International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 2 Issue 12, December - 2013

Aluminium Toxicity and Stress Resistance : Transgenic Approach

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Abstract

Aluminium toxicity and tolerance mechanisms differ strikingly with its chemical form, and the study of aluminium related processes is complicated by the complex chemistry of aluminium. Therefore the experimental results may differ with experimental conditions such as pH and coexisting ions, even when the same concentration of Al is used. Therefore, germplasmsthose are collected from acid soil areas shows best tolerance or sensitivity and thus suitable for entry scheme in Al-tolerance breeding. To identify tolerant somatic cell variants, genotypes with high aluminium tolerance as well as genetic engineering methods, in vitro techniqueshavebeen used.Stimulation of Al-tolerant genes and proteins due to Al exposure provides an impetus reason to the researchersfor molecular studies in plants. In this direction, the first approach was the expression of a *Pseudomonas aeruginosa* citrate synthase gene in tobacco. However, the molecular and genetic bases are still not well understood. Aluminium resistance genes have yet to be cloned from any species, with the exception of ALMT1 from wheat.

Key words: Aluminium, Genetic engineering, Molecular studies, ALMT1.

1. Introduction

Aluminum, third most abundant metal, represents approximately 8% of the total mineral components in the earth's crust (Verstraeten, *et al.*, 2008). It has a ubiquitous presence in the human environment and thus, in acid soils, its toxicity shows the primary growth-limiting factor in plants (Foy, 1992). In poor Ca and Mg soils, it becomes more severe (Vitorello, *et al.*, 2005). Aluminum, in its Al^{3+} cationic form, is very inimical to agriculture, as it becomes toxic in nature (pH below 5) which injures plant root cells and interfere nutrient and water uptake in crop plants(Samac andTesfaye, 2003), thus damaged root system and inhibits plant root growth(Barceló and Poschenrieder, 2002).

Although Al toxicity has become one of the active area for research; however, physiological, molecular and genetic basis are still not wellunderstood.The cellular components and processes have been proposed to be most affectedby Al toxicity.Some of the most affected components are; cell nuclei, cell division and mitosis (Silva,*et al.*, 2000), uptake of Caand other ions (Ryan,*et al*., 1993; Liu and Luan, 2001), composition, physical properties and structure of the plasma membrane (Zhang,*et al*., 1997; Ishikawa and Wagatsuma, 1998), oxidative stress (Yamamoto,*et al.*, 2003) and cytoskeletal dynamics (Sivaguru,*et al*., 1999).

A number of studies haveproposed thatAl^{3+}induces the formation of ROSand oxidative stress which results in Al toxicity. This all helps in the synthesis of oxidative stress response proteins. Moreover, it enhances the enzymes activity (Catalase and SOD) and lipid peroxidation in *Pisumsativum* (Yamamoto, *et al*., 2001) *Glycine max* (Cakmak and Horst, 1991), and *Nicotianatabacum* (Ono, *et al*., 1995; Yamamoto, *e al*., 1997; Ikegawa, *et al*., 2000).It is also suggested that the oxidative stress brings to changes in the expression of various genes in *Arabidopsis* (Sugimoto and Sakamoto, 1997; Richards, *et al*., 1998), tobacco (Ezaki, *et al*., 2000) and wheat (Snowden and Gardner, 1993; Cruz-Ortega, *et al*., 1997; Hamel, *et al*., 1998). For example, Al induces the expression of genes that encode blue-copper proteins, glutathione S-transferase and peroxidases.

Ezaki, *et al*. (2000) observed that due to over-expression of some induced proteins, there was an increase in Al tolerance and increased tolerance to oxidative stress in *Arabidopsis*. Study in yeast has also shown that rather than organic acid efflux, over-expression of genes is the reason for Al tolerance (MacDiarmid and Gardner, 1998).

2. Breeding for resistance

2.1 Conventional

To improve nutrient use efficiency and to enhance crop productivity, a diverse set of approaches like molecular breeding, agronomyis required. Multiple aspects are required or have to be considered for the simultaneous crop improvement like crop performance, evaluated in a multidisciplinary approach as in biotic and abiotic stress, etc.(Parry and Hawkesford, 2012).

2.2 Transgenic approaches for increasing Al resistance

It is estimated that, by the year 2050, world population will beraised by two billion people. To fulfill their demands like food, fibre, fodder and other animal feed, crop production will need to be increased by more than 50% and this can be done by increasing cropping intensity and yields, even in the challengingcultivating conditions like acids soil lands, unsuitable for farming.Acid soils are widely distributed in Asia, sub-Sarahan Africa and other regions where, there is heavy population.Thisproblem of acid soils is most severe in the humid tropics. In Colombia, for example, 70% of the agricultural land is acidic. Plant crops which shows high sensitivity to soil acidity (example; maize, soybean, cotton, etc.) are unable to grow well in the tropics (Fuente, *et al.*, 1997 and Barinaga, 1997).

As the land area cannot be increased and aerable land is limited, it is imperative to use acid soils for cultivation. Hence, transgenic crops are in great need for such areas.For enhancing Al tolerance, a wealth of studies can be sited in literature on the expression of genes in plants. For this, two different approaches have been taken: 1) Al-induced plant genes' expression and 2) expression of genes to increase organic acid production. However, it is doubtful that alteration in the single gene expression will confer high levels of Al tolerance, due to the complex physiological effect of Al on plant cells. Some studies have revealed that combinations of transgenes can increase Al tolerance in plants (Ezaki,*et al.*, 2001).

Moreover, it may also be possible to identify microbial genes that confer Al tolerance in their host and enhance Al tolerance in plants. For example, two genes, on encoding for an *Arabidopsis* blue copper-binding protein and other a tobacco putative GDP-dissociation inhibitor, have shown Al tolerance to yeast (Ezaki,*et al*., 2000).

3. Role of genetic engineering in Al tolerance

3.1Germplasm sources

Germplasm of some species (e.g. in barley and alfalfa) are limited for Al tolerance. Whereas some of the Al-tolerant crop varieties show a large number andmost of them are obtained and developed from highly acidic soils lands of the world (Hede, *et al*., 2001; Stodart, *et al.*, 2007; Caniato, *et al.*, 2011). For example, out of 250 bread wheat landraces, originated from 21 countries, 25 accessions were found to be Al tolerant and were collected from highly acid soils area of Nepal. Adaptation,natural selection orhuman selection by early agriculturalists might be the reason for such associations (Stodart, *et al.*, 2007; Caniato, *et al*., 2011). Therefore, it may be logical and appropriate strategy of collecting germplasms from acid soil areas for Al-tolerance species.

3.2 Genes for Al tolerance

To reduce Al stress, the identification of stress-regulated genes plays an important role (Abate, *et al*., 2013). Some of the Al stress regulated genes, for example, could play an important role against oxidative stress or in alleviating phosphate deficiency. It is to be noted that due to the precipitation with Al, nutrients deficiency, especially phosphate, takes place in plants(Houde and Diallo, 2008).As studied in many plant species like rye (*Secalecereale*), triticale (*Triticale* ssp.) and sicklesenna(*Cassia tora*), Al exposure induces Al-resistance related genes and proteins (Li,*et al*., 2000a, b; Ma,*et al*., 1997a).

In some cases, when Al-sensitive cultivar and Al-tolerant cultivar werecrossed, it was observed that resistance against Al toxicity is due to a single, dominant locus while two loci are responsible for resistance, in some other cases (Garnin and Carver, 2003). Wheat, in its inheritance, is one of the most and longtime studied plants for Al-resistance, and genetic studies have shown that Al-tolerance in wheat plant is controlled by multi-genes (Takagi, 1983; Aniol, 1990; Carver and Ownby, 1995).For example,wheat genotype- Atlas 66was reported, having a complex genetic mechanism with several genes for Al resistance(Berzonsky, 1992).Alt2 or$Alt_{BH}$is one of the loci in wheat (Milla and Gustafson, 2001), mapped on the long arm of chromosome-4D.

Rye (*Secalecereale*) hasalso shown an excellent resistance to abiotic stresses including Al, when compared to its close relative wheat(Aniol and Gustafson, 1984). In fact, a greater number of Al tolerant loci are detected than in wheat (Aniol and Gustafson, 1984; Gallego and Benito, 1997; Hede, *et al*., 2001). Another member of the sametribe, Barley (*Hordeumvulgare*), has also shown Al-resistance locus, Alp, on the long arm of chromosome-4 (Minella and Sorrells, 1992). Sorghum (S*orghum bicolor*

L. Moench) exhibits a simple pattern of inheritance with a single locus (Magalhaes, 2002; Magalhaes, *et al*., 2003). BnALMT1 and BnALMT2, two genes from rape (*Brassica napus*),have shown Al tolerance and homology to ALMT1 from wheat(Panda, *et al*., 2009). Al resistance in rice was identified under the control of two genes, *STAR1* and *STAR2* (Huang, *et al*., 2009).

Triticale is a hybrid, obtained by crossing wheat and rye synthetically, whose tolerance against Al toxicity is believed to be inherited from rye and is largely grown on acid soils in South America, Europe and Australia (Pfeiffer, 1993). It is believed that genes necessary for Al tolerance are carried on the short arm of chromosome 3R (Aniol and Gustafson, 1984). A Brazilian genetic study reveals that oat (*Avena sativa*) has only one or two dominant genes against Al with tolerance genotype carrying AlaAla (Ezaki, *et al*., 2000). Under Al stress, expression mechanism of two *GST* genes in Arabidopsis *AtGST1* and *AtGST11* was studied(Ezaki, *et al*., 2004).

In the early eighties, Luis Herrera-Estrella was the first to develop transgenic plants. Heacquainted the citrate synthase (CSb) gene in rice and corn (Barinaga, 1997).When transgenic CSb plants weregrown under extreme acidic conditionswith high aluminum concentration, lower root growth inhibition was found as compared to the untransformed plants. It might be because the citrate synthase produced by transgenic plants was preventing Al uptake (Prakash, 1997). Moreover, the Mexican scientists had introduced a bacterial CSb gene into tobacco and papaya and found these genetically engineered plants more tolerant to the insidious metal (Prakash, 1997).

Thus, transgenic plants may help in using the soils for cultivationthat were once inhospitable because of toxicity(Barinaga, 1997). This technology of genetic engineering seems to have great potential in enhancing agricultural yield and productivity, specially, in developing countries where there is urgent requirement of more food and where toxic effects of aluminum are at their worst; thus helps in fighting with problems of the real world.

4. Transformation

Production ofa successful and productive transgenic plant depends on 1)characteristics of the gene expression cassettes, 2)DNA deliver method into the recipient cellsand 3) tissue culture and selection techniques used to regenerate fertile plants from the transformed cells (Repellin, *et al.*, 2001). Systematic optimization of the above steps would help in the development of transformation protocols for plants. Certain criteria must be accomplished to demonstrate the transgenic nature of a plant(Potrykus, 1990) such as detailed information for phenotypic, genetic and molecularcharacterization of the primary transformant and its offspring. However, it is not mandatory that stable transmission and expression of the transgenic plant would always be achieved, but it is an essential factor for developing commercial cultivars.

5. Conclusion

More than 50% of the world's aerable lands are acidic. Al, in its cationic form, Al^{3+}, gets easily solubilized in this acidic soil, thus becomestoxic and represents plant growth inhibition asprimary factor in plants. Therefore,Al toxicity has become one of the active areas for researchglobally, to determine the mechanisms responsible for Al toxicity and tolerance.Presence of Al in plant root cells interfere a number of biological activities, thus altering cellular mechanisms. To cope up, traditional agricultural strategies, like, selection and breeding processes, may help in developing Al tolerant plant cultivars. Conventional plant breeding, along with classical cytogenetic techniques, represented the main method of cereal crop improvement. However, designing appropriate screening method remains a challenging task for characterizing Al tolerant species. A number of studies have been done to achieve the goal of developing crop varieties better suited for cultivation with Al toxicity in acid soil.In this regard, biotechnological tools have played an enormous role in providing suitable protocols for finding the genes from unrelated sources that become available to be introduced asexually into plants.Recombinant DNA techniques helped in the collection of different genes from microbes, plants and animals, some of which may be useful for crop improvement. Nutritional quality of rice and dough functionality in wheat have been improved by the use of biotechnological tools. Genomics, proteomics and bioinformatics; other tools of biotechnology, would also help in collecting the required information for the improvement of current cereal.

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