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# Interaction between Aluminum Toxicity and Calcium Uptake at the Root Apex in Near-Isogenic Lines of Wheat (*Triticum aestivum* L.) Differing in Aluminum Tolerance[1]

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**Aluminum (Al) is toxic to plants at pH < 5.0 and can begin to inhibit root growth within 3 h in solution experiments. The mechanism by which this occurs is unclear. Disruption of calcium (Ca) uptake by Al has long been considered a possible cause of toxicity, and recent work with wheat (*Triticum aestivum* L. Thell) has demonstrated that Ca uptake at the root apex in an Al-sensitive cultivar (Scout 66) was inhibited more than in a tolerant cultivar (Atlas 66) (J.W. Huang, J.E. Shaff, D.L. Grunes, L.V. Kochian [1992] Plant Physiol 98: 230–237). We investigated this interaction further in wheat by measuring root growth and Ca uptake in three separate pairs of near-isogenic lines within which plants exhibit differential sensitivity to Al. The vibrating calcium-selective microelectrode technique was used to estimate net Ca uptake at the root apex of 6-d-old seedlings. Following the addition of 20 or 50 $\mu$M $AlCl_3$, exchange of Ca for Al in the root apoplasm caused a net Ca efflux from the root for up to 10 min. After 40 min of exposure to 50 $\mu$M Al, cell wall exchange had ceased, and Ca uptake in the Al-sensitive plants of the near-isogenic lines was inhibited, whereas in the tolerant plants it was either unaffected or stimulated. This provides a general correlation between the inhibition of growth by Al and the reduction in Ca influx and adds some support to the hypothesis that a Ca/Al interaction may be involved in the primary mechanism of Al toxicity in roots. In some treatments, however, Al was able to inhibit root growth significantly without affecting net Ca influx. This suggests that the correlation between inhibition of Ca uptake and the reduction in root growth may not be a mechanistic association. The inhibition of Ca uptake by Al is discussed, and we speculate about possible mechanisms of tolerance.**

Aluminum (Al)[3] toxicity is believed to be the major factor limiting plant growth in acid soils. The primary symptom of Al stress is inhibition of root growth, which can begin to occur within 3 h in solution experiments (Ownby and Popham, 1989). The root apex appears to be a critical site for toxicity (Ryan et al., 1993), and although many physiological effects of Al stress have been documented, the mechanism causing the inhibition of growth remains unclear (for reviews, see Foy et al., 1978; Roy et al., 1988; Taylor, 1988a).

Al is known to affect directly membrane structure and permeability (Vierstra and Haug, 1978; Haug, 1984; Zhao et al., 1987; Caldwell, 1989; Chen et al., 1991), and disruption of membrane transport processes can severely limit nutrient accumulation in the long term (Taylor, 1988a). However, for an observed response to Al stress to be implicated in the inhibition of root growth, it must occur within the first few hours of exposure. Al is especially disruptive to Ca acquisition (Foy et al., 1978; Roy et al., 1988; Taylor, 1988a), and, in some species, the symptoms of prolonged Al stress are similar to those of Ca deficiency (Foy et al., 1967). Ca movement into cells is mediated by membrane-spanning channels (Tester, 1990), and the disruption of Ca uptake by Al is consistent with competitive inhibition (Lindberg, 1990; Huang et al., 1992a; Rengel and Elliot, 1992). Using ion-selective microelectrodes, Huang et al. (1992a, 1992b) showed that the inhibition of Ca uptake by Al in wheat (*Triticum aestivum* L.) roots was greater in the Al-sensitive cv Scout 66 than in the Al-tolerant cv Atlas 66. Because the response was rapid and reversible, Huang and co-workers suggested that Al acts at the outer face of the plasmalemma to block channels that mediate Ca influx. Rengel and Elliot (1992) presented evidence that in protoplasts prepared from *Amaranthus* coleoptile tissue Al specifically binds to the verapamil-binding site on the Ca channels.

Many plant species and cultivars show an inheritable tolerance to Al stress, and these species and cultivars have become important resources for investigating Al toxicity and tolerance. Relative tolerance to Al stress is a dominant trait in wheat and appears to be controlled by one or more genes (Aniol and Gustafson, 1984; Fisher and Scott, 1987). Although useful, in all of the studies to date, cultivars with different genetic backgrounds or even different species have been compared. Therefore, distinguishing which are primary effects and which are secondary symptoms of Al stress is difficult, and identification of the physiological basis for toxicity can be hindered by the natural variation between the cultivars used. Indeed, some of the conflicting data concern-

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[3] Al hydrolyzes in solution and, apart from the $Al^{3+}$ cation, which dominates speciation at pH<5, various hydroxy-Al and polynuclear species can also occur (Kinraide, 1991). Although accumulating evidence suggests that $Al^{3+}$ is the most toxic Al species under acid conditions (Kinraide et al., 1992), in this text, we denote aluminum as Al without implying a particular Al species.

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ing Al stress (Roy et al., 1988; Taylor, 1988a, 1988b) may be attributed to such comparisons.

Pairs of near-isogenic lines of wheat that differ in sensitivity to Al have been developed in several laboratories. Because the Al-sensitive and Al-tolerant plants within each of these lines share between 94 and 99% of their genome, any differential response to Al stress has a relatively high probability of being directly involved with the causes of toxicity and/or tolerance. We obtained three different pairs of near-isogenic lines and measured the effect of Al on Ca uptake in the short term (<1 h) and the effect of Al on root growth over 24 h. Net Ca flux was measured at the root apex with the vibrating Ca-selective microelectrode technique. Our aim was to determine whether the differential effect of Al on Ca uptake in Scout 66 and Atlas 66 was also observable in these near-isogenic lines. We also examined the effect of the trivalent cation La on Ca uptake in roots of Scott 66 and Atlas 66.

## MATERIALS AND METHODS

### Seeds and Growth Conditions

Wheat (*Triticum aestivum* L. Thell) seeds were sterilized in 0.5% NaClO for 20 min, washed in deionized water, and germinated on damp filter paper in the dark for 2 or 3 d (depending on the wheat line). Seedlings were then planted in black polyethylene pots (8 to 12 per pot) containing 900 mL of control solution, which consisted of (in mM) 1.0 NaCl, 0.2 KCl, 0.2 $CaCl_2$ (pH 4.5), and were placed for 3 d in a controlled environment chamber with a 16-h 20°C and 8 h 15°C day/night regimen. Metal halide lamps provided a light intensity of 580 $\mu$mol $m^{-2}$ $s^{-1}$ at the level of the shoots. Solutions were changed after the first or second day. Al was diluted from a 10 mM $AlCl_3$ stock in 0.2 mM HCl, and La was diluted from a 10 mM $LaCl_3$ stock.

The Al-sensitive cv Scout 66 and the Al-tolerant cv Atlas 66 were obtained from Dr J. Peterson, University of Nebraska. The three near-isogenic wheat lines were developed in different laboratories.

#### *Line 1: $E_6$-tol and $E_6$-sens*

Line 1 was kindly provided by Drs. P. Randall and E. Delhaize from Commonwealth Scientific and Industrial Research Organization Division of Plant Industry, Canberra, Australia. The wheat lines $E_6$-tol and $E_6$-sens were derived from crosses between Carazinho, an Al-tolerant cultivar, and Egret, an Al-sensitive cultivar (E. Delhaize and P. Randall, personal communication). Briefly, $F_1$ progeny resulting from a Carazinho × Egret cross were backcrossed three times to Egret or derivatives of Egret. In the third backcross population, an $F_2$ group segregating for Al tolerance was identified using hematoxylin staining, and a pair of lines differing in tolerance to Al was selected from the group such that each member of the pair had the same $F_1$ parent and were homozygous tolerant or homozygous sensitive (Fisher and Scott, 1987). The tolerant line was crossed to the sensitive line, and the resulting $F_1$ progeny was backcrossed to the sensitive parent an additional three times. The pair of lines $E_6$-tol (homozygous tolerant) and $E_6$-sens (homozygous sensitive) was developed from individual seedlings that were selected from the $F_2$ population that resulted from the last backcross. Bulk seed was collected by self-fertilization of these lines.

#### *Line 2: $W_2F$-RR (Tolerant) and $W_2F$-SS (Sensitive)*

This line was provided by Drs. R. Gardiner and T. Richardson, Department of Cellular and Molecular Biology, University of Auckland, New Zealand. The Al-tolerant cv Waalt was isolated by screening a population of seeds from the cv Warigal. Larkin (1987) showed that a single dominant gene was responsible for the difference in Al sensitivity. Waalt and Warigal were crossed, and a heterozygous plant in the $F_2$ population was backcrossed to the sensitive Warigal. Progeny from this cross segregated 1:1, and sensitive and tolerant plants were intercrossed. The progeny of this cross also segregated 1:1, as expected. Sensitive plants ($W_2F$-SS) were grown, and tolerant progeny were selfed to obtain homozygous resistant plants ($W_2F$-RR). These lines will share $^{15}/_{16}$ of their genome on average.

#### *Line 3: $OK_{tol}$ and $OK_{sens}$*

Line 3 was provided by Drs. B.F. Carver and E.L. Smith, Department of Agronomy, Oklahoma State University, Stillwater, OK. Near-isogenic lines OK91G106 and OK91G108 (referred to in this work as $OK_{tol}$ and $OK_{sens}$, respectively) were derived from a backcrossing program between Century, an Al-sensitive cultivar, and Atlas 66, an Al-tolerant cultivar, with Atlas 66 serving as the donor parent. The lines $OK_{tol}$ and $OK_{sens}$ represent single plant selections from the selfed progeny of a single Al-tolerant backcross 3 ($BC_3$ $F_1$) individual and are true breeding for Al tolerance and sensitivity, respectively.

### Growth Experiments

Seedlings were placed in plastic Petri dishes (12 cm in diameter) containing 80 mL of solution, and the main root was secured to the bottom of the dish with Plexiglas[4] blocks smeared with silicon grease. A notch was filed into one side of each block so that it could straddle the root without causing injury. Root elongation was determined under a microscope (×40 magnification) with an eyepiece micrometer by measuring the distance of the root tip from a stationary reference line. Where possible, measurements were made along the middle of the root to avoid errors associated with root curvature. The accuracy using this technique was estimated to be 100 $\mu$m per measurement.

Measurements of root growth and Ca fluxes were performed in shallow dishes where the solution was unstirred for minutes or even hours. In shallow dishes, the sensitivity of roots to a given Al concentration is considerably lower than when seedlings are exposed to Al treatments in constantly aerated or stirred conditions (compare with Kinraide et al., 1992).

[4] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may be suitable.

### Ca Flux Measurements

The vibrating Ca electrode measures the concentration gradient of Ca in the unstirred layer near the root surface. Net flux of Ca at the surface of the root can be estimated from the concentration gradient by assuming a diffusion profile. The superior spatial and temporal resolution of this technique allows localized measurements of Ca flux at the root apex, the primary site of toxicity.

Detailed descriptions of the technique can be obtained elsewhere (Newman et al., 1987; Küthreiber and Jaffe, 1990; Ryan et al., 1990; Henriksen et al., 1992; Kochian et al., 1992). Briefly, borosilicate glass capillaries (A-M Systems Inc.; World Precision Instruments Inc.) were pulled to a tip diameter of 2 to 5 μm with a two-stage Flaming/Brown Micropipette Puller. The electrodes were dried and silanized with tributylchlorosilane. After cooling, electrodes were backfilled with 100 mM $CaCl_2$, and a small column of Ca-selective ionophore (Fluka Chemical Co., Ronkonkoma, NY) was introduced into the electrode tip to a final length of about 20 to 80 μm. Electrodes with resistances <2 GΩ in a 0.2 mM $CaCl_2$ solution were used and calibrated in 0.02, 0.2, and 2.0 mM $CaCl_2$.

Seedlings were positioned in Petri dishes containing control solution as described above and allowed to recover from handling for at least 3 h. Solutions were changed hourly during this period. A Petri dish was placed on the stage of an inverted Zeiss IM 35 microscope (×200 magnification), and the solution was removed by suction and replaced with 80 mL of test solution. Two to four minutes were required to maneuver the Ca-selective electrode to the measurement position, 50 μm from the root surface and between 1.5 and 2.0 mm from the tip of the root cap. Vibration frequency of the electrode was 0.3 Hz with an amplitude of 30 μm (between 50 and 80 μm from the root surface).

Data were collected automatically and stored on computer at a rate of approximately one point per 2 s. Each replicate measurement represents the mean Ca flux during a 7- to 8-min period. After a further 30 min, the treatment solution was changed, and a second measurement was made. The average Ca concentration in the unstirred layers was monitored throughout the experiment by comparing the absolute voltage of the electrode with the voltage and known concentration in the bulk solution. Net Ca flux ($J_{Ca}$) (pmol $cm^{-2}$ $s^{-1}$) was calculated, by assuming cylindrical diffusion, from

$$J_{Ca} = \frac{\Delta V \cdot C \cdot D_{Ca}}{S \cdot r_1 \cdot \log(r_2/r_3)} \quad (1)$$

where $\Delta V$ is the voltage difference of the electrode between the extremes of vibration (μV), $C$ is the Ca concentration at the point of measurement (μM), $D_{Ca}$ is the self-diffusion coefficient for Ca in solution ($7.5 \times 10^{-6}$ $cm^2$ $s^{-1}$), $S$ is the slope of the electrode calibration (mV $decade^{-1}$), $r_1$ is the radius of the root (cm), and $r_2$ and $r_3$ are the radial distances of the electrode at the extremes of its oscillation, measured from the center of the root. The efficiency of the vibrating electrode in detecting a standing Ca gradient was estimated to be 65% from the procedure outlined by Küthreiber and Jaffe (1990) modified to account for the Ca activity coefficient.

### Cell Wall Preparation

Morphologically intact root cell walls were prepared as described by Huang et al. (1992b). Briefly, intact 3-d-old wheat seedlings were washed alternately in 0.5% Triton X-100 and 95% ethanol for 24 h (approximately 2- to 3-h exposures). They were then washed in 0.2 mM $CaCl_2$ (pH 4.5) for 3 h and placed in Petri dishes, as described above for intact seedlings, for at least 60 min before being used.

## RESULTS

### Root Growth

The effect of Al treatment on root elongation is shown in Table I. As expected, root growth in sensitive plants was inhibited significantly more than in the Al-tolerant plants. A 24-h treatment with 20 μM Al inhibited growth in the tolerant lines by approximately 20 to 30% compared to controls and by 50 to 70% in the sensitive lines. Exposure to 50 μM Al inhibited growth by approximately 50 to 60% in the tolerant plants and 70 to 80% in the sensitive plants.

### Time Dependency of Ca Fluxes

Mean Ca influx at the root apex ranged from 0.2 to 5.0 pmol $cm^{-2}$ $s^{-1}$, and in control conditions fluxes were generally higher in Scout 66 and Atlas 66 than in the near-isogenic lines. Within each pair of near-isogenic lines, however, the average fluxes measured on Al-tolerant and -sensitive plants were similar in control conditions. Fluxes varied with time, and, in some roots, they appeared to change in a regular pattern (Fig. 1A). These "oscillations" had a period of 2 to 5 min and were observed in about 20% of all measurements (>100 roots). The average Ca flux calculated during a 7- to 8-min measuring period was relatively constant, and replacing the control solution did not affect the mean flux.

Addition of Al did affect Ca fluxes, and the responses observed in the short term typically fell into three main categories (Fig. 1). In approximately 30% of measurements, a net Ca influx was maintained for most of the measuring period (Fig. 1A). More commonly, an initial efflux of Ca was observed, which gradually turned to an influx within 10 min (Fig. 1B). Less frequently, and only with the higher Al concentration, an efflux was maintained throughout the measuring period (Fig. 1C). We tested the possibility that the Ca efflux represented Al/Ca exchange from the root cell wall by performing similar experiments on morphologically intact root cell wall preparations. In control solution, no net Ca flux was observed in the wall preparations (Fig. 2). Addition of 50 μM Al to preparations from Scout 66 and $OK_{sens}$ roots caused a strong efflux of Ca, which decreased with time in an exponential-like fashion. After 15 to 20 min, the Al-induced Ca efflux had decreased to a zero net flux. The data from both cultivars were pooled, and a plot of ln(flux) versus time generated a straight line ($r = 0.955$) with a slope of 5.3 min, which is equivalent to the time constant of decay (data not shown).

When on two occasions control solution was reintroduced to the wall preparation following Al treatment, a transient influx of Ca was observed (data not shown). Similar re-

**Table I.** *Net root growth following 24-h treatment in 0, 20, or 50 μM $AlCl_3$*

The basal solution was (in mM) 1.0 NaCl, 0.2 KCl, 0.2 $CaCl_2$ (pH 4.5), and solutions were changed approximately every 2 to 3 h. Data represent the means and SE from five replicate roots and the percentage of growth relative to Al-free controls.

| Line or Cultivar | Net Root Growth after 24 h | | | | |
|---|---|---|---|---|---|
| | 0 μM Al | 20 μM Al | | 50 μM Al | |
| | *mm* | *mm* | % | *mm* | % |
| Atlas 66 | 8.5 ± 0.8 | 6.5 ± 1.0 | 76 | 4.2 ± 0.7 | 49 |
| Scout 66 | 7.0 ± 1.0 | 1.8 ± 0.45 | 26 | 1.9 ± 0.3 | 27 |
| Near-isogenic lines | | | | | |
| $E_6$-tol | 17.0 ± 0.9 | 12.9 ± 0.2 | 76 | 6.7 ± 0.4 | 39 |
| $E_6$-sens | 17.4 ± 0.8 | 4.5 ± 2.0 | 26 | 3.0 ± 1.3 | 17 |
| $W_2F$-RR | 6.5 ± 0.4 | 5.1 ± 0.5 | 78 | 3.0 ± 0.4 | 46 |
| $W_2F$-SS | 9.0 ± 0.5 | 3.4 ± 1.0 | 38 | 1.75 ± 0.3 | 19 |
| $OK_{tol}$ | 12.3 ± 1.0 | 8.0 ± 0.4 | 65 | 4.7 ± 0.3 | 38 |
| $OK_{sens}$ | 10.6 ± 0.4 | 5.5 ± 0.15 | 52 | 2.8 ± 0.1 | 26 |

sponses were also observed in root preparations from Al-tolerant seedlings. These results demonstrate that Al/Ca exchange is occurring in the root apoplasm and that Ca fluxes, measured within 15 min following the addition of Al, will include a contribution from the cell wall. This will tend to overestimate the apparent inhibition of net Ca fluxes by Al.

Ca fluxes were also measured following a 40-min exposure to Al to avoid exchange occurring within the cell wall. In Al-tolerant plants, Ca fluxes were either unaffected or were stimulated by a 40-min exposure to Al (Fig. 3). For the Al-sensitive plants, 20 μM Al had little effect on Ca fluxes, whereas 50 μM Al inhibited influx. A three-factor analysis of variance was used to analyze the results for the three near-isogenic lines shown in Figure 3. The factors were (a) pairs of near-isogenic lines; (b) relative sensitivity to Al; and (c) Al concentration, with six replicate roots per treatment (Table II). All factors were considered fixed and orthogonal. Two outcomes were significant; the main effect of sensitivity to Al ($P_{0.001}$) and the lines/concentration interaction ($P_{0.05}$). The results demonstrate that Al-tolerant and -sensitive plants reacted differently to Al treatment. Ca uptake at the root apex was inhibited more by Al in sensitive plants than in tolerant plants.

The effect of La on Ca fluxes in Scout 66 and Atlas 66 was also examined. After a 40-min treatment with 38 μM $LaCl_3$, net Ca uptake was inhibited by approximately 50% in both cultivars (Table III). No statistical difference was observed between the cultivars. (N.B. This concentration of La is the equivalent to the concentration of trivalent Al cation present in 50 μM $AlCl_3$ at pH 4.5.)

## DISCUSSION

The ion-selective electrode technique estimates net Ca fluxes from the extracellular Ca gradient adjacent to the root surface. It is possible, therefore, that a proportion of the measured gradient is not caused by transmembrane transport but is due to apoplasmic exchange or to mass flow. Henriksen et al. (1992) concluded that the ion movement due to transpirationally driven water flux would not significantly interfere with the estimation of fluxes using ion-selective microelectrodes. However, we have established that the measured Ca fluxes will have contributions from cell wall exchange as well as from transmembrane fluxes during the first 15 min of Al exposure in unstirred conditions. This is consistent with several previous studies demonstrating the displacement of Ca from the apoplasm by Al and other cations (Wagatsuma, 1983; Lynch et al., 1987; Caldwell, 1989; Rengel and Robinson, 1989).

Numerous reports have demonstrated an interaction between Al toxicity and Ca nutrition in wheat and other crops (see the introduction). Many of those experiments were conducted over days and even weeks, and the results have uncertain bearing on the inhibition of root elongation, which can begin within hours. Recent work with roots and protoplasts has shown that the reduction in Ca uptake by Al is rapid and consistent with competitive inhibition (Huang et al., 1992a, 1992b; Rengel and Elliot, 1992). We compared the effect of Al treatment on Ca flux in cv Scout 66 and cv Atlas 66, and the results in Figure 3 are qualitatively similar to those presented by Huang et al. (1992a, 1992b). In the present work, 20 μM Al inhibited Ca influx by approximately 35% in Scout 66 and had a negligible effect on Atlas 66. In comparable conditions (0.2 $CaCl_2$, pH 4.5), Huang et al. (1992a) observed a 50% inhibition of Ca influx in Scout 66 and a 15% inhibition in Atlas 66. These differences may, in part, be attributable to the extra salts in our experimental solutions. The apparent stimulation of Ca influx by 50 μM Al in Atlas 66 was surprising, and similar responses were observed in tolerant plants of the near-isogenic lines. The reasons for this are unknown.

Experiments performed on the three near-isogenic lines produced a similar pattern between Al-tolerant and -sensitive plants. Following treatment with 50 μM Al, Ca uptake in sensitive plants was significantly lower than in the tolerant plants. It is interesting that the effect in the near-isogenic lines was not as marked as for Scout 66 and Atlas 66, and this may indicate different sensitivities in the parent lines. It

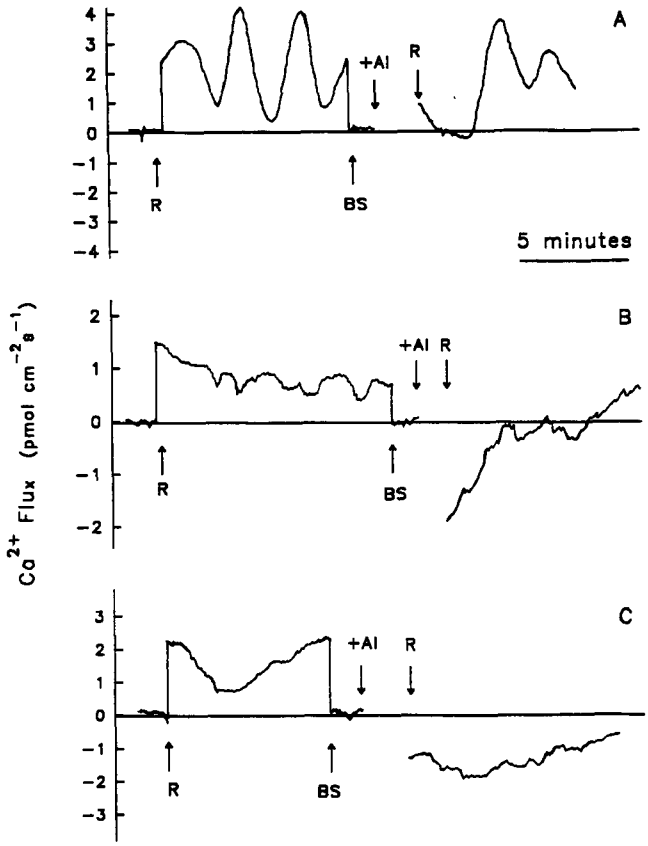


**Figure 1.** Net Ca fluxes at the apex of 6-d-old wheat roots measured with the vibrating Ca-selective microelectrode. A, B, and C represent three responses of Ca flux to Al addition. At positions marked "R," the electrode was moved from the bulk solution (BS) to the measuring position near the root. At positions marked "+Al," control solution was replaced with a similar solution containing 50 $\mu$M $AlCl_3$. Positive values depict net influx and negative values efflux of Ca.

should be noted that the genes encoding Al tolerance (and therefore the mechanism of tolerance) may or may not be similar in the three near-isogenic lines used in these experiments. Notwithstanding this, these results do show a general correlation between the Al-induced inhibition of Ca influx and a subsequent reduction in root growth. They support the general hypothesis that an Al/Ca interaction may be a primary source of toxicity in roots. However, because the relationship between the magnitude of Ca influx and the rate of root growth is unknown, we cannot be certain that the greater inhibition of root growth in Al-sensitive plants is a direct consequence of the lower Ca influx. Indeed, despite the general correlation mentioned above, some data presented here suggest that the relationship is not direct. For instance, in Al-sensitive cultivars, 20 $\mu$M Al caused a 50 to 70% inhibition of growth after 24 h (Table I), whereas the measured effect on Ca uptake was small. Furthermore, 50 $\mu$M Al inhibited growth in most Al-tolerant plants, yet the Ca fluxes were either unaffected or slightly stimulated. Although these comparisons may be misleading because of the different times at which the measurements were made (growth measurements followed a 24-h exposure to Al, and fluxes were measured after a 40-min exposure), the mechanistic association between growth and Ca uptake remains unclear and deserves further investigation. It remains possible that the differences in Ca fluxes observed in the Al-tolerant and -sensitive plants reflect secondary responses to Al stress.

### Inhibition of Ca Influx

There are several ways in which Al might inhibit Ca uptake at the root apex, and the main possibilities are discussed below. Although it is unclear to what extent the inhibition of Ca uptake by Al is the primary cause of Al toxicity, we can consider the likely mechanisms involved and speculate why tolerant plants are more resistant to the inhibition of Ca uptake by Al (Taylor, 1988b; Rengel and Elliot, 1992).

#### *Al Could Inhibit Influx by Reducing the Concentration of Ca Near Transport Proteins on the Membrane Surface*

Cations in the rhizosphere, and especially trivalent cations, are electrostatically attracted to the negatively charged surface of membranes and can accumulate there to much higher concentrations than occurs in the bathing solution (McLaughlin, 1977). This reduces the membrane surface potential, because the fixed anions on the membranes are shielded or neutralized (McLaughlin, 1977; Akeson et al., 1989), and decreases further accumulation of other cations toward the membrane surface. The potential involvement of surface

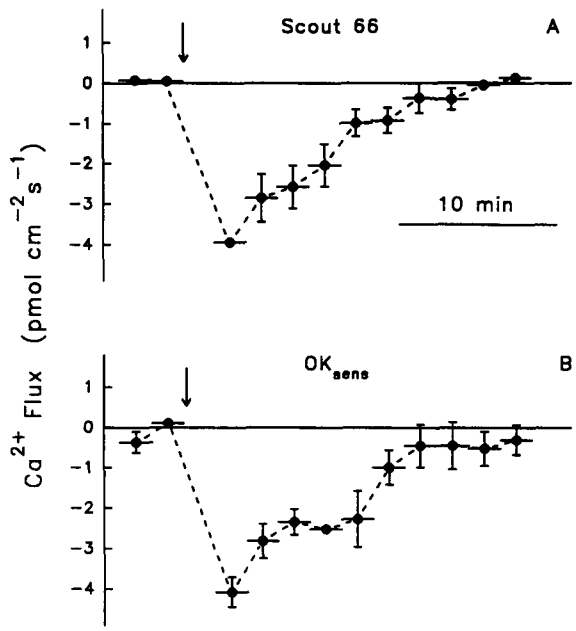


**Figure 2.** Net Ca fluxes measured in morphologically similar root cell wall preparations. Seedlings (6 d old) of Scout 66 (A) and $OK_{sens}$ (B) were treated alternatively with 95% ethanol and 0.5% Triton X-100 during 24 h. Roots were washed for 3 h in 0.2 mM $CaCl_2$ (pH 4.5) and treated as described for live seedlings. The arrows indicate addition of 50 $\mu$M $AlCl_3$ (similar background solution). The results show the mean flux and SE from four replicate roots measured during 2-min periods (horizontal bars).

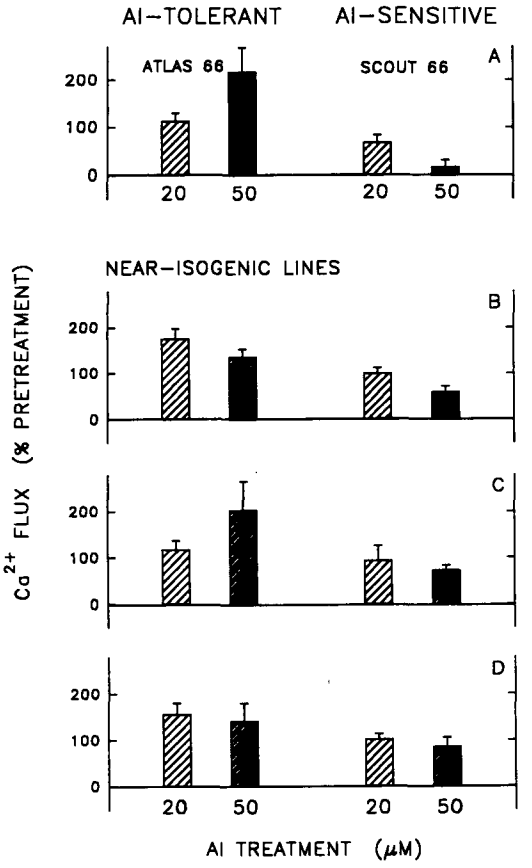


**Figure 3.** Relative change in Ca flux following a 40-min treatment in 20 or 50 μM $AlCl_3$. Results are expressed as a percentage of pre-Al values, calculated from individual roots, and show the mean and SE from six replicates. A, Comparison of cv Atlas 66 and cv Scout 66; B–D, comparison of the Al-tolerant and Al-sensitive plants from the three near-isogenic lines $E_6$-tol/$E_6$-sens (B), $W_2$FF-RR/$W_2$FF-SS (C), and $OK_{tol}$/$OK_{sens}$ (D). The results for Al-tolerant plants are shown in the two left columns, and those for Al-sensitive plants are shown in the two right columns.

**Table II.** *Results of the analysis of variance applied to the Ca flux data collected from the three pairs of near-isogenic lines*

The three orthogonal factors are (a) near-isogenic lines (three levels), (b) sensitivity to Al (two levels), and (c) Al concentration (two levels). There are six replicates roots per treatment. All *F* ratios were calculated from the residue. SS, Sum of squares; df, degrees of freedom; MS, mean square; NS, not significant; Sig, significant.

| Source | SS | df | MS | *F* ratio | Pr ($P_{0.05}$) |
|---|---|---|---|---|---|
| a (lines) | 280.775 | 2 | 140.387 | 0.0298 | NS |
| b (sensitivity) | 74949.139 | 1 | 749499.139 | 15.909 | Sig |
| c (Al concentration) | 5706.7055 | 1 | 5706.7055 | 1.2113 | NS |
| a × b | 15768.319 | 2 | 7884.1595 | 1.6735 | NS |
| a × c | 32732.503 | 2 | 16366.252 | 3.474 | Sig |
| b × c | 2391.8888 | 1 | 2391.8888 | 0.5077 | NS |
| a × b × c | 8364.0697 | 2 | 4182.0349 | 0.8877 | NS |
| Residue | 282663.510 | 60 | 4711.0585 | | |

**Table III.** *Effect of La on net Ca uptake at the apex of wheat roots*

Seedings were treated in 38 μM La for 40 min before measurement of fluxes with the vibrating Ca-selective microelectrode. Bathing solution consisted of (in mM) 1.0 NaCl, 0.2 KCl, 0.2 $CaCl_2$ (pH 4.5). Results are the means and SE from six replicate roots.

| Treatment | Net Ca flux | |
|---|---|---|
| | Scout 66 | Atlas 66 |
| | *pmol* $cm^{-2}$ $s^{-1}$ | |
| Control | 1.40 ± 0.24 | 1.40 ± 0.30 |
| +La | 0.68 ± 0.19 | 0.66 ± 0.10 |
| % Inhibition | 51.4 | 52.9 |

potential in ion transport is well established (Mozhayeva and Naumov, 1970; Theuvenet and Borst-Pauwels, 1976), and in some cases the interaction can closely resemble competitive inhibition (Gage et al., 1985). Because this is a purely coulombic interaction, Al toxicity could be one example of a general phenomenon of ionic stress. Indeed, many polyvalent cations (e.g. scandium and gallium) are known to inhibit root growth (Kinraide, 1991). It has been suggested that roots with naturally lower cation-exchange capacity in the cell wall may be less susceptible to metal-ion stress, because the accumulation of toxic cations in the cell wall would be smaller (Vose and Randall, 1962; Foy et al., 1967). The same reasoning has also been extended to the membrane itself (Wagatsuma and Akiba, 1989), and Kinraide et al. (1992) showed that the predicted activity of $Al^{3+}$ at the membrane surface of wheat roots, in a range of ionic environments, was correlated with the inhibition of root growth. If tolerance is related to a lower surface potential, Al-tolerant plants should be similarly tolerant to all cation stresses. However, in the present work, we found that La, unlike Al, inhibited Ca uptake in Al-tolerant and -sensitive plants equally. Furthermore, La has been shown to inhibit root growth similarly in Al-sensitive and Al-tolerant cultivars of wheat (Parker, 1988; Kinraide et al., 1992), as has the organic trivalent cation tris(ethylenediamine)-cobolt(III) (our unpublished data). The simplest interpretation of these results is that the mechanism of tolerance is not equally effective for all trivalent cations, and this argues against a mechanism of tolerance based on differences in membrane surface potential.

### *Al Could Be Blocking Ca Channels Mediating Ca Influx*

La is a potent blocker of Ca channels and, like Al, inhibits root growth in the micromolar concentration range. The hydrated radius of $Al^{3+}$ (4.75 Å) is close to that of $La^{3+}$ (4.52 Å), and Al could be blocking Ca channels in a similar manner. Schroeder (1988) demonstrated that Al blocked inward-rectifying K channels in guard cells, and Rengel and Elliot (1992) argued that Al could bind to the verapamil-specific site on Ca channels in protoplasts of *Amaranthus*. However, as mentioned above, La inhibited Ca influx similarly in Scout 66 and Atlas 66; therefore, the actions of Al and La may be dissimilar. Alternatively, it is possible that Al and La do block Ca channels in a similar manner, but the mechanism of tolerance is specific for Al ions. Perhaps differences exist in

the structure of the Ca channel such that Al is able to bind and block them more effectively or even pass through them more easily in sensitive plants. Alternatively, tolerant plants may protect channels by detoxifying Al in the rhizosphere, for example, by releasing a chelator (e.g. an organic acid) that has a higher binding affinity for Al than for La.

### *Al May Trigger Changes in the Concentration of Cytoplasmic Ca or Other Secondary Messengers*

In animal cells, regulation of ion channels by extracellular signals can often involve GTP-binding proteins and similar pathways are now being reported in plant cells (Fairly-Grenot and Assmann, 1991). Evidence that Al interacts with GTP-binding proteins in plant cells was presented by Rengel and Elliot (1992) for protoplasts of *Amaranthus*. They proposed that this interaction may, in part, be responsible for the Al-induced inhibition of Ca uptake in that system. Changes in cytoplasmic Ca concentrations appear to affect many metabolic processes (Hepler and Wayne, 1985; Kauss, 1987), and local increases in Ca concentration could inhibit the activity of Ca channels at the plasmalemma (Brehm and Eckert, 1978). Haug (1984) proposed that Al may bind to calmodulin and disrupt its normal interaction with Ca. Lynch et al. (1989) demonstrated that high concentrations of NaCl rapidly elevated cytoplasmic Ca in protoplasts from maize roots and suggested that the phosphoinositide pathway may be involved in initiating a metabolic response to salt stress. Other ionic stresses may cause a similar response. Relative tolerance to Al stress may be related to the initiation of this response (e.g. external detoxification of Al or differential binding of Al to the plasmalemma) or to differences in the metabolic response to the signal. However, until the effect of Al on Ca homeostasis is directly compared in Al-sensitive and -tolerant plants, and preferably with near-isogenic material, it will be unclear how important this response is to Al toxicity and whether it is involved in the expression of tolerance.

### *Al May Be Causing a Nonspecific Disruption to the Plasmalemma*

Al readily binds to artificial membrane vesicles and to isolated plasmalemma preparations and may compete with Ca for binding sites on the membrane surface (Hanson, 1984; Haug, 1984; Deleers, 1986; Kinraide and Parker, 1987; Akeson et al., 1989; Caldwell, 1989). This can change the physical properties of membranes (Vierstra and Haug, 1978; Haug, 1984; Zhao et al., 1987; Chen et al., 1991) and perhaps inhibit transport activity by modifying the environment of membrane-bound proteins (Haug, 1984; Caldwell, 1989). Relative tolerance may be related to the degree of Al binding, and some evidence suggests that root cell membranes isolated from an Al-sensitive cultivar of wheat have a greater binding affinity for Al than those isolated from an Al-tolerant cultivar (Caldwell, 1989).

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