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Ca^{2+} Ultrastructural Distribution in Root Apical Cells of Wheat Under Aluminum Stress

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Aluminum (Al) is the most abundant metal in the earth's crust. Most of the Al is bound by ligands or occurs in other nonphytotoxic forms such as aluminosilicates and precipitates. However, solubilization of Al is enhanced by low pH. Soil acidification can develop naturally, but it can be accelerated by acid rain resulted from atomspheric contamination. Al toxicity has become one of the primary environmental stresses limiting crop productivity on acid soils (Delhaize and Ryan 1995 ; Kong et al. 1999).

It is generally assumed that Al^{3+} is the major phytotoxic species of aluminum. Inhibition of root growth is one of the most easily recognized symptoms of Al toxicity, and this has become a widely accepted measure of Al stress in plants. The root apex (root cap, meristem, and elongation zone) accumulates more Al and attracts greater damage than the mature root tissues (Delhaize and Ryan 1995). Indeed, only the apical 2 to 3 mm of maize roots (root cap and meristem) need be exposed to Al for growth inhibition (Ryan et al. 1993). Since Al^{3+} is a potent inhibitor of Ca^{2+} uptake by plant roots, and it appears to block Ca^{2+}-permeable channels in the plasma membrane of cultured tobacco cells, leading to a decrease of cytoplasmic calcium concentration ($[Ca^{2+}]_{cyt}$) (Huang et al. 1992), the symptoms of prolonged Al stress in some plants are similar to those of Ca^{2+} deficiency. Alternatively, Jones et al. (1998) found that Al exposure resulted in prolonged elevations in tip-localized $[Ca^{2+}]_{cyt}$ in root hairs of *Arabidopsis thaliana*. They suggested that although exposure of root to toxic levels of Al cause an alteration in cellular Ca^{2+} homeostasis, this may not be a required event for Al toxicity. Despite the conflict of above studies, we don't know the effect of Al on ultrastructural distribution of Ca^{2+}, and this distribution change may be harmful to Ca^{2+} acting as a secondary messenger to initiate and regulate metabolic process. In present study, the relationships between Ca^{2+} ultrastructural distribution and Al stress were examined in root apical cells of wheat. Al treatment were applied to 3-d-old seedlings and Ca^{2+} was localized by cytochemical method.

MATERIALS AND METHODS

Seeds of wheat (*Triticum aestivum* L., cv.Baipi 224) were surface sterilized in 0.25%

sodium hypochlorite for 15 min, rinsed three times with deionized water, and grown for 3 d in germination trays containing Hoagland's solution.Then the seedlings were placed into a dilute nutrient solution supplemented with 100 μ mol/L Al as $AlK(SO_4)_2$ for 12 hr at an initial pH of 4.5 (Hoddinott et al.1991). The control seedlings were grown continuously in Hoagland's solution.

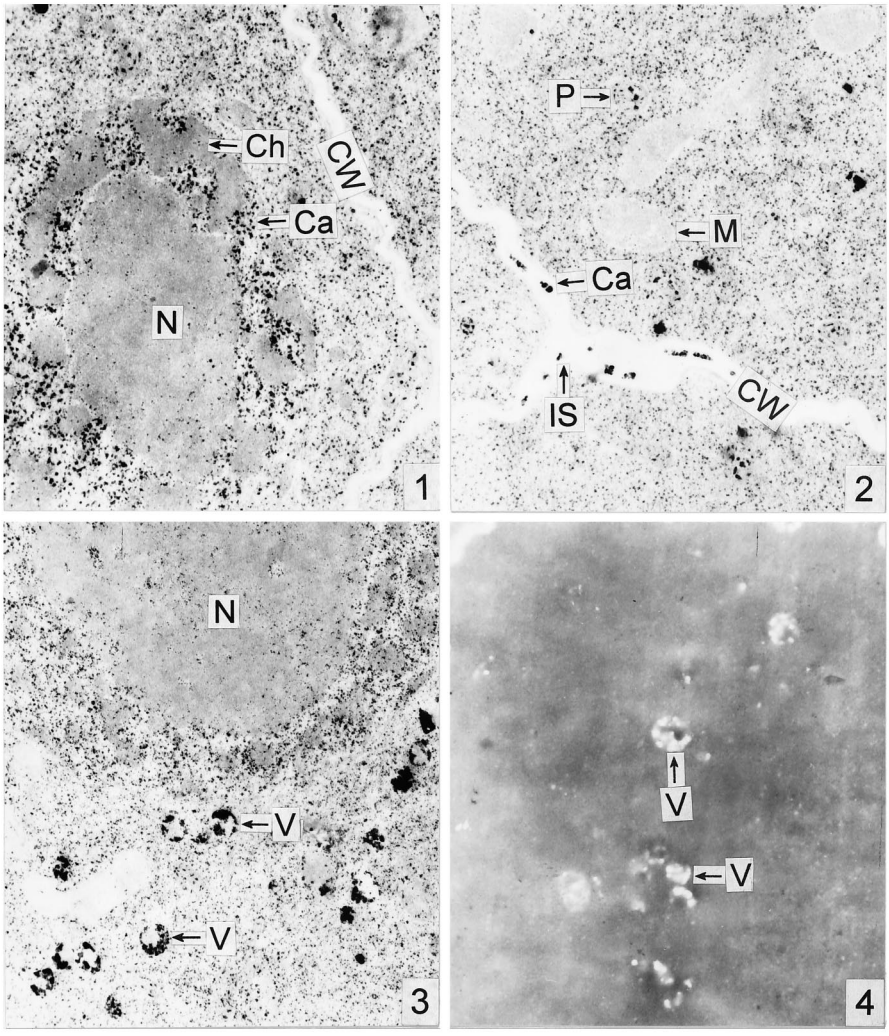
The root tips were cut and used to locate Ca^{2+} with the cytochemical method of potassium antimonate (Wick and Hepler 1982). The samples were dehydrated in an ethanol series and embedded in Spurr resin, then sectioned with ultramicrotome (LKB-2088). The sections were not stained by any other chemicals and observed, photographed under a JEM 100CX/II transmisson electron microscope (TEM). In order to verify the localization of Ca^{2+}, control treatment involving the chelation of Ca^{2+} with EGTA was performed. Grids with tissue sections previously examined by TEM were incubated in a solution of 0.1 mol/L, pH8.0 EGTA for 1 hr to remove calcium precipitates, and then observed and photographed again with TEM.

RESULTS AND DISSCUSSION

For root apical sections treated with potassium antimonate, the calcium antimonate precipitates, which are indicators of Ca^{2+} localization, appeared as electron-dense particles when observed under TEM, the same as observed in previous Ca^{2+} localization studies (Wick and Hepler 1982). Abundant Ca^{2+} precipitates distributed in the apical cells of root grown in normal environment. The precipitates localized mainly in nucleus and cytoplasm. In nucleus, there were more and larger Ca^{2+} precipitates in nuclear matrix than in chromatin (Fig.1). Alternatively, in cytoplasm, many smaller Ca^{2+} precipitates distributed in cytoplasmic matrix, while larger precipitates in vacuoles (Fig.3). A few Ca^{2+} precipitates appeared in proplastids, but almost no precipitates in mitochondria (Fig.2). Furthermore, some large Ca^{2+} precipitates localized in intercellular space (Fig.2). After the tissue sections were treated with EGTA, there were transparent holes in the vacuoles, corresponding to where the electron-dense precipitates were localized before the treatment (Fig.4). This result showed that the location of precipitate is a reliable indicator of Ca^{2+} localization.

Under Al stress, the shoot growth was similar to the shoot in normal environments, but root extension was minimal. The root twisted and its tip become thickened and turn brown. Root growth appeared to be a more sensitive indicator of Al stress than shoot growth.

The content and distribution of Ca^{2+} changed obviously under Al stress for 12 hr. The characteristical change was that many Ca^{2+} precipitates localized near the inner side of plasma membrane. These precipitates arranged along the plasma membrane and formed a circle in accordance with the shape of root cell. The number of Ca^{2+} precipitates in cytoplasmic matrix decreased significantly and restricted to appear in some regions (Fig.5,6). The Ca^{2+}



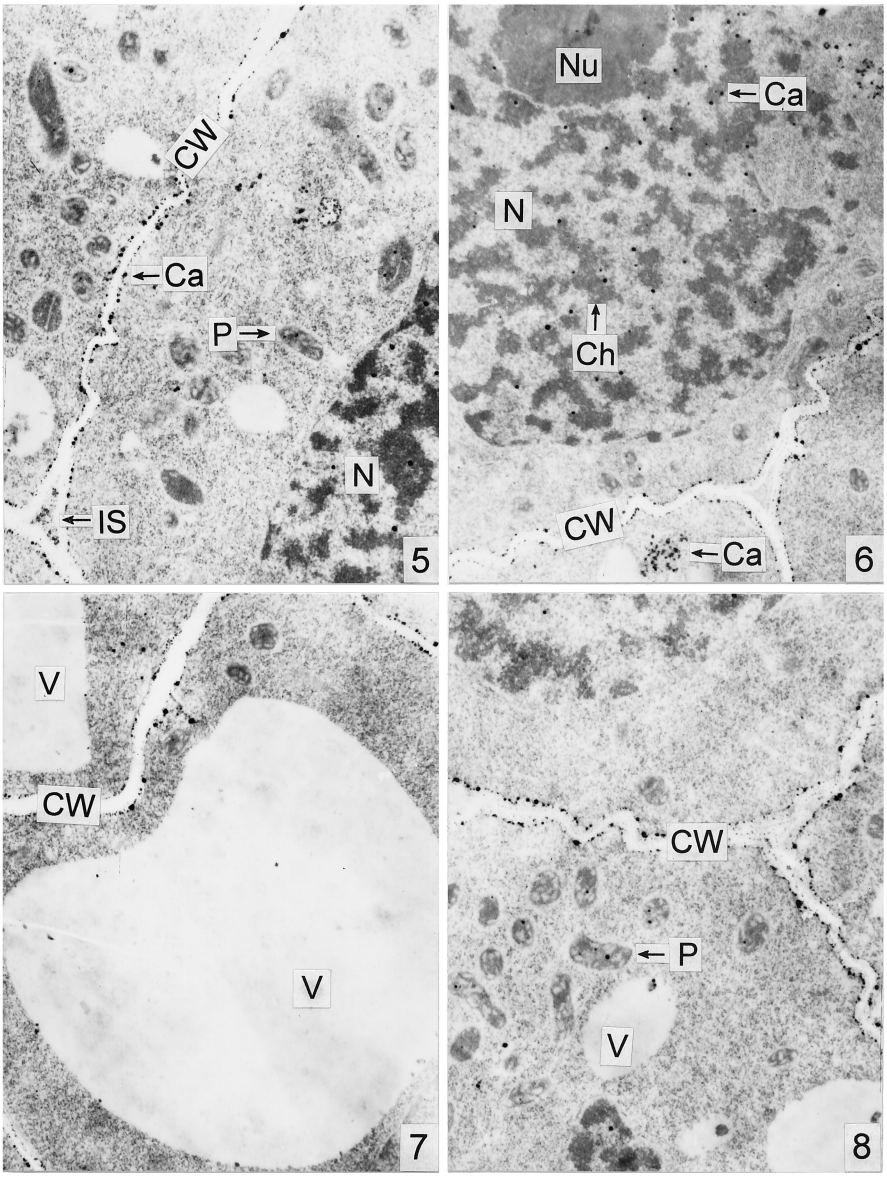
Figures1-4. The localization of Ca^{2+} in the apical cells of wheat roots grown under normal environment. Ca: Ca^{2+} precipitate; Ch: Chromatin or chromosome; CW: Cell wall; IS: Intercellular space; M: Mitochondria; N: Nucleus; Nu: Nucleolus; P: Proplastid; V: Vacuole;

Figure 1. The calcium antimonate precipitates visualized in nucleus and cytoplasm, ×15000

Figure 2. Showing the Ca^{2+} precipitates in intercellular spaces and proplastids, but rare precipitates in mitochondria, ×15000

Figure 3. Showing the Ca^{2+} precipitates in small vacuoles, ×10800

Figure 4. Sections treated with EGTA, showing electronic transparent areas at the same sites where the Ca^{2+} precipitates located before the treatment, ×10800



Figures 5-8. The localization of Ca^{2+} in the apical cells of wheat root grown under Al stress. The abbreviations are the same as Figures 1-4.

Figure 5. The Ca^{2+} precipitates arranged in the inner side of plasma membrane. There still were precipitates in interecellular space and some areas of cytoplasm, ×15000

Figure 6. Showing the Ca^{2+} precipitates in nucleus, ×10800

Figures 7,8. Showing the Ca^{2+} precipitates in vocuoles and proplastids, ×15000

precipitates in proplastids was similar to those in normal environment (Fig.5,8). In nuclei, the amount of Ca^{2+} precipitates was also reduced. The precipitates were distributed mainly in chromatin and chromosomes, and few appear in nucleolus and nuclear matrix (Fig.6). Vacuolization was reported under Al stress (Bennet et al. 1987; Delhaize and Ryan 1995), and this phenomennon was also observed in present study. The volume of vacuoles increased, but the amount of Ca^{2+} precipitates decresaed (Fig.7,8). Alternatively, a few Ca^{2+} precipitates were still observed in intercellular space.

It is now fairly established that calcium plays a key role in plant growth and development. Changes in cellular Ca^{2+}, acting through Ca^{2+}-modulated proteins and their target, regulate a variety of cellular processes in response to many stimulin (Bush 1995). $[Ca^{2+}]_{cyt}$ often shows significant changes in plant cell under the influence of various stress signals such as touch, wind stimulation, cold and heat shock, wounding, hypersensitive response, salinity, and mechanical stimulation (Bush 1995; Xu and Health 1998; Gong et al.1998). To date, the primary cause of Al rhizotoxicity has remain elusive. There were conflicting reports regarding to whether Ca^{2+} influx into plant cell is inhibited by Al. Huang et al. (1992) proposed the inhibition of Ca^{2+} uptake in roots by Al as a possible mechanism for Al toxicity, but some later researchs suggested that the inhibition of root growth is not caused by reduction of calcium uptake (Ryan et al.1994; Jones 1998a). Furthermore, Jones et al. (1998b) found that Al exposure even resulted in prolonged elevations in tip-localized $[Ca^{2+}]_{cyt}$ in *Arabidopsis thaliana* root hairs. They suggested that the phytotoxic action of Al in root hairs is not through blockage of Ca^{2+} influx into the cytoplasm. From the results presented here, it appears that exposure of root tips to toxic levels of Al disturbed the uptake and ultrastructural distribution of Ca^{2+}. Under Al stress, the significant decreased amount of Ca^{2+} precipitates in nucleus, cytoplasmic matrix and vacuoles suggested that the Ca^{2+} influx was inhibited. On the other hand, the Ca^{2+} precipitates rearranged along the inner surface of plasma membrane, and this indicated that the ultrastructural distribution of Ca^{2+} precipitates also changed. As mentioned above, Ca^{2+} plays an important role in plant growth and development. The changes of the amount and subcellualr distribution of Ca^{2+} are most likely to affect the root tip growth process, such as cell division and expansion, and eventully inhibite the growth of root. This may be one of the reasons for phytotoxicity of Al in the soil solution.

The phytotoxic effects of Al on roots can be partically or completely overcome by increasing the concentration of Ca^{2+} in soil (Foy et al.1978). This phenomennon is not solely due to changes in external Al activity, but probably involves more specific interaction at the membrane surface. The ultrastructural distribution changes of Ca^{2+} under Al stress supplied with extra Ca^{2+} are likely to be fruitful areas for future research into the cellular basis of Al toxicity in plants.

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