Root Behavior in Response to Aluminum Toxicity

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Abstract Roots have an extraordinary capacity for adaptive growth which allows them to avoid toxic soil patches or layers and grow into fertile sites. The response of roots to aluminum toxicity, a widespread problem in acid soils, is an excellent model system for investigating the mechanisms that govern this root behavior. In this review, after a short introduction to root growth movement in response to chemical factors in the soil, we explore the basic mechanisms of Al-induced inhibition of root growth. The actinomyosin network and endocytic vesicle trafficking are highlighted as common targets for Al toxicity in cell types with quite different origins: root tip transition zone cells, tip-growing cells like root hairs or pollen tubes, and astrocytes of the animal or human brain. In the roots of sensitive plants, the perception of toxic Al leads to a change in root tip cell patterning. The disturbance of polar auxin transport by Al seems to be a major factor in these developmental changes. In contrast, Al activates organic acid efflux and the binding of Al in a nontoxic form in Al-resistant genotypes.

1 Introduction

Individual terrestrial higher plants are sessile, living anchored to the substrate by their roots. Migration to better, more fertile soil conditions is only possible for their genetic information (pollen) or their offspring (seeds), which have different mechanisms of

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dissemination. Slow movement away from the original placement is also possible as clones by vegetative propagation, e.g., through the formation of stolons or rhizomal growth (Hart 1990).

Investigations into plant movements have so far mainly focused on aerial plant parts. Different mechanisms can be distinguished: those based on turgor changes (e.g., nyctinasty and thigmonasty), or those based on differential growth (such as phototropism and epinasty). An exception is gravitropism, another growth-based movement, which has mainly been investigated in roots. However, bending in response to gravitational stimulus is far from being the only movement available to roots (Barlow 1994). Hydrotropism, the directed growth of roots in relation to the gradient of soil water potential, is a well-established growth-based movement of roots in response to an essential chemical soil factor (water) (Ponce et al. 2008). The availability of other essential nutrients can also induce changes in the orientation of root growth in order to improve acquisition. Phosphorus and nitrogen are the best-studied examples (Desnos 2008). The movement of roots into nutrient-rich soil patches implies complex morphogenetic events, such as root hair formation, the induction of new laterals, or—in certain species—proteoid root formation. These trophomorphogenetic responses are controlled directly by the nutrient concentration in the external medium or indirectly by the nutrient status of the plant, or by both (Forde and Lorenzo 2001).

Avoiding toxic soil conditions by altering root growth patterns is a further mechanism that allows plants to move away and try to escape from inadequate growth conditions. Two different scenarios can be envisaged: (1) heterogeneous soil contamination with small hotspots of high toxicant concentrations embedded in less toxic soil, and (2) extended toxic layers in the subsoil.

A heterogeneous distribution of potentially toxic concentrations of metal ions is frequently observed in soils polluted by mining activities. The observation that less Cd was taken up by *Brassica juncea* from soil with a heterogeneous Cd distribution than from uniformly polluted soil supports the view that plants are able to sense the spot contamination and avoid growth into contaminated sites (Manciulea and Ramsey 2006). Contrastingly, *Thlaspi caerulescens*, a metal hyperaccumulating species with unusually high Zn requirements (Tolrà et al. 1996), exhibits zincophilic root foraging patterns, i.e., preferential growth into hot spots with high Zn concentrations (Haines 2002). The efficiencies of both avoidance and foraging responses seem to depend on the root system size of the species. While a negative correlation between species root biomass and precision of placement has been observed in foraging studies on nutrient-rich patches (Wijesinghe et al. 2001), larger root systems seem to be more effective at avoiding toxic spots than small ones (Manciuela and Ramsey 2006). A well-developed tap root system can also be very useful for avoiding the relatively uniform topsoil contamination produced by (for example) smelting activities or after years of applying copper sulfate to vines or hopyards.

In contrast, subsoil acidity is a typical scenario where the extension of roots into the deep soil is hampered by the presence of a layer of soil with high metal availability extending from several decimeters below the soil surface. Crop plants used in tropical and subtropical agriculture and forest stands affected by natural acidification or that due to acid rain are the plants of most concern in this context

(Jentschke et al. 2001; Kochian et al. 2004). Aluminum is considered to be the main toxic factor in acid soils with pH values of less than 4.5. More than 50% of the world's arable land is acidic, so Al toxicity should be considered one of the most important ion toxicity stressors in crop production worldwide. Intensive research into the mechanisms of Al toxicity and Al tolerance mechanisms has been carried out over the last few decades in order to provide the scientific background needed to speed up breeding programs in order to improve crop productivity in acid soils. Aside from this evident practical reason, the responses of plants to Al toxicity are also being used as highly informative model systems. The Al-induced alterations allow fundamental aspects of root stress perception and transduction to be investigated, as well as basic mechanisms of adaptative growth in roots, which are characterized by an enormous capacity for plastic responses to changing physical and chemical conditions in the soil.

2 Aluminum-Induced Inhibition of Root Growth

Root growth is a primary target for Al toxicity in plants. Maintenance of root elongation rate under Al stress is frequently used for Al tolerance screening purposes in hydroponics (Llugany et al. 1994; Ma et al. 2005; Narasihmamoorthy et al. 2007). Monitoring root elongation rates of maize varieties during the first minutes and hours upon exposure (Llugany et al. 1995) reveals various response patterns (Fig. 1): (1) The *threshold of toxicity* model, where a threshold time of 15–45 min and a threshold concentration (usually of a few μM) is required before Al-induced inhibition of elongation is detectable; (2) the *hormesis* response, where a transient Al-induced stimulation of root elongation followed by inhibition is observed, and; (3) the *threshold of tolerance* response, where a fast inhibition of elongation is followed by a recovery in the growth rate (Barceló and Poschenrieder 2002).

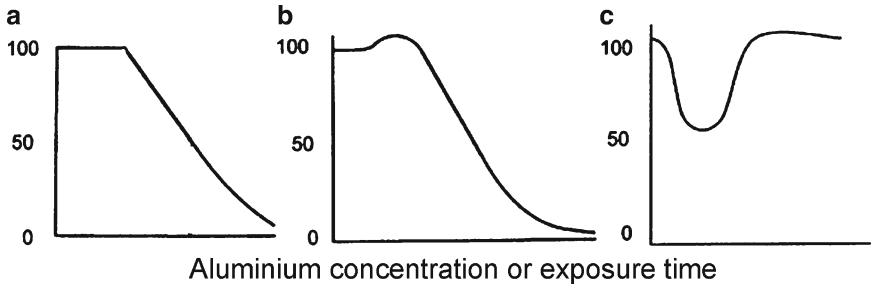

Fig. 1a–c Models for relative root elongation response (%) as a function of Al concentration or exposure time. **a** Toxicity threshold response: the greatest Al concentration or exposure time that does not have an observable effect on root elongation is an indicator of plant Al resistance. **b** Hormesis: growth stimulation by low Al concentrations or short exposure times due to the alleviation of another stress factor (e.g., proton toxicity). **c** Threshold of tolerance response: after the perception of Al-induced stress and the inhibition of elongation, defense mechanisms are activated (e.g., pattern 2 of organic acid efflux)

In the first response pattern, the threshold concentration and the time needed for growth inhibition are indicators of the Al tolerance of the plant. The need for a lag time of usually more than 15 min before elongation inhibition is detectable in sensitive plants (Llugany et al. 1995; Blamey et al. 2004) does not imply that key processes governing root growth cannot be affected even more rapidly (see Sects. 4 and 5).

The second pattern, a transient Al-induced stimulation of root elongation, is a clear hormetic effect, i.e., a positive response to a potentially toxic factor due to the alleviation of another stress suffered by the target organism. In experimental systems where plants are exposed to Al in nutrient solutions with low pH in order to maintain high Al^{3+} activity, proton toxicity is most probably the additional stress factor alleviated by Al (Llugany et al. 1994). The ameliorating effect of the trivalent Al^{3+} on the toxicity of monovalent H^+ can be attributed to competition among these cations in binding to the cell wall and plasma membrane surface, leading to site-specific amelioration at biological ligand targets and to alterations of the plasma membrane surface potential. Effects on the plasma membrane surface potential, in turn, influence the bioavailability of the intoxicating and ameliorating cations (Kinraide 2006; Kinraide and Yermiyahu 2007).

A threshold for tolerance response is observed in species with an inducible Al resistance mechanism, e.g., Al-induced secretion of organic acid anions following pattern II behavior (Ma 2000) (see Sect. 5). This response implies that the initial inhibition of root elongation is reversible upon the activation of the resistance mechanisms leading to the removal of the toxic Al species from the early targets that were responsible for the inhibition of elongation. In fact, even in sensitive plants, the initial inhibition of root elongation after short-term exposure to Al can be completely reversed by transferring the plants to Al-free medium (Kataoka and Nakanishi 2001). The duration of Al treatment after which full recovery of growth can be achieved in Al-sensitive plants varies between 15 and 120 min according to species and experimental conditions (Kataoka and Nakanishi 2001; Amenós 2007; Kikui et al. 2007). The observation that recovery is accelerated in solutions containing organic acids or high Ca concentrations (Alva et al. 1986) supports the view that lowering the Al concentration in the tips is crucial to the resumption of root elongation (Rangel et al. 2007). Recent investigations, however, suggest that malate secretion can stimulate regrowth in roots of sensitive wheat, even without decreasing root-tip Al concentrations (Kikui et al. 2007).

3 Mechanisms of Al-Induced Inhibition of Root Growth

Root growth is a complex process which implies not only the maintenance of cell viability, the production of new cells, and their enlargement, but also cell patterning, morphogenetic processes and coordination by hormonal signals (Barlow 2002; Osmont et al. 2007). As the Al-induced inhibition of root elongation is observable within minutes upon exposure, mechanistic research has mainly focused on the processes of cell enlargement. Cell division makes a negligible contribution to the

root length in the short term, and Al-induced morphogenetic alterations are visible after prolonged exposure. Therefore, these processes have warranted less attention. However, recent investigations have demonstrated the relevance of alterations in cell patterning, morphogenetic processes and hormonal regulation in the primary responses of roots to Al toxicity (Doncheva et al. 2005).

3.1 Al-Induced Inhibition of Cell Expansion

Expansion growth of root cells occurs in the elongation zone, located in the subapical root zone a few millimeters from the apex. Turgor-driven expansion requires loosened and extensible primary cell walls, intact plasma membrane, and an adequate water supply to maintain the water potential gradient (Barceló et al. 1996). Cell integrity is a prerequisite for cell expansion. This begs the question of whether Al-induced cell death can account for fast inhibition of root elongation.

Aluminum is not a Fenton-type metal, but it clearly exhibits prooxidant activity (Exley 2004). Aluminum-induced oxidative stress in roots has been found in many investigations (Cakmak and Horst 1991). Aluminum-induced cell death has been observed after hours of exposure to extremely high Al concentrations (Pan et al. 2001; Šimonovičová et al. 2004). Such lethal distress treatments, however, provide scarce information on the dynamics of Al-induced inhibition of root growth. Vital staining of root tips of plants suffering from Al-induced inhibition of root elongation under less drastic conditions has revealed that massive cell death due to loss of cell compartmentation is not a primary cause of the inhibition of root elongation (Corrales et al. 2008). As an example, Fig. 2 shows root tips of a maize (Fig. 2a) and a cucumber plant (Fig. 2b) suffering from a 30–40% inhibition of relative root elongation rate in comparison to the untreated control (Fig. 2c). Note that only a few cells stain with propidium iodide, i.e., have damaged plasma membranes (Fig. 2). Time-dependent studies also demonstrated that cell death and protein oxidation in Al-exposed maize plants occurred later than inhibition of root elongation (Boscolo et al. 2003). Fast, locally induced formation of reactive oxygen species (ROS) can, however, play a crucial role in both stress signaling and cell wall alterations, leading to cell wall stiffening and inhibition of cell expansion.

3.1.1 Cell Wall Expansion and Al Binding

Large amounts of Al accumulate in the cell walls and intercellular spaces of root tips. This apoplastic Al comprises between 85 and 99.9% of the total Al fraction in roots (Ma 2007). Besides Al precipitation on the root surface and in intercellular spaces, an exchangeable form of Al bound to the negative charges of the pectin substances can be identified (Blamey et al. 1993), or it can be found in a more tightly bound form (Eticha et al. 2005).

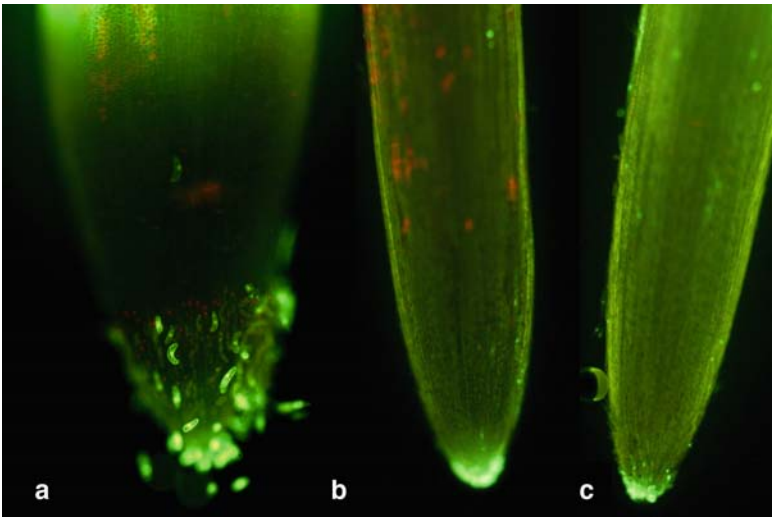

Fig. 2a–c Root tips double stained with fluorescein diacetate (green fluorescence of intact living cells) and propidium iodide (orange fluorescence of cells with damaged plasma membranes). **a** Root tip of maize plant exposed to 50 μM Al and suffering a relative root elongation inhibition of 40%. Only a few cells in the meristem and the transition zone are damaged. **b** Root of a cucumber plant exposed to 7 μM Al suffering a 30% inhibition of relative root elongation. Only a few cells in the elongation zone are damaged. **c** Control cucumber plant without damage. (Unpublished data and modified from Corrales et al. 2008)

Al-induced stiffening of cell walls has been observed in different experimental systems (Gunsé et al. 1997; Tabuchi and Matsumoto 2001; Ma et al. 2004). In vitro studies with maize coleoptiles floating on Al solutions (Llugany et al. 1992; Barceló et al. 1996) or dead root tips treated with Al (Ma et al. 2004) did not reveal Al-induced cell wall stiffening. This supports the view that Al-induced stiffening of cell walls is a biochemical process and not merely physical crosslinking of pectin substances by trivalent Al^{3+}. Cell wall expansion requires both the loosening of the wall matrix and the synthesis of new wall components. The binding of Al to the newly formed material, which is required for the elongation process, may lead to a deterioration in the mechanical properties of the walls, hampering cell elongation (Ma et al. 2004; Ma 2007).

Other polar wall constituents, such as the hydroxyproline-rich glycoprotein (HRGP), have received scant attention in Al toxicity research. Higher extensin concentrations were observed in Al-sensitive than in Al-resistant wheat (Kenzhebaeva et al. 2001). The binding of Al to extensin was observed both in vitro and in vivo (Kenjebaeva et al. 2001). The crosslinking of HRGPs by reactive oxygen species in combination with callose deposition induced by the ethylene precursor ACC has been shown to be an important mechanism for inhibiting cell expansion (de Cnodder et al. 2005). Aluminum-induced enhancement of ethylene evolution clearly precedes the inhibition of root growth in bean seedlings (Massot et al. 2002). Taken together, these results suggest that crosslinking of HRGPs—either directly by Al or indirectly

through Al-induced enhancement of ethylene-derived, apoplastic ROS—plays an important role in the inhibition of root cell elongation (Laohavisit and Davies 2007). Therefore, reactive oxygen species participate in the Al-induced inhibition of elongation by inducing crosslinking reactions in proteins or cell wall phenolics rather than through a general breakdown of membrane integrity due to lipid peroxidation reactions. The inner cortical cell layers (Pritchard 1994) drive root elongation. However, cell wall rigidification of the epidermal cell layers could hamper this expansion process (Jones et al. 2006). Cracks in the epidermal layer (frequently observed after a few hours of Al exposure) are the visible consequence. Furthermore, Al-induced ROS can disturb Ca homeostasis through ROS-activated Ca channels (Kawano et al. 2004)

3.1.2 Plasma Membrane, Cytoplasm, and Tonoplast

Although cell walls make the initial contact with high Al concentrations in the soil solution, and most root-tip Al is localized in the apoplast, the primary toxic effects of Al on cell expansion are not restricted to impaired cell wall extensibility. Aluminum-induced impairment of the hydraulic conductivity (Gunsé et al. 1997) of the plasma membranes (PMs) and the tonoplasts of root cells have severe consequences for cell expansion. The importance of this toxic effect of Al on hydraulic conductance is reflected in the prominent changes in aquaporin gene transcription induced by Al within both plant roots and animal cells (Milla et al. 2002; Mathieu et al. 2006; Kumari et al. 2008). The PM responds very quickly to Al toxicity. Depolarization of PM has been observed immediately upon exposure to Al in root cells and Characeae (Sivaguru et al. 1999; Kisnierienë and Sakalauskas 2005). The cell membrane provides potential binding sites for Al, such as carboxyl and phosphate groups. The affinity of Al for the surfaces of phosphatidylcholine (PC) vesicles is 500 times higher than that of Ca (Akeson et al. 1989). The binding of Al to the plasma membrane can account for changes in key properties of this membrane, such as fluidity and lateral lipid phase separation. Decreased hydraulic conductivity of PM (Gunsé et al. 1997), changes in membrane potential and ion channel activity, alteration of Ca homeostasis (Rengel and Zhang 2003), and inhibition of H^+–ATPase (Ahn et al. 2001) are rapid consequences. All of these effects are characteristics of Al toxicity syndrome (Ma 2007; Poschenrieder et al. 2008). The exact sequence of events signaling the presence of Al at the plasma membrane, leading to adaptive root growth responses or inducible resistance mechanisms or both, is still not clearly established (see Sect. 4).

Classically, the plasma membrane was considered impermeable to trivalent cations. Aluminum was thought to penetrate into the symplast only after long-term exposure. As the inhibition of root elongation is a fast process, most research efforts have focused on the apoplast and membrane surface binding. In fact, studies with Al^{3+} or Ga^{3+} (used as an Al analog) have shown that the influx rate of these trivalent cations is slow. Rates on the order of 20–250 pmol m^{-2} s^{-1} have been reported (Reid et al. 1996; Ritchie and Raghupati 2008). However, even these slow rates allow small

amounts of potentially toxic Al to enter the symplasm within minutes. This has now been clearly demonstrated by several investigations (Lazof et al. 1996; Vázquez et al. 1999; Taylor et al. 2000; Silva et al. 2000). The mechanisms and the chemical species that enable Al to pass through the plasma membrane are still unknown. Based on results with Al-tolerant accumulator species like *Fagopyrum* and *Melastoma* (Ma and Hiradate 2000; Watanabe et al. 2001), it was postulated that ionic Al^{3+} is taken up by a passive mechanism facilitated by an as-yet unidentified transporter and driven by a favorable electrochemical gradient. The gradient is maintained due to the immediate chelation of the incoming Al^{3+} by citrate or oxalate (Ma 2007).

Membrane transport of Al via endocytosis appears to be another path for Al intake. Internalization of aluminum into endosomal/vacuolar vesicles in cells of the distal transition zone of *Arabidopsis* roots has been visualized by fluorescence microscopy (Illéš et al. 2006). The presence of Al in the distal transition zone of maize and *Arabidopsis* was detected approximately 3 h after Al was supplied to the small root tip vacuoles (Vázquez et al. 1999; Illéš et al. 2006). This implies Al transport across the tonoplast. In *Arabidopsis*, chelated Al can be transported through the tonoplast by a half-type ABC transporter (Larsen et al. 2007).

Due to the low uptake rates of Al across the plasma membrane and the compartmentation of Al into the vacuole, combined with the close-to-neutral pH of symplastic solutions, it can be expected that the free activity of Al^{3+} in the cytoplasm is extremely low. However, even subnanomolar concentrations of Al can efficiently compete with Mg for binding to ATP (Ma 2007). In fact, the toxicity of symplastic Al would largely depend on the relative affinity for Al of toxicity targets and of protective ligands that are able to detoxify Al. Symplastic toxicity targets include (among others) ATP, GTP, nucleic acids, glutamate, endosomal vesicle transport and the cytoskeleton (Sect. 5). Organic acids, especially citrate and oxalate, are well-identified organic ligands that can prevent Al binding to these targets.

3.2 Effects of Aluminum on Cell Division

Pioneering work by Clarkson (1965) demonstrated that Al toxicity strongly affects root developmental features, and he pointed to the inhibition of cell division as a primary cause of Al-induced inhibition of root growth. The binding of Al to nucleic acid in root tips was demonstrated more than 40 years ago (Matsumoto et al. 1976; Morimura et al. 1978). More recent investigations revealed severe toxic effects of Al on root tip cell nuclei and cell division. Chromosome bridges, breaks and nuclear dissolution have been described in maize or onion roots (de Campos and Viccini 2003). Most of the early investigations were performed after several days of exposure to Al. As Al was thought to enter the symplasm only after long-term exposure, while Al-induced inhibition of root elongation can be observed after less than 1 h under Al stress, further investigations focused mainly on cell walls and root cell elongation (Horst 1995).

In recent years there has been a renewed interest in Al-induced alteration of the cell cycle for several reasons. On the one hand it is now well established that small amounts (at least) of Al can penetrate into the symplast quite rapidly (see Sect. 3.1.2). On the other hand, alterations of the cell cycle could be induced by Al in an indirect way, through a signaling cascade, without the need for Al to reach the nuclei of meristematic cells directly. Moreover, the strong influence of Al is not restricted to inhibition of the main root length. The fast developmental changes in response to Al seem to imply a complex coordination of cell patterning events that include inhibition of root cell elongation, inhibition of root cell division, and even stimulation of root cell division (Doncheva et al. 2005).

Lumogallion, a highly specific fluorescence stain for Al, revealed the presence of Al in root tip nuclei after only 30 min of exposure to low Al concentrations (Silva et al. 2000). Aluminum-induced inhibition of the cell cycle in root tips has been observed to occur even more quickly than this. Figure 3 shows the effects of Al in different zones (Fig. 3a) of root tips of maize plants. After only 5 min of exposure to Al followed by a 2-h labeling period, strong inhibition of the incorporation of fluorescent-labeled desoxybromouridine into the cells of the apical meristem is observable (Fig. 3b). Confocal microscopy of the apical meristems of control and Al-treated plants revealed a high number of S-phase cells in controls (Fig. 3d) and a virtual halting of cell cycle activity in the Al-treated plant (Fig. 3e).

This rapid negative effect on cell cycling in the apical meristem of maize root is not due to a general caryotoxic effect of Al in the root tips (Doncheva et al. 2005). On the contrary, the Al treatment quickly stimulated cell cycle activity in the subapical part of the root, in the transition zone (Fig. 3b). After 30 min an incipient protuberance with many dividing cells was observable. After longer Al exposure (3 h) the initial of a new lateral at a short distance from the apex of the main root was distinguished (Fig. 3c). This sequence of events shows that the plant is able to detect excess Al and react to it by adaptive root growth within minutes.

Stimulation of cell division by low Al concentrations has mainly been described in cell culture experiments. Cell cycle activity and cyclin-dependent kinase type A activity were stimulated in the Al-tolerant cell lines of *Coffea arabica*, while inhibition was observed in an Al-sensitive line (Valadez-Gonzalez et al. 2007). Aluminum-induced enhancement of cell division has also been described in human or animal osteoblasts and blood cells (Quarles et al. 1991; Yao et al. 1994) and in yeast (Zheng et al. 2007). The response is concentration-dependent and exposure to higher Al levels causes inhibition of mitosis and cell death. The Al-induced cell activation has been related to Al binding to an extracellular cation-sensing G-protein-coupled receptor (CaR) that is responsible for the perception of extracellular Ca^{2+} (Pi et al. 2005). The expression of a plasma membrane protein for extracellular Ca^{2+} sensing (CAS) has also been described in stems, leaves and stomata of *Arabidopsis* (Han et al. 2003). No ortholog exists in animal species. However, CAS apparently uses the same mechanism to increase intracellular Ca^{2+} by the phosphoinositide/Ca^{2+} pathway (Hofer 2005).

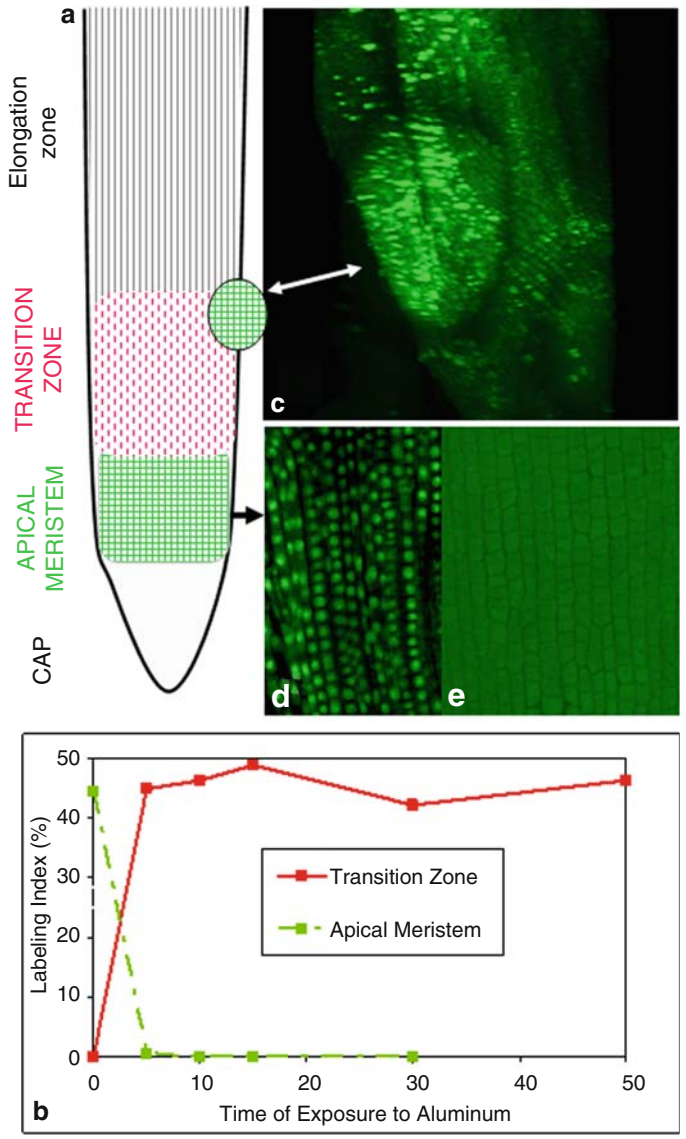

Fig. 3a Model of a maize root tip showing different developmental zones. **b** Labeling index (% of cells with S-phase nuclei) in the apical meristem and the transition zone cells of root tips of maize plants exposed to Al for different times followed by a 2 h bromodeoxyuridine (BrDU) labeling period. **c** Confocal image showing the formation of a lateral root initial close to the transition zone in a maize root exposed to Al for 3 h; S-phase nuclei exhibit green fluorescence due to BrDU labeling. **d** Apical meristem of a control root tip. **e** Apical meristem of a root tip exposed to Al for 30 min; no S-phase nuclei are detectable. (Unpublished data and modified after Doncheva et al. 2005)

3.3 Root Transition Zone: Site for Al Perception and Al Signal Transduction

Investigations on the spatial sensitivity to Al in different root tip zones revealed the transition zone (1–2 mm from the tips of maize roots) to be the main target of Al toxicity (Sivaguru and Horst 1998; Rangel et al. 2007). The transition zone is located between the meristem and the elongation zone (Fig. 3a). Distinctive features of the cells in the transition zone should be responsible for the perception of Al. Transition zone cells have a specific architecture that has been related to their exceptional capacity for sensing environmental factors (Baluška et al. 2001b, 2004).

Studies into the gravitropic responses of maize roots revealed a high sensitivity to extracellular Ca in the transition zone (Ishikawa and Evans 1992). Different membrane proteins are responsible for Ca binding and Ca transport in plant cells: the abovementioned CAS (Han et al. 2003); Mca1, a plasma membrane protein from *Arabidopsis* that enhances Ca influx into the cytoplasm upon distortion of the plasma membrane (Nakagawa et al. 2007); ROS-activated Ca channels (Mori and Schroeder 2004); other voltage dependent and independent Ca channels, and Ca efflux transporters (White and Broadley 2003). However, it is still unclear whether the high environmental sensitivity of the transition zone is related to a site-specific distribution of Ca receptors and/or Ca channels. The interference of Al with Ca homeostasis is well established (Rengel and Zhang 2003). Aluminum causes an increase in cytosolic Ca. This can be due to enhanced entry from the apoplast or enhanced release from intracellular storage sites, or both (Ma 2007). Aluminum-induced disturbance of Ca homeostasis can also be brought about by the interference of Al with the phosphoinositide cascade (Jones and Kochian 1995; Ramos-Diaz et al. 2007). Aluminum inhibits phospholipase C, which in turn affects the synthesis of phosphatidic acid.

The cytoskeleton plays a crucial role in driving the impressive changes in cell architecture that occur during the transition from mitotic to elongating cells. The fast impact of Al on the actin cytoskeleton has been documented in detail (Grabski and Schindler 1995; Blancaflor et al. 1998; Ahad and Nick 2007). Using high Al concentrations, Sivaguru et al. (1999) reported the most conspicuous effects of Al on the cytoskeleton in the epidermal and outer cortex cells of the distal transition zone in maize root tips. Under less severe toxicity, we have scored the most prominent Al-induced alterations on F-actin in the central, stelar part of the transition zone and, to a lesser extent, in the central part of the meristem zone (Amenós et al., unpublished). Actin filaments were also an early target of Al in the meristem cells of *Triticum turgidum* roots (Frantzios et al. 2005).

4 Al Toxicity Mechanisms: Common Features in Plant and Animal Cells?

The characterization of the structural and functional differences between transition zone cells and cells in less sensitive root zones is of fundamental interest when assessing primary mechanisms of Al toxicity in roots. Another approach arises

from the question: what are the common features shared by the different highly Al-sensitive cell types? Besides root transition zone cells, examples of highly Al-sensitive cell types include plant cells that experience tip growth, like root hairs (Jones et al. 1995; Care 1995), pollen tubes (Konishi and Miyamoto 1983; Zhang et al. 1999) and filamentous algae (Alessa and Oliveira 2001), as well as astrocytes of the animal and human nervous systems (Suarez-Fernandez et al. 1999).

4.1 Actin–Myosin Network and Vesicle Trafficking: Common Targets for Al Toxicity in Plant and Brain Cells

Effects of Al on polar growing cells can be extremely fast. In *Vaucheria longicaulis*, a filamentous alga, cytoplasmic streaming was inhibited by more than 50% after 30 s of Al exposure, and the movement of cell organelles was completely inhibited after only 3 min (Alessa and Oliveira 2001). The movement of cell organelles should not be considered a passive flow movement but rather an active organelle translocation due to the actomyosin transport network (Peremyslov et al. 2008). Rigor has also been observed in the actin filament network as a fast Al-induced alteration in suspension-grown soybean cells (Grabski and Schindler 1995). In this system, the fast Al effects were not related to alterations in ion fluxes, and it was hypothesized that the formation of nonhydrolyzable Al–ATP or Al–ADP complexes and its binding to actin/myosin could be responsible for the stiffness of the network. Knocking out myosin genes XI-2 and XI-K severely affects Golgi-derived vesicle trafficking and root hair development (Peremyslov et al. 2008). Class VIII myosins play the role of endocytic motors in plants, and endocytosis is a fundamental process in cell tip growth (Šamaj et al. 2004, 2005).

Astrocytes in the brain are specific targets for Al toxicity (Levesque et al. 2000; Aremu and Meshitsuka 2005). Astrocytes play a crucial role in the functioning of neurons (Aremu and Meshitsuka 2006). Among others, clathrin-dependent endocytosis of GLT-1, a glutamate transporter that is predominantly expressed in astrocytes, seems to be important for the maintenance of local glutamate concentrations in synapsis. Impaired astroglial function leads to inhibition of glutamate clearance and excitotoxicity. Astroglia can respond to external stimuli by generating Ca waves that release the neurotransmitter glutamate, enhancing the activity in the synapses of nearby neurons. The signal can also be spread across distances through gap junctions. By altering the organization of the actin network, aluminum disturbs connexin trafficking and therefore the formation of gap junctions of two hemichannels in adjacent cells (Theiss and Meller 2002). In root tips of plants, Al also inhibits cell-to-cell transport via plasmodesmatal connections (Sivaguru et al. 2000). Plasmodesmata are located in the actomyosin-enriched domain of the cell periphery (Baluška et al. 2000). As Ca waves regulate fast changes in plasmodesmatal permeability (Baluška et al. 2001a), an Al-induced rise in intracellular Ca can be expected to account for plasmodesmata closure and inhibition of cell-to-cell trafficking.

Glutamate also plays a role in the response to Al in plant cells. Effects of glutamate on membrane depolarization, depolymerization of microtubules and root growth inhibition are similar to those of Al. However, the effects of glutamate occurred more rapidly than those of Al, and Al did not further enhance glutamate action. These observations suggest that glutamate or a glutamate-like substance is involved in the early signaling response to Al toxicity in plants (Sivaguru et al. 2003). The glutamate receptor GLR3.3 is required for Ca^{2+} transport into *Arabidopsis* cells in response to glutamate by a mechanism that can be considered homologous to the fundamental component of neuronal signaling (Qui et al. 2006). This glutamate-receptor-mediated Ca^{2+} influx also seems to be responsible for the glutamate-specific alterations in root branching (Walch-Liu et al. 2006). These root architectural changes are similar to those observed in Al-stressed plants.

Altogether, these observations reveal striking similarities in the responses to Al between Al-sensitive plant and animal cells. Tip-growing plant cells, such as root hairs, pollen tubes or filamentous algae, transition zone cells in plant root tips, and astrocytes are very different in terms of origin, morphology and function. However, a common characteristic of all of them is a high activity of vesicle trafficking. In both the quickly expanding tip-growing cells (Ishida et al. 2008) and the transition zone cells, intense vesicle trafficking is required to provide the new components for the expanding cell walls, among other reasons (Illéš et al. 2006). Vesicle trafficking in astrocytes is essential for the astrocyte-to-neuron communication in the brain (Potokar et al. 2007). Actomyosin network integrity is crucial to the correct functioning of this endocytic and exocytic transport. The fast impact of Al on this network can be considered the common toxicity target in both plant and animal cells. Moreover, in both root transition zone cells (Illéš et al. 2006) and astrocytes (Levesque et al. 2000), endocytosis appears to be an important mechanism for the entry of Al into cells. Therefore, the high Al sensitivities of cells with high endocytic activity may be due to the fact that the actomyosin network is a primary target for Al toxicity, as well as the preferential accumulation of Al in these cells.

5 Coordination of Root Developmental Features Under Al Stress

From this brief glance into the mechanisms of Al toxicity mechanisms, it has become clear that the response of plant roots to this important stress factor is not simply a disruption of cell elongation and a cessation of root growth due to the loss of cell viability. The perception of Al by transition zone cells induces a signaling cascade that can lead to changes in root architecture. The inhibition of main root extension and the induction of lateral roots are key processes in this adaptive growth response.

Inhibition of cell cycle activity in the root apical meristem and activation of cell division for lateral initiation are coordinated events in determinate root growth (Shishikova et al. 2008). Determinate root growth can be constitutive or inducible.

Constitutive determinate root growth is characteristic of certain species like Cactaceae. In these species, the apical meristem function is lost with age, and root hairs and laterals emerge very close to the tip. Exhaustion of the root apical meristem is temporally related to the onset of lateral development. This loss of meristem function has been described as being a physiological root decapitation (Dubrovsky 1997). Phosphorus deficiency (Sánchez-Calderon et al. 2005) and glutamate (Walch-Liu et al. 2006) have been found to induce determinate root growth. The exhaustion of the apical meristem induced by these factors requires several days and is reversible at the beginning. A stimulation of lateral root development close to the tip has also been observed in roots suffering from Cu^{2+} or Al^{3+} toxicity after a few days of exposure to the toxic factor (Llugany et al. 2003; Doncheva et al. 2005). However, the inhibition of the cell cycle in the apical meristem and stimulation of cell division in the subapical region can be observed after only a few minutes of exposure to Al. Similar effects can be induced when NPA (naphthylphthalamic acid), a auxin transport inhibitor, is locally applied to the transition zone of maize root tips (Doncheva et al. 2005).

Lateral roots originate from pericycle cells at a variable distance from the main root apex. Usually laterals emerge from the root zone, where a clearly differentiated vascular cylinder can be distinguished. However, early lateral root primordia initiation can arise close to the root tip (Dubrovsky et al. 2000). Cell division activity in the pericycle cells is restricted by the E2F–RB pathway. Auxin triggers cell division in these stem cells. In addition, an auxin-derived signal seems to be required for the proliferation of a new lateral (Vanneste et al. 2007). The role of polar auxin transport and its relation to differential gene expression in the patterning of morphogenetic events has mainly been investigated in plant shoots (Bowman and Floyd 2008). However, there is increasing evidence for a similar role of polar auxin transport in the development of the roots (Vanneste et al. 2007). In *Arabidopsis*, the patterning of root stem cells is mediated by PLETHORA genes (PLT) (Aida et al. 2004). The expression of PLT can be induced by maximum auxin concentrations.

Based on this, the plastic response of roots to environmental factors could be regulated by direct or indirect interactions between the environmental factor and the mechanism of polar auxin transport, leading to changes in the local auxin gradients and therefore to changes in developmental patterns; e.g., the induction of lateral root formation. It is now well established that polar auxin transport is mediated by a polar distribution of the auxin efflux transporter protein (PIN) (Wisniewska et al. 2006). Endocytotic cycling is considered a highly regulated mechanism for polar PIN localization (Benjamin and Scheres 2008).

Within this scenario, the mechanism responsible for the strong influence of Al on root architecture could be directly related to the toxic action of Al on the actomyosin network that governs vesicle trafficking required for polar auxin transport. The potential key molecule for this toxic action of Al could be small GTPases that are involved in vesicle trafficking and PIN localization (Molendijk et al. 2004). Aluminum fluoride (AlF^{4-}) is a well-known activator of trimeric G proteins, while it inhibits small GTPases.

6 Aluminum Tolerance

Plants adapted to grow in soils with high Al^{3+} activity must have efficient mechanisms for either Al exclusion or tolerance to high Al tissue concentrations (Barceló and Poschenrieder 2002; Ma 2007). Figure 4 summarizes some of these mechanisms. Internal detoxification of Al can be achieved by binding Al to strong chelators like oxalate, citrate, or phenolic substances and Al compartmentation in vacuoles (Vázquez et al. 1999). A constitutively expressed gene (*AlS1*) coding for a half-type ABC transporter protein has been identified in *Arabidopsis*. Located at the tonoplast, this transporter could be important for the compartmentation of chelated Al into the vacuoles (Larsen et al. 2007). It has been suggested that a phloem-located PM transporter protein that is inducible by Al removes the potentially toxic Al from sensitive parts of the root (Larsen et al. 2005). In rice, a gene coding for a possible Al efflux protein (Als1) located in the PM of root tip cells has recently been identified (Ma 2007). Rice mutants defective in this PM protein have higher cytoplasmic Al concentrations than the wild type. Even plants that can withstand the hyperaccumulation of Al in their shoots, such as members of the Melastomataceae or tea plants, must prevent the access of phytotoxic Al species to the sensitive cells in the transition zone. Different mechanisms have been proposed to operate in Al exclusion: plant-induced pH changes in the rhizosphere, production of mucilage and border cells, fewer binding sites in root tip cell walls, lower PM permeability, or enhanced Al efflux. The best-characterized mechanism, however, is the root-tip-located exudation of low molecular weight organic substances with a high affinity for Al (Kidd et al. 2001; Ryan et al. 2001; Kochian et al. 2005). Organic acid exudation seems to be the most widespread mechanism. Two exudation patterns in response to Al can be distinguished: pattern 1 exudation which is activated by Al almost immediately, and pattern 2, where a lag time of several hours is required before the Al-stimulated exudation of organic acids is detectable (Ma et al. 2001). The presence of an efficient, Al-activable, organic acid efflux system in root tips is responsible for the Al resistance (Fig. 4). In contrast, organic acid metabolism seems of minor importance (Ma 2007). Aluminum-activated malate efflux in wheat (*TaALMT1*) (Saski et al. 2006), in *Arabidopsis thaliana* (*AtALMT1*) (Hoekenga et al. 2006), and in *Secale cereale* (*ScALMT1*) (Fontecha et al. 2007; Collins et al. 2008) is related to Al resistance. Reversible phosphorylation is important in the transcriptional and posttranscriptional regulation of *ALMT1* (Kobayashi et al. 2007). In maize, *ZmALMT1* is not, however, involved in the specific Al-activated efflux of citrate (Piñeros et al. 2008). Aluminum-activated citrate efflux in barley and in sorghum is mediated by a protein of the MATE (Multidrug And Toxic compound Extrusion) efflux pump family (Furukawa et al. 2007; Magalhaes et al. 2007; Wang et al. 2007).

How Al activates these organic acid efflux systems has not yet been clearly established. Delhaize et al. (2007) recently proposed two hypothetical models for Al^{3+}-activated organic acid efflux by ALMT and MATE family proteins: model 1, where a direct interaction of Al with the membrane transporter occurs (e.g., TaALMT1

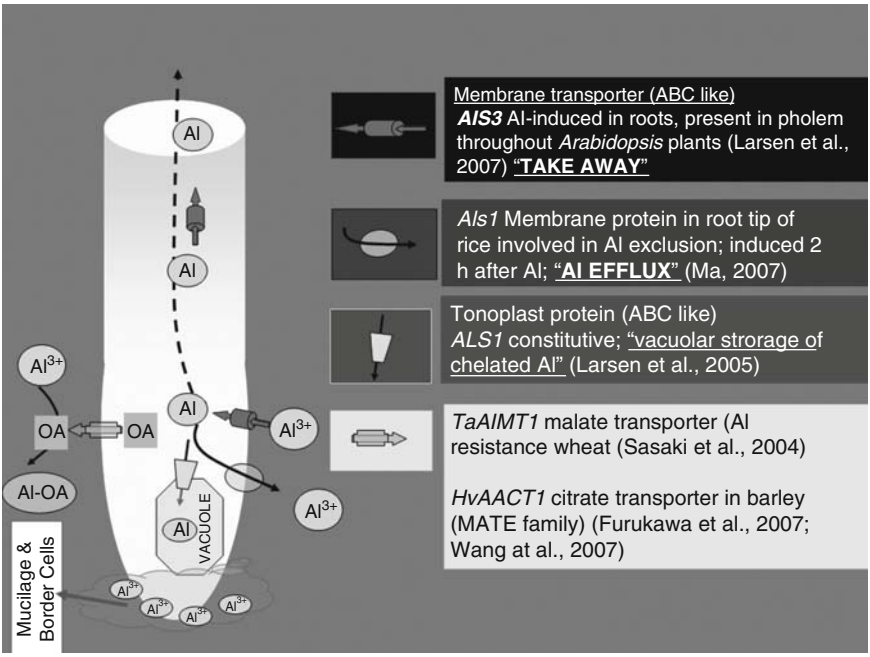

Fig. 4 Mechanisms for Al exclusion and compartmentation in root tips. Distribution of membrane transporter proteins involved in Al efflux, Al phloem transport and Al vacuolar transport are shown along with transporters for organic acid anions. Mucilage and border cells help to stop Al^{3+} from reaching the sensitive root tip (modified after Ma 2007)

in wheat), and model 2, which implies an Al-activated signal transduction cascade. This second model corresponds to Al-activated malate efflux in *Arabidopsis* and *Brassica* and to Al-activated citrate efflux in sorghum. In this pattern 2 response, Al induces the expression of the proteins either by binding to specific PM receptors or by activating a nonspecific stress response. Interaction of Al with these new proteins would then promote the organic acid efflux (Delhaize et al. 2007).

7 Conclusions and Outlook

During the last decades of intense research, substantial advances have been made in our understanding of the molecular mechanisms that are responsible for the resistance of plants to Al toxicity. The identification of Al resistance genes has provided new strategies for improving the breeding of crops adapted to acid soils with Al toxicity problems.

Besides this evident practical progress, the plant response to Al toxicity is becoming a very illustrative model system for basic research—not only in the field of membrane transport systems, but also in the area of studies into the mechanisms governing root developmental features. The information summarized in this review highlights the endocytic process as a common target for Al toxicity in very different cellular systems: tip-growing plant cells like pollen tubes, root hairs and filamentous algae, cells in the transition zones of plant roots, and astrocytes in the brain. Taken together, this information suggests the hypothesis that cells with high endocytotic activity are especially vulnerable to Al. Future research should clarify if his high Al sensitivity is due to enhanced Al entry into these cells via an endocytic uptake mechanism. Investigations into the differences in the Al-activated signal transduction cascades that can lead to adaptive root growth in Al-sensitive genotypes, while activation of anion efflux is induced in resistant genotypes of pattern 2 species, will help to establish the primary mechanism of Al perception in plant roots.

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