academicJournals

Vol. 8(9), pp. 711-722, 18 March, 20123
DOI: 10.5897/AJARx12.003
ISSN1991-637X©2013AcademicJournals
http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Review

Aluminium toxicity tolerance in cereals: Mechanisms, genetic control and breeding methods

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Accepted 11 March, 2013

Aluminium (Al) toxicity is one of the major factors constraining crop production on 67% of the total acid soil area in the world. Al toxicity restricts root growth and affects nutrient and water absorption with resultant stunted growth and reduced grain and biomass yield of crops. Cereals occupy about half of the world's cropland area and, therefore, take the lion share of the global Al-toxicity constraint. Al-toxicity is more serious in tropical environments where the soil is highly resistant to improvement by lime application. In addition, in these environments, the use of adequate lime and organic fertilizer sources is constrained by various technological and socio-economic constraints. Development and use of Al-tolerant crop varieties is economically feasible and an environmentally friendly management option that can complement other non-genetic management options. This paper introduces the importance of soil acidity and development of Al-toxicity. It also summarizes limitations of non-genetic management options and the need to complement these approaches with tolerant varieties. Further account is given on the effects of Al-toxicity on plant growth and development and yield. Organic acid exudation and other tolerance mechanisms of the globally important cereals and their genetic control are also discussed in details. The authors believe that the screening methods outlined in this review provide basic information and insights about Al-tolerance breeding in cereal crops.

Key words: Al-tolerance, genetic control, cereals, Al-toxicity.

INTRODUCTION

Soil acidity is one of the most important factors that affect crop production worldwide. Acid soils (pH < 5.5 in surface layer) constitute 3,950 million ha or 30% of the world's total ice free land or about 40% of the arable land (von Uexk¨ull and Mutert, 1995). In Africa 22% or 659 million ha of the total 3.01 billion ha land area has soil acidity problem (von Uexk¨ull and Mutert, 1995; Malcolm and Andrew, 2003).

Al-toxicity is the single most important contributing factor constraining crop production on 67% of the total acid soil area in the world (Eswaran et al., 1997). Aluminium is the most abundant metal and the third most common element in the earth's crust (Delhaize and Ryan, 1995; Vitorello et al., 2005). In soils, it mostly exists as structural constituent of primary and secondary minerals especially of the aluminosilicates. Nonetheless, despite its abundance, Al is not known to be used in any living organisms (Vitorello et al., 2005). As the soil gets acidic, the silicon will be leached leaving aluminium in the solid forms as aluminium oxyhydroxides, such as boehmite and

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gibbsite. These forms release the phytotoxic aluminium species, Al^{3+} also known as $Al(H_2O)_6^{3+}$ into the soil solution (Abebe, 2007; Miyasaka et al., 2007). Even though, there are several forms of Aluminium species in the soil, Al^{3+} and monomeric Al-hydroxyl species ($AlOH^{2+}$ and $Al(OH)_2^{+}$) are the most phytotoxic ones (Miyasaka et al., 2007). The trivalent Al^{3+} is dominant in soil solutions when the soil pH is less than *5*. The most common and immediate toxic effect of Al^{+3} in plants is inhibition of root growth which happens within few minutes to few hours after exposure to micro molar concentrations of Al (Barcelo and Poschenrieder, 2002).

Root inhibition could be exhibited on primary and lateral root apexes, and such roots become thick and develop brown colour (Vitorello et al., 2005; Wang et al., 2006; Claudio et al., 2008). Supersession and abnormal root morphology directly hinders nutrient uptake as well as water absorption. Consequently, plants show stunted growth and become susceptible to drought (Vitorello et al., 2005; Wang et al., 2006; Miyasaka et al., 2007).

The yield loss associated with Al-toxicity varies depending on soil Al saturation, the crop species and the specific variety used. For instance, Al-tolerant maize variety gave 61% higher grain yield than the Al-sensitive variety, and with lime treatment, yield increment of 208 and 82% was obtained for Al sensitive and Al- tolerant varieties of maize, respectively (The et al., 2006).

Applications of lime, manure, compost, and use of tolerant crop species or varieties are the most common methods used to overcome the impact of Al-toxicity. Nevertheless, in the context of acid soils of tropical Africa, utilization of lime, manure and other organic fertilizer sources have their own technical and or socio economic constraints.

Lime has been extensively used to ameliorate acid soils of temperate areas. In these areas, soil acidity develops mainly as a consequence of heavy use of chemical fertilizers and environmental pollution (Rao et al., 1993). In the tropics, several experimental reports also indicate significant yield increment with application of lime (The et al., 2006). However, the highly acidic soils of this region have strong buffering capacity against amendment by lime. Such soils demand heavy dose and need deeper incorporation to ameliorate the sub surface acidity. Most of resource poor farmers in the tropics, however, are constrained by unavailability, transport and high cost of this bulky dose (Rao et al., 1993). In addition, since lime incorporation to the subsoil is hardly possible, even when surface soil is neutralized, difficulty of ameliorating the subsoil restricts root growth of plants to surface soil and make them vulnerable to drought (Little, 1989; Foy, 1992). Runoff pollution and adverse effects of lime on rotation crops are also other side effects of lime application (Wang et al., 2006).

Use of organic matter seems an applicable strategy to resource poor farmers of the tropics who cannot afford purchase of large volume of lime and fertilizers. However, regular and high volume application of manure and compost to the highly acidic soils is limited by competing uses of organic matter sources for fuel, animal feed and construction (Schlede, 1989; Buresh et al., 1996; IFPRI, 2010). On the contrary, in the tropics, the use of acid forming fertilizers on cultivated land and expansion of crop production to forest inhabited areas accelerate development of soil acidity and Al-toxicity (Giller et al., 1996).

Cereals, the predominant staple food crops of the world population, are cultivated roughly on half of the world's crop land (Dyson, 1999). By the year 2025, the world's farmers are expected to produce about 3 billion tons of cereals to feed the human population of around 8 billion, and this requires an average world cereal yield of about 4 metric tons/ha (Dyson, 1999). The current average cereal yields in Africa is below 1 ton per ha (Langyintuo, 2011).
The use of tolerant crop varieties is considered to be the best complement to non-genetic management option for combating Al-toxicity problem (Rao et al., 1993; Abebe, 2007). This article attempts to review tolerance mechanisms, genetic control and screening methods of Al-toxicity tolerance in cereals. The reviewers believe that the paper gives better insight to basic information on Al-tolerance breeding and recent developments on the subject.

EFFECTS OF Al-TOXICTY ON GROWTH AND DEVELOPMENT OF CEREALS

Inhibition of root tip growth or root elongation, a phenomena well described as root pruning, is the most common symptom of Al toxicity (Vitorello et al., 2005; Miyasaka et al., 2007). Callose formation in roots of maize (Horst et al., 1997; Eticha et al., 2005) and lignin deposition in cortical cells of roots in wheat (Miyasaka et al., 2007) are also reported as early markers of aluminium toxicity in such crops.

Long term exposure of plants to Al results in reducing fine branching, suppression of root hairs development and subsequently in reduction root biomass. Abnormal root morphology such as observable cracks in the root apex , and thickening of root as a consequence of uneven and radial expansion of cells of cortex, are also root related to long term symptoms in Al sensitive plants (Vitorello et al., 2005; Miyasaka et al., 2007).

Supersession and abnormal root morphology directly impact nutrient uptake as well as water absorption. Consequently, deficiency of calcium, magnesium, potassium, iron, molybdenum and phosphorus is common symptom in plants grown on soils with Al toxicity problem (Vitorello et al., 2005; Wang et al., 2006; Miyasaka et al., 2007). Because of its inhibitory effect on root development, Al toxicity decreases tolerance of plants to drought and use of subsoil nutrients (Little, 1989; Carver and Ownby, 1995).

Suppression of photosynthetic capacity of shoots is also one of the consequences of Al toxicity. This is associated with cellular and ultrastructural modifications in leaves, reduced stomatal opening and CO_2 assimilation, reduced chlorophyll concentration, chlorosis and leaf necrosis (Vitorello et al., 2005; Chen, 2006; Miyasaka et al., 2007; Chen et al., 2010). #

The overall effect of Al toxicity is significantly expressed on biomass and grain yield of crops. Growth of several tropical crops in acid soil areas was reduced by 50% or above when compared to growth of plants grown on limed soil that had soil aluminium saturation of greater than 60% (Kamprath, 1984). Gallardo et al. (1999) also reported that grain yield reduction of 50 and 30% in Al sensitive and tolerant varieties of barley, respectively. On wheat liming increased shoot weight and grain yield of Al-sensitive genotype by 60% and ear number by 32% (Tang et al., 2001). In maize, acid soil tolerant variety gave 61% higher grain yield than the sensitive variety. With lime treatment, yield increment of 208 and 82% was obtained for Al sensitive and Al- tolerant varieties, respectively (The et al., 2006).

MECHANISMS OF Al-TOXICITY TOLERANCE IN CEREALS

Wheat is probably the first crop in which genetic variation for tolerance of the then “Crestamento” (the present day symptom of Al toxicity) was discovered. This discovery happened in Brazil, a country that owns the largest area of acid soil in the world (David and Brett, 2003). Subsequent studies carried out on inter and intra-specific genetic variability for Al-toxicity tolerance indicated presence of wide genetic variability among and within species of major cereals (Howeler and Cadavid, 1976; Marschner, 1991; Foy et al., 1993; Ring et al., 1993; Foy, 1996; Khan and McNeilly, 1998; Pinto-Carnide and Guedes-Pinto, 1999, 2000 Lisitsyn, 2000; Zhao et al., 2003; Liu et al., 2007; Stodart et al., 2007; Portaluppi et al., 2010; Raman et al., 2010; Kabir et al., 2011).

In general, exclusion of Al from root apex and detoxification of Al in the root and shoot symplasm are two known mechanisms of Al tolerance in plants. In cereals and grass species, exclusion mechanism is the most common mechanism (Ma et al., 2001; Kochian et al., 2004).

Al-induced exudation of organic acids

The first report on Al-induced exudation of organic acids came from the work of Miyasaka et al. (1991), who demonstrated Al-tolerant snap bean cultivar exuded 10 fold higher citric acid than the Al sensitive cultivar. Among cereals, exudation of organic acid (malate) in Al tolerant genotypes was first reported on wheat (Delhaize et al., 1993b; Basu et al., 1994; Ryan et al., 1995a, b). Very recently, the second mechanism of Al-tolerance that involves efflux of citrate has been reported from Brazilian wheat cultivars (Ryan et al., 2009). In tolerant barley varieties, citrate secretion operates as a mechanism of Al-tolerance instead of malate (Zhao et al., 2003). Even though its contribution to Al-tolerance has not been confirmed, exudation of citrate in response to Al exposure was also reported in rice (Ishikawa et al., 2000; Ma et al., 2002a). In rye and triticale citrate and malate release contribute to Al-tolerance (Li et al., 2000a; Ma et al., 2002b). In Maize, Al-induced citrate and malate exudations were observed in Al-tolerant maize plants in contrast to Al-sensitive ones and the level of citrate was 2 to 4 fold more than the level of malate (Renato and Paulo, 1997). Similarly, Radhouane and Brahim (2009), Maron et al. (2008, 2010), indicated possible involvement of Al-induced citrate exudation in high level of Al-resistance in maize. In sorghum, Al-activated root citrate exudation correlated with level of Al-tolerance in two sorghum cultivars (Magalhaes, 2002; Kochian et al., 2005). These organic acids detoxify Al ion by chelating and preventing it not to bind to the negatively charged sites of the cell wall and plasma membrane of roots. Plant roots cannot take such Al-carboxylate complex (Miyasaka et al., 1991; Delhaize et al., 1993b; Kochian et al., 2005). In wheat malic acid externally added to nutrient solutions could protect Al-sensitive seedlings from toxic Al concentrations (Delhaize et al., 1993b).

The organic acids are exuded from the first few millimetres of root apices of Al- tolerant genotypes and the amount is dependent on the concentration of external Al (Rincon and Gonzales, 1992; Delhaize et al., 1993b). These parts of the roots are the most sensitive part where tolerant genes are likely to be expressed. As a result, after exposure to Al, sensitive genotypes accumulate several fold more Al in their root apex than Al-tolerant genotypes. Such difference is not observed on the matured zones of the root (Delhaize et al., 1993a; Delhaize and Ryan, 1995). The amount of organic acids exuded from tolerant genotypes is dependent on the concentration of Al in the external medium. In wheat, malic acid exudation was triggered by Al concentration of as low as 10 µM (Delhaize et al., 1993b).

The time to start exudation of organic acid after Al exposure varies among tolerant genotypes of different species. For instance, in Al-tolerant genotypes of wheat malic acid exudation was detected after 15 min of exposure to Al (Delhaize et al., 1993b). But the rate of malate release did not increase overtime. Thus the rapid release of malate indicates the fact that its activation starts at protein level rather than gene level (Kochian et al., 2005). So far, convincing difference has not been reported in the activity of enzymes involved in the synthesis of the organic acids between Al-sensitive and Al-tolerant genotypes. Hence, the difference between exudation of organic acids is likely associated with

activation of a transport channel protein (Delhaize et al., 1993b; Ryan et al., 1995b; Kochian et al., 2005). On the other hand, in tolerant genotypes of rye and triticale, there is a lag phase after Al-exposure or before exudation starts (Li et al., 2000a; Ma et al., 2002b). The exudation then increases over time. In this case, it is likely that the exudation is activated at gene level (Kochian et al., 2005).

Currently, the association of carboxylate exudation with activation of transmembrane channel has been well established. Organic acids exist as anion in the cytoplasm in the cytosol. Upon exposure to external toxic aluminium species, anion channel across the plasma membrane become activated to release the organic acids to the outside environment. (Kochian et al., 2005).

Other exudates and exclusion mechanisms

In addition to organic acid exudation, other exudates and exclusion mechanisms have been reported as additional or alternate mechanism of Al-tolerance in different crop species. In Al-tolerant wheat cultivar called Atlas, high level of Al-tolerance was exhibited through high exudation of malate and phosphate to the rhizosphere (Didier et al., 1996; Pellet et al., 1997). The other less tolerant cultivar, ET3, resisted the toxicity only by exudation of malate which is conferred by the gene *TaALMT1*. And this study indicated Al-tolerance in the cultivar Atlas was conditioned by at least two genes: *TaALMT1* gene and another gene that encodes for exudation of phosphate. Similarly, in barley, the accumulation Al-phosphate on the root surface of the tolerant cultivar Dayton was reported to be two times more than the Al-sensitive cultivar Kearney (Wang et al., 2006). It is stated that phosphate plays important role by complexing with Al, and alkalinisation of the *rhizosphere* pH by binding proton (Pellet et al., 1997).

Exudation of aromatic secondary plant metabolites called phenolic compounds could also be involved in Al toxicity tolerance. In Al-tolerant maize, exudation of Al-induced flavonoid type phenolics catechin and quercetin from 10 mm root tip is reported (Kidd et al., 2001). The high exudation rate of the catechin at Al- sensitive area of the root and high stability constant of the Al complex with pentahydroxy-flavones and flavanpentols strongly supported the role of the flavonoid type phenolics in Al tolerance in maize (Kidd et al., 2001; Barcelo and Poschenrieder, 2002). Exudation of *de novo* synthesised polypeptides in response to Al exposure is also reported in wheat.

These exudates bind Al and co-segregates with the Al-tolerant phenotype in F2 population (Basu et al., 1997; Basu et al., 1999).

Binding of Al to root tip mucilage is also proposed to protect root tip from Al by binding the toxic Al. The presence of mucilage on the root tip of wheat minimized Al injury (Horst et al., 1982). Similarly, Henderson and Ownby (1991) observed strong correlation between mucilage volume and Al-tolerance of winter wheat cultivars. In Maize, however, despite strong binding of Al to mucilage, the root tips were not prevented from Al injury (Li et al., 2000b).

Internal detoxification

Though internal detoxification mechanism is common in plants like tea, buckwheat etc, few reports are available on the use of this mechanism in cereals. Plants that can accumulate silicon in their system can release the silicon so as to detoxify aluminium by forming aluminosilicate compounds in root apoplast (Cocker et al., 1998). In cereals this mechanism is reported in sorghum, where Al and silicon are complexed in the outer wall of endodermis of roots (Hodson and Sangster, 1993). Suicidal death of cells affected by Al is also reported as detoxification mechanism in wheat among cereals (Delisle et al., 2001). Such hypersensitive reaction of cells is common mechanism in plant defence against pathogens in order to prevent spread of the pathogen to other healthy cells and tissue (Miyasaka et al., 2007). The other detoxification mechanism involves sequestration of Al in vacuole or other organelles so as to prevent its toxic effect in the cytoplasm (Miyasaka et al., 2007). Such mechanisms are presumed to operate in tolerant barley and wheat varieties (Taylor et al., 1997).

GENETIC CONTROL OF Al-TOLERANCE IN CEREALS

Studies on genetic control of Al toxicity are active areas of research for most of the globally important cereals. In wheat, earlier reports presumed that Al toxicity in wheat is controlled at least by two major loci (Didier et al., 1996; Pellet et al., 1997; Garvin and Carver, 2003). The two genes proposed were genes that codes for malate, and phosphate exudation to the rhizosphere (Didier et al., 1996; Pellet et al., 1997). A major aluminium tolerance gene in wheat, *ALMT1* latter renamed as*TaTAALMT1*, is known to confer an Al-activated efflux of malate from root apices (Sasaki et al., 2004; Raman et al., 2005a). This gene is mapped to chromosome 4DL using 'Chinese spring' deletion lines. Absence or loss of this gene resulted in loss of Al-tolerance and malate exudation (Raman et al., 2005a). Hence, it was suggested that Al-tolerance in diverse range of wheat genotypes to be primarily controlled by *TaALMT1* located at Alt_{BH} (Raman et al., 2005a). Very recently, with discovery of a new mechanism of Al-tolerance that involve efflux of citrate in root apices of Brazilian wheat cultivars, another gene that resides on chromosome 4BL has been identified (Ryan et al., 2009). They also indicated that the citrate efflux is controlled by single gene which could explain 50% of the

phenotypic variation in citrate efflux. In addition, Navakode et al. (2009) located two major Al-tolerance QTL on chromosome arm 4DL and 3BL which could, respectively, explain 49 and 31% of the phenotypic variance present in the population of 'Chinese Spring' wheat cultivar. These findings indicated that the trait is controlled by major and minor genes in wheat.

In Barley, Lima Echart et al. (2002) indicated that the F2 generation analysed with haematoxylin staining followed the Mendel's segregation ratio of 3:1 for Al toxicity tolerant to susceptible plants; revealing the fact that the trait is controlled by single dominant gene.

It is generally agreed that Al tolerance in barley is conditioned by the *Alp*locus which is located on the long arm of chromosome 4H. This locus is associated with Al-induced efflux of citrate from root apices of tolerant barley varieties (Wang et al., 2006). A gene encoding a multidrug and toxic compound extrusion protein is proposed as a candidate gene for Al-tolerance in Barley (Wang et al., 2007). In addition, quantitative trait loci that could explain 50% of the phenotypic variation are also associated with the same chromosomal location (Jian Feng et al., 2004). Similarly, Raman et al. (2005b), identified quantitative trait loci for root elongation under aluminium stress on 3H, 4H, 5H and 6H chromosomal locations.

Alike other cereals, aluminium tolerance in rye is effected by efflux of organic acids. Segregation ratio of 3:1 (tolerant to sensitive) was found in three F2 populations analysed indicating the fact that the trait is controlled by single dominant locus (Matos et al., 2005). So far, four independent loci *Alt1, Alt2, Alt3 and Alt4* located on chromosome arms 6RS, 3RS, 4RL and 7RS, are known to confer Aluminium toxicity tolerance in this crop (Matos et al., 2007). Specifically, the *Alt4* locus contains cluster of genes homologous to the single copy Al-activated malate transporter (*TaALMT1*) (Collins et al., 2008). Tolerant and sensitive rye genotypes contain five and two genes of the clusters at the locus, respectively. Out of these, two *ScALMT1-M39.2* and one *ScALMT1-M77* genes are highly expressed in the root tip (Collins et al., 2008).

The multi-allelic locus *Alt SB* that conditions wide range of variation controls Al-tolerance in sorghum (Caniato et al., 2007). These authors also suggested possibility of presence of additive or co-dominant effect of different loci that can explain transgressive segregation observed in some tolerant lines. Magalhaes et al. (2007), identified the gene encoding a member of the multidrug and toxic compound extrusion (MATE) family which is an aluminium-activated citrate transporter, as responsible gene for the major sorghum aluminium tolerance locus, Alt(SB). They also suggested polymorphisms in regulatory regions of *Alt*(SB) are likely to contribute to large allelic effects, acting to increase Alt(SB) expression in the root apex of tolerant genotypes.

In contrast to the above situation, Al tolerance trait in rice and maize is mainly controlled by quantitative genes (Kochian et al., 2005). In maize, several Al-regulated genes such as cell wall related, low phosphate response and Al-activated citrate release were expressed in response to exposure of Al tolerant and sensitive genotypes. The response, however, was higher to tolerant genotype. This phenomena indicates involvement of several genes in Al tolerance (Maron et al., 2008). Al-activated efflux of citrate from roots is well characterized and most important mechanism of Al tolerance in maize. The responsible gene for this is likely to be the member of the multidrug and toxin extrusion (MATE) family (Jorge (and Paulo, 1997; Maron et al., 2008). Very recently, a study that involved cloning and characterization of two MATE family members indicated ZmMATE1 to underlie the largest maize Al tolerance QTL found on chromosome 6 and is described to be a functional homolog of the Al tolerance genes in sorghum and barley (Maron et al., 2010).

In rice, root growth under Al stressed condition is controlled by several quantitative trait loci (QTLs) genes. Two-three QTLs of largest effect, however, are identified to explain phenotypic variation for Al-tolerance (Ma et al., 2002a; Nguyen et al., 2002). A recent study identified two genes STAR1 and STAR2 which function as bacterial-type ATP binding cassette (ABC) transporter to control Al-tolerance in rice (Huang et al., 2009). The mechanism, however, is not yet clear enough.

GERMPLASM AND SCREENING METHODS FOR Al-TOLERANCE

Germplasm sources

Most of Al-tolerant crop varieties developed so far are obtained from highly acidic soils of the world (Hede et al., 2001; Stodart et al., 2007; Caniato et al., 2011). For instance among 250 bread wheat landraces originating from 21 countries, all of 25 accessions collected from highly acid soils area of Nepal were found to be Al-tolerant. The most likely reasons for such associations are natural selection and adaptation or human selection by early agriculturalists (Stodart et al., 2007; Caniato et al., 2011). Hence, evaluation germplasm collected from acid soil areas is considered to be the logical and appropriate entry strategy in Al-tolerance breeding.

Mutation treatment can also be used to rapidly increase genetic variability for Al-tolerance for screening programmes. In barley, mutagenic treatment with N-methyl-N-nitroso urea (MNH) and sodium azide yielded thirteen mutants with increased level of Al-tolerance (Nawrot et al., 2001). Similarly, EMS-mutagenized Al- sensitive Arabidopsis mutant, *als3-1* could result in seedlings that could sustain root growth in an Al-containing environment that is highly toxic (Kelly et al., 2006).

In vitro techniques that help to identify tolerant

somaclonal variants as well as genetic engineering methods have also been applied to obtain genotypes with enhanced aluminium tolerance (Deborah and Tesfaye, 2003; Dharmendra et al., 2011). For instance, acid soil/Al-tolerant variants of sorghum, rice, maize could be obtained from somaclonal variation under *in vitro* condition (Foy et al., 1993; Duncan et al., 1995; Jan et al., 1997; Sibov et al., 1999).

After appropriate germplasms are found the screening of plants adapted to Al-toxicity or acid soil problems can be carried out under field condition or under controlled condition. Under controlled condition, acid soils, sand culture, solution culture or hydroponics and *in vitro* techniques that involve cell culture or somaclonal variation can be used (Rao et al., 1993; Hede et al., 2001; Deborah and Tesfaye, 2003; Dharmendra et al., 2011).

Screening methods

Nutrient solution culture

Solution culture is the most common screening medium for Al tolerance (Rao et al., 1993; Worland et al., 1994; Hede et al., 2001; Deborah and Tesfaye, 2003; Raman and Gustafson, 2011). The screening is done by comparing root growth of seedlings in a pair of hydroponic solutions with and without Al (Magalhaes et al., 2004; Sasaki et al., 2004; Famoso et al., 2010). This technique offers several advantages such as direct access to the root system, simplified control over nutrient availability and pH, light condition and non-destructive measurements of tolerance (Carver and Ownby, 1995).

Al concentrations used in solution culture depend on several factors. Nutrient composition and associated ionic strength of nutrient solutions affect the concentration of Al to be used in screening (Famoso et al., 2010). The toxic form of Al is the free Al^{3+}. Speciation of added Al compounds to this form however depends on presence of other ions. For instance, the concentration of free Al^{3+} is lower in nutrient solutions that have more levels of SO_4^{2-} and HPO_4^{2-}, than nutrient solutions with less level of these ions. Al^{3+} strongly interacts with SO_4^{2-} and HPO_4^{2-} and is converted to non-toxic forms through precipitation (Famoso et al., 2010). The most common nutrient solutions used in screening of cereals are Magnavaca's nutrient solution for maize, sorghum and wheat, and Yoshida's nutrient solutions for rice (Yoshida et al., 1976; Magnavaca et al., 1987; Magalhaes et al., 2004; Sasaki et al., 2004; Magalhaes et al., 2007). Recently, Famoso et al. (2010) developed a modified Magnavaca's nutrient solution which has reduced ionic strength. This medium reduced the precipitation effect of Al and increased availability of important nutrients by reducing interaction of Al with other mineral ions. Blamey et al. (1991) recommend use of low-ionic-strength nutrient solution combined with a low Al concentration so as to emulate ionic strength and aluminium activity in real soil composition.

In addition to the nutrient solution, plant species and objective of screening also affects the concentration of Al to be used (Hede et al., 2001). In screening of genotypes, Al-tolerant species such as rye are evaluated with higher aluminium concentration as opposed to sensitive species which are screened with lower concentration. If the screening is to identify a variety which is more tolerant than a tolerant variety under production, higher Al concentrations can be used. If the purpose is to characterize tolerance of genotypes, a lower Al concentration should be used to discriminate the germplasm (Hede et al., 2001).

The screening method also affects the concentration of aluminium to be used. For instance, Hede et al. (2002) found that with the haematoxylin method, the most proper Al concentration for discriminating rye genotypes was 50 mg/L. At higher Al concentrations very few rye plants showed root re-growth. With the root growth method, the genotypes were best separated by using the lowest Al concentration (4 mg Al/L).

Since aluminium can form complexes with important nutrients such as P and S at low pH, all important nutrients in the solution cannot be used by the plant. Hence, seedlings depend on their reserve food. This indicates the importance of selecting Al-concentration which does not completely inhibit root growth (Deborah and Tesfaye, 2003). In addition, since plant exudates can rapidly change the pH, there is a need to regular monitoring so as to adjust or change the medium (Deborah and Tesfaye, 2003). Use of a buffer homopipes has been found to be useful in stabilizing this fluctuation of pH (Kinraide and Sweeney, 2001).

The bioassay for Al toxicity tolerance in nutrient solution can be made in two methods.

Root tip staining: There are several of root tip staining methods. Among these, haematoxylin staining of root tips is widely used and a powerful method (Polle et al., 1978; Deborah and Tesfaye, 2003; Raman and Gustafson, 2011). Eriochromecyanine and lumogallion root staining methods are also used to discriminate tolerant and sensitive materials of barley and a forage species *Medicagotruncatula*, respectively (Junping et al., 2006; Narasimhamoorthy et al., 2007). These stains identify Al-sensitive genotypes by forming complexes with Al-accumulated in root tips of such genotypes. With haematoxylin, seedlings are treated with an acidic Al solution for 1 to 24 h; and then will be rinsed in water to remove unbound Al; and will be stained in 0.2% haematoxylin with 0.02% $NaIO_3$ or KI; finally the roots are rinsed to remove excess stain and finally rated for degree of tolerance (Deborah and Tesfaye, 2003). Most sensitive genotypes accumulate more aluminium in their root and therefore their intensity of staining (purple coloration) is

higher. Nitrobluetetrazolium (NBT) reduction staining is also reported to correlate with degree of Al tolerance in wheat, rye, maize, and rice and is the first marker that identifies tolerant genotypes instead of the sensitive ones (Maltais and Houde, 2002; Raman and Gustafson, 2011).

Root growth: root growth measurements are also widely used assays to discriminate Al-tolerant and sensitive genotypes under nutrient solution culture (Baier et al., 1995; Carver and Ownby, 1995). The root growth method considers two Al tolerance parameters: Root growth (RG) and a root tolerance index (RTI) (Baier et al., 1995). The RG parameter measures root growth under Al stress. Roots that elongate or grow under this situation identifies tolerant genotypes. RTI or Relative Root Growth (RRG) is the ration of root growth under Al stress to root growth without Al stress [RTI (RRG) = Root growth under aluminium stress/Root Growth without aluminium stress] (Hede et al., 2001; Raman and Gustafson, 2011). RG under Al stress can be a combination of two genes/ alleles controlling Al-tolerance. RTI removes the effect genes controlling root vigour by taking relative growth of the genotype in Al solution compared to its potential growth without Al. Hence, RTI is specific and better parameter that measures Al-tolerance (Hede et al., 2001).

When the two assaying methods are compared, root staining evaluates accumulation of aluminium in the roots, disregarding possibility of accumulation of aluminium in other plant parts. Hence, it may misclassify plants when there is genetic difference among plants in absorption, and spots of aluminium accumulation. RTI, however, avoids this complexity as it measures the genetic potential of plants to overcome the known effect of root growth inhibition (Raman and Gustafson, 2011). Hence, it is often used and a reliable method in screening of genotypes for Al-tolerance (Baier et al., 1995; Hede et al., 2002). The drawback of RTI where researchers should be cautions is that genotypes have slow growing roots may appear more tolerant because their RTI value can be higher than the ones with fast growing roots (Deborah and Tesfaye, 2003). The root growth or measurement method has also shortcomings. It is time consuming and the response is dependent on concentration of ions or nutrient status of the solution, genetic vigour and age of seedlings (Raman and Gustafson, 2011). These shortcomings can be overcome by using seedlings with similar vigour (Baier et al., 1995). Since seed age is also very important for plant and root vigour, seeds should be regenerated before evaluating for Al tolerance and other traits that may be affected by seed age (Hede et al., 2002).

In addition to the staining and root growth method, other parameters can also be used to identify tolerant genotypes. For instance, aluminium induced callose formation on root tips after exposure to aluminium is used a marker associated with Al-tolerance in several crops (Zhang et al., 1974; Basu et al., 1997; Horst et al., 1997; Basu et al., 1999; Massot et al., 1999). In nutrient solution culture such markers can be detected easily. Furthermore, parameters such as root and shoot biomass or dry weight and other yield related traits can be used as useful indicators of Al-tolerance (Mugwira et al., 1976).

In vitro screening method

Compared to screening under field condition, *in vitro* techniques are fast and can be done early (Dharmendra et al., 2011). The underlying principle for development of tolerant materials from callus culture is that tolerance at cell culture level operates in whole-plants under field conditions. Screening is done by evaluating callus development from different genotypes under acid medium containing various concentrations of aluminium along with aluminium free acidic medium (Deborah and Tesfaye, 2003; Dharmendra et al., 2011). This technique has been used to identify Al-tolerant plants. However, there are authors who question its economic feasibility for some species (Dall'Agnol et al., 1996).

There are also several technical challenges with *in vitro* screening. In order to simulate problems of acid soils with Al-toxicity problem, the pH has to be reduced to about 4 (Conner and Meredith, 1985b). However, under low pH of 4, agar does not solidify when autoclaved. In order to overcome this, high concentration of Gelrite (up to 14 g/L) (Jan et al., 1997) and 5 to 9g/l is used (Ramgareeb et al., 1999). Since aluminium forms precipitation with various nutrients in the medium, the availability and activity of the toxic aluminium species becomes far lower than the concentrations anticipated (Ramgareeb et al., 1999).

These authors investigate how to achieve anticipated Al^{3+} availability and activity by using MINTEQA2, a chemical equilibrium speciation model, and recommended use of phosphate in standard MS medium, with 1 mM $SO4^{2-}$ and no EDTA at pH 4. Alfalfa and sorghum are two of the few cases where *an in vitro* technique found to successful in regenerating tolerant materials (Conner and Meredith, 1985a; Parrott and Bouton,1990).

Soil based screening method

Even though, screening can be directly done on acid soils under controlled environment, soil based screening is usually preceded by preliminary screening in solution culture.

It is recommended to conduct soil based screening on soils taken from target production area or can represent the target production area (Carver and Ownby, 1995). Typically, the plants are grown on soil limed to non-toxic level, and unlimed soil and tolerance index or relative values for root length, root dry-matter, shoot length, shoot dry-matter are computed as [Relative value = value under

limed/value under unlimed] (Foy et al.,1987; Hill et al., 1989; Foy and Murray, 1998; Deborah and Tesfaye, 2003; Liu, 2005). The advantage of soil based screening methods compared to nutrient solution culture is that it takes into consideration other soil factors that may influence Al tolerance (Ring et al., 1993).

Field evaluation and selection

The need to develop Al-tolerant crop varieties/species is to make target acid soil areas productive through production Al-tolerant crops. Hence, evaluation of selected varieties for yield and other economically important traits before recommendation for production in wide area is imperative. Field evaluation is usually conducted in pair of lime amended and naturally acidic plot for all genotypes evaluated and the data are analysed and reported as the ratio of grain yield or the trait of interest in unlimed to the lime amended plot (Carver and Ownby, 1995; Johnson et al., 1997). In field evaluation, plant diseases, soil heterogeneity and other stresses can complicate or affect the output.

Molecular marker-assisted breeding for Al-tolerance in cereals

Molecular markers are verified to be used as tools in marking major and minor loci controlling aluminium tolerance. Some of the molecular markers used are random amplified polymorphic DNAs (RAPDs) (Senft and Wricke, 1996; Masojc´ et al., 2001), restriction fragment length polymorphisms (RFLPs) (Tang et al., 2000; Nguyen et al., 2002), simple sequence repeats (SSRs) (Raman et al., 2002; Wang et al., 2007), amplified fragment length polymorphisms (AFLPs) (Nguyen et al., 2002; Raman et al., 2002), and cleaved amplified polymorphic sequences (CAPS) (Miftahudin et al., 2005; Raman et al., 2005a). Molecular markers for Al-tolerance have been applied in breeding programmes to monitor expression of the desired alleles in different genetic background and also in genetic diversity studies (Raman and Gustafson, 2011).

Devos and Gale (2000) indicated extensive synteny or colinearity among genomes of rice, wheat, barley, rye, oat, maize and sorghum with comparative mapping study. This indicates possibility of assessing Al-tolerance loci in cereals using set of common markers linked to Al-tolerance (Raman and Gustafson, 2011).

Currently, diagnostic markers associated with candidate genes *TaALMT, HvMATE,* ZmMATE1 and *SbMATE* have been developed (Raman and Gustafson, 2011). These genes are mainly correlated with Al-tolerance in wheat, barley, maize and sorghum, respectively. These markers also help to determine mechanism of tolerance involved. The MATE family confers Al tolerance through exudation of citrate whereas the *TaALMT* employs malate exudation.

CONCLUSION

Al-toxicity is a major crop production constraint associated with strongly acidic soils of the world. Cereals, as widely produced and consumed crop, are affected by Al-toxicity worldwide. Even though, liming could alleviate Al-toxicity problem in temperate areas where the problem is restricted mainly the surface soil, in the tropics, its use is constrained by high application rate needed to improve the highly weathered surface and subsurface acidity. When the lime is available, incorporation to the subsurface is difficult due to slow mobility of lime and technical limitations to mix the lime mechanically. Consequently, amelioration by lime restricted to the surface soil. On such fields, root growth is restricted to the surface soil because of toxic effect of aluminium and hence nutrient and moisture absorption will be hampered. Hence, plants become vulnerable to drought.

Amendment through use of compost and manure in the tropics is also constrained by competing use of organic matter sources for fuel and animal feed. Use of Al-tolerant varieties is economical and environmentally friendly option that complements other non-genetic management options specifically in the tropics. Development of tolerant varieties needs knowledge on available mechanisms of tolerance, genetic control of the genes responsible for tolerance, and breeding methods.

The predominant mechanism of Al-tolerance in cereals involves exclusion of the toxic species of aluminium by chelating it with organic acids which are mainly malate and citrate depending on the crop species. The gene coding for *TaALMT1* (aluminium-activated malate transporter) and another gene which codes for the multidrug and toxic compound extrusion (MATE) are responsible for Al-activated malate and citrate exudation, respectively. Inheritance of Al-tolerance in cereals other than maize and rice are mainly major gene controlled with involvement of some modifying genes. In Maize and rice, the inheritance is mainly quantitatively inherited.

Breeding for Al-tolerance starts with use of appropriate germplasm and involves utilization of various screening methods. Use of germplasm collected from acid soil problem areas is the common starting point. The screening methods need to be able to discriminate the genotypes and represent the target production environment. The early screening activities are usually carried out on seedling under controlled environment with controlled treatment of Al^{3+} and the emphases is to phenotype tolerance and select tolerant genotypes. Various methods are used to measure and quantify tolerance with primary focus on root and shoot growth and development. Screening on acid soil is an intermediate step before field evaluation and it to evaluate

and the genotypes under an environment closer to the field condition. Under field genotypes are evaluated for more agronomic traits, yield and yield components. Recent advances in molecular marker studies indicated extensive synteny or colinearity among genomes of many cereals. Development diagnostic markers associated with candidate genes *TaALMT, HvMATE*, ZmMATE1 and *SbMATE* also helps enhance efficiency of the conventional breeding.

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