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Aluminum Toxicity in Roots[1]

Correlation among Ionic Currents, Ion Fluxes, and Root Elongation in Aluminum-Sensitive and Aluminum-Tolerant Wheat Cultivars

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ABSTRACT

The inhibition of root growth by aluminum (Al) is well established, yet a unifying mechanism for Al toxicity remains unclear. The association between cell growth and endogenously generated ionic currents measured in many different systems, including plant roots, suggests that these currents may be directing growth. A vibrating voltage microelectrode system was used to measure the net ionic currents at the apex of wheat (*Triticum aestivum* L.) roots from Al-tolerant and Al-sensitive cultivars. We examined the relationship between these currents and Al-induced inhibition of root growth. In the Al-sensitive cultivar, Scout 66, 10 micromolar Al (pH 4.5) began to inhibit the net current and root elongation within 1 to 3 hours. These changes occurred concurrently in 75% of experiments. A significant correlation was found between current magnitude and the rate of root growth when data were pooled. No changes in either current magnitude or growth rate were observed in similar experiments using the Al-tolerant cultivar Atlas 66. Measurements with ion-selective microelectrodes suggested that H^+ influx was responsible for most of the current at the apex, with smaller contributions from Ca^{2+} and Cl^- fluxes. In 50% of experiments, Al began to inhibit the net H^+ influx in Scout 66 roots at the same time that growth was affected. However, in more than 25% of cases, Al-induced inhibition of growth rate occurred before any sustained decrease in the current or H^+ flux. Although showing a correlation between growth and current or H^+ fluxes, these data do not suggest a mechanistic association between these processes. We conclude that the inhibition of root growth by Al is not caused by the reduction in current or H^+ influx at the root apex.

Al^2 toxicity has been identified as one of the most important factors limiting plant growth in acid soils (5). Although a wide range of Al-related effects have been described in plants (5, 30, 32), it is unclear whether any represent primary responses to Al toxicity. An early and dramatic symptom of Al phytotoxicity is inhibition of root growth, and with extended exposure (days), the roots thicken and become stubby and darker in color.

The vibrating probe technique (12) has been used by many investigators to demonstrate a correlation between cell growth and endogenously generated transcellular ionic currents (8, 13, 27, 28). In several single-cell systems exhibiting tip growth (pollen tubes, root hairs, algal rhizoids, fungal hyphae), an inward current of between 0.1 and 5.0 $\mu A\ cm^{-2}$ enters the tip region and a smaller, more diffuse current leaves the cell farther back. It is generally thought that separation of membrane-bound transport systems (separated either spatially or operationally) generate these transcellular currents (8, 28), a net influx of cations (or efflux of anions) being localized at the tip and a net efflux of cations (or influx of anions) occurring back from the tip. Clues to the identity of these ions can be obtained by manipulating the ionic composition of the bathing solution and monitoring changes in the measured current. However, this procedure is not always conclusive and may potentially give misleading results, especially in the case of Ca^{2+} (7; see "Discussion").

Because an association between transcellular currents and polarized growth has been described in a diverse range of plant systems, some workers have suggested that these currents, or perhaps the ions carrying the currents, are directing growth in some way (13, 27). Root elongation is not a true example of tip growth. Apart from being multicellular, they have two meristems: the root meristem proper and a second meristem directing new cells forward to maintain the root cap. However, roots do elongate in a polarized manner, and current patterns, similar to those described above for tip-growing systems, have been detected around roots of seedlings from at least five different families (20, 33). Net current enters the root apex (root cap, meristem, and part of the elongation zone) and leaves through the mature tissue. In roots of *Zea mays*, Miller and Gow (21) found that treatments that stimulated root growth (low pH, fusicoccin) were associated with an increase in the inward current at the root tip and elongation zones, whereas treatments that inhibited root growth (high pH, IAA) reduced the current magnitude. Furthermore, Miller *et al.* (23) showed that localized areas of

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[2] Apart from the Al^{3+} cation, aluminum has the potential to form various hydroxy-aluminum and polynuclear species in solution. The available evidence suggests that the Al^{3+} cation is phytotoxic, but it is unclear whether other hydroxy-aluminum species are also toxic (15). In this text, we denote aluminum as Al, without implying a specific aluminum species.

inward current were associated with sites of emerging lateral roots.

Kochian and Shaff (16) measured the net currents around the root apex of the Al-sensitive wheat cv "Scout 66" and the Al-tolerant cv "Atlas 66" and showed that Al addition (10 μM Al, 0.4 mM $CaCl_2$, pH 4.5) reduced the current magnitude and root elongation at approximately the same time in Scout 66. Treatment with 10 μM of Al had no effect on either the net apical current or root growth in Atlas 66 seedlings, although higher concentrations did begin to inhibit these processes. The authors suggested that the ion transport processes creating these currents are involved in root growth and that the Al-induced alterations of these processes may indicate a primary response to Al toxicity.

In this paper, we report further investigations of Al-induced effects on the apical currents of wheat (*Triticum aestivum* L.) roots and describe the use of ion-selective microelectrodes to identify the current-carrying ions. By simultaneously measuring root elongation, we attempted to correlate the onset of Al toxicity with changes in ion transport processes at the root apex.

MATERIALS AND METHODS

Plant Material

Two cultivars of winter wheat (*Triticum aestivum* L.) were selected for their extreme differences in Al sensitivity: Altas 66 is Al tolerant and Scout 66 is Al sensitive. (Both cultivars were generously supplied by J. Peterson, Wheat, Sorghum and Forage Research Laboratory, University of Nebraska.) Seeds were surface sterilized with 0.5% sodium hypochlorite for 45 min, rinsed in distilled water for 15 min, and germinated at 25°C on damp filter paper for 2 d in the dark. Eight seedlings were selected for uniformity, transferred to polyethylene cups with mesh bottoms, and covered with black polyethylene beads. The cups were then placed over 900 mL of 0.6 mM $CaCl_2$ solution, pH 4.5 (adjusted with 0.1 M HCl), and aerated for 2 d in a controlled environment chamber with a day/night regimen of 16 h at 20°C and 8 h at 15°C, Light intensity was 580 $\mu E\ m^{-2}\ s^{-1}$ at the level of the shoots. On the third day the growth solution was changed to 0.1 mM $CaCl_2$, pH 4.5, and experiments were conducted on the fourth day in a similar solution. In all cases, the Al treatment was 10 μM $AlCl_3$ with 0.1 mM $CaCl_2$, pH 4.5.

Measurements of Ionic Currents

For a comprehensive discussion of the vibrating voltage microelectrode technique, see the work reported by Jaffe and Nuccitelli (12) and Nuccitelli (28). The vibrating probe measures the voltage difference (ΔV, volts) at the extremes of its amplitude (x, cm), and by measuring the resistivity of the bathing solution (r, Ω cm), the current density (I, A cm^{-2}) can be calculated from Ohms law:

$$I = \frac{\Delta V}{rx} \quad (1)$$

For a discussion concerning the possible limitations of these assumptions, see the papers by Lucas and Kochian (19) and Ferrier and Lucas (4). Before use, the vibrating probe was calibrated in an artificially generated electric field of known magnitude. The resistivity of the 0.1 mM $CaCl_2$ solution was approximately 28 kΩ cm, and, when required, corrections for changes in solution resistivity were made after the addition of Al. The amplitude of vibration was 30 μm.

Intact seedlings (main root 80–100 mm long) were used for both the net current and ion flux measurements. They were placed in a 145-mm diameter Petri dish containing approximately 80 mL of 0.1 mM $CaCl_2$ (pH 4.5) and gently secured to the bottom of the dish with plexiglass blocks and silicon grease. The blocks (8 × 4 × 4 mm) had a notch cut into one face, which allowed them to be placed over the roots without causing damage. In addition to the root, the seed and shoot were held gently with dental wax. The mucigel around the root apex was then removed by gently applying suction with a fire-polished glass pipette. A pipette was hand drawn to a tip diameter of about 50 to 100 μm and positioned near the cell surface with a micromanipulator. The root was then left to recover for at least 2 h before measurements were started. The Petri dish containing the seedling was placed on the stage of a Zeiss IM35 inverted microscope and the probe positioned such that the direction of vibration was perpendicular to the longitudinal axis of the root and the center of vibration was maintained at 100 μm from the root surface. If the root is approximated by a cylinder with a 200-μm radius, the current measurements will underestimate the actual current occurring at the root surface by a constant value of approximately 30%. The results are presented in the uncorrected form.

Because the roots were actively growing, care was taken to maintain the probe at a constant position relative to both the root surface and root tip. The probes were regularly drawn out to the bulk solution to monitor and correct for baseline drift. Apart from the scan of current densities along some roots in the initial experiments, all measurements were made at two positions near the root apex. The first was midway along the root cap, and the second was near the meristematic region of the root apex, where the root cap meets the apex proper (or approximately adjacent to the quiescent center). The results collected from these positions were qualitatively similar, and, for clarity, only the results from the second position will be presented here. An average current value was calculated from the measurements collected during a 5- to 10-min period.

Because many roots required more than the 2 h allowed for recovery from handling, measurements were typically made in control solution for 5 to 7 h before Al was added to obtain reliable pretreatment estimates of current magnitude and root growth. Currents and root growth were then monitored for an additional 4 to 7 h after the first addition of Al. Solutions were gently aspirated off and renewed every 2 to 3 h with the control solution (0.1 mM $CaCl_2$, pH 4.5) or with a similar solution containing 10 μM Al.

Measurement of Ion Fluxes

The net flux, J_i, of an ion, i, is given by Equation 2, in which $(d\mu_i)/(dx)$ is electrochemical potential gradient, u_i is the

mobility of ion i, and C_i is the concentration.

$$J_i = -u_i C_i \cdot \frac{(d\mu_i)}{(dx)} \quad (2)$$

An ion-selective microelectrode can provide an estimate for $(d\mu_i)/(dx)$ by measuring the electrochemical potential difference, $\Delta\mu_i$, between two points radial from the root surface, a distance Δx apart (where $\Delta\mu_i = z_i F \Delta V$, and ΔV is the measured voltage, F is the Faraday constant and, z_i, the valency at the ion). The value of $(\Delta\mu_i)/(\Delta x)$ can be substituted into Equation 2 to calculate the net flux. The theory of the ion-selective electrode technique and the construction of the electrodes has been described previously (19, 26). The H^+-, K^+-, and Ca^{2+}-selective cocktails were purchased from Fluka Chemical Company, and the Cl^--selective cocktail was from Orion Research Incorporated.

The general experimental procedure is similar to that described for the net current measurements. The seedlings were left to recover for 2 h before measurements began. Readings were made with the pipette positioned 50 and 100 μm from the root surface, adjacent to the quiescent center as described above. The results presented here were calculated with planar geometry, which, for a cylindrical wheat root of 200 μm radius, will underestimate the flux occurring at the root surface by approximately 30%. Because the errors associated with both the current and the flux measurements were similar and constant, their magnitudes can be compared directly without the need for an additional correction ($I_i = z_i \cdot F \cdot J_i$; where I_i is the current carried by ion i, z_i is its valency, J_i is its flux, and F is the Faraday constant). The flux value was the average from three to six replicate measurements, and other aspects of the experimental protocol are identical with those described above.

The electric field surrounding most organs or cells in an aqueous environment is usually weak, and its contribution is often ignored in the calculation of net fluxes. There is some concern that the presence of a sufficiently large electric field may confound the measurements of ion fluxes using the technique described here. In this work, an electric field of 10 to 80 mV cm^{-1} was consistently measured near the root surface at the apex, and the potential interference of these fields with the measurement of ionic fluxes needed consideration. Previous investigators (26, 31) have concluded that the main source of interference from an electric field will not come from errors in estimating the $(d\mu_i)/(dx)$ term in Equation 2 but from uncertainty in the value of the ion concentration, which is assumed to be known independently. In the present work, the voltage difference measured between the bulk solution and the zone around the root apex was found to be <2.0 mV, and therefore, the associated offset in the electrode calibration will be relatively small (<9% when $z = 1$).

Root Growth Rate

Root elongation was monitored during the course of an experiment by measuring the distance between the root tip and a stationary reference point with an eyepiece micrometer. Where possible, these measurements were made along the center axis of the root to minimize errors due to root curvature. The resolution of these length measurements was approximately 50 μm. Growth rate was estimated by dividing each increment in length by the time elapsed, and, for data presentation, growth rate was plotted at a point midway between the times at which root length was measured.

RESULTS

Net Ionic Currents

The vibrating voltage probe was used to measure the currents along the length of horizontally positioned roots. Typically, a net current of 1.0 to 4.0 μA cm^{-2} entered the apical 2.0 to 3.0 mm, and a smaller net outward current was observed in the more mature root regions. The magnitude of the current and the crossover point were not constant but varied between roots and to some extent with time. Figure 1 shows representative current patterns along Scout 66 roots measured in control solution. The current pattern for Atlas 66 roots was the same as that shown for Scout 66.

Net ionic currents at the root apex were also monitored through time under control conditions (data not shown). The average magnitudes of inward current (1.0–4.0 μA cm^{-2}) and growth rate (0.5–0.8 mm h^{-1}) were found to be relatively constant over 10 h in both cultivars.

Effect of Al on Net Ionic Currents

Exposure of Al-tolerant Atlas 66 roots to a solution containing 10 μM Al (pH 4.5) had no effect on either the average net current measured at the apex or on the growth rate for up to 7 h. Figure 2 shows the results from two of five replicate experiments. In the Al-sensitive Scout 66 seedlings, exposure to 10 μM Al caused the rate of root elongation and the net inward current to start declining within 1 to 3 h. Figure 3 shows the simultaneous current and growth measurements from four of the eight replicate experiments conducted. In six

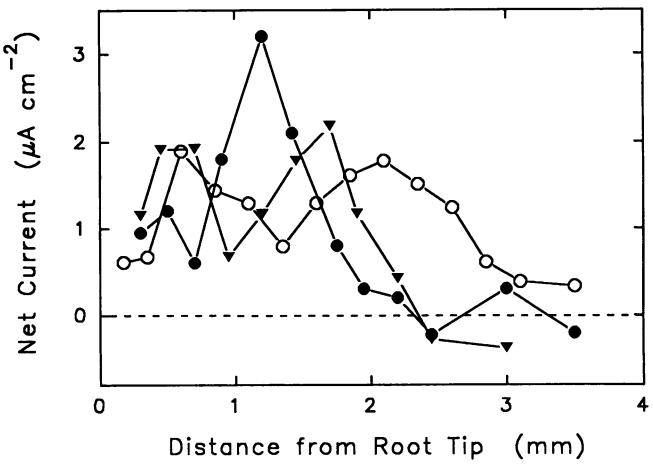

Figure 1. Representative profiles of the net ionic current density measured along 6-d-old Scout 66 roots with a vibrating voltage probe. The distance at which net inward current (positive) turned to a net outward current (negative) was not constant but varied between roots and also with time.

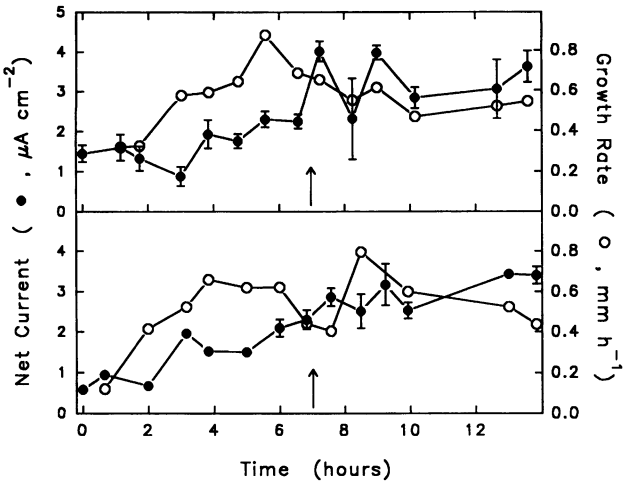

Figure 2. Results from two replicate experiments showing the lack of effect of 10 μM Al (in a solution of 0.1 mM $CaCl_2$, pH 4.5) on either the rate of root growth (O) or net ionic currents (●) measured at the apices of Al-tolerant Atlas 66 roots. Arrows indicate when Al was first introduced into the chamber. The external solution was renewed every 2 to 3 h with fresh solution. Each data point is the mean ± SE for three to six measurements.

of the eight experiments, a close relationship was observed between growth rate and current, and plots of current against growth rate generated significant correlations (*e.g.* Fig. 3, A and B, *r* values are shown). The correlation became very significant when data were pooled from all experiments, as shown in Figure 4 ($r = 0.57$, $n = 98$, $P < 0.01$). Note that a residual inward current entering the root apex was always detected, even after root growth had been severely inhibited by Al treatment.

Despite the general correlation found between net current and growth rate, calculated over the entire length of an experiment (Figs. 3 and 4), there were indications that the onset of these changes, in response to Al exposure, was not simultaneous in all roots. In two of the eight experiments, a decrease in growth rate preceded a sustained reduction in current by nearly 2 h (Fig. 3, C and D).

To further examine the temporal relationship between net current and root growth, the data from all experiments were normalized and plotted to show the relative changes occurring after the addition of Al (Fig. 5). Normalization involved calculating pre-Al values for current and growth rate from each experiment. All measurements collected for the 3- to 4-h period before Al addition were averaged, and every data point subsequent to Al addition was then divided by this initial value and plotted against time. Regressions through the growth rate and current data were not significantly different from one another (Fig. 5). When data are pooled from all experiments, therefore, Al appears to inhibit current and growth rate in a similar manner.

Ion Fluxes at the Root Apex

Ion-selective microelectrodes were used to estimate the net fluxes of K^+, Cl^-, Ca^{2+}, and H^+ at the root apex. These results and calculations of an average equivalent current for each ion are summarized in Table I. Data in Table I suggest that the large net H^+ influx accounts for most of the measured current (*cf.* with Figs. 2 and 3), with small variable contributions from Ca^{2+} influx and Cl^- efflux. K^+ efflux was small and not significantly different from 0 in many cases. The direction of these fluxes was generally stable for many hours under control conditions, but the magnitudes sometimes varied with time. Figure 6 shows control data from Scout 66 roots during a 10-h period; the typical patterns of K^+ and Cl^- efflux and Ca^{2+} and H^+ influx are shown.

Effect of Al on Net Ion Fluxes

Exposure of roots to Al for up to 7 h had no detectable effect on K^+ or Cl^- fluxes in either cultivar (data not shown). The addition of 10 μM Al to the Al-tolerant Atlas 66 seedlings had no effect on the net H^+ fluxes measured at the apex (data

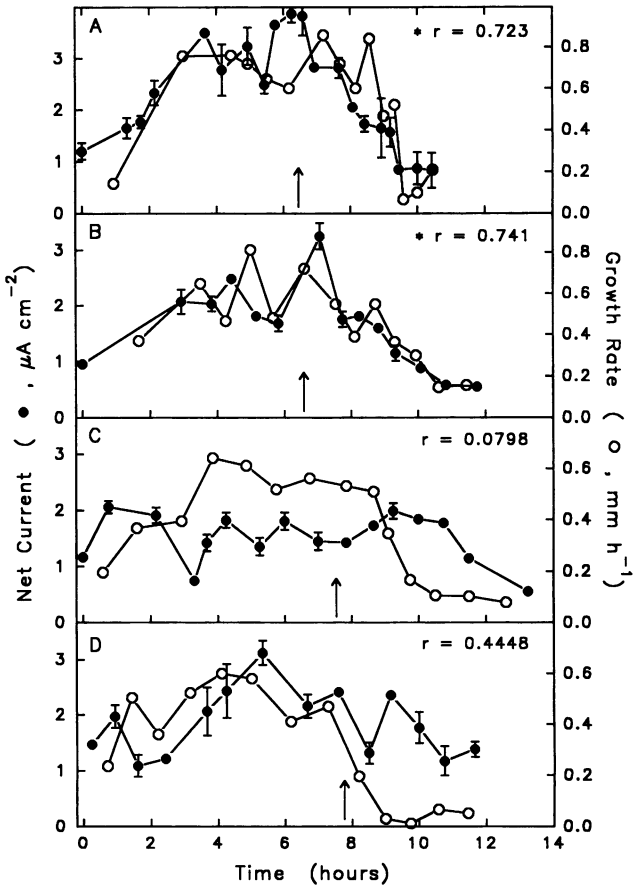

Figure 3. Results from four of eight replicate experiments showing the effect of 10 μM Al (in 0.1 mM $CaCl_2$, pH 4.5) on the rate of root growth (O) and net ionic current (●) measured at the apices of Al-sensitive Scout 66 roots. Arrows indicate when Al was first introduced into the chamber, and solutions were renewed every 2 to 3 h. The extent to which the magnitudes of growth rate and current are correlated in each experiment is given by *r*, the correlation coefficient, where an asterisk (*) indicates a significant correlation ($P = 0.05$). Each data point is the mean ± SE for three to six measurements.

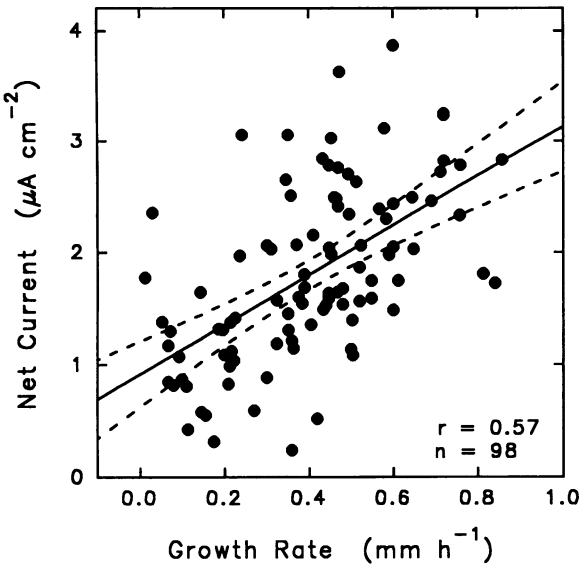

Figure 4. Correlation between root growth rate and net ionic current. Data were pooled from eight replicate experiments with Scout 66 roots, and ionic current at the apex was plotted as a function of root growth rate. The external solution was 0.1 mM $CaCl_2$ at pH 4.5. The correlation coefficient, *r*, was significant at P = 0.01 and the 95% confidence limits of the regression are shown.

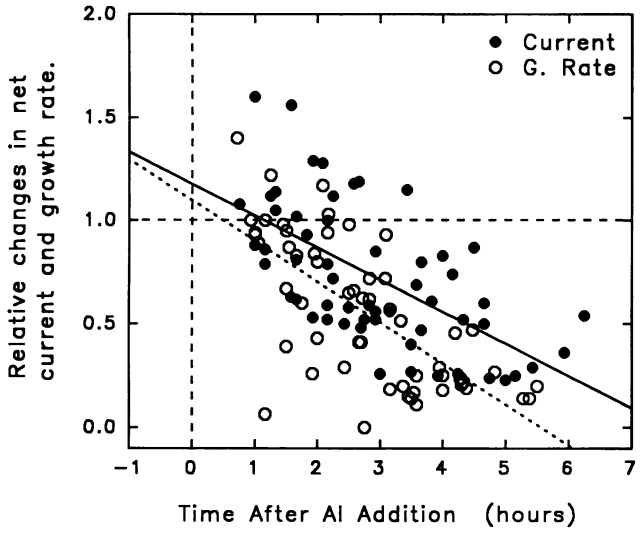

Figure 5. Data from eight replicate experiments with Scout 66 roots were pooled, normalized, and plotted against time to show the relative changes in growth rate (O) and net current (●) occurring after the addition of Al. Pre-Al values for growth rate and current were calculated for each experiment by averaging all measurements collected during a 4-h period before the addition of Al. Each measurement subsequent to Al addition was then divided by the pre-Al value and plotted against time. The solid line is the regression through the normalized current data, and the dotted line is the regression through the normalized growth rates. A statistical comparison of regression lines found no difference between the slopes or intercepts of these lines (P = 0.01).

Table I. *Average Net Ion Fluxes Detected at the Root Apex*

A summary of the net ion fluxes measured at the root apex with ion-selective microelectrodes and the equivalent current produced by these fluxes (see "Materials and Methods"). Positive fluxes represent a net influx, and negative fluxes represent a net efflux. Positive current is defined as the net inward movement of positive charge. The bathing solution was 0.1 mM $CaCl_2$, pH 4.5.

Ion	Flux Measurements		Equivalent Current
	Range	Approximate mean	
	pmol cm^{-2}s^{-1}		*μA cm^{-2}*
K^+	−5.0 to +0.2	−2.0	−0.2
Cl^-	−40.0 to 0.0	−8.0	+0.8
Ca^{2+}	−0.1 to +20.0	+4.0	+0.8
H^+	+10.0 to +60.0	+25.0	+2.5
			Net current +3.9

not shown). Net H^+ flux in the Al-sensitive Scout 66 began to decrease within 3 h of Al addition, and the data from four of the eight replicate experiments using Scout 66 seedlings are plotted in Figure 7. In half of the experiments, a significant correlation was found between the magnitude of the H^+ fluxes and growth rate calculated over the entire experiment (*e.g.* Fig. 7, a and b; *r* values as shown). In other experiments, the changes in H^+ flux and growth rate following Al addition were not coincidental (growth rate decreasing before flux), and no significant correlations were detected (*e.g.* Fig. 7, c and d). On most occasions, a small residual H^+ influx was detectable despite the Al-induced inhibition of root growth.

As was found for the current measurements, a significant correlation ($P < 0.01$) existed between root growth and the magnitude of H^+ flux when data were combined from all experiments (data not shown). Notwithstanding, the changes

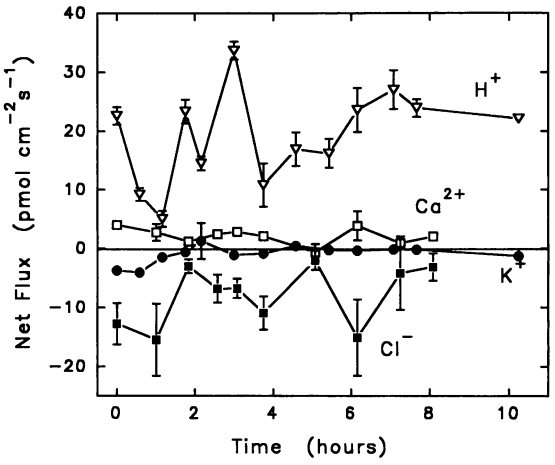

Figure 6. Representative fluxes of H^+, K^+, Ca^{2+}, and Cl^- at the apices of Scout 66 roots measured through time. The external solution was 0.1 mM $CaCl_2$ at pH 4.5. Positive values refer to a net influx of an ion and negative values to a net efflux. Flux measurements underestimate the fluxes occurring at the root surface by a constant value of approximately 30% (see "Materials and Methods"). Each data point is the mean ± SE from three to six measurements.

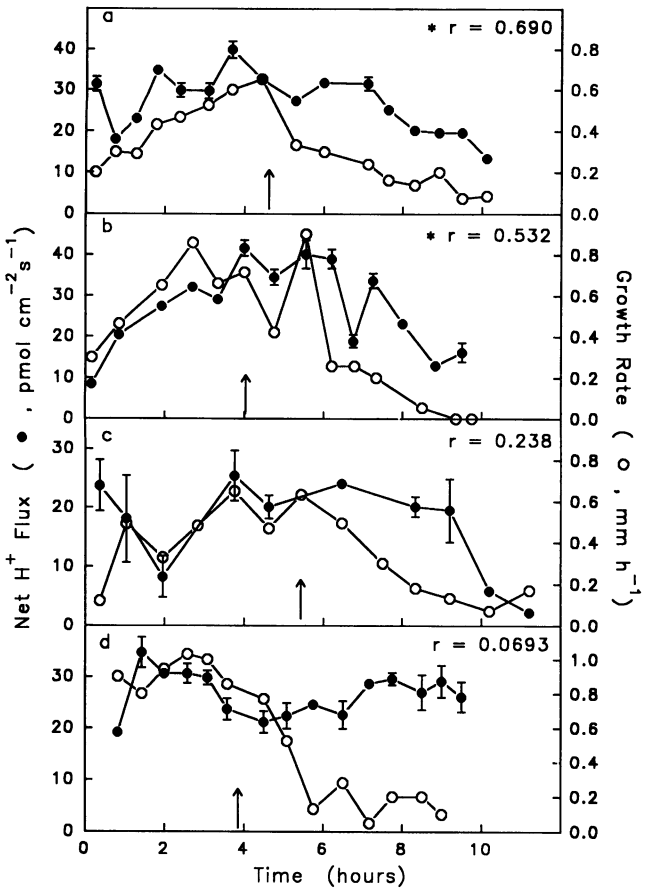

Figure 7. Results from four of eight replicate experiments showing the effect of 10 μM Al (in 0.1 mM $CaCl_2$, pH 4.5) on the growth rate (O) and net H^+ fluxes (●) at the apices of Al-sensitive Scout 66 roots. Arrows indicate when Al was first introduced into the chamber. Solutions were renewed every 2 to 3 h. The extent to which the magnitude of the fluxes were correlated with growth rate is indicated by the correlation coefficient, *r*, where an asterisk (*) indicates a significance at P = 0.05. Flux measurements underestimate the fluxes occurring at the root surface by a constant value of approximately 30% (see "Materials and Methods"). Each data point is the mean ± SE for three to six measurements.

in H^+ flux and growth rate in response to Al exposure did not occur simultaneously on every root measured. This was examined further with normalized data (Fig. 8) following the same procedure described above for the current measurements. Regression lines were drawn through the normalized data, and an analysis of covariance was performed to compare the lines. No difference was found between their slopes, but the intercepts were significantly different from one another ($P < 0.05$). This shows that Al began to inhibit root growth before H^+ fluxes declined. After 5 h, growth had slowed considerably, but net H^+ flux was still 60% of the initial flux.

The measured Ca^{2+} fluxes were often small and variable in control conditions. Addition of Al had no detectable effect on Ca^{2+} fluxes in Atlas 66 seedlings. The results with Scout 66 were similar, and in five of the seven experiments no detectable effect of Al on Ca^{2+} fluxes was observed (data not shown). In two cases, the addition of Al caused an instantaneous reduction in Ca^{2+} influx (data not shown).

DISCUSSION

Apical Currents and Growth

The pattern and magnitude of net currents reported here, measured under control conditions, are similar to those reported previously for roots (1, 16, 20, 22, 33). The addition of Al to Al-tolerant Atlas 66 had no effect on either the growth rate or measured currents (Fig. 2), which is also in agreement with previous observations (16). However, in Scout 66, similar Al treatments began to inhibit the currents within 2 to 3 h and, in six of the eight experiments, a significant correlation was found between the decline in current magnitude and the reduction in root growth (*e.g.* Fig. 3, A and B). Furthermore, normalization of the pooled data also suggested that current and growth were inhibited in a similar manner following the addition of Al (Fig. 5). There were, however, indications from individual roots that this association was not causal (*e.g.* Fig. 3, C and D). In two of the eight experiments, a large reduction in growth rate was observed before any sustained decrease in current; the reverse would be expected if the flow of current was an important effector of growth. Therefore, despite the general correlation

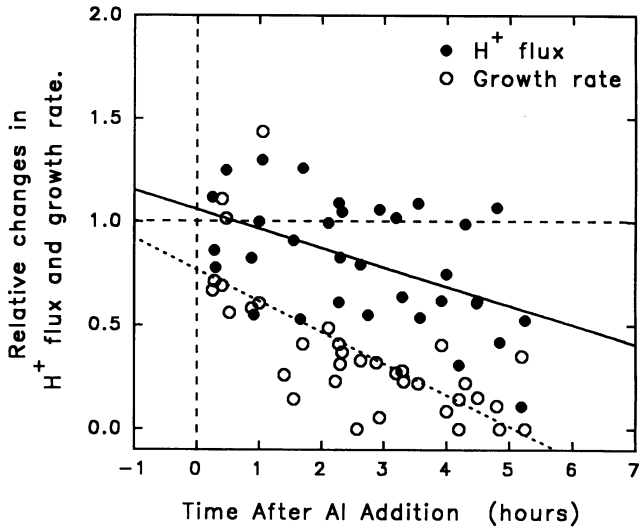

Figure 8. Data from eight replicate experiments with Scout 66 roots were pooled, normalized, and plotted against time to show the relative changes in growth rate (O) and net H^+ fluxes (●) occurring after the addition of Al. Pre-Al values for growth rate and H^+ flux were calculated for each experiment by averaging all measurements collected during a 4-h period before the addition of Al. Each measurement subsequent to Al addition was then divided by the pre-Al value and plotted against time. The solid line is the regression through the normalized H^+ flux data and the dotted line is the regression through the normalized growth rates. An analysis of covariance between the regressions found the slopes to be the same, but the intercepts were significantly different (P = 0.05). Flux measurements underestimate the fluxes occurring at the root surface by a constant value of approximately 30% (see "Materials and Methods").

established between these processes, we believe that these exceptions are sufficient to reject the hypothesis that growth is directly dependent upon the magnitude of current in this system. Instead, we agree in general terms with the conclusions of Kropf *et al.* (17) and Harold (7) based on their work on the water mold *Achlya*. They concluded that, although no mutual dependence exists between current and growth, an indirect association is preserved by the continued separation of transport sites during growth. We suggest that the Al-induced decrease in ionic current cannot be responsible for the inhibition of growth in these roots.

Identity of the Current-Carrying Ions

The general pattern of H^+ and Ca^{2+} influx and Cl^- and K^+ efflux at root apices agrees, in part, with previous studies using the same technique (in wheat [24] and maize [31]). It is also encouraging to note that the net current predicted from summing the ion fluxes in Table I (*i.e.* +3.9 μA cm^{-2}) is similar to the current measured with the vibrating voltage probe. The conclusion that the inward current is carried primarily by protons is consistent with the results of some previous studies of roots in which H^+ fluxes were not measured directly (1, 14, 21). Inhibition of H^+ influx in Scout 66 roots by Al occurred within 1 to 3 h, and in more than half the experiments performed, this coincided with the inhibition of root growth (*e.g.* Fig. 7, a and b). When data were pooled from all eight experiments, a significant correlation was found between these processes. However, no significant correlation was observed between these measurements in about 50% of the individual experiments, and in three of the eight replicates, growth rate was clearly inhibited before H^+ influx (*e.g.* Fig. 7, c and d). Again, this is the reverse of what would be expected if net H^+ flux were an important growth-determining process. Furthermore, when data from all experiments were normalized (Fig. 8), a statistical analysis showed that Al inhibited growth rate before the H^+ fluxes. Therefore, although they indicate a general association between H^+ flux and growth, these results do not suggest a direct mechanistic association. The inhibition of root growth by Al is unlikely to be caused by the reduction in H^+ fluxes at the root apex.

A Role for Cl^- or Ca^{2+}?

Miller and Gow (21) concluded that in roots of *Zea mays* Ca^{2+} influx was not contributing to the net inward current because removal of external Ca^{2+} and addition of 1.0 mM EGTA increased the inward current density around the root apex. They suggested, instead, that external Ca^{2+} might be regulating the current loop. However, it is possible that the EGTA treatment was harmful to the membranes and that the measured stimulation of current indicated a wounding response (11, 23).

In the present work, results shown in Table I suggest that Ca^{2+} and Cl^- fluxes did contribute to the measured currents. The processes resulting in net efflux of Cl^- (and K^+) around the root apex are unclear, but the lack of any effect of Al on these processes in Scout 66 roots indicates that they are not involved with the Al toxicity response (data not shown). It is well documented that Al can interfere with Ca^{2+} nutrition in plants (5, 30, 32), and some evidence suggests a competitive interaction between Al and Ca^{2+} at the membrane surface (6, 10, 18). However, in the present work, Al had no effect on Ca^{2+} influx in five of seven experiments. In the remaining two experiments, Al addition caused a reduction in Ca^{2+} flux. The Ca^{2+} measurements were approaching the limits of detection of the system (31), and we cannot draw any solid conclusions from these results. It is possible that small but significant changes in Ca^{2+} flux went undetected in other experiments due to the temporal variability in the flux measurements.

Using the same technique, Huang *et al.* (10) were able to examine the interaction between Al treatment and Ca^{2+} fluxes in these same cultivars by performing the experiments in a greatly reduced external Ca^{2+} concentration. This has the advantage of increasing the signal-to-noise ratio of the ion-selective microelectrode. With a solution containing 20 μM Ca^{2+}, they found that Ca^{2+} influx near the root apex was inhibited by 5 μM Al in Scout 66, but not Atlas 66, roots. This differential effect of Al on Ca^{2+} fluxes indicates an important difference between these cultivars and offers a promising area in which to investigate the mechanism of Al tolerance in other species.

Ion Fluxes at the Root Apex

Although the alkalinity around root apices has been reported previously (33), our lack of knowledge about the ion transport processes in this region forces us to speculate about the mechanisms involved. One explanation for this zone of H^+ influx (or OH^- release) would be a localization of H^+ co-transport systems. Being a metabolically active part of the root, the apex is an important sink for phloem unloading, and, although the recent evidence tends to support a mostly symplastic mechanism for phloem unloading at the root tip (3, 25), some unloading may be occurring into the apoplasm as well (29). The measured H^+ influx, therefore, may indicate the cotransport of unloaded sugars and amino acids back into the cytoplasm. It is also possible that, because of the rapid rate of cell division and elongation, the membranes in cells of the root apex are simply more permeable to protons. The delivery and release, by exocytosis, of membrane and cell wall precursors at the plasmalemma could weaken the membrane or influence local pH. Furthermore, protons may be required for cell wall formation, suggesting that not all of the measured H^+ influx will necessarily cross the plasmalemma. Alternatively, the release of dissociated organic acids or HCO_3^- in sufficient amounts would also give the appearance of a net H^+ influx. In the acidic environment of the cell wall, the acid anions and HCO_3^- would become protonated, increasing the local pH and creating a H^+ gradient.

External calcium is also necessary for cell wall expansion, and the bulk of cellular calcium in plants resides in the apoplasm (2, 9). This suggests that a proportion of the measured calcium uptake into this region of active cell division and expansion will not cross any membrane but remain in the apoplasm and be incorporated into the developing cell wall and membranes.

CONCLUSIONS

The current patterns measured around the apex of growing wheat roots are consistent with those reported elsewhere for roots and many single-cell, tip-growing systems. Addition of toxic levels of Al began to inhibit root elongation and current magnitude within 1 to 3 h in Al-sensitive Scout 66, but not in Al-tolerant Atlas 66, roots, and a significant correlation was found between root growth and inward current. We attribute the bulk of this current to H^+ influx, with smaller contributions from Ca^{2+} influx and Cl^- efflux. However, the Al-induced inhibition of both current and H^+ influx at best coincided with, and at worst lagged behind, the reduction in root growth. Therefore, it is suggested that the apical current and H^+ flux, although closely associated with growth, do not regulate it. We conclude that the inhibition of root elongation by Al is not directly caused by the disruption of these currents or H^+ fluxes.

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