Effect of aluminum on cell wall, plasma membrane, antioxidants and root elongation in triticale

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Abstract

Two triticale cultivars ZC 237 (Al-resistant) and ZC 1890 (Al-sensitive) were used to investigate the effects of 30 to 100 μM Al on antioxidative enzyme activity, lipid peroxidation and cell wall composition. In ZC 1890, the root elongation was significantly inhibited after 1-h exposure to 50 μM Al, the changes in hemicellulose fraction were clearly detected after 2-h Al exposure, while the peroxidase (POD) and superoxide dismutase (SOD) activities significantly increased after 6-h exposure, and the malondialdehyde (MDA) content after 12-h exposure. The similar patterns were also found in ZC 237. Treatment of ZC 1890 with 1 mM citrate for 30 min after 3-h exposure to Al resulted in significant decrease of Al bound to cell-wall and recovery of root elongation. These results suggested that Al affected cell wall before the damage of plasma membrane, but this was not the primary cause of root elongation inhibition.

Additional key words: lipid peroxidation, oxidative stress, toxicity, *Triticosecale.*

Introduction

Aluminum (Al) toxicity is a major growth-limiting factor for crop production in acid soils. The initial and most dramatic symptom of Al toxicity is the inhibition of root elongation (Kochian 1995, Matsumoto 2000). However, the mechanisms of Al toxicity have not been elucidated, although it has been well established that root apex is the major site that incurred the greatest injury and a wide range of potential effects of Al to root tip growth has been established (Zheng and Yang 2005).

Several lines of evidence support the view that the apoplast plays an important role in Al toxicity (Horst 1995). It was reported that 85 - 90 % of the total Al accumulated by barley roots was tightly bound to cell walls (Clarkson 1967). Up to 99 % of the total Al was associated with the cell wall in giant algal cells of *Chara corallina* (Taylor *et al*. 2000). Le Van *et al*. (1994) analyzed the cell-wall composition of squash roots exposed to Al and observed some Al-induced changes in the amount of cell-wall polysaccharides. The Al bound to the cell walls and changes in cell wall composition may cause a modification of the mechanical properties of the cell wall, leading to the inhibition of root growth and development. Tabuchi and Matsumoto (2001) reported that Al modified the metabolism of cell-wall compositions and decreased the mechanical extensibility, which makes the cell wall thick and rigid, thereby inhibiting the growth of wheat roots. The evidence from Ma *et al*. (2004) indicated that the Al-dependent changes in the cell wall viscosity and elasticity were involved in the inhibition of root growth. These results suggested that the cell wall, due to its high density of negative charges, is an important site for binding of Al, thus leading to the inhibition of root elongation.

On the other hand, increasing evidence also suggested that plasma membrane is the major target for Al toxicity due to the negativity of carboxyl groups and phosphate groups in the plasma membrane (Matsumoto 2000, Zheng and Yang 2005). It was reported that Al induced enhancement of lipid peroxidation and antioxidative enzyme activities in soybean root apices (Cakmak and Horst 1991). Up to now, these phenomena have been reported in phospholipid liposomes (Oteiza 1994),

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Abbreviations: f.m. - fresh mass, MDA - malondialdehyde, NBT - nitroblue tetrazolium chloride, POD - peroxidase, RRE - relative root elongation, SOD - superoxide dismutase, TBA - thiobarbituric acid.
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tobacco cell culture (Yamamoto *et al*. 2002), detached rice leaves (Kuo and Kao 2003) and barley root apices (Tamás *et al*. 2003, Šimonovičová *et al*. 2004). In *Arabidopsis*, Al induced the expression of several genes (*e.g*. for superoxide dismutase, peroxidase) that are induced by oxidative stress (Richards *et al*. 1998). The evidence for a link between Al and oxidative stress is to be found in transgenic plants expressing Al-induced genes which ameliorate oxidative stress-caused damages (Ezaki *et al*. 2000). These results confirmed that the oxidative stress is an important reaction of plants to toxic level of Al ions.

Despite the increasing evidence on the effect of Al on cell wall composition and oxidative stress, little information is available about which process is an earlier event. This maybe due to the fact that only one or several aspects of Al toxicity were taken into account in the most of previous studies. In the present study, the effects of Al on root growth, plasma membrane lipid peroxidation, and changes of cell-wall composition in two cultivars of triticale differing in Al resistance were investigated.

Materials and methods

Plants and treatments: Seeds of triticale (× *Triticosecale* Wittmack) cultivars ZC 237 (Al-resistant) and ZC 1890 (Al-sensitive) were fully imbibed with distilled water and then germinated at 25 °C in the dark. The germinated seeds were transferred to a net tray floated on a container filled with 5 dm^3 of 0.5 mM $CaCl_2$ solution at pH 4.5. The solution was renewed daily. For the dose-response experiment, seedlings with seminal roots of 4 - 5 cm length were exposed to 0.5 mM $CaCl_2$ solution containing 0, 30, 50, or 100 μM $AlCl_3$ for 24 h (for detail see Yang *et al*. 2005) and the length of the longest root was measured. For the time-course experiment, seedlings were exposed to 0.5 mM $CaCl_2$ solution containing 0 or 50 μM $AlCl_3$ and root elongation was estimated at 0, 0.5, 1, 2, 3, 6, or 12 h. The relative root elongation (RRE) was calculated as (root elongation at Al treatment/root elongation without Al) × 100. A desorption experiment with citric acid (1 mM, pH 4.5) was conducted with seedlings treated with or without Al for 3 h. The seedlings were placed in citric acid solution which was replaced once during a 30 min desorption process. After washing the seedlings with distilled water three times, half of the seedlings were subjected to Al analysis and cell wall extraction. The other half of the seedlings was incubated in the solution without Al for additional 9 h. The root lengths were estimated at 0 and 12 h. All the experiments were conducted in the growth chamber with a 14-h photoperiod, day/night temperature of 26/23 °C, irradiance of 150 μmol(photon) m^{-2} s^{-1} and a relative humidity of 70 %.

Measurement of enzyme activities and lipid peroxidation: Root apices (0 - 10 mm) were cut from seedlings exposed to 0, 30, 50, or 100 μM $AlCl_3$ for 24 h or exposed to 50 μM $AlCl_3$ after 0, 0.5, 1, 2, 3, 6, or 12 h treatment for the measurement of superoxide dismutase (SOD), peroxidase (POD) activities, and malondialdehyde (MDA) content.

Root apices were homogenized in 3 cm^3 of 50 mM phosphate buffer (pH 7.8), then centrifuged for 20 min at 1 000 *g*, the supernatant was collected for the measurement of SOD and MDA. SOD activity was assayed by using the nitroblue tetrazolium chloride (NBT) method. The assay mixture in 3 cm^3 contained 50 mM phosphate buffer, 130 mM L-methionine, 750 μM NBT, 100 μM Na_2-EDTA, 20 μM riboflavin, enzyme extract and water. One unit (U) of SOD is the amount of enzyme that inhibits NBT photoreduction by 50 %, monitored at 560 nm on spectrophotometer (*UV-2450*, *Shimadzu*, Japan).

To measure POD activity, root apices were homogenized in water. POD activity was measured with guaiacol as the substrate in a total volume of 4 cm^3. The reaction mixture consisted of acetic acid buffer (pH 5.0), 0.1 % guaiacol, 0.08 % H_2O_2 and enzyme extract. The increase in absorbance due to tetraguaiacol formation was recorded at 470 nm.

For the measurement of lipid peroxidation, the thiobarbituric acid (TBA) test which determines MDA as an end product of lipid peroxidation was used. The reaction mixture consisting of 2.5 cm^3 of 0.5 % TBA made in 5 % trichloroacetic acid and 1.5 cm^3 of enzyme extract, was heated at 100 °C for 15 min and then quickly cooled down on ice. The absorbance of the supernatant was measured at 532 nm. Correction of non-specific turbidity was made by subtracting the absorbance value taken at 600 nm.

Analysis of cell wall components: Cell wall polysaccharides were fractionated according to the method by Nishitani and Masuda (1979). Root apices were ground in a mortar with a pestle, and then washed twice with cold water, acetone, a mixture of methanol and chloroform (1:1, v/v), and ethanol. The cell-wall material was treated with 10 U cm^{-3} of α-amylase (*Sigma*, St. Louis, MO, USA) for 3 h at 37 °C, and thoroughly washed with water. The cell wall preparation was successively extracted 3 times (15 min each) at 90 °C with 50 mM EDTA (pH 6.8), and 3 times (18 h each) at 25 °C with 17.5 % (m/v) NaOH. These extractions yielded the pectin and the hemicellulose fractions, respectively. The hemicellulose fraction was neutralized with acetic acid and dialyzed against water. The residue after 17.5 % NaOH extraction was washed with 0.1 M acetic acid and water (cellulose fraction). The cellulose fraction was dissolved in 72 % (v/v) sulfuric acid for 1 h at 25 °C with stirring. Total sugars were determined by the phenol-sulfuric acid method (Dubois *et al*. 1956).

Cell wall extraction and Al determination: The content of Al bound to the root cell walls was estimated by homogenizing the frozen root apices (15 for each sample) with 0.5 cm^3 ice-cold distilled water in an Eppendorf tube using a plastic grinder according to Ma *et al*. (2004). For total Al determination, the excised root apices were placed in a 1.5 cm^3 Eppendorf tube containing 1 cm^3 of 2 M HCl. The tubes stood for at least for 24 h with occasional shaking. The Al concentrations in the extracts were determined via inductively coupled plasma atomic emission spectrometry (*IRIS/AP* optical emission spectrometer, *Thermo Jarrel Ash*, San Jose, CA, USA).

Statistical analysis: All data presented are the mean values. The measurements were done with ten replicates for root growth experiment, three replicates for all enzyme activities and MDA content, and three replicates for cell wall composition and Al content. Statistical analysis was carried out by one-way *ANOVA* using Student's *t*-test to test the significance of differences.

Results

Root elongation was significantly inhibited in both ZC 237 and ZC 1890 after 24-h exposure to 30, 50 or 100 μM Al. The inhibition of root elongation in ZC 237 at 30 μM Al was similar to that in ZC 1890 at 100 μM, thus ZC 237 was more Al resistant than ZC 1890 (Fig. 1*A*). The SOD and POD activities, as well as the MDA content, were also significantly enhanced after 24-h exposure to various concentrations of Al in both cultivars (Fig. 1*B*,*C*,*D*), indicating the occurrence of Al-induced oxidative stress.

The time-course experiment showed that the inhibition of root elongation in ZC 237 was significant after 3-h exposure to 50 μM Al, while the enhancement of oxidative stress, indicated by the increase of SOD and POD activities, was evident after 12-h exposure. In ZC 1890, the inhibition of root elongation was significant after 1-h exposure, however, the significant enhancement of SOD and POD activities occurred after 6-h exposure, and even after 12-h exposure for MDA content (Fig. 2). These results indicated that oxidative stress could not be the cause of Al-induced root growth inhibition.

In the roots of ZC 237, the polysaccharide content of pectin, hemicellulose and cellulose did not change significantly after the exposure to Al for 3 h, but pectin and hemicellulose fraction increased after 6-h exposure. However, in the roots of ZC 1890, the hemicellulose content increased significantly after 2-h Al exposure and thereafter (Fig. 3). These results indicated that Al changed the synthesis or degradation of cell wall components in root apex and the sensitive one was more easily to be effected.

After exposed to 50 μM Al for 3 h, then transferred to $CaCl_2$ solution without Al for 9 h, the root growth of ZC 1890 was significantly inhibited when compared to the root growth without Al exposure (Fig. 4*A*). However, when the Al treated roots were transferred to $CaCl_2$ solution containing 1 mM citric acid for 30 min, the root elongation increased by 70 % compared with Al treated root during the following 9 h growth in $CaCl_2$ solution. Citrate washing itself had no effect on the elongation of roots not subjected to Al treatment. The total and cell wall Al contents with citrate desorption treatment were decreased by 38 and 30 %, respectively, when compared to that of without citrate desorption treatment (Fig. 4*B*).

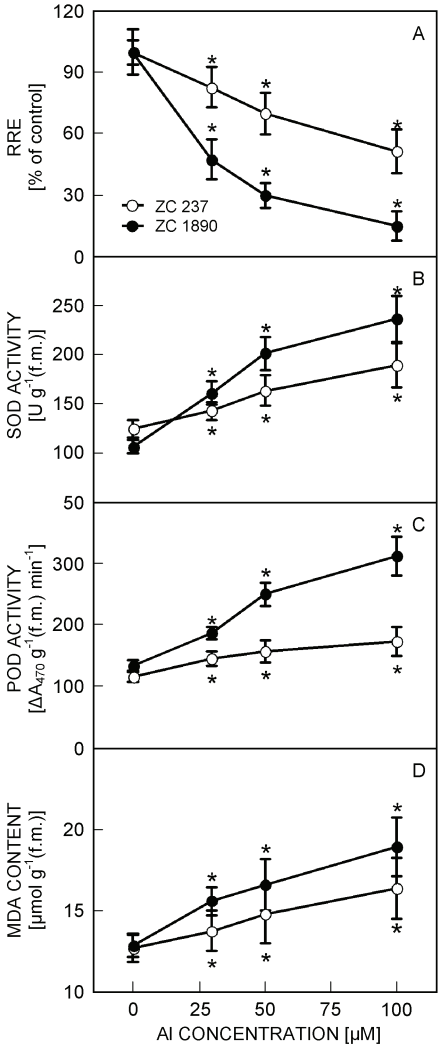

Fig. 1. Effect of different Al concentrations on relative root elongation, RRE (*A*), SOD (*B*) and POD (*C*) activities, and MDA content (*D*) in apical root apices of triticale ZC 237 and ZC 1890. Three-day-old seedlings were exposed to 0.5 mM $CaCl_2$ (pH 4.5) solution containing 0, 30, 50, or 100 μM $AlCl_3$ for 24 h. Root elongation in control treatments: 2.1 ± 0.14 cm (ZC 237), 2.0 ± 0.09 cm (ZC 1890). *Asterisks* represent the significant differences between Al treatment and control at $P = 0.01$.

Discussion

Al-induced oxidative stress and changes in cell wall properties have been suggested as the two major factors leading to Al toxicity (Matsumoto 2000, Yamamoto *et al*. 2003, Zheng and Yang 2005). The data presented in this study indicate that the oxidative stress is a later event of Al toxicity (Fig. 2), and the modification of cell wall composition especially the increment of hemicellulose component is well correlated with the inhibition of root elongation (Fig. 3).

Although Al itself is not a transition metal and cannot catalyze redox reactions, the Al-induced oxidative stress

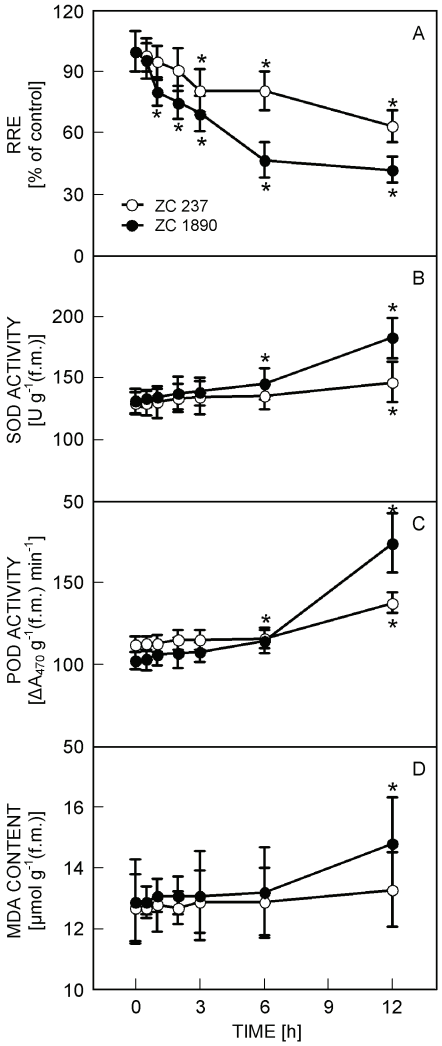

Fig. 2. Effect of treatment duration on relative root elongation, RRE (*A*), SOD (*B*) and POD (*C*) activities, and MDA content (*D*) in root apices of ZC 237 and ZC 1890. Three-day-old seedlings were exposed to 0.5 mM $CaCl_2$ (pH 4.5) solution containing 50 μM Al. Root elongation in control plants at 0.5, 1, 2, 3, 6, or 12 h: 0.05 ± 0.01, 0.10 ± 0.01, 0.18 ± 0.02, 0.27 ± 0.02, 0.52 ± 0.04 or 1.03 ± 0.15 cm (ZC 237), 0.04 ± 0.01, 0.10 ± 0.01, 0.17 ± 0.02, 0.25 ± 0.02, 0.47 ± 0.04 or 0.98± 0.08 cm (ZC 1890). *Asterisks* represent the significant differences between Al treatment and control at $P = 0.01$.

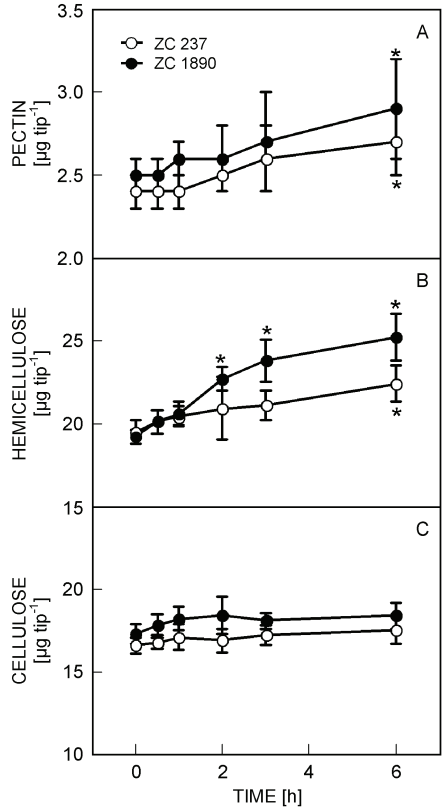

Fig. 3. The amounts of root apices cell wall polysaccharides in pectin (*A*), hemicellulose (*B*) and cellulose (*C*) fractions in ZC 237 and ZC 1890 at different times in the presence or absence of Al. Three-day-old seedlings were exposed to 0.5 mM $CaCl_2$ (pH 4.5) solution containing 50 μM Al. The root apices (0 - 10 mm) were excised for determination cell wall compositions. The amount of cell-wall polysaccharides are expressed as glucose equivalent. *Asterisks* represent the significant differences between Al treatment and control at $P = 0.01$.

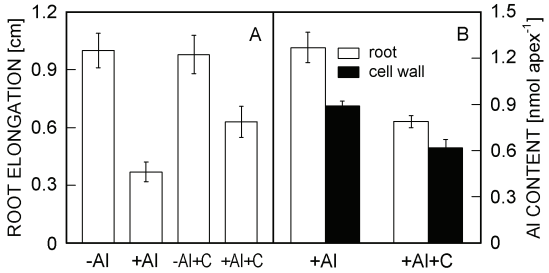

Fig. 4. Effect of citrate desorption on Al-induced inhibition of root elongation, Al content in root apical section and the cell wall of ZC 1890. *A*: 3-d-old seedlings were exposed to 0.5 mM $CaCl_2$ solution without or with 50 μM Al for 3 h, followed by desorption with 1 mM citric acid (pH 4.5) for 30 min, and then the roots were exposed to 0.5 mM $CaCl_2$ (pH 4.5) without Al for a further 9 h. *B*: 3-d-old seedlings were exposed to 0.5 mM $CaCl_2$ solution with 50 μM Al for 3 h, followed by desorption with 1 mM citric acid for 30 min. The Al contents in root apices and cell wall fraction were determined *via* ICP-AES.

has been observed in many plant species (Cakmak and Horst 1991, Yamamoto *et al*. 2001, Kuo and Kao 2003, Šimonovičová *et al*. 2004). Furthermore, it has even been suggested that Al-enhanced oxidative stress is a decisive event for inhibition of cell growth by Al (Yamamoto *et al*. 2002). In the present paper, the oxidative stress triggered by Al was examined in the root apices of Al-resistant and Al-sensitive triticale cultivars, facilitating the comparison of results. The activities of SOD and POD increased dramatically with increasing Al concentration in both cultivars, but the magnitude was higher in ZC 1890 than that in ZC 237 (Fig. 1). MDA is routinely used as an indicator of lipid peroxidation. The MDA content increased significantly at all three Al levels (Fig. 1), verifying the occurrence of lipid peroxidation. This result indicates that Al resulted in the oxidative stress in triticale. However, the time-course experiment showed that the inhibition of root elongation could be detected much earlier than the oxidative stress as indicated by the activities of SOD, POD and MDA content (Fig. 2). Consistently, using pea roots, Yamamoto *et al*. (2001) demonstrated that butylated hydroxyanisole (BHA), a lipophilic antioxidant, decreased lipid peroxidation in pea roots, but did not prevent root growth inhibition by Al stress. Boscolo *et al*. (2003) also reported that the oxidative stress took place only after a sustained inhibition of root growth by Al stress in two inbred lines of maize. Therefore, it is reasonable to conclude that Al-induced oxidative stress was the secondary response of Al toxicity, not the primary cause of root growth inhibition in triticale roots.

Cell wall is the first barrier for any ions to enter the cell, so for the Al. When a root grows in acid soil, the apoplast or cell wall is the first part of a plant to come in contact with Al. Several studies have shown consistently that most of Al is bound to the cell wall (Chang *et al*. 1999, Ma *et al*. 1999, Taylor *et al*. 2000). In the present study, when root elongation was inhibited after 3-h exposure to Al in ZC 1890, 71 % of the total Al was found in the cell wall (Fig. 4), which confirm that cell wall is also the major binding site for Al in triticale. Furthermore, desorption experiments with citrate provided evidence that Al binding in the apoplast or cell wall was the main reason resulted in root growth inhibition since removing of Al bound in apoplast by citrate significantly restored the root regrowth in ZC 1890 (Fig. 4). These results suggested that cell wall plays an important role in the expression of Al toxicity.

The chemical and mechanical properties of the cell wall were reported to be modified by Al because Al binds strongly to the cell wall, thus causing growth inhibition (Tabuchi and Matsumoto 2001, Ma *et al*. 2004). In the present study, the time-course of root elongation and changes in cell-wall polysaccharides at 50 μM Al were investigated. Inhibition of root elongation could be detected 1 h after Al treatment in ZC 1890. Such rapid inhibition by Al was also reported in wheat (Wallace and Anderson 1984, Ryan *et al*. 1992), maize (Llugany *et al*. 1995) and mungbean roots (Blamey *et al*. 2004). Analyses of root cell walls in ZC 1890 revealed that Al-induced changes in the hemicellulose fraction were significant after 2 h, and 6 h for pectin fraction (Fig. 3). It is noteworthy that the Al effect on the amount of cell-wall fractions was not found during the 1st h treatment. This clearly indicates that the early stage of growth inhibition was not explained merely by the amounts of cell-wall fractions or wall components.

References

Blamey, F.P.C., Nishizawa, N.K., Yoshimura, E.: Timing, magnitude, and location of initial soluble aluminum injuries to mungbean roots. - Soil Sci. Plant Nutr. **50**: 67-76, 2004.

Boscolo, P.R.S., Menossi, M., Jorge, R.A.: Aluminum-induced oxidative stress in maize. - Phytochemistry **62**: 181-189, 2003.

Cakmak, I., Horst, W.J.: Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). - Physiol. Plant. **93**: 463-468, 1991.

Chang, Y.C., Yamamoto, Y., Matsumoto, H.: Accumulation of aluminum in the cell wall pectin in cultured tobacco (*Nicotiana tabacum* L.) cells treated with a combination of aluminum and iron. - Plant Cell Environ. **22**: 1009-1017, 1999.

Clarkson, D.T.: Interactions between aluminum and phosphorus on root surfaces and cell wall material. - Plant Soil **27**: 347-356, 1967.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F.: Colorimetric method for determination of sugars and related substances. - Anal. Chem. **28**: 350-356, 1956.

Ezaki, B., Gardner, R.C., Ezaki, Y., Matsumoto, H.: Expression of aluminum-induced genes in transgenic *Arabidopsis* plants can ameliorate aluminum stress and/or oxidative stress. - Plant Physiol. **122**: 657-665, 2000.

Horst, W.J.: The role of the apoplast in aluminium toxicity and distance of higher plants: A review. - Z. Pflanzenernähr. Bodenk. **158**: 419-428, 1995.

Kochian, L.V.: Cellular mechanisms of aluminum toxicity and resistance in plants. - Annu. Rev. Plant Physiol. Plant mol. Biol. **46**: 237-260, 1995.

Kuo, M.C., Kao C.H.: Aluminum effects on lipid peroxidation and antioxidative enzyme activities in rice leaves. - Biol. Plant. **46**: 149-152, 2003.

Le Van, H., Kuraishi, S., Sakurai, N.: Aluminum-induced rapid root inhibition and changes in cell-wall components of squash seedlings. - Plant Physiol. **106**: 971-976, 1994.

Llugany, M., Poschenrieder, C., Barceló, J.: Monitoring the aluminium-induced inhibition of root elongation in four maize cultivars differing in tolerance to aluminium and proton toxicity. - Physiol. Plant. **93**: 265-271, 1995.

Ma, J.F., Shen, R.F., Nagao, S., Tanimoto, E.: Aluminum targets elongating cells by reducing cell wall extensibility in wheat roots. - Plant Cell Physiol. **45**: 583-589, 2004.

Ma, J.F., Yamamoto, R., Nevins, D.J., Matsumoto, H., Brown, P.H.: Al binding in the epidermis cell wall inhibits cell

elongation of okra hypocotyl. - Plant Cell Physiol. **40**: 549-556, 1999.

Matsumoto, H.: Cell biology of aluminium toxicity and tolerance in higher plants. - Int. Rev. Cytol. **200**: 1-46, 2000.

Nishitani, K., Masuda, Y.: Growth and cell wall changes in azuki bean epicotyls. I. Changes in wall polysaccharides during intact growth. - Plant Cell Physiol. **20**: 63-74, 1979.

Oteiza, P.L.: A mechanism for the stimulatory effect of aluminum on iron-induced lipid peroxidation. - Arch. Biochem. Biophys. **308**: 374-379, 1994.

Richards, K.D., Schott, E.J., Sharma, Y.K., Davis, K.R., Gardner, R.C.: Aluminum induces oxidative stress genes in *Arabidopsis thaliana*. - Plant Physiol. **116**: 409-418, 1998.

Ryan, P.R., Shaff, J.E., Kochian, L.V.: Aluminum toxicity in roots: correlation among ionic currents, ion fluxes and root elongation in aluminum-sensitive and aluminum-tolerant wheat cultivars. - Plant Physiol. **99**: 1193-1200, 1992.

Šimonovičová, M., Tamás, L., Huttová, J., Mistrík I.: Effect of aluminium on oxidative stress related enzymes activities in barley roots. - Biol. Plant. **48**: 261-266, 2004.

Tabuchi, A., Matsumoto, H.: Changes in cell-wall properties of wheat (*Triticum aestivum* L.) roots during aluminum-induced growth inhibition. - Physiol. Plant. **112**: 353-358, 2001.

Tamás, L., Huttová, J., Mistrík, I.: Inhibition of Al-induced root elongation and enhancement of Al-induced peroxidase activity in Al-sensitive and Al-resistant barley cultivars are positively correlated. - Plant Soil **250**: 193-200, 2003.

Taylor, G.J., McDonald-Stephens, J.L., Hunter, D.B., Bertsch, P.M., Elmore, D., Rengel, Z., Reid, R.J.: Direct measurement of aluminum uptake and distribution in single cells of *Chara corallina*. - Plant Physiol. **123**: 987-996, 2000.

Wallace, S.U., Anderson, I.C.: Aluminum toxicity and DNA synthesis in wheat roots. - Agron. J. **76**: 5-8, 1984.

Yamamoto, Y., Kobayashi, Y., Devi, S.R., Rikiishi, S., Matsumoto, H.: Aluminum toxicity is associated with mitochondrial dysfunction and the production of reactive oxygen species in plant cells. - Plant Physiol. **128**: 63-72, 2002.

Yamamoto, Y., Kobayashi, Y., Devi, S.R., Rikiishi, S., Matsumoto, H.: Oxidative stress triggered by aluminum in plant roots. - Plant Soil **255**: 239-243, 2003.

Yamamoto, Y., Kobayashi, Y., Matsumoto, H.: Lipid peroxidation is an early symptom triggered by aluminum, but not the primary cause of elongation inhibition in pea roots. - Plant Physiol. **125**: 199-208, 2001.

Yang, J.L., Zheng, S.J., He, Y.F., Tang, C.X., Zhou, G.D.: Genotypic differences among plant species in response to aluminum stress. - J. Plant Nutr. **28**: 949-961, 2005.

Zheng, S.J., Yang, J.L.: Target sites of aluminum phytoxicity. - Biol. Plant. **49**: 321-331, 2005.