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REVIEW

Morphological and structural responses of plant roots to aluminium at organ, tissue, and cellular levels

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Abstract

Toxic effects of aluminium are primarily root-related. This review deals with growth, morphological, and ultrastructural responses of root to aluminium, their diversity along the root axis, and in the root tissues. The cell elongation seems to be most sensitive and responsible for early inhibition of root elongation. Longer Al treatment is required to reduce cell division or to interfere with nucleic acids in the root apex. Alterations of root morphology include root thickening, disturbances of root peripheral tissues, and initiation of lateral roots closer to the root tip. Ultrastructure alterations depend strongly on position of the cells with respect to the Al source, and on their developmental stage. Cell elongation and cell ultrastructure including organisation of cytoskeleton are most sensitive within the distal part of the transition zone of the root apex. This correlates with the rate of uptake and accumulation of Al along the root apex. Recognising the diverse responses and the most sensitive sites within the root apex can help in elucidating the mechanism(s) of Al effects on plants.

Additional key words: root apex, tissue and cellular sensitivity.

Introduction

Phytotoxic effects of aluminium have been studied since their experimental confirmation at the beginning of the 20th century. The reason for the interest is a wide occurrence of soils with low pH, in which aluminium acquires chemical form that can be taken up by plants and have toxic effects. Soils often contain toxic concentrations of Al ions (10 to 100 μM) which may have negative impact on crop yields. The Al toxicity also influences distribution of plant species in the natural environments (Kinzel 1982). Therefore, the interest is concentrated on understanding the mechanisms of Al effects and, on the mechanisms by means of which a plant "protects itself" against the Al toxicity at the cellular level (Taylor 1995). Concomitantly, considering the complex network of reactions and interactions would allow us to define responses of whole plants to the presence of Al in the root zone (Bennet and Stewart 1999).

The main symptom of Al toxicity is the inhibition of root elongation as a result of interaction of Al with root cells and their components. Recent knowledge on principles of Al toxicity and mechanisms of plant resistance to Al are documented in several review articles. In their early hypothesis, Bennet and Breen (1993) suggested the sequence of toxicity stimulus: perception by the root cap cells, signal transduction, and physiological response within the root meristem. The more recent reviews did not confirm the crucial role of root cap, and root meristem was considered as the most sensitive site on the root axis (Kochian 1995, Delhaize and Ryan 1995).

At cellular level, the possible sites of Al effects may occur in the cell wall (apoplast), on plasma membrane, or in cytoplasm (symplast). Possible mechanisms of Al toxicity within the apoplast as reviewed by Delhaize and Ryan (1995), and Horst (1995) involve interactions of Al

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Abbreviation: DTZ - distal part of the transition zone within the root apex.

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with cell wall constituents, *e.g.*, binding of Al to pectin or proteins that might lead to lowering extensibility or hydraulic conductivity of the cell wall. Displacing other ions from critical sites on the cell wall constituents or plasma membrane may disturb intracellular physiological processes. Effects of Al on plasma membranes may include modifications of its structure, induction of callose synthesis, disturbances of ion transport and Ca^{2+} homeostasis (Horst 1995, Marschner 1995). Within the symplast, the toxicity may result from interaction of Al with the processes depending on Mg^{2+} ions, with cytoskeleton, or with the processes of signal transduction. After a longer treatment, Al may bind to the molecules of nucleic acids (Matsumoto 2000). In the review articles, all these possibilities of Al interaction with plant cells have been considered. However, none of them could be unambiguously assigned as the primary event or generally occurring in plants exposed to aluminium stress (Kochian 1995, Delhaize and Ryan 1995, Matsumoto 2000).

The aim of this review was to summarise recent knowledge of aluminium effects on morphology, cytological characteristics, and cell ultrastructure of root apex. The emphasis was put on diversity in the responses of the root cells to aluminium toxicity, with respect of their tissue type, developmental stage, and position within the root. Diverse ultrastructural responses of the cells belonging to the same tissue and, apparently of the same age are also included. Differences in the rate of Al uptake and accumulation along the root axis as one of the factors influencing the diversity in structural responses are also discussed.

Effects of Al on root growth: the cellular aspects

With the exception of plants adapted to acid soils with increased concentrations of Al in available form, the inhibitory effect of Al on root elongation has been confirmed in each experimental work. The extent of root

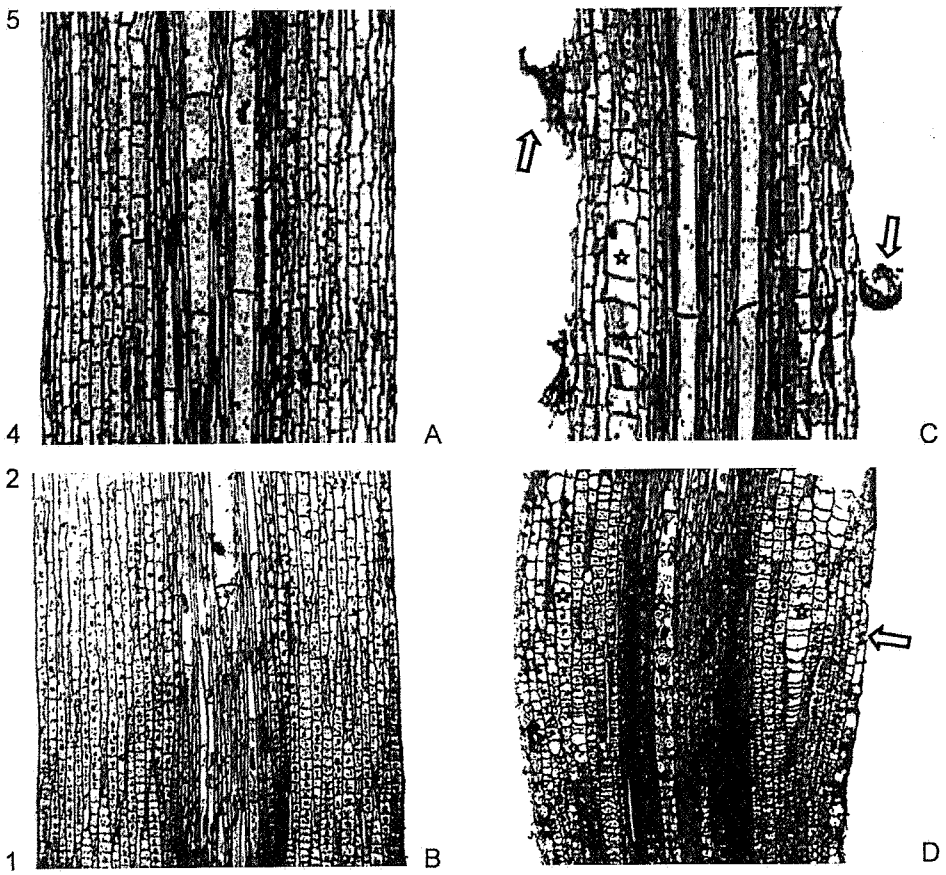

Fig. 1. Longitudinal sections through segments of control (*A, B*) and stressed (*C, D*) roots with 50 μM Al^{3+} for 24 h, at the positions 1 to 2 (*B, D*) and 4 to 5 mm (*A, B*) from the root tip. Radial expansion of the cortical cells in Al-treated roots (*asterisks* in *C* and *D*) is evident. Disintegration of epidermal cells appears in root apex (*arrows* in *D*). In the older part of the root (*C*) peripheral root tissues are destroyed and only their remnants can be seen (*arrows* in *C*) on the root surface.

growth inhibition always depends on Al concentration, length of plant exposure, and on genetically fixed tolerance or sensitivity of the tested plants. Root growth can be strongly inhibited. Bennet (1995) for instance, found growth rate reduction within the whole root system of the wild grass *Lolium perenne*.

Root elongation is the result of division and elongation of the root cells. Decrease in mitotic activity was induced by Al in the root tips of *Allium cepa* (Clarkson 1965), *Allium sativum* (Roy *et al.* 1989), *Vigna unguiculata* (Horst *et al.* 1983), *Avena sativa* (Marienfeld *et al.* 1995), *Hordeum vulgare* (Nichol and Oliveira 1995), *Triticum aestivum* (Votrubová *et al.* 1997), *Zea mays* (Budíková, unpubl. data). The Al-induced reduction of the number of proliferating cells is accompanied with the shortening of the region of cell divisions in maize (Ryan *et al.* 1993). After a longer exposure (14 and 24 h) the cell division can be affected due to interaction of Al with DNA (Matsumoto *et al.* 1976, 1977). Rapid entry of Al into the cells and its co-localisation with cell nuclei (30 min) was observed in the root cells of soybean. In spite of that, there is still lack of an unambiguous evidence of Al entry into the nuclei (Silva *et al.* 2000). Recent knowledge on interaction of Al with nuclear constituents suggest that the lethal toxicity of Al may be associated with inhibition of DNA replication (Matsumoto 2000).

Decrease in mitotic activity occurred several hours after exposure of roots to aluminium, *e.g.* 6 to 8 h in onion (Clarkson 1965) and 24 h in wheat (Votrubová *et al.* 1997). Modifications of fragmoplast and mitotic spindle microtubules which are involved in cell division, occur after 24 h of Al treatment (Sivaguru *et al.* 1999b) while the inhibition of root elongation has mostly been recorded in a time period shorter than 1 h. As the cell cycle takes usually several hours to be completed, and the effect of Al on DNA synthesis appeared after several hours (Sampson *et al.* 1965, Matsumoto *et al.* 1977), it is evident that Al interaction with cell elongation but not with cell division must play a primary role in the initial stages of the inhibition of root elongation (Horst and Klotz 1990, Horst 1995, Kochian 1995).

Morphometric evaluation of the cell size within the growing zone of the root has confirmed the inhibitory effect of Al on their elongation. Correlation between the cell length and elongation of the whole root was shown (Bennet 1998). Cell length was reduced in both meristematic and elongation zones of the barley root (Nichol and Oliveira 1995), in root cortex of wheat (Sasaki *et al.* 1997) and maize (Budíková 1999). Reduced length of the root cells is accompanied with their radial expansion (Sasaki *et al.* 1997, Votrubová *et al.* 1997) particularly in the cells of inner cortex (Fig. 1). This may suggest that Al affects orientation of the cellular growth. The altered growth orientation together with unusual periclinal divisions (Horst *et al.* 1999, Sivaguru *et al.* 1999a, Vázquez *et al.* 1999) may result in local thickening of Al-treated roots. There are indications that the mechanisms of changing the cell growth orientation are associated with the effects of Al on the cytoskeleton. Similar growth inhibition and thickening of *Arabidopsis thaliana* roots accompanied by disorganisation of microtubule cytoskeleton were induced by the inhibitors of protein kinases and phosphatases, suggesting the effects of Al on mechanisms of the regulation of cell growth polarity (Baskin *et al.* 1997).

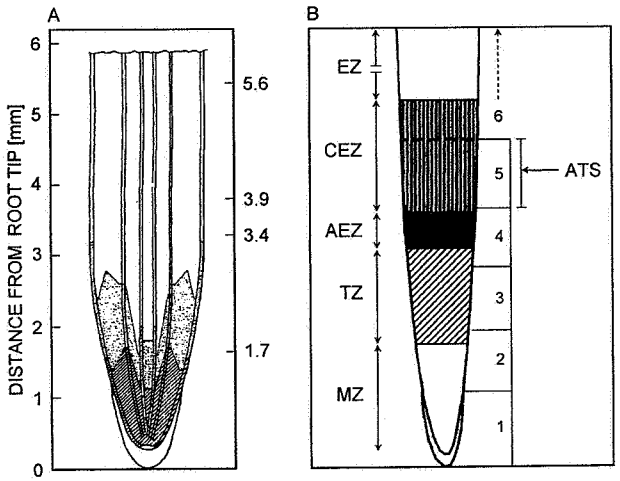

Fig. 2. Two schemes representing the growth pattern and the positions of Al-treated segments (ATS) in maize root apex. *A* - Maize cv. CE-380, the cross-hatched area represents the meristem (according to Luxová 1981), the dotted area represents the transition zone (according to Fig. 2, Baluška *et al.* 1990). *B* - Maize cv. Lixis showing the extent of meristematic (MZ), transition (TZ), apical elongation (AEZ), central elongation (CEZ), and elongation (EZ) zones. (According to Fig. 1, Sivaguru and Horst 1998). Distances from root tips (DFT) in mm, respective to the margins of the root zones (*B*) are given in the right hand side of the scheme *A* (the scales of both schemes have been unified). The material is copyrighted by Springer-Verlag (*A*) and the American Society of Plant Biologists (*B*), and it is reprinted with their kind permission.

Root elongation driven by cell division and cell elongation is accomplished within the first 7 mm in maize root tip (Luxová 1981, Webster and MacLeod 1996). The location of these processes within this root region predetermines the elongation rates of the individual consecutive 1 mm-long segments. The strongest Al-induced inhibition of maize root elongation occurred in the segments 1 to 4 mm behind the root tip (Budíková *et al.* 1997, Budíková and Čiamporová 1998). According to previous analyses (Luxová 1981, Baluška *et al.* 1990), this region involved the meristem, the transition zone, and the beginning of rapid elongation (Fig. 2), at the moment of Al application. Similarly, the strongest inhibition of elongation occurred in the apical segments 2 to 5 mm

behind the maize root apex after 2 h of Al treatment (Blancaflor *et al.* 1998).

Although there is almost a general consent in Al affecting primarily the root cell elongation, there are different explanations of this effect. Interference of Al with growth processes within the cell wall, *e.g.* wall loosening and signal transduction necessary for the growth were suggested (Rengel 1996, Samuels *et al.* 1997). Disturbances in the endogenous auxin levels and interaction between the auxin and the cytoskeleton could be the reasons not only for morphological alterations but also for inhibition of root elongation. Direct or indirect interaction of Al with factors influencing organisation of the cytoskeleton, *e.g.* with ATP, ADP, actin (Grabski and Schindler 1995) and, effect of Al on the calcium level in the cytoplasm have also been considered (Jones *et al.* 1998a, Sivaguru *et al.* 1999b).

Effects of Al on root morphology

With long-term Al application also the architecture of the root system can be changed. Lateral roots appeared very close to the apex of the axial roots (Kinzel 1982, Čížková 1995, Clune and Copeland 1999). Roots of the seedlings growing in media with increased Al concentrations may appear stunted and thickened (Moustakas *et al.* 1992), irregularly curved and may have a changed colour (Eleftheriou *et al.* 1993, Budíková *et al.* 1997). Toxic concentrations of Al induced formation of cracks on the root surface of pea, soybean, maize, and wheat (Wagatsuma *et al.* 1987, Delhaize and Ryan 1995, De Lima and Copeland 1994, Budíková *et al.* 1997). The cracks (Fig. 1) result from disintegration and consequent death of the cells of epidermis, hypodermis, and sometimes also of the next cortical layers. An uneven expansion of the cells within the root tissues may exert mechanical stress causing destruction of peripheral root tissues. Occurrence of the root surface injury was limited to the apical region between about 0.5 mm from the tip (where the epidermal cells stop being covered with root cap cells) and about 15 mm. Locally thickened region between 15 and 20 mm from the maize root apex appeared after 24 h Al treatment. No morphological changes of the primary maize roots occurred further in proximal direction (Budíková *et al.* 1997, Budíková and Čiamporová 1998, 2000).

The growth of root hairs is reduced or ceased in the presence of toxic Al concentrations. The severity of inhibition depends on both degree of genotype tolerance and physiological activity of the hair (Care 1995, Jones *et al.* 1998a,b). Inhibition of hair growth was induced with higher Al concentration in a resistant *Arabidopsis thaliana* mutant than in the wild type. Aluminium induced increased concentrations of cytoplasmic calcium only in the tip of the root hair while the cytoplasmic streaming was not ceased (Sattelmacher *et al.* 1993, Jones *et al.* 1998a). Disturbance of the cytoplasmic Ca concentration homeostasis was suggested to be responsible for hair growth inhibition (Jones *et al.* 1998b).

Effects of Al on root cell ultrastructure

Effects of Al on root cell ultrastructure depend on the type of tissue, on the developmental stage and, particularly on the position of the cell with respect to the source of the toxic ions. Almost regularly the cells at the root cap periphery, the cells of root epidermis and outer cortex undergo more drastic changes than the cells of inner cortex and central cylinder. The higher sensitivity of peripheral as well as of younger cells is apparently related to the rate of uptake and accumulation of Al within the apoplast and symplast, along the root axis as will be shown later.

An early response of root cells to Al (in maize primary roots within 2 h, at 50 μM Al^{3+}, Budíková, unpubl. data) is the increase of vacuolar volume in the cytoplasm (Fig. 1). Vacuolation was observed in root cap, epidermis, and cortex (Bennet and Breen 1987, Ikeda and Tadano 1993, De Lima and Copeland 1994, Marienfeld *et al.* 1995, Budíková *et al.* 1997, Votrubová *et al.* 1997, Clune and Copeland 1999, Vázquez *et al.* 1999). Dark deposits were observed inside the vacuoles of root cap cells in Al-treated seedlings of *Thinopyrum bessarabicum* (Eleftheriou *et al.* 1993). Vázquez *et al.* (1999) detected Al in similar vacuolar deposits located in Si-containing vacuoles of maize root cells. The amount of Al in the vacuolar deposits increased with increasing exposure length of maize root to Al stress what might lead to reducing toxic effects of Al in the cytoplasm and to recovery of the root growth.

Thickened cell walls (Fig. 3) are frequently observed in Al-treated roots (Ikeda and Tadano 1993, Marienfeld *et al.* 1995, Budíková *et al.* 1997, Vázquez *et al.* 1999). Numerous vesicles indicated deposition of polysaccharide material into the cell wall via extreme exocytosis in epidermal and cortical cells of Al-treated soybean roots. These cells were evidently condemned to disintegration (Danilova *et al.* 1992). Synthesis and accumulation of

callose which is a rapid and frequently occurring response to Al (for review see Horst 1995) may contribute to the increase in cell wall thickness. Dark deposits in the walls of maize root meristem cells were analysed and revealed the presence of aluminium together with other elements, P, S, Ca, Zn and Mg after 4 h but not after a longer exposure (Vázquez *et al.* 1999).

In the cells of root cap particularly Golgi bodies are sensitive to Al. Structural modifications such as producing vesicles from only one side of the cisternae stack, disintegration of Golgi cisternae together with a lower frequency of Golgi bodies in the cells lead to reduction of mucilage secretion of the peripheral cap cells (Bennet and Breen 1987, Johnson and Bennet 1991, Crawford and Wilkens 1997). For the secretory functions of the cap cells, Ca^{2+} is required. Aluminium changes Ca^{2+} homeostasis and thereby reduces secretion of mucilage (Marschner 1995). The mucilage with its strong binding ability of aluminium may play a role in protecting the root from external Al toxicity (Hecht-Buchholz and Foy 1981, Bennet and Breen 1993, Li *et al.* 2000). The importance of mucilage in Al toxicity can involve also the role of mucilage in providing a pathway for apoplasmic transport of signal substances (Marschner 1995).

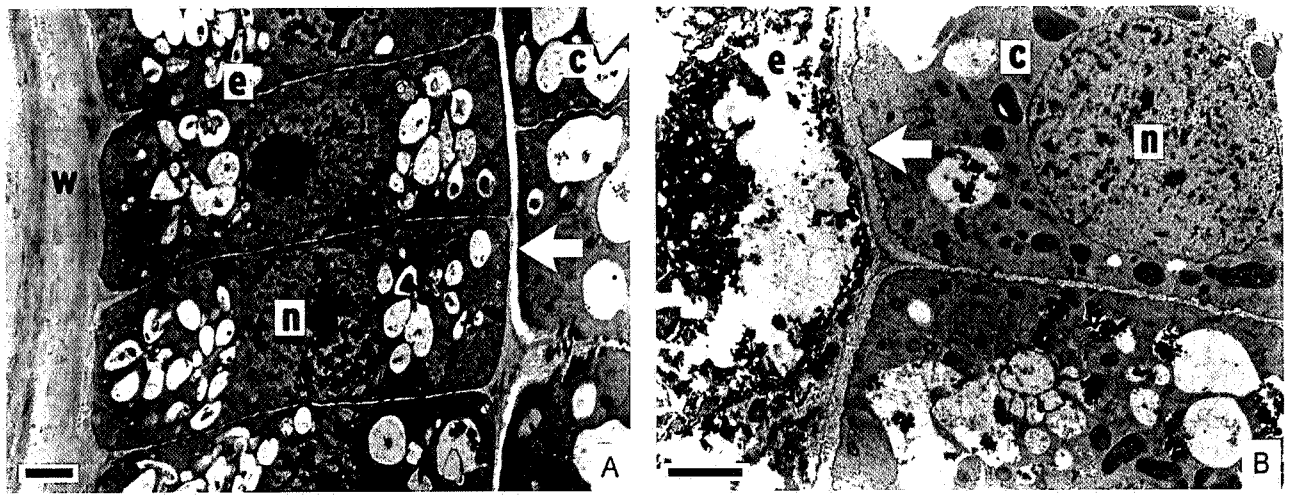

Fig. 3. Part of epidermal (e) and the outermost cortical (c) cells in control (*A*) and Al-treated (*B*) maize roots (cv. CE-380, 50 μM Al^{3+} for 24 h). Al induced destruction of the epidermal cell and thickening of the cell walls as can be seen comparing the corresponding tangential walls between e and c (*arrows*) in control and Al-treated cells. n = nucleus, w = outer epidermal cell wall. *Bars* represent 2 μm.

Interestingly, after shorter exposures (2 to 12 h), there were no visible structural alterations of nucleus, mitochondria or plastids in the columella cells or in the cells of inner cortex and central cylinder (Ikeda and Tadano 1993, Marienfeld *et al.* 1995, De Lima and Copeland 1994, Budíková *et al.* 1997, Čiamporová 2000). In columella cells the number and size of starch grains in the amyloplasts may decrease early (Bennet and Breen 1987, Eleftheriou *et al.* 1993). Only after longer exposures (most often 24 h) disintegration of organelles and of the whole cytoplasmic contents were observed in peripheral root cells. In order to document the development of structural injuries induced by aluminium with respect to time, various shorter exposures will be necessary to investigate. The fact that no transition steps of the drastic damage have been reported so far, may indicate very rapid course of structural damage under aluminium toxicity.

Plant cell cytoskeleton appears to be extremely sensitive to aluminium toxicity. Aluminium induced morphological alterations similar to those induced by cytochalasin B known as inhibitor of actin cytoskeleton, in rhizoids of *Riccia fluitans* (Alfano *et al.* 1993). Grabski and Schindler (1995) suggested the growth inhibition to be a result of disturbance of actin network dynamics. Al-induced gene encoding fibrin-like cytoskeletal protein was isolated from wheat root tips. This protein can bind to actin fibres and induce their stabilisation (Cruz-Ortega *et al.* 1997). Rearrangement and other alterations of the cytoskeleton (depolymerisation of microtubules, fragmentation of actin and disintegration of actin cytoskeleton) appeared very early after Al administration in root apices of wheat (Sasaki *et al.* 1997) and maize (Sivaguru *et al.* 1999a, Horst *et al.* 1999). To induce cytoskeleton alterations within the maize root elongation zone longer exposure to Al was necessary. Orientation of the microtubule cytoskeleton did not change in the cells of root epidermis and outer cortex even after 12 h stress duration (Blancaflor *et al.* 1998). The authors suggest damage to the cytoskeleton dynamics induced by aluminium.

Uptake and localisation of Al along the root axis and in the root tissues

A number of examples show relatively rapid flux of Al into the root apoplast depending on the external conditions (Al concentration, pH) and genetic characteristics of the plant (for reviews see Horst 1995, Rengel 1996). Differences in cell wall constituents between di- and monocotyledons facilitate higher uptake in the monocotyledons. In general, the tolerant genotypes take up less Al than the sensitive ones (Rincón and Gonzáles 1992, Aniol 1996). In addition, physiological activity of the cells can play a role in Al uptake: the growing cells in cell culture were more active in Al uptake than the cells in stationary phase (Vitorello and Haug 1996, Sivaguru *et al.* 1999b).

Regardless of a plant species, most of Al accumulates in the apical part of the roots (for review see Kochian 1995). For instance, the highest Al content was detected in the 0 - 2 mm tip of *Picea abies* roots (Godbold and Jentschke 1998), in 0 - 5 mm root tips of soybean (Kataoka *et al.* 1997) and maize cultivars (Llugany *et al.* 1994), in 1 - 2 cm tip of bean and barley roots (Waisel *et al.* 1970), in 0 - 10 mm tip of barley, maize, oat, rice, and pea roots (Wagatsuma *et al.* 1987). In wheat the highest Al content was found within 0 - 5 mm from the root tip in both sensitive and tolerant lines (Yeremiyahu *et al.* 1997, Sasaki *et al.* 1997), while Samuels *et al.* (1997) obtained similar results only with the sensitive cultivar. An opposite was found in the tolerant cultivar: higher Al content in the older region (5 - 15 mm) and lower in the youngest tissues (0 - 5 mm from the root tip).

Recently, the detailed determinations have shown the distal part of transition zone (DTZ, Fig. 2) of the maize root apex to be the most active region in Al uptake (Sivaguru *et al.* 1999a,b, Kollmeier *et al.* 2000, Marienfeld *et al.* 2000). The DTZ in maize occurs between the first and second mm behind the root tip. This zone is mostly constituted of cells immediately after cessation of their dividing activity and in preparation for rapid elongation (Baluška *et al.* 1990, 1996, Ishikawa a Evans 1993). In DTZ, aluminium was detectable in the cell walls of epidermal cells after several seconds and, in other root tissues by 60 minutes (with a gradual increase of uptake: epidermis >> outer cortex > middle cortex > inner cortex > stele). The rate of uptake and accumulation of Al decreased along the root in proximal direction. The longitudinal gradient in Al uptake may be related to gradual changing the cell wall composition, resulting in decrease in both relative proportion of pectins and cation exchange capacity. Thus, in the walls of older cells the amount of sites available for Al binding may diminish (Godbold and Jentschke 1998). This has been demonstrated experimentally by NaCl-induced increase of pectin amount in the cell walls that lead to an increase in Al uptake and sensitivity in plants (Horst *et al.* 1999) as well as in cell cultures (Schmohl and Horst 2000).

Although there is less evidence of Al entering the cells, the symplasmic toxicity is generally accepted. Evidence has been obtained using analyses of Al in the fractions of cellular components (for review see Delhaize and Ryan 1995), X-ray microanalytical demonstration of Al (Rasmussen 1968, Waisel *et al.* 1970, Vázquez *et al.* 1999), method of secondary ion mass spectrometry (Lazof *et al.* 1994), staining with specific dyes morin (Tice *et al.* 1992) or lumogallion (Kataoka *et al.* 1997, Silva *et al.* 2000) and demonstrating the Al-compounds using confocal microscopy, or laser microscope mass analysis. The latter method revealed to be highly sensitive and it was used to localise Al in the cytoplasm of maize and broad bean sensitive cultivars (Marienfeld *et al.* 2000). Concentration of Al^{3+} ions in the cytoplasm can not reach high values as pH of the cytoplasm does not conform their stability and, complexes of Al with potential ligands present in the cytoplasm are formed rapidly. Nevertheless, Al can exert toxic effects due to its high binding activity with physiologically important molecules including nucleic acids (for reviews see Delhaize and Ryan 1995, Matsumoto 2000).

In the root cells of soybean, Al was detected after 30 min treatment (Lazof *et al.* 1994). Within the sensitive DTZ of maize root, Al was detectable inside the cells of epidermis and inner cortex after 3 h Al-treatment. In contrast, aluminium was detected only inside the epidermal and outer cortical cells of the dicotyledonous *Vicia faba* roots (Marienfeld *et al.* 2000). With prolongation of the exposure the Al content increased in the cells of the sensitive soybean cultivar. The highest levels were detected not only in peripheral but also in internal cell layers within the meristem. The localisation of Al corresponded with the localisation of cell nuclei (Silva *et al.* 2000).

Sensitivity of the elongation along the root axis

Development of more sensitive methods and analytical approaches to studying root responses to Al toxicity allowed to identify the most sensitive root region. Evidence is increasing (Sivaguru and Horst 1998) that it is the DTZ. If aluminium was applied locally to this zone (Fig. 2), the highest accumulation of Al and callose occurred specifically in this DTZ. Also the strongest growth reduction of the elongation zone (2.5 to 5 mm

from the root tip) occurs after the local Al application onto the DTZ in spite of a minor contribution of DTZ itself to the whole root elongation. None of the other maize root segments (1 mm long) within the 5 mm long apex, revealed as high sensitivity as DTZ (Sivaguru and Horst 1998, Kollmeier *et al.* 2000). Distinguishing the specific and sensitive DTZ have brought several suggestions of the mechanisms of Al effects on root elongation including auxin transport and interactions with cytoskeleton or, effects of Al on the cell wall/plasma membrane/cytoskeleton continuum (Sivaguru *et al.* 1999a, Horst *et al.* 1999, Kollmeier *et al.* 2000, Schmohl and Horst 2000).

Structural responses of the individual root tissues

Differences in the responses of individual tissues to aluminium are evident for instance when comparing tissues of the root cap and of the root itself. These differences can be explained by the position of the tissues as well as by their inherent physiological characteristics. Cells on the periphery of the root cap and of the root itself, the epidermis and 1 to 2 outer cortical layers which get into immediate contact with the stress factor are most drastically injured after exposure to aluminium (Figs. 3, 4). They are the first tissues where vacuolation increases, cell walls thicken, and after a longer exposure the whole cytoplasmic content disintegrates irreversibly (Bennet and Breen 1987, Wagatsuma *et al.* 1987, Eleftheriou *et al.* 1993, De Lima and Copeland 1994, Budíková *et al.* 1997, Votrubová *et al.* 1997, Čiamporová 2000).

Cells with different mode of growth can also respond diversely to Al toxicity. This can be documented by Al-induced disturbance of cytoplasmic Ca^{2+} homeostasis in the cells increasing their volume by diffuse growth in culture (Jones *et al.* 1998a) while increase of Ca level occurs specifically in the tip of root hairs for whose the tip growth is characteristic (Jones *et al.* 1998b).

The position of the tissues in the root is not the only reason for differences in their responses to Al. This can be demonstrated with tissues in the maize root tip. After Al application on the sensitive DTZ, both responses depolymerisation of microtubules and Al-induced callose synthesis occurred first in peripheral layer of the root cortex (1 h) but not in the outermost epidermal cells (Sivaguru *et al.* 1999a). In the elongation zone, microtubule orientation did not change in the cells of epidermis and outer cortex while depolymerisation was observed in the cells of inner cortex (3 h) localised in a greater distance from the stress source (Blancaflor *et al.* 1998). Obviously, the early response of the more inner root cells must be mediated by some rapidly transduced signal(s) and some specific receptors which induce alterations even without a direct contact with the toxic element. The basis of such tissue specificity is not yet known.

Responses of cells in different developmental stages

Studying structural responses in a certain tissue we can find evidence of different responses in the cells of different developmental stage. In the roots such differences can well be followed along the cell files particularly of the more sensitive peripheral tissues. Within a file of epidermal cells, the cell ultrastructure was most severely damaged in the distal part. Disintegration of cell cytoplasm occurred between 0.5 mm from the root tip up to the region which often appears thickened, *i.e.* at about 15 mm from the root tip (Budíková *et al.* 1997, Čiamporová 2000). Structure of the cells occurring further in the proximal part of the files was not affected (Budíková, unpubl. data). The highest sensitivity of cytoskeletal elements was observed between 1 and 2 mm from the root tip (Sivaguru *et al.* 1999a,b, Horst *et al.* 1999). The highest accumulation of both aluminium and callose occurred also in this stage of cell ontogeny (Kollmeier *et al.* 2000). Higher sensitivity of ontogenetically young cells comparing to mature cells was distinct also with respect to membrane responses to Al. With increasing distance from the maize root tip the rapidity and degree of membrane depolarisation decreased and Al had no effect on membrane polarisation at the root base (Pavlovkin and Mistrík 1999). Similarly, different responses were obtained in Al-induced quantitative changes of peripheral proteins between the root tip and the root base (Mistrík *et al.* 2000).

To this type of diversity also different responses of cells with different growth activities can be included as has been shown with Al uptake (Vitorello and Haug 1996). Al-induced changes in cytoplasmic calcium concentration were recorded only in the growing root hairs (Jones *et al.* 1998b) but not in those which had completed their elongation (Sattelmacher *et al.* 1993).

One of the reasons for the diverse sensitivity of the cells of the same tissue but differing in their age may be the quality of their cell walls. In meristematic cells the cell walls seem to create impermeable barrier for Al

movement (Rasmussen 1968). During maturation the pores between cell wall microfibrils enlarge (for review see Peterson and Cholewa 1998) thus facilitating both entry and contact of Al with the cells. During continuing maturation processes, the cell walls again become impermeable barriers due to gradual changing the cell wall composition, resulting in decrease in both relative proportion of pectins and cation exchange capacity. This can lead to the frequently observed low sensitivity of mature root cells to aluminium toxicity.

The different (mostly higher) sensitivity of the cells within DTZ when compared to the tissues towards either the root apex or the more mature ones could be related to their specific metabolic properties (Baluška *et al.* 1990). Interestingly, these cells are extremely sensitive also to some other environmental stimuli (Ishikawa and Evans 1990, 1993). Molecular principles and mechanisms of either Al effects on plant cells or diversity in the cell responses are not yet known.

Different responses of individual cells

The diversity in the responses to Al of the cells of different tissues or of different developmental stages can be expected with relation to the specific physiological and biochemical characteristics of the particular cell type and age. Less expected would be a diversity within the cells belonging to the same tissue and being apparently of the same developmental stage. In cross sections of maize root two types of structural responses to 24 h Al stress occurred in epidermis: cells with almost unaltered structure and protoplast integrity and, cells with changed shape and extremely dense cytoplasm, in which organelles could not be distinguished (Budíková *et al.* 1997, Čiamporová 2000). Such diverse responses could be observed immediately behind the region where the epidermal cells were not covered by the root cap cells any more, *i.e.* at about 0.5 mm from the root tip (Čiamporová 2000). Non-uniform responses were observed also within the cortical tissue of barley root (Ikeda and Tadano 1993). Swelling of only some cells occurring at the same distance from the root tip was observed in wheat root cortex (Votrubová *et al.* 1997). In maize root cortex, single cells or, a small number of cells within one cell file, occurred with severely damaged structural integrity (Fig. 4) while the neighbouring cells revealed unaffected structure (Čiamporová 2000).

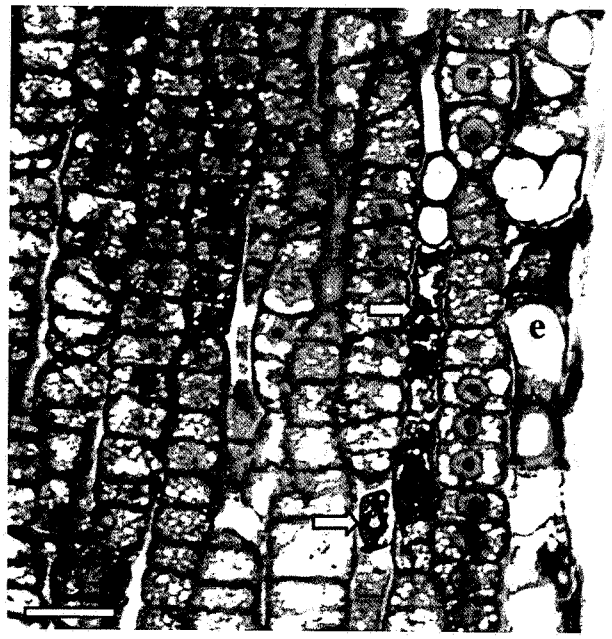

Fig. 4. Longitudinal section of the files of epidermal (e) and cortical cells at 1 mm from the root tip in maize (cv. CE-380) root stressed with 50 μM Al^{3+} for 24 h. Note the increased vacuolation in the epidermis and several cortical seriously damaged cells (*arrows*) in the cortex surrounded by undisturbed cells. *Bar* represents 20 μm.

Based on mere structural manifestation of cells it is not possible to give explanation for diversity in the individual cell responses to a stress. Aluminium toxicity was not the only stress type inducing such diverse responses in the treated tissues. A similar "mosaic-like" response of cell structure was found under copper toxicity in wheat roots (Ouzounidou *et al.* 1995). Non-uniform occurrence of apoptotic cells was induced by salinity in barley roots (Katsuhara 1997). We could assume that not all the cells within a tissue, at the same distance from the root tip are identical. A certain individuality of the cells has been shown for instance with relation to localisation of some enzymes in single cortical cells of broad bean roots (Beneš 1983). Under Al or other stresses such individual response can be of importance due to rapid death of some cells which had accumulated the toxic ion(s) enabling thus survival of the cells in the surrounding tissue.

Conclusions

Al-induced changes in numerous characteristics like root morphology, cell ultrastructure, growth rate, Al uptake and accumulation, and callose formation have indicated that the transition zone (particularly its distal part) plays the main role in expression of Al toxicity to the root apex. Towards the base the sensitivity of the root declines. In

radial direction, the decreasing sensitivity of root tissues is mostly centripetal, in agreement with increasing distance from the source of the Al ions. However, there must be some other circumstances determining higher sensitivity of the more distant cells, *e.g.*, of hypodermis or inner cortex comparing to epidermis or outer cortex, respectively, at least in the earliest phases of the stress exposure. Elucidating the principles of cellular responses to Al is difficult also due to different sensitivity of adjacent cells within the same tissue, apparently of the same developmental stage.

Diverse responses of roots at any level of their organisation to Al toxicity may be of importance for plant adaptation. At cellular level the mechanisms may include the ability of preserving cell and tissue integrity in both mature root zones where primordia of lateral roots are initiated and central cylinder where the important vascular systems are differentiated. Also accumulation of the toxic Al ion in the individual cells accompanied by their disintegration may allow the surrounding tissue to survive the stress.

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