Plant Physiol. (1997) 113: 595–602

Water Transport Properties of Roots and Root Cortical Cells in Proton- and Al-Stressed Maize Varieties[1]

Benito Gunsé, Charlotte Poschenrieder, and Juan Barceló*

Laboratorio de Fisiología Vegetal, Facultad de Ciencias, Universidad Autónoma de Barcelona, E-08193 Bellaterra, Spain

Root and root cell pressure-probe techniques were used to investigate the possible relationship between Al- or H^+-induced alterations of the hydraulic conductivity of root cells (L_{Pc}) and whole-root water conductivity (L_{Pr}) in maize (*Zea mays* L.) plants. To distinguish between H^+ and Al effects two varieties that differ in H^+ and Al tolerance were assayed. Based on root elongation rates after 24 h in nutrient solution of pH 6.0, pH 4.5, or pH 4.5 plus 50 μM Al, the variety Adour 250 was found to be H^+-sensitive and Al-tolerant, whereas the variety BR 201 F was found to be H^+-tolerant but Al-sensitive. No Al-induced decrease of root pressure and root cell turgor was observed in Al-sensitive BR 201 F, indicating that Al toxicity did not cause a general breakdown of membrane integrity and that ion pumping to the stele was maintained. Al reduced L_{Pc} more than L_{Pr} in Al-sensitive BR 201 F. Proton toxicity in Adour 250 affected L_{Pr} more than L_{Pc}. In this Al-tolerant variety L_{Pc} was increased by Al. Nevertheless, this positive effect on L_{Pc} did not render higher L_{Pr} values. In conclusion, there were no direct relationships between Al- or H^+-induced decreases of L_{Pr} and the effects on L_{Pc}. To our knowledge, this is the first time that the influence of H^+ and Al on root and root cell water relations has been directly measured by pressure-probe techniques.

Al toxicity is considered a major problem for crop production on acid soils (Foy, 1984). High activity of Al in soil solutions has also been proposed as an important factor in the forest decline in Europe (Ulrich et al., 1980). The primary toxicity effects of Al occurs in roots (Ryan et al., 1993). Root stunting, as a consequence of Al-induced inhibition of root elongation, and an increase of root diameter are typical symptoms of Al toxicity (Clarkson, 1969). In plants growing in fields with subsoil acidity, the capacity to explore the soil for nutrients and water is substantially restricted. As a result, plants may suffer from severe water stress after only a few days without rainfall (Foy, 1984). The reduced volume of the root system may not be the only reason for Al-induced water deficiency in plants (Barceló and Poschenrieder, 1990). The Al-induced decrease of both the L_{Pc} (Zhao et al., 1987) and the overall L_{Pr} have been reported (Kruger and Sucoff, 1989; Barceló et al., 1996).

To date, it is not clear whether the Al-induced decrease of L_{Pr}, which has been observed in long-term studies, is a direct consequence of the primary effects of Al on root membrane permeability, which have been found after only a few minutes or hours after exposure to Al. It is likely that many of the effects of Al are mediated by its ability to bind to the carboxyl and phosphate groups, of which there are many in the cell wall and membrane, respectively. Changes in cell wall and membrane properties can be well studied using root and cell pressure probes. To date, however, this has not been attempted in Al-treated roots.

In the present study root and root cell pressure-probe techniques were used to investigate the possible relationship between the Al-induced decrease of water permeability of root cells and whole-root water conductivity. Al toxicity occurs only at a low pH. A high H^+ concentration may also influence root and root cell water relations. To clearly separate H^+ and Al effects, the study was performed using two maize varieties, which, according to their root elongation rates, differ in Al and H^+ tolerance. However, because pressure-probe techniques are challenging, this first approach addresses only the possible influence of Al- and H^+-induced changes of cell water relations on the hydraulic properties of whole roots using mature root zones. The technically more difficult investigation of cell water relations in the elongation zone in relation to Al-induced root growth inhibition will be performed in the future if the present study validates this kind of approach.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Experiments were performed on two maize (*Zea mays* L.) varieties, which, according to previous short-term studies (Llugany et al., 1995; Poschenrieder et al., 1995), differ in Al and H^+ tolerance: var Adour 250, which is Al-tolerant and H^+-sensitive, and var BR 201 F, which is Al-sensitive and H^+-tolerant. Surface-sterilized seeds were germinated on filter paper (4 d) moistened with 0.4 mM $CaCl_2$. Seedlings were transferred to a continuously aerated nutrient solution (5-L plastic beakers, three plants per beaker) of the

[1] This work was supported by the European Union grant no. EC TS3 CT92–0071 and by Dirección General de Investigación Científica y Tecnológica, Spain, grant no. PB94-0738-C02-01.

* Corresponding author; e-mail j__barcelo@cc.uab.es; fax 34–3–581–20–03.

Abbreviations: β, elastic coefficient of root pressure probe with attached root (MPa m^{-3}); ϵ_c, cell elastic coefficient (MPa); L_{Pc}, cortex cell hydraulic conductivity (m s^{-1} MPa^{-1}); L_{Pr}, root hydraulic conductivity (m s^{-1} MPa^{-1}); P_c, cell turgor (MPa); P_r, root pressure (MPa); π_i, osmotic pressure of the cell sap (MPa); π_r, average osmotic pressure of the roots (MPa); superscripts en and ex, endosmotic and exosmotic values, respectively; $T_{1/2}$, half-time of relaxation(s).

following composition (in μM): 200 $CaSO_4$, 100 $MgSO_4$, 400 KNO_3, 300 NH_4NO_3, 15 $MnSO_4$, 0.38 $ZnSO_4$, 0.16 $CuSO_4 \cdot 5H_2O$, 10 Fe-EDTA, 5 NaH_2PO_4, 16 H_3BO_3, and 0.06 $(NH_4)_6Mo_7O_{24}$, pH 6.0. The nutrient solution was completely renewed every 3 d. After 7 d, the formation of adventitious roots was induced by submerging the basal part of the stem into the nutrient solution. When functional adventitious roots appeared (4 d), the remains of the seed and the primary root were excised to favor the development of vigorous adventitious roots. These roots were allowed to grow for a further 7 d, and the plants were then transferred to treatment solutions and exposed for 24 h to pH 6.0 or 4.5 or to pH 4.5 plus 50 μM Al. Plants were grown in a controlled environment chamber under the following conditions: 16 h of light/8 h of darkness; PPFD 155 μmol $m^{-2}\,s^{-1}$; day/night temperature 24/18°C; and RH 50/80%.

Because Al toxicity symptoms in plants correlate better with the activity of Al^{3+} or the sum of monomeric Al species in solution than with the total Al concentration (Taylor, 1988), these putative, toxic Al species were determined in the nutrient solution. The sum of the monomeric Al species in the solution with a nominal concentration of 50 μM Al was 32 μM, as analyzed by the short-term aluminon method (Kerven et al., 1989). According to GEOCHEM speciation (Parker et al., 1987) the Al^{3+} activity of the solution was 11 μM.

Root Elongation and Root Cell Volume Measurements

Just before and after the 24-h treatments the length of the longest adventitious root was measured. Root elongation was calculated as the difference between initial and final lengths. The volume of root cortex cells, necessary for the calculation of cell water relation parameters (see below), was determined on freehand cross-sections and longitudinal sections made at a distance of 50 mm from the tip, i.e. at the same distance at which cell pressure-probe measurements were performed. Sections were mounted in water and photographs were taken to determine the lengths and diameters of the cells in the different layers with a digitizer pad (KD 4610 Graphtec Corp., Yokohama, Japan) connected to a computer (PC 486DX33).

Root Pressure-Probe Measurements

Root pressure and root hydraulic conductivity were determined on excised adventitious roots of plants that had been exposed to different pH and Al treatments for 24 h, using the hydrostatic method described by Steudle and Jeschke (1983). In brief, excised roots, submerged in continuously aerated treatment solution, were connected via a silicone seal (Xantopren plus, Bayer, Leverkusen, Germany) to a 250-μm-diameter capillary filled with 0.4 mM $CaCl_2$. The end of the capillary was connected to the root pressure probe (Steudle, Bayreuth, Germany) filled with silicone oil (AS4, Wacker Chemie, Munich, Germany). The meniscus between the water and oil phases was observed by a stereomicroscope (Wild M3Z, Leica Heerbrugg AG, Heerbrugg, Switzerland). The root pressure was continuously monitored on a chart recorder. The inverse of elastic extensibility of the system was determined as the change of the pressure (P) in response to a change in volume (V) ($\beta = \Delta P/\Delta V$) caused by pushing the meniscus forward and backward. Relaxations of the root pressure were obtained by increasing (exosmotic flow) or decreasing (endosmotic flow) the volume of liquid in the head of the probe. L_{Pr} was calculated according to the equation of Azaizeh and Steudle (1991):

$$L_{Pr} = \frac{1}{A_r}\frac{ln2}{\beta T^r_{½}}, \quad (1)$$

where A_r is the surface area of the root (assuming the root shape to be cylindrical and excluding the tip) and $T^r_{½}$ is the half-time for the relaxation of root pressure.

The π_r of the root was measured in a zone from 30 to 50 mm from the tip as follows. The root segments were frozen in liquid nitrogen. After thawing, the sap was expressed, a fraction was collected on filter paper (Whatman no. 2), and the osmotic potential of the solution was measured with an HR 33T Dew Point microvoltmeter (Wescor, Logan, UT).

Cell Pressure-Probe Measurements

The cell pressure-probe device was used to determine P_c, $T_{½}$, ϵ_c, and L_{Pc} as described by Azaizeh et al. (1992). Intact roots were exposed to treatment solution during the measurements. Hydrostatic experiments were performed on mature root cortex cells, 50 mm from the tip. Cells from the six outer cell layers were analyzed. Cell turgor was determined by insertion of the capillary tip (diameter 4–8 μm) into the cells. After the cell pressure was monitored, endo- and exosmotic experiments were made as in the experiments with whole roots (see above). For calculation of the hydraulic parameters (ϵ_c and L_{Pc}), mean values of the cell volume (V_c) and cell surface area (A_c) for each layer were determined from root cross-sections (see above). The osmotic pressure of the cell sap (π^i) was estimated from turgor pressure. The hydraulic conductivity of the cell membranes was calculated according to the equation of Azaizeh et al. (1992):

$$L_{Pc} = \frac{V_c}{A_c}\frac{ln2}{T_{½}(\epsilon_c + \pi_i)}. \quad (2)$$

Root Al Content

The Al contents of whole adventitious roots of plants grown under the same conditions as those used for the pressure-probe measurements were determined by inductively coupled plasma emission spectrometry as previously described (Poschenrieder et al., 1995).

Statistics

Water relation parameters and root elongation rates were determined on at least five plants per variety and treatment. Root Al content was determined on two independent samples for each variety and treatment. Given results represent means ± SD. Data were analyzed by anal-

ysis of variance, and the significance of differences between treatments and varieties was determined at the 5% level.

RESULTS

Root Elongation, Cell Volume, and Al Content

When exposed to pH 6.0, both maize cultivars exhibited similar root elongation rates. Acid treatment (pH 4.5) affected root elongation more in Adour 250 than in BR 201 F. When additionally exposed to Al, the elongation rates decreased more in BR 201 F than in Adour 250 (Fig. 1A).

In the proton-sensitive, Al-tolerant Adour 250, acid pH significantly decreased the volume of cells in layers 2, 4, and 5. The supply of Al did not cause any further effect on cell volumes (Fig. 2A). In the proton-tolerant, Al-sensitive BR 201 F, cell volume was unaffected by the high H^+ concentration, whereas Al tended to increase the volume of the root cortex cells (Fig. 2B). However, statistically significant differences were only found in cell layers 1 and 6 between plants growing at pH 6.0 and Al-treated plants.

In all treatments, even in solutions without Al supply, BR 201 F showed higher root Al contents than Adour 250 (Fig. 1B).

Root Water Relations

Under control conditions (pH 6.0), variety BR 201 F showed significantly lower P_r than did Adour 250 (Fig. 3A). Supply of a high H^+ concentration (pH 4.5) did not affect P_r in the H^+-tolerant BR 201 F or in the H^+-sensitive Adour 250. Al significantly decreased P_r in Adour 250 but not in BR 201 F. When exposed to pH 6.0, Adour 250 showed a significantly higher π_r than did BR 201 F. The low-pH treatment caused a significant increase of π_r only in BR 201 F. No effect of Al on π_r was observed in either variety (Fig. 3B).

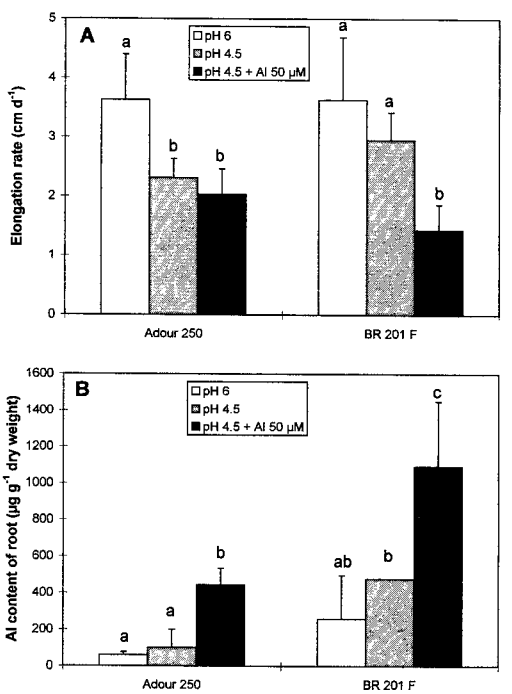

Figure 1. A, Root elongation rates (cm d^{-1}) of *Z. mays* varieties Adour 250 and BR 201 F exposed to different pH and Al treatments (means ± SD, n = 8). Within a variety, bars with the same letter are not significantly different (P = 0.05). B, Al content (µg g^{-1} dry weight) of roots (means ± SD, n = 2); values with the same letter are not significantly different (P = 0.05). Values without SD bar, SD was smaller than the line used to draw the bar.

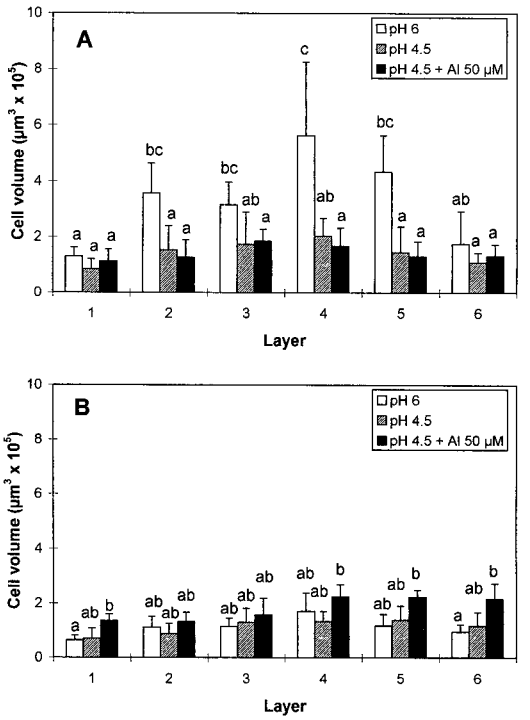

Figure 2. Average cell volume in different root cortex cell layers (50 mm from tip) of Adour 250 (A) and BR 201 F (B) exposed to different pH and Al treatments (means ± SD; n = 8). Values with the same letter are not significantly different (P = 0.05).

Exposure of Adour 250 to an acid pH caused a significant decrease of L_{Pr} and an increase in the half-time of water exchange ($T_{½}$) (Table I). The simultaneous supply of high H^+ and Al concentrations only decreased $L_{Pr}{}^{ex}$, whereas the effect on $L_{Pr}{}^{en}$ was not significant statistically. The high H^+ concentration reduced $L_{Pr}{}^{en}$ more than $L_{Pr}{}^{ex}$, as indicated by the significant decrease of the $L_{Pr}{}^{en}/L_{Pr}{}^{ex}$ ratio. When Al was supplied in addition to H^+, this polarity in water movement was cancelled, in spite of the asymmetric behavior of $T_{½}$, which was increased by Al during influx but not during efflux experiments.

In BR 201 F Al, but not acid pH alone, decreased L_{Pr} (Table I). $L_{Pr}{}^{en}$ was more affected than $L_{Pr}{}^{ex}$ and a significant decrease of the $L_{Pr}{}^{en}/L_{Pr}{}^{ex}$ ratio was observed. Acid pH increased $T_{½}$.

Cell Water Relations

In Adour 250 the high H^+ supply increased P_c and decreased L_{Pc} in the first cortex cell layer, whereas no significant effects were found in the inner layers (Fig. 4, A and B). Al did not affect P_c, whereas L_{Pc} tended to increase

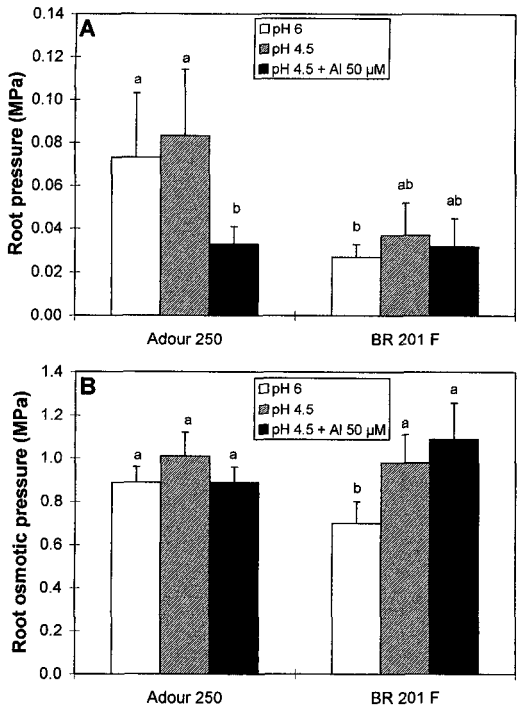

Figure 3. P_r (A) and root π_r (B) in Adour 250 and BR 201 F exposed to different pH and Al treatments (means ± SD; n = 5). Values with the same letter are not significantly different (P = 0.05).

in Al-treated plants. This effect was statistically significant in layers 1 and 3. The average values for L_{Pc} of the six outer cortex cell layers were 0.097×10^7 and 0.182×10^7 m s^{-1} MPa^{-1} for the pH 4.5 and the pH 4.5 plus 50 μM Al treatments, respectively. This 88% increase in L_{Pc} contrasts with the 22 or 58% decrease of L_{Pr}^{en} or L_{Pr}^{ex} of the entire root (Table I). In cortical cells no differences between the influx and efflux experiments were observed. In all cell layers and treatments L_{Pr}^{en}/L_{Pr}^{ex} ratios were close to unity (data not shown). Either a decrease or no effect of H^+ and Al on $T_{½}$ and ϵ_c was observed (Fig. 4, C and D), although there was a significant increase of ϵ_c in the first cell layer in plants exposed to pH 4.5.

Acid pH did not influence P_c in BR 201 F, whereas Al significantly affected P_c in the third layer (Fig. 5A). With the exception of the third cell layer, the high H^+ concentration did not significantly influence L_{Pc}, whereas the Al treatment decreased L_{Pc} values in cell layers 1, 3, and 4 (Fig. 5B). The average L_{Pc} of the six outer cortex cell layers decreased from 0.138×10^7 m s^{-1} MPa^{-1} in plants exposed to pH 4.5 alone to 0.069×10^7 m s^{-1} MPa^{-1} when they were additionally supplied with 50 μM Al, i.e. Al induced a 50% decrease of the average L_{Pc}. Nevertheless, the Al-induced decreases of root L_{Pr}^{en} and L_{Pr}^{ex} were only 29 and 21%, respectively. Half-times of the water-exchange values were hardly influenced by any of the treatments (Fig. 5C). Al supply tended to increase ϵ_c values (Fig. 5D), and this effect was statistically significant in cell layers 1, 3, and 6.

At pH 6.0, good correlations between cell volume and the cell elastic modulus ϵ_c were found in both varieties (Fig. 6). In plants exposed to pH 4.5, the correlation was worse and remained significant only in the H^+-tolerant BR 201 F. In H^+-sensitive and Al-tolerant Adour 250 exposed to pH 6.0, ϵ_c was significantly lower for a given cell volume than in plants exposed to pH 4.5. The opposite was true for BR 201 F. Nevertheless, in BR 201 F the difference was small and tended to disappear for high cell volumes. However, in both varieties the correlation between the cell volume and ϵ_c disappeared in plants that were exposed to Al (Fig. 6, inset). In the Al-sensitive BR 201 F, ϵ_c values were considerably higher than in Adour 250 in the presence of Al.

DISCUSSION

The root elongation rate is widely used as a reliable indicator for varietal differences in Al tolerance (Hanson and Kamprath, 1979). According to the root elongation

Table I. *Effects of acid pH with or without Al on L_{pr}, β, and $T_{½}$ in hydrostatic endosmotic and exosmotic experiments with roots of two maize varieties using the root pressure probe (values in parentheses are SD; n = 5–10)*

Values within a column and variety followed by the same letter are not significantly different (P > 0.05).

Treatment	L_{Pr}^{en}	L_{Pr}^{ex}	L_{Pr}^{en}/L_{Pr}^{ex}	$T_{½}^{en}$	$T_{½}^{ex}$	$T_{½}^{en}/T_{½}^{ex}$	β^{en}	β^{ex}	β^{en}/β^{ex}
	ms^{-1} MPa^{-1} × 10^7			s	s		MPa m^{-3} × 10^{-8}		
Adour 250 (Al-tolerant and H^+-sensitive)									
pH 6.0	3.5a	4.8a	0.6ab	21.1a	22.4ab	0.9a	6.08a	4.82a	1.3a
	(1.7)	(1.0)	(0.2)	(5.4)	(7.4)	(0.1)	(2.82)	(1.80)	(0.1)
pH 4.5	0.9b	1.9b	0.4b	39.8b	55.3b	0.8a	10.3a	10.8a	1.0ab
	(0.3)	(0.4)	(0.03)	(1.7)	(8.1)	(0.2)	(1.78)	(1.66)	(0.1)
pH 4.5 + 50 μM Al	0.7b	0.8c	0.7a	63.4c	27.8a	2.8b	9.50a	11.3a	0.9b
	(0.3)	(0.4)	(0.1)	(5.0)	(4.0)	(0.9)	(1.56)	(1.94)	(0.1)
BR 201 F (H^+-tolerant and Al-sensitive)									
pH 6.0	2.4a	2.1a	1.1ab	12.0a	12.1a	1.0a	16.3	18.0a	0.9ab
	(0.4)	(0.3)	(0.3)	(1.8)	(2.0)	(0.1)	(2.6)	(9.8)	(0.04)
pH 4.5	2.1a	2.1a	1.0a	25.8b	22.4a	1.2a	10.0a	8.8b	1.1a
	(0.4)	(0.6)	(0.1)	(3.9)	(3.9)	(0.2)	(3.6)	(1.9)	(0.1)
pH 4.5 + 50 μM Al	1.5b	1.7a	0.9b	13.2a	13.3a	1.0a	14.0a	18.0a	0.8b
	(0.1)	(0.2)	(0.0)	(1.1)	(1.2)	(0.1)	(1.7)	(1.5)	(0.1)

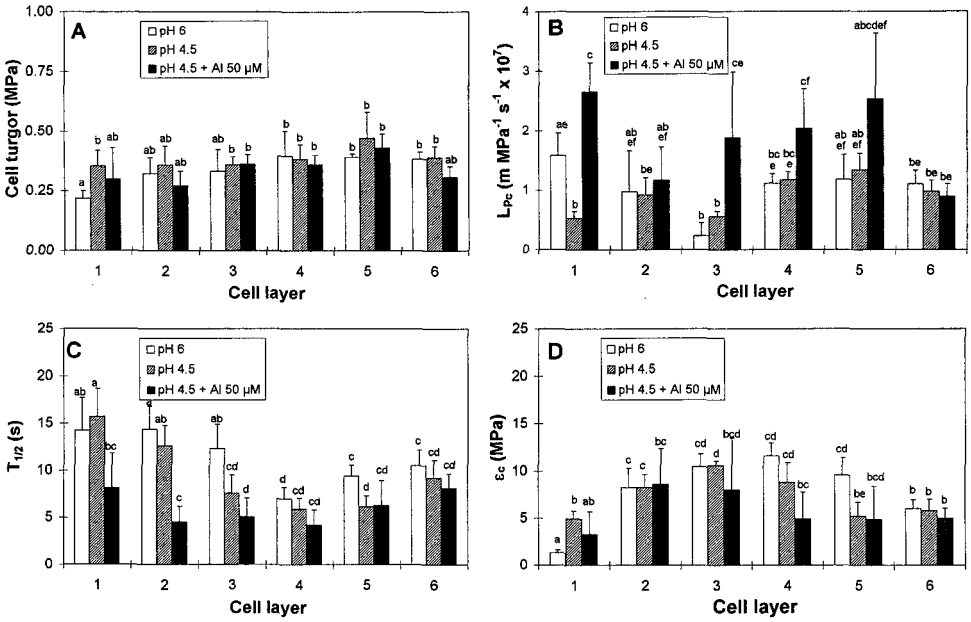

Figure 4. P_c (A), L_{Pc} (B), $T_{½}$ (C), and ϵ_c (D) of cells from the six outer cortex cell layers (50 mm from tip) in roots of Adour 250 exposed to different pH and Al treatments (means ± SD; n = 5–10). Cell layers are numbered from the outside to the inside. Values with the same letter are not significantly different (P = 0.05).

rates shown in Figure 1A, Adour 250 was more tolerant to Al and less tolerant to high H^+ concentrations than BR 201 F. The lack of influence on the root cell volume of Al in Adour 250 and of H^+ in BR 201 F, as well as the H^+-induced decrease of cell volume in Adour 250, is in line with the varietal differences in Al and H^+ tolerance stated above. The Al-induced tendency to increase rather than to decrease the cell volume in BR 201 F may not be considered an Al-induced stimulation of cell growth, i.e. indicative of Al tolerance in BR 201 F, but a consequence of Al injury. Swelling of root cortex cells has been described as an early consequence of Al toxicity (Hecht-Buchholz et al., 1990). The varietal differences in H^+ and Al tolerance that we observed in this study on adventitious roots confirm previous results obtained on seedling roots in these varieties (Llugany et al., 1995; Poschenrieder et al., 1995).

Under control conditions (pH 6.0) the maize varieties used in this study showed relatively low P_r values compared with literature data (Steudle and Frensch, 1989; Frensch et al., 1992; Peterson et al., 1993). This may have been due to the low ionic strength of our nutrient solution, which was used to mimic the composition of solutions in acid soils and to maintain a high Al^{3+} activity.

Cation toxicity caused by H^+ in Adour 250 and by Al in BR 201 F did not affect P_r and π_r (Fig. 3). No significant decrease of P_c was observed in any variety and treatment. These results indicate that in the mature root zones of cation-stressed plants active ion pumping to the xylem was maintained and no general breakdown of root membrane integrity occurred. Previous reports have also shown that even severely Al-intoxicated roots maintained negative membrane electric potentials and normally functioning plasmalemma H^+-ATPase and K^+ uptake (Kochian, 1995).

However, the negative effect of Al on L_{Pc} in Al-sensitive BR 201 F (Fig. 5) indicates Al-induced alterations of membrane properties. Toxic concentrations of Hg^{2+} and Zn^{2+} are thought to decrease L_{Pc} in *Characea* cells by blocking water channels (aquaporins) (Rygol et al., 1992; Henzler and Steudle, 1995). A salt-induced decrease of L_{Pc} in maize roots has also been related to a blocking of the water channels by salt (Steudle and Henzler, 1995).

Al tends to bind to the phosphate or carboxyl groups rather than to -SH groups. Therefore, an interaction of Al with the polar headgroups of the membrane surface would be a likely cause for the decreased water permeability of membranes in BR 201 F than a direct blockage of water channels, which seems to be caused by -SH reagents. Al toxicity has been found to cause lipid phase changes in membranes of *Thermoplasma acidophilum* (Vierstra and Haug, 1978). Both the binding of Al to membranes and the Al-induced decrease of membrane fluidity have been observed in microsomes (Suhayda and Haug, 1986; Matsumoto et al., 1992). In our more complex, experimental system a direct interaction of Al with cell membranes cannot be excluded. The opposite effects of Al on L_{Pc} in Adour 250 and BR 201 F (Figs. 4B and 5B) provide indirect evidence for differences between Al effects on plasmalemma membranes in the mature root zones of these maize varieties. However, these differences may merely reflect varietal differences in Al uptake and/or Al speciation and do not prove varietal differences in membrane properties.

Root L_{Pr} and cell L_{Pc} values were similar to those found in other maize varieties grown in nutrient solutions at a higher ionic strength (Azaizeh and Steudle, 1991; Azaizeh et al., 1992). Cation toxicity caused by acid pH in Adour 250

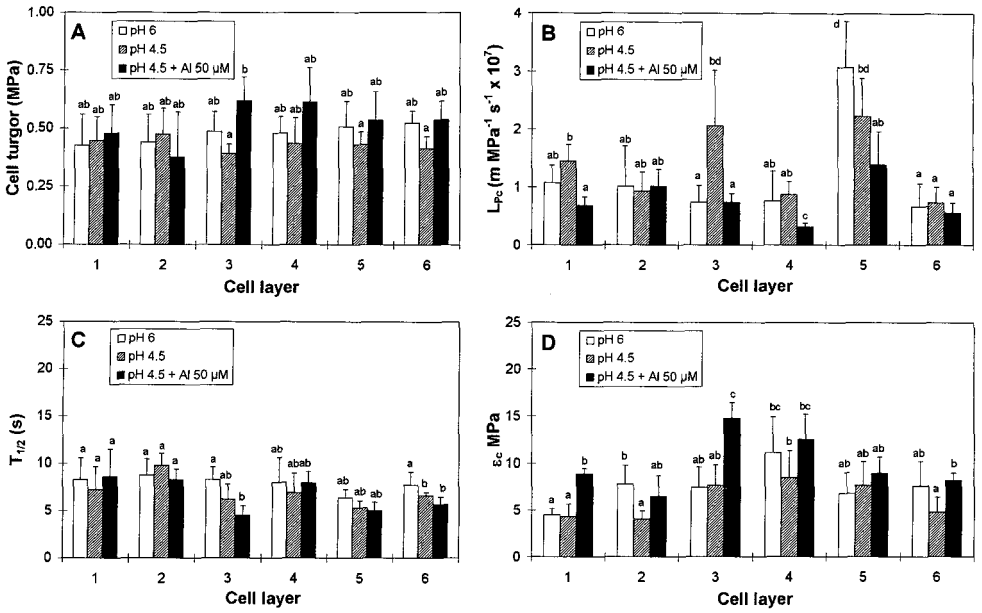

Figure 5. P_c (A), L_{Pc} (B), $T_{½}$ (C), and ϵ_c (D) of cells from the six outer cortex cell layers (50 mm from tip) in roots of BR 201 F exposed to different pH and Al treatments (means ± SD; n = 5–10). Cell layers are numbered from the outside to the inside. Values with the same letter are not significantly different (P = 0.05).

and by Al in BR 201 F significantly decreased L_{Pr}. However, the decrease of L_{Pr} was not directly related to the H^+- or Al-induced alterations of L_{Pc}. In the Al-sensitive BR 201 F the average L_{Pc} of the six outer cortex cell layers was much more affected by Al than L_{Pr}. In the H^+-sensitive Adour 250 the average L_{Pc} was less affected by acid pH than was L_{Pr}. Moreover, in Adour 250 the Al-induced increase of L_{Pc} did not yield higher L_{Pr}.

Wounding experiments on young maize roots similar to those used by us have shown that the unsuberized endodermis is not a major barrier to water movement into the stele, but the major barrier to the radial movement of water is the membranes and apoplast of all of the living tissue (Peterson et al., 1993). In our experiments L_{Pr} was generally larger than L_{Pc} and water flow seemed to be mainly apoplastic. A predominant radial water flow around cells would explain the stronger effect of Al toxicity on L_{Pc} than on L_{Pr} in Al-sensitive BR 201 F. The observation that in the Al-tolerant Adour 250 the positive effect of Al on L_{Pc} did not yield higher L_{Pr} also supports the hypothesis that under hydrostatic conditions the water flow in maize is predominantly apoplasmic (Steudle et al., 1987; Jones et al., 1988). Similar results have been reported for salt-stressed maize roots in which the ameliorative effect of Ca on L_{Pc} was much stronger than on L_{Pr} (Azaizeh and Steudle, 1991; Azaizeh et al., 1992). Other stress factors, such as N or P deficiency (refs. in Radin and Matthews, 1989) and anoxia (Birner and Steudle, 1993), have also been found to decrease L_{Pr}. Unfortunately, data from studies combining both cell and root pressure probes are not available, and the relative importance of the stress-induced alterations of L_{Pc} on L_{Pr} in plants exposed to nutrient deficiency or anoxia remains to be established.

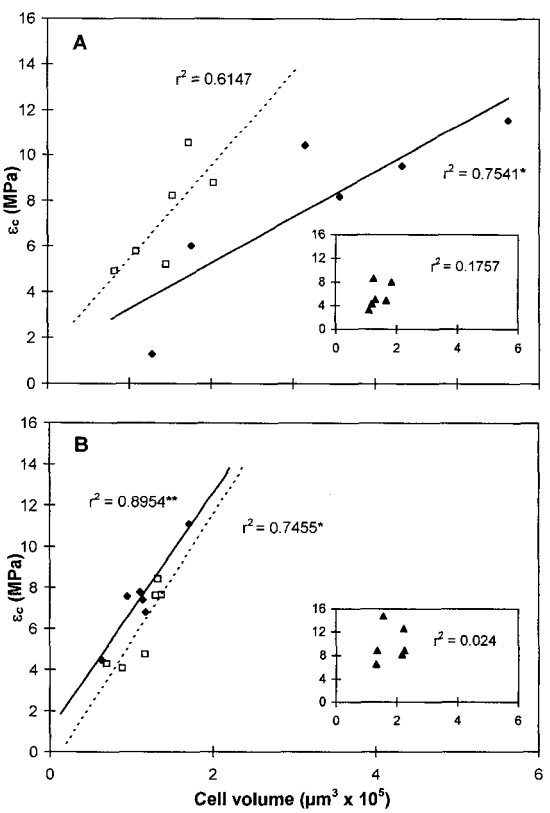

Figure 6. Correlation between ϵ_c and volume in cortex cells of Adour 250 (A) and BR 201 F (B) exposed to pH 6.0 (◆), pH 4.5 (□), or pH 4.5 plus 50 μM Al (inset; ▲). Correlation coefficients marked ** or * are significant at the 5 and 10% probability level, respectively.

There is evidence that L_{Pr} is less affected in salt-tolerant species than in sensitive ones (Steudle, 1994). However, comparative studies within a species using varieties differing in tolerance have not yet been performed. To our knowledge, the present work is not only the first report of cell and root pressure-probe measurements in H^+- and Al-stressed plants but also the first approach using these techniques to demonstrate the varietal differences of the effects of ion toxicity on L_{Pr} and L_{Pc}.

The use of the cell pressure probe also revealed a strong influence of H^+ and Al toxicity on the mechanical properties of cell walls. Proton and Al toxicity caused changes of the volume dependence of ϵ_c (Fig. 6). It is well known that, in relative terms, small cells are more extensible than larger ones. This volume dependence of ϵ_c is increased at a higher P_c (Steudle et al., 1977). No statistically significant effect of acid pH on P_c (Fig. 4A) was observed in acid-sensitive Adour 250, except in the outermost cell layer (level 1). Nevertheless, an acid pH increased the volume dependence of ϵ_c (Fig. 6A) and, for a given cell volume, root cell walls were less elastic in acid-stressed than in control plants of Adour 250. In contrast, Al, but not acid pH, caused cell wall stiffening in the Al-sensitive, acid-tolerant BR 201 F (Fig. 6B). Although these effects of cation toxicity on cell walls in mature tissues may be of little importance for the root hydraulic conductivity, a similar influence of H^+ and Al on the mechanical properties of cell walls in elongating root tissues might play a prominent role in stress-induced inhibition of root elongation (Pritchard and Tomos, 1993).

In conclusion, our results show that maize varieties that differ in tolerance to H^+ and Al also differ in effects of these cations on L_{Pc} and L_{Pr}. However, the decrease of L_{Pr} caused by cation toxicity was not directly related to the H^+- or Al-induced alterations of L_{Pc}. Al tolerance was associated with varietal differences in the response to Al of both cell hydraulic conductivity and mechanical properties of cell walls. Further investigations are required to determine whether this was due to varietal differences in Al-sensitive binding sites at the plasmalemma and in the apoplast (Horst, 1995) or merely a consequence of varietal differences in Al uptake and Al speciation in the roots.

It must be taken into account that this investigation was performed on mature root zones (50 mm from the tip). In maize the root apex does not seem to contribute to L_{Pr} (Frensch and Steudle, 1989). The root apex (up to 21 mm from the tip) is hydraulically isolated from the mature root (Peterson and Steudle, 1993) and may exhibit different responses to Al and H^+ stress. Further investigations of the root apex are in progress. They should reveal whether varietal differences in H^+- and Al-induced alterations of cell water relations in elongating cells are related to differential responses in root elongation, a process that is considered a primary target of Al toxicity (Ryan et al., 1993; Horst, 1995; Kochian, 1995).

Received June 24, 1996; accepted October 28, 1996.

LITERATURE CITED

Azaizeh H, Gunsé B, Steudle E (1992) Effects of NaCl and $CaCl_2$ on water transport across root cells of maize (*Zea mays* L.) seedlings. Plant Physiol **99:** 886–894

Azaizeh H, Steudle E (1991) Effects of salinity on water transport of excised maize (*Zea mays* L.) roots. Plant Physiol **97:** 1136–1145

Barceló J, Poschenrieder CH (1990) Plant water relations as affected by heavy metal stress: a review. J Plant Nutr **13:** 1–37

Barceló J, Poschenrieder CH, Vázquez MD, Gunsé B (1996) Aluminium phytotoxicity: a challenge for plant scientists. Fert Res **43:** 217–226

Birner T, Steudle E (1993) Effects of anaerobic conditions on water and solute relations, and on active transport in roots of maize (*Zea mays* L). Planta **190:** 474–483

Clarkson DT (1969) Metabolic aspects of aluminium toxicity and some possible mechanisms for resistance. *In* IH Rorison, ed, Ecological Aspects of the Mineral Nutrition of Plants. Oxford, UK, pp 381–397

Foy CD (1984) Physiological effects of hydrogen, aluminum, and manganese toxicities in acid soil. *In* F Adams, ed, Soil Acidity and Liming, Ed 2. American Society of Agronomy, Madison, WI, pp 57–97

Frensch J, Stelzer R, Steudle E (1992) NaCl uptake in roots of *Zea mays* seedlings: comparison of root pressure probe and EDX data. Ann Bot **70:** 543–550

Frensch J, Steudle E (1989) Axial and radial hydraulic resistance to roots of maize (*Zea mays* L.). Plant Physiol **91:** 719–726

Hanson WD, Kamprath, EJ (1979) Selection for aluminium tolerance in soybean based in seedling-root growth. Agron J **71:** 581–586

Hecht-Buchholz CH, Brady DJ, Asher CJ, Edwards DG (1990) Effects of low activities of aluminium on soybean (*Glycine max*). II. Root cell structure and root hair development. *In* ML Van Beusichem, ed, Plant Nutrition—Physiology and Applications. Kluwer Academic, Dordrecht, The Netherlands, pp 335–343

Henzler T, Steudle E (1995) Reversible closing of water channels in *Chara* internodes provides evidence for a composite transport model of the plasma membrane. J Exp Bot **46:** 199–209

Horst WJ (1995) The role of apoplast in aluminium toxicity and resistance of higher plants: a review. Z Pflanzenernaehr Bodenkd **158:** 419–428

Jones H, Leigh RA, Wyn Jones RG, Tomos AD (1988) The integration of whole-root and cellular hydraulic conductivities in cereal roots. Planta **174:** 1–7

Kerven GL, Edwards DG, Ascher CJ, Hallman PS, Kokot S (1989) Aluminium determination in soil solution. II. Short-term colorimetric procedures for the measurement of inorganic monomeric Al in the presence of organic acid ligands. Aust J Sci Res **27:** 91–102

Kochian LV (1995) Cellular mechanisms of aluminum toxicity and resistance in plants. Annu Rev Plant Physiol Plant Mol Biol **46:** 237–260

Kruger E, Sucoff E (1989) Aluminium and the hydraulic conductivity of *Quercus rubra* L. root systems. J Exp Bot **40:** 659–665

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

Matsumoto H, Yamamoto Y, Kasai M (1992) Changes of some properties of the plasma membrane-enriched fraction of barley roots related to aluminium stress: membrane associated ATPase, aluminum and calcium. Soil Sci Plant Nutr **38:** 411–419

Parker DR, Zelazny LW, Kinraide TB (1987) Improvement of the program GEOCHEM. Soil Sci Soc Am J **51:** 488–491

Peterson CA, Murrmann M, Steudle E (1993) Location of the major barriers to water and ion movement in young roots of *Zea mays* L. Planta **190:** 127–136

Peterson CA, Steudle E (1993) Lateral hydraulic conductivity of early metaxylem vessels in *Zea mays* L. roots. Planta **189:** 288–297

Poschenrieder CH, Llugany M, Barceló J (1995) Short-term effects

of pH and aluminium on mineral nutrition in maize varieties differing in proton and aluminium tolerance. J Plant Nutr **18:** 1495–1507

Pritchard J, Tomos AD (1993) Correlating biophysical and biochemical control of root cell expansion. *In* JAC Smith, H Griffiths, eds, Water Deficits, Plant Responses from Cell to Community. Bios Scientific, Oxford, UK, pp 53–72

Radin JW, Matthews MA (1989) Water transport properties of cortical cells in roots of nitrogen- and phosphorus-deficient cotton seedlings. Plant Physiol **89:** 264–268

Ryan PR, DiTomasso JM, Kochian LV (1993) Aluminium toxicity in roots: an investigation of spatial sensitivity and the role of the root cap. J Exp Bot **44:** 437–446

Rygol J, Arnold WA, Zimmermann U (1992) Zinc and salinity effects on membrane transport in *Chara connivens*. Plant Cell Environ **15:** 11–23

Steudle E (1994) Water transport across roots. Plant Soil **167:** 79–90

Steudle E, Frensch J (1989) Osmotic responses of maize roots. Water and solute relations. Planta **177:** 281–295

Steudle E, Henzler T (1995) Water channels in plants: do basic concepts of water transport change? J Exp Bot **46:** 1067–1076

Steudle E, Jeschke WD (1983) Water transport in barley roots. Measurements of root pressure and hydraulic conductivity of roots in parallel with turgor and hydraulic conductivity of root cells. Planta **158:** 237–248

Steudle E, Oren R, Schulze ED (1987) Water transport in maize roots. Measurement of hydraulic conductivity, solute permeability, and of reflection coefficients of excised roots using the root pressure probe. Plant Physiol **84:** 1220–1232

Steudle E, Zimmermann U, Lüttge U (1977) Effect of turgor pressure and cell size on the wall elasticity of plant cells. Plant Physiol **59:** 285–289

Suhayda CG, Haug A (1986) Organic acids reduce aluminum toxicity in maize root membranes. Physiol Plant **68:** 189–195

Taylor GJ (1988) The physiology of aluminum phytotoxicity. *In* H Sigel, A Sigel, eds, Metal Ions in Biological Systems, Vol 24: Aluminum and Its Role in Biology. Marcel Dekker, New York, pp 123–163

Ulrich B, Mayer R, Khanna PK (1980) Chemical changes due to acid precipitation in loess derived soil in central Europe. Soil Sci **130:** 193–199

Vierstra R, Haug A (1978) The effect of Al^{3+} on the physical properties of membrane lipids in *Thermoplasma acidophilum*. Biochem Biophys Res Commun **84:** 138–143

Zhao XJ, Sucoff E, Stadelmann EJ (1987) Al^{3+} and Ca^{2+} alteration of membrane permeability of *Quercus rubra* root cortex cells. Plant Physiol **83:** 159–162