Interactive Effects of Al³⁺, H⁺, and Other Cations on Root Elongation Considered in Terms of Cell-Surface Electrical Potential[1]

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ABSTRACT

The rhizotoxicities of Al³⁺ and of La³⁺ to wheat (*Triticum aestivum* L.) were similarly ameliorated by cations in the following order of effectiveness: H⁺ ≈ C³⁺ > C²⁺ > C¹⁺. Among tested cations of a given charge, ameliorative effectiveness was similar except that Ca²⁺ was slightly more effective than other divalent cations and H⁺ was much more effective than other monovalent cations. H⁺ rhizotoxicity was also ameliorated by cations in the order C³⁺ > C²⁺ > C¹⁺. These results suggest a role for cell-surface electrical potential in the rhizotoxicity of Al³⁺, La³⁺, H⁺, and other toxic cations: negatively charged cell surfaces of the root accumulate the toxic cations, and amelioration is effected by treatments that reduce the negativity of the cell-surface electrical potential by charge screening or cation binding. Membrane-surface activities of free Al³⁺ or La³⁺ computed according to a Gouy-Chapman-Stern model correlated well with growth inhibition, which correlated only poorly with Al³⁺ or La³⁺ activities in the external medium. The similar responses of Al-intoxicated and La-intoxicated roots to ameliorative treatments provide evidence that Al³⁺, rather than AlOH²⁺ or Al(OH)₂⁺, is the principal toxic species of mononuclear Al. Comparisons of the responses of Al-sensitive and Al-tolerant wheats to Al³⁺ and to La³⁺ did not support the hypothesis that varietal sensitivity to Al³⁺ is based upon differences in cell-surface electrical potential.

Aluminum rhizotoxicity (a world-wide problem in acidic soils [29]) is strongly influenced by H⁺ and other cations in the rooting media. At constant $\{Al^{3+}\}_E$,[2] divalent cations ameliorate toxicity much more effectively than do monovalent cations (3, 13), but H⁺ is even more effective than divalent cations. H⁺ increases of less than 50 μM in the pH range 4 to 5 were significantly ameliorative for four dicotyledonous plants (15). This amelioration of Al toxicity at lower pH may be attributed to a H⁺ amelioration of Al³⁺ toxicity or to

reduced activities of AlOH²⁺ and Al(OH)₂⁺ (12, 15), species whose toxicity has not been established.

It may be impossible to differentiate formally the two hypotheses for H⁺ amelioration of Al toxicity or to establish the rhizotoxicity of all of the Al species (12), but in this article we shall argue that Al³⁺ is toxic and that Al³⁺ toxicity, specifically, is ameliorated by H⁺ and other cations. The argument will rest upon new data that support the hypothesis that the negatively charged cell surfaces in the root accumulate Al³⁺ and other toxic cations, and that amelioration is effected by treatments that reduce the negativity of the cell-surface electrical potential.

Negative charges, located in the cell wall and on the plasma membrane, are carried on the carboxylate groups of cell-wall pectins, the residues of acidic amino acids in membrane proteins, and the phosphate groups of membrane phospholipids (16, 19, 23). The charges on the cell surface create electrical potential gradients that interact with the distribution of ions (5, 18, 19). Salts in the bathing medium reduce the negative surface potential in two ways: by cation binding and by charge screening. Because of the binding of H⁺ with carboxylate, phosphate, and amino groups, charge reversal of the plasma membrane surface can occur as the pH there drops below 4 (22). Divalent and polyvalent cations can also bind, and the latter can cause charge reversals at the membrane surface (1, 21). Charge screening occurs because coulombic attractions concentrate cations around the cell-surface negative charges. The effectiveness of the cations in charge screening increases with cation valence according to basic electrostatic models (5, 18, 19).

Amelioration of Al toxicity by H⁺ and other cations can be interpreted as evidence of the influence of cell surface charge on Al toxicity, but another line of evidence also suggests the same conclusion. Several reports indicate that within closely related taxa, higher varietal sensitivity to Al corresponds to higher varietal cation exchange capacity of whole roots (8, 27, and citations in those articles and in ref. 4). One recent study found an opposite relationship (4), but in that study titratable acidity was measured in isolated cell walls. These results raise the issue of the relative importance of cell walls and cell membranes in determining Al sensitivity. A recent line of investigation indicates that the surface negativity and

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[2] Abbreviations: $\{Al^{3+}\}_E$, chemical activity of Al³⁺ in the external rooting medium; $\{Al^{3+}\}_o$, chemical activity of Al³⁺ at the plasma membrane surface; TMA⁺, tetramethylammonium; TEC³⁺, tris(ethylenediamine)cobalt(III); RRL, relative root length.

the binding of basic dyes to protoplasts correspond to the Al sensitivity of the parent roots (30).

MATERIALS AND METHODS

For most of the experiments, caryopses of wheat (*Triticum aestivum* L. cvs Atlas 66 [Al tolerant] and Scout 66 and Tyler [both Al sensitive]) were incubated in 1% NaClO for 15 min and then rinsed with four changes of water for at least 30 min. The seeds were then germinated in thin layers of water in Petri dishes. After 2 d, eight seedlings were transferred to each of the plastic pots containing, unless otherwise noted, 900 mL of 0.4 mM $CaCl_2$ at pH 4.5. $AlCl_3$, when used, was added from a 10 mM stock solution in 0.2 mM HCl. Adjustment of pH was done with HCl exclusively. Precautions were taken to avoid the formation of polynuclear Al (*cf.* ref. 12 for a discussion of methods and precautions suitable for Al rhizotoxicity studies). The seedlings were then incubated in the dark at 25°C with aeration. After 2 d the lengths of the two longest roots per seedling were measured.

The RRL for each solution was computed according to the formula RRL = $100(L_{Al} - L_S)/(L_C - L_S)$, in which L_{Al} was the mean root length in the Al solution, L_C was the mean root length in the corresponding Al-free control, and L_S was the mean root length in Al sufficient to saturate growth inhibitory processes (12). (The formula was also applied to test solutes other than Al.) We consider the RRL to be the appropriate measure, but in most cases we provide values for L_S and L_C so that readers can compute root lengths. Unless noted, each experiment was performed twice, and each datum point in the figures is a mean of two values.

The concentrations and activities of free Al^{3+} at the plasma membrane surface ($[Al^{3+}]_o$ and $\{Al^{3+}\}_o$, respectively) were computed according to a Gouy-Chapman-Stern model. The Gouy-Chapman theory is expressed in the equation

$$\sigma^2 = (0.00351)\sum_i C_{i\infty}(\exp\{-z_iE_o/25.7\} - 1) \tag{1}$$

where σ is the charge density at the membrane surface in C/m^2; 0.00351 is the value of the quantity $2\epsilon_r\epsilon_oRT$ at 25°C and for concentrations expressed in M (ϵ_r is the permittivity of the solution, ϵ_o the permittivity of a vacuum, R the gas constant, and T the temperature); $C_{i\infty}$ is the concentration of the *i*th ion at infinite distance from the membrane (*i.e.* in the external medium); z_i is the charge on the *i*th mobile ion; E_o is the electrical potential in millivolts at the membrane surface; and 1/25.7 is the value of F/1000RT at 25°C, where F is the Faraday constant (5, 18).

The derivation of Equation 1 incorporates the statement

$$C_{ix} = C_{i\infty}\exp(-z_iE_x/25.7) \tag{2}$$

in which the subscript *x* refers to conditions at distance *x* from the membrane surface. Equation 2 may be used to compute the surface concentration of ions when *x* = 0. The equation incorporates the assumption that the activity coefficient for each ion remains constant at all distances from the membrane surface. For that reason concentration may be written in place of activity. McLaughlin (18) and Barber (5) discuss this and other assumptions in the Gouy-Chapman theory. We have computed activity coefficients, using ion

concentrations in the external medium, according to the Davies equation (17).

The Stern modification of the Gouy-Chapman theory considers ion binding at the membrane surface (16). σ is variable because of this binding, which can be represented as a Langmuir-type adsorption by a surface ligand Q of an ion S.

$$[Q^xS^y] = K_{Q,S}[Q^x][S^y] \tag{3}$$

where x and y are charges, $K_{Q,S}$ is an association constant in units M^{-1}, $[S^y]$ is the concentration of S at the membrane surface in units M, and $[Q^xS^y]$ and $[Q^x]$ are in moles per square meter.

σ may be computed from a knowledge of the concentration of surface ligands, the association constants for the ligands and ions, and the surface concentration of the ions. The ions H^+ and Ca^{2+} are always important in our experiments, and our model assumes a negatively charged ligand, R^-, and a neutral ligand, P^o, corresponding to aggregates of ligands such as phosphatidylserine and phosphatidylcholine, for example. Considering only these ions and ligands, σ may be computed as follows (see ref. 16):

$$\sigma = \{R_T(-1 + K_{R,Ca}[Ca^{2+}])/(1 + K_{R,H}[H^+] + K_{R,Ca}[Ca^{2+}])$$
$$+P_T(K_{P,H}[H^+] + 2K_{P,Ca}[Ca^{2+}])/(1 + K_{P,H}[H^+] \tag{4}$$
$$+ K_{P,Ca}[Ca^{2+}])\}F$$

where R_T and P_T are the total surface concentrations of R and P with and without bound ions. The equation actually used in the model was expanded for all additional important ions. Multiplication by the Faraday constant is required to convert units from equivalents per square meter to coulombs per square meter.

A combination of the equations above may be considered a rendition of the Gouy-Chapman-Stern model (16). The model we employ in this article, together with the values for the parameters, was derived from the literature without reference to our own data. Subsequently, the model was applied to our data without adjustment of parameters. The parameter values are as follows: $R_T = 10^{-6.84}$, $P_T = 10^{-5.92}$, $K_{R,H} = 3800$, $K_{R,Na} = 0.63$, $K_{R,TMA} = 0.05$, $K_{R,Ca} = 7$, $K_{R,Mg} = 6$, $K_{R,Al} = 1500$, $K_{R,La} = 120$, $K_{R,TEC} = 20$, $K_{R,Cl} = 0$, $K_{P,H} = 132$, $K_{P,Na} = 0.63$, $K_{P,TMA} = 0.05$, $K_{P,Ca} = 7$, $K_{P,Mg} = 6$, $K_{P,Al} = 1500$, $K_{P,La} = 120$, $K_{P,TEC} = 20$, $K_{P,Cl} = 0$. (Al refers to Al^{3+} only.)

Estimates of K values for H^+, Na^+, TMA^+, Ca^{2+}, and Mg^{2+} were derived from several sources (7, 16, 24). $K_{H,P}$ was assigned the value of the reciprocal of the first dissociation constant for phosphoric acid. A value for $K_{P,Al}$ was taken from Akeson *et al.* (2) and was used for $K_{R,Al}$ as well. P_T was assigned half the value of 2.4×10^{-6} mol/m^2 for phosphatidylcholine in pure vesicles (2). Our value for R_T was selected to obtain a computed value for E_o of -48.7 mV at pH 6.7 in a medium of 1 mM NaCl and 0.1 mM $CaCl_2$ (-48.8 mV was the zeta potential observed by Abe and Takeda [1] for barley protoplasts under similar conditions). Similarly, our values for $K_{R,La}$ and $K_{P,La}$ were selected to obtain an E_o value of 0 mV at 0.3 mM La^{3+} (in conformity with Abe and Takeda's measured values). Our values for $K_{R,TEC}$ and $K_{P,TEC}$ were selected to obtain an E_o value of -4.5 mV in 1 mM TEC (in

conformity with Abe and Takeda's values with the large trivalent cations hexaaminecobalt[III] and spermidine).

Once our parameters had been chosen, our model was checked against some additional published values. For example, raising Na$^+$ to 6 mM from 1 mM reduced our computed E$_o$ from -48.7 to -39.1 mV compared with Abe and Takeda's change from -48.8 to -38.9 mV, and a reduction of the pH of that 6 mM Na$^+$ solution to 3.6 changed our computed E$_o$ to −4.3 mV compared with Abe and Takeda's measured value of −2.1 mV. Furthermore, our computed value of −29.8 mV for E$_o$ in 0.4 mM CaCl$_2$ at pH 4.7 is comparable with other published values for zeta potentials of root-cell protoplasts in low-salt solutions at pH 4.7 (30).

A consequence of a high affinity between Al^{3+} and a surface with mixed neutral and negative ligands (P^o and R$^-$) is that low levels of external Al^{3+} will induce a positive potential. In our model the crossover from negative to positive occurs at 20.3 μM AlCl$_3$ in 0.4 mM CaCl$_2$ at pH 4.5. This is in agreement with the predictions of Akeson et al. (2) and the results of Obi et al. (21), who observed a marked increase in the membrane adsorption of trivalent metals as the ionic radius of the metal decreased. Their metal of smallest ionic radius (Yb^{3+}) had a radius much larger than that of Al^{3+}.

RESULTS

Cation Amelioration of Al^{3+} and La^{3+} Toxicity

In this section new data will be presented together with reassessed published data and previously obtained unpublished data. In 1987 (13) results were published of experiments in which Tyler wheat seedlings were incubated in solutions containing 0.4 mM CaCl$_2$ at pH 4.3 supplemented with AlCl$_3$ and one of the salts NaCl, MgCl$_2$, or additional CaCl$_2$. The final solutions were factorial in {Al^{3+}}$_E$ at approximately 0.00, 1.62, 3.24, 4.86, and 6.48 μM and [NaCl]$_E$ at 0, 10, 20, 30, and 40 mM or [MgCl$_2$]$_E$ or additional [CaCl$_2$]$_E$ at 0, 0.8, 1.6, 2.6, and 3.6 mM. New species computations used equilibrium constants for Al compiled by Nordstrom and May (20) and other equilibrium constants compiled by Lindsay (17). From that study and other experiments it was determined that the ameliorative effectiveness of cations (at constant {Al^{3+}}$_E$) was in the order Ca^{2+} > Mg^{2+} ≈ Sr^{2+} >> K$^+$ ≈ Na$^+$, but no interpretation from an electrostatic viewpoint was offered.

However, electrostatic theory does provide an explanation of the results because the theory predicts that the ameliorative treatments would reduce the activity of Al^{3+} at the cell surface. The results of the experiments just described were reanalyzed by computing free Al^{3+} at the membrane surfaces for each of the culture solutions using, without adjustment of parameters, the Gouy-Chapman-Stern model described above. Data from these and additional experiments to be described later have been incorporated into Figure 1, which presents RRL as a function of {Al^{3+}} in the external medium and at the membrane surface. It can be seen readily that {Al^{3+}}$_E$ fails to predict RRL and that {Al^{3+}}$_o$ is much more closely related to root elongation. Nonlinear regression analyses with the equation RRL = 100/exp([ax]^b) (14) yielded values for R^2 of 0.353 for x = {Al^{3+}}$_E$ and 0.740 for x = {Al^{3+}}$_o$.
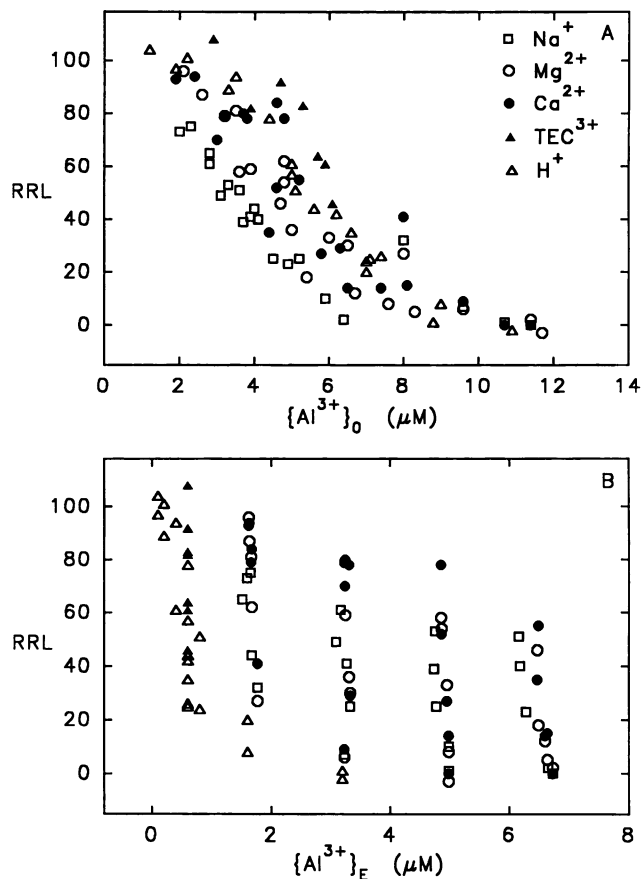

Figure 1. RRL as a function of Al^{3+} activity in the rooting medium ({Al^{3+}}$_E$) and at the root-cell membrane surface ({Al^{3+}}$_o$) as computed by a Gouy-Chapman-Stern model. The symbol code in A refers to the ameliorative cations for both panels. Data points denoted ▲ were derived from Figure 3A and points denoted △ were derived from Figures 4 (Scout 66) and 6A.

Similar experiments with La^{3+} and Tyler wheat were performed at the same time as the preceding experiments but were not published. In one experiment (repeated three times) the solutions were factorial in {La^{3+}}$_E$ at 0.0, 1.5, 3.0, 4.5, and 6.0 μM and in [NaCl]$_E$ at 0, 10, 20, 30, and 40 mM. In this experiment {Ca^{2+}}$_E$ = 0.4 mM and the pH was 4.3. In a similar experiment (repeated three times) the solutions were factorial in {La^{3+}}$_E$ as above and in [CaCl$_2$]$_E$ at 0.4, 1.2, 2.0, 3.0, and 4.0 mM at pH 4.3. Finally, in an unreplicated experiment, the solutions were factorial in [LaCl$_3$]$_E$ at 0, 2, 5, and 10 μM and in [MgCl$_2$]$_E$ at 0, 1, 2, and 3 mM. [CaCl$_2$]$_E$ was 0.4 mM and the pH was 4.3 throughout. The results of these experiments were analyzed by computing free La^{3+} at the membrane surface according to the Gouy-Chapman-Stern model. Data from these and additional experiments to be described later have been incorporated into Figure 2, which presents RRL as a function of {La^{3+}}$_E$ and {La^{3+}}$_o$. Here, too, it can be seen that estimated membrane-surface {La^{3+}} correlates well with RRL and that {La^{3+}} in the medium does not. Nonlinear regression analyses with the equation RRL = 100/exp([ax]^b) yielded values for R^2 of 0.384 for x = {La^{3+}}$_E$ and 0.762 for x = {La^{3+}}$_o$.
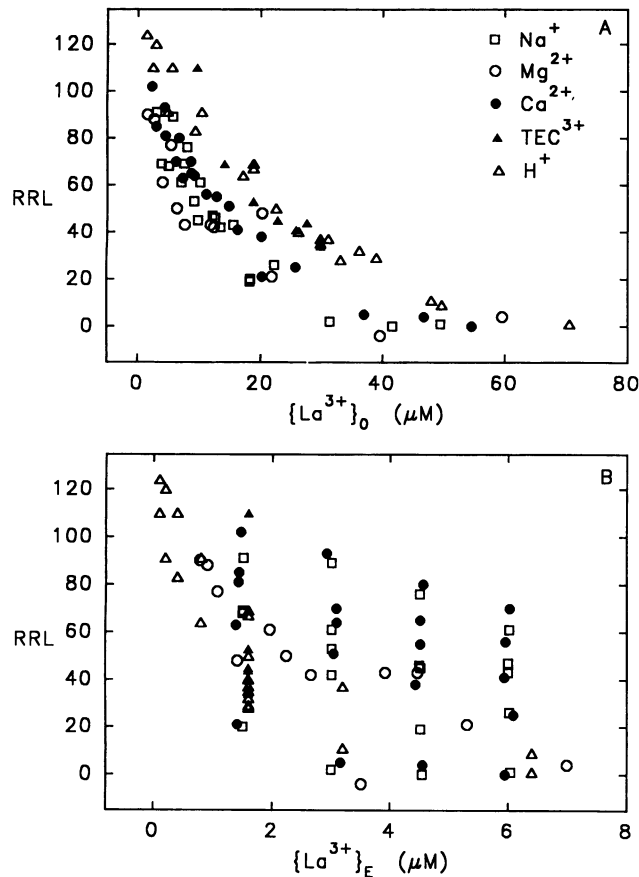
Figure 2. RRL as a function of La^{3+} activity in the rooting medium ($\{La^{3+}\}_E$) and at the root-cell membrane surface ($\{La^{3+}\}_o$) as computed by a Gouy-Chapman-Stern model. The symbol code in A refers to the ameliorative cations for both panels. Data points denoted ▲ were derived from Figure 3B and points denoted △ were derived from Figures 5 and 7A.

New experiments were performed to test further the electrostatic hypothesis of cation amelioration. The large monovalent cation TMA^+ binds to membrane ligands so weakly (24) that amelioration by this ion would confirm that charge screening alone plays some role in the phenomenon. An experiment repeated three times with Scout 66 wheat assessed root elongation in 0, 0.2, 0.4, 0.8, 1.6, and 3.2 μM $\{Al^{3+}\}_E$ in a background of 0.4 mM $CaCl_2$ alone or together with 20 mM NaCl or 20 mM TMACl, all at pH 4.5. Na^+ and TMA^+ were nearly equally effective in ameliorating Al^{3+} toxicity. At 0.8 μM $\{Al^{3+}\}_E$, for example, RRL was 42% in $CaCl_2$ alone but was 82% in NaCl and 71% in TMACl.

Theory predicts that a trivalent cation should be more effective than divalent cations. Specifically, one might expect significant amelioration at 0.1 mM because the divalent and monovalent cations were at least partially ameliorative at 1 and 10 mM, respectively. The trivalent cation TEC^{3+} does not hydrolyze at acidic pH and has much less tendency to bind than do smaller elemental trivalent cations (6). This ion was not rhizotoxic at concentrations less than 0.1 mM. Consequently, we performed the experiments presented in Figure

3 using Scout 66 wheat. The toxicity imposed by 0.6 μM $\{Al^{3+}\}_E$ or 1.6 μM $\{La^{3+}\}_E$ was overcome by 0.1 mM TEC^{3+}. The data from the experiments of Figure 3 were incorporated into Figures 1 and 2.

H^+ Amelioration of Al^{3+} and La^{3+} Toxicity

In four dicotyledonous species H^+ appeared to ameliorate Al toxicity at constant $\{Al^{3+}\}_E$ in the rooting medium (15). Occasional weak indications of H^+ amelioration in Tyler wheat have been observed (fig. 7 of ref. 14), but generally no pH effect upon Al^{3+} sensitivity was seen in published (15) or unpublished experiments. However, the Tyler wheat from a more recent seed lot did appear to respond to pH in the same way as did Scout 66 (Fig. 4). Experiments to test the H^+ amelioration of Al^{3+} and La^{3+} rhizotoxicity were done in two stages. In the first, the response of RRL to the rhizotoxicants was assessed at pH 4.2 and 4.5 to determine appropriate activities for further study (Figs. 4 and 5). From these experiments it was determined that high sensitivity to changes in external pH in Scout 66 could be obtained at 0.6 μM Al^{3+} and 1.6 μM La^{3+}. These activities (together with zero-level controls) were then employed across the pH range 4.2 to 4.8 (Figs. 6 and 7). In each case RRL increased with decreasing pH. The data from the experiments of Figures 4, 5, 6, and 7 were incorporated into Figures 1 and 2.
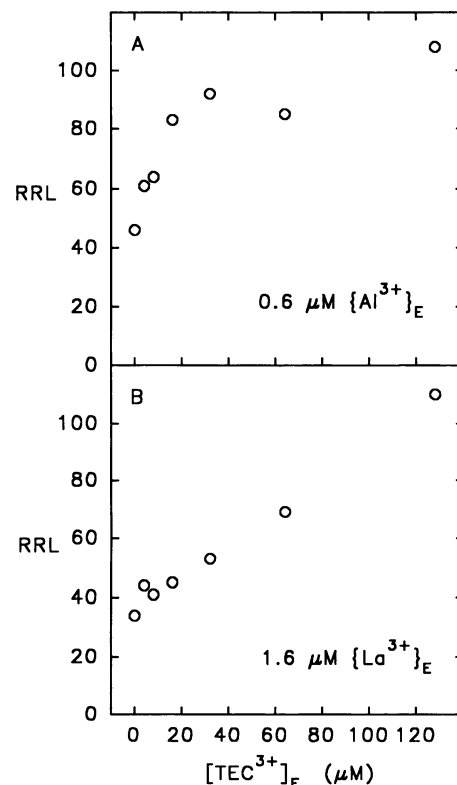

Figure 3. Amelioration of Al^{3+} and La^{3+} rhizotoxicity by TEC^{3+}. The experiment was performed twice with Scout 66. An RRL value of 0% corresponded to a root length of 27.3 mm (A) or 32.8 mm (B). An RRL value of 100% varied with the $[TEC^{3+}]_E$ ranging from 82.0 mm at 32 μM TEC^{3+} to 65.8 mm at 128 μM TEC^{3+}.

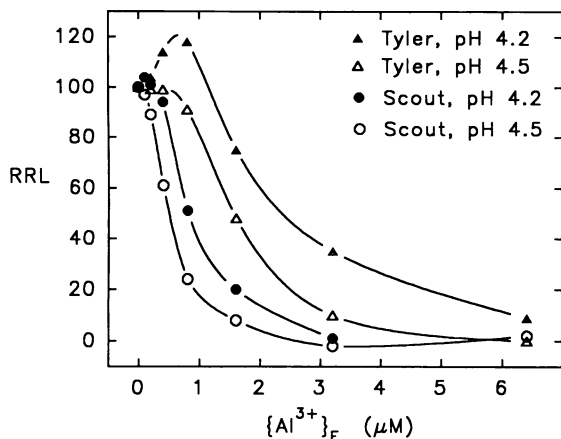**Figure 4.** RRL in response to external {Al³⁺} and pH. The experiment was performed twice. An RRL value of 0% corresponded to 29.5 mm for Scout 66 wheat and to 21.5 mm for Tyler wheat. An RRL value of 100% corresponded to 56.6 mm (Scout 66 at pH 4.2), 74.2 mm (Scout 66 at pH 4.5), 47.0 mm (Tyler at pH 4.2), or 63.2 mm (Tyler at pH 4.5).

Cation Amelioration of H⁺ Toxicity

Cations will substantially offset the growth-inhibitory effects of low pH. The increased Ca^{2+} requirement at low pH is dramatic. Reasonably good growth in wheat (Scout 66 and Tyler) can be obtained at pH 3.6 in 20 mM $[CaCl_2]_E$ (unpublished data), at pH 4.3 in 0.4 mM $CaCl_2$ (present study), and at pH 8.0 in 0.025 mM $CaCl_2$ (11). Na^+ is also ameliorative at low pH. In 2 mM $\{Ca^{2+}\}_E$ at pH 3.7 root lengths of Tyler wheat seedlings after 2 d exposure were 23, 26, 32, and 43 mm in solutions supplemented with 0, 10, 20, and 30 mM NaCl, respectively. Low levels of Al^{3+} and La^{3+} also appear to stimulate elongation at pH 4.2 but not, at least in the case of Al^{3+}, at pH 4.5 (Figs. 4 and 5). Root elongation was progressively stimulated by low $\{Al^{3+}\}_E$ as pH decreased in
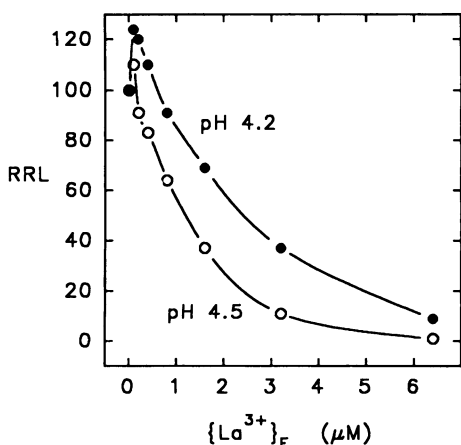

Figure 5. RRL in response to external {La³⁺} and pH. The experiment was performed twice with Scout 66. An RRL value of 0% corresponded to 34.0 mm, and 100% RRL corresponded to 55.9 mm at pH 4.2 and 74.9 mm at pH 4.5.
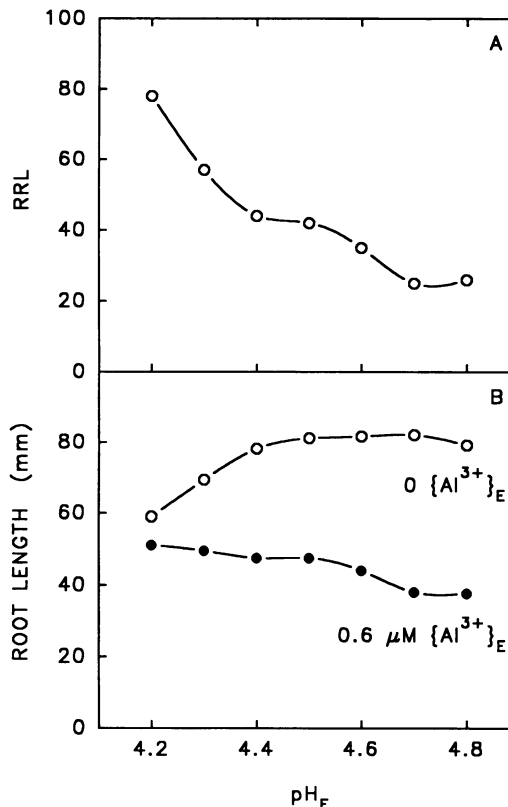

Figure 6. Root lengths and RRLs in response to external {Al³⁺} and pH. The experiment was performed twice with Scout 66. An RRL value of 0% corresponded to 23.6 mm, and 100% RRL corresponded to the zero-Al data in B.

the rooting medium of the extremely pH-sensitive Purple-top cultivar of turnip (15).

That the growth-enhancing effects of Al^{3+} and La^{3+} were usually small may be attributable to the fact that those cations are themselves rhizotoxic at very low levels. For that reason the relatively nontoxic TEC^{3+} was assessed for ameliorative effectiveness. At 80 μM, growth at pH 4.2 was nearly doubled (Fig. 8). Not only does Figure 8 illustrate cation amelioration of H^+ toxicity, it probably illustrates another example of H^+ amelioration of cation toxicity referred to in the previous section. $[TEC^{3+}]_E$ greater than about 0.1 mM was toxic, but the toxicity was relatively less at the lower pH.

Responses of Scout 66 and Atlas 66 to Al³⁺ and La³⁺

The wheat cultivars Scout 66 and Atlas 66 differ greatly in their sensitivity to Al^{3+} (28). If the difference in sensitivity to Al were based upon cell-surface charge we would expect the two cultivars to show a similar differential sensitivity to La^{3+}. Figure 9 confirms the common experience of others with respect to Al sensitivity, but Atlas was at best marginally less sensitive than was Scout to La^{3+}. In this experiment Scout and Atlas seedlings were incubated in the same pots to reduce variation among solutions caused by varietal influences upon the bulk-rooting medium. These results are similar to those reported by Parker (25): Tyler wheat was much less tolerant
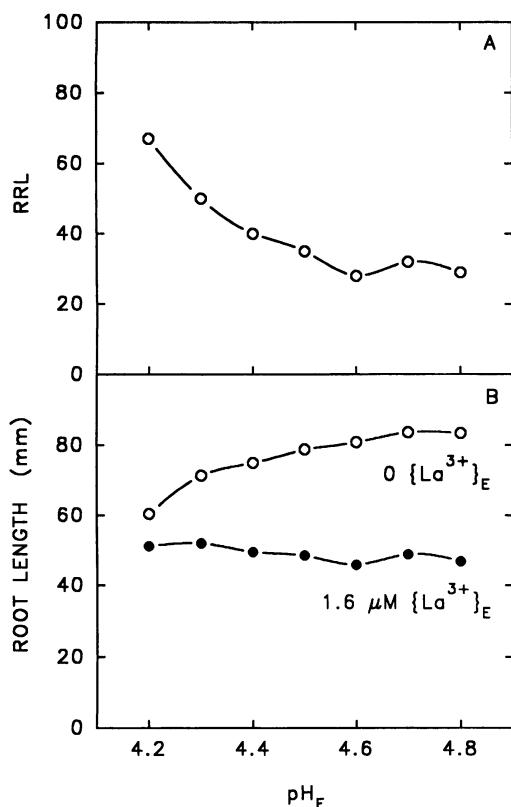
Figure 7. Root lengths and RRLs in response to external $\{La^{3+}\}$ and pH. The experiment was performed twice with Scout 66. An RRL value of 0% corresponded to 32.1 mm, and 100% RRL corresponded to the zero-La data in B.
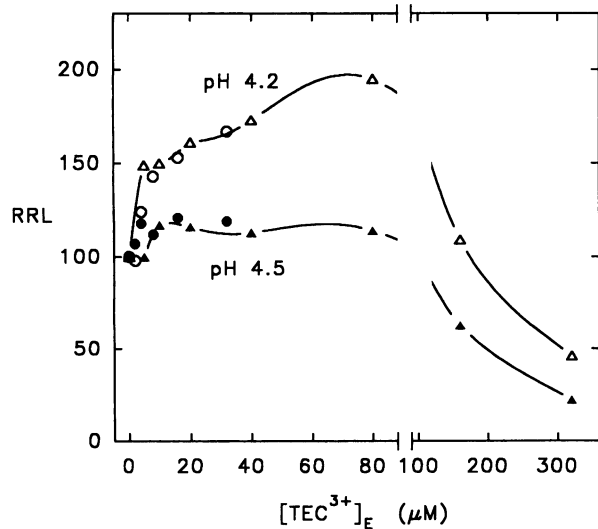

Figure 8. RRL in response to TEC^{3+} and pH. Two experiments were performed once with Scout 66. An RRL value of 0% corresponded to 25.0 mm (circles) or 30.0 mm (triangles), and 100% RRL corresponded to 46.9 mm (circles) or 57.9 mm (triangles) at pH 4.2 or 62.1 mm (circles) or 83.6 mm (triangles) at pH 4.5.
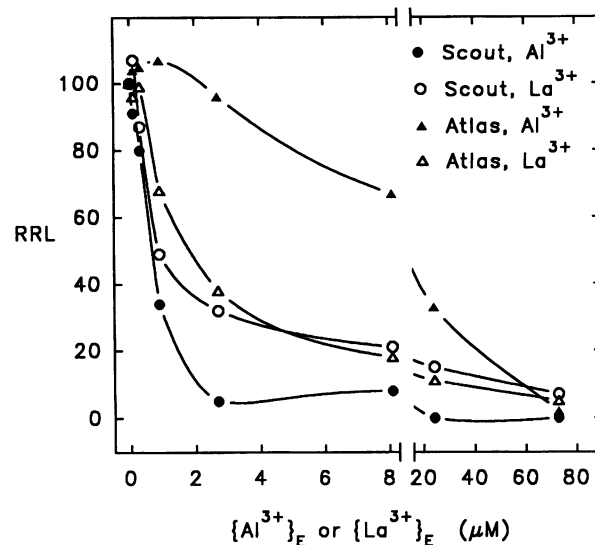

Figure 9. Comparison of Scout 66 and Atlas 66 sensitivity to external $\{Al^{3+}\}$ or $\{La^{3+}\}$. The experiment was performed twice. An RRL value of 0% corresponded to 28.0 mm for Scout 66 and 20.0 mm for Atlas 66, and 100% RRL corresponded to 75.4 mm for Scout 66 and 68.4 mm for Atlas 66.

of Al^{3+} than was Seneca wheat, but the two cultivars were similarly sensitive to La^{3+}.

DISCUSSION

Theoretical models predict, and measurements confirm, that the cell-surface potential becomes less negative as the cation concentration of the external medium increases; the effectiveness of the cations increases with increasing charge (5, 6, 18, 21). This occurs because of the higher charge-screening effectiveness of more highly charged ions and because of their greater tendency to bind to surface ligands. In addition, there are some ion-specific effects because of the influence of ionic radius and other factors upon ion binding. Our observation that cations ameliorate Al^{3+} and La^{3+} toxicity in the order $H^+ \approx C^{3+} > C^{2+} > C^{1+}$ can be readily explained in this way: H^+ readily binds to surface ligands (7, 22) so that it reduces surface electrical potential as effectively as TEC^{3+}. TEC^{3+} screens very effectively because of its 3+ charge (Eq. 1) but is presumed to bind ineffectively, for its charge, because of its large size (6). Apparently the combined screening and binding capacity of TEC^{3+} is greater than the combined screening and binding capacity of the divalent cations. The monovalent cations screen and bind poorly. The consequence of a less negative cell-surface potential is a lower activity of Al^{3+} or La^{3+} at the cell surface.

In general we have conceptualized the Gouy-Chapman-Stern model to be appropriate for the surface of the plasma membranes and the Donnan equilibrium to be an appropriate model for the cell walls with the understanding that neither model is wholly adequate for either phase and that at points of contact one phase may influence the other. We have analyzed our data according to a Donnan equilibrium model as well as the Gouy-Chapman-Stern model, and the results

are qualitatively similar to those presented in Figures 1 and 2. At present we do not know which phase is more important to the mechanism of Al^{3+} toxicity because we do not know the site of the lesion. We assume that the lesion is unlikely to be caused directly by cell-surface free Al^{3+}; some cell-surface ligand probably binds Al^{3+} before injury, but binding will be a function of cell-surface $\{Al^{3+}\}$.

Previous attempts to interpret cation amelioration of Al toxicity have considered a direct (*e.g.* competitive) cation inhibition of Al^{3+} binding at a surface ligand whose degree of Al saturation determines the degree of intoxication (10, 13). This hypothesis is different than the one considered in the present article. Conceivably, Al^{3+} binding at the critical ligand is not directly inhibited by ameliorative cations (or at least not some of them, such as TEC^+). In that case the direct-inhibition hypothesis would fail but the surface-potential hypothesis could still apply. Probably both mechanisms play some role: the ameliorating cations reduce surface negativity so that $\{Al^{3+}\}_o$ and the degree of Al saturation of a critical Al^{3+}-binding ligand are reduced. Furthermore, the ameliorating cations also may directly inhibit Al^{3+} binding at the critical ligand.

Al hydrolysis creates considerable uncertainty in the study of Al rhizotoxicity. A strict attribution of toxicity to Al^{3+} is not possible because Al^{3+} is always in equilibrium with its hydrolysis products, as follows (12, 20).

$$\{Al^{3+}\} = \{AlOH^{2+}\}\{H^+\}10^5 = \{Al(OH)_2^+\}\{H^+\}^2 10^{10.1} \quad (5)$$
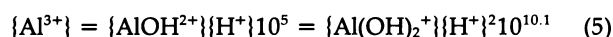
The commonly observed amelioration of Al toxicity with declining pH at constant $\{Al^{3+}\}_E$ could be attributed to H^+ amelioration of Al^{3+} toxicity or to the reduced activities of $AlOH^{2+}$ and $Al(OH)_2^+$. At present, judgments must be based upon indirect evidence. The arguments in favor of Al^{3+} toxicity ameliorated by H^+ *versus* amelioration because of reduced levels of $AlOH^{2+}$ and $Al(OH)_2^+$ are these: (a) Polyvalent cations (charge > 2) are generally rhizotoxic at low concentrations (12). Examples include $Al-O_4Al_{12}(OH)_{24}(H_2O)_{12}^{7+}$ (Al_{13} for short), La^{3+}, poly-L-lysine, polymyxin B, spermine, and undetermined species of indium, gallium, and scandium that may be polyvalent and polynuclear. The least toxic polyvalent cation in our experience is TEC^{3+}, which is toxic only at 10^{-4} M or higher. The data indicate that the rhizotoxicity of polyvalent cations increases with charge and decreases with size. By these criteria Al^{3+} should be highly toxic. (b) Al-binding ligands other than OH^- (*e.g.* SO_4^{2-}, F^-, and organic anions; ref. 12) yield nontoxic monovalent and divalent Al complexes, and it may be reasonable to expect $AlOH^{2+}$ and $Al(OH)_2^+$ to be nontoxic also. (c) The H^+ amelioration of La^{3+}-intoxicated roots demonstrates a direct amelioration of trivalent cation toxicity rather than an amelioration caused by reduced activities of hydrolysis products; La^{3+} does not hydrolyze in acidic media. This suggests that Al^{3+} toxicity could be directly ameliorated by H^+ also.

Cell surface electrical potential may help to explain four phenomena associated with Al toxicity. (a) Cations may ameliorate Al^{3+} toxicity by reducing cell-surface negativity and thus the cell-surface activity of Al^{3+} (present study and ref. 9). (b) H^+ binding and consequent reduction of cell-surface negativity and Al^{3+} activity may account for the

apparent toxicity of mononuclear hydroxy-Al (present study and ref. 15). (c) Al^{3+} binding and consequent reduction of cell-surface negativity may account for Al^{3+} inhibition of cation uptake, particularly the uptake of divalent cations (2, 27). (d) Differential cell surface charge could be the basis of differential varietal sensitivity to Al. However, our data (Fig. 9) and Parker's observations that Seneca and Tyler wheats are differentially sensitive to Al^{3+}, but not to La^{3+} (25) and Al_{13} (26), do not support the hypothesis for wheat.

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