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This paper is dedicated to the memory of Professor Jacek Augustyniak

Review

Effect of aluminium on plant growth and metabolism

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Aluminium toxicity is one of the major factors that limit plant growth and development in many acid soils. Root cells plasma membrane, particularly of the root apex, seems to be a major target of Al toxicity. However, strong interaction of Al^{3+}, the main Al toxic form, with oxygen donor ligands (proteins, nucleic acids, polysaccharides) results in the inhibition of cell division, cell extension, and transport. Although the identification of Al tolerance genes is under way, the mechanism of their expression remains obscure.

Soil chemical factors that limit root growth in acid soils, diminish crop production, include Al, Mn and various cations, and also deficiency or unavailability of Ca, Mg, P, Mo, and Si. These effects are further complicated by interactions of Al with other ions in different plant genotypes and under stress conditions (Foy, 1992).

Cytotoxicity of Al has been well documented in plants (Delhaize & Ryan, 1995; Horst *et al.*, 1999; Kollmeier *et al.*, 2000; Marienfeld *et al.*, 2000). It is generally known that plants grown in acid soils due to Al solubility at low pH have reduced root systems and exhibit a variety of nutrient-deficiency symptoms, with a consequent decrease in yield. In many countries with naturally acid soils, which constitute about 40% of world arable soil (LeNoble *et al.*, 1996), Al toxicity is a major agricultural problem, and is intensively studied in plant systems.

The effects of aluminium on plant growth, crop yield, uptake and nutrients distribution in vegetative and reproductive parts are still

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not fully understood. This review discusses recent information on aluminium toxicity with an emphasis on plant response to Al stress.

CHEMICAL NATURE OF ALUMINIUM AND ITS OCCURENCE IN PLANTS

Al is present in water, soil and air but most of it is incorporated into aluminosilicate soil minerals and only very small quantities (at submicromolar levels) appear in soluble forms capable of influencing biological systems (May & Nordstrom, 1991). Different forms of aluminium occur in soil solution: $Al(OH)^{2+}$ and $Al(OH)_2^{+}$ at pH 4–5, Al^{3+} at pH 5.5–7, and $Al(OH)_4^{-}$ at pH 7–8. Other complex ions $AlO_4Al_{12}(OH)_{24}(H_2O)_{12}^{7+}$ (Al_{13}) and Al^{3+} are almost certainly toxic, but no rhizotoxicity has been detected for $AlSO_4^{+}$ and $Al(SO_4)_2^{-}$ or Al-F (e.g. AlF^{2+} and AlF_2^{+}). The status of $Al(OH)^{2+}$ and $Al(OH)_2^{+}$ is uncertain although experimental results have appeared indicating Al-OH toxicity (Kinraide, 1997). 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) opinion toxicity has been convincingly demonstrated only for Al_{13} and Al^{3+}.

Intensification of the process of Al compounds solubilization is connected with the degree of soil acidification caused by the washing out of alkaline metals ions (Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}) from the soil and a decrease in the pH of soil solutions.

Al ions translocate very slowly to the upper parts of plants (Ma *et al.*, 1997a). Most plants contain no more than 0.2 mg Al g^{-1} 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 g^{-1} dry mass in old leaves (Matsumoto *et al.*, 1976). Approximately 400 species of terrestrial plants, belonging to 45 families, have so far been identified as hyperaccumulators of various toxic metals (Baker *et al.*, 2000).

VISUAL AND CELLULAR SYMPTOMS OF ALUMINIUM TOXICITY

Inhibition of root and shoot growth is a visible symptom of Al toxicity. The earliest symptoms concern roots. Shoots in contrast to the situation observed for Mn toxicity are less affected (Chang *et al.*, 1999). Root stunting is a consequence of Al-induced inhibition of root elongation. Roots are usually stubby and brittle and root tips and lateral roots become thick and may turn brown (Mossor-Pietraszewska *et al.*, 1997). Such roots are inefficient in absorbing both nutrients and water. Young seedlings are more susceptible than older plants. Al apparently does not interfere with seed germination, but does impair the growth of new roots and seedling establishment (Nosko *et al.*, 1988).

The common responses of shoots to Al include: cellular and ultrastructural changes in leaves, increased rates of diffusion resistance, reduction of stomatal aperture, decreased photosynthetic activity leading to chlorosis and necrosis of leaves, total decrease in leaf number and size, and a decrease in shoot biomass (Thornton *et al.*, 1986).

Blancaflor *et al.* (1998) have studied Al-induced effects on microtubules and actin microfilaments in elongating cells of maize root apices, and related the Al-induced growth inhibition to stabilization of microtubules in the central elongation zone. With respect to growth determinants (auxin, gibberelic acid and ethylene), Al apparently interacts directly and/or indirectly with the factors that influence organization of the cytoskeleton, such as cytosolic levels of Ca^{2+} (Jones *et al.*, 1998), Mg^{2+} and calmodulin (Grabski *et al.*, 1998), cell-surface electrical potential (Takabatake & Shimmen, 1997), callose formation (Horst *et al.*, 1997), and lipid composition of the plasma membrane (Zhang *et al.*, 1997).

Recently, Yamamoto *et al.* (2001) have shown that peroxidation of lipids is a relatively early event following Al exposure and appears to partly influence the Al-induced production of callose, but not the Al-induced inhibition of root elongation. By comparison, the loss of plasma membrane integrity is a relatively late event and seems to be a consequence of the cracks in the root formed by the inhibition of root elongation.

SIGNAL TRANSDUCTION PATHWAY

Stress recognition activates signal transduction pathways that transmit information within individual cells and throughout the plant. These pathways lead to the expression of genes and resultant modification of molecular and cellular processes. In plants, there is little research on Al signalling mediated by second messengers.

Experimental data suggest the existence of a cascade pathway under Al stress. An increase in cytoplasmic Ca^{2+} level in wheat root apexes may be related to the expression of Al toxicity (Zhang & Rengel, 1999). Osawa & Matsumoto (2001) suggested that protein phosphorylation is required for the signal transduction in Al-activated malate efflux and that malate could pass through organic anion-specific channels. Because of its rapidness and specificity to Al, Al-induced malate efflux is a useful system for studying how the Al signal is transmitted into the cell that expresses physiological responses underlying Al-toxicity or tolerance.

Protein phosphorylation plays an important role in the regulation of various biological activities in plants and provides a signal transduction pathway for mediating extracellular stimuli into cells. The mitogen-activated protein kinase (MAPK) cascade is one of the major pathways for transmitting signals such as light, temperature stress, mechanical stress, wounding, pathogen elicitors, drought, salt, hormone signalling, nutrient deprivation and Al stress (Ligterink & Hirt, 2001; Osawa & Matsumoto, 2001). Osawa & Matsumoto (2001) demonstrated using various inhibitors of protein phosphorylation/dephosphorylation that the inhibition of Al-responsive malate efflux in wheat is associated with protein phosphorylation, possibly related to an organic anion-specific channel or its upstream signalling by a K-252a (a broad range inhibitor of protein kinases)-sensitive protein kinase. Using in-gel kinase assay with myelin basic protein (MBP) as an artificial substrate, these authors observed activation of a 48-kDa protein kinase in the root apex treated with 200 μM Al. The activity of this kinase was elevated from 0.5 to 5 min after the addition of Al, and it diminished after 5 min. This suggested that transient activation of the 48-kDa protein kinase might be involved in the early physiological response to Al. The activity of the 48-kDa kinase was approximately 10-fold higher after the treatment with Al than without Al, and the Al-induced activation was lost within 5 min. Al transiently activates this protein kinase quickly enough to precede the initiation of malate efflux. This protein kinase phosphorylated MBP, indicating that this kinase may be categorized in the MAP kinase group.

In yeast, expression of a MAP kinase gene complemented Al tolerance in an Al-sensitive mutant, indicating that MAP kinase may be associated with the expression of physiological responses involved in Al-resistance (Schott & Gardner, 1997). Although it remains unknown whether the 48-kDa protein kinase is directly involved in the pathway for malate efflux, it appears that this 48-kDa MAP kinase plays a significant role in the transduction of the Al signal and expression of some physiological responses in the root apex of Al-resistant wheat (Osawa & Matsumoto, 2001).

Molecular genetic approaches involving the use of reporter gene expression have been explored and appear very promising for dissecting stress signal transduction pathways in

plants. Knight & Knight (2001) discuss two aspects of abiotic stress signalling pathways networks, namely cross-talk and specificity. Cross-talk is an instance of two signalling pathways from different stressors that converge. Specificity of a signalling pathway enables distinction between two or more possible outcomes and thus links a particular stimulus exclusively to a particular end response and not to any other. Both cross-talk and specificity can occur within a particular pathway.

Molecular and biochemical studies suggest that abiotic stress signalling in plants involves receptor-coupled phosphorelay, phosphoinositol-induced Ca^{2+} changes, mitogen-activated protein kinase cascades and transcriptional activation of stress-responsive genes (Xiong & Zhu, 2001). In addition, protein posttranslational modifications and adapter or scaffold-mediated protein–protein stress interactions are also important in abiotic signal transduction.

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 (Takabatake & Shimmen, 1997). 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 (Gunsé *et al.*, 1997).

Although a primary response to Al has been localized to root apex (Kochian, 1995; Taylor, 1995; Sivaguru *et al.*, 1999), 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 (Lazof *et al.*, 1994). However, Horst (1995) and Rengel (1996) focused their attention on the apoplast. Recent findings on the cell wall – plasma membrane – cytoskeleton continuum (Miller *et al.*, 1997) 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 (Marienfeld *et al.*, 2000). The major portion of absorbed Al is localised in apoplast ranging from 30–90% of the total tissue Al content (Rengel,1996). 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 (Rengel, 1996; Silva *et al.*, 2000; Taylor *et al.*, 2000) it seems that most of the Al accumulates in the cell wall. Rengel & Reid (1997) 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) 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 (β-1,3-glucan) formation is a sensitive marker for genotypic Al toxicity (Horst *et al.*, 1997). 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) 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 (Sivaguru *et al.*, 2000).

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 (Turnau, 1996).

ALUMINIUM TOXICITY AND TOLERANCE

The current state of knowledge concerning Al toxicity and differential Al tolerance in plants has been covered in several reviews and new papers are constantly being published, indicating the importance of the problem (Roy *et al.*, 1988; Taylor, 1991; 1995; Kochian, 1995; Cocker *et al.*, 1998; Matsumoto, 2000; Osawa & Matsumoto, 2001).

Al is reported to interfere with cell division in root tips and lateral roots, increase cell wall rigidity by crosslinking pectins, reduce DNA replication by increasing the rigidity of the double helix, fix P in less available forms in soils and on plant root surfaces, decrease root respiration, interfere with a number of enzymes, decrease deposition of cell wall polysaccharides, decrease production and transport of cytokinins, modify structure and function of plasma membranes, reduce water uptake, and interfere with the uptake, transport, and metabolism of several essential nutrients. In cowpea a toxicity treshold was observed at 0.1 μM Al and complete growth inhibition at > 40 μM (Taylor *et al.*, 1998).

Mechanisms of Al tolerance have been broadly classified as those which prevent Al uptake by roots and those which detoxify Al already accumulated in the cell (Rengel, 1996). Plant species and genotypes within species vary widely in the tolerance to mineral stresses frequently combined with tolerance to other stresses. For example, Al-tolerant plants may be more drought tolerant and require lower inputs of lime and P fertilizer than less tolerant genotypes (Little, 1988). The Al tolerance of tribe *Triticeae* generally follows the order: rye > triticale > wheat > barley (Aniol & Gustafson, 1984). However, the mechanisms responsible for the high Al tolerance in both rye and triticale are not understood (Ma *et al.*, 2000).

Although several mechanisms have been proposed for Al tolerance (Delhaize & Ryan, 1995; Kochian, 1995; Ma *et al.*, 2000; Matsumoto, 2000; Osawa & Matsumoto, 2001), recently secretion of organic acids from roots has repeatedly been shown to play an important role in the external and the internal Al neutralization (Ma, 2000; Ma *et al.*, 1997b). Some organic acids form a stable complex with ionic Al, thereby preventing binding of Al with intra- and intercellular compounds in roots (Li *et al.*, 2000).

The major organic anion released in response to Al is malate in wheat (Ryan *et al.*, 1995), citrate in leguminous crops (Yang *et al.*, 2000), both citrate and malate in triticale (Ma *et al.*, 2000), and oxalate in buckwheat (Ma *et al.*, 1997a). Osawa & Matsumoto (2001) found that root apex of Al-resistant wheat cv. Atlas lost organic anions citrate and succinate as well as malate immediately after exposure to Al. A recent molecular approach established that citrate efflux is enhanced by overproduction of citrate in transgenic tobacco and papaya plants (de la Fuente *et al.*, 1997). However, in genetically Al-resistant plants, organic anion efflux is highly specific to Al. Making a resistant plant which can release or-

ganic anions only in the presence of Al would be a preferable strategy in preventing excess carbon loss (Osawa & Matsumoto, 2001).

Many pieces of evidence show that the increase of organic acid release is induced 0–12 h after exposure to Al. Based on previous studies on Al-induced release of organic acids, two patterns can be classified. One is that there is no discernible delay between the addition of Al and the onset of release of organic acids. For example, in an Al-tolerant genotype of wheat, ET3, Al-stimulated secretion of malate from both intact roots and excised root apexes was observed within 20 min after exposure to Al (Ryan *et al.*, 1995). Similarly, Osawa & Matsumoto (2001) showed that malate efflux started 5 min after the addition of Al in wheat. In buckwheat, the secretion of oxalic acid occurred within 30 min after the exposure to Al (Ma *et al.*, 1997a). The efflux rate in this pattern is the same at any time after the exposure to Al. The other pattern is that there is a marked lag phase between the addition of Al and the onset of organic acid release. In *Cassia tora*, secretion of citrate in response to Al was increased after 4 h (Ma, 2000). In an Al-resistant cultivar of maize, a considerable lag phase before maximal citrate efflux is observed (Pellet *et al.*, 1995). Therefore, the efflux rate of organic acids in this pattern varies with the time after exposure to Al, being initially low and high at a later time. The secretion pattern observed in triticale (ST2) belongs to the latter pattern (Ma *et al.*, 2000). Since there are marked differences in the lag time required for the induction of efflux of organic anion between plant species, the regulatory mechanism of organic anion efflux in response to Al stress is still lacking.

A large number of processes could contribute to Al exclusion from the meristematic cell region, including increased secretion of mucilage (Crawford & Wilkens, 1997), polypeptides (Basu *et al.*, 1999), inorganic phosphate (Pellet *et al.*, 1996), and organic acids (Larsen *et al.*, 1998). The involvement of rhizosphere alkalinization (Degenhardt *et al.*, 1998), efflux of Al from the symplast (Ezaki *et al.*, 1999), and decreased cell-surface negativity (Wagatsuma & Akiba, 1989) are also possible.

Different mechanisms seem to be involved in the secretion patterns of organic acids (Delhaize & Ryan, 1995). Organic acids have been suggested to be secreted through an anion channel located on the plasma membrane (Ryan *et al.*, 1995; Piñeros & Kochian, 2001); the rapid secretion of organic acids upon Al exposure suggests that gene induction is not involved. However, gene induction may be involved in the cases of a lag phase in the excretion of organic acids. The R genes may be related to the biosynthesis of organic acids, to the formation of anion channels on the plasma membrane and/or tonoplast, or to the transport of, e.g., citrate from mitochondria (Ma *et al.*, 2000). According to Delhaize & Ryan (1995) activation of the anion channel by Al might be due either to: (i) a direct action on the channel protein, causing a change in its conformation and thus increasing its mean opening time or conductance, (ii) interaction with specific receptor of the membrane or (iii) entrance to cytoplasm and altering channel protein through a signal transduction pathway. Al-specific carrier protein has not been found.

Organic acids extracted from roots have different ability to precipitate Al. Hue *et al.* (1986) give the following list of acids in the decreasing order of the ability to precipitate Al: oxalic acid > citric acid > malic acid > succinic acid.

P_i efflux from roots may be considered another potential Al-resistance mechanism in plants *via* the formation of Al–P_i complexes in the rhisosphere (Taylor, 1991).

The mechanism responsible for decreased cell division in root exposed to Al is not clear, but a direct effect associated with Al binding to DNA or other nuclear material cannot be excluded (Matsumoto, 1991; Silva *et al.*, 2000).

Al-sensitive soybean seedlings exposed to Al^{3+} solution of low concentration (1.45 μM)

within 30 min accumulate in nuclei higher amounts of Al than resistant genotypes (Silva *et al.*, 2000). This result is consistent with other literature data concerning higher Al concentrations.

Al stress results in a decrease in the total adenine nucleotide level and the adenylate pool sizes. This may lead to a change of energy state (Lorenc-Plucińska & Ziegler, 1996). Hamilton *et al.* (2001) described on induction of vacuolar ATPase and mitochondrial ATP synthase by Al in an Al-resistant cultivar of wheat. These enzymes were reported to play a role in Al resistance.

Proline is thought to play a cardinal role as an osmoregulatory solute in plants subjected to hyperosmotic stresses, primarily drought and soil salinity. Indeed, the accumulation of this imino acid may be part of a general adaptation to adverse environmental conditions, having been documented in response to several stresses including exposure to Al (Mossor-Pietraszewska, unpublished data). Proline stabilizes cellular structures as well as scavenges free radicals (Hare & Cress, 1997).

The possibility that Al is detoxified by formation of stable metal–protein complexes has been raised. Many authors showed inducible synthesis of a cytosolic Al binding protein (Basu *et al.*, 1999; Snowden *et al.*, 1995; Somers & Gustafson, 1995; Wu *et al.*, 2000).

Although productions of phytochelatins confers heavy metal tolerance in plants (Cobbet, 2000), however, phytochelatins do not contribute to Al tolerance, most likely because they do not bind Al effectively (Larsen *et al.*, 1996). Al tends to bind to the phosphate or carboxyl groups rather than to –SH groups characteristic for chelatins (Gunsé *et al.*, 1997). However, Snowden *et al.* (1995) and Wu *et al.* (2000) suggested that plant metallothionein-like protein and phytochelatins may play a role in Al tolerance. An Al-induced polypeptide (TA1-18) was identified in wheat that shows homology to a pathogenesis related (PR) protein (Cruz-Ortega & Ownby, 1993). Basu *et al.* (1999) observed differences between wheat cultivars in the amount of polypeptide exuded in response to Al stress, and showed enhanced accumulation of 12-, 23-, and 43.5-kDa polypeptides in the Al-resistant cv. Maringa. A greater association of Al with high molecular mass fraction (including the Al-induced polypeptides) from Al-resistant cultivars suggested that these polypeptides may have significant Al-binding capacity, and co-segregate with Al resistance. Thus it is hypothesized that specific proteins as well as the exudation of organic acids may be involved in Al-stress defence mechanisms.

Among the various effects induced by Al in biological systems, either *in vitro* or *in vivo*, is the destruction of membrane polyunsaturated fatty acids depending on oxygen free radicals (AOS). Different types of environmental stress commonly result in enhanced generation of AOS. Al stress and oxidative stress are strongly linked in plants. Ezaki *et al.* (2000) described the construction of transgenic *Arabidopsis* lines expressing nine Al-induced genes: 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. Two of these genes, *AtBCB* and *parB*, and a peroxidase gene from *Arabidopsis* (*AtPox*) also conferred increased resistance to oxidative stress. These authors conclude that these Al-induced genes can protect against Al toxicity, and also provide genetic evidence for a link between Al stress and oxidative stress in plants.

CHANGES IN GENE EXPRESSION DURING ALUMINIUM STRESS

Plants have both a constitutive (present in most phenotypes) and an adaptive (present only in tolerant phenotypes) mechanism for coping with elevated metal concentrations, both under genetic control.

Over 20 genes induced by Al stress have been isolated from a range of plant species, including wheat (Aniol, 1995; Delhaize *et al.*, 1999), rye (Gallego & Benito, 1997), rice (Nguyen *et al.*, 2001), soybean (Bianchi-Hall, *et al.*, 1998), tobacco (Ezaki *et al.*, 1997), and *Arabidopsis* (Richards *et al.*, 1998). Most of the Al-induced genes seem to be general stress genes that are induced by a range of different plant stresses. It has been proposed that there are common mechanisms for gene induction by Al and oxidative stress. By analogy with other stress genes, these genes may play a role in protecting cells against Al stress.

Genetic variation in the response to Al toxicity has been found not only among plant species but also among cultivars within species (Aniol & Gustafson, 1984). In hexaploid wheat, major genes influencing tolerance to Al are located on the short arm of chromosome 5A and the long arms of chromosomes 2D and 4D (Aniol, 1995; Aniol & Gustafson, 1984). In rye these genes are located on chromosomes 3R, 4R, and the short arm of 6R (Aniol & Gustafson, 1984). Gallego & Benito (1997) found that Al tolerance is controlled by at least two major dominant and independent alleles in rye: *Alt1* and *Alt3*, located on chromosomes 4R and 6R. DNA markers linked to Al tolerance loci were also selected in rye (Gallego *et al.*, 1998). In triticale genes required for the complete expression of Al tolerance are located on the short arm of chromosome 3R (Ma *et al.*, 2000). These genes are also necessary for the release of organic acids.

The use of restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSRs or microsatellites), amplified fragment length polymorphisms (AFLPs), and various other molecular systems has made possible a tremendous advancement in the production of high-density linkage maps and in the power of utilizing linkage studies for localizing genes in plants (Gallego *et al.*, 1998; Ma *et al.*, 2001; Nguyen *et al.*, 2001; Wu *et al.*, 2000). Developing maps based on molecular markers is one of the steps in the genomic studies of important plants. Several techniques have been used to create genetic and physical maps in plants.

A molecular linkage map, together with 104 AFLP markers and 103 RFLP markers, was constructed to map quantitative trait loci (QTLs) and epistatic loci for Al tolerance based on the segregation for relative root length (RRL) in rice (Wu *et al.*, 2000).

The identification of DNA markers diagnostic of Al tolerance can accelerate the development of cultivars that can remain productive even under Al stress, and may be the starting point for identifying the specific genes responsible for differences in the response of plant genotypes to toxic Al levels.

Efforts have been made to obtain Al-tolerant plants using biotechnological techniques, e.g. transgenic rice (Wu *et al.*, 2000), tobacco and papaya (de la Fuente *et al.*,1997), and *Arabidopsis* (Ezaki *et al.*, 2000).

INTERACTIONS OF ALUMINIUM 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 (Foy, 1988). 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 (Taylor, 1988). 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 (Jones *et al.*, 1998). There is a wealth of information indicating

that Ca and Mg accumulation in plants is depressed by Al much more significantly than the uptake of other important mineral nutrients (Rengel & Robinson, 1989). 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 (Huang *et al.*, 1996), 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 (Ślaski *et al.*, 1996). 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) 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 μM) 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) under physiologically and environmentally relevant conditions. Their data demonstrate 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 (Lukaszewski & Blevins, 1996). LeNoble *et al.* (1996) 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 (Cocker *et al.*, 1998).

CONCLUDING REMARKS

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. Aniol, A. (1995) Physiological aspects of aluminum tolerance associated with long arm of chromosome 2D of the wheat (*Triticum aestivum* L.) genome. *Theor. Appl. Genet.* 91, 510-516.
2. Aniol, A. & Gustafson, J.P. (1984) Chromosome location of genes controlling aluminum tolerance in wheat, rye and triticale. *Can. J. Genet. Cytol.* 26, 701-705. MEDLINE
3. Baker, A.J.M., McGrath, S.P., Reeves, R.D. & Smith, J.A.C. (2000) Metal hyperaccumulator plants: A review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils; in *Phytoremediation of Contaminated Soil and Water* (Terry, N. & Banuelos, G., eds.) pp. 85-107, Lewis Publisher, Boca Raton.
4. Basu, U., Good, A.G., Aung, T., Slaski, J.J., Basu, A., Briggs, K.G. & Taylor, G.J. (1999) A 23-kDa, root exudate polypeptide co-segregates with aluminum resistance in *Triticum aestivum*. *Physiol. Plant.* 106, 53-61.
5. Bianchi-Hall, C.M., Carter, T.E., Rufty, T.W., Arellano, C., Boerma, H.R., Ashley, D.A. & Burton, J.W. (1998) Heritability and resource allocation of aluminum tolerance derived from soybean PI 416937. *Crop Sci.* 38, 513-522.
6. Blancaflor, E.B., Jones, D.L. & Gilroy, S. (1998) Alterations in the cytoskeleton accompany aluminum-induced growth inhibition and morphological changes in primary roots of maize. *Plant Physiol.* 118, 159-172. MEDLINE
7. Chang, Y.-C., Yamamoto, Y. & Matsumoto, H. (1999) Accumulation of aluminium in the cell wall pectin in cultured tobacco (*Nicotiana tabacum* L.) cells treated with a combination of aluminium and iron. *Plant Cell Environ.* 22, 1009-1017.
8. Cobbett, C.S. (2000) Phytochelatins and their roles in heavy metal detoxification. *Plant Physiol.* 123, 825-832. MEDLINE
9. Cocker, K.M., Evans, D.E. & Hodson, M.J. (1998) The amelioration of aluminium toxicity by silicon in higher plants: Solution chemistry or an *in planta* mechanism? *Physiol. Plant.* 104, 608-614.
10. Crawford, S.A. & Wilkens, S. (1997) Ultrastructural changes in root cap cells of two Australian native grass species following exposure to aluminum. *Aust. J. Plant Physiol.* 24, 165-174. MEDLINE
11. Cruz-Ortega, R. & Ownby, J.D. (1993) A protein similar to PR (pathogenesis related) proteins is elicited by metal toxicity in wheat roots. *Physiol. Plant.* 89, 211-219.
12. Degenhardt, J., Larsen, P.B., Howell, S.H. & Kochian, L.V. (1998) Aluminum resistance in the *Arabidopsis* mutant *alr-104* is caused by an aluminum-induced increase in rhizosphere pH. *Plant Physiol.* 117, 19-27. MEDLINE
13. de la Fuente, J.M., Ramírez-Rodríguez, V., Cabrera-Ponce, J.L. & Herrera-Estrella, L. (1997) Aluminum tolerance in transgenic plants by alteration of citrate synthase. *Science* 276, 1566-1568. MEDLINE
14. Delhaize, E. & Ryan, P.R. (1995) Aluminum toxicity and tolerance in plants. *Plant Physiol.* 107, 315-321. MEDLINE
15. Delhaize, E., Hebb, D.M., Richards, K.D., Lin, J.M., Ryan, P.R. & Gardner, R.C. (1999) Cloning and expression of a wheat (*Triticum aestivum* L.) phosphatidylserine synthase cDNA: Overexpression in plants alters the composition of phospholipids. *J. Biol. Chem.* 274, 7082-7088. MEDLINE
16. Ezaki, B., Koyanagi, M., Gardner, R.C. & Matsumoto, H. (1997) Nucleotide sequence of a cDNA for GDP dissociation inhibitor (GDI) which is induced by aluminum (Al) ion stress in tobacco cell culture (accession no. AF012823) (PGR 97-133). *Plant Physiol.* 115, 314. MEDLINE
17. Ezaki, B., Sivaguru, M., Ezaki, Y., Matsumoto, H. & Gardner, R.C. (1999) Acquisition of aluminum tolerance in *Saccharomyces cerevisiae* by expression of the *BCB* or *NtGDI1* gene derived from plants. *FEMS Microbiol. Lett.* 171, 81-87. MEDLINE
18. Ezaki, B., Gardner, R.C., Ezaki, Y. & Matsumoto, H. (2000) Expression of aluminum-induced genes in transgenic *Arabidopsis* plants can ameliorate aluminum stress and/or oxidative stress. *Plant Physiol.* 122, 657-665. MEDLINE
19. Foy, C.D. (1988) Plant adaptation to acid, aluminum-toxic soils. *Commun. Soil Sci. Plant Anal.* 19, 959-987.
20. Foy, C.D. (1992) Soil chemical factors limiting plant root growth. *Adv. Soil Sci.* 19, 97-149.
21. Gallego, F.J. & Benito, C. (1997) Genetic control of aluminium tolerance in rye (*Secale cereale* L.). *Theor. Appl. Genet.* 95, 393-399.
22. Gallego, F.J., Lopez-Solanilla, Figueiras, A.M. & Benito, C. (1998) Chromosomal location of PCR fragments as a source of DNA markers linked to aluminium tolerance genes in rye. *Theor. Appl. Genet.* 96, 426-434.
23. Grabski, S., Arroyo, E., Bush, B. & Schindler, M. (1998) Regulation of actin tension in plant cells by kinases and phosphatases. *Plant Physiol.* 116, 279-290. MEDLINE
24. Gunse, B., Poschenrieder, Ch. & Barcelo, J. (1997) Water transport properties of roots and root cortical cells in proton- and Al-stressed maize varieties. *Plant Physiol.* 113, 595-602. MEDLINE
25. Hamilton, C.A., Good, A.G. & Taylor, G.J. (2001) Induction of vacuolar ATPase and mitochondrial ATP synthase by aluminum in an aluminum-resistant cultivar of wheat. *Plant Physiol.* 125, 2068-2077. MEDLINE
26. Hare, P.D. & Cress, W.A. (1997) Metabolic implications of stress-induced proline accumulation in plants. *Plant Growth Regul.* 21, 79-102. MEDLINE
27. Horst, W.J. (1995) The role of the apoplast in aluminum toxicity and resistance of higher plants: A review. *Z. Pflanzenernaehr Bodenkd.* 158, 419-428.
28. Horst, W.J., Puchel, A.-K. & Schmohl, N. (1997) Induction of callose formation is a sensitive marker for genotypic aluminium sensitivity in maize. *Plant Soil* 192, 23-30.
29. Horst, W.J. Schmohl, N., Kollmeier, M., Baluska, F. & Sivaguru, M. (1999) Does aluminium inhibit root growth of maize through interaction with the cell wall-plasma membrane-cytoskeleton continuum? *Plant Soil* 215, 163-174.
30. Huang, J.W., Pellet, D.M., Papernik, L.A. & Kochian, L.V. (1996) Aluminum interactions with voltage-dependent calcium transport on plasma membrane vesicles isolated from roots of aluminum-sensitive and -resistance wheat cultivars. *Plant Physiol.* 110, 561-569. MEDLINE
31. Hue, N.V., Craddock, G.R. & Adams, F. (1986) Effect of organic acids on Al toxicity in subsoils. *Soil Sci. Soc. Am. J.* 50, 28-34.
32. Jones, D.L., Kochian, L.V. & Gilroy, S. (1998) Aluminum induces a decrease in cytosolic calcium concentration in BY-2 tobacco cell cultures. *Plant Physiol.* 116, 81-89. MEDLINE
33. Kinraide, T.B. (1997) Reconsidering the rhizotoxicity of hydroxyl, sulphate, and fluoride complexes of aluminum. *J. Exp. Bot.* 48, 1115-1124. MEDLINE
34. Knight, H. & Knight, M.R. (2001) Abiotic stress signaling pathways: Specificity and cross-talk. *Trends Plant Sci.* 6, 262-267. MEDLINE
35. Kochian, L.V. (1995) Cellular mechanism of aluminum toxicity and resistance in plants. *Annu. Rev. Plant Physiol. Mol. Biol.* 46, 237-260. MEDLINE
36. Kollmeier, M., Felle, H.H. & Horst, W.J. (2000) Genotypical differences in aluminum resistance of maize are expressed in the distal part of the transition zone. Is reduce basipetal auxin flow involved in inhibition of root elongation by aluminum? *Plant Physiol.* 122, 945-956. MEDLINE
37. Larsen, P.B., Tai, C.Y., Kochian, L.V. & Howell, S.H. (1996) *Arabidopsis* mutants with increased sensitivity to aluminum. *Plant Physiol.* 110, 743-751. MEDLINE
38. Larsen, P.B., Degenhard, J., Tai, C., Stenzler, L.M., Howell, S.H. & Kochian, L.V. (1998) Aluminum-resistance *Arabidopsis* mutants that exhibit altered pattern of aluminum accumulation and organic acid release from roots. *Plant Physiol.* 117, 9-18. MEDLINE
39. Lazof, D.B., Goldsmith, J.G., Rufty, T.W. & Linton, R.W. (1994) Rapid uptake of aluminum into cells of intact soybean root tips. A microanalytical study using secondary ion mass spectroscopy. *Plant Physiol.* 106, 1107-1114. MEDLINE
40. LeNoble, M.E., Blevins, D.G., Sharp, R.E. & Cumbie, B.G. (1996) Prevention of aluminium toxicity with supplemental boron. I. Maintenance of root elongation and cellular structure. *Plant. Cell Environ.* 19, 1132-1142.
41. Li, K.F., Ma, J.F. & Matsumoto, H. (2000) Pattern of aluminum-induced secretion of organic acids differs between rye and wheat. *Plant Physiol.* 123, 1537-1543. MEDLINE
42. Ligterink, W. & Hirt, H. (2001) Mitogen-activated protein (MAP) kinase pathways in plants: Versatile signalling tools. *Int. Rev. Cytol.* 201, 209-275. MEDLINE
43. Little, R. (1988) Plant soil interaction at low pH: Problem solving genetic approach. *Commun. Soil Sci. Plant Anal.* 19, 1239-1257.
44. Lorenz.-Plucinska, G. & Ziegler, H. (1996) Changes in ATP levels in Scots pine needles during aluminium stress. *Photosynthetica* 32, 141-144.
45. Lukaszewski, K.M. & Blevins, D.G. (1996) Root growth inhibition in boron-deficient in aluminum stressed squash may be a result of impaired ascorbate metabolism. *Plant Physiol.* 112, 1135-1140. MEDLINE
46. Ma, J.F. (2000) Role of organic acids on detoxification of aluminum in higher plants. *Plant Cell Physiol.* 41, 383-390. MEDLINE
47. Ma, J.F., Zheng, S.J., Matsumoto, H. & Hiradate, S. (1997a) Detoxifying aluminum with buckwheat. *Nature* 390, 569-570. MEDLINE
48. Ma, J.F., Hiradate, S., Nomoto, K., Iwashita, T. & Matsumoto, H. (1997b) Internal detoxification mechanism of Al in hydrangea: Identification of Al forms in the root apices. *Plant Physiol.* 113, 1033-1039. MEDLINE
49. Ma, J.F., Taketa, S. & Yang, Z.M. (2000) Aluminum tolerance genes on the short arm of chromosome 3R are linked to organic acid release in triticale. *Plant Physiol.* 122, 687-694. MEDLINE
50. Ma, X.-F., Ross, K. & Gustafson, J.P. (2001) Physical mapping of restriction fragment length polymorphism (RFLP) markers in homoeologous groups 1 and 3 chromosomes of wheat by *in situ* hybridization. *Genome* 44, 401-412. MEDLINE
51. Marienfeld, S., Schmohl, N., Klein, M., Schroeder, W.H., Kuhn, A.J. & Horst, W.J. (2000) Localisation of aluminium in root tips of *Zea mays* and *Vicia faba*. *J. Plant Physiol.* 156, 666-671. MEDLINE
52. Matsumoto, H. (1991) Biochemical mechanism of the toxicity of aluminum and the sequestration of aluminum in plant cells; in *Plant-Soil Interactions at Low pH* (Wright, R.J., Baligar, V.C. & Murrmann, R.P., eds.) pp. 825-838, Kluwer Academic Publishers, Dordrecht, Netherlands.
53. Matsumoto, H. (2000) Cell biology of aluminum toxicity and tolerance in higher plants. *Int. Rev. Cytol.* 200, 1-46. MEDLINE
54. Matsumoto, H., Hirasawa, E., Morimura, S. & Takahashi, E. (1976) Localization of aluminum in tea leaves. *Plant Cell Physiol.* 17, 627-631. MEDLINE
55. May, H.M. & Nordstrom, D.K. (1991) Assessing the solubilities and reaction kinetics of aluminous minerals in soil; in *Soil Acidity* (Urlich, B. & Sumner, M.E., eds.) pp. 125-148, Springer-Verlag, Berlin.
56. Miller, D., Hable, W., Gottwald, J., Ellard-Ivey, M., Demura, T., Lomax, T. & Carpita, N. (1997) Connections: The hard wiring of the plant cell for perception, signaling and response. *Plant Cell* 9, 2105-2117. MEDLINE
57. Mossor-Pietraszewska, T., Kwit, M. & Legiewicz, M. (1997) The influence of aluminium ions on activity changes of some dehydrogenases and aminotransferases in yellow lupine. *Biol. Bull. Poznan* 34, 47-48.
58. Nguyen, V.T., Burow, M.D., Nguyen, H.T., Le, B.T., Le, T.D. & Paterson, A.H. (2001) Molecular mapping of genes conferring aluminum tolerance in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 102, 1002-1010.
59. Nosko, P., Brassard, P., Kramer, J.R. & Kershaw, K.A. (1988) The effect of aluminum on seed germination and early seedling establishment, growth and respiration of white spruce (*Picea glauca*). *Can. J. Bot.* 66, 2305-2310.
60. Osawa, H. & Matsumoto, H. (2001) Possible involvement of protein phosphorylation in aluminum-responsive malate efflux from wheat root apex. *Plant Physiol.* 126, 411-420. MEDLINE
61. Pellet, D.M., Grunes, D.L. & Kochian, L.V. (1995) Organic acid exudations as an aluminum-tolerance mechanism in maize (*Zea mays* L.). *Planta* 196, 788-795. MEDLINE
62. Pellet, D.M., Papernik, L.A. & Kochian, L.V. (1996) Multiple aluminum-resistance mechanisms in wheat: Roles of root apical phosphate and malate exudation. *Plant Physiol.* 112, 591-597. MEDLINE
63. Pineros, M.A. & Kochian, L.V. (2001) A patch- clamp study on the physiology of aluminum toxicity and aluminum tolerance in maize. Identification and characterization of Al3+-induced anion channels. *Plant Physiol.* 125, 292-305. MEDLINE
64. Rengel, Z. (1996) Uptake of aluminium by plant cells. *New Phytol.* 134, 389-406.
65. Rengel, Z. & Robinson, D.L. (1989) Aluminum effects on growth and macronutrient uptake by annual ryegrass. *Agron. J.* 81, 208-215.
66. Rengel, Z. & Reid, R.J. (1997) Uptake of Al across the plasma membrane of plant cells. *Plant Soil* 192, 31-35.
67. Richards, K.D., Schott, E.J., Sharma, Y.K., Davis, K.R. & Gardner, R.C. (1998) Aluminum induces oxidative stress genes in *Arabidopsis thaliana*. *Plant Physiol.* 116, 409-418. MEDLINE
68. Roy, A.K., Sharma, A. & Talukder, G. (1988) Some aspects of aluminum toxicity in plants. *Bot. Rev.* 54, 145-177.
69. Ryan, P.R., Delhaize, E. & Randall, P.J. (1995) Characterization of Al-stimulated efflux of malate from apices of Al-tolerant wheat roots. *Planta* 196, 103-110. MEDLINE
70. Schott, E.J. & Gardner, R.C. (1997) Aluminum- sensitive mutants of *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* 254, 63-72. MEDLINE
71. Silva, J.R., Smyth, T.J., Moxley, D.F., Carter, T.E., Allen, N.S. & Rufty, T.W. (2000) Aluminum accumulation at nuclei of cells in the root tip. Fluorescence detection using lumogallion and confocal laser scanning microscopy. *Plant Physiol.* 123, 543-552. MEDLINE
72. Sivaguru, M., Baluska, F., Volkmann, D., Felle, H.H. & Horst, W.J. (1999) Impacts of aluminum on the cytoskeleton of the maize root apex. Short-term effects on the distal part of the transition zone. *Plant Physiol.* 119, 1073- 1082. MEDLINE
73. Sivaguru, M., Fujiwara, T., Samaj, J., Baluska, F., Yang, Z., Osawa, H., Maeda, T., Mori, T., Volkmann, D. & Matsumoto, H. (2000) Aluminum-induced 1F255-3-beta-D-glucan inhibits cell-to- cell trafficking of molecules through plasmodesmata. A new mechanism of aluminum toxicity in plants. *Plant Physiol.* 124, 991-1005. MEDLINE
74. Slaski, J.J., Zhang, G., Basu, U., Stephens, J.L. & Taylor, G.J. (1996) Aluminum resistance in wheat (*Triticum aestivum*) is associated with rapid Al-induced changes in activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in root apices. *Physiol. Plant.* 98, 477-484.
75. Snowden, K.C., Richards, K.D. & Gardner, R.C. (1995) Aluminum-induced genes. Introduction of toxic metals, low calcium, and wounding and pattern of expression in root tips. *Plant Physiol.* 107, 341-348. MEDLINE
76. Somers, D.J., Gustafson, J.P. (1995) The expression of aluminum stress induced polypeptides in a population of wheat (*Triticum aestivum* L.). *Genome* 38, 1213-1220. MEDLINE
77. Takabatake, R. & Shimmen, T. (1997) Inhibition of electrogenesis by aluminum in characean cells. *Plant Cell Physiol.* 38, 1264-1271. MEDLINE
78. Taylor, G.J. (1988) The physiology of aluminum tolerance; in *Metals Ions in Biological Systems* (Sigel, H., ed.) vol. 24, Aluminum and Its Role in Biology, pp. 165-198, Marcel-Dekker, New York.
79. Taylor, G.J. (1991) Current views of the aluminum stress response; The physiological basis of tolerance. *Curr. Top. Plant Biochem. Physiol.* 10, 57-93.
80. Taylor, G.J. (1995) Overcoming barriers to understanding the cellular basis of aluminum resistance. *Plant Soil* 171, 89-103.
81. Taylor, G.J., Blamey, F.P.C. & Edwards, D.G. (1998) Antagonistic and synergistic interactions between aluminum and manganese on growth of *Vigna unguiculata* at low ionic strenght. *Physiol. Plant.* 104, 183-194.
82. Taylor, G.J., McDonald-Stephens, J.L., Hunter, D.B., Bertsch, P.M., Elmore, D., Rengel, Z. & Reid, R.J. (2000) Direct measurement of aluminum uptake and distribution in single cells of *Chara corralina*. *Plant Physiol.* 123, 987-996. MEDLINE
83. Thornton, F.C., Schaedle, M. & Raynal, D.L. (1986) Effect of aluminum on the growth of sugar maple in solution culture. *Can. J. For. Res.* 16, 892-896.
84. Tuman, K. (1996) Role of arbuscular mycorrhiza in plant resistance to heavy metals. *Biol. Bull. Poznan* 33 (Suppl.), 65.
85. Wagatsuma, T. & Akiba, R. (1989) Low surface negativity of root protoplasts from aluminum-tolerant plant species. *Soil Sci. Plant Nutr.* 35, 443-452.
86. Wu, P., Liao, C.Y., Hu, B., Yi, K.K., Jin, W.Z., Ni, J.J. & He, C. (2000) QTLs and epistasis for aluminum tolerance in rice (*Oryza sativa* L.) at different seedling stages. *Theor. Appl. Genet.* 100, 1295-1303.
87. Xiong, L. & Zhu, J.-K. (2001) Abiotic stress signal transduction in plant: Molecular and genetic perspectives. *Physiol. Plant.* 112, 152-166.
88. Yamamoto, Y., Kobayashi, Y. & Matsumoto, H. (2001) Lipid peroxidation is an early symptom triggered by aluminum, but not the primary cause of elongation inhibition in pea roots. *Plant Physiol.* 125, 199-208. MEDLINE
89. Yang, Z.M., Sivaguru, M., Horst, W.J. & Matsumoto, H. (2000) Aluminum tolerance is achieved by exudation of citric acid from roots of soybean (*Glycine max* L. Merr.). *Physiol. Plant.* 110, 72-77.
90. Zhang, G., Slaski, J.J., Archambault, D.J. & Taylor, G.J. (1997) Alteration of plasma membrane lipids in aluminum-resistant and aluminum-sensitive wheat genotypes in response to aluminum stress. *Physiol. Plant.* 99, 302-308.
91. Zhang, W.H. & Rengel, Z. (1999) Aluminum induces an increase in cytoplasmic calcium in intact wheat root apical cells. *Aust. J. Plant Physiol.* 26, 401-409. MEDLINE