SYMP-01

Recent advances in the physiological and molecular mechanism of Al toxicity and tolerance in higher plants

Sung-Ju Ahn[1] and Hideaki Matsumoto[2]

[1] Department of Plant Biotechnology, College of Agriculture & Life Science, Chonnam National University, Gwang-Ju 500-757, Korea
[2] Research Institute for Bioresources, Okayama University, Chuo 2-20-1, Kurashiki, Okayama 710-0046, Japan

1. GENERAL ASPECTS OF AL STRESS IN ACID SOILS

1.1 Introduction to acid soils

Acid soils occupy approximately 30 % or 3950 million ha of the world's ice-free land area and occur mainly in two global belts where they have developed under moisture regimes. Sixty-seven percent of the acid soils support forests and woodlands and approximately 18 % are covered by savanna, prairie and steppe vegetation. Only 4.5 % (179 million ha) of the acid soil area is used for growing arable crops. The factors involved in producing acid soils are prolonged leaching by rain water, soil-forming processes, and climacteric conditions (Sanchez and Benites, 1987). The level of soil acidification generally reflects the degree of weathering and leaching it has experienced (Baligar, Beaner, and Ahlrichs, 1998).

In addition to the natural factors that affect weathering, management practices, such as acidifying effects of acid-forming N fertilizer, removal of cations by harvesting crops, increase leaching and run-off of cations, and cultivations of leguminous crops (N_2-fixation), have resulted in the lowering of the natural pH (Baligar and Fageria, 1997). Furthermore, the acidity of the soil is gradually increasing as a result of changes in the environment problems including acid rain. Under such situations, attention has been paid to suppress the population growth in the world, especially the burst of population in the developing countries where the acid soils dominate. Worldwide, 20 % of maize, 13 % of rice and 5% of wheat is grown in acid soil (Kochian, 2000). In the past few decades, scientists in agronomy, physiology and molecular biology have contributed to our understanding of the mechanism of aluminum (Al) toxicity and the tolerance of plants to Al toxicity for the promotion of agricultural products in acid soils. Several important review papers have been published (Foy et al., 1978; Haug, 1984; Taylor, 1988, 1991; Delhaize and Ryan, 1995; Kochian, 1995; Matsumoto, 2000, 2002a, 2002b; Matsumoto et al., 2001).

1.2 Characteristics of acid soils

The main factor in producing acid soils is the thousands and even millions of years of leaching by rain water, which is both a good solvent and is slightly acidic. The water adds protons (H^+ ions) while removing the more soluble nutrients and gradually dissolving most primary and secondary minerals. Under the most intensive weathering this ultimately results in soils that consist of little except oxides and hydroxides of iron and aluminum plus some kaolinite and quartz and produces soils that are acidic

to great depths. Most of the acid soils are very low in exchangeable bases (K, Ca, Mg), which are important for plant nutrition. This is mainly due to the low exchange capacity of this soil, leaching losses, and crop removal. The adverse effects of most acid soils are not caused by the H^+ ions concentration but by deficiencies in Ca and Mg and toxicities of Al and Mn (Baligar, Fageria, and Elrashidi, 1998). Deficiencies of micronutrients are also commonly observed. Most of the acid soils are very low in P. Added P fertilizers are readily converted to compounds that are hardly soluble such as aluminum phosphate. P-fixation (insolubilization) is high in soils containing allophane, an amorphous, very reactive alumino-silicate (Baligar, Fageria, and Elrashidi, 1998).

Exchangeable Al and Mn are the predominant toxic elements in most of the acid soils. Most of the Oxisols and Ultisols are saturated with Al ranging from 4 to 94 % of the cation exchange site. Globally, soils with Al problems dominate (67 % of the total acid soil) the acid soil region. In South and North America, Africa, and South and Southeast Asia subsoil Al problems are seen in 86, 68, and 84 % of the acid soil lands, respectively (Baligar, Beaner, and Ahlrichs, 1998). Manganese is easily soluble at pH values lower than 5.5 and exhibit toxicity in acid soils.

2. AL TOXICITY IN ACID SOIL

Plant productivity is greatly limited by environmental stresses, such as temperature stress, drought stress, flooding and metal toxicity. Particularly, Al is the third most abundant element in the earth's crust and solubilization of Al is enhanced by acidic environments; therefore, Al is the most growth-limiting factor (in many acid soils throughout the world) (Foy, 1988). For this reason, Al toxicity in plants has been recognized for at least a century, the specific mechanism by which Al inhibits root elongation is yet to be determined (Horst, 1995Kochian, 1995 Taylor, 1995 Matsumoto, 2000 for recent reviews). In particular, the inhibition of root growth caused by Al is well known as an early symptom of Al phytotoxicity in acid soil (Horst, 1995; Kochian, 1995; Taylor, 1995; Matsumoto, 2000). The root apex, which is the primary target sites for Al, plays a central role in the expression of Al-tolerance and toxicity (Ryan et al. 1993; Sivaguru and Horst, 1998, Ahn et al., 2001, 2002). Nevertheless, the mechanism of tolerance to Al and Al-toxicity in plant cells is poorly understood (Kochian, 1995; Matsumoto, 2000).

2.1 Inhibition of growth at the root apex

Recent advances have led to a general understanding that the root apex plays a central role in Al toxicity (Ryan et al., 1993; Kochian, 1995; Sivaguru and Horst, 1998). Morphological changes in the root are induced within hours of Al treatment. The root apex becomes thick with cracks. Inhibition of root cell elongation in the elongation zone is the major outcome of the inhibition of elongation. Shortening of the root elongation zone by Al is accompanied with an increase in the diameter and a decrease of the cell length in the second and third layers of the cortex in the elongation zone of wheat root (Sasaki et al., 1996). The Al-induced inhibition of longitudinal cell elongation and accompanying cell swelling in theelongation zone might be related to the disorder of the microtubules (MTs). Cortical MTs are known to be involved in the orientation of cellulose microfibriles. Indeed, the disappearance of the cortical MTs in elongating cells of wheat roots was observed under Al stress

(Sasaki et al., 1997). Al-induced inhibition of root elongation in Al-sensitive maize occurred by Al within 30 min (Llugany et al., 1995). However, when Al is selectively applied to the elongation zone or to the entire root except the apex, growth is unaffected. Ryan et al. (1993) showed that exposure of only the terminal 2-3 mm of a maize root to Al, the meristem and root cap, was enough to inhibit root growth. Most recently Ahn et al. (2001, 2002, 2004) found that the central elongation zone, which is located 2-4 mm from the tip, was preferentially inhibited by Al using highly sensitive growth-measuring method. Bennet and Breen (1991) suggested that Al might inhibit root growth indirectly, via a signal-response pathway including, hormones, and secondary messengers in the root cap. Ryan et al. (1993), however, found that Al-induced inhibition of root growth was unaltered by decapping maize roots, indicating that the root cap has a minor role in fighting Al toxicity. Therefore, research on the mechanism of Al toxicity should be directed to interaction of Al-induced changes within the root apex.

2.2 Physiological and molecular aspects of al toxicity

The primary toxic responses to Al are stunted root growth, poor root hair development, swelling of root apices, and stubby and brittle roots. These result in the decrease in translocation of water and nutrient elements from roots to shoots (Delhaize and Ryan, 1995; Kochian, 1995). However, direct measurement of growth may not be suitable to find the earliest symptoms of Al toxicity, because a number of physiological and biochemical processes in the plant cell might be affected before growth inhibition. Convincing arguments have been advanced on the toxicity mechanisms operating in the root apoplast (Horst, 1995; Rengel, 1996), symplasm (Jones and Kochian, 1995; Kochian, 1995; Jones and Brassington, 1998), and the plasma membrane (Barceló et al., 1996; Ahn et al., 2001, 2002), as well as complex interactions among the changes in cell wall, plasma membrane and cytoskeleton continuum (Sivaguru et al., 1999).

2.2.1 Apoplast

It is important to elucidate the Al entry into cytoplasm and to differentiate quantitatively between apoplastic and symplastic Al separately to understand the mechanism of Al toxicity (Tice et al., 1992). So far, different techniques have been used to fractionate Al in each cell constituent. Al, as a polyvalent cation under acidic conditions, binds strongly to negative charges in the Donnan Free Space of the root-cell apoplasm. Al accumulates in the apoplasm of epidermal and cortical root cells in various species, since endodermis acts as a distinctive barrier. A plausible candidate of the binding site of Al in the apoplast is a carboxyl base in the pectin. Although Al binds to the negative-changed sites of pectin, the binding capacity of pectin varies with the plant species, and the pectin content is extremely different between monocots and dicots. Blamey et al. (1983) showed that Al reduced the water permeability of an artificial Ca pectate membrane substantially and instantaneously. The interaction of Al with cell wall constituents such as enzymes, extensin, and xyloglucan seems to affect the functional integrity of the cell wall. In vitro and in vivo Al binding experiments suggested that extensin has the highest capacity to bind Al among cell wall proteins (Kenjebaeva et al., 2001).

It is well established that citrate, which has a strong chelating capacity for Al, is used to desorb Al

from the apoplasm, where Al is assumed to be present. Taylor et al. (2000) showed that accumulation of Al in the cell wall dominated total uptake (71-318 μg $m^{-2}min^{-1}$), although transport across the plasma membrane was detectable (71-540 ng $m^{-2}min^{-1}$) within 30 min of ^{26}Al isotope exposure to a *Chara carallina.* They suggested that cell wall is the major site of Al accumulation. Therefore, we may need to reconsider intracellular Al without separation of the cell wall from the cytosol.

2.2.2 Symplasm

It has long been thought that an important part of the growth suppression by Al involves disruption of cell division within 60 min, but whether sufficient quantity of Al moves into the symplasm within this period is uncertain. Lazof et al. (1994) detected Al in the symplasm of soybean roots after only 30 min of exposure to Al. This demonstrates that Al can move into cells before root growth is inhibited suggesting a possibility that the site of Al toxicity is symplasm. It has been shown by Matsumoto et al. (1976), Matsumoto (1991) and Silva et al. (2000) that intracellular Al binds to cell nuclei and DNA. These results suggest that the template activity of DNA and/or chromatin for RNA synthesis is repressed by Al. This is caused by Al-induced structural alteration of DNA and chromatin through the association of the negative charged phosphate with positively charged Al^{3+} (Morimura and Matsumoto, 1978). There is little doubt that Al could cause considerable damage in the symplasm. Nonetheless, the importance of Al binding at the nucleus in the root growth response remains in question. Sivaguru et al. (1999) found that the actively dividing log-phase cells contain faint and large phragmoplasts and unusually enlarged nuclei after 6 h Al treatment. After a 24-h Al treatment, no phragmoplast and spindle MTs (SMT) were observed in the cells having metaphase plate chromosomes. The disintegration of SMT and disorganization of phragmoplasts caused by Al might prevent cell division directly at the metaphase. Recently mitochondrial dysfunction was found to be caused by Al toxicity (Yamamoto et al., 2002) in cultured tobacco cells (*Nicotiana tabacum*, non-chlorophyllic cell line SL). The accumulationof Al in tobacco cells caused instantaneous repression of mitochondrial activities and, after a lag of about 12 h, triggered reactive oxygen species (ROS) production, respiration inhibition, ATP depletion, and the loss of growth capability almost simultaneously. The presence of an antioxidant, butylated hydroxyanisol, during Al treatment prevented not only ROS production but also ATP depletion and the loss of growth capability in tobacco cells. These events were also observed in the pea roots subjected to Al stress (Yamamoto et al., 2001).

2.2.3 Plasma membrane

Although the apoplastic and symplastic target sites of Al in plant cells are under debate (Horst, 1995; Kochian, 1995; Rengel, 1996), several studies have focused attention on the plasma membrane as having a key function under Al stress. The plasma membrane is the first candidate for the target of Al because of its outermost location in the cell and high content of phosphorus as phospholipid. One of the biochemical changes of plasma membrane is the Al-dependent lipid peroxidation in the root tip of soybean (*Glycine max*). A close relationship existed between lipid peroxidation and inhibition of root elongation induced by Al and/or Fe toxicity and/or Ca deficiency (Cakmak and Horst, 1991).

Enhanced lipid peroxidation by oxygen free radicals is a consequence of primary action of Al on membrane structure. Al has a 560-fold higher affinity for the phosphatidylcholine surface than Ca^{2+}. The plasma membrane properties such as surface negativity or zeta potential have been reported to be altered by Al, and may be important to protect the passive movement of Al into root cells (Miyasaka et al., 1989; Wagatsuma and Akiba, 1989; Kinraide, 1994; Yermiyahu et al., 1997; Kinraide et al., 1998). The difference in the negative electrical charge of the plasma membrane among plant species is expected to be the difference in the attraction of the positively charged Al ions (Kinraide et al., 1992). Al may alter phospholipid profile thereby affecting the lipid-mediated signaling (Jones and Kochian, 1997). Such alterations to membrane electrical properties may destabilize the plasma membrane. Yermiyahu et al. (1997) elucidated the mechanisms of the underlying differences in surface-charge density using the plasma membrane vesicles isolated from whole-root, root tips, and tip-less roots of wheat (Scout 66 and Atlas 66). The surface charge of the plasma membrane depends on both biotic and abiotic factors such as the external pH. Al has been shown to inhibit calmodulin-stimulated, membrane-bound ATPase activity, which regulates the H^+fluxes across the plasma membrane and the maintenance of trans-membrane potential (Siegel and Haug, 1983; Matsumoto et al., 1992). Recently, Kinraide and co-workers modified the Gouy-Chapman-Stern model and developed a computer program to demonstrate the near-equal-binding constants of $H^+$and Al to the negatively charged surface sites of the plant cell membranes. The computed Al activities on the surface of the plasma membrane were correlated with the surface charge, which is in turn correlatedwith root growth. It has been generally accepted that differences in the resting surface potential among plant species may play an important role in determining the uptake of cations, including Al, and thus contribute to genotypic differences in the Al sensitivity.

Since the proposal of Vose and Randall (1962) that the negative surface-charge densities may play an important role in Al tolerance mechanisms, intensive research has been made in this area by studying cell surface electrical properties of plasma membrane in relation to H^+efflux and influx upon Al treatment. A few correlations have been obtained between surface potential and Al tolerance in plants such as wheat (Kinraide, 1988, 1994; Kinraide et al., 1992, 1998; Yermiyahu et al., 1997) and maize and barley (Wagatsuma and Akiba, 1989). Recently Ahn et al. (2001) found that the depolarization of the surface potential (zeta potential) occurred mostly at the root apex where most of the Al was localized in Al-treated squash and that this is the first circumstantial evidence for a zone specific depolarization of the plasma membrane surface potential coupled with inhibition of H^+-ATPase activity.

It is common and reasonable to consider that the plasma membrane is the first potential target for Al toxicity because of rich in phosphates as phospholipids. Also, the ion transport processes and permeability of the plasma membrane is altered by Al. In an Al-sensitive cultivar of barley in 100 μM Al, the influx of Ca^{2+}, NH_4^+ and K^+ was inhibited by 69 %, 40 % and 13 % but the flux of NO_3^- and PO_4^{3-} was enhanced by 44 % and 17 %, respectively (Nichol et al., 1993). In cortex cells in the intact roots of Northern red val, Al significantly altered the activation energy required to transport water (+32 %), urea (+9 %), and monoethyl urea (-7 %) across cell membrane (Zhao et al., 1987). However, it remains unclear to what extent Al-related changes in membrane permeability interfere with Al

uptake of the transmembrane itself. We need to address these important questions and develop a broad understanding of the nature of Al transport across biological membranes by obtaining quantitative information about the uptake and distribution of Al at the cellular level. At present, we do not know which molecular forms of Al are capable of crossing membranes, what the transport rate of Al might be, or the time required for initiating the transport. The mechanistic basis of Al transport and the overall subcellular distribution remain speculative. Our understanding of Al transport across biological membranes has been hindered by several factors, including the complex aqueous coordination chemistry of Al, its propensity to bind tightly to cell walls, the lack of an affordable and suitable isotope, and the lack of sensitive analytical techniques for detecting the low levels of Al associated with subcellular compartments. Nevertheless, recently, Taylor et al. (2000) measured the rates of Al transport across membranes in single cells of *Chara corallina* using the rare ^{26}Al isotope, an emerging technology (accelerator mass spectrometry), and a surgical technique for isolating subcellular compartments. These studies suggest that Al is capable of crossing a biological membrane, but that significant barriers prevent direct, and unambiguous measurements of transport rates.

2.2.4 Ca and Al toxicity

It has been postulated by numerous authors that Al may interfere with cellular Ca^{2+}-dependent signal transduction cascades that may be necessary for both cell division and cell elongation (Rengel, 1992; Delhaize and Ryan, 1995; Kochian, 1995; Matsumoto, 2000). It is not surprising that mechanisms of Al toxicity involve Al-Ca interactions which inhibit Ca^{2+} uptake, displace Ca^{2+} from the apoplasm, and disrupt Ca^{2+} homeostasis in the cytoplasm.

In studies using Al-resistant and Al-sensitive wheat cultivars, Huang et al. (1992) showed that Al-induced inhibition of the root apical Ca^{2+} influx correlated well with the inhibition of root growth. The genotypic differences in Al-induced inhibition of Ca^{2+} translocation and root growth were found to be primarily those in the root apex. However, Ryan et al. (1994) found that low concentrations of Al inhibit root growth without inhibiting Ca^{2+} uptake, and that the addition of other cations improves Al-induced suppression of root growth without inhibiting Ca^{2+} uptake.

One of the plausible mechanisms for the inhibitory action of Al on Ca^{2+} metabolism is the displacement of apoplasmic and membrane Ca^{2+} by Al due to the competition for ligands (Rengel, 1992). However, many different cations (e.g. H^+, Mg^{2+}, Sr^{2+}, and La^{3+}) alleviate Al toxicity by a mechanism that is independent of the changes in ionic strength. This indicates that the Ca displacement hypothesis fails to explain Al toxicity and that amelioration of Al toxicity by cations occurs because of the reduction of the negative potential difference on the membrane surface (Kinraide et al., 1992; Ahn et al., 2001). More work is needed to clarify the relationship between Al and Ca.

Studies using Ca^{2+}-sensitive fluorescent dyes and Ca^{2+}-imaging techniques showed Al-induced changes in cytosolic Ca^{2+}levels ($[Ca^{2+}]_{cyt}$) in several plant systems. Many reports published so far demonstrated an increase in cytosolic Ca^{2+} activity as a consequence of Al toxicity, e.g. in intact wheat (Zhang et al., 1998; Zhang and Rengel, 1999), excised barley roots (Nichol and Oliveira, 1995), Arabidopsis root hairs (Jones, Gilroy, et al., 1998) and protoplasts isolated from wheat roots (Lindberg

and Strid, 1997). On the other hand, Jones, Kochian, and Gilroy (1998) showed Al-induced rapid decrease of $[Ca^{2+}]_{cyt}$ in BY-2 tobacco cell culture. They suggested that a block of Ca-permeable channels by Al might be crucial to the phytotoxic nature of Al. However, regardless of an increase or a decrease in $[Ca^{2+}]_{cyt}$, all plants tested so far responded to the Al toxicity stress by altering the cytosolic Ca^{2+} homeostasis (as predicted by Rengel, 1992).

Recently the expression of Ca^{2+}-ATPase genes has been investigated by semi-quantitative RT-PCR in *Arabidopsis* (Ler-0) and it was found that the expression of the plasma membrane-localized Ca^{2+}-ATPase gene (ACA8) was more rapidly and transiently (within 0.5 h) induced by Al stress compared with other Ca^{2+}-ATPase genes. However, these expression levels were deduced after 1 h in all Ca^{2+}-ATPases (Ahn et al., unpublished results). These results suggest that Al-induced changes of cytoplasmic Ca^{2+} concentration are regulated temporally by Ca^{2+}-ATPases.

2.2.5 Callose formation

Callose formation on plasma membrane is a specific phenomenon under Al stress. In a soybean cell suspension, Al induced callose formation at as low as 5 μM and as early as 10 min after Al addition (Horst, 1995). In addition, Ahn et al. found that Al-induced callose levels were highest in root apex after 3-h Al treatment (unpublished data). Although the longer duration of Al treatment induced higher amount of callose, the difference in the magnitude of callose formation between the 3rd- and 6th-h of Al treatment was not large. Thus, callose formation is thought to be a potent parameter of Al toxicity and tolerance. Callose is synthesized by (1-3)-β-D-glucane synthase on the plasma membrane and activated by external Ca^{2+} ion. Callose itself might prevent "wall-loosening processes" and cell-wall extension (Eklund and Eliasson, 1990). Recently, Sivaguru et al. (2000) found that callose accumulated in plasmodesmata, caused the inhibition of cell-to-cell trafficking of molecules through plasmodesmata in wheat root. In general, Al-induced callose formation often associated tightly with the root growth inhibition in several monocot plants including wheat and maize (Horst, 1995 for a review).

2.3 Gene expression under Al toxicity

Recent studies on Al resistance mechanisms are focused on a molecular genetic study. Since several cDNAs whose transcripts accumulate after Al treatment in wheat (*Triticum aestivum*), were obtained by Snowden and Gardner (1993), over 20 induced by Al stress have been isolated from a range of plant species, including wheat (Cruz-Ortega et al., 1997; Hamel et al., 1998), tobacco (*Nicotiana tabacum* Ezaki et al., 1995, 1996) and *Arabidopsis thaliana* (Sugimoto and Sakamoto, 1997; Richards et al., 1998). Most of these Al-induced genes are general stress-inducible genes, whose expression is turned on by oxidative stress, pathogen infection, phosphate starvation, heat shock, other metal stresses and hormone treatments. It is therefore suggested that a common gene induction mechanism exists among these different stresses. However, the biological roles of Al-induced genes in Al stress and the induction mechanisms of these genes by Al stress are still unclear. Ezaki et al. (2000) recently expressed plant Al-induced genes in both yeast (*Saccharomyces cerevisiae*) cells and Arabidopsis. The two genes, the Arabidopsis blue copper-binding protein gene (*AtBCB*) and a tobacco GDP dissociation

inhibitor gene (*NtGDI1*), conferred Al resistance in yeast (Ezaki etal., 1999). These two genes and two others, a tobacco glutathione *S*-transferase (GST) gene (*parB*) and a tobacco peroxidase gene (*NtPox*), also ameliorated Al toxicity in Arabidopsis over a narrow range of Al concentration. These four genes have different biochemical functions, suggesting that there are several different Al tolerance mechanisms in plants. By characterizing these genes in terms of their Al resistance mechanisms we may be able to develop new strategies for Al resistance in plants in addition to the release of organic acids.

3. MECHANISM OF AL TOLERANCE

The acute and remarkable symptom caused by Al is the inhibition of the root elongation (Ryan et al., 1993). Therefore, Al tolerance in crop plants is mainly evaluated by the avoidance capability for the Al-caused inhibition of root elongation. The inhibition of the cell elongation may be responsible for Al-induced inhibition of root elongation, which occurs within a short term (within 1 h) (Blancaflor et al., 1998). In view of potential problems on long-term Al stress, which damages crop plants leading into the whole growth inhibition, the Al tolerance involves the tolerance to Al-induced secondary stresses (oxidative stress, drought, nutrient uptake, etc). However, the study on the early minimal inhibition of the root elongation is the most important for avoidance of Al toxicity, because it may offer the opportunity to escape from toxic Al-contaminated sites, which are dispersed inequality in acid soils. In this paragraph, the mechanisms of temporal Al-tolerance are discussed, separated from the Al-derived secondary stress tolerance, in physiological and genetic aspects.

3.1 Physiological aspects

3.1.1 Release of organic anions

Enhanced release of organic anions such as citrate, malate and oxalate is one of the adaptive mechanisms involved in the reduction of Al accumulation in the root apex (Ma et al., 2001; Ryan et al., 2001). These organic anions have a high chelating capacity for Al, and decrease the opportunity of binding the phytotoxic form of Al (e.g. Al^{3+}) with negatively charged components in cell walls and plasma membrane in the root apices (Suhayda and Haug, 1984; Hue et al., 1986; Ownby and Popham, 1989). Among these organic anions, citrate has a highest binding constant for Al (Hue et al., 1986). Releasing a large amount of organic anions from root apices is considered to increase the Al-resistance in several crop species and cultivars, although the types of the organic anions differ with the plant species. The major organic anions released in response to Al are malate in wheat, citrate in leguminous crops including snapbean (*Phaseolus vulgaris* L.), soybean (*Glycine max*) and *Cassia tora*, both citrate and malate in maize (*Zea mays* L.) and rye (*Secale cereale* L.),and oxalate in buckwheat (*Fagopyrum esculentum* Moench) (Miyasaka et al., 1991; Delhaize, Ryan, and Randall, 1993; Ryan et al., 1995; Ma, Zheng, and Matsumoto, 1997; Ma, Zheng, Matsumoto, et al., 1997; Li, Ma, and Matsumoto, 2000; Yang et al., 2000; Kollmeier et al., 2001; Silva et al., 2001). However, there are marked differences in the time lag for the efflux of organic anions in these crop species. A few hours were required for citrate releases after exposure to Al in leguminous crops and rye, but no interval for the release of organic anions in wheat, maize and buckwheat (Ma, 2000). The difference in

the pattern of organic anion efflux among crop species may be attributed to the differences in the induction time after exposure to Al.

To clarify the mechanism of organic-anion release in Al-resistant crop plants, the pathway involved in the release of organic anions has been examined. Recent experiments with channel inhibitors or the patch-clamping analysis revealed that Al-responsive release of organic anions is mediated by the Al-induced activation of anion channels on the plasma membrane in root apical cells (Ryan et al., 1995; Papernik and Kochian, 1997; Ryan et al., 1997; Zheng et al., 1998; Kollmeier et al., 2001; Piñeros and Kochian, 2001; Zhang et al., 2001). Al-activated anion channels were detected more clearly in Al-resistant cultivars than in Al-sensitive cultivars, which may be responsible for the enhanced efflux of organic anions in the former (Kollmeier et al., 2001; Zhang et al., 2001). However, it is unknown whether the number of anion channels per cell or secondary messengers that modulate anion channel opening regulate the permeability of organic anions in response to Al.

Recent progress supports the idea that several factors besides Al modulate the channel-mediated efflux of organic anions. In wheat, Al-induced malate release may be regulated in part by depolarization of the plasma membrane (Papernik and Kochian, 1997) or protein phosphorylation (Osawa and Matsumoto, 2001). Recently we characterized the Al-induced K^+ efflux from the root apex of wheat (Osawa and Matsumoto, 2002), which plays an essential role in the charge-balance transport in Al-induced malate efflux (Ryan et al., 1995). Since K^+efflux itself shifts the membrane potential toward a negative direction, it might act as a signaling pathway to open the Al-dependent anion channels activated by hyperpolarization (Ryan et al., 1997; Piñeros and Kochian, 2001). In the roots of *Arabidopsis thaliana*, Cu^{2+}-induced citrate efflux was partially decreased by the inhibition of K^+ efflux, which suggests that K^+ efflux is required for citrate efflux (Murphy et al., 1999). The results of our experiments suggest that Al independently regulates the release of K^+ and malate, and that the Al-induced K^+ efflux is mediated by activating ion channels that are sensitive to both TEA and lanthanides. Several lanthanides including La^{3+} and Yb^{3+} have ionic properties similar to that of Al in an aqueous solution, and these lanthanides can partially induce malate release in Al-resistant wheat genotype ET8, suggesting that Al-induced malate release is in part regulated by common factors shared for trivalent ions (Kataoka et al., 2002).

As mentioned above, the release of organic anions in crop plants is one of the intensive research areas for the Al-resistance mechanisms. However, it is highly possible that some mechanisms except for the organic anion release are involved in the Al-resistance. For example, the high Al resistance of a tropical forage grass (*Brachiaria decumbens* Stapf.) cannot be explained by the release of organic anions from the roots (Wenzl et al., 2001). Possible Al-resistance mechanisms other than the release of organic anions are mentioned below.

3.1.2 Release of non-organic anions for chelating Al

Some Al-resistant wheat cultivars release not only malate but also Al-chelatable substances in the presence of Al. Polypeptides, having a binding capacity for Al, are inducibly released from the root of the Al-tolerant wheat cultivar (Basu et al., 1994b). The release of phosphate from the root apex of Al-resistant wheat cultivar alleviates Al toxicity (Pellet et al., 1997). In maize root tips, the exudation

of flavonoid-type phenolics (catechin and quercetin), which is enhanced by the silicon pretreatment, has a potential role in alleviating Al-toxicity (Kidd et al., 2001).These results suggest that the release of Al-chelatable substances as well as the organic anion efflux contribute to the Al resistance.

3.1.3 Role of mucilage

Root cap, where abscisic acid and somatotropin mucosubstances (mucilage) are produced, protects the root meristem at the root apex. Thus, it is anticipated that the root cap may have a direct role in protecting roots from Al-injury (Horst et al., 1982). However, the root cap itself appeared not to have a direct role in Al-tolerance, since removal of root cap hardly affected the Al-induced inhibition of theroot elongation in wheat (Ryan et al., 1993) and maize (Li, Ma, Hiradate, et al., 2000). It is hypothesized that a large amount of organic anions released from the root might be retained in the unstirred layer formed by the mucilage (Henderson and Ownby, 1991). Mucilage contains around 25-35 % of total Al accumulated in the root apex in wheat (Archambault et al., 1996a) and maize (Li, Ma, Hiradate, et al., 2000). Mucilage produced from border cells, which is actively detached from the root cap, plays a potential role in protecting root tips from the cell damage caused by Al (Miyasaka and Hawes, 2001). These results suggest that the root cap may indirectly contribute to the resistance to Al.

3.1.4 Regulation of pH

The proportion of polyvalent Al ions decreases with the rise of pH in the soil solution. Therefore, one hypothesisis that the altered uptake-pattern of nutrients such as nitrate ion and ammonium ion may increase the rhizosphere pH, thus reducing the Al toxicity (Taylor and Foy, 1985a, 1985b). It is uncertain whether the control of rhizosphere pH mediated by nutrient uptake is involved in the Al resistance mechanism, since the expression of Al toxicity seems to precede the change of the N uptake pattern. Moreover, Al-induced change of the root surface pH around the apex showed similar pattern in all cultivars differing in Al-resistance in snapbean or wheat (Miyasaka et al., 1989; Ryan et al., 1992).In both Al-sensitive and Al-resistant maize cultivars, Al diminished the increase of the surface pH around the distal part of transition zone at the root apex, suggesting that alkalization itself is insufficient for conferring Al resistance (Kollmeier et al., 2000). By contrast, physiological experiments revealed that an Al-induced increase in root surface pH is a plausible explanation for the increased Al-resistance in the Arabidopsis mutant *alr*-104 (Degenhardt et al., 1998). Since it is difficult to directly measure the cell-wall pH, which has an important role in cell elongation, and the root surface pH is easily influenced by the uptake of ions or efflux of organic anions, whether Al-resistance is acquired by the control of the rhizosphere pH is a controversial subject.

3.1.5 Cell Wall

Cell walls, which have many negatively-charged sites for chelating Al, accumulate most of the Al in the root apex (Archambault et al., 1996b; Taylor et al., 2000). Therefore, the Al-binding sites in the cell wall components are possible sites for suppressing of cell elongation. Binding of Al to the epidermal cell wall in the hypocotyls of okra (*Abelmoschus esculentus* Moench) inhibits the

auxin-induced cell elongation (Ma et al., 1999). The high Al-binding capacity of the cell wall glycoproteins, especially extensisns, in the root tips may be involved in the binding of Al in the apoplast (Kenjebaeva et al., 2001). Among the cell wall components, pectin is a major site of Al accumulation in cultured tobacco cells (Chang et al., 1999). Using suspension-cultured maize cells, whose pectin content was modulated by NaCl treatment, or transgenic potato plants that overexpress pectinmethyl esterase, the binding of Al to the cell wall pectin-matrix and the degree of methylation in the pectin-matrix were found to be associated with the expression of toxicity of Al and resistance to Al (Schmohl and Horst, 2000; Schmohl, Pilling, et al., 2000). Al decreases the mechanical extensibility of the cell wall, and thus inhibits the root elongation in wheat (Tabuchi and Matsumoto, 2001). However it is still unclear why the Al-resistant crop plants maintain the loosening and reconstruction of the cell wall in the presence of Al. The role of the cell wall in resistance to Al needs to be clarified by identifying the Al-binding sites in the epidermal cell wall, which affect critical components in the cell wall loosening and reconstruction (Ma et al., 1999).

3.1.6 Tolerance to Al

Some crops and woody plants can retain a large amount of Al inside the cells without any growth reduction. This means that the cell has a mechanism for detoxifying the Al invading it. In the leaf of hydrangea (*Hydrangea macrophylla*), Al in the cytoplasm is combined with citrate at a molar ratio of 1:1 (Ma, Hiradate, et al., 1997). In buckwheat, Al in the cytoplasm is combined with oxalate at a molar ratio of 1:3 (Ma, Zheng, Matsumoto, et al., 1997). In buckwheat, accumulated-Al in the leaf is not readily retranslocated to other parts of the organ and is sequestrated into the vacuoles (Shen and Ma, 2001). In these Al-accumulator plants, it seems that intracellular organic anions have a significant role in reducing Al toxicity.

Several researches also support the idea that vacuoles have a significant role in sequestrating Al. Using the rare ^{26}Al isotope synthesized by an accelerator mass spectrometry, isolated vacuoles in single cells of *Chara corallina*were found to contain very low, but a significant amount of Al, suggesting that Al is sequestered into the vacuoles within a short time (Taylor et al., 2000). Energy-dispersive x-ray microanalysis has revealed that Al in the vacuoles may form a complex with phosphorus or silicon in the root tip cells of maize (Vázquez et al., 1999). In the leaf of tea (*Camelina sinensis*), an Al-accumulator plant, Al-tannin complexes were detected by the NMR analysis (Nagata et al., 1992). X-ray microscopy analysis also supported the hypothesis that Al may bind with the condensed tannin in the root tip cells of Al-tolerant legume forage (*Lotus pedunculatus*) (Stoutjesdijk et al., 2001).

3.2 Genetic aspects

3.2.1 Genetic research of Al tolerance

Although physiological and biochemical studies on the toxicity of Al to the crop plants and their resistance to Al, have been advanced at the individual, organic, and cellular levels, the mechanisms of the Al-induced suppression of root elongation remains unknown. This might be ascribed to the lack of information about the Al responses at the molecular level. It is well known that many plant genes are

expressed in response to environmental stresses including wounding, pathogen infection, high salinity, drought, temperature, and others. It is highly probable that Al tolerant crop species also respond to Al stress at gene level. In near-isogenic Al-tolerant wheat lines produced by repeating backcrossing, Al resistance is considered to be controlled by a single genetic locus, which determines the Al-induced malate efflux (Delhaize, Craig, et al., 1993; Delhaize, Ryan, et al., 1993). Also in various cultivars of wheat, a short time is sufficient to induce the Al toxicity/resistant responses including the enzyme activity (Slaski et al., 1996), protein synthesis (Delhaize et al., 1991; Picton et al., 1991; Rincon and Gonzales, 1991; Cruz-Ortega and Ownby, 1993; Basu et al., 1994a; Somers et al., 1996; Taylor et al., 1997) and membrane function (Zhang et al., 1996). These results indicated that the Al-triggered changes in the physiological responses might be controlled at the level of gene expression.

3.2.2 Al-induced genes

Recently, Al-responsive genes have been isolated from several crop plants using differential screening method. These Al-inducible genes were the genes for metallothionein-like protein (Snowden and Gardner, 1993), phenylalanine ammonia-lyase (Snowden and Gardner, 1993; Hamel et al., 1998), peroxidase (Ezaki et al., 1996; Hamel et al., 1998; Richards et al., 1998), auxin-inducible protein (Ezaki et al., 1995), 1,3-β-glucanase (Cruz-Ortega et al., 1997), glutathione S-transferase (Ezaki et al., 1995; Richards et al., 1998), blue-copper binding protein (Richards et al., 1998), oxalate oxidase (Hamel et al., 1998; Delisle et al., 2001), cysteine proteinase (Hamel et al., 1998) and ABC transporter (Sasaki et al., 2002). However, many of these genes havethe characteristic of being responsive to pathogen infection or oxidative stress. Several of these genes may contribute to the adaptive mechanism, since the expression of the genes seems to be indirectly related to promote apotosis-like cell death in epidermal cells at the meristematic and elongation zone and thereby protect cortex and stele cells for the maintenance of root growth (Delisle et al., 2001). The structural and functional analyses of Al-specific responsive genes are important for the clarification of Al toxicity and resistance mechanisms (Matsumoto, 2000).

3.2.3 Al-tolerance genes

Genetic researches on the near-isogenic Al-resistant lines of crop cultivars have revealed the gene locus involved in Al-tolerance. In wheat, the major Al-tolerance gene was mapped on the chromosome 4DL (Aniol, 1990; Riede and Anderson, 1996). It is a matter of debate whether Al-tolerance in wheat is controlled by single gene (Riede and Anderson, 1996) or at least 2 or 3 genes (McKendry et al., 1996; Johnson et al., 1997), although it may vary with the cultivar. Physiological studies on ditelosomic lines of Chinese Spring, a moderately Al-tolerant wheat cultivar, have revealed that at least two independent genes on different chromosome arms affect Al-activated malate efflux, suggesting that this process is controlled by two or more genes (Papernik et al., 2001).Al-resistance acquired by the release of organic anions in triticale is linked to chromosome 3R (Ma et al., 2000). A single dominant gene locus *Alp* involved in the Al-tolerance in barley was mapped on the long arm of chromosome 4H (Tang et al., 2000). By contrast, multiple genes may regulate the Al-tolerance in rice (Khatiwada et al., 1996 Nguyen et al., 2001). Identification of the genes involved in the Al-tolerance

and its utilization as a resistance marker, would contribute to the selection of the Al-tolerant crop cultivars.

3.2.4 Gene transformation

There is a possibility that crop plants utilize the function of other organisms for improving Al-resistance. For example, enhanced secretion of the phytase of *Aspergillus niger*s in transgenic Arabidopsis plant resulted in improved growth by making the organic forms of phosphorus utilizable (Richardson et al., 2001). Overexpression of the *Pseudomonas aeruginosa* citrate synthase in tobacco (*Nicotiana tabacum* L.) or papaya (*Carica papaya*) plants enhanced citrate efflux, thus improving the Al-resistance (De la Fuente et al., 1997). Similarly Al-resistance or phosphorus (P)-acquisition was increased by overexpressing enzymes involved in the organic acid biosynthesis in other transgenic plants (Koyama et al., 1999; Tesfaye et al., 2001). However, re-examination by Delhaize et al. (2001) revealed that the strategy based on the overexpression of citrate synthase is not readily reproducible for the improvement of Al-tolerance or low P-tolerance in transgenic plants. In genetically Al-resistant plants, organic anion efflux is highly specific to Al. Production of a resistant plant that can release organic anions only in the presence of Al, preventing the excess carbon loss, would be a more preferable strategy.

A budding yeast strain, which can grow well at a low pH, is often used as a conventional gene-expression system for screening the Al-tolerance gene. The budding yeast expressing the wheat's phosphatidylserine synthase, which is involved inphospholipid biosynthesis, was highly resistant to Al (Delhaize et al., 1999). The competitive inhibition of Mg uptake and transport by Al inhibited the yeast's growth (MacDiarmid and Gardner, 1998). Expression of a blue-copper-binding protein (*BCB*) or the GDP dissociation inhibitor (*NtGDI1*) enhanced the resistance to both Al and oxidative stress in the yeast and Arabidopsis (Ezaki et al., 1999 Ezaki et al., 2001). However, the direct function of those gene products on the Al-tolerance mechanism in yeast remains unknown. Moreover, Al-tolerance in yeast cells, like other cultured cells, may differ from that in the root elongation of crop plants, because thecapacity of cell division rather than cell expansion contributes to the yeast's growth. Therefore, it should be reevaluated whether the physiological responses that acquire the Al-tolerance in cultured cells also confer the Al-tolerance in higher plants (Yamamoto et al., 2001).

Several Al-resistant cell lines and transformed plants so far obtained have tolerance to multiple environmental stresses including oxidative stress that induces programmed cell death. The enhancement of the tolerance to cell death, which is the final response to Al toxicity, seems to be inappropriate in the research field of A-tolerance. Cell elongation, which is an earlier response than cell death, is a more appropriate indicator for elucidating the Al-tolerance. Clarification of the mechanism of Al-induced inhibition of the cell elongation is essential for the production of "real"Al tolerant crop plants.

4. CONCLUDING REMARKS AND FUTURE SCOPES

Al toxicity has one of the most deleterious effects on plant growth in acid soils. Increasing environmental problems such as acidification of soils, lakes and ponds and continued burst of the

global population warn that we will be confronted with a food crisis in the near future. Under such situations, the research for improving crop production in acid soil has been advanced intensively in the past few decades. The information on toxicity of Al and tolerance to Al is accumulating rapidly. The elucidation of the mechanism of Al tolerance is very important from the practical point of view. The exclusion of Al and intracellular tolerance mechanism, especially the former has been studied intensively. The understanding of the mechanism of the toxicity of Al and tolerance to it are complicated problems and several important problems still remain unsolved. However, the knowledge obtained concerning the cell response to the short-term effect of Al is expected to help us understand the response of the plant to Al, and to improve crop products in acid soils in the future.

REPRESENTATIVE REFERENCES

Ahn SJ, Matsumoto H. (2006) The role of the plasma membrane in the response of plants roots to aluminum toxicity. Plant Signaling & Behavior 1:37-45.

Matsumoto H, Osawa H, **Ahn SJ**. (2006) Aluminum toxicity syndrome and tolerance mechanism of crop plant in acid soils. (CROPS: Growth, Quality and Biotechnology, WFL PUBLISHER) p: 1314-1330.

Yamaguchi M, Sasaki T, Sivaguru M, Yamamoto Y, Osawa H, **Ahn SJ**, Matsumoto H. (2005) Evidence for the Plasma Membrane Localization of Al-Activated Malate Transporter (ALMT1). Plant Cell Physiology 46:812-816.

Shen H, He LF, Sasaki T, Yamamoto Y, Zheng SJ, Ligaba A, Yan XL, **Ahn SJ**, Yamaguchi M, Sasakawa H, Matsumoto H. (2005) Citrate Secretion Coupled with the Modulation of Soybean Root Tip Under Aluminum Stress: Up-regulation of Transcription, Translation and Threonined - oriented Phosphorylation of Plasma Membrane H^+-ATPase. Plant Physiology 138:287-296.

Sivaguru M, Yamamoto Y, Rengel Z, **Ahn SJ**, Matsumoto H. (2005) Early events responsible for aluminum toxicity symptoms in suspension-cultured tobacco cells. New Phytologist 165:99-109

Ahn SJ, Shin, R, Schachtman P. (2004) Expression of KT/KUP Genes in Arabidopsis and the Role of Root Hairs in K^+ Uptake. Plant Physiology 134:1135-1145.

Sasaki T, Yamamoto Y, Ezaki B, Katsuhara M, **Ahn SJ**, Matsumoto H. (2004) A wheat gene encoding an aluminium-activated malate transporter. Plant Journal 37:645-653.

Ahn SJ, Rengel Z, Matsumoto H. (2004) Aluminum-induced plasma membrane surface potential and H^+-ATPase activity in near-isogenic wheat lines differing in tolerance to aluminum. New Phytologist 162:71-79.

Zhu MY, **Ahn SJ**, Matsumoto H. (2003) Inhibition of growth and development of root border cells in wheat by Al. Physiol Plant 117: 359-367.

Nian H, **Ahn SJ**, Yang ZM, Matsumoto H. (2003) Effect of phosphorus deficiency on aluminium-induced citrate exudation in soybean (*Glycine max*). Physiol Plant 117: 229-236.

Nian H, Yang ZM, **Ahn SJ**, Cheng ZJ, Matsumoto H. (2002) A comparative study on the aluminum- and copper-induced organic acid exudation from wheat roots. Physiol Plant 116: 328-335.

Ahn SJ, Sivaguru M, Chung GC, Rengel Z, Matsumoto H. (2002) Aluminum-induced growth inhibition is associated with impaired efflux and influx of H^+ across the plasma membrane in root apices of squash (*Cucurbita pepo*). J Expt Botany 53: 1959-1966.

Ahn SJ, Sivaguru M, Osawa H, Chung GC and Matsumoto H. (2001) Aluminum inhibits the H^+-ATPase activity by permanently altering the plasma membrane surface potentials in squash roots. Plant Physiol. 126: 1381-1390.