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Molecular Basis of Aluminium Toxicity in Plants: A Review

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ABSTRACT

Aluminium toxicity in acid soils having pH below 5.5, affects the production of staple food crops, vegetables and cash crops worldwide. About 50% of the world's potentially arable lands are acidic. It is trivalent cationic form *i.e.* Al^{3+} that limits the plant's growth. Absorbed Aluminium inhibits root elongation and adversely affects plant growth. Recently researches have been conducted to understand the mechanism of Aluminium toxicity and resistance which is important for stable food production in future. Aluminium resistance depends on the ability of the plant to tolerate Aluminium in symplast or to exclude it to soil. Physiological and molecular basis of Aluminium toxicity and resistance mechanism are important to understand for developing genetically engineered plants for Al toxicity resistance. This paper provides an overview of the state of art in this field.

Keywords: Aluminium Toxicity; Acid Soils; Malate; Citrate; Wheat; Field Crops

1. Introduction

It is estimated that around 30% of the world's total land area consists of acid soils, and about 50% of the world's arable lands are acidic [1,2]. Moreover, up to 60% of the acid soils in the world occur in developing countries like in South America, Central Africa and Southeast Asia due to which, food production is critical. Aluminium (Al) is the third most abundant metal found in earth's crust after oxygen and silicon [3]. Al toxicity in acid soils affects the production of staple food crops, particularly grain crops, by decreasing their yield and vigor. Although the poor fertility of acid soils is due to some of the mineral toxicities (Al and Mn) and deficiencies (P, Ca, Mg and Mo), Al toxicity is the most important factor, being a major constraint for crop production on 67% of the total acid soil area [4].

Acidic soil occurs naturally in tropical and subtropical zones, but in temperate zones, it is the result of acid rain in the industrial regions of the USA, Canada and Europe [5] and this is an increasing problem. Al toxicity is considered as one of the most important limiting factors in agricultural production worldwide [1]. Many of the soils used for agriculture, particularly those in developing countries where forests have been cleared, are considered sufficiently acidic that they restrict the growth of many susceptible plant species. A description of the types of soils that are acid and their distribution is provided by [1].

The naturally occurring forms of Al are stable and do not interact with the biological processes of living organisms. But, as the soil pH drops below 5, Al is solubilized into toxic forms like $[Al(H_2O)_6]^{3+}$ which is now presenting in 40% of the arable lands in the world. Excess Al^{3+} in soil enters roots [6-8], thus interfering with a wide range of physical and cellular processes, resulting in the inhibition of root growth and function which finally affects other plant parts and related processes and thus reducing crop yields. Not only in plants but, it is also found that it causes problems in humans, also as in nervous system, lungs, and kidney. Even some studies also showed that the people exposed to high levels of aluminum may develop Alzheimer's disease, Parkinson's disease etc.

Al adversely affects the uptake and transport of essential nutrients, thus affecting the cell division in root tip and lateral roots, increases the rigidity of DNA double helix by reducing DNA replication, cross links pectin, increases cell wall rigidity, reduces root respiration and interferes with enzyme activity related to sugar phos-

phorylation and the deposition of cell wall polysaccharides. Aluminium toxicity has remained a hot spot in the field of research these days. A number of scientists are highly indulged in studying the physiology, genetics and molecular biology of Al resistance in crop plants.

Fortuitously, crop plants have evolved resistance mechanisms that enable them to tolerate toxic levels of Aluminium in acid soils [7,9-13]. Resistance to Al can be achieved via exclusion of Al from the root apex and/or via intracellular tolerance by sequestration of Al in the plant's symplasm (*i.e.* "true tolerance"). Although recent evidence for an Al resistance mechanism involving internal detoxification and sequestration is starting to emerge, the most compelling evidence has focused on a resistance mechanism based on chelation and exclusion of extracellular Al via Al-activated root organic acid release [12,14-16]. An early study in snap bean (*Phaseolus vulgaris*) [17], followed by a more extensive characterization of the same phenomena in wheat (*Triticumaestivum*) [18,19], showed that Al-tolerant genotypes exhibit a strong, Al-activated exudation of Al-chelating organic acids (citrate in snap bean and malate in wheat), which is absent or much smaller in the Al-sensitive genotypes.

2. Aluminium Induced Changes in Physiology and Morphology of Plant

Al toxicity is associated with gross changes in root morphology. Briefly, Al toxicity results in inhibited root elongation, which yields swollen root apices and poor or no root-hair development. This extensive root damage results in a reduced and damaged root system which limits water and mineral nutrient uptake [11,20]. Long-term exposure to Al results in deficiency of some essential nutrients such as phosphorous, calcium, magnesium, potassium and iron [21] which can be easily detected in plants as deficiency symptoms. The most common responses in shoot and leaves to Al toxicity are curling or rolling of young leaves, small and dark green colouredleaves, reduced stomatal opening, purpling of stems, decreased photosynthetic activity, yellowing and death of leaf tips, chlorosis and foliar necrosis [3]. Interplay of Al to other nutrients like Ca and K is reliable indicator of the quality response than the Al status alone. This all ultimately results in reduced plant biomass [22]. The degree of toxicity reported in the literature varies widely depending on the plant species, growth conditions, Al concentrations, and the duration of the exposure.

2.1. Effects of Al on Root Cap Function and Root Development

Aluminium is mainly solubilized in its predominant form *i.e.* Al^{3+} form in soil. This is the main toxic form of Al for crop plants. Aluminium interacts with cell wall, plasma membrane, particularly of the root apex, and rarely with cytoplasm of the plant cell to form Al-complex. Root is the primary site for Al toxicity. In its presence, roots usually become stubby and brittle, root tips and lateral roots become thick and may turn brown which is the visible symptom of Al toxicity [23]. As the earliest symptoms of toxicity are concerned with roots, thus root growth inhibition can be used as a tool for the measurement of Al toxicity. It was reported that a greater amount of Al absorbed by plant roots penetrate the boundary between the root apex and root cap and get accumulated in the nuclei and cytoplasm of the cells that are adjacent to this area. While some of the Al passed through the epidermis cortex, it seems to be that endodermis of the cell prevents Al from entering into the central cylinder. He added that some Al might have by-passed the epidermis by entering the root apex and passing through meristematic cells of the central cylinder. Effects of Al on plant root can be examined by observing the different segments of the root cell.

2.2. Al Toxicity at Cellular Level

The complexity of the many cellular processes involved in root growth inhibition, the precise Al toxicity targets in this complex chain of events remain elusive. Al binding to plasma membrane phospholipids surrounding trans-membrane transporters may induce local charge disturbances and alter local ion concentrations, thus effects ion movement to binding sites in membrane transport proteins. One of the most noticeable consequences of root Al exposure is an almost instantaneous depolarization of the plasma membrane [24,25]. This change in the trans-plasma membrane electrochemical potential may be due to both direct and indirect interactions of Al with a number of different ion transport pathways [26].

2.2.1. Cell Wall

Al primarily and predominantly gets accumulated in the root apoplast which covers 30% - 90% of the total absorbed Al of peripheral cells in plant [27,28] which is then slowly translocated to other central tissues [29,30]. The primary binding of Al^{3+} in the apoplast is probably the pectin matrix, with its negatively charged carboxylic groups [30,31]. Al absorption in the plant cell wall reduces the movement of water and solutes through the apoplasm which finally and directly decreases nutrient acquisition by the root [32]. Consequently, the strong and rapid binding of Al can alter cell-wall structural and mechanical properties, making it more rigid, leading to a reduction in the mechanical extensibility of the cell wall required for normal cell expansion in the root elongating zone [13].

X-ray microanalysis and secondary ion mass spectro-analysis techniques are used to determine the amount

of Al which is associated with apoplastic binding sites. The net negative charge on the cell wall determines its Cation Exchange Capacity (CEC) and consequently the degree to which Al interacts with the cell wall. Aluminium crosses links with pectin and increases cell wall rigidity thus leading to decrease in the mechanical stability and ultimately decrease in cell growth. Cell wall cations are strongly replaced by Al, finally resulting in drastic change in cell wall structural and mechanical properties [30,33-35].

2.2.2. Apoplast

Isotopic tracer studies using Mg^{2+} demonstrated the existence of the apoplasmic pathway for Mg^{2+} ions in the cortex of mycorrhizal roots of Norway spruce [36]. Further, entry of Mg^{2+} into the endodermis was faster through the apoplasmic than the symplasmic pathway [36]. Given this importance of the apoplasmic pathway for Mg uptake and transport, it should be borne in mind that in Al^{3+} toxicity large amounts of Al (85% - 99.9% of total cellular Al) accumulate in the cell walls and intercellular root spaces [14,37,38]. More specifically, binding of Al to the negative charges on the pectin substances in the cell wall was observed [32]. Such binding of Al on the cell wall and precipitation of Al in the apoplast may decrease loading of Mg^{2+} ions into the apoplast and movement via the apoplasmic pathway.

2.2.3. Plasma Membrane

Plasma membrane is a dynamic quasi fluid structure which forms the external boundary of the cells. Membranes viewed as quasi-fluid structures are those in which proteins are inserted into the lipid bilayers. Al can interact strongly with the negatively charged plasma membrane. Depending on pH and other factors, Al can bind either to proteins or to the lipids. It can displace other cations like Ca that may form bridges between the phospholipid head groups of the membrane bilayer [39]. As aluminum has greater affinity for the choline head of phosphotidyl choline, a lipid constituent of the plasma membrane, it displaces other cations that are present on negatively charged plasma membrane and then binds itself. Depolarization of the plasma membrane is one of the consequences of the Al toxicity [25]. Al interaction with plasma membrane could lead to depolarization of the trans-membrane potential [40] and/or reduction of H^+-ATPase [41] which, in turn, can alter the activities of ions near the plasma membrane surface and impede the formation and maintenance of the trans-membrane H^+ gradient [13]. Moreover, Al changes in plasma membrane can modify the uptake of several cations (e.g., Ca, NH^{4+}) [42-44]. These changes are related to direct Al^{3+} interactions with plasma membrane ion channels [43] and changes in membrane potential. This may cause nutritional imbalances induced by Al exposure.

Displacement of cations by Al results in the excessive synthesis of callose(β-1, 3-glucane) on the plasma membrane by β-1, 3-glucanesynthetase. Accumulation of callose may lead to the further cellular damage by inhibiting intercellular transport through plasmodesmatal connections [45]. Thus the callose formation can be taken as a parameter of Al sensitivity and is positively correlated with pectin content [30]. Increase in the pectin content results in higher Al content of the cell, clearly indicating that pectin plays a major role in the binding of Al. Therefore, Al-sensitivity is found in cells having high pectin content while the cells with less pectin content seems to be Al-resistant. Accumulation of Al depends on the degree of dissociation of carboxylic and hydroxylic groups of the pectin [46]. Aluminium directly or indirectly interacts with a number of different ion transport pathways resulting in change in the transplasma membrane electrochemical potential [26].

It has been well established that Al can enter the symplasm of root cells quite rapidly [47,48] and can be sequestrated in the vacuole after 30 min [38]. Indeed, putative plasma membrane-localized Al transporter, Nrat1 (Nrampaluminium transporter 1), has been identified recently in rice [49], but it remains unclear whether it is specific for Al^{3+} or can transport other cations also. The Al entry into the cytoplasm affects the homeostasis of various ions, such as H^+ [50], Ca^{2+} [51,52], and K^+ [53].

2.2.4. Plasma Membrane H^+-ATPase

Activity of the plasma membrane H^+-ATPase is inhibited by the presence of Al which ultimately affects the formation of trans-membrane H^+ gradient [54,55]. For secondary ion transport processes trans-membrane H^+ gradient plays a major role. Therefore disruption of H^+ gradient could indirectly alter the ionic status and ion homeostasis of root cells.

2.2.5. Cytoskeleton

The orientation of cytoskeleton provides a template both for cell division and cell wall biosynthesis [56]. It is believed that because of the central importance of cytoskeletal components (microtubules and microfilaments) in cell division, it is a main target for Al toxicity [57-66]. Cytoskeletal dynamics can be disrupted either by direct-interaction with cytoskeletal elements (*i.e.*, microtubules and actin filaments) or indirectly by altering cytosolic Ca^{2+} levels that are involved in cytoskeletal stabilization. Al exposure can disrupt both the organization of microtubules and microfilaments in root cells [59-64]. For example, exposure to Al results in the disruption and reorganization of cortical microtubules. Likewise, Al induced a significant increase in the tension of the actin filaments of soybean (*Glycine max*) cells [67].

2.2.6. Mitochondria

A recent study demonstrated that *Rhodotorulaglutinis* has 2.5 to 3 folds more mitochondria in Al-resistant yeast strain than the wild-type strain [68]. The Mg^{2+} ions are essential for normal functioning of mitochondria as their deficiency often results in mitochondrial disintegration [69], reactive oxygen species (ROS) production, and photo-oxidative damage in many plant species [70]. Plant cells also have numerous mitochondria; however, no direct correlation has yet been established between abundance of mitochondria and Al resistance. Mg ion might be an important component of characterizing the physiology of Al resistance. Al^{3+} toxicity may also provoke similar mitochondrial dysfunction [71] and ROS production in many plant species [72-76] presumably by causing Mg deficiency inside the mitochondria or by substituting Mg for Al^{3+} in Mg^{2+}-dependent enzymes [77,78]. Thus, mitochondrial Mg porters could be the target site for Al^{3+} toxicity.

2.2.7. DNA/Nuclear Damage

After interaction with cell wall and cell membrane, Al interacts with the structures within the nucleus detrimentally affecting DNA composition, chromatin structure and template activity. Al reduces DNA replication by increasing the rigidity of the double helix. [79,80] reported that the application of Al (0.2 - 1.0 mM) inhibits cell division and cell viability.

3. Effect of Aluminium on Shoot

Ten barley cultivars were tested and screened out for Al tolerance by growing them for 25 days in the greenhouse in pots containing acid soil and Al toxic Tatum subsoil [81]. It was reported that relative shoot dry weight averaged 28.6% for tolerant and 14.1% for sensitive cultivar groups. In shoots of sensitive cultivar, Al concentration at pH 4.4 was found to be three times higher than in those of the tolerant group. 15 Durum wheat (*Triticum durum* Desf.) cultivars were also tested for Al tolerance at pH 5.7 [81]. Concentrations of Al and phosphorous were significantly higher in shoots of sensitive lines as compared to the tolerant one, grown in acid soils. For the first time, they demonstrated that Al tolerant group of wheat was able to increase pH in nutrient solutions comparatively to Al sensitive cultivars, when both are tested with or without Al. [82] tested two cultivars of *Coleus blumei* in nutrient solution containing 0 - 24 mg/L Al and in Al-toxic tatum subsoil under greenhouse conditions. Inhibitory effects of Al-toxicity were observed on shoot growth, that were cultured in nutrient solution having Al concentration 8 mg or above, while inhibition of root growth was observed in solution having Al concentration 16 mg/L or above.

4. Nutritional Imbalance

Nutritional imbalances induced by Al exposure were reported in several plant species by many researchers. Al interferes with the uptake, transport and utilization of most of the mineral elements. Under Al stress, the uptake of many cations including Ca^{2+} (69%), Mg^{2+}, K^{+} (13%) and NH^{4+} (40%) is inhibited while the influx of the anions of nitrate (44%) and phosphate (17%) get enhanced. Mineral nutrition was most often accompanied by increased H^{+} release in Sorghum [83], Maize [84], Wheat and Soybean. [85] reported that Mg^{2+} was more effective than Ca^{2+} in alleviating Al stress in monocotyledons whereas vice-versa for the dicotyledons. Al-sensitive cultivars were characterized by chlorosisin Al stress in nutrient solution, decreased Fe concentrations in tops, decreased Ca and Mg in shoots and roots both, a tendency towards accumulation of Al, P and Fe in roots, and reduced Mn in tops. Eleven families of pteridophytes presented different nutritional imbalances, mostly in Ca, Mg, P, K depending on Al accumulation [86]. In maize, Al resulted in negative effects on the uptake of micro (Mn and Zn) and macronutrients (Ca and Mg) [87] and K [88] than the Al-sensitive genotypes. Both sensitive and tolerant genotypes of wheat had presented a decrease in K and Mg contents in roots, whereas Ca, Al, Si contents increased [89]. It was reported that NO^{3-} uptake by soybean was decreased when Al concentration in solution increased from 10 to 50 μM [90] whereas, Al reduced Cl^{-} and NO^{3-} uptake in maize [91].

It is also observed that Al toxicity is closely related to nitrogen metabolism [92]. It was noted that nitrate reductase activity was higher in Al tolerant cultivars, when grown in Al treated nutrient solution [93]. Al interfered with the binding of the cations in the cell wall by the same order of magnitude as their respective influxes whereas phosphate binding was strongly enhanced [94]. Some suggested that Ca^{2+} that plays an important role in mechanism of resistance against Al toxicity is particularly inhibited by Al^{3+} [95]. Due to the deficiency or reduction of Ca^{2+} ion transport and disruption of cellular Ca^{2+} homeostasis, root growth of a plant is inhibited. Al toxicity appears as an induced Ca deficiency or reduced Ca transport problem. Excess Al even induces iron (Fe) deficiency symptoms in rice (*Oryza sativa* L.), sorghum and wheat [92,96,97].

Deficiency of Phosphorous

Phosphorous is the main component of several biological compounds such as nucleic acids, phospholipids and ATP. It also acts as a metabolite involved in energy transfer, the activation of proteins and the regulation of metabolic processes [98,99]. Inorganic phosphate is the primary source of phosphorous for plants. It enters into

the equilibrium reactions defined by P-sorption isotherm [100]. Even in the most fertile soils, P_i concentration in soil solutions rarely exceeds 8 μM [101]. For the increment in the P_i concentration, plants have adapted a number of morphological and biochemical strategies. Highly branched root systems with more root apices are highly capable of acquiring phosphorous. Therefore, the surface area of roots in contact with the soil, increased in some species by an increase in diameter of roots when the plants are P-stressed [15] and both the density and length of root hairs may increase [102-104]. As Al-toxicity highly affects root development, as a result phosphate deficiency occurs in plant whose adverse effect can be seen in plants growth. Deficiency of phosphorous in plant that grows on acid soils or in nutrient solution is caused because of Al interference with phosphorous.

5. ROS (Reactive Oxygen Species)

Aerobic processes such as respiration and photosynthesis led to the formation of ROS in mitochondria, chloroplasts, and peroxisomes [12]. Different types of ROS are found in plant cell but the common property among them is that they all have the capacity to cause oxidative damage to proteins, DNA and lipids and ultimately results in the death of a cell. ROS are mainly synthesized as signaling molecules in plants and are involved in regulating development and pathogen defense responses. But their production in higher amounts can affect the cell metabolism. 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. Large number of swollen mitochondria with many vacuoles, structural disturbances of the plasma membrane, and preapoptotic nuclear structures were some of the characteristic features of Al treated tobacco cells, confirming that Al signaling follows the mitochondrial pathway of cell death. 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 dehydroascorbatereductase, dehydroascorbatereductase, glutathione-S-transferase (GST) and glutathione reductase, which help to detoxify the ROS.

Different types of anti-oxidative defense components are present in cellular compartment that cause scavenging of ROS. But the equilibrium between production and scavenging of ROS may be perturbed by a number of adverse environmental factors: Al-toxicity is one of them. Al exposure leads to oxidative stress [74,105-107]. Under environmental stress, for example, Al-stress plant cells generate ROS by activating various oxidases and peroxidases. Because Al ions form electrostatic bonds preferentially with oxygen donor ligands (e.g., phosphate groups or carboxylate), cell wall pectin and the outer surface of the plasma membrane seem to be major targets of Al [108].

"Oxidative burst" is the condition when there is a rapid increase in ROS concentration. Toxicity of ROS has often been monitored by measuring lipid peroxidation. Polyunsaturated fatty acids within the lipids are preferred target of ROS attack. Enhancement in lipid peroxidation occurs under prolonged Al-stress and results in formation of highly toxic oxygen free radicals [105].

Anti-Oxidant Enzymes

Antioxidant enzymes that act as scavengers of ROS are activated during Al-stress [109]. Enzymatic ROS scavenging mechanism that acts as the first line of defense system in plants, include-superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione peroxidase (GPX), and CAT. SODs act as the first line of defense against ROS, dissimulating superoxide to H_2O_2. APX, CAT and GPX subsequently detoxify H_2O_2 [110].

6. Mechanism of Aluminium Tolerance in Plants

Al toxicity and tolerance mechanisms differ in plants according to its chemical form, and the study of Al related processes are complicated by the complex chemistry of Al. There are several recent reviews/researches that discuss mechanisms of Al tolerance and toxicity in plants by [7,9,15,16,111-115]. Al toxicity affects a number of cellular components such as composition, physical properties and structure of the plasma membrane [116,117], cell nuclei, mitosis and cell division [Silva, *et al*., 2000], uptake of Ca^{2+} and other ions [118] and cytoskeletal dynamics [56] and many more. Primary target of Al toxicity is the disturbance of cytoplasmic Ca^{2+}-homeostasis [52] and may be involved in the inhibition of the cell division. In wheat root apices, it was found that Al inhibits Ca^{2+}-dependent phospholipase C, which acts on the lipid substrate phosphatidylinositol-4, 5-biphosphate. It was hypothesized that phosphoinositide signaling pathway might be the initial target of Al [119].

7. Cultural Practices

7.1. Altering Soil pH

Liming has been attempted for checking soil acidity and provides resistance against Al toxicity. However, use of lime as a main application for managing acid soils is either too costly or takes many years to be effective particularly where the acidity occurs at depth.

7.2. Crop Rotation

On acid soils, Al sensitive species can be replaced by Al tolerant species to maintain crop production. For example, replacement in pastures alfalfa from Al sensitive to more Al-tolerant pasture species but the drawback with this approach is that the nutritional quality of the alternative pastures might not match that of alfalfa.

7.3. Breeding for Resistance

Breeders can use even little variation within a species to enhance the Al tolerance of elite genotypes. For example, a small number of major dominant genes control Al tolerance in wheat (*Triticum aestivum*) and breeders exploit this property in breeding programs to enhance Al tolerant cultivars [120-122]. Root growth inhibition is the first symptom of Al toxicity and the use of defined concentrations of Al in hydroponic culture has proven to be a reliable measure of Al tolerance for a number of species. The hematoxylin stain is also one of the useful parameter in determining the Al tolerance of plants [123].

8. The Complex Role of Organic Acids in Detoxifying Al^{3+}

It is estimated that over a dozen of Al-tolerant plant species secrete organic acids from their roots in response to Al-stress. Al resistance is correlated with the Al-induced secretion of organic acids like citrate, oxalate or malate [124-127]. Some of the organic acid that are secreted by plants such as citrate, oxalate, malate etc., which are anionic in nature, form complexes with Al^{3+} in order to protect plant roots [15]. Al-resistant cultivars of snapbean (*Phaseolus vulgaris*) excreted eight folds more citrate from the roots than did an Al-sensitive genotype, is the first evidence in the field of Al-resistance mechanism [12,17]. Malate is released from the roots of Al-tolerant cultivars of wheat (*Triticum aestivum*), citrate from Al-tolerant cultivars of snapbean (*Phaseolus vulgaris*), maize (*Zea mays*), *Cassia tora* and soyabean (*Glycine max*) and oxalate from buckwheat (*Fagopyrum esculentum*) and taro (*Colocasia esculenta*). Some plant species such as Al-tolerant triticale, rapeseed (*Brassica napus*), oats (*Avenasativa*), radish (*Raphanus sativus*) and rye (*Secale cereal*) release both malate and citrate [15,17, 128-135].

It was found that Mn, La, Cd, or Pb treatment did not induce the secretion of organic acids. Even P deficiency during Al treatment was also not responsible for Al-induced secretion of organic acids. Constitutive phosphate secretion might operate in conjunction with the Al-induced secretion of organic acids to confer Al resistance in certain wheat genotypes [136]. In Cassia toraor buckwheat, neither P deficiency nor application of La or Yb induced secretion of organic acid [126,127,132]. It was reported that La failed to induce secretion of malate in Al-resistant cultivars of wheat [137]. Aluminum resistance in the arabidopsis mutant *alr*-104 is caused by an Al-induced increase in rhizosphere pH [138]. Thus, it was observed that the secretion of organic acid from roots was a specific response to Al stress [129,139,140]. In these examples, it was observed that the efflux of organic acids occurs primarily from the root apices and this proves somewhere that the plant root system is most susceptible to Al toxicity. Moreover, the finding that Al-tolerant genotypes exude more organic acid than the Al-sensitive genotypes supports the hypothesis that the organic acid efflux is an Al tolerance mechanism.

5- or 6-membered ring structures of organic acid with Al^{3+} to protect the plants from Al toxicity.

Thus, there are now a number of evidences which proves that organic acids play an important role in the Al tolerance mechanisms in different plant species. Some organic acids are able to form non-toxic complex with Al^{3+}. It was observed a range of different organic acids that chelate the Al and therefore protect the roots from Al toxicity in hydroponic culture [141]. They found that an organic acid with hydroxyl and carboxyl groups shows greatest protection from Al toxicity. Al tolerance mechanisms suggested that organic acids can be divided into external and internal detoxification and some plant species are using both types of mechanisms.

Two patterns of organic acid secretion have been observed. In first pattern, no discernible delay was observed between the addition of Al and the onset of organic acid release. For example, in beet and buckwheat, the secretion of malate and oxalate respectively was detectable within 15 - 30 min after exposure to Al [15,128,132]. In second pattern, delayed secretion of organic acids, for several hours after exposure to Al^{3+}, was observed. For example, after 4 hours exposure to Al, maximal efflux of citrate occurs in *C. tora* [130] and in rye, malate and citrate efflux increases steadily during 10 hour period [134]. It is believed that in first pattern, transporter on the plasma membrane might be activated by Al to initiate anion efflux and there is no need of novel proteins. Some authors suggested that in Pattern I, Al activates a pre-existing transport mechanism for malate and a role for anion-channels in the transport of the organic acid. While little information is available about the mechanism of Pattern II as it is estimated that in second pattern, protein induction is required. These proteins could be in-

volved in transporting organic acids out of the root cells and/or in the synthesis of organic acids.

Some of the major aspects of this resistance mechanism include [12]:

1) Al forms complexes with carboxylate that are not further transported into roots or across the membranes [142,143].

2) Exogenous Al^{3+} induces secretion of carboxylate [124].

3) Over expression of genes encoding enzymes involved in organic acid synthesis such as citrate synthase and malate dehydrogenase results in enhanced Al-resistance in some plant species [144-146].

4) Al-resistance co-segregates with Al-induced malate release in wheat and *Arabidopsis* [18,19,147].

5) An Al gated anion channel in maize (*Zea mays*) and wheat root tip protoplasts has been identified via electrophysiological experiments, and exhibits the properties necessary for it to be the transporter mediating Al-activated carboxylate release [43,148-150].

It is also observed that the amount of organic acid released from root apices need not to detoxify all Al in the soil surrounding the root system. Detoxification of Al that immediately surrounds the root apices is quite sufficient. However, it is also required to replace organic acids that are broken down by microorganisms as well as to replace organic acids that diffuse away from the root apex. It is suggested that when organic acid moves through an acid soil, it acts as a protective sheath around the root apex.

Anion Channels and the Efflux of Organic Anions

At pH 7 of the cytoplasm, organic acids are dissociated in their anionic forms from their protons. Both the concentration gradient for the organic anions and the electrochemical gradient across the plasma membrane helps in the efflux of organic anions out of the cells. As organic anions are the charged molecules, they move through the hydrophobic lipid bilayer of the plasma membrane. Organic anions might be released in two conditions, 1) if the plasma membrane gets damaged, because of any natural phenomenon which results in cell leakage and finally cell death, 2) efflux of organic acids due to Al toxicity. However, second one is a controlled process and can be stopped or reduced when Al is removed from the medium. Thus, rupture of the plasma membrane is not actually responsible for the efflux because, only one or two organic anions are exuded from roots, in response to Al. Furthermore, it is also observed that Al tolerant genotypes efflux more organic ions than the sensitive genotypes, where Al toxicity results in damage to the plasma membrane (**Figure 1**).

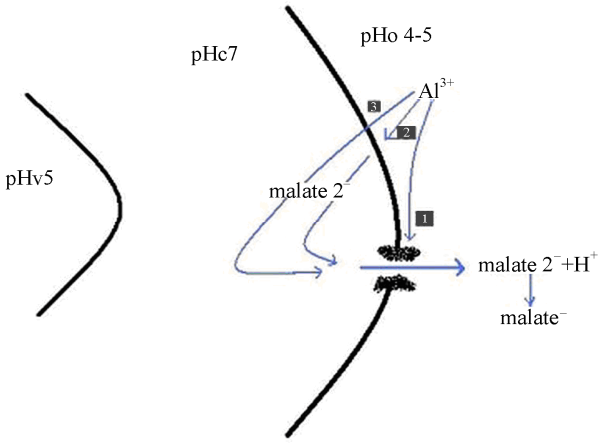

Figure 1. Mechanism of Al activated efflux in the plant root tip cells. Three possibilities are shown where Al interacts (1) with the channel protein directly or (2) with any component of the plasma membrane or (3) it enter the cell to trigger the opening of the channel for malate efflux. The malate has the capacity to chelates Al^{3+} thus makes it nontoxic.

9. Carboxylate Transporter

For translocation of organic anions across the plasma membrane, difference in pH or H^+ activity between the cytoplasm and the apoplasm plays a major role. Without any stress, the pH in the cytoplasm is (7.3 - 7.6); vacuoles (4.5 - 5.9); mitochondria (7.0); chloroplasts (7.2 - 7.8), and in apoplasm (5.5) [151]. Thus, it is found that the cytoplasm is neutral when compared with vacuoles and the apoplasm.

Detoxification of Al in the rhizosphere by organic acids occurs in the apoplasmtherefore, organic acid must be transported from the cytosol to the apoplasm. As mentioned above, in cytoplasm, organic acid anions are formed at neutral pH (7.3 - 7.6); these organic acid anions are transported out of the root cells. Al exposure triggers the release of a specific carboxylate from a cytoplasm that contains a number of different carboxylate species. Therefore, activation of a particular carboxylate transporter is required that presumably resides in the root cell plasma membrane. In maize and wheat, this transport system has been identified as an anion channel [43,148, 149,152]. Anion channels are the membrane bound transport proteins that allow the passive flow of anions down their electrochemical gradient [15]. Using the patch clamp technique, protoplast isolated from root tips of Al-resistant wheat, anion channels have been identified in the plasma membrane that are specifically activated by extracellular Al^{3+}. These anionic channels are permeable to malate and chloride [148,149]. Anion channel is more active and open more frequently in root tip protoplast of the Al-tolerant wheat genotype compared with those from the sensitive one. In root tip cells

from Al-resistant maize, a similar anion channel has been identified where Al-activated root citrate release is correlated with resistance [43,150].

Three possibilities have been proposed for, how Al can activate these anion channels [6].

1) Interaction of Al directly with the channel protein triggers its opening.

2) After entering into the cytoplasm Al directly or indirectly activates the channel through signal cascade.

3) Interaction of Al with a specific receptor on the membrane surface with membrane itself for the initiation of a secondary messenger cascade which then activates the channel.

It is still unclear, what is the reason behind release of large amount of organic acid from the Al-tolerant genotype than Al-sensitive genotype. Researchers are assuming that there might be differences in the number of channel proteins in the membrane of each genotype, in their permeability to organic anions or in their activation by Al [15].

10. Al-Resistant Mechanism Involves Detoxification of Internal Al through Organic Acids

In some plant species that are highly tolerant, accumulation of high concentration of Al in the above ground herbage is observed without showing symptoms of Al toxicity *i.e.* they have the ability of accumulating Al in its shoots and roots. For example—*Hydrangea macrophylla* is an ornamental plant whose sepals turn from red to blue with increasing Al concentration [130]. This blue color is due to the formation of a complex of Al with two compounds, delphinindin-3-glucoside and 3-caffeolylquinic acid that are present in *Hydrangea macrophylla* sepals [153].

Al can bind to oxygen ligand with a greater affinity than any other element. Al^{3+} form of Al has 10^7 times stronger binding affinity with ATP than does Mg^{2+} for binding sites on ATP [154]. Therefore, for the resistance from Al, plants must possess effective mechanism for the detoxification of internal Al^{3+}. Three Al accumulator species have been identified recently that are involved in the detoxification of internal Al^{3+} by forming Al organic acid complexes.

Al, in leaves, exists primarily as a 1:1 Al-citrate complex. A second Al-accumulator in buckwheat (*Fagopyrum esculentum*), in which complexes of Al-oxalate as 1:3, have been identified [127,155].

Al undergoes a ligand exchange from oxalate to citrate when it is transported into the xylem and is exchanged back with oxalate when in the leaves. Detoxification of Al is subsequently followed by the storage of Al-organic acid complex in the vacuole [14]. Tonoplast localized mechanism mediating the transport of Al into the vacuole, as well as the nature of substrate (*i.e.* free Al versus Al carboxylate complexes) remain unknown [12].

11. Other Al—Tolerance Mechanisms

Where release of organic acids is one of the most important mechanisms used by plants against Al-toxicity, some plant species do not rely on these mechanisms. For example, *Brachiaria decumbans* does not secrete organic acids in response to Al [156]. As Al toxicity is largely dependent on pH, thus, increase the pH around root apices by any mechanism, and may provide protection against toxicity. For example, Al-tolerant Arabidopsis mutant (*alr*1) exhibit an Al-induced increase in pH, surrounding the root apex which results in decrease in Al^{3+} activity [138]. Efflux of phosphate is also one of the Al tolerance mechanisms. Phosphate combines with Al and form complexes and released along with malate, from the root apices of Atlas [136].

A number of studies had shown that Al toxicity may be due to oxidative stress and this induces the synthesis of proteins typical of oxidative stress responses. For example, Al induces the expression of genes that encode glutathione S-transferase, peroxidases and blue-copper proteins. It was observed that in Arabidopsis there was an increase in Al tolerance as well as increased tolerance to oxidative stress due to over-expression of some these induced proteins [157]. Study in yeast has also shown that Al tolerance is based on over-expression of genes rather than organic acid efflux [158].

12. Discussion

Al toxicity is the primary growth-limiting factor for plants in acid soils [159] and is most severe in soils with low base saturation, poor in Ca and Mg [5]. The primary limitations on acid soils are toxic levels of aluminum (Al) and manganese (Mn), as well as suboptimal levels of phosphorous (P). This extensive root damage results in a reduced and damaged root system and limited water and mineral nutrient uptake [9]. Although Al resistance has been a successful and active area of research; however, the underlying molecular, genetic and physiological principles are still not well understood. The cellular components and processes which have been proposed to be affected by Al are wide ranging and some of the most important include; cell nuclei, mitosis and cell division [147], composition, physical properties and structure of the plasma membrane [116,117], uptake of Ca and other ions [118,160], phosphoinositide-mediated signal transduction and cytoplasmic calcium homeostasis [20,52], oxidative stress [105], cytoskeletal dynamics [56] and the cell wall-plasma membrane-cytoskeleton continuum [61].

Malate exudation mechanism by wheat has been investigated most thoroughly [9] while citrate seems to be

the most common organic acid anion exudated by Al-tolerant maize and snapbean [11]. In all three species secretion was greater (up to 10-fold) in Al-resistant cultivars than in Al-sensitive ones. Oxalate exudation in response to Al has also been observed in maize, but no differences between sensitive and tolerant varieties were detected [161].

Thus, it is observed that after Al^{3+}exposure, exudation of organic acids may occur either immediately (pattern I) or after a time delay (pattern II) [15,150]. But for long-term efflux of organic acid anions, continuous synthesis of organic acids inside the root cells is required [162] and for organic acid synthesis and metabolism, cytoplasmic Mg^{2+} activity plays important role in activation of many enzymes, such as, citrate synthase [163], malate dehydrogenase [164], malate synthase [165], iso-citrate dehydrogenase [166,167], malic enzyme [168], PEPC (phosphor enol pyruvate carboxylase) [169-171], and pyruvate kinase [172]. Even alleviation in Al^{3+} toxicity was found by addition of miliM concentrations of Mg in the external medium, by enhancingexudation of citrate in soybean [173] and rice bean [174]. It was also observed that pre-treatment with Mg^{2+} enhanced the secretion of citrate within an hour compared with seedlings without Mg^{2+} pre-treatment [175,176]. The Mg^{2+} activity inside the cytoplasm are directly involved in the regulation of H^+-ATPase activity [177,178]. Thus, pretreatment with Mg^{2+} undersealed the citrate synthesis mechanism for release of organic acid. Somewhere, proton pumps (H^+-ATPase and H^+-PPase), located in the plasma membrane and the tonoplast, respectively, also play important role by driving H^+ from the cytoplasm to either the apoplasm or the vacuole [179]. Hence, disturbance in H^+-ATPase activity by Al^{3+} toxicity [180-182] would affect cytoplasmic pH regulation [48,50].

13. Conclusion

Al toxicity is an important growth limiting factor for plants in acid soils which is comprised in a large area of fertile land, particularly in pH-5 or below. The morphological and physiological symptoms of Al toxicity in plants are often clearly recognizable. Tolerance to Al toxicity is clearly visible through differences in structure and function of plant parts. Metallic toxicity in tolerant and sensitive plant genotype was studied to determine specific gene(s) responsible for tolerance level and kind of amino acids which act as metallic chelator and detoxifier, level and forms of enzyme and changes in root permeability to ions and molecules and its mechanisms.

14. Future Challenges

Although considerable progress has been made in understanding Al-tolerance mechanisms based on organic acid efflux, much is still to be learned of the molecular mechanisms underlying the activation of anion-channels by Al. For instance, we need to better understanding of the processes involved in how a cell initially senses Al that then leads to channel gating and organic acid efflux. In addition, the genes encoding these anion channels need to be cloned. As indicated above, there are clearly Al tolerance mechanisms operating in plants that do not rely on organic acids but to date little is known about these mechanisms. Some progress has been made in genetically modifying plants to enhance their Al tolerance and future work is needed to ensure that sufficient levels of Al tolerance are obtained to be useful for agriculture.

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