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Cell-wall composition modulates aluminium toxicity

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Abstract

The pectin content of *Zea mays* suspension cells was modified through long-term and short-term pretreatments. After 2 h of treatment at various Al concentrations a significant positive correlation between pectin and Al contents could be found. There was a close positive correlation between Al contents and the relative callose induction in the maize cells. Investigating the spatial Al sensitivity of root apices of *Zea mays* and *Vicia faba* and combining the data for both species revealed a close positive relationships between pectin and Al contents, and pectin contents and relative callose induction. In transgenic lines of potato differing in the expression of pectin methylesterase it could be demonstrated that higher expression of this enzyme resulted in stronger inhibition of root elongation and higher Al contents of root tips. The results presented support our view that the binding of Al to the cell-wall pectin-matrix represents an important step in the expression of Al toxicity.

Introduction

Aluminium (Al) primarily affects root growth by interfering with processes decisive for the regulation of growth in the root apex (Ryan *et al*., 1993; Delhaize *et al*., 1993). The mechanism of Al-induced inhibition of root growth and the reasons for the spatial differences in Al sensitivity between apical root zones are still not well understood (Horst, 1995). Over the last years, evidence has been accumulated supporting the hypothesis that the root apoplast plays an important role in the expression of Al toxicity and Al resistance (Blamey *et al*., 1990; Horst, 1995). To investigate the role of the cell wall for the expression of Al toxicity we used plant species of different cell-wall types (Carpita and Gibeaut, 1993), cell suspension-cultures with modified pectin contents, and transgenic potato plants differing in degree of expression of pectin methylesterase (PME).

Materials and methods

Zea mays suspension cells were cultured, modified in cell-wall composition, and incubated as described by Schmohl and Horst (2000). Seeds of maize (*Zea mays* cv Helix) and broad bean (*Vicia faba* cv Herz Freya) were germinated between filter paper. For the experiments 10-d-old seedlings were incubated for 2 h in nutrient solution with 50 µM $AlCl_3$ or 10 µM digitonin. Aluminium and callose contents of each 1-mm segment (0-5 mm from root tip) were determined. Potato plants (*Solanum tuberosum* cv. Desiree) and derived transgenic lines were provided by J. Fisahn (MPI Golm, Germany) including anti-sense inhibited and over-expressing PME (from *Petunia inflata*). Plants were treated in nutrient solution for 24 h at pH 4.3 with 10 and 25 µM Al. The determinations of pectin and callose were performed as described in Schmohl & Horst (2000). Statistical analysis was carried out with SAS Release 6.12. Coefficients of determination from regression analysis are given according to their level of significance as ***, ** or * for $p< 0.001$, 0.01 and 0.05.

Results

Due to the different pre-treatments of cell suspension-cultures of *Zea mays* the pectin content was modified. After 2 h of incubation with Al a close positive relationship between total Al contents and the pectin contents was found (data not shown). There was also a close positive correlation (r^2= 0.715***) between Al contents and relative callose induction.

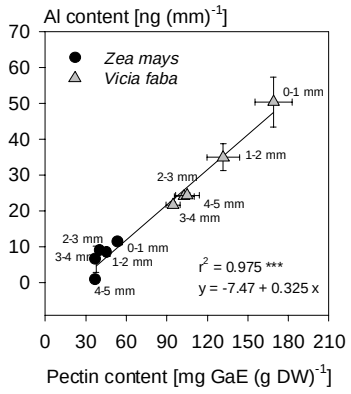

Figure 1. Correlation between pectin and Al contents of different zones of the root apex of *Zea mays* and *Vicia faba*. Incubation of intact plants with 50 µM Al at pH 4.3 for 3 h. Means ± SD of 7 (Al) or 12 (pectin) independent replicates, respectively.

Since it is established that not all parts of a root are similarly affected by Al, individual 1-mm root segments of maize and bean were studied. In both plant species, pectin contents decreased from the first apical mm to more distal root zones. Total pectin contents were higher and the gradient was steeper in bean than in maize. After Al application (50 µM, 3 h) Al contents showed a gradient with the highest Al contents in the first apical 1-mm zone in both plants. The Al contents were higher and the

gradient was steeper in bean than in maize. Combination of the data for both species revealed a close positive relationships between pectin and aluminium contents (Fig. 1) and Al contents and relative callose induction (r^2 = 0.864***, not shown).

In transgenic potato plants antisense-inhibited or over-expressing (PME) genotypical differences could be observed. Root growth was more inhibited in lines over-expressing PME than in the wild type (Fig. 2). The Al contents of the lines showed a similar pattern: plants over-expressing PME showed higher contents (Fig. 2). Antisense inhibition did not significantly influence Al sensitivity. There was a close negative correlation between relative root elongation and Al contents (r^2= 0.845***) as well as a close positive correlation between Al contents and callose induction (r^2=0.717***) (not shown).

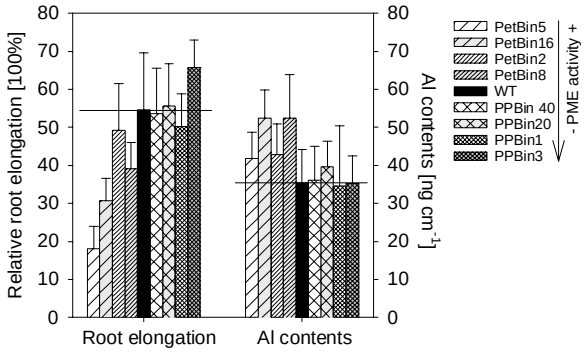

Figure 2. Effect of Al supply on relative root elongation and Al contents of 9 transgenic lines of potato differing in the expression of PME. Incubation for 24 h at 25 µM Al at pH 4.3. Means ± SD of 10 (RRE) or 5 (Al) independent replicates.

Discussion

Aluminium strongly binds to the cell wall of root epidermal and cortical cells. Blamey *et al*. (1990) as well as Grauer and Horst (1992) concluded that the binding of Al to Al-sensitive binding sites determines Al-induced inhibition of root elongation. The main source of apoplastic binding sites are pectins. Since cell cultures and root segments with high pectin contents were more Al-sensitive, we conclude that binding of Al in the apoplast especially to the pectin matrix is modulating Al toxicity. Considering the difficult transfer of Al through the plasma membrane (Rengel, 1996) it seems likely that apoplastic Al itself contributes to Al toxicity. Godbold and Jentschke (1998) could demonstrate that in *Picea abies* Al accumulation in the apoplast is a marker for Al-induced inhibition of root growth. They could also show that the accumulation of Al was determined by the cation exchange capacity of the cell wall and especially the degree of dissociation of carboxylic and hydroxylic groups of the pectins. The amount of carboxylic groups of pectins is regulated by PME. Indeed, the degree of expression of this enzyme in potato plants affected Al sensitivity: lines with higher expression of PME were more Al-sensitive (higher Al contents in root tips, stronger inhibition of root growth). These results indicate that binding of Al in the apoplast is modulated by the pectin content and the degree of methylation of pectins.

High Al accumulation in the root apoplast appears to be characteristic of Al sensitivity (Rincon and Gonzales, 1992). It is not yet possible to say whether binding of Al to the pectic matrix of the root apoplast induces inhibition of root elongation directly or indirectly by enhancing the flux of Al into the symplast, thus leading to symplastic lesions. Sivaguru *et al*. (1999) and Horst *et al*. (1999) were able demonstrate that Al treatment resulted in alterations of the cytoskeleton primarily in the most Al-sensitive zone in maize roots, the distal part of the transition zone. Cause and effect in the interactions of cell wall and cytoskeleton is still a matter of debate (Nick, 1999). Although symplastic Al can not be excluded as a cause for the alterations of the cytoskeleton, we believe that binding of Al to the apoplastic face of the cytoskeleton - plasma-membrane - cell-wall continuum (Wyatt and Carpita, 1993) could explain theses changes to the cytoskeleton. Binding of Al to apoplastic sites may create a physical stress which is transferred to the cytoskeleton, leading to a disturbance of processes necessary for cell elongation, such as, ordered deposition of cellulose fibrils (Carpita and Gibeaut, 1993).

In conclusion, the presented results using quite varying experimental conditions and materials support our view that the pectin matrix in the apoplast of root apical cells plays an important role in the expression of Al toxicity and Al resistance.

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