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Quantitative changes in maize membrane proteins induced by aluminium

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Abstract

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was used for monitoring Al-induced changes in polypeptide composition of membrane proteins isolated from 3-d-old maize seedlings subjected to aluminium stress. Analysis of peripheral membrane proteins isolated from maize root showed an Al-induced increase in accumulation of 14 polypeptides with apparent molecular mass from 10 to 135 kDa. Qualitative differences were found between peripheral membrane proteins isolated from root tip (increased accumulation of 4 polypeptides with M_r 42 000 - 135 000) and from root base (increased accumulation of 10 polypeptides with M_r 10 000 - 59 000). On the other hand, no Al-induced changes were observed in peripheral membrane proteins isolated from maize coleoptile and integral membrane proteins isolated either from root or coleoptile. These results indicate that peripheral membrane proteins undergo considerable changes during 24-h Al treatment while integral membrane proteins pattern is stable.

Additional key words: coleoptile, polypeptide patterns, root, stress proteins, *Zea mays*.

Introduction

Aluminium toxicity is a serious problem in acid soils. At soil pH mildly acidic or neutral, Al is primarily in the form of insoluble aluminosilicates or oxides. However, as soils become more acidic, Al^{3+} is released into the soil solution (Rao *et al.* 1993). Due to the fact that aluminium is the third most abundant element in the earth's crust, comprising about 7 % of its mass, potential for soils to be Al toxic is considerable.

The major symptom of Al toxicity is a rapid inhibition of root growth which can occur within 1 - 2 h after exposure to Al (Ryan *et al.* 1992). Other visible symptoms of Al toxicity include swelling of the root tip, and sloughing off of the epidermis (Delhaize and Ryan 1995, Budíková *et al.* 1997). Root lesions caused by Al can cause disruption of membrane structure and function (Stass and Horst 1995), membrane potential depolarization (Olivetti *et al.* 1995), alteration of ion fluxes (Miyasaka *et al.* 1989), as well as changes in synthesis of organic acids (Pellet *et al.* 1996), polysaccharides and proteins (Campbell *et al.* 1994). Although a wide range of Al-related changes have been identified in plants it is still unclear which of them are primary responses to Al exposure (Rengel 1996).

Several recent studies have shown Al induced synthesis and accumulation of different proteins (Basu *et al.* 1994, Richards and Gardner 1994). The isolation and characterization of seven genes induced by Al has been reported during screening of wheat cultivars (Al-resistant cv. Waalt and Al-sensitive cv. Warigal) by Snowden and Gardner (1993) and Richards *et al.* (1994). Six of these genes showed an Al-induced increase in transcript levels in both Al-sensitive and Al-resistant wheat cultivars, while the induction of the seventh gene, *wali2* occurred only in Al-sensitive cv. Warigal. Some of these genes encode plant metallothionein-like protein (*wali1*), enzymes such phenylalanine ammonia-lyase (*wali4*), proteinase inhibitor (*wali3* and *wali5*), function of *wali2* and *wali7* is still unknown. Due to the fact that six of these genes were induced in both Al-resistant and Al-sensitive genotypes it seems that they are involved in responses to Al stress and not in Al tolerance. Increased transcript levels of some of these genes (*e.g. wali1, wali3, wali4,* and *wali5*) after 2-d treatment with other metals like Cd, Zn, Cu, Ga, and by wounding (Snowden

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Abbreviations: BHT - butylated hydroxytoluene; BSA - bovine serum albumine; DTT - dithiothreitol; EDTA - ethylenediamine tetraacetic acid; PVP - polyvinylpyrrolidone.

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et al. 1995) confirm the prevalent view that these genes probably represent a part of a suite of stress genes that are induced not only by Al treatment but also by treatment with other toxic metals, wounding, UV-radiation, and plant pathogens (Cruz-Ortega and Ownby 1993, Didierjean *et al.* 1996, Tamás *et al.* 1997).

In our previous study (Huttová *et al.* 1998), we described considerable changes in polypeptides composition of cytoplasmic proteins isolated from maize root and coleoptile after 24 h exposure of maize seedlings to 100 μM Al. The most pronounced changes were found in cytoplasmic proteins isolated from maize coleoptile, where Al increased accumulation of polypeptides with molecular mass of 14, 17.5, 20, 24.5, 28, 30, and 37.5 kDa. In cytoplasmic proteins isolated from maize root Al treatment induced accumulation of only two polypeptides (16 and 22 kDa), and simultaneously reduction of three polypeptides. Because no correlation was found between sensitivity of analysed plant parts and the extent of changes in polypeptide composition of cytoplasmic proteins we concluded that these changes are part of more general plant responses to environmental stresses, than in playing a role in Al tolerance.

The present study was focused on characterization of Al induced changes in membrane proteins isolated from maize seedling root tip, mature region of root, and coleoptile. Changes in composition of peripheral and integral membrane proteins isolated from microsomal fraction of different parts of plants grown 24 h in the presence of 100 μM Al are compared with the sensitivity of these plant parts to Al and with the changes induced by Al in the fraction of cytoplasmic proteins.

Materials and methods

Maize (*Zea mays* L. cv. TO 360) caryopses after surface sterilization with 12 % H_2O_2 were germinated in dark at temperature of 25 °C and relative humidity of 98 %. Two-day-old maize seedlings with 0.5 cm long primary seminal roots were planted on the surface of the 0.3 % agar solid medium containing 1 mmol dm^{-3} $CaCl_2$ (control plants) and 100 μmol dm^{-3} $AlCl_3$ with 1 mmol dm^{-3} $CaCl_2$ (Al-treated plants), pH 4.5, in 5 dm^3 glass jars. The jars were incubated in a growth chamber (*E 8, Conviron*, Winnipeg, Canada) at 25 °C, 65 % RH, and irradiance of 150 - 200 μmol m^{-2} s^{-1} for 12-h photoperiod. After 24 h treatment, the terminal 1 cm root segment and 2 cm long segment from root base, and coleoptiles were harvested and frozen immediately in liquid nitrogen. Samples were ground to a fine powder in a cold mortar in liquid nitrogen and the resulting powder was rehomogenized in extraction buffer (50 mmol dm^{-3} Tris buffer, pH 8.0, 5 mmol dm^{-3} EDTA, 1 mmol dm^{-3} DTT, 3 % PVP) with homogenizator (*DIAX 900, Heidolph GmbH*, Keilheim, Germany). The homogenate was centrifuged at 1 500 *g* for 10 min and 14 000 *g* for 10 min. The microsomal membrane fraction was isolated from resulting supernatant by ultracentrifugation at 150 000 *g* for 30 min (*L8-M, Beckman Instruments*, Palo Alto, USA). The supernatant was removed and pellet was washed with 10 mmol dm^{-3} Tris-maleate buffer, pH 7.3 containing 0.15 mol dm^{-3} NaCl, 1 mmol dm^{-3} EDTA, and 10 mg dm^{-3} BHT (Yoshida 1984) and centrifuged at 150 000 *g* for 30 min to obtain peripheral membrane proteins (supernatant) and integral membrane proteins (pellet). The integral membrane proteins were fractionated by temperature-induced phase separation in *Triton X-114* (Pryde and Phillips 1986) and peripheral membrane proteins after buffer exchange were fractionated using anion exchange column (*Bio-Scale Q2, BIO RAD*, Hercules, USA) equilibrated with 25 mmol dm^{-3} Tris buffer, pH 8.0. The adsorbed proteins were eluted with a linear 0 - 1.0 mol dm^{-3} NaCl gradient in the same buffer. Fractions eluted from the column were precipitated overnight at -20 °C with 4 volumes of ice cold acetone. Proteins were solubilized and separated under denaturing conditions on 10 - 20 % gradient polyacrylamide slab gels using the discontinuous buffer system (Laemmli 1970) and silver stained (Heukesloven and Dernick 1985). Protein concentrations were determined by the slightly modified method of Lowry *et al.* (1951) with bovine serum albumin as the standard. The apparent molecular masses of polypeptides were calculated based on the mobilities of protein standards obtained from *Serva* (Heidelberg, Germany) with gel documentation system (*UVP 5000*, England).

Results

SDS-PAGE analysis of membrane proteins isolated from microsomal fraction of maize root tip showed different effect of Al on polypeptide composition of integral and peripheral membrane proteins. Although no qualitative changes were induced by Al in both of membrane protein fractions, the fraction of peripheral membrane proteins (Figs. 1, 2) was characterized by Al-induced accumulation of polypeptides, while fraction of integral membrane proteins (Figs. 4, 5) by reduction of the quantity of some polypeptides. Surprisingly no Al-induced changes were

found in polypeptide composition of membrane protein fractions isolated from maize coleoptile (Figs. 3 and 5).

In the fraction of peripheral membrane proteins isolated from root tip Al induced accumulation of

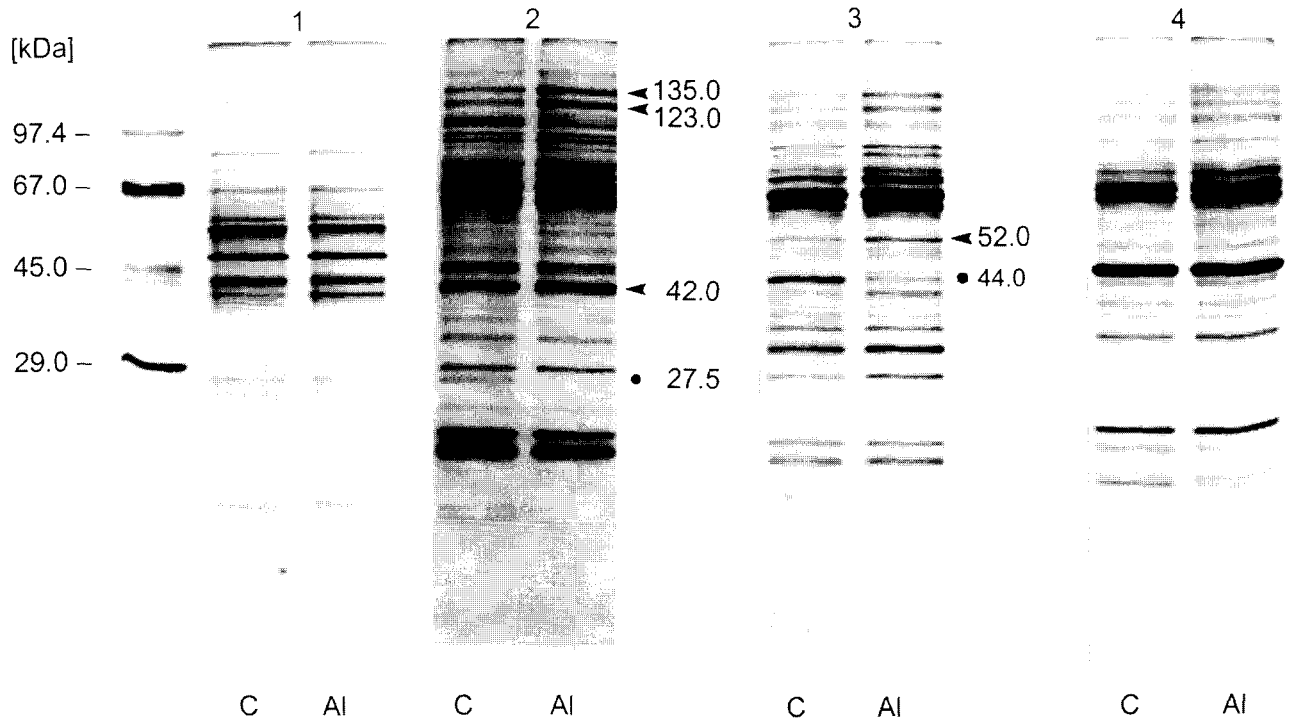

Fig. 1. SDS-PAGE analysis of peripheral membrane proteins isolated from root tip of maize seedlings grown for 24 h in the presence (Al) and absence (C) of 100 μmol dm^{-3} $AlCl_3$. The peripheral membrane proteins were fractionated using an anion exchange column *Bio-Scale Q2* and eluted with linear 0 - 1.0 mol dm^{-3} NaCl gradient (lane *1 - 4*). The molecular mass of marker proteins are indicated on the left. *Arrowheads* indicate induced, and *dots* reduced polypeptides.

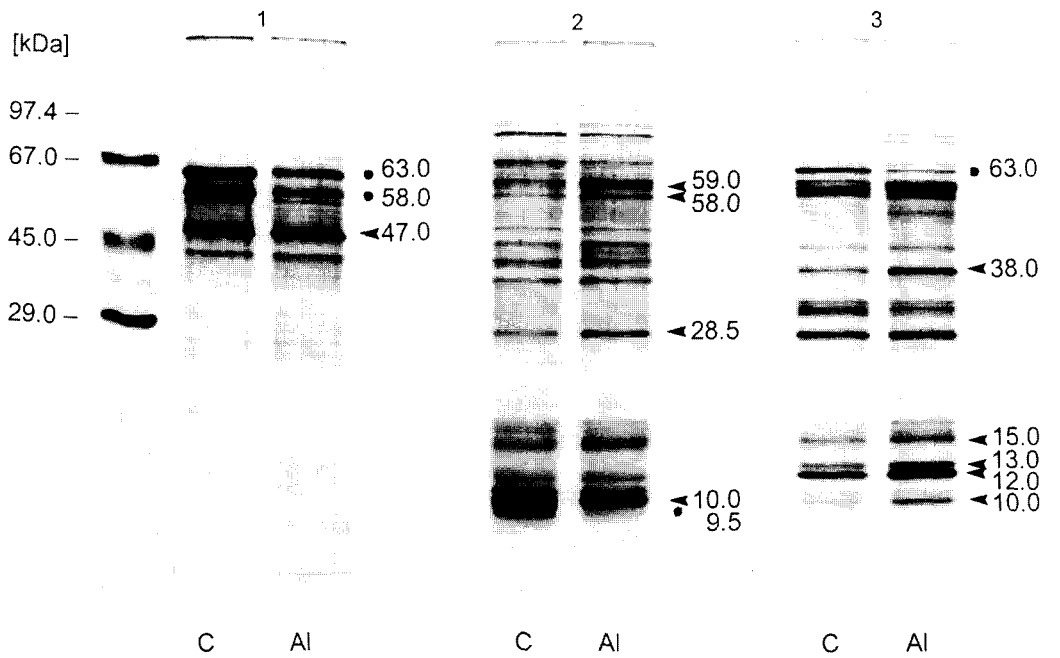

Fig. 2. SDS-PAGE analysis of peripheral membrane proteins isolated from mature zone of root of maize control and Al-treated maize seedlings. See Fig. 1 for details.

4 polypeptides with molecular mass of 135, 123 and 42 kDa (Fig. 1, line *2*) and 52 kDa (Fig. 1, line *3*). The presence of two polypeptides with molecular mass 44 and 27.5 kDa was in the same time reduced in line *2* and *3* (Fig. 1). Most pronounced Al-induced changes were found in the fraction of membrane proteins

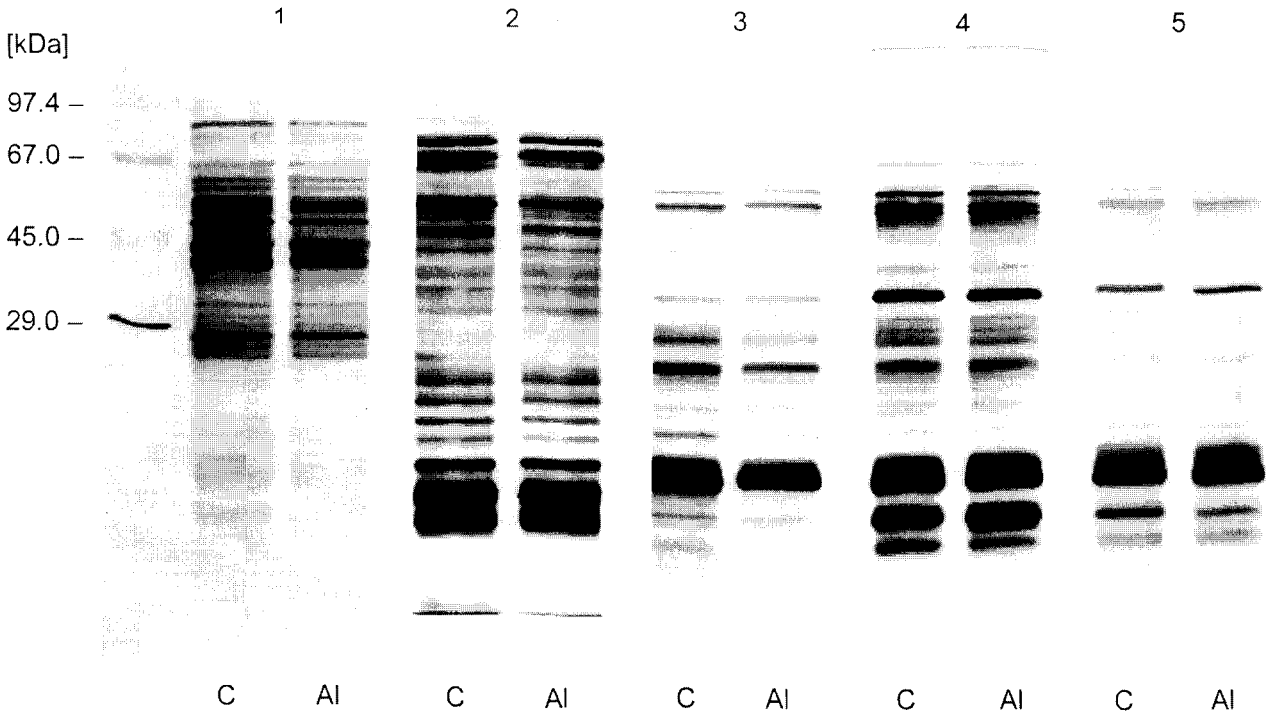

Fig. 3. SDS-PAGE analysis of peripheral membrane proteins isolated from coleoptile of control and Al-treated maize seedlings. See Fig. 1 for details.

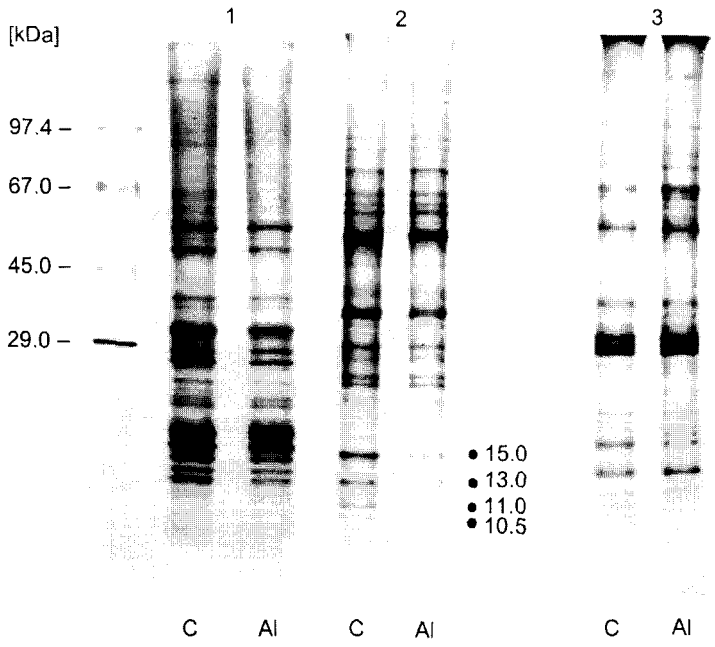

Fig. 4. SDS-PAGE analysis of integral membrane proteins isolated from root tip of control and Al-treated maize seedlings. The integral membrane proteins were fractionated by temperature-induced phase separation in *Triton X-114* (lane *1* - *3*). See Fig. 1 for details.

isolated from segments of the root base (Fig. 2). In the three fractions of peripheral membrane proteins (Fig. 2, lines *1* - *3*) which we obtained after separation on anion exchange chromatography column, accumulation of ten polypeptides with molecular mass of 10, 12, 13, 15, 28.5, 38, 47, 58 and 59 kDa was observed. In less extent reduction of 9.5, 58 and 63 kDa polypeptides followed the Al-induced changes in these three fractions of peripheral membrane proteins. Polypeptide composition of peripheral membrane protein fractions (Fig. 3) as well as integral membrane fractions (Fig. 5) isolated from maize coleoptile showed no differences between Al-treated and control maize seedlings.

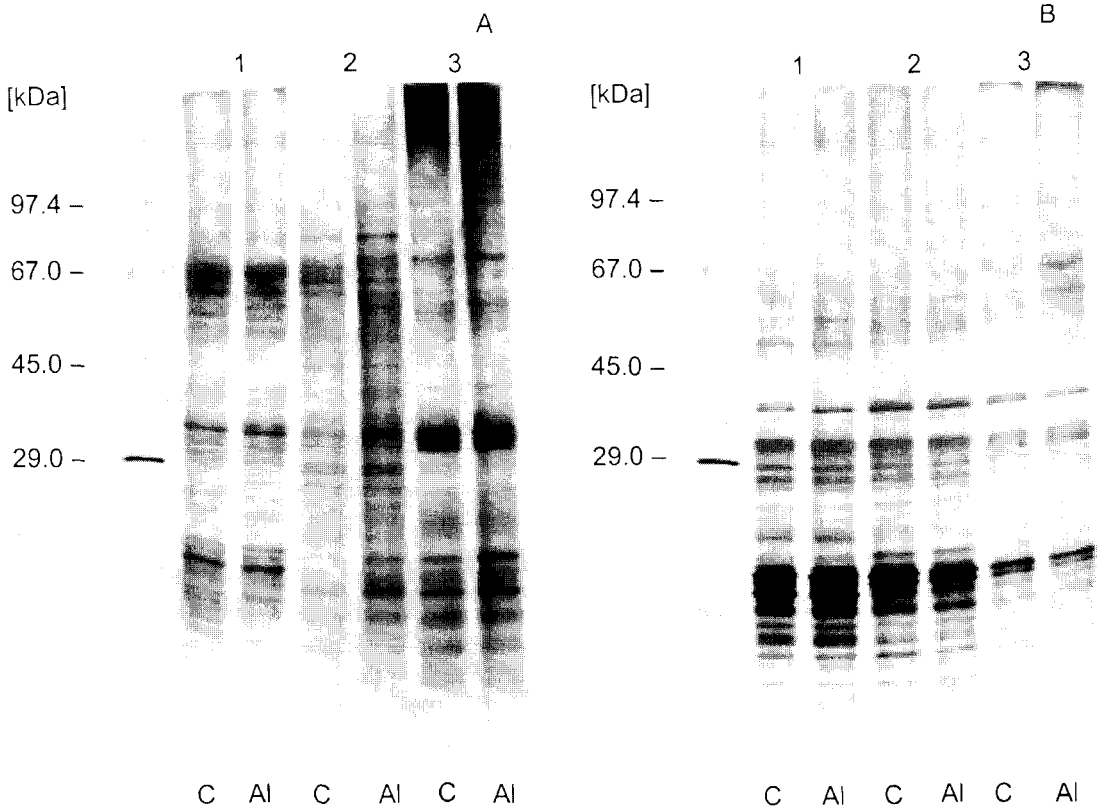

Fig. 5. SDS-PAGE analysis of integral membrane proteins isolated from root base (*A*) and from coleoptile (*B*) of control and Al-treated maize seedlings. See Figs. 1 and 4 for details.

Discussion

Recent evidence indicates that Al triggers changes at the transcript levels of numerous genes. The time course of gene induction by Al treatment varies in time; as described by Richards *et al.* (1998), some of genes show only transient induction in the first 2 h of Al-treatment, and some show a longer induction period for at least 48 h. Consistently with Snowden and Gardner (1993) and Richards and Gardner (1994), some of Al-induced genes in seedlings of *Arabidopsis thaliana* belong to the family of genes induced by different kinds of stresses including heavy metals, Al stress, oxidative stress, and pathogen treatments (Richards *et al.* 1998). Other general phenomenon is that the sensitivity of individual organs as well as sensitivity of individual cells in the same organ differs markedly which results in changes in gene expression. In maize the most sensitive part of the plant is the root apex (Mistrík *et al.* 1997), nevertheless, we found the most pronounced changes induced by Al in cytoplasmic proteins isolated from maize coleoptile (Huttová *et al.* 1998). In the root apex Al-induced increased accumulation of only one polypeptide with apparent molecular mass of 16 kDa, and 14 kDa polypeptide was found to be accumulated in the root base tissues. In general Al-induced changes in cytoplasmic fraction of maize root proteins represented rather inhibition than increased accumulation of individual polypeptides. Similar results were described by Basu *et al.* (1994) in cytoplasmic protein pattern isolated from roots of Al-sensitive and Al-resistant wheat cultivars. Al stress had no effect on polypeptide pattern of cytoplasmic proteins and only one membrane protein with an apparent molecular mass of 51 kDa was significantly accumulated in the Al-resistant wheat cultivar. This accumulation was significantly greater in root tips when compared to the

rest of the root.

Quite different results were found in our experiments with maize plants. Exposure of maize seedlings to 24-h Al stress induced marked changes in the root fraction of membrane proteins especially in the peripheral membrane proteins. Regardless of the origin of root segments, Al induced an increased accumulation of 14 peripheral membrane proteins in root, while in coleoptile neither peripheral nor integral proteins pattern was changed by Al treatment. Due to the fact that our experiments were only performed on maize cultivar with high sensitivity to Al we can not explain these changes with respect to Al-resistance.

Similar results have been described in cereal crops and alfalfa (Snowden *et al.* 1995). In Al-tolerant alfalfa (*Medicago sativa* L.) plants, Campbell *et al.* (1994) observed synthesis of low molecular mass proteins which probably play a role in intracellular binding of Al, and thus allow plants to make biochemical and structural adjustments which enable them to cope with Al-toxicity. Protective roles of two aluminium induced genes in Al and oxidative stresses were described in *Saccharomyces cerevisiae* by Ezaki *et al.* (1998), and in young plants of *Arabidopsis thaliana* by Richards *et al.* (1998). The most of the expressed genes encoded enzymes involved in reactions suppressing oxidative stress. These results confirm the view that Al ions attack primarily cell membrane phospholipids and membrane proteins by inducing oxidative stress and by enhanced peroxidation causes damage of cell membranes in both animal and plant cells (Cakmak and Horst 1991). The question if the Al-induced inhibition of root elongation is the consequence of secondary oxidative stress remains open due to the observed 2-h lag phase before any significant change in expression of oxidative-stress related genes, while cell division and elongation stops under Al stress within minutes (Delhaize and Ryan 1995).

Based on our results, we can conclude that Al-treatment of maize seedlings induces quantitatively different changes in cytoplasmic and membrane bound proteins isolated from roots and coleoptiles. The slight changes, representing the accumulation of two, and reduction of five polypeptides observed in the fraction of cytoplasmic proteins isolated from maize roots (Huttová *et al.* 1998) does not correspond with the accumulation of 14 and reduction of 6 polypeptides in the fraction of peripheral membrane proteins during 24 h of Al stress. On the other hand, the most pronounced changes in cytoplasmic proteins found in coleoptile are in great contradiction to the none of changes observed in coleoptile peripheral membrane proteins. Comparing the changes in cytoplasmic and membrane proteins pattern it seems that the extent of changes induced by Al in roots in peripheral membrane proteins is consistent with the sensitivity of roots to Al. The cytoplasmic fraction of proteins showed the most pronounced changes in proteins isolated from coleoptile where no visible injury or inhibition of growth was induced by Al. The analysis of polypeptide patterns of integral membrane proteins regardless of the analysed root parts or coleoptile showed a considerable stability to Al stress.

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