs recognition of calmodulin and changes its internal dynamics by binding to calmodulin directly. Other evidence also exists that Al toxicity is not the cause of interaction between Al and CaM (Richardt *et al.* 1985, You and Nelson 1991, Jorge *et al.* 2001). Thus, the role of CaM in Al toxicity should be viewed cautiously until more information becomes available.

The formation of callose

The synthetic pathway for callose has not been completely elucidated. One of the proposed pathways involves the Ca^{2+}-activated enzyme (β-1,3-glucane synthetase which is associated with the PM. Since Al induces a transient rise of cytosolic Ca^{2+} (see above), an increase of callose accumulation under Al stress is not unexpected. Indeed, it has been well documented that Al stress caused callose deposition in roots of soybean (Wissemeier *et al.* 1987), Norway spruce (*Picea abies* L.) (Jorns *et al.* 1991) and *Phaseolus vulgaris* L. cell cultures (Taylor 1995), and that Al-induced callose formation is negatively correlated with Al-resistance in the root tips of maize (Llugany *et al.* 1994, Horst *et al.* 1997) and in protoplasts of *Avena sativa* and wheat but not in *Hordeum vulgare* (Schaeffer and Walton 1990). Interestingly, an increase in callose accumulation was detected only after 2 h of Al-treatment (Taylor 1995). Therefore, callose deposition has been proposed as one of the early indicators for Al toxicity (Massot *et al.* 1999).

The Al-dependent synthesis and accumulation of callose in sieve plates, pit fields, and developing cell plates of higher plants seals those cells and tissue and this has been considered as a mechanism to prevent Al from penetrating into the apoplast (Marschner 1991). Yet in at least one study, a negative correlation was found between the callose concentration and relative root elongation rates of three soybean genotypes differing in Al sensitivity (Wissemeier *et al.* 1992). Thus the question arises whether Al-dependent synthesis of callose is a protective response, whether fit is damaging to cell function or whether fit is a harmless consequence of Al stress. Evidence for the former case was provided by Sivaguru *et al.* (2000) who found that the Al induced the accumulation of callose in the plasmodesmata of root cells in wheat inhibited root elongation by blocking cell-to-cell trafficking of molecules.

Cytoskeleton

Plant cells require dynamic cytoskeleton-based networks for various cell activities (*e.g.*, differentiation and cell division) as well as cell wall biosynthesis (Sivaguru *et al.* 1999a). Al treatment often induces the apices of roots and the tips of root hairs to swell (Jones *et al.* 1995) and this phenomenon has been attributed to Al somehow interfering with the function of components of the cytoskeleton (Jones and Kochian 1995). So, it is possible that the microtubules and microfilaments are important targets for Al stress in plants.

Microfilaments: Grabski and Schindler (1995) reported that Al induced a significant increase in the tension within the trans-vacuolar actin network in suspension-cultured soybean cells. Based on these results they proposed that the dynamics of the actin network is disrupted by Al and this could have important consequences on many other cellular processes. It is known that actin is regulated, in part, through the action of profilin, and that the concentration of profilin in plants is regulated by its binding to the lipid anchor phosphatidylinositol-4,5-bisphosphate (PIP_2) (Drøbak *et al.* 1994, Darnowski *et al.* 1996). Whether some interaction between Al and PIP_2 is capable of disrupting the actin component of the cytoskeleton is still unclear but others have reported an inhibition of phospholipase C activity by Al both of which are components of the inositol triphosphate signal transduction pathway as discussed by Jones and Kochian (1995).

In addition to the disruption of actin cytoskeletal dynamics, Al also increased the rigidity of actin network perhaps due to the formation of either Al-ATP or Al-ADP complexes (Grabski and Schindler 1995) which are several magnitudes more stable than the corresponding Mg complexes. This supports one hypothesis that Al toxicity results from the displacement of Mg from nucleotide di- or triphosphate complexes (Grabski and Schindler 1995).

Microtubules: The orientation of the microtubules is closely related to cell expansion (Matsumoto 2002). MacDonald *et al.* (1987, 1988) found that Al strongly promoted tubulin assembly into microtubules and inhibited subsequent Ca-induced depolymerization of the microtubules. They speculated that Al displaced the Mg which is believed to bind at GTP and GDP receptor sites resulted in dramatic reducing of microtubule-dependent GTP hydrolysis. They also suggested that even at very low levels of Al in cytoplasm might be sufficient to disrupt the sensitive dynamics of microtubule formation and disassembly, which in turn could result in cellular malfunction. Similarly, Blancaflor *et al.* (1998) reported that initial stabilization of cortical microtubules (cMTs) after 3 h Al treatment in the EZ cells of maize root apex (3 - 4 mm distance from the root tip) correlates well with the growth inhibition. On the other hand, Sivaguru and Horst (1998) reported that not stabilization but depoly-merisation of cMTs observed in the DTZ (1 - 2 mm distance form root tip) of the maize root tip as early as 1 h Al treatment. Therefore, fit has not been clearly demonstrated whether Al-induced depolymerisation of cMTs in the DTZ or Al-induced stabilization in the EZ is the cause of Al toxicity. With this background, Sivaguru *et al.* (1999b) found that the impacts of Al on suspension-

cultured tobacco *(Nicotiana tabacum* L. cv. Samsun) cells were distinct, namely, Al-induced depolymerisation of cMTs of logarithmic phase cells and stabilization of cMTs of stationary phase cells. Sivaguru *et al.* (1999b) concluded that the previously conflicting reports on the question were not surprising, because logarithmic and stationary phase cells are comparable, respectively, to the DTZ region cells and the EZ region cells of maize root apex. Recently, Schwarzerová *et al.* (2002) reported that Al had a rapid effect on the microtubular cytoskeleton of the suspension tobacco cell lines BY 2 and VBI-0. Moreover, the cells were more sensitive to Al during exponential phase as compared to stationary cells. During the first hours of exposure, Al induced the formation of additional bundles of cMTs, but the thickness of the individual bundles decreased. Prolonged exposure resulted in disorientation of cMTs. These changes in cMTs preceded the decrease of cell viability by several hours and were accompanied by an increase in the levels of α-tubulin (in its tyrosinated form) and elements of the tubulin-folding chaperone CCT. Based on these findings, Schwarzerová *et al.* (2002) concluded that the microtubular cytoskeleton is one of the early targets of Al toxicity. Nonetheless, more convincing evidence can be obtained from investigating the root system itself. Therefore, simultaneous measurements of Al-induced alteration in both DTZ cMTs and EZ cMTs are promising.

Nucleus

The rapid inhibition of root elongation under Al stress has been primarily been ascribed to the inhibition of root cell elongation rather than cell division (Kochian 1995, Matsumoto 2000), with the latter being a potential longer-term and lethal consequence of Al toxicity (Matsumoto 2000). Al was detected in nuclei of root hair cells by staining and by chemical determination of Al in purified nuclei prepared from Al-treated pea roots (Matsumoto *et al.* 1976, Matsumoto and Morimura 1980, Matsumoto 1988). Matsumoto (1991) also suggested that the target of Al might be phosphate groups of DNA or RNA in nuclei. A similar result was obtained in root tips of wheat (Rincón and Gonzales 1992). It is possible that the binding of Al to DNA or to chromatin could condense the DNA and inhibit cell division by reducing its capacity to provide a viable template for transcription (Matsumoto *et al.* 1976, 1980, 1988). Thus, the strong electrostatic interaction between the negatively charged phosphate groups and the positively charged Al polymer could prevent not only cell division but the synthesis of all proteins. However, the usefulness of many of the early experiments was questioned for their use of high Al concentrations and long exposure times. Some opinions indicated that penetration of Al into the nuclei was likely to be observed in extreme cases where tissue damage had already occurred. Yet a recent study using the Al-sensitive stain lumogallion and confocal laser scanning microscopy has clearly demonstrated that Al can accumulate in the nuclei of meristematic cells of soybean root tips within a 30 min exposure to low activity (1.45 μM) of Al (Silva *et al.* 2000). Thus we need to reconsider the role of the interaction of Al with nuclei in the mechanism of Al toxicity. In the future more sensitive technologies for measuring elements *in situ* will help us understand the biochemical and anatomical target of Al toxicity. For example, the accelerator mass spectrometry (AMS) has the potential to use ^{26}Al to detect Al down to the picograms per milligram range (Masaoka *et al.* 2002).

Conclusions

Aluminum toxicity is one of the most deleterious factors for plant growth on acid soils. Application of lime to acid soils to increase the soil pH is one strategy for alleviating Al toxicity. However, this technique is problematic from the economical and environmental points of view and benefits of applying lime to deeper soil layers can be delayed by years (De la Fuente and Herrera-Estrella 1999). A complementary strategy to manage acid soils is to use Al-tolerant germplasm and identifying the causes of Al toxicity may help crop breeders select genotypes with increased tolerance to Al. However, research on mechanisms of Al toxicity has been hampered by the complexity of Al chemistry in solution, and multiplicity of possible sites within the root where Al might act to inhibit root growth and function (Kochian and Jones 1997). Indeed, depending on the concentration and duration of exposure, Al may, and probably does, have multiple target sites within the plant. Notably, these sites may differ among plant species.

Plants growing at Al stress environment must have evolved mechanisms by which to increase their tolerance through both physical adaptations and interactive molecular and cellular changes that begin after the onset of stress. The first step in switching on such molecular responses is to perceive the stress as it occurs and to relay information about it through a signal transduction pathway. Therefore, some Al-dependent responses might be the effect of Al toxicity rather the cause. We need

more information on Al signal perception and subsequent signal transduction under stress and the crucial sites within the tissue or cells that induce Al stress. The question remains open whether these primary sites of Al toxicity are in the apoplast or symplast or whether both phases are important. Such questions may be difficult to resolve when each symptom of Al toxicity is investigated separately. Thus, simultaneous investigation of various targets, both apoplastic and symplastic targets, could be useful in future work. There has been one study so far (Rengel 2000) in which Al-caused depolarization of the transmembrane potential and an increase in cytosolic Ca^{2+} contents were measured simultaneously in the same cells. We believe that this direction will help to continuing our progress in understanding the target sites of Al toxicity.

References

Ahn, S.J., Sivaguru, M., Chung, G.C., Rengel, Z., Matsumoto, H.: Aluminium-induced growth inhibition is associated with impaired efflux and influx of H^+ across the plasma membrane in root apixes of squash (*Cucurbita pepo*). - J. exp. Bot. **376**: 1959-1966, 2002.

Ahn, S.J., Sivaguru, M., Osawa, H., Chung, G.C., Matsumoto, H.: Aluminum inhibits the H^+-ATPase activity by permanently altering the plasma membrane surface potentials in squash roots. - Plant Physiol. **126**: 1381-1390, 2001.

Akeson, M., Munns, D.N.: Lipid bilayer permeation by neutral aluminum citrate and by three alpha hydroxy carboxylic acids. - Biochem. biophys. Acta **984**: 200-206, 1989.

Akeson, M., Munns, D.N., Burau, R.G.: Adsorption of Al^{3+} to phosphatidylcholine vesicles. - Biochem. biophys. Acta **986**: 33-40, 1989.

Archambault, D.J., Zhang, G., Taylor, G.J.: Accumulation of Al in root mucilage of an Al-resistant and an Al-sensitive cultivar of wheat. - Plant Physiol. **112**: 1471-1478, 1996.

Basu, A., Basu, U., Taylor, G. J.: Induction of microsomal membrane proteins in roots of an aluminum-resistant cultivar of *Triticum aestivum* L. under conditions of aluminum stress. - Plant Physiol. **104**: 1007-1013, 1994.

Basu, U., Good, A.G., Taylor, G.J.: Transgenic *Brassica napus* plants overexpressing aluminium-induced mitochondrial manganese superoxide dismutase cDNA are resistant to aluminium. - Plant Cell Environ. **24**: 1269-1278, 2001.

Bennet, R.J., Breen, C.M.: The aluminium signal: new dimensions to mechanisms of aluminium tolerance. - In: Wright, R.J., Baligar, V.C., Murrmann, R.P. (ed.): Plant-Soil Interactions at Low pH. Pp. 703-716. Kluwer Academic Publ., Dordrecht 1991.

Berridge, M.J.: Inositol trisphosphate and diacylglycerol: two interacting second messengers. - Ann. Biochem. **56**: 159-193, 1987.

Blamey, F.P.C.: The role of the root cell wall in aluminum toxicity. - In: Ae, N., Arihara, J., Okada, K., Srinivasan, A. (ed.): Plant Nutritent Acquisition. New Perspectives. Pp. 201-226. Springer-Verlag, Tokyo 2001.

Blamey, F.P.C., Dowling, A.J.: Antagonism between aluminium and calcium for sorption by calcium pectate. - Plant Soil **171**: 137-140, 1995.

Blancaflor, E.B., Jones, D.L., Gilroy, S.: Alterations in the cytoskeleton accompany aluminum-induced growth inhibition and morphological changes in primary roots of maize. - Plant Physiol. **118**: 159-172, 1998.

Boscolo, P.R.S., Menossi, M., Jorge, R.A.: Aluminum-induced oxidative stress in maize. - Phytochemistry **62**: 181-189, 2003.

Cakmak, L, Horst, W.J.: Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*).- Physiol. Plant. **83**: 463-468, 1991.

Čiamporová, M.: Morphological and structure responses of plant roots to aluminium at organ, tissue, and cellular levels. - Biol. Plant. **45**: 161-171, 2002.

Clarkson, D.T.: The effect of aluminium and some trivalent metal cations on cell division in the root apices of *Allium cepa.* - Ann. Bot. **29**: 309-315, 1965.

Coté, G.G., Crain, R.C.: Biochemistry of phosphoinositides. - Annu. Rev. Plant Physiol. Plant mol. Biol. **44**: 333-356, 1993.

Cruz-Ortega, R., Cushman, J.C., Ownby, J.P.: cDNA clones encoding 1,3-(β-glucanase and a fimbrin-like cytoskeletal protein are induced by Al toxicity in wheat roots. - Plant Physiol. **114**: 1453-1460, 1997.

Darnowski, D.W., Valenta, R., Parthasarathy, M.V.: Identification and distribution of profilin in tomato (*Lycopersicon esculentum* Mill.). - Planta **198**: 158-161, 1996.

De la Fuente, J.M., Herrera-Estrella, L.: Advances in the understanding of aluminum toxicity and the development of aluminum-tolerant transgenic plants. - Adv. Agron. **66**: 103-120, 1999.

Delhaize, E., Ryan, P.R.: Aluminum toxicity and tolerance in plants. - Plant Physiol. **107**: 315-321, 1995.

Devi, S.R., Yamamoto, Y., Matsumoto, H.: An intracellular mechanism of aluminum tolerance associated with high antioxidant status in cultured tobacco cells. - J. inorg. Biochem. **97**: 59-68, 2003.

Drøbak, B.K., Watkins, P.A.C., Valenta, R., Dove, S.K., Lloyd, C.W., Staiger, C.J.: Inhibition of plant plasma membrane phosphoinositide phospholipase C by the actin-binding protein profiling. - Plant J. **6**: 389-400, 1994.

Ezaki, B., Gardner, R.C., Ezaki, Y, Matsumoto, H.: Expression of aluminum-induced genes in transgenic arabidopsis plants can ameliorate aluminum stress and/or oxidative stress. - Plant Physiol. **122**: 657-665, 2000.

Ezaki, B., Tsugita, S., Matsumoto, H.: Expression of a moderately anionic peroxidase is induced by aluminum treatment in tobacco cells: Possible involvement of peroxidase isozymes in aluminum ion stress. - Physiol. Plant. **96**: 21-28, 1996.

Ezaki, B., Yamamoto, Y., Matsumoto, H.: Cloning and sequencing of the cDNAs induced by aluminium treatment and Pi starvation in cultured tobacco cells. - Physiol. Plant. **93**: 11-18, 1995.

Fiskesjö, G.: Occurrence and degeneration of "Al-structure" in root cap cells of *Allium cepa* L. after Al-treatment. - Hereditas **112**: 193-202, 1990.

Foy, C.D.: The physiology of plant adaptation to metal stress. - Iowa State J. Res. **57**: 355-391, 1983.

Grabski, S., Schindler, M.: Aluminum induces rigor within the actin network of soybean cells. - Plant Physiol. **108**: 897-901, 1995.

Gunse, B., Poschenrieder, C., Barceló, J.: Water transport properties of roots and root cortical cells in proton- and Al-stressed maize varieties. - Plant Physiol. **113**: 595-602, 1997.

Hartwell, B.L., Pember, F.R.: The presence of aluminium as a reason for the difference in the effect of so-called acid soil on barley and rye. - Soil Sci. **6**: 259-279, 1918.

Haug, A., Vitorello, V.: Aluminium coordination to calmodulin: thermodynamic and kinetic aspects. - Coordination Chem. Rev. **149**: 113-124, 1996.

Horst, W.J.: The role of the apoplast in aluminium toxicity and resistance of higher pants: A review. - Z. Pflanzenernähr. Bodenk. **158**: 419-428, 1995.

Horst, W.J., Asher, C.J., Cakmak, I., Szulkiewica, P., Wissemeier, A.H.: Short-term responses of soybean roots to Al. - J. Plant Physiol. **140**: 174-178, 1992.

Horst, W.J., Pöschel, A.K., Schmohl, N.: Induction of callose formation is a sensitive marker for genotypic aluminium sensitivity in maize. - Plant Soil **192**: 23-30, 1997.

Horst, W.J., Schmohl, N., Baluška, F., Sivaguru, M.: Does aluminium affect root growth of maize through interaction with the cell wall-plasma membrane-cytoskeleton continuum? - Plant Soil **215**: 163-174, 1999.

Horst, W.J., Wanger, A., Marschner, H.: Mucilage protects roots from aluminum injury. - Z. Pflanzenphysiol. **105**: 435-444, **1982**.

Huang, J.W., Grunes, D.L., Kochian, L.V.: Aluminum effects on the kinetics of calcium uptake into cells of the wheat root apex. - Planta **188**: 414-421, 1992a.

Huang, J.W., Shaff, J.E., Grunes, D.L., Kochian, L.V.: Aluminum effects on calcium fluxes at the root apex of aluminum-tolerant and aluminum-sensitive wheat cultivars. - Plant Physiol. **98**: 230-237, 1992b.

Huck, M.G.: Impairment of sucrose utilization for cell wall formation in the roots of aluminum-damaged cotton seedlings. - Plant Cell Physiol. **13**: 7-14, 1972.

Ishikawa, S., Wagatsuma, T.: Plasma membrane permeability of root-tip cells following temporary exposure to Al ions is a rapid measure of Al tolerance among plant species. - Plant Cell Physiol. **39**: 516-525, 1998.

Ishikawa, S., Wagatsuma, T., Takano, T., Tawaraya, K., Oomata, K.: The plasma membrane intactness of root-tip cell is a primary factor for Al-tolerance in cultivars of five species. - Soil Sci. Plant Nutr. **47**: 489-501, 2001.

Jones, D.L., Gilroy, S., Larsen, P.B., Howell, S.H., Kochian, L.V.: Effect of aluminum on cytoplasmic Ca^{2+} homeostasis in root hairs of *Arabidopsis thaliana* (L.). - Planta **206**: 378-387, 1998.

Jones, D.L., Kochian, L.V.: Aluminium inhibition of the inositol 1,4,5-triphosphate signal transduction pathway in wheat roots: a role in aluminium toxicity? - Plant Cel1 **7**: 1931-1922, 1995.

Jones, D.L., Shaff, J.E., Kochian, L.V.: Role of calcium and other ions in directing root hair tip growth in *Limnobium stoloniferum.* I. Inhibition of tip growth by aluminum. - Planta **197**: 672-680, 1995.

Jorge, R.A., Menossi, M., Arruda, P.: Probing the role of calmodulin in Al toxicity in maize. - Phytochemistry **58**: 415-422, 2001.

Jorns, A.C., Hecht-Buchholz, C., Wisseier, A.H.: Aluminum-induced callose formation in root tips of Norway spruce [*Picea abies* (L.) Karst.]. - Z. Pflanzenernähr. Bodenk. **154**: 349-353, 1991.

Kataoka, T., Furukawa, J., Nakanishi, T.M.: The decrease of extracted apoplast protein in soybean root tip by aluminium treatment. - Biol. Plant. **46**: 445-449, 2003.

Kenzhebaeva, S.S., Yamamoto, Y., Matsumoto, H.: Aluminum-induced changes in cell-wall glycoproteins in the root tips of Al-tolerant and Al-sensitive wheat lines. - Russ. J. Plant Physiol. **48**: 441-447, 1999.

Kinraide, T.B.: Use of Gouy-Chapman-Stern model for membrane-surface-electrical potential to interpret some feature of mineral rhizotoxicity. - Plant Physiol. **106**: 1583-1592, 1994.

Kochian, L.V.: Cellular mechanisms of aluminum toxicity and resistance in plants. - Annu. Rev. Plant Physiol. Plant mol. Biol. **46**: 237-260, 1995.

Kochian, L.V., Jones, D.L.: Aluminum toxicity and resistence in plants. - In: Yokel, R., Golub, M.S. (ed.): Research Issues in Aluminum Toxicity. Pp. 69-90. Taylor and Francis Publ., Washington 1997.

Kollmeier, M., Felle, H.H., Horst, W.J.: Genotypical differences in aluminum resistence of maize are expressed in the distal part of the transition zone. Is reduced basipetal auxin flow involved in inhibition of root elongation by aluminum? - Plant Physiol. **122**: 945-956, 2000.

Kuo, M.C., Kao, C.H.: Aluminum effects on lipid peroxidation and antioxidative enzyme activities in rice leaves. - Biol. Plant. **46**: 149-152, 2003.

Lazof, D.B., Goldsmith, J.G, Rufty, T.W., Linton, R.W.: Rapid uptake of aluminum into cells of intact soybean root tips: a microanalytical study using secondary ion mass spectrometry. - Plant Physiol. **106**: 1107-1114, 1994.

Le Van, H., Kuraishi, S., Sakurai, N.: Aluminum-induced rapid root inhibition and changes in cell-wall components of squash seedlings. - Plant Physiol. **106**: 971-976, 1994.

Li, X.F., Ma, J.F., Syuntaro, S., Matsumoto, H.: Mucilage strongly binds aluminum but dose not prevent roots from aluminum in *Zea mays.* - Physiol. Plant. **108**: 152-160, 2000.

Lindberg, S., Strid, H.: Aluminum induces rapid changes in cytosolic pH and free calcium and potassium concentrations in root protoplasts of wheat (*Triticum aestivum*). - Physiol. Plant. **99**: 405-414, 1997.

Lindberg, S., Szynkier, K., Greger, M.: Aluminium effects on transmembrane potential in cells of fibrous roots of sugar beet. - Physiol. Plant. **83**: 54-62, 1991.

Llugany, M., Massot, N., Wisseier, A.H., Poschenrieder, C., Horst, W.J., Barceló, J.: Aluminum tolerance of maize cultivars as assessed by callose production and root elongation. - Z. Pflanzenernähr. Bodenk. **157**: 447-451, 1994.

Llugany, M., Poschenrieder, C., Barceló, J.: Monitoring of aluminium-induced inhibition of root elongation in four maize cultivars differing in tolerance to aluminium and proton toxicity. - Physiol. Plant. **93**: 265-271, 1995.

MacDonald, T.L., Humphreys, W.G., Martin, B.R.: Promotion of tubulin assembly by aluminum ion *in vitro*. - Science **236**: 183-186, 1987.

MacDonald, T.L., Martin, R.B.: Aluminum ion in biological systems. - Trends biol. Sci. **13**: 15-19, 1988.

Marienfeld, S., Stelzer, R.: X-ray microanalyses in roots of Al-treated *Avena sativa* plants. - J. Plant Physiol. **141**: 569-573, 1993.

Marschner, H.: Mechanisms of adaptation of plants to acid soils. - Plant Soil **134**: 1-20, 1991.

Masaoka, Y., Saito, A., Arakawa, Y., Matsuzaki, H., Miyazaki, C., Kobayashi, K.: Al^{26} microanalysis in the leaf cell organelles of ryegrass and barley by accelerator mass spectrometry (AMS). - J. Plant Nutr. **25**: 343-354, 2002.

Massot, N., Llugany, M., Poschenrieder, C., Barceló, J.: Callose production as indicator of aluminum toxicity in bean cultivars. - J. Plant Nutr. **22**: 1-10, 1999.

Matsumoto, H.: Changes of the structure of pea chromatin by aluminum. - Plant Cell Physiol. **29**: 281-287, 1988.

Matsumoto, H.: Biochemical mechanism of the toxicity of aluminium and the sequestration of aluminum in plant cells. - In: Wright, R.J., Baligar, V.C., Murrmann, R.P. (ed.): Plant-Soil Interactions at Low pH. Pp. 825-838. Kluwer Academic Publ., Dordrecht 1991.

Matsumoto, H.: Cell biology of aluminum toxicity and tolerance in higher plants. - Int. Rev. Cytol. **200**: 1-46, 2000.

Matsumoto, H.: Plant roots under aluminum stress: Toxicity and tolerance. - In: Waisel, Y., Eshel, A., Kaflcaf, U. (ed.): Plant Roots. Pp. 821-838. Marcel Dekker, New York 2002.

Matsumoto, H., Hirasawa, E., Torikai, H., Takahashi. E.: Localization of absorbed aluminum in pea root and its binding to nucleic acids. - Plant Cell Physiol. **17**: 127-137, 1976.

Matsumoto, H., Morimura, S.: Repressed template activity of chromatin of pea roots treated by aluminum. - Plant Cell Physiol. **21**: 951-959, 1980.

May, H.M., Nordsrom, D.K.: Assessing the solubilities and reaction kinetics of aluminuous minerals in soils. - In: Ulrich, B., Sumner, M.E. (ed.): Soil Acidity. Pp. 125-148. Springer-Verlag, Berlin - Heidelberg 1991.

Miyasaka, S.C., Hawes, M.: Possible role of root border cells in detection and avoidance of aluminum toxicity. - Plant Physiol. **125**: 1978-1987, 2001.

Moody, S.F., Clark, A.E., Bacic, A.: Structure analysis of secreted slime from wheat and cowpea roots. - Phytochemistry **27**: 2864-2875, 1988.

Nichol, B.E., Oliveira, L.A.: Effects of aluminum on the growth and distribution of calcium in roots of an aluminum-sensitive cultivar of barley (*Hordeum vulgare*). - Can. J. Bot. **73**: 1849-1858, 1995.

Nichol, B.E., Oliveira, L.A., Glass, A.D.M., Siddiqi, M.Y.: The effects of aluminum on the influx of calcium, potassium, ammonium, nitrate, and phosphate in an aluminum-sensitive cultivar of barley (*Hordeum vulgare* L.). - Plant Physiol. **101**: 1263-1266, 1993.

Obi, I., Ichikawa, Y., Kakutani, T., Senda, M.: Electrophoresis, zeta potential and potential and surface charges of barley mesophyll protoplasts. - Plant Cell Physiol. **30**: 129-135. 1989a.

Obi, I., Ichikawa, Y., Kakutani, T., Senda, M.: Electrophoretic studies on plant protoplasts from various sources. - Plant Cell Physiol. **30**: 439-444, 1989b.

Olivetti, G.P., Cumming, J.R., Etherton, B.: Membrane potential depolarization of root cap cells precedes aluminum tolerance in snap bean. - Plant Physiol. **109**: 123-129, 1995.

Ono, K., Yamamoto, Y., Hachiya, A., Matsumoto, H.: Synergistic inhibition of growth by aluminum and iron of tobacco (*Nicotiana tabacum* L.) cells in suspension culture. - Plant Cell Physiol. **36**: 115-125, 1995.

Osawa, H., Matsumoto, H.: Possible involvement of protein phosphorylation in aluminum-responsive malate efflux from wheat root apex. - Plant Physiol. **126**: 411-420, 2001.

Oteiza, P.L.: A mechanism for the stimulatory effect of aluminum on iron-induced lipid peroxidation. - Arch. Biochem. Biophys. **308**: 374-379, 1994.

Piñeros, M., Tester, M.: Plasma-membrane Ca^{2+} channels in roots of higher plants and their role in aluminum toxicity. - Plant Soil **155**: 119-122, 1993.

Rengel, Z.: Role of calcium in aluminum toxicity. - New Phytol. **121**: 199-513, 1992a.

Rengel, Z.: Disturbance of cell Ca^{2+} homeostasis as a primary trigger of Al toxicity syndrome. - Plant Cell Environ. **15**: 931-938, 1992b.

Rengel, Z.: Uptake of aluminium by plant cells. - New Phytol. **134**: 389-406, 1996.

Rengel, Z.: Relationship between cytosolic calcium activity and toxicity of aluminium to plant cells. - In: International Symposium on Impact of Potential Tolerance of Plants on the Increased Productivity under Aluminum Stress. Pp. 15-18. Institute of Bioresources, Okayama University, Kurashiki 2000.

Rengel, Z., Elliott, D.C.: Mechanism of aluminum inhibition of net $^{45}Ca^{2+}$ uptake by *Amaranthus* protoplants. - Plant Physiol. **98**: 632-638, 1992.

Rengel, Z., Zhang, W.H.: Role of dynamics of intracellular calcium in aluminium toxicity syndrome. - New Phytol. **159**: 295-314, 2003.

Richardt, G., Federolf, G., Habermann, E.: The interaction of aluminum and other metal ions with calcium-calmodulin-dependent phosphodiesterase. - Arch. Toxicol. **57**: 257-259, 1985.

Rincón, M., Gonzales, A.: Aluminum partitioning in intact roots of aluminum-tolerant and aluminum-sensitive wheat (*Triticum aestivum* L.) cultivars. - Plant Physiol. **99**: 1021-1028, 1992.

Ryan, P.R., Delhaize, E., Jones, D.L.: Function and mechanism of organic anion exudation from plant roots. - Annu. Rev. Plant Physiol. Plant mol. Biol. **52**: 527-560, 2001.

Ryan, P.R., Ditomaso, J.M., Kochian, L.V.: Aluminium toxicity in roots: An investigation of spatial sensitivity and the role of the root cap. - J. exp. Bot. **44**: 437-446, 1993.

Ryan, P.R., Kinraide, T.B., Kochian, L.V.: Al^{3+}-Ca^{2+} interactions in aluminum rhizotoxicity I. Inhibition of root growth is not caused by reduction of calcium uptake. - Planta **192**: 98-103, 1994.

Ryan, P.R., Reid, R.J., Smith, F.A.: Direct evaluation of the Ca^{2+}-displacement hypothesis for Al toxicity. - Plant Physiol. **113**: 1351-1357, 1997.

Schaeffer, H.J., Walton, J.D.: Aluminum ions induce oat protoplasts to produce an extracellular (1,3)(β-D-glucan. - Plant Physiol. **94**: 13-19, 1990.

Schildknecht, P.H.P.A., Vidal, B.C.: A role for the cell wall in Al^{3+} resistance and toxicity: crystallinity and availability of negative charges. - Int. Arch. Biosci. **2002**: 1087-1095, 2002.

Schmohl, N., Pilling, J., Horst, W.J.: Pectin methylesterase modulates aluminium sensitivity in *Zea mays* and *Solanum tuberosum.* - Physiol. Plant. **109**: 419-427, 2000.

Schofield, R.M.S., Pallon, J., Fiskesjö, G., Karlsson, G., Malmqvist, K.G.: Aluminum and calcium distribution

patterns in aluminum-intoxicated roots of *Allium cepa* do not support the calcium-displacement hypothesis and indicated signal-mediated inhibition of root growth. - Planta **205**: 175-180, 1998.

Schwarzerová, K., Zelenková, S., Nick, P., Opatrný, Z.: Aluminum-induced rapid changes in the microtubular cytoskeleton of tobacco cell lines. - Plant Cell Physiol. **43**: 207-216, 2002.

Silva, L.R., Smith, J., Moxley, D.F., Carter, T.E., Allen, N.S., Rufty, T.W.: Aluminum accumulation at nuclei of cells in root tip. Fluorescence detection using lumogallion and confocal laser scanning microscopy. - Plant Physiol. **123**: 543-552, 2000.

Šimonovičová, M., Tamás, L., Huttová, J., Mistrík, I.: Effect of aluminium on oxidative stress related enzyme activities. - Biol. Plant. **48**: 261-266, 2004.

Sivaguru, M., Baluška, F., Volkmann, D., Felle, H.H., Horst, WJ.: Impacts of aluminum on the cytoskeleton of the maize root apex. Short-term effects on the distal part of the transition zone. - Plant Physiol. **119**: 1073-1082, 1999a.

Sivaguru, M., Fujiwara, T., Samaj, J., Baluška, F., Yang, Z., Osawa, H., Maeda, T., Mori, T., Volkmann, D., Matsumoto, H.: Aluminum-induced 1,3-β-D-glucan inhibits cell-to-cell trafficking of molecules through plasmodesmata: a new mechanism of Al toxicity in plants. - Plant Physiol. **124**: 991-1005, 2000.

Sivaguru, M., Horst, W.J.: The distal part of the transition zone is the most aluminum-sensitive apical root zone of maize. - Plant Physiol. **116**: 155-163, 1998.

Sivaguru, M., Yamamoto, Y, Matsumoto, H.: Differential impacts of aluminium on microtubule organization depends on growth phase in suspension-cultured tobacco cells. - Physiol. Plant. **107**: 110-119, 1999b.

Tabuchi, A., Matsumoto, H.: Changes in cell-wall properties of wheat (*Triticum aestivum*) roots during aluminum-induced growth inhibition. - Physiol. Plant. **112**: 353-358, 2001.

Taylor, G.J.: The physiology of aluminium phytotoxicity. - In: Siegel, H. (ed.): Metal Ions in Biological Systems. Pp. 123-163. Marcel Dekker, New York 1990.

Taylor, G.J.: Overcoming barriers to understanding the cellular basis of aluminum resistance. - Plant Soil **171**: 89-103, 1995.

Taylor, G.J., McDonald-Stephens, J.L., Hunter, D.B., Bertsch, P.M., Elmore, D., Rengel, Z., Reid, R.J.: Direct measurement of aluminum uptake and distribution in single cells of *Chara corallina.* - Plant Physiol. **123**: 987-996, 2000.

Tice, K.R., Parker, D.R., DeMason, D.A.: Operationally defined apoplastic and symplastic aluminum fractions in root tips of aluminum-intoxicated wheat. - Plant Physiol. **100**: 309-318, 1992.

Van Breemen, N.: Acidification and decline of Central European forests. - Nature **315**: 16, 1985.

Vázquez, M., Poschenrieder, C., Corrales, L, Barceló, J.: Changes in apoplastic aluminum during the initial growth response to aluminum by roots of tolerant maize variety. - Plant Physiol. **119**: 435-444, 1999.

Wagatsuma, T.: Characterization of absorption sites for aluminum in the roots. - Soil Sci. Plant Nutr. **29**: 499-515, 1983.

Wagatsuma, T., Jujo, K., Ishikawa, S., Nakashima, T.: Aluminum-tolerant protoplasts from roots can be collected with positively charged silica microbeads: A method based on differences in surface negativity. - Plant Cell Physiol. **36**: 1493-1502, 1995.

Wissemeier, A.H., Dieming, A., Hergenroder, A., Horst, W.J., Mix-Wanger, G.: Callose formation as parameter for assessing genotypical plant tolerance of aluminium and manganese. - Plant Soil **146**: 67-75, 1992.

Wissemeier, A.H., Klotz, F., Horst, W.J.: Aluminum induced callose synthesis in roots of soybean (*Glycine max* L.). - J. Plant Physiol. **129**: 487-492, 1987.

Yamamoto, Y., Kobayashi, Y., Devi, S.R., Rikiishi, S., Matsumoto, H.: Aluminum toxicity is associated with mitochondrial dysfunction and the production of reactive oxygen species in plant cells. - Plant Physiol. **128**: 63-72, 2002.

Yamamoto, Y., Kobayashi, Y., Devi, S.R., Rikiishi, S., Matsumoto, H.: Oxidative stress triggered by aluminum in plant roots. - Plant Soil **255**: 239-243, 2003.

Yermiyahu, U., Rytwo, G, Brauer, D.K., Kinraide, T.B.: Binding and electrostatic attraction of lanthanum (La^{3+}) and aluminum (Al^{3+}) to wheat root plasma membranes. - J. Membr. Biol. **159**: 239-252, 1997.

You, G, Nelson, D.J.: Al^{3+} versus Ca^{2+} ion binding to methionine and tyrosine spin-labeled bovine brain calmodulin. - J. inorg. Biochem. **41**: 283-291, 1991.

Zhang, W.H., Rengel, Z.: Aluminium induces an increase in cytoplasmic calcium in intact wheat root apical cells. - Aust. J. Plant Physiol. **26**: 401-409, 1999.