Advances in Molecular Biology (1): 1-12, 2007
İstanbul Kültür University, Printed in Turkey
www.advmolbiol.org

Review

Aluminum toxicity and resistance in higher plants

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Abstract

Aluminum (Al) is the major element in the soil and exists as a stable complex with oxygen and silicate. When the soil pH is below 5, Al is solubilized in the soil water and absorbed by plant roots. Absorbed Al inhibits root elongation severely within hours. Al toxicity is a very important limitation to worldwide crop production, because 50% of the world's potentially arable lands are acidic. Thus, many research has been conducted to understand the mechanism of Al toxicity and resistance which is important for stable food production in future. Al resistance can be achieved by mechanisms that facilitate Al exclusion from the root apex and/or by mechanisms that confer the ability of plants to tolerate Al in the plant symplasm. However, despite intense research efforts, there are many aspects of Al toxicity and resistance remain unclear. In this review, Al toxicity and resistance mechanisms are described with the physiological and molecular basis.

Key words: Aluminum, toxicity, resistance, exclusion, detoxification.

Introduction

Although aluminum (Al) is not regarded as an essential nutrient, it is one of the most abundant mineral in the soil, comprising approximately 7%. At neutral or weakly acidic pH, Al exists in the form of insoluble aluminosilicate or oxide. When the soil becomes more acid, Al is solubilized into a phytotoxic form (Matsumoto, 2000). $Al(H_2O)_6^{3+}$ which is known as Al^{3+} is dominant in acid soil below pH 5 and is the most toxic form. Al toxicity is the primary growth-limiting factor for plants in acid soils (Foy, 1992) and is most severe in soils with low base saturation, poor in Ca and Mg (Vitorello *et al.*, 2005).

It has been estimated that over 50% of the world's potentially arable lands are acidic (Von Uexküll and Mutert, 1995). Furthermore, up to 60% of the acid soils in the world occur in developing countries in South America, Central Africa and Southeast Asia, where food production is critical. Soil acidity is a natural occurrence in tropical and subtropical zones. But in temperate zones, it is an increasing problem and the result of acid rain in the industrial regions of the USA, Canada and Europe (Vitorello *et al.,* 2005). Although the poor fertility of acid soils is due to a combination of mineral toxicities (Al and Mn) and deficiencies (P, Ca, Mg and Mo), Al toxicity is the most important factor, being a major constraint for crop production on 67% of the total acid soil area (Eswaran *et al.*, 1997).

Al toxicity and tolerance mechanisms differ strikingly with its chemical form, and the study of Al-related processes is complicated by the complex chemistry of Al. Therefore the experimental results may differ with experimental conditions such as pH and coexisting ions, even same concentration of Al is used (Matsumoto, 2000). The cellular components and processes which have been proposed to be affected by Al are wide ranging and some of the most important include; cell nuclei, mitosis and cell division (Silva *et al.*, 2000), composition, physical properties and structure of the plasma membrane (Zhang *et al.*, 1997; Ishikawa and Wagatsuma, 1998), uptake of Ca^{2+} and other ions (Ryan and Kochian, 1993; Liu and Luan, 2001), phosphoinositide-mediated signal transduction and cytoplasmic calcium homeostasis (Jones and Kochian, 1995; Rengel and Zhang, 2003), oxidative stress (Yamamoto *et al.*, 2003), cytoskeletal dynamics (Sivaguru *et al.*, 1999) and the cell wall-plasma membrane-cytoskeleton continuum (Horst *et al.*, 1999).

Because of the complex chemistry of Al, molecular, genetic and physiological bases are still not well understood. Despite the interest from many

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Received: January 11, 2007; Accepted: February 8, 2007.

researchers, Al resistance genes have yet to be cloned from any species, with the exception of *ALMT1* from wheat (Sasaki *et al.*, 2004).

Although this review will cover Al toxicity and resistance generally, our aim is to focus especially on the mechanisms of toxicity and to point out aspects which have been largely ignored in this field.

Mechanisms of aluminum toxicity

Al interferes with a wide range of physical and cellular processes. Potentially Al toxicity could result from complex Al interactions with apoplastic, plasma membrane and symplastic targets. According to the literature it is difficult to give a definite time for Al toxicity, because some Al-toxic symptoms and responses are detectable within seconds to minutes after exposure to Al, others are only noticeable after long-term exposure (Kochian *et al*., 2005).

Aluminum toxicity symptoms

The most evident symptom of Al toxicity is root growth inhibition, which can be detected within 30 min. to 2 hours, even as micromolar concentrations of Al (Barceló and Poschenrieder, 2002). Although the seed germination is not affected by Al, root and seedling developments are reduced (Nosco *et al.*, 1988). Cells which are affected by Al are the root apex (root cap, meristem and elongation zone), more specifically the distal part of the transition zone within apex, root hairs and branch initials (Sivaguru and Horst, 1998). The root apex accumulate more Al within minutes and play a major role in the Al-perception mechanism (Matsumoto, 2000). Inhibition of root growth is considered to be primarily the result of inhibited cell elongation and expansion, prior to inhibiting cell division (Frantzios *et al.*, 2001; Ciamporova, 2002; Vardar *et al.*, 2004). Prolonged exposures lead to Al interactions with the root cell division and the cytoskeleton (Silva *et al.*, 2000).

Much of the Al absorbed by roots penetrate root apex and root cap. According to Rout *et al.* (2001) some Al passed through the epidermis and cortex, but considerable amounts were retained in cortical cells. Although a large fraction of the Al interacts with apoplastic targets, a small fraction enters the symplasm and interacts with symplastic targets. Severity of Al toxicity depends on the concentrations of Ca^{2+} and other cations in the external solution, the ionic strength of solutions, pH, the presence of chelators, cell type and plant genotype (Kinraide and Parker, 1987).

Al toxicity is associated with severe changes in root morphology. Briefly, it results in curved, swollen, cracked, brownish, stubby and stiff root apices (Vardar *et al.*, 2006). Fine branching and root hairs are reduced. Uneven and radial expansion of cells of the cortex cause root thickening and mechanical stress on the epidermis (Ciamporova, 2002). This extensive root damage results in a reduced and damaged root system and limited water and mineral nutrient uptake (Barceló and Poschenrieder, 2002).

Although symptoms of Al toxicity are also manifested in the shoots, these are usually regarded as a result of root damage. The most common responses in shoots to Al toxicity are cellular modifications in leaves, reduced stomatal opening, decreased photosynthetic activity, chlorosis and foliar necrosis (Vitorello *et al.*, 2005). Long-term exposure to Al and inhibition of root growth generally lead to P, K, Ca and Mg deficiencies (Haug and Vitorello, 1996). The ultimate consequence is reduced plant biomass. With the exception of Al-accumulating plants little Al is transported into the shoot (Watanabe and Osaki, 2002).

Researchers have regarded the cell wall, plasma membrane, signal-transduction pathways, root cytoskeleton and DNA/nuclei as potential Al targets which are associated with root growth.

The cell wall

X-ray microanalysis and secondary ion mass spectroanalysis have indicated that a significant fraction of Al is associated with apoplastic binding sites in walls of the root periphery cells (Vazquez *et al.*, 1999). The net negative charge of the cell wall determines its cation exchange capacity (CEC), and consequently the Al interaction degree with the cell wall. Among the many components of the cell-wall network, pectins have been proposed to be a critical site for Al-cell wall interactions (Blamey *et al.*, 1993). Al interactions lead to the displacement of other cations (e.g. Ca^{2+}) fundamental for cell-wall stability and rapid callose synthesis on plasma membrane which incorporate into apoplasm (Tabuchi and Matsumoto, 2001).

It is proposed that the accumulation of Al in the cell wall exerts a detrimental effect on root growth and function in three ways. First the decrease in apoplastic sorption of basic cations, which have limited ability to displace bound Al, reduces nutrient acquisition per unit root length. Second the Al sorbed in the cell wall reduces cell expansion, thus reducing root elongation.

This would also reduce nutrient uptake through decreased root proliferation through the soil. Third, sorption of Al in the cell wall reduces the movement of water and solutes through the apoplasm, directly decreasing nutrient acquisition by the root (Blamey, 2001).

Consequently, the strong and rapid binding of Al can alter cell-wall structural and mechanical properties, making it more rigid, leading to a reduction in the mechanical extensibility of the cell wall required for normal cell expansion in the root elongating zone particularly (Kochian *et al.*, 2005).

The plasma membrane

Negatively charged plasma-membrane surface is the first potential target for Al^{3+} (Kinraide *et al.*, 1998). As Al has a more than 560-fold greater affinity for the choline head of phosphatidylcholine than other cations such as Ca^{2+} have, Al^{3+} can displace other cations that may form positively charged bridges between the phospholipid head groups of the membrane bilayer (Akeson and Munns, 1989). A positively charged layer would retard the movement of cations and increase the movement of anions to the transport proteins of the plasma membrane in proportion to the charges carried by these ions (Nichol *et al.*, 1993). As a consequence, the phospholipid fluidity and the charges of the plasma membrane are altered. Thus, Al interactions at the plasma membrane can modify the structure of the plasma membrane as well as the ionic environment near the surface of the cell; both can lead to disturbances of ion-transport processes, which can perturb cellular homeostasis (Kochian *et al.*, 2005).

One of the early symptoms of Al toxicity is quickly activated callose (β-1,3-glucane) accumulation in the apoplast (Massot *et al.*, 1999). Since callose synthesis depends on the presence of Ca^{2+}, it has been suggested that Al displacement of Ca^{2+} from the membrane surface may increase the apoplastic Ca^{2+} pool required to stimulate callose synthesis. Under Al stress, callose accumulation may lead to further cellular damage by inhibiting intercellular transport through plasmodesmatal connections (Sivaguru *et al.*, 2000).

Al can significantly inhibit the activity of the plasma membrane H^+-ATPase, impending formation and maintenance of the trans-membrane H^+ gradient. Consequently, Al disruption of the H^+ gradient could indirectly alter the ionic status and ion homeostasis of root cells (Kochian *et al.*, 2005).

Electrophysiological approaches were subsequently used to demonstrate that Al^{3+} interacts directly with several different plasma-membrane channel proteins, blocking the uptake of ions such as Ca^{2+}, K^+, Mg^{2+} and NH^+_4 (Piňeros and Tester, 1997). In addition to directly altering ion permeation through channels, extracellular Al can also modulate the transporter's activity via changes in the membrane potential. For example, Al-induced membrane depolarizations can alter voltage-dependent Ca^{2+} channel transport by indirectly modulating and shifting the activation thresholds of distinct transport pathways, such as hyperpolarization-activated (Very and Davies, 2000) and depolarization-activated (Piňeros and Tester, 1997; Thion *et al.*, 1996) Ca^{2+} channels.

Al effects on signal-transduction pathways

Al interactions with signal-transduction pathways, in particular disruption of intracellular Ca^{2+} and pH homeostasis, have been proposed to play crucial roles in Al toxicity (Ma *et al.*, 2002). Al can also interact with and inhibit the enzyme phospholipase C of the phosphoinositide pathway associated with Ca^{2+} signalling (Jones and Kochian, 1997). Guanine nucleotide-binding proteins (G proteins) and a phosphatidylinositol-4,5-diphosphate (PIP_2)-specific phospholypase C are probable interaction sites for Al ions. Following interiorization of Al by the cell, metal interactions decrease the accumulation of inositol phosphate, especially that of inositol-1,4,5-triphosphate (IP_3), concomitant with disorders of intracellular Ca homeostasis (Rengel, 1992). These alterations would ultimately reflect in any of the physiological and morphological changes described above. Al also may play a role in the regulation of protein phosphorylation and/or dephosphorylation (Matsumoto, 2000).

Reactive oxygen species (ROS) such as superoxide anions and hydrogene peroxide that result from photosynthesis and oxidative metabolism can be involved in a number of stress responses (Foyer *et al.*, 1994). It has been shown that Al exposure is associated with peroxidative damage of membrane lipids due to the stress-related increase in the production of highly toxic oxygen free radicals (Cakmak and Horst, 1991). Phosphatidylserine is the most susceptible substrate for Al to facilitate lipid peroxidation (Xie and Yokel, 1996). A close relationship existed between lipid peroxidation and inhibition of root elongation rate. Enhanced lipid peroxidation by oxygene free radicals is a consequence of the primary effects of Al on membrane

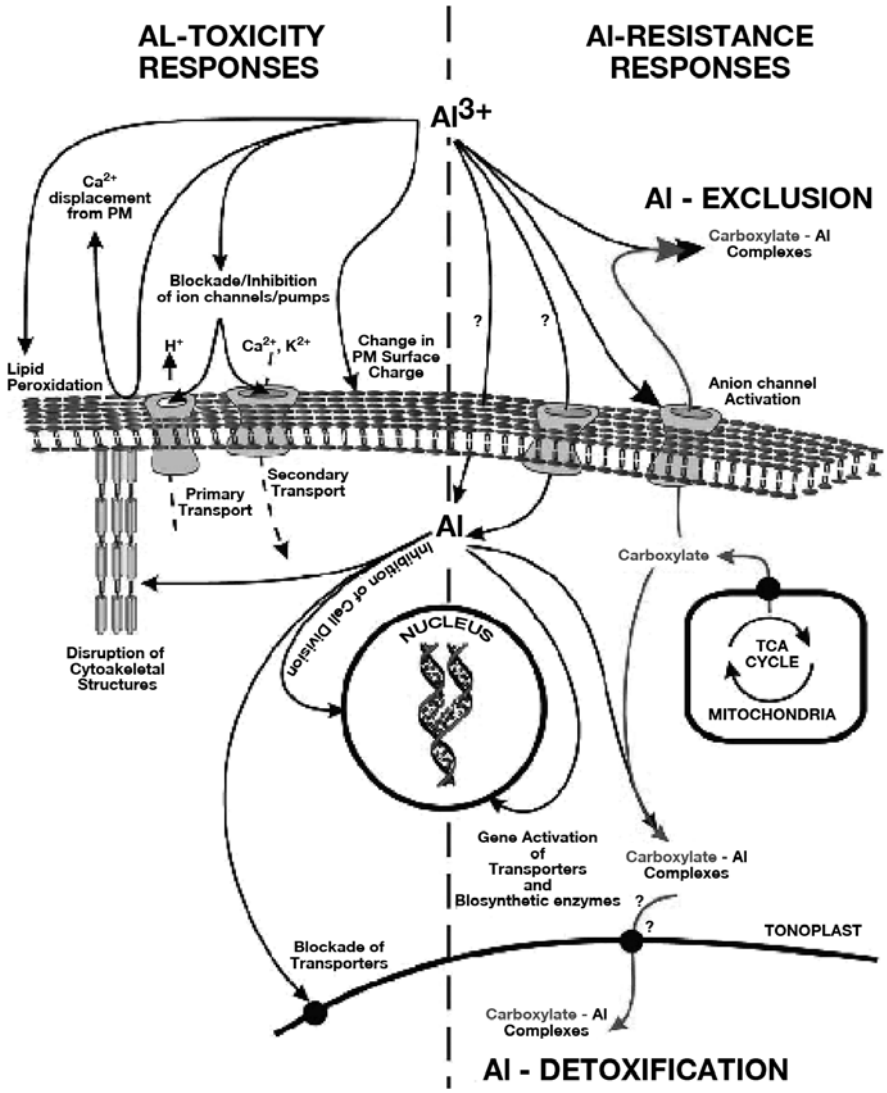

Figure 1. Possible mechanism of aluminum toxicity and resistance in plants. Aluminum toxicity targets described in the text are illustrated on the left side of the diagram. For clarity, the interactions of aluminum with the cell wall were not shown on the right side, aluminum resistance mechanisms (aluminum exclusion and internal aluminum detoxification) are based on the formation of aluminum complexes with carboxylates. The aluminum exclusion mechanism involves the release of carboxylate anions via an Al-gated anion channel at the plasma membrane. The internal aluminum detoxification mechanism involves chelation of cytosolic aluminum by carboxylate anion with the subsequent sequestration into the vacuole via unknown transporters (Kochian *et al*., 2005)

structure (Cakmak and Horst, 1991) but Al-induced lipid peroxidation does not occur rapidly enough to be an initial mechanism of Al toxicity (Yamamoto *et al.*, 2001).

The root cytoskeleton

The cytoskeleton is the potential cytosolic target for Al toxicity, because of the central importance of cytoskeletal components in cell division and expansion of a growing root. Al could disrupt cytoskeletal dynamics either via a direct interaction with cytoskeletal elements (i.e. microtubules and actin filaments) or indirectly, via alteration of signaling cascades such as cytosolic Ca^{2+} levels that are involved in cytoskeletal stabilization. Plant cells require dynamic cytoskeleton-based networks both for cell division and cell-wall biosynthesis (Sivaguru *et al.*, 1999). It has been well documented that Al exposure inhibited longitudinal cell expansion and induced lateral cell swelling by disrupting both the organization

of microtubules and microfilaments in elongation zone cells of root (Frantzios *et al.*, 2001; Sivaguru *et al.*, 2003). For example, exposure to Al results in the disruption and reorganization of cortical microtubules (Sasaki *et al.*, 1997). The disintegration of spindle microtubules and disorganization of phragmoplasts caused by Al might block cell division directly at metaphase under Al stress (Sivaguru *et al.*, 1999). Likewise, Al induced a significant increase in the tension of the actin filaments of soybean (*Glycine max*) cells. This may result from the formation of nonhydrolyzable [Al^{3+}-ADP] or [Al^{3+}-ATP] complexes whose binding to actin/myosin. Al^{3+} can bind to nucleoside triphosphates approximately 107 times better than Mg^{2+} and the rate of hydrolysis for Al^{3+}-ATP or Al^{3+}-GTP complexes is considerably lower than that for the physiological Mg^{2+} complex (10^5 times slower), supporting the hypothesis that toxicity is a result of Al^{3+} displacement of Mg^{2+} from nucleoside di- or triphosphate complexes. Such Al-induced cellular structural changes are likely to result in and underlie the morphological changes and structural malformations observed in Al-stressed roots (Grabski and Schindler, 1995).

DNA/nuclei

Prolonged exposures can lead to Al interactions with structures within the nucleus, detrimentally affecting DNA composition, DNA replication by increasing rigidity on the double helix and chromatin structure (Silva *et al.*, 2000). Al can bind to nucleoside triphosphates with an association constant 10^7 times that of Mg^{2+} (Grabski and Schindler, 1995). Therefore, Al prefers binding to DNA compared to histone and nonhistone proteins. The binding to DNA was inhibited by 70 % in the presence of an equal amount of histone to DNA (Matsumoto *et al.*, 1976). Al affected the mechanisms controlling the organization of the microtubular cytoskeleton, as well as tubulin polymerization which induced the delay of the microtubule disassembly during mitosis, resulting in the persistence of preprophase microtubule bands in the late prophase cells and a disturbance in the shortening of kinetochore microtubule bundles in anaphase cells. Al also affected the disorder of chromosome movements carried out by the mitotic spindle (Frantzios *et al.*, 2000). Nuclear changes were nucleoli (Bennet *et al.*, 1985). These types of increase in size and frequency of vacuoles in interactions of Al with the nucleus can result in the disruption of the cytoskeleton and cell division processes. The above putative mechanisms of Al toxicity are summarized in the model shown in Figure 1.

Mechanisms of aluminum resistance

Because of the agronomic importance, breeding crops with Al resistance has been a successful and active area of research; however, the underlying molecular, genetic and physiological principles are still not well understood. Despite the interest from many researchers, no Al resistance genes have yet been cloned from any plant (Kochian *et al.*, 2005).

It has been known that plants which exist in the presence of potentially toxic Al concentrations must be able to avoid direct contact of vital structures and metabolic processes with high activities of Al^{3+} ions. The physiological mechanisms of Al resistance can either be mediated via exclusion of Al from the root apex or via intracellular tolerance of Al transported into the plant symplasm (Kidd *et al.*, 2001; Kochian *et al.*, 2005). Either extracellular precipitation or detoxification of Al^{3+} may be implied in exclusion.

Aluminum exclusion

Aluminum tends to form strong complexes with oxygene donor ligands. Large experimental evidences have shown that complexation with chelating root exudates or binding to mucilage play a main role in the prevention of the accumulation of phytotoxic Al in both apoplast and symplast (Barceló and Poschenrieder, 2002). In plants, Al makes complexes with phosphate and carboxylates secreted from the root apex, but strong complexes can also be formed with phenolic substances, pectates, mucopolysaccharides or siderophores (Winkler *et al.*, 1986).

Aluminum exclusion via root carboxylate exudation

Al chelation by carboxylate exudates reduces the activity of free Al ions and consequently, their binding to the root cell wall and/or plasma membrane. The kinds of carboxylates secreted by Al-exposed roots vary depending on the Al tolerant plant species, but secretion of citrate and malate are the most commonly cited ones.

Malate exudation mechanism by wheat has been investigated most thoroughly (Kochian, 1995) while citrate seems to be the most common organic acid anion exudated by Al-tolerant maize and snapbean (Barceló and Poschenrieder, 2002). In all three species secretion was greater (up to 10-fold) in Al-resistant cultivars than in Al-sensitive ones. Oxalate exudation in response to Al has also been observed in maize, but

no differences between sensitive and tolerant varieties were detected (Kidd *et al.*, 2001). Among the organic acid anions, the most potent chelator of Al^{3+} is citrate which can be synthesized in a large amount through photosynthesis (Larsen *et al.*, 1998). After chelation, Al-citrate transport through the plasmalemma seems to be very slow (Kochian, 1995). In long-term studies an Al-resistant cultivar of snapbean excreted 8-fold more citrate from the roots than did an Al-sensitive genotype (Miyasaka *et al.*, 1991). This is supported by the observations in wheat that Al-resistant genotypes release malate and accumulate significantly less Al in the first few milimeters of root apex compared with Al-sensitive genotypes (Delhaize *et al.*, 1993a). Related to these studies, high levels of Al-activated release of carboxylates have been correlated with Al-resistance in a large number of plant species. Some of the major aspects of this resistance mechanism include:

- A correlation between Al resistance and Al-activated carboxylate release in many plant species (Kochian *et al.*, 2005).
- Al-carboxylate complexes are not transported into roots or across membranes (Akeson and Munns, 1990).
- Activation of carboxylate release is triggered specifically by exogenous Al^{3+} (Ryan *et al.*, 1995a).
- The rates of Al-activated carboxylate release are dose-dependent on the Al activity in the rhizosphere (Delhaize *et al.*, 1993b; Piñeros *et al.*, 2002).
- In some cases, overexpressions of genes encoding enzymes involved in organic acid synthesis, such as citrate synthase and malate dehydrogenase can result in enhanced Al resistance (Tesfaye *et al.*, 2001).
- An Al-gated anion channel in maize and wheat root tip protoplasts has been identified via electrophysiological experiments and exhibits the properties necessary for it to be the transporter mediating Al-activated carboxylate release (Piñeros *et al.*, 2002; Zhang *et al.*, 2001).

The Al-citrate 1:1 complex is not phytotoxic (Kochian, 1995). At a 1:1 ratio the Al-oxalate complex also had little toxic effects in Al-sensitive wheat and the complex prevented Al accumulation in the root tip (Ma *et al.,* 2001). In contrast, Al-malate treated roots stained for Al (i.e. Al was taken up) and root elongation was inhibited, but Al-malate was less toxic than $AlCl_3$. This graduation of efficiency of organic acid anions in preventing Al toxicity and uptake is good agreement with the stability constants (Barceló and Poschenrieder, 2002).

The interesting contradiction of this mechanism is whether it is inducible at the level of gene expression. An Al-inducible resistance mechanism is seen in some plant species such as rye, triticale and *Cassia tora*. In these species the rate of exudation increases over the first 12-24 hours of Al exposure. This means Al-activated carboxylate exudation increases slowly (Li *et al.*, 2000). However, root malate exudation is very rapidly activated by Al exposure in wheat and the rate of malate efflux does not increase over time. Therefore, in species like wheat, Al apparently activates an already expressed carboxylate transporter and gene activation does not seem to play a role. In species where the rate of carboxylate exudation apparently increases with time, it is possible that induction of Al-resistance genes contributes to this increased capacity (Kochian *et al.*, 2005).

In many plant species, exudation of specific carboxylate anions is activated by Al exposure rapidly. Thus, an important part of this Al-resistance mechanism is the activation of a particular carboxylate transporter that presumably exists in the root cell plasma membrane (Kochian *et al.*, 2005). In wheat, Al activates malate release almost instantly and increased carboxylate snythesis is not involved (Osawa and Matsumoto, 2001). Even though Al exposure activates a large and continuous efflux of malate in the Al-resistant genotype, no differences in root tip malate concentration or in malate dehydrogenase activity in Al-resistant to sensitive genotypes have been observed (Ryan *et al.*, 1995a, b).

The termodynamic conditions for carboxylate transport from the cytosol to the external solution suggest that ion channels could be the primary transporter involved in this resistance response. The organic acids in the cytosol exist primarily as anions ($malate^{2-}$ and $citrate^{3-}$) and due to the large negative-inside transmembrane electrical potential in plant cells, there is a very strong gradient directed out of the cell for anions (Kochian *et al.*, 2005). Thus, an anion channel that opens upon exposure to Al would be sufficient to mediate this transport. Anion channels that are specifically activated by extracellular Al^{3+} have recently been identified using the patch-clamp technique with protoplasts isolated from root tips of Al-resistant wheat (Zhang *et al.*, 2001) and maize (Kollmeier *et al.*, 2001; Piñeros *et al.*, 2002). In maize

the most important discovery was that the anion channel could be activated in isolated plasma-mambrane patches, where the anion channel is operating in isolation from cytosolic factors (Piñeros and Kochian, 2001; Piñeros *et al.*, 2002).

These features needed for Al activation of the anion channel are contained within the channel protein itself, or are close by in the membrane (e.g., an associated membrane receptor). As shown in Figure 1, there are three possible ways that Al could activate a plasma-membrane anion channel involved in carboxylate exudation:

1- Al interacts directly with the channel protein, causing a change in conformation and increasing its mean open time or conductance;

2- Al interacts with a specific receptor on the membrane surface or with membrane itself, which through a series of secondary messages in the cytoplasm, changes channel activity; or

3- Al enters the cytoplasm and alters channel activity either directly binding with the channel or indirectly through a signal transduction pathway (Matsumoto, 2000; Kochian et al., 2005).

Phenolic compounds

Several comparative studies including different species showed that there were no correlation between Al resistance and the amount of organic efflux (Ishikawa *et al.*, 2000). These results support that exudation of organic acids may not be the only mechanism of Al exclusion.

Root exudation of phenolic compounds has been described by many authors (Marschner, 1995). Phenolics can reverse the toxic effects of Al on hexokinase (Taylor 1988) and on root elongation (Wagatsuma *et al.*, 2001). However, they are less efficient at equimolar concentrations than citrate in complexing Al (Ofei-Manu *et al.*, 2001). Thus, phenolics in complex formation with Al has deserved much less consideration than organic acid anions. However, by a deprotonation reaction, the phenolics in presence of carboxylic groups from organic acids can strenghen the interaction between Al^{3+} and the organic acid anion ligand, increasing the effective stability constant for the Al-organic acid anion complex (Driscoll and Schecher, 1988). It has also been argued that phenolics may favor Al binding by organic acid anions by inhibiting rhizosphere microorganisms that degrade organic acids.

Recent investigations revealed that Al induced exudation of the flavonoid type phenolics catechin and quercetin from 10 mm root tips in an Al resistant maize variety (Kidd *et al.*, 2001). Stimulation of exudation of these flavonoid-type phenolics was in good agreement with protection of root elongation against Al. Investigations on a larger number of maize varieties and on other species are required in order to see if this exudation of flavonoid-type phenolics is a particularity of certain Al-resistant maize varieties or a common property of a larger group of Al resistant species (Barceló and Poschenrieder, 2002).

Rhizodepositions

The meristem and root cap where Al toxicity appeared dominantly are coated with mucilage. Mucilage consists of enormous molecules which are glucose, galactose, arabinose and uronic acids (Matsumoto, 2000). Mucilage and border cells have been implicated in Al resistance mechanisms (Horst *et al.*, 1982). Higher mucilage production was observed in the Al-persistant wheat cultivar Atlas 66 than in a sensitive cultivar (Puthota *et al.*, 1991). Mucilage blocks the entry of Al into the root by bounding it in rhizosphere. Archambault *et al.,* (1996) found that Al bound to the mucilage of wheat root accounted for approximately 25-35% of Al remaining after desorption in citric acid.

In snapbean cultivars higher Al resistance was related to better border cell viability and to higher mucilage production by the border cells of the Al resistant cultivar (Miyasaka and Hawes, 2001). According to Delisle *et al.,* (2001), at equal effect concentrations early cell death is rapidly seen in the Al resistant wheat cultivar, but not in the Al sensitive one. This early cell death response differs from the formation of the detached living border cells found in Al resistant snapbeans. This limited cell death seemed to contribute to Al resistance and cannot be attributed to the oxalate-mediated H_2O_2 burst occuring later as a second wave response that may be implied in Al trapping in the cell wall. This early death response in the Al resistant wheat was limited to a few cells in the elongation zone and showed similarities to the hypersensitive response of tolerant plants to potential pathogens (Barceló and Poschenrieder, 2002).

Internal aluminum detoxification

Although exclusion from root tips and restriction of Al transport to upper plant parts seem to be the most important mechanism in Al resistance, there are numerous species that tolerate relatively high Al concentrations that is based on the complexation and

detoxification of Al after its entry the plant. This discovery has come from research on plants that can accumulate Al to high levels in the shoot. High shoot accumulation of Al with ligands in an innocuous form (soluble or solid) occurs in leaf vacuoles or in the apoplast. Among the ligands that form stable complexes with Al, organic acid anions, phenolic substances and silicon may be implied in Al detoxification inside shoot tissues (Barceló and Poschenrieder, 2002).

High citrate concentrations have been reported in *Hydrangea macrophylla* leaves whose sepals turn from red to blue due to Al accumulation in the sepals when the soil is acidified (Takeda *et al.*, 1985). It can accumulate more than 3000µg g^{-1} Al dry weight in its leaves (Ma *et al.*, 1997a). Identification of Al chelates by ^{27}Al NMR indicates that Al is complexed in a 1:1 Al-citrate complex in leaves. Citrate should bind Al very tightly in the cytosol with a pH of around 7 and protect the cytosol against Al injury. Ma and colleagues (1997b) also studied a second Al accumulator, buckwheat (*Fagopyrum esculentum*) whose Al resistance due to Al-activated oxalate exudation from the root apex (Zheng *et al.*, 1998). However buckwheat also accumulates Al to very high levels in its leaves, as high as 15,000 µg Al g^{-1} dry weight when the plant is grown on acid soils. Most of the Al in both roots and leaves was complexed in a 1:3 Al-oxalate complex (Ma *et al.*, 1998). Subsequently, it has been proposed that Al is transported in the xylem sap complexed with citrate, while oxalate would be the storage form of Al in leaf vacuoles (Watanabe *et al.*, 2000). These findings suggest that the Al undergoes a ligand exchange from oxalate to citrate when it is transported into the xylem, and is exchanged back with oxalate in the leaves. Leaf compartmental analysis showed that 80 % of the Al in buckwheat leaves was stored in vacuoles as a 1:3 Al-oxalate complex (Shen *et al.*, 2002). On the right side of Figure 1 where the different possible Al-resistance mechanism depicted, this internal detoxification mechanism is shown to involve Al chelation in the cytosol and subsequent storage of the Al-carboxylate complex in the vacuole. The tonoplast-localized mechanisms mediating the transport of Al into vacuole, as well as the nature of its substrate (i.e., free Al versus Al-carboxylate complexes) remain unknown.

More recently, barley plants transformed with a gene (*ALMT1*) encoding a putative malate transporter were found to be more resistant to Al (Delhaize *et al.*, 2004). This perhaps makes more sense, given that this could increase exudation without necessarily changing cytoplasmic metabolite concentrations (Vitorello *et al.*, 2005)

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