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Mechanisms of Aluminum Tolerance in Wheat[1]

An Investigation of Genotypic Differences in Rhizosphere pH, K^+, and H^+ Transport, and Root-Cell Membrane Potentials

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ABSTRACT

Control of rhizosphere pH and exclusion of Al by the plasma membrane have been hypothesized as possible mechanisms for Al tolerance. To test primarily the rhizosphere pH hypothesis, wheat cultivars (*Triticum aestivum* L. 'Atlas 66' and 'Scout'), which differ in Al tolerance, were grown in either complete nutrient solution, or 0.6 millimolar $CaSO_4$, with and without Al at pH 4.50. A microelectrode system was used to simultaneously measure rhizosphere pH, K^+, and H^+ fluxes, and membrane potentials (E_m) along the root at various distances from the root apex. In complete nutrient solution, the rhizosphere pH associated with mature root cells (measured 10–40 millimeters from the root apex) of Al-tolerant 'Atlas 66' was slightly higher than that of the bulk solution, whereas roots of Al-sensitive 'Scout' caused a very small decrease in the rhizosphere pH. In $CaSO_4$ solution, no significant differences in rhizosphere pH were found between wheat cultivars, while differential Al tolerance was still observed, indicating that the rhizosphere pH associated with mature root tissue is not directly involved in the mechanism(s) of differential Al tolerance. In Al-tolerant 'Atlas 66', growth in a $CaSO_4$ solution with 5 micromolar Al (pH 4.50) had little effect on net K^+ influx, H^+ efflux, and root-cell membrane potential measured in cells of mature root tissue (from 10–40 mm back from apex). However, in Al-sensitive 'Scout', Al treatment caused a dramatic inhibition of K^+ influx and both a moderate reduction of H^+ efflux and depolarization of the membrane potential. These results demonstrate that increased Al tolerance in wheat is associated with the increased ability of the tolerant plant to maintain normal ion fluxes and membrane potentials across the plasmalemma of root cells in the presence of Al.

Approximately 40% of the world's cultivated lands, and up to 70% of the potentially arable lands, are acidic (4). In many of these acidic soils, aluminum (Al) toxicity is a major factor limiting root growth. Plant species and genotypes within species differ widely in tolerance to Al (2, 22, 24), and some of these differences are heritable (2). Plant breeding for Al tolerance could increase yields on such acidic soils, as well as minimize the inputs required, such as lime.

An increased understanding of the mechanisms involved in Al tolerance could help in the breeding of plants that are adapted to acidic soils. There are many hypotheses regarding mechanisms of Al avoidance or internal tolerance (for detailed reviews, see refs. 2, 22); this paper will focus on two such mechanisms. First, control of rhizosphere pH has been proposed as a means of Al avoidance, because Al solubility is very pH dependent (2, 3, 22). Aluminum tolerance in wheat, barley, rye, and triticale is associated with an increased pH of the growth medium (3, 14), or an increased resistance towards lowering the pH of a mixed NH_4^+/NO_3^- solution (22, 24).

However, controversy exists over whether the observed pH difference is the cause or the effect of differential Al tolerance. Wagatsuma and Yamasaku (28) found no positive correlation between Al tolerance in barley and pH changes in the bulk nutrient solution induced by the plant in response to manipulation of nitrogen (N) sources. Taylor (23) found similar results for winter wheat.

A second hypothesis of Al avoidance in plants is the exclusion of Al by the root plasma membrane (2, 22, 26). Wagatsuma (26) found that anaerobiosis greatly increased Al uptake by roots, and he proposed that cell membranes function as an important barrier to the passive movement of Al. He also found that there was a correlation between the resistance of membranes to N_2 gas injury and the tolerance of various species to Al (26).

Aluminum has been reported to alter the properties of biological membranes. Membrane fluidity in the microorganism, *Thermoplasma acidophilum*, was decreased by Al (25). Also, Zhao *et al.* (30) found that Al altered the combined permeabilities of the plasmalemma and tonoplast in the root cortical cells of red oak, suggesting that Al affected the architecture of membrane lipids.

Once Al^{3+} enters the cytoplasm, formation of an Al-calmodulin complex has been hypothesized as a key lesion (4, 20). Aluminum, in micromolar concentrations, interfered with calmodulin-stimulated ATPase activity and proton transport in plasma-membrane enriched vesicles (20). Mat-

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sumoto (12) found that the extrusion of protons from barley roots decreased markedly after treatment with Al, and that the proton transport activity of membrane vesicles isolated from barley roots was also inhibited by Al. However, Kinraide (5) found that wheat roots exhibiting severe Al toxicity symptoms had intact membranes, and were capable of vigorous proton extrusion.

Our first objective was to test the hypothesis that a plant-induced pH increase in the rhizosphere is the cause of differential Al tolerance in wheat. We have developed a microelectrode system that allowed us to accurately quantify rhizosphere pH, while simultaneously measuring root cell membrane potentials and net ionic fluxes associated with individual cells at the root surface of intact seedlings (15). With this system, rhizosphere pH was measured near the root surface of Al-tolerant and sensitive wheat cultivars grown either in complete nutrient solution or in a $CaSO_4$ solution, in the presence and absence of Al. Microelectrode techniques permitted characterization of rhizosphere pH at various positions along the root.

The second objective of this research was to characterize the differential effects of Al on potassium (K^+) and proton (H^+) fluxes, and transmembrane potentials (E_m), using Al-tolerant and Al-sensitive wheat cultivars. Although this approach did not enable us to precisely differentiate between Al-exclusion and internal tolerance mechanisms of Al-tolerance, it did yield useful information concerning the effect of both rapid and long term exposures of Al on these membrane transport parameters in tolerant and sensitive cultivars of wheat.

MATERIALS AND METHODS

Plant Material

Cultivars of winter wheat (*Triticum aestivum* L.) were selected to represent the extremes in Al tolerance: 'Scout' is Al-sensitive, while 'Atlas 66' is Al-tolerant (24). Seeds were surface sterilized in 10% sodium hypochlorite solution for 45 min, rinsed in tap water for 15 min, and germinated on filter paper saturated with 0.6 mM $CaSO_4$ for 3 d in the dark at approximately 25°C. Subsequently, eight seedlings were selected for vigor and uniformity, transferred to polyethylene cups with polyethylene mesh bottoms, and covered with black polyethylene beads. The cups with seedlings were placed over 0.9-L of aerated nutrient solution, and grown for 4 to 6 d under a photon flux density of 370 μmol m^{-2} s^{-1}, in a water bath maintained at 25°C.

Nutrient Solutions

The two winter wheat cultivars were grown either in a modified Steinberg solution (24) or 0.6 mM $CaSO_4$ solution. For the Steinberg solution, the concentrations of macronutrients were, in mM: Ca, 1.0; Mg, 0.3; K, 0.8; NH_4-N, 0.3; NO_3-N, 3.3; S, 0.1; and P, 0.1. The concentrations of micronutrients were, in μM: Mn, 2.0; B, 6.0; Zn, 0.5; Cu, 0.15; Mo, 0.10; and Fe as FeHEDTA, 20. Aluminum was added as $Al_2(SO_4)_3$ at a concentration of 0 or 74 μM. For the $CaSO_4$ solution, Al was added at a concentration of 0 or 5 μM. The pH for both growth solutions was adjusted initially to 4.50, using H_2SO_4.

Measurement of Rhizosphere pH and Net K^+/H^+ Fluxes

Liquid membrane-type neutral carrier-based H^+- and K^+-selective microelectrodes (tip diameter = 0.5 μm) were constructed as previously detailed (11) using Fluka H^+ and K^+-selective cocktail (Catalog No. 95291 [H^+], No. 60031 [K^+], Fluka Chemical Co.). We have recently developed a technique that enables us to quantify net ionic fluxes associated with individual root epidermal cells, based on the measurement of radial ion activity gradients in the unstirred layer at the root surface with ion-selective microelectrodes. These steady state gradients are the result of ion transport at the root surface (influx or efflux) and the diffusion of ions either toward or away from the root (see ref. 15 for a detailed description of the experimental procedures). Briefly, the intact seedling was housed in a Plexiglas chamber which was attached to the stage of an Olympus compound microscope mounted on its back on the surface of a vibration-damped table (Kinetic Systems Inc.). The ion-selective microelectrode was mounted in a pressure-relieved holder on the preamplifier of a model FD 223 high input resistance dual electrometer (World Precision Instruments, Inc.). The preamplifier was then mounted onto a Narashige hydraulically driven micromanipulator (Model MO-204, Narashige USA) which was attached to the microscope stage so that the microelectrode could be lowered vertically into the solution and reach chosen radial distances from the horizontally oriented root (usually 50 and 100 μm from the root surface).

The root and vertically positioned ion-selective microelectrodes were viewed under moderate magnification (60–150×) with the Olympus microscope. To measure net K^+ and H^+ fluxes (and rhizosphere pH), the appropriate experimental solution was introduced into the chamber, displacing the previous solution, and then flow was halted. The Plexiglas chamber was constructed to minimize mixing of the solution surrounding the root due to mechanical vibration and convection; we have found that steady state ion activity gradients are established at the root surface approximately 5 min after flow is stopped. Subsequently, the K^+ and H^+ activities in the unstirred layer were measured at 50 and 100 μm from the root surface and the net fluxes at the root surface were determined from the following equation derived from diffusion analysis of the spatial symmetry of the K^+ and H^+ activity gradients:

$$J_i = \frac{2\pi D_i(C_1 - C_2)}{\ln (R_1/R_2)}$$

where J_i is the net flux of ion i per unit length of root (in μmol cm^{-1} s^{-1}), D_i is the self-diffusion coefficient for i (in cm^2 s^{-1}), C_1 and C_2 are the ion activities at the two positions, and R_1 and R_2 are the respective distances from the positions where the ion activities were measured to the center of the root. The appropriate conversion factors were then used to obtain flux values in terms of μmol g^{-1} h^{-1}. The net ionic fluxes determined in this study were measured at the root apex and 10, 20, 30, and 40 mm back from the root apex. For the rhizosphere pH measurements, we determined that the H^+ fluxes were constant between 0 and 100 μm from the root surface. Thus, the pH at the root surface was calculated

from the measurements of H^+ activity at 50 and 100 μm from the root surface using the above equation. A number of measurements of rhizosphere pH were made at the root surface to verify the validity of this approach.

Measurements of rhizosphere pH at various positions along the root were made with seedlings grown either in modified Steinberg or $CaSO_4$ solution, with and without Al (pH measurements were made in the same solutions). Because the cultivar-specific growth responses to Al were similar whether seedlings were grown in full nutrient or $CaSO_4$ solutions, measurements of net K^+ and H^+ fluxes were conducted only on $CaSO_4$-grown plants. The uptake solutions for these measurements consisted of 50 μM K^+ (as K_2SO_4), 0.6 mM $CaSO_4$, and (±)5 μM Al (as $Al_2[SO_4]_3$) at pH 4.50.

At least three separate measurements of rhizosphere pH and K^+/H^+ transport were made at each position along the root and a minimum of two roots were measured for each cultivar treatment. Analysis of variance was calculated with the main effects of cultivar, Al, and distance from the root apex, as well as the interactions between these effects. A probability level of 0.05 or less was considered significant.

Electrophysiological Studies

The microelectrode system was constructed such that membrane potentials and K^+/H^+ fluxes could be measured simultaneously. Membrane potentials were measured using a WPI model KS-750 amplifier (World Precision Instruments, Inc.), and microelectrodes (tip diameter = 0.5 μm) made from single-barrelled borosilicate glass tubing and filled with 3 M KCl (adjusted to pH 2 to reduce tip potentials). The reference electrodes for both membrane potentials and K^+/H^+ flux measurements were also 3 M KCl-filled micropipettes and were placed in the solution away from the measured root to minimize contamination with K^+ diffusing from the reference electrodes. Cells of the root epidermis and cortex were impaled using a separate hydraulically driven Narashige micromanipulator mounted at a second position on the microscope stage.

Calculation of Al Speciation

Equilibrium speciation of Al was calculated in the $CaSO_4$ solution, using the PC-based GEOCHEM program (17, 21) and the Al^{3+} thermodynamic constants outlined in Table I. It is important to detail the thermodynamic constants used, because the speciation results of GEOCHEM are very sensitive to these values. The thermodynamic constant for amorphous $Al(OH)_3$ was chosen, because gibbsite was not expected to form in this solution under the short term conditions of these experiments. Solution pH was varied in the calculations to determine the effect of pH on Al speciation in $CaSO_4$ solution (Table II). Aluminum speciation in the complete nutrient solution was not calculated, because of the difficulty in characterizing aluminum phosphate interactions (16).

RESULTS

Rhizosphere pH

The Al-tolerant wheat cultivar 'Atlas 66', when grown in complete nutrient solution, had a root tolerance index (RTI) of 0.92 (±0.06).[2] The RTI for a particular Al^{3+} activity is defined as the root length of plants grown with Al relative to that of plants grown without Al (24). The RTI of 'Scout' was 0.50 (±0.07), confirming the differential Al tolerance of these two winter wheat cultivars, as noted by Taylor and Foy (24).

Between 10 and 40 mm from the root apex, 'Atlas 66' grown in Steinberg solution without Al caused a small increase in the rhizosphere pH relative to the bulk solution (Fig. 1A). In the presence of Al, 'Atlas 66' did not greatly change the rhizosphere pH in this root zone, relative to the bulk solution. In contrast, Al-sensitive 'Scout' slightly acidified the rhizosphere in this region of the root, relative to the bulk solution, in the presence or absence of Al (Fig. 1B). (Analysis of variance revealed a significant cultivar effect [P = 0.0001]).

Near the root apex, both 'Atlas 66' grown with or without Al, and 'Scout' grown without Al, increased the rhizosphere pH by about 0.1 to 0.2 units relative to the bulk solution pH of 4.50 (Fig. 1, A and B). However, 'Scout' grown with Al only slightly raised the pH (above the bulk solution pH) at the surface of the root apex.

When the two cultivars were grown in 0.6 mM $CaSO_4$ solution with or without Al, 'Atlas 66' had a root tolerance index of 0.98 (±0.08), while Al-sensitive 'Scout' had a RTI of 0.41 (±0.02). These results again confirm the differential Al tolerance of the two wheat cultivars, as observed earlier in the modified Steinberg solution.

Between 10 and 40 mm from the apex, the rhizosphere pH of both cultivars, when grown in $CaSO_4$ solution, was relatively unchanged from that of the bulk solution (Fig. 2, A and B). Furthermore, the inclusion of 5 μM Al in the growth solution had little effect on the rhizosphere pH for either cultivar over this root region.

Both wheat cultivars, when growth in $CaSO_4$ solution with

[2] Means are followed by standard errors of the mean in parentheses.

Table I. *Thermodynamic Constants Used for Computed Speciation of Al in Growth Solutions*

Reaction	log K	Reference
$Al^{+3} + SO_4^{2-} = AlSO_4^+$	3.2	Lindsay (10)
$Al^{+3} + 2SO_4^{2-} = Al(SO_4)_2^-$	5.1	Kinraide and Parker (7)
$2Al^{+3} + 3SO_4^{2-} = Al_2(SO_4)_3$	−1.9	Lindsay (10)
$Al^{+3} + H_2O = AlOH^{2+} + H^+$	−5.0	Lindsay (10), Parker *et al.* (18)
$Al^{+3} + 2H_2O = Al(OH)_2^+ + 2H^+$	−9.3	Lindsay (10), Parker *et al.* (18)
$Al^{+3} + 3H_2O = Al(OH)_3 + 3H^+$	−15.0	Lindsay (10), Parker *et al.* (18)
$Al^{+3} + 4H_2O = Al(OH)_4^- + 4H^+$	−23.3	Lindsay (10), Parker *et al.* (18)
$Al^{+3} + 3H_2O = Al(OH)_3$ (amorph.)	−9.7	Lindsay (10)

Table II. *Free Al^{3+} and Complexed Al Species in 0.6 mM $CaSO_4$ Solution as Determined by GEOCHEM Model*[a]

pH	free Al^{3+}	Bound to SO_4^{2-}	Bound to OH^-
	% of total Al activity		
4.75	32.6	16.0	51.4
4.70	36.0	17.7	46.3
4.65	39.3	19.3	41.4
4.60	42.5	20.8	36.7
4.55	45.4	22.2	32.4
4.50	48.1	23.6	28.4
4.45	50.5	24.7	24.8
4.40	52.7	25.8	21.6
4.35	54.6	26.7	18.7

[a] Parker *et al.* (17); Sposito and Mattigod (21).

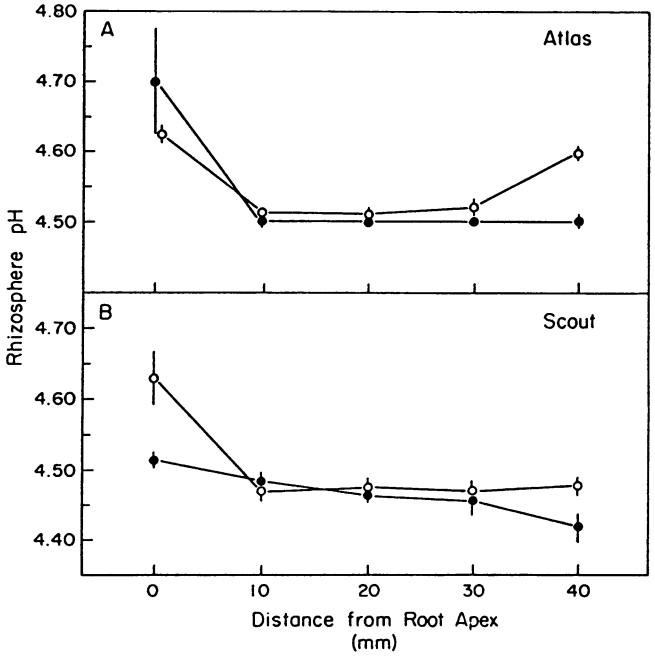

Figure 1. Rhizosphere pH measured at various distances from the root apex for (A) 'Atlas 66' and (B) 'Scout' seedlings grown in a modified Steinberg nutrient solution (pH 4.50), in the presence (●) and absence (○) of 74 μM Al. The values presented in this and subsequent figures represent the rhizosphere pH at the root surface. Error bars presented here and in subsequent figures represent ± the standard error of the mean. Points that lack error bars do so because the standard errors were smaller than the symbols used.

Al, tended to increase the rhizosphere pH at the root apex by about 0.1 pH units relative to the bulk solution (Fig. 2, A and B). When Al was included in the growth solution, the pH increase at the apex was maintained in 'Atlas 66', whereas 'Scout' only slightly increased the pH at the root apical surface. These results indicate that rhizosphere pH patterns for the two cultivars were similar whether grown in $CaSO_4$ or complete nutrient solution, with and without Al.

Speciation of Al in 0.6 mM $CaSO_4$, at a range of pH values between 4.35 and 4.75, is shown in Table II. It is apparent from Table II that as solution pH increases, the activity of free Al^{3+} decreases.

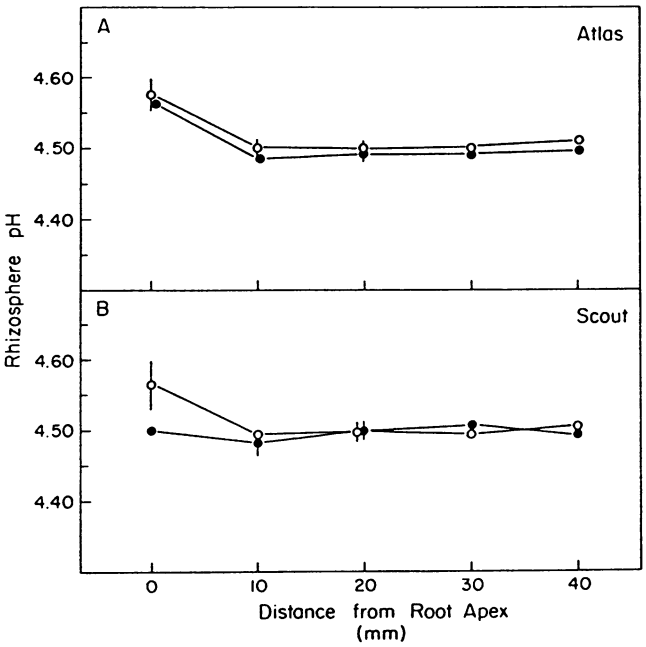

Figure 2. Rhizosphere pH measured at various distances from the root apex for (A) 'Atlas 66' and (B) 'Scout' grown in a 0.6 mM $CaSO_4$ solution (pH 4.50), in the presence (●) and absence (○) of 5 μM Al.

Measurement of K^+/H^+ Transport and Root Cell Electrical Properties

Net K^+ uptake into roots of Al-tolerant 'Atlas 66', measured at 10 to 40 mm from the root apex, was relatively insensitive to the presence of Al in the growth medium (Fig. 3A). In contrast, root K^+ influx in 'Scout' was dramatically reduced by the presence of Al in the growth medium (Fig. 3B). (Analysis of variance showed a significant cultivar effect [$P = 0.0355$], and Al effect [$P = 0.0001$]).

Often, net K^+ uptake was smaller and more variable at the root apex for both cultivars in the presence and absence of Al. Occasionally the variability at the apex was large; for example, in Figure 3, a net K^+ efflux was observed at the root apex of 'Atlas 66' in the presence of Al, whereas in 'Scout', a net K^+ uptake was measured for the same conditions. We have observed this same phenomenon without Al at the root apices of other plants (corn, barley, peas) and feel that is reflects physiological changes resulting from cell division and differentiation, and is not necessarily due to Al exposure.

Similar patterns were found for H^+ efflux as for K^+ uptake in the two cultivars exposed to K_2SO_4 (Fig. 4). When 'Atlas 66' was grown in the presence of 5 μM Al, net H^+ efflux measured at 10 to 40 mm from the root apex was slightly greater than that measured in plants grown without Al (Fig. 4A). In contrast, the inclusion of Al in the growth medium was associated with a moderate reduction in net H^+ efflux in this same region of the root for 'Scout' (Fig. 4B). It should be noted here that because we are quantifying H^+ fluxes by measuring the H^+ activity gradient over a small distance (from 50–100 μm from root) in an acidic medium, fairly large H^+ efflux values will result in quite small changes in rhizosphere pH. For example, for a net H^+ efflux of 2.00 μmol g^{-1} h^{-1} determined in a solution of pH 4.50, the pH difference

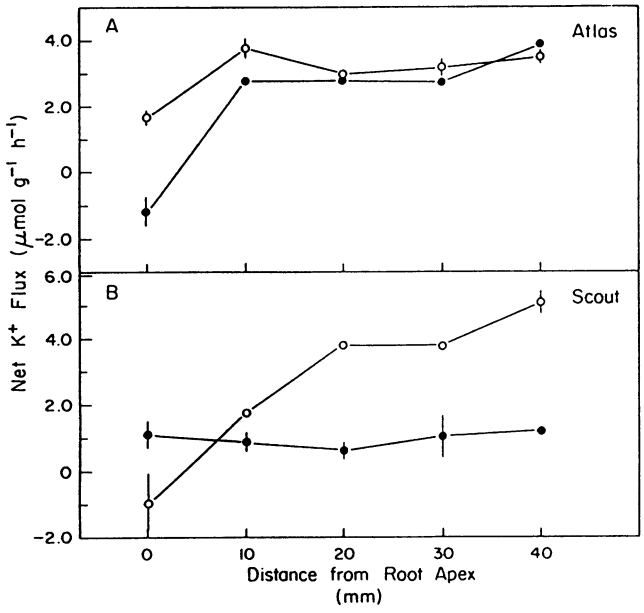

Figure 3. Net potassium fluxes measured at various distances from the root apex for (A) 'Atlas 66' and (B) 'Scout' grown in 0.6 mM $CaSO_4$ solution (pH 4.50), in the presence (●) and absence (○) of 5 μM Al. Potassium (and proton) fluxes presented here and in subsequent figures were measured in a solution consisting of 25 μM K_2SO_4, 0.6 mM $CaSO_4$, and ±5 μM Al (pH 4.50). A positive value denotes a net uptake, while a negative value denotes a net efflux.

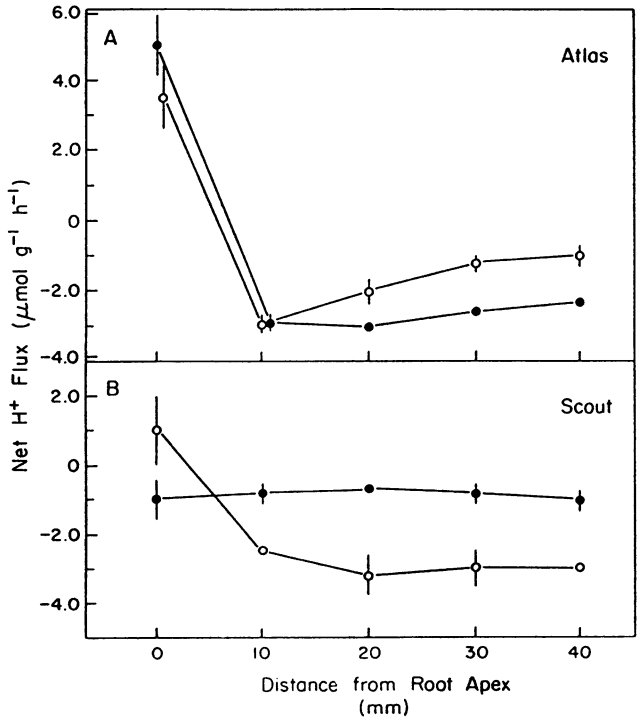

Figure 4. Net proton fluxes measured at various distances from the root apex for (A) 'Atlas 66' and (B) 'Scout' grown in 0.6 mM $CaSO_4$ solution (pH 4.50), in the presence (●) and absence (○) of 5 μM Al. A positive value denotes a net uptake, while a negative value denotes a net efflux.

measured between 50 and 100 μm from the root surface is only 0.007 of a pH unit. Hence, the measurements of H^+ efflux presented in Figure 4 were made close to the limits of detection of our microelectrode system. Therefore, although we are confident that Al exposure resulted in a moderate inhibition of H^+ efflux in the Al-sensitive 'Scout', caution must be exercised in assessing the degree of inhibition caused by Al-exposure.

At the root apex, 'Atlas 66' grown with or without Al, and 'Scout' grown without Al, had a large net H^+ uptake (Fig. 4). These results correlate with the pH increase of the root apical rhizosphere observed earlier for the same cultivar treatments when measured in $CaSO_4$ solution (Fig. 2). At the root apex of 'Scout' grown with Al, a slight net H^+ efflux was observed, which again agrees with the rhizosphere pH results presented in Figure 2B.

The influence of 5 and 50 μM Al on E_m, the K^+-induced depolarization of E_m, and net K^+ and H^+ fluxes was determined and representative traces are presented for the two cultivars grown in $CaSO_4$ (Fig. 5) and $CaSO_4$ plus 5 μM Al (Fig. 6). The K^+ and H^+ fluxes are presented in the inserts above each E_m trace.

A number of electrophysiological measurements were made

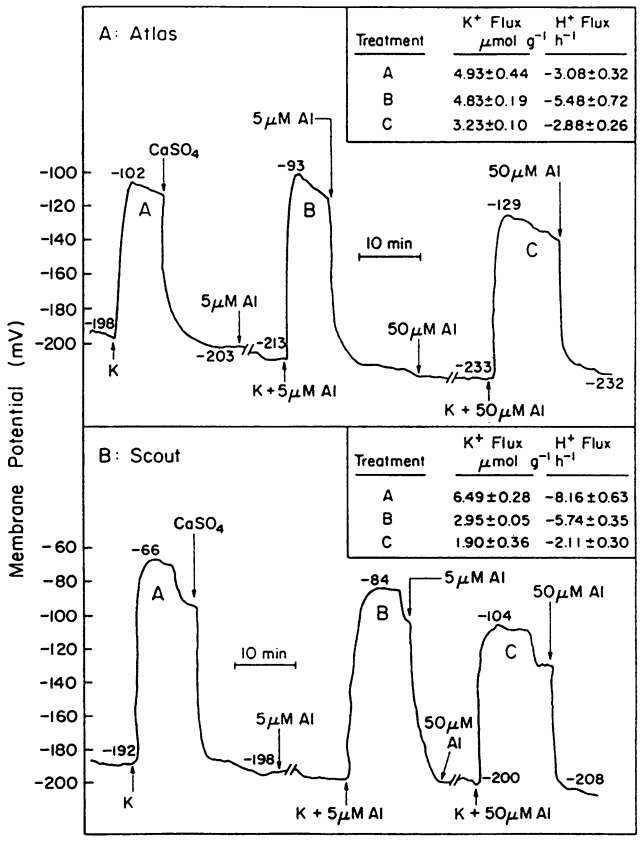

Figure 5. The influence of 5 and 50 μM Al on root transmembrane potentials (E_m) and net K^+ and H^+ fluxes measured in (A) 'Atlas 66' and (B) 'Scout' grown in 0.6 mM $CaSO_4$ solution (pH 4.50) without Al. The net K^+ and H^+ fluxes were measured at time points A, B, and C on the figure and are presented in the inset. A positive value denotes a net uptake, while a negative value denotes a net efflux.

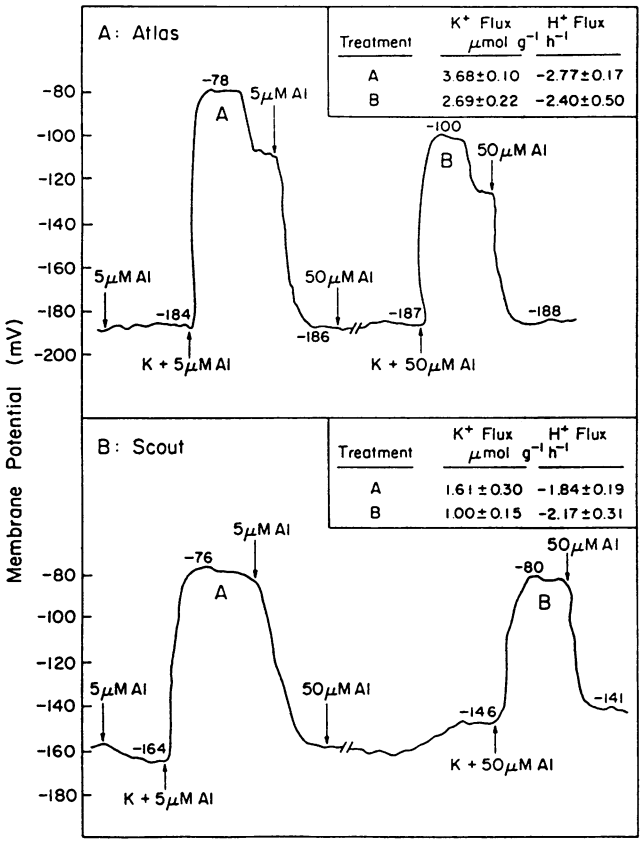

Figure 6. The influence of 5 and 50 μM Al on root transmembrane potentials and net K^+ and H^+ fluxes measured in (A) 'Atlas 66', and (B) 'Scout' grown in 0.6 mM $CaSO_4$ + 5 μM Al (pH 4.50). The net K^+ and H^+ fluxes were measured at time points A and B on the figure and are presented in the inset. A positive value denotes a net uptake, while a negative value denotes a net efflux.

on at least three roots of 'Atlas 66' grown with or without Al, and 'Scout' grown without Al, and resting potentials ranged from −184 to −213 mV for these treatments. In contrast, 'Scout' grown with Al, had a resting E_m that ranged from −128 to −180 mV. The E_m traces presented in Figures 5 and 6 were chosen as representative responses for each cultivar and treatment. The presence of 5 or 50 μM Al in the perfusion solution alone did not greatly affect membrane potentials, although exposure to Al often elicited a gradual hyperpolarization of E_m (see Fig. 5).

The short-term effect of Al on net K^+ uptake and net H^+ efflux is evident when both Al and K^+ are added to the perfusion solution of 'Atlas 66' and 'Scout' grown without Al (Fig. 5). Aluminum at a concentration of 5 μM in the perfusion solution had little effect on net K^+ uptake in Al-tolerant 'Atlas 66', whereas exposure of Al-sensitive 'Scout' to 5 μM Al caused a greater than 50% reduction in K^+ uptake. Additionally, this Al exposure elicited a small decrease in magnitude of the K^+-induced depolarization of E_m in 'Scout', while it had no effect on the same parameter in 'Atlas 66'. The presence of 5 μM Al in the perfusion solution increased H^+ efflux in 'Atlas 66', whereas it decreased H^+ efflux in 'Scout' (Fig. 5). Aluminum at a concentration of 50 μM in the perfusion solution resulted in moderate decreases in net K^+ uptake and H^+ efflux in 'Atlas 66', while it much more dramatically inhibited both fluxes in 'Scout' (Fig. 5).

The long-term effect of Al on net K^+ uptake and H^+ efflux is seen when the wheat cultivars were grown in solutions containing 5 μM Al (Fig. 6). Net K^+ uptake is considerably lower in 'Scout' grown with Al, compared to 'Scout' grown without Al, or to 'Atlas 66' grown with or without Al (*cf.* Figs. 5 and 6). Net H^+ efflux is also reduced to a greater degree in 'Scout' grown with Al, compared to the other cultivar treatments, although considerable variability in H^+ flux values was often observed (Figs. 5 and 6).

DISCUSSION

Rhizosphere pH

Measurements of plant-induced pH changes in bulk solution (3, 12, 23, 24, 28) do not allow the characterization of localized pH changes at the root surface. Also, the technique of visualizing rhizosphere pH based on placing roots in agar containing pH indicator dyes (1, 5) is limited to qualitative approximations of rhizosphere pH, without much spatial resolution. Moreover, because of difficulties in using agar at the acidic pH values needed for Al studies (pH 4.50), the roots are usually placed in agar plates at much higher pH values (pH 6.3). The use of micro-pH electrodes in our experiments allowed us to obtain a precise quantification of rhizosphere pH with a high degree of spatial resolution along the root *in situ*, at an acidic pH of 4.50.

In complete nutrient solution, Al-sensitive 'Scout' slightly acidified the rhizosphere associated with fully differentiated regions of the root (10–40 mm from the root apex), in comparison with the bulk solution. In contrast, 'Atlas 66' tended to slightly increase the rhizosphere pH in this region of the root, particularly in the absence of Al. If these rhizosphere pH patterns are maintained over long periods, the cumulative result could account for the differences in pH of the growth solution reported previously for these cultivars (3, 14, 24).

For wheat seedlings grown in $CaSO_4$ solution, the rhizosphere pH measured from 10 to 40 mm from the root apex did not greatly differ between cultivars. However, differential effects of Al were still observed in these wheat cultivars, indicating that differences in rhizosphere pH in this region of the root are not the primary cause of Al tolerance.

The region of the root where pH effects are probably most critical is the root cap and root apical meristem. Bennet *et al.* (1) found that corn roots treated with Al showed increased H^+ efflux near the root apex and root cap, compared to the control roots, which were slower to exhibit H^+ efflux. They hypothesized that the primary site of Al injury is in the root cap, and Al effects on cell division and differentiation in the root meristem are mediated via hormones produced in the root cap (1). Kinraide (5) found a different pattern with wheat; he showed that control wheat roots did not acidify the apical regions, while Al-treated roots often acidified the rhizosphere around the apex.

In our research, 'Atlas 66' grown in complete nutrient solution or in $CaSO_4$ with or without Al, and 'Scout' grown

without Al, significantly increased the pH of the root apical rhizosphere by about 0.15 units relative to the bulk solution. However, the stunted roots of Al-sensitive 'Scout' grown with Al, only slightly raised the rhizosphere pH in this region of the root. These results for 'Scout' agree with those of Kinraide (5), in which the Al-sensitive 'Tyler' wheat cultivar was used.

However, it is unlikely that this observed difference in apical rhizosphere pH between the two cultivars could account for the differences in Al-tolerance, for two reasons. First, the difference is only observed after growth on a solution containing aluminum. When Al is absent from the growth solution, no differences in apical rhizosphere pH between the cultivars were seen. Thus, it is more likely that the differences in rhizosphere pH are results of differential Al-tolerance between the cultivars (*e.g.* a product of reduced root growth in 'Scout'). Second, although there is considerable disagreement in the literature as to the species of Al which are phytotoxic, there is a general agreement that Al^{3+} is toxic (8, 18). Table II shows that an increase in pH from 4.50 to 4.60 (the situation at the apical rhizosphere for 'Atlas' grown in $CaSO_4$ solution with or without Al) results in only a small decrease in free Al^{3+} activity from 48 to 42%. Such a slight decrease would still result in free Al^{3+} of approximately 2 μM, a level shown to inhibit root growth in a sensitive cultivar (6).

Wiesenseel *et al.* (29) studied the electric field surrounding growing root tips, and presented data consistent with a proton current (net H^+ uptake) entering the root in the zone of root elongation. He hypothesized that this natural electric current could be critical for the control of localized growth (29). Thus, the reduction of growth in Al-sensitive wheat roots due to the presence of Al, could either be the cause or the result of the decreased H^+ uptake that we measured near the root apex of 'Scout' exposed to Al.

Our observations of differential Al tolerance in wheat cultivars grown in $CaSO_4$ solution agree with the results of Kinraide *et al.* (6) for barley cultivars. These results demonstrate that the relative rates of NH_4^+ and NO_3^- uptake are not a causal factor in Al tolerance, as suggested by Foy (2). Instead, these results agree with those of Taylor (23) and Wagatsuma and Yamasaku (28), which showed that plant-induced pH changes of the external solution in response to varying N sources did not affect Al tolerance.

Potassium and Proton Fluxes

Ion-selective microelectrodes allowed us to characterize net K^+ and H^+ fluxes along the root. Net K^+ uptake was fairly constant along the root in the region consisting of mature, fully differentiated cells (from 10 and 40 mm from the root apex). In this region of the root, net K^+ uptake was not greatly affected by the presence of Al in the growth medium for Al-tolerant 'Atlas 66'. However, in this same region of the root, Al in the growth medium dramatically reduced net K^+ uptake for Al-sensitive 'Scout'. Additionally, exposure of 'Scout' roots grown without Al to Al in the uptake solution resulted in an immediate and dramatic inhibition of K^+ uptake, while Al-tolerant 'Atlas 66' was unaffected by the same treatment. These results strongly indicate that distinct differences exist in the root-cell plasmalemma and/or plasmalemma ion transport systems between the two cultivars and these differences are associated with the different Al-tolerance observed.

These results for Al-sensitive 'Scout' agree with those of Matsumoto and Yamaya (13), in which Al depressed K^+ uptake by peas, based on measurements of solution depletion of K^+. It was suggested that Al was complexing with ATP in the root cell symplasm, which in turn could inhibit a number of metabolically coupled processes. In support of this hypothesis, Pfeffer *et al.* (19) found that Al reduced ATP levels in corn, as revealed by ^{31}P-NMR studies.

The results of Al-sensitive 'Scout' also agree with those of Wagatsuma *et al.* (27), in which Al treatment decreased the K^+ content in the zone of root elongation in pea, maize, and rice. In contrast to Matsumoto and Yamaya's hypothesis (13), these authors felt that the binding of Al to the plasma membrane induced phase separation, which allowed leakage of K^+ out of the cells (27). Our data do not allow us to distinguish between these two hypotheses (13, 27); however, they demonstrate the effect of differential Al tolerance on net K^+ uptake by wheat roots.

The effect of differential Al tolerance on net H^+ efflux of roots exposed to K_2SO_4 showed a similar pattern to that of net K^+ uptake. In Al-tolerant 'Atlas 66', net H^+ efflux in the zone of maximum K^+ uptake is slightly greater in the presence of Al in the growth medium. However, in this same region of the root, Al-sensitive 'Scout' showed a moderate decrease in net H^+ efflux, when Al was present in the growth medium. This decrease in net H^+ efflux was usually associated with a decrease in net K^+ uptake. Kochian and Lucas (9) have observed that although K^+ uptake is generally considered to be influenced or associated with an electrochemical potential gradient for protons across the plasmalemma, a controversy exists concerning the mode and coupling of K^+ uptake with H^+ efflux (see also ref. 15). Evidence in support of a direct coupling between the two fluxes was not found for wheat roots in the current study, because H^+ efflux often varied considerably over time, while the associated K^+ uptake values tended to be fairly constant.

Our observation that Al inhibited H^+ efflux in the mature root zone of the sensitive cultivar exposed to K_2SO_4 is in agreement with the findings of Matsumoto (12), where Al inhibited H^+ extrusion from the roots of barley in the presence of KCl. Our results appear to be in contradiction to some earlier reports of Al-induced stimulation of H^+ efflux (see, for example, 1, 5, 24). However, this topic is marked by confusion in the literature, primarily because of the differences in experimental tissues and conditions employed by different researchers. For example, in some of the previous studies (1, 5), seedlings were grown under acidic conditions (pH 4.0–4.5), whereas physiological parameters such as H^+ efflux were measured on agar plates at pH values near neutrality. Also, the age of the root tissue used (root tips *versus* intact roots or mature root tissue) can affect the experimental results. As we have demonstrated, H^+ fluxes at the root apex are quite different than those measured in mature root tissue and exhibit markedly different responses to Al-exposure in tolerant *versus* sensitive wheat cultivars (Figs. 2 and 4). Finally, in many reports, seedlings were grown and H^+ efflux was determined in solutions containing both NO_3^- and NH_4^+, where

confounding effects arise from acidification due to NH_4^+ absorption *versus* alkalinization due to NO_3^- uptake, complicating data interpretation. In our studies, we have attempted to simplify our experimental system by: (a) conducting our investigations in a simple salt solution (0.6 mM $CaSO_4$), after demonstrating that differential Al-tolerance occurs under this condition; (b) making all measurements at the same pH as that in the growth solutions; and (c) measuring rhizosphere pH and ion transport at varying distances along the roots of intact plants, to determine spatially related differences in response to Al (root apex *versus* mature root tissue).

Transmembrane Potentials

Results based on the measurements of root cell membrane potentials showed that Al did not have a dramatic, immediate effect on the plasma membrane of wheat, in terms of root-cell electrical properties. However, 'Scout' grown in a medium containing 5 μM Al did exhibit a moderate reduction in the resting E_m compared with 'Scout' grown without Al, or 'Atlas 66' grown with or without Al. From these results, one might speculate that the initial phytotoxic effect of Al does not occur at the outer face of the plasma membrane. However, these electrophysiological results are somewhat puzzling, when contrasted with the rapid inhibition of both H^+ and K^+ transport by Al in 'Scout' grown without Al. In contrast to the electrophysiological results, the observations that root ion transport processes exhibit almost instantaneous cultivar-specific differences in response to initial Al-exposure supports, at least circumstantially, the hypothesis proposed by Wagatsuma (26), that differential Al tolerance is primarily due to differences in the properties of the plasma membrane. Therefore, the relative constancy of the membrane potential following exposure of the roots to Al suggests that the root cell membrane potential, which is a complex parameter resulting from the operation of all of the active and passive ion transport systems functioning across the plasmalemma, may not be a useful parameter for studying Al-induced toxicity.

Several of our results concerning Al effects on E_m and H^+ efflux are somewhat different from those presented recently by Kinraide (5). First, he measured resting membrane potential of about −100 mV in both Al-cultured and control wheat roots. These E_m values are much smaller than the ones presented here, and could be the result of wounding during the process of root excision, prior to measurement of E_m. In our research, intact roots of whole plants were used, and a resting E_m of approximately −200 mV was found for 'Atlas 66' roots grown with or without Al, and 'Scout' roots grown without Al, while a value of about −160 mV was found for 'Scout' roots grown with Al. Second, Kinraide (5) found that Al-sensitive 'Tyler' wheat roots, exhibiting severe Al toxicity symptoms, were capable of vigorous proton extrusion, particularly in the presence of fusicoccin or acetic acid, which both have been shown to stimulate H^+ efflux. In the present study, H^+ efflux was measured in the presence of 50 μM K^+, and it was found to be depressed in 'Scout' roots grown with Al. However, we did not study the influence of fusicoccin or acetic acid on H^+ efflux in 'Scout' roots exhibiting Al toxicity symptoms.

CONCLUSION

The results presented in the current study strongly indicate that for cultivars of wheat exhibiting differential Al tolerance, differences in rhizosphere pH do not play a critical role in the mechanism(s) of Al tolerance. However, based on electrophysiological evidence and measurements of K^+ and H^+ fluxes, it appears likely that increased Al tolerance in wheat is associated with the increased ability of the tolerant plant to maintain "normal" ion fluxes and membrane potentials across the plasmalemma of root cells in the presence of Al.

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LITERATURE CITED

1. **Bennet RJ, Breen CM, Fey MV** (1987) The effects of aluminium on root cap function and root development in *Zea mays* L. Environ Exp Bot **27:** 91–104
2. **Foy CD** (1988) Plant adaptation to acid, aluminum-toxic soils. Commun Soil Sci Plant Anal **19:** 959–987
3. **Foy CD, Burns GR, Brown JC, Fleming AL** (1965) Differential aluminum tolerance of two wheat varieties associated with plant-induced pH changes around their roots. Soil Sci Soc Am Proc **29:** 64–67
4. **Haug A** (1984) Molecular aspects of aluminum toxicity. CRC Crit Rev Plant Sci **1:** 345–373
5. **Kinraide TB** (1988) Proton extrusion by aluminum-intoxicated wheat roots. Plant Physiol **88:** 418–423
6. **Kinraide TB, Arnold RC, Baligar VC** (1985) A rapid assay for aluminium phytotoxicity at submicromolar concentrations. Physiol Plant **65:** 245–250
7. **Kinraide TB, Parker DR** (1987) Non-phytotoxicity of the aluminum sulfate ion, $AlSO_4^+$. Physiol Plant **71:** 207–212
8. **Kinraide TB, Parker DR** (1989) Assessing the phytotoxicity of mononuclear hydroxyaluminum. Plant Cell Environ **12:** 479–488
9. **Kochian LV, Lucas WJ** (1988) Potassium transport in roots. Adv Bot Res **15:** 93–178
10. **Lindsay WL** (1979) Chemical Equilibria in Soils. John Wiley & Sons, New York
11. **Lucas WJ, Kochian LV** (1986) Ion transport processes in corn roots: An approach utilizing microelectrode techniques. *In* WG Gensler, ed, Advanced Agricultural Instrumentation: Design and Use. Martinus Nijhoff Publ, Boston, pp 402–425
12. **Matsumoto H** (1988) Inhibition of proton transport activity of microsomal membrane vesicles of barley roots by aluminium. Soil Sci Plant Nutr **34:** 499–506
13. **Matsumoto H, Yamaya T** (1986) Inhibition of potassium uptake and regulation of membrane-associated Mg^{2+}-ATPase activity of pea roots by aluminium. Soil Sci Plant Nutr **32:** 179–188
14. **Mugwira LM, Patel SU** (1977) Root zone pH changes and ion uptake imbalances by triticale, wheat, and rye. Agron J **69:** 719–722
15. **Newman IA, Kochian LV, Grusak MA, Lucas WJ** (1987) Fluxes of H^+ and K^+ in corn roots. Characterization and stoichiometries using ion-selective microelectrodes. Plant Physiol **84:** 1177–1184
16. **Nordstrom DK, May HM** (1989) Aqueous equilibrium data for mononuclear aluminum species. *In* G Sposito, ed, Aluminum. CRC Press, Boca Raton, FL (in press)
17. **Parker DR, Zelazny LW, Kinraide TB** (1987) Improvements to the program GEOCHEM. Soil Sci Soc Am J **51:** 488–491

18. **Parker DR, Kinraide TB, Zelazny LW** (1988) Aluminum speciation and phytotoxicity in dilute hydroxy-aluminum solutions. Soil Sci Soc Am J **52:** 438–444
19. **Pfeffer PE, Tu S-I, Gerasimowicz WV, Cavanaugh JR** (1986) *In vivo* ^{31}P NMR studies of corn root tissue and its uptake of toxic metals. Plant Physiol **80:** 77–84
20. **Siegel N, Haug A** (1983) Calmodulin-dependent formation of membrane potential in barley root plasma membrane vesicles: A biochemical model of aluminum toxicity in plants. Physiol Plant **59:** 285–291
21. **Sposito G. Mattigod SV** (1980) GEOCHEM: A computer program for the calculation of chemical equilibria in soil solutions and other natural water systems. Kearney Foundation of Soil Science, University of California, Riverside
22. **Taylor GJ** (1987) The physiology of aluminum tolerance. *In* H Sigel, ed, Metal Ions in Biological Systems, Vol 24, Aluminum and its Role in Biology. Marcel-Dekker, New York, pp 165–198
23. **Taylor GJ** (1988) Mechanisms of aluminum tolerance in *Triticum aestivum* (wheat). V. Nitrogen nutrition, plant induced pH, and tolerance to aluminum; correlation without causality? Can J Bot **66:** 694–699
24. **Taylor GJ, Foy CD** (1985) Mechanisms of aluminum tolerance in *Triticum aestivum* L. (wheat). I. Differential pH induced by winter cultivars in nutrient solutions. Am J Bot **72:** 695–701
25. **Vierstra R, Haug A** (1978) The effect of Al^{3+} on the physical properties of membrane lipids in *Thermoplasma acidophilum*. Biochem Biophys Res Commun **84:** 138–143
26. **Wagatsuma T** (1983) Effect of nonmetabolic conditions on the uptake of aluminum by plant roots. Soil Sci Plant Nutr **29:** 323–333
27. **Wagatsuma T, Kaneko M, Hayasaka Y** (1987) Destruction process of plant root cells by aluminum. Soil Sci Plant Nutr **33:** 161–175
28, **Wagatsuma T, Yamasaku K** (1985) Relationship between differential aluminum tolerance and plant-induced pH change of medium among barley cultivars. Soil Sci Plant Nutr **31:** 521–535
29. **Weisenseel M, Dorn A, Jaffe LF** (1979) Natural H^+ currents traverse growing roots and root hairs of barley (*Hordeum vulgare* L.). Plant Physiol **64:** 512–518
30. **Zhao X-J, Sucoff E, Stadelmann EJ** (1987) Al^{3+} and Ca^{2+} alteration of membrane permeability of *Quercus rubra* root cortex cells. Plant Physiol **83:** 159–162