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Al Partitioning Patterns and Root Growth as Related to Al Sensitivity and Al Tolerance in Wheat[1]

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Studies of Al partitioning and accumulation and of the effect of Al on the growth of intact wheat (*Triticum aestivum* L.) roots of cultivars that show differential Al sensitivity were conducted. The effects of various Al concentrations on root growth and Al accumulation in the tissue were followed for 24 h. At low external Al concentrations, Al accumulation in the root tips was low and root growth was either unaffected or stimulated. Calculations based on regression analysis of growth and Al accumulation in the root tips predicted that 50% root growth inhibition in the Al-tolerant cv Atlas 66 would be attained when the Al concentrations were 105 μM in the nutrient solution and 376.7 μg Al g^{-1} dry weight in the tissue. In contrast, in the Al-sensitive cv Tam 105, 50% root growth inhibition would be attained when the Al concentrations were 11 μM in the nutrient solution and 546.2 μg Al g^{-1} dry weight in the tissue. The data support the hypotheses that differential Al sensitivity correlates with differential Al accumulation in the growing root tissue, and that mechanisms of Al tolerance may be based on strategies to exclude Al from the root meristems.

Al is a major growth-limiting factor of plants in acid soils (Foy, 1988; Kochian, 1995) because Al solubility in the soil solution increases as the soil pH decreases. Al inhibits plant growth by interfering with the regulatory processes of root growth and development (for reviews see Foy, 1988; Taylor, 1988a; Kochian, 1995). Nonetheless, varieties of the same species have developed strategies to avoid or tolerate Al stress. These strategies are genetically controlled (Foy, 1988) and several genes may be involved (Berzonsky, 1992). The mechanism(s) of differential Al sensitivity is a subject of much discussion and debate and has been reviewed recently (Taylor, 1988a, 1988b; Kochian, 1995).

To better understand the principles of Al tolerance mechanisms and Al sensitivity, it is necessary to elucidate whether the concentration of Al in the tissue is responsible for the onset of root growth inhibition and to understand how Al is taken up and transported by the roots at both the cellular and tissue levels. Although Al binds mainly to the components of the cell wall (Zhang and Taylor, 1990, 1991), there is evidence that Al is transported across the root plasma membrane after a short exposure of the tissue to Al (Lazof et al., 1994). Al accumulation inside the cell may be required for growth inhibition (e.g. binding to DNA, microtubules, enzymes, etc.); however, Al may inhibit growth by disrupting the signal transduction pathways without entering the protoplast.

The primary site of Al toxicity is the root meristem (Foy, 1988; Bennet and Breen, 1991; Ryan et al., 1993), and recently, Rincón and Gonzales (1992) and Delhaize et al. (1993a) have found that the major site of Al accumulation in wheat is the growing root region. Their observations indicate that a differential Al accumulation between the root tips of sensitive and tolerant wheat cultivars correlates with the differential sensitivity to Al.

The objective of this study was to spatially and temporally characterize the differential Al accumulation between the roots of Al-tolerant and Al-sensitive wheat cultivars and to explain the relationship between the content of Al in the root tissues and growth.

MATERIALS AND METHODS

Atlas 66 seeds (Cargill Hybrid, Fort Collins, CO) were surface-sterilized with 5% (w/v) commercial bleach and 0.1% (w/v) SDS for 5 min and then rinsed well with distilled water and deionized water. Scout 66 seeds (kindly provided by Dr. James Petterson, Department of Agronomy, University of Nebraska, Lincoln) and Tam 105 seeds (Texas Foundation Seed Stock, College Station, TX) were coated with Heptachlor. Before germination the seeds were put on autoclaved paper towels saturated with 0.1 mM $CaCl_2$ and placed in a refrigerator for 24 h. The seeds were then transferred to a growth chamber and kept in the dark at 23°C for 3 d. The paper towels were kept saturated with 0.1 mM $CaCl_2$. The seedlings were grown further hydroponically as described previously (Rincón and Gonzales, 1992), except that the hydroponics were set under fluorescent lights (117 μmol photons m^{-2} s^{-1}) with a light/dark cycle of 16/8 h at room temperature. The NS consisted of 0.4 mM $CaCl_2$, 0.65 mM KNO_3, 0.25 mM $MgCl_2$, and 0.08 mM NH_4NO_3 (pH 4.2).

Al Treatment

To determine the Al accumulation in intact roots, 5-d-old seedlings were floated on 200 mL of aerated NS containing

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Abbreviations: Al, total aluminum; Al^{3+}, ionic aluminum; NS, nutrient solution.

$AlCl_3.6\ H_2O$ (Sigma) at different concentrations for various periods of time. The roots were rinsed briefly with deionized H_2O and transferred to 200 mL of aerated ice-cold H_2O for 10 min. The roots were excised in consecutive segments from the apex, including the cap (in mm): 0 to 2, 2 to 5, and 5 to 15.

In some experiments a 30-min wash in ice-cold 0.5 mM citric acid (Sigma; anhydrous) at pH 4.5 (adjusted with 5 N NaOH) was used to remove Al from the free space and the cell wall compartments as described by Zhang and Taylor (1989, 1990). To determine Al accumulation in excised root tissue, 5-d-old roots were excised prior to the Al treatment as described above.

Al^{3+} Chemical Activity

Table I shows the Al^{3+} activities and ionic strength of the NS as calculated by the computer program GEOCHEM-PC version 2 (Parker et al., 1995) at different total Al concentrations used in this study.

Effect of Al on Growth

The primary roots of 5-d-old seedlings were measured to the nearest millimeter using a ruler and the seedlings floated on NS with or without Al. After 24 h of Al exposure the primary roots were measured again and 0- to 2-mm root tips were excised from the primary and seminal roots for Al content determination. Regression analyses were performed to explain the relationships between root growth and tissue Al content, and between Al concentration in the solution and tissue Al content.

Al Determination

Al analysis was done by ion chromatography as described by Rincón and Gonzales (1992). Briefly, the tissue was dried in an oven at 75°C for 24 to 48 h and then digested with HNO_3 (70%; Baker Instra analyzed; VWR Scientific, Media, PA) and H_2O_2 (50%; Fisher Scientific) (1:1, v/v) at 75°C for 30 to 60 min. Ion chromatography was performed with an HPLC model DX 500 (Dionex, Houston, TX) equipped with a full control Peaknet software/interface system (Dionex).

All samples and Al standards were contained in polypropylene tubes that were soaked in 20% (w/v) HNO_3 for 48 h and rinsed with distilled H_2O and ultrapure water (Milli-Q, Millipore). All solutions were prepared with ultrapure water. All treatments were duplicated or triplicated and all experiments were repeated at least twice.

Table I. *Total Al concentration, Al^{3+} activity, and ionic strength of the NS*

The activity of Al^{3+} in the NS (pH 4.2; see "Materials and Methods") and the ionic strength were estimated using the computer software program GEOCHEM-PC version 2.

Total Al	Al^{3+} Activity	Ionic Strength
μM	μM	mM
0	0	2.71
0.5	0.26	2.72
1.0	0.53	2.72
5.0	2.63	2.74
10	5.24	2.77
25	13	2.86
50	25.75	2.99
75	31.72	3.09
100	31.65	3.12

RESULTS

Al Partitioning in Intact Roots Exposed to Various Al Concentrations

Figure 1 illustrates the Al partitioning along the intact roots of the tolerant cv Atlas 66 exposed to increasing Al concentrations for 6 h. Al accumulation in the more mature 5- to 15-mm root region was 1.8 times that in the 0- to 2-mm root tips at an external Al concentration of 5 μM (Fig. 1). The same Al accumulation pattern was observed with increasing concentrations of Al. The magnitude of differential Al accumulation between the 0- to 2-mm region and the mature regions declined with the increasing Al concentration in the NS; for instance, the Al content in the mature region (5 to 15 mm) was 4 times that in the 0- to 2-mm root tip when the Al concentration in NS was 25 μM Al, but the Al content was the same in all root regions when the Al concentration in the NS was 100 μM.

Figure 2 shows Al partitioning along the intact roots of the Al-sensitive cv Scout 66 when exposed to either 10 μM or 50 μM Al. A differential Al accumulation among the various root regions was evident. When the Al concentration in the NS was 10 μM, Al accumulation in the 0- to 2-mm tips was 1.2 times higher than in the 2- to 5-mm segments and 6 times higher than in the 5- to 15-mm segments. When the Al concentration in the NS was 50 μM, Al accumulation in the 0- to 2-mm tips was 1.3 times higher than in the 2- to 5-mm segments and 3 times higher than in the 5- to 15-mm segments. Figure 3 shows the results of time-course experiments of Al accumulation in cv Scout 66 intact roots. Al continued to accumulate throughout the 24-h time course in the 0- to 2-mm, 2- to 5-mm, and 5- to 15-mm root regions. Al concentration in the different regions was about the same after 1 h of Al exposure but differed after 6 h of Al exposure, and the differences increased throughout 24 h.

Effect of Different Concentrations of Al on Root Growth and Correlation of Growth and Al Accumulation in the 0- to 2-mm Root Region

The experiments described above and elsewhere (Rincón and Gonzales, 1992; Delhaize et al., 1993a) indicate that differential Al sensitivity in wheat is related to the accumulation of Al in the root meristems. To determine the lowest tissue concentration of Al that inhibits growth, seedlings of both Al-tolerant and Al-sensitive cultivars were exposed to different concentrations of Al for 24 h, and the root growth and Al content in the 0- to 2-mm root tips were determined. The effects of Al on root growth and Al concentration in the 2-mm root tips of both the Al-tolerant cv

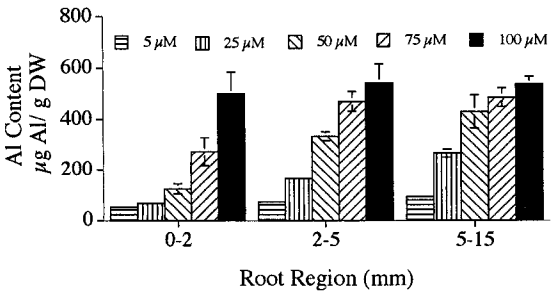

Figure 1. Al partitioning in intact roots of Al-tolerant cv Atlas 66 exposed to various Al concentrations. A, Intact roots were submerged in aerated NS in the absence (controls) or in the presence of different Al concentrations for 6 h at room temperature. The results obtained from the intact roots exposed to 5 μM Al are from experiments conducted in different days from those in which the roots were exposed to 25 to 100 μM. Mean ± SD are of two separate experiments. No Al was detected in the controls. DW, Dry weight.

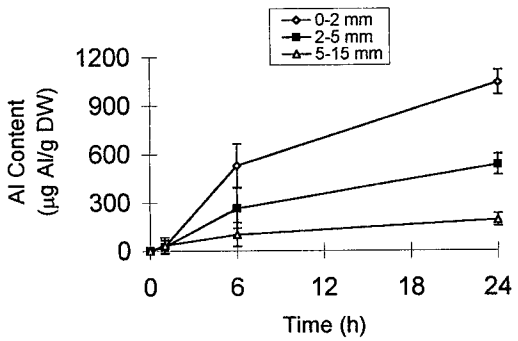

Figure 3. Time course of Al accumulation in the intact roots of Al-sensitive cv Scout 66. The intact roots were incubated in NS containing 50 μM Al for 1, 6, and 24 h. Mean ± SD are of two separate experiments. No Al was detected in the control. DW, Dry weight.

Atlas 66 and the Al-sensitive cv Tam 105 are illustrated in Figures 4 and 5, respectively. Al stimulated root growth in both the Al-tolerant and Al-sensitive cultivars when the Al concentrations in the NS were low (Figs. 4A and 5A). The tissue Al content that corresponded with stimulation or no inhibition of root growth is referred to as the stimulatory Al content. The stimulatory Al content was often low and in some experiments it was difficult to determine because of the detection limits of the ion chromatograph.

In the tolerant cv Atlas 66, the stimulatory Al content varied from 0 to 6.5 μg Al g^{-1} dry weight when the Al concentration in the NS was between 1 μM and 5 μM. Doubling the Al concentration in the NS from 5 μM to 10 μM caused slight growth inhibition (4%) and a 7-fold increase in the Al content in the tissue (i.e. from 6.5 μg Al g^{-1} dry weight to 46.6 μg Al g^{-1} dry weight). Root growth inhibition of 51% was observed when the Al content in the tips was 348.5 μg Al g^{-1} dry weight (inhibitory Al content) at an external Al concentration of 100 μM. The inhibitory Al content in the tissue represents a 54-fold increase over the stimulatory Al content. Regression analyses indicated that the relationships between root growth and the tissue Al content and between the tissue Al content and Al concentration in the NS were linear (Fig. 4, B and C).

Figure 5 displays data from similar experiments performed with the Al-sensitive cv Tam 105. Stimulation of growth was observed at Al concentrations in the NS of 0.5 μM and 1 μM (Fig. 5A). Root growth inhibition of 54% was observed when the Al content in the tips was 481 μg Al g^{-1} dry weight at an external Al concentration of 10 μM. Regression analyses of these data indicated that the relationships between root growth and the tissue Al content and between the tissue Al content and the Al concentration in the NS were best described by a polynomial equation (Fig. 5, B and C).

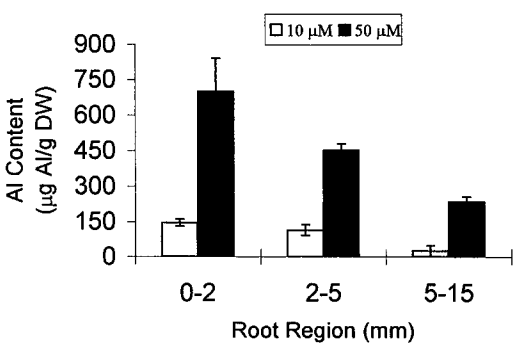

Figure 2. Al partitioning in the intact roots of Al-sensitive cv Scout 66 exposed to different Al concentrations. Intact roots were submerged in aerated NS in the absence (control) or in the presence of 10 and 50 μM Al for 6 h at room temperature. Mean ± SD are of two separate experiments. No Al was detected in the controls. DW, Dry weight.

Removal of Exchangeable Al by Citric Acid

Figure 6 illustrates the results of experiments in which a 30-min citric acid wash was used to remove "exchangeable" Al, presumably from the free space and cell wall (Zhang and Taylor, 1989, 1990). In cv Atlas 66 citric acid removed exchangeable Al from all three root regions, 0 to 2, 2 to 5, and 5 to 15 mm, and Al partitioning along the roots was the same in both the water- and citric acid-washed roots; in both washes the Al content in the 2- to 5-mm and 5- to 15-mm root regions was higher than in the 0- to 2-mm tips. The proportion of Al removed by citric acid decreased as the time of Al accumulation increased. Citric acid removed 84% of the Al accumulated in the 0- to 2-mm region in 1 h; however, the fraction of Al removed by citric acid declined to 55% at 3 h and to 32% at 24 h. As the time of Al absorption increased Al either became tightly bound to the cell wall or entered a compartment that was inaccessible to citric acid. The rates of Al accumulation for both water- and citric acid-washed root tissue were calculated by linear regression analysis and are shown in Table II. During the first 6 h the apparent rate of Al accumulation in the 0- to 2-mm root tips washed with citric acid was 63% of that in the root tips washed with water. After 6 h the rates of Al accumulation dropped and the values were the same in both citric acid- and water-washed tissue. The drop in the rates of Al accumulation with time was also

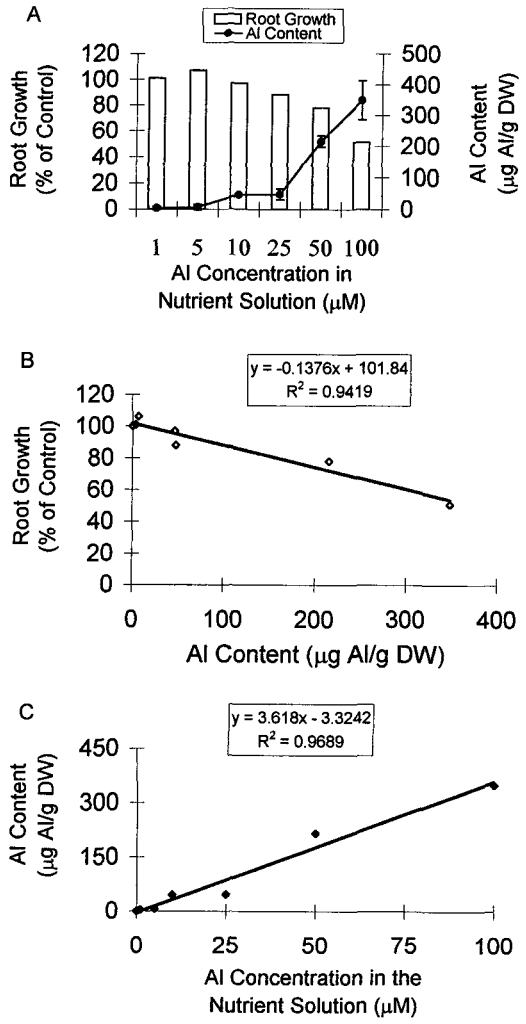

Figure 4. Root growth and Al accumulation in the meristematic region (0 to 2 mm) of the Al-tolerant cv Atlas 66. A, The intact roots were submerged in the aerated NS in the absence and in the presence of different concentrations of Al for 24 h at room temperature. Growth of the intact primary root was determined and 0- to 2-mm tips from the seminal and primary roots were excised and pooled for Al determinations. The control roots grew 16.6 mm in 24 h. Mean ± SD are of two experiments. B, Linear regression analysis of the data presented in A. C, Linear regression analysis of the tissue Al content and the Al concentration in the NS. DW, Dry weight.

observed in the more differentiated 2- to 5-mm and 5- to 15-mm root regions.

Al Accumulation in Intact and Excised Tolerant Root Tips

A time course of Al accumulation in excised 0- to 2-mm root tips of cv Atlas 66 exposed to 50 μM Al is shown in Figure 7. We included data from Figure 6A to compare the rates of Al accumulation in excised root tips with that in intact tissue. Al accumulation in the excised root tips increased with time, and the rate of Al accumulation was much higher than in the intact tissue. The rate of Al accumulation between 0 h and 3 h was 130.8 μg Al g^{-1} dry weight h^{-1} and linear (r^2 = 0.96) in the water-washed excised root tips. Citric acid removed 30% of the Al accumulated at 1 h, however, after 3 h removal of exchangeable Al by citric acid varied from 7% to 14%. The rate of Al accumulation between 0 h and 3 h was 114.2 μg Al g^{-1} dry weight h^{-1} and also linear (r^2 =0.99). After 6 h the rates of Al accumulation in both water- and citric acid-washed tissues were linear and dropped to 21.8 μg Al g^{-1} dry weight h^{-1} (r^2 = 1.00) and 18.4 μg Al g^{-1} dry weight h^{-1} (r^2 =0.96), respectively.

DISCUSSION

In this study we have characterized Al partitioning in roots of both an Al-tolerant and an Al-sensitive wheat

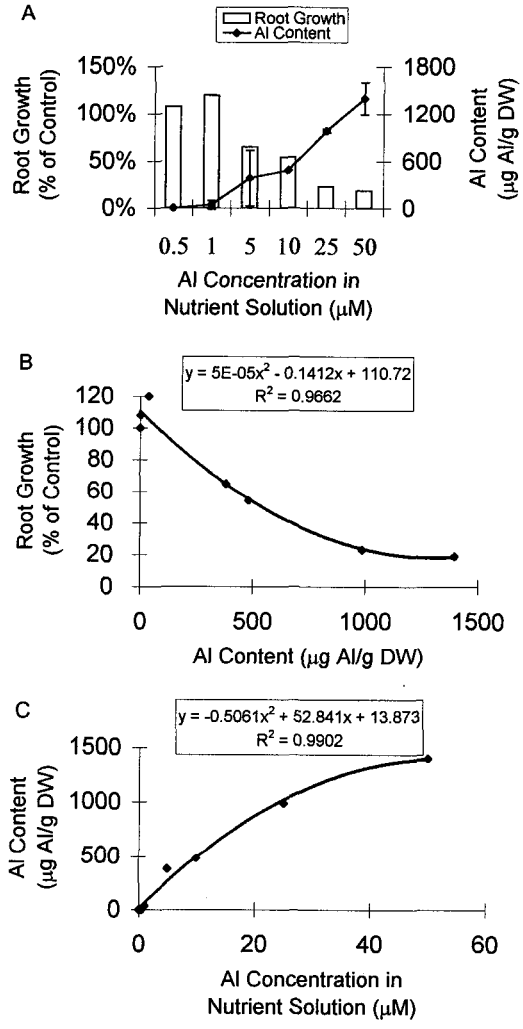

Figure 5. Root growth and Al accumulation in the meristematic region (0 to 2 mm) of Al-sensitive cv Tam 105. A, The intact roots were submerged in the aerated NS in the absence and in the presence of different concentrations of Al for 24 h at room temperature. Growth of the intact primary root was determined and 0- to 2-mm tips from the seminal and primary roots were excised and pooled for Al determination. The control roots grew 12.6 mm in 24 h. Mean ± SD are of two experiments. B, Polynomial regression analysis of the data presented in A. C, Regression analysis of the tissue Al content and the Al concentration in the NS. DW, Dry weight.

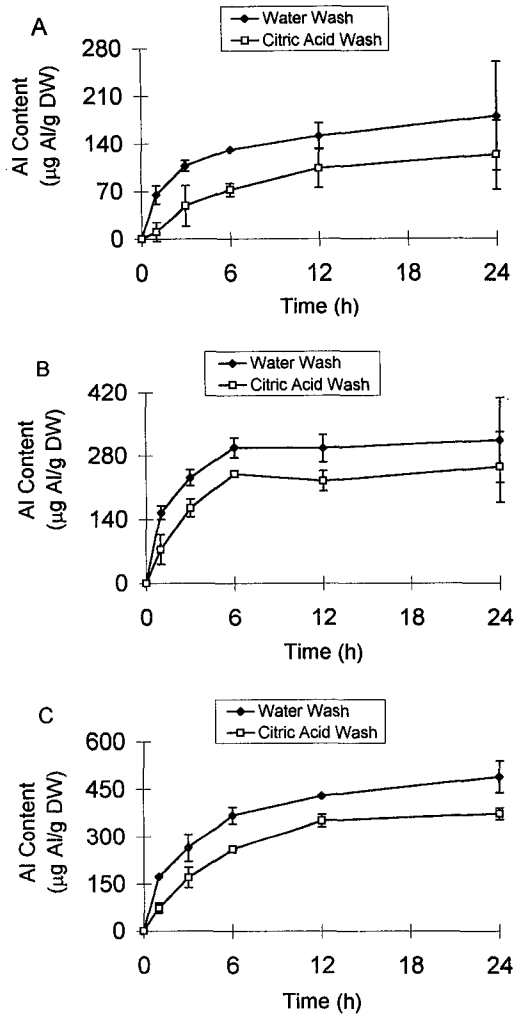

Figure 6. Time course of Al accumulation in intact roots of Al-tolerant cv Atlas 66. Intact roots were submerged in the aerated NS in the presence of 50 μM Al for different periods of time. Following Al exposure, the roots were transferred to ice-cold 0.5 mM citric acid (pH 4.5) for 30 min or to ice-cold water for 10 min. The roots were then excised in different lengths. A, Al content in the 0- to 2-mm tip. B, Al content in the 2- to 5-mm region. C, Al content in the 5- to 15-mm region. Mean ± SD are of two experiments. DW, Dry weight.

cultivar. We present quantitative data that support the hypothesis that Al accumulation in the root-growing regions is related to Al sensitivity.

In the Al-tolerant cv Atlas 66, Al accumulation in the apical 0- to 2-mm root region was always lower than in the more mature regions at concentrations between 1 μM and 75 μM (Fig. 1). However, at a high Al concentration (100 μM) the accumulation of Al in all regions was approximately the same. On the other hand, Al accumulation in the apical 0- to 2-mm root region of the Al-sensitive cv Scout 66 was always higher than in the mature regions (Figs. 2 and 3). The same pattern was observed in the Al-sensitive cv Tam 105 (Rincón and Gonzales, 1992).

Rincón and Gonzales (1992) and Delhaize et al. (1993a) reported results that support the hypothesis that differential Al sensitivity between the Al-tolerant and Al-sensitive cultivars of wheat was related to a differential Al accumulation in the root tips. The results presented here clearly demonstrate that Al sensitivity, measured as an effect of Al on root growth, correlated with the concentration of Al in the root tips (Figs. 4 and 5). In the tolerant cv Atlas 66, the accumulation of Al in the 0- to 2-mm root tips strongly correlates with the concentration of Al in the NS ($r^2 = 0.97$; Fig. 4C), and more importantly, there was a strong linear relationship between root growth and tissue Al concentration ($r^2 = 0.94$; Fig. 4B). Low Al concentration in the 0- to 2-mm root tissue caused either a small stimulation or no inhibition of root growth. The growth-stimulatory tissue Al content varied from 0 to 6.5 μg Al g^{-1} dry weight. Stimulation of growth in other wheat cultivars and other plant species by Al has been observed (discussed by Foy, 1988; Rincón and Gonzales, 1991; Kinraide, 1993), which might be due to an alleviating H^+ toxicity (Kinraide, 1993), increasing the PO_4^{3-} uptake (Macklon and Sim, 1992; Nichol et al., 1993), and redistributing the PO_4^{3-} pool inside the plant (Miranda and Rowell, 1989). Based on the regression analyses shown in Figure 4, B and C, it was calculated that in Atlas 66 a 10% inhibition of root growth would correspond to 86.05 μg Al g^{-1} dry weight Al in the root tips and

Table II. *Rates of Al accumulation in intact roots of the Al-tolerant cv Atlas 66 at different time periods*

The rates of Al accumulation were calculated by regression analysis of the data in Figure 6. The numbers in parentheses are the coefficients of determination (r^2).

Root Region	Time Period	Al Accumulation	
		Water wash	Citric acid wash
mm	*h*	*μg Al g^{-1} dry wt h^{-1}*	
0–2	0–6	19.6 (0.83)	12.4 (0.96)
	6–24	2.7 (0.90)	2.7 (0.98)
2–5	0–6	44.1 (0.83)	38.6 (0.94)
	6–24	0.96 (0.85)	1.07 (0.41)
5–15	0–6	55.2 (0.88)	41.9 (0.96)
	6–24	6.5 (0.96)	5.7 (0.74)

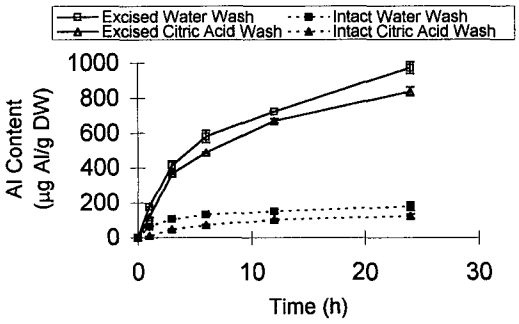

Figure 7. Time course of Al accumulation in excised cv Atlas 66 root tips (0 to 2 mm). Excised root tips were submerged in the aerated NS containing 50 μM Al for different periods of time. Following Al exposure, the tips were transferred to ice-cold 0.5 mM citric acid (pH 4.5) for 30 min or to ice-cold water for 10 min. The results from Figure 6A are plotted for a comparison (dotted lines). Mean ± SD are of two experiments. DW, Dry weight.

to 24.7 μM Al in the NS, and 50% root growth inhibition would correspond to 376.7 μg Al g^{-1} dry weight in the root tips and to 105 μM in the NS.

The relationship between growth and tissue Al concentrations was not linear in the Al-sensitive cv Tam 105 (Fig. 5); the estimated Al concentration, which would cause 50% root growth inhibition, corresponded to 546.2 μg Al g^{-1} dry weight (Fig. 5B) and to a NS Al concentration of 11.4 μM (Fig. 5C). Saturation of growth inhibition occurred when the tissue Al concentration was higher than 1 mg Al g^{-1} dry weight. The inhibitory Al content in cv Tam 105 was 145% of that in cv Atlas 66. These results however, differ from those reported by Tice et al. (1992); they calculated that the inhibitory Al content that caused 50% root growth was approximately 160 μg Al g^{-1} dry weight in both the Al-sensitive cv Tyler and the Al-tolerant cv Yecora Rojo. The apparent discrepancy between their results and ours may be because of the differences in the experimental conditions (duration of the Al treatment, NS composition, seedling age, etc.) and because of the degree of sensitivity to Al exhibited by the Al-sensitive cvs Tyler and Tam 105 and the Al-tolerant cvs Yecora Rojo and Atlas 66. Our results clearly demonstrate that the Al-sensitive cv Tam 105 exhibits higher rates of Al accumulation than the Al-tolerant cv Atlas 66 (Figs. 4 and 5). However, further research is needed to establish the cellular compartment(s) where Al is accumulated and the signal transduction mechanism that leads to root growth inhibition.

Al accumulation in the 0- to 2-mm root tips of the tolerant cv Atlas 66 was faster during the first 6 h of Al exposure than during the time interval of 6 h to 24 h (Fig. 6; Table II). The rates of Al accumulation in the 2- to 5-mm and 5- to 15-mm root regions were higher than in the 0- to 2-mm root tips. The low Al content in the growing root region of the Al-tolerant wheat cv Atlas 66 may be due to an Al efflux mechanism coupled to an Al-induced organic acid and a PO_4^{-3} efflux. Reves and Rincón-Zachary (1995) have reported that in cv Atlas 66, the loss of Al from excised 0- to 2-mm is evident and depends on the metabolism. Delhaize et al. (1993b) and Ryan et al. (1995a, 1995b) identified and characterized an Al-induced malate efflux from the root tips of Al-tolerant cultivars of wheat. Al did not induce malate efflux in Al-sensitive cultivars (Delhaize et al., 1993b; Ryan et al., 1995a). Also, in Al-tolerant cultivars of snapbeans and corn, exudation of citric acid was evident (Miyasaka et al., 1991; Pellet et al., 1995). Furthermore, Al-induced PO_4^{-3} exudation from wheat root tips has been observed (Pellet et al., 1996). These results support the working hypothesis that malate, citrate, and PO_4^{-3} chelate external Al; consequently, the external chemical activity of Al in the rhizosphere and its accumulation in the root tips are reduced (Kochian, 1995). Active Al-Pi and Al-malate (or citric acid) efflux may be involved in excluding Al from tolerant roots (Taylor, 1988b, 1991; Lindberg, 1990), however, direct experimental evidence to support these Al-exclusion mechanisms is lacking. In summary, there may be several strategies operating in the root cells of Al-tolerant cultivars to lower the tissue Al content in the growing region.

We also compared the effectiveness of a citric acid wash and a water wash to remove the desorbable Al from the root surface. The fraction of Al removed by citric acid decreased as the time of Al uptake increased (Fig. 6). Thus, under the conditions of these experiments, Al entered a compartment that was inaccessible to exchange (tightly bound in the cell wall or in the symplast). Zhang and Taylor (1989, 1990) and Tice et al. (1992) identified the cell wall as the main site of Al accumulation. Zhang and Taylor (1989, 1990) used citric acid washes to remove Al from the cell wall and showed that over a 3-h period there were two phases of Al uptake; a rapid, linear phase followed by a slower phase of accumulation. The authors suggested that the second linear phase of Al uptake represented transport of Al across the plasma membrane.

In cv Atlas 66 the rates of Al accumulation in the 0- to 2-mm region dropped with time in both water- and citric acid-washed root tips (Fig. 6A; Table II). The rates of Al accumulation in the presence of 50 μM Al were 19.6 and 12.4 μg Al g^{-1} dry weight h^{-1} during the first 6 h for water- and citric acid-washed root tips, respectively, and they dropped to 2.7 μg Al g^{-1} dry weight h^{-1} between 6 h and 24 h. In cv Tam 105 the rate of Al accumulation in the presence of 10 μM Al in the NS was almost twice as high as in the cv Atlas 66 root tips during the early phase of Al uptake (i.e. 31.6 and 34.3 Al g^{-1} dry weight h^{-1} for water- and citric acid-washed tips, respectively), and as in cv Atlas, the fraction of Al removed by citric acid decreased with time (data not shown).

Ownby and Popham (1989) showed that inhibition of root growth by Al in Al-tolerant cv Atlas 66 could be completely reversed by removing Al from the solution or treating the roots with 2 mM citric acid, whereas in Al-sensitive cultivars the recovery of root growth by citric acid was partial. Recently, Lazof et al. (1994) used secondary ion MS to estimate symplastic Al concentrations at 71 nmol g^{-1} fresh weight (1.92 μg Al g^{-1} fresh weight) in intact roots of an Al-sensitive cultivar of soybean within 30 min of Al exposure. Thus, it is possible that the initial phase of root growth inhibition may be due to Al interference with the growth processes that occur in the cell wall (e.g. cell wall loosening) and with the signal transduction pathways that are involved in cell growth. However, it remains to be demonstrated whether apoplastic or symplastic Al is responsible for the initiation of root growth inhibition.

Most ion transport studies are done with excised root tissue because of the convenience of conducting transport experiments under laboratory conditions (Huang et al., 1992). Usually, the excised root segments are allowed to recover from the initial "injury" of excision during a washing or aging period. The assumption is that aged root tissue behaves like intact root tissue in terms of ion transport (Gronewald and Hanson, 1980). The results in Figure 7 clearly show the differences in Al accumulation between excised and intact 0- to 2-mm root tips of the Al-tolerant cv Atlas 66 at the external Al concentration of 50 μM. The data show a 2.8-fold increase of the Al content in the excised tissue at 1 h Al uptake as compared with the intact root

tips. The tissue Al concentration in the excised tips reached the inhibitory concentration observed in intact Al-sensitive cv Tam 105 root tips (Fig. 5). However, this apparent increase of the Al content in the tips may be due to an increased Al binding to the cut surface. If the apparent increase in Al accumulation in the excised tips were due to Al binding to the cut surface, then citric acid should remove the bound Al. However, a 30-min citric acid wash had little effect on the shape of the time-course curve or on the removal of Al from the tissue (Fig. 7). The citric acid wash removed 36% of the Al accumulated in 1 h, but after 3 h the citric acid wash removed only a small fraction of the accumulated Al (7–16%). These results indicate that in excised tissue Al either binds to the cut surface very tightly or enters the symplasm where it is not available for chelation by citric acid.

A preliminary experiment showed that Al uptake was greater in aged excised 0- to 2-mm root tips of cv Atlas 66 than in freshly excised root tips (data not shown). Ion transport researchers have interpreted that increased K^+ uptake in washed excised roots is due to a "recovery" of cellular activities (e.g. respiration, protein synthesis, etc.) that regulate ion transport mechanisms damaged by the initial injury of excision. The H^+-ATPase activity increases during the washing period and, consequently, the cells regain the electrical and pH gradients across the plasma membrane, and influx of K^+ and other ions is restored. Miyasaka et al. (1989) reported that Al induced hyperpolarization of wheat root cells. A higher electrical gradient across the membrane (negative inside and positive outside) could increase Al uptake. Does Al uptake depend on the activity of the H^+-ATPase? More research is needed to explain the nature of Al uptake into root cells.

Based on the results shown in Figure 7, we conclude that caution must be exercised in interpreting results when excised root tissue is used in studies of Al sensitivity, Al transport, and the effects of Al on root growth. Therefore, whenever possible, intact roots should be used to assess Al sensitivity. In summary, the data reported here support the hypotheses (a) that differential Al accumulation in the growing root tissue is related to differential Al sensitivity; (b) that inhibition of root growth is related to the Al content in the root tissue; and (c) that mechanisms of Al tolerance may be based on strategies to reduce or to restrict Al absorption in the root meristems and are the subject of ongoing studies.

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