Available online at www.joac.info

ISSN: 2278-1862

Journal of Applicable Chemistry

2013, 2 (3): 447-474

(International Peer Reviewed Journal)

Aluminium Toxicity in Plants - A Review

Satish A. Bhalerao [1*] and Damodar V. Prabhu[2]

1. Department of Botany, Wilson College, Mumbai- 400007, **INDIA**
2. Department of Chemistry, Wilson College, Mumbai - 400007, **INDIA**

Email: drsatishbhalerao@yahoo.com, drsatish16@rediffmail.com

Received on 26th April and finalized on 2nd May 2013.

ABSTRACT

Aluminium (Al) is the most abundant metal in the earth's crust, comprising about 7% of its mass. A large amount of Al is incorporated into aluminosilicate soil minerals and very small quantities appear in the soluble form. Aluminium toxicity is one of the major factors that limit plant growth and development in many acid soils which differs strikingly in their chemical form. Al has been shown to interfere with cell division in plant roots, decrease root respiration, increase cell wall rigidity and interfere with the uptake and transport of Ca, Mg, K, P and water supply to plants, alter cell-wall Donnan free space, the plasma membrane, membrane transport proteins etc. Al toxicity is mainly associated with severe changes in root morphology, resulting in curved, swollen, cracked, brownish, stubby and stiff root apices. It has been known that plants which exist in the presence of potentially toxic Al concentrations must be able to avoid direct contact of vital structures and metabolic processes with high activities of Al ions. The physiological mechanisms of Al resistance can either be mediated via exclusion of Al from the root apex or via intracellular tolerance of Al transported into the plant symplasm. The approaches like metal uptake and transportation in various plant parts, mechanism behind the interaction with mineral nutrients, specific genes responsible for tolerance levels and kinds of organic and amino acids which act as metal chelators and detoxifiers, level and forms of enzymes, and changes in root permeabilities to ions and molecules and their mechanisms are used to study Al toxicity in tolerant and sensitive plant genotypes.

Keywords: Aluminium toxicity, resistance, tolerance, exclusion, root morphology.

INTRODUCTION

Aluminium (Al) ranks third in abundance among the Earth's crust elements after oxygen and silicon and comprises approximately 7% of its mass. A large amount of Al is incorporated into aluminosilicate soil minerals and very small quantities appear in the soluble form capable of influencing biological systems [1]. Al bioavailability and in consequence, toxicity, is mainly restricted to acid environments. Acid soils (with a pH of 5.5 or lower) are among the most important limitations to agricultural production. The production of staple food crops, in particular grain crops, is negatively influenced by acid soils [2]. Some agricultural practices, as removal of products from the farm, leaching of nitrogen below the plant root zone, inappropriate use of nitrogenous fertilizers, and build-up in organic matter, are causing further acidification of agricultural soils.

Different forms of aluminium occur in soil solution: $Al(OH)^{2+}$ at pH 4-5, Al^{3+} at pH 5.5-7, and $Al(OH)^4$ at pH 7-8. Other complex ions $AlO_4Al_{12}(OH)_{24}(H2O)_{12}{}^{7+}$ (Al_{13}) and Al^{3+} are almost certainly toxic, but no rhizotoxicity has been detected for $AlSO4^+$ and $Al(SO_4)_2^-$ or Al-F (e.g. AlF_2^+ and AlF^{2+}). The status of $Al(OH)^{2+}$ is uncertain although experimental results have appeared indicating Al-OH toxicity [3]. The following Al species are toxic for wheat roots in the following increasing order: $AlF_2^+ < AlF^{2+} < Al^{3+} < Al_{13}$. According to Kochian's, 1995 [4] opinion toxicity has been convincingly demonstrated only for Al_{13} and Al^{3+}. When pH drops below 5.5, aluminosilicate clays and aluminium hydroxide minerals begin to dissolve, releasing aluminium-hydroxy cations and $[Al(H_2O)6^{3+}]$ and (Al^{3+}), that then exchange with other cations. Under these conditions, Al^{3+} also forms the mononuclear species $Al(OH)^{2+}$, $Al(OH)_3$, and $Al(OH)_4$ [5]. The mononuclear Al^{3+} species and Al_{13} are considered as the most toxic forms [4,6]. It has been estimated that over 50% of the world's potentially arable lands are acidic [7]. Soil acidity is a natural occurrence in tropical and subtropical zones. But in temperate zones, it is an increasing problem and the result of acid rain in the industrial regions of the USA, Canada and Europe [8]. Although the poor fertility of acid soils is due to a combination of mineral toxicities (Al and Mn) and deficiencies (P, Ca, Mg and Mo), Al toxicity is the most important factor, being a major constraint for crop production on 67% of the total acid soil area [9].

Al toxicity and tolerance mechanisms differ strikingly with its chemical form, and the study of Al related processes are complicated by the complex chemistry of Al. Therefore the experimental results may differ with experimental conditions such as pH and coexisting ions, even though the same concentration of Al is used [10]. The cellular components and processes which have been proposed to be affected by Al are wide ranging and some of the most important include; cell nuclei, mitosis and cell division [11], composition, physical properties and structure of the plasma membrane [12,13], uptake of Ca^{2+} and other ions [14], phosphoinositide-mediated signal transduction and cytoplasmic calcium homeostasis [15,16], oxidative stress [17], cytoskeletal dynamics [18] and the cell wall-plasmamembrane-cytoskeleton continuum [19]. Because of the complex chemistry of Al, molecular, genetic and physiological bases are still not well understood. Despite the interest from many researchers, Al resistance genes have yet to be cloned from any species, with the exception of *ALMT1* from wheat [20].

The problem of Al phytotoxicity is exacerbated by the use of ammonium fertilizers and acid rain [7]. The main symptom of Al toxicity is a rapid inhibition of root growth, which may translate to a reduction in vigor and crop yields [21,2]. Plants have different mechanisms to resist or tolerate the toxic effect of Al in response to this stress. These resistance mechanisms in plants have been classified as a) external or exclusion through the exudation of organic acids from the radical apexes and subsequent chelation of the root in the rhizosphere and b) internal or Al-tolerant since Al chelation is produced inside the cell and then later stored and compartmentalized in organelles like the vacuole [2, 22]. Several investigations have reported that Al interferes with the cell division of radical apexes, increases rigidification of the cell wall by crossing with pectins, reduces DNA replication by increasing the rigidification of the double helix [23], interferes with the signal transduction pathways, alters cytoplasmic Ca^{2+} levels [24] and inhibits phospholipase C (PLC) activity of the phosphoinositide pathway associated with Ca^{2+} signaling [25, 26]. The genetic bases for Al resistance have been studied in a limited number of species, such as wheat, oats, rye, triticale, sorghum [27, 28, 29]. Although the resistance seems to be a multigenic trait in most of the plants studied, in some species it seems to be codified by a simple dominant locus [30]. In species like wheat, barley and rye, loci for Al resistance have been mapped on the long arm of chromosomes 4D, 4H and 4R, respectively [31, 32, 33], suggesting that this position may be conserved for Al resistance in cereals.

Al ions translocate very slowly to the upper parts of plants [34]. Most plants contain no more than 0.2 mg Al/gm of dry mass. However, some plants, known as Al accumulators, may contain over 10 times more Al without any injury. Tea plants are typical Al accumulators: the Al content in these plants can reach as high as 30 mg/gm of dry mass in old leaves [35]. Approximately 400 species of terrestrial plants, belonging to 45 families, have so far been identified as hyperaccumulators of various toxic metals [36].

Aluminium Toxicity

Cytoplasmic Ca^{2+}: Disturbance of cytoplasmic Ca^{2+} homeostasis is believed to be the primary target of Al toxicity [16] and may be involved in the inhibition of the cell division or root elongation by causing potential disruptions of Ca^{2+}-dependent biochemical and physiological processes [16,37,38]. In wheat root apices, Jones D. L et al., [39] found that Al inhibits Ca^{2+}-dependent phospholipase C, which acts on the lipid substrate phosphatidylinositol-4,5-biphosphate. The authors hypothesized that phosphoinositide signaling pathway might be the initial target of Al. In accordance, Zhang et al. [40] found Al-induced inhibition of genes related to phosphoinositide signaling pathway and hypothesized that the gene inhibition could result in disruption of this pathway. Also, it was reported that components of the actin-based cytoskeleton interact directly with phospholipase C in oat [41]. Most workers reported an increase in cytoplasmic Ca^{2+} when plants were exposed to Al [42,43,44]. However, Jones et al. [45] reported a decrease in cytoplasmic Ca^{2+} in tobacco cell cultures in the presence of Al. Furthermore, Zhang and Rengel [43] reported an increase in cytoplasmic Ca^{2+} in two lines with different tolerance to Al and correlated it with the inhibition of root growth in both lines. Moreover, Ma et al. [42] correlated cytoplasmic Ca^{2+} with root growth response. Moreover, alteration in cytoplasmic Ca^{2+} homeostasis can occur within few minutes (20–30 minutes) in root hair tips of *Arabidopsis thaliana* [45]. It is certain that Al exposure influences cytoplasmic Ca^{2+} homeostasis, but it is still unclear if it is a primary cause of Al induced inhibition of root growth or a secondary effect. The source of Ca^{2+} for the increase of cytosolic Ca^{2+} activity could be extracellular and/or intracellular but is still insufficiently documented, as well the effects on increased cytosolic Ca^{2+} (for review see [16]).

Effect on Leaves: Aluminium toxicity is a potential growth-limiting factor for plants grown in acid soils in many parts of the world [46,47,48,49]. The symptoms of aluminium toxicity are not easily identifiable. In plants, the foliar symptoms resemble those of phosphorous (P) deficiency (overall stunting, small, dark green leaves and late maturity, purpling of stems, leaves, and leaf veins, yellowing and death of leaf tips). In some cases, Al toxicity appears as an induced calcium (Ca) deficiency or reduced Ca transport problem (curling or rolling of young leaves and collapse of growing points or petioles). Excess Al even induces iron (Fe) deficiency symptoms in rice (*Oryza sativa* L.), sorghum and wheat [50,51,52].

Callose: The induction of callose (1,3-*β*-D-glucan) formation in Al-exposed roots has been reported in many plant species [53,54,55,56,57]. Al-induced callose formation in root tips is recognized as an excellent indicator of Al sensibility [58,59,60,61], and some works negatively correlated root elongation with callose formation during Al exposure [58,62]. Recently, it was reported that Al induced callose accumulation not only in the root meristematic regions but also in mature zones, in both wheat and rye genotypes [63,64]. In maize roots, Jones et al. [53] found a close spatial and temporal coordination between Al accumulation and callose production in roots. Also, in wheat, callose accumulation in root tissues was progressive with Al-exposure, and, contrarily to the tolerant genotype, the sensitive one presented callose deposition at inner cell layers [63,64]. Still, Tahara et al. [58] reported that, in some Myrtaceae species, induction of callose formation was not accompanied by root growth inhibition and suggested that callose formation is a more sensitive indicator to Al than root elongation.

Since Al induces a transient rise of cytosolic Ca^{2+}, an increase of callose accumulation under Al stress is not unexpected. Cytosolic Ca^{2+} is one of the prerequisites for the induction of callose synthesis, but not the only factor modulating increases in callose synthesis and deposition [44]. Callose formation, as response to Al, is described in sensitive and, to a lesser extent, in tolerant roots [57, 59]. In a less extent, callose deposition has been considered as a mechanism to prevent Al from penetrating into the apoplast. Also, this accumulation is reported to inhibit the symplastic transport and cell communication by blocking plasmodesmata, avoiding Al-induced lesions in the symplast [65]. However, callose deposition in sensitive roots has also been shown to lead to uncontrolled rigidity of cell walls [53] leading ultimately to protoplast degradation.

Effect on Roots: Aluminium does not affect the seed germination but helps in new root development and seedling establishment [66]. Root growth inhibition was detected 2–4 days after the initiation of seed germination [67]. Vanpraag and Weissen [68] reported that plant species and ecotypes growing on acid

soils had become very resistant to the inhibitory effects of aluminium on root absorption and growth in course of time and phenological evolution. The major Al toxicity symptom observed in plants is inhibition of root growth [67, 69, 70,71,72]. The roots exhibit greater signs of cellular damage than other parts of the plant [73, 74]. Al toxicity could be observed in the root system particularly in root-tips and in lateral roots; lateral roots become thickened and turn brown [75,76]. The root system as a whole is corraloid in appearance with many stubby lateral roots but lacks fine branching [77]. The toxicity appears to be determined by the availability of certain monomeric species of Al to the plant roots [78,79]. Losses of phytoactive, monomeric Al can occur by polymerization of Al as the pH and the Al concentrations rise [79,80,81] to make complex formation or chelation with phosphate and organic acids [78,79]. Kinraide et al. [82] demonstrated rapid assay for aluminium phytotoxicity at submicromolar concentrations of Al to *Trifolium pratense*. Wagatsuma et al. [83] noted the role of aluminium on root cells of various crops. They reported that the cells of the epidermis and outer cortex of maize (Al-sensitive) in the portion approximately 1 cm from the root-tip were damaged, and the walls of these cells were abnormal and partially detached in barley (a plant highly sensitive to Al); more pronounced abnormality and detachment of the cell walls involved almost the whole cortex, and few cortex cells remained alive in oats (Al-tolerant) after 6 days' exposure to the Al treatment. They also reported that in the case of peas, the roots were elongated due to a low level of Al treatment. Aluminium was absorbed in large amounts in the tip portion of the root. In the tip portion, the K content decreased with the increase of the Al content, but the Ca content was almost constant. Bennet et al. [84] reported that an anisotropic growth response of cortical cells with 20-h root exposure to Al was associated with the collapse of the conducting tissue of the stele and disintegration of the outer cells of the root.

Oxidative Stress: Al-induced oxidative stress and changes in cell wall properties have been suggested as the two major factors leading to Al toxicity [85, 86]. Oxidative stress occurs when any condition disrupts the cellular redox homeostasis. The reactive oxygen species (ROS) have the capacity to oxidize cellular components such as lipids, proteins, enzymes, and nucleic acids, leading to cell death. Metals are known to act as catalysts in ROS production and to induce oxidative damage in plants. Al itself is not a transition metal and cannot catalyze redox reactions; however, Al exposure leads to oxidative stress [17,53,87,88]. Because aluminium ions form electrostatic bonds preferentially with oxygen donor ligands (e.g., carboxylate or phosphate groups), cell wall pectin and the outer surface of the plasma membrane seem to be major targets of aluminium [86]. Al binding to biomembranes leads to rigidification [39], which seems to facilitate the radical chain reactions by iron (Fe) ions and enhance the peroxidation of lipids [17].

Al induction lipid peroxidation has been reported for some species, including barley [89], sorghum [90], triticale [91], rice [88], greengram [92], and wheat [93]. Yamamoto et al. [86] found that, for *Pisum sativum* seedlings treated with Al in a simple Ca solution, Al accumulation, lipid peroxidation, and callose production had a similar distribution on the root apex surface and was accompanied by root growth inhibition. However, the loss of membrane integrity was only detected at the periphery of the cracks on the surface of the root apex. Furthermore, Yamamoto et al. [17] concluded that the Al enhancement of lipid peroxidation is an early symptom of Al accumulation and appears to cause partly callose production, but not root growth inhibition. Later, however, in maize, Al treatment did not induce lipid peroxidation, indicating that lipids are not the primary cellular target of oxidative stress in maize [87]. So, it seems that cellular target of oxidative stress depends on plant species.

Plant cells are equipped with a defensive system composed by enzymatic antioxidants such as catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPOX), superoxide dismutase (SOD), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione-S-transferase (GST), and gluthatione reductase (GR) and nonenzymatic antioxidants such as ascorbate (AsA), glutathione (GSH), α-tocopherol, and carotenoids that help to detoxify the ROS. Some workers reported ROS production and alterations in the antioxidant system as a consequence of Al exposure. In pea seedlings, ROS production is detected in root apex after two hours of Al exposure and increased with time exposure [17]. In maize roots, Al treatment also led to increase in ROS production rate in all epidermal cells, only within 10min of Al exposure and continued to increase during Al exposure [53]. APX and SOD

activity increased in roots of both Al-resistant and Al sensitive triticale cultivars (with higher magnitude in the sensitive one), but changes were detected first in the sensitive cultivar (6 h) and then in the resistant (12 h) [91]. Boscolo et al. [87] reported for maize root tips an increase of SOD and APX activities. Furthermore, these authors found that SOD and APX activity is inversely proportional to root growth rate and, therefore, suggested that the increase of O_2^- and H_2O_2 production is related to Al toxicity.

An increase in SOD, APX, and GR activities was reported for green-gram seedlings, whereas a decrease in CAT activity and glutathione and ascorbate contents was also found at higher Al concentrations [92]. These authors justified the decrease in CAT activity due to the fact that this enzyme is photosensitive and, therefore, needs constant synthesis and suggested that glutathione and ascorbate may be able to detoxify the ROS directly [92]. Devi et al. [94] found an increase in manganese superoxide dismutase (MnSOD) activity in both sensitive and tolerant cell lines of tobacco and in AsA and GSH contents, mostly in the tolerant line. These data indicated that AsA and GSH seem to be in part responsible for the tolerance mechanisms of the tolerant line to Al. Activities of SOD, CAT, and APX also increased in roots of plants and in cultured tea cells exposed to Al [95]. However, plants of this species provide a complex scenario compared with other models, as aluminium may show a stimulatory effect on plant growth. That increase seemed to result in increased membrane integrity, since lipid peroxidation reduced with Al exposure [95].

These findings reporting increase of antioxidants (enzymatic and nonenzymatic) are accompanied with others that prove gene regulation associated with oxidative stress. For example, Ezaki et al. [96] expressed nine genes derived from *Arabidopsis*, tobacco, wheat, and yeast in *Arabidopsis* ecotype Landsberg. An *Arabidopsis* blue-copper-binding protein gene (*AtBCB*), a tobacco glutathione-S-transferase gene (*parB*), a tobacco peroxidase gene (*NtPox*), and a tobacco GDP-dissociation inhibitor gene (*NtGDI1*) conferred a degree of resistance to Al: significative differences in relative root growth and decrease in Al content and oxidative damages. They also showed that overexpression of three Al-induced genes in plants conferred oxidative stress resistance. Furthermore, overexpression of the *parB* gene simultaneously conferred resistance to both Al and oxidative stresses. Therefore, Ezaki and coworkers concluded that some of the genes induced during Al exposure and oxidative stresses play protective roles against both stresses. Cancado et al. [97] identified a maize Al-inducible cDNA encoding a glutathione-Stransferase (GST). Expression of that gene (GST27.2) was upregulated in response to various Al concentrations in both Al-tolerant and Al-sensitive maize lines.

Recently, using Alsensitive *Medicago truncatula* cultivar Jemalong genotype A17, 324 genes were upregulated and 267 genes were downregulated after Al exposure [98]. Upregulated genes were enriched in transcripts involved in cell-wall modification and abiotic and biotic stress responses, while down regulated genes were enriched in transcripts involved in primary metabolism, secondary metabolism, protein synthesis and processing, and the cell cycle. Known markers of Al-induced gene expression including genes associated with oxidative stress and cell wall stiffening were differentially regulated in that study [98]. For maize plants, Al exposure led to alteration in gene expression, mostly in the Al-sensitive genotype. Although Al-sensitive genotype showed changes in the expression of more genes, several Al-regulated genes exhibited higher expression in the tolerant genotype [99]. So, it is clear that expression of some genes confers Al resistance and contributes to reduce oxidative stress.

Effect on Plant Physiology and Morphology: Aluminium is one of the most abundant elements in the earth's crust, and toxic for many plants when the concentration is greater than 2–3 ppm with a soil pH < 5.5 [100]. A significant correlation between low pH and high Al concentration has also been shown in acidified freshwater, where this metal may reach levels of 0.3–1.6 mM [101] and cause serious metabolic derangement in some hydrophytes [102]. In general, young seedlings are more susceptible to Al than older plants [103]. So far as physiology is concerned, Al has been shown to interfere with cell division in plant roots; fix phosphorous in less available forms in the soil and in or on plant roots; decrease root respiration; interfere with certain enzymes governing the deposition of polysaccharides in cell walls; increase cell wall rigidity (cross-linking pectins) and interfere with the uptake, transport and with some essential nutrients (Ca, Mg, K, P) and water supply to plants [49,104,105] alters cell-wall Donnan free space [106, 107], the plasma membrane [108], membrane transport proteins [109] and regulates the activity of many enzymes

[110,111] and metabolic pathway for repair mechanism [112]. Trim [113] reported that Al is known to form strong complexes to precipitate nucleic acids. Soileau and Engelstad [114] and Soileau et al. [115] indicated that chemical factors were more important than physical factors in limiting cotton root growth in an acid (pH 4.4) fragile soil. Al becomes soluble or exchangeable and also toxic depending on the soil pH and many other factors including the predominant clay minerals, organic matter levels, concentrations of other cations, anions and total salts, and the plant species [116].

Dickson [101] reported that there was a significant correlation between low pH and high aluminium concentration in fresh water, and metal may reach levels of 0.3–1.6 mM. It also causes serious metabolic derangement in some hydrophytes [102]. Berggren and Fiskesjo [117] reported aluminium toxicity in *Allium cepa* with reference to root growth and morphology. Further, Severi [118] analyzed the aluminium toxicity in *Lemna minor* with reference to citrate and cytokinin metabolism. Physiological mechanisms due to Al toxicity have been focused on field crops and other herbaceous plants [77]. Plieth et al. [119] reported that low pH elevation in cytosolic calcium were inhibited by aluminium as a potential mechanism for aluminium toxicity. They observed that plant roots responded to external low pH by a sustained elevation in cytosolic free calcium concentration [Ca^{2+}] (C) in the presence of aluminium. They also suggested that a primary toxic effect of aluminium might impair calcium-mediated plant defense responses against low pH.

Cell Wall, Plasma Membrane and Nutrient Imbalances: Al accumulation is primarily and predominantly in the root apoplast (30–90% of the total absorbed Al) [91, 120] of peripheral cells and is only very slowly translocated to more central tissues [121,122]. The primary binding of Al^{3+} in the apoplast is probably the pectin matrix, with its negatively charged carboxylic groups [122,123]. Several workers reported increase of pectin levels in Al sensitive genotypes [122,123,124,125], and some also detected increase in Al content in the same sensitive genotypes [122,125]. These findings indicated that pectin plays a major role in the binding of Al and suggested that some of the additional Al accumulation in sensitive genotypes bound in the newly formed cell wall pectin [122,123]. Binding of Al to the pectin matrix and other cell wall constituents could alter cell wall characteristics and functions such as extensibility [126], porosity, and enzyme activities thus leading to inhibition of root growth [122]. Another mechanism for Al toxicity targeted to the apoplast invokes a rapid and irreversible displacement of Ca^{2+} from cell wall components by Al ions [126]. Accumulation of Al occurs predominantly in the root apoplast. Nevertheless, Al accumulates also in the symplast and with a fast rate [127]. Recently, Xia et al. [128] reported a transporter, *Nrat1* (Nramp aluminium transporter 1), specific for Al^{3+} localized at the plasma membrane of all rice root tips cells, except epidermal cells. These authors inferred that the elimination of the *Nrat1*enhanced Al sensitivity, decreased Al uptake, increased Al binding to cell wall and concluded that this transporter is required for prior step of final Al detoxification through sequestration of Al into vacuoles. Furthermore, given its physicochemical properties, Al can interact strongly with the negatively charged plasma membrane. For instance, Al can displace other cations (e.g., Ca^{2+}) that may form bridges between the phospholipid head groups of the membrane bilayer [129]. Furthermore, Al interaction with plasma membrane could lead to depolarization of the transmembranar potential [130] and/or reduction of H^+-ATPase [131] which, in turn, can alter the activities of ions near the plasma membrane surface and impede the formation and maintenance of the transmembrane H^+ gradient [2]. Moreover, Al changes in plasma membrane can modify the uptake of several cations (e.g., Ca^{2+},Mg^{2+}, K^+, NH_4^+) [132,133,134]. These changes are related to direct Al^{3+} interactions with plasma membrane ion channels [135] and changes in membrane potential.

Nutritional imbalances induced by Al exposure were reported for several plant species. Eleven families of pteridophytes presented different nutritional imbalances (mostly in Ca, Mg, P, K) depending on Al accumulation [136], and in maize, Al had negative effects on the uptake of macro and micronutrients, with Ca and Mg being the macro nutrients and Mn and Zn the micronutrients more affected [134]. Also, the maize Al-tolerant genotypes accumulated higher concentration of Ca, Mg [134], and K [137] than the sensitive genotypes. In wheat, both sensitive and tolerant genotypes presented a decrease in K and Mg contents in roots, whereas Ca, Al, Si contents increased [63]. However, the sensitive wheat genotype

showed more nutritional imbalances and Al accumulation than the tolerant one in both roots and shoots [63]. Al exposure led to an increase of Ca accumulation in rye-sensitive genotype, contrary to the tolerant rye genotype [64]. However, other studies reported different results in Al-induced nutritional imbalances in maize: Lidon et al. [138] inferred that all elements in roots, except K, Mn, and Zn, increased in Al-treated roots and that in shoots Ca and Mg had little variation. Poschenrieder C. et al. [133] reported that only the specific absorption rate of B was correlated to the Al induced root growth inhibition. Al exposure led to decrease in K, Mg, Ca, and P contents and uptake in rice plants, and, as observed in maize, the tolerant cultivar presented less negative effects in nutrient content than the sensitive one [139]. In tomato cultivars, Al exposure decreased the content of Ca, K, Mg, Mn, Fe, and Zn in roots, stems, and leaves [140]. Zobel et al. [141] related changes in fine root diameter with changes in concentration of some nutrients, as N, P, and Al. It seems that the differential tolerance to Al may be due to their differences in uptake, ability to keep adequate concentrations and to use the nutrients efficiently. Differences in nutrient uptake, accumulation, and translocation are evident between plant species and within each species. Furthermore, since each author utilized different Al concentrations, diverse nutritive solutions and time exposures, it is difficult to make a general and accurate model of Al-induced nutritional unbalances.

Others: Al-induced effects/damages are first detected in the root system [142,143]. Changes in the root system may affect nutrient uptake, which can lead to nutritional deficiencies in shoots and leaves [144]. Except for Al-accumulator plants, Al accumulates more in roots than in leaves [145]. In some species, Al-induced alterations in leaves were considered indirect, since Al accumulation was not detected in leaves [144]. Nevertheless, alterations in leaves induced by Al exposure were reported for many species. Several works reported leaves biomass reduction [146], thickness [145], lipid peroxidation [147], nutritional imbalances [148], changes in the photosynthetic performance [149], and changes in chlorophyll contents [146, 147, 149, 150], among others. Reductions in carbon dioxide (CO_2) assimilation rate due to Al toxicity are reported for several species [144, 149, 150], and some works indicated that Al exposure induced damage of the photosystem-II [147, 151]. Very few works focused on the consequence of Al treatment in the carbohydrate metabolism. The effects of Al exposure on Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) content and activity are still unclear, and the few reports available were performed in citrus [149, 150] and in wild rice [152].

Cytogenetic Effects of Aluminium

Al tolerant genes: The toxic effects of aluminium on plants first take place in the roots, and the mechanisms have been reported [153, 154, 74]. Al tolerance in certain barley populations is controlled by one major, dominant gene [155]. Al tolerance is controlled by a single gene in certain wheat populations [156]. Lorezeski and Ohm [157] reported the occurrence of different Al-tolerant genes in the two wheat cultivars IAS-58 and Norteno. Subsequently, Campbell and Lafever [158, 159] stated that Al tolerance in wheat was not simply inherited and that the expression of Al tolerance was additive with high values of heritability. Rhue et al. [160] reported that in the case of diploid *Zea mays*, Al tolerance is controlled at a single locus by a multiple allelic series. In diploid *Hordeum vulgare*, Al tolerance is controlled by a single dominant gene, located on chromosome-4 [161]. Al tolerance in barley, however, is expressed at a much lower level of Al concentration in the medium as compared to wheat, and it might be that only one subcellular compartment is involved in Al tolerance in barley [161].

Effect of Al on nuclear activity: Foy [49] reported that aluminum interfered with cell division in root tips and lateral roots, increased cell wall rigidity by cross-linking pectins, and reduced DNA replication by increasing the rigidity of the double helix. Minocha et al. [162] reported that the application of aluminium (0.2–1.0 mM) inhibited cell division and cell viability. They also reported that aluminium treatment resulted in a severe inhibition of DNA synthesis within 16 h–24 h. Matsumoto et al. [163] suggested that the binding of Al to DNA was a potential cause for inhibition of cell division. Bennet et al. [84] reported that nuclear changes were obtained with a low level of Al due to chromatin condensation of the nucleus

and an increase in size and frequency of vacuoles in the nucleoli. They also considered ultrastructural features as possible indicators of increased nuclear activity involving RNA synthesis [164]. Aluminium interfered in the function of the Golgi apparatus in the peripheral cap cells of intact roots and the quiescent centre [165,166] and in mitotic activity [167] and DNA synthesis [168]. Bennet et al. [169] reported significant alterations in cell volume of the root cap and disruption of golgi apparatus activity in the peripheral cap cells at the lowest Al concentration (0.5 mg/L). Aluminium treatment also resulted in a redistribution of amyloplasts to the proximal halves of central cap cells as well as alterations in the linear arrangement of these cells and rapid efflux of H+. Frantzios et al. [170] reported that Al affected the mechanisms controlling the organization of the microtubule cytoskeleton, as well as tubulin polymerization and which induced the delay of the microtubule disassembly during mitosis, resulting in the persistence of preprophase microtubule bands in the late prophase cells and a disturbance in the shortening of kinetochore microtubule bundles in anaphase cells. They also indicated that Al affected the disorder of chromosome movements carried out by the mitotic spindle. After prolonged Al treatments chromatin condensation was inhibited. The microtubule cytoskeleton was a target site of Al toxicity in mitotic root-tip cells of *Triticum turgidum* as observed by Frantzios et al. [170].

Effect of Aluminium on Metabolism: In general, many plant species are resistant or can be tolerant to certain amounts of metals. This is probably achieved through trapping of these metals with metal-binding proteins. Many of the biochemical effects of Al on plants are probably associated with the alteration of root membrane structure and function [171]. Plant membranes are visualized as arrangements of semi fluid proteins and lipids. Aluminium can bind either proteins or lipids, depending on pH and other conditions. Vierstra and Haug [172] found that Al decreased lipid fluidity in membranes of *Termoplasma acidophilium.* Gomez-Lepe et al. [173] found Al in the cell membrane proteins on the inner epidermal cells of onion. Foy and Fleming [50] reported that chlorosis seemed to be due to Al-induced interference in the uptake and/or use of iron, copper and potassium. Under Al stress in nutrient solution, the Al-sensitive cultivar was characterized by chlorosis, decreased Fe concentrations in tops, decreased Ca and Mg in both shoots and roots, a tendency towards accumulation of P, Al and Fe in roots, and reduced Mn in tops. Gallagher et al. [174] noted that nitrate reductase activity was higher in Al tolerant cultivars grown in nutrient solution having aluminium. Al toxicity was also closely related to nitrogen metabolism [50]. Aluminium (100 mM) was found to inhibit the influx of the cations of calcium (69%), ammonium (40%) and potassium (13%) and enhance the influx of the anions of nitrate (44%) and phosphate (17%). Aluminium interfered with the binding of the cations in the cell wall by the same order of magnitude as their respective influxes whereas phosphate binding was strongly enhanced [175]. They also reported that aluminium was bound to the plasma membrane phospholipids, forming a positively charged layer that influenced ion movement to the binding sites of the transport proteins.

Huang et al. [176] suggested that Al^{3+} induced inhibition of ion fluxes, particularly Ca^{2+} which played an important role in mechanisms of Al^{3+} toxicity due to binding of cations or screening of the negative charges on the plasma membrane, thus reducing the activity of Al^{3+} close to the cell surface. Ryan et al. [177] showed that only the meristem was sensitive to Al^{3+}. Miyasaka et al. [178] found that there was a net K^+ efflux and H^+ influx at the root apex (first 1 cm), whereas in the rest of the root these fluxes were reversed. In general, aluminium adversely affected several physiological activities producing a severe physiological stress which increased peroxidase activity [179]. Increased peroxidase activity might be linked to a decreased growth rate, as found in plants after treatment with aluminium [180]. Aluminium effectively interfered with the metabolism of cell wall polysaccharides and calcium-producing fissures in the case of *Lemna minor* [181, 182]. Severi [118] reported that the presence of aluminium had the tendency to decrease the multiplication rate of *Lemna minor* L. with significantly increased guaiacol peroxidase activity. Schier and McQuattie [183] compared the application of nitrogen to Al toxicity in non-mycorrhizal and ectomycorrhizal pitch pine (*Pinus rigida* Mill) seedlings. They observed that the application of nitrate or ammonium had no significant effect to Al toxicity in non-mycorrhizal seedlings. Symptoms like thick and stunted roots of ectomycorrhizal pitch pine seedlings were obtained at ambient N

levels due to Al toxicity. Al toxicity at ambient ammonium– N was reduced by elevating the level of NO_3–N or NH_4–N.

Mechanism of Al Toxicity: Al interferes with a wide range of physical and cellular processes. Potentially Al toxicity could result from complex Al interactions with apoplastic, plasma membrane and symplastic targets. According to the literature it is difficult to give a definite time for Al toxicity, because some Al-toxic symptoms and responses are detectable within seconds to minutes after exposure to Al, others are only noticeable after long-term exposure [2].

Aluminum toxicity symptoms: The most evident symptom of Al toxicity is root growth inhibition, which can be detected within 30 min. to 2 hours, even at micromolar concentrations of Al [143]. Although the seed germination is not affected by Al, root and seedling development are reduced [184]. Cells which are affected by Al are the root apex (root cap, meristem and elongation zone), more specifically the distal part of the transition zone within apex, root hairs and branch initials [185]. The root apex accumulates more Al within minutes and plays a major role in the Al-perception mechanism [10]. Inhibition of root growth is considered to be primarily the result of inhibited cell elongation and expansion, prior to inhibiting cell division [186]. Prolonged exposures lead to Al interactions with the root cell division and the cytoskeleton [11]. Much of the Al absorbed by roots penetrate root apex and root cap. According to Rout *et al., 2001* [23] some Al passed through the epidermis and cortex, but considerable amounts were retained in cortical cells. Although a large fraction of the Al interacts with apoplastic targets, a small fraction enters the symplasm and interacts with symplastic targets. Severity of Al toxicity depends on the concentrations of Ca^{2+} and other cations in the external solution, the ionic strength of solutions, pH, the presence of chelators, cell type and plant genotype [187].
Al toxicity is associated with severe changes in root morphology. Briefly, it results in curved, swollen, cracked, brownish, stubby and stiff root apices [188]. Fine branching and root hairs are reduced. Uneven and radial expansion of cells of the cortex cause root thickening and mechanical stress on the epidermis [186]. This extensive root damage results in a reduced and damaged root system and limited water and mineral nutrient uptake [143]. Although symptoms of Al toxicity are also manifested in the shoots, these are usually regarded as a result of root damage. The most common responses in shoots to Al toxicity are cellular modifications in leaves, reduced stomatal opening, decreased photosynthetic activity, chlorosis and foliar necrosis [8]. Long-term exposure to Al and inhibition of root growth generally lead to P, K, Ca and Mg deficiencies [189]. The ultimate consequence is reduced plant biomass. With the exception of Al-accumulating plants little Al is transported into the shoot [190]. Researchers have regarded the cell wall, plasma membrane, signal-transduction pathways, root cytoskeleton and DNA/nuclei as potential Al targets which are associated with root growth.

Cell wall: X-ray microanalysis and secondary ion mass spectroanalysis have indicated that a significant fraction of Al is associated with apoplastic binding sites in walls of the root periphery cells [191]. The net negative charge of the cell wall determines its cation exchange capacity (CEC), and consequently the degree of interaction of Al with the cell wall. Among the many components of the cell-wall network, pectins have been proposed to be a critical site for Al-cell wall interactions [192]. Al interactions lead to the displacement of other cations (e.g. Ca^{2+}) fundamental for cell-wall stability and rapid callose synthesis on plasma membrane which incorporate into apoplasm [193].
It is proposed that the accumulation of Al in the cell wall exerts a detrimental effect on root growth and function in three ways. First the decrease in apoplastic sorption of basic cations, which have limited ability to displace bound Al, reduces nutrient acquisition per unit root length. Second the Al sorbed in the cell wall reduces cell expansion, thus reducing root elongation. This would also reduce nutrient uptake through decreased root proliferation through the soil. Third, sorption of Al in the cell wall reduces the movement of water and solutes through the apoplasm, directly decreasing nutrient acquisition by the root [194]. Consequently, the strong and rapid binding of Al can alter cell-wall structural and mechanical properties,

making it more rigid, leading to a reduction in the mechanical extensibility of the cell wall required for normal cell expansion in the root elongating zone particularly [2].

The plasma membrane: Negatively charged plasma-membrane surface is the first potential target for Al^{3+} [195]. As Al has more than 560-fold greater affinity for the choline head of phosphatidylcholine than other cations such as Ca^{2+} have, Al^{3+} can displace other cations that may form positively charged bridges between the phospholipid head groups of the membrane bilayer [196]. A positively charged layer would retard the movement of cations and increase the movement of anions to the transport proteins of the plasma membrane in proportion to the charges carried by these ions [132]. As a consequence, the phospholipid fluidity and the charges of the plasma membrane are altered. Thus, Al interactions at the plasma membrane can modify the structure of the plasma membrane as well as the ionic environment near the surface of the cell; both can lead to disturbances of ion-transport processes, which can perturb cellular homeostasis [2].
One of the early symptoms of Al toxicity is quickly activated callose (β-1,3-glucane) accumulation in the apoplast [197]. Since callose synthesis depends on the presence of Ca^{2+}, it has been suggested that Al displacement of Ca^{2+} from the membrane surface may increase the apoplastic Ca^{2+} pool required to stimulate callose synthesis. Under Al stress, callose accumulation may lead to further cellular damage by inhibiting intercellular transport through plasmodesmatal connections [65]. Al can significantly inhibit the activity of the plasma membrane H^+-ATPase, impending formation and maintenance of the trans-membrane H^+ gradient. Consequently, Al disruption of the H+ gradient could indirectly alter the ionic status and ion homeostasis of root cells [2]. Electrophysiological approaches were subsequently used to demonstrate that Al^{3+} interacts directly with several different plasma-membrane channel proteins, blocking the uptake of ions such as Ca^{2+}, K^+,Mg^{2+} and NH^+_4 [198]. In addition to directly altering ion permeation through channels, extracellular Al can also modulate the transporter's activity via changes in the membrane potential. For example, Al induced membrane depolarizations can alter voltage dependent Ca^{2+} channel transport by indirectly modulating and shifting the activation thresholds of distinct transport pathways, such as hyperpolarization-activated [199] and depolarization-activated [198] Ca^{2+} channels.

Al effects on signal-transduction pathways: Al interactions with signal-transduction pathways, in particular disruption of intracellular Ca^{2+} and pH homeostasis, have been proposed to play crucial roles in Al toxicity [200]. Al can also interact with and inhibit the enzyme phospholipase C of the phosphoinositide pathway associated with Ca^{2+} signalling [201]. Guanine nucleotide-binding proteins (G proteins) and a phosphatidylinositol-4,5-diphosphate (PIP2)-specific phospholypase C are probable interaction sites for Al ions. Following interiorization of Al by the cell, metal interactions decrease the accumulation of inositol phosphate, especially that of inositol-1,4,5- triphosphate (IP3), concomitant with disorders of intracellular Ca homeostasis [202]. These alterations would ultimately reflect in any of the physiological and morphological changes described above. Al also may play a role in the regulation of protein phosphorylation and/or dephosphorylation [10].
Reactive oxygen species (ROS) such as superoxide anions and hydrogen peroxide that result from photosynthesis and oxidative metabolism can be involved in a number of stress responses [203]. It has been shown that Al exposure is associated with peroxidative damage of membrane lipids due to the stress-related increase in the production of highly toxic oxygen free radicals [180]. Phosphatidylserine is the most susceptible substrate for Al to facilitate lipid peroxidation [204]. A close relationship existed between lipid peroxidation and inhibition of root elongation rate. Enhanced lipid peroxidation by oxygen free radicals is a consequence of the primary effects of Al on membrane structure [180] but Al-induced lipid peroxidation does not occur rapidly enough to be an initial mechanism of Al toxicity [86].

The root cytoskeleton: The cytoskeleton is the potential cytosolic target for Al toxicity, because of the central importance of cytoskeletal components in cell division and expansion of a growing root. Al could disrupt cytoskeletal dynamics either via a direct interaction with cytoskeletal elements (i.e. microtubules and actin filaments) or indirectly, via alteration of signaling cascades such as cytosolic Ca^{2+} levels that are

involved in cytoskeletal stabilization. Plant cells require dynamic cytoskeleton-based networks both for cell division and cell-wall biosynthesis [18]. It has been well documented that Al exposure inhibited longitudinal cell expansion and induced lateral cell swelling by disrupting both the organization of microtubules and microfilaments in elongation zone cells of root [65]. For example, exposure to Al results in the disruption and reorganization of cortical microtubules [205]. The disintegration of spindle microtubules and disorganization of phragmoplasts caused by Al might block cell division directly at metaphase under Al stress [18]. Likewise, Al induced a significant increase in the tension of the actin filaments of soybean (*Glycine max*) cells. This may result from the formation of nonhydrolyzable [Al^{3+}-ADP] or [Al^{3+}-ATP] complexes whose binding to actin/myosin. Al^{3+} can bind to nucleoside triphosphates approximately 107 times better than Mg^{2+} and the rate of hydrolysis for Al^{3+}-ATP or Al^{3+}-GTP complexes is considerably lower than that for the physiological Mg^{2+} complex (105 times slower), supporting the hypothesis that toxicity is a result of Al^{3+} displacement of Mg^{2+} from nucleoside di- or triphosphate complexes. Such Al-induced cellular structural changes are likely to result in and underlie the morphological changes and structural malformations observed in Al-stressed roots [206].

DNA/nuclei: Prolonged exposures can lead to Al interactions with structures within the nucleus, detrimentally affecting DNA composition, DNA replication by increasing rigidity on the double helix and chromatin structure [11]. Al can bind to nucleoside triphosphates with an association constant 107 times that of Mg2+ [206]. Therefore, Al prefers binding to DNA compared to histone and nonhistone proteins. The binding to DNA was inhibited by 70 % in the presence of an equal amount of histone to DNA [207]. Al affected the mechanisms controlling the organization of the microtubular cytoskeleton, as well as tubulin polymerization which induced the delay of the microtubule disassembly during mitosis, resulting in the persistence of preprophase microtubule bands in the late prophase cells and a disturbance in the shortening of kinetochore microtubule bundles in anaphase cells. Al also affected the disorder of chromosome movements carried out by the mitotic spindle [170]. Nuclear changes were observed in nucleoli [84]. These types of increase in size and frequency of vacuoles in interactions of Al with the nucleus can result in the disruption of the cytoskeleton and cell division processes.

Mechanism of Al Resistance: Because of the agronomic importance, breeding crops with Al resistance has been a successful and active area of research; however, the underlying molecular, genetic and physiological principles are still not well understood. Despite the interest from many researchers, no Al resistance genes have yet been cloned from any plant [2]. It has been known that plants which exist in the presence of potentially toxic Al concentrations must be able to avoid direct contact of vital structures and metabolic processes with high activities of Al^{3+} ions. The physiological mechanisms of Al resistance can either be mediated via exclusion of Al from the root apex or via intracellular tolerance of Al transported into the plant symplasm [2]. Either extracellular precipitation or detoxification of Al^{3+} may be implied in exclusion.

Aluminum exclusion: Aluminum tends to form strong complexes with oxygen donor ligands. Large experimental evidences have shown that complexation with chelating root exudates or binding to mucilage play a main role in the prevention of the accumulation of phytotoxic Al in both apoplast and symplast [143]. In plants, Al makes complexes with phosphate and carboxylates secreted from the root apex, but strong complexes can also be formed with phenolic substances, pectates, mucopolysaccharides or siderophores [208].

Aluminum exclusion via root carboxylate exudation: Al chelation by carboxylate exudates reduces the activity of free Al ions and consequently, their binding to the root cell wall and/or plasma membrane. The kinds of carboxylates secreted by Al-exposed roots vary depending on the Al tolerant plant species, but secretion of citrate and malate are the most commonly cited ones. Malate exudation mechanism by wheat has been investigated most thoroughly [4] while citrate seems to be the most common organic acid anion exudated by Al-tolerant maize and snapbean [143]. In all three species, secretion was greater (up to 10-

fold) in Al-resistant cultivars than in Al-sensitive ones. Oxalate exudation in response to Al has also been observed in maize, but no differences between sensitive and tolerant varieties were detected [209]. Among the organic acid anions, the most potent chelator of Al^{3+} is citrate which can be synthesized in a large amount through photosynthesis [210]. After chelation, Al-citrate transport through the plasmalemma seems to be very slow [4]. In long-term studies an Al-resistant cultivar of snap bean excreted 8-fold more citrate from the roots than did an Al-sensitive genotype [211]. This is supported by the observations in wheat that Al-resistant genotypes release malate and accumulate significantly less Al in the first few millimeters of root apex compared with Al sensitive genotypes [212]. Related to these studies, high levels of Al-activated release of carboxylates have been correlated with Al-resistance in a large number of plant species. Some of the major aspects of this resistance mechanism include:

• A correlation between Al resistance and Al activated carboxylate release in many plant species [2].
• Al-carboxylate complexes are not transported into roots or across membranes [196].
• Activation of carboxylate release is triggered specifically by exogenous Al^{3+} [213].
• The rates of Al-activated carboxylate release are dose-dependent on the Al activity in the rhizosphere [214, 216].
• In some cases, overexpressions of genes encoding enzymes involved in organic acid synthesis, such as citrate synthase and malate dehydrogenase can result in enhanced Al resistance [215].
• An Al-gated anion channel in maize and wheat root tip protoplasts has been identified via electrophysiological experiments and exhibits the properties necessary for it to be the transporter mediating Al-activated carboxylate release [216].

The Al-citrate 1:1 complex is not phytotoxic [4]. At a 1:1 ratio the Al-oxalate complex also had little toxic effects in Al-sensitive wheat and the complex prevented Al accumulation in the root tip [217]. In contrast, Al-malate treated roots stained for Al (i.e. Al was taken up) and root elongation was inhibited, but Al-malate was less toxic than $AlCl_3$.

This graduation of efficiency of organic acid anions in preventing Al toxicity and uptake is in good agreement with the stability constants [143]. The interesting contradiction of this mechanism is whether it is inducible at the level of gene expression. An Al-inducible resistance mechanism is seen in some plant species such as rye, triticale and *Cassia tora*. In these species, the rate of exudation increases over the first 12-24 hours of Al exposure. This means Al activated carboxylate exudation increases slowly [218]. However, root malate exudation is very rapidly activated by Al exposure in wheat and the rate of malate efflux does not increase over time. Therefore, in species like wheat, Al apparently activates an already expressed carboxylate transporter and gene activation does not seem to play a role. In species where the rate of carboxylate exudation apparently increases with time, it is possible that induction of Al-resistance genes contributes to this increased capacity [2].

In many plant species, exudation of specific carboxylate anions is activated by Al exposure rapidly. Thus, an important part of this Al-resistance mechanism is the activation of a particular carboxylate transporter that presumably exists in the root cell plasma membrane [2]. In wheat, Al activates malate release almost instantly and increased carboxylate snythesis is not involved [219]. Even though Al exposure activates a large and continuous efflux of malate in the Al-resistant genotype, no differences in root tip malate concentration or in malate dehydrogenase activity in Al-resistant to sensitive genotypes have been observed [213]. The thermodynamic conditions for carboxylate transport from the cytosol to the external solution suggest that ion channels could be the primary transporter involved in this resistance response. The organic acids in the cytosol exist primarily as anions ($malate^{2-}$ and $citrate^{3-}$) and due to the large negative inside transmembrane electrical $citrate^{3-}$ potential in plant cells, there is a very strong gradient directed out of the cell for anions [2]. Thus, an anion channel that opens upon exposure to Al would be sufficient to mediate this transport. Anion channels that are specifically activated by extracellular Al^{3+} have recently been identified using the patch-clamp technique with protoplasts isolated from root tips of Al-resistant wheat [220] and maize [221, 216]. In maize, the most important discovery was that the anion channel could be activated in isolated plasma-mambrane patches, where the anion channel is operating in isolation from cytosolic factors [135, 216].

These features needed for Al activation of the anion channel are contained within the channel protein itself, or are close by in the membrane (e.g., an associated membrane receptor). There are three possible ways that Al could activate a plasma membrane anion channel involved in carboxylate exudation:
1- Al interacts directly with the channel protein, causing a change in conformation and increasing its mean open time or conductance;
2- Al interacts with a specific receptor on the membrane surface or with membrane itself, which through a series of secondary messages in the cytoplasm, changes channel activity; or
3- Al enters the cytoplasm and alters channel activity either directly binding with the channel or indirectly through a signal transduction pathway [10, 2].

Phenolic compounds: Several comparative studies including different species showed that there were no correlation between Al resistance and the amount of organic efflux [222]. These results support that exudation of organic acids may not be the only mechanism of Al exclusion. Root exudation of phenolic compounds has been described by many authors [223]. Phenolics can reverse the toxic effects of Al on hexokinase [224] and on root elongation [225]. However, they are less efficient at equimolar concentrations than citrate in complexing Al [226]. Thus, phenolics in complex formation with Al have got much less consideration than organic acid anions. However, by a deprotonation reaction, the phenolics in presence of carboxylic groups from organic acids can strenghen the interaction between Al^{3+} and the organic acid anion ligand, increasing the effective stability constant for the Al-organic acid anion complex [127]. It has also been argued that phenolics may favor Al binding by organic acid anions by inhibiting rhizosphere microorganisms that degrade organic acids. Recent investigations revealed that Al induced exudation of the flavonoid type phenolics catechin and quercetin from 10 mm root tips in an Al resistant maize variety [209]. Stimulation of exudation of these flavonoid-type phenolics was in good agreement with protection of root elongation against Al. Investigations on a larger number of maize varieties and other species are required in order to see if this exudation of flavonoid-type phenolics is a particularity of certain Al-resistant maize varieties or a common property of a larger group of Al resistant species [143].

Rhizodepositions: The meristem and root cap where Al toxicity appeared dominantly are coated with mucilage. Mucilage consists of enormous molecules which are glucose, galactose, arabinose and uronic acids [10]. Mucilage and border cells have been implicated in Al resistance mechanisms [228]. Higher mucilage production was observed in the Al resistant wheat cultivar Atlas 66 than in a sensitive cultivar [229]. Mucilage blocks the entry of Al into the root by bounding it in rhizosphere. Archambault *et al.*, (1996) found that Al bound to the mucilage of wheat root accounted for approximately 25-35% of Al remaining after desorption in citric acid. In snapbean cultivars higher Al resistance was related to better border cell viability and to higher mucilage production by the border cells of the Al resistant cultivar [230]. According to Delisle *et al.*, 2001 [231], at equal effect concentrations early cell death is rapidly seen in the Al resistant wheat cultivar, but not in the Al sensitive one. This early cell death response differs from the formation of the detached living border cells found in Al resistant snap beans. This limited cell death seemed to contribute to Al resistance and cannot be attributed to the oxalate-mediated H_2O_2 burst occuring later as a second wave response that may be implied in Al trapping in the cell wall. This early death response in the Al resistant wheat was limited to a few cells in the elongation zone and showed similarities to the hypersensitive response of tolerant plants to potential pathogens [143].
Internal aluminum detoxification: Although exclusion from root tips and restriction of Al transport to upper plant parts seems to be the most important mechanism in Al resistance, there are numerous species that tolerate relatively high Al concentrations that is based on the complexation and detoxification of Al after its entry the plant. This discovery has come from research on plants that can accumulate Al to high levels in the shoot. High shoot accumulation of Al with ligands in an innocuous form (soluble or solid) occurs in leaf vacuoles or in the apoplast. Among the ligands that form stable complexes with Al, organic acid anions, phenolic substances and silicon may be implied in Al detoxification inside shoot tissues [143].
High citrate concentrations have been reported in *Hydrangea macrophylla* leaves whose sepals turn from red to blue due to Al accumulation in the sepals when the soil is acidified [232]. It can accumulate more

than 3000µg/gm Al dry weight in its leaves [233]. Identification of Al chelates by 27Al NMR indicates that Al is complexed in a 1:1 Alcitrate complex in leaves. Citrate should bind Al very tightly in the cytosol with a pH of around 7 and protect the cytosol against Al injury. Ma and colleagues, 1997b [234] also studied a second Al accumulator, buckwheat (*Fagopyrum esculentum*) whose Al resistance due to Al-activated oxalate exudation from the root apex [235]. However buckwheat also accumulates Al to very high levels in its leaves, as high as 15,000 µg Al/gm dry weight when the plant is grown on acid soils. Most of the Al in both roots and leaves was complexed in a 1:3 Al-oxalate complex. Subsequently, it has been proposed that Al is transported in the xylem sap complexed with citrate, while oxalate would be the storage form of Al in leaf vacuoles [236]. These findings suggest that the Al undergoes a ligand exchange from oxalate to citrate when it is transported into the xylem, and is exchanged back with oxalate in the leaves. Leaf compartmental analysis showed that 80 % of the Al in buckwheat leaves was stored in vacuoles as a 1:3 Al oxalate complex [237]. The internal detoxification mechanism can involve Al chelation in the cytosol and subsequent storage of the Al-carboxylate complex in the vacuole. The tonoplast-localized mechanisms mediating the transport of Al into vacuole, as well as the nature of its substrate (i.e., free Al versus Al-carboxylate complexes) remain unknown. More recently, barley plants transformed with a gene (*ALMT1*) encoding a putative malate transporter were found to be more resistant to Al [238]. This perhaps makes more sense, given that this could increase exudation without necessarily changing cytoplasmic metabolite concentrations [8]

Interaction of Al with Other Ions: The mutual interactions of metals are very important for plant growth and development and determine the availability of metal ions under different soil conditions, such as pH or redox potential. Al toxicity is a complex event which may be manifested as a deficiency of P, Ca, Mg or Fe [239]. Solubility of Al can be increased or decreased depending on the presence of other elements in the soil-plant system.

Calcium transport into root is more intensive at the root apex, which is also the primary site of Al accumulation and toxicity [224]. The interactions between Al and Ca are probably the most important factors affecting Ca uptake and transport in plants grown in acid soils (pH < 5.5). With increased Al levels Ca concentration in shoots and roots in wheat decreased dramatically [240]. Mossor-Pietraszewska 2001 indicates that Ca and Mg accumulation in plants is depressed by Al much more significantly than the uptake of other important mineral nutrients [241]. Possibly, this is due to the Al-induced alteration in the properties and architecture of the membrane lipid bilayer. Thus, the Al inhibition of Ca^{2+} transport may be involved in the initial phase of Al toxicity. Al either inhibits Ca^{2+} transport into the symplasm of root cells, or displaces Ca^{2+} from the critical metabolic sites in the apoplasm. It is known that Al^{3+} can effectively inhibit Ca^{2+} transport into roots, algal cells, protoplasts, and membrane vesicles [242], e.g. by blocking Ca^{2+} and K^{+} channels. In many plants Al tolerance appears to be closely associated with phosphorus-use-efficiency. Al markedly increases the redox potential of root tissues, decreases the contents of high energy bond P, and increases contents of mineral P in the roots. Al binding by organic acids prevents the formation of P–Al complexes, which results in an increased availability of P in the root cell. Therefore, Al-tolerant plants have a lower demand for P. The concentrations of soluble Al and Mn frequently reach phytotoxic levels in acid soils.

Taylor *et al.,* 1998 [243] have examined the effect of combinations of Al and Mn on growth and metal accumulation in cowpea. Low concentration of Al in solution (1 to 8 µM) had little effect on Mn accumulation in roots and shoots, while higher concentrations (up to 100 µM) decreased the accumulation of Mn in shoots. Similarly, low concentration of Mn (0.1 to 6 µM) had little effect on Al accumulation, while higher concentrations (up to 50 _M) increased the accumulation of Al in both roots and shoots. The objective of this research was to investigate the combined effects of Al and Mn, when both are supplied at low concentrations under conditions of low ionic strength as found in soil solution of acid soils. In contrast with previous reports, evidence for antagonistic, synergistic, and multiplicative effects of Al and Mn on growth, metal uptake, and expression of foliar symptoms was obtained by Taylor *et al.*, 1998 [243] under physiologically and environmentally relevant conditions. Their data demonstrates that the effects of toxic metals cannot be considered in isolation. Symptoms of boron deficiency and Al toxicity are very similar

and generally associated with impaired membrane function and root growth [244]. LeNoble *et al.,* 1996 [245] reported that supplement of B protects against Al inhibition of root growth. Protection was apparent at all levels of organization examined: primary root and lateral root lengths, primary root cell elongation, cell production rate, tissue organization and cell structure, primary root morphology and maturation. Protection against Al inhibition was also apparent for shoot growth. Silicon can ameliorate Al toxicity in plants under some conditions and in a variety of species. Explanations for the mechanism of Al detoxification by Si are controversial: a Si-induced increase in pH of soil solution, reduced bioavailability of Al *via* the formation of aluminosilicate species in the external growth media bathing the roots, or an internal *in planta* detoxification mechanisms [246].

Al Resistance: It has been known for a long time that many plant species, including crop plants, show wide variability with respect to their resistance to Al, and this has been exploited to obtain Al-resistant varieties. The understanding of the mechanisms and genetics of Al resistance has advanced considerably over the last decade and traditional screening and breeding programs have resulted in considerable success over the years. Because of the large areas of acid soils and the importance of this constraint in Brazil, the collection of acid-soil resistant Brazilian plant varieties are among some of the best in the world.
One problem, however, is how to evaluate Al resistance. The most widely used methods use relative root growth in Al at low pH compared to growth in low pH alone. However, as discussed above, there are several problems associated with this approach [247]. Aluminum resistance studies should take the detrimental effect of acidity into consideration and appropriate controls designed. Screening methods for identifying Al-resistant plants and their inherent limitations were reviewed by Samac and Tesfaye, 2003 [248]. They pointed out that slower growing plants tend to be selected as Al-resistant [249], which is consistent with the previously mentioned link between cellular sensitivity to Al and rate of growth.
These observations raise questions as to the correlation between adaptation to soils with high levels of Al and screening for Al resistance in nutrient solutions. Thus, knowledge on the mechanisms of Al toxicity is also important because it can contribute to the development of more accurate screening procedures via improved criteria for the determination of resistance and also for the development of Al-resistant plants.

Genetics and inheritance of Al resistance: The inheritance and genetics of Al resistance has been examined mostly in cereals of the Triticeae. From these studies it was found that in some species, such as in wheat or rye, that Al resistance is determined by one or a few genes [250], whereas in other species such as rice or maize it is multigenic and quantitative [251]. However, there is an increasing awareness that Al resistance is more likely a multigenic trait. Until recently, no Al-resistance gene had been cloned. However, Sasaki et al., 2004 [252] cloned a gene with properties of an Al-induced channel and subsequently transformed barley and obtained high levels of resistance [253]. This may be the first Al resistance gene to have been cloned.

Cellular mechanisms of Al resistance: The mechanisms for resistance, like those for toxicity, are not entirely known, but at least one mechanism, the secretion of organic acids, is now reasonably well established and understood [251]. Good evidence for this mechanism initially came from three independent groups that demonstrated that malate-secretion is enhanced in Al-resistant cultivars as compared to Al-sensitive ones [245, 255].
Several studies have attempted to express either citrate synthase or malate dehydrogenase, with the intended purpose of increasing organic acid exudation [256, 257], or general stress genes [258]. However, the increases in Al resistance have been modest or results have not been reproducible [259]. The latter is not entirely surprising given that it would not be expected that a single enzyme should change the levels of highly regulated metabolites such as organic acids. From this, it follows that if Al resistance is indeed determined by organic acid exudation then it is not surprising that multiple genes are involved.
More recently, barley plants transformed with a gene (*ALMT1*) encoding a putative malate transporter were found to be more resistant to Al [253]. This perhaps makes more sense, given that this could increase exudation without necessarily changing cytoplasmic metabolite concentrations. However, the results

presented above must be contrasted with the fact that transformation of plants with several different general stress genes can also confer Al resistance to these plants, some of which have no obvious relation to any mechanism of Al resistance [257, 258]. One fact that must be looked at carefully is the relation between rate of cell growth and Al toxicity and the fact that most if not all of these genes could be expected to affect cell growth rates since organic acids are important in metabolism

Despite the large number of studies in support of an organic acid mechanism of resistance, this issue is probably far from over. There are several observations that do not fit the model. The most important is that several Al-sensitive plants have high levels of organic acid secretion [251]. Rice plants also did not show increased Al resistance, despite increased organic acid efflux [252]. How good is the correlation between organic acid exudation and resistance to Al? How well has it been quantified? These are important questions which have been discussed by Mariano and Keltjens, 2003 [260]. There is of course, the case in which there are Al-resistant plants which do not show enhanced organic acid exudation, such as Signal grass (*Brachiaria decumbens*) for example [261]. Similar results have also been found in some soybean cultivars [262]. Therefore, there is clearly evidence for the existence of other resistance mechanisms also.

Unfortunately, the mechanisms of Al resistance in species native to acid soils are much less studied. Such species are commonly divided into Al excluders and accumulators. The accumulation of Al, and thus, internal mechanisms of resistance have received more attention [263]. Exclusion mechanisms of plants native to acid-soil regions are largely unknown, although CIAT is undertaking an effort to examine this in *Brachiaria*species [264].

Al Uptake and Transport: Although aluminium is not recognized as an essential element for plant growth, it may, nevertheless, fulfill some fundamental role in the physiology of plants adapted to acid environments with a high concentration of soluble Al [265]. Some plants have the ability to accumulate enormous amounts of Al in their foliage without any evidence of injury or toxicity. Jackson [266] concluded that correlations between Al contents in the foliage of crop plants and Al toxicity were more the exception than the rule. He also stated that toxic effects of Al may result from excess Al in the growth medium with little or no change in the Al contents in the foliage.

Aluminium accumulation in tolerant plants: Aluminium-tolerant plants may be grouped according to Al accumulates within their tissues [77]. In one group, Al concentrations in the shoots are not consistently different from those of Al-sensitive plants, but in the root Al concentrations are lower in certain tolerant cultivars of wheat, barley, soybean and pea [267]. In such cases, Al tolerance apparently involves an exclusion mechanism. In a second group of plants, Al tolerance is associated with less Al in plant shoots, entrapment of more Al in roots or both in wheat, barley and potato [77] and grass and cabbage [268]. In a third group, Al tolerance is directly associated with Al accumulation by the tops; such plants have high internal tolerance to Al particularly pine trees, tea and mangroves [77].

Aluminium uptake at root level: Henning [269] reported that much of the Al absorbed by wheat roots penetrated the boundary between root apex and root cap and accumulated in the nuclei and cytoplasm of cells adjacent to this zone. Some Al passed through the epidermis and cortex, but considerable amounts were retained in cortical cells. Although the endodermis seemed to prevent movement of Al into the central cylinder, he suggested that some Al might have bypassed the epidermis by entering the root apex and passing through meristematic cells of the central cylinder. Wallace and Rommey [270] reported that threshold concentrations of Al toxicity were 30 mg/kg in soybean leaves and 20 mg/kg in rice roots. Malavolta et al. [271] stated that Al toxicity in sorghum was associated with 640 mg/kg of Al in lower leaves and 1220 mg/kg in upper leaves.

Duncan [272] found that sorghum genotypes were tolerant to low soil pH (and probably Al), and contained lower concentrations of Al, Fe and Mn than those that were more sensitive. Wagatsuma [273] reported the mechanism of Al uptake by plant roots in relation to non-metabolic conditions. Under normal conditions, Al was absorbed in an exchangeable manner at almost all the Ca existing sites on the cell walls of roots. The metabolic inhibitors like chloroform gas and 2,4-dinitrophenol (DNP) increased the Al uptake by roots

significantly. Further, Wagatsuma [274] also noted the characterization of absorption sites for aluminium in the roots of *Cucurbita pepo, Vicia faba, Glycine max, Lycopersicon esculentum* and *Pisum sativum*. Among the plant species, Al content in the roots was positively correlated with the cation exchange capacity (CEC) of the dry root powder. Al content of the dry root powder was considerably higher than that of the excised roots which were treated with Al. He also indicated that in most of the cases, Al was bound to the pectic substances in the cell walls but a part of Al entered the protoplast and combined with nucleic acids and acid soluble phosphates. A higher concentration of Al was found in nuclei and other cell compartments of root tissue in tolerant wheat genotypes than in sensitive genotypes, and tolerant plants survived accumulating higher Al in cellular components than sensitive genotypes of *Cucurbita pepo, Vicia faba, Glycine max, Lycopersicon esculentum* and *Pisum sativum* [275, 276].

Aluminium and nutrient uptake: Bennet et al. [165] noted the aluminium toxicity in *Zea mays* and observed nutrient disorders involving the uptake and transport of P, K, Ca and Mg. Phosphorous transport between roots and shoots diminished with increased Al concentration in roots. Aluminium changed the Ca and Mg concentrations in plants which were primarily connected in the uptake and transportation. The positive correlation of P and Al in roots of sorghum was reported [277]. Poor plant growth with Al toxicity was a result of phosphorous starvation [278]. Wagatsuma et al. [83] reported that the concentration of Al was high in the roots and generally low in the tops. In sensitive plants, Al was considerably deposited in the root-tips; the root elongation was retarded and finally the top growth inhibited. Nalewajko and Paul [279] demonstrated that the addition of Al (250 µg/L) significantly decreased the microbial phosphate uptake in water samples from two Canadian lakes. Pettersson et al. [280] indicated that aluminium exerted toxic effect in *Anabaena cylindrica* causing phosphate starvation. Husaini and Rai [281] observed a pH-altered reduction in uptake and assimilation of nitrate and phosphate in the cyanobacterium *Nostoc linckia* under aluminium stress. Further, Husaini et al. [282] reported that a pH-dependent inhibition of Mg^{2+} and Ca^{2+}- ATPase activities of *Nostoc linckia* and *Chlorella vulgaris* exposed to either $AlCl_3$ or $AlCl_3^+$ NaF. DeGraaf et al. [283] analysed the aluminium toxicity and tolerance in three heathland species on the basis of Al accumulation and growth rate. They reported that Al concentrations increased with increasing Al concentrations in the nutrient solution in all the three health and species (*Arnica montana, Cirsium dissectum* and *C. vulgaris*). Application of Al for 1 h to individual 1 mm section of root apex only inhibited root elongation. Aluminium-induced prominent alterations in both the microtubular and the actin cytoskeleton were found especially in the apical 1–2 mm zone using monoclonal antibodies as reported by Horst et al. [284]. They also indicated that NaCl- adapted plants with higher pectin content accumulated more Al in their root apices and these were more Al-sensitive indicating more severe inhibition of root elongation and enhanced callose induction by Al.

Uptake and Distribution of Aluminium: Al ions are taken up by plants mostly through the root system, and only small amounts penetrate the leaves. Most authors now agree that generally the active metal uptake processes involve ion-specific carriers with energy expenditure but a specific Al carrier has not yet been found. Plasma membrane represents the primary target of Al toxicity [285]. The primary effects of Al on root membrane permeability may appear only after a few minutes or even hours after exposure to Al. It is likely that these effects are mediated by Al ability to bind to the carboxyl and phosphate groups of the cell wall and membrane, respectively [286].

Although a primary response to Al has been localized to root apex [4, 38], the mechanism of the Al-induced growth inhibition remains poorly understood and controversial. Some evidence points to Al entrance to root symplast in considerable quantities possibly affecting growth of the membrane from the cytosolic side [287]. However, Rengel, 1996 [288] focused their attention on the apoplast. Recent findings on the cell wall -plasma membrane- cytoskeleton continuum [289] call for a reassessment of this debate. Since the cellular site of Al toxicity is still unresolved, symplastic *versus* apoplastic targets are being intensively discussed [127]. The major portion of absorbed Al is localised in apoplast ranging from 30–90% of the total tissue Al content [288]. This seems to grossly overestimate the symplastic part of Al due to apoplastic contaminations or insufficient desorption. Although many research groups have suggested

integration of Al with many cellular sites: cell wall, plasma membrane, or DNA [288, 11] it seems that most of the Al accumulates in the cell wall. Rengel & Reid,1997 [120] reported using giant cells of the alga *Chara corallina* that 99.99% of the total cellular Al accumulates in the cell wall, and according to Chang *et al., 1999* [123] this concerns mainly the part of cell wall pectin which remains in the protoplast even after enzymatic digestion of the wall. These authors even hypothesize that Al may bind to the pectin newly produced during Al treatment.

Quantitative information on the uptake and cellular distribution of Al is required to understand the mechanisms of Al toxicity. At present, we do not know which molecular forms of Al are capable of crossing membranes what the rates of Al transport are. The mechanistic basis of Al transport and the overall subcellular distribution remain speculative. Induction of callose (ß-1,3-glucan) formation is a sensitive marker for genotypic Al toxicity [59]. Callose is accumulated in the cell wall around plasmodesmata in response to the damage caused by Al in the roots of various plants. Larsen *et al., 1996* [290] observed increasing callose deposition in wild-type *Arabidopsis* seedling roots with increasing Al concentrations over the range of 0 to 100 μM $AlCl_3$. Callose may cause the blockage of cell–to–cell transport by blocking plasmodesmata [65]. Ectomycorrhizal fungi may influence seedling absorption and tolerance to Al and heavy metals in soils. The mechanism by which ectomycorrhiza influences absorption of metal ions may be associated either with fungal mantle protection of roots or the modification of rhizosphere by the fungal associate. Both the cell walls and the cytoplasm of fungal tissue are the main accumulation sites for metal ions resulting in decreased metal transfer from the fungus to the root [291].

Aluminum (Al^{3+}) tolerance mechanisms: Species can vary in their ability to grow in acid soils with severe Al phytotoxicity [30]. Al tolerance mechanisms have been classified into two main types: a) those that exclude Al from the root cells and b) those that allow Al to be tolerated once it has entered the plant cells [143]. Species in tropical areas are very resistant to Al stress and some of these species can accumulate high concentrations of Al in the leaves, greater than 1% of their dry weight [30]. By contrast, cereals like *Secale cereale*, *Zea mays*, *Hordeum vulgare*, *Triticum aestivum*, X *Triticosecale*, *Sorghum bicolor* and *Avena sativa* do not accumulate high concentrations of Al internally but rather use the Al exclusion mechanism through organic acid exudation [27, 29,214, 216, 218,]. This would be one of the most widely used mechanisms by most of the species studied.

External tolerance mechanism (exclusion): Some species detoxify Al in the rhizosphere by exuding organic acid from their roots. The organic acids commonly secreted are malate, citrate and oxalate. Malate and citrate are present in all cells given that they are involved in the mitochondrial respiratory cycle [30]. Organic acid levels vary between species, cultivars and even between tissues of the same plant under identical growth conditions. In addition, organic acid biosynthesis and accumulation increase drastically in response to environmental stress [292]. It has been observed that tolerant genotypes exude a greater amount of organic acids that sensitive genotypes, which would support the notion that organic acid exudation is an Al tolerance mechanism [69]. However, it has been reported that Al-sensitive species of wheat show a greater accumulation in the cortical tissue (5 to 10 times more) than the tolerant genotypes exposed for the same period of time [212].

Some organic acids such as citrate, malate and oxalate are able to form stable complexes with Al, where the Al-citrate complex bond is strongest, followed by the Al-oxalate and Al-malate complexes, which are insoluble and not available for plants [30]. This is because Al is a metal that tends to form strong complexes with the oxygen donor ligand [143]. The transport of these organic acids from the radical cells is mediated by the anionic channel activity in the plasma membrane [293]. These anionic channels might be Al-activated, which was demonstrated using the patch-clamp technique on isolated protoplasts of wheat and maize radical apexes [30]. Using anionic channel inhibitors such as niflumic acid would support the existence of these channels as elements for organic acid exudation in response to Al [135, 221]. Conversely, it has been observed that Al also induces the exudation of certain phenolic compounds, such as catequin and quercitin, from maize radical apexes, forming stable complexes with these compounds; it

is likely that they can contribute to Al tolerance, but more research is required to confirm this hypothesis [30].

Internal tolerance mechanism (inclusion): Another proposed detoxification mechanism is internal tolerance or inclusion [4]. Once the Al enters the cell, the Al^{3+} cation concentration free in the cytoplasm will be very low due to the high pH of the cytoplasm (pH 7.0). However, it has been indicated that these internal Al concentrations can be dangerous [294]. Al also exhibits a high affinity for the oxygen ligand, which allows it to compete with other ions for metabolically important sites, despite a large difference in their concentrations [30, 293]. It has been observed that the Al-ATP bond is less strong than the bond of Al citrate or Al-oxalate complexes. This may indicate that organic anions are able to protect plants by Al chelation in the cytosol. The metallic anion complex could then be transported around the plant for its storage [30]. This mechanism immobilizes, compartmentalizes or detoxifies the Al from the simplast [295]. The formation of less toxic Al complexes seems to be a prerequisite for tolerating the high concentrations of internal Al that have been observed in such plants as *Hydrangea macrophylla, Fagopyrum esculentum* and *Melastoma malabathricum*, able to accumulate high concentrations of Al [30, 143].
In *Fagopyrum sculentum*, the organic anions chelate the Al in different tissues, as in the radical cells and the vacuole of the cells of the leaves [30]. Moreover, Al-accumulating plants have been identified; these hyperaccumulators accumulate more than 1000 mg kg^{-1} of Al in the leaves [296]. It has been reported that a high Al accumulation in stems involves the transport of Al-soluble complexes through the xylem and the subsequent innocuous accumulation, solid or soluble, in the vacuole of the leaves or in the apoplast. The unstained Al in the leaves of various accumulators suggests that Al can be transported to the phloem [143].

Biochemistry of Al Phytotoxicity: To evaluate meaningful biochemical effects of toxic metals, one must examine conditions (different metals and their concentrations) which are phytotoxic in nature [297]. Aluminium toxicity is strongly influenced by acid soils (pH 5.0) but can occur at pH levels as high as 5.5 [298]. Woolhouse [299] noted that aluminium inhibited the activities of ATPase in plants. He also found that the ATPase activity of cell wall preparations from roots of an acid soil ecotype of *Agrostis tenuis* was inhibited less by Al than that of a preparation from a calcareous soil ecotype of the same species. He also suggested that structural changes in these enzymes might be responsible for differential Al tolerance of the ecotypes. Foy and Fleming [50] reported that under Al stress in nutrient solutions, the Al-sensitive cultivar was characterized by chlorosis, decreased Fe concentrations in tops, decreased Ca and Mg in both tops and roots, a tendency toward accumulation of P, Al and Fe in roots and reduced Mn in tops. Aluminium induced changes in the uptake of most macro element cations by plant roots, including reductions in the uptake of calcium [300], magnesium [268] and potassium [301]. Foy and Fleming [50] found negative effects of Al on the nitrate reductase activity (NRA), the first enzyme involved in the NO_3^- assimilation in plants. Further, Keltjens and vanUlden [302] compared the effect of Al on nitrogen uptake, nitrate reductase activity and protein release in two sorghum cultivars differing in Al tolerance.
Prolonged Al stress induced an enhancement of lipid peroxidation [118] and caused formation of highly toxic oxygen free radicals [303]. An increase in the activities of superoxide dismutase and peroxidase and a decrease of catalase activity indicates the presence of an antioxidant scavenging system in Al-treated roots [303]. Plucinska and Karolewski [304] reported a significant decrease of the anabolic reduction charge (ARC: NADPH/($NADP^+$ + NADPH)) and an increase of the redox status (NAD(P)H/$NAD(P)^+$), catabolic reduction charge (CRC:NADH/(NAD^++NADH)) and phosphorylation capacity expressed as $NADPH^+/NAD^+$ ratio in the presence of 4.0 mM Al treatment in hydroponic culture of Scots Pine seedlings. Subsequently, Plucinska and Ziegler [112] indicated that the longer exposure to Al ions led to a drastic decrease in AdN (total adenylate) and ATP pool-levels with a corresponding rise in ADP and AMP content and great depression both in ATP/ADP and AEC (adenylate energy charge) and inhibition of metabolic activity [305]. Pavlovkin and Mistrik [306] studied the effect of Al on the electrical membrane potential (Em) of outer cortex root cells of 3-day-old maize seedlings. They indicated that Em values of root cells ranged between –115 and – 146 mV. The membrane potential was rapidly and significantly

depolarized by Al. The depolarization was concentration-dependent and reached the maximum at 150 mM Al. The extent of membrane depolarization by 100 mM Al decreased continuously from the apex to the base of the root. Both the P-ATPase activator fusicoccin and glucose diminished the depolarizing effect of Al on electrical membrane potential. The roots exposed to Al retarded K^+ efflux from root tip segments and had no effect on K^+ efflux from segments of the root base as reported by Pavlovkin and Mistrik [306]. Gunse et al. [307] tested two maize (*Zea mays*) cultivars on root growth by using Al and the role of ethylene metabolism. They suggested that Al-resistant genes were not constitutively expressed in the absence of Al in the growing medium, but activated upon exposure to Al. Enhanced ethylene formation does not seem to play a role either in the Al-induced inhibition of root elongation or in the induction of the resistance mechanism.

CONCLUSIONS

Aluminium toxicity is an important growth-limiting factor for plants in many acid soils, particularly in pH of 5.0 or below. Aluminium toxicity in plants is often clearly identifiable through morphological and physiological symptoms. Differential tolerances to Al toxicity almost certainly involve differences in the structure and function of roots. Aluminium interferes with cell division in roots, decreases root respiration and uptake and use of water and nutrients, particularly calcium and phosphorous and metabolic pathway. Other promising approaches to studying metal toxicity in tolerant and sensitive plant genotypes are to determine the metal uptake and transportation in various plant parts, the mechanism behind the interaction with mineral nutrients, specific genes responsible for tolerance, levels and kinds of organic and aminoacids which act as metal chelators and detoxifiers, level and forms of enzymes, and changes in root permeabilities to ions and molecules and its mechanisms.

Although aluminium has been shown to be a genotoxic metal, the molecular mechanism of Al toxicity to plants is not well understood. Al is a complicated ion in terms of chemical form and exerts a divergent biological function. The destructive influence of Al has been shown at different levels of plant organization. Many questions concerning plant response to Al can be posed but very few answers can be given. Al entrance to the root apex, particularly to the distal part of the transition zone still more precisely to cell symplasts is crucial and all factors environmental and cellular intervening with Al transport play an essential role. However, the question arises to what extent the general mechanism of signal transduction of stresses is involved and to what extent Al interferes with DNA metabolism. The recent accumulating data on gene maps, including molecular markers, in different plants and gene homology should facilitate answers to the questions on Al toxicity and tolerance.

REFERENCES

[1] H.M. May, D.K. Nordstrom, Assessing the solubilities and reactions kinetics of aluminuous mineral in soils, in *Soil Acidity*, Springer, Berlin, Germany, **1991**, B. Ulrich and M. E. Summer, Eds., 125-148.
[2] L.V. Kochian, M.A. Pi˜neros, Hoekenga O.A., *Plant and Soil*, **2005**, 274 (1-2), 175-195.
[3] T.B. Kinraide, *J. Exp. Bot*., **1997**, 48, 1115-1124.
[4] L.V. Kochian, Annual Review of Plant Physiology and Plant Molecular Biology, **1995**, 46, 237-260.
[5] S.K. Panda, H.Matsumoto, *Botanical Review*, **2007**, 73 (4), 326-347.
[6] T.B. Kinraide, *Plant and Soil*, **1991**, 134(1), 167-178.
[7] H.R. Von Uexküll, E. Mutert, In: R.A. Date, N.J. Grundon, G.E. Raymet, M.E. Probert (Ed.), Plant-Soil interactions at low pH Principles and Management, Kluwer Academic Publ. Dordrecht, Netherlands, **1995**, 5-19.
[8] V.A. Vitorello, F.R.C. Capaldi, V.A. Stefanuto, Braz *J Plant Physiol*, **2005**, 17, 129-143.

[9] H. Eswaran, P. Reich, F. Beinroth, In: Moniz AC (Ed.), Plant-Soil Interactions at low pH, Brazilian Soil Science Society, **1997**, 159-164.
[10] H. Matsumoto, *International Review of Cytology*, **2000**, 200, 1-47.
[11] Silva, T. Smyth, D. Moxley, T. Carter, N. Allen, T. Rufty, *Plant Physiol*, **2000**, 123, 543-552.
[12] G.C. Zhang, J.J. Slaski, D.J. Archambault, G.J. Taylor, *Physiol Plant*, **1997**, 99, 302-308.
[13] S. Ishikawa, T. Wagatsuma, *Plant Cell Physiol*, **1998**, 39, 516-525.
[14] K. Liu, S. Luan, *Plant Cell*, **2001**, 13, 1453-1465.
[15] D.L. Jones, L.V. Kochian, *Plant Cell*, **1995**, 7, 1913-1922.
[16] Z. Rengel, W.H. Zhang, *New Phytol*, **2003**, 159, 295-314.
[17] Y. Yamamoto, Y. Kobayashi, S.R. Devi, S. Rikiishi, H. Matsumoto, *Plant Soil*, **2003**, 255, 239-243.
[18] M. Sivaguru, F. Baluska, D. Volkmann, H.H. Felle, W.J. Horst, *Plant Physiol*., **1999**, 119, 1073-1082.
[19] W.J. Horst, N. Schmohl, M. Kollmeier, F. Baluska, M. Sivaguru, *Plant Soil*, **1999**, 215, 163-174.
[20] T. Sasaki, Y. Yamamto, B. Ezaki, M. Katsuhara, S.J. Ahn, P.R. Ryan, E. Delhaize, H. Matsumoto, *Plant J*., **2004**, 37, 645-653.
[21] Z. Rengel, *Plant Cell Environ*, **1992**, 15, 931-938.
[22] S. Ramgareeb, J.A. Cooke, M.P. Watt, *J. Plant Physiol*, **2004**, 161, 1245-1258.
[23] G. Rout, S. Samantaray, P. Das, *Agronomie*, **2001**, 21, 3-21.
[24] D.L. Jones, L.V. Kochian, S. Gilroy, *Plant Physiol*, **1998**, 116, 81-89.
[25] D.L. Jones, L.V. Kochian, *The Plant Cell*, **1995**, 7, 1913-1922.
[26] Ramos-Díaz, L. Brito-Argaes, T. Munnik, S.M.T. Hernandezsotomayor, *Planta*, **2007**, 225, 393-401.
[27] I.C. Nava, C.A. Delatorre, I.T. Delima Duate, M.T. Pacheco, L.C. Federezzi, *Euphytica*, **2006**, 148, 353-358.
[28] G. Fontecha, J. Silva-Navas, C. Benito, M.A. Mestres, F.J. Espino, M.V. Hernándezriquer, F.J. Gallego, *Theor Appl Genet*, **2007**, 144, 249-260.
[29] F.F. Caniato, C.T. Guimaraes, R.E. Shaffert, V.M.C. Alves, L.V. Kochian, A. Borem, P.E. Klein, J.V. Magalhaes, *Theor Appl Genet*, **2007**, 114, 863-876.
[30] D.L. Jones, P.R. Ryan, *Nutrition*, **2003**, 656-664.
[31] Y. Tang, M. Sorrells, L. Kochian, D. Garvin, *Alp. Crop Sci*, **2000**, 40, 778-782.
[32] J. Miftahudin G., Scoles, J.P. Gustafson, Theor. *Appl. Genet*, **2002**, 104, 626-631.
[33] T. Miftahudin, K. Chikmawati, G. Ross, J. Scoles, J.P. Gustafson, *Theor Appl. Genet*, **2005**, 110, 906-913.
[34] J.F. Ma, S.J. Zheng, H. Matsumoto, S. Hiradate, *Nature*, **1997a**, 390, 569-570.
[35] H. Matsumoto, E. Hiraseva, S. Morimura, E. Takahashi, *Plant Cell Physiol*., **1976**, 17, 627-631.
[36] A.J.M. Baker, S.P. McGrath, R.D. Reeves, J.A.C. Smith, In: N. Terry, G. Banuelos (Ed.), Phytoremediation of Contaminated Soil and Water, Lewis Publisher, Boca Raton, **2000**, 85-107.
[37] S. Silva, Ph.D. thesis, Biologia Universidade de Aveiro, Aveiro, Portugal, **2011**.
[38] M. Sivaguru, F. Baluˇska, D. Volkmann, H.H. Felle, W.J. Horst, *Plant Physiology*, **1999**, 119(3), 1073-1082.
[39] D.L. Jones, L.V. Kochian, *FEBS Letters*, **1997**, 400(1), 51-57.
[40] J. Zhang, Z. He, H. Tian, G. Zhu, X. Peng, *Journal of Experimental Botany*, **2007**, 58(8), 2269-2278.
[41] C.H. Huang, R.C. Crain, *Planta*, **2009**, 230(5), 925-933.
[42] Q. Ma, Z. Rengel, J. Kuo, *Annals of Botany*, **2002**, 89(2), 241-244.
[43] W.H. Zhang, Z. Rengel, *Australian Journal of Plant Physiology*, **1999**, 26(5), 401-409.
[44] P. Bhuja, K. McLachlan, J. Stephens, G. Taylor, *Plant and Cell Physiology*, **2004**, 45(5), 543-549.
[45] D.L. Jones, S. Gilroy, P.B. Larsen, S.H. Howell, L.V. Kochian, *Planta*, **1998**, 206(3), 378-387.
[46] C.D. Foy, J.A. Duke, T.E. Devine, *J. Plant Nutr*., **1992**, 15, 527–547.
[47] C.D. Foy, T.E. Carter, J.A. Duke, T.E. Devine, *J. Plant Nutr*, **1993**, 16, 305–325.

[48] C.D. Foy, *J. Plant Nutr*, **1996**, 19, 1361–1380.
[49] C.D. Foy, In: J.L. Hatfield, B.A. Stewart (Ed.), Advances in Soil Sciences: Limitations to Plant Root Growth, Springer Verlag, New York, **1992**, 19, 97–149.
[50] C.D. Foy, A.L. Fleming, *J. Plant. Nutr*, **1982**, 5, 1313–1333.
[51] R.R. Furlani, R.B. Clark, *Agron. J.*, **1981**, 73, 587–594.
[52] R.B. Clark, H.A. Pier, D. Knudsen, J.W. Maranville, *J. Plant Nutr*, **1981**, 3, 357–374.
[53] D.L. Jones, E.B. Blancaflor, L.V. Kochian, S. Gilroy, Plant, Cell and Environment, **2006**, 29(7), 1309–1318.
[54] E.D. Mariano, W.G. Keltjens, *Journal of Plant Nutrition*, **2005**, 28(2), 323-333.
[55] A.C. Jorns, C. Hechtbuchholz, A.H. Wissemeier, Zeitschrift Fur Pflanzenernahrung Und Bodenkunde, **1991**,154, 349–353.
[56] K.A. Schreiner, J. Hoddinott, G.J. Taylor, *Plant and Soil*, **1994**, 162(2), 273–280.
[57] D. Eticha, C. Th´e, Welcker, C., L. Narro, A. Staß, W.J. Horst, Field Crops Research, **2005**, 93(2-3), 252–263.
[58] K. Tahara, M. Norisada, T. Hogetsu, K. Kojima, *Journal of Forest Research*, **2005**, 10(4), 325–333.
[59] W.J. Horst, A.K. P¨uschel, N. Schmohl, *Plant and Soil*, **1997**, 192(1), 23–30.
[60] N. Massot, M. Llugany, C. Poschenrieder, J. Barcel´o, *Journal of Plant Nutrition*, **1999**, 22(1), 1–10.
[61] B. Meriga, B.K. Reddy, G. Jogeswar, L.A. Reddy, P.B.K. Kishor, *Current Science*, **2003**, 85(3), 383–386.
[62] N.E. Nagy, L.S. Dalen, D.L. Jones, B. Swensen, C.G. Fossdal, T.D. Eldhuset, *New Phytologist*, **2004**, 163(3), 595–607.
[63] S. Silva, O. Pinto-Carnide, P. Martins-Lopes, M. Matos, H. Guedes-Pinto, C. Santos, Environmental and Experimental Botany, **2010**, 68(1), 91–98.
[64] S. Silva, C. Santos, M. Matos, O. Pinto-Carnide, *Environmental and Experimental Botany*, **2011**, 75, 89–97.
[65] M. Sivaguru, T. Fujiwara, J. Samaj, F. Baluska, Z. Yang, H. Osawa, T. Maeda, T. Mori, D. Wolkmann, H. Matsumoto, *Plant Physiology*, **2000**, 124(3), 991-1005.
[66] P. Nosko, P. Brassard, J.R. Kramer, K.A. Kershaw, *Can. J. Bot.*, **1988**, 66, 2305–2310.
[67] R.J. Bennet, C.M. Breen, M.V. Fey, *Plant and Soil*, **1991**, 134, 153–166.
[68] H.J. Vanpraag, F. Weissen, *Plant and Soil*, **1985**, 83, 331–338.
[69] E. Delhaize, P.R. Ryan, *Plant Physiol.*, **1995**, 107, 315–321.
[70] L.V. Kochian, Annu. *Rev. Plant Physiol. and Plant Mol. Biol.*, **1995**, 46, 237–260.
[71] H. Marschner, *Plant and Soil*, **1991**, 134, 1–20.
[72] P.R. Ryan, J.M. DiTomaso, L.V. Kochian, *J. Exp. Bot*., **1993**, 44, 437–446.
[73] M. Rincon, R.A. Gonzales, *Plant Physiol.*, **1992**, 99, 1021–1028.
[74] T. Wagatsuma, M. Kaneko, Y. Hayasaka, *Soil. Sci. Plant Nutr*., **1987**, 33, 161–175.
[75] T.B. Kinraide, *Plant Physiol.*, **1988**, 88, 418–423.
[76] A.K. Roy, A. Sharma, G. Talukder, *Bot. Rev*., **1988**, 54, 145–177.
[77] C.D. Foy, R.L. Chaney, M.C. White, *Annu. Rev. Plant Physiol.*, **1978**, 29, 511–566.
[78] R.J. Bartlett, D.C. Riego, *Plant and Soil*, **1972,** 37, 419–423.
[79] F.P.C. Blamey, D.G. Edwards, C.J. Asher, *Soil Sci.*, **1983**, 136, 197–207.
[80] B.W. Bache, G.S. Sharp, J. *Soil Sci*., **1976**, 27, 167–174.
[81] C.F. Jr. Baes, R.E. Mesmer, Wiley Interscience, New York, **1976**, 112–118.
[82] T.B. Kinraide, R.C. Arnold, V.C. Baligar, *Physiol. Plant*., **1985**, 65, 245–250.
[83] T. Wagatsuma, T. Kyuuda, A. Sakuraba, Bull. Yamagata Univ. Agric. Sci., **1987**, 10, 355–359.
[84] R.J. Bennet, C.M. Breen, M.V. Fey, S. Afr. Tydskr. Planik., **1985**, 51, 355–362.
[85] S.J. Zheng, J.L. Yang, Biologia *Plantarum*, **2005**, 49(3), 321–331.
[86] Y. Yamamoto, Y. Kobayashi, H. Matsumoto, *Plant Physiology*, **2001**, 125(1), 199–208.
[87] P.R.S. Boscolo, M. Menossi, R.A. Jorge, *Phytochemistry*, **2003**, 62(2), 181–189.

[88] M.C. Kuo, C.H. Kao, *Biologia Plantarum*, **2003**, 46(1), 149–152.
[89] T. Guo, G. Zhang, M. Zhou, F. Wu, *J. Chen, Plant and Soil*, **2004**, 258(1-2), 241–248.
[90] P.H.P. Peixoto, J. Cambraia, R. Sant'Anna, P.R. Mosquim, M.A. Moreira, Revista Brasileira de Fisiologia Vegetal, **1999**, 11, 137–143.
[91] Q. Liu, J.L. Yang, L.S. He, Y.Y. Li, S.J. Zheng, Biologia Plantarum., **2008**, 52, (1), 87–92.
[92] S.K. Panda, L.B. Singha, M.H. Khan, *Bulgarian Journal of Plant Physiology*, **2003**, 29, 77–86.
[93] M.A. Hossain, A.K.M.Z. Hossain, T. Kihara, H. Koyama, T. Hara, *Soil Science and Plant Nutrition*, **2005**, 51(2), 223–230.
[94] S.R. Devi, Y. Yamamoto, H. Matsumoto, *Journal of Inorganic Biochemistry*, **2003**, 97(1), 59–68.
[95] F. Ghanati, A. Morita, H. Yokota, *Plant and Soil*, **2005**, 276(1-2), 133–141.
[96] B. Ezaki, R.C. Gardner, Y. Ezaki, H. Matsumoto, *Plant Physiology*, **2000**, 122(3), 657– 665.
[97] G.M.A. Cancado, V.E. De Rosa, J.H. Fernandez, L.G. Maron, R.A. Jorge, M. Menossi, *Functional Plant Biology*, **2005**, 32(11), 1045–1055.
[98] D. Chandran, N. Sharopova, S. Ivashuta, J.S. Gantt, K.A. VandenBosch, D.A. Samac, *Planta*, **2008**, 228(1), 151–166.
[99] L.G. Maron, M. Kirst, C. Mao, M.J. Milner, M. Menossi, L.V. Kochian, *New Phytologist*, **2008**, 179(1), 116–128.
[100] A.M. Balsberg Pahlsson, *Physiol. Plant.*, **1990**, 78, 79–84.
[101] W. Dickson, Verh. Int. Verein. Limnol, **1978**, 20, 851–856.
[102] Pettersson, L. Hallbom, B. Bergman, *Physiol. Plant*, **1985**, 63, 153–158.
[103] N. Thawornwong, A. van Diest, *Plant and Soil*, **1974**, 41, 141–159.
[104] A.L. Fleming, J.W. Schwartz, C.D. Foy, *Agron. J*., **1974**, 66, 715–719.
[105] C.D. Foy, In: F. Adams (Ed.), Soil Acidity and Limiting, Second Edition, Amer. Soc. Agron., Madison, Wisconsin, **1984**, 57–97.
[106] C.S. Cronan, *Tree Physiol*., **1991**, 29, 511–566.
[107] G. Jentschke, H. Schlegel, D.L. Godbold, *Physiol. Plant*., **1991**, 82, 266–270.
[108] A.A. Meharg, *Physiol. Plant.*, **1993**, 88, 191–198.
[109] C.A. Suhayda, A. Haug, *Physiol. Plant*, **1986**, 68, 189–195.
[110] J.J. Slaski, *J. Plant Physiol.*, **1990**, 136, 40–44.
[111] G. Tyler, A.M. Pahlsson, G. Bengtsson, E. Baath, L. Tranvik, *Water Air Soil Pollut*, **1989**, 47, 189–215.
[112] G.L. Plucinska, H. Ziegler, *Acta Physiol. Plant*, **1995**, 17, 225–232.
[113] A.R. Trim, *Biochem. J*., **1959**, 73, 298–304.
[114] J.M. Soileau, O.P. Engelstad, *Soil Sci. Soc. Am. Proc*., **1969,** (I), 33, 915–919.
[115] J.M. Soileau, O.P. Engelstad, J.B. Martin, *Soil Sci. Soc. Am. Proc*., **1969,** (II), 33, 915–919.
[116] E.J. Kamprath, C.D. Foy, In: O.P. Engelstad (Ed.), Fertilizer Technology and Use, 3rd edition, Soil Sci. Soc. Am., Madison, Wisconsin, **1985**, 91–151.
[117] D. Berggren, and G. Fiskesjo, *Environ. Toxicol. Chem.*, **1987**, 6, 771–779.
[118] Severi, *Environ. Exp. Bot*., **1997**, 37, 53–61.
[119] Plieth, B. Sattemacher, U.P. Hansen, M.R. Knight, *Plant J*., **1999**, 18, 643–650.
[120] Z. Rengel, R.J. Reid, Plant and Soil, **1997**, 192(1), 31–35.
[121] S. Marienfeld, H. Lehmann, R. Stelzer, *Plant and Soil*, **1995**, 171(1), 167–173.
[122] N. Schmohl, W.J. Horst, *Plant, Cell and Environment*, **2000**, 23(7), 735–742.
[123] Y.C. Chang, Y. Yamamoto, H. Matsumoto, *Plant, Cell and Environment*, **1999**, 22(8), 1009–1017.
[124] Le Van Hoa, S. Kuraishi, N. Sakurai, *Plant Physiology*, **1994**, 106(3), 971–976.
[125] A.K.M. Zakir Hossain, H. Koyama, T. Hara, *Journal of Plant Physiology*, **2006**, 163(1), 39–47.
[126] Tabuchi, H. Matsumoto, *Physiologia Plantarum*, **2001**, 112 (3), 353–358.
[127] S. Marienfeld, N. Schmohl, M. Klein, W.H. Schr¨oder, A.J. Kuhn, W.J. Horst, *Journal of Plant Physiology*, **2000**, 156(5-6), 666–671.
[128] J. Xia, N. Yamaji, T. Kasai, J.F. Ma, Proceedings of the National Academy of Sciences of the United States of America, **2010**, 107(43), 18381–18385.

[129] M.A. Akeson, D.N. Munns, R.G. Burau, *Biochimica et Biophysica Acta*, **1989**, 986(1), 33–40.
[130] T.B. Kinraide, P.R. Ryan, L.V. Kochian, *Plant Physiology*, **1992**, 99(4), 1461–1468.
[131] S.J. Ahn, M. Sivaguru, G.C. Chung, Z. Rengel, H. Matsumoto, *Journal of Experimental Botany*, **2002**, 53(376), 1959–1966.
[132] B.E. Nichol, L.A. Oliveira, A.D.M. Glass, M.Y. Siddiqi, *Plant Physiology*, **1993**, 101(4), 1263–1266.
[133] C. Poschenrieder, M. Llugany, J. Barcelo, *Journal of Plant Nutrition*, **1995**, 18(7), 1495–1507.
[134] E.D. Mariano, W.G. Keltjens, *Journal of Plant Nutrition*, **2005**, 28(2), 323–333.
[135] M.A. Pi˜neros, L.V. Kochian, *Plant Physiology*, **2001**, 125(1), 292–305.
[136] E. Olivares, E. Pe˜na, E. Marcano, *Environmental and Experimental Botany*, **2009**, 65(1), 132–141.
[137] Giannakoula, M. Moustakas, P. Mylona, I. Papadakis, T. Yupsanis, *Journal of Plant Physiology*, **2008**, 165(4), 385–396.
[138] F.C. Lidon, H.G. Azinheira, M.G. Barreiro, *Journal of Plant Nutrition*, **2000**, 23(2), 151–160.
[139] R.J. de Mendonca, J. Cambraia, J.A. de Oliveira, M.A. Oliva, *Pesquisa Agropecuaria Brasileira*, **2003**, 38(7), 843–848.
[140] L. Simon, T.J. Smalley, J. Benton Jones Jnr., F.T. Lasseigne, *Journal of Plant Nutrition*, **1994**, 17(2-3), 293–306.
[141] R.W. Zobel, T.B. Kinraide, V.C. Baligar, *Plant and Soil*, **2007**, 297(1-2), 243–254.
[142] S. Doncheva, M. Amen´os, C. Poschenrieder, J. Barcel´o, *Journal of Experimental Botany*, **2005**, 56(414), 1213–1220.
[143] J. Barcel´o, and C. Poschenrieder, *Environmental and Experimental Botany*, **2002**, 48(1), 75–92.
[144] M. Moustakas, G. Ouzounidou, E.P. Eleftheriou, R. Lannoye, *Plant Physiology and Biochemistry*, **1996**, 34(4), 553–560.
[145] Konarska A., *Acta Physiologiae Plantarum*, **2010**, 32(1), 145–151.
[146] R. Azmat, S. Hasan, *Pakistan Journal of Botany*, **2008**, 40(2), 779–784.
[147] X.B. Zhang, P. Liu, Y.S. Yang, G.D. Xu, *Botanical Studies*, **2007**, 48(4), 435–444.
[148] T.R. Guo, G.P. Zhang, Y.H. Zhang, *Colloids and Surfaces B*, **2007**, 57(2), 182–188,.
[149] H.X. Jiang, L.S. Chen, J.G. Zheng, S. Han, N. Tang, B.R. Smith, *Tree Physiology*, **2008**, 28(12), 1863–1871.
[150] L.S. Chen, Y.P. Qi, B.R. Smith, X.H. Liu, *Tree Physiology*, **2005**, 25(3), 317–324.
[151] M. Reyes-D´ıaz, C. Inostroza-Blancheteau, R. Millaleo, E. Cruces, C. Wulff-Zottele, M. Alberdi, M. de la Luz Mora, Journal of the American Society for Horticultural Science, **2010**, 135(3), 212–222.
[152] Y. Cao, Y. Lou, Y. Han, J. Shi, Y. Wang, W. Wang, F. Ming, *Molecular Biology Reports*, **2011**, 38(8), 4839–4846.
[153] D.T. Clarkson, In: H. Rorison (Ed.), Ecological Aspects of the Mineral Nutrition of Plants, Blackwell Scientific Public, **1969**, 381–397.
[154] H. Matsumoto, S. Morimura, *Plant Cell Physiol.*, **1980**, 21, 951–959.
[155] D.A. Reid, In: R.A. Nilan (Ed.), Barley Genetics II. Proc. 2nd Int. Barley Genetics Symp. Pullman, Washington state Univ. Press, **1971**, 409–413.
[156] P.C. Kerridge, W.E. Kronstad, *Agron. J*., **1968**, 60, 710–711.
[157] E.J. Iorezeski, H.W. Ohm, *Agron. Abst*., **1977**, 7, 59–69.
[158] L.G. Campbell, H.N. Lafever, Proc. Fifth Int. Wheat Genet. Symp, New Delhi, India, **1978**, 963–977.
[159] L.G. Campbell, H.N. Lafever, *Cereal Res. Commun.*, **1981**, 9, 281–297.
[160] R.D. Rhuem, C.O. Groganm, E.W. Stockmeyerm, H.L. Everettm, *Crop Sci.*, **1978**, 1063–1067
[161] O. Stolen, S. Anderson, *Hereditas*, **1978**, 88, 101–105.
[162] R. Minocha, S.C. Minocha, S.L. Long, W.C. Shortle, *Physiol. Plant*., **1992**, 85, 417–424.
[163] H. Matsumoto, S. Morimura, E. Takahashi, *Plant Cell Physiol.*, **1977**, 18, 987–993.

[164] E.G. Jordan, J.N. Timms, A.J. Trewavas, In: N.E. Tolbert (Ed.), The Biochemistry of Plants, 1, Academic Press, New York, **1980**, 489–588.
[165] R.J. Bennet, C.M. Breen, M.V. Fey, *S. Afr. J. Plant Soil*, **1985**, 2, 1–7.
[166] R.J. Bennet, C.M. Breen, M.V. Fey, *S. Afr. J. Plant Soil,* **1985**, 2, 8–17.
[167] D.T. Clarkson, *Ann. Bot*., **1965**, 29, 309–315.
[168] S.U. Wallace, I.C. Anderson, *Agron. J*., **1984**, 76, 5–8.
[169] R.J. Bennet, C.M. Breen, M.V. Fey, *Environ. Exp. Bot*., **1987**, 27, 91–104.
[170] G. Frantzios, B. Galatis, P. Apostolakos, *New Phytol*, **2000**, 145, 211–224.
[171] C. Hechi-Buchholz, C.D. Foy, In: R. Brouwer (Ed.), Structure and Function of Plant Roots, Martinus Nijhoff / Dr. W. Junk Publishers, The Hague, **1981**, 343–345.
[172] R. Vierstra, A. Haug, *Biochem. Biophys. Res. Commun*., **1978**, 84, 138–143.
[173] B.E. Gomez-Lepe, O.Y. Lee-stadelman, J.A. Patta, E.J. Stadeland, *Plant Physiol.*, **1979**, 64, 131–138.
[174] L.W. Gallagher, K.M. Soliman, C.O. Qualset, R.C. Huffaker, D.W. Rains, *Crop Sci*., **1980**, 20, 717–721.
[175] B.E. Nichol, L.A. Oliveira, A.D.M. Glass, M.Y. Siddiqi, *Plant Physiol.*, **1993**, 101, 1263–1266.
[176] J.W. Huang, D.L. Grunes, L.V. Kochian, *Planta*, **1992**, 188, 414–421.
[177] P.R. Ryan, J.E. Shaff, L.V. Kochian, *Plant Physiol.*, **1992**, 99, 1193–1200.
[178] S.C. Miyasaka, L.V. Kochian, J.E. Shaff, C.D. Foy, *Plant Physiol*., **1989**, 91, 1188–1196.
[179] J.L. Peters, F.J. Castillo, R.L. Heat, *Plant Physiol*., **1989**, 89, 153–158.
[180] Cakmak, W.J. Horst, *Physiol. Plant.*, **1991**, 83, 463–468.
[181] M.G. Huck, *Plant Cell Physiol*., **1972**, 13, 7–14.
[182] Severi, Atti. Soc. Nat. e Mat. di Modena , **1991**, 122, 95–108.
[183] G.A. Schier, C.J. McQuattie, *J. Plant Nutr*, **1999**, 22, 951–965.
[184] P. Nosco, P. Brassard, J.R. Kramer, K.A. Kershaw, *Can J Bot*., **1988**, 66, 2305-2010.
[185] M. Sivaguru, W.J. Horst, *Plant Physiol*., **1998**, 116, 155-163.
[186] M. Ciamporova, *Biol Plant*., **2002**, 45, 161-171.
[187] T.B. Kinraide, D.R. Parker, *Plant Physiol*., **1987**, 83, 546-551.
[188] F. Vardar, E. Arcan, N. Gozukirmizi, *Advances in Food Science*, **2006**, 28, 85-88.
[189] Haug, V. Vitorello, Coord Chem Rev., **1996**, 149, 113-124.
[190] T. Watanabe, M. Osaki, *Commun Soil Sci Plant Anal*., **2002**, 33, 1247-1260.
[191] M.D. Vazquez, C. Poschenrieder, I. Corrales, J.B. Barceló, *Plant Physiol*., **1999**, 119, 435-444.
[192] F.P.C. Blamey, C.J. Asher, G.L. Kerven, D.G. Edwards, *Plant and Soil*, **1993**, 149, 87-94.
[193] Tabuchi, H. Matsumoto, *Physiol Plant*, **2001**, 112, 353-358.
[194] F.P.C. Blamey, In: N. Ae, J. Arihara, K. Okada and A. Srinivasan (Ed.) Plant Nutrient Acquisition, New Perspectives, Springer Verlag, New York, **2001**, 201-226.
[195] T.B. Kinraide, U. Yermiyahu, G. Rytwo, *Plant Physiol*., **1998**, 118, 505-512.
[196] M.A. Akeson, D.N. Munns, *J. Plant Nutr*, **1990**, 13, 467–484.
[197] N. Massot, M. Llungany, C. Poschenrieder, J. Barceló, *J. Plant Nutr*., **1999**, 22, 1-10.
[198] M.A. Piñeros, M. Tester, *J Exp Bot*., **1997**, 48, 551-577.
[199] A.A Very, J.M. Davies, Proc Natl Acad Sci. USA, **2000**, 97, 9801-9806.
[200] J.F. Ma, Z. Rengel, J. Kuo, *Annals Bot*., **2002**, 89, 241-244.
[201] D.L. Jones, L.V. Kochian, *FEBS Lett*, **1997**, 400, 51–57.
[202] Z. Rengel, *New Phytol*, **1992**, 121, 499–513.
[203] C.H. Foyer, P. Descourvieres, K.J. Kunert, *Plant Cell Environ*, **1994**, 17, 507-523.
[204] Xie, C.X. and Yokel, R.A., **1996**, Aluminum facilitation of iron mediated lipid peroxidation is dependent on substrate, pH and aluminum and iron concentrations, *Arch Biochem Biophys*., 327, 222- 226.
[205] M. Sasaki, Y. Yamamato, H. Matsumoto, *Soil Sci. Plant Nutr*, **1997**, 43, 469-472.
[206] S. Grabski, M. Schindler, *Plant Physiol*., **1995**, 108, 897–901.
[207] H. Matsumoto, E. Hirasawa, H. Torikai, E. Takahashi, *Plant Cell Physiol*., **1976**, 17, 127-137.

[208] S. Winkler, W. Ockels, H. Budzikiewicz, H. Korth, G. Pulverer, Z Naturforschung. C., **1986**, 41, 807-808.
[209] P. Kidd, M. Llugany, C. Poschenrieder, B. Gunse, J. Barceló, *J Exp Bot.*, **2001**, 52, 1339–1352.
[210] P.B. Larsen, J. Degenhardt, C.Y. Tai, L.M. Stenzler, S.H. Howell, L.V. Kochian, *Plant Physiol.*, **1998**, 117, 9-18.
[211] S. Miyasaka, J. Bute, R. Howell, C. Foy, *Plant Physiol*., **1991**, 96, 737–743.
[212] E. Delhaize, S. Craig, C.D. Beaton, R.J. Bennet, V.C. Jagadish, P.J. Randall, *Plant Physiol*., **1993a**, 103, 685–693,
[213] P.R. Ryan, E. Delhaize, P.J. Randall, *Planta*., **1995 b**, 196, 103–110.
[214] E. Delhaize, P.R. Ryan, P.J. Randall, *Plant Physiol.*, **1993b**, 103, 695–702.
[215] M. Tesfaye, S. Temple, D. Allan, C. Vance, D. Samac, *Plant Physiol.*, **2001**, 127, 1836–1844.
[216] M.A. Piňeros, J.V. Magalhaes, V.M. Carvalho Alves, L.V. Kochian, *Plant Physiol*., **2002**, 129, 1194–1206.
[217] J.F. Ma, P.R. Ryan, E. Delhaize, *Trends Plant Sci*., **2001**, 6, 273-278.
[218] X. Li, J. Ma, H. Matsumoto, *Plant Physiol*., **2000**, 123, 1537–1544.
[219] H. Osawa, H. Matsumoto, *Plant Physiol*., **2001**,126, 411–420.
[220] W. Zhang, P. Ryan, S. Tyerman, *Plant Physiol*., **2001**, 125, 1459–1472.
[221] M. Kollmeier, P. Dietrich, C. Bauer, W. Horst, R. Hedrich, *Plant Physiol*., **2001**, 126, 397–410.
[222] S. Ishikawa, T. Wagatsuma, R. Sasaki, P. Ofei-Manu, *Soil Sci Plant Nutr*., **2000**, 46, 751–758.
[223] H. Marschner, Mineral nutrition of Higher Plants (2nd Ed), Academic Press, London, **1995**.
[224] G.J. Taylor, In: H. Sigel, A. Sigel (Ed), Metal Ions in Biological Systems, 24, Marcel Dekker, New York, **1988**, 165-198.
[225] T. Wagatsuma, S. Ishikawa, T. Akimoto, K. Tawaraya, P. Ofei-Manu, In: W.J. Horst, M.K. Schenk, A. Bürkert, N. Claassen (Eds), Plant Nutrition-Food Security and Sustainability of Agroecosystems, Kluwer Acad. Publ, Dordrecht, **2001**, 454-455.
[226] P. Ofei-Manu, T. Wagatsuma, S. Ishikawa, K. Tawaraya, *Soil Sci Plant Nutr*., **2001**, 47, 359-375.
[227] C.T. Driscoll, W.D. Schecher, In: H. Sigel (Ed), Metal Ions in Biological Systems, 24, Marcel Dekker, New York, **1988**, 59-122.
[228] W.J. Horst, A. Wagner, H. Marschner, *Z Pflanzenphysiol*, **1982**, 105, 435-444.
[229] V. Puthota, R. Cruz-Ortega, J. Jonson, J. Ownby, In: R.J. Wright, V.C. Baligar and R.P. Murrmann (Eds), Plant-Soil Interactions at Low pH, Kluwer Academic Publ., Dordrecht, **1991**, 779-789.
[230] S.C. Miyasaka, C. Hawes, *Plant Physiol*., **2001**, 125, 1978-1987.
[231] G. Delisle, M. Champoux, M. Houde, *Plant Cell Physiol.*, **2001**, 42, 324-333.
[232] K. Takeda, M. Kariuda, H. Itoi, *Phytochemistry*, **1985**, 24, 2251-2254.
[233] J.F. Ma, S. Hiradate, K. Nomoto, T. Iwashita, H. Matsumoto, *Plant Physiol.*, **1997a,** 113, 1033–1039.
[234] J. Ma, S. Zheng, H. Matsumoto, S. Hiradate, *Nature*, **1997b**, 390, 569–570.
[235] S. Zheng, J. Ma, H. Matsumoto, *Plant Physiol*., **1998**, 117, 745–751.
[236] T. Watanabe, M. Osaka, T. Tadano, Proceedings Int. Symposium on Impact of Potential Tolerance of Plants on the Increased Productivity under Aluminum Stress. Kurashiki, Japan, **2000**, 47-50.
[237] R. Shen, J. Ma, M. Kyo, T. Iwashita, *Planta*, **2002**, 215, 394–398.
[238] E. Delhaize, P.R. Ryan, D.M. Hebb, Y. Yamamoto, T. Sasaki, H. Matsumoto, Proc Natl Acad Sci. USA., **2004**, 101, 15249-15254.
[239] C.D. Foy, Commun. *Soil Sci. Plant Anal*., **1988**, 19, 959-987.
[240] D.L. Jones, L.V. Kochian, S. Gilroy, *Plant Physiol*., **1998**, 116, 81-89.
[241] Z. Rengel, D.L. Robinson, *Agron. J*., **1989**, 81, 208-215.
[242] J.W. Huang, D.M. Pellet, L.A. Papernik, L.V. Kochian, *Plant Physiol.*, **1996**, 110, 561-569
[243] G.J. Taylor, F.P.C. Blamey, D.G. Edwards, *Physiol. Plant*, **1998**, 104, 183-194.
[244] K.M. Lukaszewski, D.G. Blevins, *Plant Physiol*., **1996**, 112, 1135-1140.
[245] M.E. LeNoble, D.G. Blevins, R.E. Sharp, B.G. Cumbie, *Plant. Cell Environ,* **1996**, 19, 1132-1142.

[246] K.M. Cocker, D.E. Evans, M.J. Hodson, *Physiol. Plant*, **1998**, 104, 608-614.
[247] D.B. Lazof, M.J., Holland, *Aust. J. Plant Physiol*., **1999**, 26, 147-157.
[248] D.A. Samac, M., Tesfaye, *Plant Cell Tissue Organ Cult*, **2003**, 75, 189-207.
[249] M. DallAgnol, J.H. Bouton, W.A. Parrott, *Crop Sci*., **1996**, 36, 64-70.
[250] W.A. Berzonsky, *Genome*, **1992**, 35, 689-693.
[251] L.V. Kochian, O.A. Hoekenga, M.A. Pineros, *Annu. Rev. Plant Biol*., **2004**, 55, 459-493.
[252] T. Sasaki, Y. Yamamoto, B. Ezaki, M. Katsuhara, S.J. Ahn, P.R. Ryan, E. Delhaize, H. Matsumoto, *Plant J*., **2004**, 37, 645-653.
[253] E. Delhaize, P.R. Ryan, D.M. Hebb, Y. Yamamoto, T. Sasaki, H. Matsumoto, Proc. Natl. Acad. Sci. USA, **2004**, 101,15249-54.
[254] U. Basu, A. Basu, G.J. Taylor, *Plant Physiol.*, **1994**, 106,151-158.
[255] D.M. Pellet, D.L. Grunes, L.V. Kochian, *Planta*, **1995**, 196, 788-795.
[256] H. Koyama, A. Kawamura, T. Kihara, T. Hara, E. Takita, D. Shibata, *Plant Cell Physiol*., **2000**, 41, 1030-1037.
[257] M. Tesfaye, S.J. Temple, D.L. Allan, C.P. Vance, D.A. Samac, *Plant Physiol.*, **2001**, 127, 1836-44.
[258] B. Ezaki, R.C. Gardner, Y. Ezaki, H. Matsumoto, *Plant Physiol*., **2000**, 122, 657-665.
[259] E. Delhaize, D.M. Hebb, P.R. Ryan, *Plant Physiol*., **2001**, 125, 2059-2067.
[260] E.D. Mariano, W.G. Keltjens, *Plant Soil*, **2003**, 256, 469-479.
[261] P. Wenzl, G.M. Patino, A.L. Chaves, J.E. Mayer, I.M. Rao, *Plant Physiol*., **2001**, 125, 1473-1484.
[262] H. Nian, Z.M. Yang, H. Huang, X.L. Yan, H. Matsumoto, *J. Plant Nutr*., **2004**, 27, 2047-2066.
[263] T. Watanabe, M. Osaki, Commun. *Soil Sci. Plant. Anal*., **2002**, 33, 1247-1260.
[264] M. Ishitani, I. Rao, P. Wenzl, S. Beebe, J. Tohme, *Field Crops Res.*, **2004**, 90, 35-45.
[265] J.P. Grime, J.S. Hodgson, In: I.H. Rorison (Ed.), Ecological Aspects of the Mineral Nutrition of Plants, Blackwell Series, Oxford, **1969**, 381–397.
[266] W.A. Jackson, In: R.W. Pearson, F. Adams (Ed.), Soil Acidity and Liming, Am. Soc. of Agron, Madison, USA, **1967**, 43–124.
[267] E.L. Klimashevskii, Yu. A. Markova, S.M. Zyabkina, G.K. Zirenki, T.E. Zolotukhin, S.E. Pavolva, Fiziol. Biokhim. Kul't. Rust, **1976**, 8, 396–401.
[268] D.O. Huett, R.C. Menary, *Aust. J. Agric. Res*., **1980**, 31, 749–761.
[269] S.J. Henning, Ph.D. thesis, Oregon State Univ., Corvallis, Oregon, USA, **1975**.
[270] Wallace, E.M. Rommey, *Commun. Soil Sci. Plant Anal*., **1977**, 8, 791–794.
[271] E.E. Malavolta, L.M. Coutinho, G.C. Vinni, N.V. Alejo, N.J. Novacs, V.L. Furlani Netto, An. Esc. Super. Agric. Luiz de Quiciroz Univ. Sao Paulo, **1979**, 36, 173–202.
[272] K.K. Duncan, *J. Plant Nutr*, **1981**, 4, 21–32.
[273] T. Wagatsuma, *Soil Sci. Plant Nutr*, **1983**, 29, 323–333.
[274] T. Wagatsuma, *Soil Sci. Plant Nutr*, **1983**, 29, 499–515.
[275] G. Naidoo, J.D. McSteward, R.J. Lewis, *Agron. J*., **1978**, 70, 489–492.
[276] G. Niedziela, A. Aniol, *Acta Biochem. Pol.*, **1983**, 30, 99–105.
[277] K. Ohki, *Plant and Soil*, **1987**, 98, 195–202.
[278] W.S. Ligon, W.H. Pierre, *Soil Sci.*, **1932**, 34, 307–321.
[279] C. Nalewajko, B. Paul, Can. J. Fish *Aquat. Sci.*, **1985**, 42, 1946–1953.
[280] Pettersson, L. Halboom, B. Bergman, *Plant Physiol*., **1988**, 86, 112–116.
[281] Y. Husaini, L.C. Rai, *J. Plant Physiol*., **1992**, 139, 703–707.
[282] Y. Husaini, L.C. Rai, N. Mallick, *BioMetals*, **1996**, 9, 277–283.
[283] M.C.C. DeGraaf, R. Bobbink, P.J.M. Verbeek, J.G.M. Roelofs, *Water Air Soil Pollut*., **1997**, 98, 229–239.
[284] W.J. Horst, N. Schmohl, M. Kollmeier, F. Baluska, M. Sivaguru, *Plant and Soil*, **1999**, 215, 163–174.
[285] R. Takabatake, T., Shimmen, *Plant Cell Physiol.*, **1997**, 38, 1264-1271.
[286] B. Gunsé, Ch. Poschenrieder, J. Barceló, *Plant Physiol*., **1997**, 113, 595-602.

[287] D.B. Lazof, J.G. Goldsmith, T.W. Rufty, R.W. Linton, *Plant Physiol*., **1994**, 106, 1107-1114.
[288] Z. Rengel, *New Phytol*, **1996**, 134, 389-406.
[289] D. Miller, W. Hable, J. Gottwald, M. Ellard-Ivey, T. Demura, T. Lomax, N. Carpita, *Plant Cell*, **1997**, 9, 2105-2117.
[290] P.B. Larsen, C.Y. Tai, L.V. Kochian, S.H. Howell, *Plant Phy soil.*, **1996**, 110, 743-751.
[291] K. Turnau, *Biol. Bull. Poznañ, (Suppl.)*, **1996**, 33, 65.
[292] J. Lopez-Bucio, M.F. Nieto-Jacobo, V. Rramí'frez-rodrí'fguez, Herrera-Estrella, L., *Plant Sci*., **2000**, 160, 1-13.
[293] J.F. Ma, P.R. Ryan, E. Delhaize, *Trends in Plant Sci.*, **2001**, 6, 273-278.
[294] D.L. Jones, *Plant Soil*, **1998**, 205, 25-44.
[295] P. Guo, G. Bai, B. Carver, R. Li, A. Bernardo, M. Baum, *Mol. Genet. Gen.*, **2007**, 277, 1-12.
[296] S. Jansen, M. Broadley, E. Robbrecht, E. Smets, *Bot. Rev*., **2002**, 68, 235-269.
[297] J.D. Cunningham, D.R. Keenay, J.A. Ryan, *J. Environ. Qual*., **1975**, 4, 460–462.
[298] C.D. Foy, In: E.W. Carson (Ed.), The Plant Root and its Environment, Charlottesville, Univ. Press, Virginia, **1974**, 601–642.
[299] H.W. Woolhouse, In: I.H. Rorison (Ed.), Ecological aspects of the mineral nutrition of plants, Blackwell Publishers, Edinburg, Oxford, **1969**, 357–380.
[300] R.E. Johnson, W.A. Jackson, *Soil Sci. Soc. Am. Proc*., **1964**, 28, 381–386.
[301] J.R. Cumming, R.T. Eckert, L.S. Evans, *Can. J. Bot*., **1985**, 63, 1099–1103.
[302] W.G. Keltjens, P.S.R. van Ulden, *Plant and Soil*, **1987**, 104, 227–234.
[303] Cakmak, W.J. Horst, *Physiol. Plant*, **1991**, 83, 463–468.
[304] G.L. Plucinska, P. Karolewski, *Acta Soc. Bot. Pol.*, **1994**, 63, 167–171.
[305] G.L. Plucinska, H. Ziegler, *Photosynthetica*, **1996**, 32, 141–144.
[306] J. Pavlovkin, I. Mistrik, *Biologia*, **1999**, 54, 473–479.
[307] B. Gunse, C. Poschenrieder, J. Barcelo, *Environ. Exp. Bot*., **2000**, 43, 73–81.