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Quantitative changes in maize cytoplasmic proteins induced by aluminium

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Abstract

Three-day-old maize seedlings were subjected to 100 μM $AlCl_3$ for 24 h. Cytoplasmic proteins were isolated from root tips, root base and from coleoptiles. After fractionation of cytoplasmic proteins on anion chromatography column *Bio-Scale Q2* sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis was used to monitor Al-induced changes in polypeptide composition of particular fractions. Four (root) and 7 (coleoptile) fractions were eluted from the column with linear 0 - 1.0 M NaCl gradient. In fraction 1 of cytoplasmic proteins from root tips Al induced accumulation of polypeptide with molecular mass of 16 kD and simultaneous reduction of two polypeptides (67.5 and 60 kD). In fraction 1 isolated from mature zone of maize roots Al-induced accumulation of 22 kD polypeptide and reduction of 67.5, 60, and 14 kD polypeptides. Most pronounced changes were revealed in coleoptile. In three protein fractions increased accumulation of polypeptides with molecular mass of 14, 17.5, 20, 24.5, 28, 30, and 37.5 kD were observed. In the remaining three root or four coleoptile fractions of cytoplasmic proteins, no differences were found between Al-treated and control maize seedlings.

Additional key words: Al-induced stress, coleoptile, polypeptide patterns, root, *Zea mays* L.

Introduction

In many acidic soils, aluminium ion (Al^{3+}) is a major factor limiting plant growth and yield of crops. 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. Generally, some symptoms of the Al toxicity appear after a short-term exposure

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

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(measurable within minutes, *e.g.*, changes in plasmalemma characteristics, synthesis of callose, *etc.*) followed by the long-term responses (hours or days). A very sensitive response of plants to Al is the inhibition of root elongation which occurs in Al-sensitive wheat cultivars after less than 2 h of Al supply (Ryan *et al.* 1992). The physiological reasons for this inhibition are not yet fully understood (Horst 1995). 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). Al can induce disruption of membrane structure and function (Stass and Horst 1995), plasma membrane depolarization (Olivetti *et al.* 1995), alteration of ion fluxes (Miyasaka *et al.* 1989), disruption of DNA synthesis and mitosis (Horst and Klotz 1990) as well as changes in synthesis of organic acids (Pellet *et al.* 1996), polysaccharides and proteins (Campbell *et al.* 1994). Recent evidence indicates that Al triggers changes at the transcript levels of numerous genes coding proteins thought to have protective functions against damage caused by this toxic element. The isolation and characterization of seven genes induced by Al has been reported by Snowden and Gardner (1993) and Richards and Gardner (1994). Six of these genes show an Al-induced increase in transcript levels in two different wheat cultivars with different sensitivity to Al, while the induction of the seventh gene (*wali2*) occurred only in Al-sensitive cultivar. Increased transcript levels of some of these genes (*e.g. wali1, wali3, wali4,* and *wali5*) have been observed after 2-d treatment with other metals like Cd, Zn, Cu, Ga, and by wounding (Snowden *et al.* 1995). It means that *wali* genes previously identified in wheat root tips as a response to Al are not specific to Al stress. They probably represent a part of a suite of stress genes that are induced not only by Al treatment but also by treatment with toxic metals, wounding and plant pathogens (Cruz-Ortega and Ownby 1993, Diderjean *et al.* 1996, Tamás *et al.* 1997).

In our previous study (Tamás *et al.* 1997) we described accumulation of identical extracellular proteins in the apoplast of barley leaves after both inoculation with powdery mildew and heavy metal treatments including Al. Because the Al induced changes in protein pattern were very similar to those in powdery mildew inoculated leaves it seems that accumulation of these proteins belong to the category of non-specific stress responses. The present study was undertaken to characterise Al-induced changes in cytoplasmic protein composition isolated from three parts of maize seedlings differing in their sensitivity to Al: root tips, representing the most sensitive part of the roots, and less sensitive mature region of roots with no visible injury after 24 h of Al-treatment, or coleoptile growth of which was unaffected by the concentration of Al used. Relationship between protein pattern changes of the analysed plant parts with structural changes induced by Al in the same maize cultivar are discussed.

Materials and methods

Maize seeds (*Zea mays* L. cv. TO 360), after surface sterilization with 12 % H_2O_2 were germinated in dark at temperature of 25 °C and relative humidity of 98 %. Two-d-old maize seedlings with 0.5 cm long primary seminal roots were planted on

the periphery of the 0.3 % agar solid medium containing 1 mM $CaCl_2$ (control plants) and 100 μM $AlCl_3$ (Al-treated plants), at pH 4.5, in 5 dm^3 glass jars. The jars were incubated in a growth chamber (*E8, Controlled Environment Ltd.*, Winnipeg, Canada) at temperature of 25 °C, relative humidity of 65 % RH and irradiance of 150 - 200 μmol(PAR) 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 mM Tris buffer, pH 8.0; 5 mM EDTA; 1 mM DTT; 3 % PVP) with homogenizator (*DIAX 900, Heidolph GmbH*, Keilheim, Germany). The homogenate was centrifuged at 1 500 *g* for 10 min and resulting supernatant re-centrifuged at 14 000 *g* for 10 min. The cytoplasmic protein fraction was isolated from resulting supernatant by ultracentrifugation at 150 000 *g* for 30 min (*L8-70M, Beckman Instruments*, Palo Alto, USA). The supernatant was regarded as the cytoplasmic fraction. After passing through *Sephadex G-25* proteins were fractionated using anion exchange column (*Bio-Scale Q2* from *Bio Rad Laboratories*, Hercules, USA) equilibrated with 25 mM Tris buffer, pH 8.0. The adsorbed proteins were eluted with a linear 0 - 1.0 M NaCl gradient in the same buffer. Fractions eluted from the column were precipitated overnight at -20 °C with 2 volumes of ice cold acetone. Proteins were solubilized and separated under denaturing condition on 12 % polyacrylamide slab gels using the discontinuous buffer system (Laemmli 1970), and silver stained (Heukesloven and Dernick 1985). Protein concentrations were determined by slightly modified method of Lowry *et al.* (1951), with BSA 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*).

Results and discussion

Recent evidence indicates that Al triggers changes at the transcript level of numerous genes encoding proteins thought to have protective functions against damage caused by toxic elements. In our experiments we analysed the polypeptide composition of cytoplasmic proteins isolated from two parts of maize primary seminal roots with different sensitivity to Al (Figs. 1 and 2). Four fractions of proteins were isolated after separation of protein extracts on anion exchange chromatography column. Three of them, lanes 2, 3, and 4 showed no changes of polypeptide composition induced by Al. Small but distinct changes were observed in root tip cytoplasmic proteins in fraction 1 (Fig. 1, lane 1). Al induced an enhanced accumulation of polypeptide with molecular mass of 16 kD and reduced the amount of 60, and 67.5 kD polypeptides. Reduction of the same two polypeptides with molecular mass of 60 and 67.5 kD was also observed in cytoplasmic protein fraction isolated from the root base (Fig. 2, lane 1). In contrast to polypeptide composition in the lane 1 of root tip cytoplasmic proteins Al enhanced accumulation of 22 kD polypeptide and reduced the amount of 14 kD polypeptide in root base.

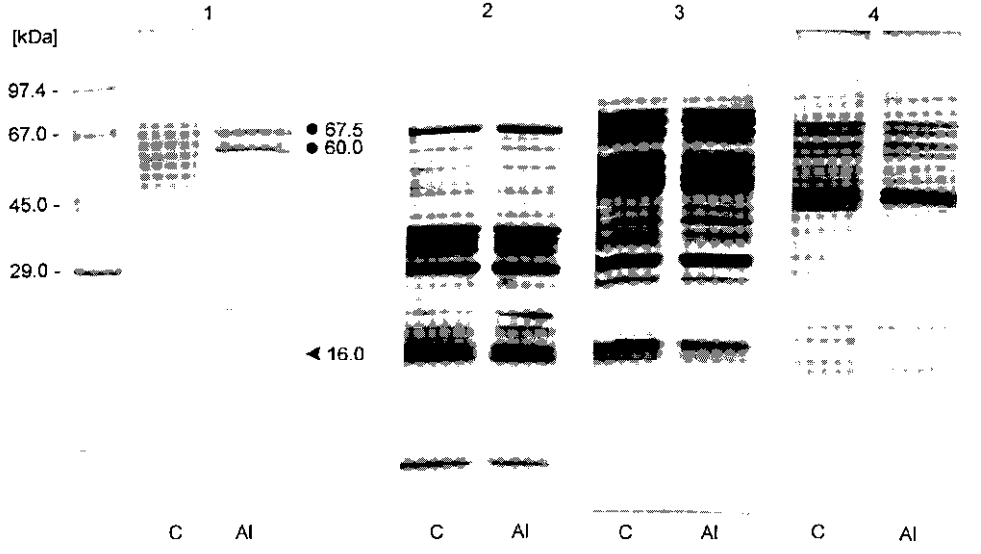

Fig. 1. SDS-PAGE analysis of cytoplasmic proteins isolated from root tips of maize seedlings grown for 24 h in the presence (Al) and absence (C) of 100 μM $AlCl_3$. The cytoplasmic proteins were fractionated using an anion exchange column *Bio-Scale Q2* and eluted with linear 0 - 1.0 M NaCl gradient (lanes 1 - 4). The molecular mass [kD] of marker proteins are indicated on the left. *Arrowheads* indicate induced, and *dots* reduced polypeptides.

Similar changes in polypeptide patterns of proteins isolated from root tips and mature parts of wheat roots have also been described by Basu *et al.* (1994a). Accumulation of the polypeptide of microsomal fraction of roots with molecular mass of 51 kD, occurred in 5 mm long root tips within 24 h of exposure of wheat plants to Al, and was significantly lower in the rest of root. Working with the same species, Rincon and Gonzales (1991) showed that several high, intermediate, and low molecular mass poplypeptides are induced in the root tips of an Al-sensitive and an intermediate cultivar after exposure to Al. In accordance with the prevailing view that plants perceive Al stress in the apical region of root system (Ryan *et al.* 1993), these polypeptides are strategically located in root tips to play a role in Al resistance.

Similar results have been described in cereal crops and alfalfa (Snowden *et al.* 1995). In Al-tolerant alfalfa 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. Ownby and Hruschka (1991) noted three cytoplasmic proteins (27, 33, and 55 kD) which were induced or enhanced only in Al-tolerant winter wheat cultivar, and hypothesized that they may be directly involved in Al-tolerance in wheat. One protein with molecular mass of 18.6 kD that was enhanced 50-fold in both sensitive and tolerant wheat cultivar by Al treatment was

very similar to 18.7 kD protein produced in Al-tolerant alfalfa clones but not detected in the sensitive clones.

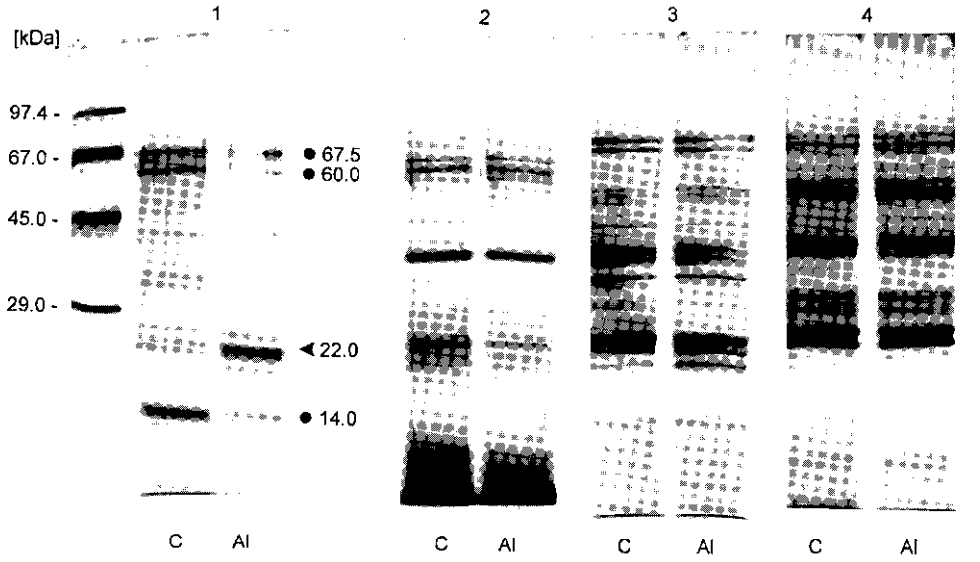

Fig. 2. SDS-PAGE analysis of cytoplasmic proteins isolated from mature zone of root of maize seedlings grown for 24 h in the presence (Al) and absence (C) of 100 μM $AlCl_3$.

In our experiments we also analysed the effect of Al on polypeptide composition of cytoplasmic proteins isolated from maize coleoptile (Fig. 3). In contrast to 4 fractions of root cytoplasmic proteins we got 7 fractions after the separation of cytoplasmic protein extract from coleoptile on anion exchange column. Al treatment induced quantitative changes in three fractions. In fractions 1, 2, and 4 (Fig. 3, lanes 1, 2, 4) increased accumulation of polypeptides with molecular masses 14, 17.5, 20, 24.5, 28, 30, and 37.5 kD was observed. No changes in polypeptide composition were observed in fractions 3, 5, 6 and 7 (Fig. 3, lanes 3, 5, 6, 7). In comparison with polypeptide patterns of root cytoplasmic proteins only enhancement of the amount of 7 polypeptides occurred in coleoptiles while decrease of polypeptides dominated in root cytoplasmic proteins.

The different behaviour of the root and leaf cytoplasmic protein composition under Al treatment might be the result of different concentrations of Al in the root and leaf tissues. It is well known that the primary site of Al influx into the root cells is the apical part of the root (Delhaize *et al.* 1993). In intact wheat roots, Samuels *et al.* (1997) have determined 3-times higher accumulation of Al in meristematic 2-mm root tip of sensitive wheat cultivar than in the more mature 5- to 15-mm root region. This accumulation of Al in root tips is accompanied by structural changes in particular root cells and tissues. The light and electron microscopy of the same maize

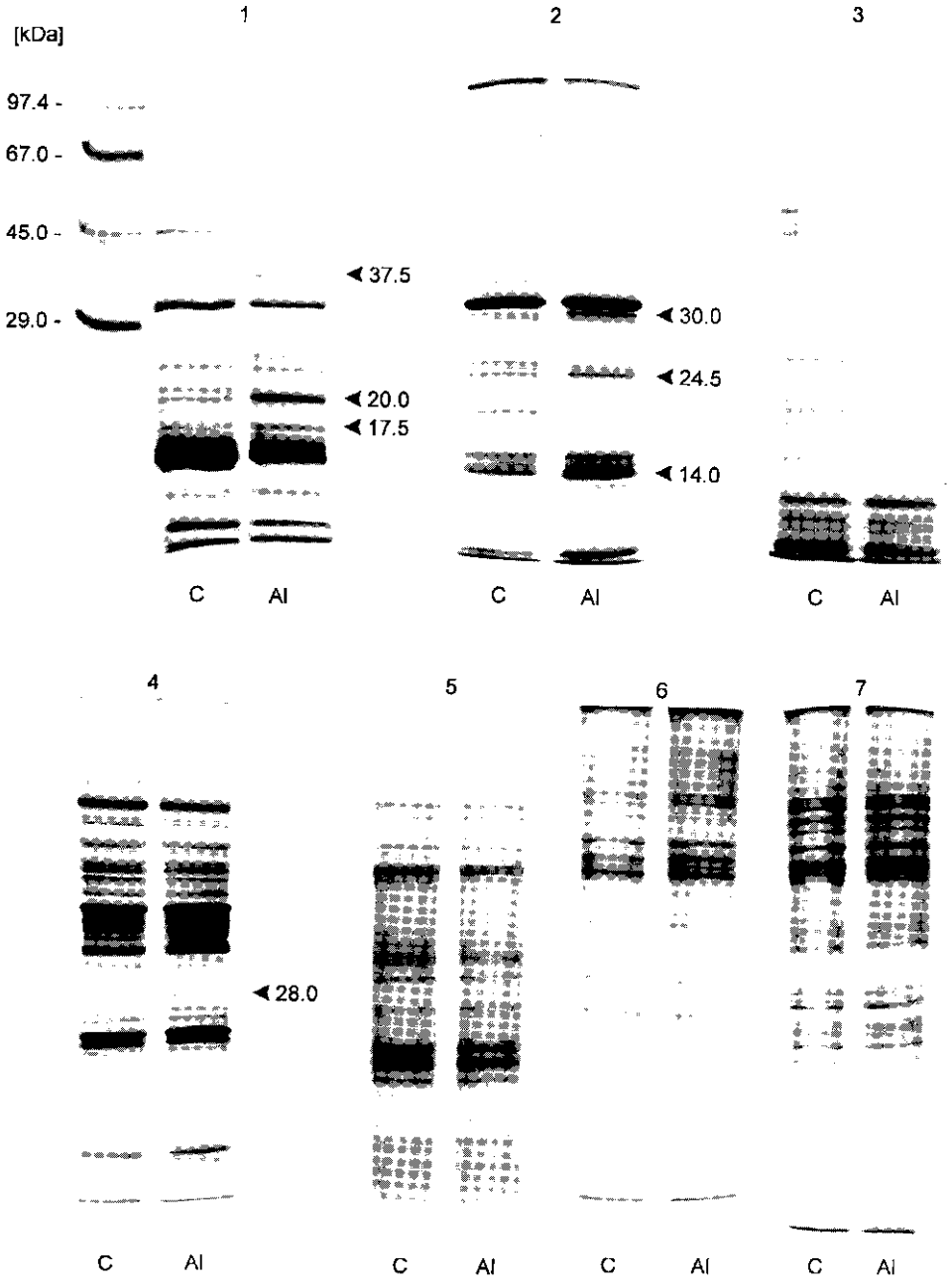

Fig. 3. SDS-PAGE analysis of cytoplasmic proteins isolated from coleoptile of maize seedlings grown for 24 h in the presence (Al) and absence (C) of 100 μM $AlCl_3$. The cytoplasmic proteins were fractionated using an anion exchange column *Bio-Scale Q2* and eluted with linear 0 - 1.0 M NaCl gradient (lanes 1 - 7). The molecular mass of marker proteins are indicated on the left (in kD). *Arrowheads* indicate induced, and *dots* reduced polypeptides.

cultivar showed that 50 μM concentration of Al caused a serious structural damage to root tip tissues including formation of transversal cracks in peripheral tissues of maize root tips (up to 15 mm behind the root apex) which led to the collapse of rhizodermal and peripheral cortical cells (Budíková *et al.* 1997). All these root tip injuries appeared within 24 h exposure of maize roots to 50 μM Al. At the same time scale no morphological and anatomical changes have been observed in older parts of root. The structural changes of roots correlated with Al localization which was the highest in apical 15 mm. Due to the fact that 100 μM Al had only a negligible effect on growth of maize coleoptile but the same concentration inhibited the root growth up to 80 % we suggest that the differences observed in polypeptide patterns of coleoptile cytoplasmic proteins are not directly connected to Al toxicity. The possible explanation might be a secondary stress induced by Al causing disorders in uptake and distribution of ions especially Mg^{2+}, Ca^{2+}, K^{+} and water (Nichol *et al.* 1993).

We can conclude that Al treatment of maize seedlings induces quantitatively different changes in cytoplasmic proteins of roots and coleoptiles. The slight changes, representing the accumulation of one, and reduction of two polypeptides were observed in fraction of cytoplasmic proteins isolated from 10 mm long root tips. This region of maize root represented the root region which showed significant surface injuries including inhibition of elongation growth and formation of transversal cracks in peripheral cell layers. Very similar changes in polypeptide composition were observed in cytoplasmic proteins isolated from root base representing more mature region of maize root. This region did not show any visible signs of Al induced injury. The most dramatic changes in polypeptide composition were revealed in the fraction of cytoplasmic proteins isolated from maize coleoptile. The amount of seven low molecular mass polypeptides increased during 24 h exposure of maize seedlings to 100 μM $AlCl_3$. The differences found in polypeptide compositions between root and leaf cytoplasmic proteins are the subject of ongoing studies.

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