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Review

Aluminum stress signaling in plants

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Aluminum (Al) toxicity is a major constraint for crop production in acidic soil worldwide. When the soil pH is lower than 5, Al^{3+} is released to the soil and enters into root tip cell ceases root development of plant. In acid soil with high mineral content, Al is the major cause of phytotoxicity. The target of Al toxicity is the root tip, in which Al exposure causes inhibition of cell elongation and cell division, leading to root stunting accompanied by reduced water and nutrient uptake. A variety of genes have been identified that are induced or repressed upon Al exposure. At tissue level, the distal part of the transition zone is the most sensitive to Al. At cellular and molecular level, many cell components are implicated in the Al toxicity including DNA in nucleus, numerous cytoplastic compounds, mitochondria, the plasma membrane and the cell wall. Although it is difficult to distinguish the primary targets from the secondary effects so far, understanding of the target sites of the Al toxicity is helpful for elucidating the mechanisms by which Al exerts its deleterious effects on root growth. To develop high tolerance against Al stress is the major goal of plant sciences. This review examines our current understanding of the Al signaling with the physiological, genetic and molecular approaches to improve the crop performance under the Al toxicity. New discoveries will open up new avenues of molecular/physiological inquiry that should greatly advance our understanding of Al tolerance mechanisms. Additionally, these breakthroughs will provide new molecular resources for improving the crop Al tolerance via molecular-assisted breeding and biotechnology.

Introduction

Aluminum (Al) toxicity is a serious factor limiting crop productivity in acid soil. Al is one of the major constituents of soil and it dissolves in the soil in various ionic forms among these Al^{3+} is the most toxic form. Apart from Al^{3+} cation, Al has the potential to form various hydroxy-Al and polynuclear species in solution. When the soil pH drops below 5.0 Al^{3+} is solubilized in the soil. Approximately 70% of soil in world is problem soil contaminated with acid, alkali, heavy metals etc. However acid soil is the most frequently encountered limiting production of most of the world's staple food. It has been estimated that approximately 50% of the arable land is negatively impacted by the Al toxicity due to acidic soil. Considerable measures must be taken to overcome this problem.

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Occurrence of Aluminum

Aluminum (Al) is a member of boron group of chemical element with atomic number 13. In the earth crust it is the most abundant metallic element and third most abundant of all element (after oxygen and silicon). The Al release from soil minerals under acidic conditions occurs as $Al(OH)^{2+}$, $Al(OH)^{3+}$ and $Al(H_2O)^{3+}$ that commonly effect on Al toxicity.[1]

An Overview of Al Toxicity in Plant

The most easily affected region of Al toxicity is the root in plant. The Al toxicity is due to the inhibition of root growth. Root elongations a process of cell division, but Al phytoxicity block the mechanism of cell division. As a result of this root become stunted and brittle, root hair development is poor and the root apices become swollen and damage.[2] Al causes extensive root injury leading to poor ion and water uptake.[3] The root apex i.e., root cap, meristem and elongation zone is highly sensitive to Al and accumulates Al very easily. As a result it attracts greater physical damage than the mature region of the root tissue. Primary toxic effects of Al are localized to the distal transition zone in the root tip.[4] In this root zone meristematic cells exit the division phase and prepare for F-actin dependent rapid cell elongation.[5,6] Cell division in the meristem and cell elongation in the elongation zone is inhibited by the primary effects of Al occurring in the adjacent transition zone, in which these processes are less active.

Al is so reactive that there are many potential Al binding sites including the cell wall, the plasma membrane surface, the cytoskeleton and nucleus that could target of injury. Al strongly binds to the cell wall of root epidermal and cortical cells.[7] The extent to which Al can bind to the cell wall components depends on the density of negative charges and ultimately determines the cation exchange capacity (CEC). In addition to rapid accumula-

tion of Al in the cell wall and apoplast of the root apex, Al rapidly accumulates in the plasma membrane as well the symplasm of sensitive plant affecting many processes of root growth.[8-10] Plasma membrane is rich in phospholipids, representing sensitive target of the Al phytotoxicity.

Al can alter the function of plasma membrane by interacting with the lipid thus inducing lipid peroxidation. Al can bind principally to phospholipids within the membrane. Several reports have been described the Al mediated interference with membrane lipid, as a result of which there is an increase in the highly toxic reactive oxygen free radicals. More over due to Al toxicity there is change in the membrane potential and this change in membrane potential is directly correlates with changes in the membrane surface potential i.e., zeta potential. In one sentence the shifting of plasma membrane potential to Al-induced depolarization. Calcium uptake has been strongly affected due to Al toxicity. Cytoplasmic Ca^{2+} is known to regulate many processes in cell growth and metabolism. The disruption of cytoplasmic Ca^{2+} homeostasis is another mechanism hypothesized to cause Al injury.[9,11,12] The Al-dependent disruption of cytoplasmic Ca^{2+} homeostasis may be directly or indirectly involved in the inhibition of the cell division or root elongation. Al might disrupt Ca-dependent metabolism by maintaining Ca^{2+} levels in the cytoplasm or by preventing Ca^{2+} transients from occurring altogether. The evidence supporting this hypothesis is indirect at best. For instance, callose (I-3-P-glucan) synthesis in plants requires an increase in Ca^{2+}, and several polyvalent metal cations, including Al, so it induces callose synthesis in roots within 30 min.[13] This phenomenon of callose synthesis shows a rapid link between Al stress and changes in $[Ca^{2+}]$. Calcium uptake rapidly recovers when Al is removed from the solution. Calmodulin (CaM) plays a pivotal role in cellular metabolism and there is some evidence that interactions between Al and CaM could be an important cause of cell toxicity.

According to Sivaguru et al.[4] Al induced the accumulation of callose in the plasmodesmata of root cell in wheat, thus blocking the cell to cell trafficking. Plant cell requires dynamic cytoskeleton based network for proper functioning of cell differentiation and cell division. Al toxicity disrupts the structure of cytoskeleton. In addition to this microtubules and actin filaments are also the target of Al. Some evidences were reported which suggested the physiological injuries due to the Al^{-} toxicity. In two recent studies,[14,15] it was proved that Al induced the decrease in the chlorophyll content and photosysthetic rate. The impact of Al toxicity in photosynthesis is indirect. Due to Al toxicity there is disturbance in the chloroplast architecture. Moreover, there is decrease in photosynthesis due to reduction of electron transport in photosystem II (PSII).

Al inhibited the efflux of H^{+} from barley roots.[16] Decrease activities of K^{+}, Mg^{+} and ATPase of plasma membrane were scored due to Al stress. Increase in the ATP and PPi dependent H^{+} pumps of the tonoplast membrane of barley. In the nucleus, binding of Al to DNA or to chromatin could condense DNA molecules and inhibit the cell division by reducing its capacity to provide a viable template for transcription.[16] Al has been shown to accumulate in the symplast.[17] The nuclei of the root tip cells shows accumulation of Al within 30 min of Al treatment in a sensitive genotype.[18] The mitochondrial activity was repressed in cultured tobacco cell and pea roots treated with Al and this is followed by inhibition of respiration, depletion of ATP and production of reactive oxygen species at later stages.[19] It is proved that exposure to Al could affect production of reactive oxygen species (ROS) in plants because Al stress causes peroxidation of lipids in the plasma membrane, the effect that could be due to ROS and Al induces the expression of several genes encoding antioxidative enzymes such as glutathione S-transferase, peroxidase and superoxide dismutase (SOD). Long-term treatment of green gram (*Vigna radiata*) with Al resulted in greatly increased levels of peroxide and lipid peroxidation in the leaves.[20] The imposition of biotic and abiotic stresses can give rise to further increases in ROS levels. Metals, including Al, are known to act as catalysts in ROS production and to induce oxidative damage in plants.[19-22] Large number of swollen mitochondria with many vacuoles, structural disturbances of the plasma membrane, and pre-apoptotic nuclear structures were some of the characteristic features of Al treated tobacco cells, confirming that Al signaling follows the mitochondrial pathway of cell death.[24]

Al toxicity affected severely the mitochondrial respiratory functions and altered the redox status studied in vitro and also the internal structure, which caused finally cell death in tobacco cells.[23] Increase in the vascular and total cell volume with out the change in the nuclear volume has been observed due to 24 hour of Al treatment. A marked increase in the surface area of Golgi complex and endoplasmic reticulum was identified under Al stress. Plant cells are well equipped with complex non enzymatic antioxidants such as ascorbate, glutathione, tocopherol and carotenoid, and with enzymatic antioxidants such as catalase, ascorbate peroxidase, guaiacol peroxidase, superoxide dismutase (SOD), mono dehydro ascorbate reductase, dehydro ascorbate reductase, glutathione-S-transferase (GST) and glutathione reductase, which help to detoxify the ROS.[21,24] Signal transduction is also affected by Al, a key signal transduction enzyme designated as phospholipase C (PLC) is inhibited by Al toxicity. This suggests that Al may interfere with the phosphoinositide signaling pathway.[25]

Al Toxicity and Tolerance Mechanism

Over the past decades many laboratories around world have focused their efforts on identifying and characterizing the mechanisms employed by plants that enable them to tolerate toxic levels of Al in acid soils. This research revealed that there are two main classes of Al tolerance mechanism. Some are those that operate to exclude Al from the root apex and other are those that allow the plant to tolerate Al accumulation in the root and shoot symplasm. As proposed by Taylor,[12] the tolerance strategies identified can be separated into those in exclusion of Al from the root apex and mechanisms that allow the plant to tolerate Al within cells. A wealth of studies provide very strong evidence that Al^{-} tolerant genotypes of wheat, corn, sunflower, soybean and common bean, among other exclude Al from root by exertion of organic acids that chelate Al.[27-31] The study of these tolerance mechanisms in plant become an interesting and essential topic of research. Production of organic acid (OA) plays a vital role in the mechanism of Al detoxification. Activation of organic acid efflux occurs rapidly

with any measurable delay after exposure to Al in several plants including wheat, in which it is well studied.[30,32] Of the organic acid, citrate has the highest binding activity for Al followed by citrate, malate and succinate.[33] Rice bean roots can specifically release citrate to alleviate Al toxicity.[34] In order to determine the key step involved in the Al-stimulated citrate efflux, several anion channel inhibitors and citrate carrier inhibitors as well as a protein synthesis inhibitor were used. The results indicated that de novo protein synthesis (possibly of the citrate carrier and anion channel themselves) rather than citrate biosynthesis is the critical step leading to citrate efflux in roots.

There is strong evidence that malate exudation from wheat and citrate exudation from corn roots in response to Al occurs by activation of an anion channel located in the plasma membrane.[15,30,35] Al might directly bind and then activate a membrane protein or an associated receptor, or it might indirectly activate the channel via cytosolic components. The two most important families of channel proteins are the chloride channel family and a subset of the ATP-binding cassette (ABC) protein super family. In yeast *(Saccharomyces cerevisiae*), Pdr12, an ABC protein, assists the carboxylate efflux. In cowpea, root cap mucilage was shown to bind to Al and the mucilage removal increases the Al sensitivity of root,[36] Henderson and Ownby[37] correlated the amount of mucilage produced by wheat root to Al tolerance and suggested that mucilage aided in forming a diffusion barrier to Al or concentrated organic acids that chelated Al. The mucilage from maize roots has been shown to bind Al,[38] but did not give satisfactory protection of roots from Al toxicity. This lack of protection is due distance between site of formation of mucilage and the Al sensitive zone i.e., distal part of the transition zone (DTZ).

The Al tolerance of canola (*Brassica napus*), *Arabidopsis thaliana*, tobacco (*Nicotiana tabacum*) and alfalfa (*Medicago sativum*) have been reported to be enhanced by increasing organic acid biosynthesis through overexpression of citrate synthase or malate dehydrogenase genes derived from plants or bacteria. Other potential mechanism of Al exclusion has been identified than organic acid (OA) efflux, this mechanism is the exudation of phenolic compounds. Phenolics, which are characterized as organic compounds containing one or more hydroxylated aromatic rings, represent a broad range of plant compounds including alkaloids, flavonoids, terpenoids and glycosides. They reportedly form strong complexes with Al^{3+} at neutral pH and were implicated in internal Al detoxification in tea and other Al-accumulating species.[39] For the better understanding of Al tolerance mechanisms, genes which are conferring the tolerance should be studied. Many studies must be done because in this field physiological as well as molecular level of study is essential. With respect of genetic analysis of Al tolerance, the work has been done in cereals especially among members of the Triticeae (e.g., wheat, rye). Among these the tolerance gene in wheat has been first focussed. The ALMT1 gene encoding a malate transporter from wheat (*Triticum aestivum*) can confer Al tolerance in transgenic tobacco cells. Delhaize et al.[40] generated transgenic barley (*Hordeum vulgare*) plants expressing the ALMT1 gene to exude malate and withstand Al stress. In wheat, the most extensively studied Al-resistant sources all have the Brazilian ancestor, Polyssu, in their pedigrees.[41] BH 1146 and Atlas 66 have been used widely in inheritance and gene expression studies[42,43] and they both can be traced back to Polyssu although Atlas 66 was developed in the USA. More recently, a Chinese wheat landrace, FSW, was found to have Al resistance similar to Atlas 66, but FSW has a different haplotype pattern for the markers derived from ALMT1.[60,62] Inheritance of Al resistance in wheat has been well studied. A major QTL on 4DL has been identified in wheat cultivars BH 1146, Atlas 66 and Chinese Spring.[43] Markers are available for screening this QTL in wheat materials[44] In addition, diagnostic markers for ALMT1 gene were reported[44,45] and also mapped on the 4DL QTL region of Atlas 66.[38]

However, some studies demonstrated that more than one gene might be involved in Al resistance of wheat. Berzonsky[42] reported that Al resistance in Atlas 66 was determined by a complex genetic mechanism involving several genes. Near-isogenic lines containing a single Al resistance gene from Atlas 66 show only partial Al resistance, providing indirect evidence to support this assumption. Further study of the near-isogenic lines suggested that at least two genetic loci might contribute to Al resistance in Atlas 66.[54] More recently, Zhou et al.[46] reported a minor QTL for Al resistance on chromosome 3BL of Atlas 66, in addition to the major QTL on 4DL. The two genes BnALMT1 and BnALMT2 from rape (*Brassica napus*) show homology to ALMT1 from wheat and shows Al tolerance. Low level of tolerance mechanism by five genes i.e., Arabidopsis blue copper-binding protein gene(*AtBCB*), tobacco GST(*parB*), tobacco peroxidase gene(*NtPox*), a tobacco guanosine diphosphate-dissociation inhibitor gene(*NtGDI*) and F9E10.5, has been defined. Al-tolerance genes in the moderately tolerant wheat Chinese Spring are located in chromosome arms 6AL, 7AS, 2DL, 3DL, 4DL and 4BL and in chromosome 7D. In self-incompatible rye, the long arm of chromosome 4 contains a major Al resistance locus called Alt3.[47] In rice, also Al tolerance mechanism has been extensively studied. Many varieties of rice has been characterised for QTL and Twenty-seven QTLs important for Al tolerance, as estimated by relative root growth, were identified in the five studies. Rice chromosome 3 (linkage block 3C) is homologous to triticeae 4L; genetic markers linked to Al tolerance loci common with wheat, barley. The two genes *WAK1* (wall associate kinase) from *Arabidopsis thaliana* and wali3, wali5 and wali61 (protease inhibitors), and part of plant Asn synthetases (wali7); respectively, confers tolerance for Al stress. Triticale is a synthetic wheat/rye hybrid that is largely grown on acid soils in Europe, South America and Australia.[48] Its Al tolerance is considered to be inherited from rye. A short arm of chromosome 3R carries genes necessary for Al tolerance. Using wheat-rye addition lines, major genes influencing Al tolerance in rye were located on chromosomes 3R, 4R, and the short arm of 6R.[49] The Al-induced genes encoding proteins that function to overcome oxidative stress e.g., glutathione S-transferase, peroxidase, blue copper-binding protein, phenylalanine ammonia lyase, 1,3-(β-glucanase, or cysteine proteinase) has been previously reported. Altolerance is genetically controlled by few major genes.[50,51] But the research reports on the Al tolerance in oat are very few. Genetic studies made in Brazil indicate that Al in oat is controlled by one or two dominant genes with

the tolerance genotype carrying AlaAla. In addition, expression of these Al-induced genes in transgenic Arabidopsis plants conferred Al tolerance.[52] Basu et al.[53] reported that transgenic *Brassica napus* overexpressing *MnSOD* gene acquires an Al resistance phenotype.

The identification of stress-regulated genes provides new tools to reduce Al stress. Among the candidate genes regulated by Al stress, several could play a role in alleviating phosphate deficiency and provide energy to fight oxidative stress. Nutrient deficiency, and especially phosphate, occurs in the presence of Al due to the precipitation of Al phosphate.[36] The mechanism of expression of two *GST* genes in Arabidopsis *AtGST1* and *AtGST11*, under Al stress was elucidated by Ezaki et al.[54] An approximately 1-kb DNA fragment of the gene was fused to a β-glucuronidase (*GUS*) reporter gene (*pAtGST1::GUS* and *pAtGST11::GUS*) and introduced in *Arabidopsis thaliana* and significant tolerance to Al stress has been observed. The constructed transgenic lines showed a time-dependent gene expression to a different degree in the root and/or leaf by the Al stress. The *pAtGST1::GUS* gene was induced after a short Al treatment (maximum expression after a 2-h exposure), while the *pAtGST11::GUS* gene was induced by a longer Al treatment (approximately 8 h for maximum expression). Since the gene expression was observed in the leaf when only the root was exposed to Al stress, a signaling system between the root and shoot was suggested in Al stress. Regulation of Al tolerance by alternative oxidase (AOX) in tobacco has been observed through an overexpression approach.[55] Recently, two genes *STAR1* and *STAR2*, were identified by Huang et al.[57] which are responsible for Al tolerance in rice. Both, *STAR1* and *STAR2* are expressed mainly in the roots and are specifically induced by the Al exposure.

Endocytosis and Endocytic Vesicle Recycling as Primary Target of the Al Toxicity?

Any explanation of the Al toxicity in plants must deal with the fact that shoots are less sensitive than roots; and that in the roots only very small region of the root apex, the distal portion of the transition zone, is the most sensitive portion of the whole root.[4,63-65] In other words, the primary target must be either a molecule which is expressed only in this very particular root apex zone, or a process which is accomplished very actively only in cells of this root apex zone. Recent studies highlight high rates of endocytosis and endocytic vesicle recycling in cells of the this root apex zone.[66,67] Moreover, these cells internalize Al via endocytosis[64] and the endosomal Al affects endosomes, endocytic vesicle recycling[64,68,69] and all the processes linked to this phenomenon including the actin cytoskeleton,[63,68] nitric oxide (NO) production[64] and the polar auxin transport.[69-71] Close interactions between high Al-sensitivities, endocytic vesicle recycling, prominent NO production and signaling as well as dynamic and abundant actin cytoskeleton are characteristic not only for root cells of the transition zone[63,64,68] but also for apices of the tip-growing cells such as pollen tubes and root hairs[73-75] (review in ref. 76). The higher Al toxicity is also linked to higher proportion of recycling pectins including the deesterified JIM5-positive pectins and the boron cross-linked RGII pectins.[77-79] As these cell wall pectins are internalized together with the PIN proteins transporting auxin and auxin itself,[80-82] it is not surprising that the Al toxicity affects strongly the polar auxin transport in cells of the transition zone.[69-71] Finally, also in animals/humans, Al is neurotoxic and the Al-sensitive neurons are also active in endocytosis and endocytic vesicle recycling (reviewed in ref. 76). So rather unexpected unification of the Al toxicity phenomena in biology is possible as common features of the Al toxicity emerge for both animal and plant cells.[76]

Future Directions and Conclusions

Al^{3+} solubilized in acidic soil is extremely toxic in terms of root elongation, and is believed to be the primary factor inhibiting plant growth. Therefore, intensive research has been conducted in order to ascertain the mechanisms inherent to the Al toxicity and tolerance, on scales from the global to the molecular. Many of the biological activities of the plant are altered via the Al toxicity. So through selection and breeding process strategies, it is possible to develop Al tolerant plant. Better understanding of the Al tolerance mechanisms involving internal detoxification of Al with organic acids and the sequestration of the Al-OA complexes in the vacuole will be needed. Also, deeper understanding of the role of mitochondria and biochemical mechanisms involved in Al stress signaling needs to be achieved. Designing appropriate screening method remains the most challenging aspect of developing and characterizing Al tolerant plant. Over the past decades many researches has been done for significant progress towards the goal of developing crops better suited for cultivation with Al toxicity in acid soil. Several physical aspects of the Al cytotoxicity have been uncovered. Screening assays based on Al-accumulation in root cells and excluding of Al from the root should be extensively studied for better response of plant to the Al-phytoxicity. With further identification of molecular markers linked with Al-tolerance gene it is possible to develop better Al tolerant crop. However the nutrient deficiencies associated with the Al toxicity in acid soil need to be addressed in developing new Al-stress tolerant plant lines. These technologies will prove useful in environmental cleanup procedures as well as in restoration of soil fertility. These measures in the field of research can be able to solve the problem of food scarcity due to abiotic stress and thus give food security to the malnourished population in the developing third world countries.

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