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THE TOXIC ALUMINUM REACTION IN CORN AND BARLEY ROOTS: AN ULTRASTRUCTURAL AND MORPHOLOGICAL STUDY

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M.S. degree in Horticulture

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THE TOXIC ALUMINUM REACTION IN CORN AND BARLEY ROOTS: AN ULTRASTRUCTURAL AND MORPHOLOGICAL STUDY

By

Ian Bruce McLean

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Horticulture

1979

ABSTRACT

THE TOXIC ALUMINUM REACTION IN CORN AND BARLEY ROOTS: AN ULTRASTRUCTURAL AND MORPHOLOGICAL STUDY

By

Ian Bruce McLean

The progressive gross morphological and ultrastructural changes in the root tips of corn (Zea mays L., cv. Spring Gold) and barley (Hordeum vulgare, cv. Coho) due to Al toxicity were studied. Roots from plants grown in solution culture with 10^{-3}M $AlCl_3$ were examined by scanning and transmission electron microscopy. Constriction of the corn root cortex followed by transverse shearing, reduced root elongation, root cap cell sloughing, cytoplasmic degeneration, and changes in the endoplasmic reticulum (ER) and ribosomal frequency were observed. Barley roots exhibited cortical constriction, reduced elongation, and root cap cell sloughing and degeneration. Longitudinal cortical fissures, abundant surface debris, and damaged root hairs were observed. The frequency of ribosomes on cortical cell ER increased. A possible cause of cell elongation failure involving protein synthesis is postulated.

ACKNOWLEDGEMENTS

Appreciation and love are felt for my wife, Elly, and children, James, Stephanie, and Heather, for their selfless sacrifice of material comforts and time together during my studies.

The support and friendship of my adviser, Dr. H. Paul Rasmussen and the other members of my committee, Drs. Gary Hooper and John Bukovac, has been enjoyed and valued.

Gratitude is expressed to the United States Government and the people of Michigan for the freedom and financial support received during this degree.

The gracious assistance of Bonna Davis in the layout and typing of this thesis was invaluable and most appreciated.

Readers:

The paper-format utilized in this thesis meets the requirements stipulated by the Horticulture Department and the University. The thesis body was prepared for publication in the Journal of the American Society for Horticultural Science and follows the manuscript style for this journal.

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THE TOXIC ALUMINUM REACTION IN CORN AND BARLEY ROOTS: AN ULTRASTRUCTURAL AND MORPHOLOGICAL STUDY

The Toxic Aluminum Reaction in Corn and Barley Roots: An Ultrastructural and Morphological Study

Ian B. McLean and H. Paul Rasmussen
Michigan State University, East Lansing

Abstract. The progressive gross morphological and ultrastructural changes in the root tips of corn (Zea mays L., cv. Spring Gold) and barley (Hordeum vulgare, cv. Coho) due to Al toxicity were studied. Roots from plants grown in solution culture with 10^{-3} M $AlCl_3$ were examined by scanning and transmission electron microscopy. Constriction of the corn root cortex followed by transverse shearing, reduced root elongation, root cap cell sloughing, cytoplasmic degeneration, and changes in the endoplasmic reticulum (ER) and ribosomal frequency were observed. Barley roots exhibited cortical constriction, reduced elongation, and root cap cell sloughing and degeneration. Longitudinal cortical fissures, abundant surface debris, and damaged root hairs were observed. The frequency of ribosomes on cortical cell ER increased. A possible cause of cell elongation failure involving protein synthesis is postulated.

Losses in economic crops due to Al toxicity occur worldwide where the soil pH is below 5.0. Rainfall leaching, crop harvesting, and application of acid-forming fertilizers contribute to soil acidification (6). Modification of surface soil pH by heavy applications of lime has been attempted. However, the A2 and lower horizons frequently remain untenable to roots. Water and nutrients located in those horizons are unavailable to affected plants which are subsequently susceptible to relatively short dry periods. The cultivation of a susceptible species under such conditions may be impracticable (14).

Aluminum toxicity causes stunted roots and tops in many crop species. Common symptoms include severely stunted primary and lateral roots which become stubby and discolored. Tops are dwarfed and may appear deficient in P and be partially chlorotic (8).

Surveys have established wide inter- and intraspecific variability in resistance to Al (7, 14, 16). The variability and the economic impact of yield reduction in acid soils has motivated scientific interest in the toxic reaction. Two hypotheses of Al toxicity predominate in the literature. First, a chronic internal P deficiency caused by symplastic precipitation of $Al(OH)_2H_2PO_4$, and second, inhibition of mitosis by a nuclear Al-DNA interaction (2, 3, 23).

This study investigated the progressive gross morphological and ultrastructural changes in barley and corn roots subjected to toxic levels of Al in nutrient solution. Barley and corn are crop species respectively sensitive and resistant to Al (16).

MATERIALS AND METHODS

Corn (Zea mays L., cv. Spring Gold) and barley (Hordeum vulgare, cv. Coho) seeds were germinated between sheets of moist polyethylene-backed paper in the dark at 24 C. Germinated seeds were transferred to a nutrient culture as described by Rasmussen et al. (21), and 200 ppm fungicide (Benlate) was added. The germinated seeds in nutrient culture were placed in a growth chamber at 24 C under 40 μE incandescent and 210 μE fluorescent illumination. After 5 days, either 10^{-3}M $CaCl_2$ or 10^{-3}M $AlCl_3$ was added to the nutrient cultures, resulting in pH 6.2 and 3.7 respectively. The terminal 1 cm of primary roots was excised from the 5 day old plants at hourly intervals from 0-12 hr, and at 12 and 18 hr, and prepared for both transmission (TEM) and scanning electron microscope (SEM) observation. Three roots in each of two replications were prepared for each instrument.

Root segments including the terminal 1.5 mm and a 1.5 mm section from the zone of elongation were excised for TEM examination. They were

killed and fixed in 2% acrolein and 1% $K_2Cr_2O_7$ in .05M Na cacodylate buffer at pH 7.2 for 2 hr, rinsed in buffer, and post-fixed in 2% OsO_4 for 2 hr. Following fixation, tissues were dehydrated for 20 min at each step in a 10, 25, 50, 75, 90, 95 and 100% ethanol series, transferred to propylene oxide, and embedded in Epon 812 resin. Polymerization of the resin was done at 70 C for 24 hr. Blocks were thin sectioned (Porter-Blum MT-2 ultra microtome) and stained in uranyl acetate (saturated solution) for 45 min and lead citrate (.4%) for 7 min prior to TEM examination at 60 KV accelerating voltage (Philips 201). The frequency of ribosomes per .4 μm endoplasmic reticulum (ER) in root cap and epidermal cells at each sampling time was determined from the TEM micrographs at 26,400X magnification and a simple linear regression line was fitted to the data (19).

Preparation of roots for SEM studies was by dehydration through the same ethanol series used for TEM for 30 min at each step. The samples were transferred to a critical point dryer (Denton DCP-1) and dried in liquid CO_2. The dried roots were mounted on SEM stubs with double-sided sticky tape and sputter coated with 20-30 nm of Au (Film-Vac. Inc. mini-coater). SEM observation was performed (ISI S-III microscope) at 15 KV accelerating voltage and 0 or 35° tilt.

RESULTS AND DISCUSSION

Examination of Al-treated corn and barley roots in the SEM and TEM revealed rapid tissue degeneration in less than 24 hr.

1. CORN ROOT.

A. External Morphology.

The terminal 1 cm of the untreated corn primary root appeared smooth and of uniform diameter to within 1-2 mm of the root cap. The root tip

tapered as it approached the root cap, which was compact and composed of clearly defined cells (Fig. 1A). In Al treated roots a similar condition existed until 6 hr after treatment, when a constriction of the root 2-5 mm posterior to the root cap was observed (Fig. 1B). A shrinkage of cortical and epidermal cell volume was evident. A similar constriction in the zone of elongation of onion root was reported by Clarkson and Sanderson (4). After 9 hr in Al root cap cells appeared disordered and poorly adhered (Fig. 1C). Transverse cracks appeared in the epidermis and exterior ranks of cortical cells where the constriction had previously occurred (Fig. 1D). The separation of epidermal and deeper cortical cells, and disorganization of the root cap increased through 12 hr (Fig. 2A, B). After 30 hr the cortex was sheared transversely and appeared as bands separated longitudinally over a 5 mm segment. The root cap was an amorphous mass of irregularly shaped cells (Fig. 2C). Root elongation was greatly reduced. The deformed root cap and bands of cortex contributed to a stubby, spatulate appearance similar to that reported for other species (11, 13, 22).

This progression of Al damage confirmed the observation of Hatch (9) who showed that the stele continued to elongate at a reduced rate, producing stress sufficient to crack and shear the non-elongating cortex and epidermis. Differential growth on either side of the endodermis reflected the presence of Al in the respective tissues. Continued elongation of the stele supported previous reports of the failure of Al to penetrate the endodermis in large amounts (9, 20).

B. Cytological Observations.

Untreated corn root cap and epidermal cells contained many dictyosomes and parallel sheets of rough ER which were generally located near the

cell wall. Numerous mitochondria, ribosomes and small vacuoles were present throughout the cytoplasm (Fig. 3). Nucleoli were present in nuclei which contained areas of dark-staining material.

Cortical cells in the zone of elongation contained abundant cytoplasmic ribosomes but little rough ER and few dictyosomes. Mitochondria were numerous and the nuclei appeared identical to those in the root cap (Fig. 4).

Deterioration of the root ultrastructure after Al treatment was observed. The cytoplasm of root cap cells contained remnants of membrane, ribosomes and vacuoles after 12 hr (Fig. 5A, B). Organelle structure was severely deformed compared to untreated cells (Fig. 3). Root cap cells frequently contained large central areas of cytoplasmic debris and a band of organelle remnants lying near the cell wall after 24 hr. The tonoplast was not evident and no alteration of the cell wall was observed (Fig. 6).

Cells immediately interior to the epidermis and root cap retained some organized structure after 12 hr (Fig. 5A, 7). Dictyosomes and mitochondria appeared intact even though the cytoplasm appeared abnormal as evidenced by distorted ER, irregular vacuoles, and a general reduction in electron density (Fig. 7).

Changes in the ER were observed. Normal cells contained multiple sheets of ER in which the two membranes were parallel (Fig. 8A). After 6 and 12 hr the two membranes were distended and irregular, and some sections of smooth ER were present (Fig. 8B, C).

The frequency of ribosomes associated with the ER increased over 24 hr in the Al treated cells. Groups of 3-5 ribosomes were common in the untreated cells, with an average of 7 ribosomes per .4 μm of rough ER (Fig. 9a). The groups in Al-treated cells became larger and more

closely packed over time, reaching greater than 13 ribosomes per .4 μm ER by 24 hr (Fig. 9B). The slopes of simple linear regression lines fitted to the ribosome count data revealed a progressive increase in the ribosome frequency on the ER of the cells exposed to Al. There was a small increase in the simple linear regression line for the control (Fig. 10). This confirmed the suggestion by Hatch (9) that ribosomes appeared to "jam" along corn root ER in toxic Al conditions. The implications of the ER and ribosome changes are discussed below.

2 BARLEY ROOT.

A. External Morphology.

The terminal 1 cm of barley roots at time 0 tapered towards the root cap. Root hairs were present to within 1.5 mm of the tip, and root cap cells were clearly defined (Fig. 11A). A constriction of the root beyond 2.5 mm from the root cap and a reduced number of root hairs was observed after 4 hr in Al (Fig. 11B). The shrinkage was similar to that observed in corn (Fig. 1B). Sloughing of root cap cells was evident after 12 hr. Surface debris had accumulated on the root tip by this time and some epidermal cells had shrunk radially (Fig. 11C).

The epidermis appeared severely damaged after 30 hr and root elongation had virtually ceased (Fig. 12A). Longitudinal fissures separated epidermal and cortical cells, some of which had plasmolysed. This was similar to a report of plasmolysed cells in the elongation zone of onion after Al treatment (4). Some root hairs were fractured at the base (Fig. 12B). Large numbers of sloughed cells and other debris covered the length of the root. Some of this material may have been dried cell contents since transverse cracks in epidermal cell walls were observed (Fig. 12B).

Bacterial cells were observed on this debris and their exudate may have bound stray particulate matter in the nutrient culture to the root.

B. Cytological Observations.

The ultrastructure of untreated barley root cells was similar to that of corn. The root cap cells contained numerous dictyosomes, rough ER, mitochondria and small vacuoles (Fig. 13). Cortical cells contained some dictyosomes and mitochondria, numerous ribosomes, but little rough ER (Fig. 14).

The root cap ultrastructure showed evidence of damage 4 hr after Al treatment and was deformed after 6 hr (Fig. 15). Fragments of ER and mitochondria indicated their degeneration, and ribosomes were not evident. Some cortical cells were also degenerated after 6 hr and no intact dictyosomes or mitochondria remained. Myelin-like figures symptomatic of membrane breakdown were observed (Fig. 16).

Inflation and distortion of the rough ER was not observed. However, a 60% increase in the ribosome frequency from 13 to 21 ribosomes per .4μm ER was evident only 1 hr after Al treatment (Fig. 17 A, B, C). The slope of simple linear regression lines fitted to the ribosome frequency data of root cap and epidermal cells showed that the slope for the Al treatment was 8.7 times greater than control (Fig. 10). Damage to root cap and outer cortical cells by 6 hr was so severe that no ribosome counts could be obtained. This result confirmed the evidence from corn of an association between Al treatment and ribosome frequency along the ER.

Several parallels existed in the reactions of corn and barley roots to Al. The root cap and epidermis of both species were rapidly penetrated and destroyed. Damage to the elongation zone took different forms but

cortical and epidermal cell elongation was effectively terminated in both species.

The ribosome-ER association was altered more severely in barley. Both the slope of the regression line and the increased ribosome frequency after 1 hr were greater than in corn. This more severe reaction confirmed previous reports of greater barley sensitivity to Al (7, 16).

3. HYPOTHESES OF Al TOXICITY.

Aluminum exists as $Al(H_2O)_6^{3+}$ in acid solution and readily precipitates P as $Al_6(OH)_{12}(H_2PO_4)_6$. An Al-P reaction also occurs on the surface of polymerized Al hydroxy aggregates at higher pH (10, 12).

Early research into Al toxicity pointed to P deficiency as the cause of the plant reaction. Plant symptoms of Al toxicity which could be removed by addition of P to the growth medium were reported (1). In acid soils, $Al(H_2O)_6^{3+}$ enters the root intercellular space and adsorbs onto the cell wall or middle lamella. Precipitation of P by the adsorbed Al leads to a reduced supply of P for uptake to the shoots. Electron microprobe and elemental analysis has confirmed this model (15, 20). However, the rapidity of the plant reaction and the absence of symptoms in solution cultures devoid of both P and Al suggests an additional, more direct role for Al.

The failure of mitosis in the root meristem of onion under toxic Al conditions led Clarkson (3) to suggest an Al-DNA interaction. He suggested that Al prevented DNA replication during the mitotic cycle. This hypothesis has been supported by reports of Al binding to isolated DNA of pea, and identification of Al in nuclei by staining techniques (17, 18). However, the termination of cell division does not explain

the failure of cell elongation or the rapid degeneration of root cap and epidermal cells.

The rapidity and breadth of disruption to cell processes by Al suggests another causal factor of toxicity. Phosphorus is an integral part of RNA nucleotides and therefore of ribosomes. Cell membranes contain P in their phospholipid component. The changed configuration of corn root ER membranes and the increased frequency of ribosomes associated with ER in cells of Al-treated corn and barley roots suggests interference in these structures and their activities. Aluminum may directly disrupt protein synthesis at both the ER-ribosome and free polysome sites. This would account for the prompt cessation of elongation since enzymes are required for loosening of the cell wall fibers (5). If the replacement of other enzymes which require constant renewal was interrupted, then rapid cell degeneration would ensue.

The high r^2 of .93 and .71 for corn and barley after Al treatment suggests constant interference of ribosome movement on the ER. Conversely, the lower r^2 of .38 and .26 for Ca-treated corn and barley suggest that movement of ribosomes on the ER is not inhibited, and normal exit follows required protein synthesis.

Aluminum has been found to precipitate with P outside the cell causing a P deficiency. A second Al-P interaction in the nuclear DNA which would prevent mitosis has been suggested. This third Al-P interaction in the RNA and ER causing disruption to protein synthesis would explain impeded cell elongation and rapid cell degeneration. The extraction of RNA and confirmation of its association with Al is indicated. An understanding of how Al enters the cell and remains in solution at cellular pH levels is also required before Al toxicity may be fully elucidated.

Figure 1. Scanning electron micrographs of primary corn roots before and after Al treatment.

A) Normal control root with compact root cap (RC).
B) Constricted cortex and epidermis in zone of elongation (arrow) after 6 hr Al treatment.
C) Shearing of cortex and epidermis (arrow) and sloughing of root cap (RC) after 9 hr treatment.
D) Cracks several layers deep in cortex of elongation zone (arrow) after 9 hr Al treatment.
Arrows in (C) and (D) point to the same location on the same root.

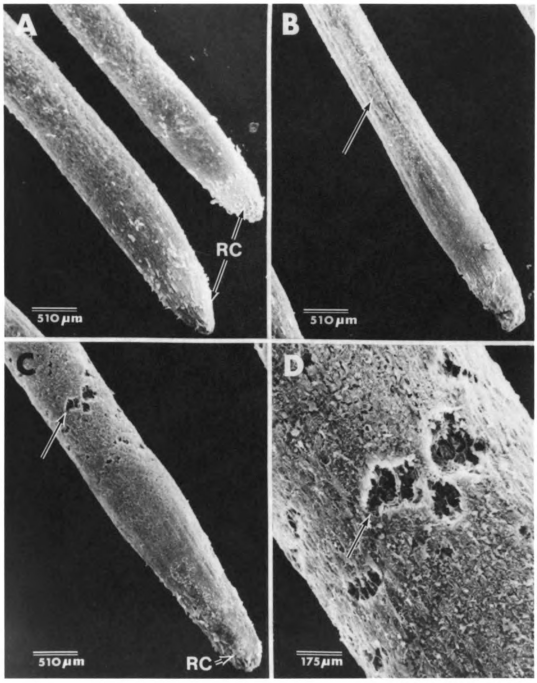

A
RC
510 µm
B
510 µm
C
510 µm
RC
D
175 µm

Figure 2. Scanning electron micrographs of severe Al damage to corn roots.

A) Deepening cracks in cortex (arrow) and sloughing root cap (RC) after 12 hr Al treatment.
B) Fissures (arrow) in cortex formed as cells were pulled apart.
C) Final appearance of primary root after 30 hr Al treatment. The cortex appeared as bands distributed longitudinally along the root (arrow) and the root cap (RC) was deformed and sloughing.

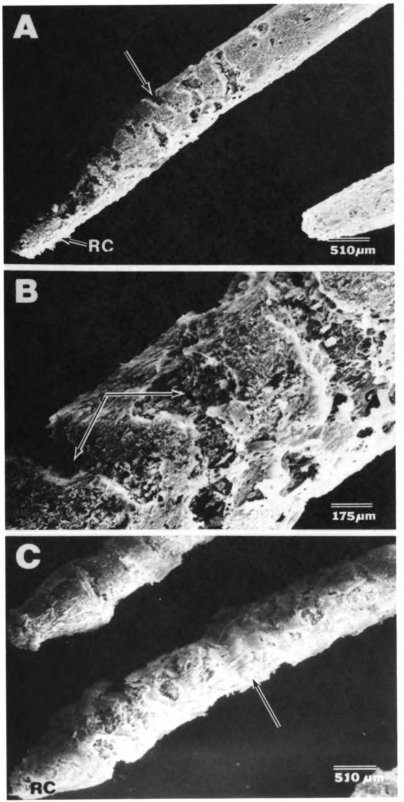

A
RC
510μm
B
175μm
C
RC
510 μm

Figure 3. Transmission electron micrograph of a corn root cap cell. Structures evident were cell wall (CW), dictyosomes (D), rough endoplasmic reticulum (ER), mitochondria (M), plasmalemma (P), small vacuoles (V) and ribosomes (R).

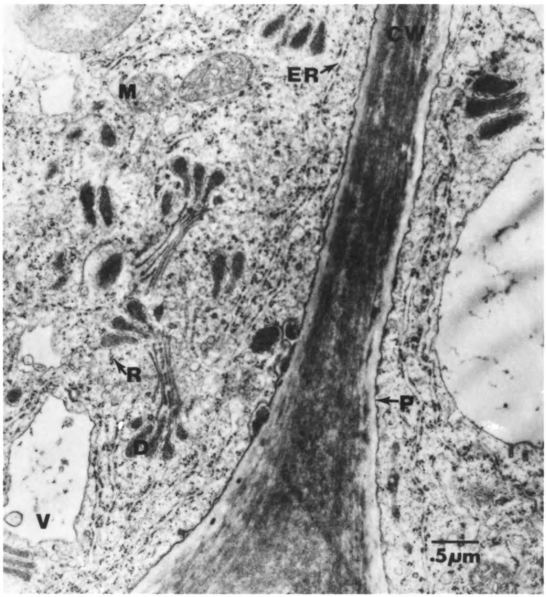

CW
ER
M
R
P
V
.5µm

Figure 4. Transmission electron micrograph of untreated corn root cortical cells. Note the cell wall (CW), rough endoplasmic reticulum (ER), mitochondrion (M), nucleus (N), nucleolus (Nu), ribosome (R) and vacuole (V).

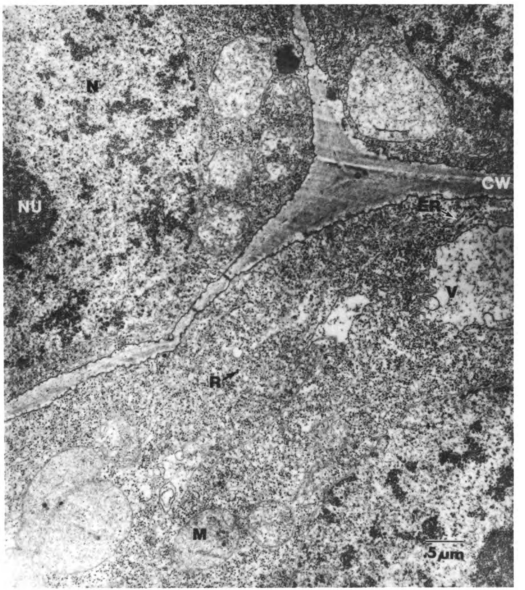

N
NU
CW
ER
V
R
M
.5µm

Figure 5. Transmission electron micrographs of corn root tip after 12 hr Al treatment.

A) Root cap cell (arrow) with large vacuole (V) and degenerated cytoplasm (C). Apical cell (Ap) appeared intact.
B) Cytoplasm of root cap cell with remnants of membrane (M), ribosome (R) and vacuole (V).

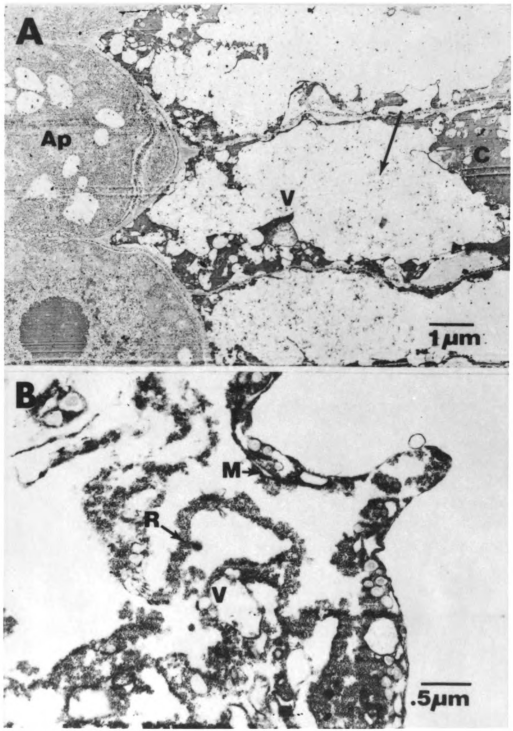

A
Ap
V
C
1µm
B
M
R
V
.5µm

Figure 6. Transmission electron micrograph of a corn root cap cell after 24 hr Al treatment. Note large central area of cytoplasmic debris (Cy), cell wall (CW), dictyosome (D), distended rough endoplasmic reticulum (ER), mitochondrion (M), plasmalemma (Pl), polysome (P) and vacuole (V).

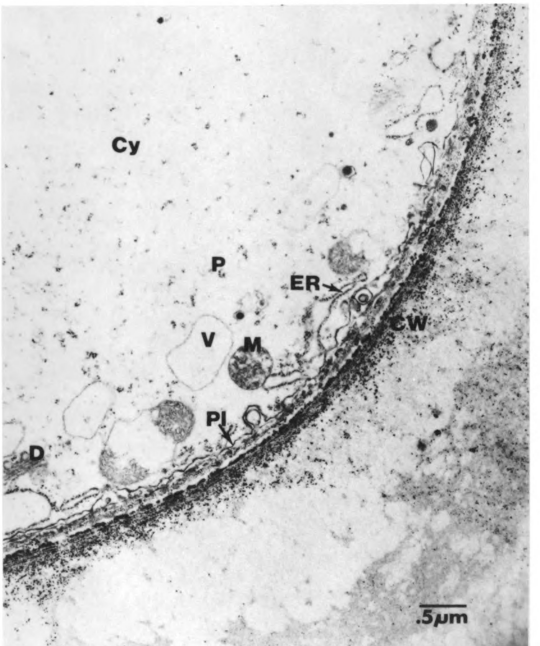

Cy
P
ER
CW
V
M
Pl
D
.5µm

Figure 7. Transmission electron micrograph of a corn cortical cell after 12 hr Al treatment. Dictyosome (D), rough endoplasmic reticulum (ER), mitochondrion (M) and nucleus (N) retain some structure.

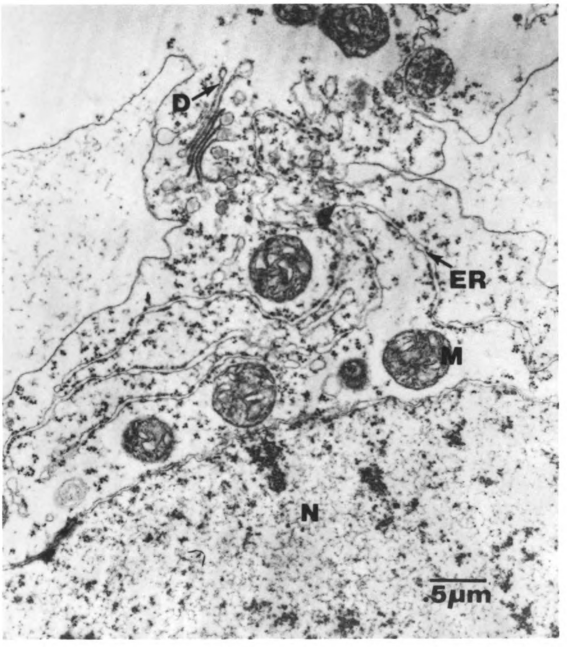

D
ER
M
N
5µm

Figure 8. Transmission electron micrographs of endoplasmic reticulum in corn root cap and epidermal cells.

A) Untreated root cap cell with multiple sheets of rough endoplasmic reticulum (ER) in which the membranes are parallel.
B) Rough endoplasmic reticulum (ER) of epidermal cell with inflated distortions after 6 hr Al treatment.
C) Endoplasmic reticulum of epidermal cell showing smooth (SER) and rough (RER) forms and disconnections (arrow) after 12 hr Al treatment.

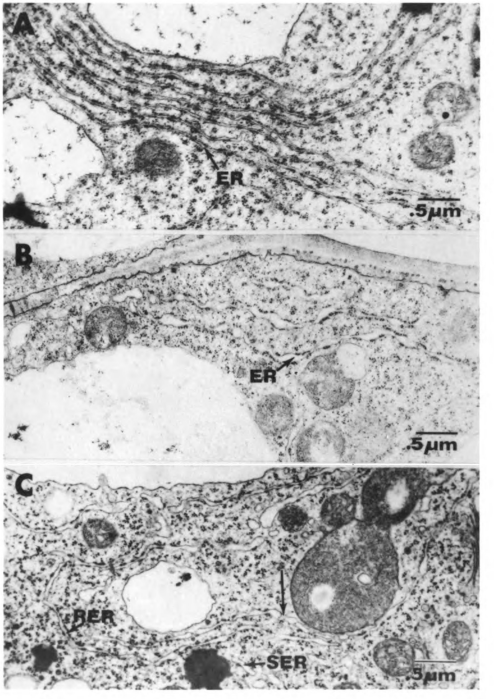

A
ER
.5μm
B
ER
.5μm
C
RER
SER
.5μm

Figure 9. Transmission electron micrographs of ribosomes and endoplasmic reticulum in corn root cells.

A) Untreated cell with infrequent groups of ribosomes (R) associated with the endoplasmic reticulum (ER).
B) Numerous groups of ribosomes (R) associated with endoplasmic reticulum (ER) after 24 hr Al treatment.

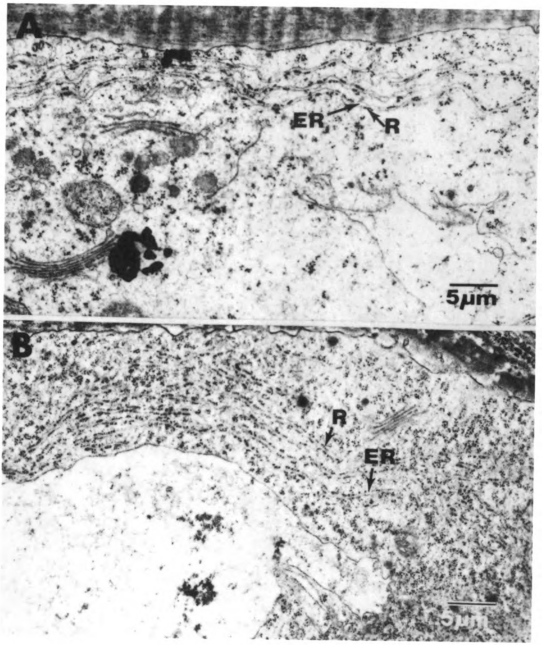

A
ER
R
5µm
B
R
ER

Figure 10. Simple linear regression of ribosome frequency on corn and barley root endoplasmic reticulum.

1) Barley root following Al treatment:

$$\hat{Y} = 19.5 + 1.38X$$
$$r^2 = .71$$

2) Barley root control:

$$\hat{Y} = 12.7 + .158X$$
$$r^2 = .38$$

3) Corn root following Al treatment:

$$\hat{Y} = 7.18 + .25X$$
$$r^2 = .93$$

4) Corn root control:

$$\hat{Y} = 7.24 + .053X$$
$$r^2 = .26$$

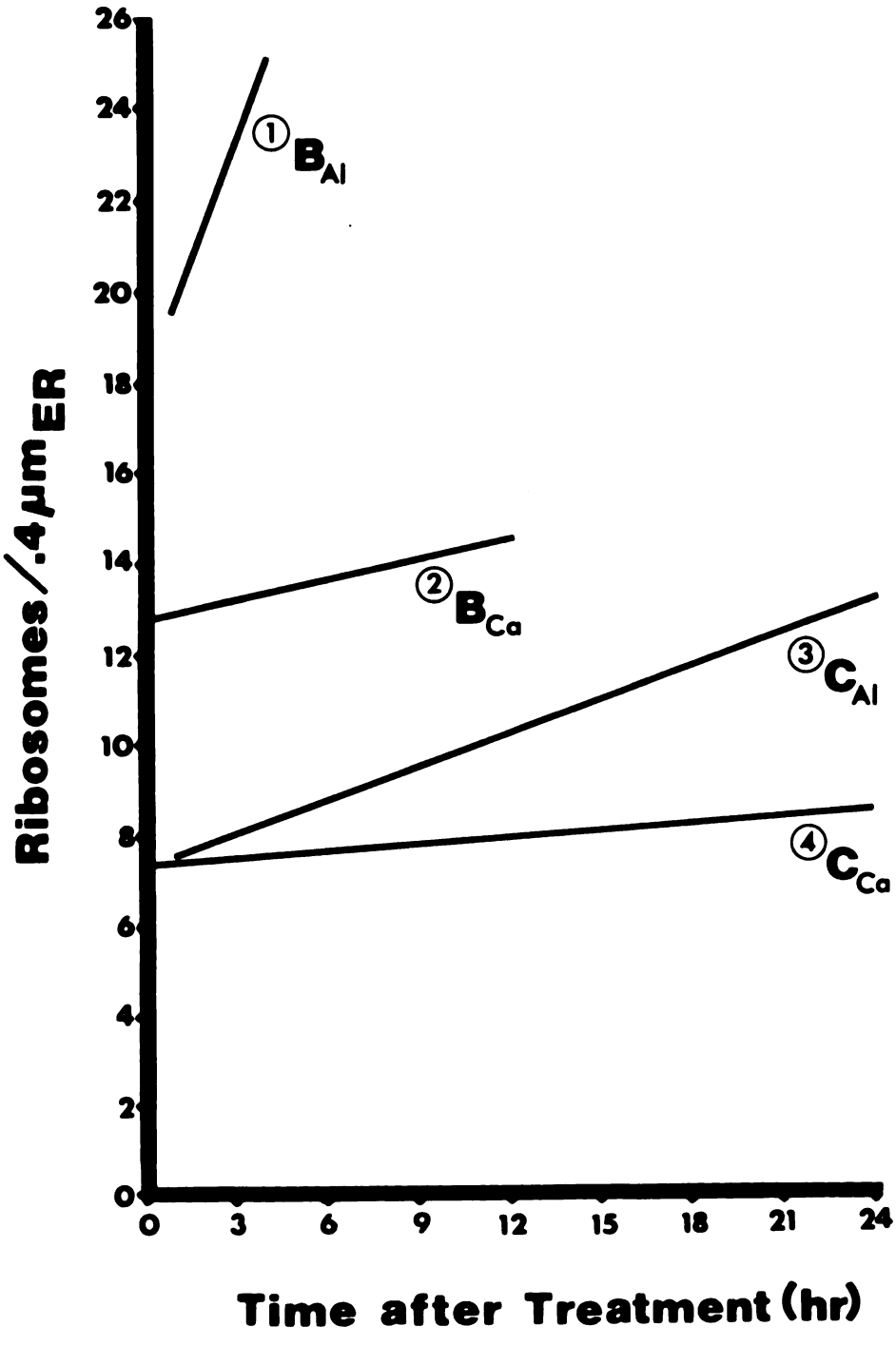

Ribosomes/.4μm ER
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
3
6
9
12
15
18
21
24
Time after Treatment (hr)
① B Al
② B Ca
③ C Al
④ C Ca

Figure 11. Scanning electron micrographs of barley root.

A) Untreated root with clearly defined root cap cells (C) and root hairs (RH).
B) Root showing constriction (arrow) and reduced numbers of root hairs (RH) after 4 hr Al treatment.
C) Sloughing root cap cells (RC), surface debris (arrow) and shrunken epidermal cells (E) present after 12 hr Al treatment.

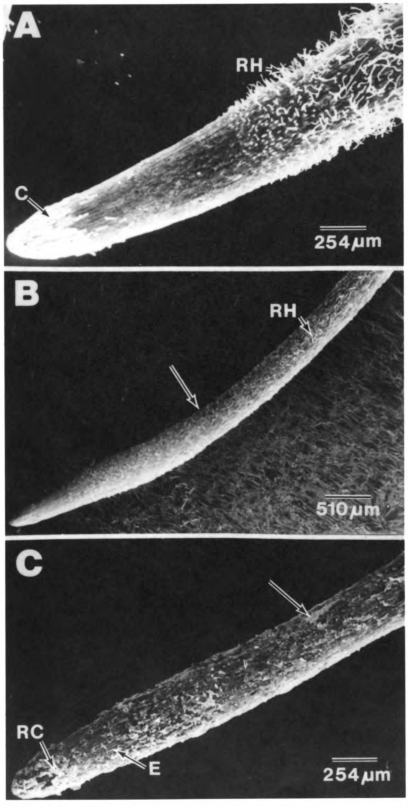

A
RH
C
254 µm
B
RH
510 µm
C
RC
E
254 µm

Figure 12. Scanning electron micrographs of a barley root after 30 hr Al treatment.

A) Severely damaged epidermis and cortex showing longitudinal fissures (F). Sloughed cells and other debris adhered to the surface (arrow). Some root hairs (RH) remained.
B) Fissures caused by epidermal and cortical cell shrinkage (arrow). Transverse cracks (Cr) appeared in some epidermal cells. Root hairs (RH) were frequently broken off at the base.

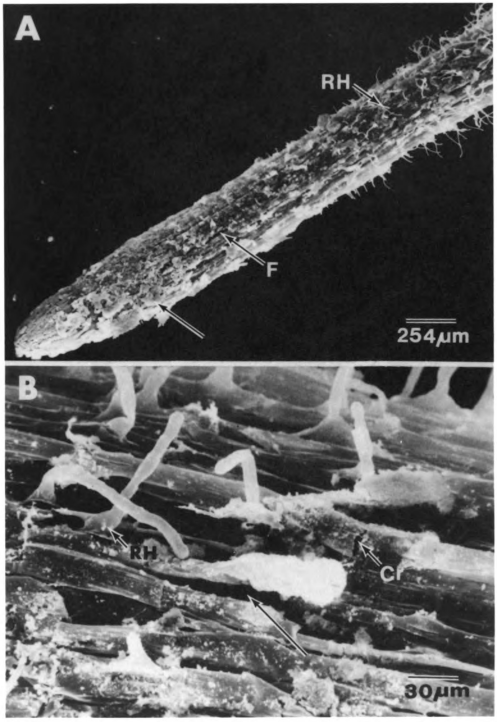

A
RH
F
254µm
B
RH
Cr
30µm

Figure 13. Transmission electron micrograph of an untreated barley root cap cell. Cell wall (CW), dictyosomes (D), rough endoplasmic reticulum (ER), mitochondrion (M), ribosome (R) and vacuole were evident.

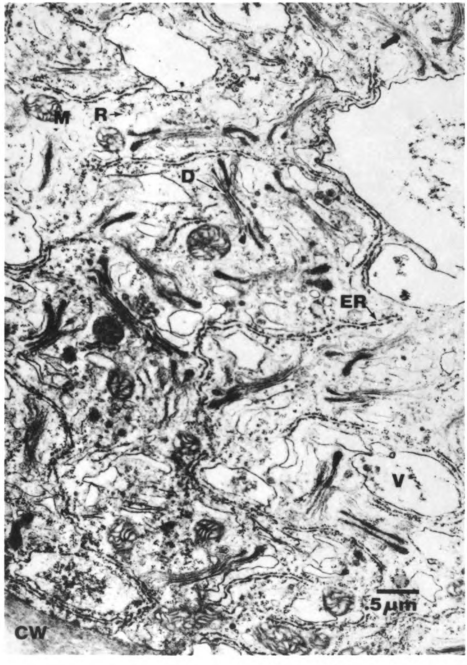

M
R
D
ER
V
.5 µm
CW

Figure 14. Transmission electron micrograph of untreated barley root cortical cells. Dictyosome (D), mitochondrion (M) and numerous ribosomes (R) were observed.

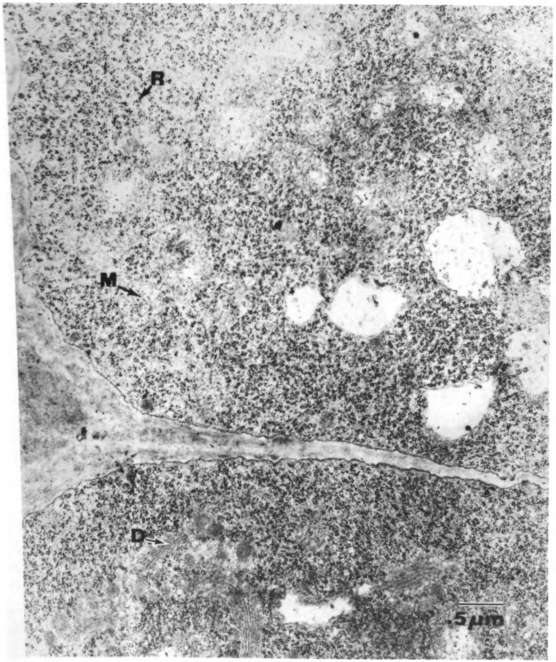

R
M
D
.5µm

Figure 15. Transmission electron micrograph of a barley root cap cell after 6 hr Al treatment. Remnants of an amyloplast (Am), endoplasmic reticulum (ER), mitochondrion (M), plasmalemma (Pl) and vacuole (V) were evident. The cell wall (CW) appeared intact.

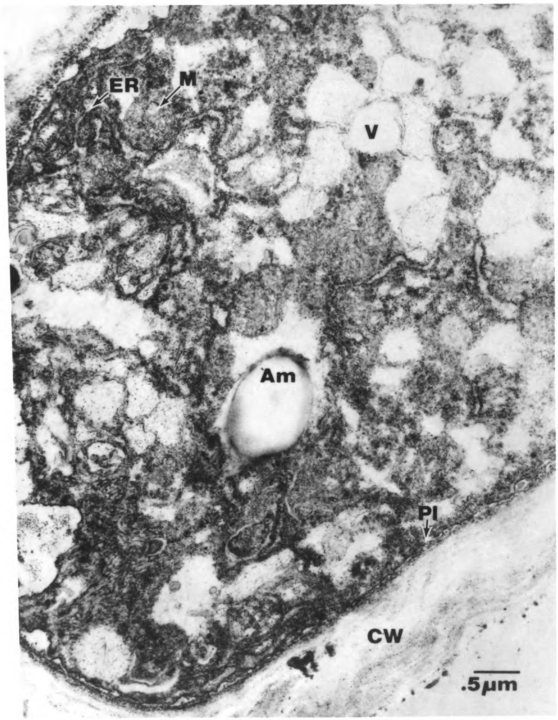

ER
M
V
Am
Pl
CW
.5µm

Figure 16. Transmission electron micrograph of barley root cortical cells after 6 hr Al treatment. Myelin-like figure (My) was evident in degenerated cytoplasm. Note cell wall (CW) and remnants of ribosome (R) and vacuole (V).

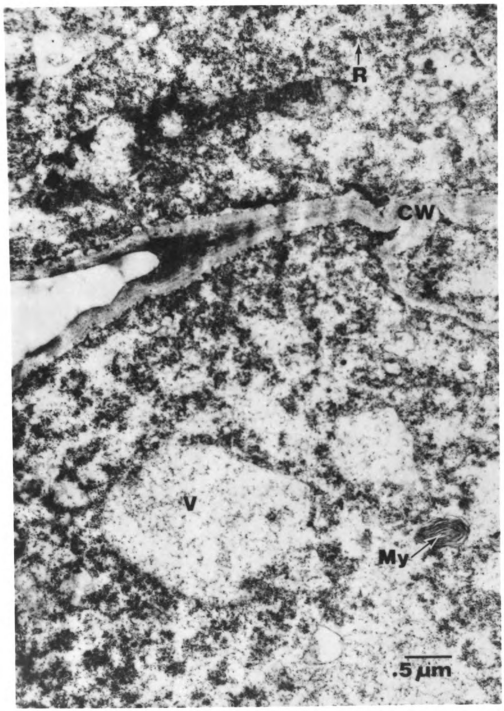
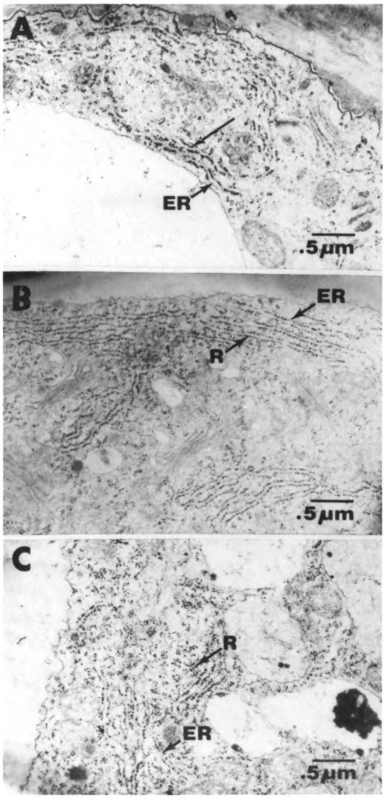

R
CW
V
My
.5 µm

Figure 17. Transmission electron micrographs of endoplasmic reticulum of barley root cap cells.

A) Untreated cell showing groups of ribosomes (arrow) along the endoplasmic reticulum (ER).
B) Increased frequency of ribosomes (R) on endoplasmic reticulum (ER) after 1 hr Al treatment.
C) An almost continuous array of ribosomes (R) on the endoplasmic reticulum (ER) after 4 hr treatment.


A
ER
.5μm
B
ER
R
.5μm
C
R
ER
.5μm

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APPENDICES

APPENDIX I

GENERAL LITERATURE REVIEW

GENERAL LITERATURE REVIEW

A. ALUMINUM AS AN ELEMENT

Aluminum is the third most abundant element in the outer lithosphere and exists generally in alumino-silicate rock-forming minerals. Considerable quantities are found in all soils except certain sands and peats (49, 68). The concentration in natural water is usually less than 100 ug per liter. Higher concentrations may occur at a pH less than 4.7 as the hydrated Al cation, and at pH higher than 8.5 as the aluminate anion (41, 68).

The hydrated form, $Al(H_2O)_6^{3+}$, predominates in acid solutions. As pH increases deprotonation of one water molecule leads to the formation of $AlOH(H_2O)_5^{2+}$, which polymerizes via a double OH^- bridge into $Al_2(OH)_2(H_2O)_8^{4+}$. This process leads to gibbsite formation. Ionic Al in natural water is generally complexed with F^- or OH^-, or SO_4^{2-} at low pH. Polymerized hydroxy aggregates of colloidal or sub-colloidal size predominate in the range of pH 4.7 - 6.5 (41). Richburg and Adams (85) suggested that $Al_6(OH)_{15}^{3+}$ was the major species at pH 5.5 - 6.0.

A complex relationship between soluble Al and pH exists in soils. The actual value of the ionic product $Al(OH)_3$ must be considered in order to place their values on a consistent physicochemical basis (8). Evans and Kamprath (16) found the Al concentration in the soil solution of mineral soils related to the percent Al saturation of the effective cation exchange capacity (CEC). The concentration of soluble salts further affected solubility.

In organic solution the Al concentration was related more closely to exchangeable Al than percent Al saturation. The Al concentration in soil solution decreased with increasing organic matter at a given pH (8).

In 30 acid soils at pH of 4.0, the Al concentration ranged from 1.5 to 23 ppm, at pH 4.5 from 0 to 12 ppm with most less than 5 ppm, and at pH 4.9 from 0 to 2 ppm with most less than 1 ppm (77). The Al concentration in soil solutions above pH 5.5 is less than 0.1 ppm (60). Therefore, prediction of soil solution Al concentration is not possible at a given pH, but toxic Al levels may exist in soils below pH 5.0.

The interaction between soil Al and P is critical to plant growth due to possible Al toxicity per se and P deficiencies from precipitation in the soil matrix. In a moderately acidic solution (pH 4.0) with a high P concentration, the precipitation reaction is:

$$6\ Al^{3+} + 6\ H_2PO_4^- \longrightarrow Al_6(OH)_{12}(H_2PO_4)_6 + 12\ H^+ \qquad (1)$$

or

$$Al_6(OH)_{12}^{6+} + 6\ H_2PO_4^- \longrightarrow Al_6(OH)_{12}(H_2PO_4)_6 \qquad (2)$$

From eq. (1) the reduction in pH upon addition of Al^{3+} to nutrient solutions is evident. Equation (2) is the reaction to form the smallest possible polymer unit. The phosphate ion tetrahedron reacts with two different polymers to increase polymer size. In the first stage of precipitation phosphate reacts with both Al^{3+} and the polymer reducing pH and turbidity. The second stage involves a continued drop in Al concentration due to polymer formation (45, 46).

In slightly acidic to neutral solutions (pH 6-7), low in P, phosphate is adsorbed on the surface of stable Al hydroxides. The surface-reactive amorphous Al hydroxides are not limited in activity above pH 5 and are the real factors governing the P concentration in solution (45, 46).

B. ALUMINUM AND PLANTS

Soil acidity and high Al levels frequently limit plant growth in the high rainfall areas of eastern and south-eastern United States and other parts of the world. Basic elements such as Ca and Mg are removed from the soil by leaching, erosion and harvest. Fertilizers such as $(NH_4)_2SO_4$ intensify acidification (20).

Lime has been applied to acid soils to raise pH above 5.5 and precipitate Al, but often the subsurface layers were not penetrated sufficiently and the pH remained low (21, 22). For example, MacLeod and Jackson (67) found that liming had a negligble effect on pH and therefore upon water soluble and exchangeable Al below 23 cm, and that 9200 kg ha^{-1} of lime was required to influence soil pH at this depth and lower. Subsequent poor root development in the acidic subsurface layers effectively prevented plant access to water and nutrients in the A2 and deeper horizons. Plants with root systems largely confined to the limed layer were susceptible to relatively short dry periods, particularly at critical stages of plant growth (22, 62).

1. Calcicoles and Calcifuges.

Wide variability exists in the ability of plants to grow at low pH. Two broad plant categories are evident. Calcicoles are species inhabiting calcareous soils which are 100% base saturated due to the reaction:

$$CaCO_3 + H_2\text{-micelle} \longrightarrow Ca\text{-micelle} + H_2O + CO_2 \qquad (3)$$

Soil pH is controlled primarily by hydrolysis of $CaCO_3$:

$$CaCO_3 + 2\ H_2O \longrightarrow Ca(OH)_2 + H_2CO_3 \qquad (4)$$

The strong base, $Ca(OH)_2$, exceeds the influence of the weak acid, H_2CO_3, and pH generally ranges from 7.0 - 8.3. Calcicoles grow only on soils with high pH due to their sensitivity to Al. Calcifuges are rare or absent

on calcareous soils and may grow in conditions which cause Al toxicity in calcicoles. On calcareous soils calcifuges suffer from lime chlorosis (18, 90, 91).

2. Differential Tolerance.

Most important crop species are sensitive to Al, but a range of reactions exists. Sensitive species include barley, beet, cabbage, cotton, lettuce, mustard, oats, radish, rye, sorghum, timothy and turnip. Tolerant or resistant species include buckwheat, corn, redtop and soybean (25, 62, 66). Surveys have revealed intraspecific variability in crop species including barley, cotton, rye, snap bean, soybean, triticale and wheat (1, 21, 22, 30, 48, 75, 83, 84).

Resistance is apparently genetically controlled. Natural and breeding selection resulted in an association of geographic location and the degree of tolerance. Generally, cultivars developed in high rainfall, low soil pH areas of the world have greater Al tolerance (21, 23, 31, 52 84). Wide variability in barley and snap bean tolerance facilitated their use as biological indicators of Al levels (62).

3. Aluminum Accumulators.

Aluminum accumulation is thought to be a primitive character and has been reported for numerous woody genera (4, 7). Hutchinson (50) reported an estimated mean content of .02% Al in herbaceous dry matter of many species. However, accumulator species could take up prodigious amounts. For example, young leaves of the tea bush contained approximately 100 ppm Al with up to 5000-16000 ppm in leaves about to fall (6). Aluminum accumulated in the tea leaf epidermis (69). Foliar Al levels of 321-2173 ppm were reported in Pinus species which normally inhabit very

acid soils in eastern Australia (47). Aluminum influences flower color of Hydrangea macrophylla. Chenery (5) found that a delphinidin flower pigment which was pink in an acid cell-sap turned blue in the presence of excess Al.

4. Beneficial Effects.

Beneficial effects of Al on plant growth have been reported. Increased seed production and dry weight of oats and corn were observed with small amounts of added Al (72, 89). Hackett (36) reported stimulated root growth in 5 ppm Al in seedlings of the calcifuge Deschampsia flexuosa (L.) Trin. Stimulated growth of Valencia orange and lemon in 2.5-5.0 ppm Al was reported (58). However, reports of the beneficial effect of Al on plant growth remain isolated.

5. Other Metal Toxicities.

Manganese and Fe toxicity in plants has been reported. Manganese toxicity may occur in acid soils and mine spoils with pH below 5.5, and also at high pH in soils under reducing conditions. Symptoms appear in shoots and include marginal chlorosis and necrosis of leaves, leaf puckering and necrotic leaf spots. Excess Mn caused "crinkle leaf" in cotton, "stem streak necrosis" in potato and "internal bark necrosis" in apple trees. Plant roots may be injured and turn brown following severe injury to the tops. Wide variability in species and cultivar tolerance to Mn toxicity has been documented (27).

Iron toxicity was reported in some acid soils at pH below 6.0. Symptoms included dark green foliage and overall stunted growth. Roots were thickened and contained large deposits of inorganically bound

phosphates. Necrotic spots on leaves of rice and sugarcane were attributed to Fe accumulation. Differential species and cultivar tolerance to Fe has been reported (27).

Phytotoxicity has been observed for a few other heavy metals. Zinc, Cu and Ni toxicities frequently occurred. Lead, Co, Be, As and Cd toxicities under unusual conditions have also been reported (27).

C. PLANT REACTIONS TO ALUMINUM.

1. Symptoms of Toxicity.

Reactions to Al differ qualitatively and quantitatively between and within plant species. However, a coherent pattern has emerged from data of numerous species and cultivars.

Typically plants are stunted above and below ground in toxic levels of Al. Specifically, barley, clover, corn, flax, lettuce, potato and sunflower developed both stunted tops and roots (39, 44, 56, 65, 66, 73, 87).

1A. Root System.

The primary injury from Al is to the root system where proliferation is greatly reduced. de Waard and Sutton (93) found that the roots of black pepper eventually died off centripetally. Less severe reactions result in severely stunted primary and lateral roots. Clarkson and Sanderson (13) found that elongation of onion roots in 10^{-3}M Al slowed progressively after 3-4 hr and ceased altogether after 8 hr. Reduction in elongation has also been observed in corn, sunflower and sugar beet (40, 44, 48). Cessation of elongation was followed by brown discoloration and thickening leading to a stubby and spatulate appearance of the tip in corn,

cotton and sunflower (44, 59, 86). Dissociation and sloughing of the root cap were early symptoms in corn, while disorganization of the root cap, root apex and vascular elements was observed in wheat (17, 40). Hatch (40) found that the cortex and epidermis of corn roots sheared perpendicular to the root axis after elongation had been arrested. Evidently the stele continued to elongate, albeit at a reduced rate. A related phenomenon was the collapse of cells in the cortex in the zone of elongation of onion (13).

Lateral root development patterns are markedly altered by Al. Root length and frequency of lateral root emergence were reduced in corn (59). Hutchinson et al. (48) found that the emergence of lateral roots of sugar beet seedlings grown at 4, 8 and 12 ppm Al extended down to the region of the root apex. Lateral roots of flax grown at 5 ppm Al remained as abortive stubs close to the primary root (73). Lateral roots of barley in 20 ppm Al either failed to penetrate the cortex or formed an irregular mass of cells as a small tumor (82). Lateral roots of wheat penetrated the cortex but became a shapeless mass of tissue with irregularly arranged cells due to inhibition of differentiation of the root cap and vascular elements (17).

Extended exposure to 10^{-3}M Al of Agrostis tenuis demonstrated the cumulative effect of these abnormalities. Lateral roots were initiated close to the apical meristem along with failure of the main axis to elongate. These first order laterals in turn failed to develop and gave rise to second order laterals. This process continued to higher orders. The laterals of successive orders developed close to the apical meristems of the preceding order and all the roots were of similar diameter. Consequently the root system failed to effectively ramify in the soil

under natural conditions and predisposed the plant to dessication (12).

Cellular abnormalities were observed in roots subjected to toxic levels of Al. Hatch (40) found that corn root epidermal and cortical cells in the zone of elongation exhibited increased vacuole size after 8 hr and coalescence of most vacuoles occurred by 24 hr. He also observed changes in the ribosome distribution. There appeared to be "jamming" of ribosomes along the endoplasmic reticulum and an increase in the number of polysomes in the cytoplasm. Nuclear abnormalities were observed in the meristematic cells of cotton roots. Large numbers of binucleate cells were present indicating inhibition of cell division (86). Clarkson (12) observed no mitotic figures in onion roots which had ceased elongating, and concluded that cell division was blocked. However, "sticky" chromosomes, manifested mainly by the formation of anaphase bridges, have been reported for onion (57).

Elemental analysis revealed changes in the root composition under toxic Al conditions. Rios and Pearson (86) found a higher concentration in cotton roots grown at 2.5 ppm Al. Similarly, barley roots had increasing P concentrations with increasing Al in the nutrient solution (65). Reduced Ca levels in roots and/or tops were observed in alfalfa, barley, cotton, rye, perennial ryegrass, soybean, sunflower, triticale and wheat (24, 28, 29, 34, 44, 55). Clarkson and Sanderson (14) found that 100 uM Al reduced Ca uptake in barley to 31-37% of control plants. However, inhibition of root growth was not directly dependent on reduced Ca uptake, and in cotton roots similarly treated the Al toxicity symptoms remained (55). Inhibition of Cu uptake was reported for citrus, lettuce, potato and wheat (3, 39, 42, 56, 58). Absorption of Mg and Zn, and K at high Al levels, was inhibited in potato (56).

The general debility and irregular tissue of roots damaged by Al toxicity has been associated with increased root susceptibility to fungal attack (32). For example, Hoffer and Trost (43) found that corn roots were severely attacked by Fusarium moniliforme Sheldon. Older cotton roots were subject to bacterial decomposition after growing in excess Al (86).

1B. Leaves and Stems.

The leaves and stems of plants grown at toxic Al levels were generally stunted and economic crop yield was reduced. Millikan (73) found that shortened internodes and reduced leaf area caused the stunting of flax.

Leaf symptoms reflected the root disorder. Petiole collapse associated with decreased Ca was symptomatic of Al toxicity in soybean (1, 28). Young barley leaves developed tip dieback while the older leaves became yellow and brittle (65). Leaf margins of sunflower turned brown and the cotyledons died (44). Leaf cupping, chlorosis, and in extreme cases purpling has also been reported (82).

Elemental analysis of barley shoots revealed lower P with increased Al concentration in the growth medium. This was contrary to the root situation (65). Calcium was lower in the leaves of cotton and soybean (24, 28).

2. Distribution of Al in the Plant.

The distribution of Al in roots has been investigated using staining, autoradiographic and electron microprobe techniques. Aluminum penetration into the root has been established. The initial phase of Al adsorption in onion occurred in the mucigel (13). McCormick and Borden

(64) found high levels of Al in the mucilaginous material at the barley root surface. An adsorption intensity gradient in the vertical axis was evident. Adsorption along the root surface decreased from a high at the root cap to indiscernible amounts 3-4 mm back. The root cap was particularly susceptible to entry and passage of Al. Aluminum entered the root cap of sugar beet and accumulated in the cells between the root and the cortex. After 10 days in 4 ppm Al the root cap was separated from the apex by a thick Al phosphate layer and the cap cells had disintegrated (53).

Radial penetration behind the root cap diminishes centripetally. Rasmussen (80) found that corn root epidermal cells largely excluded Al from the cortex. Aluminum which had entered the root was generally found in the cortex but most of it failed to penetrate the endodermis (13, 40, 53, 95). Clarkson and Sanderson (13) found that the Al analog Sc failed to penetrate further than 3 ranks of cortical cells at a point 3-4 mm from the root apex of onion. However, a large accumulation of Al in or on the endodermis, as well as slight penetration into the stele, has been reported for sugar beet (54). Rasmussen (80) found that the rupture of the endodermis by emerging lateral roots provided a pathway for Al movement into the vascular tissue and on to the aerial parts of the corn plant.

Cellular deposits of Al have been observed in the plasmalemma of barley root cells, and the cell wall of root hair and root tip cells of pea. Aluminum has been found in the nuclei of onion and pea (13, 64, 70).

3. Hypotheses of Al Toxicity.

Historically the cause of toxicity in acid soils remained controversial for several decades. Hutchinson (49) reviewed the early debate

over the pre-eminence of Al or pH in the toxic reaction. Several workers noted the decrease in pH of soils following repeated applications of $(NH_4)_2SO_4$ fertilizer. In an effort to elucidate the causal factor plants were grown in soil, soil extract, and nutrient solutions treated with Al. In fact, soluble Al was the main cause of growth depression. Benefits accrued from application of basic materials or acid phosphate to soil came primarily from the precipitation of Al (2, 35, 37, 38). Other workers concluded that native Al levels were too low to account for plant injury and that toxicity was due to precipitation and subsequent deficiency of P, low pH, or unfavorable percentage base saturation (61, 76). The toxicity of Al to plant growth is now accepted and several hypotheses regarding its action have been proposed.

3A. Al-P Interaction.

Increased P concentrations were observed in roots of barley, cotton and perennial ryegrass grown in toxic Al conditions (10, 79, 86). However, Clarkson (10) observed that the increased P was not incorporated into phosphorylated compounds but remained in an inorganic form in barley. This was in marked contrast to a report regarding untreated barley roots in which as much as 80% of the P was in an organic form 15 min after adsorption (63).

Fixation of P by an adsorption-precipitation reaction in the roots leading to P deficiency has been proposed (10, 78, 81, 94). Aluminum firmly adsorbs to isolated root cell wall material, perhaps adsorbing to the free carboxyl groups of polygalacturonic acid chains in the middle lamella. It has been suggested that Al may be precipitated on the root or cell surface as hydroxide. Phosphorus could then be precipitated on

the hydroxide surface as $Al(OH)_2H_2PO_4$. This process would reduce the P available for active uptake from soils where Al and P arrive at the root surface continuously (11). Electron microprobe analysis confirmed that Al and P have similar distribution in corn roots (80).

Aluminum may affect root metabolism directly. Clarkson (10) found that incorporation of ^{32}P into sugar phosphates was markedly reduced while the pool size of ATP and other nucleotide triphosphates was increased. The rate of ATP synthesis was similar in treated and untreated roots. He suggested that Al and P may react within the mitochondria resulting in a decrease in the rate of sugar phosphorylation due to inhibition of hexokinase.

3B. Al-Ca Interaction.

Calcium is involved in numerous plant processes. Calcium affected the uptake of K, P and other ions, and influenced the activity of certain enzymes. Its involvement in cell division, elongation, cell wall stability, and membrane binding and/or stabilization has been reported (19, 51). Therefore, any inhibition in Ca uptake leading to suboptimal plant Ca levels could have multiple deleterious effects.

Clarkson and Sanderson (14) reported that Al inhibited the uptake of Ca. They suggested that the control of Ca uptake and translocation occurred in the epidermis and outer rank of cortical cells of barley roots. The entry of Al into the intercellular space could cause displacement of cations of lower valence, thereby lowering the amount of exchangeable Ca available for uptake. The superficial location of Al could therefore restrict entry of Ca into the stele through the intercellular pathway.

3C. Al-DNA Interaction.

Several workers suggest that the precipitation of P does not provide the whole answer to Al toxicity. Clymo (15) stated that the kinetics of the toxic reaction, wherein deleterious change is almost immediate and recovery follows a considerable lag, precludes a simple blockage of exchange sites. Clarkson (9) reported that the cessation of onion root elongation due to Al toxicity was closely correlated with the disappearance of mitotic figures. Abnormal mitotic figures were not observed. This inhibition of mitosis could not be duplicated by the withdrawal of P in the absence of Al in the nutrient solution. Also, inhibited roots required up to 7 days to restore cell division after withdrawal of Al and addition of P. He postulated that some Al-sensitive mechanism of cell division had been permanently damaged by short exposures to Al.

Aluminum was associated with nuclei of onion and pea by autoradiographic and aluminum staining techniques respectively (13, 70). Clarkson (12) suggested that the mitotic cycle of onion was blocked during the DNA synthesis period. This point of blockage would explain the lack of mitotic figures and fit the timing of the mitotic cycle. However, some DNA synthesis was still possible (88).

An association between DNA and Al was observed in pea root nuclei. Aluminum was recovered from the chromatin of isolated nuclei which had been treated in 10^{-3}M $AlCl_3$ for 1 day. Almost 90% of the Al was associated with the DNA following separation of the DNA from chromatin proteins. These workers suggested that partial displacement of histone *in vivo* could increase accessibility of Al to DNA, thus stabilizing the double helix and limiting template activity (71). Morimura and Matsumoto (74)

found that the absorption spectrum of DNA purified from pea seedlings did not shift after treatment with Al, indicating that Al may bind to the DNA phosphate. They suggested that the numerous binding sites on an Al polymer may allow binding to phosphates of two strands of DNA causing stabilization of the double strands. Such stabilization may inhibit mitosis and hence cell division.

4. Mechanisms of Resistance.

Various mechanisms of resistance have been proposed. Clymo (15) found that Al EDTA was much less toxic and more mobile than Al^{3+}. Clarkson (13) suggested that an unidentified chelating agent may prevent Al from being in a cationic form, thus avoiding injury.

Hydrolysis of Al at the root cell surface removes Al from solution due to its solubility product of 10^{-33} (33). The precipitation of free Al by excess hydroxyl ions produced in the roots could provide a mechanism for resistance (13).

Low CEC favors the uptake of monovalent and divalent cations, and has been associated with Al resistance. The lower CEC of resistant plants may increase monovalent cation uptake at the expense of the polyvalent Al (92).

Differential tolerance of two wheat cultivars was associated with plant-induced pH changes at the root surface. In nutrient culture the sensitive cultivar lowered the pH while the resistant cultivar raised it, resulting in a differential of pH 0.7 in the nutrient solution. Such a pH change is sufficient to significantly alter the solubility of Al (26).

These hypotheses suggest that resistance to Al is based on its elimination from the cell biochemical processes. This may be accomplished

by either blocking entry of Al to the cell or inactivating it chemically after entry, or a combination of both factors.

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APPENDIX II

GEL ELECTROPHORESIS OF CORN AND BARLEY ROOT WATER-SOLUBLE PROTEINS

GEL ELECTROPHORESIS OF CORN AND BARLEY ROOT WATER-SOLUBLE PROTEINS

Scanning and transmission electron micrographs of Al treated corn and barley roots showed severe structural damage, including retarded cell elongation and changes in the ribosome/endoplasmic reticulum association. It was postulated that the protein synthesizing process was altered. Water-soluble proteins were extracted from the root and examined by gel electrophoresis to detect any changes in the protein profile after Al treatment.

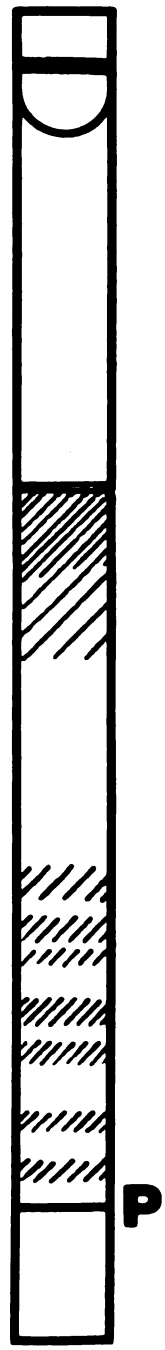
Water-soluble proteins were extracted from 1 g corn or barley primary root tips by the method of McCown et al. (3). Samples were ground, strained and centrifuged at 20,000X g for 30 min at 4 C. The supernatant was dialyzed at 4 C against .05M tris glycine buffer at pH 8.3, frozen in liquid N_2 and lyophilized, and the protein estimated by the Folin reaction (2).

Acrylamide gel electrophoresis was adapted from Davis (1). The protein was dissolved in 20% sucrose in .05M tris glycine buffer at pH 8.3, and 100 ug samples were layered onto the sample gel. The running gel was 13 cm long and electrophoresis was conducted at .4 watts/tube for approximately 6 hr. Gels were stained for 1 hr in Coomassie Blue R and destained for 24 hr in 7% acetic acid with 1 amp current across 12 gels.

Protein bands were identified in samples from Ca and Al treated roots (Fig. 1). However, no change in the water-soluble protein profile after Al treatment was observed for either corn or barley.

Two possible inferences may be drawn. First, no qualitative or quantitative change in the water-soluble proteins had occurred. This seems unlikely given the electron microscope data. Second, qualitative changes were below the resolution of the gels. Refined protein extraction and purification, and higher resolution gel electrophoresis, such as isoelectric focusing, will be required to reveal apparently subtle protein changes.

Figure 1. Diagram of gel after electrophoresis of corn root water-soluble proteins. Note leading protein band (P).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