THE REGULATION OF ROOT DEVELOPMENT BY ALUMINIUM IN *Zea mays* L.

(VOLUME 1)

by

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PREFACE

The experimental work described in this thesis was carried out in the Department of Botany, University of Natal, Pietermaritzburg, from June 1982 to December 1985, under the supervision of Professor C. M. Breen, formerly of the Department of Botany, and Dr. M. V. Fey of the Department of Soil Science and Agrometeorology.

These studies represent original work by the author and have not been submitted for degree purposes to another University. Where use was made of the work of others it has been acknowledged in the text.

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ABSTRACT

This investigation confirmed that plant reaction to Al was directed through the disruption of intercellular co-ordination existing between the cell populations comprising the root meristem.

The first detectable response to Al in the root involved disruption of Golgi apparatus function in the cap periphery. Ultrastructural changes in the cap periphery coincided with the presence of Al in these cells. Conspicuous physiological changes involving cell enlargement, cell metabolism and root elongation were identified in cells initially remote from the site(s) of Al uptake. Communication between Al-damaged peripheral root cap cells and the cell populations of the cap and proximal meristems, quiescent centre and region of cell growth was therefore indicated. A decrease in amyloplast numbers in response to increasing Al concentration coincided with diminished Golgi apparatus activity in the peripheral cap. These changes preceded detectable reductions in mitotic activity, indicated by decreases in cap volume and root length, and it was consequently suggested that control of intercellular activities in the root meristem may be directed through polysaccharide metabolism.

Low concentrations of Al ($\leq 1{,}25$ mg dm^{-3}) produced a positive growth response in the primary root. This coincided with an increase in mean cap volume and these events were interpreted as an Al-induced release of the proximal meristem from growth inhibition originating in the cap. Experiments which followed the effect of decapping on the morphology of quiescent centre cells in the presence and absence of Al supported the existence of a Golgi apparatus-derived morphogen involved in the control of Golgi apparatus secretory activity and plastid differentiation. In contrast to the controls (0 ABA), treatment of decapped roots with 2×10^{-4} and 5×10^{-5} M abscisic acid inhibited the development of Golgi apparatus secretory activity and plastid differentiation. It is postulated therefore, that an early response to Al may involve inhibition of the basipetal movement of an endogenous growth inhibitor originating in the cap, which may be ABA.

The presence of Al in the nutrient solution caused an efflux of H^+ from the root. Some of the more adverse plant responses to Al were initiated at Al concentrations where the molar ratio of charge between Ca^{2+} and Al^{3+} favoured Al. An hypothesis for an electrophysiological plant response to Al involving membrane hyperpolarisation by Al ions is developed.

Chemical analysis of plant fractions did not connect Al-induced nutrient disorders with the primary expression of Al toxicity.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Aluminium (Al) is the most abundant metal cation present in the earth's crust (McLean, 1976; Lindsay, 1979), amounting to some 7,5 percent by mass, with an estimated average concentration of 81 000 mg kg^{-1} (Haug, 1984). There is, however, no defined requirement for Al in living organisms with the possible exception of an obscure rôle in the pigmentation of some flowers and fruits (Chenery, 1948a). The low concentrations of Al frequently encountered in many living organisms are considered indicative of rejection of the element by biological systems (da Silva and Williams, 1976), which accords with the extensive literature attempting to correlate Al uptake and the pathophysiological responses occurring in plants and animals (Foy et al., 1978; Haug, 1984; Evans, 1984).

It is indicative of the complexity of these responses that it is almost 70 years since Al was first implicated in retarding the growth of some plants when grown on acid soils (Hartwell and Pember, 1918), yet there remains considerable speculation on the mechanism of toxicity and the physiological rôle of Al in the plant is poorly defined (Foy and Fleming, 1978; Hecht-Buchholz, 1983; Siegel and Haug, 1983; Wallace and Anderson, 1984).

The possibility of exploiting genetic variability to increase the tolerance of agronomically important plant species to Al has not been overlooked (Duvick et al., 1981; Lafever, 1981; Furlani et al., 1983) and it is therefore surprising that as yet there are no reports of cultivars being bred specifically for Al tolerance (Mendes et al., 1984). Breeding programmes depend on the number of genotypes which can be tested and, although advances have been made in developing screening procedures for selecting for Al

tolerance (Rhue and Grogan, 1976; Konzak et al., 1976; Furlani and Clark, 1981), the lack of unanimity with respect to criteria on which tolerance is assessed (Mendes et al., 1984) reinforces the argument that without information on the physiological response to Al, programmes will suffer from a lack of selectivity.

An ambiguity exists in the literature which simultaneously connects the considerable diversity of symptoms arising from Al treatment (Foy et al., 1978) with the low mobility of Al in plant tissue (Jackson, 1967). In the present investigation, the hypothesis will be considered that the physiological reaction to Al involves several responses which occur at different rates and at different levels of scale. Relevance will therefore be attached to identification of the primary site of Al action and the separation of secondary consequences of Al injury. It is pertinent to this concept that the rapidity with which some symptoms of Al toxicity are manifest (Clarkson, 1965) cannot be reconciled with low mobility unless the site(s) of Al uptake and injury are nearly coincidental. Considerable relevance will therefore be attached to the root apex, since Clarkson (1965) has intimated that the most obvious consequence of Al treatment involving root elongation arises from an Al-induced decline in mitotic activity. It is, however, pertinent that not all the cell populations comprising the root meristem are mitotically active (Ellmore, 1982). Co-ordination of function of the root is nevertheless dependent on the integration of activities between these cell populations. A rôle for Al in disrupting the co-ordination of intercellular activities has not previously been considered.

This thesis is therefore directed at the identification of Al uptake site(s) in the root of Zea mays, identification of the primary response to Al and to tracing the sequence of events which characterize secondary responses to Al.

1.2 LITERATURE REVIEW

1.2.1 Aluminium Toxicity and Plant Growth

A close association between Al treatment and reduced levels of: mitotic activity in the root meristem (Clarkson, 1965; Morimura et al., 1978; Horst et al., 1983), oxygen uptake by roots (Clarkson, 1969), DNA synthesis (Matsumoto et al., 1976; Naidoo et al., 1978; Wallace and Anderson, 1984), water uptake (Lance and Pearson, 1969; Lee and Pritchard, 1984), nodulation and nitrogen fixation in legumes (Munns, 1965a) has been reported for many plant species. Aluminium also causes abnormalities in root morphology, some of which are illustrated in Figure 1. These arise from an increase in lateral root initiation along the root axis (Clarkson, 1969) and an increase in root diameter, while finely branched roots are often absent. Moreover, the presence of Al has been implicated in a wide range of nutrient disorders involving the uptake and transport of phosphorus (P) (Wright, 1943; Foy and Brown, 1963; Clarkson, 1967; Andrew and Vanden Berg, 1973), calcium (Ca) (Foy et al., 1972; Foy, 1974), potassium (K) (Clark, 1977), magnesium (Mg) (Clark, 1977; Grimme, 1982) as well as several trace elements (Foy et al., 1978).

Aluminium toxicity is therefore characterized by a diversity of plant symptoms. Little is known of the initial site(s) of injury, but the responses involving P metabolism, chemical complexing of DNA and biochemical changes arising from disturbed Ca nutrition are generally accepted as likely targets for Al. The evidence on which these hypotheses are based is nevertheless incomplete and even contradictory.

1.2.1.1 Phosphorus Metabolism

The concept of chemical inactivation of phosphorus by Al in the plant (Wright, 1943; Wright and Donahue, 1953) is developed around the reactivity of aluminium and phosphate ions in soils, where the initial reaction products are known to include sparingly soluble (Hsu, 1982) or insoluble (Lindsay,1979) phosphate minerals. The internal precipitation of P within the plant as a primary mechanism of Al toxicity has received considerable support from the observation that both Al and P coincidentally accumulate preferentially in the roots of many plants (Clarkson, 1967; Andrew and Vanden Berg, 1973), possibly in close association (McCormick and Borden, 1974) and that Al-treated plants may exhibit foliar symptoms consistent with extreme P deficiency (Foy et al., 1978).

Phosphorus is fundamental to many metabolic pathways within the plant which may also involve other ions as activators of enzyme systems or in electron transfer reactions (Clarkson and Hanson, 1980). The importance of P in plant metabolism has prompted Foy and Fleming (1978) to imply that separation of the effects of Al toxicity and P deficiency may be impossible, while Adams (1980) has indicated that elucidation of Al toxicity may be dependent on a complete understanding of Al-P interactions within the plant.

Several authors have found the concept of inactivation of P by Al a less satisfactory explanation of Al toxicity. Wallihan (1948) was unable to find evidence for the formation of aluminium phosphate precipitates in the roots of clover (Trifolium repens L.). This accords with the data of Clarkson (1967) who showed that the ratio of Al to P retained in the root varied with time, the Al and P concentrations in solution, and did not correspond to any known aluminium phosphate compound. Moreover, many of the physiological responses to Al are extremely rapid (Clymo, 1962; Clarkson, 1965) and diminished mitotic activity has for example, been demonstrated in the root

meristem with 2 hours' exposure to Al (Clarkson, 1965). It is difficult to connect this observation to the internal precipitation of P, since Clarkson (1969) has shown that the inclusion of P in the Al treatment did not influence the inhibition of mitosis in the root meristem.

1.2.1.2 Inactivation of DNA

Clarkson (1965) found that Al treatment caused a rapid inhibition of onion (Allium cepa L.) root elongation, which was parallelled by a decline in the frequency with which mitotic figures were encountered in the root meristem. Sampson et al., (1965) concluded that Al acted by blocking the incorporation of P into high molecular weight DNA fractions. Aluminium has also been reported to decrease both the rate of DNA synthesis (Sirover and Loeb, 1976) and DNA template activity (Morimura and Matsumoto, 1978). Aluminium has been detected in the nuclei of root meristem cells of Al-treated roots (Matsumoto et al., 1976; Naidoo et al., 1978).

Wallace and Anderson (1984) have however, demonstrated a twofold effect for Al in wheat (Triticum aestivum L.) where the response was seen to primarily be directed at root growth, followed by a decline in DNA synthesis. These observations are of considerable interest, since it can be argued that if the action of Al involved a blockage of the cell cycle during the period of DNA synthesis, as suggested by Clarkson (1969), then an Al-induced failure of DNA synthesis must precede a reduction in root growth. The findings of Wallace and Anderson (1984) are therefore clearly contradictory to the idea that inactivation of DNA represents a fundamental response to Al.

1.2.1.3 Calcium Nutrition

Aluminium interferes in the uptake and transport of Ca (Foy et al., 1972; Foy, 1974; Clark, 1977). Evidence also exists to show that the ameliorating effect of lime in combating

Al on acid soils reflects, at least partly, improved Ca supply (Munns, 1965a; Adams, 1981; Abruna-Rodrigues et al., 1982), and these observations have been interpreted as evidence for a specific requirement for Ca in retarding the consequences of Al toxicity. A critical need exists for Ca on the exterior surface of the plasmalemma as well as a recognised, but unidentified structural rôle in cell membranes, where physiologically Ca serves to prevent membrane damage and leakiness (Clarkson and Hanson, 1980).

These observations have prompted suggestions that Al may act either by reducing the Ca concentration at important membrane sites (Foy et al., 1972) or that reduced uptake may affect biochemical systems dependent on Ca for regulation (Siegel and Haug, 1983). The application of Al to barley (Hordeum vulgare L.) roots has indicated that plasmalemma degeneration may be an early indication of the presence of toxic levels of Al (Hecht-Buchholz and Foy, 1981). Activation of calcium-regulated processes in many instances involves calmodulin (Caldwell and Haug, 1981), and Siegel and Haug (1983) have shown that Al/calmodulin interactions disrupted the calmodulin-stimulated ATPase activity involved in the maintenance of trans-membrane potentials.

That these observations represent primary sites of injury is clearly dependent on demonstrating that the action of Al involves direct inhibition of Ca uptake, either through in-activation of uptake mechanisms or competition at common uptake sites. Evidence for this is poor at present since Horst et al.(1983) have shown that short-term Al exposure, although depressing root elongation, was not connected to the induction of Ca or P deficiencies over the course of the experiment.

1.2.1.4 Agronomic and Environmental Factors

There is no shortage of published information to illustrate that the presence of toxic levels of Al in soils is an agronimic factor of substantial importance (Hartwell and Pember, 1918; Magistad, 1925; McLean and Gilbert, 1927; Jackson, 1967; Foy et al., 1978). Low pH soils, which mobilise soil Al and where Al toxicity might be anticipated, are known to occur extensively in Central and South America, Eastern North America, South Central Africa and Eastern Asia (Bouton et al., 1981). Recent estimates indicate that 40% of the arable soils and up to 70% of the land area potentially available for food production are subject to Al toxicity (Haug, 1984). Many of these regions are located in areas where the acid soil properties associated with Al toxicity are not economically treatable with conventional liming practices (Foy and Fleming, 1978). Moreover acidic sub-soil horizons are known to restrict rooting depth so that plants grown on these soils are at higher risk to drought (Foy et al., 1978).

Besides natural soil acidity, industrial development in the form of acid precipitation which has increased substantially over wide areas of North America and Western Europe (Evans, 1984), and agricultural practices (Williams, 1980; Perl et al., 1982) have contributed to the acidification of areas previously not subject to Al toxicity.

In presenting a problem of global proportions, it remains a matter of experience that many soils high in Al, notably in sub-tropic or tropic zones, support extensive and divergent vegetation types. Rorison (1960) may have been the first to suggest that Al is an ecological factor of considerable importance in determining the exclusion of some plant species from acid soils. Elucidation of physiological factors involved in adaptation to acid soils remains to be achieved, but varietal differences in Al tolerance have been reported for many crop plants, including the cereals and grain (Foy et al., 1965;

Foy et al., 1967; MacLeod and Jackson, 1967; Kerridge et al., 1971; Rhue and Grogan, 1976; Rhue, 1979; Furlani and Clark, 1981; Farina et al., 1982; Mendes et al., 1984), legumes (Munns, 1965a; Armiger et al., 1968; Bouton et al., 1981; Davies, 1981; Edwards et al., 1981), potatoes (Lee, 1971) and tropical root crops (Abruna-Rodrigues et al., 1982).
Because some of the factors controlling plant response to Al may be genetically controlled (Foy et al., 1978), it has been suggested that a reasonable alternative to soil amelioration could include development of plant genotypes exhibiting greater Al tolerance (Duvick et al., 1981; Lafever, 1981).

1.2.1.5 Tolerance Mechanisms

Various mechanisms have been proposed to explain differential Al tolerance, but little agreement exists on the physiological pathway(s) involved (Foy and Fleming, 1978; Foy et al., 1978). It is however, possible that plants have become adapted in different ways to excess Al, and plant response to Al may be additionally complicated by co-existing mechanisms of tolerance within a single species. These observations are supported by the lack of agreement with respect to the assessment of Al tolerance and, although Clarkson (1965) suggested that depression of root elongation was possibly the most obvious effect of Al uptake, doubt persists regarding the selection of descriminatory growth parameters for Al tolerance (Mendes et al., 1984). Indices of performance suggested have included top growth and root mass (Foy and Brown, 1964; Lutz et al., 1971; Clark, 1977) seminal root length (Stockmeyer and Everett, 1978; Stockmeyer et al., 1978); total root length (Furlani, 1981), the relative seminal root length of plants grown simultaneously in nutrient solutions with and without Al (Rhue and Grogan, 1976; Rhue and Grogan, 1977), and also include qualitative assessments based on visual symptoms of toxicity (Clark and Brown, 1974a; Stockmeyer and Everett, 1978; Rhue, 1979; Mendes et al., 1984).

1.2.1.5.1 Exclusion At The Root Surface

Since varietal differences in Al concentrations present in the roots of a number of plant species coincided with an ability to tolerate Al (Foy, 1974; Horst et al., 1983), several investigators have suggested that Al tolerance must result from a mechanism which restricts entry of Al into root cells (Henning, 1975; Foy et al., 1978; Rhue, 1979). This argument is reinforced by comparative trials on wheat Triticum aestivum) where Al tolerance was associated with an ability to resist Al in the growth medium, although little difference could be detected between tolerant and sensitive varieties once Al entered the root (Henning, 1975).

No clear idea exists as to how Al is prevented from entering the root. Henning (1975) has intimated that structural features of the plasmalemma are involved in differential Al uptake. If the cell membranes function as a "molecular sieve", it is difficult to conceive what permits Al to enter the root of tolerant plants, albeit at considerably higher Al treatment levels. An alternative hypothesis involves the possibility that a degree of Al tolerance is conferred on the root by the chemical properties of the mucilagenous secretions of the root which have a demonstratable affinity for binding Al (Horst et al., 1982).

Plants have the capacity to influence the pH of the rhizosphere (Barber, 1974), where the direction and magnitude of the pH changes are usually connected to imbalances between anion and cation uptake (Dodge and Hiatt, 1972; Mugwira and Patel, 1977). The suggestion that Al tolerance may coincide with an ability to increase the pH of the growth medium and thus decrease the mobility of Al ions (Foy et al., 1978) has thus far only been demonstrated in a limited number of species (Foy et al., 1967; Foy and Fleming, 1982). Attempts to establish this concept on a broader base are contradictory and have either failed to distinguish differences in tolerance on

the basis of pH changes (Foy et al., 1972), or have connected Al treatment with acidification of the nutrient solution, although Al tolerance may in some cases correspond to smaller decreases in pH (Clark and Brown, 1974b; Clark, 1977). More recently, Lee and Pritchard (1984) have associated a net proton efflux from the Al-stressed roots of Trifolium repens with restricted anion (NO_3^-) uptake. This observation has parallels with the results of Foy and Fleming (1982) who demonstrated that increased nitrate reductase activity in the presence and absence of Al, coincided with Al tolerance and an ability to increase the pH of the nutrient solution in wheat (Triticum aestivum cv. UC 44 - 111).

Recovery from initial Al shock has been reported in a number of plants (Hecht-Buchholz, 1983; Horst et al., 1983; Mendes et al., 1984). The probable mechanisms involved are not clear, although recovery may reflect the resistance of the primary root meristem to irreversible damage (Fleming and Foy, 1968). Conditions where Al tolerance may arise from an ability to adjust to the presence of Al in the growth medium (Aniol, 1984) are difficult to reconcile with the exclusion of Al at the root surface and may imply that a proportion of the Al entering the root is detoxified with time.

1.2.1.5.2 Accumulation Within The Plant

A number of species including several woody dicotyledonous plants of tropical origin, have been shown to be capable of accumulating substantial amounts of Al in the above-ground tissues (Chenery 1948a, b; Webb, 1954). Plants where the concentration of Al in the aerial fraction exceeds 1000 mg kg^{-1}, have been termed "Al accumulators" (Chenery and Sporne, 1976). The ability to tolerate large quantities of Al without ill effect implies the existence of physiological pathways involved in preventing the precipitation of Al within the cells which might be anticipated in association with P (Jones, 1961) or as a consequence of the formation of solid-

phase $Al(OH)_3$ within the physiological pH range. The nature of these pathways is unknown, although Jones (1961) has suggested that chelation with organic acids may be involved. Support for this idea was provided by Chenery (1948a) who has drawn attention to the prevalence of acid buffer systems in Al-accumulating plants.

1.2.1.5.3 Nutrient Uptake And Utilization

Aluminium tolerance has also been connected to plant nutrient efficiencies involving the acquisition and use of a number of essential elements including P (Foy et al., 1967; Foy et al., 1972; Clark and Brown, 1974b; Tiwari et al., 1984), Ca (Foy et al., 1967; Foy et al., 1972; Clark, 1977), Mg, Mn and K (Clark, 1977). The significance of these observations as possible mechanisms of Al tolerance is not well defined. Rorison (1960) and Clarkson (1969) have, however, intimated that physiological adaptation to dystrophic soils may include the ability to acquire nutrient elements under conditions of limited supply. Plant advantages with respect to nutrient utilization may therefore, represent a secondary response to the soil condition.

1.2.2 The Chemistry of Aluminium

da Silva and Williams (1976) have attached considerable importance to abundance as a factor in determining the evolution of biological requirements for inorganic ions. Aluminium is a significant inorganic constituent of the environment, present in minerals as alumino silicates or oxides (Lindsay, 1979), quantitatively exceeding the levels in many soils (Lindsay, 1979) of the biologically important elements Ca, Mg and K (Clarkson and Hanson, 1980). Yet not only has no functional requirement evolved for Al, but evidence exists to implicate the presence of Al to the detriment of the chemistry of life. This idea is supported by the exclusion, with the exception of boron (B) of all remaining group III elements of the periodic classification

(Moeller, 1952) from inclusion in living organisms. Significantly, it is not abundance which determines the incorporation of B in plants in trace quantities in preference to Al. Boron is however, chemically distinguished from Al, Gallium (Ga), Indium (In) and Thallium (Tl) by strong electronegative properties which favour hydrolysis to form the borate ion $B(OH)_4^-$ (Baes and Mesmer, 1976), while the remaining elements demonstrate increasing electropositive characteristics.

1.2.2.1 The Hydrolysis of Aluminium

The extent to which hydration occurs is determined by the strength of the electric field surrounding the ion, which is in turn a function of the ionic charge and crystal radius (charge density). A clear progression exists (Table 1) between Al and the biologically important elements Mg, Ca, K which is a basis for considering cellular interactions which may determine the exclusion of Al from plant cells.

Table 1 : Selected chemical properties of the biologically important elements K, Ca, Mg compared to Al. Data derived from (1) Nightingale (1959); (2) Baes and Mesmer (1976); (3) Lindsay (1979).

Element	Ionic Charge	Ionic radius Å (1)	Hydrated radius Å (1)	Charge density	Solubility of hydroxide
K	+	1,33	3,31	0,75	soluble(2)
Ca	2^+	0,99	4,12	2,02	moderately soluble(2)
Mg	2^+	0,65	4,28	3,07	solid phase above pH 9 - 10(2)
Al	3^+	0,50	4,75	4,75	solid phase between pH 5,0 - 8,0(3)

The degree of hydration increases with decreasing ionic radii and increasing ionic charge (Nightingale, 1959). Aluminium occurs only in the trivalent state and is characterized among the elements under consideration by the smallest ionic radius (Baes and Mesmer, 1976). Predictably, the progression of increasing ionic properties with correspondingly less tendency to form aqueous complexes follows the series $Al < Mg < Ca < K$ and the Al^{3+} ion is extensively hydrolysed to yield polynuclear hydroxide complexes (Baes and Mesmer, 1976) while hydration of the K^{+} ion is limited (Nightingale, 1959) to the extent that KOH is often considered a completely dissociated electrolyte (Baes and Mesmer, 1976). The strongly hydrated ions are associated with more marked increases in hydrated ionic radii (Table 1) so that Al has the largest hydrated radius in the series Al to K. It may also be significant that, although the difference in crystal radii is substantial, Ca and Al hydrated radii differ by only 13% and Mg shows a proportionately smaller difference. These observations imply that in aqueous solution, hydrated ionic size is unlikely to be a criterion in selecting between Ca, Mg and Al.

The low solubility of aluminium hydroxide within the physiological pH range has been suggested as a fundamental reason for exclusion of Al from living cells (Hutchinson, 1945; da Silva and Williams, 1976). Possibly one of the most important anomalies in the study of Al toxicity arises from this, since it is inconceivable that the rapid expression of the symptoms of Al toxicity (Clymo 1962; Clarkson, 1965) can be connected to chemical immobilisation of the element. The corollary to this argument requires that the anticipated precipitation of $Al(OH)_3$ be prevented in cells expressing a toxic response to Al.

da Silva and Williams (1976) have also emphasised "avoidance of risk" in determining biological requirements. It is therefore possible that the diversity of hydrolysis

products associated with Al is a more powerful criterion in selecting against Al in favour of progressively lower hydration levels shown by Mg, Ca and K. This concept may be particularly significant in non-specific rôles which include charge neutralisation, where a degree of substitution may be expected between elements.

1.2.2.2 Speciation of Aluminium Ions in Solution

Considerable controversy surrounds the hydrolysis of Al^{3+} (Baes and Mesmer, 1976; Lindsay, 1979), and the identification of stable hydrolysis products has been influenced by the slow rate of reaction, interference by transient and permanent precipitation, and the presence of at least one stable polymeric ion species which is difficult to characterise (Baes and Mesmer, 1976).

At pH's above 3 (Baes and Mesmer, 1976), it is suggested that the Al^{3+} is 6 co-ordinated with water to form $Al(H_2O)_6^{3+}$. Omitting for simplicity the hydrated water from the hydrolysis species, incrementing pH yields the following predicted mononuclear hydrolysis products (Lindsay, 1979)

$$Al^{3+} \rightleftharpoons Al(OH)^{2+} + H^+$$
$$\rightleftharpoons Al(OH)_2^+ + 2H^+$$
$$\rightleftharpoons Al(OH)_3 + 3H^+$$
$$\rightleftharpoons Al(OH)_4^- + 4H^+$$
$$\rightleftharpoons Al(OH)_5^- + 5H^+$$

of which the existence of only $Al(OH)^{2+}$ and $Al(OH)_4^-$ are established with certainty (Baes and Mesmer, 1976). It is relevant however, that Lindsay (1979) has suggested that, if the hydrolysis species are plotted as a function of pH on the basis that Al^{3+} is in equilibrium with $Al(OH)_3$ (gibbsite), then solid phase hydroxide will dominate between pH5 and 8. It is therefore significant that Al toxicity has been most frequently associated

with pH levels below 5,5 (Foy and Fleming, 1978).

1.2.2.3 Ionic Charge and the Expression of Toxicity

The activity of the Al ion increases markedly below pH 5,0 (McLean, 1976) and, although Pavan and Bingham (1982) have demonstrated that the growth of coffee (Coffea arabica L.) is negatively correlated with increases in the activity of the Al ion, evidence connecting Al toxicity directly to the charge on the Al ion is poor. Studies of root response to ionic species have variously implicated Al^{3+} (Gapon and Voshchinskaya, 1941; Blamey et al., 1983), $Al(OH)^{2+}$ (Moore, 1974), $Al(OH)_2^+$ (Blamey et al., 1983), while yield reductions in maize, attributed to Al toxicity, have been reported at near neutral pH (Farina et al., 1980). Jones (1961) has also drawn attention to the possibility of Al toxicity arising from the presence of negatively charged aluminate ions at alkaline pHs, and this idea is supported by the data of McCain and Davies (1983) who report an inhibiting effect of AlO_2^- ion on root growth at pH 8,8. Interestingly, evidence does exist to suggest that Al complexed by a range of organic materials (Bartlett and Riego, 1972), and polymeric Al species (Blamey et al., 1983) may produce markedly diminished plant responses to the element.

1.2.3 The Plant Root

1.2.3.1 Regulation of Root Development

Plant root development is sensitive to a wide variety of environmental stimuli (Feldman, 1984a) and root activity may fluctuate appreciably during root life (Ellmore, 1982). Root response to mechanical impedance (Goss and Russell, 1980), flooding (Konings and Jackson, 1979) and light (Burström, 1960) which may involve a number of morphological or physiological reactions are, in common with Al (Clarkson, 1965), most noticeably expressed through a decline in root growth.

The root apex (Luxová, 1981) is the site of origin for all new root cells which contribute to primary tissues. Longitudinal section through the root apex reveals a highly organized pattern of longitudinal files of cells that radiate from a region of the meristem directly adjacent to the root cap (Feldman, 1984b). Organization of the root apex primarily involves the bidirectional production of cells which contribute to the formation of the axial structure of the root in the proximal direction and the maintenance of the root cap located at the distal end of the root.

Co-ordination of the activities of cells comprising the root apex is complicated by fundamental differences in cell cycle times between the cell populations (Clowes, 1961; De la Torre and Clowes, 1972) which comprise the meristematic region of the root. These differences principally involve the length of the G_1 phase of the cell cycle, that is, the length of the period immediately preceding DNA synthesis (Clowes, 1972a; Barlow, 1975). The most conspicuous differences in cell cycle times (Clowes, 1958) occur between the cells of the quiescent centre which divide rarely and the mitotically active cells of the cap and proximal meristems.

In rapidly growing roots, the proximal meristem functions as the ultimate source of cells for the root proper (Feldman, 1984b). The cap meristem which generates the cells of the root cap (Ellmore, 1982) is characterized by the presence of the most actively dividing cells in the root (Clowes, 1971; 1980). The quiescent centre is delineated by the mitotically active cells of the cap and proximal meristems (Figure 2). The activities of these cell populations must therefore represent a finely balanced equilibrium, since alterations in the rate of cell division may be expected to influence the size of the quiescent centre. Changes in the size of the quiescent centre may be of fundamental importance in regulating root development, since statistically significant correlations have emerged between the size of the quiescent centre and a number

of anatomical features, including the complexity of the root vascular system (Feldman, 1977; Charlton, 1980).

Definition of how the activities of the cells of the root apex are co-ordinated and how the distinctive organization arises are less than satisfactory. Experiments involving the excision of the root cap result in a temporary increase in root elongation (Pilet, 1972) which is seen as support for the concept that low levels of inhibitory substances originate in the cap (Gibbons and Wilkins, 1970). Removal of the root cap also results in a marked increase in the mitotic activity of tissue directly basal to the root cap, including the quiescent centre (Barlow, 1974a; Feldman, 1976). These observations are subject to the alternative interpretation (Clowes, 1972a; Barlow, 1974a; Clowes, 1975; Barlow and Hines, 1982; Clowes, 1984) that the intact cap imposes a physical constraint on the adjacent tissues of the proximal meristem and the quiescent centre.

Cytokinins have also been implicated in quiescent centre metabolism (Feldman, 1975; Feldman, 1979a), although Feldman (1984b) has intimated that the phenomenon of quiescence may be too complex to be explained only in terms of cytokinins. A possible rôle for ethylene in the organization of the root meristem has also been suggested by experiments with mechanically impeded roots, since a decline in axial growth (Vicia faba) coincided with marked increases in ethylene evolution (Kays et al., 1974).

1.2.3.2 The Root Cap

Haberlandt (1914) originally considered the principal function of the root cap to include protection of the sub-terminal, apical meristem of the root. More recently, the cap has emerged as a unique tissue region of the root, since it is involved in the perception of a number of stimuli, including gravity (Barlow, 1974b) and light (Pilet and Ney, 1978), which are then translated into growth or regulatory

responses (Feldman, 1984a).

Clowes (1972b) has also demonstrated that the root cap exercises an important function in controlling the cycling rate of cells within the root meristem. Statistically significant correlations have emerged between the volume of the root cap and the size of the quiescent centre (Barlow and Rathfelder,1984). Since in roots with the "closed" construction, the quiescent centre does not normally contribute cells to the root cap (Barlow, 1974a) this observation is interpreted as re-inforcing the argument that the cap is involved in imposing quiescence on the cells of the quiescent centre. Although the mechanism remains uncertain (Barlow and Pilet, 1984; Clowes, 1984), this observation has implications for the regulation of root development, as there are proposals that the quiescent centre in some way regulates mitotic activity in root meristems (Torrey, 1972; Barlow, 1976).

In actively growing roots, cap cells constantly move from the cap meristem at the base of the cap to the tip where they are sloughed off (Barlow, 1975). The life of cap cells is subject to considerable debate. Phillips and Torrey (1972) with Convolvulus, Barlow (1978a) with Zea and Harkes (1973) with Avena, report that the cap is renewed totally every 6 - 9 days. Clowes (1976) argues in favour of a much more rapid (22 - 33 hours) renewal of cap cells. Notwithstanding these differences, it is relevant that in seed plants, no other tissue has a higher constant turnover rate than the root cap (Feldman, 1984a). Since economy of function is an important biological criterion, the rapid turnover which characterizes cap cells may alert us to an as yet unexplored function of the root cap.

Differentiation of cap cells coincides with the passage of cells through the spatial gradients of the root cap. Some of the most notable changes include an increase in cell size,

changes in plastid structure involving starch accumulation, formation of amyloplasts (Juniper and French, 1970; Barlow,1975), and the appearance, frequency and activity of the Golgi apparatus and endoplasmic reticulum (Barlow, 1975). The secretion of mucilagenous polysaccharide materials from cap cells occurs while intracellular starch diminishes in size, which suggests that the mucilagenous secretions may be partly synthesized at the expense of polysaccharide materials present in the amyloplasts (Ellmore, 1982).

Although evidence is not unequivocal, gravity perception is thought to occur in specialized cells located in the central portion of the root cap. These cells are characterized by the presence of large starch-containing amyloplasts which are believed to have a gravity-sensing function (Audus, 1975; Juniper, 1976; Jackson and Barlow, 1981). It is therefore significant that the bending response initiated in roots by the gravitropic signal emanating from the cap occurs in cells remote from those in which the signal is perceived (Shen-Miller et al., 1978). This observation indicates that communication occurs between the cells of the root apex and evidence exists to support the idea of chemical control (Ellmore, 1982) which, in the case of gravitropism, largely revolves around the occurrence of growth regulating substances which may be released from the root cap in response to the gravitropic signal (Jackson and Barlow, 1981).

1.2.4 RÉSUMÉ

The literature contains many examples which illustrate the diversity of plant symptoms arising from the presence of Al in the growth medium. It is increasingly clear, however, that the physical, chemical and biological mechanisms which relate to the plant systems that interact with Al are not clearly understood. Some of the more obvious responses to Al involve the root meristem. Meristems are characterized by the complexity of the spatial distribution of the rates of cell division and cell growth, and little information exists to indicate how control of intercellular activity

is maintained. The possibility that Al acts through the disruption of the co-ordinated function of the meristem has not previously been considered.

CHAPTER 2

MATERIALS AND METHODS

2.1 PLANT MATERIAL

This investigation included two cultivars of Zea mays: TX 24, Saffola Seeds (Pty.) Limited, and HL 1, Plant Improvement Section, Summer Grains Centre, selected for previously demonstrated differences in acid tolerance (Farina et al., 1982).

2.2 PLANT GROWTH

2.2.1 Nutrient Solution Culture

Plants grown in liquid culture have frequently formed the basis of physiological investigations of plant response to Al (Clarkson, 1965; Rhue and Grogan, 1976; Clark, 1977; Pavan and Bingham, 1982; Horst et al., 1983; Wallace and Anderson, 1984). Choice of growth medium is undoubtedly influenced by the relative simplicity with which individual factors, suspected of affecting plant response to Al, may be manipulated (Blamey et al., 1983). Furthermore, well-stirred nutrient solutions provide a relatively homogenous experimental system which can be quantitatively characterized (Asher and Edwards, 1978).

In contrast, the complex and dynamic nature of root/soil interactions precludes precise experimental control of the root environment. Interpretation of plant response to soil acidity problems involving the presence of toxic concentrations of exchangeable Al may be surprisingly difficult, since the addition of soil ameliorants (e.g. lime) may simultaneously increase Ca and Mg supply, alleviate Al (and sometimes Mn) toxicities and alter the availability of some plant nutrients through pH changes (Blamey et al., 1983). Roots of plants grown in soil are also not easily studied (Warncke and Barber, 1974), and contamination with soil minerals may effectively exclude these tissues from meaningful

chemical analysis.

Many of the difficulties associated with the application of solution culture techniques involve changes in the mobility of nutrient ions which reflect the somewhat artificial root environment, reinforced by rapid fluctuations in concentration arising from nutrient uptake by test plants (Asher and Edwards, 1978). It is therefore reassuring to find that nutrient uptake rates for plants (Zea mays) grown in nutrient solutions, are similar to those observed in the field (Warncke and Barber, 1974). It is nevertheless significant that Clark (1982) has highlighted the difficulties involved in adequately defining the growth parameters required for the successful application of nutrient solution culture techniques.

Factors which are reported to affect the expression of Al toxicity in nutrient culture studies include: Al concentration, solution pH, temperature and the concentration of nutrient cations, particularly Ca and Mg. Rhue and Grogan (1976) have intimated that "almost any degree of toxicity can be obtained by varying one or more of these factors". The extent to which these factors may influence the present investigation is examined in the ensuing sections.

2.2.2 Preparation of the Nutrient Solution

The full strength nutrient solution contained the following plant nutrients (mg dm^{-3}) :

N 36; P 3; K 40; Ca 40; Mg 10; S 14;
Na 1,5; Mn 0,11; Zn 0,05; Cu 0,02;
Fe 5,5; Mo 0,03; B 1,9.

The composition of the nutrient solution is similar to that derived by Rhue and Grogan (1976) for screening cultivars of Zea mays for Al tolerance. Where experimental requirements have necessitated modifications to the nutrient solution proposed by these authors, changes have been described in detail.

Nutrient solutions were produced by dilution with constant agitation of the concentrated stock solutions outlined in Table 2. Analytical reagent quality chemicals were used throughout.

Table 2 : Chemicals used in the preparation of full-strength nutrient solutions.

Solution No.	Chemical	Concentration (g 10 dm^{-3})	Dilution (ml 25 dm^{-3})
1	$CaCl_2 .2H_2O$	183,40	200
2	$MgSO_4 .7H_2O$	253,39	100
3	KNO_3	51,00	250
	$(NH_4)_2 SO_4$	6,60	
	$KH_2 PO_4$	13,60	
	$NH_4 NO_3$	80,02	
4	$MnSO_4 .H_2O$	3,38	25
	$CuSO_4 .5H_2O$	0,75	
	$ZnSO_4 .7H_2O$	2,30	
	NaCl	17,52	
	$Na_2 MoO_4 .2H_2O$	0,73	
	$H_3 BO_3$	6,20	
5	Fe CDTA		140

Solutions were added in the sequence indicated by the solution number. Salts in solution 3 and 4 were combined to make fewer stock solutions. (Note: Ca salts were separated from high concentrationsof SO_4 or PO_4 salts. so as to avoid exceeding the solubility levels for these ions in combination.)

Young seedlings are also known to respond more favourably to less concentrated nutrient solutions (Clark, 1982). Experiments which involved limited growth periods were therefore conducted with half-strength nutrient solutions.

2.2.2.1 Fe CDTA

Fe CDTA was prepared by dissolving (69,2g) CDTA (cyclohexane - 1,2 - diaminetetracetic acid - Merck Art 8424) in approximately 8 litres deionised water containing (36,2g) KOH and adding (49,8g) solid $FeSO_4 .7H_2O$ to the CDTA solution so as to completely dissolve the Fe salt. Solution pH was adjusted to 5,5 by small additions of M KOH. The solution was made to final volume (10 dm^3) before oxidation to the ferric state by aerating overnight (Hewitt, 1966).

The effectiveness of various chelated Fe compounds in preventing the precipitation of Fe in nutrient culture work is extensively discussed by Hewitt (1966). Simultaneous pH dependent equilibria may also exist between the chelating agent, nutrient cations (other than Fe) and Al. pH stability criteria for CDTA (Halvorson and Lindsay, 1972; Norvell, 1972) indicate that Fe CDTA dominates stability criteria at pH's below 5,5. This chelating agent is therefore unlikely to be an effective competitor for nutrient ions (with the possible exception of Cu) within the experimental pH range. The low affinity of CDTA to bind Al (Norvell, 1972) over the pH range 4,0 - 9,0 is particularly noteworthy, and was the basis for the application of CDTA to this investigation.

2.2.2.2 Nitrogen

The supply of nitrogen was aimed to achieve nearly equimolar levels of NO_3^- and NH_4^+ ions in the nutrient solution through the use of NH_4NO_3 as the principal source of nitrogen. A degree of control can be exercised over nutrient solution pH by regulating the supply of NO_3^- and NH_4^+ - nitrogen (Clark, 1982;

Jones, 1982), since nitrate-fed plants raise the pH, whilst ammonium ion supply depresses it (Smith and Raven, 1979). Nutrient solutions used in the study of Al toxicity commonly feature high concentrations of NO_3^- - N (Moore et al., 1976; Rhue and Grogan, 1976; Clark, 1977; Wallace and Anderson, 1984). It is therefore important that enhanced Al uptake has been connected to NO_3^- - N supply (McCain and Davies, 1983). The precise physiological mechanism on which this observation is based remains speculative, but these authors (McCain and Davies, 1983) consider it probable that nitrate-based acidic solutions will over-estimate the toxic effect of Al. There is also evidence to indicate that the NH_4^+ ion is the predominant source of N in many acidic soils (Theron, 1951; McCain and Davies, 1983). An increase in the supply of NH_4^+ - N in nutrient solutions used in investigation of Al toxicity may therefore be more representative of the soil condition.

2.2.2.3 Calcium

Calcium was present as $CaCl_2 .2H_2O$. Selection of this Ca carrier was dictated by the solubility of the salt and the flexibility in experimental procedure of adjusting Ca concentrations in the nutrient solution without corresponding changes in the levels of anions which are also macro-nutrients. The presence of relatively high Cl^- (79,2 mg dm^{-3}) levels is defended on the basis that chloride is best considered an inert anion (possibly essential in small amounts for photo-system II) which is readily transported and tolerated by plants over a wide concentration range (Clarkson and Hanson, 1980).

2.2.2.4 Aluminium

Aluminium was added when required as $Al_2(SO_4)_3 .18H_2O$. The stock solution was prepared by dissolving 61,75g $Al_2(SO_4)_3 .18H_2O$ in 5 dm^3 deionised water. Dilutions to the required concentration were made on the basis that 1 ml of this solution contained 1 mg dm^{-3} Al.

2.2.3 Nutrient Solution pH

The pH of the nutrient solution prior to adjustment, was observed to range between pH 5,0 - 5,2 in the absence of Al, or pH 3,8 - 4,0 with Al added. The pH of the nutrient solution was adjusted to 4,6 without ageing of the solution (Rhue and Grogan, 1976) immediately prior to planting, by the addition of small quantities of either 0,1 M HCl or 0,1 M KOH. Except where otherwise indicated (Chapter 7), no further adjustments to pH were made, although changes in nutrient solution pH were monitored. Gradual acidification of the nutrient solution was associated with most treatments (Figure 3). The exceptions involved cultivar HL 1 where increases in solution pH occurred in the absence of Al, while somewhat smaller pH changes in the same direction also occurred at the lowest Al level (Figure 3).

Precise control of solution pH using conventional solution culture techniques is difficult to attain (Islam et al., 1980). The effect of low solution pH on plant growth in solution culture experiments has yielded conflicting results. Islam et al. (1980) have indicated that optimum growth is achieved in a number of plant species at pH 5,5, whereas Clark (1982) has indicated that deleterious growth effects have not been noted in Zea with a solution pH of 4,0.

The position in studies of Al toxicity is additionally complicated by the effect of pH on Al ion speciation, notably with respect to solid phase $Al(OH)_3$ dominating equilibria between pH 5,0 and 8,0 (Lindsay, 1979). Since it is clearly necessary to maintain Al in solution, it is significant that support for the view of Clark (1982) comes from the work of Loneragen and Dowling (1958), Munns (1965b) and Andrew (1976) who reported minimal growth effects at pH levels as low as 4,0, provided the plants were adequately supplied with essential mineral elements.

2.2.4 Selection of Calcium and Magnesium Concentrations

The selection of appropriate concentrations of Ca and Mg may therefore be influenced by the need to maintain an as yet undefined but adequate concentration of the elements in solution to prevent a pH-induced growth depression (Munns, 1965b; Hewitt, 1966), which might otherwise be expected at the pH range ($\leq$ 4,6) commonly encountered in nutrient solution culture studies of plant response to Al (Rhue and Grogan, 1976; Clark, 1977; Pavan and Bingham, 1982; Mendes et al., 1984).

Calcium and Mg also exercise important but undefined rôles in the expression of Al toxicity, and increases in the concentration of these elements, not directly related to pH have been observed to alleviate some symptoms of toxicity (Munns, 1965a; Foy et al., 1969; Rhue and Grogan, 1977). Calcium may also be effective in influencing the uptake of P, Zn and Fe (Adriano et al., 1971). Wide divergences nevertheless exist in the selection of concentrations for Ca and Mg in studies of Al toxicity, and Ca levels in nutrient solutions have been reported to range between 4 mg dm^{-3} (Konzak et al., 1976), and 200 mg dm^{-3} (Asher and Edwards, 1978).

There have however, been few attempts (Rhue and Grogan, 1977) to experimentally define the requirements for Ca and Mg. The results of a randomised factorial experiment, based on the full-strength nutrient solution in which Ca and Mg concentrations were varied to provide Ca (mg dm^{-3}) 20; 30; 50; 85 and Mg (mg dm^{-3}) 6; 10; 18; 30 in a nominal ratio of Ca : Mg of 3 : 1, and incorporating two levels of Al (0; 10 mg dm^{-3}) are presented in Tables 3 and 4.

Table 3 : The effect of different nutrient solution concentrations (mg dm^{-3}) of Ca and Mg, in the absence of Al, on the relative (% of maximum recorded value) yield of roots, tops and tissue concentrations of Ca and Mg. Plants grown for 14 days in full-strength nutrient solution.

Soln. Conc. (mg dm^{-3}) Ca / Mg		Relative Data (% of maximum observed value)						
		D.M. Yield pot^{-1}		Ca		Mg		pH
		Root	Top	Root	Top	Root	Top	
20	6	74	83	83	72	63	85	4,7
30	10	76	92	83	84	84	92	4,5
50	18	82	84	94	94	98	100	4,8
85	30	100	100	100	100	100	96	4,4
LSD 0,05		13	11	30	12	12	11	
0,01		18	15	40	16	16	15	

Significant increases in yield (roots and tops) and tissue concentrations of Ca (plant tops only) and Mg (roots and tops) were attributed to increased Ca and Mg supply in the absence of Al (Table 3). The levels of Ca (roots) and P, K, Mn, Zn, Cu, Fe (roots and tops) were not altered by changes in Ca/Mg supply.* Visual symptoms of Fe deficiency, confirmed by the foliar application of ferrous sulphate (Clark, 1982) were, however, evident at the two highest levels of Ca/Mg. Notwithstanding the absence of analytical data in support of this observation, and the progressive increase in yield observed over the course of the experiment, Ca and Mg levels were selected (40 mg dm^{-3} Ca; 10 mg dm^{-3} Mg) to avoid possible complication arising from interactions between Ca/Mg and Fe. No clear evidence emerged to indicate a relationship between solution pH at the termination of the experiment and

* Relative yield data for these elements omitted for simplicity from Tables 3 and 4.

yield or nutrient uptake (Table 3).

Table 4 : The effect of Al (10 mg dm^{-3}) in the nutrient solution on the relative (% of control, 0 Al) plant concentrations of P, K, Ca, Mg, Mn, Zn and Cu

	Relative (%) Concentration							
	P	K	Ca	Mg	Mn	Zn	Cu	
Control (0 Al)	100	100	100	100	100	100	100)	roots
Al (10 mg dm^{-3})	73	98	34	16	6	64	66)	
Control (0 Al)	100	100	100	100	100	100	100)	tops
Al (10 mg dm^{-3})	43	84	38	68	18	43	52)	

Aluminium in the nutrient solution reduced plant concentrations of P, Ca, Mg, Mn, Zn and Cu in the roots and tops (Table 4). Root K levels were unaffected by Al, but leaf concentrations were reduced in Al-treated plants, which may be indicative of a rôle for Al in influencing K transport. The only statistically significant plant response to increased Ca/Mg supply in the presence of Al involved an increase in root Mg concentration. This observation may seem to contradict the results of Rhue and Grogan (1976; 1977) and may indicate instead that the Al stress level (10 mg dm^{-3}) was excessive. Notwithstanding this, leaf tissue concentrations of Ca (0,23%), Mg (0,21%), Mn (28 mg kg^{-1}) and Zn (35 mg kg^{-1}) recorded in this experiment for solution Ca/Mg levels of 30 : 10 (mg dm^{-3}) and above in the presence of 10 mg dm^{-3} Al were all in excess of the levels which are reported to reduce plant growth rates (Islam et al., 1980). This reinforces the selection of nutrient solution concentrations for Ca and Mg, since plant response to Al is unlikely to be influenced by limiting concentrations of the above nutrient elements (Ca, Mg, Mn Zn).

2.2.5 Significance of Interactions between Aluminium and Phosphorus

Munns (1965b) has intimated that elucidation of interactions of Al in the plant may be dependent on nutrient solution levels of Al and P uncomplicated by precipitation of insoluble reaction products. Some investigators, possibly influenced by the ameliorating effect of increased P levels on plant response to Al (Foy and Brown, 1963), have been encouraged to avoid interactions between these elements by omitting P from nutrient solutions which contain Al (Konzak et al., 1976). Where P is included in the solution, Asher and Edwards (1978) have observed that the levels of P and Al present frequently exceed the solubility product for aluminium phosphate. In consequence, the concentration of these elements with respect to the expression of Al toxicity may be greatly overstated.

Interpretation of interactions between Al and P which occur in nutrient solutions may be influenced by controversial questions concerning the existence of soluble aluminium phosphate complexes within the range of experimental conditions.

Hsu (1982) has provided evidence to support the existence of these products and has suggested that basic aluminium phosphate $Al(OH)_{3-x}(PO_4)_x$ may form in preference to variscite ($AlPO_4 .2H_2O$) as a result of competition between OH^- and PO_4^{3-} for the Al^{3+} ion. Conditions which favour formation of soluble basic aluminium phosphates are applicable to many nutrient culture solutions and include low concentrations of Al (< 13 mg dm^{-3}), P (P/Al molar ratio $< 0,9$) and a pH range of 4 - 8 (Hsu, 1982).

The reaction products of Al and P in dilute solutions may therefore represent plant available P reserves (Hsu, 1977). Little is, however, known of the rôle of these substances in short-term nutrient culture studies. A comparison between cultivars TX 24 and HL 1, grown in full-strength nutrient solutions with either KH_2PO_4 (control), or amorphous aluminium phosphate (0,15g dm^{-3}) without Al added, or amorphous aluminium phosphate (0,15g dm^{-3}) with Al

added to give concentrations of 5 and 10 mg dm^{-3}, indicated that aluminium phosphate per se is an acceptable P source, since dry matter yields were either unaffected (cv. TX 24), or increased (cv. HL 1) as a consequence of these changes (Figure 4). Yields of plant roots and tops were, however, reduced when Al was present in combination with aluminium phosphate (Figure 4) which suggested that Al was the overriding factor controlling the utilization of aluminium phosphate P reserves. Furthermore, the initial precipitation associated with some nutrient solution formulations (Rhue and Grogan, 1976) may not permanently deprive the experiment of either P or Al. With the exception of cv. TX 24 (root concentration only), P levels in the plant fractions were lower when aluminium phosphate was the P source, and were further reduced by the presence of Al in the nutrient solution (Figure 4). Plant concentrations of K, Ca and Mg were not affected by P source (data not shown), but the levels of all these elements were reduced by the presence of Al.

Phosphorus acquisition by plants from aluminium phosphates must involve a coincidental dissociation of Al into the nutrient solution. Calculations based on P tissue concentrations noted in these experiments indicate that the release of Al may have been as high as 4,5 mg dm^{-3}. Root concentrations of Al (Table 5) do not completely support this contention, since Al uptake appears substantially reduced, notably when aluminium phosphate was the only source of Al.

An increase in nutrient solution pH over the control (KH_2PO_4) was, however, noted with time in both cultivars when P was supplied as aluminium phosphate without added Al (Figure 4). The addition of Al to the nutrient solution was more usually connected to gradual acidification of the solution (Figures 3 and 4.) Increases in solution pH are therefore unlikely to be initiated by the release of Al from aluminium phosphate and more probably arise in response to altered ionic balances in the plant (Riley and Barber, 1969), brought about by lower P uptake. Increases in nutrient solution pH may nevertheless, represent a factor controlling

Al ion speciation which could conceivably influence Al uptake through the formation of a solid phase $Al(OH)_3$ at pH's above 5,0 (Lindsay, 1979).

Table 5 : Root concentrations of Al for plants grown in full-strength nutrient solutions containing either KH_2PO_4 or aluminium phosphate to which Al had been added to give concentrations of 0; 0,5; 5; 10 mg dm^{-3}. Plant growth period 14 days.

P Source		Root Al conc. mg kg^{-1}			
		Aluminium phosphate		KH_2PO_4	
		TX 24	HL 1	TX 24	HL 1
Al added ($mg\ dm^{-3}$)	0	99	661	-	-
	0,5	-	-	559*	3082*
	5	4358	3825	4375*	3248*
	10	4225	3538	5248*	4624*

* (data derived from Chapter 8.)

2.2.6 Depletion of Nutrients by Plant Growth

Changes in solution composition will arise as a consequence of nutrient uptake by plants. The rate of depletion of the nutrient solutions will be determined by the initial concentration of the ion, the volume of solution provided for each plant and the growth rate of the plant. A serious criticism arising from many investigations has involved the high initial concentration of some ions employed in an attempt to maintain adequate total amounts of nutrients in conveniently small volumes of solution (Asher and Edwards, 1978). Nutrient solution formulations frequently employ

ionic concentrations well above those encountered in the soil solution (Hewitt, 1966; Asher and Edwards, 1978). Evidence (Clark, 1982) also indicates that the growth rate of Zea does not respond to P added above relatively low levels, and increased growth was not reported for P concentrations outside the range 2 - 4 mg dm^{-3} supplied at 0,76 dm^{3} per plant over a ten day growing cycle.

The concentration of anions and cations in displaced soil solutions taken from tropical soils are generally much lower than those encountered in temperate soils (Asher and Edwards, 1978). Plants can tolerate a range of mineral element concentrations (Hewitt, 1966; Clark, 1982) and it is therefore significant that only the P level in the full-strength nutrient solution exceeded the concentration range expected to be encountered in the soil solution (Table 6).

Table 6 : Comparison of nutrient solution concentrations with the levels of anions and cations encountered in soils of temperate and tropical origin (Asher and Edwards, 1978).

	Nutrient soln.	Modal Conc. Temperate Soils	Range for 6 Tropical Soils 0 - 10 cm
		mg dm^{-3}	
Anions			
nitrate	61	550	0,6 - 32
sulphate	28	115	0,1 - 190
phosphate	6	0,1	0,2
Cations			
Mg	10	75	1 - 21
Ca	40	76	0,4 - 26
K	40	30	4 - 31

Nutrient solutions were not renewed during the course of the experiments. The extent of changes in composition arising from plant growth over a 15 day period, based on the analysis of the nutrient solutions at commencement and termination of the experiment are presented in Figure 5 for P, K, Ca and Mg (mean values obtained from 27 pots). In addition, analysis of plant fractions at the conclusion of the experiment indicated that the uptake of nutrient ions had depleted the solution concentrations by the amounts shown (%): N 22; Mn 25; Zn 38; Cu 14; Fe 2. These data suggest that insofar as can be determined from Table 6, the nutrient levels remaining in solution after 15 days were still adequate.

2.2.7 Plant Containers

The nutrient solution was contained in plastic buckets fitted with lids pre-drilled to give twenty-four 19 mm diameter holes. Each lid had a plastic screen fastened across the bottom. A single, pregerminated grain was placed in each of twenty-three holes, resting on the screen, with the radicle protruding through the screen into the nutrient solution. The remaining hole was left free to facilitate the measurement of solution pH by inserting a combination electrode directly into the nutrient solution. The nutrient solution was maintained in contact with the screen so that the roots were submerged at all times. The volume of solution in each bucket was 25 litres (1,08 litres/plant) and the solution level was maintained by the daily addition of sufficient de-ionised water to offset losses due to evaportranspiration.

The growth of *Zea* is inhibited by a lack of aeration (Clark, 1982). Continuous aeration of the nutrient solution from aquarium air pumps was therefore provided. Uniform distribution of air across the plant container was achieved through the use of formed rigid plastic aeration tubes.

Plant support was provided by removable frames covered with plastic mesh (10 mm diameter) which fitted across the top of

the plant containers. Plant containers were located on moveable trolleys, placed on a wooden platform above floor height.

Contamination of the nutrient solutions by agents used in the manufacture of plastic materials has been discussed by Hewitt (1966). It is significant that Al is not listed as a potential contaminant. Prior to use, all plastic pots used in these experiments were, however, filled with deionised water acidified to pH 2,5 with HCl and allowed to stand for seven days. Equipment was thoroughly washed, refilled with deionised water and allowed to equilibrate for fourteen days before the contents of the pots were analysed. The presence of (mg dm^{-3}) Ca $<$ 0,18; Mg $<$ 0,01; Zn $<$ 0,02 were detected. Levels of P, K, Mn, Cu, Fe and Al were below the detection limits of available analytical equipment. Nitrogen, Mo and B were not determined.

2.2.8 Plant Growth Conditions

Grains were washed and soaked overnight (16 h) in deionised water. Imbibed grains were placed between layers of moist paper towels in petri dishes and maintained at a temperature of 25°C in the dark. Germination usually occurred within 36 - 48 h. Cultivar HL 1 was consistently slower (12 h) than cv. TX 24 to germinate. Germinated seeds were transferred directly to the nutrient solution once the radicles were 7 - 10 mm long.

Artificial light was supplied to give a 14 h light, 10 h dark regime with alternating temperatures of 28 : 23°C. The light source was Gro-lux W.S. (731,3 Wm^{-2}) and incandescent (113,4 Wm^{-2}) lamps to give a light intensity of 300 μ E m^{-2} s^{-1} at plant height.

The plant growth period varied with experimental requirements but did not exceed fifteen days.

2.2.8.1 Decapped Root Experiments

In Zea, the root cap is delimited from the root by a

distinct boundary between the two cell populations (Barlow, 1975). The root apex is also sufficiently large and robust to allow surgical manipulation of the cap leading to partial or complete removal.

Plant growth procedure employed in experiments, which involved the removal of the root cap of the primary root, differed from that already described, in the following respects: Pregerminated grains of Zea mays cv. TX 24 were planted along the upper edge of 66 X 22 cm filter paper. Grains were arranged with the radicles pointing downwards. The paper was loosely rolled and placed vertically in a glass container with 5 cm depth of half-strength nutrient solution. This 'Rag-Doll' technique has been more fully described by Konzak et al. (1976).

Plants were grown in the dark for three days at 25°C to give a radicle length of 6 - 10 cm. Plants were then selected for a straight root axis and the main root was decapped under a binocular microscope by making a shallow scalpel cut in line with the apical dome and easing the cap away from this point (Feldman, 1975; Hillman and Wilkins, 1982).

Experiments were carried out directly on plants with decapped roots which were transferred after the decapping operation to glass vessels containing half-strength nutrient solution, supported on plastic screens and grown with continuous aeration under the conditions indicated.

2.3 HISTOCHEMISTRY

2.3.1 Detection of Aluminium

The detection of Al in plant tissue at the cellular level is complicated by the low atomic mass (26,98), the absence of a radioactive tracer for Al, and the idea that Al and P co-precipitate within root cells as insoluble aluminium phosphates (Wright, 1943). Methods which have been applied include

histochemical procedures (Hoffer and Carr, 1923; McLean and Gilbert, 1927; Kalovoulos and Misopolinos, 1983), electron microprobe X-ray analyses (Rasmussen, 1968), and the indirect determination of Al by the application of electron-dense materials (Mo) to local concentrations of P. This procedure relies on the assumption that localisation of P can be attributed to the presence of Al (McCormick and Borden, 1972; 1974).

Many of these techniques (Hoffer and Carr, 1923; McCormick and Borden, 1974) are characterized by severe chemical pretreatment of plant material. The effect of these treatments on the distribution of Al ions must be considered in assessing the reliability of experimental procedures, since Clarkson (1967) has shown that Al was extracted from cell wall material by dilute HCl (10^{-3} M). Furthermore, comparisons involving SEM/EDX analysis of Al-treated root material (cv. TX 24), subject to freeze-drying and critical point drying, indicated that root caps of freeze-dried material contained on average 43,6% more Al. Material for critical point drying was fixed overnight in 6% glutaraldehyde, buffered in sodium cacodylate to pH 7,2 and dehydrated in graded alcohol (Anderson, 1951). This result differs from those reported by McCormick and Borden (1974) who concluded that glutaraldehyde was without effect on the intensity of blue colour development in the Mo/P procedure. These observations suggest that Al is preferentially extracted during fixation, while glutaraldehyde has little effect on tissue P levels. While freeze-dried material may be satisfactory with respect to the absence of chemical redistribution of Al ions, adequate structural preservation of freeze-dried material was only achieved with considerable difficulty. In this respect, results accord with those of Rasmussen (1968). Preservation of structural integrity of test tissue is a prerequisite for the identification of Al uptake sites in the root.

2.3.1.1 The Use of Hematoxylin* Stains

Aluminium in plant tissue has a long association with hematoxylin, and Hutchinson (1945) has indicated that many Al-accumulating plants have been identified following the use of the plant material in the traditional technology of dyeing. Hematoxylin is widely used in biological microtechniques (Gill et al., 1974) and a number of investigators (Hoffer and Carr, 1923; McLean and Gilbert, 1927; Wright and Donahue, 1953) have applied these procedures to the detection of Al in plant tissue. Some criticisms with respect to the severity of these experimental techniques have already been made. More recently, Polle et al. (1978) have used a half-oxidised hematoxylin formulation (Gill et al., 1974) to stain living plants (Triticum aestivum) for the visual assessment of Al tolerance.

Confirmation of the applicability of this screening technique has been reported from Japan (Takagi et al., 1981).

In aqueous solution, hematoxylin is colourless and subsequent histochemical use is dependent on oxidation to hematein (Gill et al., 1974). Theoretical considerations involved in the oxidation of hematoxylin are extensively discussed by Gill et al. (1974) as a basis for proposals involving the use of $NaIO_3$ as an oxidant. Hematoxylin formulations which include $NaIO_3$ do not require boiling to release oxygen (Gill et al., 1974).

Hematein carries a negative charge (Gill et al., 1974) and functions as a basic (cationic) dye. The subsequent chemical combination of the dye to form an insoluble, coloured compound (mordant) is dependent on the presence of positively charged metallic ions. The aluminium/hematein lake arising from these

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* Hematoxylin is extracted from the Central American logwood (Haematoxylen campechianum L.) (Baker, 1958) and is not to be confused with haematoxylen, which is logwood containing some 10% hematoxylin (Gill et al., 1974).

reactions is characterized by a dense, violet/blue colour (Gill et al., 1974).

2.3.1.2 Sensitivity of Hematoxylin Staining

Uncertainty exists with respect to the sensitivity of hematoxylin staining procedures applied to studies of Al in plant tissue, since positive identification of Al compounds present in plant tissue remains to be achieved. These have been variously considered to include insoluble aluminium phosphates (Wright, 1943; Wright and Donahue, 1953; Adams, 1980), amorphous aluminium hydroxides (Clarkson, 1967) and complexes between Al and a range of organic materials which include mucilagenous polysaccharides (Horst et al., 1982) and nucleic acids (Matsumoto et al., 1976; Naidoo et al., 1978). Possible interferences arising from the presence of cationic substances must also be considered.

It is therefore reassuring to find that Dalal (1972) has established on a quantitative basis, that determination of Al present in soil extracts by hematoxylin proved six times more sensitive than the standard aluminon method (Frink and Peech, 1962). Moreover, hematoxylin was considerably less sensitive to the presence of interfering ions (P, Ca, Mg), while colour response of Al to hematoxylin was enhanced by Fe and Mn. The recovery of Al was also not seriously affected by the presence of complexing agents which included fluoride, oxalate, citrate and E.D.T.A.

2.3.1.3 Experimental Procedure

Hematoxylin stain was prepared by dissolving Merck 4305 hematoxylin (2g dm^{-3}) and $NaIO_3$ (0,2g dm^{-3}) in deionised water (Gill et al., 1974; Polle et al., 1978).

The staining procedure followed that recommended by Polle et al. (1978). Roots of whole plants were briefly rinsed (1 minute) in flowing deionised water before soaking for 60 minutes in vigorously aerated deionised water (2 dm^3). The plants were then transferred to the staining bath and the roots submerged in hematoxylin solution (1 dm^3) with aeration for fifteen minutes. Staining was followed by a brief rinse (1 minute) in flowing deionised water and washing in aerated deionised water (2 dm^3) for a further thirty minutes. The development of an intense violet/blue colour was considered indicative of the presence of Al. The intensity of stain was found to diminish in plants allowed to stand in deionised water for periods in excess of 6 h.

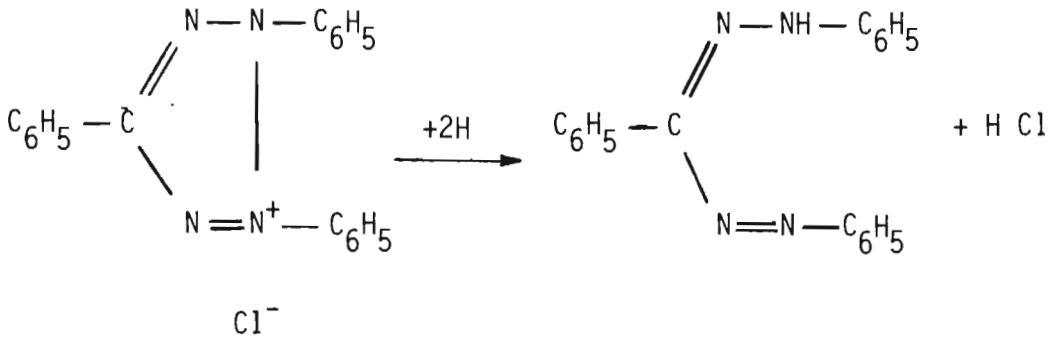
2.3.2 Identification of Respiratory Gradients within the Root Apex

Gradients in respiratory activity between the cell populations comprising the root apex may be expected to reflect the differences reported in mitotic activity (Clowes, 1961), and changes in physiological function associated with cellular differentiation, occurring notably in the cells of the root cap (Barlow, 1975). Methods used to estimate respiratory activity in plant organs, extensively reviewed by Rees (1980), are frequently based on measurements of gaseous exchange (O_2 or CO_2) (Yemm, 1965). These procedures may not be suitable for detecting changes in respiration which may occur between cell populations as a consequence of Al treatment, and it is relevant that Clarkson (1969) was unable to relate reduced O_2 uptake by the root (Hordeum vulgare) to a decline in mitotic activity following Al treatment.

2.3.2.1 Demonstration of Dehydrogenase/Diaphorase Activity with Tetrazolium Salts

The histochemical demonstration of sites of dehydrogenase/diaphorase enzyme activity through the use of tetrazolium salts is widely practised (Barka and Anderson, 1963;

Pearse, 1972). These procedures depend on the tetrazolium salt acting as an electron acceptor (Pearse, 1972) during the controlled release of respiratory energy from metabolites, which occurs along a chain of enzyme catalysed oxidation reduction reactions, culminating in the reduction of molecular oxygen to water (Storey, 1980). The histochemical usefulness of these substances is further dependent on the conversion by reduction of the water-soluble, near-colourless tetrazolium salt to a water-insoluble, highly-pigmented formazan (Barka and Anderson, 1963; Pearse, 1972) as outlined in the equation:



Tetrazolium salt (TTC)
(colourless)

Formazan
(bright red)

The histochemical development of a number of tetrazolium salts is reviewed by Pearse (1972). Differences between salts, which may reflect changes in Redox potentials (Barka and Anderson, 1963) have been exploited in the identification of the location of dehydrogenase/diaphorase enzyme systems, since the salts characteristically act as electron acceptors at different points along the respiratory chain (Barka and Anderson, 1963; Pearse, 1972; Horobin, 1982). It is pertinent that triphenyl-tetrazolium chloride (TTC) used in these experiments receives electrons from cytochrome 3 (Barka and Anderson, 1963; Pearse, 1972), that is, at the end of the respiratory chain immediately preceding the reduction of oxygen to water.

2.3.2.2 Triphenyl Tetrazolium Chloride (TTC)

The Redox potential reflects the ease with which tetrazolium salts are reduced and salts with low Redox potentials (TTC) are not generally considered for the fine location of enzymes (Pearse, 1972). Triphenyl-tetrazolium chloride is however, readily available since it is widely used to test seed viability (Barka and Anderson, 1963; Gurr, 1965). Furthermore, as the objectives of this investigation included a wider spectrum of application extending to the cell populations of the root apex, development of appropriate techniques were considered worthwhile.

Factors other than Redox potential likely to increase the rate of reduction of TTC include higher salt concentrations and mildly alkaline pH levels (Barka and Anderson, 1963). An element of caution is also required since these factors have been connected to toxicity, altered enzyme kinetics and damage to mitochondria (Barka and Anderson, 1963; Pearse, 1972).
The rate of entry of TTC into the tissue is, however, unlikely to limit the reaction rate, since Pearse (1972) has noted that the diffusion constant is sufficiently high to permit rapid penetration.

Some compromise between TTC concentration and solution pH may therefore be required to achieve optimum red formazan colour development with acceptably short treatment times. The following procedure was found to give reproducible results when applied to the roots of Zea mays.

2.3.2.3 Experimental Procedure

Tetrazolium solution (4,6 m M) was prepared by dissolving 2 : 3 : 5 triphenyl-tetrazolium chloride (TTC) (BDH 34072 ; MW = 334,81) in phosphate buffer.

Phosphate buffer was prepared from 0,067 M Na_2HPO_4 and 0,067 M KH_2PO_4 to give pH 7,2 (Vogel, 1955).

Roots of intact plants were submersed in TTC solution for 1,5 h at 25°C in the dark.

Note 1: The concentration of TTC is considerably lower than that used for testing seed viability (Gurr, 1965) and approximates the optimum range considered by Glick and Nayyar (1956).

Note 2: Temperature markedly influenced colour development. At 20°C the development of red formazan was found to be negligible even after 4 h root treatment.

Note 3: The red colour developed in root tissue was permanent and was not diminished in intensity by 6 h soak in deionised water.

2.3.3 Distribution of Muco-polysaccharide Materials in Root Tissue

2.3.3.1 Methylene Blue Extinction (M.B.E.) Test

Gurr (1958) has noted that acidic substances are stained by methylene blue with increasing intensity at pH values above their isoelectric point, while below this point, the capacity to bind the stain diminishes sharply. The ability of plant tissue to bind methylene blue at pH values below 4,0 has therefore been considered indicative of the presence of acid muco-polysaccharides (Gurr, 1958).

Polysaccharide materials have been widely associated with high molecular-weight mucilagenous root cap slimes (Wright and Northcote, 1974; Chaboud, 1983). The degree of affinity shown by root tissue for the basic dye (methylene blue) was considered a basis for the identification of polysaccharide materials and for the subsequent characterization on a comparative basis of the effect of Al on the synthesis of these substances.

The following procedure was found to give

reproducible results.

2.3.3.2 Experimental Procedure

A 1% aqueous solution of methylene blue was diluted by adding 0,64 ml to 100 ml deionised water (Gurr, 1958) and adjusting the pH to 3,98. The roots of intact plants were rinsed in deionised water and then stained in the methylene blue solution for 45 minutes at 27°C. The capacity of the tissue to bind methylene blue at this pH was considered indicative of the presence of acid muco-polysaccharides.

Stained material was stable in deionised water for up to 1 h. The stain lacked permanence in root tissue temporarily mounted (Section 2.4.1) in glycerine/water mixtures.

2.4 LIGHT AND ELECTRON MICROSCOPY

2.4.1 Freeze Sectioning for Light Microscopy

Pre-stained (Sections 2.3.1.3; 2.3.2.3; 2.3.3.2) root material used for histochemical investigation was sectioned frozen on a Reichert, Thermo-Electric Cold Stage Microtome. Temporary mounts for sections were provided in a 50% glycerine/water mixture. Sections were viewed on an Olympus B H - 2 microscope and photographed on Kodachrome 25 film.

Freeze sectioning was applied in these studies in order to avoid interferences arising from chemical agents employed in conventional fixation, dehydration and embedding procedures. For the same reason, the use of "anti-freeze" media, extensively described by Knox (1970) and Benes (1973) as aids to freeze-sectioning of plant material, were also avoided. The procedure involved freezing of root tip material directly in deionised water immediately prior to sectioning. This technique is considered reliable with respect to the possible redistribution of ions (Lauchli, 1973), but material frozen in this way may present

difficulty with respect to damage arising from the formation of ice crystals within the tissues (O'Brien and McCully, 1981). The freezing rate of root tips was not determined but, in an attempt to minimize freezing damage, material was frozen at the maximum rate provided by the capacity of the equipment available.

Knox (1970) has indicated that freezing and/or thawing damage may be most noticeable in differentiated tissues with rigid cell walls further characterized by the highly vacuolated nature of the cytoplasm, and it is therefore significant that freeze-sectioning techniques have previously been most successfully applied to meristematic tissue (Knox, 1970; O'Brien and McCully, 1981), which was the type of tissue which was of particular interest in this study.

2.4.2 Fixation, Dehydration and Embedding of Material

Root segments ($\leq$ 2mm long) for study at the light and transmission electron microscope levels were fixed in 6% glutaraldehyde in 0,05 M sodium cacodylate buffer at pH 7,2 for a minimum of 24 h, with post-fixation (2 h) in 2% OsO_4 in sodium cacodylate buffer (Hayat, 1981).

Residual glutaraldehyde which is reported to precipitate in the presence of OsO_4 (Hayat, 1981), was removed from the specimens by 2 X 30 minute washes in 0,05 M cacodylate buffer prior to the post-fixation treatment. Excess OsO_4 was washed from root material by successive rinses (2 X 30 minutes) in 0,05 M cacodylate buffer.

All free water from fixed and washed specimens was extracted by dehydration in graded alcohol involving successive treatments of 8 - 10 minutes duration in 10%; 20%; 30%; 40%; 50%; 60%; 70%; 80%; 90% and 100% (three washes) ethanol, followed by two 30 minute steps in 100% propylene oxide.

Material was embedded in a mixed resin (Hayat, 1981)

comprising one part Epon 812, one part Araldite 6005 and three parts DDSA (dodocenylsuccinic anhydride). Prior to use, DMP - 30 (2, 4, 6 tri (dimethyl aminomethyl phenol) (5 drops per ml) was added to the resin as an accelerator. Uniform infiltration of tissue by the embedding medium was achieved by gradually decreasing the proportion of solvent (propylene oxide) and proportionately increasing the concentration of embedding medium according to the schedule outlined in Table 7.

Table 7 : Epon/Araldite embedding schedule applied to segments of root tissue.

Resin Change	Propylene oxide %	Embedding resin %	Infiltration time (h)	temp °C
1	75	25	2	room temp.
2	50	50	2	"
3	25	75	overnight	"
4	-	100	24	"
5	-	100	48	70°

Sections (1 μm) for light microscopy were cut with glass knives. Ultra-thin (gold/silver) sections were cut with diamond knives and mounted on copper grids for transmission electron microscope viewing on a Joel 100 CX electron microscope at an accelerating voltage of 80 k V.

2.4.3 Staining Resin Embedded Material for Light Microscopy

Resin embedding media are considered to provide advantages for light microscopy, including lower distortion and higher information content after staining, over the older paraffin wax techniques (Bennett et al., 1976). In sharp contrast to the

situation with wax-embedded sections, conventional staining procedures are almost useless with epoxy sections (O'Brien and McCully, 1981), and successful procedures for staining epoxy sections are essentially limited to the use of cationic dyes (Bennett et al., 1976; O'Brien and McCully, 1981). Resin-embedded plant tissue is strongly stained by these materials (e.g. azure II, methylene blue, toluidene blue), although the colour contrasts are usually poor (O'Brien and McCully, 1981). The problem is aggravated in studies of Al toxicity, since evidence (Chapter 3) indicates that the plant response to Al may include a diminished ability of root tissue to bind methylene blue.

Methylene blue and Ladd Multiple Stain (70955) nevertheless proved the most successful of the stains tested when applied to root apical tissue, and these stains were used in this investigation. Ladd Multiple Stain was, however, found to lack permanence, and sections faded appreciably within a few weeks. Stained sections were preserved by mounting in DPX Mountant (BDH 36029). Material was viewed on an Olympus B H - 2 microscope. Contrast in black and white photomicrographs was improved, notably in the case of root tissue from Al-treated plants, by the use of red (methylene blue) or green (Ladd Multiple Stain) filters. Material was photographed on Agfapan 25 film.

2.4.4 Staining Sections for Transmission Electron Microscopy

Electron opacity of sectioned material was selectively enhanced by staining for 15 minutes duration in aqueous solutions of lead citrate and uranyl acetate (Reynolds 1963; Hayat, 1981).

2.5 CHEMICAL ANALYSIS OF PLANT MATERIAL

2.5.1 Experimental Procedure

The procedures involved in the preparation and dry ashing of plant material for chemical analysis have been adapted

from the methods more fully described by Hunter (I.S.F.E.I.P. 1971 and 1972).

Plant material was dried at 70°C and milled to pass a 0,84 mm stainless steel screen before storing in tightly stoppered glass bottles.

A 0,5g sample of dried and milled plant material was ashed overnight (16 h) in a pyrex glass beaker at 450°C. The ashed sample was cooled, moistened with deionised water and about 2 ml concentrated HCl was added prior to evaporating to dryness. The residue was taken up in 15 ml M HCl, filtered and used with suitable dilutions to determine K, Ca, Mg, Mn and Al by atomic absorbtion spectrophotometry (Allan, 1970) and P, colourimetrically using the molybdovanadate procedure (Technicon, 1972).
A nitrous oxide-acetylene flame was used to determine Al. Strontium (2500 mg dm^{-3}) was used to suppress interferences between elements in the determination of Ca and Mg.

CHAPTER 3

ALUMINIUM UPTAKE AND TRANSPORT

3.1 INTRODUCTION

Little is known of the physiological factors which control Al uptake and transport in plants. The mobility of Al in most plants is, however, considered to be low (Jackson, 1967) and, with the exception of Al-accumulating plants (Chenery and Sporne, 1976), plants treated with Al in the growth medium are frequently characterized by high root concentrations of Al, with little or no movement into the leaves (Rhue, 1979; Chapter 8).

The failure of Al to be transported through the vascular system has been ascribed to the internal precipitation of insoluble aluminium phosphates (Jones, 1961; Middleton and Smith, 1982) at the cell surface and within the root-free space (Haynes, 1980). It is, however, pertinent to this suggestion that conclusive evidence is lacking to indicate that plant interactions between Al and P represent a primary response to Al (Wallihan, 1948; Clarkson, 1967). Furthermore, Al tolerance was observed to be poorly correlated with mobility of the element in plants drawn from a wide range of ecological conditions (Hou and Merkle, 1950).

Depression of root elongation as a result of reduced mitotic activity remains the most obvious effect of Al uptake (Clarkson, 1965). Low mobility of Al in the plant consequently attaches significance to the root apex as a site of Al uptake, since it may be expected that the rapidity with which a decline in the rate of cell division is expressed in Al-treated plants (Clarkson, 1965) cannot be reconciled with low mobility, unless the sites of uptake and action are nearly coincidental.

Published research on Al uptake is infrequent (McLean and Gilbert, 1927; Wright and Donahue, 1953; Rasmussen, 1968; Kalovoulos and Misopolinos, 1983) and the function of the root

epidermis in controlling entry of Al into the root is controversial (Wright and Donahue, 1953; Rasmussen, 1968). Clarkson (1967) has also demonstrated (Allium cepa) that the initial high rate of Al uptake was not maintained with time, and this may mean that relatively long Al treatment periods, featured in a number of earlier studies (Rasmussen, 1968; Kalovoulos and Misopolinos, 1983), may provide little information on the initial site(s) of uptake. Identification of the site(s) of Al uptake and accumulation in the root nevertheless remains fundamental to the elucidation of the mechanism of Al toxicity, and it is therefore the purpose of this Chapter to trace the chronological pattern of Al uptake by the primary root (Zea mays), and to suggest mechanisms for the control of uptake and mobility of Al in Al-treated plants.

3.2 EXPERIMENTAL TREATMENT

3.2.1 Experiment 1

This experiment was designed as a rapid means of locating Al uptake sites in large numbers of unsectioned roots. Plants (cvs. TX 24 and Hl 1) were grown in full-strength nutrient solutions (Sections 2.2.2; 2.2.8) for seven days, $Al_2(SO_4)_3$ solution was added in advance of harvest to give 0 h (control); 1 h; 2 h; 5 h; 24 h; 96 h and 168 h root exposure to 8 mg dm^{-3} Al in solution. A minimum of twenty plants was sampled from each treatment and the roots stained in hematoxylin (Section 2.3.1.3).

3.2.2 Experiment 2

This experiment investigated at the light microscope level the chronological sequences involved in the distribution of Al between the cell populations of the primary root apex, as well as Al uptake by emerging lateral roots, evident with 48 h total root exposure to Al. Plants (cv. TX 24) were grown in full-strength nutrient solution (Sections 2.2.2; 2.2.8) for seven days, $Al_2(SO_4)_3$ solution was added in advance of harvest to give 0 h (control); 0,5 h; 2 h; 12 h; 20 h and 48 h root exposure to

8 mg dm^{-3} Al in solution. A minimum of five roots was examined from each treatment. Root material, prestained in hematoxylin was sectioned frozen (Sections 2.3.1.3; 2.4.1).

3.2.3 Experiment 3

The rôle of the root cap in restricting entry of Al into the cells of proximal meristem and quiescent centre was investigated by growing plants (cv. TX 24) with (control) or without root caps in a half-strength nutrient solution (Section 2.2.8.1) containing 8 mg dm^{-3} Al for 20 h at 22°C. Roots, prestained in hematoxylin, were sectioned frozen (Sections 2.3.1.3; 2.4.1).

3.2.4 Experiment 4

The distribution of acid muco-polysaccharide materials was determined in Al-treated roots and the controls (0 Al) by transferring three day-old seedlings (cv. TX 24) with intact roots to half-strength nutrient solutions containing either no Al (control) or 8 mg dm^{-3} Al as $Al_2(SO_4)_3$. Plants were grown for 20 h at 27°C prior to staining the roots in methylene blue solution (Section 2.3.3.2). Material was sectioned frozen (Section 2.4.1).

3.2.5 Experiment 5

Plants (cvs. TX 24 and HL 1) were grown for fourteen days in full-strength nutrient solutions (Sections 2.2.2; 2.2.8) which contained Al added at the levels of 0 (control); 2 and 4 mg dm^{-3}. Without removing the plants from the nutrient solution, the root system was excised at the junction with the grain, and the cut, basal end inserted into a plastic tube and sealed (Anderson, 1975). Xylem fluid was collected after 2 h. The limited quantity of exudate produced required that fluid from each treatment (23 plants) be combined for pH measurement. Nutrient solution pH was also determined at the conclusion of the experiment.

3.3 RESULTS

3.3.1 Location of Aluminium Uptake Sites

3.3.1.1 Experiment 1

The results of Al uptake studies with unsectioned primary roots are presented in Figures 6A to 6D and 7A and 7B.

In the control treatments (0 Al), roots were notable for the complete absence of stained tissue (Figure 6A). Al uptake by Al-treated roots, as indicated by the development of an intense violet/purple stain, was initiated in the distal regions of the root apex and was visually apparent within 1 h.* The pattern of Al uptake was not significantly altered, except in the intensity of colour development in stained tissue during the first 5 h (Figures 6B and 6C) and this was considered indicative that roots continued to rapidly absorb Al during the initial stages of the experiment. In all cases, Al uptake was limited to the apical few mm of the root except where root tissues showed obvious damage (Figure 6C). The remainder of the root axis remained free of stain.

Aluminium treatments exceeding 24 h (Figure 6D) were associated with the development of lateral roots along the main axis, extending ultimately (96 h and 168 h) to within a few mm of the root tip (Figures 7A and 7B). The development of lateral roots influenced the overall pattern of Al uptake, since the entry of Al was rapid but localised once the emerging root tip had penetrated the epidermis. Differences in the location of the sites of Al uptake in the root did not emerge between cultivars.**

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* The cost of printing colour illustrations has necessitated the omission of some treatments from the plates. Limited numbers of colour prints of these treatments are available on request.

** Cv. TX 24 preferred for subsequent cytological studies as the root material proved easier to handle.

3.3.1.2 Experiment 2

The results of a time-course study of Al uptake by the cell populations of the root apex are presented in Figure 8. The extent of the cell populations comprising the root apex and primary growth region of the root axis as referred to in this experiment, has been defined by Barlow (1975) and Luxová (1981), and is further illustrated in Figure 9.

Figure 8A, the control treatment with no Al added, confirms the complete absence of stain from all tissue. The distribution of stained tissue (Figures 8B to 8D) showed that the initial sites of Al uptake includedthe root cap and mucilagenous secretions covering the epidermal cells of the root apex. The presence of Al in the peripheral cap cells could be demonstrated with 30 minutes exposure to 8 mg dm^{-3} Al. Al was also rapidly absorbed onto the outer surface of the root epidermis (Figures 8B and 8C).

Aluminium spread rapidly through the cells of the root cap (Figures 8C and 8D) although the cap initials were the last group of cells within the root cap to be reached with 12 h treatment.*

Prolonged Al treatment led to the accumulation of appreciable quantities of Al in the outer cortical cells (Figure 8D). These changes were apparent within 12 h and were accompanied by gross morphological disorganization of the root apex, leading ultimately to disintegration of the outer cells of the root in the apical 1,5 - 2,0 mm (Figure 8D). Severe cellular disorganization and failure of the epidermis did not, however, result in appreciable quantities of Al being found in the

* The cost of printing colour illustrations has necessitated the omission of some treatments from the plates. Limited numbers of colour prints of these treatments are available on request.

inner cortical regions or the stele (Figure 8D).

Transverse sections of the root axis taken 5 mm from the root tip to include developing lateral roots* (48 h) indicated that Al uptake was limited to defined areas of the emerging root tip. The cortical cells of the main root did not respond to hematoxylin stain, thereby confirming the absence of Al from these tissues.

3.3.2 The Effect of Cap Removal

3.3.2.1 Experiment 3

The distribution of Al in the root apex as shown by hematoxylin staining in decapped and whole roots, is illustrated in Figures 10A and 10B. The pattern of Al uptake was considerably altered in decapped roots with the accumulation of substantial amounts of Al in the cells of the quiescent centre and the proximal meristem with 20 h treatment with 8 mg dm^{-3} Al. Significantly, penetration of Al in the decapped root did not extend to the inner cortical regions or the central vascular tissue (Figure 10B). The absence of Al from the quiescent centre and substantial areas of the proximal meristem of the control (Figure 10A) may implicate the root cap in protection of these tissues from Al.

Furthermore, neither the control nor the decapped root showed the level of cellular disorganization found in the previous experiment, and the epidermis remained intact for the period of treatment. Aluminium was nevertheless found in considerable quantities in the epidermal and outer cortical cells of the root (Figures 10A and 10B), thereby confirming that failure

* The cost of printing colour illustrations has necessitated the omission of some treatments from the plates. Limited numbers of colour prints of these treatments are available on request.

of the epidermis was not a prerequisite for entry of Al into the root.

The movement of Al into the cap columella and cap initials of the control (Figure 10A) was less rapid than in the previous experiment.

3.3.3 Acid Muco-polysaccharide Materials

3.3.3.1 Experiment 4

The apical 2 - 3 mm of intact roots showed a strongly preferential capacity to bind methylene blue, while the remainder of the root axis had only a limited capacity to take the stain (Figure 7C). In longitudinal section there were parallels between the cell populations stained with methylene blue (Figure 10C) and the regions of the root associated with Al uptake and accumulation (Figures 8B to 8D and Figure 10A) namely, the cells of the root cap and epidermal and outer cortical cells. Cells of the quiescent centre and proximal meristem were found to be stained with methylene blue (Figure 10C) but not hematoxylin in intact roots (Figures 8B to 8D and Figure 10A). Substantial amounts of Al were, however, found in these cells in decapped roots (Figure 10B). Significantly, the inner cortex and stele had only a limited capacity to bind methylene blue. Reference to Figures 10A and 10B shows that these tissues do not accumulate Al in decapped or intact roots.

Aluminium was also shown to reduce the roots' capacity to bind the stain. With 20 h treatment with 8 mg dm^{-3} Al, intensive methylene blue staining was limited to the root cap and the root meristem proximal to the cap/root junction (Figure 10D). In contrast to the control (Figure 10C), only isolated stain of low intensity was evident in the outer cortical-epidermal regions of Al-treated roots (Figure 10D).

3.3.4 Xylem Exudate pH

3.3.4.1 Experiment 5

This experiment demonstrated that cultivars TX 24 and HL 1 both maintained the pH of xylem sap exudate at levels well above those reflected by the nutrient solutions (Table 8). Furthermore, the pH of the xylem exudate was regulated to narrow limits (pH 5,33 - 5,63) and was not apparently influenced by either the final pH of the nutrient solution or the presence of Al. Important differences in mean pH over the range of experimental treatments did not emerge between cultivars TX 24 (pH 5,53) and HL 1 (pH 5,52).

Table 8 : The effect of different concentrations of Al (0; 2; 4 mg dm^{-3}) in the nutrient solution on the pH of xylem exudates collected from 14 day-old plants of *Zea mays* (cvs. TX 24 and HL 1). Technique described in Section 3.2.5

Solution Al mg dm^{-3}	TX 24		HL 1	
	pH nutrient solution	pH xylem exudate	pH nutrient solution	pH xylem exudate
0	4,57	5,63	4,93	5,46
2	4,42	5,33	4,14	5,58
4	4,15	5,62	4,15	5,54

3.4 DISCUSSION

The results of this investigation differ from previous research on Al uptake in *Zea* (Rasmussen, 1968; Kalovoulos and Misopolinos, 1983) and *Triticum* (Wright and Donahue, 1953) with respect to identification of the chronological pattern of Al uptake by the primary root and in the interpretation of biochemical factors which may control Al uptake and transport in the plant. This information provides a basis for speculating on the probable location of the site of Al injury in the root, and indeed on the mechanism of Al toxicity.

3.4.1 Aluminium Uptake

Experiments with unsectioned roots clearly indicate that Al uptake sites are located in the root apex, although an Al-induced loss of apical dominance may be expected to give rise to secondary uptake through the emerging lateral root tips. Aluminium treatment times (96 h and 168 h) which were associated with secondary uptake, approximate the experimental conditions of previous investigations (Rasmussen, 1968; Kalovoulos and Misopolinos, 1983) which supports the view that these reports may provide little information on the initial site of uptake. The present time-course experiments have nevertheless, connected the peripheral cap cells with the primary site of Al uptake. Plant reaction to toxic levels of Al is known to be extremely rapid (Clymo, 1962; Clarkson, 1965) and since these cells are the first to take up Al, the peripheral cap must also be considered as a primary target for Al injury. Aluminium is also rapidly adsorbed onto the outer surface of the root apex where it may be complexed by mucilage (Horst *et al.*, 1982).

Earlier researchers (Wright and Donahue, 1953; Rasmussen, 1968) have differed in their interpretation of the effectiveness of the epidermis in preventing entry of Al into the root. While Al concentration may be relevant, this investigation has demonstrated that Al does enter the cells of the root through the

epidermis, in the apical regions only. Over the remainder of the primary root axis, secondary uptake is limited to emerging lateral root tips. Significance is also attached to the observation that the morphological disturbances of the root apex, leading to fracture of the epidermis, which occur with extended Al treatment times (Experiment 2), does not increase the entry of Al into the inner cortical or stelar tissues. This may imply that the integrity of the epidermal structure is not, as suggested by Rasmussen (1968), the primary factor controlling Al uptake.

Chronologically, Al does enter the cells of the root some time after it has been detected in the root cap. Mobility of Al in the root cells is also very low, and penetration does not extend beyond the outer cortical layers. This observation may explain the association reported between Al accumulation sites and the outer surface of the root (Rasmussen, 1968; McCormick and Borden, 1974). These factors also suggest that the presence of Al in root tissues proximal to the root cap may not represent a primary site of Al injury.

Differences in Al uptake emerged between Experiments 2 and 3. These experiments differed with respect to temperature and the age of the plants under investigation. Penetration of Al into the inner cap cells proceeded more slowly in the second experiment, which featured a lower temperature and a shorter growth period. Clarkson (1967) has indicated that Al uptake is not influenced by temperature. The corollary to this observation would be that Al uptake may be affected by the physiological stage of development of the plant, although the mechanism remains to be investigated.

3.4.2 Root Biochemistry

Aluminium accumulates preferentially in well-defined areas of the root, including the root cap, epidermis and outer cortical cells of the root apex. Furthermore, the movement of Al to the neighbouring cell populations of the inner cortex and central vascular cylinder may be restricted, or even non-existent.

The immobility of Al in plant tissue has previously been connected to the internal precipitation of insoluble compounds arising from interactions between Al and phosphorus (Wright, 1943). Chemical immobilisation of Al is, however, difficult to reconcile with the rapidity with which some of the toxic consequences of Al are expressed, and a significant decline in mitotic activity has, for example, been observed (Allium cepa) within two hours of Al treatment (Clarkson, 1965). It can therefore be argued that Al must be retained in solution in cells expressing a rapid toxic reaction to Al. This view is subject to the interpretation that uptake and mobility of Al are determined by unique properties of the tissues involved.

It is therefore attractive to consider that Al uptake and distribution in the root may be determined by the biochemistry of the cell populations involved. With the exception of the root meristem proximal to the cap junction, there are clear parallels between control roots stained with methylene blue, and the regions of the root associated with Al uptake. Significantly, removal of the root cap permitted entry of Al into those cells of the root meristem proximal to the cap which were stained with methylene blue, thus confirming the potential of these cells for Al uptake and accumulation. The appearance of Al in these cells in intact roots may therefore be time dependent, and this may explain the apparent anomalies between this work and the detection of Al in the nuclei of mitotically active cells of the root (Morimura et al., 1978; Naidoo et al., 1978). The limited capacity of the cells of the inner cortex and stele to bind methylene blue, and the exclusion of Al from these tissues, even in decapped roots, supports the hypothesis that Al uptake is a function of cell biochemistry.

The ability of plant tissue to bind methylene blue at pH values below 4,0 is indicative of the presence of acid mucopolysaccharide materials (Gurr, 1958). Obrouchevá (1975) has connected the rapid synthesis of uronic acid in the root meristem with the region of cell division. Polyuronic acids are also

present in the mucigel (Wright and Northcote, 1974). The biosynthetic function of the cells of the root apex may therefore be a factor in the expression of Al toxicity. The precise mechanism is uncertain, but it is reasonable to expect that it will include avoidance of the formation of insoluble Al compounds.

The noticeable effect of Al in reducing the capacity of the root to bind methylene blue stain may result from Al acting directly on the site of synthesis of the muco-polysaccharides, or from a more general decline in the metabolic activity of the root. Since the symptoms of Al toxicity become apparent before the mitotically active cells of the cap and proximal meristems receive Al, it is tempting to postulate that toxicity may be effected through action at the site of synthesis of the muco-polysaccharides. This aspect is discussed further in Chapter 4.

3.4.3 Transport of Aluminium

These experiments have demonstrated that Al uptake is not characterized by significant mobility of Al in cortical cells of both primary and secondary roots. Rasmussen (1968) has, however, postulated that the development of lateral roots may provide an alternative path of entry into the conducting tissue of primary roots. Transport of Al to the aerial fractions of the plant along this pathway is, however, dependent on Al being retained in solution in the xylem stream. The measurement of xylem sap pH in *Zea mays* contradicts this notion, and accords with the low concentrations of Al found in the leaves of *Zea* (Chapter 8). Small amounts of Al do, however, reach the aerial fraction (Chapter 8), and Jones (1961) has intimated that this may be dependent on complexing of Al by organic acids (malic and oxalic) which are effective in preventing precipitation of Al at physiological pH levels.

Metabolic control of intracellular pH is a fundamental physiological process (Smith and Raven, 1979). Aluminium is known to influence a wide range of metabolic pathways (Foy *et al.*, 1978) and it is therefore surprising that the pH of the xylem sap

was unaltered by Al treatment. This result does, however, accord with the finding that intracellular pH does not respond greatly to changes in the soil condition or plant nutrition patterns (Smith and Raven, 1979).

3.4.4 The Root Cap: Evidence for a Transmitted Response

An apparently anomalous situation exists in the interpretation of Al uptake studies with decapped roots. Removal of the root cap permitted entry of Al directly into the cells of the quiescent centre and adjacent tissues of the proximal meristem, and a protective function for the cap over these tissues may therefore be implied. Barlow (1974a) has shown that removal of the cap stimulates the cells of the quiescent centre to enter mitosis and, since repeated divisions of these cells ultimately lead to regeneration of a new cap, the rôle of the root cap in delaying or restricting entry of Al into the quiescent centre may be fundamental to the recovery of some root meristems from initial Al shock (Horst *et al.*, 1983; Mendes *et al.*, 1984).

Decapped roots treated with Al were, however, also slower than the controls (with caps) to demonstrate the morphological disturbances which occurred in the primary growth region, 1,5 - 2,0 mm from the root apex. This observation is clearly subject to an alternative interpretation which includes a stress signal emanating from Al-damaged cap cells which is transmitted to the tissues where cellular disorganization occurs. This hypothesis accords with the absence of Al from the inner cortical tissues of the root where Al-induced changes in the pattern of cell enlargement are most apparent (Chapter 4).

There is also considerable evidence to illustrate the rapidity with which mitotic activity declines following Al treatment. Reductions in the rate of cell division have been reported in 2 hours, followed by complete inhibition of cell division within 5 - 6 hours of Al treatment (Clarkson, 1965; Horst *et al.*, 1983). The present experiments do, however, indicate

that in intact roots, considerably longer times may be required before Al is detected in the cells of the cap (12 h) and proximal meristems. This observation implies that in Al-treated roots, a decline in activity of the cap and proximal meristems may depend on Al-induced changes occurring in the root cap. This aspect is discussed further in Chapter 7, but clear parellels exist with the finding of Clowes (1972b) who unequivocally demonstrated that the root cap exercises a function in regulating the activity of the root.

3.5 CONCLUSION

Aluminium uptake was initially directed at the peripheral root cap cells and the mucilagenous secretions surrounding the root apex. Aluminium spread rapidly through the cells of the root cap, but the cap initials were the last cells to be reached. Epidermal cells of the root apex were not an effective barrier to Al, and it was suggested that Al uptake and distribution between cell populations was determined by cell biochemistry, notably the presence of acid muco-polysaccharide materials. Aluminium entry in the root apex was also conspicuously slower than the root cap, and the distribution of Al was limited to the epidermal and outer cortical regions.

Decapped root experiments have indicated that the distribution of Al between the cells of the root apex was altered by cap removal, and that the expression of some symptoms of Al toxicity involving cellular enlargement was delayed by this operation.

Aluminium treatment caused a loss of apical dominance in the primary root. Initiation of lateral root primordia observed with Al treatment times in excess of 24 h, provided sites for secondary Al uptake along the main root axis. Aluminium uptake by lateral roots was rapid once penetration of the epidermis had occurred, but distribution of Al was limited to the developing root tip. pH measurement of xylem sap exudate has confirmed that Al

reaching the central vascular cylinder is unlikely to be transported in an ionic form.

Little evidence emerged of Al reaching actively dividing cells of the proximal meristem during the first 20 h of root exposure to Al. The concept of Al acting directly on cell division is consequently questioned.

The present experiments have, however, implied a fundamental rôle for the root cap in the expression of Al toxicity, which has not previously been considered. Preliminary evidence from these studies favours the hypothesis that the expression of toxicity involves the transmission of a response from Al-damaged cap cells to cell populations which may be initially remote from the site(s) of uptake. The mechanisms of regulation remains to be considered in subsequent Chapters, but Juniper (1972) has proposed the presence in the root cap of two integrated perception systems. Organization of these systems is dependent on starch metabolism within the differentiating cells of the root cap. Experiments on Al uptake have implied a connection between Al toxicity and the biochemistry of the muco-polysaccharides, and it is conceivable that the interference by Al in root function could be directed along this pathway. This aspect is discussed further in Chapter 7.

An understanding of plant reaction to the presence of Al may therefore depend on demonstrating the physiological changes which arise in the root apex in response to the presence of Al (Chapter 4).

CHAPTER 4

THE RESPONSE OF THE APICAL REGIONS OF THE PRIMARY ROOT TO ALUMINIUM

4.1 INTRODUCTION

Aluminium uptake studies (Chapter 3) have indicated a fundamental significance for the peripheral cap in the expression of Al toxicity, since these cells are the first to take up Al. Paradoxically, mitoses are absent from the cap periphery (Barlow, 1975) and an understanding of the physiology of plant response to Al is further complicated by the observation that Al may initially be absent from the cells of the cap and proximal meristems (Chapter 3).

An increasingly animated view of root function does, however, require that the root meristem should not be considered as a single meristem, but rather as a number of discrete cell populations, not all of which are meristematic (Ellmore, 1982). Co-ordination of function of the root is nevertheless dependent on the integration of activities between the cell populations comprising the root meristem.

The interpretation of the results of Al uptake studies (Chapter 3) may therefore depend on the identification of an as yet unexplored regulatory function for the peripheral cap cells in co-ordinating intercellular activities. This hypothesis accords with the wider functional involvement for the root cap in regulating root development (Chapter 1).

The mechanism of control and the effect(s) of Al on the cell populations of the root meristem and primary growth region are unknown. Although the root apex has been connected to the accumulation of plant growth substances (Torrey, 1976), the possible failure of cell division resulting secondarily from impairment of some other function by Al has received little attention. Furthermore, Clarkson (1969) was unable to relate reduced levels of mitoses

to lower respiration rates in Al-treated roots of barley (Hordeum vulgare) and onion (Allium cepa).

Aluminium treatment is, however, known to produce morphological disturbances in the cell populations of the root apex (Hecht-Buchholz, 1983; Chapter 3) and Fleming and Foy (1968) have intimated that varietal differences in the response of wheat (Triticum aestivum) cultivars to Al may directly reflect the resistance of the meristem to damage.

Very little information exists on the cellular changes occurring in Al-treated roots. It is therefore the aim of this Chapter to identify Al-induced alterations in respiratory activity between cell populations, and to examine morphological changes occurring in the cell populations of the root meristem and growth region of the primary root in response to Al.

4.2 EXPERIMENTAL TREATMENT

4.2.1 Experiment 1

This experiment was intended as a time-course study of the physiological response shown by the cell populations of the root apex to Al, and to the identification at an ultrastructural level of the cellular changes associated with Al treatment.

Plants (cv. TX 24) were grown in full-strength nutrient solutions (Sections 2.2.2; 2.2.8) for 7 days. Aluminium sulphate solution was added in advance of harvest to give 0 h (control); 2 h; 5 h; 6,5 h; 12 h; 20 h; 48 h root exposure to 8 mg dm^{-3} Al in solution.

The roots of plants (10) from each treatment were treated with TTC (Section 2.3.2.3) and root material was sectioned frozen (Section 2.4.1). Root tips (6) from each treatment were also processed for light and electron microscopy (Sections 2.4.2; 2.4.3; 2.4.4) which included investigation of the cells comprising the root

cap (Juniper, 1972), quiescent centre (Clowes, 1956), proximal meristem (Feldman and Torrey, 1975) and growth region (Luxová,1981).

4.2.1.1 Cell Enlargement Studies

Micrographs of longitudinal sections taken to include the central cylinder were prepared for the apical 2 mm of the primary root, projected for additional magnification and analysed in 0,5 mm segments to cover the following cell populations:

i) epidermis
ii) outer cortex, cell file 2
iii) mid-cortex, cell file 5, and
iv) quiescent centre.

Procedures for the measurement of cell dimensions and the calculation of cell volumes have been described by Barlow (1974a).

Micrographs of transverse sections 1,5 mm from the root tip were also prepared to assess the effect of cell enlargement in mid-cortical tissues on the structure of the stele.

4.2.2 Experiment 2

This experiment was intended to determine the effect of Al on the osmotic potential of cell sap expressed from the cells of the root apex. Plants (cv. TX 24) were grown in full-strength nutrient solutions (Sections 2.2.2; 2.2.8) for 6 days. Aluminium sulphate ($Al_2(SO_4)_3$) solution was added in advance of harvest to give 0 h (control); 1 h; 5 h; 12 h; 20 h root exposure to 8 mg dm^{-3} Al. Sixty-nine plants were sampled from each treatment. Roots of plants were rinsed in deionised water. The sap was expressed from the distal 20 mm of the primary root and immediately frozen in solid carbon dioxide (dry ice). Sap was thawed, centrifuged and the osmolality determined with the minimum delay on a Westcor Vapor Pressure Osmometer.

4.2.3 Statistical Treatment of Results

The availability of plant material was a limitation to the number of measurements made during these experiments. Because of the importance attached to the interpretation of changes in osmotic potential (Experiment 2), this experiment was repeated to provide a second set of results. Ranges covering two experiments are given for the measurements made.

Changes in cell volume were considered to be so massive (Experiment 1) that the frequency of measurements was limited to the number of cells found within the defined cell populations of single roots sampled from each treatment.

4.3 RESULTS

It is pertinent to note that the calculated Al^{3+} activity of 8,4 X 10^{-5} mol dm^{-3} (P.G. SOIL DRIP Programme, University of Natal, unpublished) corresponding to an Al concentration of 8 mg dm^{-3} Al used in these experiments coincides with the highest level of toxicity reported by Pavan and Bingham (1982) in coffee (Coffea arabica).

4.3.1 Respiratory Activity of the Root Meristem

In the absence of Al, the highest rate of respiratory activity, as indicated by the intensity of formazan colour development, was associated with the cap periphery and epidermis (Figure 11A). Figure 11A also shows an area of intense respiratory activity in the cells proximal to the cap/root junction, corresponding to the region of the root forming the distal cells of the quiescent centre.

Aluminium treatment (8 mg dm^{-3}) was effective in rapidly altering the pattern of activity and, with 2 h treatment time, reduced levels of respiration were evident in the peripheral cap cells, epidermal cells of the root apex and the quiescent centre (Figure 11B). In contrast, limited exposure to Al (2 h) stimulated the cells of the central cap (columella), cap (cap initials)

and proximal meristems to increased respiratory activity (Figure 11B). Increased activity was, however, short-lived (Figure 11C and Figure 11D) and with longer Al treatment times (12 h), only isolated respiratory activity could be detected.

Results with prolonged Al treatment (48 h) were variable. Some roots continued to show respiratory activity of low intensity, mainly in the areas proximal to the quiescent centre and including the pole of the stele (Figure 11D), while in others, little activity could be detected.

Factors affecting the intensity of red (formazan) colour development are discussed (Section 2.3.2.2). The absence of colour development in Al-treated roots should be interpreted as a decline rather than cessation of respiratory activity.

4.3.2 Ultrastructure of the Root Cap

The overall effect of Al on the cell structure of the root cap is illustrated in the low magnification electron micrographs of Figure 12. In the control treatments (0 Al), the peripheral cap cells were found to be rich in mitochondria with well developed endoplasmic reticulum (E.R.) and nuclei and amyloplasts were present. Vacuoles were present but were generally small. Cells of the cap columella were essentially non-vacuolate, contained amyloplasts with large starch grains and E.R. Cells of the cap meristem (cap initials) were characterized by an absence of amyloplasts although plastids were numerous and many showed evidence of starch accumulation (Figure 12A).

Progressive vacuolation of the cells of the root cap was one of the most easily identifiable responses to Al. Vacuolation was initiated in the peripheral cap cells where it was evident with 2 h treatment (8 mg dm^{-3} Al) and was followed by severe disorganization of the cytoplasmic contents of peripheral cap cells with longer treatment times (20 h) (Figure 12F). Vacuolation of the cap columella and cap meristem proceeded more

slowly. There was, however, evidence of vacuolation of the cells of the cap columella within 6,5 h (Figure 12D) and of an increase in vacuolation of the cells of the cap meristem with 20 h Al treatment (Figure 12B).

The occurrence of cytoplasmic organelles was also reduced in Al-treated cells; this included an almost total disappearance of E.R. with prolonged Al treatment. Comparison between Figures 13B and 15C suggests that the disappearance of E.R. was preceded by severe swelling of the E.R. Aluminium treatment was also associated with an altered appearance of the amyloplasts which showed a notable increase in the intensity of stain evident in the starch bodies (Figures 12C to 12F). There was little evidence of starch accumulation in plastids of the cap meristem of Al-treated roots (Figure 12B). The presence of Al was not connected to alterations in the structure of mitochondria (Figures 13C, 14C and 15B).

No change in the nuclear structure of the cap meristem could be detected during the first 20 h of this experiment (Figures 12A and 12B). Cells contained well-defined nuclei, the nuclear membranes remained intact (Figure 15A), and no apparent change in the degree of chromatin condensation as a consequence of Al treatment was observed (Figures 12A and 12B).

No evidence emerged to connect Al treatment with the rapid disruption of the plasmalemma (Figures 13C, 14C and 15C).

4.3.3 The Golgi apparatus*

The Golgi apparatus located in peripheral cap cells of the control treatment reflected structural features associated with the secretory function of these cells. Dictyosomes were

* Terminology used in this investigation has involved describing individual cisternal stacks as dictyosomes, and inter-associations of dictyosomes functioning synchronously, as the Golgi apparatus (Mollenhauer and Morré, 1980).

characterized by an easily recognizable polarity, based on the appearance of the cisternae and the size, shape and frequency of secretory vesicles (Figures 13A and 13B).

Aluminium treatment caused a rapid alteration in the morphology of the dictyosomes which was observed with 2 h treatment to include disruption of vesicle production and a change in membrane structure with many vesicles showing disintegration of the outer membranes (Figures 14A and 14B). Severe curling of the cisternae (Figure 14B) and a reduction in the number of cisternae per dictyosome from a mean of 5,5 (control 0 Al) to a mean of 3,7 (6,5 h treatment, 8 mg dm^{-3} Al) were also observed.

The relative absence of secretory vesicles from the cytoplasm of the control treatment (Figure 13C) was considered evidence for the rapidity of transfer of vesicular material across the plasmalemma. Limited (2 h) exposure to Al resulted in an accumulation of secretory vesicles around the dictyosomes (Figures 14B and 14C). The contents of the vesicles was also progressively altered in appearance from a dense, granular material (Figures 13A and 13B) to a diffuse, fibrillar material (Figure 14A, 14B, 15A and 15B). Significantly, some apparently normal secretory vesicles could still be found with the shorter (2 h) Al treatment (Figure 14C). Longer Al treatment times were, however, associated with the disappearance of vesicles containing finely granular material (Figure 15C). Similar changes to the appearance of Golgi apparatus material, accumulated between the plasmalemma and cell wall, were also noted (Figures 13C, 14C and 15C).

4.3.4 Cell Enlargement

Severe cellular disorganization, notably of the cell populations comprising the quiescent centre and growth region of the root apex was associated with treatment of the primary root with Al (Figures 16 and 17), so that with prolonged treatment (48 h), complete disruption of the organized, cytologically heterogenous root apex occurred (Figure 16D). Aluminium treatment caused a

rapid alteration in the size of the cells of the root apex. Analysis of the relative extent of these changes with time for the different cell populations (Figure 18) indicated that increases in cell volume were most notable in the cells of the mid-cortex 1 mm - 2 mm from the root tip. Cell volume changes were associated with alterations in the growth direction of these cells (Figure 19) and resulted in:

i) increased root thickness evident with 6,5 h exposure to Al (Figures 16 and 17)

ii) distortion of the longitudinal walls of immature metaxylem vessels (6,5 h) (Figure 17B), and

iii) progressive collapse of metaxylem vessels of the stele with longer treatment times (Figures 17C and 17D).

Disintegration of the epidermis (20 h) was also associated with continued growth of mid-cortical cells (Figures 16C, 16D, 17C and 17D).

A conspicuous effect of Al involved the cells of the quiescent centre, where increased mean cell volume was evident with 2 h treatment. It was considered pertinent that during the first 5 h of the experiment, cell enlargement recorded in the quiescent centre substantially exceeded that shown by neighbouring cell populations. This pattern was not maintained and a decline in growth of quiescent centre cells (6,5 h) coincided with progressive disorganization of the root apex. No measurement of quiescent centre cells was possible with 20 h treatment.

Little attention was given to the remaining cell populations since the initial growth of the outer cortical cells associated with short-term Al treatment was not maintained during the course of the experiment, and changes in the size of epidermal cells was comparatively modest.

4.3.5 Ultrastructure of Proximal Meristem

Ultrastructural features of roots treated with Al (Figure 20) included progressive vacuolation of the cells of the proximal meristem first observed with 5 h treatment (Figure 20A). In contrast to the situation in the cap meristem, nuclei of the proximal meristem showed, with extended Al treatment times, an almost total disappearance of dense chromatin from interphase nuclei (20 h) (Figure 20C) as well as improved definition of areas of the nucleolus, which was also found to become progressively granular with an increase in the frequency and size of nucleolar vacuoles. The nuclear membranes remained intact at least during the first 20 h of the experiment.

4.3.6 Osmotic Potential of the Cells of the Root Apex

Aluminium treatment (8 mg dm^{-3}) resulted in an initial (1 h) increase in the osmotic potential of expressed cell sap. This was followed (5 h) by a sharp decline, and subsequent treatments which involved longer times were connected to a continued decrease in osmotic potential (Figure 21).

4.4 DISCUSSION

These experiments confirmed the significance of the peripheral root cap cells in the expression of Al toxicity (Chapter 3). Not only are these cells the first to take up Al, but studies of root metabolism confirm that a physiological response to Al, as evidenced by a decline in respiratory activity, is initiated in the cap periphery. An Al-induced increase in vacuolation of peripheral cap cells also precedes similar changes in neighbouring cell populations of the cap and proximal meristem.

The most conspicuous activity of the peripheral cap cells involves the secretion of slimes and mucilages of polysaccharide - protein complexes (Mollenhauer and Morré, 1980). The association between Al uptake and the biochemistry of the muco-polysaccharides

(Chapter 3) may therefore serve to implicate the activity of peripheral cap cells in the uptake of Al.

4.4.1 The Golgi apparatus and the Expression of Aluminium Toxicity

The Golgi apparatus has been connected to a remarkably diverse range of functions, including synthesis of polysaccharide materials, membrane transformation, export of materials from the cell including mucilages (Mollenhauer and Morré, 1980). Furthermore, the passage of secretory products through the Golgi apparatus is obligatory and involves extensive modification and transfer to a membrane container which is competent to fuse with the plasmalemma at the time of exocytosis (Farquhar and Pallade, 1981). Rearrangement of the dictyosomes of the Golgi apparatus following gravitropic stimulation has also been observed (Gressel and Horwitz, 1982).

The secretory function of the Golgi apparatus of the peripheral cap cells has been extensively reviewed by Mollenhauer and Morré (1980), and Mollenhauer (1965) has further drawn attention to the often abrupt and synchronous morphological distinctions which occur between the dictyosomes of the cap periphery and those of the central cap columella and epidermis. These changes which essentially involve the size of dictyosomes and the appearance of vesicular contents support the idea that alterations in biochemical function occur between the cell populations concerned.

Structural changes noted in the dictyosomes of the Golgi apparatus following Al treatment are therefore of considerable interest. Mollenhauer and Morré (1980) have indicated that at least three structural responses characterize stressed Golgi apparatus:

i) change in secretory pattern

ii) change in the number of cisternae per dictyosome, and

iii) change in cisternal architecture,

while in secretory cells, the most immediate response to stress may involve the cessation of secretory activities. All these changes may be identified in the Golgi apparatus of peripheral cap cells within 2 h treatment with 8,0 mg dm^{-3} Al. Furthermore, the curling of dictyosomal cisternae as noted in these experiments with Al is indicative of severe physiological stress, since this symptom has previously only been connected to shock occurring during isolation, or as a consequence of treatment with potassium cyanide (Mollenhauer and Morré, 1980).

4.4.2 The Primary Site of Injury

Since Al elicits a number of ultrastructural responses, considerable relevance must be attached to the chronological sequence in which symptoms appear. Possibly the first consequence of Al injury concerns the transfer of secretory vesicles from the Golgi apparatus to the cell exterior. Evidence includes a rapid accumulation of secretory vesicles in the vicinity of dictyosomes evident even with limited Al treatment (2 h). This is considered indicative of interference in traffic through the Golgi complex (Mollenhauer and Morré, 1980; Newcomb, 1980), and the failure of mechanisms which control the direction of membrane or secretory components to the correct intracellular and extracellular destinations (Farquhar and Pallade, 1981) may therefore represent the primary response to Al. In this context, Al results in the disintegration of vesicle membranes. Farquhar and Pallade (1981) have noted that a function of the Golgi apparatus in the packaging and export of materials is to provide a membrane container that is competent for exocytosis of secretory products. It may therefore be argued that the transfer of secretory vesicles depends on vesicle membrane integrity. Mechanisms by which Al may influence the structure of vesicle membranes are presently unknown, although evidence supporting a rôle for Al in preventing the assembly of membrane material will be discussed in Chapter 5.

Aluminium treatment was also effective in altering the appearance of the vesicular contents of peripheral cap dictyosomes

from a dense, finely granular material to a more diffuse, fibrillar material. Similar changes in appearance were also noted in the Golgi apparatus-derived material exported from the cytoplasm and accumulated between the plasmalemma and cell wall. These observations suggest that Al is also effective in influencing the processing of secretory products within the Golgi apparatus.

Newcomb (1980) has intimated that the ultrastructural appearance of the cisternal contents may be characteristic of the product being synthesized. Progress in understanding the biochemical events associated with Golgi apparatus function is still quite limited. Secretory patterns involving the elaboration of finely granular secretory products are nevertheless characteristic of the cap periphery (Mollenhauer, 1965), where Golgi secreted materials are believed to contribute to the mucous secretions of the cap (Juniper and Roberts, 1966). The relevance of Al-induced changes in the appearance of secretory products to the expression of toxicity will be discussed further in Chapters 5 and 6. It is, however, pertinent to the sequence of events occurring in Al-treated roots that apparently normal vesicles, based on the appearance of vesicular contents, were observed to be intermingled with those of altered appearance with limited Al treatment time (2 h). This implies that the failure in vesicle transfer mechanisms may precede the changes observed in vesicular contents.

A reduction in the number of cisternae per dictyosome as a consequence of Al treatment is also strongly indicative of a decline in secretory activity of peripheral cap Golgi apparatus, since Morré (1977a) has intimated that during steady state secretion, the number of cisternae per dictyosomal stack may be expected to remain constant.

4.4.3 The Structured Relationship within the Endomembrane System

The complexity of traffic through the Golgi apparatus requires that the Golgi apparatus should not be considered as an

autonomous organelle and, for the more complex functions to proceed, the structured relationship with the E.R. and the plasmalemma must also be considered (Mollenhauer and Morré, 1980). It is significant therefore that transport out of the E.R. to the Golgi apparatus has been shown to be vectoral and energy dependent (Farquhar and Pallade, 1981). It is therefore pertinent that ultrastructural changes of the Golgi apparatus precede severe inhibition of respiratory activity in the peripheral cap cells. The idea that observed changes in respiration following Al treatment reflects a decline in energy requirements rather than a primary response to Al is further supported by the appearance of the mitochondria, which were found to be resistant to Al-induced morphological changes, even with extended treatment times. This observation is consistent with the findings of Hanson and Day (1980) who investigated the general response of mitochondria to stress.

The short-term consequences of Al are essentially directed at the Golgi apparatus, and it is therefore tempting to implicate specific functions of the Golgi apparatus directly to Al injury. Activities characteristic of the Golgi apparatus involve only the enzymes thiamine pyrophosphatase and certain glycosyl transferases (Morré and Mollenhauer, 1974), while all other enzymes show activites in the Golgi apparatus intermediate between E.R. and the plasmalemma. The glycosyl transferases have been connected to the ordered sequential addition of sugars within the Golgi apparatus (Morré, 1977b). Interference by Al in this function may be indicated by the altered appearance of the vesicular contents.

The present experiments did not confirm the plasmalemma as a primary site of Al action as suggested by Hecht-Buchholz and Foy (1981), since no change in appearance was evident with short-term exposure to Al. The activities of the Golgi apparatus almost certainly include the biosynthesis of new membrane material destined for incorporation in the plasmalemma (Newcomb, 1980; Farquhar and Pallade, 1981). The present experiments are consistent with the idea that failure of the Golgi apparatus may

precede structural changes in the plasmalemma.

4.4.4 Vacuolation

Progressive vacuolation of root cells is one of the most easily observed consequences of Al injury. Vacuolation is, however, unlikely to represent a primary response to Al since it is preceded by disturbances to the cytoplasmic organelles, notably in the cap periphery.

Membrane differentiation within the structured concept implicit within the endomembrane system is, however, seen to include the vacuolar apparatus of the cell interior (Morré and Mollenhauer, 1974). The initiation and origin of vacuoles remains to be defined with certainty and has been variously considered to involve E.R. or E.R.-derived provacuolar structures (Morré, 1977b; Chrispeels, 1980) or the Golgi/E.R./lysosome complex as possible origins of the tonoplast (Marty et al., 1980).

The results of this investigation suggest that in Al-treated peripheral cap cells, the vacuoles arise from swollen, rough E.R. as a direct consequence of Al injury. Furthermore, the extensive damage to the membranes of the Golgi apparatus may preclude the involvement of these structures in the formation of vacuoles.

4.4.5 The Quiescent Centre

The cells of the quiescent centre have become characterized by low levels of activity, judged largely by criteria associated with cell division (Clowes, 1972a) and the incorporation of radioactive materials (Barlow, 1974a). The importance of the quiescent centre has nevertheless been explicitly demonstrated through the in vitro culture of well organized roots of normal morphology from isolated quiescent centres (Feldman and Torrey, 1976).

The universal occurrence of quiescent centres in the

roots of angiosperm plants (Clowes, 1984) may underlie a fundamental rôle in root physiology which remains to be explored. The unique position of the quiescent centre to mediate in the distribution of materials exported from the root cap or transported down the stele has also prompted suggestions with regard to an as yet poorly defined rôle in the organization of the root apex (Torrey, 1972). Published research on the quiescent centre has, however, indicated a functional involvement in regulating intercellular relationships with the proximal meristem (Feldman, 1976), the development of primary vascular tissue (Feldman and Torrey, 1975) and, less clearly, as a possible site of cytokinin synthesis (Barlow, 1978b; Feldman, 1979a).

The mechanism through which quiescence is imposed on these cells remains controversial (Torrey, 1972; Barlow, 1974a; Clowes, 1975, 1984). It is, however, relevant that the constraint leading to quiescence does not in itself prevent the quiescent centre from reassuming a meristematic function (Feldman and Torrey, 1976) and there are numerous studies involving surgical manipulation of the cell populations of the root apex which attempt to define the forces creating the quiescent state (Barlow, 1974a; Feldman, 1976; Feldman and Torrey, 1976). These procedures inevitably affect many features of the integrated system of the root apex and questions concerning cellular interactions remain largely unresolved.

The rapid increase in the size of the cells of the quiescent centre of Al-treated roots is therefore of considerable interest, since this change coincides with an alteration in the function of the quiescent centre implicit in the observed decline in respiratory activity following Al treatment. It is also important that these changes are not the direct consequence of Al, since considerable time may be required before Al can be detected in these cells (Chapter 3). Similar changes are not shown by either the cap or proximal meristems with short-term Al exposure, and it may therefore be argued that Al treatment has acted through the removal of the constraint imposing quiescence

on these cells.

According to Barlow (1974a), excision of the root cap produces a similar result. In the present experiments, the root cap remained intact and metabolically active for some hours after Al treatment. These observations suggest therefore, that the root cap may represent the source of chemical control of quiescence imposed on the cells of the quiescent centre. The nature of such control is presently unknown, although Barlow and Pilet (1984) have intimated that abscisic acid (ABA) is effective in retarding cell enlargement. Furthermore, it is pertinent that a release from the quiescent state may be effected through treatment of the root with toxic concentrations of Al and removal of the root cap. Considerable emphasis has been attached to Golgi apparatus function in the peripheral cap cells as a primary site of Al action. It is therefore conceivable that Golgi apparatus function is involved in imposing quiescence on the cells of the quiescent centre. The re-establishment of a quiescent state during cap regeneration in decapped roots may therefore be allied to the resumption of this activity rather than to the presence of physical constraints associated with cap regeneration, as suggested by Barlow (1974a). This aspect will be discussed further in Chapter 5.

The high level of respiratory activity observed in the quiescent centres of the control (0 Al) roots contrasts with criteria normally connected to the low levels of activity associated with the quiescent state. These cells are also among the first to show a decline in activity in response to Al, and it is tempting to associate these observations with a fundamental, but as yet undefined, function of the quiescent centre. This aspect is considered further in Chapter 9.

4.4.6 Stability of the Nuclear Structure

These experiments do not indicate rapid structural or functional changes in the nuclei of meristematic cells which could be associated with the reported decline in mitotic activity

(Clarkson, 1965) following Al treatment. Furthermore, it is pertinent that the mitotically active cells of the cap and proximal meristems showed increased, but short-lived, respiratory activity during the first 2 h of the experiment. It seems improbable therefore that the decline in mitotic activity following Al treatment (Clarkson, 1965) could arise indirectly through failure of root metabolism. This observation accords with the results of Clarkson (1969) who was unable to connect oxygen uptake directly to reduced levels of mitosis in the Al-treated roots of Hordeum vulgare on a time basis.

Notable differences in the degree of resistance to Al-induced changes in nuclear structure emerged between the cells of the cap and proximal meristems. In the cap meristem, most nuclei showed little change and retained a balance between the distribution of dense and diffuse chromatin (Jordan et al., 1980) with up to 20 h Al treatment time. In contrast, nuclear changes in the cells of the proximal meristem were seen to involve a decrease in chromatin condensation of the nucleus and an increase in size and frequency of vacuoles in the nucleoli with time (20 h). P.W.Barlow,(pers.comm.*) has intimated that segregation of fibrillar and granular components of the nucleolus may be indicative of impaired nucleolar function. In support of this idea, Hyde (1967) has intimated that the large nucleoli of active cells may have their basic synthetic machinery obscured by the products of synthesis. Differences in the capacity of fixatives to define ultrastructural features of nucleoli may therefore relate to the extraction of protein during fixation which sharpens and clarifies the structure. It is therefore conceivable that the increasingly vacuolated appearance of the nucleoli of Al-stressed cells of the proximal meristem may reflect an inhibition of function, which produces a similar ultrastructural response through progressively reducing the products of synthesis present in the nucleolus. Differences in response to Al, noted between the nuclei of the cap and proximal meristems, may further imply that this function is not common to both cell populations.

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Barlow (personal communication) has also suggested that dispersion of chromatin observed in these experiments in the nuclei of the proximal meristem may be symptomatic of a premature ageing phenomenon. It is, however, pertinent to this thesis that these changes in nuclear structure were preceded by an alteration in Golgi apparatus function.

4.4.7 Growth Response of Cortical Cells

These experiments have demonstrated a strong element of growth stimulation arising from Al treatment, notably occurring in the cortical cells 1 mm - 2 mm from the root tip. This coincides with the region of the root associated with cellular changes initiated by gravistimulation (Shen-Miller et al., 1978).

An increase in cell volume may be expected to depend heavily on the uptake of water (Steward, 1969; O'Brien, 1982). In Al-treated roots, the short-term increase in osmotic potential of the expressed sap may indicate that the inflow of water is initiated by a rapid departure from osmotic equilibrium. It is, however, pertinent that continued growth of mid-cortical cells which occurred over the entire course of the experiment could only be maintained against the subsequent decline in osmotic potential by a change in pressure potential. Initially, decreased pressure potential may involve alterations in cell wall structure (Ray and Green, 1972; Green, 1980), which is supported by the change in growth direction shown with time in these cells. Furthermore, it is considered significant to this argument that the timing of the change in growth direction coincided with a decline in osmotic potential.

Growth stimulation of the mid-cortical cells is of such great magnitude that stresses directed towards the stele result in the collapse of the primary vascular tissue, while the continued requirement for reductions in pressure potential to accommodate further growth may ultimately involve the disintegration of the outer root cells.

The mechanism of cell growth stimulation in cortical cells of Al-treated roots is presently unknown. It may be argued that growth initiating factors in the cortex differ from those controlling cell enlargement in the quiescent centre, since removal of the root cap (Chapter 5) does not initiate a growth response in cortical cells in the absence of Al. It may also be relevant that progressive vacuolation is a feature of the cells of Al-treated roots, as one of the fundamental functions which has been assigned to the vacuole is storing water taken up during growth (Green, 1969; O'Brien, 1982). The nearly universal distribution of vacuoles in the cells of the root apex does not, however, follow the locally limited growth response of mid-cortical cells. These observations imply that water uptake and a loosening of cell wall material may be subject to separate controls during cell enlargement.

4.5 CONCLUSIONS

Within the context of the root meristem, development should be seen to include cell division, cell growth and differentiation (O'Brien, 1982). It is, however, clear from these experiments that the influence of Al extends beyond the well-documented inhibitory effect on cell division (Clarkson, 1965; Morimura *et al.*, 1978; Horst *et al.*, 1983), and that Al-induced changes in the pattern of cell growth and differentiation may include some of the most impressive plant responses to toxic levels of Al.

Histochemical and ultrastructural studies of Al-treated roots have confirmed a fundamental importance for the root cap in the expression of Al toxicity, where the earliest ultrastructural response involved the function of the peripheral cap Golgi apparatus. The mechanism of interference by Al in Golgi function is uncertain, but Morré (1977a) has indicated that the flow of secretory vesicles must be directional and selective and has intimated that control may be exercised through the zone of exclusion which surrounds the Golgi apparatus (Morré, 1977a). Significantly, Giulian and Diacumakes (1976) have suggested that the zone of exclusion is derived from

electrical properties which differ from that of the bulk cytoplasm. Evidence for an electro-physiological rôle for the Al^{3+} ion in the expression of Al toxicity will be considered further in Chapters 7 and 9.

Many of the cellular changes which have been identified in the root apex do not directly coincide with the presence of Al in the tissues involved (Chapter 3). This confirms the complexity of plant reaction to Al and indicates that the physiology of Al toxicity may involve several responses occurring at different rates and at different levels of scale. Little information exists to suggest how the initial response of peripheral cap Golgi apparatus is expressed through a decline in mitotic activity (Clarkson, 1965), a release of the quiescent centre from the quiescent state and cell enlargement initiated in mid-cortical cells.

Control of the root meristem through the root cap was first suggested by Clowes (1972b). Clowes (1972a), however, connected hypersensitivity of the cap initials in Zea mays directly to the high cycling rate of these cells. The present experiments do not support this concept with respect to Al toxicity and have instead assigned a fundamental rôle to the peripheral cap cells which will be discussed further in Chapter 7.

The existence of a stress response sequence within the root meristem presupposes intercellular communication. Raven and Rubery (1982) have indicated that communication between cells can involve chemical or electrical impulses. The rapid effect of Al on the function of the quiescent centre may be seen to favour a chemical pathway for transmission of the stimulus. This idea accords with evidence (Chapter 3) that a loss of apical dominance is an early consequence of Al treatment. Torrey (1976) has extensively reviewed data which suggests that gibberellic acid, cytokinins and ABA may be formed in the root apex, where a rôle for cytokinin in the control of lateral root initiation seems possible (Chapter 9).

CHAPTER 5

PRELIMINARY EVIDENCE FOR A GOLGI APPARATUS DERIVED MORPHOGEN

5.1 INTRODUCTION

Treatment of the primary root with Al (Chapter 4) has attached importance to the Golgi apparatus in the peripheral cap cells in the expression of Al toxicity. No evidence presently exists to indicate how Al-induced changes in secretory activity may influence root development.

The cells of the quiescent centre are, however, characterized by an inherent capacity to express a developmental pathway not normally seen in rapidly elongating roots (Feldman and Torrey, 1976). These cells respond to the removal of the root cap by entering mitosis and regenerating a new cap (Barlow, 1974a), which has many of the properties shown by normal caps (Barlow, 1974a; Barlow and Grundwag, 1974).

Golgi apparatus function is correlated with cellular development (Mollenhauer, 1965) and the dictyosomes of quiescent cells are characterized by low levels of activity indicated by their small size and absence of secretory vesicles (Clowes and Juniper, 1964). Dictyosomes located in the outermost layers of the quiescent centre increase activity in response to cap removal (Barlow and Sargent, 1978). Decapping also permits Al to enter directly into these cells (Chapter 3).

Consideration of a rôle for Al in influencing the developmental stages involved in cap regeneration of previously decapped roots may therefore be expected to provide information on the physiological reaction of plants to Al.

5.2 EXPERIMENTAL TREATMENT

Plants with decapped roots (Section 2.2.8.1) were transferred to half-strength nutrient solutions containing either no Al (control), or 8 mg dm^{-3} Al and grown at 27°C with continuous aeration, but without supplementary light. At 0 h; 6 h; 12 h; 24 h and 48 h after decapping, root tips from each treatment were processed for light and electron microscopy (Sections 2.4.2; 2.4.3; 2.4.4).

To follow the effect of cap removal and treatment with Al on the fine structure of the cells of the quiescent centre on a quantitative basis, photographs of these cells were built into mosaics and counts were made of the number of plastids, starch grains, lipid drops, dictyosomes, cisternae per dictyosomal stack and secretory vesicles for single roots sampled from each treatment.

5.3 RESULTS

In the control treatments (0 Al), changes were observed in the pattern of cell division (24 h) preceding formation of recognisable cap tissue which was evident 48 h after cap removal (Figures 22A and 22C). Aluminium treated roots failed to regenerate a cap (Figure 22D) and Al treatment was associated with severe cellular disorganization and progressive vacuolation of the cells of the root apex first apparent 24 h after cap removal (Figure 22B).

5.3.1 Golgi apparatus Function in Decapped Roots in the Absence of Aluminium

Changes in the cells of the quiescent centre arising from cap removal involved the activity and secretory pattern of the Golgi apparatus. In roots fixed immediately after decapping, the dictyosomes of the quiescent centre were characterized by an absence of secretory vesicles (Figure 23B) and contained the least number of cisternae per dictyosomal stack (Figure 24). Increases in the number of dictyosomes, secretory vesicles and cisternae per

dictyosomal stack were observed within 6 h of cap removal (Figure 24), suggesting that rapid increases in secretory activity occurred in response to cap removal. A return to steady state secretion, as defined by Morré (1977a) was, however, indicated 12 h after decapping since further increases in the number of cisternae per dictyosomal stack were not encountered with longer times after decapping.

Changes in the appearance of vesicular contents were also detected, and many vesicles containing a dense, granular material were observed in the outer cells of decapped roots within 6 h of cap removal (Figure 23D). With longer times (24 h) after cap removal, differences in Golgi apparatus morphology were evident between cells of cortical and stelar lineages, and the secretory vesicles in the latter were less well developed and rarely contained granular material (Figures 23F and 23H).

Re-establishment of full Golgi apparatus function, usually associated with the cap periphery and based on the presence of elongated secretory vesicles and the accumulation of Golgi apparatus-derived material (Morré et al., 1967) between the plasmalemma and cell wall, could only be identified 48 h after decapping.

5.3.2 The Golgi apparatus in Decapped Roots Treated with Aluminium

In contrast to the control, Golgi apparatus development was inhibited by Al and, with 6 h Al treatment time, the number of dictyosomes and cisternae per dictyosomal stack had declined (Figure 24). A temporary recovery in Golgi apparatus activity was evident with longer times after cap removal, but it was notable that at no stage did the numbers of dictyosomes, cisternae per dictyosomal stack, and secretory vesicles approach the levels found in the control treatment (Figure 24).

Secretory vesicles containing the granular secretory product were infrequently encountered and only with extended times

after cap removal (24 h). Vesicle membranes were often not clearly defined (Figure 25C) and secretory vesicles were found to accumulate in the vicinty of dictyosomes (Figures 25D and 25F). Dictyosomes with defined membrane structure were infrequent 24 h after de-capping, and occurrence coincided with isolated plastids containing large starch grains. No structural distinction was evident between the Golgi apparatus located in cells of cortical and stelar lineages. Curling of cisternae (Chapter 3) was not observed in this experiment.

5.3.3 Plastid Differentiation in Decapped Roots in the Absence of Aluminium

Undifferentiated plastids showed a high frequency of occurrence in the cells of the quiescent centre immediately following cap removal (Figure 26), and there was evidence of multiplication (Figure 23A). Plastids showed little internal organization, membrane profiles were indistinct, lipid drops and irregular patches of electron-dense material were numerous (Figure 26). Starch grains were infrequent and always small.

Plastids were observed to follow a defined develop-mental sequence with increasing intervals after cap removal. These changes involved increases in the frequency with which starch grains were encountered and coincidental declines in the number of lipid drops (Figure 26). Initially plastids found in cells of cortical and stelar origin were not morphologically distinct, but 24 h after cap removal, plastids in cells of stelar lineage were clearly distinguishable and characterized by many of the features observed in plastids immediately after cap removal (Figures 23G and 26). Many of these plastids were particularly rich in electron-dense areas (Figure 23G). Plastids in cells of cortical origin were distinguished by the presence of well-defined starch grains (Figure 23E), while lipid drops were rarely present in plastids which contained large starch grains.

5.3.4 Plastids in Decapped Aluminium-treated Roots

Unlike the control, many plastids in Al-treated roots failed to develop internal organization during the course of the experiment, and membrane profiles usually remained indistinct (Figures 25A and 25B). A conspicuous consequence of Al treatment involved the rapid increase (6 h) in the number of lipid drops (Figure 26) which could still be observed in considerable numbers, sometimes in conjunction with starch, 24 h after cap removal.

Aluminium treatment also prevented starch accumulation and, although there was initially little difference in the frequency of occurrence of starch deposition sites between the control (Figure 26) and Al-treated roots, starch grains in Al-treated cells were usually smaller (Figure 25A), and large grains were only infrequently found with extended time after cap removal (24 h) (Figure 25E).

Aluminium treatment caused the disappearance of electron-dense patches observed principally in undifferentiated plastids and, with 24 h exposure, these were no longer observed (Figure 26). No morphological distinction could be drawn between plastids located in cells of cortical and stelar origin.

5.4 DISCUSSION

Barlow (1981) has indicated that differentiation of cells within the root apex may occur in response to cap removal in the absence of cell division, so that cells showing the correct spatial arrangement may develop properties more usually associated with the cellular function of the root cap. He suggests that differentiation arises in response to cap-determining gradients of morphogens induced within the remaining cells of the root apex. Very little is known of the origin of these gradients and the ultrastructural changes in cytoplasmic organelles following decapping and in the presence of Al are pertinent to elucidating the processes involved in the re-establishment of biochemical gradients within the root apex.

5.4.1 The Control of Golgi apparatus Function in Response to Cap Removal

The behaviour of the Golgi apparatus is correlated with cellular development (Mollenhauer, 1965; Mollenhauer and Mollenhauer, 1978) and the dictyosomes of the quiescent centre are notable for the low level of activity suggested by their small size and absence of secretory vesicles (Clowes and Juniper, 1964; Mollenhauer and Morré, 1966). Abrupt increases in the secretory activity of the control treatments in response to decapping, implied by an almost linear increase in the frequency of occurrence of dictyosomes with time, an initial increase in the number of cisternae per dictyosomal stack, and distinctive changes in the appearance of vesicular contents, may be considered evidence that the loss of the cap coincides with the temporary removal of a constraint on the function of the Golgi apparatus.

Characteristic differences in the secretory patterns of the Golgi apparatus found in the cell populations of the root apex have been widely described (Mollenhauer, 1965; Mollenhauer and Morré, 1966; Barlow, 1975; Mollenhauer and Mollenhauer, 1978) and have generally been associated with cellular differentiation (Barlow, 1975). The Golgi apparatus of the peripheral cap cells are distinguished by the granular appearance of the vesicular contents (Mollenhauer and Morré, 1980). This is not characteristic of neighbouring cell populations of the cap or epidermal regions of the root apex where vesicles containing electron-dense material are usually encountered (Mollenhauer and Morré, 1980). These observations suggest that Golgi apparatus activity associated with the presence of vesicles containing a granular secretory product coincides with the spatial arrangement of cells with cap properties, as described by Barlow (1981) in decapped roots, and is of fundamental importance in root physiology. These observations also accord with the findings of Barlow and Sargent (1978) who demonstrated that the outer cells of decapped roots possess an increased capacity to incorporate ^{3}H - glucose - a property characteristic of peripheral cap cells (Barlow, 1974a) and related to Golgi apparatus function

(Northcote and Pickett-Heaps, 1966).

The re-establishment of biochemical gradients in decapped roots, as suggested by morphological differences between Golgi apparatus located in cells of the quiescent centre of cortical and stelar lineages and based largely on the occurrence of secretory vesicles containing granular material, infer that the Golgi apparatus function may be involved in re-imposing constraints on Golgi apparatus development. Furthermore, the differentiation of plastids with large, well-defined starch grains, which Barlow (1981) has considered a marker of resumed cap function, coincided with this distinctive Golgi apparatus secretory pattern. Elaboration of a granular secretory product may not, however, represent the full Golgi apparatus function of the outer cap cells, since the oval-shaped secretory vesicles and accumulation of Golgi apparatus-derived material typically associated with slime production in the peripheral cap region (Mollenhauer and Morré, 1980), was only evident with regeneration of recognizable cap tissue.

5.4.2 Inhibition of Golgi apparatus Function by Aluminium

Aluminium toxicity has been connected with inhibition of vesicle transfer and alteration to the secretory product elaborated in the dictyosomes of the peripheral cap cells (Chapter 4). A principal function of the Golgi apparatus involves the production of secretory vesicles (Morré, 1977a), and it is therefore of consequence that in decapped roots, Al action was directed at the elaboration of the granular secretory product, since vesicles containing this material were infrequently observed and only with extended times after cap removal. Inhibition of vesicle transfer, suggested by an accumulation of secretory vesicles in the vicinity of dictyosomes, was indicated only in the case of vesicles which contained granular material. Aluminium-treated roots also failed to re-establish the biochemical gradients observed in the control, and differences in Golgi apparatus morphology between quiescent centre cells of cortical and stelar lineage were not observed.

The bulk of the Golgi apparatus membranes are thought to be contributed by the endoplasmic reticulum (Morré, 1977b). The present experiments have,however, indicated that the initial reduction in the number of dictyosomes coincided with a sharp increase in the number of lipid drops present in the plastids. Lipids are important constituents of plant membranes (Vickery and Vickery, 1981) and these observations may imply a rôle for Al in preventing the assembly of membrane material, which accords with the slower development of the Golgi apparatus noted in Al-treated cells.

These experiments also suggest that growth stimulation of the cells of the root apex in response to Al treatment (Chapter 4) may be delayed in decapped roots, and that the appearance of these symptoms may correlate with the development of the secretory activity in the Golgi apparatus.

5.4.3 Plastid Differentiation in Response to Cap Removal in the Absence of Aluminium

Barlow and Grundwag (1974) have demonstrated that cap removal initiates a developmental sequence in the plastids of the cells of the quiescent centre, and have intimated that structural features of the majority of plastids immediately following cap removal are indicative of repression of plastid development. The elaboration of the membrane system and accumulation of starch in plastids found in cells of cortical lineage essentially followed the pattern described (Barlow and Grundwag, 1974).

Factors which govern plastid development are as yet unknown (Barlow and Grundwag, 1974). Re-establishment of axial gradients in plastid differentiation coincided with changes in Golgi apparatus function and, as the plastids in quiescent centre cells of stelar lineage show many of the features previously connected to repression (Barlow and Grundwag, 1974), it is inferred that gradients of morphogens are re-established before recognizable cap tissue is formed.

5.4.4 Aluminium Toxicity and Plastid Differentiation

Many plastids in Al-treated cells failed to develop in response to decapping and membrane profiles were frequently indistinct. The action of Al is, however, not considered to be primarily directed at plastid differentiation, since initially, many of the changes in plastids involving starch accumulation and associated with cap removal showed little quantitative difference between Al-treated roots and the control. Furthermore, the rôle of the electron-dense regions observed in many plastids is anomalous. Barlow and Grundwag (1974) have connected the presence of these regions, thought to represent DNA fibres (Salema and Badenhuizen, 1969), to sites of starch synthesis and accumulation. Aluminium has a clear inhibitory effect on the occurrence of these fibres which could be interpreted as inhibition of DNA synthesis by Al, which accords with the work of Wallace and Anderson (1984). Barlow (1981) has, however, shown that differentiation of apical cells in which amyloplast development was used as a marker of cap function, proceeded in the absence of mitosis which was suppressed by the use of DNA inhibitors. Amyloplast development is inhibited by Al, and a primary rôle for Al in preventing DNA synthesis therefore seems improbable. As a corollary, the arrested condition of plastids in Al-treated cells may arise as a consequence of failure of substrate induction mechanisms.

Observations with respect to the effect of Al on plastid differentiation support the idea of functional interdependence between Golgi apparatus and plastid differentiation. The presence of large starch grains was not a feature of Al-treated roots, even with extended periods after cap removal, and the occurrence of isolated plastids containing defined starch grains invariably coincided with the evidence of Golgi activity.

5.4.5 An Hypothesis for a Morphogen Originating in the Peripheral Cap

There are numerous suggestions of growth inhibiting substances originating in the root cap (Gibbons and Wilkins, 1970;

Pilet, 1975a). Experiments with decapped roots in the presence and absence of Al have inferred that the Golgi apparatus secretory activity involving a granular secretory product may be connected to the occurrence of a morphogen mediating Golgi apparatus activity and plastid differentiation. Furthermore, as the re-establishment of biochemical gradients in decapped roots in the absence of Al, interpreted in terms of Golgi apparatus and plastid morphology, occurs prior to the formation of recognizable cap tissue and precedes a return to the quiescent state, it is conceivable that a morphogen may also be involved in imposing quiescence on the cells of the quiescent centre. This idea accords with the previously expressed hypothesis that disruption of Golgi apparatus function by Al in the peripheral cap cells of intact roots precedes removal of the constraint on the growth of the cells of the quiescent centre (Chapter 4).

If Al decreases the synthesis of the morphogen by the disruption of Golgi apparatus function, a considerably wider involvement for this morphogenic substance in root physiology may be indicated, since Al toxicity has been connected to a decline in mitotic activity (Clarkson, 1965) and DNA synthesis (Wallace and Anderson, 1984). The endomembrane system, including the Golgi apparatus is, however, characterized by the absence of DNA (Morré and Mollenhauer, 1974) and furthermore, the presence of the morphogen is not in itself dependent on either DNA synthesis or cell division (Barlow, 1981). Since direct action between Al and nucleic acids appears improbable, it may be appropriate to consider that reduced mitotic activity and DNA levels following Al treatment are indicative of a functional involvement of the morphogen in controlling these activities. The precise biochemical pathway and the identity of the morphogen remain to be considered, and these aspects will be discussed in Chapters 6 and 7.

5.5 CONCLUSIONS

The development of Golgi apparatus secretory patterns, notably involving the synthesis of a granular secretory product,

occurred in quiescent centre cells in response to cap removal in the absence of Al. Morphological and biochemical (Barlow and Sargent, 1978) similarities with the secretory patterns more usually associated with the peripheral cap cells, suggest that these changes in Golgi apparatus activity in response to decapping, correlate with the establishment of biochemical gradients in the root apex which are expressed through morphological differences evident between dictyosomes and plastids located in quiescent centre cells of cortical and stelar lineages. Changes in Golgi apparatus activity occurred synchronously with the differentiation of proplastids to form amyloplasts. These changes which temporarily coincided with the spatial arrangement of cap cells, preceded the formation of recognizable cap tissue. This observation adds to the idea that positional control may be a factor in determining the pattern of differentiation and development between cell populations of the root meristem.

Aluminium was effective in inhibiting changes in Golgi apparatus activity in response to decapping so that dictyosomes with defined membrane structure were only infrequently encountered. Vesicle membranes were also not clearly defined and secretory vesicles accumulated in the vicinity of dictyosomes. Secretory vesicles containing a granular secretory product were infrequently encountered and only with extended periods after cap removal. Many plastids in Al-treated cells also failed to develop internal organization and starch accumulation was only apparent with extended Al treatment times in isolated cells where it was linked to the presence of dictyosomes.

The precise mechanism of control was not identified. These observations are, however, consistent with the existence of a Golgi apparatus-derived morphogenic substance which may be involved in the control of Golgi apparatus secretory patterns and amyloplast development in the root meristem.

The possible identity of this morphogenic material and its wider involvement in root physiology will be considered in Chapters 6 and 7.

CHAPTER 6

ABSCISIC ACID AS THE GOLGI APPARATUS MORPHOGEN

6.1 INTRODUCTION

The pattern of differentiation induced in the cells of the quiescent centre by cap removal (Barlow and Grundwag, 1974; Chapter 5) is altered considerably by the presence of Al (Chapter 5). These experiments have provided evidence that formation of amyloplasts may be under the control of a morphogenic substance associated with alterations in Golgi apparatus activity. The identity of the morphogen is unknown and, although Barlow and Grundwag (1974) have suggested that amyloplast development in response to decapping may reflect alterations in the distribution of growth substances, ions or starch precursor levels, they have been unable to demonstrate rôles for gibberellic acid, indole acetic acid or sucrose in amyloplast formation.

Identification of ABA has, however, been made in the roots of a number of plants (Pilet, 1975a; Lachno *et al.*, 1982; Audus, 1983), although the function in root physiology remains subject to lively debate (Audus, 1983).

In this Chapter, the effect on plastid differentiation and Golgi apparatus activity of treating previously decapped roots of *Zea mays* with different concentrations of ABA is considered.

6.2 EXPERIMENTAL TREATMENT

6.2.1 Plant Growth Conditions

Plants (5) of *Zea mays* cv. TX 24 with decapped roots (Section 2.2.8.1) were either transferred to half-strength nutrient solutions containing no ABA (control), or to 250 ml quantities of 5×10^{-5} M and 2×10^{-4} M ABA solutions and grown

with continuous aeration in the dark for 24 h after decapping. Root tips from each treatment were processed for light and electron microscopy (Sections 2.4.2; 2.4.3; 2.4.4). The ABA solutions were prepared by dissolving Sigma A 1012 mixed isomer ABA in the minimum quantity of 95% ethanol and diluting to the required concentration (Markhart et al., 1979).

6.2.2 Interpretation of Results

The small amount of plant material generated by this experiment reflects the need to maintain adequate concentrations of both ABA and nutrient elements at the root surface during the growth period, in acceptably small volumes of solution, dictated by the high cost of ABA. These requirements restrict the extent to which quantitative data can be subject to meaningful statistical treatment. In an attempt to overcome these deficiencies in the comparative treatment of the results of an electron microscope investigation, a wider than usual range of micrographs illustrating the range of structure encountered at both ABA concentrations, has been included.

6.3 RESULTS

The pattern of differentiation induced in the cells of the quiescent centre in response to cap removal has been described comprehensively by Barlow and Grundwag (1974) and Barlow and Sargent (1978) and in Chapter 5.

In the present experiments, the control treatment was associated with alterations in the secretory pattern of the Golgi apparatus involving the appearance of intermediate secretory vesicles described in Chapter 5, and the elaboration of a granular secretory product in quiescent centre cells of cortical lineage (Figure 27). Plastids were seen to be in a state of division immediately following cap removal (Figure 27A). Changes in the Golgi apparatus secretory pattern were associated with differentiation of plastids involving the accumulation of starch and the formation

of amyloplasts which were clearly evident in the cells of the quiescent centre and neighbouring meristem 24 h after cap removal (Figure 27D). At the light microscope level, alterations in the plane of cell division (Barlow and Grundwag, 1974) associated with cap regeneration,were also apparent at this stage.

Twenty-four hours after treatment of decapped roots with 5×10^{-5} M and 2×10^{-4} M ABA, the cells of the quiescent centre and surrounding meristem remained free of amyloplasts. Little distinction could be made between the effectiveness of the two ABA concentrations with respect to repression of plastid differentiation (Figure 28). In both cases, plastids were characterized by a lack of internal organization, lipid drops (plastoglobuli) and electron-dense regions (possible DNA fibres) although present, were infrequent, and starch grains were not often encountered and were always small (Figure 28). Plastids were numerous at both ABA concentrations, but there was no evidence of division.

ABA was also effective in preventing alterations to the secretory pattern of the Golgi apparatus. Twenty-four hours after decapping, the dictyosomes in the quiescent centre of ABA-treated roots continued to be characterized by their small size and low level of activity implied by the paucity of secretory vesicles (Figure 29). Secretory vesicles were invariably small when present and rarely contained evidence of a granular secretory product which was only occasionally observed at the lower ABA concentration. Concentration effects were associated with the development of the dictyosomes, and membrane structure showed better definition at the lower ABA level, while membrane profiles at the higher concentration were often indistinct (Figure 29).

No evidence emerged during the experiment of the initiation of cellular changes involved in cap regeneration. ABA-treated roots were also not characterized by abnormal cellular growth in the mid-cortical region of the root apex, as described in Chapters 3 and 4, in consequence of Al treatment.

6.4 DISCUSSION

6.4.1 Control of Plastid Differentiation by Abscisic Acid

Microsurgical experiments involving the removal of the root cap have been reported to result in a temporary loss of gravitropic sensitivity (Barlow, 1974b) and to initiate a coincidental developmental sequence involving differentiation of plastids located in the quiescent centre and neighbouring meristem, culminating in the formation of amyloplasts (Barlow and Grundwag, 1974) some 14 to 22 hours after decapping (Barlow and Grundwag, 1974; Audus, 1983). Resumption of gravitropic sensitivity by the root occurs synchronously with the appearance of amyloplasts (Barlow, 1974b; Audus, 1975; Jackson and Barlow, 1981) and precedes cap regeneration, which has been considered evidence for the involvement of the dense, starch-containing organelles in a gravity-sensing function (Jackson and Barlow, 1981).

Factors which govern amyloplast development are therefore of considerable interest. In intact roots, the plastids located in the quiescent centre, although clearly possessing an inherent capacity to form amyloplasts, are repressed from entering this developmental sequence (Barlow and Grundwag, 1974) by the presence of the cap and retain a simple internal structure (Clowes and Juniper, 1964), characterized by an absence of starch. In Chapter 5, where Al treatment was seen as an inhibitor of Golgi apparatus function, evidence was reported which implied that the changes initiated in the plastids of the quiescent centre by the decapping operation were controlled by alterations in Golgi apparatus secretory activity, involving the elaboration of a granular secretory product.

The mechanism of control was not identified. The present experiments are therefore significant since they demonstrate that exogenously supplied ABA is entirely effective in preventing plastid differentiation. Addicott and Carns (1983)

have also intimated that a positive response to the application of a naturally occurring substance supports the involvement of that substance in regulating the response. It is therefore proposed that ABA may be the morphogenic substance discussed in Chapter 5, which occurs in the root meristem and is associated with Golgi apparatus secretion and the control of plastid differentiation.

6.4.2 Golgi apparatus Activity and Abscisic Acid Synthesis

It is significant that in the absence of exogenous ABA, alterations in function of quiescent centre cells of cortical lineage following decapping coincided temporally with the spatial arrangement of cap cells (Chapter 5). Furthermore, the behaviour of the Golgi apparatus is known to correlate with cellular development (Mollenhauer, 1965; Mollenhauer and Mollenhauer, 1978), and characteristic differences in the secretory pattern of the Golgi apparatus found in the cell populations of the root apex have been widely reported (Mollenhauer, 1965; Mollenhauer and Morré, 1966; Mollenhauer and Mollenhauer, 1978). The Golgi apparatus of the peripheral cap cells are notable for the presence of a granular secretory product (Mollenhauer and Morré, 1980) which is absent from neighbouring cell populations of the cap and epidermal regions of the root apex (Mollenhauer and Morré, 1980).

Considerable importance must therefore be attached to the observation that decapping induces in the absence of ABA a secretory pattern in the Golgi apparatus of the quiescent centre with morphological similarities to that occurring in the peripheral cap. Furthermore, since exogenous ABA is effective in inhibiting these changes in secretory activity, and since the Golgi apparatus in ABA-treated roots retain characteristics consistent with repressed development evidenced by the absence of secretory vesicles, it is conceivable that control of Golgi apparatus secretory patterns in the root apex is effected through the basipetal movement of ABA synthesized in the outer cap.

Synthesis of ABA in the peripheral cap cells is consistent with the interpretation that root cap activity in these cells demonstrates steady state abscission through the continuous cell separation of the older cap cells (Addicott, 1982). The effect of Al on processes involved in controlling the loss of mature cells from the cap periphery will be discussed in Chapter 7. The absence of evidence in favour of cap regeneration in ABA-treated, decapped roots accords with the hypothesis involving the basipetal movement of ABA from the cap, since Pilet and Nocera-Przybecka (1978) have indicated that cap regeneration arises from the release of ABA-induced inhibition of DNA synthesis in the quiescent centre by decapping. This concept is supported by the paucity of electron-dense regions (DNA fibres) encountered in plastids of ABA-treated roots and contrasts with observations made in Chapter 5 where these regions were observed to be particularly numerous in plastids prior to cap regeneration.

6.4.3 An Alternate Rôle for Abscisic Acid in Gravistimulation

There have been many attempts to connect the root cap with the source of a basipetally-moving, water-soluble inhibitor of root growth (Gibbons and Wilkins, 1970; Pilet, 1972; Feldman, 1981; Chanson and Pilet, 1982; Feldman, 1984a) which is also able to produce a positive gravitropic response. Confidence in this explanation of root gravitropism would be increased by the identification of a suitable endogenous inhibitor of root growth (Firn and Digby, 1980). Although ABA has been regarded as a serious candidate, evidence for a positive rôle in gravitropic stimulation has yet to be demonstrated (Jackson and Barlow, 1981; Audus, 1983). Moreover, the specific action of ABA in promoting gravitropic curvature is unclear, since it has been shown to act as an inhibitor (Pilet and Chanson, 1981), and a temporary promoter (Mulkey *et al.*, 1983) of root growth in *Zea*.

Evidence which currently favours the root cap as the site of gravitropic perception is largely derived from the presence of amyloplasts within the cap cells (Jackson and Barlow, 1981).

Abscisic Acid has been positively identified in the root tip of Zea (Rivier and Pilet, 1981) and furthermore, the present experiments have implied that ABA is effective in simulating many of the functions associated with the presence of a root cap, including control of starch deposition and repression of plastid differentiation in the quiescent centre which would otherwise occur in response to cap removal. Iversen (1969) has confirmed a fundamental rôle for starch metabolism in the perception of gravitropic stimuli by demonstrating that starch depletion of intact roots of cress (Lepidium sativum L.) produces a loss of gravitropic sensitivity. It is therefore reasonable to expect that the action of ABA in gravistimulation may be primarily directed along this pathway, involving the perception and initiation of the gravitropic signal. The mechanism remains uncertain, although Ho (1983) has intimated that the rôle of ABA predominantly involves the redirection of developmental sequences. Concentration gradients arising from the basipetal movement of ABA from the outer cap may therefore function through the re-distribution of assimilates within the root meristem. The de-capping operation may therefore be interpreted as temporarily depriving the root of ABA. This simultaneously removes the developmental sequences associated with amyloplast development normally occurring in the cap and connected to gravity perception, and results in alterations in the supply gradients of assimilates associated with repressed development of proplastids in the quiescent centre, culminating in the formation of amyloplasts and resumption of gravisensitivity.

Evidence which suggests a common control mechanism between the Golgi apparatus activity and plastid differentiation (Chapter 5) also serves to implicate the Golgi apparatus in gravi-perception. It is, however, significant that neither the decapping operation, nor treatment of decapped roots with ABA produces cellular expansion in the mid-cortex, coinciding with the region of cell growth associated with the gravistimulation of roots (Shen-Miller et al., 1978). Cell enlargement has, however, been connected to inhibition of Golgi apparatus function by Al

(Chapter 4) and these observations imply that the growth response may involve interactions between ABA and an as yet unidentified growth-promoting substance. It is relevant to this hypothesis that Feldman (1981) has shown that optimum production of a substance able to produce a positive gravitropic response occurs in root caps cultured on a medium supplemented with indole acetic acid.

6.5 CONCLUSIONS

Treatment of decapped roots with different concentrations of ABA has indicated that the morphogenic substance, proposed in Chapter 5, which is associated with the development of Golgi apparatus secretory patterns and the control of plastid differentiation, may be ABA.

Some major problems concerning the proposed rôle for ABA warrant critical consideration, since the results noted in these experiments contrast with findings at Glasgow University (S.K.Hillman, pers. comm.*). The latter investigation (unpublished) concluded that treatment of decapped roots with ABA did not influence the formation of amyloplasts or cap regeneration. The investigation did not extend to include the effect of ABA on Golgi apparatus activity.

In typifying a rôle for ABA, it is pertinent that the interpretation of experiments involving treatment of intact roots with Al are consistent with the conclusions reached in this Chapter. Aluminium-induced changes in root length (Chapter 7) provide evidence that an early consequence of Al treatment involves interruption in the basipetal movement of an endogenous growth inhibitor originating in the cap. Changes in root growth proceeded concurrently with diminished Golgi apparatus activity in the peripheral cap (Chapters 4 and 7), interference in mechanisms controlling the abscission of mature cells from the cap (Chapter 7), a decline in the number of amyloplasts in the cap (Chapter 7), evidence that starch accumulation in proplastids located in the cap meristem

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is inhibited (Chapters 4 and 7), and an increase in volume of quiescent centre cells (Chapter 4).

Abscisic acid has been frequently cast as an inhibitor of root growth (Pilet, 1975a, b; Pilet and Chanson, 1981). Furthermore, the analysis of roots has confirmed the presence of ABA in the cap (Rivier et al., 1977; Suzuki et al., 1979), which is transported in a basipetal direction (Chanson and Pilet, 1982). Moreover, the loss of cells from the cap has been associated with "steady state abscission" (Addicott, 1982) while ABA has been demonstrated to be effective in preventing cell enlargement (Barlow and Pilet, 1984).

The availability of nutrient ions may be relevant to explaining the differences in experimental findings which exist between this investigation and the work conducted at Glasgow University. Many experiments provide evidence that a constant supply of Ca is required (Poovaiah, 1985), since internal Ca is not redistributed to zones of new growth. Calcium exercises an essential, but poorly defined, function in the development of plant membranes (Clarkson and Hanson, 1980). The present experiments (Chapter 5) indicate that rapid increases in Golgi apparatus activity may precede cap regeneration. It is therefore conceivable that an interruption in Ca supply following decapping may prevent or delay these changes, thereby effectively altering the developmental sequences involved. This aspect clearly requires more detailed investigation.

CHAPTER 7

REGULATION OF ROOT DEVELOPMENT BY ALUMINIUM

7.1 INTRODUCTION

Previous chapters dealing with plant response to Al have attached importance to the root cap in the expression of toxicity (Chapter 3), where the earliest ultrastructural effect was directed at the Golgi apparatus of the peripheral cap cells (Chapter 4). Reduction of root elongation, arising from diminished mitotic activity, nevertheless remains one of the most obvious consequences of toxicity (Clarkson, 1965). The structure of nuclei in cells of the cap and proximal meristems is, however, resistant to Al-induced damage (Chapter 4), and an understanding of the physiological response to Al^{3+} is additionally complicated by the observation that many of the changes identified in the roots of Al-stressed plants do not directly coincide with the presence of Al in the tissues involved (Chapters 3 and 4).

Although Clowes (1972b) has unequivocally demonstrated an interaction between the root cap and cell proliferation in the root meristem, little is known to indicate how removal of mature cap cells influences the cycling rate of the remaining cap meristem and quiescent centre (Barlow, 1975). The cell populations comprising the root apex are principally characterized by marked differences in cell cycle times (Clowes, 1961; De la Torre and Clowes, 1972; Barlow, 1975). Co-ordination of the activities of these cells must therefore represent a finely balanced equilibrium.

Interactions between cell populations have been experimentally verified (Feldman, 1977; Charlton, 1980; Barlow and Rathfelder, 1984; Clowes, 1984), but definition of how this co-ordination is achieved in actively growing roots remains less than satisfactory. How Al may disrupt intercellular relationships in the root meristem has not previously been considered. Aluminium uptake has, nevertheless, been connected to polysaccharide metabolism

(Chapter 3) and Golgi apparatus function is thought to include a morphogenic material involved in controlling plastid differentiation (Chapter 5). In this Chapter, the effects of different concentrations of Al on the function of the cap and the concomitant consequences for root development are considered.

7.2 EXPERIMENTAL TREATMENT

7.2.1 Experiment 1

Plants (cv. TX 24) were grown for seven days in full-strength nutrient solutions (Sections 2.2.2; 2.2.8). The nutrient solutions were then replaced with solutions of similar composition to which $Al_2(SO_4)_3 .18H_2O$ had been added to give root treatments of 0 (control); 0,5; 1,25; 2,0; 3,0; 5,0 mg dm^{-3} Al. pH was adjusted to 4,6 subsequent to the addition of Al. Solution pH was maintained at pH 4,6$\pm$ 0,1 over 29 h by the addition of measured volumes of 0,1 M NaOH. Hydrogen ion efflux determinations were repeated in a second experiment with 7 h Al treatment times. To assess the effect of factors not related to plant growth on nutrient solution pH, a single solution containing 5,0 mg dm^{-3} Al was prepared in which no plants were placed. The pH of this solution was monitored, but no change in solution pH was recorded over 29 h.

At the conclusion of the experiment (29 h), root tips from each treatment were processed for light and electron microscope viewing (Sections 2.4.2; 2.4.3; 2.4.4).

Measurements of cells and cell populations included determination of the volume of the root cap, mean cell volume of cells of the cap meristem, central cap columella and peripheral cap as defined in Figure 30. A minimum of five roots was examined from each treatment to determine mean values. Longitudinal sections were cut sequentially through each root tip. Median sections were then selected for further measurement so as to ensure a maximum cap length (H). Measurements were made at a magnification of X 240 following the procedures and assumptions

outlined by Barlow (1974a) and Barlow and Rathfelder (1984).

Root diameter measurements on longitudinal sections were made 1 mm from the root tip (Figure 30) by using a calibrated eyepiece on an optical microscope.

Counts were also made of the number of amyloplasts found in the root cap cells. Numerical estimates of the degree of amyloplast sedimentation in the central cap were based on the frequency with which amyloplasts were observed to be present in the proximal half of central cap cells. A minimum of five roots was examined to determine mean values from each treatment.

7.2.2 Experiment 2

To test the effect of Al treatment on root growth, plants (cv. TX 24) were grown (Sections 2.2.2; 2.2.8) for fourteen days at six Al levels (0; 0,5; 1,0; 3,0; 5,0 and 10,0 mg dm^{-3}). Nutrient solution pH was adjusted to 4,6 prior to planting. After fourteen days, the plants were harvested and the length of the primary root measured. The experiment was duplicated to provide a second set of measurements. Results were expressed as the mean value for forty-six plants which comprised each treatment.

7.2.3 Experiment 3

To identify the regions of the root where H^+ efflux was initiated by Al treatment, pregerminated grains (cv. TX 24) were planted along the upper edge of 66 X 22 cm filter paper (Section 2.2.8.1). Plants were grown for three days at 28°C, selected for a straight root axis measuring $100 \pm$ 10 mm.

Ten plants were transferred to a water culture containing either 0 Al (control) or 10 mg dm^{-3} Al as $Al_2(SO_4)_3$ for 10 minutes. Roots were rinsed in deionised water for 5 minutes before transfer to an agar block containing bromocresol purple at pH 6,0. Agar plates (3 mm) were prepared from 0,6% agar and included plant

nutrients (mg dm^{-3}) N 18; P 1,5; K 20; Ca 20; Mg 5; S 7; Na 0,75; Mn 0,05; Zn 0,03; Cu 0,01; Fe 3; Mo 0,02; B 1.

H^+ efflux from the root caused the indicator to become yellow. The method for preparation of agar plates and measurement of H^+ efflux was similar to that described in detail by Mulkey et al. (1981) and Moore (1985).

7.3 RESULTS

7.3.1 Cap Shape and Construction

Aluminium treatment was effective in altering the mean volume of the root cap over a 29 h period. An increase in Al concentration up to 1,25 mg dm^{-3} produced increases in cap volume, while further escalation in Al levels beyond this point resulted in a decrease in mean cap volume so that at the highest Al concentration (5,0 mg dm^{-3}), cap volume was not different from the control (Figure 31). Alterations in cap function arising from Al treatment were, however, indicated by changes in peripheral cap cell volume (Figure 32) and cap cell morphology (Figure 33). Changes in cap volume coincided with alterations to the number of cap cells viewed in median longitudinal section ($r = 0,59^*$)[+]. Aluminium concentrations $\leq$1,25 mg dm^{-3} produced an increase in cell numbers over the control (302). These changes preceded a drop in numbers from a root Al treatment of 1,25 mg dm^{-3} (327) with the fewest cells (270) being recorded at the highest Al level (5,0 mg dm^{-3}).

Differentiation of cells within the cap is usually accompanied by an increase in cell size (Barlow, 1975). Notwithstanding variation in the size of cells resulting in a substantial standard error, Al-induced changes in cell differentiation were supported by decreases in mean volume of peripheral cap cells, evident at Al levels above 1,25 mg dm^{-3}

[+] * significant at 5% level

(Figures 32 and 33). In contrast to the situation in the cap periphery, Al treatment produced modest but statistically insignificant increases in mean cell volume in the cap meristem (12,3 μm^3 X 10^2; 0 Al; 14,3 μm^3 X 10^2; 5,0 mg dm^{-3} Al) and central cap (54,9 μm^3 X 10^2; 0 Al; 66,9 μm^3 X 10^2; 5,0 mg dm^{-3} Al). Notwithstanding the stability of central cap cells to changes in size, the geometry of these tissues involving the longitudinal arrangement of cell files was extensively altered at Al concentrations $\geq$ 3,0 mg dm^{-3} (Figure 33).

Counts of the number of detached mature cap cells present in the mucilaginous material surrounding the root and cap showed that a decrease of 52% was associated with the presence of Al in the nutrient solution. No treatment effect connecting cell loss to Al concentration was, however, detected.

7.3.2 Root Growth

Over a fourteen day growth period, low Al concentrations ($\leq$1,0 mg dm^{-3}) were associated with an Al-stimulated growth response of the primary root, evidenced by an increase in mean primary root length over the control (Figure 34). In contrast, intensification of Al concentration beyond this point resulted in a sharp drop in root elongation (Figure 34).

7.3.3 Amyloplast Distribution

Over a 29 h period, the number of amyloplasts present in the cap showed a sharp decrease with increasing Al concentration (Figures 33 and 35). The distribution of amyloplasts in central cap columella cells was also altered by Al treatment, so that the number of amyloplasts present in the proximal half of central cap cells of Al-treated roots was higher than that found in the control. The highest frequency of non-sedimenting amyloplasts was observed at an Al concentration of 2,0 mg dm^{-3}.

7.3.4 Ultrastructural Response to Aluminium

7.3.4.1 Peripheral Cap Cells

The present experiments showed that the action of Al was primarily directed at the Golgi apparatus in the peripheral cap cells, and the previously reported effects of Al (Chapter 4) which included disruption of cisternal structure, damage to vesicular membranes and changes in the appearance of vesicle contents, were all evident at 0,5 mg dm^{-3} Al (Figure 36). A progressive deterioration in Golgi apparatus activity was also connected to increases in Al concentration (Figure 36D), and at 5,0 mg dm^{-3}, characteristic Golgi apparatus activity was no longer apparent in many peripheral cap cells (Figure 37C). Comparisons between Figures 36B amd 36C indicated that changes in Golgi apparatus activity coincided with an altered appearance and decrease in frequency with which Golgi apparatus-derived material was accumulated between the plasmalemma and cell wall. Some accumulation of Golgi material could, however, still be detected at 5,0 mg dm^{-3} Al (Figure 37C), although inhibition of transfer of mucilagenous material to the root exterior was indicated by the absence of slimes which were no longer evident on the root exterior of living roots.

Treatment with Al was associated with increased vacuolation of outer cap cells (Figure 37C).

7.3.4.2 The Central Cap Cells

Golgi apparatus secretory patterns correlate with cellular development (Mollenhauer, 1965), and the Golgi apparatus in central cap cells narrowly removed from the peripheral cap regions and distinguished by the size and appearance of the secretory vesicles (Figure 36E), showed little response to low concentrations of Al ($\leq$ 2 mg dm^{-3}). Increasing Al concentrations disrupted the Golgi apparatus in these cells, although the response was notably less dramatic than that observed

in the peripheral cap cells.

Changes in amyloplast structure which involved the size and number of starch grains were only suggested at higher Al levels ($\geq$ 3 mg dm^{-3}). In other respects, amyloplast structure was notably resistant to Al-induced changes.

Vacuolation of central cap cells was evident at an Al concentration of 5,0 mg dm^{-3}.

7.3.4.3 The Cap Meristem

Changes in nuclear structure involving the degree of chromatin condensation, appearance of the nucleolus and the structure of the nuclear membranes were not discernible after 29 h treatment with 5,0 mg dm^{-3} Al (Figures 37A and 37B). Cells of the cap meristem were, however, characterized by increased vacuolation at an Al concentration of 5,0 mg dm^{-3} (Figure 37B) and, while many proplastids were present, few showed evidence of starch deposition (Figures 37A and 37B).

7.3.5 Cell Enlargement

Alterations in the polarity of cell enlargement (Chapter 4) in the primary growth region of the root was indicated by an increase in mean root diameter evident after 29 h treatment at Al concentrations $\geq$ 1,25 mg dm^{-3} (Figure 38).

7.3.6 Efflux of H^+ from the Root

Aluminium treatments over the full experimental range were effective in more than doubling the efflux in H^+ from the roots (Figure 38), and measureable pH changes of the nutrient solution were detected within 1 h. Quantitative estimates of H^+ efflux were derived from the NaOH additions required to maintain the nutrient solution pH within the prescribed limits and on total root dry matter yield. Differences in H^+ efflux were not initially

evident at different Al concentrations (7 h) (Figure 38) but, with longer times, diminished H^+ efflux was observed at the higher Al concentrations, and the difference between 0,5 mg dm^{-3} Al (4,01 mg H^+g^{-1} root dry mass) and 5,0 mg dm^{-3} Al (2,10 mg H^+g^{-1} root dry mass) with 29 h treatment time, was almost twofold.

7.3.7 Root Zones Associated with H^+ Efflux

Acid (H^+) efflux in vertically orientated roots treated with Al was initiated simultaneously in the root apex, cap and elongation zone of the root and was evident within 3 minutes. Control roots were appreciably slower to demonstrate H^+ efflux which was only detected after 12 minutes. The lower level of colour development also implied that H^+ efflux was of a lower intensity in the control.

7.4 DISCUSSION

The present experiments add to the idea that a functional integration of intercellular activities in the root meristem may be directed through polysaccharide metabolism in the root cap. Furthermore, the absence of evidence to connect Al treatment with damage to the nuclear structure and the initial exclusion of Al from tissues containing the actively dividing cells (Chapter 4), imply the existence of a stress response link between the Golgi apparatus of the outer cap where the earliest ultrastructural response to Al was evident, and the mitotically active cells of the root meristem.

7.4.1 Aluminium Toxicity and Abscission of Cap Cells

Cap cells are constantly displaced from the cap meristem at the base of the cap to the tip where they are sloughed off (Barlow, 1975). In actively growing roots, the total number of cap cells remains nearly constant, and it has been aruged that the loss of cap cells must represent a highly organized and

co-ordinated process (Juniper, 1972; Barlow, 1975).

The response of the root cap to low concentrations of Al principally involved the peripheral cap cells where Al is effective in influencing mechanisms which control the loss of mature cap cells. The initial increase in cap volume observed at low Al concentrations, is brought about by increased cap cell numbers, and these events suggest that an Al-induced reduction in cell loss is not, under these conditions, met by a coincidental decline in the activity of the cap meristem. Decreases in cap volume and cell numbers, which occur at higher Al concentrations, presumably reflect reductions in mitotic activity.

Cell enlargement and abscission which normally proceed nearly synchronously in the outer cap, must both depend on a concomitant loosening of cell wall material. Aluminium-induced decreases in volume of peripheral cap cells, observed with heightened Al concentration, may therefore, serve to confirm the ultimate failure of the mechanisms controlling these processes.

7.4.2 Growth Response of Primary Roots

A positive growth response to low concentrations of Al has been reported for a number of plant species (Foy et al., 1978). Mechanisms whereby small amounts of Al may increase plant growth remain obscure and have been ascribed to the application of nutrient culture techniques (Adams, 1980).

It is therefore pertinent that an Al-induced increase in cap volume corresponded with an increase in mean root length. These observations attach physiological relevance to the finding (Barlow and Rathfelder, 1984) that statistical correlations exist in a number of species between cap volume and the size of the proximal meristem. The results of these experiments also imply that the relatively short-term effects (29 h Experiment 1) of Al on root development are translated into a permanent growth response still evident after fourteen days (Experiment 2).

Interpretation of the effect of Al on root growth may depend initially on changes in the root cap being reflected through a partial release of the proximal meristem from growth inhibition. Similar responses involving growth stimulation have been reported in decapped roots (Pilet, 1972). These observations accord with the numerous suggestions which have attempted to link the basipetal movement of growth inhibiting substances originating in the cap with the regulation of root development (Gibbons and Wilkins, 1970; Pilet, 1972; Feldman, 1981; Chanson and Pilet, 1982; Barlow and Pilet, 1984; Feldman, 1984a). Expression of Al toxicity may therefore be directed through the synthesis of naturally occurring endogenous root growth inhibitors.

7.4.3 Integration of Intercellular Activity

Juniper and French (1970) have highlighted the substantial amount of polysaccharide material moved through the cap by the passage of amyloplasts contained in differentiating cells. The present experiments have identified a rapid reduction with increasing Al concentration in the number of amyloplasts present in the root cap. Evidence to connect starch degradation and slime synthesis with the activity of the Golgi apparatus is not unequivocal (Barlow and Sargent, 1978). In intact roots, the secretion of mucilagenous, polysaccharide slimes from the peripheral cap cells occurs while intercellular starch grains diminish in size (Ellmore, 1982; Northcote and Pickett-Heaps,1966). Furthermore, inhibition of Golgi apparatus function in the outer cap by Al proceeded synchronously with diminished slime synthesis which accords with the view (Mollenhauer and Morré, 1980) that the secretory functions of dictyosomes in the cap periphery include the packaging and export of mucilagenous materials from the cap.

Haberlandt (1914) originally considered that these materials facilitated root movement through the soil. A considerably wider involvement in root physiology for polysaccharide metabolism is, however, indicated. Barlow and Hines (1982) have

intimated that these mucous slimes may control the separation of cells from the root cap, which view is supported by the present experiments which additionally serve to implicate Golgi apparatus in this function.

Amyloplasts located in central cap cells were resistant to Al-induced changes in structure. Evidence exists to indicate that Al was, however, effective in preventing starch deposition in proplastids located in the cap meristem. These observations suggest that control of amyloplast numbers may be effected through starch induction mechanisms functioning in the cap meristem. An involvement for the peripheral cap Golgi apparatus in these activities is indicated by the finding that extends specific secretory activities of the Golgi apparatus to include a morphogenic material which is involved in the control of plastid differentiation (Chapter 5).

Changes in Golgi apparatus activity and a decline in amyloplast numbers preceded a reduction in root length. Although the quantity of polysaccharide material moved to the cap periphery in differentiating cells must relate to the cycling rate of the cap meristem, it is not yet known how Al-induced changes in polysaccharide metabolism are translated into root growth responses. Moreover, a paradoxical situation exists in that small changes in activity produce a positive growth response, while more substantial declines, as evidenced by further reductions in amyloplast numbers, coincided with progressive reductions in root length. An understanding of these events may depend on demonstrating that the initial response to low concentrations of Al is expressed through a release of the root from growth inhibition originating in the cap. Root growth at higher Al concentrations may, however, depend on minimum levels of activity being maintained in the root cap.

7.4.4 The Central Cap Cells

Amyloplasts present in central cap cells have been widely connected to a gravi-perception function (Iversen, 1969; Barlow, 1974b; Audus, 1975; Audus, 1979; Volkmann and Sievers, 1979), where they are believed to respond to gravity in predictable ways (Juniper, 1972).

The present experiments are therefore of interest in connecting the frequency of non-sedimenting amyloplasts to Al treatment. Juniper (1972) has intimated that the relative absence of vacuoles from central cap cells may influence amyloplast sedimentation. It is pertinent to this view that the present experiments have shown that Al-induced increases in the frequency of non-sedimenting amyloplasts preceded changes in the morphology of the central cap, where an increase in vacuolation and altered cell geometry were only detected at appreciably higher Al treatment levels.

The mechanism through which Al acts on amyloplast sedimentation is unknown. The present experiments do, however, serve to implicate factors additional to gravity and structural features of the central cap in influencing the behaviour of these organelles. It may be relevant to these ideas that Hillman and Wilkins (1982) have observed that it is not only the size and apparent stage of amyloplast development which determines the ability of the amyloplasts to sediment.

7.4.5 Mechanisms of Aluminium Action

An early response to Al treatment involved the rapid efflux of H^+ ions. Similar effects are reported in gravi-stimulated roots (Mulkey *et al.*, 1981; Moore, 1985) which Mulkey *et al.* (1981) have attributed, in the case of gravi-stimulation, to an auxin-induced growth response. An alternative interpretation of these events may, however, depend on the

observation that in roots treated with different Al concentrations, H^+ efflux preceded a detectable increase in root diameter arising from changes in the polarity of cell enlargement (Chapter 4).

Evidence exists to connect a net H^+ efflux occurring against electro-chemical gradients to membrane hyperpolarisation (Poole, 1978; Smith and Raven, 1979), where membrane-bound electrogenic pumps are thought to function by stabilizing membrane potential through the transport of protons (H^+) (Spanswick, 1981). The present experiments attach importance to the charge on the Al ion, since initiation of a number of adverse plant responses to Al occurred at Al concentrations above 1,25 mg dm^{-3}. At this concentration (1,25 mg dm^{-3}), Al and Ca were present in the nutrient solution in equivalent moles of charge, that is, when $1/2 \log Ca^{2+} - 1/3 \log Al^{3+} = 0$. This observation is also consistent with the view that Al may act through the Ca dependent regulatory protein calmodulin (Siegel and Haug, 1983). Confidence in an electro-physiological plant response to Al is, however, increased by the observations that the trivalent ions Sc and Ga are also effective inhibitors of cell division in the root meristem (Clarkson, 1965). Haug (1984) reports that the effect of Al on calmodulin is highly specific to Al. Furthermore, Volkmann and Sievers (1979) also report a loss in the polarity of central cap cells arising from the application of an electric field, while Behrens et al. (1985) associated asymmetry in the gravi-electrical properties of root caps, arising from depolarisation of membrane potential, subsequent to gravi-stimulation to changes in the sedimentation of amyloplasts.

The plasmalemma and tonoplast have generally been implicated with H^+ ATPases involved in electrogenic pumping (Sze, 1984). Both these endomembrane components may, however, be slow to respond to the presence of Al (Chapter 4). It is therefore, of considerable interest that Chanson and Taiz (1985) have identified the presence of Golgi ATPase(s) which can function as proton pumps.

7.5 CONCLUSIONS

Low levels of Al produced a positive growth response in the primary root which coincided with an increase in mean cap volume. This was interpreted as evidence for an Al-induced release of the proximal meristem from growth inhibition originating in the cap. Significant alterations in mean cell volume were limited to the cap periphery where Al caused a decrease in cell volume. This implied that the action of Al was directed at differentiation of peripheral cap cells and that the observed changes in cap volume arose through interference by Al in mechanisms which control cell loss from the cap. This observation was supported by a reduction in the number of detached, mature cap cells surrounding Al-treated roots.

Ultrastructural investigation confirmed that disruption of Golgi apparatus activity was evident at the lowest Al concentration (0,5 mg dm^{-3}). These observations serve to implicate the peripheral cap Golgi apparatus in the control of cell loss from the cap and accord with the suggestion (Chapter 6) that the morphogenic material associated with Golgi apparatus secretion could be ABA. A decrease in amyloplast numbers also coincided with diminished Golgi apparatus activity in the peripheral cap. These changes preceded detectable reductions in mitotic activity, indicated by decreases in cap volume and root length which were only evident at Al concentrations in excess of 1,25 mg dm^{-3}. This suggested that control of cell cycling within the root meristem is directed through starch metabolism involving the movement of amyloplasts contained in differentiating cap cells. These experiments have confirmed that the Al-induced decline in cell division (Clarkson, 1965) is a secondary consequence of Al treatment directed along a stress response reaction.

Al treatment also increased the number of non-sedimenting amyloplasts present in central cap cells and altered the linear arrangement of these cells. Both these observations are consistent with alterations in the electrical properties of the root cap. It is therefore significant that the presence of the Al^{3+} ion was associated with an efflux of H^{+} which was considered evidence to

support membrane hyperpolarisation by Al. Some of the more adverse responses to Al, including an inhibition of cell division, were observed to be initiated at Al concentrations where the molar ratio of charge between Ca^{2+} and Al^{3+} favoured Al. This may attach relevance to the electrical properties of the Al ion in the initiation of an electrophysiological response to Al.

In developing an overall concept of plant response to Al, it is relevant to consider that Al toxicity has been connected to a wide range of nutrient disorders (Foy et al., 1978). The extent to which Al-induced changes in nutrient status contributes to an understanding of plant reaction to Al will be considered in the next Chapter.

CHAPTER 8

ALUMINIUM INDUCED NUTRIENT DISORDERS

8.1 INTRODUCTION

Aluminium toxicity has been widely linked to a number of nutrient disorders in plants treated with high levels of Al, notably those involving P (Wright, 1943; Foy and Brown, 1963; Andrew and Vanden Berg, 1973), Ca (Foy et al., 1972; Foy, 1974) Mg and Mn (Clark, 1977; Grimme, 1982). Attempts to equate induced nutrient deficiencies with the physiological expression of Al toxicity are frequent (Wright, 1943; Foy and Brown, 1963; Foy et al., 1978), and the demonstration of a differential ability to acquire nutrient elements in the presence of Al has been interpreted as an expression of varietal differences in plant response to Al (Foy et al., 1972; Clark, 1977; Krizek and Foy, 1982).

Several authors (Wallihan, 1948; Clarkson, 1965; Clarkson, 1969) have questioned the concept that interactions between Al and P, involving the precipitation of aluminium phosphates in plant roots, represents a primary response to Al, and there are also indications that the uptake sites for Al (Chapter 3) and P (Clarkson and Hanson, 1980; Barber, 1982) may not coincide. Attempts to explain the effect of Al on the uptake of nutrient cations are infrequent (Clarkson and Sanderson, 1971).

Aluminium is also known to elicit a range of responses in the root (Clarkson, 1965; Chapters 4, 5 and 7), and it is therefore possible that the alterations in chemical composition of plants arising from Al treatment are induced through changes in root physiology. The purpose of this Chapter is to investigate the effect of Al treatment on the acquisition and transport of the nutrient elements P, K, Ca, Mg and Mn.

8.2 EXPERIMENTAL TREATMENT

8.2.1 Experiment 1

To test the effect of Al on the uptake and transport of P, K, Ca, Mg and Mn, a randomised, factorial design was employed which incorporated two maize cultivars (TX 24 and HL 1), grown in full-strength nutrient solution (Sections 2.2.2; 2.2.8*) which included six levels of Al (0; 0,5; 1,0; 3,0; 5,0 and 10,0 mg dm^{-3}) with two replications. Pots were rotated on a random basis every three days. After fourteen days, the plant tops were separated from the roots and the plant material was prepared for chemical analysis (Section 2.5.1).

8.2.2 Experiment 2

The effect on P uptake and transport of different Mg and Mn levels in the absence of Al was tested by means of a randomised factorial design in which Mg and Mn levels in the full-strength nutrient solution (Section 2.2.2) were adjusted to give concentrations in combination of 1,0; 3,0; 6,0; 12,0 mg dm^{-3} Mg and 0,11 and 0,22 mg dm^{-3} Mn. Procedures were otherwise similar to those followed in the preceding experiment.

8.2.3 Experiment 3

The effect of Al on the respiration of intact roots was investigated by growing plants, initially without Al, for a total of ten days (Sections 2.2.2; 2.2.8*). $Al_2(SO_4)_3$ solution was added in advance of harvest to give 0 h; 3 h; 6 h; 10 h and 20 h root exposure to 2,0; 4,0 and 8,0 mg dm^{-3} Al.

Ten plants from each treatment were sampled and the roots of intact plants treated with tetrazolium chloride (Section 2.3.2.3).

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* Artificial light was supplied as fluorescent cool white (269 Wm^{-2}) and incandescent (180 Wm^{-2}) to give an estimated light intensity of 35 Wm^{-2} at plant height (Cathey and Campbell, 1980). Conditions were in other respects identical to those outlined in Section 2.2.8.

8.2.4 Statistical Treatment of Results

These experiments were designed to test for differences in performance between cultivars. Where statistically significant differences did not emerge, data presentation has been simplified by grouping results which are expressed as the mean values for both cultivars.

8.3 RESULTS

8.3.1 Aluminium and the Acquisition of Nutrient Elements

8.3.1.1 Experiment 1

Root Al concentration responded to an increase in solution Al (Figure 39). Foliar concentrations of Al were not statistically related to Al treatment, and were appreciably lower (mean 44 mg kg^{-1}) than root levels ($<$ 0,10 X root concentration). Highly significant cultivar differences were found in the pattern of Al uptake. Cultivar TX 24 showed a progressive increase in root Al with increasing Al in solution, whereas cv. HL 1 accumulated up to five times more Al at low solution Al levels. Higher Al concentrations (5 and 10 mg dm^{-3}) were, however, associated with diminished root Al levels in cv. HL 1 and, at these levels, cultivar differences in root Al concentrations were not significant.

Root Al concentration was correlated (negatively) with root length, only in the case of cv. TX 24 ($r = -0,81^{**}$)[+], and a trend towards a reduction in mean root length (both cultivars) with increasing solution Al concentration, was only evident with Al treatments $\geq$ 1,0 mg dm^{-3} (Figure 39). Root Al was not significantly related to dry matter yield of either tops or roots. Highly significant differences in mean root yield were, however, observed between cv. TX 24 (1,18g) and cv. HL 1 (0,79g). A loss

[+] * significant at 5% level;
** significant at 1% level.

of apical dominance, manifested by a proliferation of lateral roots along the main axis, was apparent at the two highest solution Al levels.

Cultivar HL 1 was characterized by significantly higher concentrations of P in both roots and shoots (Figure 40). Aluminium treatment initially increased root P levels where the observed increases were noticeably greater in cv. HL 1 (Figure 40). In the same direction, K increased slightly, while Ca, Mg and Mn concentrations in the root declined (Figures 41 and 42). The concentrations of all these elements were progressively diminished in the aerial fractions by Al (Figures 41 and 42).

Leaf concentrations of P and K were not correlated with the root levels of these elements. Highly significant linear relationships emerged between leaf and root levels of Ca [r = 0,81** (TX 24); 0,75** (HL 1)] and Mg [r = 0,84** (TX 24); 0,72** (HL 1)] . Leaf concentrations of P were, however, significantly correlated with root concentrations of Ca [r = 0,88** (TX 24); 0,75** (HL 1)] and Mg [r = 0,94** (TX 24); 0,82** (HL 1)] .

Distinctive differences in the effect of Al on the distribution of elements between plant fractions emerged. The decrease in distribution ratio (leaf concentration/root concentration) for P and K with increased Al concentration (Figures 40 and 41) indicated that reduced foliar levels were associated with an accumulation of these elements in the roots. It was therefore concluded that transport rather than uptake was the rate-limiting step. Although Al treatment resulted in reduced Ca and Mg levels in plant roots and tops, the distribution ratio for both elements increased notably at the highest Al stress level (Figure 42). This suggested that the transport mechanisms were not affected by Al and reduced plant concentrations reflected lower uptake. Cultivar TX 24 maintained a higher distribution

ratio for P in the presence and absence of Al.

A clear relationship between root concentrations of P and Al did not emerge from these experiments. With cv. TX 24, no significant correlation was observed, while with cv. HL 1, a barely significant [r = 0,59*] correlation existed between these elements. It is therefore important that a distinctive negative relationship between the distribution ratio for P and root Al was identified, notably in cv. TX 24
[r = -0,95** (TX 24); -0,82 (HL 1)].

8.3.2 The Effect of Different Magnesium and Manganese Levels on Phosphorus Uptake

8.3.2.1 Experiment 2

No significant differences could be observed in the effects produced by the two levels of Mn used in this experiment. Also, neither P concentration in the roots and tops, nor dry matter yields (roots and tops) of either cultivar varied significantly with Mg supply (data not shown).

Root Mg concentrations were negatively correlated with K [r = -0,71** (TX 24); -0,88** (HL 1)] and Ca [r = -0,70** (TX 24); -0,94** (HL 1)] concentrations (Figure 43) and, since the net positive charge on the root calculated as Σ root cations remained constant (Figure 43), a considerable degree of substitution between Mg, Ca and K was indicated. The distribution ratio for Mg (Figure 43) confirmed that low root Mg concentrations were associated with preferential movement of Mg into the plant tops in the absence of Al.

8.3.3 Root Respiration

8.3.3.1 Experiment 3

A reduction in the respiratory activity of intact roots was detected on the basis of a loss of intensity of red (formazan) colour development at Al concentrations of 4 and 8 mg dm^{-3} in the nutrient solution, with root exposure times of 20 h (Figure 44). Cultivar TX 24 proved superior to cv. HL 1 in maintaining root respiration in the presence of Al, and while low levels of colour development were still evident in the primary root of cv. TX 24 with 20 h exposure to 8 mg dm^{-3} Al, no corresponding activity was visually apparent in the roots of cv. HL L (Figure 44). Factors affecting the intensity of red (formazan) colour development are discussed in Section 2.3.2.2. The absence of colour development in Al-treated roots should be interpreted as a decline rather than cessation of respiratory activity.

8.4 DISCUSSION

8.4.1 The Physiology of Aluminium Uptake

In Chapter 3, biochemical features of the cell populations of the root apex were considered as potential controls over Al uptake and distribution. Furthermore, differences between cultivars did not emerge with respect to the regions of the root involved in Al uptake. Divergences in root Al concentrations between cvs. TX 24 and HL 1, noted in the present experiments, are therefore of considerable interest, since data emerges from the analysis of root material to support the idea that physiological differences may exist between cultivars which determine Al uptake. The appreciable uptake of Al observed in cv. HL 1 at low solution Al concentrations is clearly favoured by these differences, while the subsequent decline in root Al, observed at higher treatment levels, may be interpreted as an Al-induced failure of the biosynthetic pathways which control Al uptake.

It may also be argued that data from the analysis of plant roots supports the contention, discussed in Chapters 3, 4 and 7, that the action of Al is not primarily directed at the mitotically active cells of the root meristem. It is pertinent to this argument that the high root Al concentrations, initially observed in cv. HL 1, did not produce a reduction in mean root length and may even have resulted in an element of growth stimulation. On the contrary, evidence of an Al-induced decline in root length coincided with diminished root Al concentrations connected to the higher Al treatment levels in cv. HL 1.

The biochemical incorporation of Al into the root differs from the concept of a non-metabolic uptake pathway implicit in the data of Clarkson (1967) and Rhue (1976).

8.4.2 Mineral Nutrition and Aluminium Toxicity

Disordered mineral nutrition as a consequence of Al treatment has been reviewed by Foy et al., (1978). Aluminium-induced changes in the chemical composition of plants are subject to a variety of possible interpretations, including inactivitation of the processes engaged in uptake/transport, as well as competition between Al and the nutrient ions for common uptake sites.

Because of the importance attached to interactions between Al and P in plant cells (Adams, 1980), and the changes initiated through the Ca dependent regulatory protein, calmodulin (Haug, 1984) as a consequence of Al-induced Ca deficiency, it is relevant to consider whether the Al-induced changes in composition arise as a primary response to Al.

Evidence connecting Al-induced nutrient deficiencies with the primary expression of toxicity is, however, not supported by the observation (Horst et al., 1983) that short-term Al exposure, although depressing root elongation, is not connected to the induction of Ca or P deficiences over the course of the experiment. Chemical immobilisation of P by Al in the root does not accord with

the low correlations observed in these experiments between the root concentrations of these elements. The concept is also difficult to reconcile with the rapidity of expression of some symptoms of toxicity (Clarkson, 1965; Chapter 4).

Nutrient uptake, notably of P and K is also influenced by root morphology (Schenk and Barber, 1980; Barber, 1982). The effect of Al in restricting root length and altering root structure (Chapter 4) must be considered as a possible mechanism for restricting nutrient uptake. It is therefore important that these experiments have demonstrated that the action of Al is primarily directed at the transport of P and K rather than uptake. This observation implies that altered root morphology is unlikely to be a significant factor accounting for the changes in composition of Al-treated plants grown in nutrient solutions, as noted in these experiments.

8.4.3 Functional Involvement of Nutrient Cations in Phosphorus Acquisition

Relevance must, however, be attached to the accumulation of P in the roots of Al-stressed plants observed in these experiments, since similar results have been recorded for a variety of plant species (Clarkson, 1969; Andrew and Vanden Berg, 1973). The highly significant correlations observed between leaf levels of P and root concentrations of Ca and Mg may be indicative of interactions between these elements which influence P transport. Magnesium has established links in salt acquisition involving the stimulation of ATPase activity (Leonard and Hodges, 1973; Bowling, 1976; Marré et al., 1982). A functional involvement for Ca is less well defined, since Ca has been shown to be ineffective as a replacement for Mg in this rôle (Bowling, 1976).

Clarkson and Sanderson (1971) have suggested that the inhibition of Ca uptake by Al reflects a reversal of the electrical charge by the polyvalent ion at the entry point into

the root-free space. Similarities exist in the patterns of uptake between Ca and Mg (Ferguson and Clarkson, 1976), and it is tempting to consider that the altered P-status of Al-stressed plants reflects an Al-induced repression of Mg uptake.

Experiments involving the effect of Mg (and Mn) supply on P uptake and transport in the absence of Al do not, however, support this hypothesis, since low Mg levels were not connected to altered P status or changes in dry matter yield of plants over the range of the experiment. Furthermore, as the lowest root Mg levels recorded in the absence of Al are comparable to those found in plants showing a high degree of Al injury (Experiment 1), it was concluded that these levels are adequate to meet specific functional requirements for this element in the plant.

Negative correlations observed between Mg and Ca and K in the absence of Al are indicative that reduced Mg uptake can, to some extent, be compensated for by increased Ca and K uptake. It is therefore probable that a major requirement for these nutrient cations in the root involves a non-specific charge neutralization rôle. This idea accords with observations regarding the stability of root (+ve) charge and is compatible with the established non-specific stimulatory effect of some polyvalent cations on anion uptake (Viets, 1944; Franklin, 1970).

8.4.4 Root Metabolism, Ion Uptake and Transport

Clarkson (1967) has observed that in Al-stressed plants, much of the P retained in the root is not metabolised and is therefore readily exchangeable. Many aspects of P uptake and transfer remain uncertain, but Loughman (1981) has demonstrated that the rapid metabolic incorporation of the $H_2PO_4^-$ ion is a pre-requisite for the radial transfer of P across the cortex. There is furthermore, evidence to show that the steps involved in the acquisition and transport of P are selectively influenced by low oxygen supply, since the respiratory inhibitor 2,4 di-nitrophenol causes a cessation of P transport rather than uptake (Loughman, 1969).

In Chapter 4, evidence was presented for the inhibitory effect of Al on the respiration of the cell populations of the root apex, and the present experiments have confirmed that a reduction in activity can be extended to intact roots, albeit with longer root exposure times. If the observed decline in respiratory activity is seen to be selectively directed at P transport, a rapid accumulation of non-metabolised P in the roots of Al-treated plants may be anticipated. This accords with observed experimental data. The indirect action of Al on P transport through an Al-induced decline in root respiration is also consistent with the highly specific nature of Al uptake sites (Chapter 3), the demonstrated relationship between P transport and root Al concentration, and the observation that cultivar differences in the ability to maintain root respiration in the presence of Al coincided with cultivar differences in the fraction of P transported to the plant tops.

The concept that the effect of Al on P transport can be linked to an Al-induced decline in cellular metabolism clearly challenges the idea that P accumulation in Al-treated roots is independent of metabolism (Clarkson, 1969). A basis exists to reconsider Clarkson's findings, since the present experiments have indicated that Al and the respiratory inhibitor 2,4 di-nitrophenol, on which Clarkson's work was based, may produce a similar result.

8.4.5 An Hypothesis to Connect Aluminium Treatment and Anion Transport with Diminished Uptake of Calcium and Magnesium

The major anion species are actively accumulated against electrochemical gradients (Higinbotham, 1973) and, although the mechanism remains largely theoretical, there is considerable evidence to connect the function of membrane-bound electrogenic ion pumps involved with energy dependent ATPase activity (Fisher et al., 1970; Rao and Rains, 1976; Cheeseman et al., 1980; Rasi-Caldogno et al., 1980). Active ion movement coincidentally generates a negative electropotential at the cell wall to drive cation uptake on a non-selective basis. The passive uptake of Ca

and Mg occurs along these electrochemical gradients (Bowling, 1976; Mengel and Kirkby, 1979) so that the concentration of these elements in plant cells does not exceed the physical equilibrium levels (Higinbotham, 1973).

Experiment 1 indicates that Al-induced changes in plant concentrations of Ca and Mg are primarily connected to uptake, as evidenced by the linear correlations observed between leaf and root concentrations of these elements. Furthermore, in contrast to P and K, the distribution ratios for these elements indicate that a reduction in root concentration may be associated with an increase in the fraction transported to the plant top, implying that the transport mechanism remains functional.

Inhibition of Ca and Mg uptake may therefore be a secondary consequence of an Al-induced reduction in respiration which acts indirectly through active ion movement by reducing the net negative charge with the consequent de-activation of negatively charged exchange sites. This hypothesis differs from the interpretation of Clarkson and Sanderson (1971) who considered the effect of Al on Ca uptake to be primarily concerned with surface reactions involving the charge on the Al^{3+} ion. This study has, however, also demonstrated inter-relationships between plant concentrations of P, Ca and Mg, interpretation of which is consistent with the disruption of metabolically dependent transport processes. On the contrary, correlations between these elements are not adequately explained by the concept of surface charge neutralization.

8.5 CONCLUSIONS

The emphasis in the literature (Foy et al., 1978) on the rôle of Al-induced nutrient disorders in the expression of Al toxicity requires comprehensive consideration of this topic in defining the physiological response of plants to Al. The present experiments are therefore notable in that little evidence emerged to connect Al-induced changes in plant nutrient status involving P, K, Ca, Mg

and Mn with the primary expression of toxicity.

The action of Al was directed at the uptake of Ca and Mg and the transport of P and K. Cultivar differences in the ability to maintain root respiration in the presence of Al coincided with cultivar differences in P transport. An hypothesis was developed from this observation which involved interference by Al in root metabolism which had the secondary effect of depressing active ion movement, and thereby lowered the cation retention capacity of the root. This accorded with the highly specific nature of the Al uptake sites (Chapter 3) and the linear relationships observed between leaf P and root concentrations of Ca and Mg found in Al-stressed plants.

In the absence of Al, both cultivars responded to low Mg levels by taking up correspondingly more Ca and K without significantly affecting yield. It was therefore suggested that a major requirement for these ions involved charge neutralization. Furthermore, since the root Mg concentrations recorded in these experiments were comparable to those found in plants showing a high degree of Al injury, it was concluded that these Mg levels are adequate to meet specific functional requirements for this element in the plant.

Chemical analysis of plant fractions of cvs. TX 24 and HL 1 identified differences, notably at low solution Al levels, in the pattern of Al uptake. It was inferred from these observations that biochemical differences may exist which favour Al uptake by cv. HL 1. Furthermore, root Al concentrations (cv. HL 1) diminished at the higher solution Al levels, and this was considered evidence that the action of Al was directed at the biochemical pathways controlling uptake. These observations may have implications for the elucidation of possible Al tolerance mechanisms and require further consideration (Chapter 9).

A loss of apical dominance was confirmed by a proliferation

of lateral roots along the main axis, only at the two higher (5,0 and 10,0 mg dm^{-3}) Al levels. The implications of this observation with respect to root development will be discussed further in Chapter 9.

CHAPTER 9

GENERAL DISCUSSION

9.1 INTRODUCTION

Roots respond through changes in growth pattern to a wide variety of environmental stimuli (Kays et al., 1974; Pilet and Ney, 1978; Jackson and Barlow, 1981; Audus, 1983), many of which are, in common with Al, stressful. Although plants have in general not developed specialised sensory apparatus (Audus, 1979; Bentrup, 1979), environmental signals affecting root development are frequently perceived in the root cap (Kays et al., 1974; Juniper, 1976; Pilet and Ney, 1978; Audus, 1979; Jackson and Barlow, 1981; Chapter 3), where they are translated into growth or regulatory responses. These processes are thought to involve the transport of chemical signals between interacting cell populations (Wilkins, 1979), but scarcely anything is known about the mechanism(s) which control these activities (Wilkins, 1979), and the unambiguous identification of cytoplasmic structures involved in stimulus perception and transduction remains to be achieved (Bentrup, 1979; Shropshire, 1979).

In the grasses (including Zea), similarities exist in the morphology of root apices, which suggest construction according to a common morphogenic plan (Barlow and Rathfelder, 1984). This may indicate that responses to environmental conditions which are presumably adaptive, may be directed through interactive control mechanisms. This idea accords with the affinity suggested between the effect of Al, which reduced the ability of amyloplasts to sediment (Chapter 7), and gravity perception mechanisms, which are widely implicated in the movement of amyloplasts within gravity sensitive cells of the central cap (Audus, 1979). Aluminium treatment also altered the geometry of the central cap (Chapter 7).

The extent and distribution of central cap tissues has been connected to the transmission of gravitropic signals (Juniper, 1977; Pilet, 1982; Moore and Pasieniuk, 1984) and support for a loss of gravisensitivity may be indicated in Al-stressed plants, since roots seldom penetrate sub-soil horizons (Foy et al., 1978). Aluminium treatment is also known to restrict root elongation (Clarkson, 1965) and to promote lateral root development along the main root axis (Clarkson, 1969). Similar responses are reported in roots subject to physical stress (mechanical impedance)(Russell and Goss, 1974; Goss and Russell, 1980), anaerobic growth conditions (Geisler, 1965; Wample and Reid, 1975) and light (Feldman, 1984a).

A rôle for Al in disrupting the co-ordination of inter-cellular activities in the root meristem has not previously been considered. This study has involved identification of structural and physiological changes brought about by Al in the primary root. These investigations have indicated that plant reaction to Al is expressed through the disruption of mechanisms co-ordinating inter-cellular activities in the meristem and growth regions of the root. Moreover, the response to Al is directed through a control mechanism which provides communication between the peripheral cap cells where the stimulus is perceived, and the cell populations comprising the cap and proximal meristems, quiescent centre and growth region of the root, where responses to Al are detected. Although many aspects covering the integration of inter-cellular activities remain to be researched, the present study has inextricably linked Golgi apparatus function in the peripheral cap, polysaccharide metabolism in the root cap, and the presence of a basipetally-moving endogenous growth inhibitor, originating in the cap, which may be ABA, with the expression of Al toxicity.

Conclusions arising from previous investigations of intercellular activities (Gibbons and Wilkins, 1970; Clowes, 1972b; Pilet, 1972; Barlow, 1974a, b; Feldman, 1979a) rely heavily on indirect evidence arising from the microsurgical manipulation of cell populations of the root meristem. The destruction or removal of one or more cell populations simultaneously alters the spatial gradients of cells within the meristem and initiates developmental sequences which are not normally encountered. The interpretation of microsurgical experiments may therefore be complicated by the rapid re-establishment of biochemical functions within the remaining cell populations (Chapter 5) and it is pertinent that the growth stimulation associated with many experiments involving the excision of the root cap is only temporary (Jackson and Barlow, 1981). In contrast, Al acted as an inhibitor of Golgi apparatus secretory activity, normally occurring only in the peripheral cap cells. Evidence emerged to connect this secretory activity to Al uptake (Chapter 3). Treatment of the root with Al acted by simultaneously depriving the root of processes connected to the secretory activity of the Golgi apparatus and preventing the initiation of developmental sequences involved in the re-establishment of this function.

The experimental treatment used in this study therefore provides a unique opportunity to consider the developmental pathways which are connected to this specific morphologically distinctive cap activity. This Chapter is concerned with the extent to which an understanding of the physiological and structural changes induced by Al in the root can advance our views on intercellular relationships which exist between the root cap and root, and how interactions between cytoplasmic organelles in the cap may influence root morphogenesis. Finally, consideration will be directed at how these findings may alter perception of Al tolerance mechanisms.

9.2 INTERCELLULAR CO-ORDINATION IN THE ROOT MERISTEM

9.2.1 A Central Rôle for the Golgi Apparatus

In Al-treated roots, the earliest ultrastructural response involved the Golgi apparatus of peripheral cap cells (Chapters 4 and 7), where dictyosomes were sensitive to a wide range of Al concentrations (Chapter 7). These observations extend the involvement of the Golgi apparatus in root physiology for, although the Golgi apparatus appears at the hub of intracellular traffic and the multiplicity of connections in cellular activities cannot yet be documented with certainty (Farquhar and Pallade, 1981), a rôle in the integration of intercellular activities has not previously been considered.

Before a general picture of the regulatory activities of the Golgi apparatus can emerge, it is necessary to consider that the development of Golgi apparatus secretory activity correlates with cellular development (Mollenhauer, 1965). While evidence is not yet conclusive, observations (Chapter 7) support the idea that the action of Al is directed at a morphologically distinctive granular secretory product. Secretory activities involving the elaboration of a dense granular material are usually confined to the peripheral cap cells (Mollenhauer and Morré, 1980).

Considerable relevance is therefore attached to the interpretation of experiments which demonstrate the effect of cap removal and of treatment of decapped roots with Al on the developmental sequences involved in cap regeneration (Chapter 5). These experiments indicate that changes in Golgi apparatus activity, induced by cap removal, include the synthesis of a morphologically similar material to that normally found in the peripheral cap zone in cells corresponding temporally with the spatial position of cap cells. These observations give rise to the idea that the positional arrangement of cells within the root apex may be fundamental to determining this activity. In decapped roots, Al was effective in inhibiting the development of this Golgi apparatus

activity. Aluminium-treated decapped roots were also characterized by the failure of amyloplasts to develop and the absence of developmental sequences involved in cap regeneration.

Emerging from these observations is the notion that the Golgi apparatus activity, related to the synthesis of a granular secretory product, involves a morphogenic substance with a functional involvement in the control of Golgi apparatus activity and plastid differentiation.

9.2.2 The Root Cap as a Source of Growth Inhibition

There are numerous reports which connect the root cap with the source of at least one inhibitor of root growth (Gibbons and Wilkins, 1970; Pilet, 1972; Feldman, 1981; Chanson and Pilet, 1982; Barlow and Pilet, 1984; Feldman, 1984a). Identification of the cap inhibitor has still to be achieved with certainty (Jackson and Barlow, 1981), but evidence emerges from the treatment of decapped roots with ABA (Chapter 6) which is consistent with the proposal (Pilet, 1975a; Wilkins, 1979) that the cap inhibitor may be ABA.

The presence of ABA has been confirmed in the root caps of a number of plants (Rivier *et al.*, 1977; Suzuki *et al.*, 1979) but the rôle of ABA in root physiology has remained controversial (Audus, 1983). Furthermore, little is known of the conditions necessary for the formation of a cap inhibitor (Feldman, 1981).

The present studies serve to implicate the distinctive secretory activity of the Golgi apparatus in peripheral cap cells with the synthesis of an endogenous inhibitor of root growth (Chapter 7). A considerably wider functional involvement for this material also emerges from this study which has additionally implicated inhibition of Golgi apparatus activity by Al with mechanisms that control the loss of mature cells from the cap (Chapter 7), and the development of Golgi apparatus secretory

patterns and amyloplast differentiation within the root cap (Chapters 5 and 7). The integration of these activities in the control of root growth will be considered in the next section.

9.2.3 Polysaccharide Metabolism and Root Growth Promotion

There are suggestions which attempt to involve the quiescent centre in the synthesis (Torrey, 1972) and distribution of growth promoting materials (Feldman, 1979b). These ideas are consistent with the notion that the quiescent centre in some way maintains the activity of the proximal meristem (Torrey, 1972; Barlow, 1976), although the mechanism of control remains to be described.

A paradoxical situation exists with respect to the interpretation of experiments which demonstrate the effect of different Al concentrations on root elongation (Chapter 7). A positive growth response to low Al concentrations ($\leq$ 1,25 mg dm^{-3}) is consistent with the conclusion (Section 9.2.2) that an inhibitory compound normally occurs in the root cap. Further increases in Al concentration beyond this level ($>$ 1,25 mg dm^{-3}) did not reflect further releases of the root from growth inhibition but, on the contrary, were connected to a rapid decline in root elongation rates. Interpretation of these events may depend on the observation that in actively growing roots, the number of cap cells remains nearly constant (Barlow, 1975), and it can be reasoned that the mechanisms which control cell cycling in the cap meristem and the loss of mature cells from the cap periphery are in equilibrium. The cycling rate of the cap meristem must also relate to the quantity of polysaccharide materials contained in amyloplasts which are moved through the cap in differentiating cap cells.

Early responses to Al involved the failure of mechanisms controlling the loss of cells from the cap (Chapter 7), and co-incidental reductions in amyloplast numbers, brought about by increasing Al concentrations. These changes preceded reduced

levels of meristematic activity indicated in the cap and proximal meristems by reductions in cap volume and root elongation.

How changes in polysaccharide metabolism, evidenced by progressive reductions in amyloplast numbers with increasing Al treatment levels, are translated into growth responses involving the mitotically active cells of the cap and root, is not presently known. The results of these experiments do, however, give rise to the idea that minimum levels of polysaccharide metabolism may be required in the cap to maintain root growth.

Feldman (1979b) has suggested that the activity of the quiescent centre may involve the metabolism of materials originating in the cap. It may be pertinent to this suggestion that in the absence of Al, high levels of respiratory activity were detected in the distal cells of the quiescent centre adjacent to the cap boundary (Chapter 4). Aluminium treatment inhibited this function (Chapter 4), and changes in the activity of quiescent centre cells preceded an Al-induced increase in the size of these cells. Metabolism in the quiescent centre of materials exported from the cap may therefore be a factor in imposing quiescence on the cells of the quiescent centre.

Clowes (1972b) first demonstrated that the root cap controlled cell proliferation in the root meristem, although the mechanism whereby the removal of mature cap cells influenced the rate of cell division in the remaining cells of the cap meristem and quiescent centre, was not identified. Although many aspects remain to be researched, the present study has identified links not previously considered,between the secretory activity of the peripheral cap cells, polysaccharide metabolism and root growth, which are relevant to an understanding of Clowes' earlier finding. The presence of at least one inhibitor of root growth, originating in the root cap, is also indicated, although it seems pertinent to consider that the action of this material on root growth may principally be directed through the control of amyloplast differentiation.

9.2.4 Stimulus Perception in the Root Cap

An understanding of plant responses to external stimuli has presented severe experimental and intellectual challenges. Although the effect of gravity on plant organs is the most widely researched aspect involving the outcome of a linked sequence of events (Juniper, 1976; Audus, 1979; Volkmann and Sievers, 1979; Wilkins, 1979; Firn and Digby, 1980; Jackson and Barlow, 1981), agreement on the identity of organelles involved in gravity perception and the identification of mechanisms involved in stimulus transduction between reacting cell populations is still lacking (Audus, 1979; Wilkins, 1979).

Investigation of the site of graviperception has most usually involved the search for sedimentable cell particles (Audus, 1979) and amyloplasts present in central cap cells have been widely connected to a graviperception function (Iversen, 1969; Barlow, 1974b; Audus, 1975; Audus, 1979; Volkmann and Sievers, 1979). The gravi-electric effect has, for nearly thirty years, been considered as an alternative to the statolith function of amyloplasts in perceiving gravity (Volkmann and Sievers, 1979). The potential differences arising from gravistimulation have, however, been generally attributed to the transductory phase rather than to stimulus perception (Volkmann and Sievers, 1979).

Fundamental questions arise from the identification of the site of Al action in the root cap (Chapter 4), since the rationale underlying these observations may provide important clues on mechanisms involved in stimulus perception. The effects of Al on Golgi apparatus function included alterations to the appearance of the secretory products and failure of the transfer of secretory vesicles from the dictyosomes to the cell exterior (Chapters 4 and 7). An alteration in the appearance of vesicular material is consistent with the action of Al being directed through the Golgi apparatus specific glycosyl transferase enzyme systems (Morré and Mollenhauer, 1974). Mechanisms which govern the transfer of secretory vesicles to the correct extracellular

destinations are presently unknown, although Morré (1977a) has indicated that transfer must satisfy the criteria of being selective and directional.

An early consequence of Al treatment involved the rapid efflux of H^+ (Chapter 7). Similar effects are noted in gravistimulated roots (Mulkey et al., 1981; Moore, 1985), which Mulkey et al. (1981) attribute, in the case of gravistimulation, to an auxin-induced growth response. An alternative interpretation of these events may, however, depend on the observation that in roots treated with different Al concentrations (Chapter 7), H^+ efflux preceded a detectable increase in root diameter, arising from changes in the polarity of cell growth direction (Chapter 4). These findings are considered evidence (Chapter 7) for the activation of electrogenic pumping which may arise to stabilize an Al-induced depolarisation of membrane potential.

It is pertinent to this hypothesis that Behrens and Gradmann (1985) report that differences in membrane potential exist between the cells of the cap periphery and central cap tissues. Changes in membrane potential, arising from gravistimulation, are believed to contribute to the graviresponsiveness of roots (Behrens and Gradmann, 1985). Behrens et al. (1985) have also associated changes in the gravi-electrical properties of root caps arising from depolarisation of membrane potential, to changes in the sedimentation of amyloplasts. Treatment of roots with Al also affected the ability of amyloplasts to sediment (Chapter 7).

Sievers et al. (1984) argue in favour of membrane depolarisation in gravistimulated roots involving a spatially differentiated electric signal arising from the sedimentary action of amyloplasts. This does not adequately explain the differences in potential between the peripheral cap cells (Behrens and Gradmann, 1985), where amyloplasts lose the ability to sediment (Juniper and French, 1970) and the central cap. Furthermore, Al-induced changes in peripheral cap cells coincided with

alterations to the distribution of amyloplasts occurring in central cap cells. Although the mechanisms by which Al treatment can induce changes in amyloplast sedimentation is not known, it is tempting to associate these events with alterations in the electrical properties of root caps, brought about by the charge on the Al ion.

Evidence to connect Golgi apparatus activity directly to stimulus perception is presently lacking, although the re-arrangement of dictyosomes does occur in the avascular tip cells of oat (Avena sativa) coleoptiles in response to gravistimulation (Shen-Miller and Miller, 1972). It does, however, appear reasonably certain that the initial events involved in stimulus perception are closely associated with membrane structure and function (Shropshire, 1979). Moreover, the present studies attach considerable importance to the function of the Golgi apparatus and imply that the response may involve electro-physiological events initiated by the charge on the Al ion. These observations lead to the idea that Golgi apparatus activity may in some way contribute to the establishment of membrane potential differences reported in the root cap by Behrens and Gradmann (1985).

9.2.5 Cell Enlargement of Cortical Cells

There is evidence to implicate changes in cell growth patterns with the bending response of gravireactive roots (Jackson and Barlow, 1981). Cell growth studies of roots treated with Al (Chapter 4) have identified an increase in the volume of cortical cells in the region of the root where Shen-Miller et al. (1978) have found cellular changes to be initiated by gravity. Analysis of cell growth patterns and concomitant changes in root growth do not, however, identify an affiliation between the mechanisms which promote increases in cell size in response to Al and gravity. Aluminium treatment caused an increase in root diameter (Chapter 7) through a preferential increase in cell width (Chapter 4) which coincided with a decline in root elongation.

rates (Chapter 7). In contrast, gravicurvature is believed to involve an increase in cell elongation, while the importance attached to growth inhibition, arising during gravistimulation, appears to be exaggerated (Jackson and Barlow, 1981).

There are, however, reports which connect the application of pressure to the root with an increase in root thickness and a decline in root elongation rates (Wilson et al., 1977; Goss and Russell, 1980). It is generally agreed that roots impeded in their growth can develop considerable pressure (Feldman, 1984a). The mechanism which initiates an increase in pressure has not been resolved (Feldman, 1984a) but Wilson et al. (1977) have associated these changes with an increase in the size of cortical cells.

There is much evidence (Chapters 3 and 5) to link the root cap and Golgi apparatus secretory activity (Chapter 5) to an Al-induced increase in the volume of mid-cortical cells (Chapter 4). Moreover, since removal of the root cap does not in itself promote cortical cell growth (Chapters 5 and 6), a basis exists to consider that cell enlargement arises from the release of, rather than the inhibition of, the basipetal movement of materials promoting cell enlargement. The identity of this substance is presently unknown, but evidence exists (Chapter 4) to indicate that action may promote changes in osmotic potential of root cells, accompanied by a locally limited alteration in the polarity of cell growth of mid-cortical cells.

An increase in root thickness is not an early response to Al, and Al concentrations ($\geq$ 1,25 mg dm^{-3}) at which changes in root diameter can be detected, correspond with severe inhibition of Golgi apparatus activity in the cap periphery (Chapter 7). This implies that the Golgi apparatus may not be the source of materials initiating cell enlargement, but that diminished Golgi apparatus activity in some way triggers the release of these materials from a site remote from the Golgi apparatus. The

structured nature of the endomembrane system (Morré and Mollenhauer, 1974) may provide features consistent with the location of materials promoting a growth response in mid-cortical cells.

Increases in root diameter, arising from mechanical resistance to growth, are considered to be directed at dislodging obstacles to the passage of the root by deforming the soil (Feldman, 1984a). In Al-treated roots, cell enlargement of cortical cells is of sufficient magnitude to cause the collapse of primary vascular tissue (Chapter 4). Roots depend on an efficient transport system for the supply of the major organic nutrients (Wilkins, 1979), and changes to the vascular system through the expansion of cortical cells would effectively deprive the root of the bi-directional transport of these materials.

By removing increasing lengths of the root apex, Feldman (1979b) was able to implicate both the quiescent centre and proximal meristem in the control of root morphogenesis. It may be pertinent to an understanding of the physiological response of the root to environmental stress, that the location of cellular changes which involve the vascular system (1 - 2 mm from the apex) would effectively deprive the root of the co-ordinated functions of the quiescent centre and proximal meristem. Cellular changes noted in this study may therefore involve adaptive mechanisms directed at developmental sequences involved in lateral root initiation, arising from a need to explore new root growth directions.

9.2.6 The Proximal Meristem and Root Development

The agent(s) involved in the control of lateral root development have not yet been identified (Torrey, 1976), although it is not disputed that the root apex prevents the formation of lateral roots for some distance behind it (McCully, 1975). By surgical removal of increasing proportions of the root apex,

Feldman (1979b) was able to demonstrate in Zea that the loss of between 500 - 700 µm of apical tissue was required to prevent regeneration of the root apex and to initiate lateral root development. Arising from these observations (Feldman, 1979b), is the suggestion that root morphogenesis is in a large part regulated by the co-ordinated activities of the quiescent centre and proximal meristem. The mechanism of co-ordination is uncertain, but Feldman (1979a) has indicated that interactions between the quiescent centre and proximal meristem may depend on the presence of cytokinins.

The direct demonstration of the synthesis of cytokinins in roots has thus far not proved feasible (van Staden and Forsyth, 1986). Cytokinins do, however, occur in meristematic tissue, and actively dividing cells have been considered as possible sites of biosynthesis of these substances (Torrey, 1976). Cytokinins have also been found to inhibit lateral root formation (Torrey, 1976).

A loss of apical dominance is not an early response to Al, and lateral root development was only identified at Al treatment levels $\geq$ 5,0 mg dm^{-3} (Chapter 8). These observations may indicate that the proximal meristem and quiescent centre may possess an inherent resistance to an Al-induced change in function that has not previously been considered. Aluminium treatment (Chapter 4) also produced changes in nuclear/nucleolar ultrastructure in the proximal meristem consistent with an inhibition of function. The function has yet to be identified. In Al-treated roots, chronologically functional changes to the proximal meristem, which could lead to a loss of apical dominance, are preceded by a decline in activity of the quiescent centre and removal of the constraint to growth of quiescent cells (Chapter 4), while reductions in mitotic activity, as indicated by a decline in root length, are detected at appreciably lower Al concentrations (Chapter 7).

Observations concerning possible interactions between the activities of the quiescent centre and proximal meristem in controlling root development merit further investigation.

9.3 ALUMINIUM TOLERANCE

Numerous reports exist indicating differences in plant response to Al (Section 1.2.1.4), but little agreement is evident on the physiological pathway(s) involved in the expression of Al tolerance (Section 1.2.1.5).

Information on plant response to Al may be expected to add to the selectivity of breeding programmes which attempt to exploit genetic variability with the objective of increasing Al tolerance levels of agronomically important plant species. In considering the substantial land areas where acid soils occur (Section 1.2.1.4), the development of Al-tolerant cultivars could have significant economic and sociological implications.

This study contributes to the idea, which has not previously been considered, that similarities exist between plant reactions to Al and the growth responses of plant roots to a range of environmental signals. Fundamental questions arise from this observation which may affect the perception of mechanisms of Al tolerance.

The expression of Al tolerance requires that the physiological reaction to Al will include the ability of plant roots to continue to grow in the presence of potentially toxic concentrations of Al. An analysis of events involved in stress physiology indicates that the chain of plant reaction to environmental signals is characterized by three indispensable steps:

stimulus perception → stimulus transduction → physiological response.

It may therefore be argued that varietal differences in Al tolerance must arise from plant abilities to prevent or modify events involved in stimulus perception and/or stimulus transduction.

9.3.1 Exclusion Mechanisms

The present studies are of interest in providing information to demonstrate that the initiation of a growth-inhibiting responses between the peripheral cap cells and the mitotically active cells of the cap and proximal meristems are not detected at Al treatment levels $< 1{,}25$ mg dm^{-3}. A degree of Al tolerance may therefore be expected to arise from mechanisms which prevent the entry of Al into the root.

Aluminium uptake has been connected to the biochemical properties of cell populations of the root meristem (Chapter 3). Furthermore, this investigation has identified differences in the pattern of Al uptake between cultivars (Chapter 8). Dissimilarities in root Al concentrations between cvs. TX 24 and HL 1 at low Al concentrations (Chapter 8), do, however, support the idea that physiological differences exist between cultivars which determine Al uptake. The nature of these differences is presently unknown. Evidence exists (Chapters 3, 4 and 7) to connect the secretory activity of peripheral cap cells with the plant response to Al. Chemical characterization of the mucilagenous secretions of cap cells is controversial (Chaboud, 1983), but differences in chemical compositon have been reported at the species level (Oades, 1978). It is not known whether these differences correlate with Al-tolerance.

The present study has also attached considerable importance to Golgi apparatus secretory activity in the expression of Al toxicity. It may therefore be reasonable to consider whether Al uptake is influenced by inherently low levels of Golgi apparatus activity in the cap periphery. Reports (Foy *et al.*, 1978) indicate that Al toxicity (*Triticum* and *Gossypium*) is aggravated by increased temperature. Evidence is, however, presently lacking to suggest correlation between this information and changes in secretory activity.

It does seem pertinent to consider whether these factors

can be exploited to increase Al tolerance in agronomically important plant species.

There is also evidence to suggest that the Al uptake pathways are the target for Al action so that an increase in Al treatment level was associated with reduced concentrations of Al in the roots of cv. HL 1 (Chapter 8). Aluminium concentrations used to screen plants for Al tolerance (Rhue and Grogan, 1976; Konzak et al., 1976; Furlani and Clark, 1981) commonly exceed the level of Al which produced a reduction in root concentrations of the Al in cv. HL 1. Attempts to correlate root Al concentration with Al tolerance (Section 1.2.1.5.1) must therefore be interpreted with caution.

9.3.2 Transduction of the Stimulus

No information presently exists to show that stimulus transduction rates may be relevant to influencing plant reaction to Al. Similarities between plant response to Al and a variety of environmental stimuli, including gravity, have been discussed, and it may therefore be pertinent to consider that differences in graviresponsiveness have been connected to a variety of morphological and physiological features of root caps. These include the removal of starch from amyloplasts (Iversen, 1969), the length of the root cap (Pilet, 1982) and the extent and development of central cap columella tissue (Moore and Pasieniuk, 1984). Attempts to correlate graviresponsiveness with the size, number or volume of amyloplasts have not been successful (Moore and McClelan, 1985).

The mechanisms by which features of the cap affect gravisensitivity are unknown. The present studies on plant response to Al attach considerable relevance to the rôle of an endogenous growth inhibitor originating in the cap, which may be ABA. Differences exist between cultivars in the level of ABA found in the root apex (Rivier and Pilet, 1981), and it is tempting to postulate that these differences might relate to the expression of Al tolerance.

9.4 CONCLUDING REMARKS

This investigation has been concerned with the identification of structural and/or physiological responses initiated by treating the primary root of Zea mays with Al. From these studies, new insights emerge into the regulatory mechanisms which control root growth and development. Information on many aspects of the integrated co-ordination of intercellular activities in the root meristem nevertheless remains less than satisfactory, and the specific rôle of the Al ion in inhibiting Golgi apparatus secretory activity is unclear, although an electro-physiological effect is indicated. Further research directed at examining the rôle of Al (and other trivalent ions) on root development may be relevant to increasing an understanding of these events.

REFERENCES

Abruna-Rodrigues, F., Vicente-Chanler, J., Rivera, E. & Rodrigues, J. (1982). Effect of soil acidity factors on yields and foliar composition of tropical root crops. Soil Science Society of America Journal 46: 1004 - 1007.

Adams, F. (1980). Interactions of phosphorus with other elements in soils and in plants. In The rôle of phosphorus in agriculture, eds. Khasawnek, F. E., Sample, E. C. & Kamprath, E. J. pp. 655 - 680. Madison, Wisconsin: ASA-CSSA-SSSA.

Adams, F. (1981). Nutritional imbalances and constraints to plant growth on acid soils. Journal of Plant Nutrition 4: 81 - 87.

Addicott, F. T. (1982). Abscission. pp. 45 - 96. Berkley: University of California Press.

Addicott, F. T., Carns & H. R. (1983). History and introduction. In Abscisic acid, ed. Addicott, F. T. pp. 1 - 24. New York: Praeger.

Adriano, D. C., Paulsen, G. M. & Murphy, L. S. (1971). Phosphorus - iron and phosphorus - zinc relationships in corn (Zea mays L.) seedlings as affected by mineral nutrition. Agronomy Journal 63: 36 - 39.

Allan, J. P. (1970). The preparation of agricultural samples for analysis by atomic absorption spectroscopy. Australia: Varian Techtron.

Anderson, T. F. (1951). Techniques for the preservation of three dimensional structure in preparing specimens for the electron microscope. Transactions of New York Academy Science 11: 130 - 134.

Anderson, W. P. (1975). Long distance transport in roots. In Ion transport in plant cells and tissues, eds. Baker, D. A. & Hall, J. L. Ch. 8. pp. 231 - 266. Amsterdam: North Holland.

Andrew, C. S. (1976). Effect of calcium, pH and nitrogen on the growth and chemical composition of some tropical and temperate pasture legumes. I Nodulation and growth. Australian Journal of Agricultural Research 27: 611 - 623.

Andrew, C. S. & Vanden Berg, P. J. (1973). The influence of aluminium on phosphate sorption by whole plants and excised roots of some pasture legumes. Australian Journal of Agricultural Research 24: 341 - 351.

Aniol, A. (1984). Induction of aluminium tolerance in wheat seedlings by low doses of aluminium in the nutrient solution. Plant Physiology 75: 551 - 555.

Armiger, W. H., Foy, C. D., Fleming, A. L. & Caldwell, B. E. (1968). Differential tolerance of soybean varieties to an acid soil high in exchangeable aluminium. Agronomy Journal 60: 67 - 70.

Asher, C. J. & Edwards, D. G. (1978). Relevance of dilute solution culture studies to problems of low fertility tropical soils. In Mineral nutrition of legumes in tropical and subtropical soils, eds. Andrew, C. S. & Kamprath, E. J. pp. 131 - 152. Melbourne, Australia: C.S.I.R.O.

Audus, L. J. (1975). Geotropism in roots. In The development and function of roots, eds. Torrey, J. G. & Clarkson, D. T. Ch. 16. pp. 327 - 363. Third Cabot Symposium, London: Academic Press.

Audus, L. J. (1979). Plant geosensors. Journal of Experimental Botany 30: 1051 - 1073.

Audus, L. J. (1983). Abscisic acid in root growth and geotropism. In Abscisic acid, ed. Addicott, F. T. pp. 421 - 478. New York: Praeger.

Baes, C. F. & Mesmer, R. E. (1976). The hydrolysis of cations, pp. 73 - 128. New York: John Wiley & Sons.

Baker, J. R. (1958). Principles of biological microtechniques. Great Britain: Methuen. (1968) reprinting)

Barber, S. A. (1974). The influence of the plant root on ion movement in the soil. In The plant root and its environment, ed. Carson, E. W. Ch. 18. pp. 525 - 564. Charlottesville: University Press of Virginia.

Barber, S. A. (1982). Soil-plant root relationships determining phosphorus uptake. In Plant nutrition 1982, ed. Sciefe, A. Ninth Annual Colloquium. Vol. I. Commonwealth Agricultural Bureaux.

Barka, T. B. & Anderson, P. J. (1963). Histochemistry : theory and practice. New York: Harper and Row.

Barlow, P. W. (1974a). Regeneration of the cap of primary roots of Zea mays. New Phytologist 73: 937 - 954.

Barlow, P. W. (1974b). Recovery of geotropism after removal of the root cap. Journal of Experimental Botany 25: 1137 - 1146.

Barlow, P. W. (1975). The root cap. In The development and function of roots, eds. Torrey, J. G. & Clarkson, D. T. Third Cabot Symposium Ch. 2. pp. 21 - 54. London: Academic Press.

Barlow, P. W. (1976). Towards an understanding of the behaviour of root meristems. Journal of Theoretical Botany 57: 433 - 451.

Barlow, P. W. (1978a). Cell displacement through the columella of the root cap of Zea mays L. Annals of Botany 42: 783 - 790.

Barlow, P. W. (1978b). RNA metabolism in the quiescent centre and neighbouring cells in the root meristem of Zea mays. Zeitschrift für Pflanzenphysiologie 86: 147 - 157.

Barlow, P. W. (1981). Division and differentiation during regeneration at the root apex. In Structure and function of plant roots, eds. Brouwer, R.,Gasparikova, I., Kolek, J. & Loughman, B. C. Ch. 15. pp. 85 - 87. The Hague: Martinus Nijhoff.

Barlow, P. W. & Grundwag, M. (1974). The development of amyloplasts in cells of the quiescent centre of Zea roots in response to removal of the root cap. Zeitschrift für Pflanzenphysiologie 73: 56 - 64.

Barlow, P. W. & Hines, E. R. (1982). Regeneration of the root cap of Zea mays L. and Pisum sativum L. : a study with the scanning electron microscope. Annals of Botany 49: 521 - 539.

Barlow, P. W. & Pilet, P. E. (1984). The effect of abscisic acid on cell growth, cell division and DNA synthesis in the maize root meristem. Physiologia plantarum 62: 125 - 132.

Barlow, P. W. & Rathfelder, E. L. (1984). Correlations between the dimensions of different zones of grass root apices, and their implications for morphogenesis and differentiation in roots. Annals of Botany 53: 249 - 260.

Barlow, P. W. & Sargent, J. A. (1978). The ultrastructure of the regenerating cap of Zea mays L. Annals of Botany 42: 791 - 799.

Bartlett, R. J. & Riego, D. C. (1972). Effects of chelation on the toxicity of aluminium. Plant and Soil 37: 419 - 423.

Behrens, H. M. & Gradmann, D. (1985). Electrical properties of vertically growing root tip of Lepidium sativum L. Planta 163: 453 - 462.

Behrens, H. M., Gradmann, D. & Sievers, A. (1985). Membrane-potential responses following gravistimulation of roots of Lepidium sativum L. Planta 163: 463 - 472.

Benes, K. (1973). On the media improving freeze sectioning of plant material. Biologia plantarum 15: 50 - 56.

Bennett, H. S., Wyrick, A. D., Lee, S. W. & McNeil, J. H. (1976). Science and art in preparing tissues embedded in plastic for light microscopy, with special reference to glycol methacrylate, glass knives and simple stains. Stain Technology 51: 71 - 97.

Bentrup, F. W. (1979). Reception and transduction of electrical and mechanical stimuli. In The physiology of movement, eds. Haupt, W. & Feinlieb, M. E. Encyclopaedia of plant physiology Vol. 7. pp. 42 - 70. Berlin: Springer-Verlag.

Blamey, F. P. C., Edwards, D. G. & Asher, C. J. (1983). Effects of aluminium, OH : Al and P : Al molar ratios, and ionic strength on soybean root elongation in solution culture. Soil Science 136: 197 - 207.

Bouton, J. H., Sumner, M. E. & Giddens, J. E. (1981). Alfalfa, Medicago sativa L. in highly weathered acid soils. Plant and Soil 60: 205 - 211.

Bowling, D. J. F. (1976). Uptake of ions by plant roots. London: Chapman and Hall.

Burstrom, H. (1960). Influence of iron and gibberellic acid on the light sensitivity of roots. Physiologia plantarum 13: 597 - 615.

Caldwell, C. R. & Haug, A. (1981). Calmodulin stimulation of the barley root plasma membrane-bound Ca^{2+}, Mg^{2+} - ATPase. Plant Physiology 67 (Supplement) : 136.

Cathey, H. M. & Campbell, L. E. (1980). Light and lighting systems for horticultural plants. Horticultural Review 2: 491 - 537.

Chaboud, A. (1983). Isolation purification and chemical composition of maize root cap slime. Plant and Soil 73: 395 - 402.

Chanson, A. & Pilet, P. E. (1982). Transport and metabolism of 2^{14}.C abscisic acid in maize root. Planta 154: 556 - 561.

Chanson, A. & Taiz, L. (1985). Evidence for an ATP-dependent proton pump on the Golgi of corn coleoptiles. Plant Physiology 78: 232 - 240.

Charlton, W. A. (1980). Primary vascular patterns in root meristems of Pontederca cordata and their relevance to studies of root development. Canadian Journal of Botany 58: 1351 - 1369.

Cheeseman, J. M., La Fayette, P. R., Gronewald, J. W. & Hanson, J. B. (1980). Effect of ATPase inhibitors on cell potential and K^+ influx in corn roots. Plant Physiology 65: 1139 - 1145.

Chenery, E. M. (1948a). Aluminium in plants and its relation to plant pigments. Annals of Botany 12: 121 - 136.

Chenery, E. M. (1948b). Aluminium in trees. Empire Forest Review 25: 255 - 256.

Chenery, E. M. & Sporne, K. R. (1976). A note on the evolutionary status of aluminium-accumulators among dicotyledons. New Phytologist 76: 551 - 554.

Chrispeels, M. J. (1980). The endoplasmic reticulum. In The biochemistry of plants, ed. Tolbert, N. E. Vol. 1, Ch. 10. pp. 389 - 412. New York: Academic Press.

Clark, R. B. (1977). Effect of aluminium on growth and mineral elements of Al-tolerant and Al-intolerant corn. Plant and Soil 47: 654 - 662.

Clark, R. B. (1982). Nutrient solution growth of sorghum and corn in mineral nutrition studies Journal of Plant Nutrition 5: 1039 - 1057.

Clark, R. B. & Brown, J. C. (1974a). Differential mineral uptake by maize inbreds. Communications in Soil Science and Plant Analysis 5: 213 - 227.

Clark, R. B. & Brown, J. C. (1974b). Differential phosphorus uptake by phosphorus-stressed corn inbreds. Crop Science 14: 505 - 508.

Clarkson, D. T. (1965). The effect of aluminium and some other trivalent metal ions on cell division in the root of Allium cepa. Annals of Botany 20: 309 - 315.

Clarkson, D. T. (1967). Interactions between aluminium and phosphorus on root surfaces and cell wall material. Plant and Soil 27: 347 - 356.

Clarkson, D. T. (1969). Metabolic aspects of aluminium toxicity and some possible mechanisms of resistance. In Ecological aspects of the mineral nutrition of plants, ed. Rorison, I. H. British Ecological Symposium No. 9. Blackwell Scientific Publications.

Clarkson, D. T. & Hanson, J. B. (1980). The mineral nutrition of higher plants. Annual Review of Plant Physiology 31: 239 - 298.

Clarkson, D. T. & Sanderson, J. (1971). Inhibition of the uptake and long distance transport of calcium by aluminium and other polyvalent cations. Journal of Experimental Botany 22: 837 - 851.

Clowes, F. A. L. (1956). Nucleic acids in root apical meristems of Zea. New Phytologist 55: 29 - 34.

Clowes, F. A. L. (1958). Development of quiescent centres in root meristems. New Phytologist 57: 85 - 88.

Clowes, F. A. L. (1961). Duration of mitotic cycles in a meristem. Journal of Experimental Botany 12: 283 - 293.

Clowes, F. A. L. (1971). The proportion of cells that divide in root meristems of Zea mays L. Annals of Botany 35: 249 - 261.

Clowes, F. A. L. (1972a). The control of cell proliferation within the root meristems. In The dynamics of meristem cell populations, eds. Miller, M. W. & Kuehnert, C. C. Advances in experimental medicine and biology. Vol. 18, pp. 133 - 145. New York: Plenum Press.

Clowes, F. A. L. (1972b). Regulation of mitosis in roots by their caps. Nature New Biology 235: 143 - 144.

Clowes, F. A. L. (1975). The quiescent centre. In The development and function of roots, eds. Torrey, J. G. & Clarkson, D. T. Third Cabot Symposium pp. 3 - 19. London: Academic Press.

Clowes, F. A. L. (1976). Cell production by root caps. New Phytologist 77: 399 - 407.

Clowes, F. A. L. (1980). Mitosis in the root cap of Zea mays. New Phytologist 85: 79 - 87.

Clowes, F. A. L. (1984). Size and activity of quiescent centres of roots. New Phytologist 96: 13 - 21.

Clowes, F. A. L. & Juniper, B. E. (1964). The fine structure of the quiescent centre and neighbouring tissues in root meristems. Journal of Experimental Botany 15: 622 - 630.

Clymo, R. S. (1962). An experimental approach to part of the calcicole problem. Journal of Ecology 50: 707 - 731.

Dalal, R. C. (1972). Colorimetric determination of aluminium in soil extracts using haematoxylin. Plant and Soil 36: 223 - 231.

da Silva, J. J. R. F. & Williams, R. J. P. (1976). The uptake of elements by biological systems. Structure and Bonding 29: 67 - 121.

Davies, M. R. (1981). Growth and nutrition of legumes on a high country, yellow-brown earth sub soil : III The effect of lime. New Zealand Journal of Agricultural Research 24: 339 - 348.

De la Torre, C. & Clowes, F. A. L. (1972). Timing of nucleolar activity in meristems. Journal of Cell Science 11: 713 - 721.

Dodge, C. S. & Hiatt, A. J. (1972). Relationship of pH to ion uptake imbalances by varieties of wheat (Triticum vulgare). Agronomy Journal 64: 476 - 481.

Duvick, D. N., Kleese, R. A. & Frey, N. M. (1981). Breeding for tolerance of nutrient imbalances and constraints to growth in acid, alkaline and saline soils. Journal of Plant Nutrition 4: 111 - 129.

Edwards, D. G., Kang, B. T. & Danso, S. K. A. (1981). Differential response of six cowpea (Vigna unguiculata (L.) Walp.) cultivars to liming in an Ultisol. Plant and Soil 59: 61 - 73.

Ellmore, G. S. (1982). The organisation and plasticity of plant roots. Scanning Electron Microscopy 111: 1083 - 1099.

Evans, L. S. (1984). Botanical aspects of acidic precipitation. The Botanical Review 50: 449 - 490.

Farina, M. P. W., Mendes, Paula, Gevers, H. O. & Channon, P. (1982). Differential tolerance to soil acidity among several South African maize genotypes. Crop Production 11: 133 - 139.

Farina, M. P. W., Sumner, M. E., Plank, C. O. & Letzch, W. S. (1980). Aluminium toxicity in corn at near neutral soil pH levels. Journal of Plant Nutrition 2: 683 - 697.

Farquhar, M. G. & Palade, G. E. (1981). The Golgi apparatus (complex) - (1954 - 1981) - from artifact to center stage. Journal of Cell Biology 91: 77s - 103s.

Feldman, L. J. (1975). Cytokinins and quiescent centre activity in roots of Zea. In The development and function of roots, eds. Torrey. J.G. & Clarkson, D. T. Third Cabot Symposium Ch. 3. pp. 55 - 72. London: Academic Press.

Feldman, L. J. (1976). The de novo origin of the quiescent centre in regenerating root apices of Zea mays. Planta 128: 207 - 212.

Feldman, L. J. (1977). The generation and elaboration of primary vascular tissue patterns in roots of Zea. The Botanical Gazette 138: 393 - 401.

Feldman, L. J. (1979a). Cytokinin biosynthesis in roots of corn. Planta 145: 315 - 321.

Feldman, L. J. (1979b). The proximal meristem in the root apex of Zea mays L. Annals of Botany 43: 1 - 9.

Feldman, L. J. (1981). Root cap inhibitor formation in isolated root caps of Zea mays. Journal of Experimental Botany 32: 779 - 788.

Feldman, L. J. (1984a). Regulation of root development. Annual Review of Plant Physiology 35: 223 - 242.

Feldman, L. J. (1984b). The development and dynamics of the root apical meristem. American Journal of Botany 71: 1308 - 1314.

Feldman, L. J. & Torrey, J. G. (1975). The quiescent centre and primary vascular tissue pattern formation in cultured roots of Zea. Canadian Journal of Botany 53: 2796 - 2803.

Feldman, L. J. & Torrey, J. G. (1976). The isolation and culture in vitro of the quiescent centre of Zea mays. American Journal of Botany 63: 345 - 355.

Ferguson, I. B. & Clarkson, D. T. (1976). Simultaneous uptake and translocation of magnesium and calcium in barley (Hordeum vulgare L.) roots. Planta 128: 267 - 269.

Firn, R. D. & Digby, J. (1980). The establishment of tropic curvatures in plants. Annual Review of Plant Physiology 31: 131 - 148.

Fisher, J. D., Hansen, D. & Hodges, T. K. (1970). Correlation between ion fluxes and ion-stimulated adenosine triphosphatase activity of plant roots. Plant Physiology 46: 812 - 814.

Fleming, A. L. & Foy, C. D. (1968). Root structure reflects differential aluminium tolerance in wheat varieties. Agronomy Journal 60: 172 - 176.

Foy, C. D. (1974). Effects of aluminium on plant growth. In The plant root and its environment, ed. Carson, E. W. Ch. 20. pp. 601 - 642. Charlottesville: University Press of Virginia.

Foy, C. D., Armiger, W. H., Briggle, L. W. & Reid, D. A. (1965). Differential aluminium tolerance of wheat and barley varieites in acid soils. Agronomy Journal 57: 413 - 417.

Foy, C. D. & Brown, J. C. (1963). Toxic factors in acid soils : I Characterisation of aluminium toxicity in cotton. Soil Science Society of America Proceedings. 27: 403 - 407.

Foy, C. D. & Brown, J. C. (1964). Toxic factors in acid soils : II Differential aluminium tolerance of plant species. Soil Science Society of America Proceedings. 28: 27 - 32.

Foy, C. D., Chaney, R. L. & White, M. C. (1978). The physiology of metal toxicity in plants. Annual Review of Plant Physiology 29: 511 - 566.

Foy, C. D. & Fleming, A. L. (1978). The physiology of crop tolerance to excess available aluminium and manganese in acid soils. In Crop tolerance to sub-optimal land conditions, ed. Jung, G. A. Ch. 14. pp. 301 - 328. Wisconsin: ASA-CSSA-SSSA.

Foy, C. D. & Fleming, A. L. (1982). Aluminium tolerance of two wheat genotypes related to nitrate reductase activities. Journal of Plant Nutrition 5: 1313 - 1333.

Foy, C. D., Fleming, A. L. & Armiger, W. H. (1969). Aluminium tolerance of soybean varieties in relation to calcium nutrition. Agronomy Journal 61: 505 - 511.

Foy, C. D., Fleming, A. L., Burns, G. R. & Armiger, W. H. (1967). Characterisation of differential aluminium tolerance am varieties of wheat and barley. Soil Science Society of America Proceedings 31: 513 - 520.

Foy, C. D., Fleming, A. L. & Gerloff, G. C. (1972). Differential aluminium tolerance of two snap bean varieties. Agronomy Journal 64: 815 - 818.

Franklin, R. E. (1970). Effect of adsorbed cations on phosphorus absorbtion by various plant species. Agronomy Journal 62: 214 - 216.

Frink, C. R. & Peech, M. (1962). Determination of Aluminium in soil extracts. Soil Science 93: 317 - 324.

Furlani, P. R. (1981). Effects of aluminium on growth and mineral nutrition of sorghum genotypes. PhD thesis, University of Nebraska, Lincoln. Libr. Congr. Card No. Mic. 8120162 Univ. Microfilms. Michigan: Ann Arbor.

Furlani, P. R. & Clark, R. B. (1981). Screening sorghum for aluminium tolerance in nutrient solution. Agronomy Journal 72: 587 - 594.

Furlani, P. R., Clark, R. B., Ross, W. M. & Maranville, J. W. (1983). Variability and genetic control of aluminium tolerance in sorghum genotypes. In Genetic aspects of plant nutrition, eds. Saric, M. R. & Loughman, B. C. pp. 453 - 461. The Hague: Martinus Nijhoff.

Gapon, E. N. & Voshchinskaya, M. A. (1941). Exchangeable oxy-aluminium cations in podzolic soils. Dokl. Akad. Selsk. Khoz. Nauk. 5: 46 - 48. (quoted by Hutchinson 1945).

Geisler, G. (1965). The morphogenic effect of oxygen on roots. Plant Physiology 40: 85 - 88.

Gibbons, G. S. B. & Wilkins, M. B. (1970). Growth inhibitor production by root caps in relation to geotropic responses. Nature 226: 558 - 559.

Gill, G. W., Frost, J. K. & Miller, K. A. (1974). A new formula for a half-oxidized hematoxylin solution that neither overstains nor requires differentiation. Acta cytologica 18: 300 - 311.

Giulian, D. & Diacumakes, E. G. (1976). The study of intracellular compartments by micropipette techniques. Journal of Cell Biology 70: 332a (Abstr.)

Glick, D. & Nayyar, S. N. (1956). Studies in histochemistry : XLII further studies on the determination of succinic dehydrogenase in micro-gram amounts of tissue and distribution of the activity in the bovine adrenal. Journal of Histochemistry and Cytochemistry 4: 389 - 396.

Goss, M. J. & Russell, R. S. (1980). Effects of mechanical impedance on root growth in barley (Hordeum vulgare L.) III Observations on the mechanisms of response. Journal of Experimental Botany 31: 577 - 588.

Green, P. B. (1969). Cell morphogenesis. Annual Review of Plant Physiology 20: 365 - 394.

Green, P. B. (1980). Organogenesis - a biophysical view. Annual Review of Plant Physiology 31: 51 - 82.

Gressel, J. & Horwitz, B. (1982). Gravitropism and phototropism. In The molecular biology of plant development, eds. Smith, H. & Griersen, D. Botanical Monographs Vol. 18, Ch. 15. pp. 405 - 433. Oxford: Blackwell.

Grimme, H. (1982). The effect of Al on Mg uptake and yield of oats. In Plant nutrition 1982, ed. Sciefe, A. Ninth International Colloquium Vol. I. Commonwealth Agricultural Bureaux.

Gurr, E. (1958). Methods of analytical histology and histochemistry. Section 7. London: Leonard Hill.

Gurr, E. (1965). The rational use of dyes in biology. London: Leonard Hill.

Haberlandt, G. (1914). Physiological plant anatomy. M. Drummond (Transl.) 4th German Edition. London: Macmillan.

Halvorson, A. D. & Lindsay, W. L. (1972). Equilibrium relationships of metal chelates in hydroponic solutions. Soil Science Society of America Proceedings 36: 755 - 761.

Hanson, J. B. & Day, D. A. (1980). Plant mitochondria. In The biochemistry of plants, ed. Tolbert, N. E. Vol. 1. Ch. 8. pp. 315 - 358. New York: Academic Press.

Harkes, P. A. A. (1973). Structure and dynamics of the root cap of Avena sativa. Acta botanica neerlandica 22: 321 - 328.

Hartwell, B. L. & Pember, F. R. (1918). The presence of aluminium as a reason for the difference in the effect of so-called acid soil on barley and rye. Soil Science 6: 259 - 280.

Haug, A. (1984). Molecular aspects of aluminium toxicity. Critical Reviews in Plant Sciences 1: 345 - 373.

Hayat, M. A. (1981). Principles and techniques of electron microscopy : biological applications. London: Edward Arnold.

Haynes, R. J. (1980). Ion exchange properties of roots and ionic interactions within the root apoplasm: their rôle in ion accumulation by plants. The Botanical Review 46: 75 - 99.

Hecht-Buchholz, C. H. (1983). Light and electron microscopic investigations of the reactions of various genotypes to nutritional disorders. Plant and Soil 72: 151 - 165.

Hecht-Buchholz, C. H. & Foy, C. D. (1981). Effect of aluminium toxicity on root morphology of barley. In Structure and function of plant roots, eds. Brouwer, R., Gasparikova, I., Kolek, J. & Loughman, B. C. 2nd International Symposium Czechoslovakia, Sept. 1980. Ch. 65. pp. 343 - 345. The Hague: Martinus Nijhoff.

Henning, S. J. (1975). PhD Thesis, Oregon State University. (quoted by Foy et al. 1978).

Hewitt, E. J. (1966). Sand and water culture methods used in the study of plant nutrition. Technical Communication No. 22 (revised 2nd edition). England: Commonwealth Agricultural Bureaux.

Higinbotham, N. (1973). The mineral absorption process in plants. Botanical Review 39: 15 - 69.

Hillman, S. K. & Wilkins, M. B. (1982). Gravity perception in decapped roots of Zea mays. Planta 155: 267 - 271.

Ho, D. T-R. (1983). Biochemical mode of action of abscisic acid. In Abscisic acid, ed. Addicott, F. T. pp. 147 - 169. New York: Praeger.

Hoffer, G. N. & Carr, R. H. (1923). Accumulation of aluminium and iron compounds in corn plants and its probable relation to root-rots. Journal of Agricultural Science 23: 801 - 823.

Horobin, R. W. (1982). Histochemistry : an explanatory outline of histochemistry and biophysical staining. Stuttgart: Gustav Fisher.

Horst, W. J., Wagner, A. & Marschner, H. (1982). Mucilage protects the root meristem from aluminium injury. Zeitschrift für Pflanzenphysiologie 105: 435 - 444.

Horst, W. J., Wagner, A. & Marschner, H. (1983). Effect of aluminium on root growth, cell division rate and mineral element contents of roots of Vigna unguiculata genotypes. Zeitschrift für Pflanzenphysiologie 109: 95 - 103.

Hou, H-Y & Merkle, F. G. (1950). Chemical composition of certain calcifugous and calcicolous plants. Soil Science 69: 471 - 486.

Hsu, P. H. (1977). Aluminium hydroxides and oxyhydroxides. In Minerals in soil environments, ed. Dinauer, R. C. Madison, Wisconsin: S.S.S.A.

Hsu, P. H. (1982). Crystallization of variscite at room temperature. Soil Science 133: 305 - 313.

Hutchinson, G. E. (1945). Aluminium in soils, plants and animals. Soil Science 60: 29 - 40.

Hyde, B. B. (1967). Changes in nucleolar ultrastructure associated with differentiation in the root tip. Journal of Ultrastructure Research 18: 25 - 54.

Islam, A. K. M. S., Edwards, D. G. & Asher, C. J. (1980). pH Optima for crop growth. Plant and Soil 54: 339 - 357.

I.S.F.E.I.P. (1971 & 1972). Annual reports. International soil fertility evaluation and improvement programme. Soil Science Department, Raleigh: North Carolina State University.

Iversen, T. H. (1969). Elimination of geotropic responsiveness in roots of cress (Lepidium sativum) by removal of statolith starch. Physiologia plantarum 22: 1251 - 1262.

Jackson, M. B. & Barlow, P. W. (1981). Root geotropism and the rôle of growth regulators from the cap: a re-examination. Plant Cell and Environment 4: 107 - 123.

Jackson, W. A. (1967). Physiological effects of soil acidity. In Soil acidity and liming, eds. Pearson, R. W. & Adams, F. Ch. 2. pp. 43 - 124. Madison, Wisconsin: American Society of Agronomy.

Jones, J. B. (1982). Hydroponics : its history and use in plant nutrition studies. Journal of Plant Nutrition 5: 1003 - 1030.

Jones, L. H. (1961). Aluminium uptake and toxicity in plants. Plant and Soil 13: 297 - 310.

Jordan, E. G., Timms, J. N. & Trewavas, A. J. (1980). The plant nucleus. In The biochemistry of plants, ed. Tolbert, N. E. Vol. 1, Ch. 13. pp. 489 - 588. New York: Academic Press.

Juniper, B. (1972). Mechanisms of perception and patterns of organisation in root caps. In The dynamics of meristem cell populations, eds. Miller, M. W. & Kuehnert, C. C. Advances in experimental medicine and biology. Vol. 18. New York: Plenum Press.

Juniper, B. E. (1976). Geotropism. Annual Review of Plant Physiology 27: 385 - 406.

Juniper, B. E. (1977). The perception of gravity by a plant. Proceedings of the Royal Society, Series B. 199: 537 - 550.

Juniper, B. E. & French, A. (1970). The fine structure of cells that perceive gravity in the root tip of maize. Planta 95: 314 - 329.

Juniper, B. E. & Roberts, R. M. (1966). Polysaccharide synthesis and the fine structure of root cells. Journal of the Royal Microscopical Society 85: 63 - 72.

Kalovoulos, J. M. & Misopolinos, N. D. (1983). Aluminium detection in corn roots by the quinalizarin methods. Plant and Soil 74: 131 - 132.

Kays, S. J., Nicklow, C. W. & Simons, D. H. (1974). Ethylene in relation to the response of roots to physical impedance. Plant and Soil 40: 565 - 571.

Kerridge, P. C., Dawson, M. D. & Moore, D. P. (1971). Separation of degrees of aluminium tolerance in wheat. Agronomy Journal 63: 586 - 591.

Knox, R. B. (1970). Freeze-sectioning of plant tissues. Stain Technology 45: 265 - 272.

Konings, H. & Jackson, M. B. (1979). A relationship between rates of ethylene production by roots and the promoting or inhibiting effects of exogenous ethylene and water on root elongation. Zeitschrift für Pflanzenphysiologie 92: 385 - 397.

Konzak, C. F., Polle, E. & Kittrick, J. A. (1976). Screening several crops for aluminium tolerance. In Plant adaptation to mineral stress in problem soils, ed. Wright, M. J. pp. 311 - 327. Ithaca, New York: Cornell University.

Krizek, D. T. & Foy, C. D. (1982). Elemental content of two barley cultivars in relation to water stress and aluminium toxicity. In Plant nutrition 1982, ed. Sciefe, A. Ninth International Colloquium. Commonwealth Agricultural Bureaux. (Abstr.)

Lachno, D. R., Harrison-Murray, R. S. & Audus, L. J. (1982). The effects of mechanical impedance to growth on the levels of ABA and IAA in root tips of Zea mays L. Journal of Experimental Botany 33: 943 - 951.

Lafever, H. N. (1981). Genetic differences in plant response to soil nutrient stress. Journal of Plant Nutrition 4: 89 - 109.

Lance, J. C. & Pearson, R. N. (1969). Effect of low concentrations of aluminium on growth and water and nutrient uptake by cotton plants. Soil Science Society of America Proceedings 33: 95 - 98

Lauchli, A. (1973). Investigation of ion transport in plants by electron probe analysis : principles and perspectives. In Ion transport in plants, ed. Anderson, W. P. Ch. 1. pp. 1 - 10. London: Academic Press.

Lee, C. R. (1971). Influence of aluminium on growth and mineral nutrition of potatoes. Agronomy Journal 63: 604 - 608.

Lee, J. & Pritchard, M. W. (1984). Aluminium toxicity expression on nutrient uptake, growth and root morphology of Trifolium repens cv. 'Grassland Huia'. Plant and Soil 82: 101 - 116.

Leonard, R. T. & Hodges, T. K. (1973). Characterisation of plasma membrane associated adenosine triphosphate activity of oat roots. Plant Physiology 52: 6 - 12.

Lindsay, W. L. (1979). Chemical equilibria in soils. New York: John Wiley & Sons.

Loneragen, J. F. & Dowling, E. J. (1958). The interactions of calcium and hydrogen ions in the nodulation of subterranean clover. Australian Journal of Agricultural Research 9: 464 - 472.

Loughman, B. C. (1969). The uptake of phosphate and its transport within the plant. In Ecological aspects of the mineral nutrition of plants, ed. Rorison, I. H. British Ecological Symposium No. 9. Blackwell Scientific Publications.

Loughman, B. C. (1981). Metabolic aspects of the transport of ions by cells and tissues of roots. Plant and Soil 63: 47 - 55.

Lutz, J. A., Hawkins, G. W. & Genter, G. F. (1971). Differential response of corn inbreds and single crosses to certain properties of an acid soil. Agronomy Journal 63: 803 - 805.

Luxová, M. (1981). Growth regions of the primary root of (Zea mays L.) In Structure and function of plant roots, ed. Brouwer, R., Gasparikova, I., Kolek, J. & Loughman, B. C. 2nd International Symposium, Czechoslovakia, Sept. 1980. Ch. 2. pp. 9 - 14. The Hague: Martinus Nijhoff.

MacLeod, L. B. & Jackson, L. P. (1967). Aluminium tolerance of two barley varieties in nutrient solution, peat and soil culture. Agronomy Journal 59: 359 - 363.

Magistad, O. C. (1925). The Al content of the soil solution and its relation to the soil reaction and plant growth. Soil Science 20: 181 - 225.

Markhart, A. H., Fiscus, E. L., Naylor, A. W. & Kramer, P. J. (1979). Effect of abscisic acid on root hydraulic conductivity. Plant Physiology 64: 611 - 614.

Marré, M. T., Romani, G., Cocucci, M., Moloney, M. M. & Marré, E. (1982). Divalent cation influx, depolarisation of the transmembrane electric potential and proton extrusion in maize root segments. In Plasmalemma and tonoplast: their function in the plant cell, eds. Marré et al. pp. 3 - 13. Amsterdam: Elsevier Biomedical Press.

Marty, F., Branton, D. & Leigh, R. A. (1980). Plant vacuoles. In The biochemistry of plants, ed. Tolbert, N. E. Vol. 1, Ch. 15. pp. 625 - 658. New York: Academic Press.

Matsumoto, H., Hirasawa, E., Torikai, H. & Takahashi, E. (1976). Localization of absorbed aluminium in pea root and its binding to nucleic acids. Plant and Cell Physiology 17: 127 - 137.

McCain, S. & Davies, M. S. (1983). The influence of background solution on root responses to aluminium in Holcus lanatus L. Plant and Soil 73: 425 - 430.

McCormick, L. H. & Borden, F. Y. (1972). Phosphate fixation by aluminium in plant roots. Soil Science Society of America Proceedings 36: 799 - 802.

McCormick, L. H. & Borden, F. Y. (1974). The occurrence of aluminium-phosphate precipitate in plant roots. Soil Science Society of America Proceedings 38: 931 - 934.

McCully, M. (1975). The development of lateral roots. In The development and function of roots, eds. Torrey, J. G. & Clarkson, D. T. Third Cabot Symposium Ch. 6. pp. 105 - 124. London: Academic Press.

McLean, E. O. (1976). Chemistry of soil aluminium. Communications in Soil Science and Plant Analysis 7: 619 - 636.

McLean, F. T. & Gilbert, B. E. (1927). Aluminium toxicity. Plant Physiology 3: 293 - 302.

Mendes, Paula, Farina, M. P. W. & Channon, P. (1984). Assessment of aluminium tolerance in maize using a rapid screening procedure. South African Journal of Plant and Soil 1: 83 - 86.

Mengel, K. & Kirkby, E. A. (1979). Principles of plant nutrition. Ch. 3: pp. 97 - 147. Berne: International Potash Institute.

Middleton, K. R. & Smith, G. S. (1982). Effect of aluminium in the presence of phosphate and lime on growth and chemical composition of ryegrass and lucerne. Fertilizer Research 3: 353 - 366.

Moeller, T. (1952). Inorganic chemistry. Ch. 17. pp. 734 - 817. New York: John Wiley & Sons.

Mollenhauer, H. H. (1965). Transition forms of Golgi apparatus secretion vesicles. Journal of Ultrastructure Research 12: 439 - 446.

Mollenhauer, H. H. & Mollenhauer, B. A. (1978). Changes in the secretory activity of the Golgi apparatus during the cell cycle in root tips of maize (Zea mays L.) Planta 138: 113 - 118.

Mollenhauer, H. H. & Morré, D. J. (1966). Golgi apparatus and plant secretion. Annual Review of Plant Physiology 17: 27 - 46.

Mollenhauer, H. H. & Morré, D. J. (1980). The Golgi apparatus. In The biochemistry of plants, ed. Tolbert, N. E. Vol. 1, Ch. 12. pp. 437 - 488. New York: Academic Press.

Moore, D. P. (1974). Physiological effects of pH on roots. In The plant root and its environment, ed. Carson, E. W. Ch. 6. pp. 135 - 151. Charlottesville, Virginia: University Press of Virginia.

Moore, D. P., Kronstad, W. E. & Metzger, R. J. (1976). Screening wheat for aluminium tolerance. In Plant adaptation to mineral stress in problem soils, ed. Wright, M. J. pp. 287 - 295. Ithaca, New York: Cornell University.

Moore, R. (1985). Acid-efflux patterns of primary and secondary roots of Ricinus communis (Euphorbiaceae). Annals of Botany 55: 381 - 385.

Moore, R. & McClelan, C. E. (1985). Root graviresponsiveness and columella cell structure in carotenoid-deficient seedlings of Zea mays. Annals of Botany 56: 83 - 90.

Moore, R. & Pasieniuk, J. (1984). Graviresponsiveness and the development of columella tissue in primary and lateral roots of Ricinus communis. Plant Physiology 74: 529 - 533.

Morimura, S. & Matsumoto, H. (1978). Effect of aluminium on some properties and template activity of purified pea DNA. Plant and Cell Physiology 19: 429 - 436.

Morimura, S., Takohashi, E. & Matsumoto, H. (1978). Association of aluminium with nuclei and inhibition of cell division in onion (Allium cepa) roots. Zeitschrift für Pflanzenphysiologie 88: 395 - 401.

Morré, D. J. (1977a). Membrane differentiation and the control of secretion: a comparison of plant and animal Golgi apparatus. In International cell biology, eds. Brinkley, B. R. & Porter, K. R. pp. 293 - 303. New York: Rockefeller University Press.

Morré, D. J. (1977b). The Golgi apparatus and membrane biogenesis. In The synthesis assembly and turnover of cell surface components, eds. Poste, G. & Nicholson, G. C. Ch. 1. pp. 1 - 83. Amsterdam: North Holland.

Morré, D.J., Jones, D. D. & Mollenhauer, H. H. (1967). Golgi apparatus mediated polysaccharide secretion by outer root cap cells of Zea mays. Planta 74: 286 - 301.

Morré, D. J. & Mollenhauer, H. H. (1974). The endomembrane concept: a functional integration of endoplasmic reticulum and Golgi apparatus. In Dynamic aspects of plant ultrastructure, ed. Robards, A. W. Ch. 3. pp. 84 - 137. London: McGraw-Hill.

Mugwira, L. M. & Patel, S. U. (1977). Root zone pH changes and ion uptake imbalances by triticale, wheat and rye. Agronomy Journal 69: 719 - 722.

Mulkey, T. J., Evans, M. L. & Kuzmanoff, K. M. (1983). The kinetics of abscisic acid action on root growth and gravitropism. Planta 157: 150 - 157.

Mulkey, T. J., Kuzmanoff, K. M., Evans, M. L. (1981). Correlations between proton-efflux patterns and growth patterns during geotropism and phototropism in maize and sunflower. Planta 152: 239 - 241.

Munns, D. N. (1965a). Soil acidity and growth of a legume I. Australian Journal of Agricultural Research 16: 733 - 741.

Munns, D. N. (1965b). Soil acidity and growth of a legume II. Reactions of aluminium and phosphate in solution and effects of aluminium, phosphate, calcium, and pH on Medicago sativa L. and Trifolium subterraneum L. in solution culture. Australian Journal of Agricultural Research 16: 743 - 755.

Naidoo, G., Stewart, J. McD. & Lewis, R. J. (1978). Accumulation sites of Al in snapbean and cottonroots. Agronomy Journal 70: 489 - 492.

Newcomb, E. H. (1980). The general cell. In The biochemistry of plants, ed. Tolbert, N. E. Vol. 1, Ch. 1. pp. 1 - 54. New York: Academic Press.

Nightingale, E. R. (1959). Phenomenological theory of ion solvation. Effective radii of hydrated ions. Journal of Physical Chemistry 63: 1381 - 1387.

Northcote, D. H. & Pickett-Heaps, J. D. (1966). A function for the Golgi apparatus in polysaccharide synthesis and transport in root cap cells of wheat. Biochemical Journal 98: 159 - 167.

Norvell, W. A. (1972). Equilibria of metal chelates in soil solution. In Micronutrients in agriculture, ed. Dinauer, R. C. Ch. 6. pp. 115 - 138. Madison, Wisconsin: S.S.S.A.

Oades, J. M. (1978). Mucilages at the root surface. Journal of Soil Science 29: 1 - 16.

O'Brien, T. P. (1982). Cell growth and division. In The molecular biology of plant development, eds. Smith H. & Grierson, D. Botanical Monographs. Vol. 18, Ch. 4. pp. 49 - 109. Oxford: Blackwell.

O'Brien, T. P. & McCully, M. E. (1981). The study of plant structure, principles and selected methods. Melbourne, Australia: Termarcarphi (Pty) Ltd.

Obrouchevá, N. (1975). Physiology of growing root cells. In The development and function of roots, eds. Torrey, J. G. & Clarkson, D.T. Third Cabot Symposium Ch. 14. pp. 279 - 298. London: Academic Press.

Pavan, M. A. & Bingham, F. T. (1982). Toxicity of aluminium to coffee seedlings grown in nutrient solution. Soil Science Society of America Journal 46: 993 - 997.

Pearse, A. G. E. (1972). Histochemistry : theoretical and applied. 3rd Edition, Vol. 2. pp. 880 - 920. Edinburgh: Churchill Livingstone.

Perl, K. J., Webster, G. R. & Cairns, R. R. (1982). Acidification of a solonetzic soil by nitrogenous fertilizers. Journal of Environmental Science and Health 17: 581 - 605.

Philipps, H. L. & Torrey, J. G. (1972). Duration of cell cycles in cultured root of Convolvulus. American Journal of Botany 59: 183 - 188.

Pilet, P. E. (1972). Root cap and root growth. Planta 106: 169 - 171.

Pilet, P. E. (1975a). Abscisic acid as a root growth inhibitor: physiological analyses. Planta 122: 299 - 302.

Pilet, P. E. (1975b). Action of fusicoccin and abscisic acid on root growth. Plant Science Letters 5: 137 - 140.

Pilet, P. E. (1982). Importance of the cap cells in maize root gravireaction. Planta 156: 95 - 96.

Pilet, P. E. & Chanson, A. (1981). Effect of abscisic acid on maize root growth. A critical examination. Plant Science Letters 21: 99 - 106.

Pilet, P. E. & Ney, D. (1978). Rapid, localised light effect on root growth in maize. Planta 144: 109 - 110.

Pilet, P. E. & Nocera-Przybecka, D. (1978). Abscisic acid effect on the DNA microgradients of decapped maize roots. Plant and Cell Physiology 19: 1475 - 1481.

Polle, E., Konzak, C. F. & Kittrick, J. A. (1978). Visual detection of aluminium tolerance levels in wheat by hematoxylin staining of seedling roots. Crop Science 18: 823 - 827.

Poole, R. J. (1978). Energy coupling for membrane transport. Annual Review of Plant Physiology 29: 437 - 460.

Poovaiah, B. W. (1985). Rôle of calcium and calmodulin in plant growth and development. Horticultural Science 20: 347 - 352.

Rao, K. P. & Rains, D. W. (1976). Nitrate absorption by barley. I Kinetics and Energetics. Plant Physiology 57: 55 - 58.

Rasi-Caldogno, F., Cerana, R. & Pugliarello, M. C. (1980). Relationship between ATP level and activity of fusicoccin-stimulated H^+/K^+ - exchange system in plant tissues. Plant Physiology 66: 1095 - 1098.

Rasmussen, H. P. (1968). Entry and distribution of aluminium in Zea mays. Planta 81: 28 - 37.

Raven, J. H. & Rubery, P. H. (1982). Co-ordination of development: hormone receptors, hormone action and hormone transport. In The molecular biology of plant development, eds. Smith, H. & Grierson, D. Botanical Monographs Vol. 18, Ch. 3. pp. 28 - 48. Oxford: Blackwell.

Ray, P. M. & Green, P. B. (1972). Rôle of turgor in plant cell growth. Nature 239: 163 - 164.

Rees, T. ap (1980). Contribution of metabolic pathways to respiration. In The biochemistry of plants, ed. Davies, D. D. Vol. 2, Ch. 1. pp. 1 - 29.

Reynolds, E. S. (1963). The use of lead citrate at high pH as an electron opaque stain in electron microscopy. Journal of Cell Biology 17: 208 - 212.

Rhue, R. D. (1976). PhD Thesis, Oregon State University (quoted by Foy et al., 1978).

Rhue, R. D. (1979). Differential aluminium tolerance in crop plants. In Stress physiology in crop plants, eds. Mussell, H. & Staples, R. C. pp. 62 - 80. New York: John Wiley & Sons.

Rhue, R. D. & Grogan, C. O. (1976). Screening corn for aluminium tolerance. In Plant adaptation to mineral stress in problem soils, ed. Wright, M. J. pp. 297 - 310. Ithaca, New York: Cornell University Agr. Exp. Sta.

Rhue, R. D. & Grogan, C. O. (1977). Screening corn for Al tolerance using different Ca and Mg concentrations. Agronomy Journal 69: 755 - 760.

Riley, D. & Barber, S. A. (1969). Bicarbonate accumulation and pH changes at the soybean (Glycine max (L) Merr.) root soil interface. Soil Science Society of America Proceedings 33: 905 - 908.

Rivier, L., Milon, H. & Pilet, P. E. (1977). Gas chromatography - mass spectrometric determination of abscisic acid levels in the cap and apex of maize roots. Planta 134: 23 - 27.

Rivier, L. & Pilet, P. E. (1981). Abscisic acid levels in the root tips of seven Zea mays varieties. Phytochemistry 20: 17 - 19.

Rorison, I. H. (1960). The calcicole-calcifuge problem. Journal of Ecology 48: 679 - 688.

Russell, R. S. & Goss, M. J. (1974). Physical aspects of soil fertility - the response of roots to mechanical impedance. Netherlands Journal of Agricultural Science 22: 305 - 318.

Salema, R. & Badenhuizen, N. P. (1969). Nucleic acids in plastids and starch formation. Acta botanica neerlandica 18: 203 - 215.

Sampson, M., Clarkson, D. & Davies, D. D. (1965). DNA synthesis in aluminium treated roots of barley. Science 148: 1476 - 1477.

Schenk, M. K. & Barber, S. A. (1980). Potassium and phosphorus uptake by corn genotypes grown in the field as influenced by root characteristics. Plant and Soil 54: 65 - 76.

Shen-Miller, J., McNitt, R. E. & Wojchiechowsi, M. (1978). Regions of differential cell elongation and mitosis, and root meristem morphology in different tissuesof geotropically stimulated maize root apices. Plant Physiology 61: 7 - 12.

Shen-Miller, J. & Miller, C. (1972). Distribution and activation of the Golgi apparatus in geotropism. Plant Physiology 49: 634 - 639.

Shropshire, W. (1979). Stimulus perception. In Physiology of movement, eds. Haupt, W. & Feinleib, M. E. Encyclopaedia of plant movement. Vol. 7, pp. 10 - 35. Berlin: Springer-Verlag.

Siegel, N. & Haug, A. (1983). Calmodulin-dependent formation of membrane potential in barley root plasma membrane vesicles: a biochemical model of aluminium toxicity in plants. Physiologia plantarum 59: 285 - 291.

Sievers, A., Behrens, H. M., Buckhout, T. J. & Gradmann, D. (1984). Can a Ca^{2+} pump in the endoplasmic reticulum of the Lepidium root be the trigger for rapid changes in membrane potential after gravistimulation? Journal of Plant Physiology 114: 195 - 200.

Sirover, M. A. & Loeb, L. A. (1976). Infidelity of DNA synthesis in vitro : screening for potential metal mutagens or carcinogens. Science 194: 1434 - 1436.

Smith, F. A. & Raven, J. A. (1979). Intracellular pH and its regulation. Annual Review of Plant Physiology 30: 289 - 311.

Spanswick, R. M. (1981). Electrogenic ion pumps. Annual Review of Plant Physiology 32: 267 - 289.

Steward, F. C. (1969). Growth and development: the problem in perspective. In Plant physiology, ed. Steward, F. C. Vol. VA: XIX - XXXIV. New York: Academic Press.

Stockmeyer, E. W. & Everett. H. L. (1978). Studies on the mechanism of Al response in maize. Maize Genetics Co-op Newsletter 52: 47 - 48.

Stockmeyer, E. W., Everett, H. L. & Rhue, R. D. (1978). Aluminium tolerance in maize seedlings as measured by primary root length in nutrient solutions. Maize Genetics Co-op Newsletter 52: 15 - 16.

Storey, B. T. (1980). Electron transport and energy coupling in plant mitochondria. In The biochemistry of plants, ed. Davies, D. D. Vol. 2, Ch. 4. pp. 125 - 195. New York: Academic Press.

Suzuki, T., Kondo, H. & Fujii, T. (1979). Distribution of growth regulators in relation to light-induced geotropic responsiveness in Zea roots. Planta 145: 323 - 329.

Sze, H. (1984). H^+ - translocating ATPases of the plasma-membrane and tonoplast of plant cells. Physiologia plantarum 61: 683 - 691.

Takagi, H., Namai, H. & Murakami, K. (1981). Evaluation of a method for estimating the aluminium tolerance of wheat using haematoxylin stain. Japan Journal of Breeding 31: 152 - 160.

Technicon Auto Analyser (1972). Industrial method 144 - 171A, phosphorus in food products. New York: Technicon Industrial Systems.

Tiwari, C. C., Inbokwe, P. E., Edurg, S. & Russel, L. (1984). Evaluation of soybean germplasm for stress tolerance and biological efficiency. Soil Acidity. Soybean Genetics Newsletter 11: 61 - 62.

Theron, J. J. (1951). The influence of plants on the mineralisation of nitrogen and the maintenance of organic matter in the soil. Journal of Agricultural Science 41: 289 - 296.

Torrey, J. G. (1972). On the initiation of organisation in the root apex. In The dynamics of meristem cell populations, eds. Miller, M. W. & Kuehnert, C. C. Advances in Experimental Medicine and Biology. Vol. 18, pp. 1 - 10. New York: Plenum Press.

Torrey, J. G. (1976). Root hormones and plant growth. Annual Review of Plant Physiology 27: 435 - 459.

van Staden, J. & Forsyth, C. (1986). Maize roots, adenine and cytokinin biosynthesis : lack of a positive correlation. South African Journal of Botany 52: 85 - 90.

Vickery, M. L. & Vickery, B. (1981). Secondary plant metabolism. Ch. 3. pp. 56 - 87. London: Macmillan Press.

Viets, F. G. (1944). Calcium and other polyvalent cations as accelerators of ion accumulation by excised barley roots. Plant Physiology 19: 466 - 480.

Vogel, A. I. (1955). Quantitative inorganic analysis. 2nd Edn. Appendix A 10. pp. 868 - 872. London: Longmans Green.

Volkmann, D. & Sievers, A. (1979). Graviperception in multicellular organs. In Physiology of movement, eds. Haupt, W. & Feinlieb, M.E. Vol. 7, pp. 573 - 600. Encyclopaedia of Plant Physiology. Berlin: Springer-Verlag.

Wallace, S. U. & Anderson, I. C. (1984). Aluminium toxicity and DNA synthesis in wheat roots. Agronomy Journal 76: 5 - 8.

Wallihan, E. F. (1948). The influence of aluminium on the phosphorus nutrition of plants. American Journal of Botany 35: 106 - 112.

Wample, R. L. & Reid, D. M. (1975). Effects of aeration on the flood-induced formation of adventitious roots and other changes in sunflower (Helianthus annuus). Planta 127: 263 - 270.

Warncke, D. D. & Barber, S. A. (1974). Root development and nutrient uptake by corn grown in solution culture. Agronomy Journal 66: 514 - 516.

Webb, L. J. (1954). Aluminium accumulation in the Australian - New Guinea flora. Australian Journal of Botany 2: 176 - 196.

Wilkins, M. B. (1979). Growth-control mechanisms in gravitropism. In The physiology of movement, eds. Haupt, W. & Feinlieb, M. E. Encyclopaedia of Plant Physiology Vol. 7, pp. 601 - 626. Berlin: Springer-Verlag.

Williams, C. H. (1980). Soil acidification under clover pasture. Australian Journal of Experimental Agriculture and Animal Husbandry 20: 561 - 567.

Wilson, A. J., Robards, A. W. & Goss, M. J. (1977). Effects of mechanical impedance on root growth in barley, Hordeum vulgare L. Journal of Experimental Botany 28: 1216 - 1227.

Wright, K. E. (1943). Internal precipitation of phosphorus in relation to aluminium toxicity. Plant Physiology 18: 708 - 712.

Wright, K. E. & Donahue, B. A. (1953). Aluminium toxicity studies with radioactive phosphorus. Plant Physiology 28: 674 - 680.

Wright, K. & Northcote, D. H. (1974). The relationship of root cap slimes to pectins. Biochemical Journal 139: 525 - 534.

Yemm, E. W. (1965). The respiration of plants and their organs. In Plant physiology, ed. Steward, F. C. Vol. 4A, Ch. 3. pp. 231 - 310. New York: Academic Press.

The References are in accordance with the Style Manual for Theses, Faculty of Science, University Natal (1985), and World List of Scientific Periodicals (1963) eds. Brown, P. & Stratton, G. B. Washington: Butterworth.