

Does Salinity Reduce Growth in Maize Root Epidermal Cells by Inhibiting Their Capacity for Cell Wall Acidification?¹

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ABSTRACT

The reduction in growth of maize (*Zea mays* L.) seedling primary roots induced by salinization of the nutrient medium with 100 millimolar NaCl was accompanied by reductions in the length of the root tip elongation zone, the length of fully elongated epidermal cells, and the apparent rate of cell production: Each was partially restored when calcium levels in the salinized growth medium were increased from 0.5 to 10.0 millimolar. We investigated the possibility that the inhibition of elongation growth by salinity might be associated with an inhibition of cell wall acidification, such as that which occurs when root growth is inhibited by IAA. A qualitative assay of root surface acidification, using bromocresol purple pH indicator in agar, showed that salinized roots, with and without extra calcium, produced a zone of surface acidification which was similar to that produced by control roots. The zone of acidification began 1 to 2 millimeters behind the tip and coincided with the zone of cell elongation. The remainder of the root alkalized its surface. Kinetics of surface acidification were assayed quantitatively by placing a flat tipped pH electrode in contact with the elongation zone. The pH at the epidermal surfaces of roots grown either with 100 millimolar NaCl (growth inhibitory), or with 10 millimolar calcium \pm NaCl (little growth inhibition), declined from 6.0 to 5.1 over 30 minutes. We conclude that NaCl did not inhibit growth by reducing the capacity of epidermal cells to acidify their walls.

A better understanding of the mechanisms involved in the inhibition of plant growth by salinity may help accelerate the introduction of environmental and genetic manipulations aimed at increasing crop salinity resistance (2, 18). Plant roots provide an attractive experimental system for investigating salinity effects on growth for the following reasons: (a) they have a definable growing region in the tip, comprised of dividing and extending cells and a separate nongrowing region, with mature elongated cells, which usually begins a few millimeters behind the tip; (b) root epidermal cells can be exposed to different salt concentrations by changing the root medium and changes in growth associated ionic fluxes, *e.g.* acidification of the epidermal cell wall, can be directly assayed (16, 19), without abrading or peeling the epidermis (3).

Root and shoot elongation growth can be viewed as irreversible increases in size dependent on ongoing cell division in meristematic tissues and elongation of the cells produced.

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Salinity reduced both of these in the cortex of cotton seedling roots (12). We report here that salinity-induced reductions in maize root elongation growth and its partial restoration by increased calcium ion activity (5, 7, 13), coincide with effects on new cell production and cell elongation in the epidermis.

The underlying mechanism(s) by which salinity inhibits root growth and calcium partially restores it, are not clearly defined. Cramer *et al.* (6) argued that salinity can induce a displacement of calcium from plasmamembrane binding sites and suggested that consequent increases in membrane leakiness could be a primary cellular response to salinity stress. If salinity stress reduces plasmamembrane integrity, it could also interfere with the process of cell wall acidification, which appears to be partially dependent on ATP driven outward pumping of protons across the intact plasmamembrane (24). Wall acidification can increase the extensibility of cell wall preparations *in vitro* (4), and wall loosening is a prerequisite for wall extension in the process of cell expansion growth (22). Moreover, acidification of the growth medium accelerates elongation growth of intact maize roots (8, 15, 16) and localized regions of acidification on the epidermal surface correlate with regions of maximal cell extension growth (16, 19). Finally, the inhibition of root growth induced by treatment of roots with 2 μ M IAA inhibited surface acidification in the cell elongation zone (16). We therefore investigated the possibility that the inhibition of root elongation growth by salinity and its partial reversal by extra calcium were associated with qualitative and quantitative changes in capacity to acidify epidermal cell surfaces in the root elongation zone.

MATERIALS AND METHODS

Plant Growth

Seeds of *Zea mays* L. cv Halamish were germinated on sheets of filter paper wetted with 0.4 mM calcium chloride for 2 d in the dark at 28°C. Fifteen seedlings were then transferred to expanded polystyrene floats with the roots immersed in 1 L of aerated 0.1 strength nutrient solutions (11) and maintained in a growth room (27 \pm 2°C, 12 h photoperiod with 35 W m⁻² at plant height). The nutrient solutions were modified by additions of NaCl and extra CaCl₂ so that the seedlings received one of four treatments: Control; addition 100 mM of NaCl; addition of 10 mM CaCl₂; addition of 10 mM CaCl₂ + 100 mM NaCl. After 2 d in nutrient solution the root system, consisting of a single primary root without root hairs, was assayed.

Root growth rates were assayed *in vivo* by marking 2 d roots 2 cm from the tip with a marker pen (Stabilo 88, FRG)

and measuring the increase in length after 24 h. Results for each treatment are means of six replicate groups of five plants.

Measurement of Cell Length and Root Elongation Zone

Replicates of epidermal cells were taken 2 to 3 cm behind the tips of seedling primary roots after 2 d in the treatment solution: the area to be sampled was gently blotted with tissue paper and a thin layer of Gestetner correction fluid painted on and left to dry for 5 min. During this period, the remainder of the seedling was loosely covered by moist tissue paper to prevent drying. The imprint was lifted off by contact with clear adhesive tape and mounted on a microscope slide. Slides were viewed in a binocular microscope with an eye piece scale and the length of cells in files of three or more was assayed. A total of 67 ± 3 cells from five roots were assayed in each treatment. Dividing the rates of root elongation by the final length of the epidermal cells gave a useful comparative estimate of mean rates of cell production in a single file of cells, as influenced by the various treatments. This apparent rate of cell production cannot, however, differentiate between possible treatment effects on the number of meristematic cells and/or effects on rates of cell division.

The elongation zone was identified by marking 2 d roots at 2 mm intervals from 1 mm behind the tip, incubating them in treatment solutions for 24 h and recording the distance from the tip to the basal point beyond which no elongation occurred. Results are means of 10 roots per treatment.

Qualitative Assay of Root Surface pH

Wall acidification along the root surface was assayed by a qualitative method (16) that involved lightly pressing the intact roots of seedlings, 2 or 4 d after transfer to treatment solutions, into 0.6 or 1% w/v agar plates. The agar was prepared in the appropriate growth solution and bromocresol purple added to give 0.71 mM. The still liquid agar solution at 40°C was adjusted to pH 5 before pouring into Petri dishes, and this pH was maintained after the agar set. Agar concentration did not affect acidification patterns but plates made with 1% agar could be mounted vertically. Assay of vertically mounted roots was required to rule out the possibility that surface acidification by horizontally mounted roots was mainly a specific response to geotropic stimulation (1, 15, 16). Reductions in pH turn the agar from red to yellow and increases in pH cause the agar to turn purple. Time to the appearance of yellow and purple zones (about 10 min) and their distribution were recorded. Intact roots and excised 3 cm apical root segments, held both vertically and horizontally, were assayed. At least 24 assays were made of intact plus excised roots for each treatment at 2 and 4 d after salinization.

Quantitative Assay of Root Surface pH

A contact method (3) was used to follow the kinetics of surface pH changes in the zone of root elongation (up to 7 mm behind the tip, determined as above) and within the nongrowing zone (>7 mm behind the tip). Briefly, three intact roots were aligned in parallel on a glass plate with the regions 1 to 9 mm or 15 to 24 mm behind the tip inserted into a

preformed ring of petroleum jelly of approximately 0.9 cm i.d. A volume of 100 μ L of appropriate growth solution, with unadjusted pH at approximately 6, was injected into the well. A flat tip pH electrode (Corning) with a built-in reference was connected to an El Hamma pH meter and lowered into the well so as to just contact the moist root surfaces. The rest of the plant was covered with tissue paper wetted with appropriate growth solution to prevent drying. The surface pH changes were recorded at 3 to 5 min intervals. The means of four replicate assays are presented.

Statistics

All experiments were repeated one or more times. Typical results are presented as means \pm SD.

RESULTS

Salinity Effects on Root Growth Rate, Length of Elongation Zone, Final Length of Epidermal Cells, and Apparent Rates of Cell Production

Addition of 10 mM CaCl_2 to control solutions had little effect on rates of elongation growth of maize seedling primary roots but slightly reduced the length of the elongation zone extending from 1 mm behind the tip. Salinization with 100 mM NaCl strongly inhibited rates of root elongation and apparent rates of cell production, as well as reducing both the final length of epidermal cells and the length of the elongation zone. The inhibitory effects of salinity on each of the measured parameters were partially reversed by addition of 10 mM CaCl_2 to salinized root media (Table I).

Root Surface pH

Roots from all treatments induced acidification of indicator agar (agar turned from red to yellow) in the region of elongation starting 1 to 2 mm behind the tip. However, the yellow band in the 100 mM NaCl treatment was visibly shorter, *i.e.* it did not extend as far back along the root as in other treatments. The rest of the root, including a 1 to 2 mm region round the meristematic tip, induced alkalization of the agar (agar turned from red to purple). Similar results were obtained with roots 2 or 4 d after treatment and with roots held either vertically or horizontally during the assay. Color changes occurred within 10 min and we could not detect consistent differences in rates of color change between the different treatments.

The results of the quantitative assay of root surface acidification are shown in Figure 1. The pH in all treatments dropped rapidly from the initial pH of around 5.9 ± 1 to about 5.4 during the first 10 min of the assay and then declined more slowly. After 20 min, the pH in the treatments with additional CaCl_2 and/or 100 mM NaCl tended to stabilize at pH 5 to 5.1. The surface pH of roots grown in 0.1 Hoagland solution (control) continued to decline and was significantly lower, at pH 4.4, than that of all other treatments after 25 and 30 min.

When the pH electrode was placed in contact with a nongrowing region of the root, 2 cm behind the tip, the initial pH

Table I. Salinity Effects on Root Growth Rate, Length of Elongation Zone, Length of Epidermal Cells, and Apparent Rates of Cell Production

Germinated seedlings were grown hydroponically for 2 d in 0.1 strength Hoagland solution (control) ± 100 mM NaCl ± 10 mM CaCl₂. Figures in brackets are percent reductions compared with appropriate nonsalinized treatments. Means \pm SD.

Treatment	Growth Rate <i>mm h⁻¹</i>	Elongation Zone <i>mm</i>	Cell Length <i>μm</i>	Cell Production <i>h⁻¹</i>
Control	1.6 \pm 0.2	7.0 \pm 0.0	195 \pm 13	8.2
+NaCl	0.4 \pm 0.1 (75)	3.9 \pm 1.0	104 \pm 7 (47)	3.8 (54)
+CaCl ₂	1.6 \pm 0.3	5.8 \pm 1.0	190 \pm 12	8.4
+CaCl ₂ + NaCl	0.9 \pm 0.0 (44)	5.0 \pm 0.9	160 \pm 8 (16)	5.6 (33)

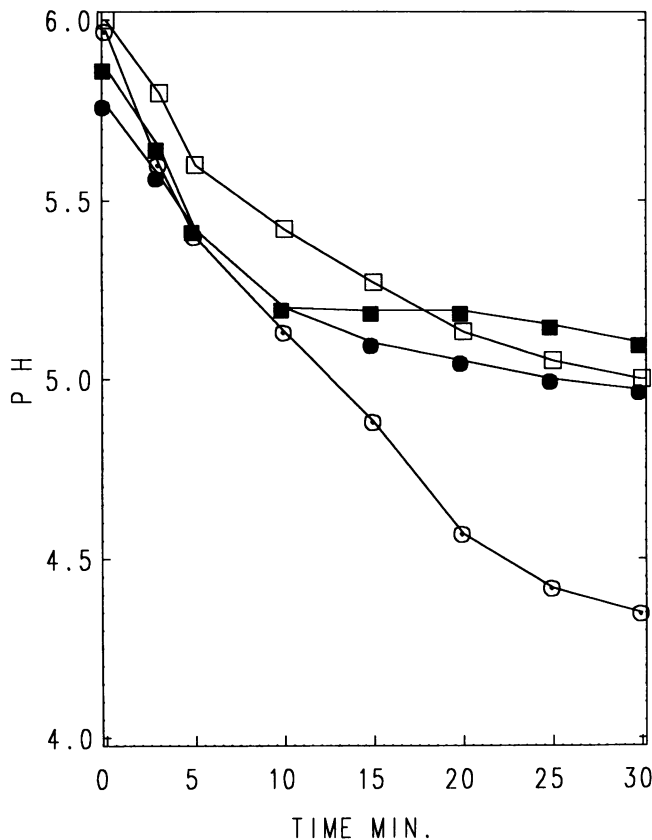


Figure 1. Kinetics of root surface acidification by the growing zone of maize root tips. Flat tipped pH electrode contacting three parallel roots of intact plants mounted horizontally in a 0.9 cm petroleum jelly well containing 100 μ L of indicated growth solution. (○), Control; (●), +100 mM NaCl; (□), +10 mM CaCl₂; (■), +10 mM CaCl₂ + 100 mM NaCl.

of 5.9 ± 0.1 was maintained in each of the treatments: If the initial pH was adjusted to 5, values for all treatments increased to 5.7 ± 0.2 during a 30 min assay period (data not shown).

DISCUSSION

Effects of Salinity and Calcium on Rates of Cell Production and Cell Length

The 75% reduction in the rate of elongation of maize roots, after equilibration for 2 d with 100 mM NaCl, was associated

with large decreases in both the apparent rate of production (54%) and length (47%) of epidermal cells. However, when 10 mM CaCl₂ was present in the salinized growth medium, root elongation rate was only reduced by 44%, and was associated with lesser reductions in both cell production (33%) and cell size (16%). Kurth *et al.* (12) made similar observations using cotton seedling roots. They found that NaCl concentrations greater than 50 mM reduced root length and also the length and production rate of cortical cells. However, in the presence of 10 mM CaCl₂, declines in root length were only induced by NaCl concentrations greater than 100 mM and were apparently associated with reductions in cell production rate, rather than cell size.

Thus, changes in cell production and cell elongation appear to underly the interactive regulation of root growth by salinity and calcium; numerous physiological and molecular mechanisms may be involved in these changes. Cell production in salinized roots of glycophytic plants could be affected by the accumulation of toxic levels of sodium ions in some of the meristematic cells; sodium accumulation and, hence, toxicity would be reduced in the presence of extra calcium (10). Alternatively, salinity-induced changes in intracellular calcium ion activities may affect the duration of the cell division cycle (12). The reductions in cell length caused by salinization could result from reductions in instantaneous rates of cell extension and/or in the duration of the period of cell extension. In either case, changes in the extensibility of the cell walls may be involved. Changes in the ionic composition of root media have been reported to cause direct or indirect changes in the extensibility of the cell walls of root (20), stem (14), and leaf (17) tissues.

Effects of Salinity and Calcium on Wall Acidification Capacity

Segmental analysis of the root growing region (not presented) showed that the region of elongation started just behind the meristematic tip. Similarly, the apical millimeter induced alkalization of the indicator agar, while the region immediately behind the apex induced surface acidification, which coincided with the zone of elongation. These results are similar to other findings based on assays of color changes in pH indicators applied to maize root surfaces (1, 16, 19). They differ however, from the findings of Miller and Gow (15): using a vibrating probe they detected a 1 mm zone of outward current at the tip of their maize roots which they believe to represent acidification. This was followed by a 2.5

mm zone of inward current, presumably in the zone of elongation. The growing region was not defined for these roots and measurements were confined to a region 0 to 5 mm from the tip. Root hairs in more distal regions prevented accurate measurements. The roots used by us were grown in nutrient solution, rather than artificial pond water; the fact that they elongated 3 times faster and had no root hairs may account for some of the differences. Finally, the interpretation of results obtained with the probe appears to be controversial since Björkman and Leopold (1), who also mapped maize root tip currents with a vibrating probe, concluded that the net current and the proton flux were not related.

It is noteworthy that the zone of acidification in our experiments was always apparent, although the roots were continuously exposed to nitrate based nutrient medium. Cells metabolizing nitrate secrete hydroxyl ions in order to maintain cytoplasmic pH (21) and this might be expected to inhibit the capacity to acidify cell walls (15). The alkalinization of the root surface along the mature regions of the root situated behind the growing zone suggests that nitrate uptake and hydroxyl secretion may be located mainly in these regions.

Although wall acidification in the growing zone was not visibly prevented by salinization, addition of NaCl and/or calcium to the growth medium reduced rates of acidification and final pH, in comparison with the control treatment (Fig. 1). However, the detailed interpretation of these results is complicated. The length of the growing zone was reduced by addition of NaCl and/or calcium (Table I) so that the proportion of fully elongated tissue in the 9 mm assay zone at the root tip (see "Materials and Methods") increased. Thus, the apparent rates of acidification in these treatments may have been reduced as a result of uptake of protons (or secretion of hydroxyl ions) by the higher proportion of fully elongated cells in contact with the electrode. The same considerations apply to wall acidification due to respiratory CO₂ accumulation and to cation-proton antiporters. In all events, the relative reduction in acidification rate induced by NaCl alone, did not differ from that induced by NaCl with extra calcium, or, by extra calcium alone: the latter treatments, however, had little or no inhibitory effects on rates of elongation growth. The lack of any clear, salinity-induced inhibition of wall acidification, is consistent with the absence of inhibitory effects of growth in saline media on ATP-driven, proton pumping by membrane vesicles isolated from roots of cotton and maize plants (9, 25).

Thus, to the extent that wall acidification is involved causally in extension growth, ongoing rates are apparently adequate, even in salinized roots. Changing the pH of the bulk solution from 6 to 5 doubled the short-term rate of elongation of maize roots (15) and a stimulation of root growth which lasted for several hours, was reported for a pH drop from 6.5 to 4.5 (8). Moreover, the pH measured at the surface of maize roots was estimated to be considerably higher than the pH adjacent to the plasmalemma (23). Thus, the equilibrium pH of 5.1, for salt-treated roots in Figure 1, probably exceeds the actual pH in the interior of the wall and is quite compatible with a causal role for *in vivo* wall acidification in root growth.

In conclusion, the results from both the qualitative and

quantitative assays indicate clearly that levels of salinity which inhibited maize root growth, did not do so by preventing acidification of cell walls in the growing zone of the root. An alternative possibility, *i.e.* that salinity and calcium interactively affect the capacity of the cell walls to respond to acidification (8, 17), remains to be investigated.

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