Plant Physiol. (1997) 115: 657–667

Possible Involvement of Al-Induced Electrical Signals in Al Tolerance in Wheat[1]

Lisa A. Papernik and Leon V. Kochian*

United States Plant, Soil, and Nutrition Laboratory, United States Department of Agriculture-Agricultural Research Service, Cornell University, Ithaca, New York 14853

The relationship between Al-induced depolarization of root-cell transmembrane electrical potentials (E_m) and Al tolerance in wheat (*Triticum aestivum* L.) was investigated. Al exposure induced depolarizations of E_m in the Al-tolerant wheat cultivars Atlas and ET3, but not in the Al-sensitive wheat cultivars Scout and ES3. The depolarizations of E_m occurred in root cap cells and as far back as 10 mm from the root tip. The depolarization was specific to Al^{3+}; no depolarization was observed when roots were exposed to the rhizotoxic trivalent cation La^{3+}. The Al-induced depolarization occurred in the presence of anion-channel antagonists that blocked the release of malate, indicating that the depolarization is not due to the electrogenic efflux of malate^{2-}. K^+-induced depolarizations in the root cap were of the same magnitude as Al-induced depolarizations, but did not trigger malate release, indicating that Al-induced depolarization of root cap cell membrane potentials is probably linked to, but is not sufficient to trigger, malate release.

Al is present in all soils, but Al toxicity is manifested only in acid conditions, in which the phytotoxic form Al^{3+} predominates. The major toxicity symptom observed in plants is inhibition of root growth (Taylor, 1988; Delhaize and Ryan, 1995; Kochian, 1995). Within plant species there is considerable genetic variation in tolerance to Al. In wheat (*Triticum aestivum* L.) Al-tolerant lines tolerate Al concentrations that are up to 10 times greater than Al levels that inhibit root growth in Al-sensitive lines (Delhaize and Ryan, 1995).

Many Al-tolerant lines of wheat release malate in response to Al (Delhaize et al., 1993; Basu et al., 1994; Ryan et al., 1995b; Huang et al., 1996), whereas Al-tolerant lines of maize (*Zea mays* L.) and snapbean (*Phaseolus vulgaris* L.) release citrate upon exposure to Al (Miyasaka et al., 1991; Pellet et al., 1995). This exudation of Al-chelating compounds from root tips serves as a mechanism of Al tolerance by lowering the activity of free Al in the rhizosphere, and thus excluding Al from the plant. The level of organic acid exudation is dependent on both the amount of Al and the duration of exposure. In wheat the rate of malate exudation is constant over time for exposure to a specific level of Al (Ryan et al., 1995a). When the Al level is increased, the rate of exudation initially increases linearly (Ryan et al., 1995a; Pellet et al., 1996) but eventually saturates (Ryan et al., 1995a). In maize the rate of citrate exudation increases over time during an exposure to a constant level of Al, but diminishes when Al is increased to phytotoxic levels (Pellet et al., 1995).

It is likely that malate exudation in wheat and citrate exudation in snapbean and maize occur via plasma membrane anion channels (Delhaize and Ryan, 1995; Kochian, 1995; Ryan et al., 1995a; Schroeder, 1995). This is because in the cytoplasm (pH approximately 7.0) both malate and citrate exist as anions. Thus, transport of either anion out of the cytoplasm and into the external solution is a thermodynamically passive process, proceeding down a fairly steep electrochemical potential gradient. Activation of a malate- or citrate-permeable anion channel would allow a large, passive efflux of either anion. Further evidence for the involvement of anion channels in Al-induced organic acid release comes from Ryan et al. (1995a), who demonstrated that several plant anion-channel antagonists that had been shown in previous patch-clamp studies to block anion currents in plant cells (Marten et al., 1992; Schroeder, 1995; Schwartz et al., 1995) inhibited Al-induced malate efflux in Al-tolerant wheat.

Recently, a number of different plasma membrane anion channels have been described in plant cells (Tyerman, 1992), including: (a) stretch-activated anion channels in broad bean (*Vicia faba* L.) guard cell protoplasts (Cosgrove and Hedrich, 1991); (b) hyperpolarization-activated anion channels encoded by tobacco (*Nicotiana tabacum* L.) cDNA (Lurin et al., 1996); (c) depolarization-activated anion channels that pass an outward current (anion influx) in protoplasts derived from wheat roots (Skerrett and Tyerman, 1994); and (d) depolarization-activated anion channels that pass an inward current (anion efflux) in epidermal cells of Arabidopsis hypocotyls (Thomine et al., 1995), tobacco protoplasts (Zimmermann et al., 1994), and guard cells (Marten et al., 1992; Hedrich and Marten, 1993; Hedrich et al., 1994; Schwartz et al., 1995; Ward et al., 1995). For some of these channels, the voltage regulation and/or kinetic behavior may be modulated by substances such as auxin (Zimmermann et al., 1994; Schroeder, 1995), ABA (Schroeder, 1995), ATP (Zimmermann et al., 1994; Thomine et al., 1995), cytoplasmic Ca^{2+} (Skerrett and Tyerman, 1994;

[1] This work was supported by U.S. Department of Agriculture/National Research Initiative Competitive Grants Program grant no. 96-35100-3213 to L.V.K.

* Corresponding author; e-mail lvk1@cornell.edu; fax 1–607–255–2459.

Abbreviations: A-9-C, anthracene-9-carboxylic acid; E_m, membrane potential; TEA, tetraethylammonium.

Schroeder, 1995; Ward et al., 1995), or extracellular malate (Hedrich and Marten, 1993; Hedrich et al., 1994). Most of the above studies focused on transport of Cl^-, but selectivity studies show that many of these channels are permeable to malate as well (Hedrich and Marten, 1993; Schroeder, 1995).

Whereas the signal transduction pathway between Al exposure and organic acid exudation is not known, the likelihood that exudation occurs via anion channels raises the intriguing question of how these channels might be gated. Recently, Olivetti et al. (1995) reported a depolarization in the E_m of root cap cells of the Al-tolerant snapbean cv Dade in response to Al, the same variety previously shown to release citrate in response to Al exposure (Miyasaka et al., 1991). They suggested that Al caused this depolarization by decreasing K^+ channel conductance. In this study we report that root cells of Al-tolerant wheat also show a depolarization in response to Al. We wondered whether this change in voltage might be a signal that leads to the exudation of organic acids when Al-tolerant plants are exposed to Al. More specifically, does this depolarization activate voltage-gated anion channels in cells of the root cap and/or apex?

We also considered an opposing explanation for the depolarization. Since the exudation of citrate (in snapbean and maize) or malate (in wheat) involves the transport of anions across the plasma membrane from the cytoplasm to the external solution, if there were no immediate compensation of charge, this would cause the E_m to depolarize. In this case, the depolarization would not be involved in the signal transduction pathway.

The objective of this study was to examine the effect of Al on E_m of cells of wheat roots. Previous studies that measured E_m of wheat roots in the presence of Al reported mixed results (Kinraide, 1988; Miyasaka et al., 1989; Huang et al., 1992; Kinraide, 1993). In this study E_m of two Al-tolerant lines of wheat (cvs Atlas and ET3) and two Al-sensitive lines of wheat (cvs Scout and ES3) were measured at three positions along the root (cap and 1 and 10 mm back from the root tip) in the presence of different concentrations of Al (as $AlCl_3$). An Al-induced depolarization was present only in the Al-tolerant lines of wheat. We also report that the depolarization observed was specific to Al and was not caused by Cl or other trivalent cations. In addition, we investigated whether the Al-induced electrical responses we observed might be involved in malate exudation triggered by Al exposure. We examined the effect of anion-channel blockers on both the depolarization and on malate exudation, and we investigated whether other compounds that depolarize the E_m and compounds that block K^+ channels were able to cause malate exudation.

MATERIALS AND METHODS

Seeds of winter wheat (*Triticum aestivum* L.) cvs Atlas 66 (Al-tolerant) and Scout 66 (Al-sensitive) were supplied by Dr. J. Peterson (University of Nebraska, Lincoln). Seeds of the near-isogenic lines ET3 (Al-tolerant) and ES3 (Al-sensitive), in which Al tolerance is due to the single gene locus, *Alt1*, were obtained from Dr. E. Delhaize (Commonwealth Scientific and Industrial Research Organization, Canberra, Australia).

Seedling Growth

For the electrophysiology studies and measurements of root elongation, seeds were surface-sterilized in 0.5% NaOCl for 15 min, rinsed in distilled water for 15 min, and germinated on moist filter paper in the dark for 2 d. Germinated seedlings were placed in polyethylene cups with mesh bottoms (two seedlings/cup) and covered with black polyethylene beads. Four or six cups were placed into precut holes on the lids of 900-mL pots. The mesh bottoms of the cups were flush with the top of 0.2 mM $CaCl_2$ solution (pH 4.5, adjusted with HCl). The pots were aerated and kept in a growth chamber with a 20°C day (16 h)/15°C night (8 h) cycle for 3 d.

Electrophysiological Studies

A single seedling was positioned in a Plexiglas chamber mounted on the stage of a compound microscope (Olympus). The microscope was mounted on its back on a vibration-damped table (model 61231, Micro-G, Technical Manufacturing Corp., Woburn, MA) inside a Faraday cage. Seedlings were equilibrated in 200 μM $CaCl_2$ solution (pH 4.5) for at least 20 min before impalements were made, unless otherwise noted. A flow-through system was used to deliver solution at a rate of approximately 4.4 mL/min throughout the measurement of root-cell E_m. Solution flowed in the direction counter to root growth. Treatment solutions (pH 4.5) all contained 200 μM $CaCl_2$ and the concentrations of $AlCl_3$, A-9-C, niflumic acid, $LaCl_3$, K_2SO_4, or TEA-Cl are detailed in the sections describing specific experiments.

E_m was measured using an impaling microelectrode made from single-barreled borosilicate glass filled with 3 M KCl (adjusted to pH 2.0 with HCl to reduce tip potentials). An amplifier (model KS-700, WPI, Microelectrodes, Inc., Londonderry, NH) and a single reference electrode (model MI-409 Micro-Reference Electrode, Microelectrodes, Inc.) filled with KCl were used to measure root-cell E_m. Cells of the root epidermis, cortex, and root cap were impaled using a hydraulically driven micromanipulator (model MO-102, Narishige USA, East Meadow, NY) mounted on the microscope stage.

Impalements were made in one of three positions: within the root cap, 1 mm back from the tip of the cap (approximately 600 μm from the apex), or 10 mm back from the tip of the cap. The seedling was mounted in a different manner, depending on the position of the impalement.

Impalements at 1- and 10-mm Positions

For these impalements, the seedling was placed in the Plexiglas chamber such that the longest root rested on platinum pins spaced ½-inch apart. Clips made out of Tygon tubing were wedged into the chamber and held the root in place on either side of a pin. The impalement was done directly over a supporting pin.

Root Cap Impalements

The root cap of wheat was usually 300 to 400 μm long, and the impalements were made at a position approximately 250 μm back from the root cap tip. It was difficult to secure the root cap in a manner so that it was sufficiently immobilized to permit impalement yet allowed for root elongation. Due to the conical shape of the cap, when the root was flush with the bottom of the chamber, the cap itself usually did not touch the bottom of the chamber. Initial attempts at impaling cells of the root cap failed because although the root was held in place, the root cap was able to move away from the impaling electrode. To solve this problem, a capillary tube was dipped into silicone adhesive/sealant (3145RTV MIL-A-46146, Dow Corning, Wolcott-Park, Rochester, NY) and then gently dragged along the floor of the chamber to create a silicone wedge. After the adhesive/sealant had cured for a few minutes, a few drops of 200 μM $CaCl_2$ were placed in the chamber. The seedling was then placed in the chamber such that the root was flush with the platform, and the root cap was flush with the silicone wedge. In effect, the silicone served as a cushion under the root cap. A notched Plexiglas block was coated with either vacuum grease or blue tac (Bostik Ltd., Leicester, UK) and used to hold the root near the cap firmly to the platform. This technique improved the success rate for impalements and permitted the root to grow; however, the success rate for root cap impalements was still low relative to the success rate for impalements in the mature region of the root. Furthermore, it was difficult to maintain the successful impalements because as the root grew, the root cap was pushed ahead and the electrode was bent. Thus, during the course of an experiment it was necessary to periodically move the electrode with the micromanipulator in the direction of root growth. This "unbending of the electrode" was done at irregular intervals. Sometimes the impalement was lost when the electrode was repositioned.

Measurement of Root Elongation

Seeds were surface-sterilized and germinated and seedlings were grown as described above. A flow-through technique was used to approximate the conditions used during the electrophysiology experiments. Three cv Scout seedlings and three cv Atlas seedlings were placed in each of three 81-cm^2 square Petri dishes that contained control solution (200 μM $CaCl_2$, pH 4.5). Notched Plexiglas blocks (Ryan et al., 1992) smeared with silicon grease were used to secure the roots of each seedling along one end of the Petri dish such that they were evenly spaced, and the tip of the longest root of each seedling was on a common starting line. Then, the control solution was removed by vacuum and replaced with the appropriate treatment solution (0, 20, or 150 μM $AlCl_3$ plus 200 μM $CaCl_2$, pH 4.5). The appropriate treatment solution was flowed via a peristaltic pump at a rate of 4.4 mL/min across each plate in a direction counter to the direction of root growth. Drainage holes allowed the solution to drain at the end of the dish away from the root tips. After 7 h, clear plastic rulers taped to the bottom of each Petri dish were used to measure the new root growth (in millimeters) from the common starting line. The experiment was replicated on two different dates, and the root growth was expressed as percent of control (100 × [growth with Al/growth without Al]).

Root-Exudation Experiments

Aseptic techniques were used throughout the root-exudation experiments to prevent microbial degradation of organic acids. All procedures that involved open containers were conducted in a sterile laminar flow hood. Seeds were sterilized by exposure to Cl_2 gas for 2 h (Huang et al., 1996) and then allowed to germinate in the dark for 1 d at 30°C on sterile Petri plates containing 1% agar and 200 μM $CaCl_2$ (pH 4.5). Two germinated seedlings were placed in sterile 125-mL flasks containing 20 mL of filter-sterilized control solution (200 μM $CaCl_2$, pH 4.5). The flasks were placed on a shaker (130 rpm) in a growth chamber with a 20°C day (16 h)/15°night (8 h) cycle for 4 d. The solutions were decanted from the flasks, and the seedlings were rinsed once with 20 mL of the sterile control solution and a second time with 20 mL of the appropriate filter-sterilized treatment solution. Then 20 mL of the same sterile treatment solution was added to each flask, and the flasks were placed on a shaker in a growth chamber during the day period (see above) for 7 h. Each treatment was replicated six times.

Treatment solutions that were used to examine the effect of compounds that either depolarized E_m or blocked K^+ channels on malate exudation contained 200 μM $CaCl_2$ and 0 μM $AlCl_3$, 50 μM $AlCl_3$, 50 μM K_2SO_4, 5 mM TEA-Cl, 100 μM tetraphenyl phosphonium chloride, or 1 mM $BaCl_2$ (all at pH 4.5). Treatments solutions that were used to examine the effect of the anion-channel blocker A-9-C on malate exudation contained 200 μM $CaCl_2$ (pH 4.5) and either 0, 50, or 100 μM A-9-C ± 75 μM $AlCl_3$. Solutions containing A-9-C were prepared from a stock solution of 5 mM A-9-C, 200 μM $CaCl_2$, and 0.1 N NaOH.

At the termination of the 7-h exudation experiment, solutions were collected and sterility was checked by streaking 20 μL of exudation solution from each flask onto Petri plates containing 1% agar and 200 μM $CaCl_2$ (pH 4.5). The plates were placed in a 30°C incubator in the dark and inspected after 2 d for microbial growth. All results reported here were free of microbial growth. The solutions were weighed and frozen (−20°C) for storage. Subsequently, the solutions were thawed, passed through Ag cartridges (On-guard, Dionex, Sunnyvale, CA) to remove Cl, refrozen, lyophilized, and resuspended in 1.2 mL of water before being analyzed for organic acids and phosphate using an ion-chromatography system (Dionex 300) that included an ion-exchange analytical column (4 mm, AS11, Dionex), eluent gradient of NaOH in 18% high-purity methanol, and a conductivity detector for detection of organic and inorganic anions.

Malate and A-9-C had similar retention times, and as a result their peaks were superimposed on chromatograms. To determine the portion of the malate peak in the 75 μM Al plus 50 μM A-9-C treatment that was actually due to

malate exudation, the A-9-C portion of the peak was mathematically subtracted out.

Anion standards for calibration of the ion-chromatography system were prepared from 2 mM stocks of each of the following: pyruvic acid (Sigma), acetic acid (Aldrich), calcium chloride (Fluka), calcium nitrate (Sigma), L-malic acid (Fluka), calcium sulfate dihydrate (Fluka), ammonium phosphate monobasic (Sigma), citric acid trisodium salt dihydrate (Sigma), DL-*iso*-citrate trisodium salt dihydrate (Fluka), *cis*-aconitic acid (Fluka), and *trans*-aconitic acid (Fluka). Each standard contained all 11 anions. Six different levels were used for calibration: 5, 10, 20, 50, 100, and 150 μM.

RESULTS

Al Effects on Root-Cell E_m

The electrical response of wheat roots to Al was monitored by impaling more than 70 intact seedlings for a total of approximately 100 measurements. When all of these measurements were analyzed, a clear pattern emerged. In approximately 85% of the cases, the E_m of root cells of Al-tolerant varieties depolarized in response to Al, whereas the E_m of Al-sensitive varieties never depolarized in response to Al (Figs. 1, A and B, and 2).

Figure 1A depicts a representative trace for an Al-induced depolarization of a root-cap cell in Al-tolerant cv Atlas. The depolarization is gradual and moderate in magnitude (approximately 30 mV). Upon removal of Al, the E_m gradually recovered to the pre-Al resting potential. In contrast to the response of Al-tolerant Atlas, root cap cells of Al-sensitive cv Scout either showed no change in E_m in response to Al (Fig. 1B) or a slight hyperpolarization was observed (not shown). Note that this Al-induced depolarization in the root cap of the Al-tolerant lines is different from a typical K^+-induced depolarization (Fig. 1C), which is rapid and large in magnitude (approximately 120 mV). Furthermore, the E_m recovers quickly after removal of K^+, unlike the slow recovery after removal of Al.

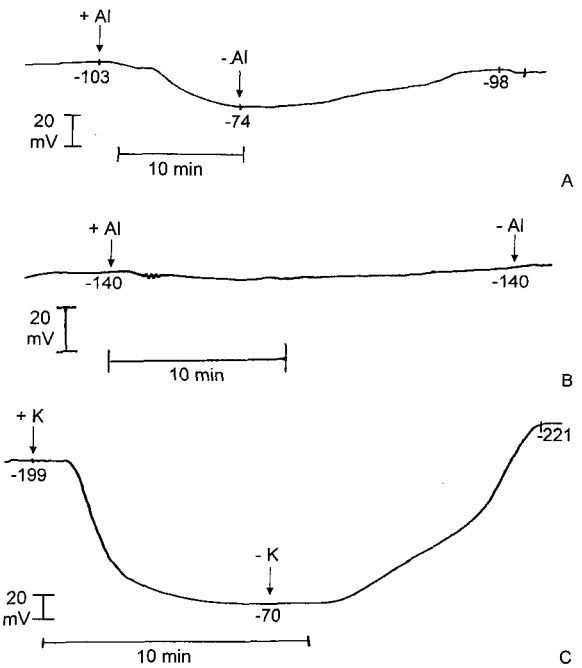

Figure 1. Effect of Al and K^+ on the E_m in wheat roots grown in 200 μM $CaCl_2$ (pH 4.5). Shown are representative traces of impalements in a root cap cell of a cv Atlas seedling exposed to 150 μM $AlCl_3$ plus 200 μM $CaCl_2$ (pH 4.5) (A), a root cap cell of a cv Scout seedling exposed to 150 μM $AlCl_3$ plus 200 μM $CaCl_2$ (pH 4.5) (B), and at a position 10 mm back from root tip in a cv Atlas seedling exposed to 50 μM K_2SO_4 plus 200 μM $CaCl_2$ (pH 4.5) (C).

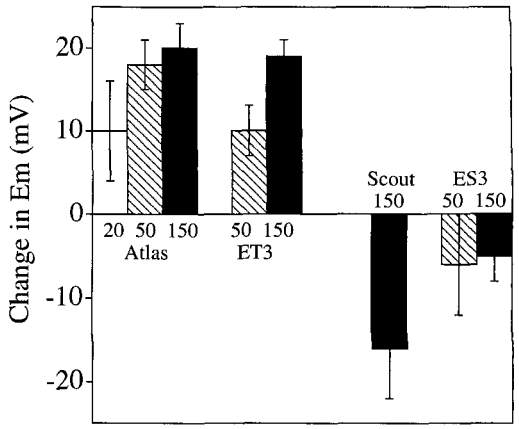

Figure 2. Effect of $AlCl_3$ on changes in the E_m of root cap cells of wheat. Plants were grown in 200 μM $CaCl_2$ (pH 4.5) and exposed to 20, 50, or 150 μM $AlCl_3$ plus 200 μM $CaCl_2$ (pH 4.5). Values for changes in E_m were the average of 7 to 18 replicates ± SE for cv Atlas, ET3, and cv Scout, and the average of 2 to 3 replicates for ES3.

As shown in Figure 2, the average depolarization of cells in the root cap of Al-tolerant cv Atlas in response to 20, 50, or 150 μM $AlCl_3$ ranged between 10 and 20 mV (n = 34). The same level of depolarization was observed in the near-isogenic Al-tolerant wheat line ET3 in response to 50 or 150 μM $AlCl_3$. In contrast, the E_m of root-cap cells of Al-sensitive cv Scout hyperpolarized an average of 16 mV in response to 150 μM $AlCl_3$, and root-cap cells of Al-sensitive wheat line ES3 hyperpolarized an average of 5 mV in response to 50 μM Al and 150 μM $AlCl_3$. These results are in agreement with those of Olivetti et al. (1995), who observed a 55-mV depolarization in the root cap of cv Dade, an Al-tolerant snapbean, in response to 150 μM $AlCl_3$. They also observed a 15-mV, but statistically insignificant, depolarization in the Al-sensitive snapbean cv Romano. In contrast, we observed on average a slight hyperpolarization of E_m of root-cap cells in the two Al-sensitive lines of wheat that we examined.

We were curious whether the depolarization in response to Al was restricted to cells of the root cap. Thus, Al effects on E_m were also studied in roots impaled at either 1 or 10 mm back from the root tip. In cv Atlas Al induced a similar depolarization of E_m at all three positions and not just in the root cap (Fig. 3; Table I), which is in contrast to snapbean, in which Olivetti et al. (1995) reported a significant Al-induced depolarization only in the root cap. Exposure to 150 μM $AlCl_3$ elicited an average depolarization of 16 to

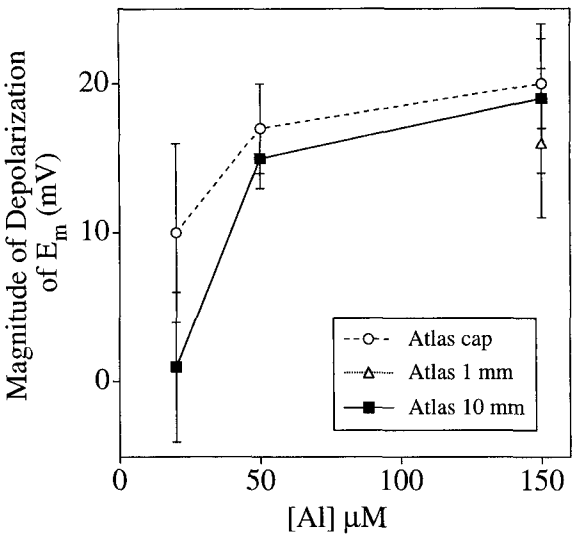

Figure 3. Effect of $AlCl_3$ on the E_m of root cells at three positions along cv Atlas roots. Plants were grown in 200 μM $CaCl_2$ (pH 4.5) and exposed to 20, 50, or 150 μM $AlCl_3$ plus 200 μM $CaCl_2$ (pH 4.5). Values for depolarization E_m were the average of 6 to 18 replicates ± SE, except for the value for 20 μM Al at 10 mm back from the tip, for which $n = 2$.

20 mV, regardless of the position impaled. Even though the magnitude of depolarization was not affected by the position of impalement, the final E_m was dependent on the position along the root. This is because the absolute magnitude of the initial resting potential increased with distance from the root tip. As shown in Table I, the average resting potential of 18 measurements in the root cap of cv Atlas was −124 mV. At 10 mm back from the root tip of cv Atlas, the mean for seven measurements was −177 mV. This phenomenon was also observed in a previous study from our laboratory (Huang et al., 1992). Thus, after the Al-induced depolarization, the E_m ranged from an average value of −104 mV at the root cap to −158 mV at 10 mm back from the tip.

To facilitate measurements of E_m, fairly high levels of Al were often used. There was a trend toward larger depolarizations with exposures to increasing levels of Al (Fig. 3). In 150 μM $AlCl_3$ depolarizations in cv Atlas averaged 19 mV, whereas when roots were exposed to 20 μM $AlCl_3$, depolarizations averaged 8 mV. This was largely because of a difference in the percentage of plants that depolarized in response to Al at 20 μM Al versus 50 or 150 μM Al. Using 50 or 150 μM $AlCl_3$, the E_m of the tolerant plants depolarized in 87% of the experiments, whereas when seedlings were exposed to 20 μM $AlCl_3$, a depolarization was observed in 55% of the experiments. To verify that a differential response between Al-tolerant and Al-sensitive wheat lines was maintained in 150 μM $AlCl_3$, root growth was assayed using a flow-through setup that provided conditions similar to those of the electrophysiology experiments.

As shown in Figure 4, in 150 μM $AlCl_3$ differential Al tolerance was maintained between cvs Scout and Atlas. Using this level of Al root growth in Al-tolerant cv Atlas was 50% of the control, whereas root growth in Al-sensitive cv Scout was inhibited by 80%. Thus, even though 150 μM $AlCl_3$ affects root growth in both cvs Atlas and Scout, root growth in Al-sensitive cv Scout was much more severely inhibited than root growth in Al-tolerant cv Atlas, indicating that an Al-tolerance mechanism in cv Atlas is functioning even at this relatively high level of $AlCl_3$. In this study both cvs Atlas and Scout appear to tolerate higher levels of Al than is usually reported (Kinraide, 1993; Ryan et al., 1994; Pellet et al., 1996). This may be due to a shorter incubation time with Al in this study, a slow flow rate that would allow a large, unstirred layer (and thus low [Al]) to develop next to the root surface, or other methodological differences.

Table I. *Effect of 150 μM $AlCl_3$ on the membrane potential at various positions along wheat roots*

Values represent means ± SE.

Position	n	E_m		
		Initial	+150 μM $AlCl_3$	Al-induced depolarization
		mV		
1 cm	7	−177 ± 9	−158 ± 10	19
1 mm	8	−137 ± 5	−121 ± 8	16
Cap	18	−124 ± 4	−104 ± 6	20

K^+ Effects on E_m

K^+-induced depolarizations were measured as a control to make sure that roots were responding normally to a

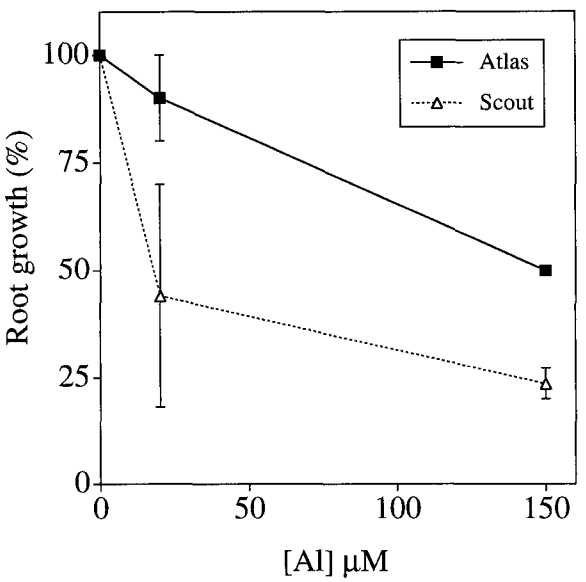

Figure 4. Effect of 0, 20, and 150 μM $AlCl_3$ (+200 μM $CaCl_2$, pH 4.5) on root growth of cvs Scout and Atlas. Percent root growth = 100 × (root length in Al/root length without Al). For each concentration, three 5-d-old seedlings of cvs Atlas or Scout were placed in a Plexiglas chamber with the tip of the longest roots at a common starting line. A flow-through system was used to supply the treatment solution at a rate of 4.4 mL/min for 7 h. New growth was measured in millimeters from the common starting line. Values represent the mean of two replicate experiments. Error bars represent ± SE.

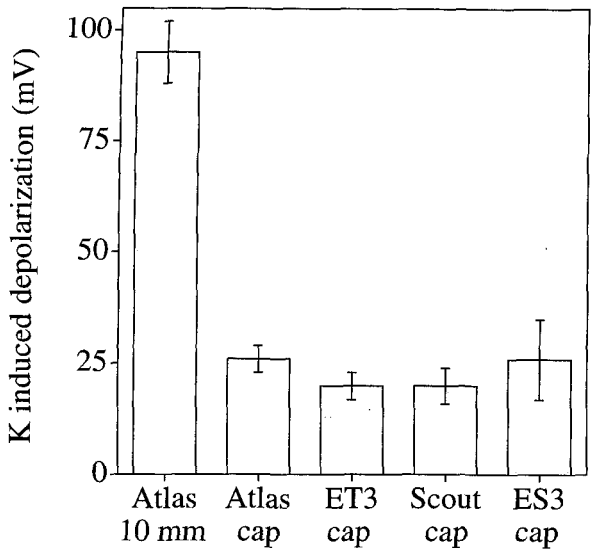

Figure 5. Effect of 50 μM K_2SO_4 (+200 μM $CaCl_2$, pH 4.5) on the E_m of roots cells for the four different wheat lines. Plants were grown in 200 μM $CaCl_2$ (pH 4.5). The values for depolarization of E_m were the average of 5 to 29 replicates ± SE.

condition that induces depolarization of E_m, and to determine whether a non-Al-induced depolarization of E_m could also elicit malate release. K^+-induced depolarizations had different characteristics than Al-induced depolarizations. There was a large difference in the magnitude of K^+ depolarizations based on position along the root. As shown in Figure 5, K^+ depolarizations in the root cap averaged 23 mV, which is much smaller than K^+ depolarizations measured in cells of the mature root (Fig. 1C). In cv Atlas the K^+ depolarization at 10 mm back from the tip averaged 95 ± 7 mV (SE, n = 10), four times larger than the K^+ depolarizations in the root cap (26 ± 3 mV [SE, n = 29]). Furthermore, there was no difference in the magnitude of the K^+ depolarizations across the four wheat lines examined; Al-sensitive and Al-tolerant varieties exhibited a similar magnitude of K^+-induced depolarization of E_m. This is in contrast to Al-induced depolarizations in wheat, in which there was a significant varietal difference (Figs. 1 and 2) but no difference based on position along the root (Fig. 3; Table I).

Anion-Channel Blockers

Since the flux of the malate anion from the cytoplasm to the external solution could cause the depolarizations observed here, we looked at the effects of Al exposure on E_m in the presence of agents that should block malate release. Two anion-channel blockers, niflumic acid and A-9-C, which have been shown to be effective at blocking anion channels and malate efflux in plant cells (Ryan et al., 1995a; Schwartz et al., 1995), were used. First, it was necessary to determine whether the anion-channel blockers themselves perturbed root E_m. Niflumic acid depolarized E_m, even in the absence of Al (data not shown), and was therefore not suitable for this experiment. A-9-C also depolarized E_m at high concentrations (300 μM), but at lower concentrations (100 μM) A-9-C had no effect on root E_m values (Fig. 6). Thus, for experiments with Al, 50 μM A-9-C was used. As shown in Figure 6, when 75 μM Al was added in the presence of the anion-channel blocker, there was still a typical Al-induced depolarization (approximately 25 mV). Since the 10-min preexposure to A-9-C may not have been sufficient for A-9-C to block anion channels before Al was added, other plants were preincubated for 2 h in 50 μM A-9-C, 200 μM $CaCl_2$, pH 4.5, and then impaled. The Al-induced depolarization in the presence of A-9-C was still observed after this 2-h preincubation in 50 μM A-9-C (data not shown).

Subsequently, we found that the exposure of cv Atlas roots to 50 μM A-9-C inhibited malate release during a 7-h exposure to 75 μM Al (Fig. 7). Malate exudation decreased 57% in the 75 μM Al plus 50 μM A-9-C treatment relative to the 75 μM Al treatment. This 57% inhibition of Al-induced malate exudation in the presence of 50 μM A-9-C is similar to levels of A-9-C blockage of anion channels reported by others. Ryan et al. (1995a) found a 65% inhibition of malate efflux by 100 μM A-9-C in response to 200 μM Al, and Schwartz et al. (1995) found an approximately 50% inhibition of anion current in broad bean guard cells in the presence of 50 μM A-9-C.

Thus, although a substantial inhibition of Al-induced malate exudation occurred in the presence of A-9-C, there was no effect on the Al-induced depolarization in the presence of A-9-C, suggesting that the Al-induced depolarization is not caused by $malate^{2-}$ release.

Specificity of the Electrical Response

In all of the experiments we conducted, $AlCl_3$ was the source of Al. Thus, it was necessary to determine whether the depolarizations were due to Al or to the high level of Cl^- supplied when roots were exposed to Al. An experiment was conducted in which equivalent amounts of Cl^- (850 μM) were supplied either as 150 μM $AlCl_3$ plus 200 μM $CaCl_2$ or as 425 μM $CaCl_2$. Changes in E_m were measured at 1 mm from the root tip. A depolarization occurred only in the presence of Al. In the presence of the high Cl without Al treatment, a small hyperpolarization (8 ± 3 mV [SE, n = 4]) was observed (data not shown). We suspect that the depolarization observed with $AlCl_3$ underestimates the magnitude of the depolarization due to Al, because it represents the summation of the Al-induced depolarization and the small Cl-induced hyperpolarization. In the Al-sensitive cultivars it is likely that the small hyperpolariza-

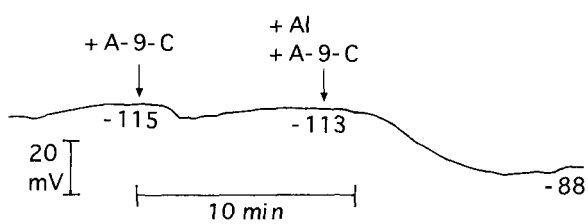

Figure 6. A trace of root-cell E_m for a cv Atlas seedling exposed to 100 μM A-9-C or 50 μM A-9-C plus 75 μM $AlCl_3$ in 200 μM $CaCl_2$ (pH 4.5).

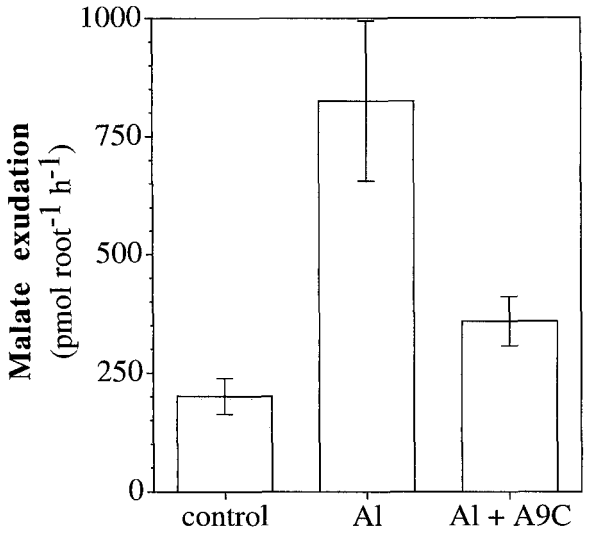

Figure 7. Effect of exposure of roots to 50 μM A-9-C on malate exudation in cv Atlas seedlings in the presence of 75 μM $AlCl_3$. Five-day-old cv Atlas seedlings were grown in 200 μM $CaCl_2$ (pH 4.5) and then exposed to either control conditions (200 μM $CaCl_2$, pH 4.5), 75 μM $AlCl_3$ (+200 μM $CaCl_2$, pH 4.5), or 75 μM $AlCl_3$ plus 50 μM A-9-C (+200 μM $CaCl_2$, pH 4.5) for 7 h. The values for malate exudation were the average of five to seven replicates ± SE.

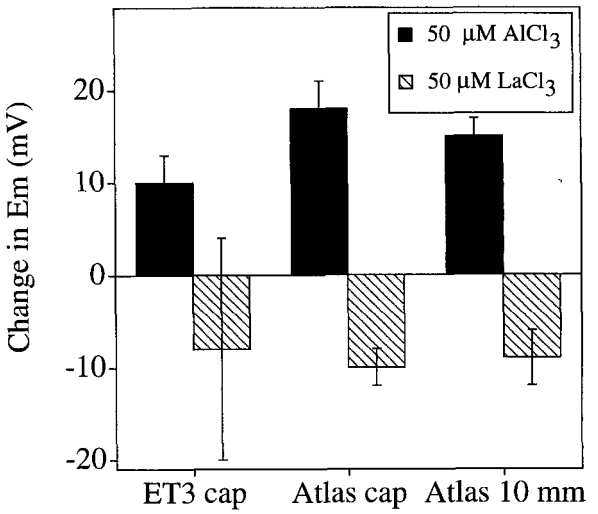

Figure 8. Effect of exposure of 50 μM $LaCl_3$ (+200 μM $CaCl_2$, pH 4.5) or 50 μM $AlCl_3$ (+200 μM $CaCl_2$, pH 4.5) on the root-cell E_m in the root cap of cv ET3 (n = 9 Al, n = 3 La), the root cap of cv Atlas (n = 9), and at 10 mm back from the root tip in cv Atlas (n = 6 Al, n = 4 La). Error bars represent ± SE.

tions observed when roots were exposed to $AlCl_3$ were due to Cl exposure and not to Al (Fig. 2).

To determine whether the depolarizations observed in Al-tolerant wheat were specific to Al, the effect of another rhizotoxic trivalent cation, La^{3+}, on root E_m was studied. La^{3+} also elicits an inhibition of root growth in wheat that is similar to the inhibition by Al^{3+}. However, both Al-tolerant and Al-sensitive wheat varieties are sensitive to La^{3+} (Kinraide et al., 1992). Also, it was recently shown in Al-tolerant wheat that La^{3+} did not trigger malate release associated with Al exclusion and tolerance (Ryan et al., 1995a). As shown in Figure 8, 50 μM $LaCl_3$ tended to hyperpolarize rather than depolarize E_m in both Al-tolerant wheat lines (cvs Atlas and ET3). In contrast, 50 μM $AlCl_3$ consistently caused a depolarization in these lines. The magnitude of the $LaCl_3$ hyperpolarizations averaged 8 to 10 mV for measurements in the root cap of cvs Atlas and ET3. These results suggest that the electrical response observed in Al-tolerant wheat is specific to Al. The hyperpolarization observed for $LaCl_3$ is similar to that seen in response to high levels of $CaCl_2$ and is probably a response to Cl^- and not to La^{3+}.

Effect of K^+-Channel Blockers

Olivetti et al. (1995) suggested that the depolarization they observed in Al-tolerant snapbean in response to Al was due to blockage of outward-rectifying K^+ channels by Al^{3+}. We examined the effect of TEA-Cl, a known K^+-channel blocker in plants (Maathuis and Sanders, 1995; Roberts and Tester, 1995; Hedrich and Dietrich, 1996; Ichida and Schroeder, 1996), on the E_m in cv Atlas and saw no depolarization after 30 min. A slight hyperpolarization was observed, which was possibly a Cl^- response. We also investigated whether K^+-channel blockers could trigger malate release. As shown in Figure 9, no malate exudation occurred even after a 7-h exposure to either 5 mM TEA-Cl or 1 mM $BaCl_2$, another known K^+-channel blocker in plants. This is in contrast to the 7-h exposure to 50 μM $AlCl_3$, which triggered a substantial increase in malate release. These results suggest that K^+ channels are not involved in an Al-induced electrical response that triggers malate exudation in Al-tolerant wheat.

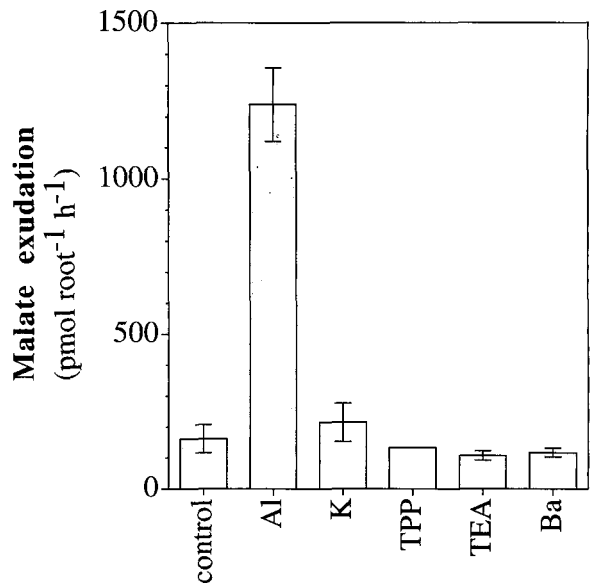

Figure 9. Malate exudation from roots of cv Atlas seedlings grown in 200 μM $CaCl_2$ (pH 4.5) during a 7-h exposure to 200 μM $CaCl_2$ and 50 μM $AlCl_3$, 50 μM K_2SO_4, 5 mM TEA-Cl, 100 μM tetraphenyl phosphonium chloride, or 1 mM $BaCl_2$, all at pH 4.5.

Effect of Depolarization on Malate Exudation

We also investigated the hypothesis that, in addition to Al, other compounds that depolarize E_m might cause malate release via activation of voltage-gated channels. As shown in Figure 9, only Al caused malate to release. Neither K^+ (as 50 μM K_2SO_4) nor 100 μM tetraphenyl phosphonium (a lipophilic cation), which both cause a significant depolarization of E_m, triggered malate release during a 7-h exposure.

DISCUSSION

Several plant species have been shown to release organic acids as a mechanism of Al tolerance, which results in the exclusion of Al from the root apex (Miyasaka et al., 1991; Delhaize et al., 1993; Pellet et al., 1995). How the presence of Al at the plasma membrane of root apical cells is perceived and then translated into the release of organic acids is unknown. Based on the recent observations by Olivetti et al. (1995) of a moderate Al-induced depolarization of E_m in the root-cap cells of Al-tolerant snapbean, the present study focuses on (a) whether this depolarization also occurred in wheat lines in which Al-induced malate release has been studied in great detail and (b) the possibility that Al-induced electrical signals are involved in this Al-tolerance response.

After determining that Al causes a depolarization in Al-tolerant but not Al-sensitive varieties of wheat, we considered two opposing hypotheses regarding the involvement of electrical signals in Al tolerance:

1. The Al-induced depolarization is caused by the movement of malate^{2-} across the plasma membrane. This hypothesis assumes that the divalent anion form of malate is the species crossing the plasma membrane and that malate transport is not charge-balanced. Under these conditions, movement of the negatively charged malate^{2-} out of the cell would depolarize the plasma membrane.

2. The Al-induced depolarization in Al-tolerant lines of wheat is an important component of Al tolerance via activation of voltage-gated anion (malate) channels. In this scenario the depolarization occurs first, and it is this electrical signal that triggers (or contributes to) the exudation of malate. This hypothesis assumes that malate exudation occurs via voltage-gated anion channels.

In the second hypothesis the cause of the depolarization is not specified. Therefore, we considered the possibility that Al might cause the depolarization by blocking outward-rectifying K^+ channels, as was suggested by Olivetti et al. (1995). We also tested whether blockage of K^+ channels in the cells of the root cap affects Al-induced malate release.

Does Blockage of K^+ Channels Affect Al-Induced Malate Release?

In studies using extracellular, vibrating K^+ microelectrodes, a small net K^+ efflux from the root tip is often observed (Kochian, 1995). Olivetti et al. (1995) postulated that Al causes a decrease in the outward conductance of K^+, and it is this prevention of the outward movement of K^+ that depolarizes E_m in root-cap cells of Al-tolerant snapbean (cv Dade). In their study it was shown that 5 mM TEA^+ depolarized the root-cap E_m, presumably by blocking channels that facilitate K^+ efflux. However, in the current study we did not find an effect of 5 mM TEA^+ on the root cell E_m. TEA^+ has been shown to consistently block K^+ channels when added to the side of K^+ channels where K^+ enters; however, there is a mixed record in the literature on the ability of TEA^+ to block K^+ channels from the opposite side. Hille (1992) reports that the delayed outward-rectifying K^+ channel is always blocked by TEA^+ from the inside, but is not blocked from the outside in all cases. For example, the delayed rectifier K^+ channels of axons in the frog node of Ranvier can be blocked by TEA^+ from either the outside or the inside, whereas external TEA^+ has no effect on the delayed rectifier K^+ channels of squid giant axons (Hille, 1992). Similarly, external TEA^+ has been shown to block inward K^+ currents of oocytes expressing the inward-rectifying plant K^+ channel KAT1, but internal TEA^+ failed to block the inward K^+ currents (Ichida and Schroeder, 1996). External TEA-Cl was shown to block a low-affinity K^+ uptake system (likely an inward K channel) in maize roots (Kochian and Lucas, 1982). At the same time, there also are reports of TEA^+ effectively blocking K^+ channels from the nonentry side. Roberts and Tester (1995) found that extracellular TEA^+ inhibited the time-dependent outward current from protoplasts from the stele of maize roots. Also, Maathuis and Sanders (1995) showed that an outward-rectifying K^+ channel in *Arabidopsis thaliana* root cells was blocked by external TEA^+.

For outward K^+ currents from cells of the root cap/root apex, the entry side of the K^+ channel is cytoplasmic. Thus, the discrepancy between our work, in which external TEA-Cl did not cause a depolarization in cells of wheat roots, and the work by Olivetti et al. (1995), in which TEA-Cl did cause a depolarization in cells of snapbean roots, is not surprising given the mixed record in the literature regarding the effectiveness of TEA exposure on the nonentry side of K^+ channels. Perhaps the inconsistency is due to differences in the ability of TEA to cross the plasma membrane and gain access to the K^+ entry side of the channel in these different species. Kochian and Lucas (1982) used a radiotracer flux approach to show that externally applied ^{14}C-labeled TEA-Cl was transported into the root symplasm of maize. Thus, it is possible that when snapbean roots are exposed to external TEA^+, a significant amount of the TEA^+ enters the symplasm and could block an outward K^+ channel at its cytoplasmic face. The long lag time (approximately 30 min) observed by Olivetti et al. (1995) between TEA^+ exposure and depolarization suggests that TEA^+ is in fact acting from the cytoplasmic side, and the lag is the time required for TEA^+ to enter the cell.

It has been shown that Al blocks inward-rectifying K^+ channels in root hairs of Al-sensitive cv Scout (Gassmann and Schroeder, 1994). However, whether Al blocks outward K^+ channels in the root cap of Al-tolerant varieties, as proposed by Olivetti et al. (1995), is unclear. Work by Ryan et al. (1995a) showed that Al induces both K^+ and malate efflux in Al-tolerant wheat; the K^+ efflux presumably charge-balances the malate exudation. A blockage of

outward-rectifying K^+ channels is inconsistent with the 2:1 ratio of K^+ release to malate release observed by Ryan et al. (1995a).

Nevertheless, we decided to test the hypothesis that blockage of K^+ channels is involved in Al tolerance in wheat. The addition of either 5 mM TEA-Cl or 1 mM $BaCl_2$, two K^+ channel blockers, failed to elicit malate release during a 7-h exposure, suggesting that simple blockage of K^+ channels is insufficient to trigger Al tolerance, at least in the form of organic acid exudation.

Hypothesis 1: The Depolarization Is Caused by Malate Exudation

We examined the possibility that the Al-induced depolarization that we observed in Al-tolerant wheat and that Olivetti et al. (1995) observed in Al-tolerant snapbean was due to the transport of negatively charged organic acids (malate or citrate) across the plasma membrane to the external solution. In the cytoplasm of root cells (pH 7.0) these organic acids will exist as divalent anions (Delhaize and Ryan, 1995). Thus, exudation of these organic acids into the rhizosphere would result in the net movement of negative charge out of the cell. If this transport were not charge-balanced, such movement of charge should cause a depolarization of the root-cell E_m.

This hypothesis was ruled out based on two pieces of evidence. First, we observed depolarizations of the same magnitude in Al-tolerant cv Atlas at all three positions along the root (root cap and 1 and 10 mm from the root tip). Al-induced malate exudation is localized primarily to the terminal 3 mm of the root (Ryan et al., 1995a; Huang et al., 1996). If the depolarization were simply due to the movement of the divalent malate anion across the plasma membrane of root cells, the magnitude of the depolarization should have been largest within the terminal 3 mm of the root, assuming that the membrane resistance is the same for cells of the root tip and 10 mm back from the tip. As shown in Figure 3 and Table I, we observed no change in the magnitude of the depolarization at the root tip (cap and 1 mm) versus 10 mm back from the tip. Second, Al-induced depolarizations occurred in the presence of the anion-channel blocker A-9-C (Fig. 6), even though A-9-C significantly inhibited malate release (Fig. 7). If the depolarization was caused by malate transport, the depolarization should have been absent or attenuated when malate transport was blocked. Thus, although we cannot rule out the possibility that the small amount of malate exudation that remained during A-9-C exposure was enough to cause the depolarization, it is likely that the Al-induced depolarization in Al-tolerant varieties of wheat is not caused by the transport of malate^{2-} across the plasma membrane of root cells.

Hypothesis 2: Malate Exudation Is Triggered by an Al-Induced Depolarization of E_m

The existence of an Al-induced depolarization in Al-tolerant, but not Al-sensitive, lines suggests that organic acid release might be triggered by the activation of voltage-gated anion channels in the plasma membrane of root cells. Exudation of these organic acid anions is thermodynamically passive, since there is both a strong downhill electrical potential gradient and concentration gradient out of the cytoplasm. Thus, it is very likely that these organic acids are transported via anion channels (Delhaize and Ryan, 1995; Kochian, 1995; Ryan et al., 1995a; Schroeder, 1995). Several anion channels in plants have been found to be voltage regulated (Marten et al., 1992; Hedrich and Marten, 1993; Hedrich et al., 1994; Skerrett and Tyerman, 1994; Zimmermann et al., 1994; Schroeder, 1995; Schwartz et al., 1995; Thomine et al., 1995; Ward et al., 1995; Lurin et al., 1996), and selectivity studies have shown that many of them are permeable to malate. Since we saw a depolarization in response to Al in Al-tolerant varieties, we considered the possibility that such a malate-permeable channel was voltage gated.

We were surprised to see Al-induced depolarizations of the same magnitude at all three positions along the root, because in Al-tolerant snapbean the Al-depolarization was observed only in the root cap and not at 10 mm back from the tip (Olivetti et al., 1995), and in Al-tolerant wheat malate exudation is localized to the terminal 3 mm of the root (Ryan et al., 1995a; Huang et al., 1996). Is the Al-induced depolarization consistent with an electrical signal that activates channels, given the discrepancy between the spatial distribution of the depolarizations versus the spatial distribution of malate exudation? As shown in Table I, the initial resting potentials vary considerably based on position, with the values becoming more negative farther from the root cap.[2] As a result, upon Al depolarization the E_m values range from -104 ± 6 mV in the root cap to -158 ± 10 mV 10 mm back from the tip, even though the magnitude of the depolarization is constant at these positions. Voltage-gated channels are activated only when the voltage shifts within a prescribed range. It is conceivable that, although Al is causing the same magnitude of depolarization all along the root, a E_m sufficiently depolarized to activate voltage-gated anion (malate) channels has only been reached in the root apex. For example, Zimmermann et al. (1994) described an anion channel in tobacco suspension cells that is activated at potentials more positive than -120 mV and has a peak amplitude at -90 mV. If a channel with similar gating characteristics were operating for malate release, then it would be activated in the presence of Al in the root apex region, but not farther back along the root.

[2] We assume in this paper that the pattern of more negative resting potentials away from the root cap is real. However, it is possible that this trend is an artifact of the measuring system. Because cell size is also a function of position along the root, the ratio of the radius of the impaling electrode to the radius of the cell decreases as one moves along the root away from the cap. Thus, any leakage around the electrode could constitute a larger fraction of the signal at the root cap than it would farther back. If, however, cells of the root cap are connected by plasmodesmata, this effect will be negated. We attempted to quantify the magnitude of this effect by intentionally impaling cells of the same size with electrodes of varying radii. However, we were unable to obtain successful impalements when the electrode configuration was altered to increase electrode radius.

Although the Al-induced depolarizations at all three positions may be consistent with hypothesis 2, experiments with K^+-induced depolarizations are not consistent with this hypothesis. As shown in the K^+-depolarization studies, the presence of a depolarization alone is not sufficient to trigger malate release (Fig. 9). K^+-induced depolarizations in the root cap (Fig. 5) are of a similar magnitude (23 mV) to those induced by Al in the same region (Fig. 2), but as shown in Figure 9, malate exudation is not triggered by K^+. If all that were required for the activation of malate channels was that the E_m decrease within a prescribed range of voltages, K^+-induced depolarizations in the root cap should have triggered malate release. Since phosphorylation status, cytoplasmic Ca^{2+}, hormones, small metabolites such as malate itself, and perhaps even external factors such as Al can modulate anion channels, it is likely that the depolarization alone is not sufficient to trigger malate release and that a second event, also requiring Al^{3+} exposure, needs to occur to facilitate malate release.

We recognize that the data presented here are correlative and do not provide specific evidence for a causal relationship between the Al-induced depolarization in Al-tolerant wheat and the Al-induced malate release in these same lines. It is possible that both events occur in Al-tolerant wheat and may even be caused by the same factor, but are still not directly coupled. However, this study at the whole-plant level suggests that the depolarization may be involved in Al tolerance and should spur on future work at the single-cell level. Patch-clamp studies would allow a more thorough and specific investigation of the Al-induced electrical response, the involvement of anion channels in Al-induced malate release, and the possible gating mechanisms of such channels.

ACKNOWLEDGMENTS

We thank Jon Shaff for helpful advice on the methodological aspects of our work, Dr. Allen Gabor for helpful discussions, and Wolcott-Park (Rochester, NY) for their donation of Dow Corning 3145RTV MIL-A-46146 adhesive/sealant.

Received March 12, 1997; accepted June 19, 1997.

LITERATURE CITED

Basu U, Godbold D, Taylor GJ (1994) Aluminum resistance in *Triticum aestivum* associated with enhanced exudation of malate. J Plant Physiol **144:** 747–753

Cosgrove DJ, Hedrich R (1991) Stretch-activated chloride, potassium, and calcium channels coexisting in plasma membranes of guard cells of *Vicia faba* L. Planta **186:** 143–153

Delhaize E, Ryan PR (1995) Aluminum toxicity and tolerance in plants. Plant Physiol **107:** 315–321

Delhaize E, Ryan PR, Randall PJ (1993) Aluminum tolerance in wheat (*Triticum aestivum* L.). II. Aluminum-stimulated excretion of malic acid from root apices. Plant Physiol **103:** 695–702

Gassmann W, Schroeder JI (1994) Inward-rectifying K^+ channels in root hairs of wheat. Plant Physiol **105:** 1399–1408

Hedrich R, Dietrich P (1996) Plant K^+ channels: similarity and diversity. Bot Acta **109:** 94–101

Hedrich R, Marten I (1993) Malate-induced feedback regulation of plasma membrane anion channels could provide a CO_2 sensor to guard cells. EMBO J **12:** 897–901

Hedrich R, Marten I, Lohse G, Dietrich P, Winter H, Lohaus G, Heldt H-W (1994) Malate-sensitive anion channels enable guard cells to sense changes in the ambient CO_2 concentration. Plant J **6:** 741–748

Hille B (1992) Ionic Channels of Excitable Membranes. Sinauer Associates, Sunderland, UK

Huang JW, Pellet DM, Papernik LA, Kochian LV (1996) Aluminum interactions with voltage-dependent calcium transport in plasma membrane vesicles isolated from roots of aluminum-sensitive and -resistant wheat cultivars. Plant Physiol **110:** 561–569

Huang JW, Shaff JE, Grunes DL, Kochian LV (1992) Aluminum effects on calcium fluxes at the root apex of aluminum-tolerant and aluminum-sensitive wheat cultivars. Plant Physiol **98:** 230–237

Ichida AM, Schroeder JI (1996) Increased resistance to extracellular cation block by mutation of the pore domain of the Arabidopsis inward-rectifying K^+ channel KAT1. J Membr Biol **151:** 53–62

Kinraide TB (1988) Proton extrusion by wheat roots exhibiting severe aluminum toxicity symptoms. Plant Physiol **88:** 418–423

Kinraide TB (1993) Aluminum enhancement of plant growth in acid rooting media: a case of reciprocal alleviation of toxicity by two toxic cations. Physiol Plant **88:** 619–625

Kinraide TB, Ryan PR, Kochian LV (1992) Interactive effects of Al^{3+}, H^+, and other cations on root elongation considered in terms of cell-surface electrical potential. Plant Physiol **99:** 1461–1468

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

Kochian LV, Lucas WJ (1982) Potassium transport in corn roots. IV. Characterization of the linear component. Plant Physiol **79:** 771–776

Lurin C, Geelan D, Barbier-Brygoo H, Guern J, Maurel C (1996) Cloning and functional expression of a plant voltage-dependent chloride channel. Plant Cell **8:** 701–711

Maathuis FJM, Sanders D (1995) Contrasting roles in ion transport of two K^+-channel types in root cells of *Arabidopsis thaliana*. Planta **197:** 456–464

Marten I, Zeilinger C, Redhead C, Landry DW, Al-Awqati Q, Hedrich R (1992) Identification and modulation of a voltage-dependent anion channel in the plasma membrane of guard cells by high-affinity ligands. EMBO J **11:** 3569–3575

Miyasaka SC, Buta JG, Howell RK, Foy CD (1991) Mechanism of aluminum tolerance in snapbeans. Root exudation of citric acid. Plant Physiol **96:** 737–743

Miyasaka SC, Kochian LV, Shaff JE, Foy CD (1989) Mechanisms of aluminum tolerance in wheat. An investigation of genotypic differences in rhizosphere pH, K^+, and H^+ transport, and root-cell membrane potentials. Plant Physiol **91:** 1188–1196

Olivetti GP, Cumming JR, Etherton B (1995) Membrane potential depolarization in root cap cells precedes aluminum tolerance in snapbean. Plant Physiol **109:** 123–129

Pellet DM, Grunes DL, Kochian LV (1995) Organic acid exudation as an aluminum-tolerance mechanism in maize (*Zea mays* L.). Planta **196:** 788–795

Pellet DM, Papernik LA, Kochian LV (1996) Multiple aluminum-resistance mechanisms in wheat. Roles of root apical phosphate and malate exudation. Plant Physiol **112:** 591–597

Roberts SK, Tester M (1995) Inward and outward K^+-selective currents in the plasma membrane of protoplasts from maize root cortex and stele. Plant J **8:** 811–825

Ryan PR, Delhaize E, Randall PJ (1995a) Characterisation of Al-stimulated efflux of malate from the apices of Al-tolerant wheat roots. Planta **196:** 103–110

Ryan PR, Delhaize E, Randall PJ (1995b) Malate efflux from root apices and tolerance to aluminium are highly correlated in wheat. Aust J Plant Physiol **22:** 531–536

Ryan PR, Kinraide TB, Kochian LV (1994) Al^{3+}-Ca^{2+} interactions in aluminum rhizotoxicity. I. Inhibition of root growth is not caused by reduction of calcium uptake. Planta **192:** 98–103

Ryan PR, Shaff JE, Kochian LV (1992) Aluminum toxicity in roots. Plant Physiol **99:** 1193–1200

Schroeder JI (1995) Anion channels as central mechanisms for signal transduction in guard cells and putative functions in roots for plant-soil interactions. Plant Mol Biol **28:** 353–361

Schwartz A, Ilan N, Schwarz M, Scheaffer J, Assmann SM, Schroeder JI (1995) Anion-channel blockers inhibit S-type anion channels and abscisic acid responses in guard cells. Plant Physiol **109:** 651–658

Skerrett M, Tyerman SD (1994) A channel that allows inwardly directed fluxes of anions in protoplasts derived from wheat roots. Planta **192:** 295–305

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

Thomine S, Zimmermann S, Guern J, Barbier-Brygoo H (1995) ATP-dependent regulation of an anion channel at the plasma membrane of protoplasts from epidermal cells of Arabidopsis hypocotyls. Plant Cell **7:** 2091–2100

Tyerman SD (1992) Anion channels in plants. Annu Rev Plant Physiol Plant Mol Biol **43:** 351–373

Ward JM, Pei Z-M, Schroeder JI (1995) Roles of ion channels in initiation of signal transduction in higher plants. Plant Cell **7:** 833–844

Zimmermann S, Thomine S, Guern J, Barbier-Brygoo H (1994) An anion current at the plasma membrane of tobacco protoplasts shows ATP-dependent voltage regulation and is modulated by auxin. Plant J **6:** 707–716