

## Review

# Aluminium toxicity tolerance in crop plants: Present status of research

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Soil acidity is one of the major constraints to agricultural production in large parts around the world. In acid soils, aluminium toxicity and consequent low phosphorous availability impair plant growth. The primary response to aluminium stress is visible in the roots. Exclusion and neutralization are two well known mechanism of aluminium tolerance in plants. Although, relative root growth in high aluminium containing solution is often used for screening, a reliable screening procedure needs to be developed. 14 genes from seven different species are reported for aluminium tolerance of which genes of the Aluminium-activated malate transporter (ALMT) and multidrug and toxic compound extrusion (MATE) families are prominent. In this review, the progress of research in identifying aluminium toxicity tolerant genes is discussed.

**Key words:** Aluminium toxicity, soil acidity, hydroponic screening, aluminium-activated malate transporter, multidrug and toxic compound extrusion.

## INTRODUCTION

Soil acidity, associated infertility and mineral toxicities are major constraints to agricultural production in several parts of the world (Pariasca-Tanaka et al., 2009). Many tropical soils are acidic because they are millions of years old and have been exposed to continuous weathering. As rain water percolates downwards, soluble nutrients such as calcium, magnesium, and potassium leaches out of the top layers of the soil, and gradually gets replaced by aluminium, manganese and hydrogen, the elements most closely associated with soil acidity. Aluminum is one of the predominant elements of the earth crust and in soils with normal pH; it is present in insoluble form and hence causes no harm to plants. The solubility of Al in neutral and alkaline soils is too low to be toxic to plants. In acidic

soils, it becomes soluble and (i) enters root where it inhibits root growth and development, (ii) reacts with soluble phosphorus (available to plants) and converts it to insoluble aluminum phosphate which is not available to plants. Even micromolar concentrations of aluminum in the soil solution can rapidly inhibit the root growth of many species (Wissuwa and Ae, 2001; Wissuwa, 2005). As rice farmers around the world begin to turn from wet paddies to dry fields in an attempt to conserve water and mitigate climate change, they are facing a new foe: aluminium (Shackford, 2012).

Aluminum can be toxic to plants in acidic conditions. Its harmful effects are diluted as traditional rice paddies are flooded, but have become an issue as farmers are trying

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Abbreviations: **ALMT**, Aluminium-activated malate transporter; **MATE**, multidrug and toxic compound extrusion.

new ways of raising their crops.

## ALUMINIUM TOXICITY

Aluminium is not essential for plants. Although, metallic aluminium is non-toxic to plants, its ionic form ( $\text{Al}^{3+}$ ), prevalent in acid soil conditions is toxic to all living cells. The ionic form of aluminium rapidly inhibits root elongation by targeting multiple cellular sites, including cell wall and plasma membrane, and various cellular processes such as signal transduction pathways and calcium homeostasis (Wissuwa et al., 2001, 2005). In response to aluminium stress, roots become stubby and brittle. The root tips and lateral roots thicken and turn brown. Such aluminium injured roots becomes inefficient in absorbing nutrients, water and the root system as a whole gets affected with many stubby lateral roots and no fine branching. Consequently, plants become susceptible to various stresses especially drought. As acid soils occupy up to 40% of the world's arable land (Pariasca-Tanaka et al., 2009), aluminium phytotoxicity may be considered as one of the major limiting factors of crop productivity in the world (Hede et al., 2001). According to Kamparath (1980), crop production is drastically reduced when aluminium saturation of the active cation exchange sites is greater than 60% and tends to be optimum when aluminium saturation is zero. When a soil is more acidic than pH 6.0 to 6.5, the availability of plant nitrogen, phosphorus, sulphur, calcium, potassium, magnesium and molybdenum, may be too low for satisfactory plant growth (Panda and Matsumoto, 2007). In general, young seedlings are more susceptible to Al than older plants.

Experiments with maize seedling roots placed in divided chambers showed that root growth is inhibited only when the apical 2 to 3 mm of the root is exposed to Al, while application of Al to any other portion of the root does not affect root growth (Ryan et al., 1993). The stubby and brittle roots after Al exposure suggest that cytoskeleton may be the target for Al toxicity (Blancaflor et al., 1998). Horst et al. (1999) reported that microtubule and microfilaments in cells show altered stability and polymerization upon exposure to Al. Studies in rice (Zhang et al., 2007) and *Triticum* (Frantzios et al., 2005) showed that actin filaments and expression of a gene encoding for actin binding protein are altered upon exposure to Al. These reports suggest that the cytoskeleton is affected upon exposure to Al. Sivaguru et al. (1999) showed that the distal transition zone of root is most sensitive to Al exposure providing support to earlier studies that the root apex is the primary target site. Oxidative stress is considered as one of the main effects of Al toxicity (Zheng and Yang, 2005). Although, Al does not act as a catalyst in the production of Reactive Oxygen Species (ROS), its ability to bind to carboxylate, phosphate, etc. groups lead to oxidative stress mediated through cell wall pectin, plasma membrane, etc. (Yamamoto et al., 2001). The resultant rigid plasma membrane leads

to enhanced peroxidation of lipids (Yamamoto et al., 2003).

Aluminium induced lipid peroxidation has been reported for barley (Guo et al., 2004), sorghum (Peixoto et al., 1999), rice (Kuo and Kao, 2003), wheat (Hossain et al., 2005), greengram (Panda and Matsumoto, 2007) etc. However, Al treatment in maize did not lead to lipid peroxidation (Boscolo et al., 2003) indicating that the target of oxidative stress due to Al exposure varied with plant species. Plant cells produce a number of enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants such as catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD) and glutathion reductase (GR) were reported to be influenced by exposure to aluminium. In maize (Jones et al., 2006) and pea (Yamato et al., 2003), increase in ROS production in response to aluminium exposure has been reported. An increase in APX and SOD activity in Al exposed maize roots has been reported. In greengram, Panda et al. (2003) reported increase in SOD, APX and GR activities and decrease in non-enzymatic antioxidants like ascorbate (ASA) and glutathion (GSH). They concluded that ascorbate and glutathion are mainly responsible for detoxification of ROS. Increase in SOD activity in the rice roots in response to Al exposure has also been reported (Meriga et al., 2010). They also observed that a pH below 4.5 reduces SOD activity.

Binding of Al to the pectic matrix, plasma membrane and other constituents of cell wall causing alteration of cell wall properties leads to decreased extensibility (Tabuchi and Matsumoto, 2001) cell wall permeability resulting in reduced root growth (Schmohl and Horst, 2000). Al also permanently replaces  $\text{Ca}^{2+}$  from cell wall causing changes in cell wall properties.

## Tolerance mechanisms of plants to aluminium toxicity

Plants have evolved different mechanisms to overcome Al stress, either by preventing  $\text{Al}^{3+}$  from entering the root or by being able to neutralize toxic  $\text{Al}^{3+}$  absorbed by the root system. The basis of which has been the focus of intense research (Kochian et al., 2004). So far the only well documented mechanism of Al resistance is the exclusion of Al from the root tip based on the release of organic acids, which chelate  $\text{Al}^{3+}$  forming stable, nontoxic complexes. Release of malate, citrate and/or oxalate from roots upon exposure to Al has been correlated with differential Al tolerance in a large number of monocot and dicot species (Maron et al., 2008). In the first mechanism, Al is prevented from moving through the plasma membrane to the cytoplasm in the root cells. This is achieved by the secretion of organic acids from the radical apex to the rhizosphere, which, in turn, modifies the pH and chelates the toxic aluminium ion (Kinraide et al., 2005). Further, these organic anions (i) compete with phosphate groups for binding sites in the soil and thus block the sorption of P to other charged sites and (ii) form stronger

complexes with  $\text{Al}^{3+}$ ,  $\text{Fe}^{3+}$  and  $\text{Ca}^{3+}$  than phosphate does and thus make the phosphorus available to plants. The second mechanism involves chelation of Al by specific proteins, short-chain organic acids, phenolic compounds and tannins that can bind and form complexes with aluminium ion ( $\text{Al}^{3+}$ ) and subsequently compartmentalize it in the vacuole thus reducing Al-toxicity in the cell (Jones and Ryan, 2004). Nevertheless, the complexity of this genetic control seems to vary among species.

In rice, no organic acid was reported, except small amount of citrate induced by Al exposure, and no significant effect on the Al detoxification in both Al-tolerant and Al-sensitive varieties. It means that rice may have different Al tolerance mechanism other than release of organic acids (Ma et al., 2002). Al tolerance is a complex trait controlled by multiple genes in rice. Control of Al tolerance in *Oryza sativa* (rice) is polygenic, thus making genetic improvement difficult for this trait (Nguyen et al., 2003). While there is considerable evidence associating organic acids in the Al tolerance mechanisms of many species, other species apparently uses mechanisms that do not rely on organic acids. For instance, *Brachiaria decumbans*, an extremely Al-tolerant species, does not secrete organic acids in response to Al and hence possess different ways of dealing with toxic levels of Al in the soil solution (Wenzl et al., 2001). Rice (*O. sativa*) has been reported to be the most Al-tolerant cereal crop under field conditions, capable of withstanding significantly higher concentrations of Al than other major cereals (Fukrei, 2011). However, despite this fact, very little is known about the physiological mechanisms of Al tolerance in rice. Two independent studies have identified increased Al accumulation in the root apex in susceptible compared with Al-tolerant rice varieties, but no difference was observed in organic acid exudation or rhizosphere pH (Yang et al., 2008). Magnesium which plays an important role in numerous physiological and biochemical processes can also ameliorate aluminium (Bose et al., 2010).

Even though over expression of magnesium transporters can alleviate aluminium toxicity in plants, the mechanisms governing such alleviation remain obscure. Possible magnesium-dependent mechanisms include: (i) better carbon partitioning from shoots to roots; (ii) increased synthesis and exudation of organic acid anions; (iii) enhanced acid phosphatase activity; (iv) maintenance of proton-ATPase activity and cytoplasmic pH regulation; (v) protection against an aluminium-induced cytosolic calcium increase; and (vi) protection against reactive oxygen species (Bose et al., 2010).

## SCREENING METHODS FOR IDENTIFYING ALUMINIUM TOLERANT PLANTS

A reliable screening procedure for Al stress is one of the most important tools required to effectively develop Al-tolerant cultivars. In the field screening, the symptoms of aluminium toxicity are not easily identifiable. The foliar

symptoms may be stunting, small, dark green leaves and late maturity (Fukrei et al., 2011), purpling of stems, leaves, and leaf veins which resemble phosphorous (P) deficiency symptoms. In some cases, curling or rolling of young leaves and collapse of growing points or petioles are observed and may be confused with calcium (Ca) deficiency. Excess Al even induces iron (Fe) deficiency symptoms in rice (*O. sativa* L.), sorghum and wheat. Aluminium does not affect the seed germination but helps in new root development and seedling establishment. Root growth inhibition was detected 2 to 4 days after the initiation of seed germination. Al tolerance screening is typically conducted by comparing root growth of seedlings in hydroponic solutions, with and without Al. Solution culture assays with, or without staining procedures are efficient methods for identifying tolerance to Al. Nevertheless, in only a few cases has Al tolerance observed in solution cultures been correlated with Al tolerance in acidic soil (Sasaki et al., 2004).

Each screening technique has distinct advantages and disadvantages and techniques also vary widely in their ease of use for screening large numbers of entries for breeding programs. With the identification of molecular markers linked with Al tolerance genes, future screening for Al tolerance may be possible based on genotype or a combination of genotype and phenotype. In addition, molecular markers may be advantageous for identifying plants with tolerance mechanisms active at different plant growth stages which would be difficult or impossible to perform with phenotypic screens (Wu et al., 2000). Hydroponic culture of rice and many other crops is commonly carried out using the chemical composition of Yoshida's solution (Shaff et al., 2010). In a hydroponic solution, Al may be found in one of the four forms: (a) as free  $\text{Al}^{3+}$ , where it actively inhibits root growth; (b) precipitated with other elements and essentially unavailable to inhibit plant growth; (c) different hydroxyl monomers of Al, which are not believed to be toxic to roots or (d) complexed with other elements in an equilibrium between its active and inactive states. The degree to which Al inhibits root growth is primarily dependent upon the activity of free  $\text{Al}^{3+}$  in solution. In rice, the high Al concentrations required to observe significant differences in root growth between susceptible and resistant varieties also complicates hydroponic Al tolerance screening due to the precipitation of Al along with other elements.

The result is that control and treatment (+Al) solutions may differ with regard to essential mineral nutrients that react with Al, leading to differences in growth not directly attributable to Al. Additionally, because the active form of Al toxic to root growth is  $\text{Al}^{3+}$ , any Al that precipitates out of solution has no effect on root growth (Famoso et al., 2010).

## Al tolerant genes

Cultivars with tolerant genes that are genetically adapted to acidic soils may offer an environmental compatible

solution, providing a sustainable agriculture system in the developing world (Fernando et al., 2006). Fourteen genes from seven different species are known to contribute to  $Al^{3+}$  tolerance and resistance, and several additional candidates have been identified. Some of these genes account for genotypic variation within species and others do not. The genes controlling efflux of organic anions such as malate and citrate from roots are members of the ALMT and MATE families which encode membrane proteins that facilitate organic anion efflux across the plasma membrane. The identification of aluminium-resistance genes provides opportunities for enhancing crop production on acid soils (Ryan et al., 2011). The gene named aluminium-activated malate transporter (ALMT) belonged to a previously uncharacterized gene family and was the first  $Al^{3+}$  resistance gene identified in any plant species. The higher expression of TaALMT1 in most  $Al^{3+}$  resistant genotypes of wheat is associated with tandemly-repeated elements in the promoter (Meyer et al., 2010). Promoter analysis demonstrated that promoters containing these multiple repeats drive higher expression than promoters without repeats (Ryan et al., 2010).

A solid understanding of the genetics and physiology of resistance in sorghum (*Sorghum bicolor*) and barley was also pivotal in identifying the first members of a second family of resistance genes. Aluminium resistance in each of these species is controlled by a major genetic locus that segregates with the  $Al^{3+}$  dependent efflux of citrate from roots. Recent studies in barley have identified another gene, HvAACT1 (*Hordeum vulgare* Aluminum Activated Citrate Transporter 1), which belongs to the multidrug and toxic compound extrusion family (MATE) and is responsible for citrate exudation in response to Al (Furukawa et al., 2007). Additionally, in *S. bicolor* (sorghum), a MATE gene (SbMATE) was identified as an aluminum-activated citrate transporter. The monogenic inheritance of genes encoding proteins responsible for transporting organic acids in cereals such as *Triticum aestivum* and *H. vulgare* facilitates the prospects of improving these species for tolerance to Al in acidic soil (Magalhaes et al., 2007). As model species, rice and *Arabidopsis* are attractive systems for mutational analysis. Rice is an important crop worldwide with a high basal level of resistance compared with other small-grained cereals (Famoso et al., 2010). The striking similarities between the resistance genes isolated from rice and *Arabidopsis* using a mutational approach is intriguing given that rice is considerably more resistant (Collins et al., 2008).

The exact mechanism of Al tolerance in rice is still unclear. It has been proven that the Al tolerance trait in rice is controlled by many genes. Unfortunately, until today there are very limited amount of Al tolerance genes that have been cloned from rice. A research was carried out to clone a rice Al tolerance gene using rice/rye micro-colinearity approach. A rice/rye syntenic relationship was identified between an Al tolerance locus region in rye and

a region in rice BAC clone originated from rice chromosome 3. Both regions were bordered by the same flanking markers B6 and BCD1230. In between both markers, there were four putative candidate genes residing in the rice BAC clone. One of the putative genes, called B11 gene, showed higher expression level in Al-tolerant rice genotype than that in Al-sensitive rice genotype under Al stress. This research has successfully cloned the Al tolerance gene candidate from Indonesian local rice genotype Hawara Bunar. Transgenic tobacco plants carrying the gene showed more tolerance to Al than that of non-transgenic tobacco plants. Bioinformatics analysis showed that the B11 protein was similar to bacterial ribosomal L32 proteins and was predicted as a transcription factor with bZIP domain and C2H2-zinc finger like motif (Miftahudin and Roslim, 2012). Several genetic studies have identified nearly 10 QTLs for aluminium tolerance in rice, but the responsible genes have not been cloned so far. ABC transporters represent a large family in plants with >120 members in both *Arabidopsis* and rice (Sugiyama et al., 2006; Verrier et al., 2008).

A recent breakthrough in uncovering the molecular basis of  $Al^{3+}$  resistance in rice was achieved when the gene underlying an  $Al^{3+}$ -sensitive mutant, star1, was identified. It is a recessive rice mutant with hypersensitivity to aluminium toxicity, which was isolated from an aluminium-tolerant cultivar of rice irradiated with gamma rays (Ma et al., 2005). This gene is unique because, unlike most plant ABC transporters, which contain both nucleotide binding domains and transmembrane domain, it only encodes a nucleotide binding domain. In two independently generated transgenic lines carrying STAR1, the tolerance to aluminium toxicity was increased to a similar level as seen in wild type rice in both hydroponic and acidic soil culture, confirming that the mutation in STAR1 is responsible for the aluminum sensitivity in the star1 mutant. A rice homolog of ALS3 involved in aluminium tolerance in *Arabidopsis* (Larsen et al., 2005), was found and then cloned and named, STAR2, from rice root cDNA. To investigate whether STAR2 is involved in aluminum tolerance in rice, knockdown transgenic lines of STAR2 was prepared by RNA interference (RNAi). The RNA accumulation of STAR2 was reduced to <10% of that in wild type rice and tolerance was significantly decreased in the RNAi transgenic lines compared with that of the vector control plants, which clearly indicate that it is also required for aluminium tolerance in rice.

Both OsSTAR1 and OsSTAR2 are predominantly expressed in roots and expression of both is specifically induced by  $Al^{3+}$  treatment. The OsSTAR1:OsSTAR2 complex localizes to vesicular membranes and transports UDP-glucose, but it is not clear how this function confers resistance. The OsSTAR proteins may release UDP-glucose to the apoplast by exocytosis and provide protection by modifying the cell walls. It is also plausible that the OsSTAR proteins confer  $Al^{3+}$  'tolerance' rather than 'resistance' by performing other functions in the

cytosol. Neither of the OsSTAR genes underlies QTLs for Al<sup>3+</sup> resistance in rice, but they are possibly responsible for its high basal level of resistance (Huang et al., 2009). In rice, Xia et al. (2010) reported the role of an aluminium transporter (Nrat1) in the transport of absorbed Al from plasma membrane to vacuoles. Recently, in rice, a tonoplast localized half-size ABC transporter has been reported to be required for internal localization of Al (Huang et al., 2012). In barley, Fujii et al. (2012) described the role of a modification in a single gene (HvAACT1) in Al tolerance.

The gene primarily produces a protein that helps to release citrate from the root pericycle cells to the xylem facilitating transport of iron from roots to shoots. However, a 1-kb insertion upstream the coding region, found only in Al tolerant lines, alters its location of expression to root tip. This results in detoxification of Al through release of citrate to the rhizosphere.

## CONCLUSION

In acid soils, Al readily enters roots and impairs root growth and development. As a result, the root system becomes inefficient in water and nutrient uptake, leading to lower crop yields. Further, soluble aluminum reacts with soluble phosphorus and makes insoluble complexes, which is not available to plants. A common agricultural practice for acid soils is to apply lime to raise soil pH. Liming acid soils does not remedy acidity in the subsoil layer. Many important crop and pasture species lack sufficient Al tolerance within their germplasm to allow effective breeding for this character. Genetic and genomic studies leading to identification of gene/QTL for aluminium tolerance focused mainly on roots. Designing an appropriate screening technique is still a challenging task. Screening techniques based on aluminium accumulation in roots may be misleading as it may not identify tolerant types that use a different mechanism. On the other hand screening, based only on root growth or biomass accumulation in Al solution, may identify highly heterotic genotypes with little tolerance to Al as tolerant type compared to a less heterotic genotype with more tolerance to Al. Although, Al is the major stress in acid soil, manganese (Mn) toxicity, P deficiency and nutrient deficiencies associated with acid soil received very little attention.

STAR1/2, the major Al tolerance gene identified in rice may represent a basal Al tolerance in rice which is probably because, unlike temporary environmental stresses such as water and temperature stresses, Al toxicity stress is continuous in acid soils. It seems therefore, that plants have acquired basic strategies to constitutively detoxify Al at different cellular levels (Yamaji et al., 2009); and hence, may not be responsible to differentiate between aluminium tolerant and susceptible genotypes. Furthermore, star1, the recessive rice mutant with hypersensitivity to aluminum toxicity, from which the STAR gene

was isolated from an aluminum-tolerant cultivar of rice was irradiated with  $\gamma$ -rays (Ma et al., 2005). Hence, further detail study and screening of a larger germplasm may be required to identify these Al tolerant genes in the natural plant population. Genes similar to STAR1 and STAR2 are known to be present in other species based on phylogenetic analysis, such as maize, arabidopsis, grape, and, interestingly, also in *Physcomitrella patens*, a model organism of initial land plant. This suggests that STAR1/2 is a universal Al tolerance mechanism conserved in land plants, although, it remains to be investigated in plant species other than rice in future.

Progress in genomic research may lead to identification of more number of tolerant genes/QTLs and accelerate development of more tolerant crops. Use of transgenic approach shows promise for highly sensitive crops like pulses.

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