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Structural changes and aluminium distribution in maize root tissues

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Abstract

Growth and structural responses of primary roots of *Zea mays* L. to aluminium chloride were studied. The treatment of seedlings with 50 μM $AlCl_3$ resulted in high accumulation of Al, partial inhibition of root growth, occurrence of surface lesions in peripheral tissues, root thickening caused by expansion of inner cortical cells, reduced root cap length, extensive vacuolation, cell distortion, and increased synthesis of callose within 24 h.

Additional key words: callose, cell viability, 2-deoxy-D-glucose, histochemistry, lignin.

Introduction

Mechanisms and the primary site of Al toxicity, in some areas one of the most limiting factors of crop productivity, have not been clarified yet. The effect of aluminium is manifested as the partial or complete growth inhibition and root morphological changes (*e.g.* Wagatsuma *et al.* 1987, Ryan *et al.* 1993, Budíková *et al.* 1997, Bennet 1998, Blancaflor *et al.* 1998), perturbation of mineral nutrition (Pintro *et al.* 1997) and metabolism. Inhibition of root growth is manifested after Al application on the root tip including the root cap and the meristem (Ryan *et al.* 1993) but no effect on the root growth was observed after Al treatment of the elongation zone where only visible injuries of rhizodermal and cortical cells were induced. Structural responses of roots such as vacuolation and lesion occurrence of peripheral root tissues are intensified with increased length of exposure to Al (Eleftheriou *et al.* 1993, Ikeda and Tadano 1993, de Lima and Copeland 1994).

To better understanding the principles of Al tolerance and Al sensitivity mechanisms, it is necessary to elucidate whether the Al presence in the tissue is responsible for the onset of root growth inhibition. Using X-ray microanalysis,

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Al was detected only in outer meristematic cells of oat and wheat roots (Marienfeld and Stelzer 1993, Ownby 1993) even after several hours of Al treatment. These authors suggested that the plasma membrane could serve as an effective barrier to the entry of Al into the cytoplasm. On the other hand, Lazof *et al.* (1994) reported that the secondary ion mass spectrometry images indicated early entry of Al into soybean root tip cells. Two methods for Al detection in plant tissues were applied on intact roots. The hematoxylin staining has been used for determination of Al in root tips (Rincón and Gonzales 1992, Ownby 1993, Ryan *et al.* 1993, de Andrade *et al.* 1997). Recently, morin has been reported as a sensitive fluorescent dye to detect aluminium in plant roots of many species (Tice *et al.* 1992, Larsen *et al.* 1996).

The aim of the work is to reveal Al distribution and the induction of callose and lignin synthesis by histochemical techniques, and to determine morphological and structural responses of maize root tips to aluminium stress.

Materials and methods

Caryopses of maize (*Zea mays* L., cv. TO 360) germinated for 72 h in darkness at 25 °C. Seedlings with roots approximately 4.5 cm long were transferred to *1*) 0.1 mM $CaCl_2$ (control), *2*) 0.1 mM $CaCl_2$ + 50 μM $AlCl_3$, *3*) 0.1 mM $CaCl_2$ + 100 μM 2-deoxy-D-glucose (DDG, inhibitor of callose synthase), or *4*) 0.1 mM $CaCl_2$ + 50 μM $AlCl_3$ + 100 μM DDG (pH 4.5). Seedlings treated for 2, 4 and 24 h were used in the following experiments. Viability of root rhizodermis was studied with 0.1 % Evans blue. Distribution of aluminium in root-tip tissues was determined by staining with hematoxylin (Rincón and Gonzales 1992) and morin (Vitorello and Haug 1996). Secondary fluorescence was used to detect lignin and callose by acridine orange (Harris and Oparka 1994) and aniline blue, respectively. Histochemical test for polyphenol detection was carried out according to Reeve (1951). For light microscopy excised root pieces (2 mm) were fixed in glutaraldehyde and OsO_4, dehydrated by ethanol and embedded in *Epon/Araldite* mixture as previously described (Budíková *et al.* 1997). Semithin sections were stained with toluidine blue and basic fuchsin. For scanning electron microscopical studies fixed apical and subapical root pieces (0.5 cm) were dehydrated, critical point dried, coated with gold and observed by *Tesla BS 300* (Prague, Czech Republic) electron microscope. Root cap length and root cap area were measured on median apical root sections by *Leica Q500MC* image analysis system *QWin* (*Leica Imaging Systems*, Cambridge, UK). The significance of differences was evaluated using *t*-test.

Results and discussion

Treatment with aluminium resulted in partial root growth inhibition, reduced root cap length and root cap area (Table 1). Reduction in cap length and the loss of organisation of cap cells was reported in Al-sensitive wheat genotype (Bennet 1998). Any marked surface defects were not found in root caps treated with 50 μM $AlCl_3$

(Fig. 1*a,b*). The visible symptoms of Al toxicity such as tissue lesions and thickened root parts were similar to those described previously (Wagatsuma *et al.* 1987, de Lima and Copeland 1994, Ishikawa *et al.* 1996, Budíková *et al.* 1997, Votrubová *et al.* 1997). Transversal cracks (Fig. 1*d*) extending over the surface of the apical region from 2 mm up to about 20 mm behind the root tip resembled those formed on root surface of many plants (Wagatsuma *et al.* 1987, Delhaize and Ryan 1995). Proximally to this part, a 5 mm long thickened zone caused by radial expansion of inner cortical cells (Fig. 2*a*) was observed. Similar results were obtained in experiments of Blancaflor *et al.* (1998) where fast reorganization of microtubules in the inner cortical cells of elongation zone coincided with later thickening of these cells. The width of these cells was 1.22-times larger than that of untreated cells in the corresponding root segment. The number of cortical cell layers in the thickened wheat root part remained the same within 24 h because expanded cells did not divide in periclinal direction. The root surface of this zone as well as of the rest of the root remained smooth and similar to control.

Table 1. The effect of 50 μM aluminium chloride on primary maize root growth (n = 50), root cap length and area (n = 8), and cortical cell width (n = 150). Seedlings were treated by $AlCl_3$ for 24 h. Means ± SE; * - differences significant at $P < 0.05$.

	Initial root length [mm]	Final root length [mm]	Root cap length [μm]	Root cap area [μm^2]	Cortical cell width [μm]
Control	45.2 ± 0.6	73.0 ± 1.0*	394.1 ± 9.7*	102.000 ± 1.4*	30.1 ± 4.8*
$AlCl_3$	45.8 ± 0.6	60.2 ± 0.8*	308.8 ± 18.8*	88.395 ± 4.7*	36.8 ± 5.8*

Control root sections at the distance of 1 mm behind the root tip revealed a typical meristematic character of all tissues (Fig. 2*b*). The extent of changes in the root cell structure followed the duration of Al exposure. The increased vacuolation of the root tip cells was visible as soon as after 2 h of Al treatment (Fig. 2*c*). With longer treatments (4 h) the vacuolation increased and some hypodermal and cortical cells died. Surface lesions and thickening of the proximally adjacent root zone were evident as soon as after 8 h of exposure. Extensive vacuolation, cell distortion, increased cytoplasmic density of many rhizodermal cells, and even cell death occurred within 24 h in the presence of Al (Fig. 2*d*).

Viability of root tip portions of control maize roots remained normal throughout the treatment (Fig. 1*e*). The peripheral cells of Al-treated roots within the distance of 2 - 20 mm lost their viability after 24 h (Fig. 1*f*) probably due to Al-induced membrane damage followed by excessive Al accumulation in this region. No decrease of viability was observed in older root portions after 24 h of Al treatment.

In control maize roots, no Al was detect along their whole axis (Figs. 1*g*, 2*e*). Low amount of hematoxylin was accumulated only in mechanically damaged root tissues. Control root sections exhibited only low autofluorescence (not shown) when stained with morin. In treated seedlings, different accumulation of Al in root tissues of different age was revealed in experiments. Hematoxylin staining was encountered in

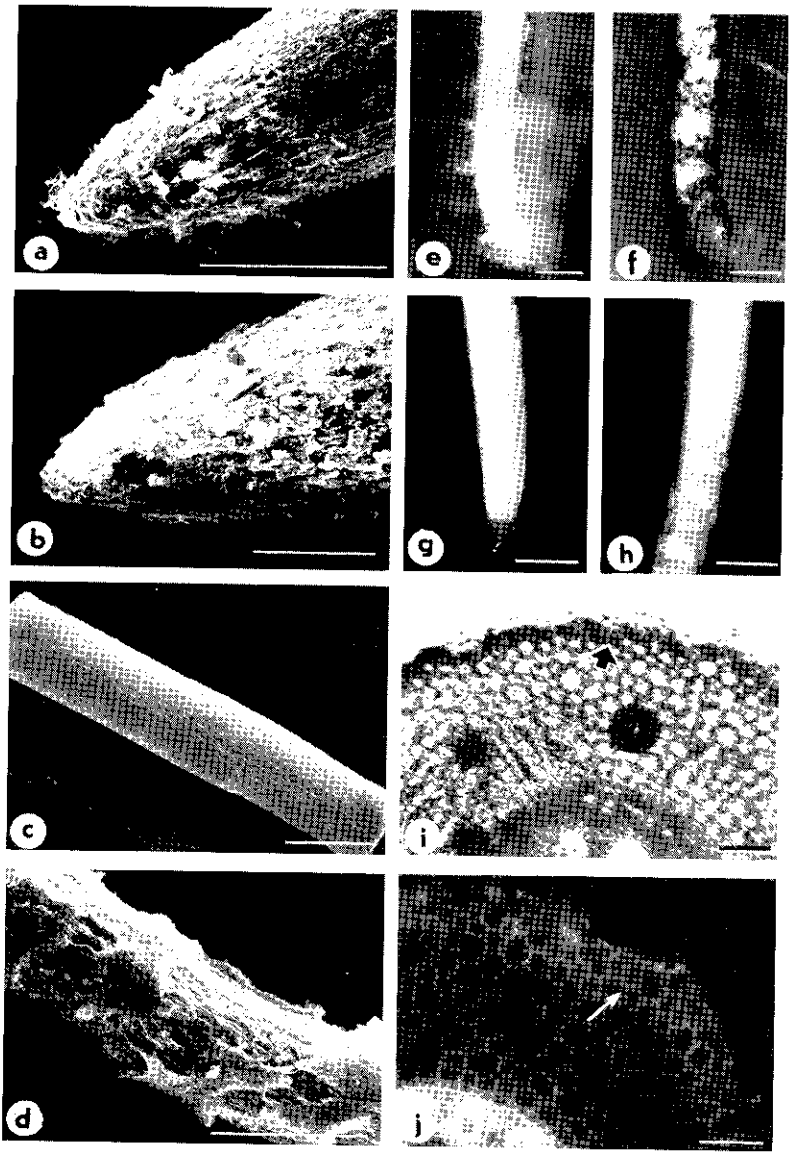

Fig. 1. The surface of control (*a*) and Al-treated (*b*) root cap. Smooth surface of control root (*c*) at the distance of 0.5 - 1 cm behind the tip. Transversal cracks (*d*) extending over the surface of the apical region. Viability of control root tested by Evans blue (*e*). The peripheral cells of the Al-treated root cap as well as those of the cracked region lost their viability within 24 h and absorbed large amounts of stain (*f*). Negative detection of Al in intact control roots with hematoxylin (*g*). Positive detection of Al in the cracked region, no staining is visible in the thickened part of the root (*h*). Induction of polyphenol (*i*) and lignin (*j*) formation in peripheral root tissues at the distance of 1 cm after 48 h treatment of aluminium (*arrows*). *Bars* represent 1 μm in the figures *a* to *h* and 50 μm in the figures *i* to *j*, respectively.

the cells of the root cap, rhizodermis, hypodermis and cortex at the distance of 1 - 3 mm behind the root tip (Fig. 2*f*). The root cap was the most heavily stained tissue of plants. Green fluorescence of Al-morin complex was revealed in outer peripheral root cap cells including edge cells (Fig. 2*g*) and peripheral cells of the cracked region (2*h*) within the root itself. The thickened region (Figs. 1*h*, 2*i*) as well as the older root parts showed no Al accumulation. Generally, the patterns of Al detection obtained by both stainings seemed to be very similar.

The callose synthase activity is closely associated with membrane damage exerted by many biotic and abiotic stresses (Schreiner *et al.* 1994). In roots grown without Al, callose could be found only in sieve elements and outer tangential hypodermal wall (Fig. 2*j*). Exposure to Al for 24 h resulted in increased callose synthesis in rhizodermal, hypodermal and cortical cells within the region of lesions. The largest deposits of non-constitutive callose were found in endodermal cell walls (Fig. 2*k*). Recently, the callose deposits were found in root cap, rhizodermis and outer cortical walls of soybean (Wissemeier *et al.* 1987) and a sensitive wheat line (Schreiner *et al.* 1994). These regions are considered to have a close association with stress perception. No qualitative changes were found in more proximal root parts including the thickened zone. Stanghellini *et al.* (1993) suggested that increased callose synthesis could be an induced structural resistance and not a sensitive response to many stresses. This hypothesis could be strengthened by further investigations in which inhibition of callose synthesis would result in an increased damage of plant tissues during Al stress.

Additional study was carried out with the use of 100 μM 2-deoxy-D-glucose (DDG). Treatment of this callose synthase inhibitor completely inhibited the callose deposition in sieve elements in control roots but did not influence the root morphology. This result corresponds with similar experiment with wheat when fewer deposits of callose after DDG treatment and almost no deposits after callose degradating enzyme treatment were detected (Schreiner *et al.* 1994). The decreased callose fluorescence in sieve elements and cortex was found after Al+DDG treatment (Fig. 2*l*) in comparison with control. The pattern of callose deposition was studied to reveal the role of callose depositions in the "cell wall loosening". This phenomenon could participate in the formation of the surface lesions. The destruction of peripheral tissues may arise as a result of mechanical stress exerted by elongation of the central cylinder (Eklund and Eliason 1990). More detailed studies showed the presence of lesions as well as the thickening in Al+DDG-treated roots therefore no correlation between callose synthesis and formation of the surface lesions was confirmed.

In control plants detection of non-constitutive polyphenols and lignin was negative along the whole root axis. In acridine orange stained roots only a green fluorescence of all peripheral tissues was observed. The staining showed the presence of lignin in vessel elements and endodermis only. After 48 h Al exposure positive reactions for both polyphenols and lignin were observed in peripheral tissues in the region of lesions as well. Pink to red staining of polyphenols was revealed in rhizodermal cell walls (Fig. 1*i*). Red fluorescence of induced lignin was revealed in rhizodermal, hypodermal and outer cortical cell walls (Fig. 1*j*) in the cracked region. The shorter time treatments induced no lignin nor polyphenols. Al-induced deposition of lignin

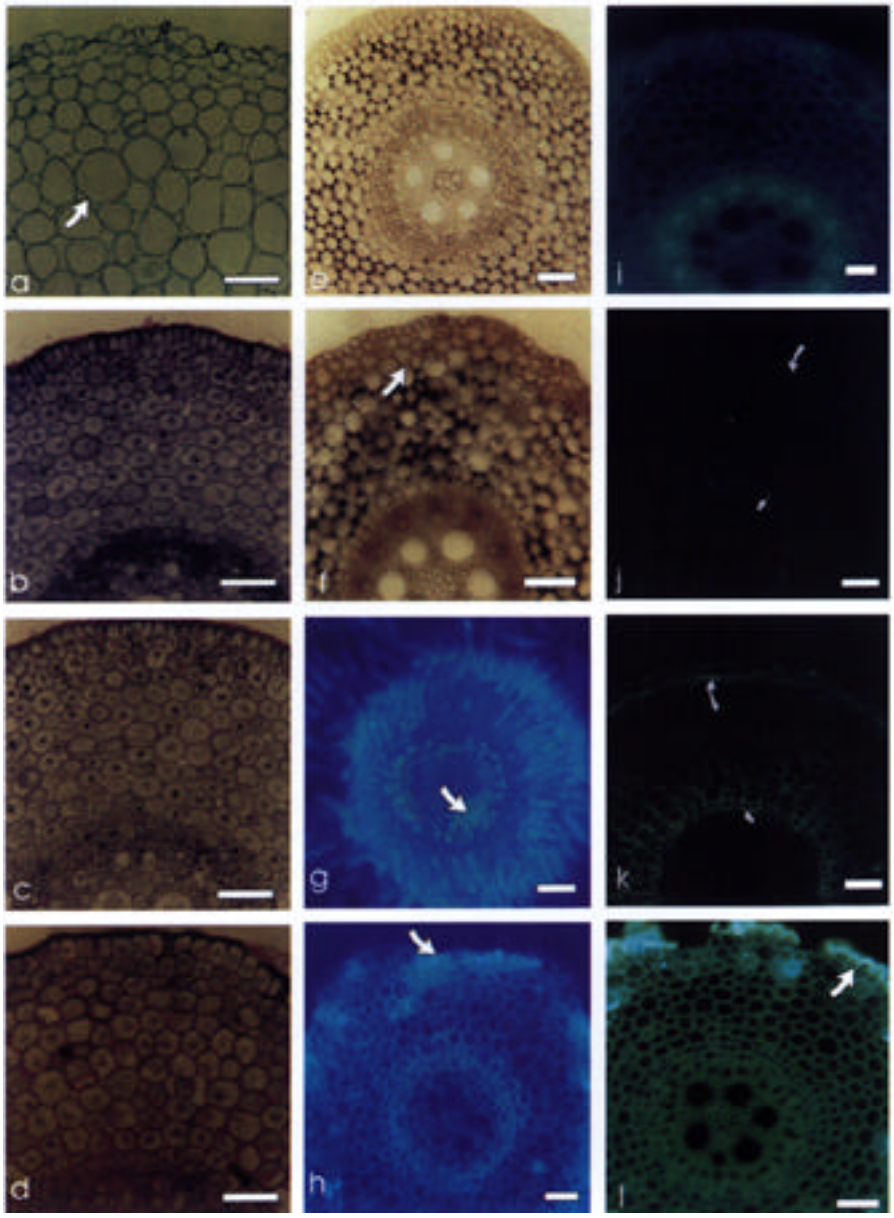

Fig. 2. Transversal section of the root thickening (*a*) at the distance of 20 - 25 mm showing radial expansion of inner cortical cells (*arrow*). Transversal sections of control (*b*) and treated (*c,d*) primary maize roots. The increased vacuolation of the root tip cells has been visible as soon as after 2 h of Al treatment (*c*). Extensive vacuolation of all peripheral tissues, cell distortion and death of many rhizodermal cells of the same root part occurred within 24 h (*d*). Hematoxylin staining of

(*Continued*)

has been reported by Sasaki *et al.* (1996). Similarly, Kpémoua *et al.* (1996) and Bach and Seitz (1997) confirmed that the activity of phenylalanine ammonia-lyase (PAL), the key enzyme of lignin synthesis, increased after long-time biotic stress in infected plant tissues.

It can be concluded that the root apex can be the primary site of Al toxicity due to severe growth inhibition, structural responses and excessive Al accumulation. The severity of Al-induced changes in the root apices followed the duration of aluminium stress. No responses were reported in basal parts of the root and the shoot as well.

References

Bach, M., Seitz, H.U.: Elicitor-induced defence responses of a suspension-cultured woody plant (*Larix decidua*) and possible mechanisms of signal transduction. - Can. J. Bot. **75**: 1243-1251, 1997.

Bennet, R.J.: The aluminium response network in wheat (*Triticum aestivum* L.)? I. The root growth reactions. - S. Afr. Tydskr. Plant Grond **15**: 38-45, 1998.

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

Budíková, S., Čiamporová, M., Ovečka, M., Polónyi, J.: Structural characterization of maize root tip cells under aluminium stress. - Acta Fac. Rer. nat. Univ. Com. - Physiol. Plant. **29**: 47-52, 1997.

De Andrade, L.R.M., Ikeda, M., Ishizuka, J.: Localization of aluminum in root tip tissues of wheat varieties differing in aluminum tolerance. - J. Fac. Agr. Kyushu Univ. **41**: 151-156, 1997.

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

De Lima, M.L., Copeland, L.: Changes in the ultrastructure of the root tip of wheat following exposure to aluminium. - Aust. J. Plant Physiol. **21**: 85-94, 1994.

Eklund, L., Eliason, L.: Effects of calcium ion concentration on cell wall synthesis. - J. exp. Bot. **41**: 863-867, 1990.

Eleftheriou, E.P., Moustakas, M., Fragiskos, N.: Aluminate-induced changes in morphology and ultrastructure of *Thinopyrum* roots. - J. exp. Bot. **44**: 427-436, 1993.

Harris, N., Oparka, K.J.: Plant Cell Biology. A Practical Approach. - Oxford University Press, Oxford 1994.

Ikeda, H., Tadano, T.: Ultrastructural changes of the root tip cells in barley induced by a comparatively low concentration of aluminum. - Soil Sci. Plant Nutr. **39**: 109-117, 1993.

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control and Al-treated roots at the distance of 1 - 3 mm behind the root tip, respectively. Control roots (*e*) show no staining. Al-treated root section (*f*) shows the presence of Al (*arrow*). Aluminium distribution in roots stained with morin. In root cap (*g*) intensive fluorescence can be observed in peripheral cap cells (*arrow*); the root cap apex is situated close to the *arrow*. In the region of lesions (*h*) Al was identified in peripheral root tissues (*arrow*). No Al was detected in the thickened region (*i*) or in more basal parts of the root. Callose deposits in sieve elements (*small arrow*) and rhizodermal cell walls (*large arrow*) of control roots (*j*). Exposure of Al for 24 h (*k*) resulted in increased callose synthesis in rhizodermal, hypodermal (*large arrow*) and cortical cells in the region of lesions. The largest deposits of callose were found in endodermal cell walls (*small arrow*). The decreased callose fluorescence in sieve elements and cortex in comparison with control was found after Al+DDG treatment (*l*) when callose deposits were observed in the damaged rhizodermis (*arrow*). *Bars* represent 50 μm.

Ishikawa, S., Wagatsuma, T., Ikarashi, T.: Comparative study of Al^{3+}, Yb^{3+}, and La^{3+} to root-tip cells differing in tolerance to high Al^{3+} in terms of ionic potentials of dehydrated trivalent cations. - Soil Sci. Plant Nutr. **42**: 613-625, 1996.

Kpémoua, K., Boher, B., Nicole, M., Calatayud, P., Geiger, J.P.: Cytochemistry of defense responses in cassava infected by *Xanthomonas campestris* pv. *manihotis*. - Can. J. Microbiol. **42**: 1131-1143, 1996.

Larsen, P.B., Tai, C.Y., Kochian, L.V., Howell, S.H.: *Arabidopsis* mutants with increased sensitivity to aluminum. - Plant Physiol. **110**: 743-751, 1996.

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

Ownby, J.D.: Mechanisms of reaction of hematoxylin with aluminium-treated wheat roots. - Physiol. Plant. **87**: 371-380, 1993.

Pintro, J., Barloy, J., Fallavier, P.: Effects of low aluminum activity in nutrient solutions on the organic acid concentration in maize plants. - J. Plant Nutr. **20**: 601-611, 1997.

Reeve, R.M.: Histochemical tests for polyphenols in plant tissues. - Stain Technol. **26**: 91-96, 1951.

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

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

Sasaki, M., Yamamoto, Y., Matsumoto, H.: Lignin deposition induced by aluminum in wheat (*Triticum aestivum*) roots. - Physiol. Plant. **96**: 193-198, 1996.

Schreiner, K.A., Hoddinott, J., Taylor, G.J.: Aluminum-induced deposition of (1,3)-β-glucans (callose) in *Triticum aestivum* L. - Plant Soil **162**: 273-280, 1994.

Stanghellini, M.E., Rasmussen, S.L., Vandemark, G.J.: Relationship of callose deposition to resistance of lettuce to *Plasmopara lactuca-radicis*. - Phytopathology **83**: 1498-1501, 1993.

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

Vitorello, V.A., Haug, A.: Short-term aluminium uptake by tobacco cells: Growth dependence and evidence for internalization in a discrete peripheral region. - Physiol. Plant. **97**: 536-544, 1996.

Votrubová, O., Černohorská, J., Hlídková, R., Krobová, J., Eliášová, K.: The effect of aluminum on growth and structure of wheat root. - J. appl. Genet. **38B**: 277-281, 1997.

Wagatsuma, T., Kaneko, M., Hayasaka, Y.: Destruction process of plant root cells by aluminum. - Soil Sci. Plant Nutr. **33**: 161-175, 1987.

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