Plant Physiol. (1992) 99, 1021–1028
0032-0889/92/99/1021/08/$01.00/0

Received for publication October 22, 1991
Accepted February 3, 1992

Aluminum Partitioning in Intact Roots of Aluminum-Tolerant and Aluminum-Sensitive Wheat (*Triticum aestivum* L.) Cultivars

Magaly Rincón[1] and Robert A. Gonzales*

The Samuel Roberts Noble Foundation, Plant Biology Division, Box 2180, Ardmore, Oklahoma 73402

ABSTRACT

Aluminum (Al) partitioning in intact roots of wheat (*Triticum aestivum* L.) cultivars that differ in sensitivity to Al was investigated. Roots of intact seedlings were exposed to Al for up to 24 hours and distribution of Al was assessed visually by hematoxylin staining or by direct measurement of concentration of Al by atomic absorption spectrophotometry or ion chromatography. Major differences in Al accumulation between Al-tolerant (Atlas 66) and Al-sensitive (Tam 105) cultivars were found in the growing regions 0 to 2 and 2 to 5 millimeters from the root apex. Al content was 9 to 13 times greater in the 0 to 2 millimeters root tips of cv Tam 105 than in the tips of cv Atlas 66 when exposed to 50 micromolar Al for 19 to 24 hours. The oxidative phosphorylation inhibitor carbonyl cyanide *m*-chlorophenylhydrazone and the protein synthesis inhibitor cycloheximide increased Al uptake by intact root tips of cv Atlas 66. Also, loss of Al from the roots of both cultivars was measured after the roots were "pulsed" with 50 micromolar Al for 2 hours and then placed in an Al-free nutrient solution for 6 hours. The 0 to 2 millimeter root tips of cv Tam 105 lost 30% of the absorbed Al, whereas the tips of cv Atlas 66 lost 60%. In light of these results, we conclude that the differential Al sensitivity in wheat correlates with the concentration of Al in the root meristems. The data support the hypothesis that part of the mechanism for Al tolerance in wheat is based on a metabolism-dependent exclusion of Al from the sensitive meristems.

Reduction of crop yields in acidic soils is due mainly to the presence of ionic Al[2] in the soil solution. At low pH, Al bound to clay minerals dissolves in the soil solution and becomes available to roots. Al toxicity is manifested primarily by inhibition of root growth, and varieties of the same species often show different degrees of sensitivity to Al (1, 2, 5, 9, 24). Tolerance to Al is genetically controlled (3, 5, 8) and regulated by several genes (3, 22). The toxic effects of Al on plants are well documented (2, 8, 10, 17, 24); however, very little is known about the mechanisms of Al toxicity and much less is known about the mechanisms that control Al tolerance. Several Al tolerance mechanisms have been proposed (8, 10, 25), including chelation of Al via formation of Al complexes with organic acids, acidic polypeptides, and/or proteins (2, 18, 23), sequestration of Al in organelles (*e.g.* vacuoles, cell walls [7, 25]), precipitation of Al by alkalinization of the rhizosphere (25), immobilization of Al by the mucigel (11, 17), changing or inducing enzyme activities to less Al-sensitive forms (22), and transport of Al out of the root tissue (25, 29).

Data on differences in Al accumulation between tolerant and sensitive cvs of wheat (*Triticum aestivum* L.) are available (1, 15, 27–29) but often conflicting. It is not clear from the literature whether the differential Al sensitivity in wheat roots is due to a differential accumulation of Al. For example, it has been reported that wheat roots of Al-tolerant cultivars accumulate more Al than those of Al-sensitive cultivars in long-term Al treatments (≥24 h) (1, 28). However, other reports indicate that there are no differences in the initial Al accumulation (<12 h) between tolerant and sensitive wheat cultivars (15, 27, 29).

In roots, one of the primary sites of Al toxicity is the meristems (4, 8, 9, 13). Thus, if the differential Al sensitivity among wheat cultivars is due to a differential Al accumulation in the meristems, then the meristematic tissue of a sensitive cultivar will accumulate more Al than the tissue of a tolerant cultivar.

The objective of this study was to assess accumulation of Al in the root meristems of two wheat cultivars that exhibit differential sensitivity to Al.

MATERIALS AND METHODS

Plant Material

Wheat (*Triticum aestivum* L.) cvs Tam 105 (Oklahoma State University, Stillwater, OK), Bounty 203-A, and Atlas 66 (Cargill Hybrid, Fort Collins, CO) were used in this study. Tam 105 seeds were obtained already treated with fungicide Vitavex-200 (Uniroyal) and Heptachlor. Bounty 203-A and Atlas 66 seeds were surface sterilized with 5% (w/v) commercial bleach and 0.1% (w/v) SDS for 5 min and rinsed well with deionized and double-distilled H_2O. Seeds were germinated on paper towels saturated with 0.1 mM $CaCl_2$ in the dark at 20°C for 3 to 5 d. Fourteen seedlings were placed on Styrofoam discs and floated on 6 L of aerated nutrient solution containing 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). The seedlings were grown for 2 to 3 d in a Conviron growth chamber under a light/dark cycle of 14 h at 25°C and 10 h at 20°C; the light intensity was 171 ± 12 μmol photons m^{-2} s^{-1}.

[1] Present address: Department of Biology, Midwestern State University, 3400 Taft Boulevard, Wichita Falls, TX 76308.

[2] Abbreviations: Al, aluminum; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DNP, 2,4 dinitrophenol.

Hematoxylin Staining of Al-Treated Roots

Hematoxylin stain was prepared as previously described by Polle *et al.* (16). It consisted of 0.2% hematoxylin (Sigma) and 0.02% $NaIO_3$, dissolution of which was aided by adding a drop of 0.1 N NaOH. Seven to 10-d-old intact seedlings were incubated in nutrient solution plus 50 μM $AlCl_3 \cdot 6H_2O$, which gave 42.5 μM Al^{3+} as calculated by the GEOCHEM program, unless otherwise indicated. At the end of the treatments, the roots were rinsed with distilled H_2O and placed in approximately 60 mL of aerated hematoxylin stain for 40 min at room temperature. The roots were then placed in aerated distilled H_2O for 10 min to remove excess stain. Roots were sectioned for microscopic examination and micrography.

Al Uptake

To determine Al accumulation in the roots, 4- to 6-d-old seedlings were placed in 2 or 6 L of aerated nutrient solution plus $AlCl_3$ at room temperature and harvested at the indicated times. The roots were rinsed briefly with distilled H_2O and kept on ice-cold H_2O during excision. The roots were excised in consecutive segments measured from the apex, including the cap (in mm): 0 to 2, 2 to 5, 5 to 15, 15 to 25, and 25 to 35.

To explore the effect of metabolic inhibitors on Al accumulation by the root tips, the intact seedlings were placed in 100 mL of aerated nutrient solution containing 50 μM $AlCl_3$ plus inhibitors for 6 h at room temperature. Stock solutions of CCCP (50 mM; Sigma) and cycloheximide (50 mg/mL;

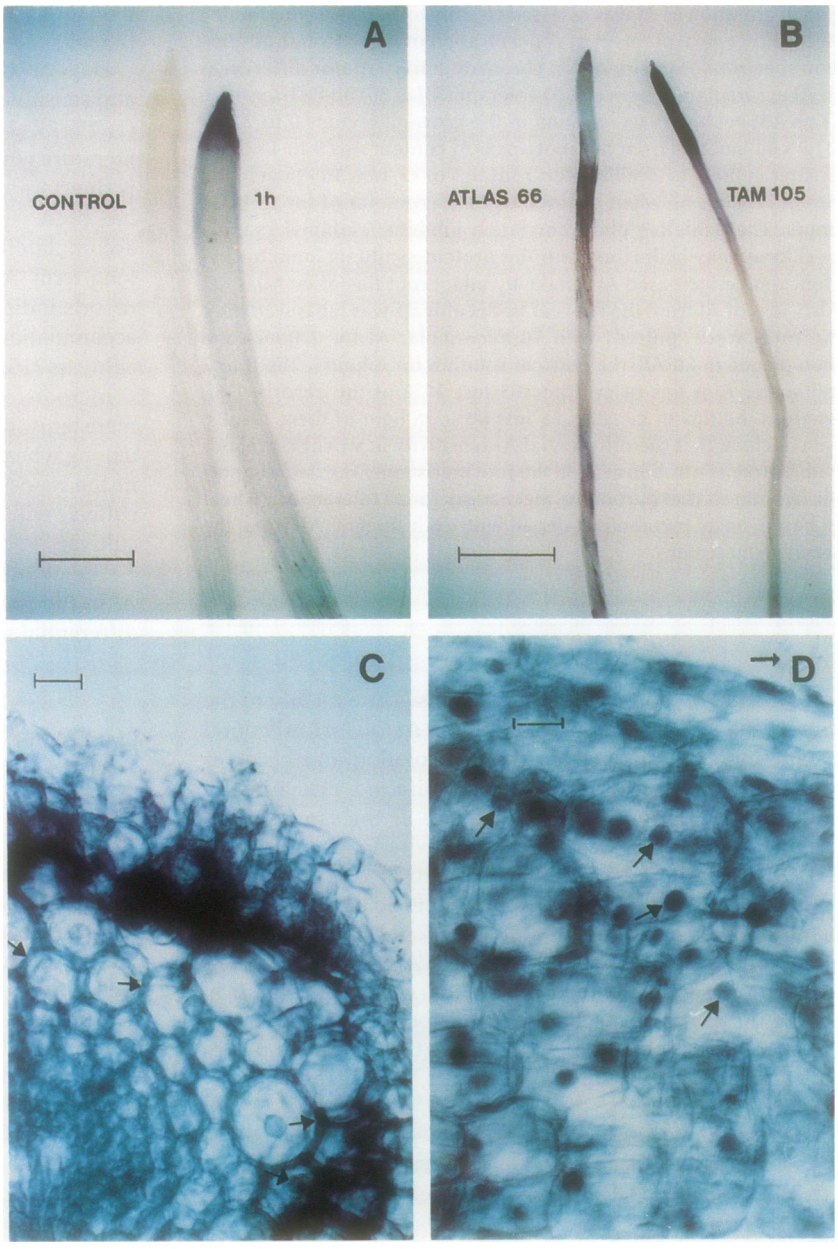

Figure 1. Detection of Al by hematoxylin staining. A, Intact seedlings of cv Atlas 66 were incubated in nutrient solution in the presence of 50 μM Al for 1 h or in the absence of Al (control) and stained with hematoxylin as described in "Material and Methods." Scale, 1 mm. B, Intact seedlings of cvs Atlas 66 and Tam 105 were incubated in nutrient solution plus 50 μM Al for 17 h and stained. Scale, 3 mm. C, Cross-section of Tam 105 root tip. The root was treated with 50 μM Al for 14 h. Arrows point to the cell walls, intercellular spaces, and nuclei. Scale, 50 μm. D, Epidermal strip of Atlas 66 root that was treated with 1 mM Al for 14 h and and stained with hematoxylin. Arrow heads point to the nuclei. Left-to-right arrow indicates the direction of the root tip. Scale, 50 μm.

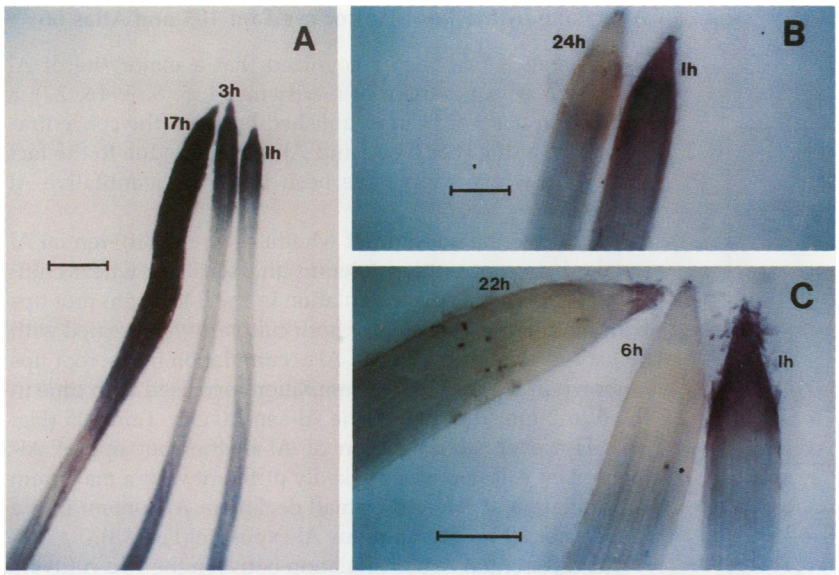

Figure 2. Time course Al accumulation in the root tips of cvs Tam 105, Bounty 203-A, and Atlas 66 as visualized by hematoxylin staining. A, Tam 105 roots were treated with 50 μM Al for 1 to 17 h. Scale, 1 mm. B, Bounty 203-A roots were treated with 20 μM Al for 1 to 24 h. Scale, 0.5 mm. C, Atlas 66 roots were treated with 50 μM Al for 1 to 22 h. Scale, 0.5 mm.

Sigma) were prepared in absolute ethanol. An equivalent volume of absolute ethanol was added to the controls.

Measurement of Al Efflux from the Tissue

After the intact roots were incubated for 2 h in 2 L of aerated nutrient solution in the presence of Al as described above, the seedlings were transferred to plastic beakers containing 200 mL of aerated Al-free nutrient solution for 6 h at room temperature and the Al remaining in the root tissue was determined as described below.

Al Determination

After treatments, the roots were excised in segments as indicated in the figure legends. The tissue was placed in polypropylene microcentrifuge tubes and dried in an oven at 75°C. The dried tissue was placed in 15-mL polypropylene tubes and digested with HNO_3 (Baker Instra-analyzed, 70%) and H_2O_2 (30 or 50%) (1:1) at 65°C for 1 to 2 h. The sample volumes were adjusted with milli-Q H_2O (Millipore filtration system) to keep the HNO_3 concentration at 14%. Al content was determined either by atomic absorption spectrophotometry with a Perkin-Elmer 2380 spectrophotometer equipped with a graphite furnace and a HGA-400 programmer or by ion chromatography. Ion chromatography was performed with a Dionex model 4505i HPLC equipped with a Al-450 full control software/interface system. Al was separated on a cation-exchange HPIC-CS2 column using 0.01 M H_2SO_4-0.2 M $(NH_4)_2SO_4$ as eluent at a flow rate of 1 mL/min. Al was detected at 310 nm following postcolumn reaction with 0.3 mM Tiron (4,5-dihydroxy-*m*-benzene disulfonic acid, disodium salt; Baker analyzed reagent) in 3 M ammonium acetate at pH 6.2. The pH was adjusted with glacial acetic acid and the postcolumn flow rate was 0.6 mL/min. The eluent and the postcolumn reagent were prepared with milli-Q H_2O and filtered through a 0.45-μm nitrocellulose filter (Millipore).

The samples and Al standards were diluted 1:10 with milli-Q H_2O and loaded onto the column by injection from a 200-μL sample loop. The samples and the Al standards were contained in polypropylene tubes that were soaked in 20% HNO_3 for 48 h and rinsed with double-distilled H_2O prior to use. The Al standards were prepared in 14% HNO_3 from a stock solution of 1 g/L (Sigma).

All treatments were duplicated or triplicated and experiments were repeated at least twice.

RESULTS

Localization of Al by Hematoxylin Stain

Figure 1 illustrates localization of Al in wheat root tissue by hematoxylin staining. When roots of the tolerant cv Atlas 66 were treated with 50 μM Al for 1 h, staining was mainly localized in the 0.2-mm apical region; the untreated root did not stain (Fig. 1A). Differences in staining between Atlas 66 and Al-sensitive Tam 105 roots could be seen when the roots of both cultivars were treated with 50 μM Al for 17 h. In Tam 105 roots, the stain intensity was greater in the apical 0 to 3 mm, whereas in Atlas 66 the stain shows Al accumulation basal to this region (Fig. 1B). The technique was also useful to visualize cellular accumulation of Al. When intact roots were treated with 50 μM Al, Al accumulated mainly in the cell walls, intercellular spaces, and nuclei of the cortical and epidermal root tip cells (Fig. 1, C and D). Under these conditions, the epidermis of Tam 105 roots showed signs of damage (Fig. 1C). No stain was observed in control roots that were incubated with hematoxylin and then sectioned for microscopic examination (data not shown). In addition, the stain intensity in the roots of the Al-sensitive cv Tam 105 increased as duration of the Al treatment increased from 1 to 17 h (Fig. 2A). However, this did not occur in the tolerant cvs Bounty 203-A and Atlas 66 (Fig. 2, B and C), where the hematoxylin stain intensity in the root tips decreased as exposure to Al continued for 6 to 24 h. The basal root region of cv Atlas 66 stained more intensely (4–7 mm from the root cap) than the apex (Fig. 1B).

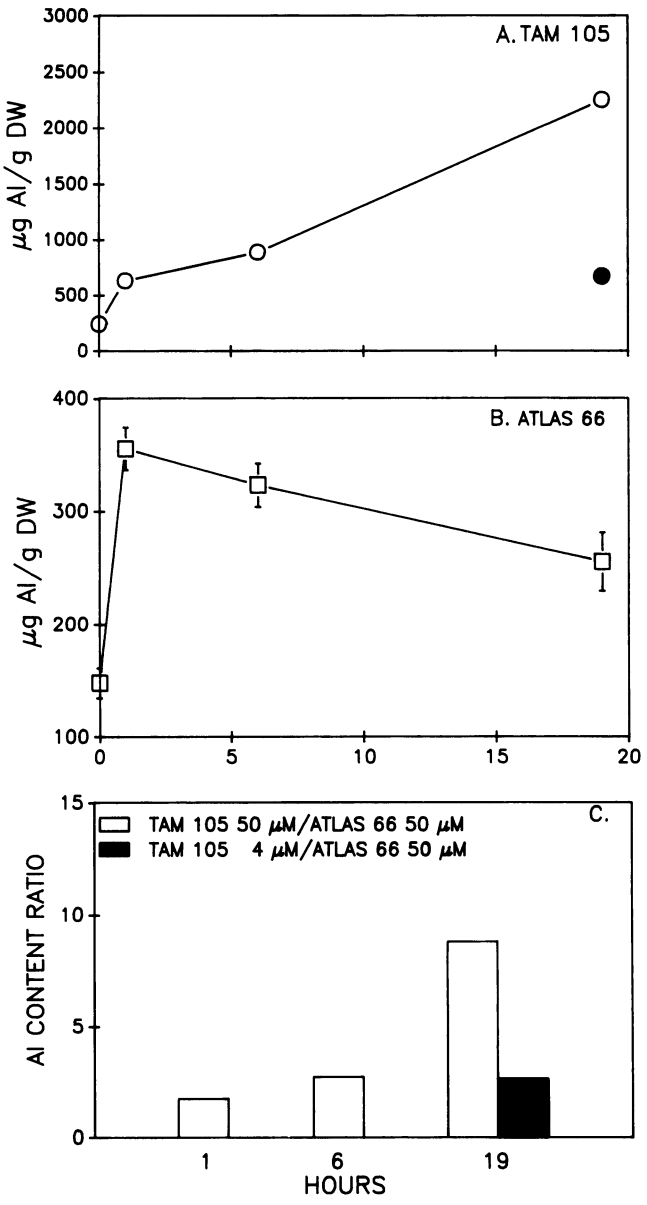

Figure 3. Time course of Al uptake by cvs Tam 105 and Atlas 66 2-mm root tips. Intact roots were incubated in aerated nutrient solution plus 50 μM Al for 1, 6, and 19 h and the Al content in the 0 to 2 mm root tips was determined by atomic absorption spectrophotometry. A, Al content in cv Tam 105 root tips. O, Al content in the root tips when the Al concentration in the solution was 50 μM; ●, Al content in the root tips when the Al concentration in the solution was 4 μM. B, Al content in cv Atlas 66 root tips. Each point is the mean ± SD of two values from one representative experiment. The SD bars are not shown when they are smaller than the symbols. C, Ratio of the Al content in Tam 105 root tips to the Al content in Atlas 66 tips at the given times. Data are from plots A and B.

Al Uptake by the Root Tips of cvs Tam 105 and Atlas 66

Although it has been recognized that a major site of Al toxicity in wheat is the root meristems (4, 5, 8, 9, 16, 27), a correlation has not been established between the concentration of Al in the root tissue and Al sensitivity due to the fact that often whole roots have been used for quantitative Al analyses.

To address the question of whether there is differential Al uptake between roots of tolerant and sensitive wheat cultivars, we followed Al accumulation in the 0 to 2 mm root tips with time. The intact roots of both cultivars were treated with 50 μM Al. The time course of Al accumulation in the root tips is shown in Figure 3. Al accumulation increased with time in the 0 to 2 mm root tips of the Al-sensitive cv Tam 105 (Fig. 3A). However, accumulation of Al in the root tips of Al-tolerant cv Atlas 66 was markedly different with a maximum accumulation at 1 h and a small decline in Al content in the tissue after 19 h of continuous Al exposure (Fig. 3B).

The differential Al accumulation between the two cultivars is better illustrated by the ratios of Al content in Tam 105 root tips to the Al content in Atlas 66 tips at the given times (Fig. 3C). At all times, the 0 to 2 mm root tips of Tam 105 accumulated more Al than the tips of Atlas 66. It is worth noting that when the roots of Tam 105 were treated with 4 μM Al, the content in the apical region was still three times higher than in Atlas 66 root tips treated with 50 μM Al.

Effect of Metabolic Inhibitors on Al Uptake by cv Atlas 66 Root Tips

The results shown in Figure 3 suggest that the 0 to 2 mm root tips of the tolerant roots have a mechanism to prevent Al accumulation in this region. Thus, the question arose whether or not this mechanism(s) was dependent on metabolic energy. To answer this question, the intact roots of Atlas 66 were treated with 50 μM Al for 6 h in the absence or in the presence of the oxidative phosphorylation inhibitor CCCP and the protein synthesis inhibitor cycloheximide. The Al content in the 0 to 2 mm tips and in the 2 to 20 mm segments was determined. The results are summarized in Table I. CCCP and cycloheximide caused an increase in Al concentration in the root tips and segments. Interestingly, the increased Al accumulation in the presence of CCCP was greater in the tips than in the segments (ninefold *versus* twofold).

Al Partitioning in the Roots of cvs Tam 105 and Atlas 66

Differences in the distribution of Al along the roots of both cvs were also investigated. The results are shown in Figure 4. The differences in accumulation of Al between Tam 105 and Atlas 66 were more accentuated in the meristematic regions, thus supporting the results of Figures 1B and 3. The Al concentration in the 0 to 2 and 2 to 5 mm tips of cv Tam 105 was 2 times higher than in the tips of cv Atlas 66 after 1 h Al exposure, and by 24 h, the Al concentration in the tips of cv Tam 105 was 13 times higher than in the tips of Atlas 66. In the more mature root regions, the differences in Al concentration between the two cultivars were not very prominent; in fact, the Al content in the mature region of the

Table I. *Effect of Metabolic Inhibitors on Al Uptake by Intact Roots of cv Atlas 66*

Five-day-old seedlings were incubated in 100 mL aerated nutrient solution containing 50 μM Al plus the indicated inhibitors for 6 h. Al was determined by ion chromatography. Data are means ± SD of two separate experiments. Values in parentheses represent percentage of controls.

Treatment	Tips (0–2 mm)	Segments (2–20 mm)
	μg Al/g dry weight	
Control	311.9 ± 82.1 (100)	402.8 ± 129.7 (100)
CCCP (10 μM)	2589.6 ± 214.6 (895)	947.7 ± 155.3 (235)
Control[a]	293.4 ± 24	ND
Cycloheximide (10 μg/mL)[a]	426.7 ± 107 (147)	ND

[a] Al was determined by atomic absorption spectrophotometry. Data are means ± SD of duplicates from one representative experiment. ND, Not determined.

sensitive roots was only twofold higher than in the tolerant roots except for the region at 15 to 25 mm from the tip, where the Al concentration in the sensitive tissue was lower than in the tolerant tissue at all times examined.

Furthermore, in the sensitive roots, the Al content in the meristematic region was higher than in the mature region, whereas in the tolerant roots, Al content in the meristems was lower than in the mature regions.

Efflux of Al from the Root Tissue

As shown in Table I, tolerant roots appeared to have a metabolism-dependent process to prevent Al accumulation in the root tips. It is possible that exclusion of Al from the root tips may operate by an efflux mechanism present in the root meristems as suggested by Zhang and Taylor (29). To test this hypothesis, a "pulse-chase"-like experiment was designed to follow the efflux of Al from the root tissue. The intact roots of both cultivars were "pulsed" with 50 μM Al for 2 h and then placed in aerated nutrient solution without Al for 6 h (chase) and the Al content in the different regions of roots was determined. Figure 5 shows the distribution of Al along the roots of both cultivars after Al pulse and chase periods. In Tam 105, the concentration of Al in the regions 0 to 2 and 2 to 5 mm from the apex were higher than in the more mature regions (Fig. 5A); in Atlas 66, Al was more evenly distributed along the roots (Fig. 5B). The Al concentration in the root apex of Tam 105 was 2.5 times higher than in the root apex of Atlas 66, supporting the results shown in Figures 3 and 4. The absolute loss of Al from the apical region in both cultivars appeared to be approximately the same. In relative terms, however, the root tips of cv Tam 105 lost 30% of the accumulated Al in 6 h, whereas the tips of cv Atlas 66 lost 60% (Fig. 5C). In contrast, in the more mature root regions of both cultivars, the loss of Al was approximately the same (50–70%).

DISCUSSION

The hematoxylin staining technique has been used to screen Al-tolerant cultivars of wheat (5, 16, 27). The method is simple and is based on the colorimetric property of hematoxylin to give a blue-purple stain when complexed with Al. Thus, visual evaluation of stained roots can be used to detect Al accumulation in the root tissue. Hematoxylin staining showed that Al accumulated mainly in the apical root region of both tolerant cv Atlas 66 and sensitive cv Tam 105 (Figs. 1 and 2). There were stain intensity differences between the sensitive and tolerant root tips, which is in agreement with previous reports (5, 16, 27). Al, at concentrations (50 μM) that inhibited root growth and changed protein synthesis patterns (19), accumulated mainly in the cell walls, intercellular spaces, and nuclei of the cortical and epidermal root tip cells (Fig. 1, C and D). Matsumoto *et al.* (14) have reported similar results in Al-treated pea roots stained with ammonium aurintricarboxylate.

The stain intensity in the sensitive root tips of cv Tam 105 increased as the duration of the Al treatment increased from 1 to 17 h (Fig. 2A), whereas the stain intensity decreased in the tolerant tips of cvs Atlas 66 and Bounty 203-A as exposure to Al continued for 6 to 24 h (Fig. 2, B and C). The physiological basis for the decreased stain intensity in the tolerant root tips remains unknown; however, formation of Al-hematoxylin complexes may be hindered by changes in the chemical composition of the root tips in response to Al, which, for example, may cause shifts in local pH. Also, synthesis of Al chelators may interfere with the Al-hematoxylin binding.

In the literature, quantitative data on differences in Al accumulation between tolerant and sensitive wheat cultivars do not correlate with the degree of Al sensitivity in the roots (1, 15, 27–29). This lack of correlation between Al sensitivity and Al concentration in the root tissue may be due to the fact that whole roots are often used for Al analyses. The differential Al accumulation between the sensitive and tolerant cultivars is most obvious only in the apical root region (Figs. 3 and 4), which parallels the qualitative observations of hematoxylin stain (Figs. 1 and 2) (16).

The differential Al accumulation was maintained even when the sensitive roots were treated with 4 μM Al; at this concentration, the Tam 105 0 to 2 mm tips absorbed three times more Al than Atlas root tips treated with 50 μM Al (Fig. 3C). However, the Al content in the mature regions of the sensitive roots was only twofold higher than in the mature regions of the tolerant roots (Fig. 4). Note that in the sensitive roots of Tam 105, the Al content in the apical region was higher than in the basal regions, whereas in the tolerant Atlas 66 roots, the Al content was lower in the apex than in the mature root regions. Accumulation of Al in the basal region of the tolerant roots may reflect Al translocation from the

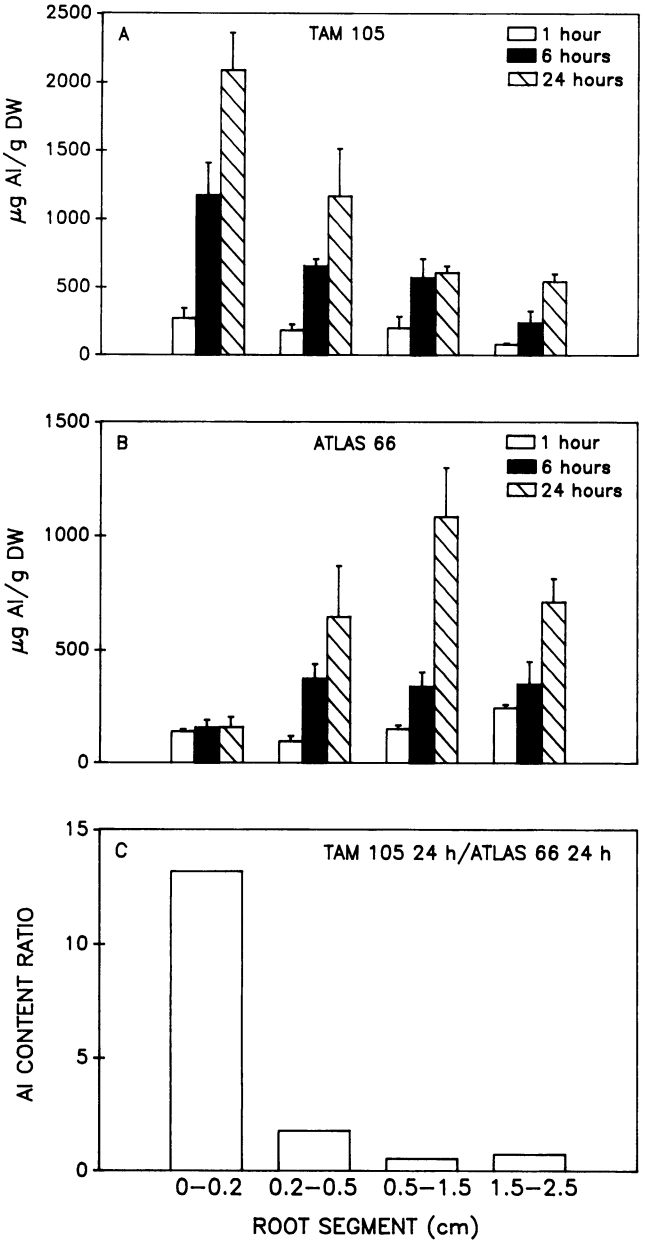

Figure 4. Al partitioning in the roots of cvs Tam 105 and Atlas 66. Intact roots of both cvs were incubated in 6 L of aerated nutrient solution in the presence of 50 μM Al for 1, 6, and 24 h. The roots were rinsed briefly with distilled H_2O and then excised in consecutive segments measured from the apex, including the root cap as indicated in the x axis. The Al content in the tissue was determined by ion chromatography. Data points are the mean ± SD of triplicate values of a representative experiment.

meristems to the differentiated root regions through the vascular tissue as suggested by Henning (cited in ref. 8). Localization of Al by energy dispersive x-ray microanalysis showed the presence of Al in the stele (M. Rincón, S. Russell, unpublished results). Translocation of Al to the basal root regions in wheat roots would be analogous to translocation of Al that occurs in Al accumulator plants (7). In these plants, Al is translocated from the roots to the tops and accumulated in the leaves where it is sequestered in vacuoles and cell walls away from the sensitive sites. Another possibility is that, in tolerant roots, Al moves (radially) through the apoplasm of the epidermis and cortex. At the endodermis, Al could enter the protoplast of the endodermal cells more readily and then be transported to the stele.

Tolerant roots of cv Atlas 66 have a mechanism to prevent Al accumulation in the root tips (Figs. 3 and 4) that depends on metabolic energy as indicated by the results shown in Table I. The Al content in the root tips of the tolerant cv Atlas 66 increased when they were treated with the oxidative phosphorylation inhibitor CCCP or with the protein synthesis inhibitor cycloheximide. The increased Al accumulation caused by CCCP was more pronounced in the 0 to 2 mm tips, where Al accumulation increased by ninefold; in the 2 to 20 mm segments, Al accumulation increased only twofold, suggesting that the meristems are dependent on metabolism to keep Al from accumulating in the sensitive sites. Similar results have been obtained with DNP and other plant species (12, 15, 26, 29). Wagatsuma (26) and Pettersson and Strid (15) suggested that DNP changed the plasma membrane permeability, increasing the passive transport of Al. Zhang and Taylor (29) proposed that the increased Al content as a result of treating the roots with metabolic inhibitors may be due to inhibition of an efflux mechanism(s) operating at the plasma membrane. There is as yet no evidence that supports the existence of Al efflux mechanisms in plants. Nevertheless, it has been suggested that Al-Pi (12) or other Al-chelate complexes (25, 29) may be actively extruded across the plasma membrane.

Recently, data on DNP-enhanced Al uptake by the cell walls isolated from both tolerant and sensitive wheat cultivars have been reported (31), implying that metabolic energy is required to prevent binding of Al to the cell wall. CCCP-induced Al accumulation in the root tips of cv Atlas 66 (Table I) may reflect Al binding to the cell walls and Al uptake across the plasma membrane due to depolarization of the membrane that may have induced ion channel opening (20). Under normal metabolic conditions, Al uptake by the cells may involve uptake by the cell walls, as suggested by Zhang and Taylor (29–31); however, once the apoplast is saturated, Al may enter the protoplast. Intracellular accumulation of Al in wheat roots has been shown by Clouse *et al.* (6). They found that Al associates with cellular components, possibly proteins, that have been fractionated from root extracts (20,000*g* supernatants) of both the sensitive cv Tam 105 and the tolerant cv Atlas 66.

Whether or not the efflux of Al indicated in the root tips (Fig. 5) is a metabolism-dependent process needs to be investigated. Nevertheless, the results shown in Figure 5, B and C suggest that the meristems of the Al-tolerant cv Atlas 66 mobilize Al out of the meristems, with the net result of

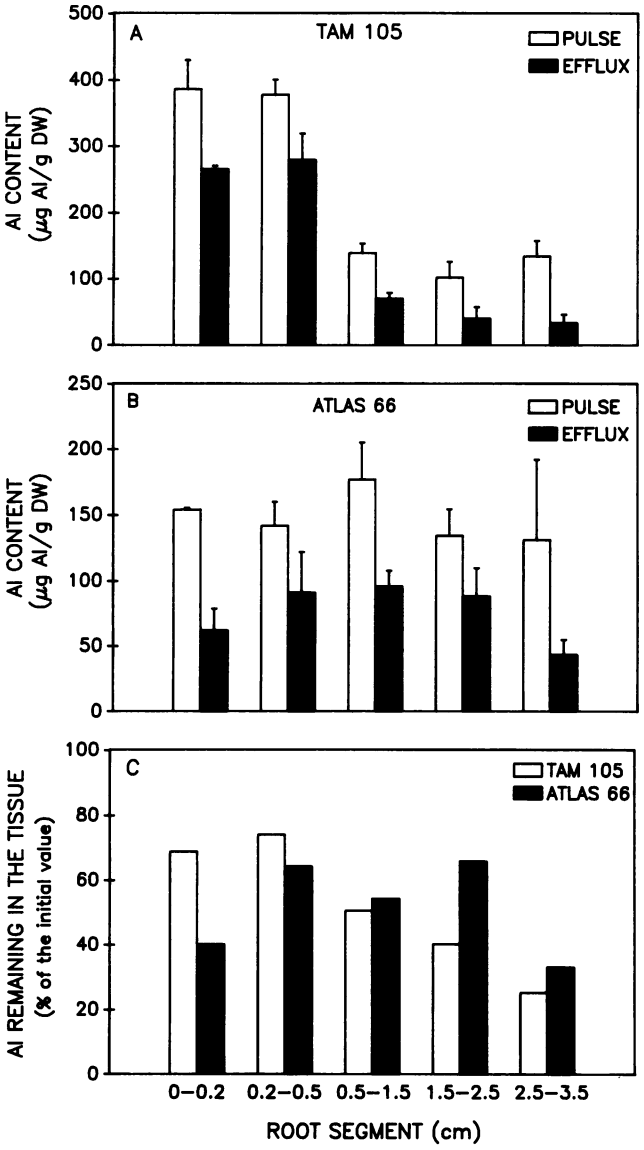

Figure 5. Loss of Al from the roots of cvs Atlas 66 and Tam 105. Intact roots of both cvs were incubated in aerated nutrient solution plus 50 μM Al for 2 h at room temperature (pulse). Then they were rinsed briefly with distilled H_2O and transferred to 200 mL of aerated Al-free nutrient solution for 6 h (efflux). The roots were excised as described in Figure 4. Al content in the tissue was determined by ion chromatography after pulse and efflux periods. A, Al content in Tam 105 roots. B, Al content in the Atlas 66 roots. Data points are the mean ± SD of triplicate values of a representative experiment. C, Al remaining in the tissue as percentage of the initial Al content.

lowering the Al concentration in the tissue to levels that allow continuous growth. However, dilution of Al due to growth cannot be ruled out. The root tips of Al-sensitive cv Tam 105 retained 70% of the accumulated Al after removing Al from the solution for 6 h (Fig. 5, A and C). Under the conditions of this experiment, the roots were unable to regrow. Moreover, Ryan and Kochian (21) reported that application of Al directly to the root apex caused growth inhibition in the wheat cultivar Scout (Al-sensitive) but not in the Al-tolerant cv Atlas 66. Application of Al to the differentiated regions of the roots had no effect on growth.

In summary, what is essential for growth under Al stress conditions is the ability of the roots to prevent accumulation of Al in the meristematic tissue.

CONCLUSIONS

Al-induced root growth inhibition may be due to association of Al with cellular components of the root apex that are critical for root growth. Based on our results, there is correlation between Al sensitivity and Al concentration in the root tips. The mechanisms that control Al accumulation appear to reside in the meristems.

Al-tolerant cultivars appear to have developed strategies to protect the root meristems against the toxic effects of Al. Our results support the hypothesis that part of the mechanism of Al tolerance in wheat is based on the metabolism-dependent exclusion of Al from the sensitive meristems.

ACKNOWLEDGMENTS

The authors thank Dr. John Hanson for his valuable discussion regarding the manuscript. They also thank Total Petroleum, Inc. (Ardmore, OK) for the use of the atomic absorption spectrophotometer.

LITERATURE CITED

1. **Aniol A** (1983) Aluminium uptake by roots of two winter wheat varieties of different tolerance to aluminium. Biochem Physiol Pflanzen **178:** 11–20
2. **Aniol A** (1984) Induction of aluminum tolerance in wheat seedlings by low doses of aluminum in the nutrient solution. Plant Physiol **76:** 551–555
3. **Aniol A, Gustafson JP** (1984) Chromosome location of genes controlling aluminum tolerance in wheat, rye, and triticale. Can J Genet Cytol **26:** 701–705
4. **Bennet RJ, Breen CM, Fey MV** (1991) The aluminium signal: new dimensions of aluminium tolerance. Plant Soil **134:** 153–166
5. **Carver BF, Inskeep WP, Wilson NP, Westerman RL** (1988) Seedling tolerance to aluminum toxicity in hard red winter wheat germplasm. Crop Sci **28:** 463–467
6. **Clouse J, Rincón M, Gonzales RA** (1991) Aluminum associates with wheat root proteins (abstract No. 753). Plant Physiol **96:** S–111
7. **Cuenca G, Herrera R, Mérida T** (1991) Distribution of aluminium in accumulator plants by x-ray microanalysis in *Richeria grandis* Vahl leaves from a cloud forest in Venezuela. Plant Cell Environ **14:** 437–441
8. **Foy CD, Chaney RL, White MC** (1978) The physiology of metal toxicity in plants. Annu Rev Plant Physiol **29:** 511–566
9. **Foy CD, Fleming AL, Burns GR, Armiger WH** (1967) Characterization of differential aluminum tolerance among varieties of wheat and barley. Soil Sci Soc Am Proc **31:** 513–521
10. **Haug A** (1984) Molecular aspects of aluminum toxicity. Crit Rev Plant Sci **1:** 345–373

11. **Horst WJ, Wagner A, Marschner H** (1982) Mucilage protects root meristems from aluminium injury. Z Pflanzenphysiol **105:** 435–444
12. **Lindberg S** (1990) Aluminium interactions with K^+($^{86}Rb^+$) and $^{45}Ca^{2+}$ fluxes in three cultivars of sugar beet (*Beta vulgaris*). Physiol Plant **79:** 275–282
13. **Marschner H** (1991) Mechanisms of adaptation of plants to acid soils. Plant Soil **134:** 1–20
14. **Matsumoto H, Hirasawa E, Torikai H, Takahashi E** (1976) Localization of aluminium in pea root and its binding to nucleic acids. Plant Cell Physiol **17:** 127–137
15. **Pettersson S, Strid H** (1989) Initial uptake of aluminium in relation to temperature and phosphorus status of wheat (*Triticum aestivum* L.) roots. J Plant Physiol **134:** 672–677
16. **Polle E, Konzak CF, Kittrick JA** (1978) Visual detection of aluminum tolerance levels in wheat by hematoxylin staining of seedling roots. Crop Sci **18:** 823–827
17. **Puthota V, Cruz-Ortega R, Johnson J, Ownby J** (1991) An ultrastructure study of the inhibition of mucilage secretion in the wheat root cap by aluminum. *In* RJ Wright, VC Baligar, RP Murrmann, eds, Plant-Soil Interactions at Low pH. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 779–787
18. **Putterill JJ, Gardner RC** (1988) Proteins with the potential to protect plants from Al^{3+} toxicity. Biochim Biophys Acta **964:** 137–145
19. **Rincón M, Gonzales RA** (1991) Induction of protein synthesis by aluminum in wheat (*Triticum aestivum* L.) root tips. *In* RJ Wright, VC Baligar, RP Murrmann, eds, Plant-Soil Interactions at Low pH. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 851–858
20. **Rincón M, Hanson JB** (1986) Controls on calcium ion fluxes in injured or shocked corn root cells: importance of proton pumping and cell membrane potential. Physiol Plant **67:** 576–583
21. **Ryan PR, Kochian LV** (1991) Aluminum toxicity in wheat and maize: investigation of the spatial sensitivity along roots (abstract No. 754). Plant Physiol **96:** S–11
22. **Slaski JJ** (1990) Response of calmodulin-dependent and calmodulin-independent NAD kinase to aluminum in root tips from various cultivated plants. J Plant Physiol **136:** 40–44
23. **Suhayda CG, Haug A** (1986) Organic acids reduce aluminum toxicity in maize root membranes. Physiol Plant **68:** 189–195
24. **Taylor GJ** (1988) The physiology of aluminum phytotoxicity. *In* AH Sigel, A Sigel, eds, Metal Ions in Biological Systems, Vol 24. Marcel Dekker, New York, pp 123–163
25. **Taylor GJ** (1988) The physiology of aluminum tolerance. *In* AH Sigel, A Sigel, eds, Metal Ions in Biological Systems, Vol 24. Marcel Dekker, New York, pp 165–198
26. **Wagatsuma T** (1983) Effect of non-metabolic conditions on the uptake of aluminum by plant roots. Soil Sci Plant Nutr **29:** 323–333
27. **Wallace SU, Henning SJ, Anderson IC** (1982) Elongation, Al concentration, and hematoxylin staining of aluminum-treated wheat roots. Iowa State J Res **57:** 97–106
28. **Zhang G, Taylor GJ** (1988) Effect of aluminum on growth and distribution of aluminum in tolerant and sensitive cultivars of *Triticum aestivum* L. Commun Soil Sci Plant Anal **19:** 1195–1205
29. **Zhang G, Taylor GJ** (1989) Kinetics of aluminum uptake by excised roots of aluminum-tolerant and aluminum-sensitive cultivars of *Triticum aestivum* L. Plant Physiol **91:** 1094–1099
30. **Zhang G, Taylor GJ** (1990) Kinetics of aluminum uptake in *Triticum aestivum* L. Identity of the linear phase of aluminum uptake by excised roots of aluminum-tolerant and aluminum-sensitive cultivars. Plant Physiol **94:** 577–584
31. **Zhang G, Taylor GJ** (1991) Effects of biological inhibitors on kinetics of aluminum uptake by excised roots and purified cell wall material of aluminum-tolerant and aluminum-sensitive cultivars of *Triticum aestivum* L. J Plant Physiol **138:** 533–539