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Sorption of Aluminum to Plasma Membrane Vesicles Isolated from Roots of Scout 66 and Atlas 66 Cultivars of Wheat

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To further elucidate the mechanisms of differential genotypic tolerance to Al, plasma membrane (PM) vesicles were isolated from whole roots, root tips, and tipless roots of Al^{3+}-sensitive and Al^{3+}-tolerant cultivars (cv) of wheat (*Triticum aestivum* L. cv Scout 66 and cv Atlas 66, respectively). Vesicles from cv Scout root tips sorbed more Al than vesicles prepared from any other source. The intrinsic surface-charge density of vesicles isolated from cv Scout was 26% more negative than vesicles from cv Atlas (−37.2 versus −29.5 millicoulombs m^{-2}). Growth experiments indicated that cv Scout is slightly more sensitive to La^{3+} than is cv Atlas, that the cultivars are equally sensitive to H^+, and that cv Atlas is slightly more sensitive to SeO_4^{2-}. The difference in sensitivity to Al^{3+} was very large; for a 50% inhibition, a 16-fold greater activity of Al^{3+} was required for cv Atlas. Using a newly developed Gouy-Chapman-Stern model for ion sorption to the PM together with growth-response curves, we estimate that the difference in surface-charge density can account for the slightly greater sensitivity of cv Scout to cationic toxicants and the slightly greater sensitivity of cv Atlas to anionic toxicants. According to our estimates the differences in PM surface negativity and Al sorptive capacity probably account for some of the difference in sensitivity to Al^{3+}, but the greater part of the difference probably arises from other tolerance mechanisms expressed in cv Atlas root tips that reduce the amount of Al^{3+} that can reach the PM.

In recent years there has been much progress in elucidating the mechanisms of differential genotypic tolerance to Al, an important rhizotoxicant in acidic soils throughout the world (Kochian, 1995). For several species the secretion of organic acids by root apices is stimulated by Al in tolerant genotypes but not in sensitive genotypes. The citrate secreted by snap beans (Miyasaka et al., 1991) and maize (Pellet et al., 1995) and the malate secreted by wheat (*Triticum aestivum* L.; Delhaize et al., 1993) chelate Al^{3+} and significantly reduce growth inhibition at organic acid concentrations of less than 0.1 mM. The stimulation of organic acid secretion by Al^{3+} is quite specific. La^{3+} is highly rhizotoxic but fails to stimulate malate secretion in wheat (Delhaize et al., 1993), and Al^{3+}-tolerant and Al^{3+}-sensitive genotypes are nearly equally sensitive to La^{3+} (Parker, 1988; Kinraide et al., 1992; Delhaize et al., 1993).

Even if organic acid secretion is a principal mechanism of tolerance, it apparently is not the sole mechanism, at least in some wheat genotypes. Genetic studies indicate that Al^{3+} tolerance can be multigenic (Aniol, 1990; Carver and Ownby, 1995). The highly tolerant wheat cv Atlas 66 not only secretes malate when challenged by Al but it also secretes phosphate constitutively at the root apices (Pellet et al., 1996). In addition, the isolated cell walls of the tolerant cvs Atlas 66 and Yecora Rojo had lower affinities for Al than the two sensitive cvs Scout 66 and Hart, despite having a higher density of carboxyl groups (Masion and Bertsch, 1997).

Another hypothesis for differential tolerance to Al^{3+} relates to negative electrical charges at the cell surface, either in the cell wall or on the surface of the PM. Treatments that reduce cell-surface negativity reduce the sensitivity of roots to cationic toxicants (Kinraide et al., 1992; Kinraide, 1994) and increase the sensitivity to at least one anionic toxicant, SeO_4^{2-} (Kinraide, 1994). Consequently, it was proposed decades ago that negative surface-charge densities may be lower in Al^{3+}-tolerant genotypes (Vose and Randall, 1962).

Several reports indicate that, within closely related taxa, higher genotypic sensitivity to Al^{3+} corresponds to higher genotypic cation-exchange capacity of whole roots (Rengel and Robinson, 1989, and refs. therein; see refs in Allan et al., 1990; Blamey et al., 1990, and refs. therein). Other studies failed to support these results. Higher titratable acidity of isolated cell walls corresponded to greater Al^{3+} tolerance (Allen et al., 1990), and in a reference to unpublished data, Wagatsuma et al. (1995) asserted that the cation-exchange capacity of terminal 1-cm root segments was not correlated with Al^{3+} tolerance in 10 plant species. (See also the study of Masion and Bertsch [1997] mentioned earlier.) Therefore, a role for cell wall electrical properties in Al^{3+} tolerance is uncertain.

It remains possible that the surface electrical properties of the PM may play a role in Al^{3+} tolerance. Wagatsuma and Akiba (1989) measured the ζ potentials (a measure of the surface electrical potential) of protoplasts isolated from five genotypes from five plant genera. In general, the PM

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Abbreviations: $[Al]_{Eq}$, equilibrium concentration of Al in the bulk-phase medium after any sorption to vesicles has been completed; $[Al]_T$, total concentration of Al in the reaction mixture whether Al is sorbed to vesicles or free in the bulk phase medium; mC, millicoulombs; PM, plasma membrane; RL, root length; RRL, relative root length; σ_0, intrinsic charge density on the plasma membrane surface (i.e. surface charge density when no solute ions are bound to the plasma membrane).

surfaces were more negative in the more Al^{3+}-sensitive genotypes. In a subsequent study (Wagatsuma et al., 1991), the stainability of root-tip protoplasts with methylene blue (supposedly a measure of surface negativity) correlated with the Al^{3+} sensitivity of four of the genotypes used before. No studies of membrane electrical properties have compared genotypes within a species, but three studies using three different pairs of wheat genotypes indicate that genotypes tolerant of Al^{3+} are not tolerant of La^{3+} (Parker, 1988; Kinraide et al., 1992; Delhaize et al., 1993). This is not consistent with a significant role for surface electrical potential in genotypic tolerance to Al^{3+}.

In addition to the likely role of root exudation and the uncertain role of cell-surface electrical properties, other factors may influence genotypic sensitivity to Al^{3+}. In general, the roots of the more sensitive genotypes take up more Al than the less sensitive genotypes (Rincon and Gonzales, 1992; Tice et al., 1992). Exclusion of Al by root exudates could account for this difference, but it is possible that the more sensitive genotypes have a greater number of Al-binding sites at the cell surfaces or that the binding sites have a higher binding affinity. Caldwell (1989) reported that the PM proteins of a more sensitive wheat genotype bound Al more strongly than those of a less sensitive genotype. This greater binding capacity may or may not be associated with a greater negative cell-surface charge density.

In the present study we measured surface electrical charges and the sorption of Al by PM vesicles isolated from whole roots, root tips, and tipless roots of the Al^{3+}-tolerant cv Atlas 66 and the Al^{3+}-sensitive cv Scout 66. Genotypic sensitivity to four ionic toxicants, Al^{3+}, La^{3+}, H^+, and SeO_4^{2-}, were also measured and considered in terms of the membrane properties.

MATERIALS AND METHODS

Preparation of Vesicles and Sorption Experiments

Sorption experiments were performed as described previously (Yermiyahu et al., 1997) with right-side-out PM vesicles isolated from approximately 10-cm, 4-d-old whole roots, 5-mm root tips, or the remaining tipless roots of wheat (*Triticum aestivum* L.). Vesicles were prepared from the Al^{3+}-tolerant cv Atlas 66 and the Al^{3+}-sensitive cv Scout 66, according to the method of Larsson et al. (1988), with slight modification. The final wash solution included 0.25 M Suc and 5 mM KCl adjusted to pH 7.2 with 1 M KOH (Yermiyahu et al., 1997). The relative purity of these PM vesicles was determined by comparing the specific activities of marker enzymes in this preparation against the specific activities of a microsomal fraction (Brauer et al., 1990). The PM vesicles were enriched more than 3-fold for each genotype in vanadate-sensitive ATPase and depleted more than 3-fold for each genotype in NADH Cyt *c* reductase, Cyt *c* oxidase, and NO_3^--sensitive ATPase relative to the microsomal fraction.

Sorption of Al and H^+ to the vesicles was measured as the reduction in concentration in media to which a concentrated suspension of vesicles (or a vesicle-free control solution) was added. Estimates of H^+ sorption take into account the H^+-generating effect of Al^{3+} hydrolysis, which is affected by changes in $[H^+]$ and $[Al^{3+}]$ (Yermiyahu et al., 1997). To measure solute reductions in the vesicle suspensions (25 μg of vesicle protein in a 500-μL reaction volume containing 0.25 M Suc, 0.5 mM KCl, and variable $[AlCl_3]$ and $[H^+]$), the assays for H^+ and Al had to be sensitive. The pH of the unfiltered 500-μL suspensions was measured with a combination glass electrode to the nearest 0.01 unit, and Al in the filtrate was assayed by inductively coupled Ar plasma emission spectrometry (model JY 46 P, Jobin Yvon Emission Instruments, Longjumeau, France) capable of detecting <0.2 μM Al at 167.02 nm (Uehiro et al., 1984). All sorption experiments were performed at least twice with three replicates within each experiment.

Estimation of σ_0

The σ_0, expressed in millicoulombs per square meter, of the PM vesicles, was determined by the 9-aminoacridine fluorescence method as described previously (Yermiyahu et al., 1997). The σ_0 is the surface-charge density when no solute ions are tightly bound to the membrane surface. The procedure calls for the progressive addition of a monovalent and a divalent salt that bind weakly or not at all to the PM surface. Consequently, we used tetramethylammonium·Cl and hexamethonium·Cl_2.

Growth Experiments

Root elongation in response to Al^{3+}, La^{3+}, H^+, and SeO_4^{2-} was measured by placing together in the same solution five or more newly germinated seedlings of both cvs Scout and Atlas into holders floated on the surface of 1-L solutions. For the Al^{3+} experiments, previously published data were used (fig. 9 in Kinraide et al., 1992). For the La^{3+} experiments, the media contained 0.35, 0.7, 1.4, 2.8, or 5.6 μM $LaCl_3$ and 0.2, 0.4, or 0.8 mM $CaCl_2$ in a factorial design, and solutions were adjusted to pH 5.0 with HCl. For the H^+ experiments, the media contained 0.2, 0.4, or 0.8 mM $CaCl_2$ adjusted to pH 3.6, 3.9, 4.2, 4.5, or 4.8 with HCl in a factorial design. For the SeO_4^{2-} experiments, the media contained 1, 10, or 100 μM Na_2SeO_4 and 0.05, 0.1, 0.2, 0.4, or 0.8 mM $CaCl_2$ in a factorial design. The SeO_4^{2-} solutions were adjusted to pH 7.0 with $NaHCO_3$, aerated overnight, and then readjusted with HCl and NaOH to pH 7.0 before adding seedlings.

The seedlings were incubated in the aerated solutions for 2 d, and then the two longest roots of each seedling were measured. RL measurements were used to compute RRL. RRL, expressed as a percentage, was computed by $100(RL_{Tox} - RL_S)/(RL_C - RL_S)$, where RL_{Tox} is the mean RL in the presence of toxicant, RL_C is the mean RL in toxicant-free solutions, and RL_S is the mean RL in high levels of toxicant sufficient to fully inhibit toxicant-sensitive growth (RL_S is nearly equal to RL at the time of seedling transfer to the test solutions). Growth data are presented as means from two replicates of each experiment.

RESULTS

Sorption of Al and H^+ to PM Vesicles from Whole Roots

The amount of Al sorbed to PM vesicles as a function of $[Al]_T$ is presented in Figure 1A. The experiment included vesicles from whole roots from two genotypes and included two levels of treatment pH. The amount of sorbed Al increased with increasing $[Al]_T$ and was greater at the higher treatment pH. The term "treatment pH" refers to the pH of the 450-μL solution to which the vesicles (or control solution) were added. The vesicles (or control solution) were added in 50 μL at pH 6.0 ± 0.2. Figure 1B presents the amount of Al sorbed as a function of $[Al]_{Eq}$. The $[Al]_{Eq}$ is comparable to the [Al] in large volumes of growth media that do not undergo significant depletions in concentration because of uptake. Sorbed Al was computed from the difference between $[Al]_{Eq}$ in the reaction mixtures with or without vesicles. Under similar solute conditions sorption of Al by the cv Scout vesicles was greater than sorption by the cv Atlas vesicles.

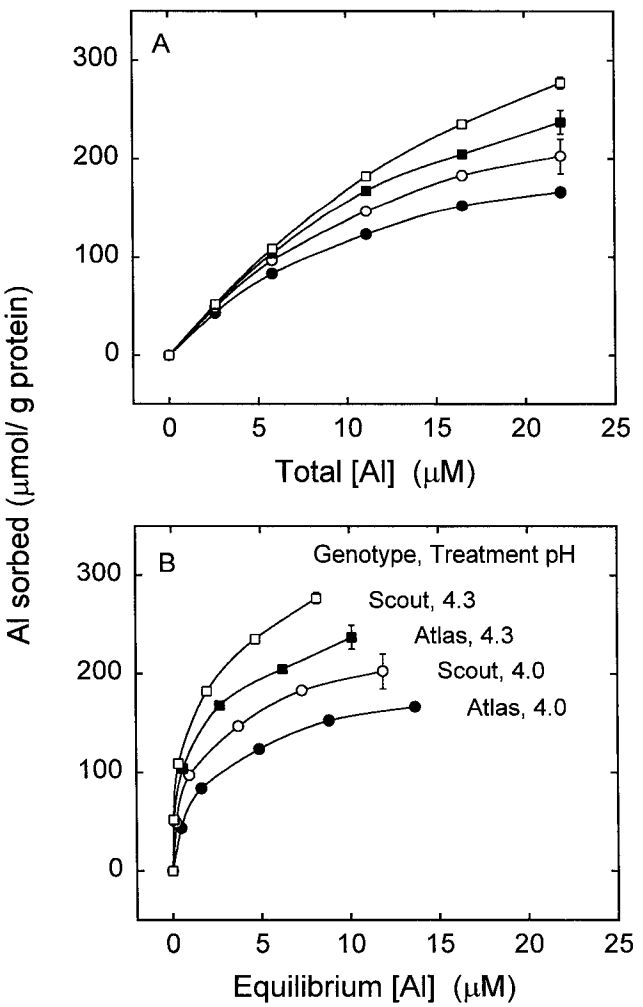

Figure 1. Al sorbed to PM vesicles isolated from whole roots as a function of genotype, treatment pH, and $[Al]_T$ in the reaction mixture (A) and $[Al]_{Eq}$ (B). Bars refer to the SES of the values at the ends of the curves.

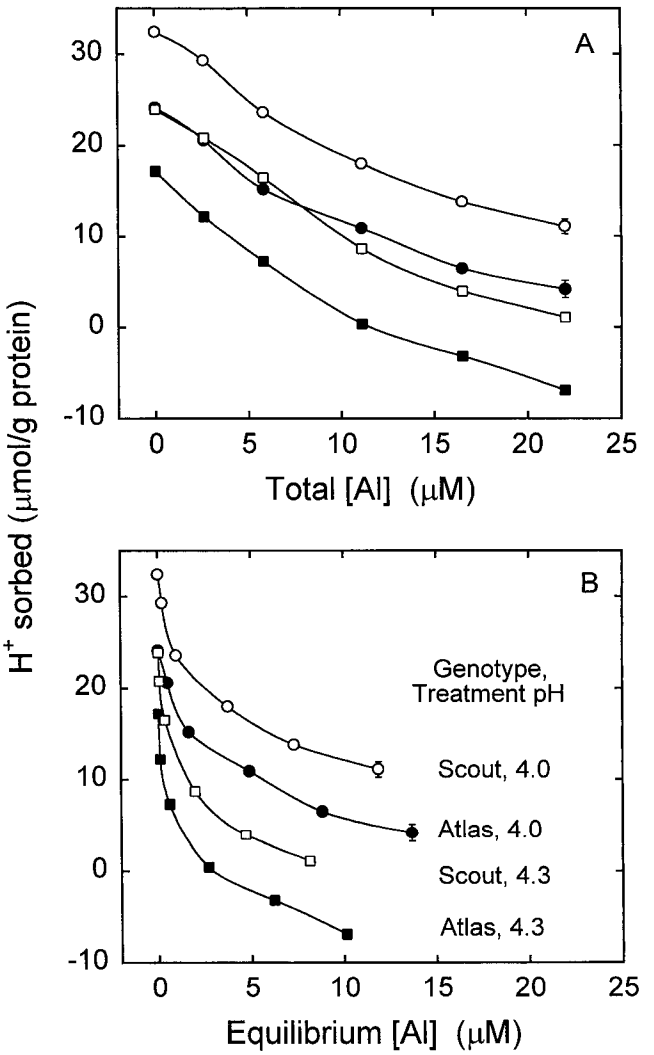

Figure 2. H^+ sorbed to PM vesicles isolated from whole roots as a function of genotype, treatment pH, and $[Al]_T$ in the reaction mixture (A) and $[Al]_{Eq}$ (B). Bars refer to the SES of the values at the ends of the curves.

The amount of H^+ sorbed to PM vesicles as a function of $[Al]_T$ in the reaction mixture is presented in Figure 2A. The amount of sorbed H^+ decreased with increasing $[Al]_T$ and was greater at the lower treatment pH. The Al^{3+}-induced release of H^+ from cv Atlas (indicated by negative values) at a treatment pH of 4.3 reflects the displacement of some H^+ initially on the PM vesicles. Figure 2B presents sorbed H^+ as a function of $[Al]_{Eq}$. Under similar solute conditions sorption of H^+ by the cv Scout vesicles was greater than sorption by the cv Atlas vesicles.

Sorption of Al and H^+ to PM Vesicles from Root Tips and Tipless Roots

Experiments similar to the preceding were performed except that vesicles were prepared from 5-mm root tips and from the remaining tipless portions of the root. Figure 3 illustrates that at three treatment pH values the sorption of

Al was greatest by vesicles from cv Scout root tips. Sorption by vesicles from tipless roots was equal for the two genotypes. Only at pH 3.7 was the sorption by vesicles from cv Atlas tips greater than the sorption by the vesicles from tipless roots. Differences in Al sorption among the vesicle types were greater at lower pH values.

The sorption of H^+ by vesicles from cv Scout and cv Atlas root tips and tipless roots is complicated (Fig. 4). Sorption by vesicles from cv Atlas tipless roots was slightly higher than sorption by vesicles from cv Scout tipless roots. These values were generally higher than values for sorption by vesicles from root tips. At low or 0 $[Al]_{Eq}$, sorption by vesicles from cv Scout tips was greater than sorption by vesicles from cv Atlas tips, but a crossover occurred at higher $[Al]_{Eq}$.

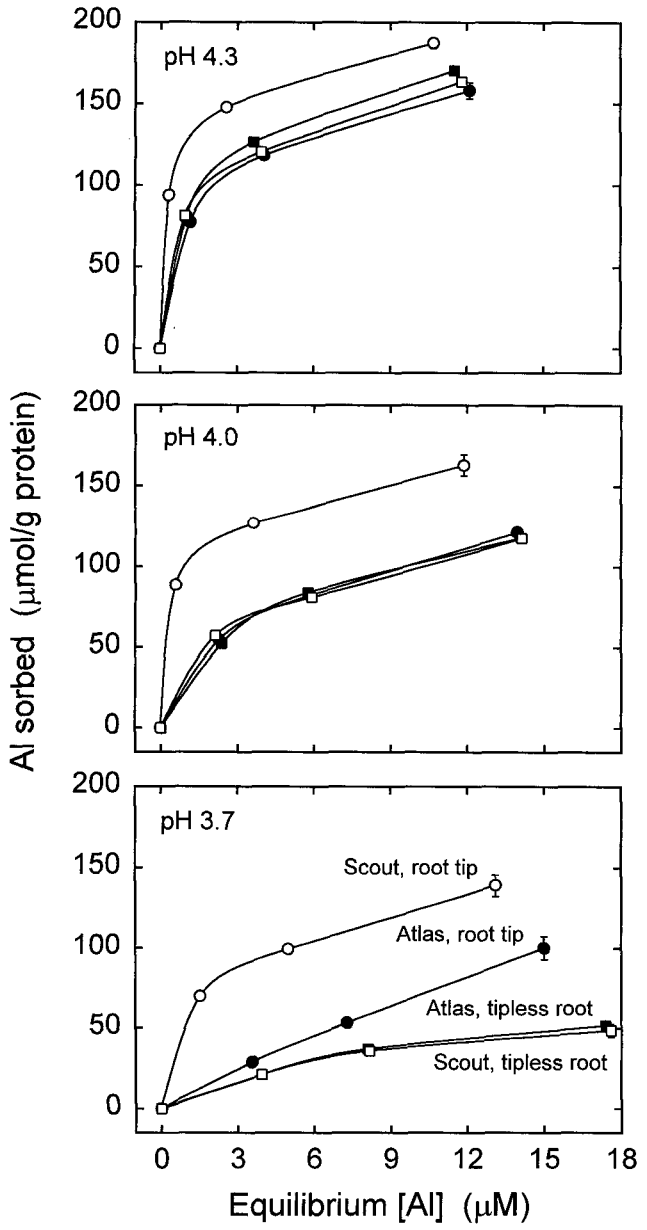

Figure 3. Al sorbed to PM vesicles isolated from root tips and tipless roots as a function of genotype, treatment pH, and $[Al]_{Eq}$. Bars refer to the SES of the values at the ends of the curves.

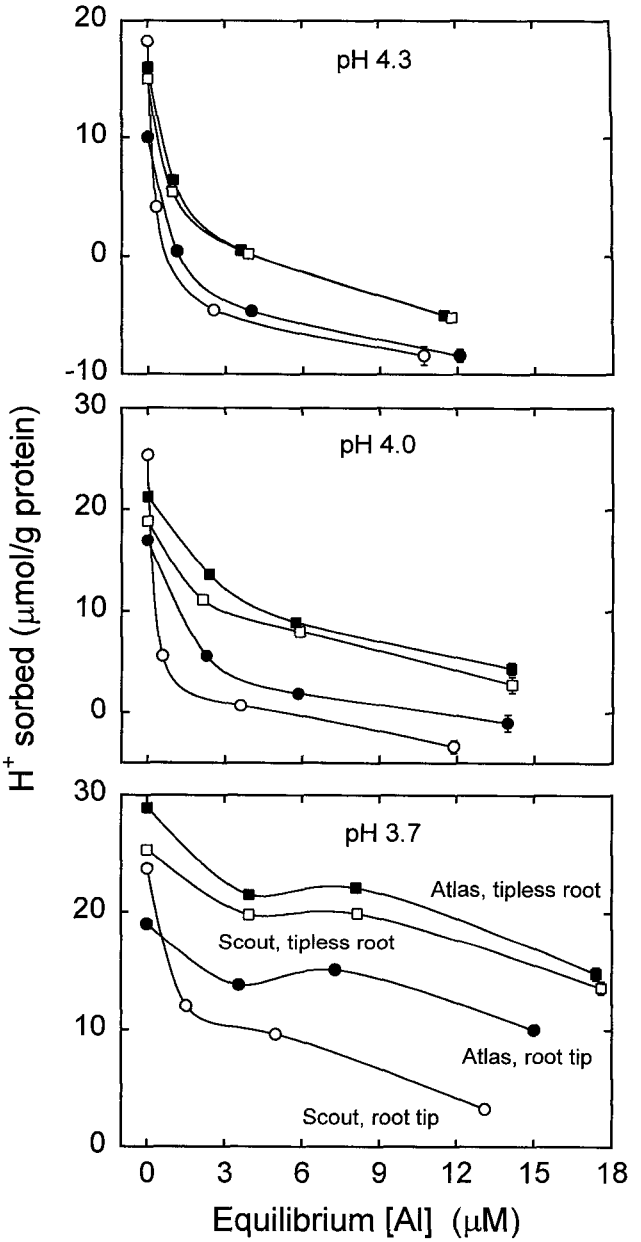

Figure 4. H^+ sorbed to PM vesicles isolated from root tips and tipless roots as a function of genotype, treatment pH, and $[Al]_{Eq}$. Bars refer to the SES of the values at the ends of the curves.

Measurement of σ_0

σ_0 was measured for PM vesicles from cv Scout and cv Atlas roots in three separate experiments. The computed σ_0 (mean ± SE in mC m^{-2}) of the PM vesicle surfaces were: from cv Scout root tips, −35.6 ± 5.8; from cv Atlas root tips, −29.4 ± 3.1; from cv Scout tipless roots, −38.3 ± 5.5; and from cv Atlas tipless roots, −29.5 ± 3.5. Apparently, there was little difference in σ_0 for root tips and tipless roots within a genotype, but the difference between genotypes

could be measured whether vesicles were isolated from tips or tipless roots. Combined measurements yielded the following values: all cv Scout (tips and tipless roots), -37.2 ± 3.6; all cv Atlas, -29.5 ± 2.2; all tips (cvs Scout and Atlas), -32.4 ± 3.2; and all tipless roots, -33.9 ± 3.5. The difference of 7.7 mC m^{-2} between cvs Scout and Atlas was significant at the 3% probability level according to a two-tailed Student's t test for paired observations.

Growth Experiments

Results of the growth experiments are presented in Figure 5. Each point represents the RRL of cv Atlas plotted on the y axis and the RRL of cv Scout plotted on the x axis. Furthermore, each point refers to measurements of seedlings from the two genotypes grown together in the same beaker. Conventional plots of root elongation versus toxicant activities can be seen in other publications (Kinraide et al., 1992), but all of the data presented in Figure 5 are from new experiments, except for the Al^{3+} data.

Figure 5 indicates that cv Atlas is much more Al^{3+} tolerant than cv Scout, which is in agreement with a previous report that a 50% inhibition of cv Atlas required a 16-fold greater activity of Al^{3+} than was required for cv Scout (Kinraide et al., 1992). In Figure 5 we see that an [Al] that reduced RRL in cv Scout to 34% increased RRL in cv Atlas to 107%. This high-toxicant tolerance by cv Atlas appears to be specific for Al^{3+}. The greater tolerance to La^{3+} by cv Atlas relative to cv Scout was slight but consistent with a previous study that also indicated a somewhat greater La^{3+} tolerance by cv Atlas (Kinraide et al., 1992). In two other studies with wheat the more Al^{3+}-tolerant genotypes were slightly more La^{3+} tolerant than the Al^{3+}-sensitive genotypes (Delhaize et al., 1993 [sensitive ES3 versus tolerant ET3]; Parker, 1988 [sensitive Tyler versus tolerant Seneca]). Resistance to H^+ by cvs Scout and Atlas was about equal in the present study, which is in agreement with a previous study (Kinraide, 1993). In contrast to results with cationic toxicants, cv Atlas appears to be more sensitive to SeO_4^{2-} than cv Scout.

A statistical test for these growth data is problematic. A two-tailed Student's t test for paired observations indicates the following mean differences ($[\Sigma(RRL_{cv\ Atlas} - RRL_{cv\ Scout})]/N$) and statistical significance: 37.0 (1.8%) for Al^{3+}, 7.0 (6.5%) for La^{3+}, -4.6 (2.3%) for H^+, and -14.8 (<0.1%) for SeO_4^{2-}. This test is not entirely appropriate and may be overly stringent, because no differences are expected at very low (RRL = 0) or very high (RRL = 100) toxicant levels. Another test was done to determine whether the data fall on a downwardly concave (e.g. Al^{3+} toxicity) or an upwardly concave (e.g. SeO_4^{2-} toxicity) curve. For the equation $RRL_{cv\ Atlas} = aRRL_{cv\ Scout}^{b}$, the curve will pass through 0 and will be downwardly concave if $b < 1$ and upwardly concave if $b > 1$. Regression analyses yielded the following values for b: 0.077 for Al^{3+}, 0.70 for La^{3+}, 1.1 for H^+, and 1.9 for SeO_4^{2-}. Only in the case of H^+ toxicity was b not statistically different from 1 (i.e. the 95% confidence intervals encompassed 1); $r^2 > 0.78$ in all cases. Therefore, there was a progressive change in relative sen-

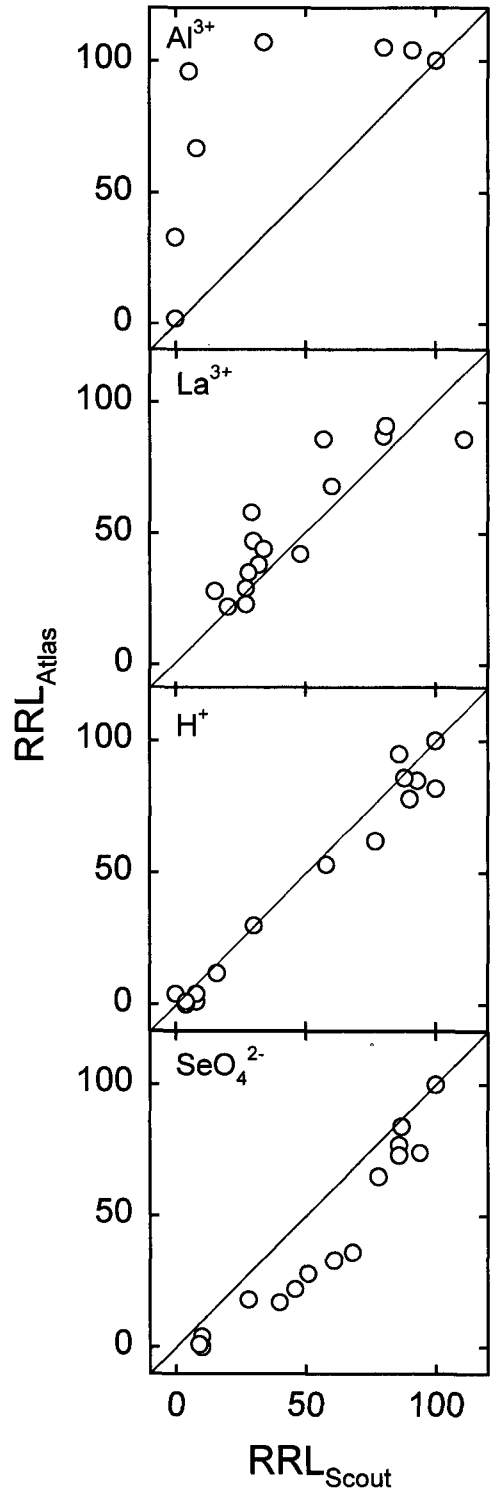

Figure 5. RRLs for seedlings of cvs Scout 66 and Atlas 66 grown *together in solutions containing the toxicants* Al^{3+}, La^{3+}, H^+, *and* SeO_4^{2-}. Points lying above the unit-slope line indicate a greater sensitivity of cv Scout to the toxicant, and points lying below the line indicate a greater sensitivity of cv Atlas.

sitivity to ionic toxicants as the ionic charge progressed from 3+ to 2−.

DISCUSSION

Sorption of Al by PM vesicles isolated from the root tips of cv Scout exceeded the sorption of Al by vesicles isolated from the tipless roots of cv Scout and from vesicles isolated from any part of the roots of cv Atlas. This finding may be related to genotypic differences in Al^{3+} sensitivity for these reasons: inhibition of root elongation by Al occurs only when the tip portion is exposed to Al (Ryan et al., 1993), whole roots exposed to Al accumulate more Al in the tips than elsewhere (Rincon and Gonzales, 1992; Tice et al., 1992), and the roots of Al^{3+}-sensitive cultivars accumulate more Al than the roots of Al^{3+}-tolerant cultivars (Rincon and Gonzales, 1992; Tice et al., 1992). The present study suggests that the PM itself may account for part of these effects, i.e. neither metabolic activity (presumably needed for organic acid synthesis and secretion) nor the presence of cell walls or multicellular structures was required for the differential sorption observed in the present study.

To explain the differential sorption of Al we considered a possible role for σ_0. Other things being equal, a higher negative σ_0 will cause a higher sorption of cations (Yermiyahu et al., 1997). More cations will bind to the PM surface, and the interfacial activity of free cations will be higher because of a more negative surface electrical potential. Estimates using the 9-aminoacridine fluorescence technique indicate that σ_0 for cv Scout was about 26% more negative than σ_0 for cv Atlas. However, a difference in Al sorption by vesicles from cv Scout root tips and tipless roots was not accompanied by a difference in σ_0, and a difference in σ_0 of vesicles from tipless roots of cvs Scout and Atlas was not accompanied by a difference in Al sorption. This indicates that sorption must be based on some factor in addition to σ_0. This is certainly possible because Al^{3+} may bind to uncharged (zwitterionic) ligands (Akeson et al., 1989), and different ligands of similar charge may have different binding strength for Al^{3+} (Tam and McColl, 1990).

Nevertheless, we estimate that the σ_0 does play a small role in the differential sensitivity of the two genotypes to ionic toxicants. Previous studies demonstrate high negative correlations between root elongation and the computed activity of toxicant ions at the PM surface (Kinraide, 1994; Kinraide et al., 1994). Treatments that reduced surface negativity reduced the toxic effects of cationic toxicants and increased the toxic effect of the anionic toxicant SeO_4^{2-}. Using a recently developed model for ion sorption by cv Scout root PM (Yermiyahu et al., 1994, 1997) together with growth-response curves, we estimate that the difference in σ_0 between cvs Scout and Atlas can account for the greater sensitivity of cv Scout to La^{3+} and the greater sensitivity of cv Atlas to SeO_4^{2-} observed in the growth experiments. However, we are unable to explain the very large difference in sensitivity to Al^{3+} by electrostatic effects alone.

The following computations serve as an example. From experimental data, root elongation by cv Scout was inhibited 50% in a medium of 1.13 μM $AlCl_3$ and 0.4 mM $CaCl_2$ at pH 4.5 (Kinraide et al., 1992). For cv Scout the model-computed membrane surface activity of free Al^{3+} is 11.8 μM and the model-computed surface concentration of bound Al is 0.0390 μmol m^{-2}. To achieve a 50% inhibition of cv Atlas root elongation experimentally, it was necessary to increase [$AlCl_3$] to 18.1 μM. For cv Atlas the model-computed membrane surface activity of free Al^{3+} is 16.5 μM and the model-computed surface concentration of bound Al is 0.0620 μmol m^{-2}. The model used to compute these values (Yermiyahu et al., 1997) was changed in only one parameter for the two genotypes: σ_0 was −37.2 mC m^{-2} for cv Scout and −29.5 mC m^{-2} for cv Atlas. This reduction in surface negativity did not come close to equalizing the computed exposure to Al (surface activity and bound concentration) of the PM of the two genotypes. Consequently, according to the sorption model, σ_0 cannot account for much of the difference in Al^{3+} sensitivity.

If we disregard σ_0 and consider only the measured differences in Al sorption (Figs. 1 and 3), it is still questionable whether we can account for the great differences in Al^{3+} sensitivity of the genotypes. To achieve a given amount of Al sorption, cv Atlas requires higher $[Al]_{Eq}$ than cv Scout, but not a 16-fold higher concentration. Inspection of Figure 3 indicates that $[Al]_{Eq}$ must be 5.7, 10.1, or 7.4 μM at treatment pH values of 4.3, 4.0, or 3.7, respectively, for cv Atlas root tip vesicles to sorb as much Al as cv Scout root tip vesicles in 1 μM $[Al]_{Eq}$. Consequently, it may be possible to account for some, but not all, of the difference in genotypic sensitivity on the basis of Al sorption characteristics of the PM.

The mechanisms of genotypic tolerance to Al^{3+} have been of interest for many years, and whether PM surface negativity plays a role in this genotypic tolerance has been an issue. We conclude from our study that the 26% higher negative σ_0 of cv Scout PM relative to cv Atlas PM very likely makes cv Scout slightly more sensitive to cationic toxicants and slightly less sensitive to anionic toxicants. In the case of Al, vesicles isolated from the Al^{3+}-sensitive tip region of cv Scout sorb more Al than vesicles from the nonsensitive, tipless region of cv Scout or from any region of cv Atlas roots. Some of that differential sorption may be attributable to differences in membrane composition rather than σ_0.

In conclusion, electrostatic sorption models for the PM can account for the slight differences in genotypic sensitivity to La^{3+} and SeO_4^{2-} but not for the great differences in genotypic sensitivity to Al^{3+}. According to our estimates, the differences in PM surface negativity and Al sorptive capacity, whatever the cause of the latter, probably account for some of the difference in genotypic sensitivity to Al^{3+}, but the greater part of that difference may arise because malate and phosphate secretion by cv Atlas root tips reduces the amount of Al^{3+} that can reach the PM (Huang et al., 1996; Pellet et al., 1996). The evidence that malate release is a general mechanism for tolerance in wheat is reasonably strong now. Correlations are strong among 36 wheat cultivars for Al-induced malate secretion versus root growth in the presence of Al (Ryan et al., 1995).

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