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Review

Fast root growth responses, root exudates, and internal detoxification as clues to the mechanisms of aluminium toxicity and resistance: a review

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Abstract

This review on aluminium stress in plants specifically addresses the dynamism of early root growth responses to Al. Three response models, threshold for toxicity, hormesis, and threshold for tolerance, are related to current knowledge on the mechanisms of Al toxicity and resistance. Aluminium exclusion by the production of root exudates and rhizodepositions, as well as internal detoxification mechanisms are considered. Special attention is paid to the central role of chelation of Al by organic acid anions and flavonoid type phenolics outside and inside plant cells. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Beside salinity, Al toxicity is among the most widespread problems of ion toxicity stress in plants. Aluminium toxicity is a major factor constraining crop performance on the acid soils that predominate under tropical climate. In addition to nutrient efficiency, especially for P and Ca, Al resistance is a fundamental trait for plants to fit into sustainable systems of crop production on acid soils (Foy, 1984; Baligar and Fageria, 1997). The humid tropics in Africa and the subhumid Brazilian Savannah Cerrado are characteristic examples where food production is being improved by such an integrated approach that combines the use of acid soil tolerant cultivars or populations with the optimization of nutrient cycling in soil (Sanchez, 1997).

Outside the tropics, enhanced Al availability has been observed as a consequence of progressive soil acidification due to air pollution from electrical power stations, industrial activities, and automobile exhaust (Smith, 1990). Aluminium toxicity has been identified as an important cofactor in forest decline (Ulrich et al., 1980) and Al resistance can be a useful characteristic in trees for forest restoration. However, the genetic solution

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for afforestation using Al tolerant varieties is complicated by the necessity to avoid the use of populations with narrow gene pools because the lack of plasticity of such populations can severely reduce the long-term survival of the stands (Hüttermann et al., 1999).

In any case, understanding the mechanisms of Al toxicity and resistance in plants is of fundamental importance for the development of fast screening procedures for the selection of Al resistant germplasm and for designing plants with good performance on acid soils. Much progress in this field has been made during the last decades and competent compilations and critical reviews on several aspects of this topic have been published (e.g. Clarkson, 1969; Foy et al., 1978; Kinzel, 1982; Foy, 1984; Haug, 1984; Taylor, 1988; Rengel, 1992; Kochian, 1995; Delhaize and Ryan, 1995; Horst, 1995; Barceló et al., 1996; Matsumoto, 2000; Ma, 2000; Ma et al. 2001; Ryan et al., 2001). This review will focus mainly on recent advances in the investigation of the dynamics of root growth responses to Al after short-term exposure and its implication in the current hypothesis on the mechanisms of Al toxicity and resistance.

2. Fast root growth responses

2.1. Timing of growth response

The toxic effects of Al^{3+} ions on plants have already been described in the first decade of the last century (Ruprecht, 1918; cited by Kinzel, 1982). It is known for years that roots usually are the most Al sensitive organs and that Al causes fast inhibition of the elongation of the main axis and restriction of lateral root development leading to a short stunted root system (Clarkson, 1965; Munns, 1965). Different hypotheses on the mechanisms of this Al-induced inhibition of root growth have been discussed during these large years of experimentation, Al-induced inhibition of phosphate availability (e.g. McLean and Gilbert, 1928; cited by Kinzel, 1982), Al interference with Ca metabolism (Rengel, 1992), Al-induced inhibition of root cell division (Clarkson, 1965) and inhibition of root cell elongation (Klimashevski and Dedov, 1975).

During the last decade, substantial advances in our understanding of Al-induced inhibition of root growth have been made because of increased research funding in this field combined with the recognition of the importance of investigations dealing with the initial events of the Al-toxicity syndrome for elucidation of primary toxicity and tolerance mechanisms. Exact timing of responses has been substantially improved by the development of different measurement methods using computer-assisted devices based on linear displacement transducer systems (Gunsé et al., 1992; Llugany et al., 1995) or video monitoring (Parker, 1995; Kidd et al., 2001b). These kinds of measurements allowed establishing the response times of roots faced with environmentally relevant Al concentrations. As a rule, sensitive plants exhibit statistically significant inhibition of root elongation after approximately 30 min to 2 h exposure.

Monitoring of root elongation during this initial time span reveals different response models (Fig. 1):

(A) threshold for toxicity curve
(B) hormesis (i.e. growth stimulation by low concentrations of an inhibitor)
(C) threshold for tolerance

The threshold for toxicity curve (Fig. 1A and D) can be observed as a function of either exposure time or Al concentration. The lag phase in the response can be interpreted as the time or concentration required for Al to interfere with key processes in root elongation growth.

Deviation from this expected behavior can frequently be seen. In the hormesis-type curve (Fig. 1B and E) growth stimulation is found either by exposure to low concentrations (below toxicity threshold) of a non-essential element or as a transient effect after short exposure times to a potentially toxic concentration. Different mechanisms can account for hormetic responses: among others, therapeutic effects alleviating an undetected disease, amelioration of latent deficiency of an essential element or stimulation of defence reactions leading to a general activation of metabolism. After long-term exposure to Al, hormesis has been related to improved Fe and P nutrition, alterations in the distribution of growth regulators, and prevention of Cu and Mn toxicities (references in Foy et al., 1978). Hormesis during the first minutes or hours after Al exposure seems often related to alleviation of proton toxicity (Llugany et al., 1995; Lazof and Holland, 1999). Comparison of root elongation in maize varieties that differ in proton and Al resistance revealed that hormesis in response to Al only occurred in plants that suffered growth reduction because of the high H^+ concentration (pH 4.3) in the nutrient medium (Fig. 1E). Hormesis was not observed in a proton tolerant variety (Fig. 1D; Llugany et al., 1995). Aluminium-induced growth stimulation in the H^+ sensitive varieties may be brought about by Al^{3+} which, as a trivalent cation, would reduce the cell surface negativity and, in consequence, the H^+ activity at the cell membrane surface (Kinraide, 1994). In this sense, the hormetic effect and the Al-induced alleviation of H^+ toxicity is being an important starting point for the investigations into the mechanisms of Al- and proton-induced inhibition of root elongation in relation to Al species and their toxic effects on the plasma membrane. The hormetic response due to alleviation of proton toxicity is relevant not only for the understanding of the mechanism of the initial root responses to Al, but also of the long-term responses in naturally selected populations as recently shown by Kidd and Proctor (2000, 2001) in studies with silver birch from different habitats.

Based on results with Al resistant maize a third type of response curve to Al can be proposed, the

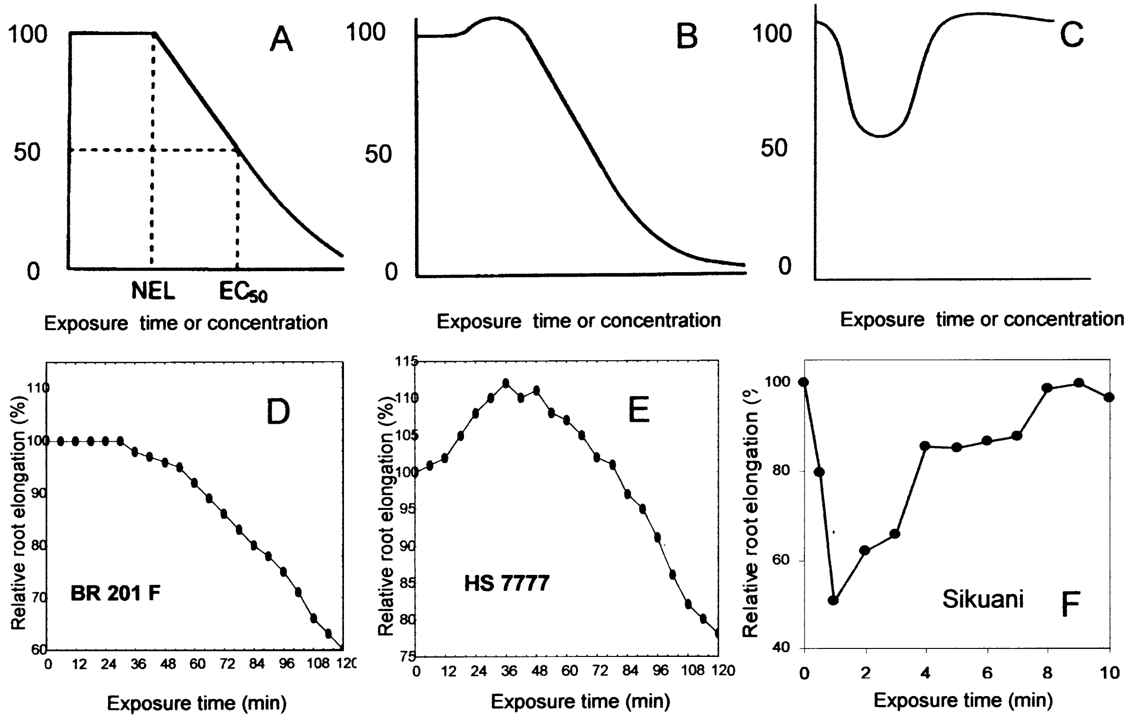

Fig. 1. Three models of response curves for root elongation to aluminum. (A) 'Threshold for Toxicity Model' with NEL, the non-effect level; (B), 'Hormesis Model' with growth stimulation for low concentrations or short exposure times and (C), the 'Threshold for Tolerance Model' with lag time or threshold concentration required for induction of tolerance mechanism. (D, E, F) are real examples from roots of different maize varieties exposed to 50 μM Al at pH 4.3: (D) Variety BR 201 F is Al sensitive and proton tolerant; (E) Variety HS 7777 is Al sensitive and proton sensitive; hormesis due to alleviation of proton toxicity by Al; (F) Sikuani is Al tolerant; a lag time of a few hours is necessary for the induction of an efficient protection mechanism; (redrawn with data from Llugany et al., 1995; Barceló et al., 2000; Kidd et al., 2001b).

threshold for tolerance model (Fig. 1C and F; Barceló et al., 2000). This model describes the observation that in certain species low Al concentrations or short exposure times can cause significant inhibition of root elongation, while higher concentrations or longer exposure times have less or no toxic effect. Birch seedlings from acid mineral soil of Sheriffmuir (Scotland) exhibited reduced root elongation rates when exposed to 74 μM Al, while 370 μM had no adverse effect (Kidd and Proctor, 2000). In tropical maize varieties C 525 M from Brazil (Gunsé et al., 2000) and Sikuani from Colombia (Kidd et al., 2001b) exposure to 50 μM Al inhibited root elongation shortly upon exposure, but after a few hours the elongation rates recovered so that after 24 h elongation rates were similar to controls. In strictly controlled solution experiments this behavior was not caused by depletion of Al in the solutions. In wheat this phenomenon has been called acclimation and was observed both, in Al-resistant and Al-sensitive varieties. This behavior may be interpreted as a shock response (Parker, 1995). In maize, however, only the Al resistant, but no the sensitive genotypes exhibit this recovering of root elongation rates. In this species the behavior seems to result from the need of a threshold concentration for the activation of the resistance mechanism (see Section 3.1). The induction of this mechanism, in turn, may require a certain time that would account for the lag period observed in the Al tolerant varieties of maize (Barceló et al., 2000).

2.2. Mechanisms of Al-induced root growth inhibition

Extensive membrane damage, peroxidation of membrane lipids, and loss of cell compartmentation are relatively late occurring events in the Al-induced root syndrome and can not be made responsible for fast root growth inhibition (Miyasaka et al., 1989; Cakmak and Horst, 1991; Gunsé et al., 1997; Vázquez et al., 1999). Far from it, Al-induced acceleration of specific cell death in the epidermis may be related to defence responses against Al toxicity (Miyasaka and Hawes, 2001; Delisle et al., 2001; see Section 3.1.3).

Investigations on the initial growth responses of roots as a function of Al speciation and exposure time and the recognition of differences in the spatial sensitivity of the root system to Al have been essential for the understanding of Al toxicity and resistance mechanisms. Selective supply of Al to different parts of the root system clearly shows that root tips are the primary sites of Al-induced injury (Ryan et al., 1993). The distal part of the transition zone has been identified as the target site in maize (Sivaguru and Horst, 1998). However, the mechanisms responsible for growth inhibition in these cells are still not clearly established.

Two major questions have been intensively discussed during the last years.

1. Is root growth inhibition brought about by inhibition either of root cell division or root cell expansion?
2. Is apoplastic or symplastic Al responsible for the toxic effect on root elongation growth?

Pioneer work by Clarkson (1965, 1969), Matsumoto et al. (1976a), Naidoo et al. (1978) showed inhibition of mitosis and binding of Al to nucleic acids in roots. It supported the view of an Al-induced inhibition of root cell division as the primary cause of toxicity. Major criticism to this hypothesis came from shorter-term investigations that found Al-induced inhibition of root elongation after 0.5 to 4 h exposure to Al (Llugany et al., 1995) while no Al could be detected inside plant cells (Delhaize et al., 1993; Marienfeld and Stelzer, 1993). Such a fast growth response and the fact that desorption of apoplastic Al by citrate allows recovering of the root growth rate sustained the hypothesis that inhibition of root growth is initially due to a reduction of root cell elongation by apoplastic Al (Horst, 1995). However, improved methods for Al detection inside cells have shown that Al can enter the symplasm within a few minutes (Lazof et al., 1996; Vázquez et al., 1999; Taylor et al., 2000; Brauer, 2001). Even when exposed to solutions with low, environmentally relevant Al^{3+} activities (e.g. 1.45 μM), Al could be detected in nuclei of root meristem cells of Al sensitive soybean plants after only 30 min exposure (Silva et al., 2000). Therefore, both apoplastic and symplastic target sites for Al may be involved in the early toxicity syndrome.

It seems likely that the effects of apoplastic Al on root cell extension cause reversible inhibition of root elongation (Lazof and Holland, 1999). Klimashevsky and Dedov had proposed cross-linking of pectic substances in cell walls as a mechanism of Al-induced inhibition of root cell extension already in 1975. More recently, cell pressure probe measurements revealed Al-induced cell wall stiffening in root cells of Al sensitive maize (Gunsé et al., 1997). In Al-sensitive wheat accumulation of hemicellulosic polysaccharides in walls of root tip cells was observed (Tabuchi and Matsumoto, 2001). However, it is unclear if the thickening and stiffening of the wall is cause or consequence of the inhibition of root elongation. Other stress factors that cause root growth inhibition such as low pH or salt stress also cause cell wall stiffening. Interestingly, Ca can overcome the toxic effects of these stress factors (Azaizeh et al., 1992) as it alleviates those of Al. It remains to be established if this is due to the stabilizing function of Ca on cell walls and plasma membrane, to the cation melioration explainable by the Gouy–Chapman–Stern Model (Kinraide, 1994), or to another mechanism.

Irreversible effects on cell shape (Gunsé et al., 1997), planes of cell division (Vázquez et al., 1999), and mitosis (Clarkson, 1965) are probably related to direct or indirect interactions of Al with the cytoskeleton and nuclei (Blancaflor et al., 1998; Sivaguru et al., 1999; Silva et al., 2000; Alessa and Oliveira, 2001; references in Barceló and Poschenrieder, 1999, and in Matsumoto, 2000). The observation that Al causes abnormal cell division planes suggests that Al interferes with the cortical actin filaments that are thought to play an early role in fixing the site of the preprophase band that is involved in the direction of the cell plate to the correct position (Verma, 2001). The mechanisms of such interactions are still unknown. The strong relation between Al and Ca and P metabolism opens a large array of possibilities of Al interference with key molecules and with the signal transduction pathways of root tip cells (for a recent review see Matsumoto, 2000).

The implications of phytohormones in the Al-induced toxic syndrome in roots are a further point of interest, especially in relation to the root cap as a source of hormones and its role in Al resistance.

After short-term exposure to Al, increased Al resistance has been found in cow pea varieties with an extended root cap (Horst, 1980). Although this resistance was not maintained under long-term field conditions, it seems possible that a huge root cap may give some advantage if the stress is short, e.g. in the field just passing a thin Al-enriched soil layer. A first hypothesis that implied a hormone signal for the transduction of the Al effect from the root cap to the elongation zone (Bennet and Breen, 1991) was discarded because plants with excised root caps were found as sensitive to Al as those with intact caps (Ryan et al., 1993). However, several recent investigations support the view that Al-induced alterations of hormone levels in roots can play a role in early responses to Al.

Inhibition of basipetal auxin transport from the meristem to the elongation zone by Al has been proposed as a cause for decreased root cell elongation (Kollmeier et al., 2000).

At Al-stress levels that inhibit root elongation in Al-sensitive maize varieties but do not cause neither extensive membrane damage by peroxidation of membrane lipids nor plant senescence and death, no differences in the endogenous levels of stress ethylene production in root tips of Al tolerant and sensitive maize varieties could be observed after 24 h exposure to Al (Gunsé et al., 2000). However, recent investigations suggest that ethylene may be involved in fast signal transduction of Al-induced enhancement of cytokinin levels in roots (Massot et al., 2002). An Al-induced transient rise in ethylene production of roots was found as soon as 5 min after exposure to Al in beans. This rise preceded a substantial increase of root cytokinin levels which was observed after only 20 min exposure to Al. Up to date, these are the fastest responses of Al-induced alterations in endogenous hormone levels ever described and the hormone responses clearly preceded the Al-induced inhibition of root elongation. However, at present, the information on Al-induced alterations of root hormones levels is far too fragmentary for establishing a general hypothesis that allows to understand the implications of hormonal regulation not only in the Al-induced inhibition of seedling root elongation but also in the complex changes that affect the morphology of the entire root system under Al stress.

3. Mechanisms of Al resistance

Mechanisms of Al-resistance usually are being classified into mechanisms of avoidance or exclusion and those of internal or protoplastic tolerance (Kochian, 1995). In any case, it seems clear that plants that perform 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 that are considered the most phytotoxic Al species. Either extracellular precipitation or detoxification of Al^{3+} by complexation with chelating root exudates or binding to mucilage may be implied in exclusion. The formation of less toxic organic Al-complexes seems also a prerequisite for the tolerance to high internal Al concentrations that have been observed in plants able to accumulate high shoot Al concentrations such as tea, buckwheat or *Hydrangea*. Aluminium as a class A metal tends to form strong complexes with oxygen donor ligands (Table 1). In plants, phosphate and organic acids have deserved most attention, but strong complexes can also be formed with phenolic substances, pectates, mucopolysaccarides or siderophores (Winkler et al., 1986). Knowledge about the relative importance of these potential ligands for binding Al in the rhizosphere, the root apoplast, and inside cells is essential for understanding Al resistance mechanisms.

At present programs such as GEOCHEM or MINEQL allow the determination of distribution of chemical species in complex solutions containing inorganic and organic ligands. Nonetheless, the fact that calculations are made for equilibrium conditions only and the uncertainty of some stability constants complicates an exact speciation of Al in complex media such as soil solutions, xylem exudates or vacuoles. In the rhizosphere an evaluation of the relative contribution of root exudates and rhizodepositions to Al detoxification is further complicated by the fact that not only the degradation of soil organic matter may provide ligands for Al binding, but also soil microorganisms can either secrete or consume potential Al ligands. Moreover, analytical problems arise when small amounts of certain organic anion ligands are to be quantified in complex solutions (Barbas et al., 1999). Therefore, most of the investigations on the role of root exudates in Al resistance are being made in single salt solutions. Taking into account the strong influence of nutrient supply (Ryan et al., 2001) and mechanical impedance (Boeuf-Tremblay et al., 1995) on the rates and composition of root exudates, this is one of the most precarious points in the hypothesis of Al resistance mechanisms based on root exudation of chelating substances.

Table 1
Stability constants for different Al–ligand and proton–ligand complexes

Ligand	Al:L	log *K*	H:L	log *K*
Malate	1:1	5.4[a]	1:1	5.1
			2:1	3.5
Oxalate	1:1	6.0	1:1	3.4
	1:2	10.9	2:1	1.0
	1:3	15.9		
Citrate	1:1	8.0	1:1	5.7
			2:1	4.3
			3:1	2.9
Catechol	1:2	6.0	1:1	13.3
			1:2	9.3
Catechin	1:3	40.9	1:1	13.3
			2:1	11.3
			3:1	9.4
			4:1	8.6

If not otherwise indicated values are for 25 °C and 0.1 M ionic strength from Smith and Martell, (1989).

[a] Value from Kochian (1995).

3.1. Exclusion of aluminium

Exclusion of Al from root tips has been found as a key process in resistance to Al in wheat (Delhaize et al., 1993). In an investigation with 12 maize cultivars a high negative correlation was found between root tip-Al concentrations and relative root elongation rates (Llugany et al., 1994). Staining intensity of root tips with haematoxylin or morin, dyes that form strong complexes with Al, as a rule agrees with Al-sensitivity of plants (Corrales, 2000; Ofei-Manu et al., 2001). Efficient exclusion of Al from tips apparently affects both apoplastic and symplastic Al and there is now large experimental evidence that root tip exudates play a fundamental role in the prevention of the accumulation of phytotoxic Al species in these compartments.

3.1.1. Exudation of organic acid anions

Several investigations describe an Al-triggered increase of organic acid anion release from root tips of Al tolerant plants (Table 2). Malate exudation by wheat is the mechanism that has been investigated most thoroughly (Kochian, 1995) while citrate seems to be the most common organic acid anion exudated by Al-tolerant species or varieties (Table 2). This process has had an taxon-independent evolution.

Table 2
Influence of Al on organic acid exudation by root tips or whole roots of different species growing in solutions with different pH and Al and Ca concentrations

Species	Organic acid	Exudation rates	Solution (μM) Al/Ca/pH	References
		Tips nmol per tip per h		
Triticum aestivum line ET3	Malate	2.0	200/200/4.2	Ryan et al., 1995
Triticum aestivum cv *Atlas 66*	Malate	0.92	200/200/4.2	Osawa and Matsumoto, 2001
Zea mays cv SA 3	Citrate	0.25	9[b]/200/4.3	Pellet et al., 1995
Zea mays tolerant selection from cv *IAC-TAIUBA*	Citrate	0.13	11.2/230/4.1	Jorge and Arruda, 1997
Zea mays cv ATP-Y	Citrate	1.3	100/200/4.3	Kollmeier et al., 2001
		Whole root *(nmol* g^{-1} *F.W.* h^{-1}*)*		
Secale cereale cv *King*	Citrate	26–70	50/500/4.5	Li et al., 2000b
	Malate	15–35		
Oryza sativa cv *Senatore*	Citrate	44	20/200/4.7	Ishikawa et al., 2000 Ma et al., 1997
Cassia tora	Citrate	106[a]	50/500/4.5	Ishikawa et al., 2000
		268[a]		
Glycine max var. *Suzunari*	Citrate	115	50/500/4.5	Yang et al., 2000
Glycine max genotype PI 416937	Citrate	49	8[b]/800/4.3	Silva et al., 2001
Miscanthus sinensis	Citrate	9.7[c]	190/45/4.0/full nutrient solution	Kayama, 2001
Galium saxatile	Citrate	70[a]	75/370/4.2/full nutrient solution	Schöttelndreier et al., 2001
Rumex acetosella	Oxalate	105[a]		
Camellia sinensis cv *Yabukita*	Citrate	0.17	400/200/4.2	Morita et al., 2001
	Oxalate	35		
Fagopyrum esculentum cv *Jianxi*	Oxalate	15	50/500/4.5	Zhengh et al., 1998
Colocasia esculenta cv *Bun-long*	Oxalate	47	900/1000/4.0/full nutrient solution	Ma and Miyasaka, 1998

[a] Assuming 7% dry weight (Ryan et al., 2001).

[b] Al^{3+} activity.

[c] $\mu mol\ l^{-1}\ h^{-1}$.

Oxalate exudation in response to Al can be detected in very Al-tolerant species (Table 2). Aluminium-induced oxalate exudation has also been observed in maize, but no differences between sensitive and tolerant varieties were detected (Kidd et al., 2001b). Aluminium-induced de novo synthesis of oxalate, mediated by the elevation of the glyoxylate oxidizing ability of the inner membrane fraction, in *Pseudomonas fluorescens* is related to oxalate release and Al resistance (Hamel et al., 1999).

Among the organic acid anions citrate forms the most stable complexes (M:L ratio 1:1) with Al (Table 1). The Al–citrate 1:1 complex is not phytotoxic and its transport through the plasmalemma seems to be very slow (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 in good agreement with the stability constants (Table 1). A central role for exudation of malate in exclusion of Al from the root apex and Al-resistance in wheat is supported by the following observations (Kochian, 1995), (a) the efflux is specifically triggered by Al^{3+} and only certain lanthanide cations such as La^{3+}, Er^{3+} or Yb^{3+} may also induce a small stimulus (Kataoka et al., 2001; Osawa and Matsumoto, 2001); (b) the efflux occurs localized in the root apex in the Al-sensitive zone; (c) addition of malate to Al-containing solutions prevent toxicity; (d) high rates of Al-triggered malate efflux cosegregate with Al-resistance (Delhaize et al., 1993); (e) decreased Al resistance in ditelosomic lines of wheat was accompanied by low Al exclusion from root tips and reduced malate exudation in comparison to the euploid parent (Papernik et al., 2001); (f) near isogenic lines of wheat differing in Al tolerance differ in capacity to activate in response to Al malate permeable channels and cation channels for sustained malate release (Zhang et al., 2001).

There is large experimental support showing that these observations may also apply to Al-triggered citrate efflux (Miyasaka et al., 1991; Pellet et al., 1995; Kollmeier et al., 2001). However, the specificity of the citrate efflux, its restriction to the root apex, and its exclusive role in prevention of Al toxicity in the root apex has been questioned. Citrate efflux can be triggered by phosphorus and iron deficiency (Marschner, 1995). In defence of both the specificity and the importance for Al resistance of Al-triggered citrate efflux in root tips it can be argued, that (a) Fe deficiency-induced citrate efflux is only observed in dicots and sedges, while Al causes citrate efflux in both dicot and monocot species; (b) plants require exposure to P-deficient solutions for several days before citrate efflux is enhanced, while Al stress induces the flux within hours (see below); (c) citrate efflux caused by P deficiency is especially high in the root hair zone or, in the case of certain lupines and species of the Proteaceae family, in proteoid roots, while Al-triggered efflux has been observed in the apex of roots of species that do not form cluster roots (Pellet et al., 1995); (d) according to the few studies performed up to date, cosegregation of P efficiency and Al-resistance is not a common observation (Schaffert et al., 2001), while some investigations observe higher citrate efflux in certain Al tolerant than in Al sensitive varieties (Miyasaka et al., 1991; Pellet et al., 1995).

Although cluster roots are formed in response to P or Fe-deficiency, and the formation is restricted to certain botanical groups, investigations into this phenomenon can help to understand the basic mechanisms of citrate exudation by roots. There is recent experimental evidence that the development of structure and function of proteoid roots are regulated by different pathways and are not within one sequence of events (Skene, 2001). Regardless the root type (cluster root or not), there are common characteristics between citrate efflux stimulated by Fe deficiency, P deficiency, and Al toxicity, the efflux does not affect tissue concentrations of citrate at the site of exudation and the efflux is inhibited by anion channel antagonists (Neumann et al., 1999). It seems likely that there are common mechanisms for citrate efflux in plants under nutrient stress. However, the time required for reaching threshold tissue concentrations for its induction and the signaling pathways for stimulation of the metabolic pathways leading to enhanced citrate production may be different. Differences in sites and frequency of efflux channels may largely account for genotypic differences in responses to these nutrient stresses.

Recent investigations in wheat and maize strongly support the view that Al stimulates the efflux of malate and citrate by activation of anion channels (Ryan et al., 1997; Pineros and Kochian, 2001; Kollmeier et al., 2001). There are, however, clear differences in the efflux patterns between malate and citrate. Timing of organic acid anion efflux revealed two patterns of release (Ma, 2000), pattern 1, where enhanced exudation is observed within a few minutes (5–30 min) upon exposure to Al and pattern 2, with a lag time of several hours (4–12 h) before a significant increase of organic acid anion concentration in exudates can be detected. Malate release follows pattern 1, while citrate is released according to pattern 2 in maize and *Cassia tora*. In Al resistant maize, the lag time in citrate exudation agrees with the threshold for tolerance model of root elongation (Fig. 1). Haematoxylin or morin staining revealed a coincidence in time between the transient accumulation of phytotoxic Al in the root tips of the resistant variety and the lag time for expression of resistance (Jorge and Arruda, 1997; Vázquez et al., 1999; Gunsé et al., 2000). Also in beans, where citrate efflux seems responsible for Al resistance (Miyasaka et al., 1991), an induction of the Al resistance mechanisms seems to be required (Cumming et al., 1992). In wheat, after short-term exposure to Al, a transient inhibition of root elongation has also been observed. However, in contrast to maize, this Al effect occurred in both Al sensitive and Al resistant wheat varieties and no transient accumulation of apoplastic Al has been reported in Al resistant varieties. This response in wheat has been interpreted as a shock response (Parker, 1995) that seems independent of the resistance mechanism based on an immediate release of malate upon exposure to Al without the need for an induction mechanism (pattern 1). In contrast, pattern 2 in maize, *Cassia tora*, soybean, and probably bean, suggests gene activation or de novo synthesis of proteins. Up to date, investigations on the role of Al-induced gene activation in Al resistance are inconclusive. Exposure to increased levels of Al activated four genes in wheat roots of both a sensitive and a resistant wheat variety. A fifth gene was only activated in the sensitive genotype (Snowden and Gardner, 1993). Unfortunately, the study was performed in a pattern 1-type species with Al concentrations that reduced growth in both varieties. Further investigations addressing both gene activation in pattern 2-type species under experimental conditions that allow distinction between sensitive and tolerant genotypes and characterization of the efflux channels and efflux patterns of potential Al chelators with metabolism and growth responses are required.

3.1.2. Phenolic compounds

Several comparative studies including different species or varieties did not find a correlation between Al resistance and the amount of organic acid efflux (Ishikawa et al., 2000; Barceló et al., 2000; Wenzl et al., 2001). This supports the view 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). However, the implication of phenolics in complex formation with Al has deserved much less consideration than organic acid anions. Phenolics can reverse the toxic effects of Al on hexokinase (ref. in Taylor, 1988) and on root elongation (Wagatsuma et al., 2001a). However, at equimolar concentrations they are less efficient than citrate in complexing Al (Ofei-Manu et al., 2001). This is especially important for simple phenols like catechol at low pH, where H^+ efficiently competes with Al^{3+} (see stability constants in Table 1) for the binding sites in 1:1 complexes. Therefore, phenolic sites in themselves are considered as not important for complexation of Al in acid environments. However, by a deprotonation reaction the phenolics in presence of carboxylic groups from organic acids can strengthen 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.

In the view of the large differences in chemical properties and biological functions of phenolic substances, the importance of phenolics in the response of plants to Al cannot be ascertained considering phenolic compounds as a homogenous group and the effect of Al on concentrations of total phenolics. Recent investigations in our laboratory found 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., 2001b). Stimulation of exudation of these flavonoid-type phenolics was in good agreement with protection of root elongation against Al. Moreover, there was a coincidence between tip exudation of catechin and quercetin and Si-induced amelioration of Al toxicity in Al sensitive maize. In Al resistant maize variety Sikuani the Al-induced exudation of catechin reached rates above 100 nmol per tip h^{-1} while that of citrate did not exceed 1 nmol per tip h^{-1}, a rate similar to those reported for citrate exudation in maize by other authors (Kollmeier et al., 2001; Gaume et al., 2001). The high exudation rate at the Al sensitive site of the roots in combination with the high stability constant for the Al complexes with pentahydroxy-flavones and flavanpentols (e.g. catechin, Table 1) strongly supports a role for the flavonoid-type phenolics in Al resistance. The ability of pentahydroxy-flavones to bind Al under the prevailing pH and ionic strength conditions of the apoplast of root tips exposed to Al is demonstrated by many studies where morin, the 3,5,7, 2′,4′ pentahydroxyflavone, has successfully been used for visualizing Al due the formation of a fluorescent complex (Gunsé et al., 2000). Aluminium-induced induction of quercetin exudation from root tips and the high structural similarity of quercetin to morin further support the role of flavonoid-type phenolics in Al detoxification by root tip exudates in maize (Fig. 2).

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.

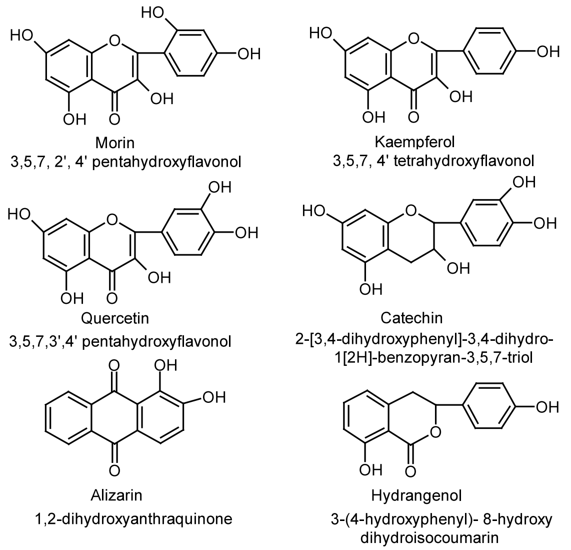

Fig. 2. Structure of some natural phenolic compounds with high affinity for Al. Morin, quercetin, and alizarin have traditionally been used in analytical chemistry for Al detection. Al-induced exudation of quercetin and catechin has been observed in Al tolerant maize (Kidd et al., 2001b). High leaf concentrations of catechin, kaempferol, quercetin or hydrangenol are found in Al accumulator plants (see Table 3).

3.1.3. Rhizodepositions

Mucilage and border cells have been implicated in Al resistance mechanisms (Horst et al., 1982; Miyasaka and Hawes, 2001). An exact evaluation of the role of these rhizodepositions in Al resistance is complicated by the fact that their production is strongly influenced by substrate impedance and composition. In the tropical root legume *Pachyrhizus ahipa* binding of Al to the negatively charged root tip mucilage, visualized by haematoxylin staining, seems to prevent Al uptake (I. Corrales et al., unpublished). Higher mucilage production was observed in the Al resistant wheat cultivar Atlas 66 than in a sensitive cultivar (Puthota et al., 1991). However, no consistent pattern of coincidence between differences in mucilage production, binding of Al to mucilage, and Al resistance in wheat or maize could be established (Li et al., 2000a; Wagatsuma et al., 2001b).

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). These authors propose that 'an Al-induced mucilage layer surrounding each of several thousand cells encapsulating the root tip could provide a significant barrier to Al uptake into the root'.

In relation to the possible role of epidermal cell turnover in Al resistance an interesting hypothesis is forecasted by Delisle et al. (2001). According to their results, at equal effect concentrations (5 μM Al in Al sensitive wheat cv. Frederck and 50 μM Al in Al resistant Atlas 66 for a 50% inhibition of root elongation) 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. Aluminium-induced cell death in the resistant wheat variety occurred in patches and only certain cells were affected. This limited cell death seemed to contribute to Al resistance and cannot be attributed to the oxalate oxidase-mediated H_2O_2 burst occurring later as a second wave response that my 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. The relation of such a resistance mechanism to the much more documented role of Al-induced malate efflux remains to be established.

3.2. Internal detoxification

Although exclusion from root tips and restriction of Al transport to upper plant parts seem to be the most important mechanisms that allow certain crops and wild plant species to grow on acid soils with high Al^{3+} availability, there are numerous species that tolerate relatively high Al concentrations not only in roots, but also in upper plant parts. Aluminium accumulator plants have been defined as those with more than 1000 mg kg^{-1} Al in leaves (Foy, 1984). Reports on high shoot Al concentrations of environmental plant samples have to be considered with caution because surface contamination by soil particles can largely contribute to such a 'shoot accumulation' of Al. There are, however, about a hundred species from more than 30 botanical families that have consistently been identified as Al accumulators. Among others, Al accumulating species can be found, in the Asteraceae, Caesalpinaceae, Euphorbiaceae, Melastomataceae, Myrtaceae, Polygonaceae, Rubiaceae, and Theaceae, mainly from tropical areas in South America and Asia (Haridasan et al., 1986; Cuenca et al., 1991; Geoghegan and Sprent, 1996; Masunaga et al., 1998; Table 3). Conifers accumulate moderate Al concentrations in needles (25–500 mg kg^{-1}; Hodson and Sangster, 1999).

High shoot accumulation of Al implies xylem transport of soluble Al complexes and the accumulation of Al in an innocuous form (soluble or solid) in leaf vacuoles or in the apoplast. Aluminon-staining of leaves of several Al accumulator plants suggests that Al can also be transported in the phloem (Haridasan et al., 1986).

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.

Silicon can protect from Al toxicity either directly by complex formation with Al inside or outside the plant or indirectly by stimulating the production of mucilage or organic acid anions in roots (Corrales et al., 1997; Kidd et al., 2001a,b). In shoots, co-localization of Al with Si is well documented in conifer needles and has also been observed in tea leaves, beech, bamboo, and Brazilian Al accumulator plants (Hodson and Sangster, 1999 and references therein). Such a co-deposition of Al and Si in cell walls may contribute to decreased symplastic Al and increased Al tolerance.

High oxalate, citrate, malate, and sometimes tartrate concentrations are typically found in Polygonaceae (e.g. *Fagopyrum esculentum* and *Rumex acetosella*; Kinzel, 1982). High citrate and oxalate concentrations have been reported in *Hydrangea* leaves and Melastoma, respectively. Identification of Al chelates by ^{27}Al NMR indicates that Al is complexed by citrate (1:1) in Hydrangea leaves, while Al–oxalate (1:3) has been found in buckwheat and Melastoma (Watanabe et al., 1998; Ma et al., 2001). Negatively charged Al complexes with organic acid anions seem to be the main transport form of Al in beech xylem sap (Michalas et al., 1992). Aluminium exposure increased the citrate concentration in xylem exudates of Melastoma and it has been proposed that in this species Al is transported in the xylem in the form of citrate, while oxalate would be the storage form of Al in leaf vacuoles (Watanabe et al., 2000). Such a ligand exchange has also been proposed in *Fagopyrum esculentum* (Ma et al., 2001).

Table 3
Concentrations of shoot Al and potential Al binding substances in selected Al accumulator species and in some highly tolerant Al excluders

Family	Organic acids, Flavonoids	Ref.[a]
Species	Leaf Al (mg kg^{-1})	
Polygonaceae	Oxalate, malate, citrate, quercetin, anthraquinones	1, 2
Fagopyrum esculentum	480–15 000	3, 4, 5, 6
Melatomataceae	Citrate, oxalate, tannate	7
Melastoma malabathricum	590–10 000	8, 9
Memecylon laurinum	12 700	10
Pternande caerulescens	16 600	10
Miconia lutescens	6800	11
Theaceae	Catechin epigallocatechin gallate	2, 12
Eurya acuminata	19 600	10
Gordonia excelsa	11 300	10
Camellia sinensis	1000–30 000	13
Caesalpinaceae	Citrate, anthraquinones	2
Chamaecrista repens	16 400	14
Chamaecrista viscosa	1000	14
Rubiaceae	Quercetin, kaempferol	2
Maschalocorymbus corymbosus	36 900	10
Uropyllum macrophyllum	23 100	10
Hydrangeaceae	Dephidin (flower) citrate, hydrangenol (leaf)	
Hydrangea spp.	400–3000	15, 19

[a] 1. Kinzel, 1982; 2. Harborne, 1967; 3. Wagatsuma et al., 1987; 4. Osaki et al., 1997; 5. Bruneton, 1999; 6. Ma et al., 2001; 7. Yoshida et al., 1994; 8. Watanabe et al., 1998; 9. Watanabe et al., 1997; 10. Masunaga et al., 1998; 11. Bech et al., 1997; 12. Nagata et al., 1992; 13. Matsumoto et al., 1976b; 14. Geoghegan and Sprent, 1996; 15. Takeda et al., 1985; 19. Yoshikawa et al., 1999.

Many Al accumulator species are woody plants in some of which deposits of aluminium succinate, oxalate and citrate have been reported in the heartwood. A considerable number of these plants has been used as mordant in vegetable dying because of high tannin contents (references in Bollard, 1983). Hydrolysable tannins have been described in the Melastomataceae (Yoshida et al., 1994). Tannins, flavonols (e.g. quercetin), flavan-3-ols (e.g. catechin) and anthocyanidins (e.g. delphinidin) may be implied in vacuolar storage of Al. Classical examples of phenolics related to Al binding in upper plant parts are tea and Hydrangea (Takeda et al., 1985; Nagata et al., 1992). Tea leaves contain large concentrations of epi-gallocatechin and epi-catechin, and their unesterified derivatives, which together can make up as much as 30% of the dry weight of green tea leaves (Wörth et al., 2000). Hydrangea is cultivated as an ornamental plant and it is known for long time that when exposed to acid substrate with high Al availability, the color of Hydrangea flowers changes from pink to blue because of the complex formation of Al with delphinidin and caffeolylquinic acid (Takeda et al., 1985). Leaves of *Hydrangea* contain hydrangenol and several other dihydroisocoumarin glycosides (Yoshikawa et al., 1999). Quercetin-3-glucoside has been identified in leaves of *Fagopyrum esculentum* (Table 3).

Aluminium-binding by phenolic compounds has deserved much less attention in plant Al resistance than in medicine and analytical chemistry. Investigations into the chemistry of water soluble, neutral compounds with a possible implication in Alzheimer disease revealed that 3-hydroxy-4-pyrone forms Al complexes with high stability over a pH range from 4 to 9 (Nelson et al., 1989). Flavonoids like quercetin are well known as antioxidants and metal antidotes. Reviewing the chemical structure of the diverse organic compounds, that classically have been used for spectrophotometric or fluorescence detection of Al (aluminon, morin, haematoxylin, alizarin, SPADNS, stilbazo, cochinilin, chromoazurol, lumogallol), certain common features are recognizable. These polycyclic compounds are characterized by any of the following features, two hydroxyl groups in an *ortho*- or *meta* position, a meta position of a hydroxyl and a carboxyl group, or an oxo group next to a hydroxyl (Holzbecher et al., 1976).

There is a clear coincidence of these functional groups of the organic chemicals with high affinity for Al used in analytical chemistry and those of the natural products observed in Al accumulating plants listed in Table 3. The role of these phenolic compounds and of their glycosidic and sulphate conjugates in internal Al detoxification in plants clearly deserves further investigation.

However, high tissue concentrations of potential ligands for Al per se cannot account for Al tolerance. High tissue concentrations of organic acid anions or flavonoid type phenolics have been reported in many plant species that are neither Al accumulators nor resistant to high substrate Al concentrations. High tissue concentrations of oxalate are usually not only found in Al accumulators from the Polygonaceae and in other calciophobe species, but also in halophytes such as Chenopodiaceae (Kinzel, 1982). High malate concentrations are typically observed in some Zn tolerant species (Ernst, 1982). The zinc hyperaccumulator *Thlaspi caerulescens* has high leaf concentrations of both citrate and malate (Tolrà et al., 1996). High concentrations of organic acid anions in leaf tissues seem to be an important, widely distributed, mechanism that allows plants to maintain cation/anion homeostasis under excess ion stress conditions. It may be considered a prerequisite necessary, but not sufficient, for metal tolerance (Tolrà et al., 1996).

Metal specificity of tolerance seems to reside in membrane related mechanisms (Ernst et al., 1992). The ion specificity of tolerance and differences between species or between organs within a species can be brought about by differential distribution of ion specific transporter systems and channels at the plasmalemma and tonoplast level, implied in exclusion, metal efflux, and subcellular compartmentation of metal ions, ligands, and metal-ligand complexes. Differences in plasmalemma ATPase have recently be found in closely related Medicago species differing in Na^+ tolerance (Sibole, 2001). Up to date transporters for Cd^{2+}, Zn^{2+}, Fe^{2+}, Mn^{2+} and Cu have been identified (Clemens, 2001) and differences in metal transporter gene expression seem responsible for differences in uptake and compartmentation of heavy metals (Kochian, 2001). The transport mechanisms of Al through the plasmalemma and tonoplast are still unknown, but the varietal differences between Al resistant and sensitive wheat or maize varieties in anion efflux channels located in the Al-sensitive zone of root tips are a clear example for the way how differences in the distribution of membrane proteins can contribute to Al resistance by exclusion (Pineros and Kochian, 2001; Kollmeier et al., 2001). Further investigations on Al tolerance related differences in tonoplast transport systems of Al and of potential Al ligands, such as the ABC-like flavone glucuronide transporters (Klein et al., 2001), will help to clarify the mechanisms of Al tolerance in Al accumulator plants.

4. Conclusions and outlook

During the last years an intense research activity has provided a fast progress in our understanding of the mechanisms of Al toxicity and tolerance. This better fundamental knowledge in combination with breeding efforts and improved agricultural management is already yielding practical results in crop productivity on acid soils in tropical regions. Nonetheless, there is still a lack of information on the basic mechanisms of Al transport through the plasma membrane and the tonoplast and on the significance and possible interrelationship between Al chelation by root exudates, Al exclusion, Al uptake, Al transport, and Al accumulation in root or leaf vacuoles. A better understanding of these processes and their relation to phosphorous efficiency and yield are required for a more efficient introduction of different crop plants into sustainable agricultural systems on acid soils.

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