THE EFFECT OF CALCIUM (Ca^{2+}) ON ALUMINUM (Al^{3+}) PARTITIONING AND UPTAKE IN INTACT WHEAT (Triticum aestivum L.) ROOTS OF AL-SENSITIVE (TAM 105) AND AL-TOLERANT (ATLAS 66) CULTIVARS

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INTRODUCTION

Aluminum toxicity is the primary factor limiting crop productivity in acid soils, which comprise up to 40% of the world's arable land particularly in the tropics and subtropics (Kochian, 1995). Aluminum which is the most common element in soils, becomes toxic to plants when the pH drops below 5 (Foy et al., 1978). It has been known for many years that nutrient deficiency and metal toxicity in plants are reduced when Ca^{2+} is present in the soil (Foy et al., 1978).

Calcium plays a key role in plant growth and development and it is well established that Ca^{2+} is an intracellular second messenger. Ca^{2+} regulates an array of cellular processes by acting through Ca^{2+} modulated proteins and their targets. The regulatory actions of calcium range from control of ion transport to gene expression and are possible because of a homeostatic system that regulates Ca^{2+} levels in the cell (Bush, 1995).

Rincón and Gonzales (1992) and Delhaize et al. (1993a,b) have reported that the primary site of Al accumulation in wheat roots is the meristem which is also the primary site of Al toxicity (Ryan et al., 1994). Differential Al sensitivity correlates with differential Al accumulation in the growing root regions (Rincón and Gonzales, 1992; Samuels et al., 1996). Addition of cations to growth solutions ameliorates Al toxicity and Ca^{2+} and Mg^{2+} are the most effective ones (Kinraide et al., 1987). However, it is not clear how Ca^{2+} and Mg^{2+} alleviate Al-toxicity symptoms. Calcium may reduce Al-toxicity by decreasing Al accumulation in the root meristems.

The primary objective of this study was to explore whether Ca^{2+} affects the uptake and partitioning of Al in intact roots of Al-sensitive and Al-tolerant wheat cultivars.

LITERATURE REVIEW

Aluminum Toxicity

Aluminum (Al) is one of the most common metals in the lithosphere and its toxicity in plants significantly reduces crop productivity in acid soils (Foy et al., 1978; Kochian, 1995). Because there are many different ways that Al interacts with extracellular and intracellular structures, the specific mechanisms of Al-toxicity are not very well understood in higher plants. Presently, many different mechanisms of Al-toxicity have been proposed by different researchers.

In order to understand the toxic effects of Al on higher plants we need to have an understanding of the chemistry of Al. Al is not toxic to plants at pH values between 6 and 7 because it is in the form of insoluble aluminosilicates or oxides (Kochian, 1995). It becomes soluble and toxic in acidic solutions ($pH<5$), where it exists as the octahedral hexahydrate, $Al(H_2O)_6^{3+}$, usually called Al^{3+} (Martin, 1988). Kinraide and Parker (1989) reported that at acidic pH ($pH<5$) the predominant toxic Al species is triskaideaaluminum [$AlO_4Al_{12}(OH)_{24}(H_2O)_{12}^{7+}$], known also as Al_{13} (Kinraide, 1991).

Inhibition of root growth is the principal and most easily recognized symptom of Al-toxicity. However, the physiological effects of Al-toxicity are manifold (Foy et al.,1978; Taylor, 1988a; Kochian, 1995), and it is difficult to separate the primary effects from secondary symptoms. The most recognized symptoms of Al-toxicity are the effects of Al on membrane structure and function, DNA synthesis and mitosis, cell elongation, and mineral nutrition and metabolism (Taylor, 1988a). The root apex is the primary site for Al-toxicity (Rincón and Gonzales, 1992; Ryan et al., 1993). Ryan et al. (1993) reported that when the apical 2 to 3 mm of maize roots (root cap and meristem) was exposed to Al, inhibition of root growth was observed. On the other hand, when Al was applied to the more mature root region, root growth was unaffected. Ownby and Popham (1989) found that micromolar concentrations of Al in simple nutrient solution inhibited root growth within one hour.

It is unclear whether apoplastic or symplastic Al is responsible for root growth inhibition. Zhang and Taylor (1989; 1990) suggested that the primary site for Al-toxicity is the apoplast. Al found in the apoplast is believed to reside in the cell wall and/or mucigel of root tips (Zhang and Taylor, 1989; Horst, 1983; Lazof et al., 1994b). However, Tice et al. (1992) reported data indicating that one half of the total Al accumulated in the root apex was located in the symplasm after removing apoplastic Al by sequential 30 minutes washes using 5 mM $CaCl_2$ (pH 4.3). In recent studies using secondary-ion mass spectrophotometry, Lazof et al. (1994a) have shown that Al is present in the symplasm of soybean roots after only 30 minutes of exposure to Al.

The plasma membrane constitutes a barrier for the entry of Al into the cytoplasm because Al is insoluble in lipid bilayer (Martin, 1988; Delhaize and Ryan, 1995; Kochian, 1995). The question is, how does Al enter the symplasm? Akeson and Muns (1990) suggested that Al probably enters the plasma membrane via endocytosis because of the tight adsorption of Al to the plasma membrane surface. It has been suggested that in animal cells Al may enter the cytoplasm by sluggishly permeating divalent cation channels normally functioning in Mg^{2+} uptake (Martin, 1988; 1992). Martin (1988) also suggested that citrate might combine with Al, forming an Al-citrate complex that enters the cytoplasm through the plasma membrane.

Aluminum Tolerance

Tolerance to Al occurs naturally in cultivars of different crop plants (Foy et al., 1978; Taylor, 1988b). Kochian (1995) describes two kind of tolerance mechanisms which appear to be present in plants. First, exclusion of Al, which is seen in Al-resistant plants that prevent Al from entering the root symplasm. Second, internal Al-tolerance, in which Al enters the symplasm, but the cells "tolerate" Al and have ways to detoxify it (e.g. internal chelation, Al efflux). Kochian refers to plants in the second category as Al-tolerant plants because they tolerate Al in the symplasm. In our laboratory we do not use the same terminology and refer to all tolerant plants as Al-tolerant plants.

Rincón and Gonzales (1992) showed that after exposure to Al, the sensitive cultivar (cv) Tam 105 accumulated about 10 fold more Al in the root tips (terminal 2 mm of root) than the Al-tolerant cv Atlas 66, while the Al-tolerant cv Atlas 66 accumulated more Al in the more mature root regions (2-5 mm and 5-15 mm) than the Al-sensitive cultivar. In addition, Samuels et al. (1996) demonstrated that inhibition of root growth correlated with accumulation of Al in the root apex. Recent experimental evidence strongly indicates that Al-tolerant plants exclude Al from their root apices (Rincón and Gonzales, 1992; Delhaize et al., 1993a; Ryan et al., 1995). Al-tolerance in plants has been correlated with the ability to release organic acids (e.g. malate, citrate) and PO_4, in response to Al (Delhaize et al., 1993a,b; Pellet et al., 1995; Ryan et al., 1995, Pellet, 1996). Delhaize et al. (1993a) reported that the *Alt1* locus in wheat was responsible for an Al-inducible release of malic acid. Citric acid is released to the external medium by maize root tissue under Al stress (Pellet et al., 1995) and it is commonly used for desorption of Al from root cell walls after experimental Al treatment (Zhang and Taylor. 1989; Lazof et al., 1994b).

Aluminum Calcium Interaction

Taylor (1988a) and Kochian (1995) state that ion transport systems in the root plasma membrane (PM) might be involved in Al-toxicity mechanism. Delhaize and Ryan (1995) summarized Al/Ca interactions related to Al-toxicity as follows: (i) Al inhibits Ca^{2+} uptake; (ii) Al displaces Ca^{2+} from the apoplasm; and (iii) Al disrupts Ca^{2+} homeostasis in the cytoplasm.

Huang et al. (1992; 1993) reported that polyvalent cations such as La^{3+}, Ga^{3+}, and Gd^{3+} inhibited Ca^{2+} transport and that Al reduced Ca^{2+} uptake and translocation in plants. Reduction of Ca^{2+} uptake by Al was more pronounced in the Al-sensitive wheat lines than in Al-tolerant lines. Huang et al. (1992; 1994) suggested that Al-toxicity may be due to Al blockage of Ca^{2+} channels in the root plasma membrane. However, in a more recent study Ryan et al. (1994) found that low concentrations (2.64 μM $AlCl_3$) of Al could inhibit root growth without inhibiting Ca^{2+} uptake in an Al-sensitive wheat line, and addition of cations such as Na^+ and Mg^{2+} improved root growth while at the same time inhibiting Ca^{2+} uptake. In related studies using PM vesicles isolated from roots of Al-sensitive Scout 66 and Al-tolerant Atlas 66 wheat cultivars, Sasaki et al. (1994) and

Huang et al. (1996) found that Al blocked Ca^{2+} influx in both cultivars at the same rate.

Kinraide et al. (1992) proposed that Al might displace apoplasmic Ca^{2+} by reducing the negative potential difference on the plasma membrane surface. The idea of Ca^{2+} displacement from the apoplast is based on the rapid interaction between Al and Ca^{2+}. Increasing the activity of divalent cations, especially Ca^{2+} and Mg^{2+}, in the growing medium ameliorates Al-induced rhizotoxicity in wheat (Kinraide and Parker, 1987). The authors concluded that the competition between the divalent cations and Al for the external binding sites might be responsible for the ameliorative effect. However, Kinraide et al. (1994) found that many different cations (including protons and trivalent cations) alleviate Al stress by a mechanism that is independent of changes in ionic strength. In other words, addition of the ameliorating cations to an Al solution is more likely to further decrease the Ca^{2+} content of the apoplasm. Based on these results, Kinraide et al. (1994) concluded that the displacement hypothesis failed in explaining Al-toxicity.

Since Ca^{2+} is very important for metabolism, Al could disturb cellular metabolism by disrupting Ca^{2+} homeostasis developed from the known antagonism between Al and Ca^{2+} (Rengel, 1992a). It is believed that Al disrupts Ca^{2+}-dependent metabolism either by keeping high Ca^{2+} concentration levels in the cytoplasm or by preventing Ca^{2+} concentration changes in the cytoplasm from occurring altogether. For example, Rengel (1992a) showed a rapid increase in Ca^{2+} concentrations in the cytosol during callose synthesis, which is an Al-toxicity symptom (Foy et al., 1978), in roots.

Importance of Calcium for Plant Metabolism

It is well known that Ca^{2+} plays a vital role in metabolism of living forms. In plants, Ca^{2+} is involved in many cellular functions, such as keeping the ionic balance of cells, plant and organelle movement, gene expression, carbohydrate metabolism, mitosis and secretion (Bush, 1995).

Ca^{2+} enters the cytosol through specific calcium channels in the plasma membrane, which are protein pores whose existence in plant cells is now clearly established by patch clamp studies (Poovaiah and Reddy, 1993; Bush, 1995). Bush (1993) has shown that the resting (unstimulated) cytosolic Ca^{2+} level, measured by using a variety of techniques such as fluorescent dyes, Ca^{2+}-sensitive microelectrodes and photoproteins, is about 30-200 nM. These concentrations of free Ca^{2+} in the cytosol change rapidly in response to endogenous stimuli, such as hormones, as well as to exogenous stimuli, such as light, temperature, salt elicitors and touch (Bush, 1993;1995; Poovaiah and Reddy, 1993; and Trewavas and Knight, 1994). The cytosolic Ca^{2+} pool is in communication with the cell wall and internal storage pools such as the vacuole and endoplasmic reticulum (ER) which may contain up to 10-1000 mM Ca^{2+} (Trewavas and Knight, 1994).

Since Ca^{2+} is involved in signal transduction pathways in plants, the mechanism for regulating the cytosolic Ca^{2+} concentration has been extensively studied (Bush 1995). Influx of Ca^{2+} into the cytoplasm is energetically passive and is mediated by Ca^{2+} channels present in the plasma membrane (Kauss, 1987; Bush, 1995). Efflux of Ca^{2+} from the cytoplasm, however, to extracellular solutions or transport from the cytoplasm into intracellular organelles requires energy and it is catalyzed by membrane proteins that utilize direct ATP hydrolysis (Ca^{2+}-ATPase) or a proton motive force (H^+-ATPase) to drive Ca^{2+} movement (Bush, 1993;1995; and Trewavas and Knight, 1994).

MATERIALS AND METHODS

Plant Material

Aluminum sensitive cultivar (cv) Tam 105 (Texas Foundation Seed Stock, College Station, Texas), and Al-tolerant cultivars (cvs) Atlas 66 (Cargill Hybrid, Forth Collins, Colorado) and Tam 202 (Texas Foundation Seed Stock, College Station, Texas) were used in this study.

Seed Germination and Seedling Growth

The Al-tolerant cv Atlas 66 seeds were surface sterilized with 5% (w/v) commercial bleach and 0.1% (w/v) sodium dodecyl sulfate (SDS) for 5 minutes and rinsed three times with distilled water and twice with deionized water (18 Mohm-cm). The Al-tolerant cv Tam 202 and Al-sensitive cv Tam 105 were treated with the fungicide heptachlor by the distributor. Seeds were germinated on autoclaved paper towels saturated with 0.1 mM $CaCl_2$ in a glass tray and placed in the refrigerator ($3^{\circ}C \pm 1^{\circ}C$) overnight. Seeds were then transferred to a growth chamber (Lab Line, Illinois) and kept in the dark at $23^{\circ}C \pm 1^{\circ}C$ for three days. Germinated seeds were placed on floating Styrofoam boats and further grown hydroponically under a light/dark cycle of 16/8 hours using fluorescent lights (117 μmol photons m^{-2} s^{-1}) at room temperature for additional two days. The hydroponic nutrient solution (NS) was prepared as described by Rincon and Gonzales (1992) and consisted of 0.4 mM $CaCl_2$, 0.65 mM KNO_3, 0.25 mM $MgCl_2$, 0.08 mM NH_4NO_3 and its pH was adjusted to 4.2 using 5.0 N HCl.

Aluminum Treatment

Six-day old seedlings were placed in 2 L of aerated nutrient solution with and without 0.4 mM $CaCl_2$ plus 10 μM $AlCl_3$ for Al-sensitive cv Tam 105 and 50 μM $AlCl_3$ for Al-tolerant cvs Atlas 66 and Tam 202 at room temperature for different periods of time. After Al treatment, the seedlings were briefly rinsed with deionized water and transferred to aerated ice cold citric acid (pH 4.5) for 30 minutes to remove the loosely bound Al from the roots. The roots were then excised in consecutive segments measured from the root apex, including the cap (in mm): 0 to 2, 2 to 5, and 5 to 15. The root segments were collected in polypropylene microcentrifuge tubes (Fisher) and dried in an oven for two days at 65°C ± 5°C.

Aluminum Determination

The content of Al in the tissue was determined as outlined after Samuels (1996). The dry root tissue was digested in 70% HNO_3:30% H_2O_2 (1:1) at 65°C for 30 minutes. Al content was determined by specific ion chromatography using a full control software/interface Dionex DX 500 HPLC, which was equipped with a GS2 guard column and CS2 cation exchange column. Al was eluted isocratically using 0.2 M $(NH_4)_2SO_4$ (Baker Analyzed, Pittsburgh, New Jersey) dissolved in 0.01 M H_2SO_4 (Baker Instra-Analyzed, Pittsburgh, New Jersey). Detection of Al was performed by post-column reaction using 3.0 M $(NH_4)C_2H_3O_2$ (Baker Analyzed, Pittsburgh, New Jersey; pH 6.2 adjusted with glacial acetic acid) and 0.3 mM Tiron (4,5-dihydroxy-1,3-benzenedisulfonic acid, disodium salt; Sigma, St. Louis, Missouri), which forms a complex with Al that absorbs UV light at a wavelength of 310 nm.

RESULTS

Figure 1 illustrates the Al partitioning pattern, Al distribution along the roots, in the sensitive cv Tam 105 in the presence and absence of Ca^{2+}. Intact roots were exposed to 10 μM $AlCl_3$ with or without 0.4 mM $CaCl_2$ for 6 hours at room temperature. Al partitioning pattern was the same in both the presence and absence of Ca^{2+}. However, in the absence of Ca^{2+}, Al content when compared to the presence of Ca^{2+} in 0-2, 2-5, 5-15 mm root sections increased by 102, 69, 76 %, respectively. Figure 2 illustrates the Al partitioning pattern in the tolerant cv Atlas 66 in the presence and absence of Ca^{2+}. Intact roots were exposed to 50 μM $AlCl_3$ with or without 0.4 mM $CaCl_2$ for 6 hours. In the presence of Ca^{2+}, Al accumulation was higher in the more mature root regions than in the apical root region. In the absence of Ca^{2+}, however, Al accumulation was higher in the root tip as it was in the sensitive cv Tam 105 (Figure 1). In the absence of Ca^{2+}, Al content in 0-2, 2-5, and 5-15 mm root sections increased by 1330, 248, and, 103 %, respectively. Figure 3 shows the Al partitioning pattern in the tolerant cv Tam 202 in the presence and absence of Ca^{2+}. Intact roots were treated as described for Atlas 66. Tam 202 Al partitioning pattern was similar to that of Atlas 66 (Figure 2) in both the absence and presence of Ca^{2+}. However, the effect of the lack of Ca^{2+} in the treatment solution was less than in Atlas 66. The increase of Al content in the 0-2, 2-5, and 5-15 mm root sections increased by 281, 10, and, 65 %, respectively.

Figure 4 shows the time course of Al accumulation in the presence and absence of Ca^{2+} in the sensitive cv Tam 105. The intact roots were exposed to 10 μM $AlCl_3$ with or without 0.4 mM $CaCl_2$ for 1, 3, and 6 hours at room temperature. Both in the presence (Fig. 4A) and in the absence (Fig. 4B) of Ca^{2+} Al partitioning pattern was the same but in the absence of Ca^{2+} Al accumulation increased in all three root sections with time. In the absence of Ca^{2+}, Al content in 0-2, 2-5, and 5-15 mm root sections increased by 88, 417, and 51 % after 1 hour, 136, 224, and 43% after 3 hours, and 127, 152, and 38 % after 6 hours, respectively.

Figure 5 shows the time course of Al accumulation in the presence (Fig. 5A) and absence (Fig. 5B) of Ca^{2+} in the tolerant cv Atlas 66. Atlas 66 intact roots were exposed to 50 μM $AlCl_3$ with or without 0.4 mM $CaCl_2$ for 1, 3,and 6 hours. In the absence of Ca^{2+}, Al partitioning pattern in Atlas 66 started to change within 3 hours and resembled the Al accumulation pattern of the sensitive cv Tam 105 (Figure 4). In the absence of Ca^{2+}, Al content in 0-2, 2-5, and 5-15 mm root sections increased by 180, 73, and 70% after 1 hour, 1299, 230, and 57% after 3 hours, and 3674, 313, and 66% after 6 hours, respectively.

Figure 6 illustrates the effect of La^{3+} on Al accumulation pattern of the sensitive cv Tam 105 in the presence and absence of Ca^{2+}. Intact roots were submerged in aerated NS containing 10 μM $AlCl_3$ and 10 μM $LaCl_3$ in the presence or absence of 0.4 mM $CaCl_2$ for 3 hours. Although La^{3+} did not change the Al partitioning pattern, it did slightly suppress Al accumulation both in the presence and absence of Ca^{2+}. However, the effect of La^{3+} on cv Tam 105 was not as pronounced as it was in the Al-tolerant cv Atlas 66 (Figure 7). In the presence of Ca^{2+}, La^{3+} did not affect the Al accumulation in 0-2 mm root section while it decreased Al content in 2-5, and 5-15 mm root sections by 5 and 14 %, respectively. In the absence of Ca^{2+}, Al content in 0-2, 2-5, and 5-15 mm root sections decreased by 17, 16, and 14 %, respectively. Figure 7 shows the effect of La^{3+} on the Al accumulation pattern of the tolerant cv Atlas 66 in the presence and absence of Ca^{2+}. Intact roots were submerged in aerated NS containing 50 μM $AlCl_3$ and 50 μM $LaCl_3$ in the presence or absence of 0.4 mM $CaCl_2$ for 3 hours. Although La^{3+} did not change the Al partitioning pattern, it did suppress Al accumulation in both the presence and absence of Ca^{2+}. In the presence of Ca^{2+}, Al content in 0-2, 2-5, and 5-15 mm root sections decreased by 46, 45, and, 30 % and in the absence of Ca^{2+}, by 14, 24, and 23 %, respectively.

Figure 8 shows the effect of different concentrations of Ca^{2+} on the Al accumulation in the sensitive cv Tam 105 intact roots. The roots were treated with various concentrations of $CaCl_2$ (400, 600, 800, 1000, and 1500 μM) plus 10 μM $AlCl_3$ at room temperature for 3 hours. Increase of Ca^{2+} concentration in the treatment solution decreased Al accumulation in all three root segments. Al content decrease was more pronounced in the meristematic (0-2 mm) and mature (5-15 mm) root regions while it was less in the elongation (2-5 mm) region. When compared to the control (400 μM), 600 μM Ca^{2+} caused a reduction in Al accumulation in the 0-2, 2-5, and 5-15 mm root sections by 38, 10, and 24 %, at 800 μM Ca^{2+} by 46, 14, and 31 %, at 1000 μM Ca^{2+} by 58, 14, and 32 % and at 1500 μM Ca^{2+} by 70, 36, and 38 %, respectively. Figure 9 shows the effect of different concentrations of Ca^{2+} on the Al accumulation of tolerant cv Atlas 66. Intact roots were treated with various concentrations of $CaCl_2$ (0, 50, 100, 200, and 300 μM) plus 50 μM $AlCl_3$ at room temperature for 3 hours. Concentration of Ca^{2+} as low as 50 μM inhibited Al accumulation in the roots and changed Al partitioning pattern from sensitive cv pattern (at 0 μM) to tolerant cv pattern (Fig. 1 & 2). The effect of increasing concentrations of Ca^{2+} on Al accumulation was more pronounced in the 0-2 mm root region. When compared to the control (0 μM), 50 μM Ca^{2+} caused a reduction in Al accumulation in the 0-2, 2-5, and 5-15 mm root sections by 68, 34, and 4 %, at 100 μM Ca^{2+} by 77, 43, and 21 %, at 200 μM Ca^{2+} by 85, 62, and 31 % and at 300 μM Ca^{2+} by 87, 71, and 46 %, respectively.

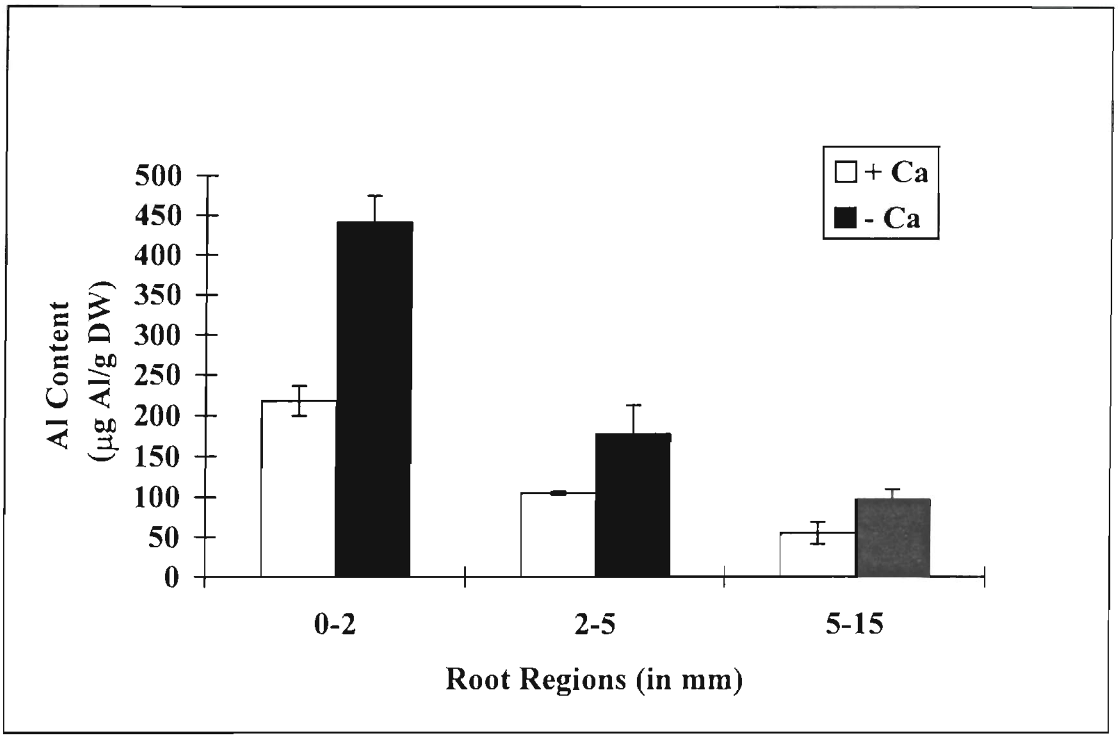

FIGURE 1.--Aluminum partitioning pattern in Al-sensitive cv Tam 105 exposed to 10 µM $AlCl_3$ for 6 hours in the presence (□) and absence (■) of 0.4 mM $CaCl_2$ in the treatment solution. Al was not detected in any of the root regions of control treatment (-Al, +0.4 mM $CaCl_2$). Mean ± SD from two separate experiments.

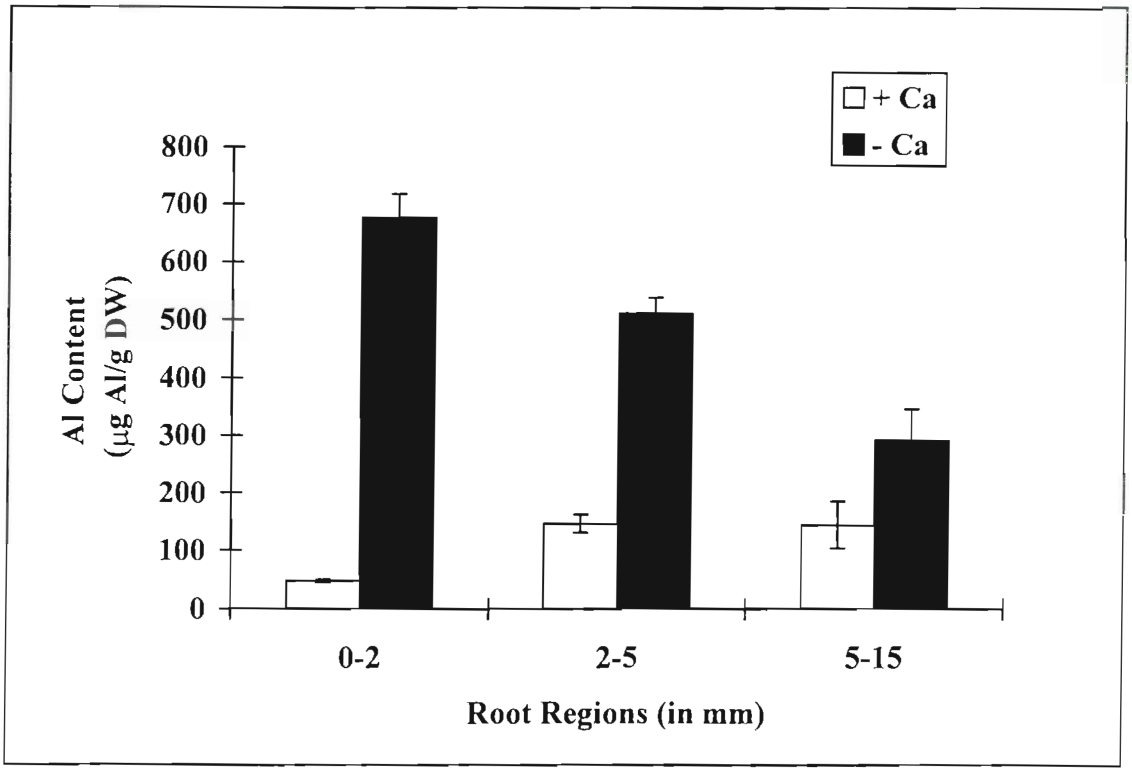

FIGURE 2.--Aluminum partitioning pattern in Al-tolerant cv Atlas 66 exposed to 50 µM $AlCl_3$ for 6 hours in the presence (□) and absence (■) of 0.4 mM $CaCl_2$ in the treatment solution. Al was not detected in any of the root regions of control treatment (-Al; +0.4 mM $CaCl_2$). Mean ± SD from two separate experiments.

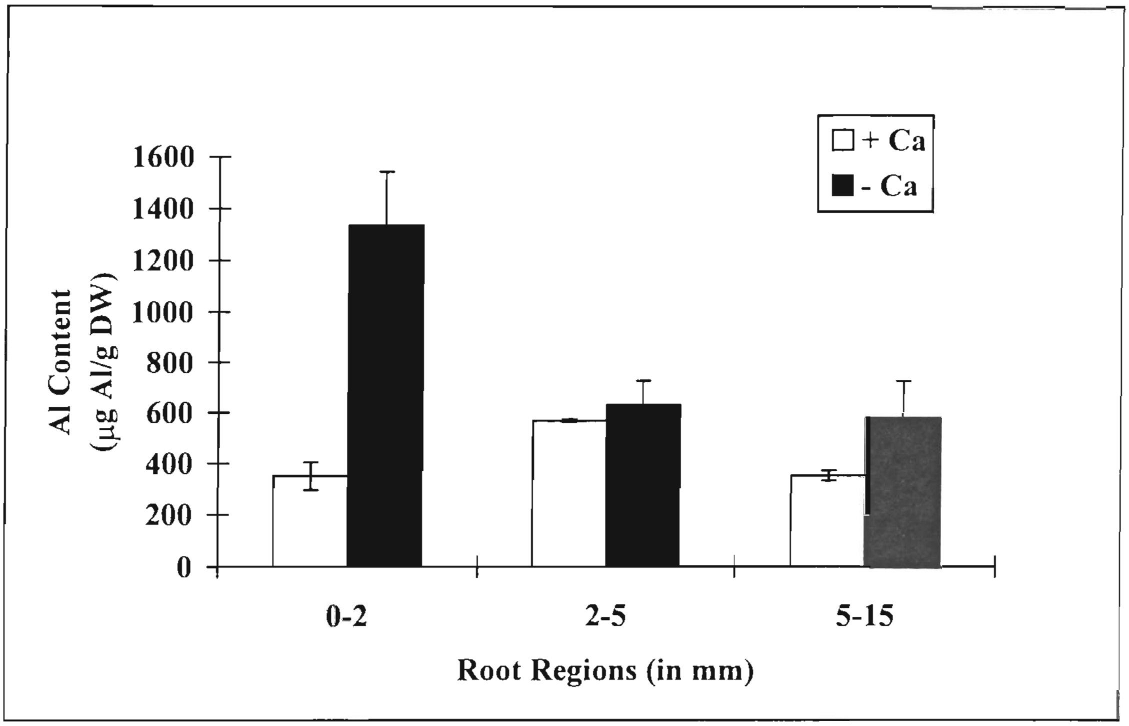

FIGURE 3.--Aluminum partitioning pattern in Al-tolerant cv Tam 202 exposed to 50 µM $AlCl_3$ for 6 hours in the presence (□) and absence (■) of 0.4 mM $CaCl_2$ in the treatment solution. Al was not detected in any of the root regions of control treatment (-Al, + 0.4 mM $CaCl_2$). Mean ± SD from two separate experiments.

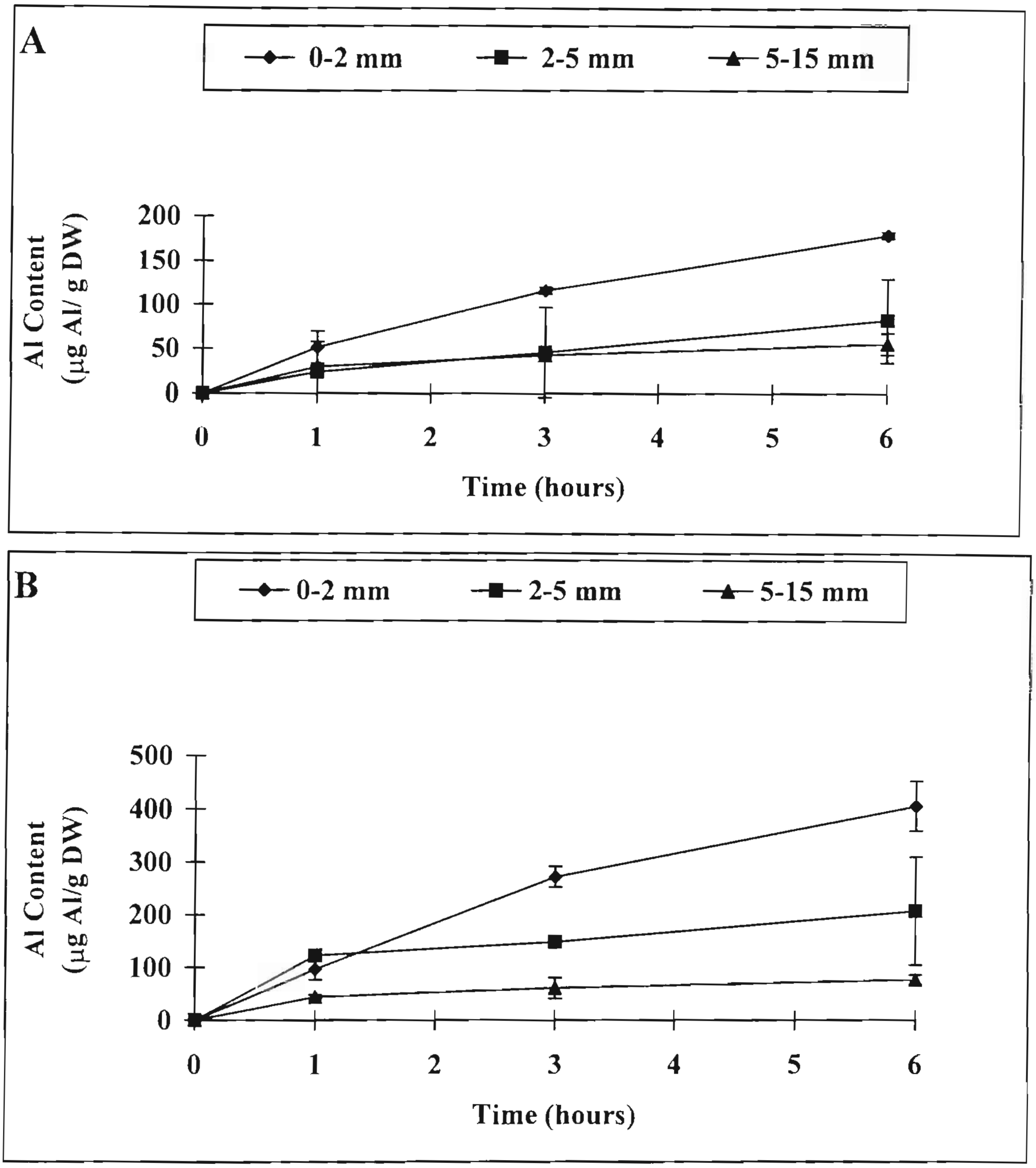

FIGURE 4.--Time course of Al accumulation in Al-sensitive cv Tam 105 exposed to 10 μM $AlCl_3$ in the presence (Fig. 4A) and absence (Fig. 4B) of 0.4 mM $CaCl_2$ in the treatment solution. Al was not detected in any of the root regions of control treatment (-Al; + 0.4 mM $CaCl_2$). Mean ± SD from two separate experiments.

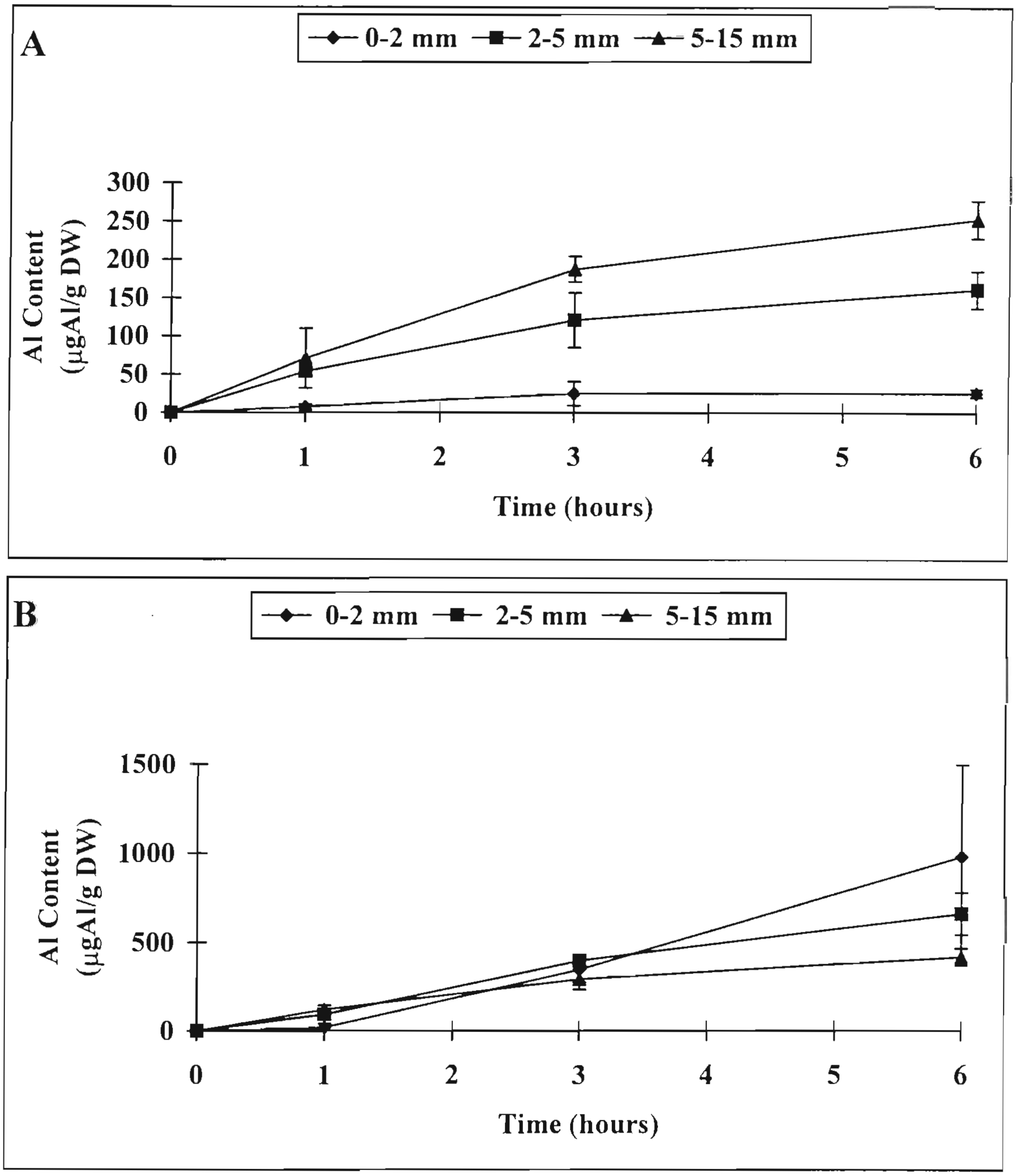

FIGURE 5.--Time course of Al accumulation in Al-tolerant cv Atlas 66 exposed to 50 µM $AlCl_3$ in the presence (Fig.5A) and absence (Fig. 5B) of 0.4 mM $CaCl_2$ in the treatment solution. Al was not detected in any of the root regions of control treatment (-Al; + 0.4 mM $CaCl_2$). Mean $\pm$ SD from two separate experiments.

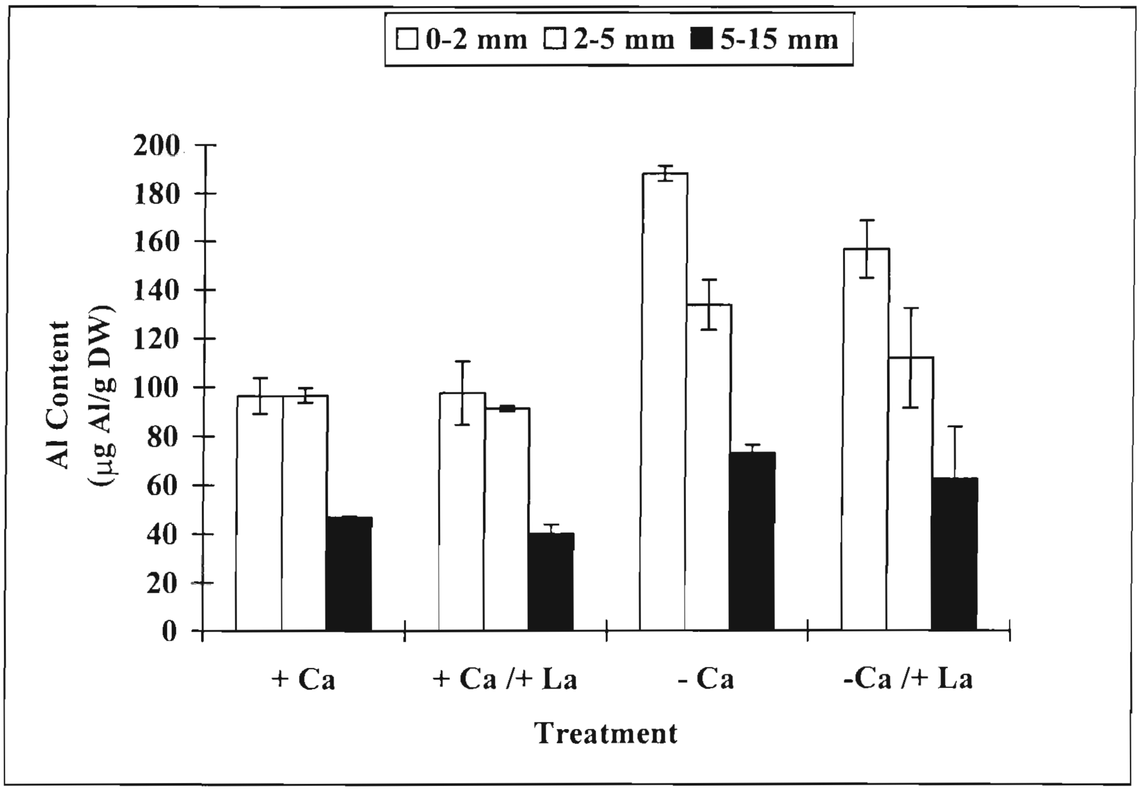

FIGURE 6.--The effect of La^{3+} on Al accumulation pattern of the Al-sensitive cv Tam 105 exposed to 10 μM $AlCl_3$ and 10 μM $LaCl_3$ in the presence (+Ca) and absence (-Ca) of 0.4 mM $CaCl_2$ for 3 hours. Al was not detected in any of the root regions of control treatment (-Al; + 0.4 mM $CaCl_2$). Mean ± SD from two separate experiments.

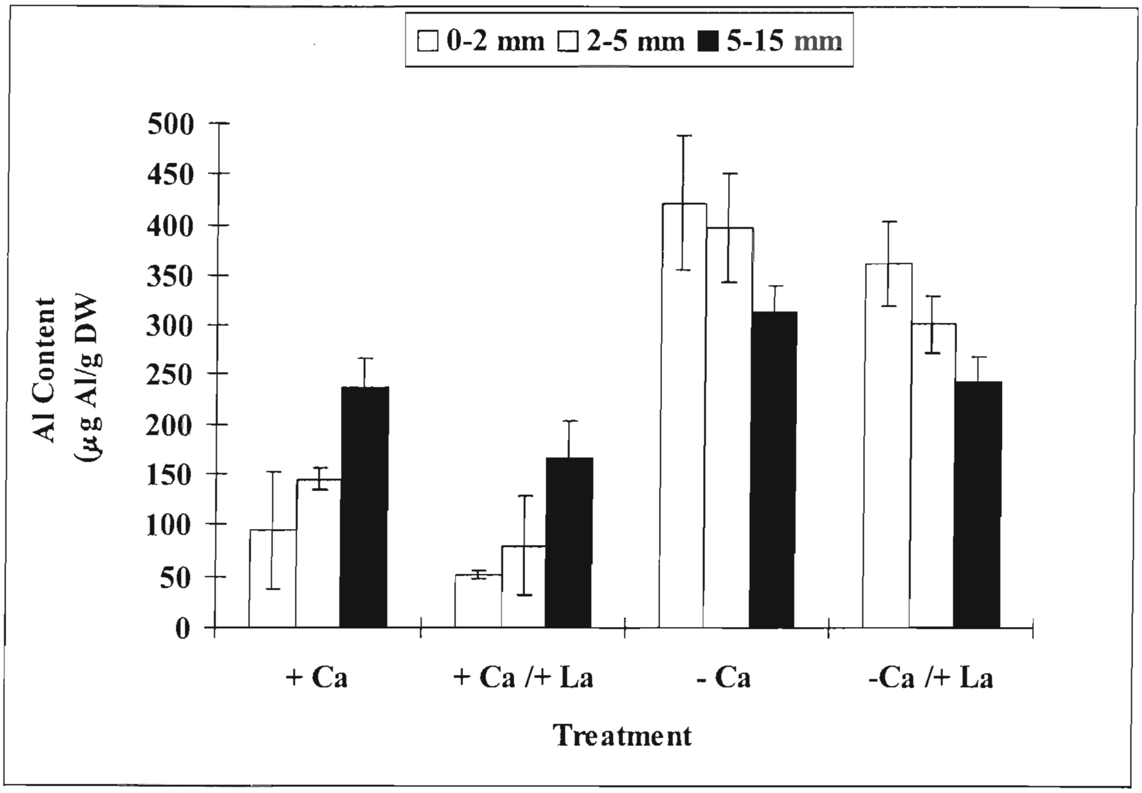

FIGURE 7.--The effect of La^{3+} on Al accumulation pattern of the Al-tolerant cv Atlas 66 exposed to 50 μM $AlCl_3$ and 50 μM $LaCl_3$ in the presence (+Ca) and absence (-Ca) of 0.4 mM $CaCl_2$ for 3 hours. Al was not detected in any of the root regions of control treatment (-Al; + 0.4 mM $CaCl_2$). Mean ± SD from two separate experiments.

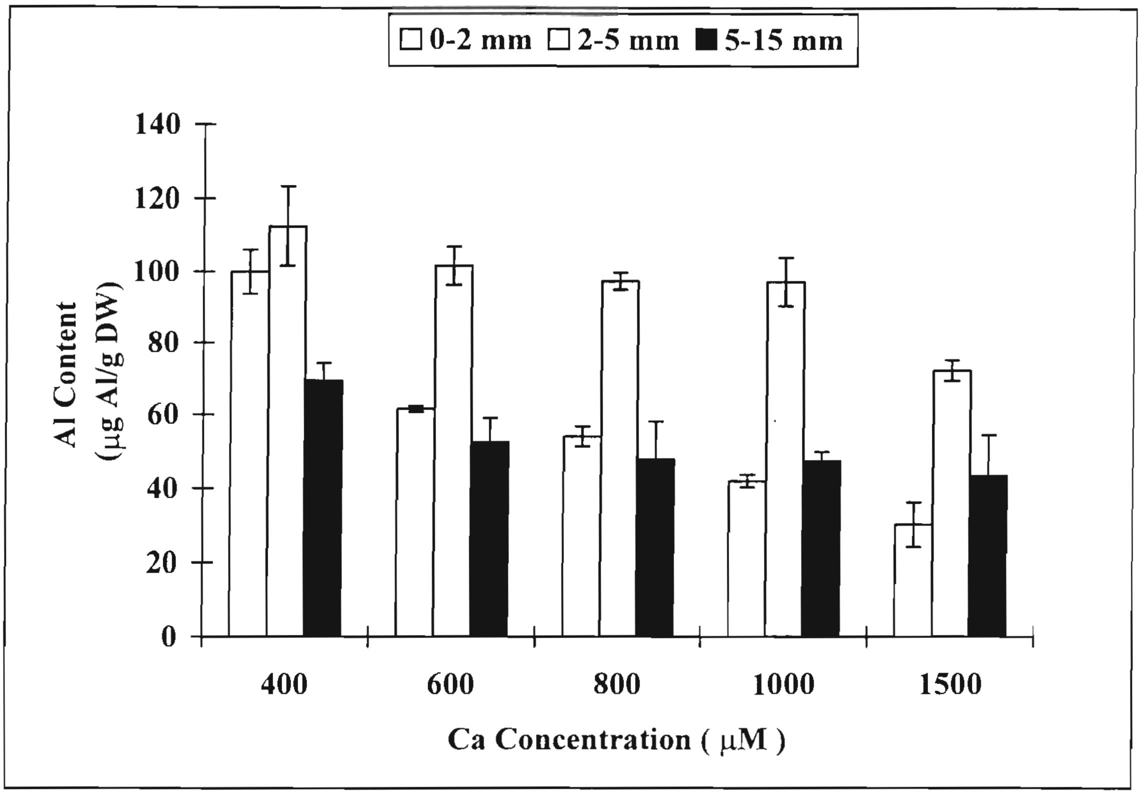

FIGURE 8.--The effect of different concentrations of Ca^{2+} on Al accumulation pattern of the Al-sensitive cv Tam 105. Intact roots of Al-sensitive cv Tam 105 were treated with 10 µM $AlCl_3$in the presence of various concentrations of Ca^{2+} for 3 hours at room temperature. Al was not detected in any of the root regions of control treatment (-Al; + 0.4 mM $CaCl_2$). Mean $\pm$ SD from two separate experiments.

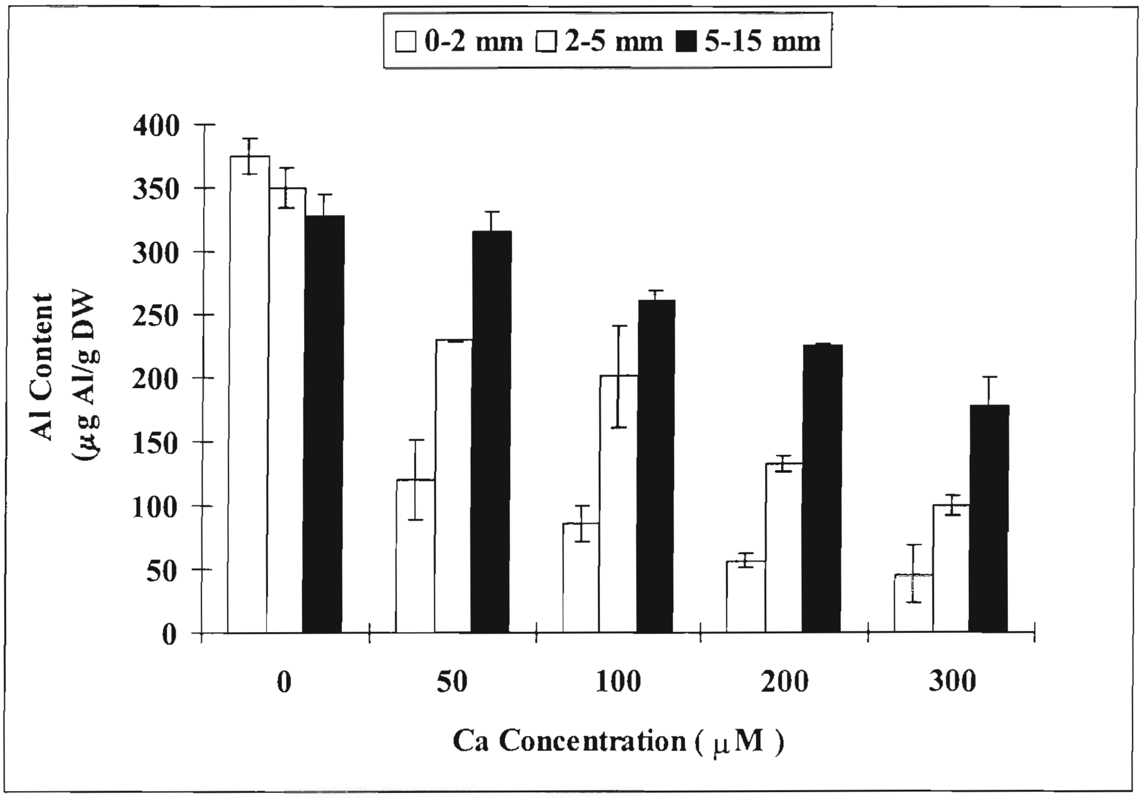

FIGURE 9.--The effect of different concentrations of Ca on Al accumulation pattern of the Al-tolerant cv Atlas 66. Intact roots of Al-tolerant cv Atlas 66 were treated with 50 µM $AlCl_3$ in the presence of various concentrations of Ca^{2+} for 3 hours at room temperature. Al was not detected in any of the root regions of control treatment (-Al; + 0.4 mM $CaCl_2$). Mean ± SD from two separate experiments.

DISCUSSION

The results of this study support the findings of Rincón and Gonzales (1992) and Samuels et al. (1996) which indicate that the Al-sensitive cv Tam 105 accumulated more Al in the root apex (0-2 mm) than the Al-tolerant cv Atlas 66, while the Al-tolerant cv Atlas 66 accumulated more Al in the more mature root regions (2-5 mm and 5-15 mm) than the Al-sensitive cultivar. Furthermore, I found that Ca^{2+}affects Al accumulation and partitioning in intact wheat roots. Lack of Ca^{2+} resulted in an increased Al uptake in both tolerant and sensitive cvs (Figure 1, 2 and 3). An interesting observation was that in the tolerant cvs Atlas 66 and Tam 202, lack of Ca^{2+} changed the "normal" Al distribution along the roots (Figure 2 and 3). The pattern resembled that of the sensitive cvs Tam 105 (Figure 1) and Scout 66 (Samuels et al., 1996) and changed in approximately 3 hours. In terms of Al partitioning pattern, the tolerant cultivar's requirement for Ca^{2+} was very low (50 μM; Figure 9) while the sensitive cv did not change its partitioning pattern even when the Ca^{2+} concentration was as high as 1500 μM (Figure 8). However, in terms of Al accumulation, the sensitive cultivar was more sensitive to Ca^{2+} because increasing concentrations of Ca^{2+} inhibited Al accumulation which may be an indicator that the sensitive cv has a higher Ca^{2+} requirement for alleviating Al-toxicity.

In the presence of Ca^{2+}, Al content was higher in all three root sections of the sensitive cv Tam 105 than it was in the tolerant cultivar (Figures 1, 2). Lower Al content in the growing root region of the tolerant cultivars may be due to an Al-induced mechanism that prevents Al accumulation in the roots. Delhaize et al. (1993b) and Ryan et al. (1995) investigated the role of organic acids in the mechanisms of Al tolerance in near-isogenic wheat lines and found that in the presence of Al, Al-tolerant wheat lines excreted 5- to 10-fold more malate than did the Al-sensitive wheat lines. They also found that the root apex was the primary site of the malic acid exudation. Secreted malate can function as a chelator and detoxifier of Al (Kochian, 1995). However, it is not clear how malate is secreted from the roots. In higher plants, anion channels have recently been suggested to play key roles in controlling cellular functions including, turgor- and osmoregulation, stomatal movements, anion transport, signal transduction, and possibly also signal propagation (Tyerman, 1992; Schroeder, 1995). Schroeder (1995) reported that opening of anion channels in the guard cell plasma membrane is stimulated by increases in cytoplasmic Ca^{2+} concentrations and that the anion channels allow malate efflux which implicates anion channels as a possible pathway for controlling and mediating malate secretion. However, the major type of anion channels in roots allows mainly influx and the question remains whether root anion channels can also permit malate efflux as in the guard cells (Schroeder, 1995). Increased Al content in the root may be due to the interference of malate efflux because Ca^{2+} was not present in the external solution, which in turn may have prevented the root apical cell anion channels from opening and excreting malate .

It is well known that the role of Ca^{2+} as a second messenger in the transduction of environmental signal depends on the maintenance of cytosolic free $[Ca^{2+}]$ at low levels (Bush, 1995). Rengel (1992b) reasoned that the internal Ca^{2+} stores in the root apex might be inadequate for the normal Ca^{2+} signalling when influx of Ca^{2+} is blocked by Al.

The time course experiment of Al-tolerant cv Atlas 66 (Figure 5) supports Rengel's idea in that the absence of Ca^{2+}, the accumulation pattern changed within 3 hours and it resembled the Al accumulation pattern of Al-sensitive cv Tam 105 which may be due to the internal Ca^{2+} stores depletion and disruption of the Ca^{2+} signalling pathway. The results do not support the idea that Al blockage of Ca^{2+} could prevent Ca^{2+} influx because in the presence of Ca^{2+} Al partitioning pattern did not change its pattern (Figure 5). However, In the presence of La^{3+}, which is a Ca^{2+} channel blocker, I expected that the tolerant cv would change its partitioning pattern (Figure 6 and 7). La^{3+} did not change the partitioning pattern but suppressed Al accumulation in both cultivars. Sasaki et al. (1994) compared the effect of Al on Ca^{2+} influx through putative Ca^{2+} channels of plasma membrane vesicles isolated from two wheat cultivars differing in Al-sensitivity and found no differences in the effects of the treatments between the sensitive and tolerant wheat cultivars, Scout 66 and Atlas 66, respectively. Sasaki et al. also reported that 20 μM La^{3+} blocked 90 % of Ca^{2+} influx, which may be the reason why La^{3+} in the external solution did not change the Al partitioning pattern in Atlas 66 (Figure 7). Increasing concentrations of Ca^{2+} suppressed Al accumulation in both wheat lines (Figures 8 and 9) which may be due to the amelioration of Al-toxicity by Ca^{2+} (Kinraide and Parker, 1987). External Ca^{2+} concentration as low as 50 μM was enough to prevent Al-tolerant cv Atlas 66 (Figure 9) from resembling the sensitive cultivar's partitioning pattern.

In conclusion, the data reported here clearly demonstrate that Ca^{2+} affects Al partitioning and uptake in intact wheat roots of both Al-sensitive and Al-tolerant cultivars. Research is needed for further clarifying the effect of Ca^{2+} on Al partitioning and uptake; for showing the effect of Ca^{2+} on growth and for finding the minimal Ca^{2+} concentration that maintains the “normal” tolerant partitioning pattern in Atlas 66.

LITERATURE CITED

Akeson, M. A., and D. N. Munns. 1990. Uptake of aluminum into root cytoplasm: predicted rates for important solution complexes, J. Plant Nutr., 13:467-484.

Bush, D. S. 1993. Regulation of cytosolic calcium in plants, Plant Physiol., 103:7-13.

_____ 1995. Calcium regulation in plant cells and its role in signaling, Annu. Rev. Plant Physiol. Plant Mol. Biol., 46:95-122.

Delhaize E., S. Craig, C. D. Beaton, R. H. Bennet, V. C. Jagadish, and P. J. Randall. 1993a. Aluminum tolerance in wheat (Triticum aestivum L.). I. Uptake and distribution of aluminum in root apices, Plant Physiol., 103:685-693.

Delhaize E., P. R. Ryan, and P. J. Randall. 1993b. Aluminum tolerance in wheat (Triticum aestivum L.). II. Aluminum-stimulated excretion of malic acid from root apices, Plant Physiol., 103:695-702.

Delhaize E., and P. R. Ryan. 1995. Aluminum toxicity and tolerance in plants, Plant Physiol., 107:315-321.

Foy, C. D., R. L. Chaney, M. C. White. 1978. The physiology of metal toxicity in plants, Annu. Rev. Plant Physiol., 29:511-66.

Horst, W. J., A. Wagner, and H. Marschner. 1982. Mucilage protects root meristems from aluminum injury, Z. Pflanzenphysiol., 105:435-444.

Huang, J. W., D. L. Grunes, and L. V. Kochian. 1992. Aluminum effects on the kinetics of calcium uptake into cells of the wheat root apex. Quantification of calcium fluxes using a calcium-selective vibrating microelectrode, Planta, 188:414-421.

_____ 1993. Aluminum effects on calcium ($^{45}Ca^{2+}$) translocation in aluminum-tolerant and aluminum-sensitive wheat (Triticum aestivum L.) cultivars, Plant Physiol., 102:85-93.

_____ 1994. Voltage-dependent Ca^{2+} influx into right-side-out plasma membrane vesicles isolated from wheat roots: Characterization of putative Ca^{2+} channel, Proc. Natl. Acad. Sci. USA, 91: 3473-3477.

Huang J. W., D. M. Pellet, L. A. Papernik, and L. V. Kochian. 1996. Aluminum interactions with voltage-dependent calcium transport in plasma membrane vesicles isolated from roots of aluminum-sensitive and -resistant wheat cultivars, Plant Physiol., 110:561-569.

Kauss, H. 1987. Some aspects of calcium-dependent regulation in plant metabolism, Annu. Rev. Plant Physiol., 38:47-72.

Kinraide T. B. 1991. Identity of the rhizotoxic aluminum species, Plant and Soil, 134:167-178.

Kinraide T. B., and D. R. Parker. 1987.Cation amelioration of aluminum toxicity in wheat, Plant Physiol., 83:546-551.

_____ 1989. Assessing the phytotoxicity of mononuclear hydroxy-aluminum, Plant Cell and Environ., 12:479-487.

Kinraide T. B., P. R. Ryan, and L. V. Kochian. 1992. Interactive effects of Al^{3+}, H^{+} and other cations on root elongation considered in terms of cell-surface electrical potential, Plant Physiol., 99:1461-14628.

_____ 1994. Al^{3+}-Ca^{2+} interactions in aluminum rhizotoxicity. II. Evaluating the Ca^{2+}-displacement hypothesis. Planta, 192:104-109.

Kochian L. V. 1995. Cellular mechanisms of aluminum toxicity and resistance in plants, Annu. Rev. Plant Physiol. Plant Mol. Biol., 46:237-260.

Lazof D. B. , J. G. Goldsmith, T. W. Rufty, and R. W. Linton. 1994a. Rapid uptake of aluminum into cells of intact soybean root tips. A microanalytical study using secondary ion mass spectrometry, Plant Physiol., 106:1107-1114.

Lazof D. B., M. Rincón, T. W. Rufty, C. T. Mackown, and T. E. Carter. 1994b. Aluminum accumulation and associated effects on $^{15}NO_3^-$ influx of two soybean genotypes differing in Al tolerance, Plant and Soil, 164:291-297.

Martin, R. B. 1988. Bioinorganic chemistry of aluminum. In H. Sigel, A. Sigel, eds, Metal Ions in Biologycal Systems: Aluminum and its Role in Biology, Vol 24. Marcel Dekker, New York, pp 2-57.

_____ 1992. Aluminum speciation in biology. In D. J. Chadwick, J. Whealan, eds, Aluminum in Biology and Medicine, New York: Wiley, pp 5-25.

Ownby J. D., and H. R. Popham. 1989. Citrate reverses the inhibition of wheat root growth caused by aluminum, J. Plant Physiol., 135:588-591.

Pellet, D. M., D. L. Grunes, L. V. Kochian. 1995. Organic acid exudation as an aluminum-tolerance mechanism in maize (Zea mays L.), Planta, 196:788-795.

Pellet, D. M., L. A. Papernik, and L. V. Kochian. 1996. Multiple aluminum-resistance mechanisms in wheat. Roles of root apical phosphate and malate exudation, Plant Physiol., 112:591-597.

Poovaiah, B. W., A. S. N. Reddy. 1993. Calcium and signal transduction in plants, Critical Review in Plant Sciences, 12:185-211.

Rengel Z. 1992a. Role of calcium in aluminum toxicity, New Phytol., 121:499-513.

_____ 1992b. Disturbance of cell Ca^{2+} homeostasis as a primary trigger of Al toxicity syndrome, Plant, Cell and Environment, 15:931-938.

Rincón M., and R. A. Gonzales. 1992. Aluminum partitioning in intact roots of aluminum-tolerant and aluminum-sensitive wheat (Triticum aestivum L.) cultivars, Plant Physiol., 99:1021-1028.

Ryan P. R., E. Delhaize, and P. J. Randall. 1995. Characterization of Al-stimulated efflux of malate from the apices of Al-tolerant wheat roots, Planta, 196:103-110.

Ryan, P. R., J. M. DiTomaso, and L. V. Kochian. 1993. Aluminum toxicity in roots: An investigation of spatial sensitivity and the role of the root cap, J. Exp. Bot., 44:437-446.

Ryan P. R., T. B. Kinraide, L. V. Kochian. 1994. Al^{3+}-Ca^{2+} interactions in aluminum rhizotoxicity. I. Inhibition of root growth is not caused by reduction of calcium uptake, Planta, 192:98-103.

Samuels, T. D. Aluminum partitioning patterns as related to aluminum sensitivity and aluminum tolerance in wheat (Triticum aestivum) roots. Wichita Falls, Texas: Midwestern State University; 1996. Thesis. 1-57.

Samuels, T. D., K. Küçükakyüz, and M. Rincón-Zachary. 1996. Aluminum partitioning patterns as related to aluminum sensitivity and aluminum tolerance in wheat (Triticum aestivum) roots. Plant Physiol., In Press.

Sasaki M., Y. Yamamoto, H. Matsumoto. 1994. Putative Ca^{2+} channels of plasma membrane vesicles are not invelvod in the tolerance mechanism of aluminum in aluminum-tolerant wheat (Triticum aestivum L.) cultivar. Soil Sci. Plant Nutr., 40:709-714.

Schroeder, J. I. 1995. Anion channels as central mechanisms for signal transduction in guard cells and putative functions in roots for plant-soil interactions. Plant Molecular Biplogy, 38:353-361.

Taylor, G. J. 1988a. The physiology of aluminum phytotoxicity. In H. Sigel, A. Sigel, eds, Metal Ions in Biologycal Systems: Aluminum and its Role in Biology, Vol 24. Marcel Dekker, New York, pp 123-163.

_____ 1988b. The physiology of aluminum tolerance. In H. Sigel, A. Sigel, eds, Metal Ions in Biologycal Systems: Aluminum and its Role in Biology, Vol 24. Marcel Dekker, New York, pp 165-198.

Tice K. R., D. R. Parker, and D. A. DeMason. 1992. Operationally defined apoplastic and symplastic aluminum fractions in root tips of aluminum-intoxicated wheat, Plant Physiol., 100:309-318.

Trewavas, A., and M. Knight. 1994. Mechanical signalling, calcium and plant form, Plant Molecular Biology, 26:1329-1341.

Tyerman, S. D. 1992. Anion channels in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol., 43:351-371.

Zhang G., and G. J. Taylor. 1989. Kinetics of aluminum uptake by excised roots of aluminum-tolerant and aluminum-sensitive cultivars of Triticum aestivum L., Plant Physiol., 91:1094-1099.

_____ 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.

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"The Effect of Calcium (Ca^{2+}) on Aluminum (Al^{3+}) Partitioning and Uptake in Intact Wheat (Triticum aestivum L.) Roots of Al-sensitive (Tam 105) and Al-toleranct (Atlas 66) Cultivars", Poster Presentation at the American Society of Plant Physiologists Convention in San Antonio, Texas (1996)

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Systematics of Seedy Plants
Molecular Biology
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Systematics of Vertebrata
Palinology
Genetics I-II
Physiology I-II

Plant Embryology
Economic Botany
Plant Soil Interaction
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Samuels, T.D., K. Küçükakyüz, and M. Rincon-Zachary. 1996. Differential Aluminum Sensitivity in Wheat (Triticum aestivum L.) as Related to Differential Aluminum Accumulation in the Root Meristems, Plant Physiol. (In Press).

ABSTRACT

In this study, I investigated the effect of calcium (Ca^{2+}) on aluminum (Al) partitioning and accumulation in intact roots of the Al-tolerant cvs Atlas 66 and Tam 202 and the Al-sensitive cv Tam 105. Six day old seedlings were treated with $AlCl_3$ in the presence and absence of Ca^{2+}. After treatments the intact roots were cut in 0-2 mm, 2-5 mm, 5-15 mm sections starting from the root apex. The results showed that in the absence of Ca^{2+} both tolerant and sensitive cultivars accumulated more Al than in the presence of Ca^{2+} in all three root segments. In the presence of Ca^{2+}, the sensitive cv Tam105 accumulated more Al in the meristematic root region, whereas, the tolerant cvs Atlas 66 and Tam 202 accumulated more Al in the more mature root regions. In the absence of Ca^{2+}, however, the tolerant cultivars' Al partitioning pattern resembles that of the sensitive cultivar. Time course experiments revealed that in the absence of Ca^{2+} the Al partitioning pattern in Atlas 66 changed within the first 3 hours of Al exposure. Lanthanum, a calcium channel blocker, was used in this study to test if the external Ca^{2+} was responsible for the change in the Al partitioning pattern. I found that in the presence of La^{3+} external Ca^{2+} did not have any effect on the change of Al partitioning pattern nonetheless, La^{3+} suppressed Al accumulation in Atlas 66 both in the presence and absence of Ca^{2+}. I also determined under the experimental conditions of this study, that the minimal external Ca^{2+} concentration needed to prevent changes in the Al partitioning pattern from "tolerant" to "sensitive" in Atlas 66 was 50 μM. In conclusion, the data reported here clearly demonstrates that Ca^{2+} affects Al partitioning and uptake in intact wheat roots of both Al-sensitive and Al-tolerant cultivars.